A biomaterials approach to Schwann cell development in neural tissue engineering

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Abstract
Schwann cells, in addition to forming myelin sheaths, have pivotal roles in regeneration of injured axons in the peripheral nervous system such as producing a natural permissive conduit between distal and proximal stumps and secreting nerve growth factors. Due to the atrophy and senescence of Schwann cells in long nerve gap, and the need to ensure the presence of nerve growth factors and basal lamina tubes for axon regeneration in a critical time, injection of Schwann cells with the aid of an engineered conduit seems to be an effective approach to induce axon regrowth. Stem cells with high differentiation and proliferation capability can provide an adequate number of Schwann cells in healthy state for regeneration purposes. Guidance of stem cells differentiation into desired lineages, control of implanted Schwann cells fate, maintenance of nerve growth factors expression, and guidance of axon regrowth are possible with the aid of biomaterials with appropriate chemical, physical, and mechanical properties. Biomaterials' surface chemistry and biomolecules interacting with cells' receptors initiate specific intracellular signaling cascades and direct cells fate. In addition, biomaterials' surface topography in association with cells contact area, focal adhesion, and cytoskeletal remodeling by mechanotransduction process influences cells behavior and induces specific differentiation. The main objective of this review is to investigate the chemical, topographical, and mechanical properties of biomaterials which influence the fate of Schwann cells and the nerve regeneration process.

KEYWORDS
biomaterials, biomolecules, peripheral nerve regeneration, Schwann cells, stem cells, surface properties

1 INTRODUCTION

The nervous system is composed of the central nervous system (CNS, brain and the spinal cord) and the peripheral nervous system (PNS, nerves throughout the body). Nervous system cells are divided into neurons, glial cells, and immune and connective cells (Painter, 2017). The neuron structure is composed of dendrites, cell body, axon, and telodendria (presynaptic terminals). Neurons are divided into three
functions of Schwann cells

Schwann cells have pivotal roles in the peripheral nervous system, such as (a) establishing myelin sheaths around peripheral axons, causing saltatory conduction and increasing signal transmitting velocity; (b) maintaining the integrity of axon; (c) cooperating with macrophages to clean Wallerian degeneration products and myelin debris after nerve injuries (aged Schwann cells have lower capability to ingest these debris and axon regeneration process does not take place in the presence of myelin debris; Painter, 2017; Xue et al., 2017); (d) guiding axonal regeneration by generating Bands of Bungner (a natural permissive nerve conduit between distal and proximal axon stumps); (e) expressing neurotrophic growth factors as trophic support of axons such as BDNF, GDNF, NGF (neuronal survival is almost dependent on neurotrophic support; Faroni et al., 2015; Lehmann & Höke, 2016; Li, Xiao, Zhang, Zhao, & Yang, 2017); (f) secreting neuropoietic cytokines (such as CNTF) and hormones like erythropoietin (Inoue et al., 2010); and (g) promoting wound healing processes (Cai et al., 2017; Clements et al., 2017; Scheib & Höke, 2013; Uz et al., 2017). As a result, an adequate number of Schwann cells in healthy state take advantage of an outstanding phenotypic plasticity (Cai et al., 2017). Interestingly, Schwann cells also have the capability to myelinate and regenerate axons in CNS when transplanted, and therefore are used in spinal cord injury repair (Cerqueira et al., 2018; Vilariño-Feltrer et al., 2016). Recently, the regenerative influence of Schwann cells on other tissues (skin wound repair and mammalian digit regeneration) has been found in the case of the critical role of secreting paracrine growth factors (Carr & Johnston, 2017). Incorporating Schwann cells in synthetic nerve conduits and maintaining their viability and functionality instead of directly injecting growth factors improves the nerve regeneration process (Gu, Ding, & Williams, 2014; Xu et al., 2017).
It has been shown that aged Schwann cells contribute to dysfunctions in mammals and restrain axon regrowth due to loss of Schwann cell plasticity (c-Jun protein as a key factor for phenotypic change of Schwann cells is not elevated in aged animals after injury) (Painter, 2017). For Schwann cells development, it is important to consider the role of cell–cell and cell–matrix interactions in cell survival and cell fate. This is more particularly observed in severe nerve injuries, where a long gap exists between distal and proximal stumps. In long nerve defects, Schwann cells atrophy and senescence reduce axon regeneration (Poppler et al., 2016; Scheib & Höke, 2013; Thomas et al., 2017). Cell–cell-mediated signals (coculture Schwann-like cells with DRG neurons) prevent the morphological change of bone marrow-derived Schwann-like cells and derive functionally mature Schwann cells in vitro studies (Cai et al., 2017). Insufficient number of Schwann cells, their low proliferation and migration capability, along with their low survival rate after transplantation, are the main causes of unsatisfactory nerve repair (Cerqueira et al., 2018; Novajra et al., 2016). Therefore, having an adequate amount of Schwann cells in a healthy state for transplantation applications is an important approach for repairing injured axons and achieving successful nerve regeneration in the critical time before neuronal death takes place (Cai et al., 2017; Faroni et al., 2015).

### 3 | STRATEGIES FOR PERIPHERAL NERVE REPAIR

Although an axon has the capability to regenerate itself, the slow rate of axon self-regenerating and the critical time for nerve repair (after which apoptosis of neurons and atrophy of non-nerve contact muscle occur; Scheib & Höke, 2013), cause the autologous nerve graft and engineering approaches by recruiting materials, cells and growth factors to be considered as efficient repair strategies for large nerve defects (Cerri et al., 2014).

Autologous treatment, as a clinical gold standard technique for nerve repair, has a low risk of immunological rejection and provides extracellular matrix and glial cells. However, there are some drawbacks in this technique such as surgical implications, extra incision, normal nerve tissue sacrifice, and diameter mismatch between donor and recipient nerve fibers, lack of donor nerve, neurona formation possibility and painful self-destructive process. Allograft nerve provides axonal regeneration and sufficient amount of Schwann cells, however, needs to use systemic immunosuppression for long period, which increases the possibility of infection and tumor formation (Kehoe, Zhang, & Boyd, 2012). Directing axonal regrowth, increasing the number and speed of regenerating axons, and creating an accelerating environment
by using engineered nerve conduit is a favorable strategy for repairing nerve defects. Engineered nerve conduits could be constructed from acellular human nerve and biodegradable biomaterials such as collagen, chitosan (Li et al., 2018), poly(glycolic acid) (PGA), polycaprolactone (PCL), with appropriate physicochemical cues (Faroni et al., 2015; Gu et al., 2014; Kehoe et al., 2012; Xu et al., 2017). Cellular nerve guidance scaffolds (conduits containing Schwann cells) are found to be functionally more efficient than acellular conduits due to secretion of neurotrophic factors for axonal regrowth (Xue et al., 2017).

Local signaling cues are very important in nerve regeneration because axons have the ability to locally synthesize translation-dependent proteins at growth cones for transmitting injury signals from a distal part and also are important for phenotypic changes of Schwann cells (Suzuki et al., 2017). Axon length is also an important factor in nerve regeneration process (Scheib & Höke, 2013). Stimulation of the axon regeneration process by transplantation of glial cells such as Schwann cells or neurotrophic factors such as NGF, GDNF, and BDNF with controlled release rate and appropriate half-life improve nerve regeneration. The capability of Schwann cells in inducing axon regeneration and lack of donor sites for direct exploitation of Schwann cells as well as low proliferation and growth factor secretion capability of old Schwann cells confirm the need for an alternative source of Schwann cells (Gu, Ding, & Williams, 2014; Painter, 2017; Uz et al., 2017; Xue et al., 2017).

4 | STEM CELLS IMPORTANCE

Cell-based therapies, for example, transplantation of Schwann cells for repairing nerve defects, have attracted much attention in regenerative medicine (Faroni et al., 2015; Painter, 2017). Stem cells, due to their potential in therapeutic applications and capability to differentiate into Schwann cells under a specific induction medium, have become a promising alternative for direct harvesting Schwann cells (Faroni et al., 2015; Thomas et al., 2017). Stem cells can be directly injected to lesion sites or become differentiated into Schwann cells in vitro and applied into the injured part with the aid of conduits.

Embryonic stem cells are pluripotent stem cells which have high proliferation potential and ability to differentiate into Schwann-like cells with high efficacy; however, the cells may increase the risk of teratoma and teratocarcinomas formation and there are ethical considerations (Bhangra, Busuttil, Phillips, & Rahim, 2016; Jiang, Jones, & Jia, 2017). Embryonic stem cells in undifferentiated state do not stimulate host immune responses due to their low level of MHC but can trigger immune responses in differentiated state (Shi, Inoue, Wu, & Yamanaka, 2017). Neural stem cells which are almost harvested from the central nervous system also are able to differentiate into Schwann cells and improve axon myelination and regeneration. However, they have major limitations such as site harvesting and tumor formation due to their high proliferation without differentiation (Heine, Conant, Griffin, & Höke, 2004; Lee et al., 2017). Mesenchymal stem cells are more attractive multipotent stem cells derived from adipose tissues, bone marrow, dental pulp, skin, hair follicle, and amniotic fluid (Hosseinirad et al., 2018). Mesenchymal stem cells have proangiogenic capabilities, immune regulatory properties, ability to differentiate into myelinating and nonmyelinating Schwann cells under specific induction environment, easy accessibility procedure, less side effects and less ethical implications in comparison with embryonic stem cells (Faroni et al., 2015; Leach & Whitehead, 2017; Painter, 2017; Thomas et al., 2017).

FIGURE 2 (a) Bone marrow-derived mesenchymal stem cells. (b) Differentiated mesenchymal stem cells toward Schwann cells phenotype through preinducing factors of beta-mercaptoethanol and retinoic acid and inducing factors of forskolin, bFGF, PDGF and neuregulin after 7 days (blue: Hoechst, green: S100β; Reprinted with permission from Zhu et al. (2014))
Bone marrow-derived mesenchymal stem cells differentiate into Schwann cells under a specific induction medium and enhance neurite growth (Mahay et al., 2008). They also enhance nerve regeneration via the direct expression of neurotrophic factors, increase Schwann cell proliferation, and stimulate Schwann cells to secrete neurotrophic factors (Bhangra et al., 2016; Wang, Ding, Gu, Liu, & Gu, 2009). However, they need an invasive and painful procedure in order to be harvested. Inducing factors that differentiate mesenchymal stem cells into Schwann cell-like cells include forskolin, bFGF, PDGF, and neuregulin (Zhu et al., 2014). Figure 2 shows bone marrow-derived mesenchymal stem cells differentiated into Schwann-like cells under a specific induction medium. It has also been shown that adipose stem cell-derived Schwann cells using a less invasive harvesting procedure have the capability to regenerate severely injured axons and exhibit better functional outcomes, compared to bone marrow-derived Schwann cells (Jiang et al., 2017; Lehmann & Höke, 2016). Transplantation of adipose-derived stem cells increases axon regeneration, but without convincing efficacy up to now (Faroon et al., 2016), and there is the risk of adipocyte formation. It is worth noting that transplantation of adipose-derived Schwann cell-like cells can enhance axon regeneration (di Summa et al., 2010), promote remyelination, prevent neurogenic muscle atrophy (Lehmann & Höke, 2016), and release growth factors (Jiang et al., 2017), and thus has better outcomes compared to transplantation of undifferentiated adipose-derived stem cells. Fetal-derived mesenchymal stem cells, especially umbilical cord blood stem cells, have strong capability of differentiating into neurons and glial cells and have advantages of noninvasive harvesting procedure and less genetic damage, although there are limited source availability and allograft reactivity probability (Jiang et al., 2017). Stem cells derived from skeletal muscle, skin, hair follicle, and dental pulp with the potential of differentiation into many cell types also exhibit nerve regeneration and myelination capabilities (Bhangra et al., 2016; Jiang et al., 2017). Different sources of stem cells and their applications in peripheral nerve regeneration were recently reviewed (Jiang et al., 2017; Moosazadeh, Bonakdar, Shokrgozar, & Faghihi, 2018), which some of them are listed in Table 1.

Induced pluripotent stem cells (iPSCs) with high differentiation potential generated from somatic cells using reprogramming factors could be a powerful source for stem cell-based therapies in nerve regeneration and myelination. iPSCs can be patient-specific derived and used as autologous cells, however, the immunogenicity of these cells is still being evaluated. For example, Zhao et al. reported T-cell mediated immune response in syngeneic mouse recipients of iPSCs (Zhao, Zhang, Rong, & Xu, 2011). In another study, lack of immune system response in the absence of immunosuppression was reported by transplantation of iPSCs-derived neural precursor cells in syngeneic minipig recipients (Strnadel et al., 2018). Also, there is a risk of genomic mutagenesis and tumorigenesis yet (Jiang et al., 2017; Shi et al., 2017). As it was reviewed by Ma et al., the inducing factors and the

TABLE 1 Different sources and types of stem cells used for peripheral nerve regeneration

<table>
<thead>
<tr>
<th>Source</th>
<th>Schwann cell differentiation medium</th>
<th>Schwann cell marker</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
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<tr>
<td>Embryonic stem cells</td>
<td>SDIA (PA6), neurosphere, FGF-2</td>
<td>GFAP, S100β</td>
<td>High differentiation potential, obtainable from autologous somatic cells</td>
<td>Ethical implications, not autologous, possibility of teratoma formation</td>
<td>Ziegler, Cigovac, Yang, Zhang, and Gokstein (2011)</td>
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<tr>
<td>Fetal-derived mesenchymal stem cells</td>
<td>Laminin, DMEM:F12, EGF, FGF2, B27</td>
<td>MBP, GFAP, S100β</td>
<td>High differentiation potential, obtainable from neural crest cells</td>
<td>Secretion neurotrophic factors and remyelination</td>
<td>Bhangra, Faria, and Anderson (2016)</td>
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<tr>
<td>Adipose-derived mesenchymal stem cells</td>
<td>Laminin, DMEM:F12/10% FBS, forskolin, PDGF, bFGF</td>
<td>MBP</td>
<td>Easily accessible, secretion neurotrophic factors and remyelination</td>
<td>Adipocyte formation probability</td>
<td>Zarinfard, Tadjall, Razavi, and Kazemi (2016)</td>
</tr>
<tr>
<td>Bone marrow-derived mesenchymal stem cells</td>
<td>αMEM, PDGF-AA, bFGF, neurospheres formation, DMEM:F12, EGF, FGF, B27</td>
<td>αMEM, all-trans-retinoic acid</td>
<td>Secretion neurotrophic factors and remyelination</td>
<td>Invasive harvesting procedure</td>
<td>Xian et al. (2017)</td>
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<tr>
<td>Biomaterials</td>
<td>Advantages</td>
<td>Limitations</td>
<td>Commercial product</td>
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<tr>
<td>Collagen</td>
<td>Biocompatible, biodegradable, low immunogenicity, versatility, component of Schwann cell natural ECM, semi-permeable to allow nutrient transfer, enhances Schwann cell adhesion and spreading, enhances cell migration, good hydrophilicity, used as surface coating to enhance cell–substrate interaction, causes long-term survival, and proliferation of cells</td>
<td>Batch to batch variation, degradation rate, long degradation period for NeuraGen® and NeuroWrap™ (36–48 months), short degradation period for NeuroMax™ and NeuroFlex™ and NeuroMend™ (4–8 months), the possibility of swelling and long degradation period cause the risk of inducing nerve compression but not seen in commercial products, risk of cracking when suturing</td>
<td>NeuraGen®, NeuroWrap™, NeuroMatrix™, NeuroFlex™, NeuroMend™</td>
<td>Dalamagas et al., 2016 Cerri et al. (2014), Gu, Ding, and Williams (2014), Novajra et al. (2016), Kehoe et al. (2012), Yue, Liu, Molino, and Wallace (2011), Chernousov, Yu, Chen, Carey, and Strickland (2008), Lou, Stowers, Tam, Xia, and Chaudhuri (2018), Sensharma, Madhumathi, Jayant, and Jaiswal (2017), Ai et al. (2014), Georgiou et al. (2013)</td>
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<tr>
<td>Hyaluronic acid</td>
<td>Biocompatible, biodegradable, nonimmunogenic, interacts with cell surface receptors such as CD44 and RHMM15, component of natural nervous system ECM, friendly environment for Schwann cells to sustain their proliferation, antibacterial effect, less glial scarring, has mechanical properties similar to soft nervous tissues, reduces extraneural scarring, surface hydrophilicity causing tight cell–cell junctions</td>
<td>Risk of swelling that applies compression to nerves, low cell adhesion due to high water content, surgical implications, its molecular weight determines its biological functions such as its angiogenic potential, low molecular weight degradation products have pro-inflammatory and high molecular weight fragments have anti-inflammatory effects, increased levels of hyaluronic acid in ECM causing several types of cancer malignancies</td>
<td>Vilarino-Feltrer et al. (2016), Thomas et al. (2017), Li et al. (2018), Yue et al. (2011), Lou et al. (2018), Sensharma et al. (2017), Lam, Truong, and Segura (2014)</td>
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<td>Zein</td>
<td>Biocompatible, biodegradable, ease of fabrication, low cost, good mechanical properties, wide resource availability, good degradation rate</td>
<td>Low resistance to pull-out of suture thread, used for small nerve defects (10 mm)</td>
<td>Wang, Yang, Wu, Zhang, and Wang (2017)</td>
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<tr>
<td>Chitosan</td>
<td>Biocompatible, permeable, biodegradable, flexible, pH dependent solubility, high processability, growth inhibition of bacteria, non-immunogenic, promotes adhesion, growth and migration of Schwann cells, supports nerve conduction and development of myelinated axons, neuroprotection effect, resistant to collapse, low growth of fibroblast, inhibits scar tissue and neuroma formation, hydrogel wall (easy to suture), transparent, low water content and surface amines enhance viability, and survival of neural precursor cells, chitosan degradation products have influence on Schwann cells behavior</td>
<td>Low compressive mechanical property, degradation rate, degree of acetylation influences Schwann cells behavior</td>
<td>Reaxon®</td>
<td>Li, Xue, et al. (2018), Gu, Zhu, et al. (2014), Zhao et al. (2017), Wang et al. (2016), Lau et al. (2018), Ao et al. (2011), Li, Xue, et al. (2018), Du et al. (2014), He et al. (2018), Farahidhosseinabadi et al. (2019)</td>
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<tr>
<td>Biomaterials</td>
<td>Advantages</td>
<td>Limitations</td>
<td>Commercial product</td>
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<tr>
<td>Carboxymethylated chitosan</td>
<td>A soluble derivative of chitosan, anti-oxidant, anti-apoptotic, attenuates caspase-3 apoptosis pathway activation, protects Schwann cells against oxidative stress of hydrogen peroxide-induced damage, promotes proliferation of Schwann cells, enhances rat sciatic nerve repair</td>
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<td>He et al. (2018), Tao et al. (2013)</td>
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<tr>
<td>Alginate</td>
<td>Biocompatible, negatively charged polysaccharide, ability in cell encapsulation, biodegradable, easy crosslinking and tunable mechanical properties, antibacterial effect, supports Schwann cells migration</td>
<td>Low and unstable mechanical properties, high degradation rate</td>
<td></td>
<td>Golafshan, Kharaziha, and Fathi (2017), Hashimoto et al. (2005)</td>
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<tr>
<td>HYAFF®</td>
<td>Benzyl ester of hyaluronic acid, biocompatible, promotes cell adhesion, complete degradability, soluble in DMSO, controllable degradation rate, supports proliferation, and adhesion of Schwann cells</td>
<td>Acidic degradation products, poor processability</td>
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<td>Sensharma et al. (2017), Vindigni, Cortivo, Iacobellis, Abatangelo, and Zavan (2009)</td>
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<tr>
<td>Gelatin</td>
<td>Biocompatible, less cytotoxicity, biodegradable, promotes nerve regeneration, inherently cell-adhesive, low cost</td>
<td>Fast degradation, poor mechanical properties</td>
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<td>Uz et al. (2017), Farzamfar et al. (2018), Hu et al. (2016)</td>
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<tr>
<td>Cellulose</td>
<td>Biocompatible, chemically modified for biological usage, nontoxic, good mechanical properties, wet-electrospinability, combined with hyaluronic acid as an anti-adhesion for neuroma management application</td>
<td>Poor biodegradability and cell adhesion</td>
<td></td>
<td>Du et al. (2014), Farzamfar et al. (2018), Luo et al. (2015), Agenor et al. (2017)</td>
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<tr>
<td>Silk fibroin/gold nanocomposite</td>
<td>Biocompatible, conductive, enhances Schwann cell proliferation and sciatic nerve regeneration</td>
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<td>Das et al. (2015)</td>
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<tr>
<td>Self-assembling peptide</td>
<td>Easily synthesized with high purity, easily modified with bioactive agent, in situ formation of scaffolds, enhances cell adhesion, ability to penetrate into brain blood barriers</td>
<td>More studies needed with regard to intelligent neural tissue engineering</td>
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<td>Koss and Unsworth (2016)</td>
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<tr>
<td>Bioactive glass</td>
<td>Doped in degradable polymers or in the form of aligned fibers inside the lumen to create guidance cues, biocompatible,</td>
<td>The ratio of glass/polymer must be optimized</td>
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<td>Novajra et al. (2016), Zhang et al. (2011)</td>
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<thead>
<tr>
<th>Biomaterials</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Commercial product</th>
<th>References</th>
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<tbody>
<tr>
<td>Polyglycolic acid (PGA)</td>
<td>Biocompatible, biodegradable, non-toxic, used as physical contact guidance for axonal regeneration, used as suture materials, provides sensory function recovery, good initial mechanical properties, wide availability of clinical data, successful in regenerating hand nerve defects, more preferable in clinical use</td>
<td>Acidic degradation products, degradation period (6–12 months), low solubility</td>
<td>NeuroTube®</td>
<td>Dalamagkas et al. (2016), Kehoe et al. (2012), Gu, Zhu, et al. (2014), Sensharma et al. (2017), Zhang et al. (2011)</td>
</tr>
<tr>
<td>Polylactic acid (PLA)</td>
<td>Biocompatible, biodegradable, used as physical contact guidance to support neurite out-growth, high porosity</td>
<td>Acidic degradation products, poor processability, higher tensile strength than natural nerve, degradation rate</td>
<td>Bhutto et al. (2016), Zhou et al. (2017), Nectow, Marra, and Kaplan (2011), Xu et al. (2014)</td>
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<tr>
<td>Polycaprolactone (PCL)</td>
<td>Biodegradable, low cost, high elasticity, good mechanical properties, high processability</td>
<td>Low cell adhesion, important to choose appropriate solvent and fabrication method, degradation rate</td>
<td>Xue et al. (2017), Dalamagkas et al. (2016), Sensharma et al. (2017), Bhutto et al. (2016), Bahrami et al. (2017)</td>
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<tr>
<td>Poly-lactic-co-glycolic acid (PLGA)</td>
<td>Biodegradable, nontoxic, ability to control degradation rate</td>
<td>Low cell adhesion, acidic degradation products, undergoes plastic deformation due to long term cyclic strain, insufficient stability</td>
<td>Xu et al. (2017), Sensharma et al. (2017), Zhang et al. (2011)</td>
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<tr>
<td>Poly-lactic-co-caprolactone</td>
<td>Biocompatible, biodegradable, transparent, semi-permeable, has preclinical data</td>
<td>Fragmentation and foreign body reactions, swelling, possibility of neuroma formation, degradation period (16 months), rigidity, severe automutilation, handling difficulties</td>
<td>Kehoe et al. (2012), Sensharma et al. (2017), Zhang et al. (2011), Nectow et al. (2011)</td>
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<tr>
<td>Polyhydroxy butyrate (PHB)</td>
<td>Biocompatible, neuroprotection effect</td>
<td>High crystallinity, brittle, long degradation period (24–30 months)</td>
<td>Dalamagkas et al. (2016), Xiao, Zhao, and Chen (2007)</td>
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<tr>
<td>Polyvinyl alcohol (PVA)</td>
<td>Soft and flexible, water content similar to natural tissue, non-toxic, good spinability, good mechanical properties, easily molded into anatomic shapes, using freeze/thaw cycles instead of harsh crosslinking agents, easily sterilized, lack of antigenicity</td>
<td>Poor cell affinity, nonbiodegradability and possibility of nerve compression</td>
<td>Salutunnel™, Salubridge™</td>
<td>Kehoe et al. (2012), Sensharma et al. (2017), Golafshan et al. (2017)</td>
</tr>
<tr>
<td>Polyglycerol sebacate (PGS)</td>
<td>Good biocompatibility, elasticity, flexibility, biodegradability, high toughness, similar mechanical properties to soft tissue</td>
<td>Degradation behavior</td>
<td>Sensharma et al. (2017), Wu, Wang, Guo, Shao, and Ma (2016)</td>
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<td>Polypyrrole (PPY)</td>
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in vitro condition of generating Schwann cells from iPSCs must be engineered and optimized to achieve safe Schwann cell-like cells (Ma et al., 2015).

In conclusion, although stem cells are able to differentiate into many cell types, their clinical performance has not been completely verified. The complexity of in vivo environment causes unsatisfactory outcomes in direct injection of un-differentiated stem cells and increases the risk of tumorigenesis; thus, it seems that in vitro predifferentiation of stem cells could obtain better clinical results. Accordingly, using the cells at the appropriate differentiation stage is important in successful nerve regeneration. Huang et al. considered the potential application of human iPSC-derived neural crest stem cells (NCSCs) and Schwann cells (NCSC-SCs) in peripheral nerve regeneration. They embedded these iPSCs-derived cells at two differentiation stages in a collagen/hyaluronan hydrogel and seeded into poly(L-lactide-co-caprolactone) conduit. They reported that the NCSCs group gained better electrophysiological and gastrocnemius muscle recovery than the NCSC-SCs group (Huang et al., 2017).

Chemical, physical and mechanical properties of culture substrates such as appropriate surface ligands, stiffness, and topographical cues regulate stem cell fate (Wen et al., 2014). Nowadays, it is more favorable to design an engineered biomaterial in order to direct differentiation of stem cells (Leach & Whitehead, 2017). There is a "triangle of control" for directing cell fate, including chemical approaches, physical and surface topography, and mechanical approaches (Bonakdar et al., 2016; Kamguyan et al., 2018; Mashinchian et al., 2015; Moghaddam, Bonakdar, Shariatpanahi, Shokrgozar, & Faghihi, 2019; Moosazadeh et al., 2019).

### 5 | BIOMATERIAL-BASED APPROACHES FOR SCHWANN CELL APPLICATIONS

For Schwann cells transplantation, they must be seeded into appropriate scaffolds in neural tissue engineering. Scaffolds are engineered biomaterials which support Schwann cells survival, proliferation, and migration. For Schwann cells differentiation and transplantation, the biomaterial’s chemical, physical, and mechanical properties have influence on Schwann cell fate, which is explained in this section.

#### 5.1 | Scaffolds for Schwann cell differentiation and transplantation

Many natural and synthetic biomaterials have been used as the base of conduits for directing and regenerating injured nerves. Table 2 shows a list of biomaterials used as nerve scaffolds with more focus on biomaterials for Schwann cell differentiation and transplantation. Biomaterials used for constructing nerve conduits must have such properties as biocompatibility, nonimmunogenicity, flexibility with good mechanical stability, good permeability allowing the diffusion of oxygen and nutrients and inhibiting the infiltration of inflammatory cells, appropriate degradation behavior, ability to promote Schwann cell migration and proliferation, ability to direct axonal regeneration to
reach distal stump, and apply no compression to regenerating nerve (Kehoe et al., 2012; Muheremu & Ao, 2015; Wang et al., 2017).

### 5.2 | Effect of chemical properties of the biomaterials on Schwann cell fate

Surface chemistry of biomaterials and biomolecules has a major influence on cellular behavior through initiating specific intracellular signaling cascades. Surface modification of the substrate, degradation behavior of Schwann cell scaffold, growth factors, and their release profile, biomolecules, drugs, and ions are chemical properties of Schwann cell environment, which affect cell fate, all of which are explained separately in detail in the following section.

#### 5.2.1 | Biomaterial surface coating

Adherence of Schwann cells to the extracellular matrix (basal lamina sheets) is necessary for their survival and their appropriate function. Low Schwann cells attachment on high water content polymers such as hyaluronic acid causes their low proliferation rate at the initial stage (Vilarino-Feltrer et al., 2016). All cells are in their niche, meaning that any cell type has its specific environment with unique chemical properties and physical architecture. In order to develop Schwann cells, it is important to mimic the ECM of the natural peripheral nervous system to create an appropriate niche for Schwann cell adhesion, migration, proliferation, differentiation, and maturation. Biofunctionalization of the substrate surface with ECM polymers of the peripheral nervous system (e.g., Laminin, collagen) or short peptide sequences (e.g., RGD) enhances adhesion, proliferation, differentiation, myelinogenic capability, migration, and survival of Schwann cells by creating appropriate ligands on the surface and initiating specific intracellular signaling cascades (Faroni et al., 2015; Gardiner, 2017; Xue et al., 2017; Zarinfard et al., 2016). ECM generates an environment with unique chemical and physical properties where cells can survive. Different types of laminins and collagens are the most momentous components of the ECM of the peripheral nervous system. Laminin (Type 2, 8, 10), interacting with β1-integrin on Schwann cells, regulates Schwann cell proliferation, function, differentiation, maturation, survival and morphogenesis (Chernousov et al., 2008; Xue et al., 2017). However, it was shown that laminin alone does not influence DRG neuron survival and it is important to present neurotrophic factors (Mahay et al., 2008). Collagen (Types I, III, V, IV) as a cell-adhesive matrix protein enhances Schwann cell adhesion and spreading (Chernousov et al., 2008). Hyaluronic acid which interacts with cell surface receptors such as CD44 and RHMM15, and fibronectin are another ECM components of the nervous system (Gu, Zhu, et al., 2014; Mukhatyar et al., 2011; Thomas et al., 2017). Accordingly, for transplantation application, it is important to develop a suitable niche where Schwann cells can be survived and maintain the nerve growth factor expression.

Razavi et al., in order to investigate the influence of laminin-coated substrate on neurotrophic secretion of Schwann cells, cultured adipose-derived stem cells on conventional plastic and also on a laminin-coated culture plate under specific induction medium (Zarinfard et al., 2016). They concluded that surface coating with laminin as the most pivotal ECM component of Schwann cells, influences the secretion of neurotrophic factors and enhances expression of Schwann cell markers (GFAP, S100), myelin basic protein and BDNF, but decreases the expression of NGF (Zarinfard et al., 2016). It is shown that the surface treatment of PDMS implants by hyaluronic-collagen conjugation increases neural cell proliferation and differentiation due to improved cell–matrix interactions, increased surface hydrophilicity and reduced mechanical mismatches between soft tissues and implanted biomaterials (Yue et al., 2011). In vivo ECM of Schwann cells has different components with specific and different functions; and therefore, deposition of individual ECM components of Schwann cells on their synthetic scaffold cannot gain satisfactory results. In this regard, acellular nerve grafts with similar structure to natural nerve have the combinational and complex structure of Schwann cell ECM polymers; however, they have some drawbacks such as the probability of inducing host response, inappropriate mechanical properties and possibility of pathogen transfer (Gu, Ding, & Williams, 2014). To overcome these problems, deposition of cell-secreted ECMs on Schwann cell scaffolds with appropriate mechanical properties is suggested, which enhances cell adhesion, proliferation, and differentiation due to the existence of collagens, glycosaminoglycan, and other ECM components without drawback of acellular nerve grafts (Gu, Ding, & Williams, 2014; Leach & Whitehead, 2017). It has been reported that coating conductive fiber-film of poly(ε-lactic acid) (PLLA) and polypyrrole with a combination of ECM polymers through directed L929 cells to secrete instructive ECMs on the surface instead of individual laminin or collagen deposition and with the aid of NGF significantly enhanced PC12 cell adhesion rate and neurite length (Zhou et al., 2017). In another study, Gu, Zhu, et al. (2014) modified chitosan/silk fibroin scaffold with cultured Schwann cell-derived ECM and indicated an enhancement in the diameter and the number of the regenerated myelinated nerve fibers.

As an imitation of natural niche of Schwann cells, it was shown that the surface treatment of PLGA conduits with the optimum dosage of Nectin-like molecule 1 (NECL1) as an adhesion molecule on natural axons enhanced Schwann cell adhesion and proliferation, elevated expression of Schwann cell marker (S100 marker) and also induced more secretion of GDNF, BDNF, CNTF factors (Xu et al., 2017). Moreover, it has been reported that knockdown of Nectin-like molecule 4 (NECL4) prevents Schwann cell differentiation and myelination (Gu, Ding, & Williams, 2014).

#### 5.2.2 | Biomaterial degradation behavior

Nerve conduits constructed from biodegradable biomaterials must have suitable degradation rate in order to provide axonal growth support, avoid collapsing, prevent chronic inflammatory responses and also completely degrade during nerve regeneration. It was reported that natural polymers crosslinking, maintains surface topography and provides guidance cues for axonal regrowth in the regeneration period, for example, cross-linked chitosan fibers with genipin, promote Schwann cell proliferation and enhance neurite growth (Lau et al., 2018). For
hyaluronic acid-based Schwann cell scaffolds, it must be considered that the effects of hyaluronic acid degradation products depend on their molecular weight; for instance, cross-linked hyaluronic acid chains degrade in high molecular weight fragments possess anti-inflammatory effects, but low molecular weight degradation products have pro-inflammatory effects (Vilariño-Feltrer et al., 2016).

Degradation products of Schwann cell scaffolds must be biocompatible and leave no adverse impacts in vivo. It has been also reported that degradation products of some Schwann cell scaffolds (e.g., chitosan scaffolds) influence the nerve regeneration process. It is shown that chitosan degradation products (chitoooligosaccharides) have influence on Schwann cell behavior, which enhances Schwann cell proliferation and axon regeneration by accelerating cell cycles (Wang et al., 2016). Moreover, it has been proved that macrophage migration to peripheral nerve injury sites at the early stage after damage is an important step in nerve regeneration and also in creating guidance cues for Schwann cell migration, due to clearing inhibitory debris, secretion growth factors such as VEGF-A within the bridge and regulation of extracellular matrix components (Cattin et al., 2015; Scheib & Höke, 2013). Chitosan degradation products through inducing CCL2 expression in Schwann cells via downregulating of miR-327, indirectly stimulate macrophage migration to axon injury sites and thus improve local regenerative microenvironment. They also induce Schwann cell proliferation through cell signaling pathway of miR-27a/FOXO1, and therefore facilitate axon regeneration in critical regeneration time window (Wang et al., 2016; Zhao et al., 2017).

5.2.3 Soluble signals

The existence of neurotrophic support is essential for nerve regeneration rate and quality. Most commonly used PNS growth factors are neurotrophins such as NGF, BDNF, NT-3, and neurotrophics such as GDNF, CNTF, and FGFs (Gu, Ding, & Williams, 2014). It is better to mention that Schwann cells express NT-3 only after nerve injuries where they have no contact with axons (Faroni et al., 2016). Growth factor carriers, for example hollow glass fibers (Novajra et al., 2016) and heparin (Li et al., 2017), as well as growth factor grafting methods on nerve guidance conduit, have important influence on nerve regeneration, in the way they control their release rate and maintain their stability and bioactivity. It has been shown that the controlled release of various growth factors in regenerative environment promotes peripheral nerve regeneration (Li, Wu, et al., 2017), while uncontrolled and fast release of different growth factors simultaneously yields results similar to those of a regenerative environment without any growth factors (Hong et al., 2018). Hong et al. produced a three-layered scaffold for sustained release of growth factors, in which the top layer contained aligned fibers of PCL, the second layer consisted of random fibers of PLGA (6535), and the third layer was constructed from random fibers of PLGA (8515) with different degradation profiles. They concluded that slow and controlled release rate of PDGF from PLGA (8515) at later differentiation stage as well as fast release rate of NT-3 and BDNF from PLGA (6535) at early proliferation stage, in combination with appropriate topographical cues (aligned fibers), promote nerve regeneration (Hong et al., 2018).

Local and systematic application of NGF, BDNF, and CNTF enhanced axonal regeneration and remyelination. However, systematically applied NGF caused hyperalgesia in adult rats (Li, Wu, et al., 2017). In addition, locally applied IGF-1, FGF, and GDNF improved axonal regrowth speed. Neuregulin-1 and its signaling pathway are important factors in Schwann cell development and survival, which enhance axonal myelination; IGF-1, VEGF, NGF, and GDNF also improve muscle re-inervation after neuromuscular system injuries (Faroni et al., 2015; Faroni et al., 2016; Gu, Ding, & Williams, 2014). Moreover, GDNF, VEGF, and betacellulin enhance Schwann cell proliferation after nerve injuries (Gu, Zhu, et al., 2014; Painter, 2017). Although Schwann cells stimulate longer and branched neurites through secretion of BDNF and NGF (Mahay et al., 2008), it should be mentioned that high concentration of BDNF has an inhibitory effect on axonal growth (Faroni et al., 2015). Due to short half-life of NGF and existence of many free growth factors in vivo, nerve conduits possess only NGF without appropriate grafting method have no satisfactory outcomes in comparison with the autograft technique (Li, Wu, et al., 2017). Li et al. loaded NGF onto a chitosan/heparin porous scaffold via electrostatic interaction and found that incorporation of heparin in the chitosan scaffold enhanced the loading amount and long-term stability of NGF. They also observed an enhancement in attachment, proliferation, and development of cultured Schwann cells in the NGF-loaded scaffold (Li, Xiao, et al., 2017). TGFβ as a growth factor incorporates in dedifferentiation of myelinating Schwann cells into mesenchymal-like cells and promotes repairing Schwann cell migration and axon regrowth (Clements et al., 2017).

Other types of soluble factors and biomolecules such as coenzymes, drugs, hormones, and ions can also affect Schwann cell proliferation and differentiation. These factors influence Schwann cell behavior via initiation specific intracellular signaling cascades. It has been shown that the sustained release of 50 mg water-soluble vitamin B5 (62% after 24 h) as a coenzyme to increase cellular mitochondrial metabolic activity, and importantly as a support for generation of acetylcholine (a neurotransmitter) from aligned fibrous scaffold of poly ε-caprolactone co-poly-l-lactic/silk fibroin composite, improves Schwann cell proliferation. Moreover, the addition of vitamin B5 to the PCL/PDLLA/Silk composite reduced surface contact angle and mechanical properties (Bhusan et al., 2016). Pyrroloquinoline quinone (PQQ) combined with transparent cellulose/soy protein-based composite nerve conduit promotes Schwann cell proliferation, migration, and nerve regeneration by influencing specific cell signaling pathway and mitochondrial function (Harris et al., 2013; Luo et al., 2015). Vinorine, as a natural alkaloid, promotes Schwann cell proliferation after nerve injuries, enhances motor function and sensation recovery, induces myelination and promotes nerve regeneration in a dose-dependent manner, by upregulating the NGF protein level and phosphorylation of extracellular signal-regulated kinase (ERK) (Guo et al., 2018).

The pharmacological approach focuses on enhancement of cells survival in injury environment, such as using N-acetyl cysteine (NAC) and acetyl-L-carnitine (ALCAR) agents (Faroni et al., 2015). Other
bionanomaterials such as Tetramethylpyrazine (Xiang et al., 2017), carboxymethylated chitosan (He et al., 2018; Tao et al., 2013) and lignin (Wang et al., 2018) also have protective effects. There exists limited pharmacological treatment for repairing nerve defects. Yin et al. determined the most effective and safe locally applied concentration of Tacrolimus (FK506), 1.786 ± 0.014 ng/mL, as an immunosuppression drug, for promotion of Schwann cell proliferation while reducing the side effects of the drug (Yin, Li, Liu, Wang, & Zhang, 2015). Optimum dosage of gabapentin as an analgesic drug loaded into the cellulose acetate/gelatin scaffold increases Schwann cell proliferation and inhibits cell apoptosis. Because it induces more secretion of nerve growth factor (NGF) by increasing levels of interleukin-1β and tumor necrosis factor-α (TNF-α) (Farzamfar et al., 2018). One study examined the promotion effect of locally-applied high concentration of methylcobalamin loaded into the poly(ε-caprolactone) scaffold on Schwann cell differentiation and regeneration of axons. They reported that methylcobalamin improves myelination and axon regeneration (Suzuki et al., 2017).

Hormones such as progesterone and allopregnanolone influence Schwann cell differentiation (Faroni & Magnaghi, 2011). In addition, thyroid hormone enhances axonal myelination. Adenosine triphosphate (ATP), acetylcholine, glutamate, and γ-aminobutyric acid as neurotransmitters influence Schwann cell receptors and regulate their interactions with neurons and differentiation (Faroni et al., 2015). Some therapeutic ions such as calcium and phosphate ions released from collagen-bioactive glass fibers improve spinal cord injury repair and regulate nerve growth. It has been shown that zinc ion, in addition to its antibacterial and neuroprotection effect, enhances Schwann cell proliferation and myelination process (Novajra et al., 2016).

### 5.3 Effect of conductive biomaterials on Schwann cells behavior

Conductivity plays an important role in transmitting signals between cells in vivo. Electrical stimulation promotes cellular activities, alters proteins adsorption on conductive biomaterials (Kotwal & Schmidt, 2001) and changes Schwann cells migration speed (Forciniti, Ybarra III, Zaman, & Schmidt, 2014). It also enhances nerve functional recovery and muscle re-innervation and also develops thicker myelin sheaths (Xu et al., 2014). It has been shown that conductive biomaterials such as polypyrrole, polyphosphazene, polyaniline, and graphene associated with electrical stimulation can accelerate axon regeneration and enhance neurite growth (Xu et al., 2014) at the early stage (Zhou et al., 2017). Schmidt et al. reported an increase in NGF secretion of electrically stimulated Schwann cells (50 mm/mm for 1 hr) cultured on an aligned porous scaffold based on PPy and PCL (Hardy et al., 2015). Graphene nano-sheets in sodium alginate/polyvinyl alcohol fibrous scaffold, promote the attachment, spreading and proliferation of PC12 cells (Golafshan et al., 2017). Major problems of conductive biomaterials such as polypyrrole, polyaniline, and polythiophene are their nondegradability, brittleness, and infusibility. Wu et al. synthesized a conductive biodegradable polyurethane based on poly(glycerol sebacate) and different contents of conductive aniline pentamer polymer, which was crosslinked with hexamethylene diisocyanate. After culturing of Schwann cells on this substrate, they demonstrated that electroactivity rather than stiffness promoted Schwann cell myelin gene expression by decreasing intracellular calcium level via inhibition of CaSR and PLCβ pathways, and facilitated sustained neurotrophin secretion of Schwann cells such as NGF (Wu et al., 2016).

### 5.4 Effect of physical and topographical properties of the biomaterials on Schwann cell fate

The niche of different types of cells has different and specific chemical, physical, and mechanical properties. Cells sense and respond to substrate topography (Hayman, Smith, Cameron, & Przyborski, 2005; Hoffman-Kim, Mitchel, & Bellamkonda, 2010; Recknor, Sakaguchi, & Mallapragada, 2006). For instance, graphene oxide coating on amine-modified glass causes more elongated cell morphology and induces myogenic differentiation due to its nano-topographical cues and protein adsorption (Ku & Park, 2013). Physical properties of biomaterials such as surface topography at both micro and nanoscales (especially at cell-scale), their porous structure and hydrophilicity play important roles in cells adhesion, cytoskeletal organization, differentiation (Yin, Pang, & Leong, 2007), migration, proliferation, and trans-differentiation by influencing cell–matrix interface and contact guidance. It has been shown that different nano-geometry and roughness of substrates lead to a different response of cells; different types of cells also respond differently to same topographies (Gentile et al., 2010; Moghaddam et al., 2019; Moosazadeh et al., 2019; Nikkah, Edalat, Manoucheri, & Khademhosseini, 2012). It is due to the different topographical features of different cell niche, which cells respond to the features similar to their natural niche appropriately. The continuous tube-like guidance cues gained more axon regeneration because of the basal lamina as the natural physical guidance of Schwann cells containing continuous tubes of laminin (~10 μm in diameter) (Spivey, Khaing, Shear, & Schmidt, 2012). In a study, Wang et al. designed a biodegradable, single component, porous zein conduit with micro-tubes inside as guidance with the aim to improve the nerve regeneration process, and enhanced nerve regeneration results such as achieving a high thickness of myelin sheaths. They provided a scaffold with appropriate topographical cues and achieved comparable sciatic nerve regeneration outcome for 10 mm defect using the autograft technique (Wang et al., 2017). Because of there are three types of nerve fibers with different diameters: A type (α: 12–20 μm, β: 5–12 μm, γ: 3–6 μm, δ: 2–5 μm), B type (<3 μm), and C type (0.3–1.3 μm), the uniaxial aligned fibrous scaffold with larger diameter of fibers (1,000 nm in comparison with 500 nm) coated with laminin (in order to mimic ECM of Schwann cells), showed better Schwann cell proliferation and differentiation due to appropriate configuration of cells cytoskeleton (Xue et al., 2017).

Research revealed that cell shape with unique nano-topographical features determines its behavior (Folkman & Moscona, 1978), and it has been demonstrated that by developing a template with similar nano-topographical cues of cell membrane features, or, in other words, by imprinting cell shape on culture substrate, can control and regulate cells fate (Mahmoudi et al., 2013; Mashinchian et al., 2014;
Moghadam et al., 2019; Moosazadeh et al., 2019). The Morphology of Schwann cells is almost Bi-polar (spindle like) and cell–matrix interactions via integrin and focal adhesion regulate intracellular signals (Chernousov et al., 2008). Stem cells, because of their high nucleus-to-cytoplasm ratio, sense and respond well to the nano-topography of the substrate by integrin-mediated mechanotransduction process and actin remodeling that leads to the expression of specific genes (Bonakdar et al., 2016; Mahmoudi et al., 2013; Mashinchian et al., 2014). Using surface patterned nerve conduit is also a method for providing efficient nerve regeneration via cell-scale biomimetic topographical cues. Richardson et al. evaluated the Schwann cell topographical features on neurite outgrowth and reported the greatest and fastest neurite outgrowth in conduit with Schwann cells imprinted topography, which is in parallel with conduit longitudinal axis (Richardson, Rementer, Bruder, & Hoffman-Kim, 2011). In cellular nerve conduits, the alignment of transplanted Schwann cells is important to generate guidance cues for axon regrowth. Georgiou et al. (2013), by applying plastic compression to a cellular collagen scaffold, stabilized Schwann cell self-alignment within the matrix without using chemical crosslinking agents and obtained better nerve regeneration outcomes.

In order to evaluate cell–substrate interactions, cell response, and topography-mediated differentiation, many works have been done to create topographical features on cell substrates such as grooves, pits, pillars, wells, and different shapes using fabrication methods such as photolithography, stereolithography, and soft lithography. In a study, Sharma et al. declared that building specific biomaterial architectures, such as micro-patterned polystyrene (PS) and poly (lactic acid) with microgrooves, had only influence on morphology, orientation, and growth of cells without significant influence on trans-differentiation of mesenchymal stem cells into Schwann cells like phenotype (Sharma et al., 2016). However, it has been shown that Substrates with aligned morphological contact guidance or anisotropic engineered biomaterials with appropriate dimensions promote the efficiency of Schwann cell differentiation and maturation, induce more secretion of neurotrophic factors and guide axonal growth (Georgiou et al., 2013; Hong et al., 2018; Lau et al., 2018; Lehmann & Höke, 2016; Xue et al., 2017; Yoshii, Oka, Shima, Taniguchi, & Akagi, 2003).

In a study, the combined effects of micro-patterned aligned topography and conductivity were examined on Schwann cell behavior (Wu, Wang, Hu, Ma, & Guo, 2018). Results indicated that both the contact guidance of narrow-ordered grooves and bioactivity have an influence on Schwann cell behavior; however, micro-patterned, in comparison to the conductive property of template, did not have a significant influence on nerve growth factor gene expression of Schwann cells (Wu et al., 2018). At the initial stage of axon regrowth, the existence of appropriate guidance cues in hollow nerve conduits such as micro-grooves or intraluminal filaments is essential for Schwann cells migration and successful nerve regeneration. Du et al. developed an aligned topography of fibrin fibers hydrogel as longitudinal intraluminal filaments within chitosan conduit and accelerated nerve regeneration process. They also increased the density and myelin thickness of the regenerated nerves by directing Schwann cells orientation and migration (Du et al., 2017). Moreover, it has been shown that chitosan-based nerve conduits with intraluminal filaments of PGA enhance Schwann cell function and nerve regeneration (Wang et al., 2005). In addition to biopolymers, some bioactive bioceramics have been used to create topographical cues for enhancing nerve regeneration. As an instance, Schwann cells attach and spread onto aligned Bioglass® 4555 fibers (Novajra et al., 2016). As an interesting study, Xue et al. (2017) attempted to show the combined effect of chemical and physical properties of scaffold on Schwann cell differentiation, and reported that uniaxial-aligned polycaprolactone (PCL) fibers coated with laminin promote Schwann cell differentiation, proliferation, and maturation due to organized actin network, appropriate morphology of cells, and surface ligands. Figure 3 shows the effect of matrix physical and chemical properties on Schwann cells differentiation.

It is important to construct a nerve conduit that does not allow transplanted glial cells to escape and prevent host inflammatory cells from the exterior to enter whilst at the same time nutrients and cytokines could be easily exchanged. The porous structure of the scaffold influences its degradation rate as well as its interaction with Schwann cells. It has been shown that appropriate porous structures with tailored properties such as a three-layered porous scaffold of hyaluronic acid-based nerve conduits with different pore architecture in each layer and crosslinked with divinyl sulfone protect transplanted Schwann cells and therefore improve their viability and distribution (Vilariño-Feltrer et al., 2016). Thomas et al., aiming to imitate physical cell–matrix interaction and low modulus of neural tissue niche, evaluated the potential application of a templated porous structure (pore size was greater than 10 μm) on the expression of S100 and myelin basic protein. They cultured Schwann cells with various forskolin (FSK, a cyclic AMP activator for inducing myelin expression) concentrations within the synthesized urea crystal templated methacrylated hyaluronic acid-based hydrogel modified with GRGDLys (cell adhesive peptide). They confirmed the expression of the two above-mentioned proteins, but in lower amounts than the non-templated hydrogel for some FSK concentration (Thomas et al., 2017). As a complete study about porous structure influence on Schwann cell behavior, it has been shown that micro-patterned radially aligned porous collagen tubular scaffold with superior cell infiltration (the internal region of tube was cell-permeable, while the external region was cell-impermeable but had permeability to protein/oxygen) in comparison to non-micropatterned porous commercial collagen conduits NeuraGen®, led to the further regulation of genes expression and creation of favorable microenvironment for Schwann cells (Cerri et al., 2014).

5.5 | Effect of mechanical properties of the biomaterials on Schwann cell fate

Mechanical properties of cells niche influence cell migration, proliferation, and differentiation (Nikkhah et al., 2012). Cells sense and respond to mechanical properties of their niche by pulling against substrate (Wen et al., 2014) and clustering transmembrane integrin; thus, the mechanical properties of substrate induce cell spreading and
specific gene expression (Leach & Whitehead, 2017). Contractility controls cell-matrix signaling and regulates cells differentiation (Wen et al., 2014). Therefore, the mechanical properties of substrates such as viscoelastic properties and stiffness have important effects on cell behavior and phenotype, based on the mechanotransduction process (Kamguyan et al., 2018).

Mechanical properties of substrates must mimic desired biological niche; for instance, stiffness has an important influence on cell proliferation and differentiation, substrate deformation due to cell contractions varying with different stiffness (Wen et al., 2014): softer biomaterials encourage neural differentiation (elastic modulus of brain tissue ECM is 0.1–1 kPa) and stiffer biomaterials encourage osteogenic differentiation (Lin, Shi, Cao, & Liu, 2016; Thomas et al., 2017; Uz et al., 2017; Yeh et al., 2017). Mechanical properties of the cell matrix, by influencing cells cytoskeleton and triggering some signaling pathways such as blocking the BMP/Smad signaling pathway, modulate the mesenchymal stem cells differentiation into Schwann cells and cause neural gene expression (Uz et al., 2017; Yeh et al., 2017). Mechanical properties of the cell matrix, by influencing cells cytoskeleton and triggering some signaling pathways such as blocking the BMP/Smad signaling pathway, modulate the mesenchymal stem cells differentiation into Schwann cells and cause neural gene expression (Uz et al., 2017). Uz et al., with the aim of preparing a 3D ECM platform for transdifferentiating bone marrow-derived mesenchymal stem cells into Schwann cells, synthesized conduits from type B gelatin with three different 3D microstructures: nanofibrous, macroporous and ladder-like structures. They concluded that, although pore size and its morphology influence the cell fate and larger pore size promotes Schwann cell migration and proliferation, the ladder-like structure with equal pore size (150 μm) induces more differentiation of stem cells into Schwann cells, and secretion of neurotrophic factors because of having low complex modulus and mechanical properties similar to Schwann cells natural ECM (Uz et al., 2017). In another study, Yeh et al. (2017) fabricated a stiffness-tunable PDMS platform with two-step thiol-ene photoreactive crosslinks which influenced cell response. The hydrogel coated on biomaterials, in addition to carrying growth factors and drugs, could decrease the mechanical mismatch between hard devices and soft tissues in neural applications (Yue et al., 2011). Moreover, alginate as a highly tailorable biomaterial influenced mesenchymal stem cell fate through mechanotransduction effect (Leach & Whitehead, 2017).

The viscous-to-elastic ratio of biomaterials is also an important factor in the evaluation of the substrates response to the forces applied by cells and the cell–substrate mechanical interactions (Kamguyan et al., 2018). It was declared that PDMS as a biomaterial for cell culture application (Halldorsson, Lucumi, Gómez-Sjöberg, & Fleming, 2015) with a low amount of the viscous-to-elastic ratio rapidly respond to mechanical pull applied by cells, and enhanced the cell–substrate mechanical interaction (Kamguyan et al., 2018). Natural ECM has viscoelastic properties and elastic crosslinked hydrogel could prevent the cells from morphological and fate changes. Stress relaxation is a key factor of appropriate ECM, and it has been shown in some studies that interpenetrating networks gain appropriate tunable characteristics for growth and maturation of cells in comparison with conventional hydrogels (Golafshan et al., 2017; Shivashankar & Mandal, 2012). Lou et al. (2018) presented a viscoelastic hyaluronic acid-based and collagen interpenetrating network hydrogel with dynamically exchangeable hydrazone crosslinking, which showed fast stress relaxation in response to a deformation, thereby promoting cell spreading cultured onto these hydrogels; for instance, cultured MSCs created protrusions with a length of up to 100 μm. Table 3

![Figure 3](image-url)
<table>
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<tr>
<th>Strategy</th>
<th>Categories</th>
<th>Mechanism of influence</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>Chemical approaches</td>
<td>Substrate surface coating</td>
<td>Laminin</td>
<td>Schwann cell natural ECM polymer; influences cell behavior via receptor interactions; laminin receptors on Schwann cells including α6β1 and α6β4 integrins; improves substrate bioactivity and surface wettability</td>
<td>Regulates Schwann cell morphology, proliferation, survival, and function; enhances differentiation of BMSCs into Schwann cells and maturation; increases secretion of neurotrophins; promotes myelin gene expression</td>
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<td></td>
<td></td>
<td>Collagen (I, III, IV, V)</td>
<td>Schwann cell natural ECM polymer; interacts with cell receptors; regulates intracellular signaling</td>
<td>Enhances Schwann cell adhesion, spreading and myelinating; increases neural cell proliferation</td>
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<tr>
<td></td>
<td></td>
<td>Hyaluronic acid</td>
<td>Enhances cell–matrix interactions; interacts with cell receptors CD44 and RHMM15; improves surface hydrophilicity; similar mechanical properties to soft tissues</td>
<td>Increases neural cell proliferation and differentiation</td>
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<td></td>
<td>Cell-derived ECM</td>
<td>The existence of different components of Schwann cells natural ECM; trigger internal cell signaling pathways via appropriate cell–matrix interactions</td>
<td>Enhance cells adhesion and differentiation</td>
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<td></td>
<td>Nectin-like molecule</td>
<td>Adhesion molecule on natural axon</td>
<td>Enhances Schwann cell adhesion and proliferation; induces more secretion of GDNF, BDNF, CNTF</td>
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<td></td>
<td>Lignin</td>
<td>Has an antioxidant activity</td>
<td>Promotes Schwann cell proliferation and myelin protein secretion</td>
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<td>Neurotrophic growth factors</td>
<td></td>
<td>NGF</td>
<td>TrkA receptor; regulates Schwann cell differentiation</td>
<td>Enhances nerve regeneration</td>
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<td></td>
<td>BDNF</td>
<td>TrkB receptor; has function in cellular proliferation before differentiation stage</td>
<td>Increases axon growth in the spinal cord; promotes locomotor activity; enhances axonal regeneration and myelination in PNS</td>
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<td></td>
<td></td>
<td>GDNF</td>
<td>GFRα receptor; survival factor for neurons</td>
<td>Improves axonal regrowth speed; promotes nerve regeneration</td>
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<td></td>
<td></td>
<td>PDGF</td>
<td>Acts as a mitogen; stimulates the neurogenesis in postmitotic stage; induce factor of differentiating mesenchymal stem cells into Schwann cell lineage</td>
<td>Promotes Schwann cell proliferation and differentiation; protects neuron from degeneration; enhances nerve regeneration</td>
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<td></td>
<td></td>
<td>VEGF</td>
<td>Useful in differentiating endothelial cell precursors to blood vessels; blood vessels</td>
<td>Has improvement effect on muscle re-innervation; enhances nerve regeneration</td>
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<th>Strategy</th>
<th>Categories</th>
<th>Mechanism of influence</th>
<th>Outcomes</th>
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<tr>
<td>IGF-1</td>
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<td>bridge distal and proximal stumps of axon and guide Schwann cell migration</td>
<td>Improves axonal regrowth speed; improves muscle re-innervation</td>
<td>Faroni et al. (2015)</td>
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<td>FGF</td>
<td></td>
<td>Activate the ERK pathway and direct the morphological change of mesenchymal stem cells during trans-differentiation toward Schwann cell-like phenotype; has a key role in neural induction of mesenchymal stem cells</td>
<td>Enhances Schwann cell differentiation; improves axonal regrowth speed; improves muscle re-innervation</td>
<td>Faroni et al. (2015), Gu, Ding, and Williams (2014), Zhu et al. (2014)</td>
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<td>TGFβ</td>
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<td>Reprograms wound Schwann cells to mesenchymal-like cells; crosstalks with the Eph signalling pathway via N-cadherin</td>
<td>Increases Schwann cell migration; promotes nerve regeneration</td>
<td>Clements et al. (2017)</td>
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<td>CNTF</td>
<td></td>
<td>CNTFRα receptor; increases the expression of myelin protein; promotes cell survival</td>
<td>Enhances myelination; regulates neurite outgrowth</td>
<td>Homs et al. (2011), Stankoff et al. (2002), Jang et al. (2010), Liu, Liu, and Bi (2014)</td>
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<td>Forskolin (FSK)</td>
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<td>A cyclic AMP activator; induce factor for differentiating mesenchymal stem cells into Schwann cell-like cells</td>
<td>Induces myelin gene expression</td>
<td>Thomas et al. (2017), Zhu et al. (2014)</td>
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<td>NT-3</td>
<td></td>
<td>TrkC receptor; c-Jun N-terminal kinase pathway; has function in cellular proliferation before differentiation stage</td>
<td>Inhibits Schwann cell myelination process; enhances Schwann cell migration; improves nerve regeneration in rat models</td>
<td>Hong et al. (2018), Yamauchi, Chan, and Shooter (2003)</td>
</tr>
<tr>
<td>Biomolecules</td>
<td>Vitamin-B5</td>
<td>A component of coenzyme A; increases cellular mitochondrial metabolic activity; highly hydrophilic</td>
<td>Improves Schwann cell proliferation</td>
<td>Bhutto et al. (2016)</td>
</tr>
<tr>
<td>Vinarone</td>
<td></td>
<td>Upregulates NGF protein level and ERK phosphorylation</td>
<td>Promotes Schwann cell proliferation and nerve regeneration</td>
<td>Guo et al. (2018)</td>
</tr>
<tr>
<td>Adenosine triphosphate (ATP), acetylcholine, glutamate, γ-aminobutyric acid</td>
<td>Neurotransmitters, interact with Schwann cell receptors</td>
<td>Regulate Schwann cell interactions with neurons and differentiation</td>
<td>Faroni et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>Carboxymethylated chitosan</td>
<td>Downregulates Bax, cytochrome c and caspase-3; upregulates Bcl-2; activates MEK/ERK, P13K/Akt and Wnt/β-catenin signaling pathways</td>
<td>Protects Schwann cells against hydrogen peroxide-induced damage; promotes proliferation of Schwann cells; induces NGF synthesis</td>
<td>He et al. (2018), Tao et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Tetramethylpyrazine</td>
<td>Has anti-oxidant and anti-inflammatory activities; apoptotic inhibition</td>
<td>Improves cells survival; enhances sciatic nerve regeneration outcomes</td>
<td>Xiang et al. (2017)</td>
<td></td>
</tr>
<tr>
<td>Pyrroloquinoline quinone (PQQ)</td>
<td>Stimulates NGF synthesis; interacts with cell signaling pathway and mitochondrial function</td>
<td>Influence on Schwann cell proliferation and migration; influence on the number of regenerated axons</td>
<td>Luo et al. (2015), Harris et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Strategy</td>
<td>Categories</td>
<td>Mechanism of influence</td>
<td>Outcomes</td>
<td>References</td>
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<tr>
<td>Drugs</td>
<td>Methylcobalamin</td>
<td>Probably regulates axonal mRNA levels and local protein synthesis; downregulates the activity of Erk1/2; promotes the lecithin synthesis</td>
<td>Promotes nerve regeneration; improves functional, electrophysiological and histological recovery; enhances differentiation and myelin basic protein expression of Schwann cells</td>
<td>Suzuki et al. (2017), Nishimoto et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>N-acetyl cysteine (NAC)</td>
<td>Acts as a free radical scavenger; protects the cytochrome oxidase system in mitochondria; increases vascularity and circulation in healing areas; improves reduction in myelin debris</td>
<td>Has neuroprotection effect; facilitates nerve recovery</td>
<td>Faroni et al. (2015), Rivera, Raymond, Groberman, Abouyared, and Angeli (2017), Karalija, Novikova, Kingham, Wiberg, and Novikov (2012)</td>
</tr>
<tr>
<td></td>
<td>Acetyl-L-carnitine (ALC)</td>
<td>Promotes the production of the antioxidant glutathione</td>
<td>Has neuroprotection effect</td>
<td>Faroni et al. (2015), Karalija et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Tacrolimus (FK506)</td>
<td>Suppresses the immune system response</td>
<td>Promotes Schwann cells proliferation when locally applied</td>
<td>Yin et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>Gabapentin</td>
<td>Probably increases the levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α); enhances the myelin debris removal after Wallerian degeneration</td>
<td>Induces more secretion of the nerve growth factor; increases Schwann cells proliferation; promotes and accelerates axonal growth and remyelination; neuroprotective and antiapoptotic properties</td>
<td>Farzamfar et al. (2018), Câmara et al. (2015)</td>
</tr>
<tr>
<td>Hormones</td>
<td>Progesterone, Allopregnanolone</td>
<td>Has influence on Schwann cell differentiation and myelination</td>
<td></td>
<td>Faroni and Magnaghi (2011)</td>
</tr>
<tr>
<td>Ions</td>
<td>Zinc</td>
<td>Has antibacterial effect; influences on sodium channels in a dose-dependent manner</td>
<td>Improves wound healing; has neuroprotection effect; enhances Schwann cell proliferation and myelination</td>
<td>Novaja et al. (2016), Zhang et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
<td>Promotes growth cone motility; as a regulator of actin filament</td>
<td>Promotes neurite elongation</td>
<td>Novaja et al. (2016), Zhang et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Cerium</td>
<td>Protects against oxidative stress</td>
<td>Has neuroprotection effect</td>
<td>Zhang et al. (2011)</td>
</tr>
<tr>
<td>Physical approaches</td>
<td>Aligned morphology</td>
<td>Direct morphology of cells and organize actin network; induce appropriate configuration of cells cytoskeleton; Mechanotransduction process; cytoskeletal changes alter nucleus shape and gene expression</td>
<td>Promote the efficiency of Schwann cell differentiation, maturation, and more secretion of neurotrophic factors; guide axonal growth; increase migration speed</td>
<td>Xue et al. (2017), Lehmann &amp; Höke, 2016, Lau et al. (2018), Hong et al. (2018), Nikkhah et al. (2012)</td>
</tr>
<tr>
<td>Mechanical approaches</td>
<td>Stiffness</td>
<td>Mechanotransduction process</td>
<td>Induces cell spreading and differentiation</td>
<td>Uz et al. (2017), Thomas et al. (2017), Wen et al. (2014), Lin et al. (2016), Yeh et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>Viscoelastic properties</td>
<td>Stress relaxation as a key factor of appropriate ECM with fast stress relaxation in response to a deformation</td>
<td>Influence cells behavior</td>
<td>Lou et al. (2018)</td>
</tr>
</tbody>
</table>
summarizes researches on the three different mechanisms of controlling Schwann cell fate and improving nerve regeneration process.

6 | CONCLUSION AND FUTURE PERSPECTIVES

Schwann cells are key regulators of the injured peripheral nervous system regeneration process. Atrophy of Schwann cells in severe nerve injuries, high amount of senescent Schwann cells, in addition to the existence of a golden time for successful nerve regeneration in order to maintain the in-contact muscle and neurons in healthy state, shows the importance of developing efficient, safe and stable procedure and biomaterials for differentiation and transplantation of Schwann cells. Combination of chemical, topographical and electrical approaches to developing an active template and scaffold that can induce proliferation, differentiation, and migration of Schwann cells and maintain their functionality seems to be an appropriate approach to enhance axon regeneration. It is important to maintain a sufficient number of Schwann cells in a healthy state and keep their phenotype in order to prevent the reduction in nerve growth factor expression with the aid of suitably engineered biomaterials. It is already confirmed that developing a scaffold which mimics the topographical, mechanical, and chemical cues of Schwann cell natural ECM could be a favorable substrate to maintain Schwann cell function. This approach combined with appropriate drugs and biomolecules is a good strategy to develop Schwann cells for successful nerve regeneration.

Regarding the culture substrate for differentiation of stem cells into Schwann cells, PDMS is an invaluable biomaterial which has such advantages as viscoelasticity, oxygen permeability, ease of fabrication, biocompatibility, optical transparency, and chemical inertness (Yue et al., 2011). A future direction of Schwann cell development by combined effect of topography and chemical-induced differentiation could be studied on stable ex-vivo differentiation of patient-specific stem cells into Schwann cells by fabricating a PDMS-based template with nano-topographical cues similar to Schwann cell membrane features and chemical induction environment. Additionally, specific engineered Schwann cell scaffold for implantation of Schwann cells are essential to axon regeneration process, in which their survival and functionality are maintained, they are sheltered against oxidative stress and inflammatory cytokines, and also their nerve growth factor secretion in injury sites is maintained.

REFERENCES


Huang, C.-W., Huang, W.-C., Qiu, X., Da Silva, F. F. F., Wang, A., Patel, S., ... Li, S. (2017). The differentiation stage of transplanted stem cells modulates nerve regeneration. Scientific Reports, 7(11), 17401.


