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Immune-cell-mediated tissue engineering strategies for peripheral nerve injury and regeneration

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During the process of peripheral nerve repair, there are many complex pathological and physiological changes, including multi-cellular responses and various signaling molecules, and all these events establish a dynamic microenvironment for axon repair, regeneration, and target tissue/organ reinnervation. The immune system plays an indispensable role in the process of nerve repair and function recovery. An effective immune response not only involves innate-immune and adaptive-immune cells but also consists of chemokines and cytokines released by these immune cells. The elucidation of the orchestrated interplay of immune cells with nerve regeneration and functional restoration is meaningful for the exploration of therapeutic strategies. This review mainly enumerates the general immune cell response to peripheral nerve injury and focuses on their contributions to functional recovery. The tissue engineering-mediated strategies to regulate macrophages and T cells through physical and biochemical factors combined with scaffolds are discussed. The dynamic immune responses during peripheral nerve repair and immune-cell-mediated tissue engineering methods are presented, which provide a new insight and inspiration for immunomodulatory therapies in peripheral nerve regeneration.

1. Introduction

Peripheral nerve injury (PNI) is commonly caused by accidents, war, natural disasters, and other factors that may result in the dysfunction of motor and sensory neurons in communicating with the central nervous system (CNS). Serious clinical issues impair the quality of life of patients and result in an enormous social burden.[1,2] Distinct from the central nervous system, the peripheral nervous system shows spontaneous regeneration ability in response to injury. This process involves dynamic complex pathological and physiological changes and elaborates on the cooperation of various cell-molecular events to establish an optimal microenvironment for regeneration and motor reinnervation.[3–5] The dynamic biochemical microenvironment homeostasis is balanced by the extracellular matrix network, blood vessels, and lymphatic vessels in connective tissues from the macroscopic level, as well as cell adhesion molecules, cytokines, chemokines secreted by Schwann cells (SCs), fibroblast cells, endothelial cells, and various immune cells at the microscopic level.[6–9] The anatomical structure of the peripheral nerve consists of bundles of longitudinal axons with or without myelinated glial cells (known as SCs) that are surrounded by three-layer membrane structures, namely, the endoneurium, perineurium, and epineurium, from the inside to the outside, respectively (Fig. 1(A)). The endoneurium is a matrix structure around the axon units. There are grouped nerve fibers called "fascicles" covered by fibroblast-like cells inside the perineurium, and single or multi-fascicles together with blood vessels, lymphatic vessels and some adipose tissues are wrapped by the epineurium that constitutes the peripheral nerve. As the outermost layer structure of the nerve, the epineurium is further divided into two layers, with the inner layer consisting of collagen bundles and elastic fibers, and the outer layer including areolar connective tissue and collagen bunches.[10,11]

According to the classification of injured nerve by Seddon and Sunderland, PNIs are classified in three types, and five types, respectively. Neuropraxia, axonotmesis, and neurotmesis are described by Seddon. Sunderland subdivided axonotmesis into three types based on the integrality of the epineurium structure.[12] Wallerian degeneration (WD) and the formation of Büngner bands are major and indispensable events for

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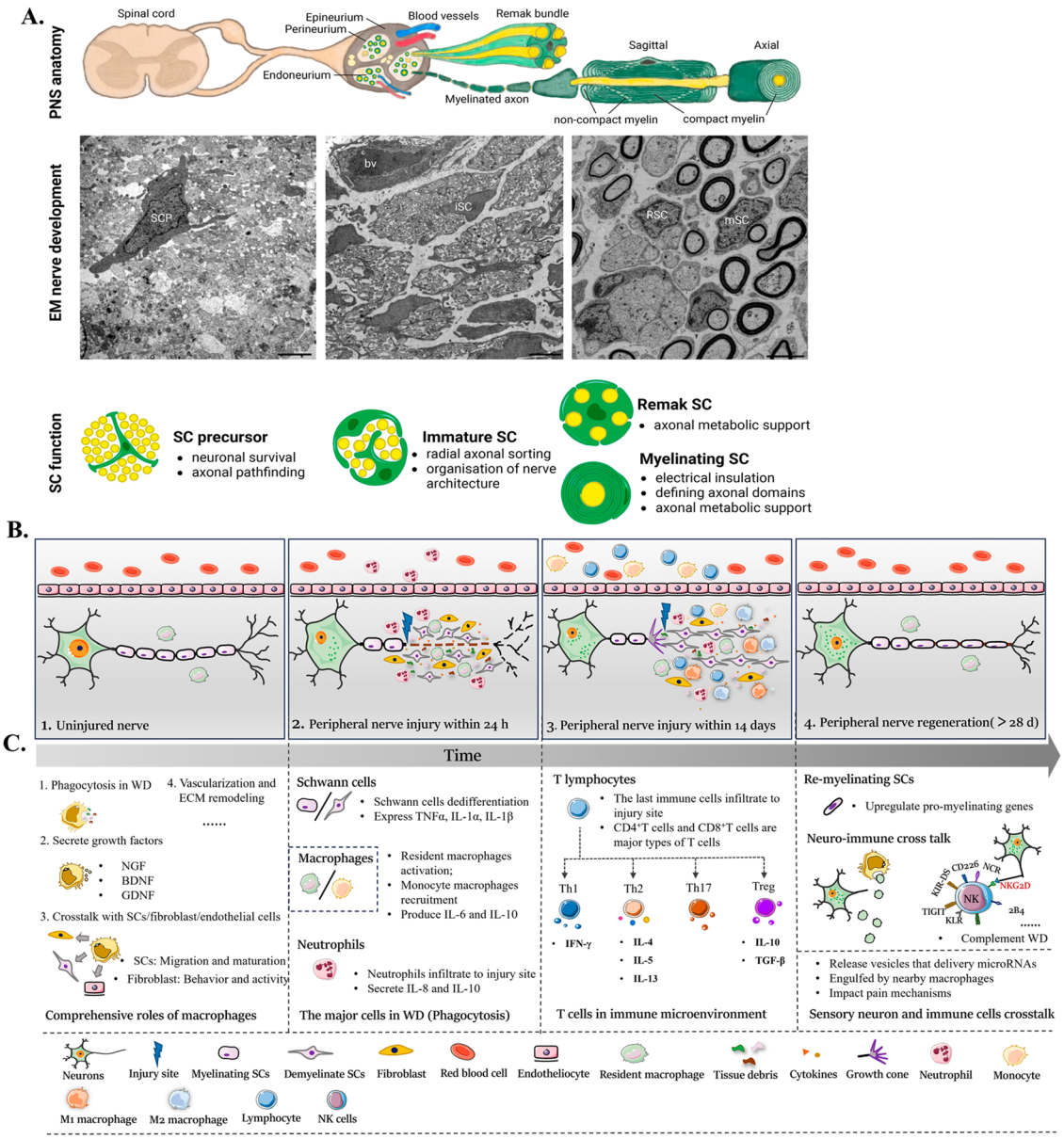

Fig. 1 The anatomy structure of the peripheral nerve (A) and the illustration of the main steps in the invasion of immune cells after peripheral nerve injury (B). The dominant immune cells and their special contributions to peripheral nerve repair (C). (A) A schematic of the peripheral nerve system and the orchestrated Schwann cells in the PNS. Reproduced from ref. 19 with the permission of Elsevier, copyright 2023.[19] The nerve architecture is organized by mature SCs that ensure the functional axonal domains. The nerve fibro consists of three layers (the endoneurium, perineurium and epineurium) along with blood vessels and lymphatic vessels to perform physiological functions. Electron micrographs exemplify the different developing stages of SCs, including SC precursors (SCP), immature SCs (iSCs), myelinating SCs (mSC) and Remak SCs (RSC) (labelled in white). (B) Following the peripheral nerve injury, the SCs dedifferentiation to repair SCs further proliferate and release factors to recruit immune cells to the injury site within 24 h. Immune cells cooperate with non-immune cells and the cytokines/chemokines to establish a local dynamic microenvironment that regulates the inflammatory response and axon regrowth. Finally, SCs undergo remyelination and encompassment of the regenerated portion of the axon; at this position, myelin is thinner than the uninjured axon. (C) This schematic diagram highlights the special roles of various immune cells (innate and adaptive immune cells) following the PNI. The functions of macrophages and T lymphocytes are described in detail.

successful nerve repair and regeneration. The process of WD includes the collapse of discontinuous axons, the breakdown of myelin, disarrayed microtubules/neurofilaments, and disassembly of the cytoskeleton. The axon of the proximal stump sprouted a growth cone that explored the microenvironment of the target tissue.[13–15] Following the nerve injury, as the major glial cells in the PNS, SCs undergo dedifferentiation, proliferation, and migration, and finally organize into a bridge along the basement membrane named Büngner bands. The reprogramming of mature myelinating and non-myelinating SCs is complex and several reviews have summarized this process.[6,16,17] The repaired SC play an essential role in the

clearance of axonal and myelin debris as well as the recruitment of immune cells to the damaged site. The other pathways of SC-mediated nerve repair include the up-regulation of a group of neurotrophic growth factors to promote axonal regeneration, up-regulating the expression of cytokines to recruit macrophages and providing guidance cues for axon regeneration. Ultimately, SCs re-myelinate newly regrown axons and accomplish the restoration of nerve function.[18,19]

Since the immune–neuro interaction has been discovered, nascent research has validated the neuroprotective and neurodestructive effects in the process of immune responses, which are crucial to injured environment homeostasis.[20,21] The functional recovery following PNI has two periods, namely, inflammation and regeneration. Cytokines, chemokines, secondary messengers, and immune cells such as macrophages, neutrophils, natural killer cells (NK), and T lymphocytes, are the major mediators of the inflammatory response.[22,23] Monocytes, neutrophils, and lymphocyte cells infiltrate the nerve lesion site in a time-dependent manner (Fig. 1(B)). It should not be ignored that the pro-inflammatory immune response remodels the distal stump microenvironment to accelerate the disintegration of nerve fibers and phagocytize tissue debris by non-neuronal cells, macrophages, and neutrophils in this process.[24] In the period of anti-inflammatory response, macrophages are polarized into the M2 phenotype, which upregulates transcription factors such as Mcf/c-maf, Mafb/MafB, and Tgfβ to promote axon regeneration. In the meantime, there is almost an absence of natural killer cells and lymphocytes, and T-cell activation is suppressed.[22,25] If the transition from pro-inflammatory to anti-inflammatory response is a failure, a state of chronic inflammation will occur and eventually lead to neuropathic pain. Therefore, the immune response following PNI should be precisely controlled to provide a better microenvironment for functional recovery.[26,27] Notably, macrophages make a significant contribution to the nerve repair process, such as interacting with SCs, axons, neurons, fibroblasts, and endothelial cells. They release neurotrophic factors to regulate the optimal environment for axon regeneration and functional recovery, as reviewed by many researchers.[23,28,29] Other immune cells, including neutrophils, NK and T cells, also act as an indispensable part of the effective immune response during peripheral repair and functional recovery (Fig. 1(C)), although the reciprocal interaction mechanism of these immune cells with nerve repair and regeneration is relative poorly understand.[30]

Given the intimate relationship between immune response and nerve repair, efforts to direct immune cell behavior or indirect methods to administer the immune microenvironment for better nerve repair and functional recovery have been widely explored.[31–34] In this review, we have systematically enumerated the general immune cell responses to peripheral nerve injury and their contributions to nerve repair. Tissue engineering-mediated strategies to regulate macrophages and T cells through physical and biochemical elements with/without scaffolds are discussed. The most recent data in dynamic immune responses during peripheral nerve repair and immune-cell-mediated engineering strategies are presented herein, which we hope will provide new insight and inspiration for immunomodulatory therapies in the peripheral nerve injury field.

2. The innate immune system especially highlights the functional role of macrophages in the pathological process following PNI

2.1 Neutrophils

Neutrophils are one of the first inflammatory cells to infiltrate the lesion site within hours to days post-injury in the peripheral nervous system (PNS).[35] Their positive markers are detectable on both days 3 and 9 post-injury in the injured sciatic nerves of mice, and the Csf3r gene was confirmed as a reliable marker gene to identify mature neutrophils in the distal nerve stump.[36,37] They are innate immune cells that originate from bone marrow stem cells with a life span of 24–48 h and can be extended during inflammatory reactions. Neutrophils are versatile and their roles include recruiting macrophages and other immune cells to the injury site by secreting pro-inflammatory cytokines (such as IL-8) and anti-inflammatory cytokines (such as IL-10), as well as various chemokines, inducing initial inflammatory reactions.[38–40] Moreover, granules released by neutrophils can promote the differentiation of invaded monocytes to macrophages, highlighting the role of neutrophils in functional macrophage polarization.[41] Following sciatic nerve injury, the myelin debris clearance is conducted by neutrophils and its depletion substantially inhibits myelin clearance in male wild-type mice and $Ccr2^{-/-}$ mice (C–C motif chemokine receptor 2, CCR2, which is expressed by macrophages and is necessary for monocyte recruitment), highlighting the phagocytosis role of these cells in WD.[42] In another study, the authors created a sciatic nerve crush injury model and histological evaluation indicated that the neutrophils accumulated at the epineurium in the WD area at 6 h after injury and reached a peak at 12 h, eventually disappearing by 1 d after injury. Neutrophil extracellular traps (NETs) formed by neutrophils restricted the macrophage infiltration into the parenchyma, further influencing the repair process in WD. The reduction of neutrophil accumulation promotes the repair process in WD by the migration inhibitory factor MIF-CXCR4-NETs axis.[43] These two distinct phenomena of neutrophils in WD may be caused by different animal and injury models. Accumulated neutrophils equally lead to hyperalgesia after nerve injury and a reduction in the recruitment of these cells will reduce mechanical hyperalgesia in post-surgical pain although its cytotoxicity in PNI remains unclear.[44] Several studies have shown that the crosstalk between neutrophils and macrophages can contribute to macrophage-mediated tissue repair as summarized by Bouchery *et al.*[45]

2.2 Macrophages and monocytes

Macrophages are mononuclear phagocytes that exist in all tissues where they act as phagocytic antigen-presenting cells

and have key roles in phagocytosing heterologous pathogens, dead cells, cellular debris, maintaining homeostasis, as well as other functional profiles, and have been well-studied over the past decades.[46–48] There are two species of macrophages in PNI, namely, tissue-resident macrophages derived from the yolk sac during embryogenesis, and monocyte-derived macrophages that originate from hematopoietic stem cells in the bone marrow, which can be recruited to the injury site by cytokines.[49] A large body of evidence has demonstrated that macrophages are the key mediators of tissue repair and provide a suitable microenvironment for regeneration in the process of peripheral nerve repair (PNR).[23,28,50] The resident macrophages along with SCs or neutrophils are the major cells that respond to nerve injury at first. The recruitment of circulating blood monocytes requires the help of chemokines or other signaling proteins, including inflammatory cytokines such as tumor necrosis factor α(TNFα), IL-1α, IL-1β expressed by SCs, monocyte chemotactic protein 1 (MCP-1, also known as CCL2) leukemia inhibitory factor (LIF), and pancreatitis-associated protein III. This event starts from 2 to 3 days after injury and peaks at about 7 days.[28,51] Interestingly, the removal of degenerated myelin by SCs and macrophages can be independent since macrophages may be recruited to the injury site in the absence of SCs.[52] Besides the important role of macrophages in the phagocytosis of myelin debris and axon fragments for balancing the dynamic microenvironment in nerve lesion sites, they also contribute to driving the PNR by simulating SC migration and maturation, remodeling extracellular matrix (ECM) organization in the injury site, promoting angiogenesis, preventing ectopic axon growth and accelerate axonal outgrowth (Fig. 1(C)).[23] Herein, we have summarized the dual functions of macrophages in pro-inflammatory and anti-inflammatory programs and highlighted their distinct effects in PNR.

2.3 Heterogeneity and plasticity of macrophages

It is considered that macrophages in different tissues have specific subpopulations, for instance, Kupffer cells in the liver, splenic macrophages in the spleen, osteoclasts in the bone, and microglia in the brain; the heterogeneity may explain the functional diversity.[29] The advances in single-cell transcriptomics enable the identification of the specific cell subsets of the tissues. Based on this technology, a recent study by Ydens *et al.* uncovered that the harbored macrophages are distinct in the epineurium and endoneurium with specific spatial characterization in the PNS. In response to the sciatic nerve crush injury, these diverse subgroups of macrophages have differences in the signature gene expression pattern, ranging from the PNS to the CNS.[53] Moreover, resident macrophages in the PNS allow prolonged monocyte-derived macrophages to be recruited following sciatic nerve injury. Further exploration showed that recruited monocyte macrophages are mainly responsible for effective debris clearance, while the resident macrophages participate in axon regrowth, confirmed by the depletion of resident macrophages, which will lead to the complete failure of axon regeneration.[54] According to specific bioactivities, macrophages can be divided into two classic groups, namely, M1 and M2 phenotypes as the pro-inflammatory and anti-inflammatory macrophages, respectively. M1 macrophages are pro-inflammatory contributors induced by lipopolysaccharides (LPS), toll-like receptor (TLR) ligands and interferon γ (IFN-γ), TNFα, and CCL2, which can aggravate inflammatory responses and the elimination of apoptotic cells and debris by secreting inflammatory factors such as IL-1α, IL-1β and IL-6.[28] In contrast, M2 macrophages can be further classified into four subtypes, namely, M2a, M2b, M2c and M2d, based on differential activation pathways. They are mainly associated with the anti-inflammatory response or pro-healing phenotype. Additionally, the stimulating factors are specific in M2 subsets as M2a is induced by IL-4 and IL-13, M2b is induced by the immune complex, M2c is induced by IL-10 and transforming growth factor-beta (TGF-β), M2d is induced by the A2AR agonist.[55–57] These subsets of M2 macrophages play anti-inflammatory roles by producing growth factors, removing apoptotic cells (M2a), promoting angiogenesis (M2b/d) and ECM synthesis (M2b/c) (Fig. 2).[58] M1 and M2 macrophages are maintained in a dynamic balance with the changes in the microenvironment during the PNI. One study explored the local delivery of IFN-γ or IL-4 in a rat model and confirmed that the pro-healing phenotypes M2a and M2c macrophages are in regenerative bias.[59] Given that the distinct subpopulations of macrophages have different effects on repair and regeneration following PNI, it will be helpful to elucidate the specific mechanism of macrophage polarization.

Macrophage plasticity in the orchestration of inflammatory and tissue damage has attracted much attention from researchers since incorrect polarization will lead to dozens of diseases.[60,61] Following PNI, M1 macrophages are dominant in WD and increase within 2 days post-injury, while M2 macrophages have a prominent action in the subsequent anti-inflammatory response and gradually replace the M1 phenotype from 3–7 days. Most studies have analyzed macrophage polarization during nerve repair and regeneration, which were mixed macrophages. The different techniques used to determine the marker genes of M1 or M2 are concomitant with distinct results, which can be seen in previous reports.[62,63] A recent study by Zhang *et al.* applied RNA sequencing technology and identified the gene profiles in dorsal root ganglions and the expression pattern of macrophages at different time points after sciatic nerve injury. Macrophage-associated miRNAs were screened and up-regulated miRNAs (miR-18a, miR-19b, miR-21, miR-29) may take part in macrophage polarization and provide an understanding of microenvironment remodeling after PNI.[64] Although the pro-regeneration capacity of M2 macrophages is well investigated, strategies always tend to attribute the macrophage fate to the M2 phenotype. The role of M1 subsets should not be ignored; particularly, elucidating the dynamic equilibrium relationship of M1 and M2 in PNR will be valuable for nerve regeneration in future investigations.

2.4 Natural killer

NK cells are innate lymphoid cells that lack antigen-specific receptors and have a crucial role in inflammation and adaptive

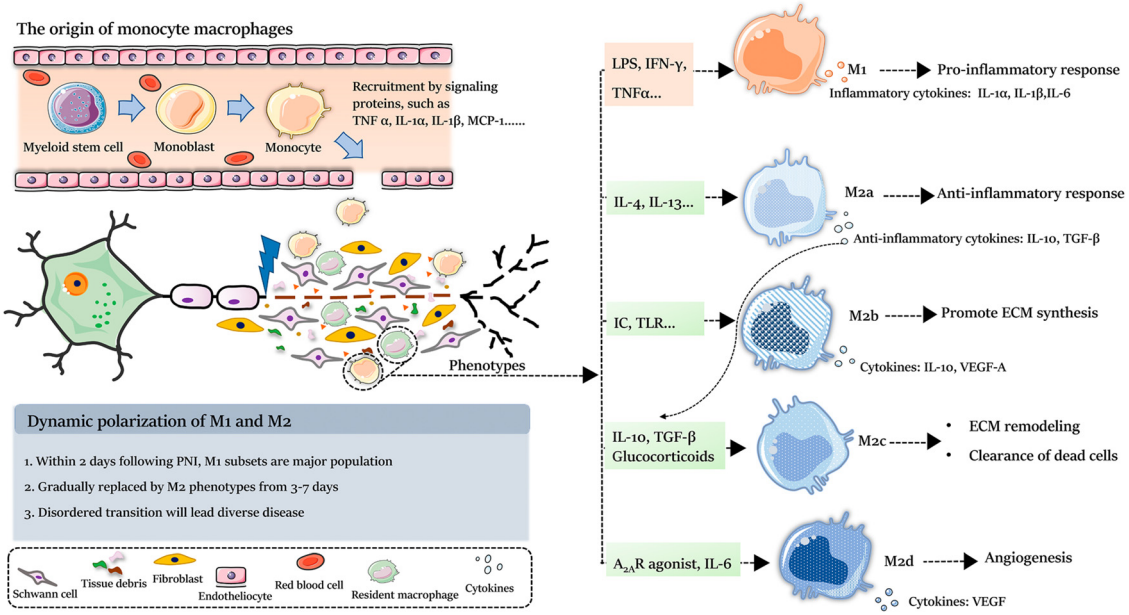

Fig. 2 The profiles of macrophages in response to the injury of the peripheral system. The monocyte macrophages originating from the myeloid stem cell are recruited to the injury site by cytokines or chemokines secreted by other immune cells and nonimmune cells. Together with resident macrophages, they can be polarized into M1 (pro-inflammatory response) or M2 (anti-inflammatory response) phenotypes. M2 macrophages are further divided into four subsets that endow macrophages with diverse roles. According to the local environment, M2 macrophages change their phenotypes with specific roles, including phagocytosis in WD, interaction with other immune cells, initiation of vascularization, ECM remodeling and so on. Moreover, the dynamic polarization balance of M1 and M2 phenotypes plays an indispensable role in nerve function recovery. IC: immunocomplex; TLR: Toll-like receptor; A2AR: adenosine A2A receptor.

immunity, producing a much faster immune reaction.[65,66] They play roles in both tumor immune supervision and induction of cascades of immune reaction synchrony with other immune cells. Depending on functional cell receptor classification, NK cell-activating receptors mainly contain killer cell immunoglobulin receptors (KIR-2DS, KIR-3DS), the NK group 2D (NKG2D, NKG2C), natural cytotoxic receptor (NCR), 2B4, and CD226; inhibitory receptors mainly contain KIR-2DL, killer cell lectin-like receptor (KLR, CD49/NKG2), and T cell immunoreceptor with Ig and ITIM domains (TIGIT).[67,68] The cross-regulation of NK cells and other immune cells, especially macrophages and T cells, has become popular in recent years. Pro-inflammatory cytokines (IL-12, TNF-α, IFN, IL-18) are secreted by macrophages that may stimulate NK cells and, in turn, promote M2 macrophages polarized or switched to the M1 phenotype, which increases the activation of NK cells. Bidirectional cross-talk between NK cells and T lymphocytes highlights that IFN-γ secreted by NK cells may mediate naive T cells to the Th1 phenotype.[67,69] Following PNI, NK cells also infiltrated the injury site in adult animals and proliferated more on day 9 than on day 3. Compared to neutrophils, NK, T, and B cells had fewer interactions with other cell types on day 3 and, similarly, on day 9 post-injury.[36] Interestingly, Davies *et al.* proposed that within days of PNI, cytotoxic NK cells infiltrate the lesion site and respond to retinoic acid early inducible protein 1 (RAE1), which is the NK group 2D (NKG2D) ligand expressed by injured sensory axons. NK cell interactions complement WD and accelerate damaged axon clearance to accomplish the function recovery of the PNS.[70] Although there is less research on NK cells, their immune-modulating role in damaged peripheral nerves should also be investigated.

3. T cells in adaptive immune response display diverse effects on nerve repair

T lymphocytes are adaptive immune cells that infiltrate the lesion site of PNI in 3 days and subsequently reach a peak level in 14–28 days.[71,72] In RAG2$^{-/-}$ (recombination activating gene 2) mice with a femoral nerve injury model in which mature T- and B-lymphocytes are absent, better motor recovery and enhanced myelination have been observed.[73] In another study, lymphocytes injected at the injury site during the acute phase of WD could improve nerve regeneration and sensory recovery. Further analysis demonstrated that in the early phase of sciatic nerve injury, lymphocyte therapy showed positive effects on regenerative processes by improving debris clearance.[74,75] The regenerative role of T cells in PNI was also verified by using acellular nerve allograft (ANA) in an athymic rat model; the axonal regeneration and recovery were significantly diminished when T cells were absent.[76] These studies indicated that T lymphocytes had an immunomodulating impact on injured nerves. Moreover, T cells also participated in the neuropathic pain following the nerve injury.[71,77] However, the role of T cells in PNR is far from resolved and the exact functions of different T cell subsets that accumulate at injury sites are largely unknown.

According to the differences in cell surface cluster differentiation antigen (CD), T cells are divided into $CD4^+$ and $CD8^+$ T cells. The $TCD4^+$ T cells subpopulation contains T-helper cells (Th1, Th2 and Th17) and regulatory T cells (Treg), and these cells are essential for maintaining homeostasis after nerve injury (Fig. 1(C)).[78,79] Generally, the Th1-mediated immune response can activate macrophages polarized into the M1 phenotype by secreting IFN-γ. They also interplay with SCs that improve the neurotrophic factor expression and promote cell proliferation. Moreover, Th2 cells could release IL-4, IL-5, and IL-13 and regulate macrophages towards the M2 phenotype to form an anti-inflammatory environment. Th17 cells are involved in neurological disorders but their role in PNI is rarely reported.[74,80] Bombeiro *et al.* used lymphocyte therapy to improve nerve regeneration in traumatic injuries. Compared to the control group, Th1 in the recipient groups revealed a higher frequency at 7 days but not 21 days, and Th17 cells also reached an early peak in the experimental groups. These phenomena imply that boosting the inflammatory response during WD could improve the conducive microenvironment for axon regeneration. Notably, compared with the control group, the recipient groups revealed higher expressions of brain-derived neurotrophic (BDNF) at 7 days and 21 days. Behavioral experiments revealed better sensory functional recovery in recipient groups by the von Frey test.[74] Additionally, Treg cells make a great contribution to maintaining immune homeostasis and regulating the inflammatory response by suppressing the activation of other immune cells.[81] They play a crucial role in the resolution of the inflammation and functional recovery after PNI by direct interaction with other cells or intermediate mediators through secreting anti-inflammatory cytokines IL-10 and TGF-β. Moreover, they can regulate neutrophil migration to the injury site, indirectly promote macrophage M2 polarization, control the activity of conventional T cells, resist neuropathic pain and shut down the overactive immune response.[82–84] In PNI, Treg-related IL-10 signaling is an intrinsic mechanism in the resistant pathophysiology of neuropathic pain, which provides a possible approach to reducing suffering after PNI.[85]

4. Immune cells-based immunoengineering strategies for repairing injured nerves

Clinical peripheral nerve injury can be caused by crushing, transection, stretching, neurological tumors or combined damage. It usually requires end-to-end epineural tension-free suturing after surgical treatment for short-distance gaps of injury. When irreducible damage happens, nerve grafts (autografts or allografts), nerve transfer (bridge the proximal nerve to target the motor endplate to accomplish earlier reinnervation) and nerve conduits can be introduced. Although autografts are the gold standard for the treatment of nerve injury due to their non-immunogenicity, this microsurgical procedure has some disadvantages, including causing secondary damage to the donor site, a limited supply of donor nerves, and mismatching between the donor and recipient sites.[86] Tissue-engineered nerve grafts provide an alternative strategy for nerve repair and have achieved significant progress. The ultimate purpose of tissue engineering strategies is to gain better nerve tissue regeneration and functional recovery. More importantly, the nerve conduits are used in clinical practice to help more people heal from injury. Recently, artificial nerve conduits with particular designs have been widely developed to improve the regeneration of the microenvironment, which further promotes the functional recovery of nerve tissue. They are scaffold-based materials that can be combined with physical properties (mechanical, surface topography, fibers/hydrogel filled in conduits lumen, bio-inspired hierarchical structure) and chemical factors (specific functional groups, immobilizing peptide, polysaccharide modification, small molecular drugs, neurotrophic factors), thus providing a suitable microenvironment for nerve regeneration.[87,88] For scaffold-free strategies, the aim is generally to reprogram a specific cell population by the direct transplant of the engineered cells or indirect delivery of a therapeutic gene into living cells at the injury site in order to promote regeneration.[89] There are already some commercial nerve conduits that have been approved by the US Food and Drug Administration (FDA) for clinical use in peripheral nerve injury, such as collagen conduits: NeuraGen® Nerve Guide (Integra LifeScience Co.) and Neuroflex (Collagen Matrix), chitosan conduits: REAXON® DIRECT (KeriMediacal).[90] As mentioned above, immune response has an indispensable role in peripheral nerve injury and functional recovery. Thus, regulating immune cells by tissue-engineering strategies will provide a new vision for nerve regeneration. It has been demonstrated that implantable materials with diverse designs could modulate the immune cells (neutrophils, macrophages, dendritic cells *et. al*) and further regulate the healing/regeneration process.[91] Herein, we review recent efforts and immunoengineering strategies in charging immune response in peripheral nerve injury and functional recovery, and we discuss the potential applications of immune cell modification in clinical treatment.

4.1 Tissue engineering scaffold-based materials to regulate the macrophage behavior

As the dominant cells in WD and nerve regeneration, it is necessary to explore strategies for controlling macrophage polarization towards an anti-inflammation phenotype and promote the restoration of function after PNI.[92,93] The scaffold-based engineering implants focused on biomimetic architecture design and microenvironmental factor modification by targeting macrophages in transection injuries. Physical (material composition, surface topological structure, roughness and hydrophilicity of implants, and degradation), chemical (functional groups/peptides or polysaccharide modification) and biotical (delivery of cytokines, antibody or chemokines) signals can have a synergetic effect on the biological behavior of macrophages.[94,95]

4.1.1 Physical signals. In terms of the physical design of implants, the small pores, low roughness, and high substrate

stiffness of biomaterials can stimulate macrophages into pro-inflammatory M1 subtypes, whereas scaffolds with a smooth shape, low stiffness, high roughness and hydrophilicity tend to trigger macrophages to polarize into anti-inflammatory M2 phenotypes.[39,96] The composition, mechanical properties, degradation, and drug-loading characteristics of scaffolds can also modulate macrophage phenotypes. For example, in terms of natural materials, our laboratory has demonstrated the degradation products of chitosan, which are known as chitooligosaccharides (COS) that stimulate CCL2 expression. CCL2 further induce macrophage migration at the injury site to reconstruct the microenvironment.[97] In another study, chitosan biomaterial without modified surfaces can specially support the PNR process by promoting macrophages polarized into M2 phenotypes.[98] Similarly, ECM and polysaccharide materials such as hyaluronic acid (HA), alginate, and heparin have been shown to modulate the plasticity of macrophages.[99,100] The physiological and anatomical structure of peripheral nerves, mainly paralleling neuron fibers, and the electrical signal conduction of nerve fibers are crucial factors of neurological recovery. The popular design of implants for peripheral nerve restoration is a topological microstructure with oriented microgrooved topography, physical fields, and conductive materials. External stimulation, such as magnetic, electrical and optical, is applied to improve the functional recovery of the nerve.[101,102]

Aligned poly(L-lactic acid-*co*-e-caprolactone) (P(LLA-CL)) nanofibers fabricated by the electrospinning technique have demonstrated a better repair effect, at least partly, *via* modulating the higher level of the M2/M1 ratio in a rat sciatic nerve defect model as compared to random fibers (Fig. 3(A)).[103] Luo and co-authors demonstrated that the multifunctional biodegradable conductive hydrogel could establish a biomimetic electrical microenvironment to facilitate macrophage polarization towards an anti-inflammatory phenotype that enhances nerve tissue repair.[104] Biodegradable waterborne polyurethanes (WPUs) and polydopamine-reduced graphene oxide (pGO) conductive nerve guidance scaffolds (NGSs) have been used for peripheral nerve repair by regulating the macrophages to M2 subsets (Fig. 3(B)).[105] In another work, poly(D,L-lactide-*co*-caprolactone) (PLCL) films with micropatterns of diverse sizes and surface-coated electrostatic adsorption of graphene oxide (GO) nanosheets were established. In particular, the micropatterns with GO film of 30 μm dimensions tend to induce macrophages into the M2 phenotype by a higher expression of arginase 1 and IL-10, thus promoting nerve repair (Fig. 3(C)).[106] A similar phenomenon was also observed by Dong *et al.* They showed that a graphene-based conductive fibrous scaffold and exogenous electrical stimulation effectively promoted PNR, partly by modulating the macrophage phenotype to M2.[107] Several reviews have summarized the materials

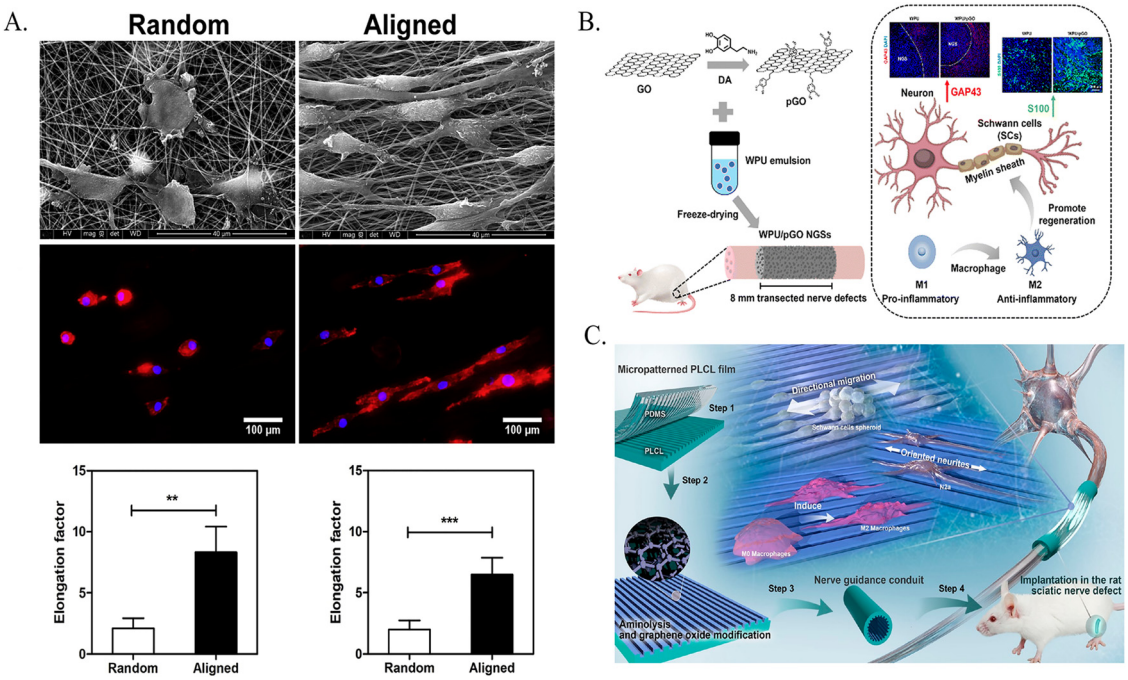

Fig. 3 Examples of tissue engineering scaffold-based materials to regulate macrophage behavior. (A) Representative macrophage morphology on random and aligned P(LLA-CL) nanofiber membranes under SEM, rhodamine–phalloidin staining and quantitative analysis of macrophage elongation from top to bottom. Reproduced with the permission of Elsevier, copyright 2019.[103] (B) Biodegradable waterborne polyurethanes (WPUs) and polydopamine-reduced graphene oxide (pGO) conductive nerve guidance scaffolds (NGSs) for peripheral nerve repair. A schematic of the WPUs/pGO NGSs for better nerve regeneration by regulating the macrophages to M2 subsets and stimulating axonal formation. Reproduced with the permission of the publisher, copyright 2023.[105] (C) The fabrication of the PLCL/GO film with micropatterns; this specific design of conduits can promote the regeneration of PNI in rats by inducing the differentiation of macrophages into M2 phenotypes and directing SCs migration along the micropatterns. Reproduced with the permission of the publisher, copyright 2020.[106]

for regulating macrophage behavior. Generally speaking, micropatterns, stiffer substrates, a combination of biomaterial composition, alignment and stiffness, as well as the precise conductivity of NGCs are factors that can induce macrophage polarization to M2 phenotypes, which further provide a positive microenvironment for nerve regeneration (Table 1).[31,108–110] The mechanism regarding the physical properties of biomaterials for regulating immune cells/systems remains elusive. A deeper understanding will be meaningful for designing a functionalized immunoregulation scaffold for better peripheral nerve recovery for *in vivo* applications.

4.1.2 Biochemical signals. Chemical modifications involve specific functional groups (such as hydroxyl (–OH), carboxyl (–COOH), amine ($–NH_2$), sulfhydryl (–SH)), crosslinking with agents (*e.g.*, genipin, carbodiimides and glutaraldehyde), immobilizing functional peptides or cytokines, and polysaccharide modification. The crosslinking agents are applied to biomaterials and can regulate the mechanical properties and stiffness of the material. Functional groups/proteins/polysaccharides connected to the scaffold can also influence the interface between the material and cells, consequently modulating macrophage response (Table 2).[126–128] For instance, genipin or formaldehyde crosslinking was performed on collagen/chondroitin sulfate nerve guide conduits (NGCs). The nerve regeneration effects were evaluated and it was shown that the genipin crosslinked group produced increased amounts of IL-10, whereas formaldehyde groups with higher levels of TNFα regulated macrophages towards a pro-repair

Table 1 Physical signals for regulating macrophage polarization

Physical signals	Results: immune response and underlying mechanisms for regeneration	Ref.
Nanofiber/topography/materials	1. Aligned poly(L-lactide) nanofibers could downregulate the proinflammatory M1 phenotype and upregulate the pro-healing M2 phenotype of macrophages 2. Inhibition of M1 polarization by JAK-STAT and NF-κB pathways	111
	1. Aligned nanofibers induced a pro-healing phenotype of macrophages and random fibers induced M1 subsets. 2. A higher ratio of M2 types and further infiltration of SCs	103
	1. HA-coated collagen nanofibers were co-cultured with macrophages and an elongated shape of macrophages was observed. 2. In *in vivo* experiments, the scaffold promoted the recruitment of anti-inflammatory M2 macrophages thus providing a better regeneration environment	112
	1. Polyethylene glycol and polycaprolactone hydrophilic nanofibers were prepared with an aligned topography 2. An increase in the hydrophilicity of aligned nanofibers induced the M2 subsets, further demonstrating that macrophages sense the biomaterials by inflammasome NOD-like receptor thermal protein domain-associated protein 3 (NLRP3)	113
	1. Biodegradable polycaprolactone scaffold with Wnt3a protein modification. 2. The functional materials can promote M2 macrophage subsets in *in vitro* experiments, promoting the early recruitment of macrophages and increasing the proportion of M2 macrophages	114
	1. Nanodiamond–polycaprolactone guidance channels; 20 mm nerve defects in a rat model 2. Inducing M1 to M2 macrophage polarization in a timely manner; activating the M2 phenotype *via* the Janus-activated kinase-STAT signaling pathway	115
	1. Scaffold fabricated by inorganic lithium–magnesium–silicon (Li–Mg–Si, LMS) bioceramics. 2. The scaffold could promote the high expression of neurotrophic factors in rat SCs in a β-catenin–dependent manner, as well as promote macrophages towards pro-regenerative M2-like cells.	116
	1. Polycaprolactone (PCL) nanofiber membranes were combined with amniotic membranes. 2. The scaffold promoted the recruitment of macrophages and polarized them into the M2 phenotype by releasing various bioactive substances. The anti-inflammatory microenvironment enhanced the ability of the nerves to regenerate.	117
Stiffness	1. On a stiff matrix (50 kPa), the expression of macrophage M1 markers increased, but this phenomenon did not occur on a soft matrix (1 kPa). 2. Material-mediated stiffness-induced macrophage polarization was mainly effected by a mechanosensitive ion channel Transient Receptor Potential Vanilloid 4 (TRPV4) through the ROS/NLRP3 pathway-regulated mechanism.	118,119
	1. Collagen-coated polyacrylamide gels with varying stiffness to direct macrophage behavior. 2. Gels (323 kPa) prime macrophages towards a pro-inflammatory phenotype, while soft (11 kPa)/medium (88 kPa) gels induced an anti-inflammatory and high phagocytic phenotype 3. On soft and medium stiffness gels, macrophages display Rho-A kinase (ROCK)-dependent migration mode.	120,121
Physical fields	1. The scaffold had gradient-magnetized iron oxide nanoparticles. High magnetic saturation produced evenly distributed micropores and a multilayer structure. 2. The scaffold could facilitate macrophage immunoregulation *in vivo* by mechanochemical signaling to accelerate the repair of injured nerves.	122
	1. A conductive scaffold with multiscale filled NGC: electrospun poly(lactide-*co*-caprolactone) (PCL)/collagen nanofibers as the sheath, rGO/PCL microfibers as the backbone, and PCL microfibers as the internal structure 2. This NGC could enhance nerve regeneration by promoting neovascularization and M2 transition.	123
	1. Poly(vinylidene fluoride-trifluoroethylene) [P(VDF-TrFE)] film covered indium tin oxide planar microelectrodes. 2. Electrical stimulation could up-regulate macrophages to M2 subsets by the increased expression of the M2 polarization receptor interleukin-4Rα, while the M1 polarization receptor toll-like receptor 4 was not affected	124
	1. Ultrasound therapy for a week (1 MHz frequency, intensity of 140 mW cm^{-2}, 20% duty cycle, 5 min per day) 2. Decreased the number of pro-inflammatory macrophages and promoted reinnervation in autograft model rats.	125

Table 2 Examples of biochemical modification to induce macrophage polarization

Biochemical signals	Materials and results	Ref.
Crosslinking reagent	1. Silk fibroin cross-linking with 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide hydrochloride) (EDC) and glutaraldehyde (GA) 2. Degradation products of GA-SF: pro-inflammatory macrophage phenotypes; EDC-SF enhances polarization towards anti-inflammatory macrophages. 3. M2 macrophages further stimulate the upregulate expression of NFG, laminins and stromal cell-derived factor-1 in SCs	134
	1. Collagen scaffold cross-linking with EDAC and genipin to modulate the stiffness of scaffolds 2. THP1 macrophages respond to cross-linking agent species rather than the bulk modulus of the scaffolds 3. EDAC cross-linking promotes a pro-inflammatory and anti-inflammatory phenotype of macrophages, while genipin cross-linking suppresses the effects.	135
Cytokines	1. Synergistic effect of electrical stimulation and cytokine on the regulation of M1/M2 polarization 2. A square waveform selectively promoted LPS/IFN-γ-induced M1 polarization and affected intracellular ion concentration 3. A sinusoidal waveform promoted both LPS/IFN-γ-induced M1, and IL-4-induced M2 polarization. Similarly, affected intracellular ion concentration and membrane receptor.	136
	1. Sciatic nerve crush injury in male C57BL/6 mice with IL-33 treatment. 2. With the 50 and 25 μg kg^{-1} doses, IL-33 could promote the macrophages toward an M2 phenotype and increase the mRNA expression of NGF, VEGF, and BDNF	137
Bionic peptides/ hydrogel	1. The multidomain peptide nanofiber hydrogel was designed to mimic various motifs of ECM components and growth factors. 2. The hydrogel could enhance the macrophage recruitment to the injury site of rat sciatic nerve crush injury and promote a multicellular pro-regeneration response.	138
	1. Self-assembly technology to fabricate bionic peptide hydrogel and entrapping with M2-derived cytokines and extracellular vesicles. 2. The scaffold could promote M2 transformation *in situ* and recruit more blood-derived M2 macrophages to promote nerve regeneration. Remodeling the local immune environment	139
	1. The hydrogel was fabricated by a tissue-mimetic silk fibroin network and bisphosphonate-alginate network. 2. Facilitated the infiltration of SCs and macrophages and regulated the polarization of macrophage, thus, providing a positive microenvironment for nerve regeneration.	140

state.[129] Apart from the crosslinking agent mediating the materials' mechanical properties, the major purpose of chemical modification is to mimic the microenvironment of cells, which will influence their proliferation, differentiation and other biological behaviors. In our previous research, we utilized genipin as the crosslinking agent to immobilize the TNF-α inhibitor onto a chitosan nerve conduit and evaluated the repair effects of functional NGC on the sciatic nerve defect in a rat model. The accelerated axonal regeneration and functional restoration effects were observed, and the scaffold could balance the harmful inflammatory stimulation to facilitate peripheral nerve repair.[130] Moreover, the combination of peptides that mimic the active constituent of ECM or bioactive factors is another key parameter for scaffold manufacture. Yang *et al.* designed an injectable hydrogel with vascular endothelial growth factor (VEGF)-mimetic peptide-encapsulated nanoliposomes. In a rat model, the specifically designed material exhibited better functional recovery effects and promoted M2 subsets that provided a pro-regenerative microenvironment for nerve repair.[131]

An additional method for administrating macrophage fates is combining biotic signals for immunomodulation. In the delivery of IFN-γ or IL-4 by polysulfone tubes, macrophages are successfully polarized into M1 and M2 phenotypes, respectively. These macrophages can affect SC migration and proliferation, thus complementing the evidence of macrophage subsets displaying diverse functions in nerve regeneration.[59] In another study, the therapeutic effects were significantly improved in rat 15-mm gap sciatic nerve defects by collagen gels with nerve growth factor NGF and IL-4, and higher densities of macrophages were recruited to the distal nerve stump. Moreover, IL-4-activated M2 subsets directly mediated angiogenesis in the regeneration process.[132] A polycaprolactone (PCL) electrospun conduit with the continuous release of collagen VI played a role in the recruitment of macrophages and promoted M2 macrophage polarization.[133] Notably, the local delivery of fractalkine or cytokines target macrophages, thereby modulating the phenotype and may be attractive candidates for peripheral nerve repair. The regeneration microenvironment of nerves is complex and multiple factors coexist. Bio-scaffolds not only have effects on macrophages but also regulate the neuronal cell or Schwann cell behavior. More importantly, the accurate modulation of the M2/M1 ratio to fit nerve regeneration by bio-scaffold should be explored in-depth.

4.2 The strategy of directly targeting immune cells for the functional recovery of injured nerves

4.2.1 Macrophages. Inspired by therapies in the cancer research field and the progress of stem cells, some researchers attempted to manipulate endogenous or exogenous cells to promote regeneration. Importantly, these cells can be applied by systemic injection at the nerve injury site and can regulate innate cellular behavior.[141–144] An encouraging method is the delivery of cells or nanoparticles for immunomodulation and gene therapy. The engineered nanoparticles have unique geometric, mechanical, and immunoprotective properties that can avoid phagocytosis by the immune system, thereby allowing

particles to adhere to immune cells and display immunomodulation effects.[145–147] In Wofford's research, poly(lactic-*co*-glycolic) acid microparticles loaded with an anti-inflammatory drug, dexamethasone (Dex), were used to target monocytes, and effectively directed the monocyte differentiation into macrophages. The released drug maintained an anti-inflammatory environment without genetic modification. In this way, macrophages can be reprogrammed to a pro-healing phenotype and they have the potential for macrophage-based cell therapy for disease and regeneration.[148,149] The delivery of cells or miRNA-mediated macrophage polarization is also considered an important mediator.[150,151] For example, Zhang *et al.* encapsulated gingiva-derived mesenchymal stem cells (GMSCs) in the methacrylate 3D-collagen hydrogel and cooperated with the decellularized small intestine submucosal extracellular matrix (SIS-ECM) to significantly accelerate functional recovery and axon regeneration in rat sciatic nerve injury, accompanied by an increased infiltration of M2 macrophages and decreased infiltration of M1 macrophages at injury site.[152] Likewise, poly(lactic-*co*-glycolic) acid (PLGA)/ECM conduits filled with epidermal neural crest stem cells (EPI-NCSCs) for repairing injured rat sciatic nerve also showed an increased expression of anti-inflammatory cytokines (IL-4, IL-13) and decreased expression levels of M1 macrophages (low expression of IL-6, TNF-α) 7 days post-injury. Thus demonstrated the EPI-NCSCs' potential for providing a suitable inflammatory microenvironment for PNI.[153] N. Iwasaki *et al.* grafted macrophages into the injury site in a rat crush model. The experiment consisted of three groups: IL-4 stimulated macrophages (IL4-MΦ), IFN-γ stimulated macrophages and no cell grafts. They demonstrated that IL4-MΦ (typical M2 subset) could stimulate axon growth by direct interaction with axons, consequently improving the regeneration and functional recovery after PNI.[154]

Exosomes are a subset of extracellular vesicles (EVs), which have diverse constituents including nucleic acids (DNA and RNA), lipids, metabolites, and amino acids; therefore, they have potential in various disease treatments. Their specific characteristics provide a strategy for delivering diverse therapeutic payloads to desired targets; the cargo involves short interfering RNAs, chemotherapeutic agents and immune modulators.[155–158] In the field of peripheral nerve injury, exosome therapy strategies have become a star method for macrophage immunomodulation (Table 3). Compared to the repair effects of macrophage-delivery microvesicles (MVs) (M1- or M0-derived MVs and M2-derived MVs), the M2-derived MVs significantly increased the infiltration and axon number of SCs *in vivo*. Moreover, the relative expression levels of miR-223 were higher in M2-derived MVs. This will hamper the migration and proliferation of SCs, and NGF and laminin protein expressions were down-regulated when miR-223 was inhibited.[159] Similarly, a study found that SC-derived exosomes could promote M2 macrophages and facilitate the axon elongation of DRG neurons. Under the ischemia-hypoxic microenvironment following PNI, oxygen-glucose-deprivation conditions induced Schwann cell exosomes-promoted M1 polarization. It further demonstrated that miR-146a-5p was the major factor that mediated the macrophage shift from M2 to M1, thereby inhibiting regeneration.[160] Recent studies using exosome strategies to modulate the immune microenvironment, especially macrophage polarization in peripheral nerve injury, and potential mechanisms are outlined in Table 3. There are also excellent reviews that summarize the exosomal miRNA delivered to the interior of macrophages for regulating its phenotypes.[161,162] These studies highlight the central role of genetic modulation and macrophage polarization for ameliorating PNR and will be beneficial for establishing more efficient

Table 3 Recent examples of exosome-mediated immune microenvironments for PNI

Exosomes	Animal model	Immune microenvironment	Mechanism and results	Ref.s
Schwann cell-derived exosomes	Rat; crush injury	SC-Exos: promote macrophage M2 polarization; OGD-SC-Exos M1 polarization	1. MiR-146a-5p was significantly decreased under the ischemia-hypoxic microenvironment 2. Inhibition on the TRAF6/NF-κB pathway	160
Lipopolysaccharide (LPS)-preconditioned mesenchymal stem cells (MSCs) exosomes	Rat; crush injury	Promote M2 macrophage polarization	1. TSG-6 served as a critical mediator in LPS pre-Exos 2. Inhibition of NF-κB and NOD-like receptor protein 3 (NLRP3)	163
MSCs exosome cooperative hydrogel stiffness	Rat; crush injury	Inhibit M1 macrophage-mediated inflammation	1. Better repair effect of injury to the peripheral nerve 2. The stiffness of the hydrogel could regulate exosome release behavior	164
Human umbilical cord MSC-derived extracellular vesicles (hUCMSC-EVs)	Rat; transection injury	Down-regulated interleukin (IL)-6 and IL-1β, up-regulated IL-10	Motor function and the regeneration of axons	165
Extracellular vesicles released by DRG neuron cell bodies	Rat; spared nerve injury	Exosomes were phagocytosed by macrophages and promoted a pro-inflammatory phenotype	1. miR-21-5p is upregulated in DRG neurons following PNI. 2. Contribute to sensory neuron-macrophage communication after damage	166
Mesenchymal stem cell-derived exosomes	Rats; facial nerve injury	Facilitate M2 subsets and reduce the M1-M2 polarization ratio	1. Decrease inflammation by p38 MAPK/NF-κb pathway 2. Promote axon regeneration and myelination.	167

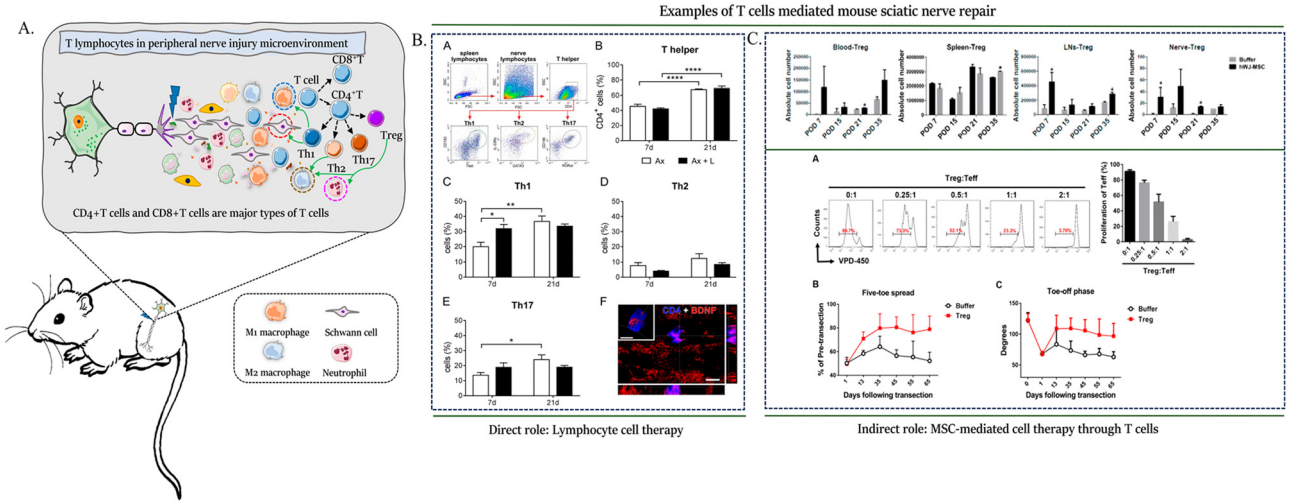

Fig. 4 T lymphocytes at the injury site and examples of T cell-mediated therapy for nerve repair. (A) A schematic of T cell character at the damaged site following PNI. The green arrows represent the diverse roles of T cell subtypes with immune/non-immune cells. These interactions may be direct or indirect ways to balance the regeneration environment at the injury site. (B) T helper cell phenotyping following the axotomized lymphocyte therapy in the mice sciatic nerve crush model. Reproduced with the permission of Elsevier, copyright 2020.[74] (C) Human Wharton's jelly-derived mesenchymal stem cells (hWJ-MSC) with immunomodulatory effects in promoting mouse sciatic nerve recovery and regeneration. Tregs are major contributors to functional recovery. Reproduced with the permission of the Creative Commons Attribution CCBY, copyright ©2020.[172]

immunomodulatory strategies to improve peripheral nerve repair and regeneration through understanding the immune response mechanism of nerve repair.

4.2.2 T lymphocytes. For nerve recovery, T cell polarization, recruitment or depletion is necessary through the effective retention, improvement or biomimicking of the neuron regeneration microenvironment by biomaterials or cell therapy (Fig. 4).[168] For instance, Bombeiro *et al.* demonstrated that in rat sciatic nerve injury models through dimethyl fumarate (DMF) treatment, increased Th2 cells and reduced Th1 cells were detected on day 7, as well as functional recovery was improved by DMF treatment. DMF is a fumaric acid ester that targets immune cells with anti-inflammatory, and immunomodulatory properties and shifts the balance of macrophages or lymphocytes to type II phenotypes. In this study, the authors highlighted the role of DMF in PNI and provided new insight into improving sciatic nerve regeneration by regulating the T cells.[75] Treg also attracted the attention of researchers as a target for nerve injury-induced dynamic mechanical allodynia or regeneration. In one study, the mice were treated with low-dose interleukin-2 (ld-IL2) in the injured sciatic nerve or the transfer of Treg to male C57BL/6J mice. The ld-IL2 treatment group increased the ratio of Treg cells at the injury site. All experiment groups effectively reversed the punctate and dynamic allodynia following PNI.[169] Mesenchymal stem cells (MSC) were also available in tissue regeneration and exhibited diverse immunomodulatory effects.[170,171] The immunomodulatory effects of MSC on sciatic nerve regeneration revealed that the modulation mechanism was partly associated with the upregulation of Treg-related cytokines such as IL-4 and IL-10, thus increasing the Treg coordination of positive or passive inflammatory microenvironments during PNR.[172] These studies indicated that remodeling T cell populations to an anti-inflammatory phenotype or targeting Treg may be an effective strategy for peripheral nerve regeneration.

5. Conclusion and prospectives

In summary, the immune response plays a bidirectional role in the repair of damaged peripheral nerves and regulates functional restoration. The appropriate inflammatory response enables the construction of a favorable microenvironment for nerve regeneration; otherwise, excessive inflammation will lead to neuropathic pain. Although there are lots of reviews that summarize the neuroimmune interactions in PNI, they mostly highlight the multi-functional roles of macrophages in the processes of repair and functional recovery. In this review, we have discussed in detail the different pathological characteristics of various immune cells following nerve injury. After axotomy, non-neuronal cells such as SCs and resident macrophages in the nerve stump segment express chemokines to recruit neutrophils and monocytes. These cells coordinate with each other and play indispensable roles in myelin and axonal debris clearance, thus providing a better environment for subsequent regeneration. Interestingly, the phagocytoses of these cells are independent events, as the clearance of debris can also proceed in the absence of SCs or macrophages. A recent study has demonstrated that NK could also participate in the process of WD and accelerate damaged axon clearance. It also secretes cytokines that mediate M2 macrophage polarization and naïve T cells to Th1 phenotypes. Neutrophils can not only recruit macrophages and other immune cells to the injury site but also promote monocytes to macrophages. It should be emphasized that there is an immune response with spatiotemporal dynamic character following the PNI. Immune cells that

infiltrate the injured site can interact with other non-immune cells and release many factors, inducing an inflammation microenvironment. In turn, the dynamic microenvironment remodeled by immune cells may provide appropriate conditions for nerve regeneration. The underlying mechanism of the pro-inflammatory to the anti-inflammatory response of various immune cells should be further explored. Moreover, the diverse subpopulations of neutrophils, macrophages, and T cells and their specific roles in resolving inflammation, promoting axon regrowth and improving functional recovery remain to be determined. In general, in-depth analyses of the immune regulation during the process of nerve repair are needed to develop means of intervention.

Tissue engineering strategies by direct or indirect immunomodulatory function have been widely explored to improve the immune microenvironment of injured nerves. Herein, we have highlighted the macrophages and T cells-mediated scaffold-based or scaffold-free tissue engineering tools to improve the regeneration microenvironment. These smart biomaterials with specific characteristics such as physical organization, chemical composition, and biotical modification contribute to the administration of the immune response that rebalances the regeneration of the microenvironment for better functional recovery. For scaffold-free strategies, the reprogramming of the immune cellular behavior or phenotypes by the delivery of functional nucleic acids or exosomes loaded with molecules may be an exciting innovation in remodeling the regenerative microenvironment. Furthermore, direct immunomodulating approaches targeting macrophages and T lymphocytes are important for improving regeneration. The local delivery of the Treg subset or stem cells to target sites has been proved. Immunosuppressive drugs, cytokine therapy, and chemokine therapy are gradually becoming alternative methods for repairing injured nerves, and the immunological principles are opening pathways for developing novel strategies for nerve regeneration. We can anticipate that the immune-cell-mediated tissue engineering strategies will accelerate and improve nerve regeneration. However, we should note that microenvironment remodeling by biomaterials is complex and regulating single immune cells or cell therapy cannot solve all problems at present. Instead of straightforward immunosuppression, elaborate immune modification is necessary. The research on clinical translation should also be evaluated. The combination of the intelligent engineering of biomedical materials and a diversified delivery system of immunomodulators will provide opportunities for effective therapeutic strategies to achieve better peripheral nerve regeneration in the future.

Author contributions

Xueying Zhao: conceptualization and writing – original draft. Hui Deng and Yuan Feng: writing – original draft. Yuehan Wang, Xiaomin Yao and Yuyang Ma: review and editing. Pengxiang Yang, Yumin Yang and Jing Jie: conceptualization, writing – original draft, review and editing, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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