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Limitations to clinically restoring meaningful peripheral nerve function across gaps and overcoming them

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Abstract

Clinically, reliably restoring meaningful peripheral sensory and motor nerve function across peripheral nerve gaps is limited. Thus, although autografts are the clinical “gold standard” repair technique for bridging nerve gaps, even under relatively good conditions, <50% of patients recover meaningful function. Due to this low recovery rate, many patients are not even provided repair surgery and, consequently, suffer permanent loss of function. This paper examines intrinsic and extrinsic changes associated with injured neurons and Schwann cells that reduce the extent of axon regeneration and recovery. It also examines how these changes can be reversed, leading to enhanced regeneration and recovery. It next examines the efficacy of platelet-rich plasma (PRP) in promoting axon regeneration and two novel techniques involving bridging nerve gaps with an autograft within a platelet-rich (PRP) collagen tube or only a PRP-filled collagen tube, which induce meaningful recovery under conditions where autografts alone are not effective. Finally, it looks at potential mechanisms by which platelet-released factors may enhance axon regeneration and recovery. This review shows that although there are many limitations to restoring meaningful function following peripheral nerve trauma, there are a number of ways these can be overcome. Presently, the most promising techniques involve using PRP.

KEYWORDS

allograft, axon regeneration, collagen tube, nerve gap, nerve trauma, peripheral nerve repair, platelet-rich fibrin, PRP

Impact statement

Restoring clinical function following peripheral nerve trauma is restricted by neuron and Schwann cell intrinsic and extrinsic limitations. Further, autografts, the current clinical “gold stand” technique for bridging nerve gaps to restore function, suffer many significant limitations in restoring meaningful functional recovery. This review discusses intrinsic and extrinsic limitations to regeneration and how they can be overcome. It also discusses how the application of platelet-rich plasma (PRP) promotes axon regeneration

and how its influences can be increased or decreased. It then discusses how, clinically, bridging nerve gaps with autograft within a PRP-filled collagen tube induces axon regeneration and recovery under currently impossible conditions. It concludes with a discussion of the potential mechanisms by which platelet-released factors may exert their influences. Understanding what limits axon regeneration and recovery and how these limitations can be overcome will lead to developing new clinical techniques that induce more extensive axon regeneration and recovery.

Introduction

Sensory nerve autografts, the clinical “gold standard” technique for restoring function across peripheral nerve gaps [1], have substantial limitations. Therefore, there is a good prognosis for reliable, meaningful sensory and motor function only when (1) the repairs are performed ≤ 5 months post-trauma [2–4], with recovery decreasing with longer delays [3–6] (2) the gaps are < 5 (cm) [7, 8], with recovery decreasing for longer gaps [2, 3, 8, 9], few axons regenerate across grafts ≥ 8 cm in length [2, 10, 11], and none across autografts > 10 cm [3, 5], and (3) patients are ≤ 20 –25 years old, with recovery decreasing with increasing ages [3, 4, 6]. Finally, there is little to no recovery when the values of two or all three variables increase simultaneously [9, 12]. Therefore, $< 50\%$ of subjects recover meaningful sensory or motor functions [13]. These findings raise the question of what underlies these limitations and how can they be reduced, leading to improved recovery.

Injury-induced intrinsic neuronal changes reduce their capacity to extend axons

Partly underlying the decreased capacity of aged and long-term axotomized neurons to extend axons are changes in their intrinsic properties [14]. These neurons lose their capacity to extend axons, and those extended regenerate only short distances [15, 16] while regenerating more slowly than normal [17]. Thus, by > 4 months post-nerve injury, only about 33% of neurons can extend an axon [15, 18], and for those that retain the capacity, it is reduced to $\sim 10\%$ of normal [16].

Reduced protein synthesis

The c-Jun transcription factor is critical for neurons' capacity to extend axons, and nerve injury induces neuronal up-regulation of c-Jun expression. However, with increasing time of axotomy, c-Jun expression decreases, paralleling the loss of neurons' capacity to extend axons [19]. This change is also associated with the down-regulation of genes for regeneration-

promoting neurotrophic factors, such as GAP-43 and $\alpha 1$ -tubulin [20], NGF [21], BDNF, and CNTF [16, 22]. Thus, the age-associated decrease in axon regeneration is due to reduced protein synthesis, which is required to induce the neurons' soma to respond to injury by triggering the regeneration process and growth cone extension [23–25]. This process also involves decreased levels of axonal translation proteins and the inability of neurons to increase the translation of regeneration-promoting axonal mRNAs released from stress granules [26]. The decrease is also associated with an increasing age-associated decrease in neurofilament mRNA levels and neurofilament proteins [27], and the loss of Nrg1, which reduces axon-Schwann cell interactions and remyelination after nerve crush, further reducing neurons' capacity to extend axons [28].

Decreased metabolism and axoplasmic transport

Neurite outgrowth from neonatal neurons *in vitro* is 40% faster than adult neurons [29]. This is attributed to an age-related decrease in cytoskeletal protein expression [30] and axoplasmic transport, which are required for axon elongation [30, 31]. This is because axon regeneration requires energy metabolism, which involves oxidative glycolysis and the formation of high-energy phosphate compounds, most importantly creatine phosphate and ATP [32]. Increasing age is also associated with a decrease in the levels of endoneurial ATP and creatine phosphate [30], which would, therefore, restrict the extent of axon regeneration.

Reversing injury-induced intrinsic neuronal changes allows neurons to extend axons by promoting neuron protein synthesis

Axon regeneration following a sciatic nerve crush is promoted by enhanced protein synthesis due to enhanced local translation and production of the protein synthesis machinery [26]. This involves dissolving stress granules, resulting in their releasing sequestered mRNAs and translation factors [33]. Further, following rat sciatic nerve injury, Nrg1 treatment increases axon diameter, myelin thickness, distance axons regenerate, and both the extent [34] and rate of recovery [35]. These effects are partly induced by neuron-released Nrg1 promoting Schwann cell differentiation, proliferation, migration, and myelination [28, 36–41].

Electrical stimulation

As mentioned, long-term axotomy results in 33% of neurons losing their capacity to extend axons. However, electrical stimulation results in a 34%–50% increase in the number of

neurons extending axons [42] and a 2.3-fold increase in the extent of axon sprouting from transected axons [43] while also increasing the speed of axon regeneration [17, 42]. This influence is exerted through various mechanisms, including direct actions on axotomized neurons [17, 44–47]. The influence of electrical stimulation is similar when applied to acute and long-term injured neurons [46, 48].

Injury-induced extrinsic neuronal changes reduce their capacity to extend axons

Reduced Schwann cell capacity to support neuron

Schwann cells release the cytokines MCP-1 and LIF [49], which recruit macrophages and convert them from the M1 to the M2 phenotype. These macrophages secrete high levels of cytokines, which promote axonal outgrowth [50]. However, nerve injury deprives Schwann cells of axon contact, causing them to become senescent and stop producing and releasing neurotrophic factors. Schwann cell development of senescence parallels the decrease in the extent of axon regeneration [8]. Thus, long autografts do not induce axon regeneration and recovery because by the time the axons reach the distal end of the autograft, the Schwann cells have become senescent and do not support axon regeneration [8].

Schwann cell senescence is also associated with a reduction in their c-Jun expression [51], loss of their injury-induced repair phenotype [8, 22, 38, 52], and their down-regulation of the genes for factors required for Schwann cells to support axon regeneration and proteins required to myelinate axons [30]. These include S100, p75, GFAP, BDNF, NGF, NT-3, NT-4, CNTF, GDNF, and small molecule trkB agonists.

Schwann cell senescence also leads to their inability to synthesize and release VEGF [53]. VEGF is essential for inducing vascularization and recruiting macrophages [54, 55] to the injury site, where the macrophage normally also releases VEGF [55, 56]. In addition, senescent Schwann cells lose their capacity to phagocytize axon and myelin debris [57], and without its removal, it inhibits axon regeneration [30]. Therefore, maximizing functional recovery requires nerve repairs be performed <3–6 months post-trauma [3, 9, 58].

Reversing injury-induced extrinsic neuronal changes by reactivating Schwann cells: applying neurotrophic factors and restoring c-Jun

Nerve injury induces Schwann cell up-regulation of Shh, which induces c-Jun expression [59–61], which leads to c-Jun

enhancing axon regeneration through autografts and *in vitro* [62]. However, with prolonged denervation and aging, c-Jun expression decreases in Schwann cells, which is associated with decreased axon regeneration [51]. Nevertheless, axon regeneration can be promoted by reactivating senescent Schwann cells by applying neurotrophic factors, which restores normal levels of Shh and c-Jun expression [63].

Reactivating Schwann cells: applying electrical stimulation

Electrical stimulation reactivates Senescent Schwann cells. This induces their expression of P0, Par-3, BDNF, NGF, and GDNF, which initiate and enhance axon regeneration and myelination [64–66].

PRP promotes axon regeneration

Platelet-released factors

Platelets contain and release an evolutionarily complex cocktail of factors, including high levels of neurotrophic and other growth factors, such as IL-10, insulin-like growth factors 1 and 2, VEGF [67], BDNF [68], transforming growth factor- β 1, HGF, and FGF. This allows platelets to play different essential roles in tissue healing and promoting axon regeneration [69–72].

In animal model studies, PRP significantly enhances the extent of axon regeneration when injected into a nerve following a nerve crush [73], is applied to sites of a nerve crush [74–78], neurorrhaphy [69, 79–82], site of rat prostatectomy [83], following nerve crush, *mycobacterium leprae* (leprosy bacteria) -induced lesion [84], sucrose-induced injury [85], autografts [86, 87], acellular allografts [88], when applied onto or injected into neurorrhaphy sites [89–92], is injected onto injured nerves [89, 93, 94], or short nerve gaps within the preserved epineurium [95], PRP exosomes are injected under the perineurium [96], and site of carpal tunnel syndrome [97–99]. However, questions have been raised about the efficacy of PRP in treating carpal tunnel syndrome [100].

PRP is similarly effective when added to vein grafts [67, 101–104], conduits composed of many different materials [105–111], and when combined with other cells, such as nMSCs [80] applied outside [86] or inside acellular allografts [88], when conduits are composed of platelet gel [112] or platelet-rich fibrin (PRF) [113, 114]. The PRP can induce axon regeneration that is as effective as autologous nerve grafts [112]. It is important to note that when PRP is applied to a rat sciatic nerve crush site, its influence is increased by surrounding the site with a collagen tube [70].

Clinically, bridging nerve gaps with an autograft within a PRP-filled collagen tube [115–118], or only a PRP-filled collagen

tube [118], induces meaningful recovery under conditions where allografts alone are ineffective. Thus, platelet-released factors alone can induce axon regeneration.

PRP-containing leukocytes

Leukocytes are reported to negatively affect axon regeneration by releasing catabolic cytokines and inducing inflammation [119, 120], while leukocyte-poor PRP (LP-PRP) exerts anabolic effects that promote axon regeneration [121, 122]. However, PRP efficacy is reported to increase with increasing leukocytes and white blood cells concentrations, and bioactivity of platelet-released factors. Platelet growth factor concentrations in leukocyte-rich PRP (LR-PRP) depend on the leukocyte concentrations, with the catabolic protease MMP-9 expressed at a considerably high concentration in the LR-PRP [121]. LR-PRP releases significantly more inflammatory mediators, such as TNF- α , IL-6, and IFN- γ than LP-PRP. However, it also increases the release of the anti-inflammatory mediators IL-4 and IL-10 [123, 124]. The combination and concentration of PRP platelets, leukocytes, and erythrocytes influence the extent of these factors' release [120].

A case report showed that LR-PRP induces meaningful recoveries despite long nerve gaps being repaired with a long repair delay, even in an older subject [118]. This influence is greater than that seen in other studies. The better recovery may be because the PRP was prepared using the Zimmer Biomet GPS III centrifuge system, which increases the platelet concentration 9.3-fold and leukocyte concentration 5-fold (Zimmer Biomet Data on File. Validation Report, GPS III Platelet Concentrator, Test new design for GPS III Buoy re-design, OT000183, 2007), which is at least two times higher than in PRP prepared using other devices [125–127].

The influence of PRP is also affected by its concentration of factors, which is influenced by how PRP is prepared [128]. FGF and TGF are rapidly released from platelets, with their concentration decreasing over time, while PDGF and VEGF are released at a constant rate for 7 days [128]. PRP from the Biomet GPS III has the highest concentrations of VEGF and MMP-9 but the lowest TGF concentration [128]. However, it has also been shown that the concentration of cytokines is not directly related to the cellular composition of PRP [128].

Angiogenesis

Proteomics analysis found that the local application of PRP significantly increases integrin β -8 (ITGB8) expression [95], which promotes angiogenesis [129, 130]. In addition to providing oxygenation to the region of the regenerating axons, Schwann cells use these new blood vessels as their pathway to migrate into the injury site, forming Schwann cell cords that

facilitate axon regeneration [55]. Thus, it has been proposed that PRP-released factors contribute significantly to axon regeneration by promoting vascularization, leading to the migration of cells by activating the FAK pathway mediated by integrin β 1 [131, 132].

Limitations of PRP

While many studies show that PRP promotes axon regeneration and recovery, the extent of the efficacy varies greatly. This is unsurprising because no standard techniques exist for preparing or applying PRP. The simplest and least expensive PRP preparation technique is single spin separation, which yields an increased platelet concentration of 2.67-fold [133], while the double spin technique increased it by 2.48 - 5.71-fold, with a mean of 3.47-fold [134]. A PRP 2.5-3.5-fold increased platelet concentration is considerably less effective in rats than a 4.5 - 6.5- or 7.5 - 8.5-fold increase, although both higher concentrations induce similar influences [135].

Working with New Zealand white rabbit 5 mm nerve gaps, PRP with a 2.5–3.5-fold increased platelet concentration induces limited axon regeneration, significantly greater with the higher concentration of 4.5–6.5-fold and 7.5–8.5-fold [95]. Although a 5-fold increased platelet concentration is recommended as the minimum to exert a meaningful physiological effect [136], the optimal concentration for maximal analgesia remains unknown.

pH

Various devices yield PRP with higher acidification than normal blood, reducing it from 7.35 to 6.8–6.5 [137, 138]. This decreases platelet aggregation by >25% [139–141] and reduces platelet sensitivity to thrombin, resulting in decreased platelet activation, which reduces PRP efficacy. Therefore, it is necessary to avoid PRP acidification during its preparation.

Glucose

Different PRP preparation devices yield PRP with glucose concentrations increased 3- to 6-fold over the starting blood [138]. Increasing PRP glucose concentration increases platelet activation [142]. Therefore, maximizing the efficacy of PRP required avoiding changes in its glucose level.

Diet and physiology affect PRP efficacy

A patient's physiology and diet can greatly affect PRP efficacy. Smoking increases platelet aggregation [143], while

alcohol consumption decreases platelet activation and aggregation [144] and reduces platelet responses to thrombin [145] and collagen. Diets including isoflavones [146], caffeine [147], quercetin, a flavonoid [148], and anthocyanins [149] reduce platelet aggregation. Conversely, diets of high saturated fats [150], simple carbohydrates [150], or excessive sugar [151] increase platelet aggregation. Platelets in patients with high blood pressure have lower concentrations of factors than platelets of patients with normal blood pressure [152] and have a decreased whole blood platelet count [153].

Platelet activation methods

The PRP efficacy is influenced by (1) whether its platelets are activated before or when PRP is applied, (2) the timing of platelet release, (3) the ratios of the various platelet released factors, and (4) their level of bioactivity [154]. Therefore, PRP that does not comply with the necessary physiological parameters will not exert maximal effects [155].

Potential mechanisms by which platelet-released factors increase axon regeneration

Platelets contain more than 300 identified factors [156, 157]. Many of these have been shown to play important roles in promoting axon regeneration and recovery. However, space limitations do now allow a discussion of these factors.

Conclusion

Over the past 70 years, little progress has been made clinically in increasing the percentage of patients who recover meaningful function following peripheral nerve injuries and repairs. Two

significant steps forward are the demonstration that, clinically, electrical stimulation and the application of PRP enhance axon regeneration and the extent of recovery. However, the efficacy of PRP varies greatly, within and between studies, which may result from differences in how the PRP is prepared and applied, as well as the patient's physiological status. Therefore, to optimize the influence of PRP, it is necessary to develop a standardized PRP preparation and application protocol. However, it is also necessary to determine which of a subject's physiological properties, such as diet, consumption of drugs, smoking, and alcohol, must be changed to allow PRP to exert its maximal influences.

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Conflict of interest

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References

- Nichols CM, Brenner MJ, Fox IK, Tung TH, Hunter DA, Rickman SR, et al. Effects of motor versus sensory nerve grafts on peripheral nerve regeneration. *Exp Neurol* (2004) **190**(2):347–55. doi:10.1016/j.expneurol.2004.08.003
- Kornfeld T, Vogt PM, Radtke C. Nerve grafting for peripheral nerve injuries with extended defect sizes. *Wien Med Wochenschr* (2019) **169**(9–10):240–51. doi:10.1007/s10354-018-0675-6
- Karabeg R, Jakirlic M, Dujso V. Sensory recovery after forearm median and ulnar nerve grafting. *Medicinski Arhiv* (2009) **63**(2):97–9.
- Ruijs AC, Jaquet JB, Kalmijn S, Giele H, Hovius SER. Median and ulnar nerve injuries: a meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair. *Plast Reconstr Surg* (2005) **116**(2):484–94. doi:10.1097/01.prs.0000172896.86594.07
- Shergill G, Bonney G, Munshi P, Birch R. The radial and posterior interosseous nerves. Results of 260 repairs. *The J Bone Jt Surg Br volume* (2001) **83-B**(5):646–9. doi:10.1302/0301-620x.83b5.0830646
- Lohmeyer JA, Sommer B, Siemers F, Mailänder P. Nerve injuries of the upper extremity-expected outcome and clinical examination. *Plast Surg Nurs* (2009) **29**(2):88–93. doi:10.1097/01.psn.0000356867.18220.73
- Pan D, Mackinnon SE, Wood MD. Advances in the repair of segmental nerve injuries and trends in reconstruction. *Muscle and Nerve* (2020) **61**(6):726–39. doi:10.1002/mus.26797
- Hoben GM, Ee X, Schellhardt L, Yan Y, Hunter DA, Moore AM, et al. Increasing nerve autograft length increases senescence and reduces regeneration. *Plast and Reconstr Surg* (2018) **142**(4):952–61. doi:10.1097/prs.0000000000004759
- Campodonico A, Pangrazi PP, De Francesco F, Riccio M. Reconstruction of a long defect of the median nerve with a free nerve conduit flap. *Arch Plast Surg* (2020) **47**(2):187–93. doi:10.5999/aps.2019.00654
- Lee YH, Chung MS, Gong HS, Chung JY, Park JH, Baek GH. Sural nerve autografts for high radial nerve injury with nine centimeter or greater defects. *J Hand Surg* (2008) **33**(1):83–6. doi:10.1016/j.jhsa.2007.10.004

11. Wolfe SW, Johnsen PH, Lee SK, Feinberg JH. Long-nerve grafts and nerve transfers demonstrate comparable outcomes for axillary nerve injuries. *J Hand Surg* (2014) **39**(7):1351–7. doi:10.1016/j.jhsa.2014.02.032
12. Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int* (2014) **2014**:1–13. doi:10.1155/2014/698256
13. Yang M, Rawson JL, Zhang EW, Arnold P, Lineaweaver W, Zhang F. Comparisons of outcomes from repair of median nerve and ulnar nerve defect with nerve graft and tubulization: a meta-analysis. *J Reconstr Microsurg* (2011) **27**(8):451–60. doi:10.1055/s-0031-1281526
14. Fawcett JW, Verhaagen J. Intrinsic determinants of axon regeneration. *Develop Neurobiol* (2018) **78**(10):890–7. doi:10.1002/dneu.22637
15. Furey MJ, Midha R, Xu QG, Belkas J, Gordon T. Prolonged target deprivation reduces the capacity of injured motoneurons to regenerate. *Neurosurgery* (2007) **60**(4):723–33. doi:10.1227/01.neu.0000255412.63184.cc
16. Gordon T. The physiology of neural injury and regeneration: the role of neurotrophic factors. *J Commun Disord* (2010) **43**(4):265–73. doi:10.1016/j.jcomdis.2010.04.003
17. Singh B, Xu QG, Franz CK, Zhang R, Dalton C, Gordon T, et al. Accelerated axon outgrowth, guidance, and target reinnervation across nerve transection gaps following a brief electrical stimulation paradigm. *J Neurosurg* (2012) **116**(3):498–512. doi:10.3171/2011.10.jns11612
18. Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. *J Neurosci* (1995) **15**(5 Pt 2):3886–95. doi:10.1523/jneurosci.15-05-03886.1995
19. Jessen KR, Mirsky R. The repair Schwann cell and its function in regenerating nerves. *J Physiol* (2016) **594**(13):3521–31. doi:10.1113/jp270874
20. Gordon T, Tetzlaff W. Regeneration-associated genes decline in chronically injured rat sciatic motoneurons. *Eur J Neurosci* (2015) **42**(10):2783–91. doi:10.1111/ejn.13070
21. Michalski B, Bain JR, Fahnstock M. Long-term changes in neurotrophic factor expression in distal nerve stump following denervation and reinnervation with motor or sensory nerve. *J Neurochem* (2008) **105**(4):1244–52. doi:10.1111/j.1471-4159.2008.05224.x
22. Saheb-Al-Zamani M, Yan Y, Farber SJ, Hunter DA, Newton P, Wood MD, et al. Limited regeneration in long acellular nerve allografts is associated with increased Schwann cell senescence. *Exp Neurol* (2013) **247**:165–77. doi:10.1016/j.expneurol.2013.04.011
23. Verma P, Chierzi S, Codd AM, Campbell DS, Meyer RL, Holt CE, et al. Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. *J Neurosci* (2005) **25**(2):331–42. doi:10.1523/jneurosci.3073-04.2005
24. Ji SJ, Jaffrey SR. Axonal transcription factors: novel regulators of growth cone-to-nucleus signaling. *Develop Neurobiol* (2014) **74**(3):245–58. doi:10.1002/dneu.22112
25. Pacheco A, Merianda TT, Twiss JL, Gallo G. Mechanism and role of the intracellular Calreticulin translation in response to axonal injury. *Exp Neurol* (2020) **323**:113072. doi:10.1016/j.expneurol.2019.113072
26. van Erp S, van Berkel AA, Feenstra EM, Sahoo PK, Wagstaff LJ, Twiss JL, et al. Age-related loss of axonal regeneration is reflected by the level of local translation. *Exp Neurol* (2021) **339**:113594. doi:10.1016/j.expneurol.2020.113594
27. Parhad IM, Scott JN, Cellars LA, Bains JS, Krekoski CA, Clark AW. Axonal atrophy in aging is associated with a decline in neurofilament gene expression. *J Neurosci Res* (1995) **41**(3):355–66. doi:10.1002/jnr.490410308
28. Fricker FR, Antunes-Martins A, Galino J, Paramsothy R, La Russa F, Perkins J, et al. Axonal neuregulin 1 is a rate limiting but not essential factor for nerve remyelination. *Brain* (2013) **136**(Pt 7):2279–97. doi:10.1093/brain/awt148
29. Lamoureux PL, O'Toole MR, Heidemann SR, Miller KE. Slowing of axonal regeneration is correlated with increased axonal viscosity during aging. *BMC Neurosci* (2010) **11**:140. doi:10.1186/1471-2202-11-140
30. Verdu E, Ceballos D, Vilches JJ, Navarro X. Influence of aging on peripheral nerve function and regeneration. *J Peripher Nervous Syst* (2000) **5**(4):191–208. doi:10.1046/j.1529-8027.2000.00026.x
31. Kerezoudi E, King RH, Muddle JR, O'Neill JA, Thomas PK. Influence of age on the late retrograde effects of sciatic nerve section in the rat. *J Anat* (1995) **187**(Pt 1):27–35.
32. Low PA, Schmelzer JD, Ward KK. The effect of age on energy metabolism and resistance to ischaemic conduction failure in rat peripheral nerve. *J Physiol* (1986) **374**:263–71. doi:10.1113/jphysiol.1986.sp016078
33. Sahoo PK, Kar AN, Samra N, Terenzio M, Patel P, Lee SJ, et al. A Ca²⁺-dependent switch activates axonal casein kinase 2 α translation and drives G3BP1 granule disassembly for axon regeneration. *Curr Biol* (2020) **30**(24):4882–95.e6. doi:10.1016/j.cub.2020.09.043
34. Joung I, Yoo M, Woo JH, Chang CY, Heo H, Kwon YK. Secretion of EGF-like domain of heregulin β promotes axonal growth and functional recovery of injured sciatic nerve. *Mol Cell* (2010) **30**(5):477–84. doi:10.1007/s10059-010-0137-5
35. Chen LE, Liu K, Seaber AV, Katragadda S, Kirk C, Urbaniak JR. Recombinant human glial growth factor 2 (rhGGF2) improves functional recovery of crushed peripheral nerve (a double-blind study). *Neurochem Int* (1998) **33**(4):341–51. doi:10.1016/s0197-0186(98)00037-0
36. Heermann S, Schwab MH. Molecular control of Schwann cell migration along peripheral axons: keep moving. *Cell Adhes and Migration* (2013) **7**(1):18–22. doi:10.4161/cam.22123
37. Gambarotta G, Fregnan F, Gnani S, Perroteau I. Neuregulin 1 role in Schwann cell regulation and potential applications to promote peripheral nerve regeneration. *Int Rev Neurobiol* (2013) **108**:223–56. doi:10.1016/b978-0-12-410499-0.00009-5
38. Ronchi G, Cillino M, Gambarotta G, Fornasari BE, Raimondo S, Pugliese P, et al. Irreversible changes occurring in long-term denervated Schwann cells affect delayed nerve repair. *J Neurosurg* (2017) **127**(4):843–56. doi:10.3171/2016.9.jns16140
39. Fricker FR, Lago N, Balarajah S, Tsantoulas C, Tanna S, Zhu N, et al. Axonally derived neuregulin-1 is required for remyelination and regeneration after nerve injury in adulthood. *J Neurosci* (2011) **31**(9):3225–33. doi:10.1523/jneurosci.2568-10.2011
40. Chang HM, Shyu MK, Tseng GF, Liu CH, Chang HS, Lan CT, et al. Neuregulin facilitates nerve regeneration by speeding Schwann cell migration via ErbB2/3-dependent FAK pathway. *PLoS One* (2013) **8**(1):e53444. doi:10.1371/journal.pone.0053444
41. Monje PV, Athauda G, Wood PM. Protein kinase A-mediated gating of neuregulin-dependent ErbB2-ErbB3 activation underlies the synergistic action of cAMP on Schwann cell proliferation. *J Biol Chem* (2008) **283**(49):34087–100. doi:10.1074/jbc.m802318200
42. Gordon T, Udina E, Verge VM, de Chaves EIP. Brief electrical stimulation accelerates axon regeneration in the peripheral nervous system and promotes sensory axon regeneration in the central nervous system. *Motor Control* (2009) **13**(4):412–41. doi:10.1123/mcj.13.4.412
43. Koppes AN, Seggio AM, Thompson DM. Neurite outgrowth is significantly increased by the simultaneous presentation of Schwann cells and moderate exogenous electric fields. *J Neural Eng* (2011) **8**(4):046023. doi:10.1088/1741-2560/8/4/046023
44. English AW, Schwartz G, Meador W, Sabatier MJ, Mulligan A. Electrical stimulation promotes peripheral axon regeneration by enhanced neuronal neurotrophin signaling. *Develop Neurobiol* (2007) **67**(2):158–72. doi:10.1002/dneu.20339
45. Zuo KJ, Shafa G, Antonyshyn K, Chan K, Gordon T, Borschel GH. A single session of brief electrical stimulation enhances axon regeneration through nerve autografts. *Exp Neurol* (2020) **323**:113074. doi:10.1016/j.expneurol.2019.113074
46. Elzinga K, Tyreman N, Ladak A, Savaryn B, Olson J, Gordon T. Brief electrical stimulation improves nerve regeneration after delayed repair in Sprague Dawley rats. *Exp Neurol* (2015) **269**:142–53. doi:10.1016/j.expneurol.2015.03.022
47. Huang J, Zhang Y, Lu L, Hu X, Luo Z. Electrical stimulation accelerates nerve regeneration and functional recovery in delayed peripheral nerve injury in rats. *Eur J Neurosci* (2013) **38**(12):3691–701. doi:10.1111/ejn.12370
48. Xu C, Kou Y, Zhang P, Han N, Yin X, Deng J, et al. Electrical stimulation promotes regeneration of defective peripheral nerves after delayed repair intervals lasting under one month. *PLoS One* (2014) **9**(9):e105045. doi:10.1371/journal.pone.0105045
49. Kroner A, Greenhalgh AD, Zarruk JG, Passos dos Santos R, Gaestel M, David S. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron* (2014) **83**(5):1098–116. doi:10.1016/j.neuron.2014.07.027
50. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* (2009) **29**(43):13435–44. doi:10.1523/jneurosci.3257-09.2009
51. Wagstaff LJ, Gomez-Sanchez JA, Fazal SV, Otto GW, Kilpatrick AM, Michael K, et al. Failures of nerve regeneration caused by aging or chronic denervation are rescued by restoring Schwann cell c-Jun. *Elife* (2021) **10**:e62232. doi:10.7554/elif.62232
52. Yi S, Yuan Y, Chen Q, Wang X, Gong L, Liu J, et al. Regulation of Schwann cell proliferation and migration by miR-1 targeting brain-derived neurotrophic factor after peripheral nerve injury. *Sci Rep* (2016) **6**:29121. doi:10.1038/srep29121

53. Pola R, Aprahamian TR, Bosch-Marce M, Curry C, Gaetani E, Flex A, et al. Age-dependent VEGF expression and intraneural neovascularization during regeneration of peripheral nerves. *Neurobiol Aging* (2004) 25(10):1361–8. doi:10.1016/j.neurobiolaging.2004.02.028
54. Van Steenwinckel J, Auvynet C, Sapienza A, Reaux-Le Goazigo A, Combadière C, Melik Parsadaniantz S. Stromal cell-derived CCL2 drives neuropathic pain states through myeloid cell infiltration in injured nerve. *Brain Behav Immun* (2015) 45:198–210. doi:10.1016/j.bbi.2014.10.016
55. Cattin AL, Burden JJ, Van Emmenis L, Mackenzie F, Hoving J, Garcia Calavia N, et al. Macrophage-induced blood vessels guide schwann cell-mediated regeneration of peripheral nerves. *Cell* (2015) 162(5):1127–39. doi:10.1016/j.cell.2015.07.021
56. Kang H, Lichtman JW. Motor axon regeneration and muscle reinnervation in young adult and aged animals. *J Neurosci* (2013) 33(50):19480–91. doi:10.1523/jneurosci.4067-13.2013
57. Painter MW, Brosius Lutz A, Cheng YC, Latremoliere A, Duong K, Miller C, et al. Diminished Schwann cell repair responses underlie age-associated impaired axonal regeneration. *Neuron* (2014) 83(2):331–43. doi:10.1016/j.neuron.2014.06.016
58. Tung TH, Mackinnon SE. Nerve transfers: indications, techniques, and outcomes. *J Hand Surg* (2010) 35(2):332–41. doi:10.1016/j.jhsa.2009.12.002
59. Zou Y, Chiu H, Zinovyeva A, Ambros V, Chuang CF, Chang C. Developmental decline in neuronal regeneration by the progressive change of two intrinsic timers. *Science* (2013) 340(6130):372–6. doi:10.1126/science.1231321
60. Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, et al. c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron* (2012) 75(4):633–47. doi:10.1016/j.neuron.2012.06.021
61. Yamada Y, Ohazama A, Maeda T, Seo K. The Sonic Hedgehog signaling pathway regulates inferior alveolar nerve regeneration. *Neurosci Lett* (2018) 671:114–9. doi:10.1016/j.neulet.2017.12.051
62. Huang L, Xia B, Shi X, Gao J, Yang Y, Xu F, et al. Time-restricted release of multiple neurotrophic factors promotes axonal regeneration and functional recovery after peripheral nerve injury. *FASEB J* (2019) 33(7):8600–13. doi:10.1096/fj.201802065rr
63. Bond CW, Angeloni N, Harrington D, Stupp S, Podlasek CA. Sonic Hedgehog regulates brain-derived neurotrophic factor in normal and regenerating cavernous nerves. *J Sex Med* (2013) 10(3):730–7. doi:10.1111/jsm.12030
64. Wan L, Xia R, Ding W. Short-term low-frequency electrical stimulation enhanced remyelination of injured peripheral nerves by inducing the promyelination effect of brain-derived neurotrophic factor on Schwann cell polarization. *J Neurosci Res* (2010) 88(12):2578–87. doi:10.1002/jnr.22426
65. Hu M, Hong L, Liu C, Hong S, He S, Zhou M, et al. Electrical stimulation enhances neuronal cell activity mediated by Schwann cell derived exosomes. *Sci Rep* (2019) 9(1):4206. doi:10.1038/s41598-019-41007-5
66. Koppes AN, Nordberg AL, Paolillo GM, Goodsell NM, Darwish HA, Zhang L, et al. Electrical stimulation of schwann cells promotes sustained increases in neurite outgrowth. *Tissue Eng A* (2014) 20(3–4):494–506. doi:10.1089/ten.tea.2013.0012
67. Park J, Kim J, Jeon W, Kim D, Rhyu I, Kim Y, et al. An inside-out vein graft filled with platelet-rich plasma for repair of a short sciatic nerve defect in rats. *Neural Regen Res* (2014) 9(14):1351–7. doi:10.4103/1673-5374.137587
68. Hontanilla B, Auba C, Gorria O. Nerve regeneration through nerve autografts after local administration of brain-derived neurotrophic factor with osmotic pumps. *Neurosurgery* (2007) 61(6):1268–75. doi:10.1227/01.neu.0000306106.70421.ed
69. Giannesi E, Coli A, Stornelli MR, Pirone A, Lenzi C, Burchielli S, et al. An autologously generated platelet-rich plasma sutureable membrane may enhance peripheral nerve regeneration after neurotomy in an acute injury model of sciatic nerve neurotmesis. *J Reconstr Microsurg* (2014) 30(9):617–26. doi:10.1055/s-0034-1372483
70. Vares P, Dehghan MM, Bastami F, Biazar E, Shamloo N, Heidari Keshel S, et al. Effects of platelet-rich fibrin/collagen membrane on sciatic nerve regeneration. *J Craniofac Surg* (2021) 32(2):794–8. doi:10.1097/scs.00000000000007003
71. Alsousou J, Ali A, Willett K, Harrison P. The role of platelet-rich plasma in tissue regeneration. *Platelets* (2013) 24(3):173–82. doi:10.3109/09537104.2012.684730
72. Alsousou J, Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. *J Bone Jt Surg Br volume* (2009) 91-B(8):987–96. doi:10.1302/0301-620x.91b8.22546
73. Sanchez M, Anitua E, Delgado D, Prado R, Sánchez P, Fiz N, et al. Ultrasound-guided plasma rich in growth factors injections and scaffolds hasten motor nerve functional recovery in an ovine model of nerve crush injury. *J Tissue Eng Regen Med* (2017) 11(5):1619–29. doi:10.1002/term.2079
74. Ding XG, Li SW, Zheng XM, Hu LQ, Hu WL, Luo Y. The effect of platelet-rich plasma on cavernous nerve regeneration in a rat model. *Asian J Androl* (2009) 11(2):215–21. doi:10.1038/aja.2008.37
75. Emel E, Ergun SS, Kotan D, Gürsoy EB, Parman Y, Zengin A, et al. Effects of insulin-like growth factor-I and platelet-rich plasma on sciatic nerve crush injury in a rat model. *J Neurosurg* (2011) 114(2):522–8. doi:10.3171/2010.9.jns.091928
76. Wu YN, Wu CC, Sheu MT, Chen KC, Ho HO, Chiang HS. Optimization of platelet-rich plasma and its effects on the recovery of erectile function after bilateral cavernous nerve injury in a rat model. *J Tissue Eng Regen Med* (2016) 10(10):E294–E304. doi:10.1002/term.1806
77. Torul D, Bereket MC, Onger ME, Altun G. Comparison of the regenerative effects of platelet-rich fibrin and plasma rich in growth factors on injured peripheral nerve: an experimental study. *J Oral Maxillofac Surg* (2018) 76(8):1823 e1–1823.e12. doi:10.1016/j.joms.2018.04.012
78. Wu CC, Wu YN, Ho HO, Chen KC, Sheu MT, Chiang HS. The neuroprotective effect of platelet-rich plasma on erectile function in bilateral cavernous nerve injury rat model. *J Sex Med* (2012) 9(11):2838–48. doi:10.1111/j.1743-6109.2012.02881.x
79. Farrag TY, Lehar M, Verhaegen P, Carson KA, Byrne PJ. Effect of platelet rich plasma and fibrin sealant on facial nerve regeneration in a rat model. *The Laryngoscope* (2007) 117(1):157–65. doi:10.1097/01.mlg.0000249726.98801.77
80. Cho HH, Jang S, Lee SC, Jeong H, Park J, Han J, et al. Effect of neural-induced mesenchymal stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model. *The Laryngoscope* (2010) 120(5):907–13. doi:10.1002/lary.20860
81. Kucuk L, Gunay H, Erbas O. Effects of platelet-rich plasma on nerve regeneration in a rat model. *Acta Orthop Traumatol Turc* (2014) 48(4):449–54. doi:10.3944/aott.2014.13.0029
82. Elgazzar RF, Mutabagani MA, Abdelal SE, Sadakah A. Platelet rich plasma may enhance peripheral nerve regeneration after cyanoacrylate reanastomosis: a controlled blind study on rats. *Int J Oral Maxillofac Surg* (2008) 37(8):748–55. doi:10.1016/j.ijom.2008.05.010
83. Liao CH, Chang CJ, Chen KC, Rajneesh CP, Tseng XW, Cheng JH, et al. Effects of platelet-rich plasma glue placement at the prostatectomy site on erectile function restoration and cavernous nerve preservation in a nerve-sparing prostatectomy rat model. *Biomed and Pharmacother* (2023) 161:114499. doi:10.1016/j.biopha.2023.114499
84. Anjayani S, Wirohadidjojo YW, Adam AM, Suwandi D, Seweng A, Amiruddin MD. Sensory improvement of leprosy peripheral neuropathy in patients treated with perineural injection of platelet-rich plasma. *Int J Dermatol* (2014) 53(1):109–13. doi:10.1111/ijd.12162
85. Park GY, Kwon DR. Platelet-rich plasma limits the nerve injury caused by 10% dextrose in the rabbit median nerve. *Muscle Nerve* (2014) 49(1):56–60. doi:10.1002/mus.23863
86. Teymur H, Tiftikcioglu YO, Cavusoglu T, Tiftikcioglu BI, Erbas O, Yigiturk G, et al. Effect of platelet-rich plasma on reconstruction with nerve autografts. *Kaohsiung J Med Sci* (2017) 33(2):69–77. doi:10.1016/j.kjms.2016.11.005
87. Ikumi A, Hara Y, Yoshioka T, Kanamori A, Yamazaki M. Effect of local administration of platelet-rich plasma (PRP) on peripheral nerve regeneration: an experimental study in the rabbit model. *Microsurgery* (2018) 38(3):300–9. doi:10.1002/micr.30263
88. Zheng C, Zhu Q, Liu X, Huang X, He C, Jiang L, et al. Improved peripheral nerve regeneration using acellular nerve allografts loaded with platelet-rich plasma. *Tissue Eng A* (2014) 20(23–24):3228–40. doi:10.1089/ten.tea.2013.0729
89. Garcia de Cortazar U, Padilla S, Lobato E, Delgado D, Sánchez M. Intraneural platelet-rich plasma injections for the treatment of radial nerve section: a case report. *J Clin Med* (2018) 7(2):13. doi:10.3390/jcm7020013
90. Ikumi A, Hara Y, Okano E, Kohyama S, Arai N, Taniguchi Y, et al. Intraoperative local administration of platelet-rich plasma (PRP) during neurolysis surgery for the treatment of digital nerve crush injury. *Case Rep Orthopedics* (2018) 2018:1–6. doi:10.1155/2018/1275713
91. Ricci E, Riva G, Dagna F, Cavalot AL. The use of platelet-rich plasma gel in superficial parotidectomy. *Acta Otorhinolaryngol Ital* (2019) 39(6):363–6. doi:10.14639/0392-100x-2093
92. Sariguney Y, Yavuzer R, Elmas C, Yenicesu I, Bolay H, Atabay K. Effect of platelet-rich plasma on peripheral nerve regeneration. *J Reconstr Microsurg* (2008) 24(3):159–67. doi:10.1055/s-2008-1076752
93. Sanchez M, Yoshioka T, Ortega M, Delgado D, Anitua E. Ultrasound-guided platelet-rich plasma injections for the treatment of common peroneal nerve palsy

associated with multiple ligament injuries of the knee. *Knee Surg Sports Traumatol Arthrosc* (2014) 22(5):1084–9. doi:10.1007/s00167-013-2479-y

94. Fahandezh-Saddi Diaz H, Rios Luna A, Villanueva Martínez M, Cantero Yubero ME, Prado R, Padilla S, et al. Surgical treatment of saphenous nerve injury assisted by plasma rich in growth factors (PRGF): lessons from a case report. *Clin Pract* (2023) 13(5):1090–9. doi:10.3390/clinpract13050097

95. Wang YS, Wang SL, Liu XL, Kang ZC. Platelet-rich plasma promotes peripheral nerve regeneration after sciatic nerve injury. *Neural Regen Res* (2023) 18(2):375–81. doi:10.4103/1673-5374.346461

96. Zhang Y, Yi D, Hong Q, Cao J, Geng X, Liu J, et al. Platelet-rich plasma-derived exosomes boost mesenchymal stem cells to promote peripheral nerve regeneration. *J Controlled Release* (2024) 367:265–82. doi:10.1016/j.jconrel.2024.01.043

97. Klifto KM, Klifto CS, Pidgeon TS. Platelet-rich plasma versus corticosteroid injections for the treatment of mild-to-moderate carpal tunnel syndrome: a markov cost-effectiveness decision analysis. *Hand* (2022) N Y:15589447221092056. doi:10.1002/micr.30263

98. Uzun H, Bitik O, Uzun O, Ersoy US, Aktaş E. Platelet-rich plasma versus corticosteroid injections for carpal tunnel syndrome. *J Plast Surg Hand Surg* (2017) 51(5):301–5. doi:10.1080/2000656x.2016.1260025

99. Wu YT, Ho TY, Chou YC, Ke MJ, Li TY, Huang GS, et al. Six-month efficacy of platelet-rich plasma for carpal tunnel syndrome: a prospective randomized, single-blind controlled trial. *Sci Rep* (2017) 7(1):94. doi:10.1038/s41598-017-00224-6

100. Raeissadat SA, Karimzadeh A, Hashemi M, Bagherzadeh L. Safety and efficacy of platelet-rich plasma in treatment of carpal tunnel syndrome: a randomized controlled trial. *BMC Musculoskelet Disord* (2018) 19(1):49. doi:10.1186/s12891-018-1963-4

101. Sabongi RG, De Rizzo LA, Fernandes M, Valente S, dos Santos J, Faloppa F, et al. Nerve regeneration: is there an alternative to nervous graft? *J Reconstr Microsurg* (2014) 30(9):607–16. doi:10.1055/s-0034-1372477

102. Firat C, Aytakin AH, Durak MA, Geyik Y, Erbatur S, Dogan M, et al. Comparison of the effects of PRP and hyaluronic acid in promoting peripheral nerve regeneration an experimental study with vascular conduit model in rats. *Ann Ital Chir* (2016) 87:362–74.

103. Roque JS, Pomini KT, Buchaim RL, Buchaim DV, Andreo JC, Roque DD, et al. Inside-out and standard vein grafts associated with platelet-rich plasma (PRP) in sciatic nerve repair. A histomorphometric study. *Acta Cir Bras* (2017) 32(8):617–25. doi:10.1590/s0102-865020170080000003

104. Zhu Y, Peng N, Wang J, Jin Z, Zhu L, Wang Y, et al. Peripheral nerve defects repaired with autogenous vein grafts filled with platelet-rich plasma and active nerve microtissues and evaluated by novel multimodal ultrasound techniques. *Biomater Res* (2022) 26(1):24. doi:10.1186/s40824-022-00264-8

105. Sahin MM, Cayonu M, Dinc SK. Effects of chitosan and platelet-rich plasma on facial nerve regeneration in an animal model. *Eur Arch Otorhinolaryngol* (2021). doi:10.1007/s00405-021-06859-6

106. Ye F, Li H, Qiao G, Chen F, Tao H, Ji A, et al. Platelet-rich plasma gel in combination with Schwann cells for repair of sciatic nerve injury. *Neural Regen Res* (2012) 7(29):2286–92. doi:10.3969/j.issn.1673-5374.2012.29.007

107. Chuang MH, Ho LH, Kuo TF, Sheu SY, Liu YH, Lin PC, et al. Regenerative potential of platelet-rich fibrin releasate combined with adipose tissue-derived stem cells in a rat sciatic nerve injury model. *Cell Transpl* (2020) 29:096368972091943. doi:10.1177/0963689720919438

108. Abbasipour-Dalivand S, Mohammadi R, Mohammadi V. Effects of local administration of platelet rich plasma on functional recovery after bridging sciatic nerve defect using silicone rubber chamber; an experimental study. *Bull Emerg Trauma* (2015) 3(1):1–7.

109. Hama S, Yokoi T, Orita K, Uemura T, Takamatsu K, Okada M, et al. Peripheral nerve regeneration by bioabsorbable nerve conduits filled with platelet-rich fibrin. *Clin Neurol Neurosurg* (2024) 236:108051. doi:10.1016/j.clineuro.2023.108051

110. Lu CF, Wang B, Zhang PX, Han S, Pi W, Kou Y, et al. Combining chitin biological conduits with small autogenous nerves and platelet-rich plasma for the repair of sciatic nerve defects in rats. *CNS Neurosci Ther* (2021) 27(7):805–19. doi:10.1111/cns.13640

111. Kim JW, Kim JM, Choi ME, Jeon EJ, Park JM, Kim YM, et al. Platelet-rich plasma loaded nerve guidance conduit as implantable biocompatible materials for recurrent laryngeal nerve regeneration. *NPJ Regen Med* (2022) 7(1):49. doi:10.1038/s41536-022-00239-2

112. Kaplan S, Pişkin A, Ayyıldız M, Aktaş A, K6Ksal B, Ulkay MB, et al. The effect of melatonin and platelet gel on sciatic nerve repair: an electrophysiological and stereological study. *Microsurgery* (2011) 31(4):306–13. doi:10.1002/micr.20876

113. Fernandes M, Valente S, Santos J, Furukawa R, Fernandes C, Leite V, et al. Platelet-rich fibrin conduits as an alternative to nerve autografts for peripheral nerve repair. *J Reconstr Microsurg* (2017) 33(8):549–56. doi:10.1055/s-0037-1603355

114. Huang ML, Zhai Z, Chen ZX, Yang XN, Qi ZL. Platelet-rich fibrin membrane nerve guidance conduit: a potentially promising method for peripheral nerve injuries. *Chin Med J* (2020) 133(8):999–1001. doi:10.1097/cm9.0000000000000726

115. Foy CA, Micheo WF, Kuffler DP. Inducing ulnar nerve function while eliminating claw hand and reducing chronic neuropathic pain. *Plast Reconstr Surg - Glob Open* (2023) 11(4):e4927. doi:10.1097/gox.0000000000004927

116. Micheo WF, Foy CA, Kuffler DP. A novel technique restores function while eliminating intractable neuropathic pain in a 71-year-old diabetic patient under challenging injury conditions. *J Reconstr Microsurg Open* (2023) 08(1):e23–e27. doi:10.1055/s-0042-1757323

117. Foy CA, Micheo WF, Kuffler DP. Functional recovery following repair of long nerve gaps in senior patient 2.6 Years posttrauma. *Plast Reconstr Surg - Glob Open* (2021) 9(9):e3831. doi:10.1097/gox.0000000000003831

118. Kuffler DP, Reyes O, Sosa IJ, Santiago-Figueroa J. Neurological recovery across a 12-cm-long ulnar nerve gap repaired 3.25 years post trauma: case report. *Neurosurgery* (2011) 69(6):E1321–6. doi:10.1227/00006123.2011.031822a9fd2

119. Moojen DJ, Everts PA, Schure RM, Overvest EP, van Zundert A, Knappe JT, et al. Antimicrobial activity of platelet-leukocyte gel against *Staphylococcus aureus*. *J Orthopaedic Res* (2008) 26(3):404–10. doi:10.1002/jor.20519

120. Nishio H, Saita Y, Kobayashi Y, Takaku T, Fukusato S, Uchino S, et al. Platelet-rich plasma promotes recruitment of macrophages in the process of tendon healing. *Regenerative Ther* (2020) 14:262–70. doi:10.1016/j.reth.2020.03.009

121. Kobayashi Y, Saita Y, Nishio H, Ikeda H, Takazawa Y, Nagao M, et al. Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations. *J Orthopaedic Sci* (2016) 21(5):683–9. doi:10.1016/j.jos.2016.07.009

122. Simental-Mendia M, Vilchez-Cavazos F, Garcia-Garza R, Lara-Arias J, Montes-de-Oca-Luna R, Said-Fernández S, et al. The matrix synthesis and anti-inflammatory effect of autologous leukocyte-poor platelet rich plasma in human cartilage explants. *Histol Histopathol* (2018) 33(6):609–18. doi:10.14670/HH-11-961

123. Braun HJ, Kim HJ, Chu CR, Dragoo JL. The effect of platelet-rich plasma formulations and blood products on human synoviocytes: implications for intra-articular injury and therapy. *Am J Sports Med* (2014) 42(5):1204–10. doi:10.1177/0363546514525593

124. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med* (2011) 39(10):2135–40. doi:10.1177/03635465111417792

125. Rittner HL, Machelska H, Stein C. Leukocytes in the regulation of pain and analgesia. *J Leukoc Biol* (2005) 78(6):1215–22. doi:10.1189/jlb.0405223

126. Machelska H, Stein C. Leukocyte-derived opioid peptides and inhibition of pain. *J Neuroimmune Pharmacol* (2006) 1(1):90–7. doi:10.1007/s11481-005-9002-2

127. Celik MO, Labuz D, Henning K, Busch-Dienstfert M, Gaveriaux-Ruff C, Kieffer BL, et al. Leukocyte opioid receptors mediate analgesia via Ca(2+)-regulated release of opioid peptides. *Brain Behav Immun* (2016) 57:227–42. doi:10.1016/j.bbi.2016.04.018

128. Dragoo JL, Braun HJ, Durham JL, Ridley BA, Odegaard JL, Luong R, et al. Comparison of the acute inflammatory response of two commercial platelet-rich plasma systems in healthy rabbit tendons. *Am J Sports Med* (2012) 40(6):1274–81. doi:10.1177/0363546512442334

129. Moyle M, Napier MA, McLean JW. Cloning and expression of a divergent integrin subunit beta 8. *J Biol Chem* (1991) 266(29):19650–8. doi:10.1016/s0021-9258(18)55042-0

130. Ma L, Shen F, Jun K, Bao C, Kuo R, Young WL, et al. Integrin $\beta 8$ deletion enhances vascular dysplasia and hemorrhage in the brain of adult Alk1 heterozygous mice. *Transl Stroke Res* (2016) 7(6):488–96. doi:10.1007/s12975-016-0478-2

131. Qin J, Wang L, Sun Y, Sun X, Wen C, Shahmoradi M, et al. Concentrated growth factor increases Schwann cell proliferation and neurotrophic factor secretion and promotes functional nerve recovery in vivo. *Int J Mol Med* (2016) 37(2):493–500. doi:10.3892/ijmm.2015.2438

132. Qin J, Wang L, Zheng L, Zhou X, Zhang Y, Yang T, et al. Concentrated growth factor promotes Schwann cell migration partly through the integrin $\beta 1$ -mediated activation of the focal adhesion kinase pathway. *Int J Mol Med* (2016) 37(5):1363–70. doi:10.3892/ijmm.2016.2520

133. Anita E, Aguirre JJ, Algorta J, Ayerdi E, Cabezas AI, Orive G, et al. Effectiveness of autologous preparation rich in growth factors for the treatment of

chronic cutaneous ulcers. *J Biomed Mater Res B: Appl Biomater* (2008) **84B**(2): 415–21. doi:10.1002/jbm.b.30886

134. Machado ES, Soares FP, Yamaguchi RS, Felipe WK, Meves R, Souza TAC, et al. A simple double-spin closed method for preparing platelet-rich plasma. *Cureus* (2022) **14**(1):e20899. doi:10.7759/cureus.20899

135. Aufiero D, Vincent H, Sampson S, Bodor M. Regenerative injection treatment in the spine: review and case series with platelet rich plasma. *J Stem Cell Res Rev and Rep* (2015) **2**:1019.

136. Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofacial Surg* (2004) **62**(4):489–96. doi:10.1016/j.joms.2003.12.003

137. Patscheke H. Shape and functional properties of human platelets washed with acid citrate. *Pathophysiology Haemost Thromb* (1981) **10**(1):14–27. doi:10.1159/000214383

138. Fitzpatrick J, Bulsara MK, McCrory PR, Richardson MD, Zheng MH. Analysis of platelet-rich plasma extraction: variations in platelet and blood components between 4 common commercial kits. *Orthopaedic J Sports Med* (2017) **5**(1):2325967116675272. doi:10.1177/2325967116675272

139. Engstrom M, Schott U, Romner B, Reinstrup P. Acidosis impairs the coagulation: a thromboelastographic study. *J Trauma Inj Infect Crit Care* (2006) **61**(3):624–8. doi:10.1097/01.ta.0000226739.30655.75

140. Flatow FA, Jr, Freireich EJ. The increased effectiveness of platelet concentrates prepared in acidified plasma. *Blood* (1966) **27**(4):449–59. doi:10.1182/blood.v27.4.449.449

141. Zavoico GB, Cragoe EJ, Jr, Feinstein MB. Regulation of intracellular pH in human platelets. Effects of thrombin, A23187, and ionomycin and evidence for activation of Na⁺/H⁺ exchange and its inhibition by amiloride analogs. *J Biol Chem* (1986) **261**(28):13160–7. doi:10.1016/s0021-9258(18)69284-1

142. Keating FK, Sobel BE, Schneider DJ. Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes mellitus. *Am J Cardiol* (2003) **92**(11):1362–5. doi:10.1016/j.amjcard.2003.08.033

143. Belch JJ, McArdle BM, Burns P, Lowe GDO, Forbes CD. The effects of acute smoking on platelet behaviour, fibrinolysis and haemorheology in habitual smokers. *Thromb Haemost* (1984) **51**(1):006–8. doi:10.1055/s-0038-1660996

144. Mukamal KJ, Massaro JM, Ault KA, Mittleman MA, Sutherland PA, Lipinska I, et al. Alcohol consumption and platelet activation and aggregation among women and men: the Framingham Offspring Study. *Alcohol Clin and Exp Res* (2005) **29**(10):1906–12. doi:10.1097/01.alc.0000183011.86768.61

145. Olas B, Wachowicz B, Saluk-Juszczak J, Zieliński T. Effect of resveratrol, a natural polyphenolic compound, on platelet activation induced by endotoxin

or thrombin. *Thromb Res* (2002) **107**(3–4):141–5. doi:10.1016/s0049-3848(02)00273-6

146. Williams JK, Clarkson TB. Dietary soy isoflavones inhibit *in-vivo* constrictor responses of coronary arteries to collagen-induced platelet activation. *Coron Artery Dis* (1998) **9**(11):759–64. doi:10.1097/00019501-199809110-00009

147. Frary CD, Johnson RK, Wang MQ. Food sources and intakes of caffeine in the diets of persons in the United States. *J Am Diet Assoc* (2005) **105**(1):110–3. doi:10.1016/j.jada.2004.10.027

148. Hubbard GP, Wolfram S, Lovegrove JA, Gibbins J. Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J Thromb Haemost* (2004) **2**(12):2138–45. doi:10.1111/j.1538-7836.2004.01067.x

149. Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Di Stefano G, González-Paramás AM, et al. One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. *J Nutr Biochem* (2014) **25**(3):289–94. doi:10.1016/j.jnutbio.2013.11.002

150. de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* (1994) **343**(8911):1454–9. doi:10.1016/s0140-6736(94)92580-1

151. Sudic D, Razmara M, Forslund M, Ji Q, Hjendahl P, Li N. High glucose levels enhance platelet activation: involvement of multiple mechanisms. *Br J Haematol* (2006) **133**(3):315–22. doi:10.1111/j.1365-2141.2006.06012.x

152. Yokogoshi H, Wurtman RJ. Meal composition and plasma amino acid ratios: effect of various proteins or carbohydrates, and of various protein concentrations. *Metabolism* (1986) **35**(9):837–42. doi:10.1016/0026-0495(86)90225-8

153. Ahmed Y, van Iddekinge B, Paul C, Sullivan MHF, Elder MG. Retrospective analysis of platelet numbers and volumes in normal pregnancy and in pre-eclampsia. *BJOG: An Int J Obstet and Gynaecol* (1993) **100**(3):216–20. doi:10.1111/j.1471-0528.1993.tb15233.x

154. Cavallo C, Roffi A, Grigolo B, Mariani E, Pratelli L, Merli G, et al. Platelet-rich plasma: the choice of activation method affects the release of bioactive molecules. *Biomed Res Int* (2016) **2016**:1–7. doi:10.1155/2016/6591717

155. Mazzocca AD, McCarthy MB, Chowanec DM, Cote MP, Romeo AA, Bradley JP, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Jt Surg* (2012) **94**(4):308–16. doi:10.2106/jbjs.k.00430

156. Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. *Blood Rev* (2015) **29**(3):153–62. doi:10.1016/j.blre.2014.10.003

157. Baidildinova G, Nagy M, Jurk K, Wild PS, ten Cate H, van der Meijden PEJ. Soluble platelet release factors as biomarkers for cardiovascular disease. *Front Cardiovasc Med* (2021) **8**:684920. doi:10.3389/fcvm.2021.684920