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Collagen for neural tissue engineering: Materials, strategies, and challenges

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ABSTRACT

Neural tissue engineering (NTE) has made remarkable strides in recent years and holds great promise for treating several devastating neurological disorders. Selecting optimal scaffolding material is crucial for NET design strategies that enable neural and non-neural cell differentiation and axonal growth. Collagen is extensively employed in NTE applications due to the inherent resistance of the nervous system against regeneration, functionalized with neurotrophic factors, antagonists of neural growth inhibitors, and other neural growth-promoting agents. Recent advancements in integrating collagen with manufacturing strategies, such as scaffolding, electrospinning, and 3D bioprinting, provide localized trophic support, guide cell alignment, and protect neural cells from immune activity. This review categorises and analyses collagen-based processing techniques investigated for neural-specific applications, highlighting their strengths and weaknesses in repair, regeneration, and recovery. We also evaluate the potential prospects and challenges of using collagen-based biomaterials in NTE. Overall, this review offers a comprehensive and systematic framework for the rational evaluation and applications of collagen in NTE.

1. Introduction

The mammalian nervous system is a highly complex physiological networks, consisting of the central nervous system (CNS) and peripheral nervous system (PNS). Damage to the nervous system significantly impacts medical care and the economy. The CNS, comprising the brain and spinal cord, exhibits a limited ability to regeneration due to the adult nervous system's inherent bias against neural differentiation and regeneration compared to the PNS [1]. In the CNS, the microenvironment that inhibits axon regeneration is more pronounced, and destroyed neuronal cell populations and disrupted synaptic connections tend to be irreversibly regenerative since mature neurons exhibit limited capability of axon outgrowth [2,3]. Medical treatments have failed to treat neuronal damage, which makes symptomatic relief the most reasonable solution for injury progression [4,5]. Axon regeneration in the CNS is adversely restricted after traumatic brain injury (TBI), spinal cord injury (SCI), and related circumstances involving fatal axonal disruption. On the contrary, PNS is prone to regenerate in certain cases to realize functional restoration, especially when the injury encompasses a relatively short distance [6]. When the damage occurs, an inhibitory microenvironment in the lesion sites will form to hinder axonal regeneration, in which case cell necrosis and synaptic disconnection are primarily irreversible since mature neurons tend to exhibit restricted regenerative potential in the CNS. Injury cascades of the CNS are further extensive to dimensional pathophysiological events such as ongoing demyelination, glial scar formation, ischemia, ionic dysregulation, and cystic cavitation [7]. In terms of the PNS, despite the optimistic regenerative capacity, functional restoration tends to be poor at the significant lesions in length (>10 mm) owing to the inadequate axonal bridging of distal targets [8]. Producing neurotrophins, and guiding axons toward their synaptic terminals by eradicating myelin debris, proliferative SC and macrophages restore neuronal function. However, the complication in CNS injury and regeneration is more pronounced. Wallerian degeneration takes place in the damage tract following the CNS injury. Nevertheless, since there was minimal macrophage recruitment after oligodendrocyte apoptosis, the

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Abbreviations

3D	Three-dimensional
BDNF	Brain derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BMMCs	Bone marrow mononuclear cells
CNS	Central nervous system
CSPGs	Chondroitin sulfate proteoglycans
DRG	Dorsal root ganglia
ECM	Extracellular matrix
EGF	Epidermal growth factor
ESC	Embryonic stem cell
FGF	Fibroblast growth factor
GAG	Glycosaminoglycan
GDNF	Glial cell derived neurotrophic factor
HA	Hyaluronic acid
HUVEC	Human umbilical vein endothelial cell
IL-1α	Interleukin-1-alpha
iPSC	induced pluripotent stem cell
NIPS	Non-solvent induced phase separation
NPC	Neural progenitor cell
NSC	Neural stem cell
NSPC	Neural stem/progenitor cell
NT-3	Neurotrophin-3
NTE	Neural tissue engineering
OPC	Oligodendrocyte progenitor cell
PCL:	Polycaprolactone
PD	Parkinson's disease
PNI	Peripheral nerve injury
PNS	Peripheral nervous system
SC	Schwann cell
SCI	Spinal cord injury
SDF-1α	Stromal-cell-derived factor 1α
TBI	Traumatic brain injury
TIPS	Thermally induced phase separation
TNF-α:	Tumor necrosis factor-alpha
VEGF	Vascular endothelial growth factor

degenerative myelin and axonal debris lasted significantly longer. Specific mechanism involved after the CNS and PNS injuries are summarized in Table 1.

The extracellular matrix (ECM) plays a pivotal role in maintaining the architectural backbone and regulating cellular crosstalk in the nervous system. Dynamic changes in normal and injured nervous tissues of ECM compositions could be potential guidance for biomaterial design in NTE. For CNS, the constitution of a typical brain ECM is distinct in its presence of a hyaluronan backbone to which chondroitin sulfate proteoglycans from the lectican family and tenascins are linked [9], which are secreted by neurons, astrocytes, oligodendrocytes and microglia residing in brain. Additional ECM constituents in the basement membranes that support the blood-brain barrier comprise laminins, collagen, fibronectin, and heparan sulfate proteoglycans produced by neurons, glia, and endothelial cells [10]. In response to TBI, certain ECM composition levels and distributions would alter. For instance, chondroitin sulfate proteoglycans (CSPGs), comprising lecticans, phosphacan, and NG2, are the most studied proteoglycan in the nervous system. In the context of TBI, aggrecan and phosphate content decrease in the injury core, while neurocan, Versican-V2, NG-2, fibronectin, Tenascin-C, Tenascin-R and osteopontin levels are relatively elevated. Moreover, certain immunoreactive molecules are activated such as laminin, perlecan, glypicans and syndecans [11]. In SCI, dynamic changes of ECM have been prevalently examined for comprehending how ECM components affect axonal restoration. It is acknowledged that specific ECM compositions play an inhibitory role in neural regeneration, especially chondroitin sulfate glycosaminoglycan-chains (GAG-chains) on proteoglycans at the lesion site. Other typically discussed scar components encompass those from fibrotic scars with basement membrane-like composition, such as collagen, laminins, and fibronectin. Furthermore, such core ECM proteins degrade in response to damage, such as matrix metallopeptidases and elastases [12]. Recent research has focused on cathepsins, another catalytic ECM-related protein group, in the spinal cord as potential ECM regulators, which serves as an illustration of the undiscovered roles in ECM along with associated proteins in CNS injury [13]. As for the PNS, ECM, secreted by Schwann cells, resides in the form of the basal lamina, which is composed of collagen type IV, laminin, fibronectin, and nidogens [14,15]. In the degenerated peripheral nerves, CSPGs and upregulated myelin-associated inhibitors of regeneration might hinder axonal outgrowth. Unlike the CNS injury, the distal portion of axons and myelin detritus are lysed and eliminated due to the activation of Schwann cells and the recruitment of macrophages, paving the way for removing inhibitory factors [16]. Notably, Collagen I, III, IV, V, VI and laminin exhibit significantly elevated deposition in Schwann and perineurial cells

Table 1
Injury mechanism and confronted repair challenges of the PNS and the CNS.

Scenario	Injury mechanism	Repairing challenges	Refs
PNS	1. Wallerian degeneration of the distal part of the axon resulting in fragmentation and disintegration of the axon in the first 18–48 h. 2. Proinflammatory cytokines secretion: tumor necrosis factor-alpha (TNF-α) and interleukin-1-alpha (IL-1α). 3. Removal of axonal and myelin debris by macrophages and SC. 4. Interleukin-1 (IL-1) and platelet-derived growth factor stimulates SC to divide, de-differentiate, and proliferate distal to the injury. 5. Stimulated SC to secrete growth factors such as nerve growth factor or insulin-like growth factor-1. 6. Band Büngner formation guiding axon and growth cones to reconnect from proximal end to distal end across the gap.	1. Long-distance regeneration (> 10 mm). 2. Re-establishment of appropriate connections between the periphery and the central nervous system.	[8, 44]
CNS	1. Wallerian degeneration in the damaged tracts. 2. Degeneration of myelin and axonal debris after the apoptosis of oligodendrocytes. 3. Inhibitory neurite growth molecules in myelin: Nogo-A, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein. 4. Glial scar formation is initiated by astrocytes with inhibitory molecules such as proteoglycans.	1. Modulation of inhibitory microenvironment for axonal regeneration in the lesion site. 2. Collaborative participation of distinct cell types for ideal axonal regeneration. 3. Aligned distribution of neuronal cells to form orientational nerve bundles. 4. Speeded reinnervation time being the most important determinant of successful clinical outcomes.	[45, 46]

during peripheral injuries and neuropathies [17].

Current medical interventions for treating neuronal damage are often inadequate and offer little more than symptomatic relief [18]. Even the most promising current strategies are limited in their ability to achieve complete damage repair or functional restoration due to a lack of understanding of neurodevelopment [19,20]. Animal models have limitations in representing human-specific features of neurodevelopment, and the use of human-induced pluripotent stem cells (hiPSCs) in a two-dimensional culture system fails to capture the complexity of the nervous system [21–24]. Therefore, various strategies have emerged to assist in neural tissue engineering, including applying specific biomaterials and integrating cells and bioactive substances to repair damaged nerves [25–32]. While these methods show promise, they require sophisticated architectural control and customization for more widespread application [33]. The fundamental principle of NTE is to create a biomimetic microenvironment that promotes neural tissue regeneration. Effective neural scaffolds in NTE should meet the following criteria: biocompatibility, controllable biodegradability, appropriate electrical conduction, an interconnected 3D porous architecture, and mimetic biophysical properties that aid in structural bridging and migratory cell guidance in lesion sites [34,35]. 3D scaffolds provide physical support for nerve regeneration, improving host tissue engraftment and enabling the regenerative tissue to attain cellular function [36–38].

The toolbox of conventional scaffold processing methods in tissue engineering includes gas foaming, freeze drying, melt moulding, electrospinning, phase separation, and rapid prototyping (RP) techniques, such as electrospinning and 3D bioprinting. Collagen, the most abundant protein in mammals, is a prevalent candidate for skin, cornea, cardiovascular, cartilage, and neural tissues for high biocompatibility and ease of manufacturing. In NTE, functionalized collagen electrospun mesh has been shown to promote the orientational migration of neural stem cells (NSCs) [39]. A tailored collagen scaffold also provided a robust platform for repair of spinal cord injury [40]. When integrated with additional bioactive components such as agarose [40], heparin sulfate [41], silk fibroin [42], and fibrin [43], it can have applications in drug screening and cancer modelling [33].

The processing techniques mentioned above have limitations in precisely altering scaffold geometry and inner channel architecture to guide cellular behaviours. In addition, these approaches restrict combined cellular participation in one-pot manufacturing, which post-cell-seeding processes could achieve. In contrast, 3D bioprinting offers the dominant advantages of customized scaffold dimensions and geometry involving various cell types, biomaterials, and growth factors. Furthermore, the selection of bioink in 3D bioprinting can regulate neuronal proliferation, differentiation, and migration in NTE. Although there are pros and cons to biofabricating neural tissues, this review will discuss collagen biosynthesis, extraction, and manufacturing approaches and evaluate current advancements in the utilization of collagen in NTE. This includes collagen scaffolds and bioprinting-mediated collagen processing in the context of neural injury repair, disease modeling, and drug screening based on neuroregenerative strategies (Fig. 1). Finally, we

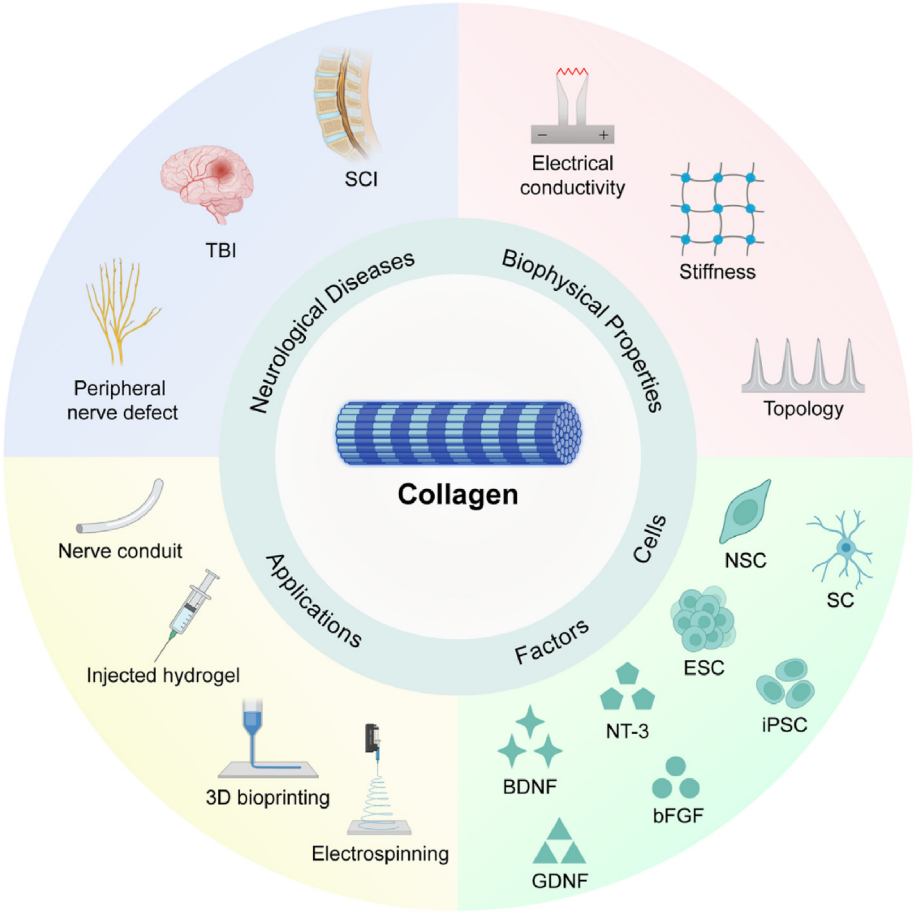

Fig. 1. Schematic drawing of collagen-based therapeutical applications, applying diverse strategies to regenerate CNS and PNS. bFGF: basic fibroblast growth factor; NT-3: Neurotrophin-3; BDNF: Brain Derived Neurotrophic Factor; GDNF: Glial Cell Derived Neurotrophic Factor; ESC: Embryonic stem cell; iPSC: induced pluripotent stem cell; SC: Schwann cell; NSC: neural stem cell; TBI: traumatic brain injury; SCI: spinal cord injury. Created with BioRender.com.

systematically review the present obstacles and possible future directions for collagen-based biomaterials for NTE. We aim to provide researchers with comprehensive knowledge and inspire thought-provoking ideas.

2. Collagen

Selecting appropriate materials with favourable physical, chemical, and mechanical properties for neural repair is indispensable because the porosity, mechanical properties, biocompatibility, and biodegradation can be highly decisive in achieving ideal repair effects for the nervous system. Various biomaterials have been applied in NTE including synthetic polymers, polysaccharides, and proteins. Table 2 summarizes current applied materials in NTE and its relative advantages and disadvantages. Collagen, the most abundant protein in mammals, accounts for approximately 25–30% of the total protein composition. Decades of studies have identified 28 distinct types of collagen, each composed of homotrimers and heterotrimers formed by three polypeptide chains (referred to as α-chains) [47]. The characteristic structural feature of all collagens refers to the triple helix - a tight right-handed helix of three α-chains, each of which comprises at least one region characterized by the repeating amino acid motif Gly-X-Y, where X and Y can be any amino acid [48]. Notably, approximately 80–90% of the collagen in the body primarily consists of collagen I, II, and III, also known as fibril-forming collagen [49]. Collagen I is the most widely distributed collagen in skin, bone, tendon, lung, and the vasculature network. The distribution of collagen II is comparatively restricted to cartilage, while collagen III is predominantly found in elastic tissues such as lungs and blood vessels [50]. The intricate hierarchical architecture determines its versatility as a building block in tissue engineering. Adaptation can fulfill a given function at all levels, resulting in a wide range of properties.

2.1. A glance of collagen: from biosynthesis to purification

In connective tissues, the fibroblast is responsible for most collagen production [68]. Our distinct processes are involved in the assembly of collagen fibrils after the transcription and translation of the procollagen α-chains are completed: (i) the importation of the rough endoplasmic reticulum, where triple-helical procollagen is sequentially formed after the modification of α-chains; (ii) the Golgi apparatus assists in the modification of procollagen, which is subsequently packaged into secretory vesicles; (iii) then extracellular cleavage of procollagen to generate collagen molecules; (iv) lysyl oxidase-catalyzed crosslinking of collagen molecules to reinforce their supramolecular structures [69–71]. Following the secretion of procollagen into the extracellular space, collagen-type-specific metalloproteinase enzymes cleave the amino- and carboxy-propeptides. Microfibrils of collagen characterize the extracellular matrix of connective tissues with a cross-striated structure [72]. Fibril-forming collagens are promising candidates for developing collagen-based biomaterials [73].

Collagen I is typically derived from pig, bovine, ovine skin, and tendon tissues for biomedical uses, whereas collagen II is mainly obtained from bovine, porcine, and chicken cartilaginous tissues. Rat-tail tendon-derived collagen was used in most preliminary collagen research due to its excellent purity and ease of extraction [74]. However, the covalently crosslinked fibrillar network restricts further applications despite the merits of excellent biocompatibility, easy availability, and high versatility. Various extraction and purification approaches are primarily carried out to tackle this challenge, including acidic solution, neutral salt, enzymatic, and alkali treatment. The main objective of these methods is to maximize the preservation of the supramolecular structure of collagen. A summary of the principles, advantages, and disadvantages of different collagen extraction methods is presented in Table 3.

2.2. Typical characterization of collagen and collagen-based scaffolds

Collagen is a thermal-responsive biomaterial that can gel in-situ at physiological temperatures and neutral pH, making it suitable for use as an injectable hydrogel [80]. However, since the absence of lysyl oxidase in vitro, the mechanical strength of spontaneously self-assembled fibrils tends to be poor under physiological conditions. Hence, appropriate crosslinking approaches are utilized to enhance tissue-matched mechanical properties and enzymatically degradable transplantation

Table 2
Advantages and disadvantages of biomaterials in NTE.

Polymer type	Polymer name	Advantages	Disadvantages	Refs.
Proteins	Collagen	1. High biocompatibility 2. Low antigenicity and cytotoxic response 3. Versatility	1. Poor mechanical properties and structural integrity in hydrogel form	[18,51,52]
	Gelatin	1. Good biocompatibility 2. Low antigenicity	1. Insufficient mechanical properties	[53,54]
Polysaccharides	Hyaluronic acid	1. High water content 2. Safe degraded products 3. Influence wound healing and metastasis	1 Lack of cell adhesion sites 2 Limited mechanical properties	[55,56]
	Alginate	1. Fast crosslinking property 2. Water solubility 3. Versatility in 3D bioprinting 4. Low cost	1 Limited biodegrading capability 2 Lack of cellular adhesive binding sites	[57,58]
	Chitosan	1. Biocompatibility and biodegradability 2. Drug delivery capacity	1. Potential toxic risks 2. Low cellular interaction 3. Limited solubility	[59,60]
Synthetic polymers	PCL	1. Biocompatibility 2. Ease of processability 3. Long-term mechanical stability	1. Potential cytotoxic effects of organic solvents 2. Limited bioactivity	[61]
	PLLA	1 High surface-to-volume ratio 2 High porosity	1. Limited bioactivity 2. Release of acidic products on degradation 3. Poor processability	[62,63]
	PLGA	1. Ease of functionalization 2. Biodegradability	1 Low bioactivity 2 Deformation on exposure to long-term strain	[64,65]
	PPy	1. Good conductivity 2. Cell adhesion properties 3. Non-mutagenic	1. Insolubility 2. Non-degradability 3. Poor processability	[66]
	Carbon nanotubes	1. Superior conductivity 2. High aspect ratio 3. Mechanical stability	1. Cytotoxicity 2. Non-biodegradability	[67]

Table 3
Summary of distinct approaches of collagen extraction.

Methods	Principles	Advantages	Disadvantages	Refs.
Acidic treatment	After lipid and protein removal, the sample is immersed in a diluted acidic solution, in which case, salt bonds between collagen molecules are broken, allowing the collagen to be solubilized. Acid-soluble collagen is obtained following salinization, dialysis, and other processes.	1. Moderate processing temperature 2. Preservation of intact triple helix structure. 3. Capability to maintain excellent biological properties.	1. Preservation of telopeptides resulting in a high risk of immunogenicity. 2. Log extraction time. 3. Low yield.	[75, 76]
Enzymatic treatment	The protease is used to restrictively cleave the terminal peptide of collagen. The remaining body part is soluble in neutral or acidic solutions, resulting in the extraction of enzyme-soluble collagen.	1. Maintenance of intact triple helix structure. 2. High bioactivity. 3. Shortened reaction time. 4. High collagen purity. 5. Satisfying physiochemical properties.	1. Risk of immunogenicity.	[77, 78]
Genetic engineering	The technology of designing, enzymatic cleavage, and splicing of human collagen genes with specific sequences, ligating vectors and then transferring them into engineered cells, and expressing the produced collagen and its analogs by fermentation. Different expression systems, such as *E. coli*, yeast, insect cells, and transgenic crops, are involved.	1. Higher bioactivity 2. Better water solubility 3. Lower risk of immunogenicity and pathogens 4. No cytotoxicity 5. Higher denaturation temperature 6. Ease of transportation and storage	1. Long process 2. High technical difficulty 3. High cost 4. Low yield.	[79]

scenarios in vivo. The basic principle of the crosslinking reaction involves modifying amine and carboxyl groups of collagen molecules, resulting in the formation of covalent bonds [68].

In tissue engineering applications of collagen-based biomaterials, immunogenicity is inevitably considered. Collagen is generally reckoned as a safe biomaterial for regenerative utilization due to low immunogenicity. Even though animal derivation may provoke an immune response, collagen is still categorized as a weak antigen [81]. Non-collagenous proteins [82], cells, and crosslinker residues mentioned may also trigger immune response [83]. Research has verified that terminal non-helical regions, known as telopeptides, might also cause an immunological response [84]. To minimize the immunological effects of collagen, telopeptides could be cleaved via proteolytic enzyme treatment, and an appropriate collagen source and extraction process can also be selected [52].

Collagen can promote cell adhesion and intracellular crosstalk with other cell types [85], leading to a cascade of cellular activity, including activating receptors as well as inducing gene transcriptions in dimensional signalling pathways in neural cells [86,87]. Regarding collagenous participation in neural cells in NTE, a previous study has demonstrated that embryo-derived neural stem cells cultured on collagen can differentiate into mature neurons with neuronal polarity and neurite outgrowth [88]. Furthermore, collagen 3D culture has also exhibited remarkable chemotropism in differentiated neurons [89].

3. Collagen-based biomaterials in the application of NTE

As one of the most competitive natural biomaterials, collagen beacons have a promising future in the therapeutic application of NTE, such as injury repair and functional restoration. Collagen-based biomaterials have been prevalent in coping with therapeutic dilemmas in NTE, such as neuroprotection and neuroregeneration. Collagen can be processed into scaffolds, microspheres, nerve conduits, and injectable hydrogels (Fig. 2). No matter what approach mentioned above should meet the following requirements: (i) providing structural bridging in lesion sites and cell-favourable microenvironment for neuronal proliferation and differentiation to facilitate axon outgrowth; (ii) releasing growth factors to stimulate neural cells to regenerate, especially in neurodegenerative diseases such as Alzheimer's, Parkinson's diseases, or spinal cord injury; (iii) encapsulate neural-related cells to accomplish therapeutic efficacy. This section will focus on the multitude of preparation formats of collagen comprising collagen scaffolds, fibrous or tubular conduits, micro/nanoparticles, and integrative collagen bioink for the regenerative solutions of NTE.

3.1. Collagen scaffolds

Recent advancements in tissue engineering have led to the development of various techniques for fabricating collagen scaffolds. Freeze-drying processing was pioneered by Yannas et al. to create porous collagen-glycosaminoglycan scaffolds, which could be packed with therapeutically active chemicals that are released as the scaffold degrades [97]. Other customized features of scaffolds include thermal-responsiveness, pH-sensitivity, and controlled drug release in injury sites [98,99].

Collagen scaffolds have shown promising outcomes in CNS repair by preventing inflammation, excitotoxicity, and oxidative stress, shielding cholinergic neurons in Alzheimer's disease, and excluding toxic plaques from the brain [100–104]. In the case of SCI, conventional stem cell therapy is limited, and physical bridging is necessary for isolated cells to functionalize in the injury site. Collagen-based scaffolds integrated with NSCs have been successful in SCI repair by ameliorating neural outgrowth and axon reconnection, and these could be integrated with highly aligned micro-channeled scaffolds. Several studies have investigated the use of native collagen, dehydrothermally crosslinked collagen implants supplemented with laminin, or EGFR inhibitors to enhance the regenerative process of adult rat NSCs and neural progenitor cells (NPCs) for SCI healing [105–108].

Collagen has been approved by the Food and Drug Administration (FDA) for clinical usage in peripheral nerve tissue repair, as it has been shown to promote nerve regeneration in various nerve scaffolds (NeuraGen [109] and Neuromaix [110]) and nerve cuffs (NeuroWrap and NeuroMend) with modulated degradation rates varying from months to years [101]. In addition, collagen scaffolds could be modified with functional proteins or growth factors to enhance nerve repair further. For instance, NGF combined with collagen scaffolds has successfully regenerated nerve gaps ranging from 5-mm to 35-mm in various animal models [6,111–113].

The selection of ideal cell sources for therapeutic neural regeneration is vital. Strategies for CNS regeneration emphasize on the transplantation of NSCs or differentiated neural cells integrated with growth factors and glial cells, which can foster neuronal differentiation in a permissive

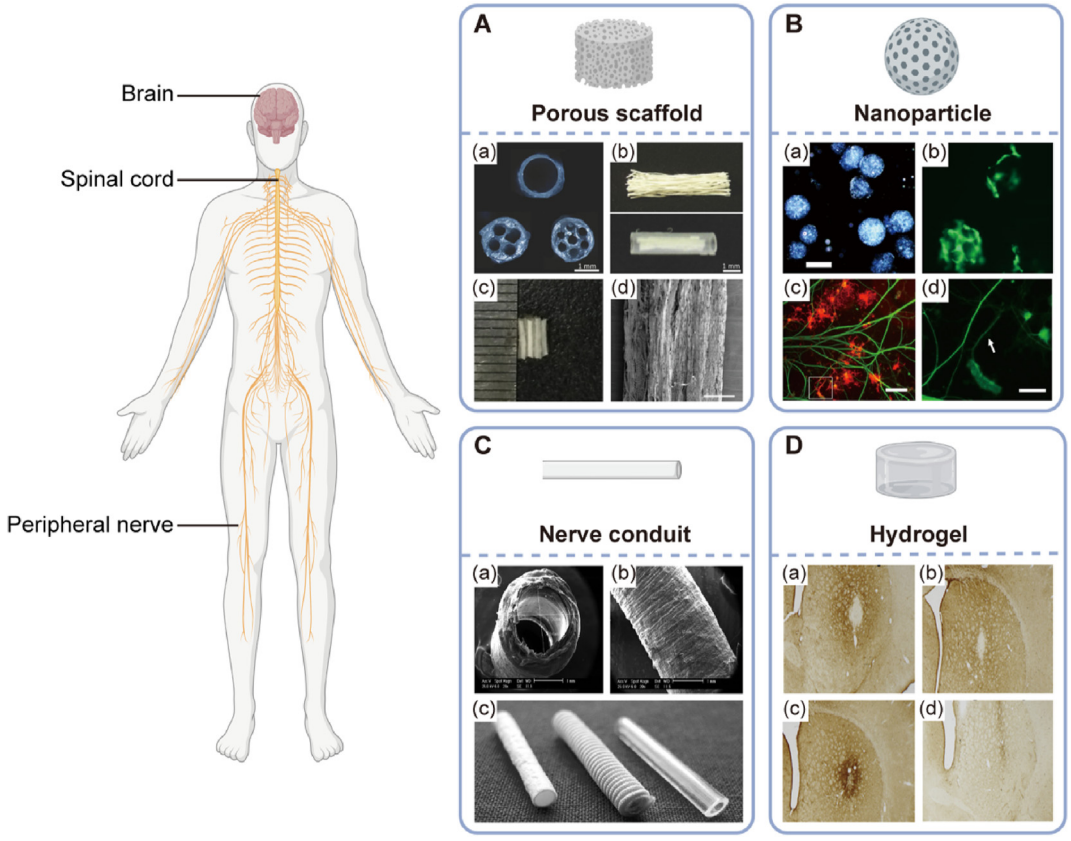

Fig. 2. Forms of collagen-based biomaterials in CNS and PNS repair application. A: Collagen porous scaffold. (a) Multichannel collagen scaffolds as peripheral nerve conduits with 1, 4, and 7 channels, respectively. Reproduced with permission. [90] Copyright 2010, Elsevier. (b) Linear ordered collagen scaffold fibers within a silicon tube. Reproduced with permission. [91] Copyright 2011, Elsevier. (c and d) Modified collagen scaffold in acute spinal cord injury repair. Reproduced with permission. [92] Copyright 2017, Elsevier. B: Collagen microsphere-based cell carrier in oligodendrocyte progenitor cell growth and differentiation. (a) Fabricated collagen microspheres. Scale bar: 200 μm. (b) 3T3 cells expanded on collagen microspheres were labeled with Calcein. Scale bar: 200 μm. (c) Co-culture of dorsal ganglions (DRGs) with oligodendrocyte progenitor cells (OPCs). Scale bar: 200 μm. (d) Magnified image depicting oligodendrocytes (OLs) wrapping DRG neurites in an arrow. Scale bar: 50 μm. Reproduced with permission. [93] Copyright 2013, Springer. C: Nerve conduits in peripheral nerve repair. (a and b) Schematic overview of electrospun collagen-based peripheral nerve conduits. Scale bar: 1 mm. Reproduced with permission. [94] Copyright 2017, Wiley-VCH GmbH. (c) Commercial peripheral nerve conduit. Left to right: Collagen I conduit; polyglycolic acid conduit; poly (DL-lactid-ε-caprolactone) conduit (Neurolac; Polyganics BV, Groningen, The Netherlands). Reproduced with permission. [95] Copyright 2009 Georg Thieme Verlag KG. D: Collagen hydrogel in rat brain recovery. A striatal microgliosis response to the implanted cells encapsulated in collagen hydrogel was detectable in the host striatum. Reproduced with permission. [96] Copyright 2013, Elsevier.

manner. As a result, the CNS and PNS responses to damage are noticeably different, and any therapeutic strategy must be tailored in accordance with this. Typically, stem cells, including iPSCs, ES, NSCs, NPCs, OPCs, and MSCs, are familiar cell sources in the CNS regeneration. In this context, stem cells are induced to differentiate in specific neuronal lineage for axonal outgrowth.

On the other hand, in PNS regeneration, SCs, astrocytes and MSCs are competitive candidates. SCs, glial cells in the PNS, are the most typically used cells, primarily aiming to support axons by releasing growth factors and isolating axons via forming the myelin sheath [114]. Besides, SCs can deposit extracellular components such as laminin and type IV collagen in addition to growth factors, which is conducive to PNI repair [115].

3.2. Manufacturing techniques of collagen-based biomaterials in NTE

Tissue engineering components, such as scaffolds and integrated technologies, have been established over time, with diverse methodologies being evolved to construct complex structures from a wide range of natural and synthetic polymers. The goal has generally been to create ideal scaffolds matching nervous tissue's physical, chemical, and mechanical features. These scaffolds should possess appropriate properties such as pores, fibres, and channels from nano to micro-scale, which are critical for NTE applications. The versatility of collagen primarily relies on different forms of collagen, including sponges, conduits, nano/microspheres, hydrogels, and nano/microfibers, which could be processed to meet specific requirements for the CNS and PNS repair. Notably, collagen is commonly fabricated as scaffolds via conventional methods (freeze drying, gas foaming, solvent casting, etc.), moulding and texturing methods (fiber mesh, photolithography, laser texturing, etc.) and rapid prototyping (microsphere sintering, 3D bioprinting, electrospinning, etc.). Conventional approaches for constructing collagen offer the advantages such as ease of operation, tunable pore size, and low cost. But they have limitations in achieving sophisticated architectures with customized sizes on the microscale and macroscale. In contrast, rapid prototyping (RP), also known as solid free-form fabrication [123], is categorized as 3D bioprinting, stereolithography, and selective laser sintering. Various processing strategies, including electrospinning, 3D bioprinting, and their combinations, have advanced significantly. These techniques facilitate control over scaffold uniformity and physical properties (i.e., porosity, hydrophilicity, hydrophobicity, and swelling ratio) and enable cell-biomaterial interaction within a favourable 3D microenvironment in a customized manner [124]. In this section, we reviewed current RP strategies in applying NTE. Table 4 summarizes the characteristics of the collagen-based manufacturing strategies used in NTE, the advantages and disadvantages of each method, and their current applications in NTE.

Table 4
Scaffold manufacturing strategies and relative applications in NTE.

Strategies	Preparation method	Advantages	Disadvantages	Application	Ref.
Freeze-drying	Polymers dissolved in a specific solvent	1. Ease of operation 2. Maintenance of bioactivity without temperature elevation 3. Tunable porosity	1. High energy cost 2. Endurable experimental duration 3. Compromised surface topology	1. Repair of particular brain trauma 2. Collagen-based SCI repair 3. Nerve grafts in peripheral nerves	[116]
Electrospinning	Solution of natural or synthetic polymers	1. Efficacy in preparing micro-/nana-scales fibrous mesh 2. High porosity and surface area to volume ratio 3. Precise control of fibre alignment 4. Convenient post-processing	1. Requirement of the high voltage field 2. Potential toxicity of the solvent	1. Guidance for directional migration of NSCs 2. Sheet rolling process for fabricating nerve conduits in PNS repair	[117]
Extrusion-based bioprinting	Cells encapsulated in homogenous, viscoelastic bioink	1. Combination of multiple types of cells, bioink, and growth factors involved in 3D milieu 2. Customized size control	1. Limited bioprinting resolution (about 100 μm)	1. In vivo neural analysis 2. SCI repair	[118, 119]
Inkjet bioprinting	Dispersion of low-viscous droplets in a controllable manner	1. High printing speed (1–10,000 droplets per second) 2. High resolution (50 μm)	1. Low cell density ($<10^6$ cells/ml)	1. Fabrication of functional neural constructs	[120, 121]
Stereolithography	Biodegradable, biocompatible polymers	1. 3D architectural integrity 2. High accuracy and resolution (25 μm) 3. No cost of possibly fabricating waste	1. Resins with cytotoxic residues 2. Costly specialized equipment	1. SCI repair	[122]

3.3. 3D bioprinting of collagen-based bioinks

The field of neural tissue engineering is continuously incorporating innovative technologies that allow researchers to precisely control functional simulation at the cellular level in vitro and simulate the development, function, and regeneration of the nervous system with better precision. 3D bioprinting is an additive manufacturing process in tissue engineering and regenerative medicine. Computer-aided design models are employed to generate 3D constructs as a prototype of biofabricating tissue or organ constructs. Notably, 3D bioprinting is one of the most prominent TE technologies that employs biocompatible hydrogels to construct precisely architected scaffolds to facilitate cellular responses. Compared to other processing techniques, one of the dominant advantages lies in the spatially and temporally efficient collaboration of bioactive molecules, distinct cell types, and dimensional biomaterial, also known as bioink in this scenario. Lee et al. were the pioneered groups in implementing collagen-based biomaterials vis 3D bioprinting technique to fabricate neural constructs [125]. Currently, there are various bioprinting approaches for NTE, including inkjet bioprinting, stereolithography (SLA), laser-assisted bioprinting, and extrusion-based bioprinting, in which specific application requirements vary for distinct demands. However, for the specialized collagen-based NTE, inkjet bioprinting and extrusion-based bioprinting have garnered the most enthusiasm amongst 3D bioprinting techniques [126]. This section outlines the manufacturing fundamentals and applicable scenarios in functional simulation and damage repair of neural tissue engineering related to collagen-oriented bioprinting approaches.

Inkjet bioprinting utilizes thermal, piezoelectric, and electrostatic printing principles to propel bioink in a controlled manner, allowing for precise and consistent cell deposition in micrometre resolution [120]. The high resolution (50 μm) of inkjet bioprinting is a significant advantage of superiority [127]. Additionally, the nozzle diameter narrows down the volume per drop to 10 pL, implying the concentration for cell seeding could be relatively high (> 5 million cells/mL) [128]. Correspondingly, narrowed nozzle size may bring about nozzle clogging; hence, bioink with low viscosities is required, which can affect the mechanical properties of the fabricated constructs. Additionally, insufficient viscosity results in cell damage due to thermal or mechanical stress [129–131]. Xu et al. developed a composite bioink formulation consisting of collage I and fibrin, where NT2 cells were encapsulated to fabricate a 3D cellular sheet via inkjet bioprinting. The printed neural construct displayed physiological viability, as evidenced by immunostaining and patch-clamp analysis [132]. Lee et al. conducted a study on bioprinting a multilayered "ring-cross" 3D neural pattern using rat embryonic neurons and astrocytes embedded in crosslinked collagen through sodium bicarbonate [133]. Similarly, Y.B. Lee et al. developed an engineered neural bioprinting platform that utilized a hybrid bioink composed of collagen, fibrin, and vascular endothelial growth factor (VEGF) to encapsulate murine neural stem cells. The results demonstrated that bioprinting of VEGF-containing fibrin promoted supportively sustained growth factor (GF) release in the collagen scaffold [134].

Extrusion-based bioprinting is the most prevalent bioprinting methodology, which employs pneumatic or mechanical (piston or screw) dispensing mechanisms to form homogenous bioink strands [121]. This method involves utilizing a syringe to print viscous bioink in a layer-by-layer fashion, which is superior owing to permitting a variety of biomaterials encapsulated with distinct cell types and growth factors in one-pot [118]. However, the printing resolution is limited to approximately 100 μm [127]. Apart from that, nozzle diameters, correspondent shear stress, and applied pressure could affect cellular viability, which could be tackled by optimizing printing parameters [119]. Chen et al. encapsulated embryonic NSCs in collagen I, heparin sulfate loaded with basic fibroblast growth factor via extrusion-based bioprinting for neural in vivo analysis [135]. Jiang et al. developed rat NSCs-encapsulated collagen and silk fibroin bioink recipe for SCI repair, in which post-transplanted reduction of glial scars, axon outgrowth, and the functional restoration of the spinal cord was significantly observed [136] (Fig. 3A).

3.4. Electrospinning of collagen-based nerve conduits

Electrospinning is a traditional manufacturing methodology that utilizes a high-voltage electric field to precisely control the deposition of natural and synthetic polymers onto a particular collecting substrate. This technique can fabricate fibrous meshes with customized fibre sizes ranging from several microns to 100 nm or less [137]. Due to remarkable efficiency, low cost, and ease of handling, electrospinning has been broadly applied in tissue engineering for the indispensable merit of manufacturing microfibers consistent with the scale of ECM. In this scenario, nano-sized fibers could be biophysical guidance for cell orientational migration. Electrospun collagen-based biomaterials take effect in two aspects: (i) providing topographical guidance as a biophysical cue for promoting aligned axonal outgrowth; (ii) functionalizing collagen scaffolds with neurotrophic biomolecules to preferably ameliorate traumatic

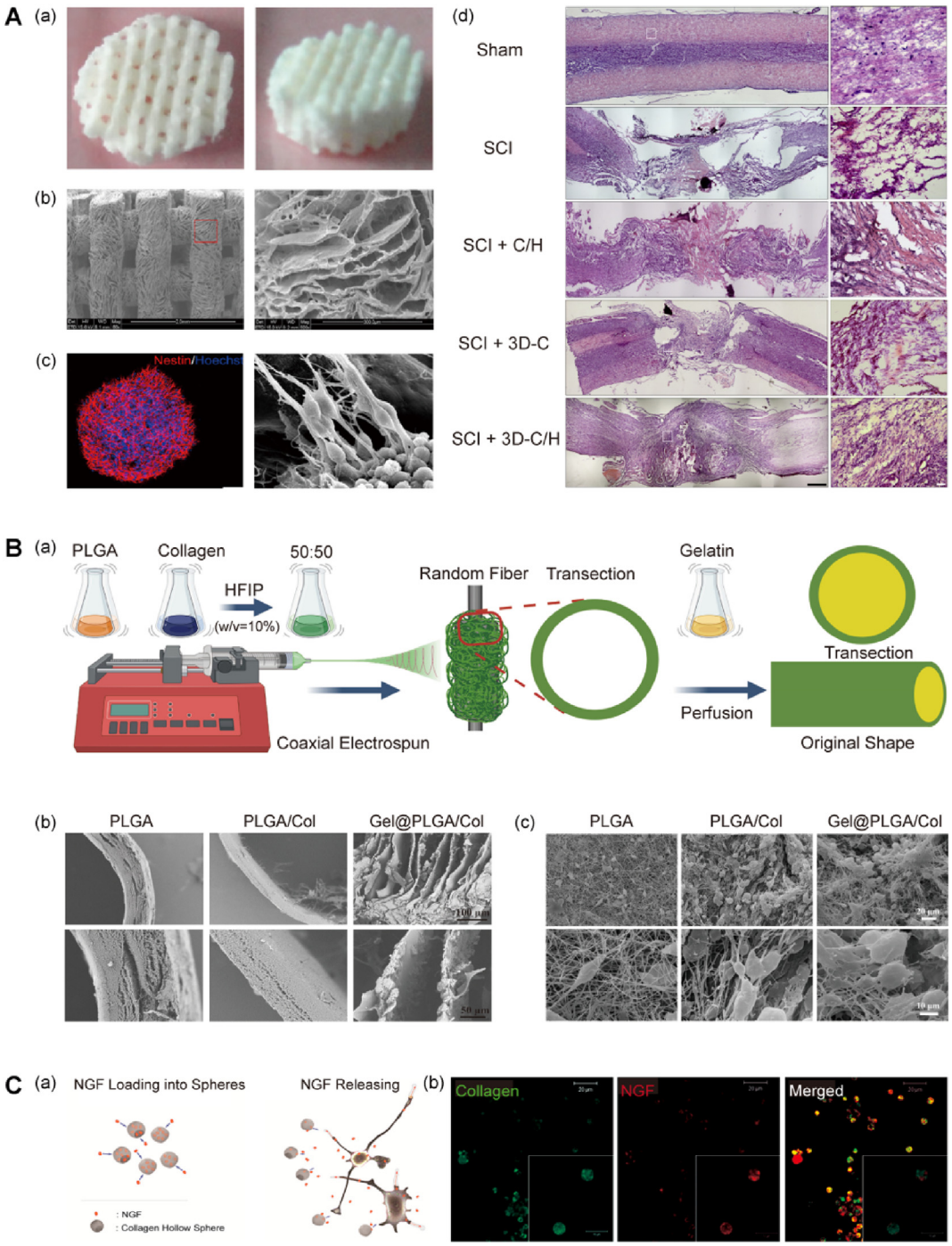

Fig. 3. Integration of collagen with advanced technologies application for neural tissue engineering. A: 3D bioprinted collagen/heparin scaffold ameliorated SCI repair. (a) Photograph of collagen/heparin construct (3D-C/H). (b) SEM image of porous 3D-C/H. (c) Left: NSC neurosphere was counterstained with Nestin (red) and Hoechst (blue). Right: SEM image of NSCs cultured on 3D-C/H. Scale bar: 50 μm. (d) Histological analysis of HE staining at 8 weeks post-injury. Higher magnification of white boxes on the left was displayed in the lesion site. Scale bar: 50 μm. Reproduced with permission. [135] Copyright 2017, Wiley-VCH GmbH. B: Sponge-reinforced electrospun PLGA and collagen-based conduit in peripheral nerve repair. (a) Graphical depiction of fabricating process of the PLGA and collagen-based conduit with gelatin perfusion (Gel@PLGA/Col). (b) Cross-section of the fabricated collagen-based conduit. SEM images of SCs cultured on nanofibrous meshes are displayed (c). Reproduced with permission. [138] Copyright 2022, Elsevier. C: Growth factor loaded collagen hollow spheres for treatment platform in neuroregeneration. (a) Graphical abstract of collagen spheres in NGF releasing pattern. (b) Confocal images of NGF-loaded collagen microspheres. Collagen spheres, green; NGF, red. Reproduced with permission. [143] Copyright 2013, American Chemical Society.

injury. For instance, Li et al. originated an electrospinning formula where collagen was functionalized with stromal-cell-derived factor 1α (SDF-1α), creating a controllable biological gradient from the rostral and caudal ends. By optimizing electrospinning parameters, radially aligned collagen/polycaprolactone (PCL) hybrid scaffolds were obtained to guide the aligned migration of NSCs, providing a robust platform for effectively guiding nerve regeneration [39]. Electrospun nanofibers could be manufactured in sheet rolling fashion to prepare nerve conduits. Zhao et al. fabricated fluffy sponge-reinforced electrospun conduits for peripheral nerve repair. The Gel@PLGA/Col conduit exhibited promising nerve

regeneration performance in a sciatic nerve defect implantation model with a large gap (Fig. 3B) [138]. When synthetic polymers are combined with aligned nanofibers, 3D nanofibrous nerve conduits with aligned nanofibers offer an optimum cell contact guidance (CCG) for axonal regeneration [139]. Besides, Kim et al. demonstrated that collagen fibres with extra topographical guidance promoted cell proliferation and neurite outgrowth along the fibres, reinstating neuronal regeneration in injury situations [140]. Liu et al. evaluated the feasibility of applying electrospun collagen I nanofibers for SCI repair. They found that neurites originated from dorsal root ganglia (DRG) migrated by the aligned pattern of electrospun collagen microfibers. These results implied that collagen could be conducive to infiltrating neuronal cells followed by neurofilament sprouting in the further strategic intervention of SCI [141]. Additionally, Zhang et al. constructed a 3D scaffold by employing an aligned electrospun fiber bundle as the inner core to dictate the aligned regeneration of axons biophysically, while sheathing collagen matrix in the outer layer to controllably release glial cell-derived neurotrophic factor (GDNF) and multiple microRNAs to rebuild neural circuitry functionally [142].

3.5. Collagen microspheres for the delivery of bioactive factors

Collagen could be processed into various configurations with therapeutic effects, such as membranes (e.g., DuraGen) and micro/nanospheres. Due to the satisfying surface-to-volume ratio, microspheres can encapsulate a greater quantity of medicine. Besides, the small size makes them easier to administer in vivo by stereotaxic surgery to a specific spot in the brain. In particular, collagen hollow microspheres have a capacity of stably and reproducibly loading growth factors involving nerve growth factor (NGF) [143] (Fig. 3C) or vascular endothelial growth factor (VEGF) [144]. In Parkinson's disease (PD), the loss of dopamine cells in the substantia nigra results in the degeneration of nigro-striatal axons. Hence, nigro-striatal regeneration route is of vital significance in PD, where collagen microspheres might serve as an effective platform for drug delivery. McRae et al. demonstrated that dopamine-loaded microspheres injected into the striatum successfully promoted neurofilament development in the striatum and functional restoration [145]. In a rat PD model, it has been recapitulated that intra-striatal implantation of GDNF-releasing microspheres increased dopaminergic neurofilament sprouting and motor function rehabilitation [146].

4. Fundamental design criteria in neural tissue engineering

One of the main challenges in neural tissue engineering (NTE) is to mimic the biological and mechanical properties of the native extracellular matrix (ECM) microenvironment. In the native tissue in vivo, cells are embedded in ECM, a highly dynamic, 3D fibrillary network comprising collagen, proteoglycans, glycoproteins, and a myriad of bioactive ligands. ECM offers an architectural scaffolding for the maintenance of tissue integrity and involves the crosstalk with residing cells, regulating diverse functions, including proliferation, migration, and differentiation, which is vital for cellular homeostasis [147]. Notably, collagen-based biomaterials are broadly employed in CNS and PNS repair as distinct scaffolds and hydrogels. Generally, parameters for therapeutic implanted biomaterials must comply with the following criteria: (i) mechanical properties that match the soft neural tissue (brain tissue is basically with a stiffness of approximately 0.5 kPa [148]) to avoid further damage to the surrounding host tissue after implantation. Scaffolds utilized for tissue bridging should support cell attachment, development, and differentiation; (ii) bioactive molecules that promote axonal regeneration and lesion bridging; (iii) electrical conductivity that enhances neural cell growth and differentiation. Besides, multi-strategies mediation for fabricating neural constitutes have been prevalent to meet dimensional requirements in diverse scales. For example, combining nanotexturing and micro-texturing can realize controlled neuron patterns in nano/micro scales [149]. Solvent casting/particulate leaching could be assisted with moulding techniques in PNI repair [150]. Furthermore, non-solvent-induced phase separation (NIPS) and microprinting could be synergized to construct nerve guides [151]. It is also reported that integrating injection moulding and thermally induced phase separation (TIPS) is effective in building nanofibrous scaffolds [63]. Besides, multi-microtubule chitosan conduits could be fabricated using novel molds and TIPS [152]. As for PNS and SCI repair, researchers constructed single-lumen and multiple-lumen nerve conduits loaded with NGF via the combination of moulding, gas foaming, and particulate leaching fabrication techniques [153]. This section will concisely elaborate on critical criteria in current designing requirements and the latest applicable instances of NTE.

4.1. Mechanical properties

Based on CNS, the brain and spinal cord are two of the most compliant tissues in the human body. Hence, implanted constructs must match the compliance of native neural tissues. Meanwhile, the endurable capability is required for bearing load forces in the motions of the spinal cord. Regarding mechanical properties, ideal transplants should be qualified to maintain structural integrity and matched conformation in the lesion cavities to eliminate potential cysts or fibrotic tissue formation. Previous studies have shown that growth cones exert traction force and respond to substrate rigidity-induced mechanical stress [154]. Soft substrates promoted neurite development and branching in CNS neuronal cells, while rigid substrates supported astrocyte growth [155]. Specifically, K Saha et al. revealed that under the mixed differentiation cultured conditions of NSCs with serum, softer gels (~100–500 Pa) greatly favoured neurons, while stiffer gels (~1–10 kPa) supported glial cultures [156]. Additional research discovered that spinal cord neurons on more rigid gels outspread more primary dendrites but shorter axons (30 kPa) [157]. The substrate stiffness-dependent axon outgrowth and traction forces of PNS (DRG) and CNS (hippocampal) neurons were identified to be distinct. Accordingly, neurite elongation from DRG neurons was maximal on substrates whose Young's modulus is approximately 1 kPa, whereas hippocampal neurites were independent of substrate stiffness [158].

Furthermore, immune-related mechanosensitivity of glial cells and foreign body reactions were investigated to confirm that compared to a substrate with a physiologic stiffness level (~100 Pa), a substrate with a shear modulus of 10 kPa remarkably triggered the overexpression of inflammatory genes and proteins between primary rat microglial cells and astrocytes (~100 Pa). Comparatively, a stiffer *in situ* implant induced considerably higher levels of glial cell activation and associated inflammation than a softer implant. Adjusting the mechanical stiffness of neural implants to counterpart native neural tissue may diminish negative responses and thus enhance biocompatibility, according to these findings [159].

The stiffness of the applied scaffolds must be finetuned to resemble native tissue and potentially activate suitable biological responses, such as adhesion, differentiation, or proliferation. Multiple strategies for differentiating stem cells into neural tissues have been reported, for instance, formulating a hydrogel recipe with an elastic modulus ranging from 1 to 10 kPa [160,161]. Besides, the elastic modulus of the spinal cord ranges between 200 and 600 kPa; thus, matching these variables can be advantageous for optimizing cellular biocompatibility and differentiating preferences [162]. Recent research aimed at inducing spinal cord axonal regeneration employed poly (2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel tubes with elastic moduli precisely fine-tuned to counterpart the characteristics of the native tissue *in vivo* (400 kPa). The excellent elastic modulus of biomaterials for peripheral nerve regeneration is approximately 0.45 MPa [163]. The latest research has employed hydrogels with elastic moduli comparable to native tissues. Additionally, the elastic modulus of applied hydrogels could be flexibly tuned by controlling the degree of crosslinking [164–166].

4.2. Biochemical properties

Consideration of the biochemical properties of scaffolds is essential due to their direct interaction with cells, which can regulate cellular behaviours. In the context of NTE, modification of scaffolds with bioactive molecules or compounds specific to neural tissue has been widely applied. These modifications have been used to optimize the functionality of injected hydrogels and bioinks in 3D bioprinting, which can enhance tissue functionalization by promoting controllable cell growth and differentiation. Lately, various growth factors integrated into the functionalization of biomaterials in NTE, including neurotrophin-3 (NT-3), fibroblast growth factors (FGF), basic fibroblast growth factors (bFGF), epidermal growth factors (EGF), brain-derived neurotrophic factors (BDNF), and glial cell-derived neurotrophic factors (GDNF) [167,168]. Incorporating bioactive molecules or compounds boosts the crosstalk of encapsulated cells with stimuli, inducing tissue-specific cellular functions and mimicking the biochemical environment of native tissues. Various modification instances of collagen-based biomaterials have been recapitulated above. For example, Sharma et al. identified that integrating guggulsterone-encapsulated drug-releasing microspheres into the bioink could stimulate neuronal differentiation in 3D bioprinted constructs [169,170]. This study highlights the potential for further application of growth factors in combination with advanced manufacturing techniques for sophisticated control over cell behaviours mediated by microcarriers and 3D bioprinting.

4.3. Electrical conductivity

The use of electrical stimulation has been shown to promote neural regeneration, making the use of electrically conductive polymers in scaffold fabrication particularly attractive for NTE. The mechanical, biochemical, and electrical features of constructed conduits regulate the efficacy of neural regeneration. Transmitting electrical signals throughout the nervous system is the essence of neurons and has a profound impact on the efficacy of neural maturation and further functional performance [171]. To achieve the electrical conductivity of fabricated scaffolds, pre-blending and post-coating are two main strategies. For instance, for the aim to enhance electrical conductivity, researchers have integrated polypyrrole, a conductive polymer, into the composite scaffolds, which was accomplished either via direct inkjet printing of polypyrrole and collagen [172] or by employing a stereolithography-based 3D bioprinter to generate silk fibroin scaffolds which were successively coated with polypyrrole through electrospinning [173,174]. Joint investigations reflected on the neurite elongation, axonal regeneration, and remyelination resulting from the additives of conductive polymers. Therefore, the fabricating methodologies of considering electrical conductivity are pivotal in NTE. Although numerous *in vitro* approaches for electrical stimulation have been used, the clinical administration of electrical stimulation by applying conductive scaffolds has not yet succeeded. Moreover, exploiting the combined advantages of novel manufacturing techniques should be considered for precise control of well-designed neural regeneration [174].

5. Therapeutic applications of collagen-based biomaterials in neural tissue engineering

With the advent of tissue engineering methodologies, the management of neural nerve injuries involving the destruction of axonal tracts has undergone a paradigm shift. When conditions such as PNI, SCI, TBI, or neurodegenerative disorders occur, the intricate neural architecture is altered, resulting in neurite growth inhibition and the loss of long-distance guidance. Collagen has been identified as an excellent candidate for addressing these challenges in NTE. In addition, by incorporating dimensional manufacturing approaches, axonal regeneration is enhanced to achieve functional recovery resulting from neurodegenerative disorders. Collagen has been extensively applied in dimensional therapeutic treatment; hence, this section aims to investigate therapeutic instances categorized by SCI injury, PNI, and TBI, which might serve as potential references for further progress in NTE (Table 5).

5.1. Traumatic brain injury

Traumatic brain injury (TBI) is defined as short- or long-term damage to the brain resulting from external mechanical forces, such as accelerating, decelerating, and rotating forces, causing direct physical disruption of the neural tissues [185]. Loss of cerebral parenchyma after TBI is a substantial barrier to functional restoration. There is currently no clinically proven method of restoring cerebral parenchyma loss brought by TBI. A penetrative TBI (such as one caused by a gunshot or stabbing) causes an irregularly shaped lesion site in the brain which the natural healing procedures are inadequately to manage. The primary trauma results in significant tissue damage and swelling that may require a craniotomy to treat. Numerous growth inhibitory substances are released during this period, including particular proteoglycans and other compounds exposed due to the collapse of the myelin sheaths and surrounding supporting cells, all hindering the axon regeneration procedure. Therefore, scientists continuously examine which therapeutic interventions can aid the transition of the inflammatory response toward the reparative profile. Since the CNS is more intrinsically complicated than the PNS, the implanted biomaterial must shift the inhibitory microenvironment to pro-regenerative one [186]. Type I collagen plays a crucial role in the development of the dura and pia maters and has emerged as a promising candidate for interventions in brain injury [187–189]. As a hydrogel, collagen can retain a significant amount of water molecules. Hydrogels are highly compatible with the mechanical properties of soft nervous tissue and are, therefore, crucial for restoring the CNS [98,190]. Typically, collagen hydrogel gelates in physiological conditions, which forges a promising path of applying collagen as injectable hydrogels in TBI repair. Collagen fibres incorporated with alginate hydrogel were loaded with iPSCs-derived neurons, where neuronal maturation and the formation of neural networks were observed [191]. Moreover, collagen-chitosan scaffolds loaded with MSCs were formulated to facilitate motor functions in a rat TBI model [192]. And another research revealed that functional hyaluronate collagen scaffolds exhibited efficacy in inducing NSCs differentiation into functional neurons in restoring the TBI [193].

Studies have shown that the implantation of collagen glycosaminoglycan scaffold (collagen-GAG) post-TBI traumatized areas is significantly smaller compared with those in the blank group. Significant DCX+ and NeuN + cells validated the presence of migrating neuroblasts and mature neurons, contributing to the functional restoration of TBI (Fig. 4A) [182]. Hoban et al. investigated GDNF-overexpressing MSC in the encapsulation of collagen gel for intra-striatal transplantation, confirming that microglial cells are reduced in the graft zone and recruiting astrocytes without compromising cell viability and GDNF secretion [96]. Zhong et al. employed hyaluronan-heparin-collagen-polyethene glycol diacrylate composite hydrogels as a “cargo carrier” loading neural progenitor cells (NPCs) to the necrotic stroke cavity, resulting in significant cell viability compared to conventional cell delivery [177]. Marta et al. formulated a semi-interpenetrating network (semi-IPN) consisting of hyaluronic acid (HA), gelatin microparticles, and collagen; when loaded with Tat-Hsp70, a neuroprotective effect is observed in the dopaminergic destruction [194]. Moreover, ventral mesencephalon grafts encapsulated in GDNF-loaded collagen scaffold significantly elevated dopaminergic cell viability, striatal re-innervation, and functional regaining [195]. This technique potentially facilitates graft longevity for future stem cell transplants in cell replacement treatments for neurodegenerative illnesses, where collagen is an appropriate biomaterial choice.

Several challenges need to be resolved to maximize the potential of collagen-based biomaterials in TBI repair. Given biodegradation and dynamic tissue remodelling, longer-term research is also essential for a more profound knowledge of how transplanted and host cells interact

Table 5
Summary of collagen-based strategies in neural tissue engineering applications. (NA: Not applicable).

Approach	Collagen-based formula	Crosslinking method	Cell type	Cell density	Cell viability	Application	Ref.
Drop-on-demand bioprinting	Agarose and collagen I	–	1. MSCs 2. HUVECs 3. Human bone marrow-derived epithelial neuroblastoma immortalized cells	1. MSCs: 1×10^6 cells mL^{-1} 2. HUVECs: 3×10^6 cells mL^{-1} 3. Neuroblastoma cells: 1×10^6 cells mL^{-1}	NA	Cancer modeling	[175]
Electrohydrodynamic jet printing	Collagen I and fibrin	EDC, NHS, $CaCl_2$ and thrombin	NSPCs	3×10^5 cells mL^{-1}	NA	SCI repair	[176]
Extrusion-based bioprinting	Collagen I, heparin sulfate, and basic fibroblast growth factor	UV cross-linking	Embryonic NSCs	NA	In vivo analysis	SCI repair	[41]
	Collagen and silk fibroin	Solidification	Rat NSCs	1×10^7 cells mL^{-1}	NA	SCI repair	[136]
Hydrogel injection	HA/heparin/collagen	Polyethylene glycol diacrylate	ES-derived NPCs	1.4×10^7 cells mL^{-1}	NA	Stroke recovery	[177]
	Collagen I sponge	–	Rat 14-day-old-embryo brain-derived NSCs	1×10^5 cells mL^{-1}	NA	Rat cerebral ischemia	[178]
	Collagen I	4S-StarPEG	MSCs	1×10^7 cells mL^{-1}	>90%	Brain cell graft platform	[96]
Microsphere	The porcine skin-derived collagen solution	Water-soluble carbodiimide	HUVECs	–	Capillary formation	Sustained release of VEGF	[144]
	Collagen I	EDC	1. OPCs 2. DRG	1. 1.5×10^4 cells/well 2. NA	~90%	Neurite myelination	[93]
Electrospinning	PCL/Collagen	–	1. DRG explants 2. SCs	NA	~90%	Nerve implants	[179]
	PCL/Collagen	–	1. Astrocytes 2. Human astrocytoma cell line	NA	NA	CNS repair	[180]
Tethered conduit	Collagen I	Thermal	1. Human HUVECs 2. SCs	1. 4×10^6 cells mL^{-1} 2. 5×10^5 cells mL^{-1}	NA	PNI repair	[181]

with collagen. Besides, practical guidance and modulation of endogenous and transplanted cell fates in collagen-based scaffolds may facilitate cell survival and enhance TBI regeneration. Furthermore, the incorporation of anti-inflammatory growth factors in a controlled manner could be pivotal in modulating the post-injury microenvironment. Thus elevating the longevity and functionality of implanted cells would be achievable. Finally, in-depth comprehension of stem cell research, regeneration mechanisms of TBI, and crosstalk of diverse cell types with collagen-based biomaterials would be conducive to the enrichment of biomaterial design principles for biomimetic biofabrication for the CNS.

5.2. Spinal cord injury repair

Spinal cord injury (SCI) is a complex traumatic disorder frequently resulting in lifelong motor and sensory dysfunction, which is a tremendously catastrophic disability among CNS traumatic disorders. Pathological activities comprising axon disruption, glial scar formation, ongoing demyelination, cystic cavitation, cell necrosis, and the long-lasting immunological storm would hinder post-injury neuronal regeneration, which is commonly irreversible [7,196]. Current strategic interventions have been employed in treating SCI, but no effective solutions for regenerating axonal outgrowth and functional restoration exist. The failure of SCI repair lies in establishing an inhibitory microenvironment, glial formation, and demyelination around the lesion site, which fundamentally impairs further axonal regeneration. Synergistic collaboration of various methodologies has seen extensive repair solutions, composed of biomaterial scaffolds transplantation as a physical provision for axonal outgrowth, modulation of neurotoxicity and immune response, and introducing neurotrophic growth factor to ameliorate glial scar formation [197–199].

Collagen has been applied in SCI repair to provide a neural-favourable microenvironment for cell proliferation and differentiation. Reproducibility and versatility of collagen were achieved by various processing approaches. Han et al. formulated a modified collagen scaffold with neuroprotective proteins for SCI repair (Fig. 4B) [183]. Ma et al. loaded collagen sponge immobilized with (bFGF) encapsulating NSCs, identifying significantly accelerated proliferation, which could be a pioneered robust platform for growth factor-functionalized collagen--scaffold-based repair strategy targeted to SCI [200]. Liu et al. explored the role of topological cues in SCI repair. Electrospun collagen nanofibers were utilized for culturing rat astrocytes and DRGs, observing astrocytes and neurites form DRGs aligned along the nanofiber axes responsive to the topographical guidance cues. Consequently, the hemi-sectioned SCI rat model was utilized to rationalize the repair outcome of electrospun collagen microfiber, where neural fiber infiltration towards the scaffold was characterized, and acute inflammatory repones were significantly downregulated [201].

The chief obstacles in SCI interventional strategies are the inhibitory microenvironment post-injury, where it is more pronounced that NSCs are prone to differentiate into glial cell lines instead of neurons in the NSC-mediated repair approaches. In SCI repair strategies, functionalizing collagen scaffold with bioactive substances, such as neural-favourable growth factors (i.e., bFGF [202], NT-3 [203], BDNF [204], GDNF [205], etc.), the antagonist of myelin-inhibitors (i.e., cetuximab, Fab fragment of cetuximab [206], etc.), and therapeutic molecules (i.e., Taxol [207], paclitaxel [208], etc.) is a relatively superior treatment for SCI repair. Li et al. modified a collagen scaffold with cetuximab, an EGFR inhibitor, to regulate NSC differentiation in the SCI model [107]. Consequent research adopted the identical modifying method to ameliorate the SCI repair of dogs [108]. Notably, Hatami et al. revealed that human embryonic stem cells (hESCs)-derived neural progenitor cells (NPCs) loaded collagen scaffolds enhance the recovery of motor functions along with targeted migrations towards the lesion cavity [105]. Composite scaffolds functionalized with growth factors, such as collagen/chitosan, are also formulated. In a study pioneered by Liu et al., bFGF-loaded collagen/chitosan (CCS/bFGF) scaffolds transplanted into

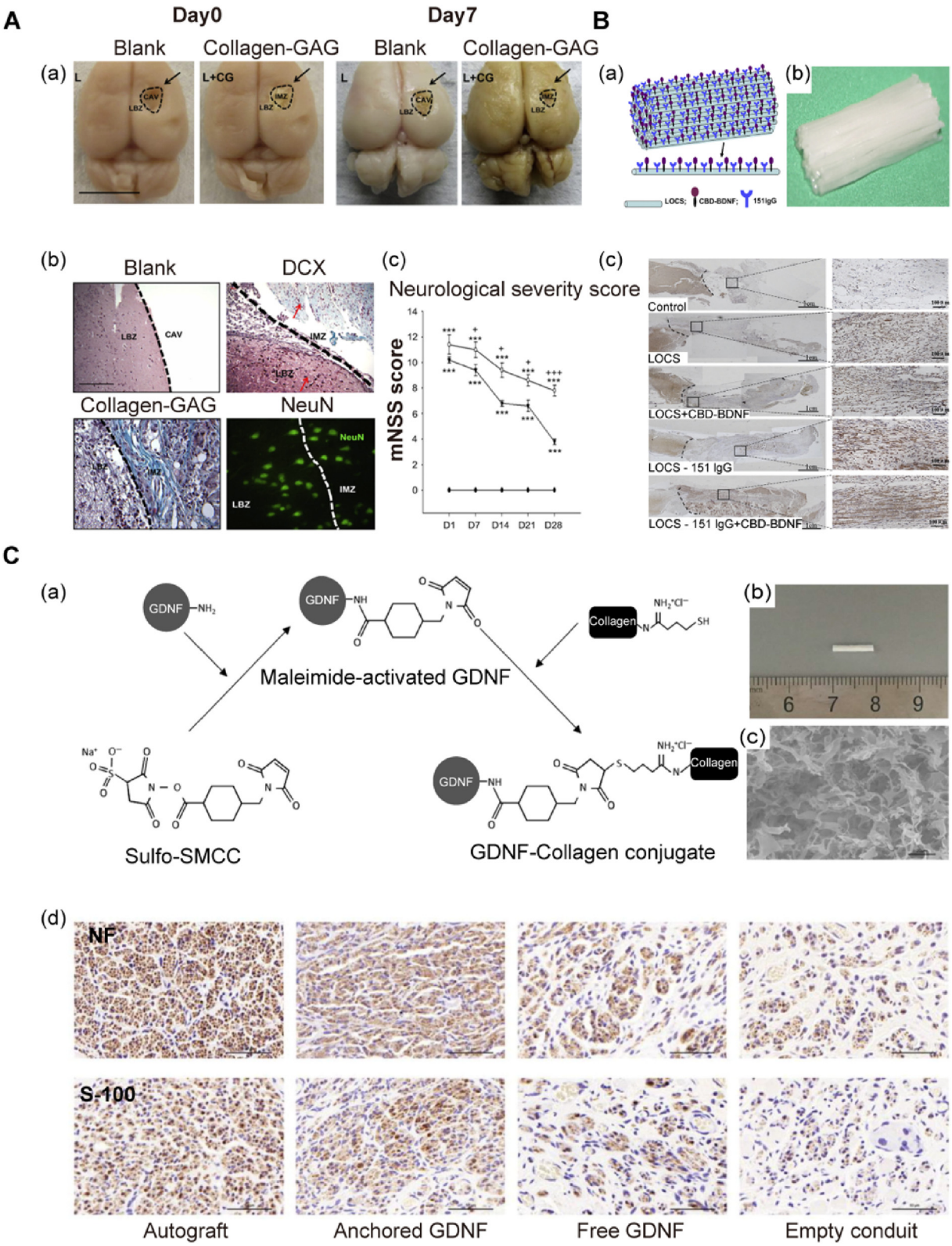

Fig. 4. Appropriate collagen-based biomaterials in neural tissue engineering. A: Collagen-based biomaterials ensure neurogenesis and functional restoration post-implantation. (a) Morphological repair effect in lesion site after collagen-GAG implantation. Scale bar: 1 mm. (b) Cell phenotypical identification in regenerated tissue. Scale bar: 75 μm. (c) Neurological severity was modulated post-collagen-GAG implantation. GAG (glycosaminoglycan), LBZ (lesion boundary zone), IMZ (intra-matrix zone), and CAV (cavity). Reproduced with permission. [182] Copyright 2012, Elsevier. B: Functionalized collagen scaffold in spinal cord injury repair. (a) Schematic illustration indicating functionalizing logic of the scaffold with neuroprotective efficacy. (b) Image of the scaffold with highly ordered, aligned micro-channel. (c) The histological evaluation proved neural regeneration in the lesion cavity after scaffold implantation. Reproduced with permission. [183] Copyright 2010, Elsevier. C: Neurotrophic factor-immobilized collagen conduits in peripheral nerve regeneration. (a) Schematic drawing of functionalizing collagen with GDNF. (b–c) Morphological display of the collagen conduit. (d) Immunohistochemical evaluation of regenerated nerve at 6 and 12 weeks after injury. Nerve fibre (NF) and S-100 were stained to identify axons and Schwann cells. Scale bar: 50 μm. Reproduced with permission. [184] Copyright 2018, Elsevier.

the T10 complete transection of the SCI rat model were identified to promote neural regeneration and axonal myelination at the lesion region [202]. Zou et al. investigated the potential of NSPC-based treatment in bridging lesion sites by transplanting exogenous neural stem progenitor cells (NSPCs) to replace necrotic cells. Human fetal brain and spinal cord-derived NSPCs were implanted through an aligned collagen sponge scaffold into a completely transected rat model. Comparing the two types of foreign cells, the spinal cord-derived neural stem progenitor cell-aligned collagen sponge scaffold (NSPC–ACSS) was noticeable in sustaining cell survival, neuronal maturation, and establishing a more constructive SCI milieu by suppressing inflammation and glial scar formation [209].

Recently, the role of biomaterials in regulating cell behaviours and modulating axonal inhibitory microenvironments has been getting more attention in NTE. Adhesive, stretchable, and SDF1α/paclitaxel spatiotemporal delivery collagen-fibrin (Col-FB) fibrous hydrogels were formulated to harness endogenous neural stem/progenitor cells for SCI repair. This aligned hybrid hydrogel also mimics the mechanical properties and topological features of the native spinal cords [176]. Besides, the covalent interaction between biomaterials and cells has also been integrated into SCI repair. Metabolic azido-labeled hNPCs conjugated on dibenzocyclooctyne-modified collagen fibres could significantly enhance cell adhesion, spreading, differentiation, and longitudinally aligned topological guidance cues in the drug-targeting context compared with non-covalent interaction [210].

Moreover, in the latest study, hNPCs were induced to differentiate into various dorsal and ventral neuronal cells on collagen scaffolds in vitro. Transplantation of human embryonic spinal cord-like tissues with dorsal and ventral neuronal characters into complete SCI models in rats and monkeys was verified to have significantly better therapeutic efficacy than undifferentiated hNPCs [211]. Furthermore, the synergistic incorporation of different cell sources is another promising fabrication window for enhanced SCI repair. Researchers have fabricated novel centimetre-scale human spinal cord neural tissue constructs with human spinal cord NPCs and astrocytes combined with a linearly ordered collagen scaffold. In this case, astrocytes could facilitate NPC adhesion and neurite outgrowth on the collagen scaffold, forming a linearly ordered spinal cord-like structure comprising mature neurons and glia cells [212].

In total, collagen scaffolds were generally functionalized to optimize binding specificity and efficacy with various bioactive and therapeutic compounds, such as growth factors and neurotrophic agents, or loaded with different cells, including human embryonic, mesenchymal, and neural stem cells. These scaffolds facilitated axonal regeneration by stimulating cell differentiation and reducing glial scar formation and astrogliosis. Moreover, functionalized collagen scaffolds enhance neuronal differentiation with neurite reconnection, displaying significantly elevated locomotor function, which reflected SCI recovery.

5.3. Peripheral nerve defect repair

Axonal regeneration is more pronounced in PNS than CNS following damages or diseases [52], in which Schwann cells (SCs) are essential for the functional repair post-injury, comprising proliferation, differentiation, migration, and potential remyelination. Additionally, immunological situations, such as recruitment and polarization of macrophages and controlled release of growth factors, are also crucial in the regenerative stages of PNS [213]. In response to peripheral injury, the endoneuria fibroblasts correspondingly secret collagen I, which aims to provide mechanical stabilization for further axonal regeneration [214,215]. Collagen has been demonstrated to be indispensable in PNS repair, positively regulating cellular behaviors of SCs and leading to rehabilitative conditions of PNS [216].

Ma et al. designed functionalized collagen conduits for peripheral nerve regeneration, facilitating sustained delivery of neurotrophic factors (Fig. 4C) [184]. Previous research has revealed that collagen V regulated SC adhesion and migration by functionalized N-terminal domain with heparin, facilitating cytoskeleton assembly and tyrosine phosphorylation of SCs [217,218]. Besides, the controlled release of collagen VI facilitates macrophage recruitment with the polarization of macrophages to the M2 phenotype, which was identified in the functional restoration route of sciatic nerve injury [219]. Given distinct processing approaches, collagen could be versatile in hydrogels, filaments, and films designed in multiple neural conduits. In vivo research employed dense, anisotropic collagen gel scaffolds encapsulated SCs demonstrating potential axonal regeneration and vascularization in repairing the 10 mm-gaping bridge of the rat sciatic nerves within 4 weeks [220]. As for collagen filaments in PNS repair, novel methodology of fabricating linear-ordered collagen (LOC) fibers [204] and functionalization of neurotrophins with the collagen-binding domain (CBD) or laminin-binding domain (LBD) [91] have seen significant success in restating neurite bridging and functional recovery in rodents and large animals [51,113,221,222]. Blending collagen with synthetic polymers or engineering topographical guiding cues could better boost axonal extension in a more controlled and reproducible manner [140]. As for the neural films, Zhuang et al. constructed a heterogeneous double-layered collagen film composed of loosely arranged collagen in the inner layer and compacted collagen fibres in the outer layer, which guaranteed intact maintenance of nerve conduits without the infiltration of foreign tissues [54]. Cerri et al. designed nerve conduits with stably gradient pores, collaboratively facilitating aligned outgrowth of neural cells, using a similar fabricating principle [223].

5.4. Drug screening and disease modelling

Traditionally, animal models have been employed to simulate the pathogenic invasion of neurological disorders. Nonetheless, these models do not faithfully recapitulate the complexity of human illness manifestations. Also, current in vitro disease models fail to elucidate dynamic crosstalk between normal cells and residing milieu. Particularly, a variety of neurological conditions afflict patients with diverse characteristics. For instance, glioblastoma (GBM) is the most prevalent malignant brain carcinoma in adults, whereas neuroblastoma (NB) is a pediatric brain tumor in the sympathetic nervous system. Recent advances in collagen-based hydrogels manufactured via 3D bioprinting have made breakthroughs in modelling pathological features in the process of tumorigenesis, especially in interpreting cell-cell and cell-matrix interactions. A recent study by Duarte Campos et al. [224] reveals that 3D bioprinted alginate-collagen I construct could serve as a platform in the NB disease model. Specifically, the scaffolds are loaded with NB cells, mesenchymal stromal cells, and human primary umbilical vein endothelial cells, followed by the physiological assessment of cellular behaviours. As a result, the NB cells formed Homer Wright-like rosettes, typical characteristics of NB tumours. Furthermore, cancer cells in collagen-based bioink maintain proliferative capabilities and secret Vimentin-rich matrices. Collective results could elucidate the platform's feasibility in precision medicine for cancer-targeted drug screening. Curtin et al. [225] formulated collagen-based scaffolds of two categories involving collagen-glycosaminoglycan (Coll-GAG) and collagen-nanohydroxyapatite (Coll-nHA). By applying two NB cell lines in the two composite collagen-based scaffolds, researchers identified the proliferation of NB cells exhibited >100-fold elevated resistance to cisplatin treatment compared with conventional 2D cultures, demonstrating the feasible functionality in the validation of miRNA-based gene delivery. Collectively, the research empowers the potential of collagen-based scaffolds in the chemotherapeutical evaluation and targeted therapies.

Liu et al. [226] displayed that ovarian cancer cell lines cultured in collagen I hydrogel scaffolds progressively transformed into multicellular spheroids with high cell viability and significantly increased expression of epithelial to mesenchymal transition (EMT) markers, including vimentin, fibronectin, and N-cadherin. In addition, the collagen-based culture system displayed a considerable activation of the Wnt/b-catenin and TGF-/Smad signalling pathways in the induction of

EMT. Microfluidics device is a prevailing instrument for simulating cellular behaviour. Anguiano et al. [227] proposed a microfluidics device loaded with collagen-Matrigel hydrogels to investigate the migration of lung cancer cells in distinct microenvironments of carcinoma invasion. The collagen-based microfluidic apparatus increased lung cancer cells' migratory profiles and dynamics.

Consequently, collagen-based hydrogels integrated with a microfluidics system would benefit the research of cancer invasion mechanisms in diverse contexts and the identification of effective cancer-targeted medications. Besides, collagen-based biomaterials could be processed into fibrous scaffolds to model several cancer types. Murakami et al. [228] fabricated collagen IV-coated fibrous silica sheets to culture squamous carcinoma cells, exhibiting highly improved cell proliferation and invasive profiles. Fabrication of fibrous scaffolds is a pivotal tool for elucidating cell-ECM crosstalk in a biomimetic milieu, further facilitating the in-depth mechanistic research of tumour progression. Apart from the applications in solid tumours, collagen-based composite scaffolds are typically employed in hematologic malignancies research. Collagen I-coated PCL scaffolds were seeded by acute lymphoblastic leukaemia (ALL) Jurkat cells. In this case, elevated cell proliferation and the typical response of drug-resistive reactions to daunorubicin and cytarabine were observed compared with bare PCL scaffolds [229]. Collagen-coated porous PLGA microspheres (PPMS) were used as microcarriers in lung cancer cell line co-culture. Results collectively indicated cell adhesion and proliferation in the PPMS-mediated microcarrier co-culture system; besides, anti-cancer drugs, including doxorubicin, cisplatin, curcumin, paclitaxel, etoposide, and gemcitabine, were applied to evaluate the potential of responsive drug resistance [230]. Consequently, collagen-PPMS could be a robust in vitro platform for lung cancer modelling, especially in drug screening. Similarly, hydroxyapatite (nHA) nanoparticles orchestrated with collagen I have progressed in the osteosarcoma microenvironment's biomimicry [231].

In summary, collagen-based biomaterial modalities exert prominence on cancer modelling and anti-cancer drug screening while selecting appropriate 3D systems combined with advantageous techniques to address specific issues remains challenging in the field of NTE [232]. Moreover, the heterogeneity of patient-derived cells and tumour microenvironment might hinder the reproducibility of 3D scaffold systems for recapitulating the efficacy of cancer-targeted therapeutics.

6. Evaluation of collagen-based biomaterials for NTE

Successful application of collagen-based biomaterial systems in NTE requires technical characterising using different techniques. For functional restoration, neuronal reconnection is the crucial prerequisite for the evaluation basis. Moreover, as the nervous system regulates locomotor activities of the body, physiological assessment post collagen implantation plays an indispensable role in functional evaluation. Notably, immunochemistry is essentially utilized for the visualization of neuronal reconnection. In this context, biomarkers such as Tuj1, NeuN, and MAP2 can indicate neuronal differentiation and axon outgrowth, while GFAP is counterstained for characterizing glial scars in the lesion sites (Fig. 5A). More importantly, physiological analysis has been extensively adapted to assess the therapeutic efficacy of collagen transplantation. Li et al. validated that paclitaxel-liposomes modified collagen microchannel scaffold resulted in electrophysiological and locomotor recovery via electrophysiological analysis. Motor evoked potential results, and Basso, Beattie, and Bresnahan (BBB) locomotor scale method could be effective in evaluating hindlimb locomotion of SCI models (Fig. 5B) [233]. For peripheral nerve repair, Masson's trichrome method and toluidine blue staining serve as versatile tools in the histological evaluation of peripheral nerve regeneration. Immunofluorescence and immunohistochemical identification of regenerated axons are also applied in the quality assessment of peripheral nerve outgrowth (Fig. 5C) [234].

7. Future perspectives and challenges

Collagen is a biomaterial with various advantages over other candidates for use in neural tissue engineering (NTE), owing to its remarkable biocompatibility, biodegradability, versatility, reproducibility, and low immunogenicity. Collagen can be applicable in multiple forms, including hydrogel-involved injected formulation utilized in traumatic brain injury and stroke, lyophilized scaffolds employed in spinal cord injury repair and peripheral nerve conduits, as well as function-enhanced hydrogels developed in cancer modeling and drug screening. Typically, collagen-based biomaterials can be controllably processed via dimensional manufacturing techniques, such as 3D bioprinting and electrospinning. More significantly, specific functionalization is conducive to re-establishing a neural-favourable microenvironment. Collaborative efforts are exerted to realize the supreme goal of repair, regeneration, and recovery. As for the technological advancement in NTE, a scaffold-free 3D bioprinting approach, also known as the Kenzan bioprinting method, emerged as an effective tool in neural regeneration, which is applied in mimicking neuronal network formation [235,236], PNI nerve regeneration [237], and the understanding of neuropathology [238]. Notably, a review recapitulates the construction of 3D neural tissue models from the perspective of spheroids and bioprinting in distinct application scenarios [239]. However, this method is relatively restricted to the bioprinting resolution and the incorporation of bioactive factors in a controllable manner compared with scaffold-based bioprinting strategies. Basically, neural regeneration requires a favourable microenvironment assisted with the growth factor additives for cell outgrowth and migration. As for neural repair in the lesion site, the scaffold-based formulation provides mechanical support and biochemical cues to facilitate neural outgrowth against axonal inhibitory microenvironment. Despite the noteworthy progress made in collagen-based interventions in NTE, the ultimate goal of translating these products from bench to bedside has not yet been fully achieved. Standardized extraction and neutralization procedures have been established in experimental and commercial settings, but these processes can be costly and time-consuming. To further advance the use of collagen-based biomaterials in NTE, promising future perspectives include stem cell-based gene therapy, drug screening, brain-computer interfaces, 3D bioprinting, and stimuli-responsiveness. (Fig. 6). Investigation of these aspects can open a new chapter in NTE and confirm the therapeutic potential of collagen-based biomaterials.

The post-hydration stage of hydrogel formulation may compromise sufficient mechanical strength and structural maintenance for further translational applications in tissue engineering. Therefore, finding benign, inexpensive, and productive methodologies as alternatives presents a challenge. Currently, collagen extracted from bovine tendons, bovine skin, porcine skin, and rat tail tendons is the primary source of collagen. However, there is no specific evidence of the differences and potential impacts on encapsulated cell behaviours in the context of 3D culture. As for further translational research, possible heterogeneity resulting from diverse collagen sources should be eliminated via in-depth analysis. More significantly, collagen originating from animal sources may be risky for disease transmission. Bovine spongiform encephalopathy (BSE) induced by ruminant collagen for human consumption has been investigated as a featured example [240]. Also commonly employed in dental surgery are bovine-derived collagen grafts, which might pose hazardous transmission of prion to patients [241]. Besides, allergic reactions caused by multi-species-derived collagen products should be considered. Specific individuals may suffer from anaphylaxis after taking marine collagen supplements, which could be the same as collagen from other sources [242]. Gelatin, the primary ingredient in the measles, mumps, and rubella vaccinations, is a derivative of bovine-derived collagen I. It is reported that children with anaphylaxis exhibited sensitivity to the vaccines mentioned above, in which scenario bovine-derived gelatin could be responsible for the incidents [243].

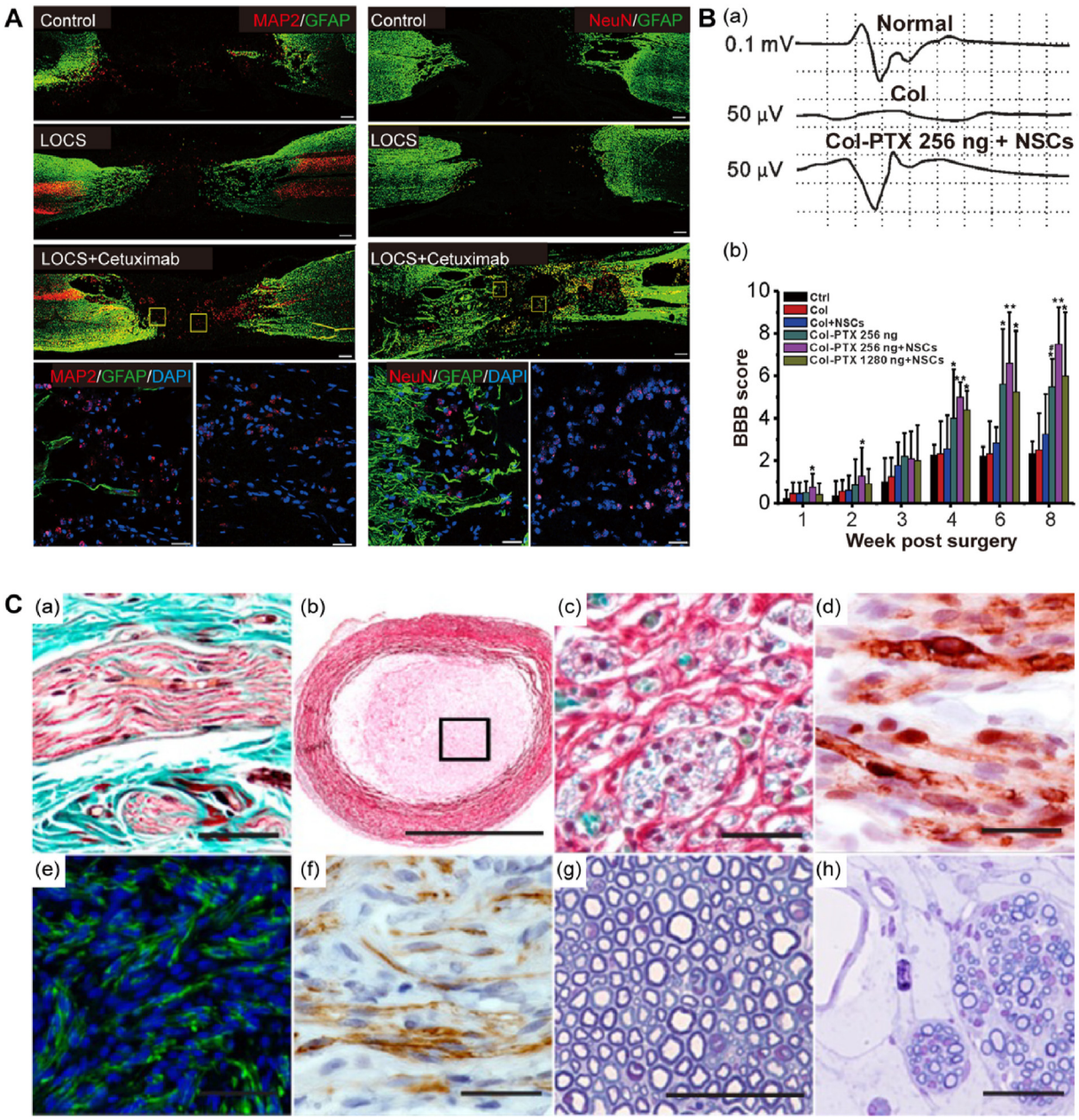

Fig. 5. Assessment of functional restoration in neural regeneration. A: Cetuximab-modified scaffold implantation (LOCS + cetuximab) facilitates neuronal regeneration in the lesion site of dogs at 9 months post-SCI injury. Confocal images displayed MAP2, NeuN-positive neuronal cells, and GFAP-positive related glial scar formation in the lesion site via immunostaining. Reproduced with permission. [108] Copyright 2017, Elsevier. B: Electrophysiological analysis revealed functional restoration for neural regeneration. (a) Motor-evoked potential reflects a functional recovery in spinal cord-injured rats. (b) Basso, Beattie, and Bresnahan (BBB) locomotor scale method reveals hindlimb locomotion of SCI models. Reproduced with permission. [233] Copyright 2018, Elsevier. C: Histological analysis as quality control in peripheral nerve regeneration. The longitudinal section of a peripheral nerve was stained with Masson's trichrome method (a). Scale bar: 50 μm. Cross-sectioned NeuraGen collagen conduit stained with the MCOLL with low (b, scale bar: 1 mm) and high magnification (c, scale bar: 100 μm). Collagen fibers are stained in red, the myelin in blue, and the nucleus darkly stained. (d) Schwann cells were characterized with S-100, featured nucleus, and cytoplasmatic positive reaction. Scale bar: 50 μm. Immunofluorescence image of neurofilament in green (e, scale bar: 100 μm) and regenerated axons in GAP-43 as brown (f, scale bar: 50 μm). Toluidine blue stained semithin cross-sectioned native nerves (g) and regenerative nerve tissue (h). Scale bar: 50 μm. Reproduced with permission. [234] Copyright 2014, Wolters Kluwer.

Collagen is highly associated with clinical outcomes, making it a prevalent biomarker for various clinical applications, including a predictor of prognosis and recurrence; in diagnosis and serving as a drug carrier for targeted therapies [244]. Although collagen-based interventions in neurotrauma and neurodegenerative diseases have shown considerable promise in preclinical research, most applications are still in the preclinical phase, particularly for treating the central nervous system [245]. Clinical trials of collagen-based scaffolds are primarily in SCI repair (Table 6). Based on the completion of preclinical studies, a clinical study incorporating collagen scaffolds with MSCs to treat complete chronic SCI was conducted (NCT02352077), in which case, surgical debridement of the scar tissues was performed, followed by the MSCs-loaded collagen implantation. In a one-year follow-up, patients showed no significant side effects, primarily demonstrating collagen scaffolds' safety and efficacy. Locomotor functions of patients were partially restored in subsequent research. Other than that, collagen

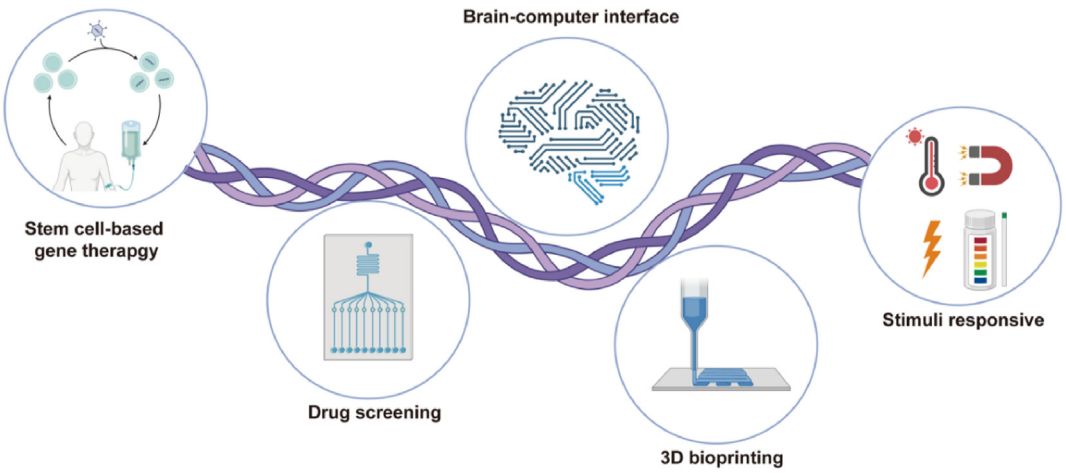

Fig. 6. Graphical diagram of promising future orientations of collagen-based biomaterials in NTE, including stem cell-based gene therapy, drug screening, brain-computer interface, 3D bioprinting, and stimuli-responsive alternatives.

Table 6
Clinical trials on collagen-based scaffolds in NTE. (NA: Not applicable).

ClinicalTrials.gov identifier	Transplant method	Injury site	Injury type	Phase	Country	Reference
NCT02510365	Necrohemorrhagic tissues removal followed by transplanted with MSCs	Thoracic and cervical	Acute complete SCI	1	China	[246]
NCT02352077	Scar tissues removal followed by transplanted with human umbilical cord MSCs or BMMCs	Thoracic and cervical	Chronic complete SCI	1	China	[247,248]
NCT02688049	Scar tissues removal followed by transplanted with MSCs or NSCs	Thoracic and cervical	Chronic complete SCI	1 and 2	China	[249]
NCT02688062	Scar tissues removal followed by transplanted with BMMCs	Thoracic	Chronic complete SCI	1 and 2	China	Clinicaltrials.gov
NCT01884376	Implantation of Neuromaix during a diagnostic nerve biopsy	Sural nerve	PNI	NA	Germany	[250]
NCT01809002	Implantation of bovine collagen-based nerve cuff	Peripheral nerve	PNI	3	America	Clinicaltrials.gov
NCT04353908	Subcutaneous collagen injection of porcine origin	Facial nerve	PNI	NA	Italy	[251]

scaffolds loaded with MSCs in a complete acute SCI clinical study were conducted (NCT02510365). Some patients showed significant recovery of sensory and motor functions through one-year follow-up. As discussed above, collagen-based nerve conduits are the only FDA-approved products for PNS repair in clinical trials. For instance, NeuraGen proved significantly effective in PNS regeneration among 43% of patients [109]. Neuromaix exhibited remarkable performance in bridging nerves of long gaps in the first clinical trial [110].

From the perspective of technological advances, integrating current fabricating strategies is promising but challenging. The primary proposition during manufacturing is mimicking native neural tissue organization and microenvironment, which not only supports but also enhances tissue development and maturation. Pores, fibres, and channels are some of the key characteristics that emerge in neural tissue and are organized in diverse ways throughout the multiple aspects of the nervous system. Therefore, more effort is being expended in reproducing these natural architectural characteristics to mimic the local microenvironment in vitro. Notably, the integration of multi-techniques should meet the requirements to spatially pattern growth factors, neurotrophic factors, and bioactive proteins in a controlled manner. Furthermore, manipulating spatial distribution and temporal release profiles of bioactive factors can mimic the convoluted developmental profiles of native tissues, which is of particular interest in scaffold fabrication for NTE.

For particular circumstances in the CNS and PNS repair, collagen-based biomaterials should meet relatively meet native characteristics in brain, spinal cord, and peripheral nerve. However, since it is troublesome in CNS repair, the dimensional properties of collagen-based biomaterials should be carefully fine-tuned to match mechanical properties and biochemical requirements. Besides, due to the intrinsic complexity of the CNS, integrating diverse cell types is crucial such as OPC, astrocytes, and microglial. For PNI repair, more concentration should be put on the technological advancements in the topological scale in guiding axonal alignment and functional enhancement based on current research. More importantly, long-distance (>10 mm) lesion gap repair is another obstacle. Therefore, incorporating distinct cell types and modification strategies would be potential in PNS repair.

8. Concluding remarks

In recent decades, the development of therapeutic strategies to address the challenges of nerve regeneration in injury repair and disease restoration has been of significant interest. To this end, a wide range of interventional approaches have been developed to target the CNS and PNS, with collagen scaffolds functionalized with neurotrophic factors emerging as a promising approach to promoting axonal outgrowth and functional restoration. In this review, we have provided an overview of collagen's preparation methods and practical advantages, with a brief description of collagen characteristics. We have also systematically summarized the current applications of collagen in NTE, categorized by manufacturing approach.

Furthermore, we evaluate the criteria applicable to TBI, SCI, and PNS repair, highlighting collagen's potential benefits in specific disease scenarios. Finally, we discuss the promising future directions and challenges that must be addressed to advance NTE utilizing collagen-based biomaterials. As no up-to-date review has assessed the diverse applications of multi-manufactured collagen in NTE, we hope this review will inspire thought-provoking ideas in interdisciplinary fields and contributes to the development of novel approaches to neural tissue engineering.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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