# Mesenchymal Stem Cells and Umbilical Cord as Sources for Schwann Cell Differentiation: their Potential in Peripheral Nerve Repair

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**Abstract:** Schwann cells are important components of the peripheral glia that form myelin, serving as the microenvironment of nerve fibers in the peripheral nervous system (PNS). Damage to the PNS induces the differentiation and activation of Schwann cells to produce factors that strongly promote axonal regrowth, and subsequently contribute to remyelination, which is crucial for the recovery of function. Although the collection and transplantation of native Schwann cells are effective for the treatment of neural diseases, isolation of Schwann cells results in new damage to other peripheral nerve segments and causes undesirable iatrogenic injury in the donor. Furthermore, the expansion of native Schwann cells to obtain a sufficient number of cells for clinical application within a reasonable period is technically difficult. Therefore, a method to induce easily accessible and highly proliferative cells to differentiate into cells with Schwann cell properties would be very practical and is highly desirable. Recently, regenerative medicine has focused on mesenchymal stem cells because they are easily accessible from various kinds of mesenchymal tissues such as the umbilical cord, bone marrow, and fat tissue. Mesenchymal stem cells are highly proliferative and it is easy to obtain an adequate number of cells. Notably, while mesenchymal stem cells are mesodermal lineage cells, they have an ability to cross oligolineage boundaries previously thought uncrossable to achieve transdifferentiation. In this review, we focus on the potential of mesenchymal stem cells, particularly umbilical cord-derived mesenchymal stem cells, to differentiate into functional Schwann cells, and discuss the prospective clinical application of these cells to PNS regeneration.

**Keywords:** Schwann cells, differentiation, nerve regeneration, myelin, mesenchymal stem cells, umbilical cord, bone marrow, fat tissue.

## INTRODUCTION

Although the peripheral nervous system (PNS) and central nervous system (CNS) are categorized into nervous tissue, nerve axons of the PNS are capable of regenerating after damage while such regeneration does not normally occur in the CNS [1]. CNS neurons, in principle, however, do have the ability to regenerate under adequate circumstances when triggered by the appropriate treatments [2, 3]. The difference in the regenerative capacity of the PNS and CNS is explained mainly by differences in the properties of their glial cells (Fig. **1**). Schwann cells, one of the most important components of the peripheral glia that forms myelin, serve as a favorable microenvironment for the repair of damaged nerve fibers in the PNS [4, 5](Fig. **1**). Normally, peripheral myelin, as well as myelinating Schwann cells, inhibit axonal regeneration, but the cellular cascades that occur after damage initiate the removal of damaged myelin and the dedifferentiated Schwann cells actively produce factors that strongly promote the growth of regenerating axons [6, 7]. On the other hand, oligodendrocytes and astrocytes are the major components of the CNS glial environment and they actively inhibit axonal regrowth. Myelin debris and reactive astrocytes are the major inhibitory factors for axonal regeneration[8, 9](Fig. **1**). The myelin in the CNS is constructed by oligodendrocytes and contains inhibitory molecules such as Nogo, oligodendrocyte-myelin glycoprotein, and myelin associated glycoprotein (MAG) on its surface[10]. These inhibitory molecules bind to the Nogo receptor on the surface of the distal tip of regenerating axons to transmit the inhibitory signal of axonal extension in cooperation with the co-receptors such as the low-affinity nerve growth factor receptor p75NTR, LINGO-1 (Leucine-rich repeat and immunoglobulin domain-containing protein 1) and TROY (Tumor necrosis factor receptor superfamily member 19) [10]. In addition, astrocytes are activated to proliferate after injury, extend the cellular processes, and form glial scars, which physically and chemically obstruct axonal regeneration [9, 11].

Schwann cells that have been collected *in vitro* and transplanted are known to support axonal regeneration in the damaged nervous system. These cells overcome the inhibitory environment to elicit axonal regeneration and construct myelin in the CNS [12, 13]. For these reasons, Schwann cells are considered one of the most suitable cell types for inducing axonal regeneration in both the PNS and CNS.

Damage to the PNS causes axonal degeneration and demyelination, which result in functional disorder. Many approaches have been used in an attempt to restore neural function. Recent tissue engineering researches have focused on the development of bioartificial nerve conduits aimed at guiding axonal regrowth [14, 15]. In this system, the nerve ends and intervening gap are enclosed within a tube composed of biologic or synthetic materials, thereby allowing axons to regrow into the distal nerve segment [16].

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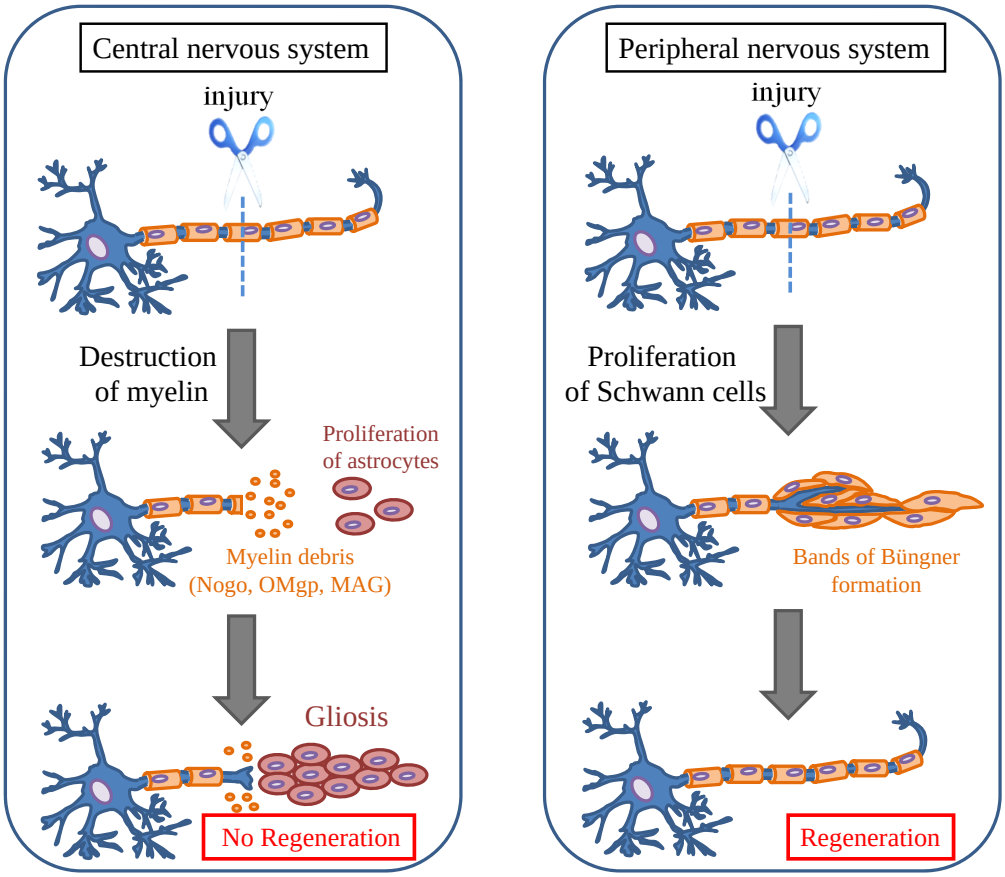


**Fig. (1).** Regenerative capacity of CNS and PNS. OMgp: oligodendrocyte-myelin glycoprotein, MAG: myelin associated glycoprotein.

Artificial nerve conduits are limited, however, when the nerve gap is long. In principle, Schwann cells are crucial for PNS regeneration, even when artificial nerve conduits are used; Schwann cells migrate out from both the proximal and distal nerve ends into the gap space and provide a cellular foothold to support axonal regrowth and to guide regenerating axons to the distal nerve segment where activated Schwann cells form cordons [17]. Axons successfully elongate over a long distance, mostly while in contact with cellular footholds such as Schwann cells, but long regrowth does not occur when only extracellular matrices are provided [18-20]. Therefore, when the gap is long, the limited Schwann cell migration results in limited regeneration efficiency.

Although Schwann cell collection and transplantation are effective for the treatment of neural diseases, isolation of Schwann cells causes new damage to other peripheral nerve segments, causing undesirable iatrogenic injury to the donor. Furthermore, expansion of Schwann cells to obtain an adequate cell number for clinical applications within a reasonable period of time is difficult. Therefore, a method to induce easily accessible and highly proliferative cells to differentiate into cells with Schwann cell properties would be very practical and is highly desirable.

In the past decade, researchers have focused on mesenchymal stem cells because these cells are easily accessible from various types of mesenchymal tissues and are highly proliferative so that an adequate number of cells can be obtained. Notably, while mesenchymal stem cells are mesodermal lineage cells, they are able to cross oligolineage boundaries that were previously thought to be uncrossable to achieve transdifferentiation [21]. In bone marrow stromal cells (BMSCs), mesenchymal stem cells differentiate into endodermal cells as well as ectodermal cells, including Schwann cells [21-25]. Such multipotency has also been clarified in umbilical cord (UC)-derived mesenchymal stem cells (UC-MSCs) and adipose-derived stem cells (ADSCs), other promising mesenchymal stem cells [26, 27]. UC-MSCs and ADSCs are also be able to differentiate into Schwann cells [28, 29]. Here in this review, we focus on the potential of mesenchymal stem cells to differentiate into functional Schwann cells and discuss the potential clinical application of mesenchymal stem cells to PNS regeneration.

## REACTION OF SCHWANN CELLS TO NEURAL DAMAGE

Damage to the PNS produces a cascade of cellular and molecular reactions in the nerve segment distal to the injury site, which is called Wallerian degeneration (originally observed by Waller, 1850) [5].

### Axonal Degradation

The first step involves the prompt degradation of axons and myelin sheaths. Proximal to the site of injury, damage to nerve axons immediately activates intracellular signaling pathways and the expression of various kinds of genes related to the cell emergency system, such as c-*fos* and c-*jun*

[30]. Free intracellular $Ca^{2+}$ and calpains also act on axonal neurofilaments and microtubules, leading to axonal degradation [31, 32].

### Myelin Degradation

Schwann cells that have lost axonal contact initiate the degeneration of their own myelin sheaths. This is an intrinsic process of self-destruction and is very important for subsequent regeneration [33-35]. Because myelin and myelinated Schwann cells contain axon growth inhibitors, rapid removal of myelin debris as well as changes of myelinated Schwann cells into an unmyelinated state is a key step for preconditioning the PNS toward regeneration [6, 7].

### Immune Cell Responses

Damage triggers the infiltration of immune cells within hours. Mast cells release histamine and chemokines, which further recruit neutrophils, monocytes/macrophages, and lymphocytes [36-38]. The most important inflammatory cells during PNS regeneration are macrophages. Two kinds of macrophages are involved in regeneration; resident endoneurial macrophages, which reside within the endoneurium of PNS and are ED-2 (CD163, a cell surface glycoprotein, 175kDa)-positive [39, 40]; and hematogenous monocytes/macrophages that are ED-1 (CD68, a single chain glycoprotein, 90-110kDa)-positive [41]. Resident endoneurial macrophages are activated within a couple of days after damage, and later, following the destruction of the blood-nerve barrier, hematogenous monocytes/macrophages begin to invade into the nerve segment distal to the injured site at 5 to 7 days after injury [42-44]. In addition to blood-nerve barrier destruction, the interaction between Schwann cells and resident macrophages also contributes to attracting hematogenous monocytes/macrophages [45]. For example, an autocrine cascade of interleukin-6 (IL-6) and leukemia inhibitory factor enhances Schwann cell secretion of monocyte chemoattractant protein-1, which directly attracts the infiltration of hematogenous monocytes/macrophages into the injured nerves [46-48]. In turn, macrophages secrete cytokines and trophic factors, such as IL-1 and insulin-like growth factor (IGF), which enhance the activation of Schwann cells as well as fibroblasts, followed by axonal growth [49].

Hematogenous monocytes/macrophages spread into the entire distal nerve segment by day 14 [43, 44]. Both the resident endoneurial macrophages and hematogenous monocytes/macrophages are involved in the removal of myelin sheaths. Importantly, as mentioned above, not only macrophages but also Schwann cells contribute to myelin removal [33].

### Schwann Cell Activation

The most characteristic feature of Wallerian degeneration is the proliferation and activation of Schwann cells within the distal nerve segment to form Schwann cell cordons, which are called bands of Büngner [5]. These Schwann cells produce various factors and cytokines that create a supportive milieu for regenerating axons in the distal segment. In the undamaged PNS, Schwann cells have a reciprocal relationship with the axons they ensheath. Axonal signals, whether acting by direct contact or by diffusible factors, regulate Schwann cell genes and control proliferation and differentiation. Conversely, Schwann cell signals regulate gene expression and intracellular axonal signaling [50]. Such a tightly regulated relationship between axons and Schwann cells is disrupted by injury, leading to subsequent Wallerian degeneration. Schwann cells quickly downregulate the expression of myelin protein genes and upregulate the low affinity neurotrophin receptor p75, as well as neurotrophins: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-4 (NT-4) [5, 51]. These neurotrophic factors are key regulatory proteins that modulate neuronal survival, axonal growth, synaptic plasticity, and neurotransmission. Schwann cells secrete a large variety of neurotrophic factors, including neurotrophins, transforming growth factor, basic fibroblast growth factor (bFGF), fibroblast growth factor-5, glial cell line-derived neurotrophic factor, osteopontin, IL-1β, IL-6 and leukemia inhibitory factor. All of these factors are thought to contribute to successful axonal regeneration [5, 52-54]. Particularly, neurotrophins and their downstream Ras/mitogen activated protein kinase and phosphoinositide-3-kinase/Akt pathways are critical for both axonal growth and neuron survival in the PNS [55, 56].

Activated Schwann cells express a variety of cell adhesion molecules, including neural cell adhesion molecules, L1, CHL1 (close homologues of L1), N-cadherin and integrins, represented by α1β1 and α6β1-integrin, that mediate interactions between Schwann cells and axons, including growth cones [4, 17, 57, 58]. In addition to these trophic factors and cell adhesion molecules, Schwann cells supply molecules of the extracellular matrix, such as fibronectin, laminin, J1/tenascin, and merosin (laminin-2), which also support the extension of regenerating axons [57]. Activated Schwann cells express connexin 46 and other connexins, coupled through junctional channels, and tight junction-related proteins [18, 59]. Finally, reinnervated Schwann cells cease dividing, show downregulated expression of the molecules related to nerve regeneration, and revert to an axon-associated phenotype [5].

### Axonal Regeneration and Re-myelination

Regenerating axons elongate into the distal nerve segment guided by Schwann cell cordons in the bands of Büngner. Along with axonal regeneration, reorganization of the endoneurial extracellular matrix occurs. In the later period of regeneration, Schwann cells ensheath axons and reconstruct myelin necessary for PNS function. During regeneration, one Schwann cell surrounds several regenerating axons, but eventually the cells segregate to form a 1:1 relationship between axon and Schwann cell, and the process of remyelination is completed [5].

## CELL BASED-THERAPY FOR PERIPHERAL NERVE REGENERATION USING SCHWANN CELLS DIFFERENTIATED FROM MESENCHYMAL STEM CELLS

In the past decade, researchers in stem cell biology and regenerative medicine have focused on mesenchymal stem cells because these cells are easily accessible from the bone

marrow, umbilical cord, or adipose tissues, and are highly proliferative so that adequate numbers of cells can be obtained for clinical application. Furthermore, mesenchymal stem cells can be collected without encountering serious ethical problems, and there is no need to use fertilized eggs or a fetus such as in case of embryonic stem cells or neural stem/progenitor cells. Thus, mesenchymal stem cells are a strong potential candidate for use in cell-based therapy, and are already applied to patients; i.e., BMSCs for arthrosis deformans and myocardial infarction [60, 61]. Therefore, mesenchymal stem cells are also expected to be applicable for neural regeneration.

Adult human mesenchymal stem cells originate from mesodermal lineage, but also differentiate into ectodermal- as well as endodermal-lineage cells. The fact that they are able to cross oligolineage boundaries previously thought to be uncrossable stimulated development of the field of regenerative medicine and promoted studies aimed at generating desired cells from mesenchymal stem cells [21]. Particularly, BMSCs have been targeted for this research. To date, various cell types such as mesodermal lineage cells (bone, cartilage, adipocytes, skeletal muscles, and cardiomyocytes), as well as endodermal lineage cells (airway epithelial cells, hepatocytes, and insulin producing cells) and ectodermal lineage cells (neuronal cells), have been induced from BMSCs *in vitro,* mostly by using cytokines, trophic factors, or gene introduction [21, 23-25, 62, 63]. Recently, pluripotent stem cells, named multilineage-differentiating stress enduring (Muse) cells, were found among adult human mesenchymal stem cells such as BMSCs, dermal skin fibroblasts, and in mesenchymal tissues such as bone marrow or dermis [64]. Muse cells are capable of differentiating into cells representative of all three germ layers from a single cell, which may explain their broad spectrum of differentiation [64].

The induction system for differentiating Schwann cells from BMSCs was first reported by Dezawa *et al.* in 2001 [22](Fig. **2**). In their report, serial administration of reagents and cytokines (described below) induced nearly 97% of BMSCs to differentiate into Schwann cell marker-positive cells, and the induced cells were shown to elicit nerve regeneration and to form myelin when transplanted into a PNS injury model. With or without some modifications, subsequent studies confirmed the successful application of this system to other kinds of mesenchymal stem cells.

Along with BMSCs, the UC and ADSCs are other promising sources of mesenchymal stem cells. Mesenchymal tissue of the UC, so-called Wharton's jelly, contains an abundance of mesenchymal stem cells. These cells have an advantage over BMSCs in that the UC derives from postnatal tissue that is discarded after birth and thus cell collection is not an invasive procedure for donors or patients. Because of the ability of UC cells to differentiate into other cell types and to proliferate, these cells are a practical and ideal source for cell-based therapies. Recently, UC-MSCs and ADSCs were shown to differentiate into Schwann cells capable of supporting neural regeneration and constructing myelin [28, 29] (Fig. **2**). The following sections discuss the induction of cells exhibiting Schwann cell properties from mesenchymal stem cells, and the potential clinical application of mesenchymal stem cells to PNS regeneration.

**• BMSCs**

BMSCs are easily accessed by aspiration of the bone marrow. They can be isolated from patients and expanded in a large scale, both from healthy donors and patients. For example, 20 to 100 ml of bone marrow aspirate yields $1x10^7$ of BMSCs within several weeks, which provides a sufficient number of cells for transplantation.

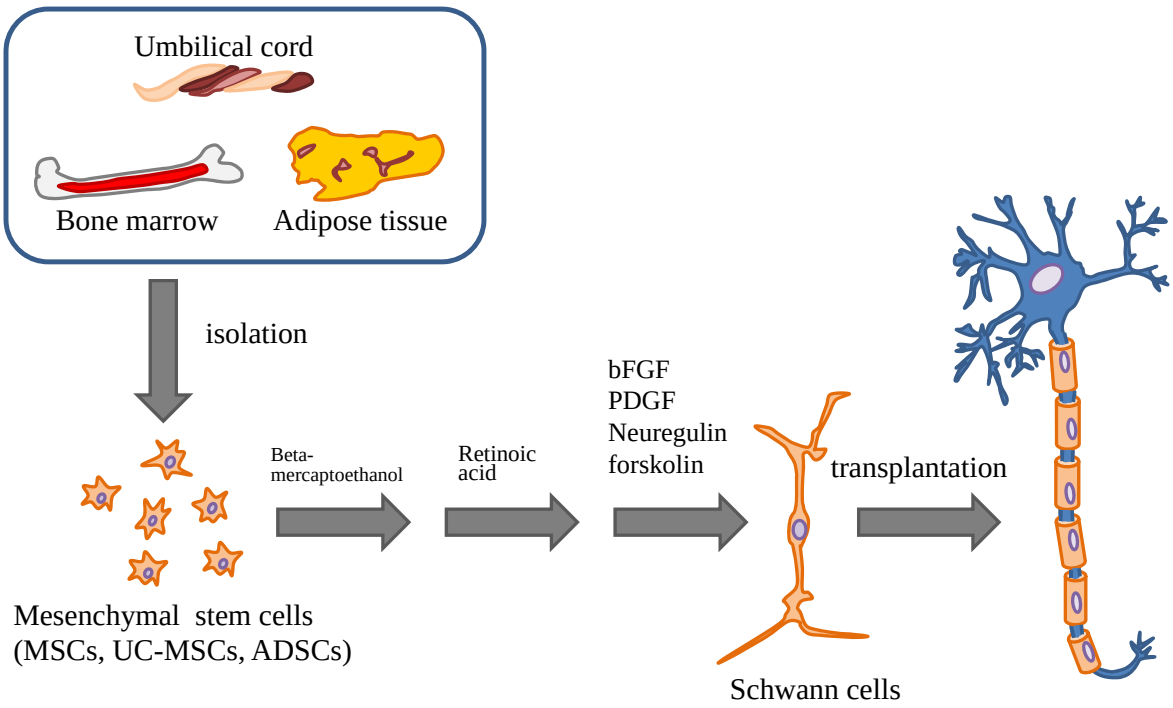


**Fig. (2).** Schwann cells can be differentiated from mesenchymal stem cells of bone marrow, umbilical cord and adipose tissue.

Dezawa *et al.,* (2001) were the first to attempt the induction of Schwann cells from mesenchymal stem cells using BMSCs [22]. The sequential treatment of rat BMSCs dispersed at a certain cell density with the reducing reagent beta-mercaptoethanol, all-trans-retinoic acid, and then a mixture of forskolin (known to upregulate intracellular cAMP concentration), bFGF, platelet-derived growth factor (PDGF), and neuregulin-1 (NRG-1 [isoforms include Type I NRG1, also called heregulin, and Type II NRG1, also called glial growth factor-2 (GGF-2)]) generated cells with Schwann cell properties with very high efficiency ( ~97%). The differentiated cells exhibited Schwann cell properties and expressed Schwann cell markers such as protein zero (P0), low-affinity nerve growth factor receptor p75, glial fibrillary acidic protein, S-100, and O4 [22]. Importantly, differentiated Schwann cells contributed to axonal regeneration as well as to reconstruction of myelin when transplanted into transected rat peripheral nerve, which is the most important property of native Schwann cells in PNS injury [22, 65].

This induction method was further reproduced by several groups, and is now widely applied to Schwann cell induction from a wide range of mesenchymal stem cells, including BMSCs, UC-MSCs and ADSCs [28, 66-69]. Schwann cells differentiated from BMSCs support axonal regeneration both *in vitro* and *in vivo*. In co-culture with dorsal root ganglion neuron, differentiated Schwann cells substantially support neurite outgrowth[70-72]. When differentiated Schwann cells are transferred into conduits and transplanted into the transected gap made in peripheral nerve, they sustain axonal regrowth and formed myelin, leading to functional recovery in nerve conduction velocity, walking track analysis, average myelin area, and myelinated axon count [22, 65, 73]. Chitosan is shown to be a favorable conduit to fill with differentiated Schwann cells for transplantation [74]. Histologically, transplanted cells were confirmed to form myelin and express myelin-related proteins such as MAG, myelin-basic protein, P0 or peripheral myelin protein 22 (PMP22) [22, 65, 75, 76].

The effectiveness of Schwann cells differentiated from BMSCs was further demonstrated in human BMSCs; under the control of immunosuppressants, these cells support axonal regeneration, construct the myelin sheath, and lead to the recovery of sciatic nerve function when transplanted into a rat sciatic nerve transection model [77]. Preclinical studies using cynomolgus monkeys demonstrated that, in behavior analysis, electrophysiology, and histologic evaluation, autologous transplantation of Schwann cells differentiated from BMSCs is effective for accelerating the regeneration of transected axons and for functional recovery of injured nerves. Furthermore, $^{18}$F-fluorodeoxyglucose-positron emission tomography scanning demonstrated no abnormal accumulation of radioactivity except in regions with an expected physiologic accumulation up to 1 year and thus confirmed the safety of this transplantation treatment [78].

These findings described above indicate that the practical advantages of BMSCs are expected to make this system applicable for neurotrauma or peripheral nerve disorders where the acceleration of regeneration is expected to enhance functional recovery.

**• UC-MSCs**

The UC-MSCs are highly advantageous as potential sources for cell-based therapies because of their easy accessibility and the few ethical problems regarding their use, as long as donors provide informed consent. UC-MSCs are proliferative cells, and cell collection is not an invasive procedure for donors or patients. They are available from cord bank. Tumorigenesis in transplantation experiments of UC-MSCs and of UC-MSC-differentiated cells is rare[79, 80], and these cells have the potential to differentiate into a wide variety of cell types as reported previously[81-84]. For example, neural cells including neurons, oligodendrocytes and astrocytes [85] and even endodermal cell types such as hepatocytes and pancreatic beta cells can be given rise to from UC-MSCs [86, 87]. Thus, along with BMSCs, UC-MSCs are considered one of the most practical sources for cell-based therapies.

Several groups have demonstrated the differentiation of functional Schwann cells from human UC-MSCs. Dezawa's group showed that the method used for BMSCs, i.e., beta-mercaptoethanol, retinoic acid and the mixture of bFGF, PDGF, and the heregulin-A1-EGF domain together with forskolin successfully induced the differentiation of Schwann cells from human UC-MSCs (UC-Schwann cells) with very high efficiency (~97%), and that UC-Schwann cell function is comparable to that of native human Schwann cells *in vivo*[29]. Transplantation into rat transected sciatic nerve under the control of immunosuppressants showed that the human UC-Schwann cells maintained their differentiated phenotype *in vivo* after transplantation and contributed to axonal regeneration and functional recovery. The transplanted UC-Schwann cells, which were pre-labeled with lentivirus green fluorescent protein, expressed MAG, PMP22, and periaxin (a marker of peripheral myelin), and immunoelectron microscopy revealed that transplanted cells formed the myelin of regenerated axons. Consistent with these results, functional recovery (measured by walking track analysis) achieved by the transplantation of UC-Schwann cells was almost identical to that of native human Schwann cells [29]. Other groups demonstrated that UC-Schwann cells differentiated by the same method produced neurotrophic factors such as NGF and BDNF [66, 67, 88]. These findings indicated that UC-Schwann cells are a viable alternative to native Schwann cells and may be applied to cell-based therapy for nerve injuries and degenerative diseases.

**• ADSCs**

Mesenchymal stem cells prepared from adipose tissue are referred to as ADSCs. The properties of ADSCs are similar to BMSCs in that they also have the capacity to differentiate into various kinds of cells [89-92]. Because humans have abundant sources for ADSCs (i.e., subcutaneous fat deposits that can be isolated by conventional liposuction procedures), they are also expected to be a practical cell source for cell-based therapy.

Terenghi's group was the first to report Schwann cell differentiation from ADSCs in 2007 [28]. They obtained Schwann cells by treating ADSCs with beta-mercaptoethanol and retinoic acid, followed by a mixture of bFGF, PDGF,

forskolin, and neuregulin (GGF-2). The differentiated cells expressed Schwann cell markers and coculture with neuronal cells induced neurite outgrowth. Haycock *et al*., (2011) reproduced these data, and further clarified that perinephric ADSCs have high potential to become Schwann cells compared with ADSCs from other sources such as subcutaneous or epididymal fat tissues [69].

Other differentiation systems have been developed as well; Vogt *et al*., (2009) demonstrated that culturing ADSCs in neurosphere culture followed by dissociation of the formed spheres and the removal of mitogen results in the differentiation of ADSCs into Schwann cell-like cells that are positive for p75, S-100, and glial fibrillary acidic protein [93]. Zhang *et al*., (2008) reported that co-culturing ADSCs with native Schwann cells is effective for Schwann cell differentiation [94].

Because the harvest of BMSCs is an invasive and painful procedure, and UC-MSCs are usually not applicable to autologous transplantation, ADSCs will be useful alternative cells for Schwann cell production and cell-based therapy.

## THE MECHANISMS OF SCHWANN CELL DIFFERENTIATION; STUDIES OF UC-SCHWANN CELLS

The method used to induce Schwann cells, namely, treatment of cells first with beta-mercaptoethanol, then with retinoic acid, and finally with a mixture of bFGF, forskolin, PDGF, and neuregulin seems to apply generally to mesenchymal stem cells. The rational for this induction system was considered in UC-Schwann cell differentiation [29].

Exposure of UC-MSCs to beta-mercaptoethanol and retinoic acid is a prerequisite because elimination of these factors fails to differentiate into Schwann cells. The beta-mercaptoethanol acts as a reducing agent on BMSCs to promote differentiation into neural-lineage cells by the synthesis of glutathione [95, 96]. Retinoic acid is a well-known factor that acts as a morphogen during development, regulating the expression of various transcription factors that are crucial for early neural determination such as MASH1 and NeuroD, and has a role in the acquisition of the responsiveness to neurotrophins [97-99].

The bFGF functions as a mitogen and accelerates Schwann cell precursors during Schwann cell transition [100, 101]. PDGF contributes to DNA synthesis and acts as a mitogen in Schwann cells [102]. Neuregulin, either heregulin or GGF, selectively induces Schwann cells from neural crest cells and promotes the survival and proliferation of Schwann cell progenitors [100]. Forskolin increases the level of intracellular cyclic adenosine monophosphate and the expression level of growth factor receptors. Therefore, the addition of forskolin to the combination of bFGF, PDGF, and heregulin might enhance cellular responses to trophic factors, leading to efficient trophic factor stimulation for Schwann cell differentiation [103].

Interestingly, gene expression studies demonstrated that UC-MSCs were initially negative for P0 and S-100B and slightly positive for Sox10 and Krox20 [29]. After the induction of UC-Schwann cells, P0 and S-100B began to be expressed, and expression of Sox10 and Krox20 was substantially upregulated. In contrast, the expression of a cell marker for the immature neural lineage Hath1 was initially positive in UC-MSCs, but decreased after the induction process, suggesting that UC-Schwann cells undergo sequential differentiation through this induction process [29].

In summary, beta-mercaptoethanol and retinoic acid might work together to trigger factors that alter the characteristics of UC-MSCs to those of neural lineage cells, and subsequent treatment with forskolin, bFGF, PDGF, and neuregulin synergistically promote the differentiation of UC-MSCs into cells with Schwann cell characteristics. Therefore, the use of these factors for inducing cells with Schwann cell properties mimics normal Schwann cell development and thus this system is generally efficient for differentiation of mesenchymal stem cells into Schwann cells.

## PERSPECTIVES

Vigorous axonal regeneration can be elicited when the injured PNS is provided a cellular foothold with the ability to support axonal growth. Compared to biomaterial nerve conduits, the number of regenerating axons is more abundant, and the regeneration speed is faster when the damaged PNS is provided with this cellular foothold. Furthermore, simple axonal regrowth is not sufficient for PNS regeneration. Reconstruction of myelin is necessary for the procurement of saltatory conduction, which is critical for functional recovery. For these reasons, Schwann cells are the most crucial cells for cell-based therapy in PNS regeneration. Transplantation of Schwann cells in combination with the use of biomaterials that are effective for supporting neural regeneration strongly promotes regeneration efficiency.

Schwann cells originate from neural crest cells [54]. Although cells with neural crest origin such as skin-derived precursors are reported to reside in adult tissues[104], these cells are technically difficult to obtain from adult tissues in large numbers for clinical application. Alternative sources are native Schwann cells that can be harvested from the adult human PNS system. It is difficult, however, to obtain an adequate amount of Schwann cells from the human PNS for clinical use. Besides, Schwann cells cannot be harvested without damaging healthy peripheral nerve. Thus, it would be more desirable to establish cells with Schwann cell characteristics from sources that are easily accessible and capable of rapid expansion.

Mesenchymal stem cells offer great potential for cell transplantation therapy because of their easy accessibility and proliferative capacity. Undifferentiated naive BMSCs or UC-MSCs have limited efficiency for PNS regeneration; compared to the transplantation of Schwann cells differentiated from BMSCs or UC-MSCs, the number of regenerating axons and the extent of functional recovery are far below that of transplantation with naive BMSCs or UC-MSCs [29, 65, 77, 78]. Therefore, it is rational to use differentiated Schwann cells, but their practical application to human PNS disorders is dependent on the technique used to control their differentiation into functional Schwann cells with high efficiency and purity. In many cases, differentiation of MSCs involves the introduction of specific genes. The basic method of differentiating Schwann cells from

MSCs (beta-mercapthoethanol -> retinoic acid -> bFGF+ PDGF+forskolin+neuregulin) [22], however, involves only the reagent and trophic factor administration and not any gene introduction.

The function of Schwann cells in neural regeneration is primarily a facilitatory effect for axonal regrowth as well as for the reconstruction of myelin to allow for saltatory conduction in the PNS. Therefore, differentiated Schwann cells must not only express markers for Schwann cells and myelin, but must also have the ability to form myelin, and this characteristic is critical for the evaluation of differentiated cells. In this sense, Schwann cells differentiated from BMSCs, UC-MSCs, and ADSCs compare favorably with native Schwann cells. Notably, differentiated Schwann cells obtained from monkey BMSCs were demonstrated to be effective and safe for PNS regeneration up to 1 year when evaluated based on general condition, tumor markers in blood analysis, and 18F-fluorodeoxyglucose-positron emission tomography scanning [78]. Furthermore, for clinical application, it is important that not only rodent cells but also human BMSCs, UC-MSCs, and ADSCs are capable of differentiating into cells with Schwann cell properties.

For these reasons, Schwann cells differentiated from human mesenchymal stem cells seem to have the most appropriate properties for clinical application.

Auto cell transplantation is an ideal therapy for regenerative medicine because it has carries less risk of rejection and infection. BMSCs and ADSCs are practical cells that are expected to be applicable for auto cell transplantation therapy in neurodegenerative diseases [105] (Fig. **3**). Some patients, however, will not be able to use their own cells due to older age or underlying disease. In such circumstances, UC-MSCs might be an alternative choice for cell-based therapy (Fig. **3**). Furthermore, UC-MSCs are superior to other cell sources in some respects because they can be noninvasively collected from many volunteers and cord banks that have been already established. A potential disadvantage of using UC-MSCs, as in allogenic transplantation, is the need for continuous immunosuppression in case of an HLA mismatch. Mesenchymal stem cells, however, suppress immunoresponses, known as immunomodulation, and in fact, allogenic BMSCs are already used in clinical application for treating graft-versus-host disease[106]. Although more studies are currently needed to demonstrate that UC-Schwann cells inherit the character of MSCs in terms of immunomodulation, UC-Schwann cells might be applicable for allogenic transplantation.

Previous studies indicate that Schwann cells also support axonal regeneration, construct myelin, and contribute to functional recovery in a spinal cord injury model [107-109]. The results suggest that Schwann cells differentiated from mesenchymal stem cells such as BMSCs, UC-MSCs, and ADSCs may also be effective for therapeutic application to spinal cord injury. In addition, other animal models of peripheral nerve injury resembling clinical cases, such as crush or laceration, may be more informative for the assessment of mesenchymal stem cells-derived Schwann cell therapies.

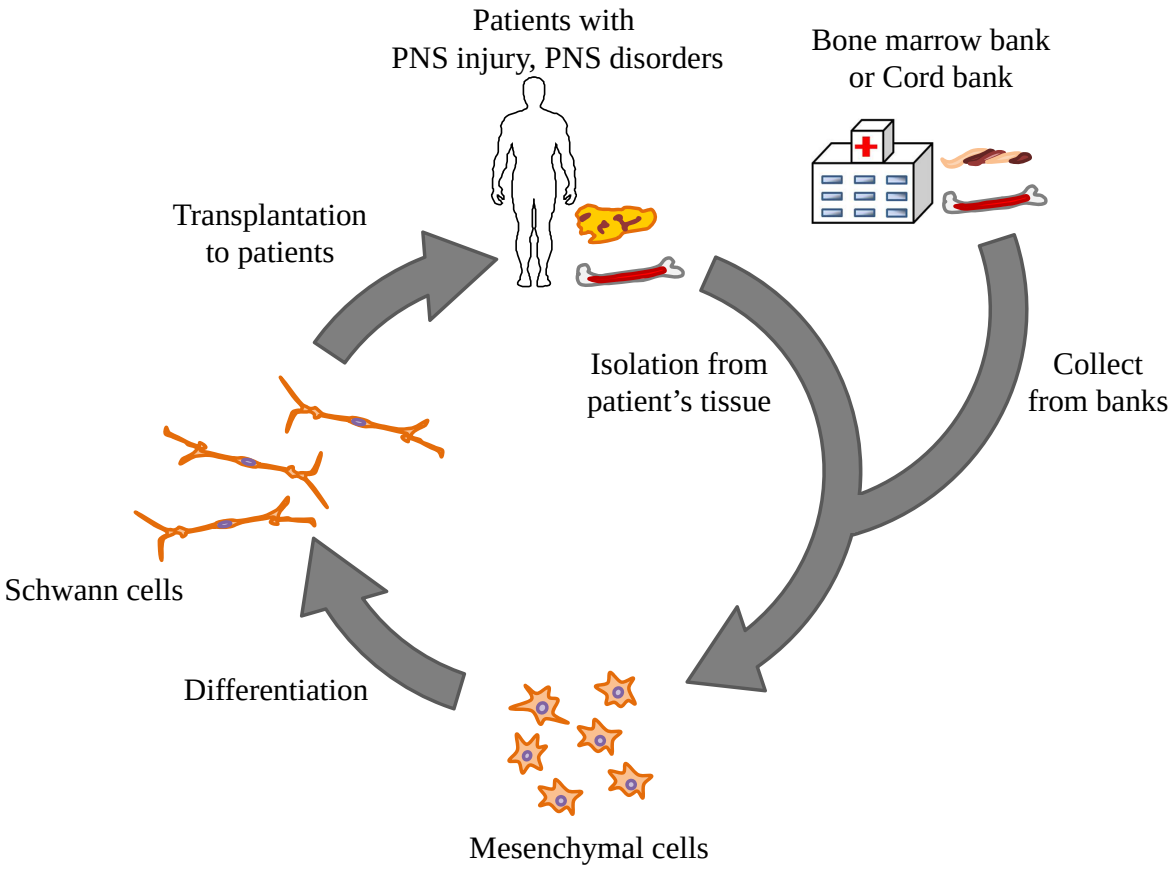


**Fig. (3).** Strategy for clinical application of Schwann cells differentiated from mesenchymal stem cells.

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