Prospects for Improving the Extent of Recovery following Peripheral Nerve Trauma: A Review

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Restoring function to damaged peripheral nerves with a gap remains challenging, with <50% of patients who undergo nerve repair surgery recovering function. Further, despite enormous efforts to improve existing techniques and develop new ones, the percentage of patients who recover function and their extent of recovery has not increased in almost 70 years. Thus, although sensory nerve grafts remain the clinical "gold standard" technique for attempting to restore function to nerves with a gap, they have significant limitations. They are effective in restoring good to excellent function only for gaps <3-5 cm, repairs performed <3-5 months post-trauma, and patients <20-25 years old. As the value of any of these variables increases, the extent of recovery decreases precipitously, and if the values of two or all three variables increase, there is little to no recovery. Therefore, novel techniques are required that increase the percentage of patients who recover function and the extent of their recovery. This review discusses the limitations of sensory nerve grafts and other techniques currently being used to repair nerves. It also discusses the use of autologous platelet-rich plasma (PRP), which appears to be the most promising technique for inducing sensory and motor recovery even when the values of all three variables are significantly greater than when sensory nerve grafts alone are not effective. Thus, there is finally the promise that patients who presently have limited to no chance of any recovery may recover good to excellent sensory and motor function. [*P R Health Sci J 2022;41(2):89-95*]

Key words: Nerve regeneration, Nerve trauma, Peripheral nerve repair, Platelet-rich fibrin, Platelet-rich plasma, Restoration of function

Sensory nerve autografts are the clinical "gold standard" for bridging nerve gaps in attempts to restore function (1). However, the extent of neurological recovery they induce is negatively influenced by increasing gap length, delay between nerve injury and repair, and patient age (2). Thus, sensory nerve grafts reliably induce good to excellent recovery only across relatively short nerve gaps (<3-5 cm) (1), when repairs are performed relatively soon after nerve trauma (<3-5 months) (3), patients are relatively young (<20-25 years old) (4), and it requires sacrificing a sensory nerve function. As the values of any of these variables (gap length (5), delay (6), and patient age (4)) increase, the extent of recovery decreases precipitously. Further, as the values of any two or all three variables increase, there is generally limited to no recovery (7). Consequently, most patients are not offered nerve repair surgery, and of those who are, less than 50% recover any sensory or motor function (7). This rate has not improved in almost 70 years (8). Therefore, new techniques are required that induce more extensive recoveries in a larger percentage of patients under all conditions.

This review examines the factors that underlie the limitations of sensory nerve grafts in promoting axon regeneration. It also examines techniques presently being applied clinically to overcome each of these limitations. It next examines studies showing that the application of autologous platelet-rich plasma (PRP) enhances the extent of axon regeneration. It then discusses two novel nerve gap repair techniques showing that a unique application of PRP to nerve gaps induces axon regeneration and good to excellent recovery even when the values of two or all three variables that limit recovery were simultaneously large, conditions where no other existing techniques are effective. Finally, it discusses the potential mechanisms by which PRP acts. It concludes by showing that function can be restored without sacrificing a sensory nerve function.

The following section addresses how each of the limitations mentioned above can be minimized, leading to improved axon regeneration and functional recovery.

I. Limitations to axon regeneration and functional recovery

One factor underlying the decreasing extent of recovery with increasing delay between nerve trauma and repair time is that

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axotomized neurons lose their capacity to extend axons (9). This is, in part, considered to result from neurons downregulating their expression of neuregulin 1 (10). There is no method for clinically up-regulating the expression of neuregulin-1 to enhance axon regeneration. However, clinically, the capacity of axotomized neurons to regenerate is restored by applying as little as one hour of electrical stimulation (11). Electrical stimulation promotes axon regeneration by increasing neuronal cAMP levels (12), causing the upregulation of BDNF, and its trkB receptor, RAGs, GAP-43, cytoskeletal proteins actin, tubulin (13, 14), and neuregulin 1 (15), while increasing local blood flow (16), induces IGF-1 synthesis (17), macrophage recruitment and activation (18, 19). An additional technique for increasing the capacity of axotomized neurons to regenerate is by applying as few as one neurotrophic factor (20), although the speed of axon regeneration is increased when multiple neurotrophic factors act together. (21).

Also limiting the extent of axon regeneration across long nerve gaps bridged with a sensory nerve graft and when nerve repairs are performed with a long delay between nerve trauma and nerve repair is Schwan cell changes. Thus, when Schwann cells lose contact with an axon, they become senescent and stop releasing neurotrophic factors which are required to promote axon regeneration (5). Similar to how the application of neurotrophic factors to axotomized neurons induces them to regenerate, those same factors reverse Schwan cell senescence and promote their up-regulation of the synthesis and release of neurotrophic factors (20). Schwann cell senescence can be reversed by a single brief period of electrical stimulation (22).

Clinically, a graft must be vascularized for axons to regenerate across gaps of >6 cm (23). However, because the vascularization of long nerve grafts is slow, by the time the regenerating axons reach the distal part of the graft, it is not vascularized and cannot support axon regeneration (6). The lack of vascularization causes the grafts to develop a necrotic environment, which inhibits axon regeneration. However, in animal models, the extent of graft vascularization, axon regeneration, and functional recovery across long nerve grafts are all significantly increased by the use of vascularized nerve grafts (24). Further, it is increased by applying vascular endothelial growth factor (VEGF) to nerve grafts, when cells within a nerve graft are induced to overexpress VEGF (25) or when VEGF is added to acellular or laminin-gel grafts (26).

Another variable contributing to the decreased capacity of axons to regenerate across nerve gaps is increasing patient's age. This is because increasing age is associated with decreased nerve injury-induced angiogenesis (27). This results in nerve grafts failing to become vascularized (28). However, in aged rats (29) and clinically (23), graft vascularization can be induced by administering VEGF, leading to extensive axon regeneration (30).

The following section examines techniques other than sensory nerve grafts currently used in animal models and clinically to restore function across nerve gaps.

II. Alternative techniques for promoting axon regeneration across nerve gaps

Allografts

A significant limitation of using sensory nerve grafts to promote functional recovery is that their use requires creating a permanent sensory deficit. This drawback led to the testing of acellular allografts (donor lengths of nerve after removing all cells and antigenicity) to avoid the need to sacrifice a sensory nerve function. Although allografts are FDA-approved and used clinically, but far less frequently than sensory nerve grafts. This is because their efficacy decreases with increasing gap length (31), being effective only for gaps ≤7 cm in length (32). In addition, they often fail to promote axon regeneration across gaps of <2 cm in length (personal clinical observations), and they are not effective if the values of two or all three variables are simultaneously large. Therefore, allografts are not recommended or FDA-approved for use in bridging "long" nerve gaps, which are considered >3 cm (5).

The limitation of acellular allografts in promoting axon regeneration and functional recovery across long nerve gaps can be reduced by: (1) by infusing them with neurotrophic factors (33), (2) autologous Schwann cells (34), and other cell types (35), or (3) filling them with autologous platelet-rich plasma (PRP) (36). A drawback in using PRP is its lack of FDA approval for this clinical use, although PRP can be used off-label. Despite these approaches improving the efficacy of allografts in promoting axon regeneration across short nerve gaps, these techniques remain less effective than autografts for long gaps (37).

Conduits

An alternative approach for bridging nerve gaps in animal models and clinically is the use of conduits composed of various materials, including fibrin (38), decellularized human umbilical artery (39), muscles (40), veins (41), and FDA-approved collagen tubes (42). Their efficacy in promoting axon regeneration is increased by filling them with muscle (43), minced peripheral nerve (44), or neurotrophic and other factors (45). However, although empty conduits can be used clinically, most of the materials used to fill them to enhance axon regeneration cannot be used clinically. Thus, clinically, empty conduits are generally not used except for what is referred to as non-critical sensory nerve repairs when the gaps are <2 cm (46).

The following section discusses evidence showing that the application of PRP enhances the extent of axon regeneration.

III. PRP

Platelets evolved as a source of over 300 different compounds (47). Their factors serve a multiplicity of functions, including promoting axon regeneration (48), hemostasis (49), wound healing (50), vascularization (51), acting as an anti-bacterial agent (52), and reducing inflammatory disorders such as sepsis and other infections (53).

Due to the host of factors contained in and released by platelets, PRP has been tested in various animal models and clinically for its efficacy in promoting axon regeneration. PRP enhances the extent of axon regeneration when clinically injected onto (54) or into nerves (55). In animal models, the extent of axon regeneration is increased by applying PRP to the site of a nerve crush (56), nerve stump anastomosis site (57), and by filling autografts (58), acellular nerve graft (36), and conduits (59) with PRP. Thus, extensive evidence shows that PRP is effective in promoting axon regeneration.

Although the application of PRP improves the extent of axon regeneration, there have been few studies examining whether it can enhance the capacity of sensory nerve grafts to promote axon regeneration or can satisfactorily induce good axon regeneration across nerve gaps without a sensory nerve graft. The following section examines two novel techniques testing this capacity of PRP.

IV. Novel PRP technique promoting extensive axon regeneration and recovery

Recent work shows that, clinically, two techniques involving unique applications of PRP are the most effective techniques for restoring function to nerves with gaps. The first involves bridging a nerve gap with a sensory nerve graft within a PRP-filled collagen tube (60). This induced excellent axon regeneration and recovery across one 9 cm and two 11 cm gaps, even when the repair was performed 2.7 years post-trauma in a and 51 years old patient (60). The second technique involved bridging a gap with only a PRP-filled collagen tube (61). This technique was effective despite the repair of a 12 cm long gap, 3.25 years post nerve trauma in a 48-year-old patient (61). Thus, recovery was restored even though the values of all three variables that typically restrict or prevent axon regeneration and recovery were singly and simultaneously large.

These studies show that platelets contain all the factors required to induce significant axon regeneration and recovery across nerve gaps even when the values of two or all three variables are simultaneously larger than when sensory nerve grafts alone are effective. Further, the recovery when using only a PRP-filled collagen tube shows that significant axon regeneration and functional recovery develop without the need to sacrifice a sensory nerve graft. Further studies are required to determine which techniques induce the most reliable and effective recovery.

It is hypothesized that, as indicated earlier in this review, PRP enhances the extent of axon regeneration is releasing neurotrophic and other factors that act directly on the axotomized neurons. Simultaneously, the same or other factors act on the senescent Schwann cells of the nerve graft and distal portion of the nerve to reactivate them, leading to their synthesis and release of regeneration-promoting neurotrophic and other factors (62).

The following section examines platelet-released factors that may participate in inducing extensive axon regeneration and their mechanisms of action.

V. Potential mechanisms of PRP promoting axon regeneration and recovery

Platelet-released VEGF may play a significant role in allowing axons to regenerate across all gaps, but especially for long nerve gaps, gaps in older patients, and long gaps in older patients. VEGF can act by inducing vascularization of the PRP/graft or the PRP alone within a gap (23, 63). However, because platelets release both VEGF and IGF-1, the combination may promote more extensive angiogenesis, axon regeneration, and functional recovery than is exerted by either alone (64).

Other potential roles VEGF can play to promote axon regeneration include being a chemoattractant for macrophages (65). This serves two purposes, first recruiting macrophages that can phagocytose the axon and myelin debris in the nerve graft, which, if not removed, inhibits axon regeneration (66, 67). Second, recruited macrophages release VEGF (65, 68), which induces further vascularization (65, 67, 69). The macrophage-released VEGF induces the development of new blood vessels, which are critical for vascularization and direct Schwann cell migration into the nerve gap site (65). VEGF also acts as a Schwann cell chemoattractant while stimulating their proliferation (5). Without Schwann cell recruitment, there are no Schwann cell-released regeneration-promoting neurotrophic factors (5, 70). Finally, VEGF acts as a neuroprotective agent by inducing neuron upregulation of NGF and GDNF, which further enhances axon regeneration (64).

Platelet releases many other factors that may also enhance axon regeneration. PDGF induces axon outgrowth across nerve gaps bridged with conduits (71). It is also a potent mitogen for Schwann cells (72, 73), which induces them to up-regulate their synthesis and release of PDGF (74), while promoting the synthesis of the extracellular matrix (75). PDGF also induces angiogenesis (76, 77), which, as just discussed, is essential for the invasion of Schwann cells to the nerve injury site and their action in inducing graft vascularization, which creates a cellular environment that supports axon regeneration.

Platelet-released IGF-1 applied to nerve crush sites induces axon regeneration (78) while promoting Schwann cell motility proliferation and differentiation (73), promotes actin

cytoskeletal remodeling (79), induces angiogenesis (78), promote the synthesis of the extracellular matrix (75), and stimulates neuron protein synthesis (80). Finally, IGF-1 enhances axon regeneration in aged animals (81).

Platelet-released IL-10 promotes axon regeneration by creating an anti-inflammatory environment, partly by inducing macrophages to transition to their M2 anti-inflammatory phenotype (82-84). This results in suppressing macrophage release of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1 (85, 86), while inducing their release of anti-inflammatory cytokines, such as IL-10 (84, 87, 88).

Platelet-released TNF-α induces NGF expression (89). NGF enhances axon regeneration (90).

Although platelet-released TGF-β1 may initially reduce axon regeneration by stimulating macrophage invasion and releasing

pro-inflammatory mediators (93), longer TGF-β1 exposure suppresses inflammation (86, 93-96), allowing for increased axon regeneration. TGF-β1 also promotes axon regeneration by inducing angiogenesis and extracellular matrix synthesis (75, 91). It also reactivates long-term denervated Schwann cells and induces their migration into nerve injury sites where they release axon regeneration-promoting factors (92-94).

Platelet-released FGF-1 promotes cell proliferation, angiogenesis, differentiation, cell migration (95) and enhances axon regeneration and functional recovery when applied to nerve neurorrhaphy sites (96) and added to conduits bridging nerve gaps (97). It acts, in part, by promoting Schwann cells proliferation (98).

The role of platelet-released BDNF is shown by it enhancing the extent of axon regeneration when applied to injured nerve neurorrhaphy sites (99), within conduits (100, 101), and when injected into injured nerves (102). BDNF applied to autographs increases the number of regenerating sensory and motor axons by up to 4-fold (25, 103, 104) and induces long-term axotomized neurons to extend axons (20). BENF also enhances Schwann cell proliferation (25, 99) and is even effective when applied following long-term axotomy (99).

Conclusions

While sensory nerve grafts remain the clinical "gold standard" for bridging nerve gaps to promote axon regeneration and recovery, their limitations are so significant that <50% of patients recover good to excellent sensory and motor function. Although various techniques increase the efficacy of sensory nerve grafts, allografts, and conduits in promoting axon regeneration, that is insufficient for restoring function to most clinical nerve injuries. However, two novel techniques involving bridging a nerve gap with a sensory nerve graft within a PRP-filled collagen tube or only with a PRP-filled collagen tube induce good axon regeneration and recovery, even when the values of two or all three variables that restrict axon regeneration are simultaneously large. In addition, one of these techniques shows that function can be restored without sacrificing a sensory nerve function. Further testing of these techniques should lead to the development of an off-the-shelf product that can be cut to the appropriate length of a nerve gap to be repaired and secured in place leading to the reliable recovery of good to excellent sensory and motor function under conditions where it is presently impossible.

Resumen

La restauración de funcionalidad en nervios periféricos luego de un trauma continúa siendo un gran reto, con < 50% recuperando funcionalidad después de un procedimiento reconstructivo. A pesar de los enormes esfuerzos para crear y mejorar técnicas existentes, el porcentaje de pacientes que recuperan su funcionalidad no ha aumentado significativamente en casi 70 años. Aunque actualmente el uso de injerto de nervios sensoriales es el método de elección para intentar restaurar nervios traumatizados con una separación, este procedimiento sufre de muchas limitaciones. Esta técnica ha sido efectiva en restaurar función buena a excelente en separaciones de nervios de <3-5 cm, reparaciones completadas antes de < 3- 5 meses después del accidente y en pacientes entre 20 a 25 años de edad. El grado de recuperación puede disminuir precipitadamente a medida que el valor de alguna de estas variables aumente, y habría poca o ninguna recuperación si dos o más variables aumenta. Es por esto que urge la necesidad de estudiar nuevas técnicas que puedan aumentar el porcentaje de funcionalidad y el grado de recuperación en esta población. El objetivo de esta revisión es analizar las limitaciones de los injertos de nervios sensoriales conjunto a otras técnicas quirúrgicas que se utilizan actualmente para reparar nervios periféricos con separación. En adición, se discutirá el uso de plasma autólogo rico en plaquetas (PRP), el cual actualmente se ha descrito como la técnica más prometedora en inducir la recuperación sensorial y motora en pacientes que requieran un injerto de nervio sensorial por lesiones periféricas, aun cuando las variables antes descritas estén aumentadas. Por lo tanto, finalmente hay un procedimiento que promete devolverle al paciente funcionalidad sensorial y motora de buena a excelente en lesiones de nervios periféricos con separación que anteriormente no tenían oportunidad de recuperar.

List of abbreviations

PRP	platelet-rich plasma
VEGF	vascular endothelial growth factor
FDA	Food and Drug Administration
BDNF	brain-derived neurotrophic factor
trkB receptor	tyrosine B receptor
RAGs	regeneration-associated genes
GAP-43	growth-associated protein-43
IGF-1	insulin-like growth factor-1
cAMP	cyclic adenosine monophosphate

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