

Practical considerations concerning the use of stem cells for peripheral nerve repair

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In this review the authors intend to demonstrate the need for supplementing conventional repair of the injured nerve with alternative therapies, namely transplantation of stem or progenitor cells. Although peripheral nerves do exhibit the potential to regenerate axons and reinnervate the end organ, outcome following severe nerve injury, even after repair, remains relatively poor. This is likely because of the extensive injury zone that prevents axon outgrowth. Even if outgrowth does occur, a relatively slow growth rate of regeneration results in prolonged denervation of the distal nerve. Whereas denervated Schwann cells (SCs) are key players in the early regenerative success of peripheral nerves, protracted loss of axonal contact renders Schwann cells unreceptive for axonal regeneration. Given that denervated Schwann cells appear to become effete, one logical approach is to support the distal denervated nerve environment by replacing host cells with those derived exogenously. A number of different sources of stem/precursor cells are being explored for their potential application in the scenario of peripheral nerve injury. The most promising candidate, transplant cells are derived from easily accessible sources such as the skin, bone marrow, or adipose tissue, all of which have demonstrated the capacity to differentiate into Schwann cell–like cells. Although recent studies have shown that stem cells can act as promising and beneficial adjuncts to nerve repair, considerable optimization of these therapies will be required for their potential to be realized in a clinical setting. The authors investigate the relevance of the delivery method (both the number and differentiation state of cells) on experimental outcomes, and seek to clarify whether stem cells must survive and differentiate in the injured nerve to convey a therapeutic effect. As our laboratory uses skin-derived precursor cells (SKPCs) in various nerve injury paradigms, we relate our findings on cell fate to other published studies to demonstrate the need to quantify stem cell survival and differentiation for future studies. (DOI: 10.3171/FOC.2009.26.2.E2)

KEY WORDS • denervation • nerve injury • nerve regeneration • Schwann cell • skin-derived precursor cell

Clinical Problem: Poor Outcome From Nerve Injuries Despite Repair

INJURIES of the peripheral nerves are common and debilitating, affecting 2.8% of trauma patients⁴⁴ and resulting in considerable long-term disability.²⁷ The assumption has been that peripheral nerve injuries recover, given the observation of spontaneous axonal regeneration following insult. While this capacity for regeneration is higher than that of the central nervous system, complete recovery is fairly infrequent, misdirected, or associated with debilitating neuropathic pain.⁵⁷ In fact, satisfactory results only tend to occur following relatively minor injuries, such as neurapraxia or axonotmesis.²¹ Nerve transection is associated with notoriously poor outgrowth compared with other injuries, particularly when the distance between injury and target is long.

Abbreviations used in this paper: BMSC = bone marrow stromal cell; GFAP = glial fibrillary acidic protein; GFP = green fluorescent protein; MBP = myelin basic protein; SKPC = skin-derived precursor cell.

Poor outcome from peripheral nerve injury is especially evident when repair is performed after a temporal delay,^{31,51,56} occurring frequently in clinical practice. Due to the nature of most nerve injuries where the nerve is left in physical continuity, the propensity for spontaneous recovery is not immediately known.³⁷ As such, surgical repair is significantly delayed in a great number of cases. Even patients undergoing immediate nerve repair are subject to a lengthy denervation of the distal nerve as a result of the low rate of regeneration (~ 1 mm/day in humans⁵⁸) and the long regeneration distances required to reach the end organ.

Elongation of regenerating axons is initially supported by resident Schwann cells that undergo a phenotypic change from myelinating to growth supportive following initial denervation.^{38,52} This switch in Schwann cell phenotype is associated with up-regulation of several growth associated genes including neurotrophic factors, p75 NTR, GFAP, GAP-43, netrin-1, and key transcription factors.^{14,38,52} Proliferating Schwann cells are a rich source of neurotrophins, cell adhesion molecules, and

cytokines that support axonal regeneration and recruit further cells into the injury site.^{14,63,68} Unless axonal contact is reestablished in a timely fashion, however, this growth supportive environment is not maintained.^{49,62,72} Denervated Schwann cells progressively lose their ability to express regeneration-assisting genes^{32,74} and in effect become “turned off.”¹⁴ As the capacity of the denervated distal nerve to support axonal regeneration is highly dependent on proliferating Schwann cells within the basal lamina tubes⁷ that guide elongating axons to their denervated target,⁵⁰ this loss of vitality and functionality in distal Schwann cells directly translates to poor muscle reinnervation outcomes.¹⁵

Role of Alternative Repair Strategies

Although placement of interposed autologous nerve grafts offers a cell-rich material through which axons can regenerate, their use is not ideal because of donor site morbidity, lack of donor tissue availability, and non-specific regeneration.^{5,43} Recent advances in tissue engineering have introduced synthetic nerve guide conduits that are capable of bridging small defects in peripheral nerves (up to ~ 3 cm in humans), but their relatively inert microenvironment reduces their value for larger or more chronic injuries.^{3,71} It appears that combined approaches with cells²⁹ or trophic factors²⁸ within synthetic tubes may extend their functionality. Indeed, delivery of Schwann cells in a variety of repair paradigms has been successful in promoting regeneration and remyelination of the injured spinal cord^{48,61} and peripheral nerve.¹⁶ However, human Schwann cells must be derived from invasive nerve biopsies in sufficient numbers for regeneration and are only available after a lengthy expansion time in vitro.¹⁷ Therefore, several groups have turned their attention to identifying more accessible sources of Schwann cell-like cells for transplant therapies.

Sources of Stem Cells for Peripheral Nerve Repair

Emphasis has been placed on exploring stem or progenitor cells that are easily accessible, rapidly expandable in culture, capable of survival and integration within the host tissue, and amenable to stable transfection and expression of exogenous genes.⁴ Table 1 summarizes many of the studies to date. Embryonic neural stem cells or cell lines have been used to repair nerve injuries with demonstration of regenerative success^{2,18,42} but suffer the drawback of being somewhat difficult to obtain. On the other hand, adult stem cells have the advantage of being available from relatively noninvasive, autologous harvest methods, and are likely the most promising choice for the majority of clinical nerve injuries. Bone marrow stromal cells have attracted the attention of several groups interested in cellular strategies to supplement nerve repair.^{10,12,20,26,54,64,70,75} These mesenchymal stem cells are harvested from the long bones, and when placed in culture medium containing the appropriate cytokine cocktail,²⁶ transdifferentiate into an adherent Schwann cell-like phenotype expressing S100 protein, GFAP, and p75.^{12,64} They have been used with artificial conduits and acellular grafts, where they have contributed to improved

electrophysiological, morphometric, and/or behavioral recovery outcomes versus vehicle controls. Although their potential to produce functional myelin in vivo has been questioned,⁶⁹ others have shown that these BMSC-derived Schwann cells are at very least capable of myelinating cultured PC12 cells in vitro,²⁶ further highlighting their therapeutic potential.

More recently, even less invasive sources of stem cells have been discovered. Adipose tissue has been identified as a niche for a multipotent stem cell with a comparable phenotypic profile to the bone marrow stromal cells, and it appears to differentiate into a myelinating Schwann cell phenotype in vitro given the appropriate medium formulation known to promote transdifferentiation of BMSCs.^{30,73} Further studies will be required to assess whether they can also translate this advantage to the injured peripheral nerve.

The skin and its associated structures pose another easily accessible source of stem cells. A large population of neural crest stem cells has been found in the bulge area of hair and whisker follicles that can differentiate into neurons, smooth muscle cells, Schwann cells, and melanocytes.⁵⁵ Cells isolated from the vibrissal follicle bulge area have been used to repair a gap created in rodent peripheral nerve, where they differentiate into Schwann cell-like cells and improve recovery.¹ Similarly, when stem cells derived from skin were transplanted into artificial nerve guidance tubes bridging a 16-mm gap in rodent sciatic nerve, there was promising improvement in behavioral, electrophysiological, and morphometric parameters measured over vehicle control.³⁴ It should be noted that cells in this study were used naive and only a small proportion differentiated into Schwann cells in the in vivo environment. The skin dermis contains neural crest-related precursor cells (termed SKPCs) that can differentiate into neural crest cell types in vitro when supplied the appropriate cues, including those with characteristics of peripheral neurons and Schwann cells.^{13,36,66,67} The SKPCs respond to neuregulins in vitro to generate Schwann cells, highlighting their potential to serve as transplantable cells for nerve injury models (where neuregulins are liberated from cells within the nerve).^{9,33} The SKPCs that are Schwann cell-like in their apparent differentiation (SKPC-Schwann cell), survive and associate with axons within both normal mouse sciatic nerve and distal to crush, where they express a myelinating phenotype.³⁸ Indeed, SKPCs appear to generate functional Schwann cells as they myelinate both sensory neurons in dorsal root ganglion cocultures in vitro and dysmyelinating shiverer mouse⁴² nerve axons in vivo.³⁸

Considerations for Optimizing Stem Cell Therapy for Peripheral Nerve Repair

Number and Method of Stem Cell Delivery

Although often not reported, the number of cells delivered to nerve injuries in animal models varies considerably between studies. While some have used as few as 4×10^3 cells,² others have transplanted 2×10^7 cells,²⁰ but there has often been little explanation for the selection of

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TABLE 1: Selected studies of transplanted stem cells for peripheral nerve repair*

Cell Source/ Type	Authors & Year	Donor/ Host Animal	No. of Cells Injected	Delivery Method	Cell Sur- vival Time	% Survival	Phenotype	Regenerative Advantage Con- ferred Over Vehicle
bone mar- row aspirate/ mesenchymal stem cell	Hu et al., 2007	Rhesus monkey	2×10^7	proximal/distal side of acellular allograft	ND	ND	ND	↑ no. of NF+ axons, improved CMAP amp/latency
	Keilhoff et al., 2006 ²⁵	Wistar rat	2×10^6 / ml	devitalized muscle conduits	≥6 wks	ND	MBP+, bipolar morphol- ogy in predifferentiated cells only	↑ no. of myelinated fibers; faster return of thermosensitivity
	Chen et al., 2007	Sprague- Dawley rat	10^6 cells	in gelatin w/in lumen of silicone tube; 15-mm gap	unable to detect due to label loss	ND	express neurotrophins; not P0, PMP22	improved SFI, im- proved CMAP amp/ latency
	Dezawa et al., 2001	Wistar rat	$1-2 \times 10^7$ cells/ml	in Matrigel, w/in hol- low fibers; 15-mm gap	≥3 wks	MD	MAG+; produced myelin	↑ axonal outgrowth achieved w/ predif- ferentiated cells
	Zhang et al., 2004	Sprague- Dawley rat	10^7	microinjected into crush-injured sciatic nerve	up to 3 wks	ND	limited expression of GFAP, S100, p75	ND
	Shimizu et al., 2007	human/ Wistar rat	$1-2 \times$ 10^6 cells/ ml	in Matrigel, w/in transpermeable tube; 10-mm gap	≥3 wks	$<12.6 \pm$ 2.98% of all MAG+ SCs	MAG+; enveloped re- generating axons; many phagocytosed	slight ↑ SFI con- ferred by transdif- ferentiated cells over naive
	Tohill & Terenghi, 2004	Sprague- Dawley rat	8×10^6 cells/ml	w/in PHB conduits; 10-mm gap	up to 15 days	ND	some differentiated to S100+ SCs	↑ outgrowth
C17.2 neonatal cerebellar granule cells ± overexpression of GDNF	Heine et al., 2004	mouse cell line/ Sprague- Dawley rat	5×10^5 cells	subepineural injec- tion into chronically denervated nerve	up to 4 mos	0.5–1%	most remained distal to repair site, very few GFAP or NF+; mesen- chymal tumor	↑ no. of axons, im- proved CMAP amp/ latency
hippocampal E17 neuronal progenitor cells	Muraka- mi et al., 2003	Fischer rat	10^5 cells	in collagen gel w/ in silicone tube; 15-mm gap	up to 10 wks	ND	some cells positive for S100/p75	superior elec- trophysiological recovery
E11 DRG/ boundary cap neural crest stem cells	Aquino et al., 2006	Rosa 26 mouse (lac-Z+)/ Sprague- Dawley rat	4×10^3 cells	intact nerve; cultured in 12-mm silicone tube & implanted in nerve gap	up to 90 days; only predifferen- tiated cells survived	ND	69.7–94.6% GFAP+ after 13 & 60 days, respec- tively; MBP+ transplanted cells ensheathed axons in tube	ND
neonatal skin/ neural crestlike precursors	Marchesi et al., 2007	Wistar rat	10^6 cells	in PBS in lumen of collagen guide; 16-mm gap	up to 2 mos	25–38%	4.5% S100+, 6.1% GFAP+	improved CMAP, SFI, no. of myeli- nated fibers
	McKen- zie et al., 2006	rodent or human/ shiverer mouse	$1-4 \times$ 10^5 cells	microinjected distal to crush injury	≥6 wks	~6.5%	70.4% of transplanted cells MBP+, associated w/ NFM+ axons	MBP positive myelin on shiverer axons
vibrissal fol- licles	Amoh et al., 2005	C57/B6- GFP/C57/ B6 mouse	ND	transplanted btwn severed sciatic/ tibial nerve stumps	detected after 2 mos	ND	GFAP+; envelop βIII tubulin+ axons	improved SFI, con- traction of gastroc- nemius
amniotic fluid/ mesenchymal stem cells	Muraka- mi et al., 2003	Sprague- Dawley rat	1.5×10^4 cells	in fibrin glue around crushed sciatic nerve	up to 10 days, none at 4 wks	ND	NT-3 and CNTF+; no expression of GFAP/ S100β	motor function recovery, improved CMAP

* CMAP = compound muscle action potential; CNTF = ciliary neurotrophic factor; DRG = dorsal root ganglion; GDNF = glial cell line-derived neurotrophic factor; MAG = myelin-associated glycoprotein; ND = not described; NF = neurofilament; NFM = neurofilament (medium chain); NT-3 = neurotrophin-3; PHB = poly-3-hydroxybutyrate; P0 = myelin protein 0; PMP22 = peripheral myelin protein 22; SC = Schwann cell; SFI = sciatic functional index; + = positive; ↑ = increase in.

cell numbers in these studies. It is admittedly difficult to compare the number of cells delivered in widely different repair paradigms, but it is fair to state that there are likely an ideal number of cells that should be determined for each cell type or repair scenario. Just as too few cells may not translate to a therapeutic effect, delivery of too many cells may also have detrimental results. This was exemplified beautifully by a study using transplanted Schwann cells delivered in 10-mm nerve gap.⁴⁰ When authors used a concentration of 20×10^6 cells/ml, there was no appreciable increase in axonal regeneration distance. Increasing the concentration to 80×10^6 cells/ml proved ideal for regeneration, whereas further increases resulted in slightly poorer regeneration. This same logic likely applies to stem cell transplantation, as they must compete for space and available resources with the cellular milieu of the regenerating nerve. Therefore, optimization strategies should take the number of delivered cells into account. Similarly, the way in which cells are delivered to the injury site has varied between studies, ranging from direct microinjection,³⁶ suspension within artificial tubes,^{10,42,54} and seeding within devitalized muscle or nerve grafts.²⁵ Although the choice of stem cell delivery method may depend on the type and extent of nerve injury in question, it may be optimized by providing transplanted cells an environment that will favor their survival and integration, such as within structured fibers¹² or biomatrices.⁸

Differentiation State of Delivered Stem Cells

Part of the appeal of using precursor or stem cells for supplementing peripheral nerve repair is their capacity for self-renewal, such that it is possible to deliver large numbers of dividing cells to the injury site.⁶⁵ By delivering stem cells into the injured nerve in a naive state, this proliferative capacity is maintained, and it is expected that cells will be prompted by the microenvironment to differentiate into the required cell type.²² In vitro studies have demonstrated that neural stem/progenitor cells in coculture with cells from the nervous system will take on a phenotype similar to their partner tissue's origin: dorsal root ganglion cultures will induce a peripheral neuron/Schwann cell/smooth muscle phenotype, and a cerebellar feeder layer will induce differentiation into CNS neurons.⁶ Nevertheless, incidence of differentiation from naive precursor cells within the peripheral nerve is rather low in many cases.^{10,11,18,46} Choosing to predifferentiate stem cells toward a desired phenotype prior to delivery into the repair site may be an effective strategy to ensure a more precise and complete therapeutic effect. It may be that cells at later developmental stages (vs embryonic stem cells, for example) possess more mature intrinsic molecular programs to direct them to their target destination.⁵³

Because it is well known that mature Schwann cells survive denervation events by secreting autocrine factors such as insulin-like growth factor, neurotrophin-3, and plant-derived growth factor-BB,²³ might an appropriately differentiated stem cell also possess similar machinery for self-preservation and thus be an ideal candidate for supplementing the injured peripheral nerve? We have found that SKPCs, when injected as naive sphere-forming

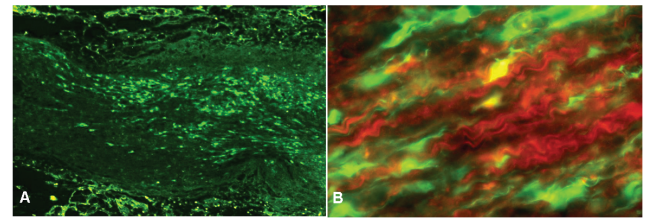


FIG. 1. Fluorescent micrographs of a longitudinal section of sciatic nerve. The SKPCs survive and differentiate within the injured peripheral nerve. Naive GFP-labeled SKPCs (green, A) injected into the transected sciatic nerve demonstrate viability after 8 weeks and integrate along host Schwann cells columns labeled by GFAP (red). The occasional double-labeled cells (yellow) on the merged image (B) are likely SKPCs expressing GFAP, suggesting their *in vivo* differentiation toward a Schwann cell phenotype. Original magnification $\times 40$ (A); $\times 400$ (B).

cells, do differentiate into GFAP-positive Schwann cells in response to cues found in the local environment of the injured peripheral nerve (Fig. 1). However, long-term survival and maintenance of Schwann cell markers is greatly improved by predifferentiating the cells to a Schwann cell phenotype prior to delivery.³⁶ On the other hand, others have reported that allowing stem cells to differentiate before delivery accelerates posttransplant cell death, perhaps owing to increased expression of major histocompatibility complex antigens or reduced proliferation rates.⁵⁹ In addition to survival of stem cells, their effect on surrounding tissues may be modified based on their level of differentiation prior to transplantation. For example, when naive adult neural stem cells were injected into a lesioned spinal cord, the resulting aberrant sprouting resulted in profound allodynia. If gliogenesis in these cells was suppressed by prior treatment with neurogenin-2, there was an overall greater functional improvement.¹⁹ One of the potentially negative consequences of stem cell therapy in any system is the tumorigenic capability of multipotent precursors. Indeed, when the regenerative potential of C17.2 neural stem cells was assessed in 3 different rat sciatic nerve injury models, there was a high incidence of tumor formation by the transplanted cells.^{18,24}

Improving Survival of Transplanted Stem Cells

Whether due to technical challenges or oversight, it is an unfortunate reality that survival of stem cells delivered to nerve injury sites is reported only infrequently. When quantified, precursor cells have shown between 0.5 and 38% survival, depending on evaluation time point and cell type.^{18,34,36,54} In our laboratory, we have also seen differences in survival based on nerve injury model and differentiation state of the cells at transplantation (unpublished observations). For example, when we delivered naive SKPCs into an acutely injured nerve, survival after 2 weeks was $\sim 10.5\%$, whereas when delivered into a nerve that had been previously chronically denervated, the number of detected SKPCs decreased to 5.8%. Seeing that 78% of the surviving stem cells in the chronic model had differentiated into GFAP-positive Schwann cells, we next used predifferentiated (Schwann cell-like) SKPCs and found that we could increase survival to $\geq 8\%$. Without quantification of survival in stem cell transplantation

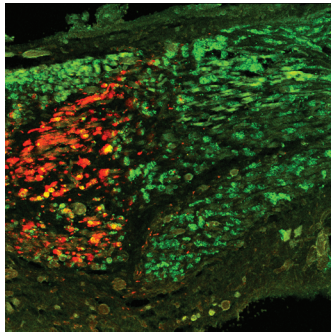


FIG. 2. Confocal image. The SKPC-Schwann cells are not immediately cleared by host immune system. Eight weeks following injection into an initially decellularized (by repetitive freeze-thawing) nerve graft bridging a 12-mm defect created in the rodent sciatic nerve, SKPC-Schwann cells (red), and ED-1-positive macrophages (green) are spatially segregated within the longitudinal extent of the nerve graft. The finding that there is very little colabeling of SKPC-Schwann cells with ED-1 positive macrophages suggests that transplanted cells are not phagocytosed in any large quantity within the host nerve. Original magnification $\times 400$.

experiments, it is difficult to determine whether they are being retained long enough and in enough numbers to confer a sufficient benefit to regeneration. The danger of exogenous cell therapy is of course cell death caused by immune system attack.⁵⁹ Although some authors have reported considerable phagocytosis of transplanted stem cells,^{54,59} this may be due to species/strain mismatching of donor and recipient, as many others have not observed this trend. In fact, we have observed a highly interesting pattern of surviving transplanted SKPCs that are spatially separate from phagocytic ED-1-positive macrophages (Fig. 2). The question of survival is mechanistically interesting, as improvement in regeneration outcomes has been also observed in the absence of detection of transplanted cells.⁴⁶

If a minimum survival time of stem cells is indeed required to observe a therapeutic effect, strategies should be devised to increase the amount of time cells remain in grafted regions. Survival and effectiveness of transplanted cells can be improved by *ex vivo* genetic manipulation or concomitant delivery of protective agents or trophic factors. Pan and colleagues⁴⁵ found that administration of granulocyte-colony stimulating factor to animals receiving transplants of amniotic fluid mesenchymal stem cells not only improved survival of transplanted cells but also augmented nerve regeneration over that of a primarily cell-based approach. Additionally, differences in the material in which stem cells are delivered have demonstrated varying capacities to support long-term cell survival.^{8,47} Finally, immunosuppressive regimens, especially in the light of interspecific transplants may protect stem cells from premature clearance from the nerve injury site.⁴⁷

Influence of Final Stem Cell Phenotype on Regenerative Success

As with survival, differentiation of stem cells within the injury site has demonstrated a mixed correlation to therapeutic effect. Some studies have demonstrated a need for differentiation to glial phenotype to observe adequate

regeneration of neural tissue, and others have shown improvement with little to no differentiation of stem cells at the assessment end point.⁶⁰ Furthermore, the glial differentiation of transplanted stem cells within the injured peripheral nerve has tended to vary between studies, even within the same cell type. Keilhoff and colleagues²⁶ could not detect Schwann cell differentiation of transplanted marrow stromal cells if delivered in a naive state, whereas Zhang et al.⁷⁵ observed at least limited expression of S100, p75, and GFAP markers in similarly obtained cells. In this case, the difference in repair paradigms (devitalized muscle graft versus crush injured nerve) may explain the disparity in the ability of these cells to differentiate, outlining the need for careful consideration of the method of delivering stem cells to the injury site. In the cases in which adequate regeneration and improvement of outcomes occurs without Schwann cell differentiation of transplanted precursors, it may be that the cells are supporting axonal growth by additional mechanisms such as the production of cytokines or harnessing the inflammatory response.⁴² Although C17.2 neural stem cells show little differentiation into a Schwann cell phenotype in the chronically denervated peripheral nerve, their secretion of various matrix metalloproteinases, capable of breaking down growth-inhibiting chondroitin sulfate proteoglycans, likely underlies their ability to elicit superior regeneration.¹⁸ Similarly, unpublished observations from our laboratory have shown that impure cultures of SKPCs at an early stage of Schwann cell differentiation secrete detectable levels of a number of neurotrophins despite lacking typical Schwann cell morphology or histological markers. Therefore the following question remains: must stem cells fully adopt a stereotypical Schwann cell phenotype to be successful adjuncts to nerve repair? Careful examination of ultimate cell fate with correlation to functional outcome is strongly recommended for future precursor transplant studies and will be required to fully answer this question for each cell type and repair strategy. It may be that, at least for some precursor cell types, there is a minimum level of differentiation to S100 β /MBP/GFAP-positive Schwann cells that is required for acceptable regeneration outcomes. If this is the case, effectiveness of precursor transplantation could be improved using technology that exists to directly alter the regenerative microenvironment by continuous delivery of neuregulins, forskolin, or other differentiation-promoting factors.³⁵

Methods for Tracking Fate of Transplanted Stem Cells

Given the evidence presented above, it is apparent that studies exploring stem cell transplantation for peripheral nerve repair should give careful thought on strategies to track the fate of transplanted cells over time. There is often little importance placed on prelabeling cells prior to delivery into the injured nerve, and as such authors cannot comment on the mechanism of any advantage conferred by cell therapy. Others have used labeling techniques that are not sufficiently robust or long-lasting to be detected at the study end points.¹⁰ Chemical markers such as bisbenzimidazole and PKH26 have been used to label Schwann cells

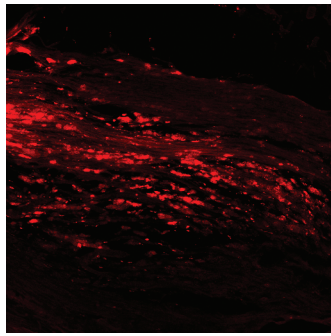


FIG. 3. Confocal image. The CM-Dil is reliable method for long-term cell fate tracking within the peripheral nerve. The SKPC-Schwann cells prelabeled for 20 minutes in 2 μ M CM-Dil retain bright labeling (red) for ≥ 8 weeks following transplantation into an acellular nerve graft bridging a gap in the rodent sciatic nerve, allowing for quantification without using additional dye techniques. Original magnification $\times 200$.

delivered to peripheral nerve injuries, but their usefulness is limited to the short term and may in fact affect the viability and phenotype of transplanted cells.³⁹ Genetic labeling with either *lacZ* or fluorescent proteins such as GFP is increasingly popular and appears to be a relatively long-lasting method that is not deleterious to transduced cells.^{12,40} We have used the lipophilic carbocyanine derivative CellTracker CM-Dil (Molecular Probes) to reliably label SKPCs within a variety of nerve injury models with no dilution or loss of signal for ≥ 10 weeks following transplantation (Fig. 3). These dyes have the advantage of being technically simple to use, rapid, and resistant to leakage to nearby cells. Emerging technologies such as quantum dots offer an exciting alternative to traditional cell labeling methods. These nanoparticles are available in a wide range of photostable colors and are resistant to chemical and metabolic degradation, making them ideal for use in long-term fate tracking of transplanted stem cells.⁴¹

Conclusions

Animal studies have demonstrated that transplantation of stem and precursor cells has the potential to serve as an adjunct therapy to common practices of surgical nerve repair. Although the application of cell-based strategies in a clinical setting is promising, optimization of cell delivery and careful investigation of the fate of transplanted cells is required to guarantee the safety and maximum efficacy of these therapies. As discussed in this review, it will be important to determine the ideal number and method of cell delivery, and elucidate the extent of transplant cell survival and differentiation that is required to elicit a therapeutic effect. Future studies should place emphasis on using reliable labeling methods to track the long-term fate of transplanted cell. Finally, while many cell types have been investigated for their potential use in cell replacement therapy, few studies have directly compared the utility of different stem cells in augmenting peripheral nerve repair. We believe that cells that are easily isolated from autologous sources such as the skin and that

can survive and differentiate to a glial phenotype within the milieu of the injured nerve provide the most promise.

Disclosure

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