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Review article

Novel approaches using mesenchymal stem cells for curing peripheral nerve injuries

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ABSTRACT

Peripheral nerve injury (PNI) is a common life-changing disability of peripheral nervous system with significant socioeconomic consequences. Conventional therapeutic approaches for PNI have several drawbacks such as need to autologous nerve scarifying, surplus surgery, and difficult accessibility to donor nerve; therefore, other therapeutic strategies such as mesenchymal stem cells (MSCs) therapy are getting more interesting. MSCs have been proved to be safe and efficient in numerous degenerative diseases of central and peripheral nervous systems. In this paper, we review novel biotechnological advancements in treating PNI using MSCs.

1. Introduction

Peripheral nerve injury (PNI) is one of the common life-altering disabilities resulting in various degrees of impairment in movement and sensation alongside autonomous function failing, apraxia and long-lasting pain [1]. Common causes of PNI are traumatic conditions, surgery and certain diseases like cancer and diabetes among which trauma-associated PNI has been found to cause significant socio-economic burden in young adults [2].

Spontaneous capability of peripheral nerve system (PNS) for repair of defective nerves is almost inadequate and current approved therapies are not satisfactory [3].The gold standard for restoring the transected nerves is bridging the distal and proximal ends of disrupted autologous nerve using neurosurgical sutures [4]. This approach is helpful if early diagnosis occurs and surgery is immediately performed; however nerve bridging is applicable in pay of consuming healthy nerves and scarring phenomena in damaged site besides difficult donor nerve accessibility and necessity for extra surgery [5]. In addition, endogenous repair system or autologous nerve grafting are not anymore capable for restoring deficient nerve in severe injuries and segmented nerves. Furthermore, permanent loss of neurodegeneration will happens in long gap disruption, mismatch nerves, axonal misdirection, and tension-depended ruptures [6]. In this context, several encouraging alternative systems such as natural or synthetic-compound nerve conduits and fabricating allograft nerve using cellular or acellular scaffold have been designed to effectively *promote nerve* regeneration. Peripheral myelinating glial cells particularly Schwann cells (SCs) are important in healing PNI but because of their little turn over, gradual expenditure over time, limited source and time-consuming harvest protocol, having alternative cells is essential [7]. Stem cells owing to self-renewal and differentiation capabilities toward neural lineages have been sound to be an appropriate choice for participating in engineered scaffolds and re-innervating disrupted nerve [8]. So far various types of stem cells had been tailored in bio-engineering medicine to improve neural regeneration but each of them offer some advantageous alongside certain downsides; therefore best source of stem cells for clinical application is still under debate [9]. Hereinafter, we will discuss recent techniques using MSCs for treatment of peripheral nerve damages.

2. Grading peripheral nerve injury

Peripheral nerve injury encompasses brachial plexus injury, peroneal nerve injury and spinal accessory nerve injury. According to clinical manifestation and injury severity, two main categories have been recognized for PNI grading. First classification was introduced by Seddon in 1943 and other was presented by Sunderland in 1951

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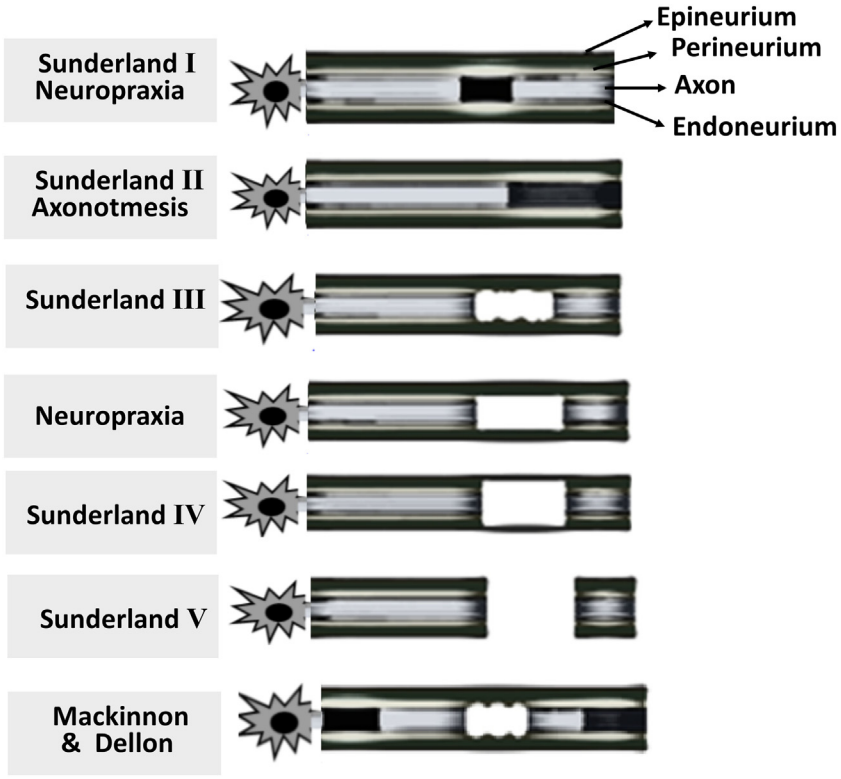

Fig. 1. Classification of nerve injuries.

describing mostly histological pattern of PNI. Seddon's classification is divided into three classes including Neurapraxia (Class I), Axonotmesis (Class II), Neurotmesis (Class III) [10] (Fig. 1).

Neurapraxia is the mildest type of nerve injury which mostly is produced by compression and segmental damages to myelin sheath resulting transient blockage in neural guidance while the elements of connected tissue (epineurium, perineurium, endoneurium) remains intact and axonal integrity is preserved. Neurapraxia is associated with partial sensory-motor impairments in distal site of damaged nerve which spontaneously is resolved after few days to 12 weeks. Neurapraxia is equivalent to Sunderland grade I injury [10].

Axonotmesis, also called 'neuroma-in-continuity', corresponds to Sunderand grade II injury. Axonotmesis is a moderate degree of nerve injury in which axon and its surrounded myelin sheath is completely damaged while both nerve continuity and surrounding connecting tissue (endoneurial core) of nerve is maintained. Wallerian degeneration in distal area of axonotmesis's lesion results in autonomic function deficit and sensory-motor impairment which is recovered after months of injury. Recovery from this form of injury is mediated by reactivation of endogenous Schwann cells in addition to healthy endoneurial tubes [11] (Fig. 1).

In the final form of Seddon's injury, neurotmesis, both the axon and connective tissue elements are destructed and due to lack of spontaneous repair, surgical intervention is required for this kind of injury.

In Sunderland grade III injury, axon and its myelin sheath is transected but perineurium is remained therefore involves both neurotmesis and axonotmesis. Sunderland grade IV and V are sever forms of PNI which epineurium is preserved in former while is lost in later.

Sunderland grade V is equivalent to neurotmesis. Sunderland grades I and II gain full recovery but grade III is recuperated partially, and grades IV and V require surgical medication [11] (Table 1).

The other pattern of injury which is a type of mixed injury (grade VI) was introduced by Mackinnon and Dellon as an addition to Sunderland's categorization in which different layers of connective tissue are damaged as a result of direct nerve injury derived from dislocations or fractures in the region of the nerves. In this form of injury both axonal loss and conduction blockage occur in some fibers. The recovery potential depends on the severity of injury (I-V) [12] (Table 1).

Generally, timing of repair process after injury is depended on distance from regeneration focal, between the lesion and target tissue, anatomical site of the injury and type of injury.

There is no method to diagnosis early stage of nerve injury. Electromyograms (EMG), nerve conduction studies (NCS) and magnetic resonance neurography (MRN) are techniques to diagnosis PNI in late stage of injury (50 days after injury) when fibril filaments are emerged in denervated muscle [12,13].

Mild and moderate injuries can cure via a broad range of non-surgical approaches such as rehabilitation, physical therapy, acupuncture, orthotic devices and massage therapy whereas serious neurotmesis and axonotmesis require different surgeries including brachial plexus, carpal tunnel, free muscle transfer, nerve entrapment, nerve transfer, nerve transplant and thoracic outlet syndrome surgery [13,14].

Other alternative approaches for non-severe PNI are electrical stimulation, thermal laser welding photochemical tissue bonding (PTB), appropriate nerve glue such as fibrin which all could shorten the time of denervation and help to nerve regeneration. In terms of allotransplantation, immunosuppressive treatments such as tacrolimus could enhance graft yielding and prevent from problems associated with allografting [13,15].

2.1. Endogenous repair following peripheral nerve injury

Subsequent to the peripheral nerve injury, there are substantial changes in differentiation status of Schwann cells (SCs) and damaged neurons in distal position of injury so that they switched from a myelin-maintenance manner to an axonal re-growth/repair one.

The process of peripheral nerve regeneration is profoundly dependent on regenerative capacity of SCs and inherent competence of peripheral neurons for outgrowth [16]. SCs because of their plasticity for

Table 1
Nerve injury classification, pathophysiology and spontaneous recovery potential.

Sunderland	Seddon	Neurophatophysiology	Recovery valence
Grade I	Neuropraxia	Segmental myelin damage, transient blockage in neural guidance, epineurium, perineurium, endoneurium and axonal integrity remains intact, partial sensory-motor impairments	Full spontaneous recovery within weeks to months
Grade II	Axonotmesis	Damage in axon and its surrounded myelin, nerve continuity and epineurium, perineurium, endoneurium are maintained, impairments in autonomic and sensory-motor functions	Full or poor spontaneous recovery (1 mm/day) depending on connective tissue integrity and distance to muscle
Grade III	Neuroma incontinuity	Axonal loss, demyelination, endoneurium is disrupted but epineurium and perineurium are intact	Poor endogenous recovery, axonal misdirection, sometimes need to surgery
Grade IV	–	Axonal and myelin damage, perineurium and endoneurium are disrupted but epineurium is preserved	Poor or no endogenous recovery, need to surgery for nerve transfer and nerve grafting
Grade V	Neurotmesis	Axonal and myelin damage, epineurium, perineurium, and endoneurium are disrupted	Poor or no endogenous recovery, need to surgery for nerve repair, nerve transfer and nerve grafting
Grade VI	Mixed injury	Various combination of nerve injuries (grade I to V)	Variable spontaneous recovery, depending upon severity of nerve injuries (grade I to V) need to surgery

various ranges of activities such as myelination, regeneration and myelinophagy are major contributors in peripheral nerve regeneration (PNR) [17]. Following injury, cross-talking between SCs, extracellular molecules (ECM) and other nearby neurons is orchestrated in favors of rebuilding damaged myelin sheath and SCs are reprogrammed into a proliferative feature and secrete plentiful healing factors for renovating disturbed nerve at a yield of 1–2 mm/day [18,19].

Generally, injury regeneration is achieved by two fundamental responses. First, myelin de-differentiation and second, phenotype alteration in SCs besides activation of innate immune responses [7].

Initial phase of repair is prompted by retrograde messages from damaged stumps toward cell bodies resulting in fragmentation of chromatin, diffusion of nuclear material and Nissl body dissolution. In this stage, the markers of immature SCs such as glial fibrillary acidic protein (GFAP), neural cell adhesion molecule (NCAM) and p75 neurotrophin receptor (p75NTR) are over-expressed while the expression of several genes related to myelin synthesis such as myelin basic protein and myelin associated glycoprotein are down-regulated. Furthermore, the activity of some transcription factors and enzymes such as Egr2 and cholesterol synthesis which are related to myelin production are decreased. In this step, SCs switch toward repair SCs and non-myelin SCs which are participated in axonal regeneration and myelinophagy of degraded myelin sheath. In second stage of reparative phase, the expression of numerous growth factors including neurotrophin-3 (NT3), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and glial cell line-derived neurotrophic factor (GDNF) are enhanced which assist in axonal re-growth and neural survival. Furthermore, in this phase, SCs by releasing chemo-attractants and inflammatory cytokines such as monocyte chemotactic protein1 (MCP-1), tumour necrosis factor α (TNFα) and nterleukin-1 result in macrophage recruitment for phagocytosis of remaining myelin debris in addition to axonal recuperation [7].

Generally, after injury, myelin SCs change their phenotype into a reparative and non-myelin cells and start to proliferate within connective tissue of basal lamina. Afterward, SCs achieve an extended bipolar figure and produce a cell layer named Bungner's band inside basal lamina which acts as a guidance trajectory for axonal regeneration. Actually, Bungner band is a connective bridge conducted by SCs, vessels and fibroblast cells. When injurious sprouts associate with SCs within Bungner band in a correct direction, the sprouts begin to growth around external layer of basal lamina to assist in axonal regeneration. Next, SCs start to re-differentiation toward myelinating phenotype to produce new myelin sheath around regenerated axons. New myelin is thinner and shorter than former healthy myelin [20,21].

One of the most important transcription factors in repair phase of PNR is c-Jun which is upregulated in order to participate in regeneration's signaling pathways [7].

In crush injuries, basal lamina sheath in SCs and axons is preserved through which regeneration promote toward distal site of injury and wounded nerve is restored within 3–4 weeks, whereas in cut injury basal lamina and layers of connective tissue is interrupted therefore axonal sprouts cannot establish an appropriate connection with the Bungner band's Cells. Hence, the sprouts developing n a misdirection place result in neuropathic pains. In this situation, surgery should be employed for rejoining the proximal and distal ends of damaged nerve [7,22].

Type of axon, length of gap and neural-promoting factors like ciliary neuro-trophic factor (CNTF) can influence spontaneous neural regrowth [5,19,23].

Endogenous repair procedure is initiated within hours post injury and is restricted to maximum 18 months after injury and then after SCs become rebellious for myelination and give poor functional outcome overtime [18,24]. Mesenchymal stem cells by secreting neural-supporting factors and anti-fibrosis agents like VGEF exert a dramatic effect on stimulating endogenous repair system for PNI. In the following sections we discus some beneficial effects of MSCs on instinct pathways of PNR [25,26].

2.2. Mesenchymal stem cells and their neuroprotective issues on different types of peripheral nerve injury

Although neurapraxia and even some types of axonotmesis could recuperate spontaneously but augmentation approaches can accelerate self-regeneration process of PNI. In this regard, mesenchymal stem cells (MSCs) have been found to be a promising trigger for restoration of nerves in both axonotmesis and neurotmesis lesions [27].

The MSCs are self-renewal cells with neuro-promoting properties which migrate into unhealthy area and by producing an enriched secretome consisting of anti-oxidant, anti-apoptosis and immunomodulatory biomolecules or by transdifferentiation into Schwann cell-like phenotype that protect neurons from extra damage and accelerate restorative phase following injury [28,29]. The MSCs are most exploited stem cells in restoring of nervous system and have been shown to be effective in remyelination procedure after embedding in various types of nerve conduits and scaffolds [30,31]. Autograft nerve bridging is associated with some drawbacks thus fabricating an artificial nerve using MSCs can be suitable for peripheral nerve cut.

Despite exact molecular and cellular mechanisms by which MSCs assist in peripheral nerve repair are not yet completely elucidated but several pathways have been recognized for this. MSCs exert its beneficial effects by reducing inflammatory responses in injury location besides decreasing Wallerian degeneration, rebuilding of myelin sheath, neovascularization, anti-fibric effect and aid in neural fibril reconstruction; Nevertheless, predominant function of MSCs is assumed

Table 2
Mesenchymal stem cells application for peripheral nerve injuries.

Cell type & differentiation status	Study model	Delivery way	Treatment efficacy	Reference
h-AT-MSCs un-differentiated	Mice model of sciatic crush (axonotmesis)	Direct intravenous administration	Improvement in fiber sprouting, reduction of inflammation, axonal regeneration using secretome of MSCs	[71]
UC-MSCs un-differentiated	Rat model of neurotmesis	Floseal® (a hemostatic sealant)	Functional and morphological recovery in both chronic and acute phases of endogenous recovery	[60]
h-UC-MSCs un-differentiated & differentiated	Rat model of neurotmesis	Poly (DL-lactide-ε-caprolactone) membrane	Motor functional recovery and regeneration of injurious nerve fiber	[72]
BM-MSCs un-differentiated	Rat model of transection of median nerve	Polycaprolactone (PCL) conduit	Higher number of myelinated fibers, larger cortical representation of median nerve	[34]
BM-MSCs un-differentiated	Rat model of small gap neurorrhaphy	Intramuscular injection	Improvement in the sciatic function index, nerve conduction velocity, myelin sheath thickness and restoration rate of gastrocnemius muscle	[73]
Amniotic MSCs un-differentiated	Sciatic nerve injury (mice)	Direct injection into injured sciatic nerve	Enhancement of angiogenesis, recovery in motor nerve conduction velocity and voltage amplitude	[74]
WJ-MSCs un-differentiated	Rat sciatic model of neurotmesis	PVA-CNTs, PVA loaded with polypyrrole (PVA-PPy), PLC	Improvement of sensory and motor function, thicken myelin sheath	[75]
AT-MSCs un-differentiated	Human patients with median & ulnar NI	Inside the nerve sheath and adjacent tissue	Significant improvement in motor and sensory recovery	[76]
BM-MSCS + SCs un-differentiated	Rat model of axonotmesis	Intra-lesion	Significant improvement in electrophysiological recordings	[77]
BM-MSCs un-differentiated	Rat sciatic model of neurotmesis	Intravenous and intra-lesion	Electromotor recovery, enhancing of fiber counts, improvement in the pace and degree of nerve regeneration	[53]
BM-MSCs un-differentiated	Rat model of laryngeal nerve (RLN) deletion	Poly(3,4-ethylene dioxythiophene) (PEDOT) scaffold	Recovery of injurious laryngeal nerve	[78]
BM-MSCs un-differentiated	Rat sciatic model of compression injury	Systemic injection intravenously	Improvement in myelin sheath thickness and axon survival	[27]
BM-MSCs un-differentiated	Rat model of neurotization surgery (neurotmesis)	Intra-lesion	Improvement in muscle function and prevent from muscle atrophy, increasing the fiber diameters and the number of motor end plates	[79]
AT-MSCs differentiated	Rat sciatic model of cut injury (neurotmesis)	A cellular nerve scaffold (ANS)	Increasing the number of regenerated myelinated nerve fibers, nerve fiber diameter, and thickness of the myelin sheath	[80]
WJ-MSCs un-differentiated	Rat sciatic model of neurotmesis	Nerve conduit	Help to nerve regeneration by increasing the expression of neurotrophic and angiogenic factors	[81]
UC-MSCs un-differentiated	Rat model of neurotmesis	Chitosan and polyethylene glycol	Increasing the numbers of fibers and vessels and enhancing percent of vessel area	[82]
AT-MSCs un-differentiated	Rat model of neurotmesis	Interpositional decellularized tube	Axonal regeneration	[83]
Canine-AT-MSCs un-differentiated	Rat model of sciatic nerve crush injury	Perineural injection	Pro-regenerative effects, motor functional recovery	[84]
BM-MSCs un-differentiated	Rat model of sciatic nerve transection	Collagen gel + silicone tubes	Functional recovery of injurious nerve	[85]
WJ-MSCs un-differentiated	Rat model of axonotmesis	Hybrid chitosan membrane	Improve post-traumatic axonal regrowth	[86]
iPBMSCs differentiated	Rat model of crush injury	PLGA + self-assembling peptide nanofibers scaffold (SAPNS)	Reducing neuromuscular junction degeneration in the target muscle, functional recovery, myelination	[87]

Abbreviations: AT = adipose tissue, UC = umbilical cord, BM = bone marrow, WJ = Wharton's jelly, iPBMSCs = induced peripheral blood MSCs.

to be via proregenerative agents for axonal regrowth and preparing a supportive microenvironment in favor of Schwann cell function [19,32]. Furthermore, MSCs, as an extra booster, loaded in scaffolds can improve efficacy of guidance scaffolds and fasten reconstruction process, recruitment of intrinsic SCs and survival of sensory and motor neurons [33]. Tubular structures filled with MSCs have been detected to repair damages of median or ulnar nerve in human [34].

Among various existing sources of MSCs, adipose tissue MSCs (AT-MSCs) are frequently used in clinical practice due to their abundance, easily harvesting, high proliferative valence, enormous pro-angiogenesis functions and less ethical restrictions [35–39]. Furthermore, AT-MSCs have been established to present strong electro-kinetic properties which make them more resistance to hypoxia and oxidative stress condition [40]. The AT-MSCs interact with endogenous SCs and by delivering a plethora of growth factors help in generation of an epineurial-like structure for regeneration of axonal lesions [41]. Likewise, AT-MSCs, when coupled with SCs and seeded onto proper conduit, can stimulate SCs for broaden myelination presumably by overexpression of CXC15, brain-derived neurotrophic factor (BDNF), glial-cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF). Additionally, AT-MSCs induce proliferation of motoneurons thereupon recover neural evoked potential even more than SCs [42,43].

The MSCs, due to their immune-privileged properties and low expression of immunological molecules such HLA-DR, have been represented to be an appropriate candidate for non-autologous grafting and eliminate requisite for immunosuppressive drugs [44,45].

MSCs in both differentiated and undifferentiated phenotypes are helpful in PNR. Although MSCs in undifferentiated stage undergo minimal risks of in vitro manipulation and have been illustrated to possess further proliferative rate compared to differentiated MSCs, but several studies have reported advantages of differentiated MSCs for PNR because of their vigorous potential for supporting neurite growth and producing neurotrophin and MBP in a great level to satisfy the PNR requirements [46,47].

The MSCs in a defined culture medium at presence of basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), glial factors and forskolin express SC markers including S100, nerve growth factor receptor (NGFR), NT3, glial fibrillary acidic protein (GFAP) and CNPase [48–50].

The MSC-derived SCs, unlike to primary Schwann cells, have a high proliferative valence which compensate dilatory division of SCs [51].

It has also been reported that MSCs embedded in a proper conduit like fibrin or collagen can locate in intra-neural venules and actively participate in neural regeneration [52]. Additionally, MSCs can assist in PNR via re-populating an acellular scaffold or by preparing an augmented neuroprotective microenvironment consist of neurotrophin-3 (NT-3), ciliary-derived neurotrophic factor (CDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and GDNF [19,53]. Likewise, MSCs, by releasing of VEGF, angiopoietin-1, IGF and HGF at a more quantity than SCs could help to neovascularization in damaged stump of nerves to feeding neo-tissue by oxygen and removing waste products. MSCs also suppress activity of caspase-3 and therefore decrease apoptosis and stimulate SCs retention. Moreover, MSCs produce ninjurin-2 which is vital for nerve guidance [54]. These above mentioned growth-enhancing factors improve survival and expansion of SCs and encourage them to shift toward renovating form [55]. SCs in combination with other stem cells such as MSCs and skin derived progenitors (SKPs) endeavor to afford more amount of neurogenesis and myelinative components with respect to SCs alone [55–57].

The MSCs also deliver a noticeable level of extracellular molecules (ECM) and synaptic molecules involved in nerve junction which take part in construction of myelin, reparation of axonal loss and recuperation of denervated muscle at a degree comparable to SCs [58,59]. Additionally, MSCs promote velocity of electrical guidance in senso-motor nerves and have great propensity for interaction with various types of biomaterials and bioactive substances while are amenable for genetic manipulation and retain their survival within repair time period [48,60–62]. MSCs, by revitalizing of neural fibers, result in thickening of the myelin sheet [63]. SCs arisen from MSCs could preserve beneficial effects of both SCs and MSCs such as myelination and restoration capabilities as well as enriched secretome [60,64,65]. The MSCs can transdifferentiate into neural lineage and then via embedding of these neuron-derived MSCs in chitosan conduit can abate fibrosis scaring in site of injury. One study has demonstrated that recovery of functional outcome in sciatic rat model is enhanced meaningfully following refreshing of MSCs medium in solid biomaterial [66].

MSCs administration is feasible in a variety of potential routes such as direct injection in injurious site via intramuscular, intrathecal pathway, systemic injection or loaded in nerve conduits and scaffolds [67] Meanwhile MSCs counteract aptly with surrounded nano-scale structures which help in creating an optimal artificial nerve. In addition, nano-formulized therapeutic agents can encapsulate MSCs and deliver them to the site of PNI [68–70]. Considered together above mentioned properties of MSCs makes them proper candidates in tissue engineering (Table 2).

2.3. Exosomes and extracellular vesicles of MSCs

Discovering a system to protect secreted MSC-originated biomolecules from degradation in unclosed system is essential. Exosomes and extra vesicles (EVs) are useful for this purpose. Exosomes are subcellular nano-size vesicles (10–100 nm) arisen from multivesicular bodies which acts as active carrier for m-RNA, iRNA, micro-RNA and participate in gene transferring and producing functional peptide/proteins in far distance from originated cells [88,89]. Exosomes/EVs owning to their natural contextures and endocytosis procedure for penetrating their components into counterpart cells have been considered as safeguard carriers for transporting paracrine factors in PNI while resolve immunogenic restrictions belong to cell-based therapies [90,91]. Additionally EVs/exosomes have been implicated to possess expediency as equal as their parent cells. Exosomes also eliminate requisite for immunosuppressive treatments in allogenic transplantation [92]. Similarly exosomes are able to pass through blood brain barrier (BBB) and based on their originated cell composition have special components for restoration of a certain tissue which prevent from systemic side effects related to cell-based medications [93]. Exosomes taken from MSCs have been demonstrated to be as constructive as their ancestor cells in neural reconstruction [94].

MSCs could interact with neural cells including SCs directly via cell to cell contact or indirectly by EV-derived bioactive factors. In this regard, it has been found that conveying of miR-133b to neural cells via MSCs-derived exosomes result in neurite outgrowth in animal model of brain injury. These secreted EVs and exosomes can be internalized inside SCs result in peripheral nerve regeneration. Given that, MSCs-derived exosome have several neuroprotective agents such as FGF, BDNF, IGF, NGF, GDNF and ciliary neurotrophic factor thereby promote recuperation of PNI. Surprisingly, the content of these secreted vesicles (EV, exosomes) is similar to the cell/tissue which arisen from and function as a selective messenger to transfer specific growth factors, enzymes, cytokines into target cell. Extracellular vesicles from MSCs participate in various cellular activities such as angiogenesis, cell repair, reduction of inflammatory responses, myelin synthesis, axonal growth and genetic reprogramming [95]. Moreover, these exosomes could conduct the direction of axonal growth and participate in regulation of neural connection. MSCs were also found to promote neural differentiation by transferring of exogenous exosome-containing miRNAs (miR-145 and miR-124) into stem cells. Accordingly, synthetic exosomes technology can eliminate several limitations associated with cell-based therapy [89,96–99].

2.4. Nerve engineering using nerve guidance conduits and MSCs

Nerve guidance conduits (NGC) are tubular structures which are inserted into insert to distal and proximal disrupted neural stumps permit them to join and acts as an artificial tubular linker between disrupted ends [100,101]. A qualified conduit mimics the function and hierarchical structure of natural nerves. Conduits are consisted of a background matrix, fillers and with or without seeded cells [102]. Fillers are comprised of filaments, sponges or gels which acts as flexible materials embedded into NGC to resemble the natural neural constituents [103]. Nevertheless, living cells are major ingredients for great repairing potential of nerve conduits. Previous investigations have illustrated that MSCs pre-seeded in conduits offer pleasant axonal configuration [61,66]. Similarly, SCs in co-culture with MSCs give rise to additive conduit function [66,104].

Through some modifications in topography and intra-structural micro-grooves of conduits or via allocation of molecules in conduit based on density gradient can alter adherence, migration and neurotrophic profile of MSCs thereby improve propagation and recanalization of artificial peripheral nerves [105,106]. In this context an improved feature of conduits is nano-functionalized nerve conduit with multichannel lumens and an enhanced surface area which prepare a more extent region for PNR. Moreover electro spinning system can print spatial design of natural ECM in conduit and provide an organized nanofibrous channel for broad distribution of neurotrophic factors as well as correct delivery of growth factors to targeted site result in axonal growth [107]. Multichannel conduits could be upgraded via axonal supportive cues such consecutive release of neurotrophic molecules and enrichment with MSCs [107]. Additionally, aligned nanoscale fibers covered by natural ECM help to correct localization of axons in repair site [108].

Although some collagen-based nerve conduits such as NeuraGen® have been shown to present adequate affinity to reparative cells like MSCs but generally collagen conduits, due to their rigid texture, high cost and their incapability to efficient attachment with surrounded cells, are not popular conduits [109]. This problem can be resolved by coating collagen conduits with key elements such as extracellular molecules, RGD tripeptide (Arg-Gly-Asp), vascular endothelial growth factor (VEGF) and bFGF to increase neovascularization subsequently enhance efficacy of nerve conduits [110–112]. Another novel strategy for elevating NGCs ability is cell-sheet system. For instance MSCs-sheet consisting of an organized row of MSCs immobilized in aligned collagen conduit capable to rehabilitee nerve guidance and neurite elongation [64]. Likewise, coating of conduits with fibronectin and laminin encourage MSCs to overabundance generation of neuro-promoting growth elements [112]. When collagen, laminin and fibronectin are loaded in hyaluronic acid matrix pre-seeded with MSCs offer plentiful benefits for axonal regrowth and induce MSCs for releasing further neurotrophic secretome result in organized distribution of cells and supplementary factors within conduit [64,113].

Decellular allograft is another material in which all of cells and immunogenic ingredients are eliminated while extracellular molecules are preserved and retain their natural physico-chemical, mechanical and spatial aligned architecture therefore SCs and other implanted cells will represent a more habitual behavior in terms of attachment, migration and bioactivity [114,115]. Of note, homogenous distribution of MSCs in acellular conduits is important for appropriate matching of interrupted stump sprouting ends [115].

Negative aspects of acellular grafts include using detergents in decellularization procedure and requirement for requiring to nerve donor [115,116]. Vein and artery conduits are natural endothelial tissues which after supplementation with palettes rich plasma or other growth-enhancing factors considerate as competent conduits for flourishing reparative phenotype of SCs and MSCs [117].

Although synthetic materials are easily accessible in broad scale, inexpensive and reproducible for urgent utilization in PNI nevertheless due to lack of cellular binding site and difficult degradability need to some modifications such as coating of adhesive molecules in surface of scaffold is imperative for utilizing them in clinical application [63,118].

Even though synthetic biodegradable polymers such as polyglycolic acid (PGA) reduce risk of nerve compression and fibrosis associated with non-degradable conduits but new generation of polymers called electro conducting constructs owing to their special traits for conducting electrical flow sounds to be more beneficial for tissue regeneration [119]. In this regard MSCs combined with COOH-functionalized multiwall carbon nanotubes implanted in polyvinyl alcohol is a stable electro conductive tube for restoration of peripheral nerve gaps and robusting electrical signals across their porous constructors and simultaneously facilitate anchoring of surrounded cells or molecules into conduit [75].

Consecutive release of fibroblast growth factor 2 (FGF2) and miR-218 in a 3D aligned nanofiber such as poly 3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV) pre-seeded with AT-MSCs has been demonstrated as an optimized engineered artificial conduit for repair of transected nerves. Furthermore, immobilization of NFG onto a prearranged nano-scale fibers impregnated in PLGA by electrospinning technique results in regular NGF releasing and conveying nerve regeneration among lesions [120–123].

Gelatin hydrogel is also a biodegradable material with strong mechanical texture and flexible structure for attachment of MSCs. When gelatin is combined with microstructure compounds it can robust adherence, expansion and migration of MSCs for neural-growth promoting procedure [59,124]. Other advantageous conduit in regenerative medicine is chitosan-based conduit. Chitosan is a natural conduit with pleasant properties such as biodegradability, biocompatibility, bactericide above and beyond anti-fibrosis [124,125]. A recent work has illustrated MSCs delivered in chitosan conduit filled with erythropoietin has a significant influence on improvement of deconstructive peripheral nerve tissue and promoting neural differentiation of MSCs. Another engineered approach for PNR is scaffolding technology.

Scaffolds are engineered materials which act as a model to guide the new tissue synthesis. Scaffolds by affecting biological and physico-chemical cell environment prepare a supportive matrix for damaged tissue. A variety of natural or synthetic scaffolds exist for regeneration of injuries in nervous system disorders [126–129].

Scaffolds after enrichment with different type of regenerative cells and pre-conditioned with essential elements are designed for special purposes in regenerating medicine. An ideal scaffold for PNR should aim to prolong cell retention in injurious site, enhance revascularization and help to homogenous distribution of growth nutrients. Scaffolds prepare an expedient milieu for restoration defective nerve and provide a suitable matrix for PNR. In this context, a recent research has demonstrated dual delivery of VEGF and NGF in a nanofibrous scaffold in a sequential manner exerts pronounced influence on repairing of PNI. In this scenario, MSCs by secreting both VEGF and NGF at a satisfactory level can accelerate this procedure and exhibit a synergist effect on regenerative pathway. Core-shell constructs are advisable material for dual delivery of growth factors in scaffolds [130–132].

Another qualified scaffold for neural regenerative purposes is polylactic glycolic acid (PLGA) which is popular for its easy handling and biocompatibility [133]. Modified multi-channel PLGA seeded with SCs or MSCs is a well-suit scaffold employed for myelin formation [134].

Another MSCs-carrying scaffold is poly (3,4-ethylenedioxythiophene) (PEDOT) which improves peripheral nerve regeneration via inducing over-expression of Notch signaling and miR-21 [135].

Chitosan-PLGA scaffold combined with MSCs has been used in primate model of PNI as an engineered allograft for renovation injurious peripheral nerve. Grafen-based scaffolds are other type of scaffolds which could accelerate mitotic cell cycle of MSCs, encourage their differentiation into SCs and quicken axonal regrowth [136,137]. In the

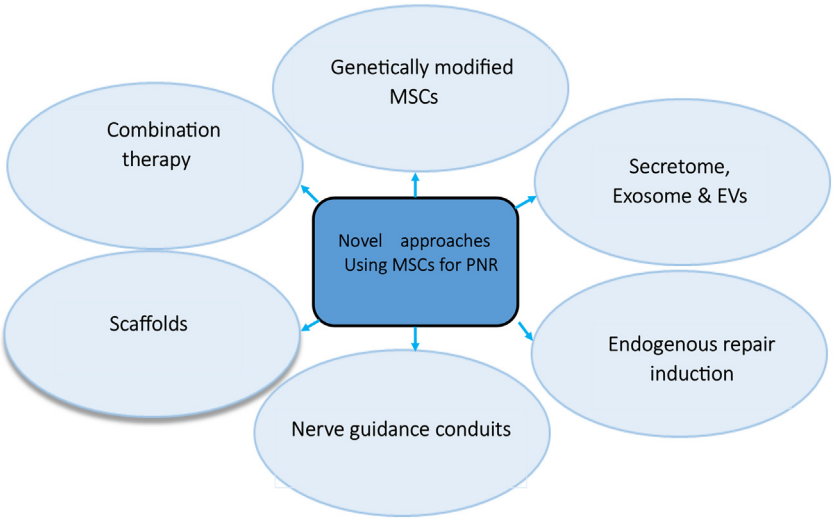

Fig. 2. Novel strategies using stem cells to enhance peripheral nerve regeneration.
MSC = mesenchymal stem cells, PNR = peripheral nerve regeneration, EV = extracellular vesicles.

same way, fibrin scaffold seeded with MSCs after insertion in autologous vein construct offer dramatic outcomes in terms of enhancing cell retention in scaffold subsequence reviving peripheral nerve damages [138,139].

Overall nano-functionalized electro-conductive scaffold such as polypyrrole is one of the optimal biomaterials for PNR. By inserting nanofibril structure in inner layer of scaffold and replacing micro-organized constructions in its external area, it can afford a close resemblance to the hierarchical pattern of natural ECM. This results in dynamic interaction among scaffold-derived components and subsequently in better regeneration [110,140].

Cell sheeting is an advanced technology in which a monolayer of primary SCs is implanted onto supportive scaffold. Subsequent decellularization of this structure will produce a natural ECM which provides enhanced function of neural cells and guidance signal. [140].

2.5. Genetically engineered MSCs for PNR

Genetically modified MSCs are becoming more popular in tissue engineering research. Genome editing is an impressive technology in which therapeutic genes are inserted in targeted cells for reprogramming the cells to express or inhibit certain genes. Schwann cells, glial cells, denervated muscle, sensory and motor neurons can be subjected for gene delivery. Main goal of gene modification is reprograming cells to over-releasing growth factors, migratory and adhesive molecules along with inhibiting defective genes [121].

Herpes and recombinant viruses are conventional and high yield vectors for gene delivery but recently AAV-PHP.eB and AAV-PHP.S derived from adeno-associated viruses have been reported as efficient capsids capable to transduction of the PNS cells with better outcome than traditional viruses [141].

Neurotrophin-3, neurotrophin-4, GNDF, BNDF, NGF, PDGF, bFGF and CNTF are principal neuroregenerative factors suitable for gene delivery in peripheral nerves. Previously mentioned growth factors are vital constituents supplying a proper environment for survival and axonal growing. MSCs engineered to hypersecrete these agents or MSCs manipulated to transdifferentiate into SC like phenotype are potent cells for augmenting beneficial effects of MSCs on neural repair [118]. In this scenario MSCs transduced with VEGF seeded in 3D fibrin scaffold have been shown to accelerate neural regenerative cues [142].

MSCs engineered to express mi-RNA34-a are designed for stimulating MSCs to differentiate toward SCs and help to neoangiogenesis in disturbed nerves in sciatic nerve gap model [143]. Ciliary neurotrophic factor and brain-derived neurotrophic factor are two major neurosupportive mediators which have been inserted into MSCs for neuroprotective performances in rats with sciatic nerve injury [144].

2.6. Other therapeutic strategies in PNR

To date, numerous therapeutic implications have been commercialized for treatment of peripheral nerve injuries which can be considered as complementary medication when combined with MSCs therapy or other therapeutic strategies for PNI. One of the novel techniques is using personalized nerve-engineered computer-controlled vehicles to alter 3D architecture of scaffold as well as its physico-chemical, mechanical and topographic properties to achieve an ideal individualized environment for tissue regeneration for each individual [145]. Nerve-on chip technology is the other reproducible in vitro method for mimicking in vivo condition of PNR and screening cell behavior in body while reduces the demand for human and animal models [146].

Co-transplantation of SCs with MSCs or neural stem cells, dorsal root ganglion neurons and skin derived progenitors have an extra influence on nerve regeneration and are a potent method to induce more functional improvements in PNI [147–150]. Moreover, co-administration of MSCs and low-intensity pulsed ultrasound lead to greater neural recovery [151].

Optogenetics is an innovative approach in which a relevant wavelength is employed to manage living neurons in their natural environment by altering conformational shape of transmembrane peptides in terms of repairing neural gaps [151,152].

Combination of MSCs and genome editing, nerve conduits and chondroitinase have been displayed to give more yield of neuroprotective influence relative to using each of them alone [153]. Vitamin E, ascorbic acid, hepatocyte growth factor (HGF-R), tropomyosin receptor kinase A, conserved dopamine neurotrophic factor, erythropoietin, and neuregulin-1 are other supplementary treatments for PNI [103,154–156].

3. Conclusion and future directions

Although peripheral nervous system is capable in full self regeneration after injury but because of insufficiency of endogenous repair in PNS and inadequacy of conventional treatments for PNI, restoration of peripheral nerve-related injuries is still of unmet clinical requisite. Mesenchymal stem cells in conjugation with nerve guidance conduits, scaffolds and gene engineering strategies are being more

widely considered as suitable alternative approaches for tissue engineering of PNI. Moreover, MSCs would envisage to be a proper candidate for SCs in clinical translation of cell based therapy for nerve injures. Despite a number of developments for fabricating engineered nerve, still several requirements are needed to be investigated before clinical translation. For example, optimized source of MSCs, dosage and number of MSCs injections, tracing techniques for monitoring behavior of MSCs in vivo and their efficacy in peripheral nerve regeneration are key items to be further studied (Fig. 2).

Conflicts of interests

The authors have no conflicts of interest to declare.

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