The Role of Schwann Cells in Peripheral Nerve Function, Injury, and Repair

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Abstract

The specialized role of the Schwann cell is reviewed in the context of peripheral nerve, spanning neural development, anatomy, signaling, and function. A particular focus of this chapter is the increasingly important role identified in many studies of Schwann cells in nerve injury and repair. We summarize a range of key studies describing these specialized roles, which include the alignment of

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Schwann cells along myelinated axons; the protection of signaling pathways between the ganglion bodies, linking the spine and target muscles; and the secretion of growth factors. Myelin structures are reviewed and their organization as nodes for physical protection and as structures that increase action potential velocities of motor and sensory systems. We focus on nerve injury, and mechanisms of nerve wound healing and repair, considering the role of the Schwann cell as it dedifferentiates, proliferates, and redifferentiates. The physical role of the glia in guiding axon regeneration and the role of neurotrophins that communicate via paracrine receptor-mediated signals are considered. Bioengineering strategies are also considered, with innovations in biomaterial scaffolds as medical devices for peripheral nerve repair, with a focus on new technologies and models for evaluation, plus new methods for Schwann and stem cell therapies.

1 Introduction

In the mid-nineteenth century, Theodor Schwann discovered that the external membrane sheathing peripheral nerve axons was composed of separate cells (Koehler et al. 2000). These cells (subsequently named after him – Schwann cells (SC)) and their remarkable ability to wrap around axons were confirmed when electron microscope resolution allowed the visualization of the myelin sheath (Bunge 1986). In the first half of the 1900s, investigators observed that when a nerve from the peripheral nervous system (PNS) was resected, Schwann cells proliferated and migrated to the site of injury, suggesting a potential role in peripheral nerve regeneration (Masson 1932). In 1965, Cravioto proposed a possible explanation for this observation: during embryonic development of the PNS, Schwann cells proliferate and accumulate near the newly formed axons to subsequently wrap around them (Cravioto 1965; Wiley-Livingston and Ellisman 1980). Schwann cell precursors (SCP) and immature Schwann cells were described in newborn animals, where it was found that these cells were essential for PNS development by providing trophic support to sensory and motor developing neurons that have not yet reached a target (Riethmacher et al. 1997; Jessen and Mirsky 2005). Discovering that the SCP phenotype differed from that of mature Schwann cells shed light on one of the main mechanisms by which Schwann cells assisted in regenerating resected axons. Schwann cells transdifferentiate into a phenotype similar to that of SCP, after losing axonal contact (Jessen and Mirsky 2005; Sherman et al. 1993; Dupin et al. 2003). The specialized repair SC, nowadays called Büngner cells, are known to secrete neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF), neurotrophin-3 (NT3), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and vascular endothelial growth factor (VEGF). They also secrete cytokines and express cell adhesion molecules, thus creating a microenvironment that favors axonal regrowth and nerve regeneration (Jessen and Mirsky 2005, 2016; Doetsch 2003; Bhatheja and Field 2006; Bandtlow et al. 1987; Meyer et al. 1992).

2 Schwann Cells in Peripheral Nerve Development, Anatomy, and Function

Schwann cells are the supportive glial cells within the peripheral nervous system and form the protective neurilemma around the axon nerve fibers. They produce a basal lamina which determines the polarity of Schwann cells, as well as myelinating axons (Simons and Trotter 2007). Schwann cells adhere to the axonal membrane by molecules of the nectin-like family (Necl or SynCAM) (Simons and Trotter 2007). Furthermore, they are responsible for producing and assembling the myelin sheath between nerve fibers and the neurilemma. The presence of the myelin sheath enhances the conduction speed of action potentials, through the formation of specialized nodes of Ranvier (Saladin 2014; Arancibia-Carcamo and Attwell 2014). These discontinuous structures are physically organized such that voltage-gated sodium and potassium ion channels are enriched only at these positions and function according to saltatory (or jumping) nerve conduction (Poliak and Peles 2003). This response is unique to myelinated axons as myelin serves as an electrical insulator. This decreases the capacitance of the axon membrane and consequently membrane electrical resistance across the internode intervals. The nodes of Ranvier are organized as gaps in the myelin sheath, exposing small but regular positions of the underlying neuronal cell membrane, which are clearly visible using low magnification electron microscopy (Poliak and Peles 2003). Myelin is a differentiation product also found in the central nervous system (CNS). However, CNS myelin is produced by oligodendrocytes and differs in biochemical composition compared to that in the PNS. In both systems, it has insulating functions, the dry mass of which is approximately 70% lipid and 30% protein. Myelin is therefore a hydrated lipoprotein and contains around 40% water (Kahan and Moscarello 1985; Chen et al. 2017). The protein component is myelin basic protein, responsible for the formation of compact myelin. PNS myelin also contains protein zero, responsible for forming and maintaining the concentric ring structures of the glial cell membrane that constitute the myelin sheath (Martini and Schachner 1997) (Fig. 1). The myelin nodes are therefore formed as a series of specialized structures and serve to propagate sodium ion diffusion through the axoplasm to the next adjacent myelinated internodes (and in turn the next node of Ranvier). This results in axon potential signaling that is unidirectional. The internodal spaces therefore determine saltatory conduction, where the signal jumps rapidly from node to node. This results in action potential velocities that are ten times faster compared to unmyelinated axons (Stampfli 1954).

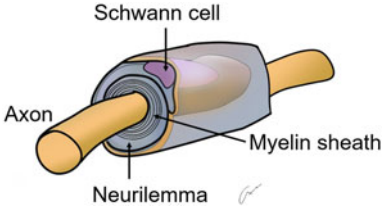

Fig. 1 Schwann cell forming a concentric myelin sheath and neurilemma structure around an axon in the PNS

3 Markers for Schwann Cells

3.1 Early Developmental Markers

One of the most important functions of Schwann cells is phenotypic plasticity, which are tightly regulated by transcriptional networks controlling proliferation, differentiation, dedifferentiation, and myelination (Jessen and Mirsky 2002). The stages of Schwann differentiation have been widely characterized. The embryonic phase of Schwann lineage can be divided in three transient cell stages (Jessen and Mirsky 2002): (i) neural crest cells (NCCs), which start migrating at embryonic day 12 (E12, mouse); (ii) Schwann cell precursors (SCPs), which arise at E12/13 in mouse development and populate the dorsal and ventral roots, the ganglions, and growing nerves (Balakrishnan et al. 2016); and (iii) immature Schwann cells (iSCs), which subsequently differentiate into myelinating or non-myelinating cells, depending on the diameter of the axon to which they are associated (Blanchard et al. 1996; Taveggia and Bolino 2018). The molecular pattern associated with each stage overlaps in part between populations; however, a consensus exists that some molecules correlate with specific developmental stages (reviewed in Jessen and Mirsky 2005). For example, SCPs express the brain fatty acid binding protein (BFABP), desert hedgehog (DHH), and cadherin 19, which are not present in NCCs. In contrast, NCCs do not interact with axons, while SCPs and iSCs associate intimately with axons. Furthermore, SCPs display a dependence on axonal neuregulin-1 (NRG1) for survival (Meier et al. 1999). The main phenotypic markers expressed by Schwann cells during development in the mouse have been described in detail by Balakrishnan et al. (2016).

Schwann cell cultures in vitro have a proliferative phenotype, characterized by a variety of markers, including p75NTR, S100β, SOX10, SOX9, AP2A1, EGR1, PAX3, SOX2, CX32, DHH, NECL4, NFATC4, POU3F1, and YY1 (Stratton et al. 2017). Although studied much less, the effect of aging is thought to modify the pattern and response to injury. It has been shown that aged Schwann cells display a reduced response to injury, such as a decreased c-jun activation and p75 NTR expression compared to young cells in mice (Painter et al. 2014). Similarly, non-myelinating Schwann cells from dental pulp show declines in GAP43 and P75 NTR levels in older mice. Myelinating Schwann cells also have a lower level of myelin basic protein compared to younger mice (Couve et al. 2018).

3.2 Myelination Markers

Transcriptional network studies revealed that Schwann cells are genetically programmed to myelinate. Classical regulators studied in detail include Krox20, Sox10, Oct-6, and NF-κB (Jessen and Mirsky 2008; Stolt and Wegner 2016). The myelination process is dependent on larger-diameter axons, where axonal neuregulin-1 is thought to be a key factor for inducing myelination (Taveggia et al. 2005). Krox20 has been proposed as a major myelin regulator, promoting

cell cycle exit and activating the myelination transcriptional factors Oct-6 and Brn-2 (Jessen and Mirsky 2008; Stolt and Wegner 2016). Sox10 is another transcription factor involved in myelination, though associated with early phases of proliferation and differentiation (Britsch et al. 2001). In contrast, Oct-6 is only required for the initial steps of myelin signaling, with Krox20 and Sox10 responsible for terminal myelin formation and maintenance (Ryu et al. 2007; Bremer et al. 2011).

Schwann cells are able to switch off myelination and reenter to cell cycle when physical axon-Schwann contact is lost following injury. This includes a down-regulation of major myelin molecules, including protein zero, myelin basic protein, and myelin-associated glycoprotein (MAG) (Jessen and Mirsky 2008). Among the most important genes involved in demyelination are Sox2 (Le et al. 2005; Roberts et al. 2017), c-jun (Parkinson et al. 2008), and Pax3 (Doddrell et al. 2012), whose expression is required to maintain Schwann cells in an immature state and exert a reciprocal inhibition to Krox20 (Parkinson et al. 2008). Other complementary factors associated with demyelination include Notch, Krox-24, and Egr-3, which participate in inhibiting pro-myelinating Krox20 signaling and promoting the expression of p75NTR, respectively (Woodhoo et al. 2009; Gao et al. 2007). Thus, an understanding of mechanisms associated with myelination and demyelination has contributed significantly to understanding regulators in differentiation and dedifferentiation during development and in response to injury.

4 Schwann Cells and Their Role Following Nerve Injury

One of the most important roles of Schwann cells is supporting nerve regeneration after injury (Saladin 2014). When an injury affects 90% of the axons within a nerve, Wallerian degeneration occurs, followed by axonal regeneration and end-organ reinnervation (Menorca et al. 2013) (Fig. 2). During Wallerian degeneration, a microenvironment is formed to encourage axonal growth. Initially, Schwann cells recruit macrophages to the site of the injury and phagocytose cell debris. Macrophages secrete pro-inflammatory cytokines, e.g., IL-1 and TNF-α, that encourage dedifferentiation and proliferation of Schwann cells. After the nerve microenvironment is cleared of debris, Schwann cells undergo a redifferentiation program, promoting the expression of neurotrophic factors (e.g., NGF and BDNF) and accelerating differentiation into so-called Büngner Schwann cells in an autocrine manner. The differentiated Büngner Schwann cells also start to physically migrate into the remaining endoneurial tubes distal to the injury and begin to align themselves to form bands of Büngner. Axons emerging from the growth cone proximal of the injury elongate along these bands of Büngner until reinnervation of the target organ. Axonal outgrowth is in part controlled by the Büngner Schwann cells through upregulation of neurotrophic factors. These small molecular weight soluble proteins diffuse and through paracrine signaling stimulate axonal growth, cell survival, and further differentiation of Schwann cells.

Later in the repair process, Schwann cells produce extracellular matrix (ECM) proteins, in particular laminin and fibronectin. Axon-membrane-integrin interactions

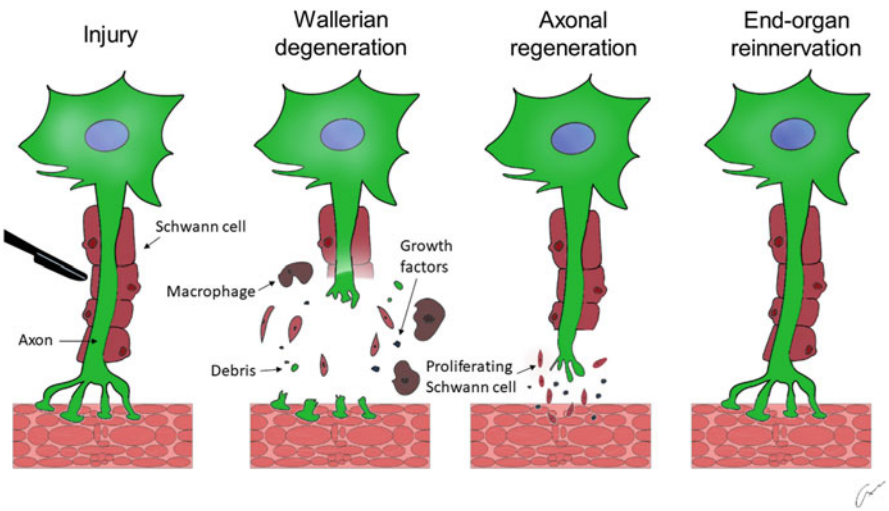

Fig. 2 Nerve regeneration process after injury in the PNS. After the nerve is severed, Schwann cells phagocytose cell debris and recruit macrophages to the site of the injury (Wallerian degeneration). Schwann cells align to form bands of Büngner, creating a path for the axons to regenerate. Finally, the axons reinnervate the target organ

are utilized by the growth cones to physically adhere to the basal lamina of the endoneurial tubes (Menorca et al. 2013; Chiono and Tonda-Turo 2015; Stoll et al. 2002; Frostick et al. 1998; Murray-Dunning et al. 2011). The axonal outgrowth process produces adenosine triphosphate (ATP) and acetylcholine, which encourages reversion of the Schwann cell phenotype, from a proliferating to a myelinating form. A maturation process is then initiated, which includes axon elongation; remyelination, which is also supported by proteins of the ECM (Chernousov et al. 2008); and ultimately functional reinnervation. Functional recovery is not always achieved because these processes are limited to small injury gap distances (typically <5 mm) and the physiological state of the endoneurial tube. If the endoneurial tube is not intact, the formation of bands of Büngner is impeded, and the formation of scar tissue will likely arise (Menorca et al. 2013; Jiang et al. 2010).

4.1 Methods for the Isolation of Schwann Cells

Due to the importance of Schwann cells in peripheral nerve regeneration, the demand for robust and reliable Schwann cell cultures for in vitro testing as well as for cell therapy applications has significantly increasing over the last 10–20 years.

Approaches utilizing cell therapy have found unequivocally that the local delivery of Schwann cells to a wound site (typically within a nerve guide device) improves nerve regeneration (Guenard et al. 1992; Hadlock et al. 2000). This is reported as being primarily due to the secretion of neurotrophic factors (e.g., NGF

and BDNF) and the presence of physical and topographic cues responsible for promoting axonal regeneration (Ide 1996). However, the benefits reported for stimulating nerve repair are impeded in practical terms by the clinical need to use autologous Schwann cells and in particular limited technically due to the very slow proliferation rate of primary Schwann cells in culture. The clinical requirement to sacrifice a healthy donor nerve adds to this practical limitation (Kaewkhaw et al. 2012). The latter requirement is typically complicated as a whole nerve (e.g., the sensory sural nerve) is necessary to source sufficient Schwann cells and additionally creates a surgical donor morbidity site. In an attempt to address this issue, methods have been developed to establish whether more efficient and selective approaches are possible for Schwann cell isolation and culture in vitro.

4.2 Methods for Obtaining and Culturing Schwann Cells

Most methods to culture Schwann cells include a pre-degeneration stage, where Schwann cells are stimulated to dedifferentiate and proliferate in situ from a fresh adult nerve (reviewed in Kaewkhaw et al. 2012). This stage requires culture from 7 to 14 days but has been found to produce inconsistent results. Schwann cells are typically cultured in vitro in a medium rich in mitogens to stimulate cell proliferation, which in parallel stimulates fibroblast growth, and consequently is a contaminating factor if pure Schwann cultures are required. Historically, several methods have been developed to overcome this issue, such as isolating cells from neonatal or embryonic nerve tissue. Fibroblast numbers are reported to be low, and the extracellular matrix is immature and not fully developed. Another approach is separation of Schwann cells from fibroblasts or fibroblasts from Schwann cells using antimitotic chemicals (e.g., cytosine arabinoside), antibodies (anti-Thy-1), and complement-mediated cell lysis or crude physical techniques (pipette jetting). However, these are time-consuming, expensive, and technically demanding, and irrespective of the method chosen, the number and yield of Schwann cells obtained are typically low (reviewed in Kaewkhaw et al. 2012).

Haastert et al. reported on the development of a more selective method to obtain pure Schwann cells. This included the use of melanocyte growth medium containing forskolin, fibroblast growth factor-2, pituitary extract, and heregulin, which stimulates Schwann cell proliferation and minimizes fibroblast growth (Haastert et al. 2007). However, it was Kaewkhaw et al. who developed an efficient protocol to obtain pure Schwann cells from adult nerve tissue, with increased purity and a reduced time in culture, based on the substitution of the essential amino acid L-valine with D-valine in the culture medium. The concept of the method was the discovery that Schwann cells metabolize D-valine preferentially over fibroblasts (when isolated from the same nerve) due to differential expression of D-amino acid oxidase (DAAO) in the Schwann cells. These differences were identified on a mRNA and protein level in both Schwann and fibroblasts. Compared to Schwann cells, fibroblasts taken from the same nerve expressed DAAO at very low levels. The discovery permitted a breakthrough for isolating and culturing highly pure Schwann cells directly from

fresh adult nerve. Average Schwann cell purities of 97% are reported after 19 days without pre-degeneration, purification, or antimitotic steps (Kaewkhaw et al. 2012). Figure 3 shows a diagram of the steps of this protocol.

In addition, Schwann cells can be obtained from stem cells. Kaewkhaw et al. reported on the differentiation of Schwann cells taken from adipose-derived stem cells. Three different anatomical sites of subcutaneous, perinephric, and epididymal adipose tissue were sourced to harvest the cells. It was described that the source of adipose stem cells correlated with Schwann cell phenotype, glial antigen expression profile, and the capacity to encourage neuronal cell differentiation. Moreover, the presence of high levels of brain-derived neurotrophic factor and nerve growth factor and low levels of neurotrophin-3 in subcutaneous and perinephric fat was shown to be more effective than the Schwann cells obtained from epididymal fat (Kaewkhaw et al. 2011).

A deeper understanding of mechanotransduction pathways and mechanosensitive ion channels in Schwann cells has inspired approaches that include physical stimulation incorporated into isolation and cultivation procedures. Tsuang et al. discovered that low-intensity pulsed ultrasound (frequency, 1 MHz; duration, 2 min; duty cycle, 20%; total treatment time, 3 min) promotes rat Schwann cell proliferation and prevents cell death (Tsuang et al. 2011). Schuh et al. utilized extracorporeal shockwave treatment (ESWT) during the isolation process and found that ESWT increases cell yield, purity, and reactivity to pro-myelination stimuli. Furthermore, ESWT has been proven as an effective method to counteract Schwann cell senescence in prolonged culture (after 105 days) (Schuh et al. 2016). Similar effects regarding increased proliferation and reactivity to pro-myelination stimuli were found by Jung et al. using optogenetic stimulation (OS). OS of 473 nm with blue LEDs elevated intracellular calcium levels, leading to the effects described above (Jung et al. 2019).

One factor less in the spotlight concerning Schwann cell culture is their strong response to substrate stiffness. In the healthy, uninjured nerve presenting all structures (axons, myelinating Schwann cells, ECM), elasticity is around 50 kPa, while tissue culture polypropylene's (TCPP) elasticity is around 100,000 kPa (Gu et al. 2012; Skardal et al. 2013). Typical Schwann cell morphology (bipolar, elongated) has been shown to be a response to stiff surfaces, such as TCPP. Gu et al. demonstrated that the ideal substrate stiffness should be around 7.45 kPa to study Schwann cell biological functions (Gu et al. 2012). Evans et al. demonstrated that the response of Schwann cells to substrate stiffness can be utilized to modulate migration: in an in vitro durotaxis model, Schwann cells displayed lower migration on mono-stiffness surfaces compared to stiffness gradients (Evans et al. 2018).

As mentioned throughout this chapter, axonal outgrowth depends on the migration of Schwann cells within an environment (Bell and Haycock 2012). For this reason, in vitro models and applications are being studied to evaluate Schwann cell proliferation, migration, and morphology. Schwann cell cultures are essential to understand their role during nerve injury; however, it is necessary to understand their potential as well as their limitations to successfully link in vitro basic research to clinical application.

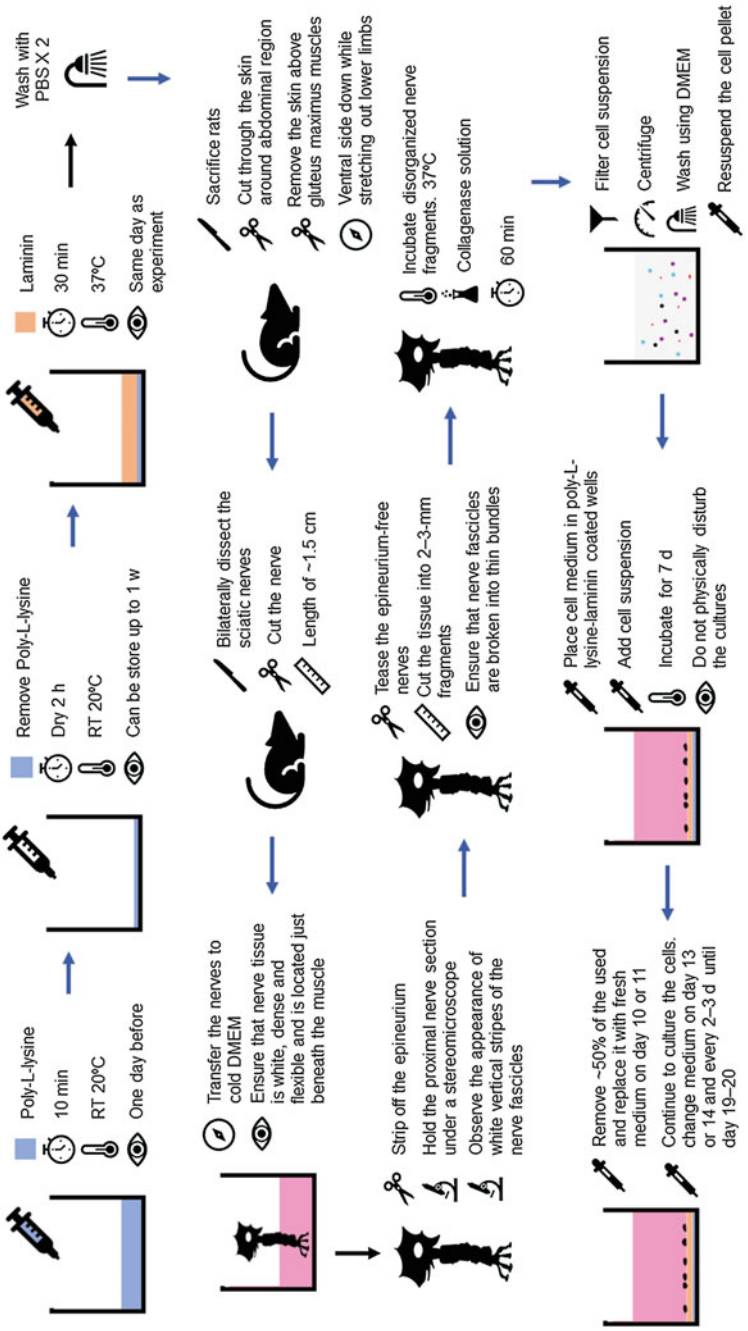

Fig. 3 Overview showing a selective protocol to isolate and purify Schwann cells from adult nerve (Kaewkhaw et al.)

5 Functional Assays for Schwann Cell Testing

5.1 Myelination Assays

The mechanisms associated with myelination and basal lamina formation in peripheral nervous system have been extensively studied, thanks to the development of in vitro co-cultures of Schwann cells with primary sensory neurons (Wood 1976; Carey and Bunge 1981; Eldridge et al. 1987). Myelination assays act as models for several myelin disorders, such as demyelination or delayed myelination. The principle underlying these assays is the cultivation of primary neurons on a layer of Schwann cells, stimulating neurite outgrowth and subsequently myelination (Hyung et al. 2015). Dorsal root ganglion (DRG) neurons are normally obtained from embryonic mouse (E13.5) or rat (E16), as a dissociated culture or in the form of organotypic explant. Other sources of primary neurons include retinal ganglion cells (Bähr et al. 1991) and motor neurons (Hyung et al. 2015).

Pioneering work carried out by Patrick Wood's group (Wood 1976; Carey and Bunge 1981; Eldridge et al. 1987) have shown that Matrigel, type I collagen, laminin, and poly-lysine (L and D) (Taveggia and Bolino 2018; Hyung et al. 2015) are important for myelination. The use of vitamins and growth factors is also important. Preliminary work established that Schwann cell-neuron co-cultures require human placental serum and chick embryo extract for myelin formation, which was enhanced by addition of ascorbic acid (Eldridge et al. 1987). Current protocols can be variable in the use of growth factors and supplements (fetal bovine serum, human placental serum, N2), but ascorbic acid and NGF seem to be necessary in all protocols to induce myelination and support neuronal growth, respectively (Taveggia and Bolino 2018; Eldridge et al. 1987; Hyung et al. 2015; He et al. 2010; Kumar et al. 2016). Newly formed myelin can be detected 1 week after the start of induction by several techniques, such as immunofluorescence, Western blotting, and transmission electron microscopy. The main markers of myelination include myelin proteins as, e.g., MBP, myelin protein zero, myelin-associated glycoprotein, PMP22, and galactosylceramide. Transcription factors associated with myelination include Sox10 and Krox20 (Taveggia and Bolino 2018; Hyung et al. 2015; He et al. 2010; Kumar et al. 2016; Wood et al. 1990; Honkanen et al. 2007).

6 In Vitro Models of Schwann Cells for the Treatment of Peripheral Nerve Injuries

Research has been conducted across a range of diverse applications for the treatment of peripheral nerve injuries. This includes use of Schwann cell cultures, as essential for stimulating axonal outgrowth. One of the most interesting characteristics of Schwann cells is their ability migrate and proliferate to form highly directional bands of Büngner, acting as guiding structures for axonal outgrowth. This ability to align has been found to be reproducible in several in vitro studies, for example, Ahmed and Brown using fibronectin fibers and neonatal rat Schwann cells, as well as

Dubey et al. using magnetically aligned collagen in a neonatal Schwann and dorsal root ganglion co-culture (Ahmed and Brown 1999; Dubey et al. 1999). Aside from natural materials, 3D in vitro peripheral nerve models have also been developed to analyze neurite formation and Schwann cell proliferation on synthetic scaffolds, e.g., aligned electrospun polycaprolactone (PCL) fibers. Results showed that when Schwann cells are co-cultured with neuronal cells, neurite formation is enhanced. Moreover, Schwann cells migrate along PCL fibers (Daud et al. 2012), and hence longer axon outgrowth is possible. Photopolymerizable polylactic acid (PLA) scaffolds can also be used to fabricate and evaluate Schwann cell growth, with results supporting that Schwann cells proliferate on similar synthetic scaffold materials (Koroleva et al. 2012).

Additionally, Schwann cells can be cultured as part of the dorsal root ganglion explants (DRGs), obtained from either rat or chick embryos. DRGs can be grown artificially in experimental nerve guide conduits (NGC) fabricated from a photocurable poly(ethylene glycol) (PEG) polymer by additive layer microstereolithography (μSL). Results showed that Schwann cells can migrate up to 9.5 mm from the DRG body after 14 days of culture in vitro. Axonal outgrowth and Schwann cell migration in such constructs are optimally visualized and quantified by lightsheet microscopy. Combining the technologies of PCL electrospun fibers and photocurable PEG NGCs, Behbehani et al. developed a 3D in vitro model to study Schwann cells and axon outgrowth from DRGs. In this study, PCL electrospun fibers were introduced inside an experimental NGC as a guidance scaffold. Isolated whole DRGs are placed on top of the fibers and cultured for up to 21 days. Results show that Schwann cells can migrate an average of 2.2 mm along fibers with average axonal outgrowth of 2.1 mm after 7 days and maximum axon lengths of >5 mm per construct observed after 21 days. These studies have two immediate areas of application: (i) in the study of complex 3D in vitro models to facilitate a range of underpinning research for neuronal-glial interactions (e.g., pharmacological studies or disease models) and (ii) for the analysis of biomedical nerve implants prior to in vivo evaluation. Initial data using the model herein interestingly supports Schwann cells in having a leading migratory role, possibly serving as guidance to regenerating axons via the deposition of extracellular matrix proteins (Behbehani et al. 2018).

Scaffolds fabricated with natural and synthetic materials are also functionalized with different molecules to stimulate Schwann cell differentiation and proliferation to aid nerve repair. Molecules used to impart biological activity to materials include growth factors; ECM proteins, such as laminin and fibronectin; and chemical functional groups. Silicone tubes functionalized with laminin, fibronectin, and fibronectin plus laminin all increase Schwann cell infiltration and migration (Bailey et al. 1993). Laminin has also been cross-linked to PCL-chitosan scaffolds, encouraging Schwann cell attachment and stimulating proliferation significantly in comparison to PCL-chitosan scaffolds with adsorbed laminin (Radoslaw et al. 2013). Moreover, PCL fibers were functionalized with neuregulin-1 (NRG1) to stimulate Schwann cell adhesion, proliferation, and directional growth. Results show positive outcomes as fibers guide the growing Schwann cells. NGR1, an important trophic factor for Schwann cells, encourages proliferation and enlargement (Tonazzini et al. 2017).

Lee and Schmidt fabricated an amine-functionalized polypyrrole to improve Schwann cell adhesion. Polypyrrole is an electrically conducting polymer with the capacity to modify the biological microenvironment through electricity. When Schwann cells were cultured on electrically stimulated polypyrrole, they show an increased secretion of brain-derived neurotrophic factor and nerve growth factor. Hence, if the surface was functionalized to improve Schwann cell adhesion, it could increase the production of these neurotrophins to enhance nerve repair (Lee and Schmidt 2015). Additionally, amine-functionalized nanodiamond is reported to encourage the growth of Schwann cells with an appropriate phenotype (Hopper et al. 2014). Polyurethane functionalized with poly(glycerol sebacate) (PGS) and aniline pentamer significantly improved Schwann cell myelin expression and sustained neurotrophin expression of nerve growth factor, brain-derived neurotrophic factor, and ciliary neurotrophic factor (CNTF) (Wu et al. 2016). CNTF is of major importance as it improves remyelination of regenerative nerves (Dinis et al. 2015).

In contrast, Arg-Gly-Asp (RGD) motifs have been added to functionalized PCL. This approach is reported to improve Schwann cell adhesion and proliferation, suggesting that Schwann cells spontaneously formed columns resembling bands of Büngner (Pérez et al. 2013). Gold nanoparticles functionalized with neuronal cell adhesion molecule L1 also stimulate Schwann cell process formation and proliferation (Schulz et al. 2013). Masand et al. functionalized a collagen hydrogels with polysialic acid (glycan) which improved Schwann cell proliferation as well as process extension (Masand et al. 2012). Electrospun scaffolds of poly(3-hydroxybutyrate) (PHB) and poly (3-hydroxybutyrate-co-3-hydeoxyvalerate) (PHBV) have also been functionalized with collagen I, H-Gly-Arg-Gly-Asp-Ser-OH (GRGDS), H-Try-Ile-Gly-Ser-Arg-NH2 (YIGSR), or H-Arg-Asn-Ile-Ala-Glu-Ile-Ile-Lys-Asp-Ile-OH (p20) peptides which are reported to recreate the native ECM motifs for nerve regeneration. Schwann cells aligned along fibers, and the biofunctionalized scaffolds, supporting differentiation along the fibers (Masaeli et al. 2014).

In summary, various in vitro studies have been conducted on Schwann cell growth and migration, using functionalized materials that resemble the native microenvironment of nerve. As mentioned above, it is important to understand how Schwann cells proliferate and migrate in vitro due to their crucial role during nerve regeneration. Furthermore, this increased understanding of Schwann cell behavior can be targeted toward in vivo studies, where the complexity of the native tissue gives valuable information on how to design nerve repair therapies using Schwann cells.

7 In Vivo Applications of Schwann Cells in Peripheral Nerve Regeneration

Since Schwann cells have been recognized as one of the driving forces in peripheral nerve regeneration, many attempts have been made to transplant them to defect sites. For this purpose, a number of preclinical models have been developed over the last

decades. Small rodents comprise the vast majority of preclinical models. The most popular model for peripheral nerve regeneration is the rat sciatic nerve defect model, with around 63% of all tissue engineering-related studies, followed by the mouse sciatic nerve and rat peroneal nerve (reviewed by Angius et al. (2012)). Gap sizes in the rat sciatic nerve defect model typically range between 6 and 10 mm. However, for long-term studies, gap sizes larger than 30 mm are not uncommon (Karimi et al. 2014). The popularity of the rat sciatic nerve defect model can be explained by its clinically relevant diameter (sciatic nerve diameter of 1–2 mm, similar to nerves in the human hand), easy and reproducible surgical procedures, as well as its availability. The mouse sciatic nerve defect model uses gap sizes between 3 and 5 mm (Goulart et al. 2014; Wang et al. 2017) and offers the advantage of readily available transgenic models and the possibility of studying large groups. Criticism on both, rat and mouse models, have been made regarding gap size as well as rapid axonal regeneration and subsequently poor translatability into the clinic (Kaplan et al. 2015; Ronchi et al. 2019). Rabbit models allow regeneration through clinically more relevant gap sizes (up to 6 cm), however lack functional assays to evaluate regenerative outcomes (Wang et al. 2011). Larger animal models (sheep, pig, monkey) allow studies of genuinely longer gaps as well as larger diameters, with the disadvantage of longer regeneration times, higher animal and housing costs, as well as ethical issues, especially with nonhuman primates (Wang et al. 2011). It is well-known that animal models are far from perfect. However, it is necessary to be aware of each model's limitations, not just to choose the correct model for the application but also to interpret the results accordingly.

Historically, the autologous nerve graft can be considered the first transplantation of Schwann cells. Within its nerve structure, it contains Schwann cells that subsequently undergo Wallerian degeneration, presenting proliferating Schwann cells in the so-called Büngner cell state. It is the gold standard in peripheral nerve regeneration; however, low success rates have driven research not only to improve the autograft itself but to develop new functionalized conduits to deliver Schwann cells to the defect site (Yang et al. 2011; Secer et al. 2008). In order to improve the autograft, it has been demonstrated by several groups in preclinical models that pre-degeneration of nerve graft has beneficial effects on the axonal outgrowth and subsequent myelination (Danielsen et al. 1994; Kerns et al. 1993). Another limiting factor of autografts is their low availability. Addressing this issue, decellularized nerve grafts, presenting nerve basement membrane and extracellular matrix components, without eliciting an immune response, have become increasingly popular (Hudson et al. 2004). While available in larger quantities, the absence of Schwann cells has been noted as negatively influencing regeneration. Recellularizing nerve allografts with autologous Schwann cells has been investigated by Jiang et al. in a 40 mm ulnar nerve defect rhesus monkey model, finding similar results compared to autologous nerve graft and improved axonal regeneration compared to the empty decellularized graft (Jiang et al. 2016). Another approach that has gained reasonable attention more recently aims to understand the mismatch between the sensory autografts in a motor defect site. Aside from structural differences influencing axonal regeneration and functional outcome (Moradzadeh et al. 2008), it has been shown in

several studies that motor and sensory Schwann cells differ in their receptors as well as secretion of neurotrophic factors (Chu et al. 2008; Höke et al. 2006; Jesuraj et al. 2012). Interestingly, the rat sciatic nerve defect model (the most common model for peripheral nerve regeneration) does not allow distinction between phenotypes due to a highly mixed motor/sensory nerve architecture. Hercher et al. analyzed in a comprehensive study the spatiotemporal behavior of homotopic and heterotopic grafts using a rat femoral nerve defect model. It was found that the gene expression of several factors such as GDNF, BDNF, TrkB, and Cadm3, among others differ throughout the 10-week observation period between phenotypically matched and mismatched grafts (Hercher et al. 2019).

Aside from understanding the limitations of autografts, the development of tissue-engineered nerve grafts containing Schwann cells has gained considerable attention. One of the first engineered Schwann cell containing grafts reported by Guenard et al. utilized Matrigel as a carrying structure, within an acrylonitrile-vinyl chloride copolymer tube. It was found that the presence of Schwann cells had a beneficial effect on axonal growth, but more interestingly, strain-mismatched Schwann cells solicited a strong immune response (Guenard et al. 1992). Over the years, a variety of scaffold materials have been tested to deliver Schwann cells, among which collagen, fibrin, PCL, PLGA, chitosan and silk fibroin, and others can be found (Schuh et al. 2018; Xie et al. 2014; Yuan et al. 2004; Hsu et al. 2005). One consistent factor determined as being essential for the success of Schwann cell transplantation is scaffold architecture. This means that the inner structures of tubes should ideally mimic nerve structures to align Schwann cells and direct axonal outgrowth. One approach to achieve bio-mimicry was by Georgiou et al., designing a self-aligning neural tissue based on collagen and Schwann cells, supporting nerve regeneration in a 15 mm rat sciatic nerve defect model (Georgiou et al. 2013). Through addition of fibrin (a known Schwann cell proliferation enhancer) to the collagen-Schwann cell blend, axonal regeneration was further improved (Schuh et al. 2018). Another approach designed by Xie et al. was based on electrospinning poly(ε-caprolactone) into a conduit containing aligned fibers and seeded with Schwann cells prior to implantation into a 14 mm rat sciatic nerve defect. Results demonstrated less axonal regeneration compared to the autograft but improved compared with an empty tube (Xie et al. 2014). Owens et al. designed a fully cellular nerve graft, containing bone marrow mesenchymal stem cells and Schwann cells, assembled by bioprinting into a multi-luminar nerve graft, finding results comparable to the autologous nerve graft (Owens et al. 2013).

Several approaches, independent of the fabrication and material, have demonstrated that the presence of Schwann cells before implantation has a beneficial effect on the outcome. One of the recurrent questions and challenges in Schwann cell transplantation is survival after implantation. While in vitro parameters such as medium composition, nutrient, and oxygen supply can be controlled and monitored, this has proven difficult for in vivo transplanted grafts. First attempts to follow Schwann cell survival in vivo were made in the early 2000s, where Schwann cells stably expressing GFP were transplanted into a defect site. Grafts were found to contain GFP Schwann cells integrated into nerve regeneration processes 2 weeks

after implantation (Tohill et al. 2004). Gambhir et al. suggested a refined approach, transducing Schwann cells using a lentiviral vector with a nuclear localization signal fused with mCherry to improve differentiation between single cells for more precise cell counts (Gambhir et al. 2016). Long-term live monitoring was also achieved by Kimura et al, using *firefly*-luciferase-labeled neural crest-like cells (Kimura et al. 2018). However, all these approaches test the survival of transplanted cells in an existing conduit. In contrast, Coy et al. presented an interesting new approach to simulate axonal behavior within a nerve conduit using mathematical modeling, giving perspectives on integrating similar algorithms into prediction of vascularization and subsequently cell survival within nerve grafts (Coy et al. 2018).

Since survival of transplanted Schwann cell cannot be assured, an upcoming hot topic of the last decade has become more and more interesting in supporting and replacing cell therapy: exosomes and microvesicles have been proven to be potent helpers in regeneration of several tissues (e.g., cardiovascular or bone and muscle, reviewed by Sun et al. (2018)). Lopez-Verrilli et al. discovered that exosome-derived Schwann cells (in the Büngner cell state) promote axonal regeneration in a rat sciatic nerve defect model by modifying the morphology of the growth cone toward a regenerative phenotype. One of the factors identified was GTPase RhoA, known to play a role in growth cone collapse and axon retraction (Lopez-Verrilli et al. 2013).

8 Alternatives to Native Schwann Cells

With the advances of cell therapy and tissue engineering, the demand for primary cells has risen. As above, Schwann cells have historically been known as difficult to isolate, since there is no readily accessible source. Furthermore, as highlighted by Wakao et al, collecting Schwann cells is inevitably damaging a nerve. Several alternative sources have been proposed, including mesenchymal stem cells, embryonic stem cells, as well as induced pluripotent stem cells (Dezawa et al. 2001; Liu et al. 2012). Recently, mesenchymal stem cells (MSCs) have gained considerable interest as an alternative to the use of native Schwann cells. MSCs have been found in several tissues including the bone marrow, umbilical cord, adipose tissue, and amniotic membrane, among others (reviewed by Hass et al. (2011)). Aside from evident advantages such as a potential to differentiate into a several mesenchymal cell types (e.g., adipocytes, osteocytes, chondrocytes, and fibroblasts (Fan et al. 2008; Väänänen 2005; Dominici et al. 2006; Strem et al. 2005)), their immunogenic phenotype lacks major histocompatibility complex class II (MHC II, hindering T-cell stimulation and subsequently immunogenic responses (Javazon et al. 2004; Barry and Murphy 2004; Chamberlain et al. 2007)).

The first group to propose generating Schwann cell-like cells from MSCs was Dezawa et al., utilizing bone marrow-derived MSCs and a three-step protocol. Cells were primed toward a neural lineage with β-mercaptoethanol and retinoic acid, followed by differentiation into Schwann cell-like cells with bFGF, PDGF, heregulin, and forskolin (Dezawa et al. 2001). Several subsequent studies demonstrated robust differentiation from a variety of MSC sources, including the adipose

tissue, umbilical cord, and amniotic membrane (Wakao et al. 2014; Chen et al. 2019). Schwann-like cells have been found to express genes, transcription factors, and proteins associated with Schwann cells, including S100ß, p75NGFR, GFAP, SOX10, Krox20, and c-jun (Kaewkhaw et al. 2011; Wakao et al. 2014; Chen et al. 2019). Furthermore, they have also been shown to express myelin and stimulate neurite outgrowth in vitro *(*Xu et al. 2008*).* The latter was partially associated with exosome and RNA transfer, as an important component of the secretome (Ching et al. 2018). In preclinical studies, the performance of Schwann-like cells was evaluated as being comparable to native Schwann cells (e.g., when seeded into decellularized nerve grafts or chitosan conduits) (Fan et al. 2014; Ao et al. 2011; Wang et al. 2012). Given the availability of Schwann-like cells differentiated from human waste material such as the adipose tissue or amniotic membrane, those cells provide a solid alternative for in vitro testing of, e.g., pharmaceuticals or potential nerve conduits, as well as for implantation to augment peripheral nerve regeneration.

9 Conclusions

The above review highlights the essential role of Schwann cells in nerve development and function and their role in regeneration following injury. A range of studies have been critiqued that have revealed our understanding of Schwann cell behavior and how they interact to axons to encourage neurite outgrowth. Research has been conducted across a wide range of in vitro and in vivo studies reporting on the use of different biomaterials and models to elucidate nerve repair therapies that either use Schwann cells as a potential therapy or identify a central role in regeneration. Furthermore, isolation protocols are discussed that have been developed to obtain purified Schwann cell cultures that overcome previous technical limitations for Schwann cell therapy. In addition, a range of alternatives to primary Schwann sources are critiqued as alternatives to this.

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