



● INVITED REVIEW

Platelet-rich plasma, an adjuvant biological therapy to assist peripheral nerve repair

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Abstract

Therapies such as direct tension-free microsurgical repair or transplantation of a nerve autograft, are nowadays used to treat traumatic peripheral nerve injuries (PNI), focused on the enhancement of the intrinsic regenerative potential of injured axons. However, these therapies fail to recreate the suitable cellular and molecular microenvironment of peripheral nerve repair and in some cases, the functional recovery of nerve injuries is incomplete. Thus, new biomedical engineering strategies based on tissue engineering approaches through molecular intervention and scaffolding offer promising outcomes on the field. In this sense, evidence is accumulating in both, preclinical and clinical settings, indicating that platelet-rich plasma products, and fibrin scaffold obtained from this technology, hold an important therapeutic potential as a neuroprotective, neurogenic and neuroinflammatory therapeutic modulator system, as well as enhancing the sensory and motor functional nerve muscle unit recovery.

Key Words: peripheral nerve injuries (PNI); Schwann cells; axons; platelet-rich plasma; biomolecules; fibrin; scaffold; intraneural; perineural; microenvironment

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Introduction

Every year, 350,000 patients are affected by traumatic peripheral nerve injuries, which accounts for \$150 billion in annual health care costs (Griffin et al., 2013). Direct tension-free microsurgical repair and/or the transplantation of a nerve autograft to bridge the gap are the gold standard treatments aimed at enhancing the intrinsic regenerative potential of injured axons (Fowler et al., 2015). However, such treatments do not recreate the suitable cellular and molecular microenvironment and in some cases, the functional recovery of nerve injuries is incomplete (Faroni et al., 2015). Platelet-rich plasma (PRP) products hold an important therapeutic potential as a neuroprotective, neurogenic, and neuroinflammatory therapeutic modulator system (Anitua et al., 2013; Kuffler, 2014; Anitua et al., 2015a; Zheng et al., 2016) and as an enhancer of sensory and motor functional nerve-muscle unit recovery (Anjayani et al., 2014; Kuffler, 2015; Sanchez et al., 2015), emerging as a biological adjuvant in peripheral nerve injuries (PNIs) and neuropathies. These autologous products are applied, as a filler of nerve conduits or vein-muscle grafts across nerve gaps post trauma by infiltrating the nerve stumps perineurally and intraneurally which is guided with ultrasound probes, or as scaffolds to bridge or wrap the injured nerve stumps (Farrag et al., 2007; Giannessi et al., 2014; Kim et al., 2014; Malahias et al., 2015). Moreover, there are non-traumatic peripheral injuries such as compression, adhesion and fibrosis (as in the case of carpal tunnel syndrome and fibrotic post-surgical side effects) (Dodla et al., 2008), where this novel approach applied may additionally diminish undesirable consequences such as fibrotic

scars and denervated organ atrophy since this adjuvant therapy can speed up the functional recovery of the nerve-muscle unit (Sariguney et al., 2008; Takeuchi et al., 2012; Wu et al., 2012; Ye et al., 2012; Sanchez et al., 2014). Therefore, PRPs may be applied to assist and synergize with the gold standard therapies in nerve regeneration and neuropathies, and may be harnessed by surgeons in the operating room and in the clinical setting as an “off the shelf” alternative.

Degeneration and Regeneration after PNI: Molecular and Cellular Events

Following a PNI, an orchestrated multicellular and pleiotropic molecular response will ensue. This response consists in the interplay among Schwann cells (SCs), resident macrophages, endothelial cells (ECs), and fibroblasts, mainly modulated by injured axons, myelin breakdown products, soluble factors, and hypoxia as main signals. It will end up regrowing and guiding axons, and reconnecting them with the target organs at a rate of about 1 mm per day in humans (Parrinello et al., 2010; Zochodne, 2012; Cattin et al., 2015) (**Figure 1**).

Disruption of the regeneration unit by the noxious agent results in loss of axonal contact with SCs whose phenotype is drastically modified, thereby contributing to SC activation or transdifferentiation. Macrophages will collaborate with the activated-dedifferentiated SCs in clearing the myelin and other tissue debris. Moreover, these SCs come into direct contact with resident fibroblasts that accumulate in large numbers at the site of injury influencing SC migration and dedifferentiation (Parrinello et al., 2010; Arthur-Farraj et al., 2012; Jessen et

al., 2015). SCs show a striking plastic response to the biological battlefield they are exposed to inside a damaged nerve and are the early detectors of damage (**Figure 1**). In a context- and time-dependent manner, transdifferentiated SCs perform a variety of cell repair tasks from phagocytosing myelin debris to secreting neurotrophic and neurotropic factors (laminin), proliferation and migration, which results in the formation of SC cords and Bungner Bands in the proximal and distal nerve segment, respectively (Gaudet et al., 2011; Zochodne, 2012; Jessen et al., 2015). Although SCs have the reputation of being the engine of peripheral nerve repair, in the nerve repair complex process, they are fuelled by axon growth cones and supportive stromal cells such as macrophages and fibroblasts, the very elements of Wallerian degeneration as a neuroinflammatory process (**Figure 1**) (Parrinello et al., 2010; Gaudet et al., 2011; Cattin et al., 2015; Chen et al., 2015; Jessen et al., 2015). Emerging evidence suggests that macrophage plasticity contributes to peripheral nerve regeneration *via* distinct mechanisms: by phagocytosing myelin debris, synthesizing trophic factors such as vascular endothelial growth factor (VEGF) and promoting angiogenesis, producing collagen type VI, modulating the proliferation and migration of SCs, and influencing the resolution of inflammation through the polarization from M1 to M2 phenotype (Mokarram et al., 2012; Cattin et al., 2015; Chen et al., 2015). Cattin et al. (2015) confirmed an idea suggested by Chen et al. (2005) that blood vessels might provide substrate or signalling for axon growth guidance and SC migration, by showing that macrophages selectively sense hypoxia in the area of nerve bridge and drive angiogenesis *via* the VEGF-secretion pathway at the nerve bridge (**Figure 1**). Despite the robust repair capacity to regrow peripheral nervous axons shown in the adult mammal (Gaudet et al., 2011; Cattin et al., 2015) and meticulous microsurgical nerve repair techniques there are some limiting factors, including the poor vascularization, the patients age, the chronic denervation of SCs, the endoneurial and perineurial fibrosis, the misguided axonal growth, the vast distance that axon growth cones must cover to reinnervate target organs/tissues, as well as their atrophy, and the rate of regeneration (Hall, 2005; Zochodne, 2012; Scheib and Hoke, 2013; Painter et al., 2014).

Plasma Rich in Growth Factors: an Injectable Scaffold to Assist in Nerve Repair

Plasma rich in growth factors (PRGFs) consist of a pool of growth factors (GFs), microparticles, and other bioactive mediators many of them trapped, through fibrin heparan sulfate-binding domains, in a three-dimensional transient fibrin matrix (**Figure 2**) (Anitua et al., 2015b). Once PRP is infiltrated intraneurally as a liquid-to-gel injectable scaffold, or wrapped around the injured nerve gap as a matrix-like viscous and malleable structure, or both, (Sanchez et al., 2015) (**Figure 3**) tissue fibrinolysis breaks the fibrin down, thereby releasing cell signalling molecules such as neurotrophic (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), VEGF, hepatocyte growth factor (HGF)) and neurotropic factors (fibrin, fibronectin, and vitronectin) (Anitua et al., 2015c).

Growing *in vitro* and *in vivo* evidence suggests that the

biomolecules conveyed by PRPs are instrumental agents that modulate early inflammation, stem cell-like myelinating SC activation, macrophage polarization, as well as the active resolution of inflammation, angiogenesis, and fibrogenesis, thereby acting as key drivers of full nerve functional recovery (Sondell et al., 1999; Jiang et al., 2013; Zheng et al., 2014; Cattin et al., 2015; Jessen et al., 2015). There are so far six lines of evidence pointing the therapeutic potential of PRPs as follows (**Figure 3**).

Neuroprotection and prevention of cell apoptosis

Several growth factors present in PRP including NGF, BDNF, PDGF, VEGF, IGF-1, transforming growth factor beta (TGFB) alone or in combination have been shown to exert an anti-apoptotic and neuroprotective effect on mesenchymal stem cells (MSCs), neurons, SCs, and human neural stem cells (Sondell et al., 1999; Lee et al., 2003; Borselli et al., 2010; Emel et al., 2011; Luo et al., 2012; Rao and Pearce, 2016). PRP fibrin scaffolds enriched with NGF, BDNF, and retinoic acid and loaded with bone marrow stromal cells (BMSCs), enhance their survival and differentiation into the neural phenotype (Zurita et al., 2010). In addition, when this PRP scaffold was transplanted into the brain, the viability and biologic activity of allogenic BMSC increased (Vaquero et al., 2013). Moreover, neuroprotective and antifibrotic beneficial effects (Cho et al., 2010; Wu et al., 2012) were reported with the injection of PRP into the corpus cavernosum in a bilateral cavernous nerve injury rat model and applying PRP in a facial nerve suture in a guinea pig model. A recent *in vitro* study on neuronal cultures from mouse model of Alzheimer's disease (Anitua et al., 2015a) showed that the neurotoxicity induced by aggregated β -amyloid added in primary neuronal cultures was significantly reduced, and the living cell number after the co-treatment with PRP increased. In addition, chronic intranasal administration of PRP in Alzheimer's disease mouse model elicits neuroprotection which is likely mediated by the activation of the antiapoptotic PI3K/Akt signalling pathway (Anitua et al., 2014).

Stimulation of angiogenesis

Borselli et al. (2010) showed in an ischemic limb rodent model with loss of neuromuscular junction (NMJ) innervation that an injectable scaffold loaded with VEGF and IGF-1 accelerated regeneration of damaged NMJs together with an enhancement of angiogenesis. In a rat model it has been reported that sciatic nerve gaps of 10 mm repaired with vein graft filled with PRP exhibited a more prominent early neoangiogenesis than sciatic nerve gaps treated with nerve autograft alone (Kim et al., 2004). In this regard, it should be taken into account that fibrin is a pivotal element within PRP that provides ECM tissue with a robust and permissive 3D matrix for angiogenesis (Hall et al., 2007).

Enhancing axonal outgrowth capacity

The crucial role played by GF within the PRP has been highlighted in a rat brain-spinal cord cocultured system, where the addition of PRP supernatant promoted an increase in the size and number of axons, a positive effect that was significantly suppressed when antibodies against IGF-1 and VEGF were added (Takeuchi et al., 2012). As a cellular carrier, two studies in acute nerve injury model in guinea pig and rabbits applied PRP and seeded the acellular carrier with either

MSCs or SCs, reporting beneficial effects on axonal counts, myelination and electrophysiological parameters (Cho et al., 2010; Ye et al., 2012). One example of the use of PRP as a filler of acellular nerve allografts (ANA PRP) represents the work of Zheng et al. (2016) that, having previously shown a dose-dependent effect of PRP on the proliferation, migration and, neurotrophic function in rat SCs cultured with PRP, they showed significant improvements in diameter, thickness, and numbers of myelinating axons as well as an enhancement of electrophysiological parameters in sciatic nerve injury repaired with autografts and ANA PRP in a rat model (Zheng et al., 2014). Using a simple inside-out vein autograft or an inside-out vein autograft filled with PRP to bridge the sciatic nerve gap in a rat model, Kim et al. (2014) observed that the number of myelinated axons, the axon diameter and myelin sheath were significantly superior when PRP was used as a filler. These results are in accord with the work of Kaplan et al. (2011), who used platelet gels as filler of collagen nerve conduit with improvement in functional and structural outcomes in an injury model of rat sciatic nerve. Using platelet-rich fibrin (PRF) as a filler of silicon nerve guidance (Lichtenfels et al., 2013) or nerve grafts (Sabongi et al., 2014) in a rat model, animals treated with PRP improved functional recovery and showed a superior sciatic functional index compared with non-treated animals. However the researchers did not find morphometric or structural improvements (Lichtenfels et al., 2013; Sabongi et al., 2014). The application of PRP as a fibrin membrane to wrap the neurotomy in an acute injury model of sciatic nerve neurotomy showed diverse positive effects. Gianessi et al. (2014) observed a stronger EMG signal, a significantly larger axonal density, and a lower scar tissue in animals treated with PRP fibrin membranes, and remains of PRP membranes were still present after 6 weeks post-surgery. In this sense, two studies reported the positive effects of using PRP as adjuvant in nerve suture. Farrag et al. (2007) reported that PRP may enhance the myelin thickness and increase the axon counts when injured nerve is sutured and assisted with PRP, whereas Sariguney et al. (2008) found no positive effects on axonal size in sutured nerves assisted with PRP. However, they showed a better functional outcome associated with improvement in the myelin thickness and the onset latency. Sanchez et al. (2008) applying PRP as both filler of the injured nerve and as a scaffold to coat the nerve crush on sheep, reported an earlier electrophysiological response, a higher axonal density, and lower muscle atrophy in treated animals compared with the saline or spontaneous regeneration groups.

Overcoming the inflammatory microenvironment

Though indirect, two important pieces of evidence in neural tissue support the antiinflammatory effect of PRP. Anitua et al. (2014) reported that astrocytes cultured with β -amyloid expressed proinflammatory cytokines, but this effect was completely blocked when the culture was supplemented with PRP, an effect mediated by the suppression of the nuclear transcription factor- κ B (NF- κ B) on astrocytes. In a mouse model of Parkinson's disease, Anitua et al. (2015a) showed that the neuroinflammatory process, mediated by microglia, was reduced, together with an improvement in motor performance, responses that were associated with a robust reduction in NF- κ B activation, nitric oxide, cyclooxygenase,

and tumor necrosis factor expression in the brain. In a rabbit model of dextrose-induced median nerve injury, the injection of PRP into the carpal tunnel of rabbits injured 4 weeks before, exerted a significant reduction in nerve swelling compared with the control group (Park and Kwon, 2014).

Dampening the denervated target muscle atrophy

Several animal studies have demonstrated that the application of PRP as a filler, a fibrin membrane, or both, induce an earlier axonal regeneration and functional recovery (Farrag et al., 2007; Sariguney et al., 2008; Emel et al., 2011; Wu et al., 2012; Gianessi et al., 2014; Kim et al., 2014; Sanchez et al., 2015). This is the case reported by Sanchez et al. (2015) on sheep, where nerves repaired with PRP were associated with an earlier electrophysiological recovery and lower muscle atrophy, suggesting that PRP application may dampen the target muscle atrophy. In addition, another recovery burden in nerve repair is scarring, which has been reported to be minimized by the repair of sciatic injured nerve assisted with PRP (Gianessi et al., 2014). Anitua et al. (2015d) showed that intramuscular injection of PRP 24 hours after the induction of limb ischemia in mice, mitigates fibrosis and muscle atrophy. These results are in agreement with the reduction of atrophy in denervated muscle reported when muscle was infiltrated with cells (Schaakxs et al., 2013), effects suggested to be mediated by the IGF-1 (Shavlakadze et al., 2005). Moreover, TGFB, an important GF within PRP, attenuates the adverse effects of chronically denervated Schwann cells, and reactivated SCs support axon regeneration *in vivo* (Sulaiman and Gordon, 2012).

The improvement of neurologic parameters in humans

In the wake of promising results in animal experimentation, PRP has been applied either as filler of nerve conduits across post traumatic nerve gaps (Kuffler, 2011, 2014), as a liquid dynamic scaffold infiltrated perineurally (Hibner et al., 2012; Anjayani et al., 2014; Malahias et al., 2015), intraneurally, or both (as in the case of a peroneal nerve palsy (Sanchez et al., 2014) and other damaged nerves. Furthermore, it also has been applied as scaffold or fibrin membranes (Kuffler, 2011, 2014; Scala et al., 2014) with beneficial outcomes and better functional recovery. Kuffler applied autologous platelet rich fibrin as a filler of a collagen tube, proceeding to bridge the 12 cm nerve gap 3.25 years after an ulnar nerve trauma, and to recovery of both muscle and sensory function (Kuffler, 2011). In a recent series of cases of surgical nerve repair, Kuffler (2014) reported functional recovery in patients under 58 years whose nerve gaps of 2–16 cm were treated with collagen tube filled with PRP, from 0.5–3 years post trauma.

In a double-blind, randomized, clinical trial, the application of perineural PRP injections in tibial and ulnar nerves has shown sensory improvement in leprosy peripheral neuropathy (Anjayani et al., 2014). In a retrospective analysis of 10 patients with persistent pudendal neuralgia, who underwent a second trans-gluteal decompression of the pudendal nerve, they injected activated PRP around the coated nerve, reporting a significant reduction in pain (Hibner et al., 2012). In a case series of 14 patients with carpal tunnel syndrome, a single ultrasound-guided injection of PRP around the median nerve led to the disappearance of pain in eight patients, and pain alleviation in three patients at three months of follow-up

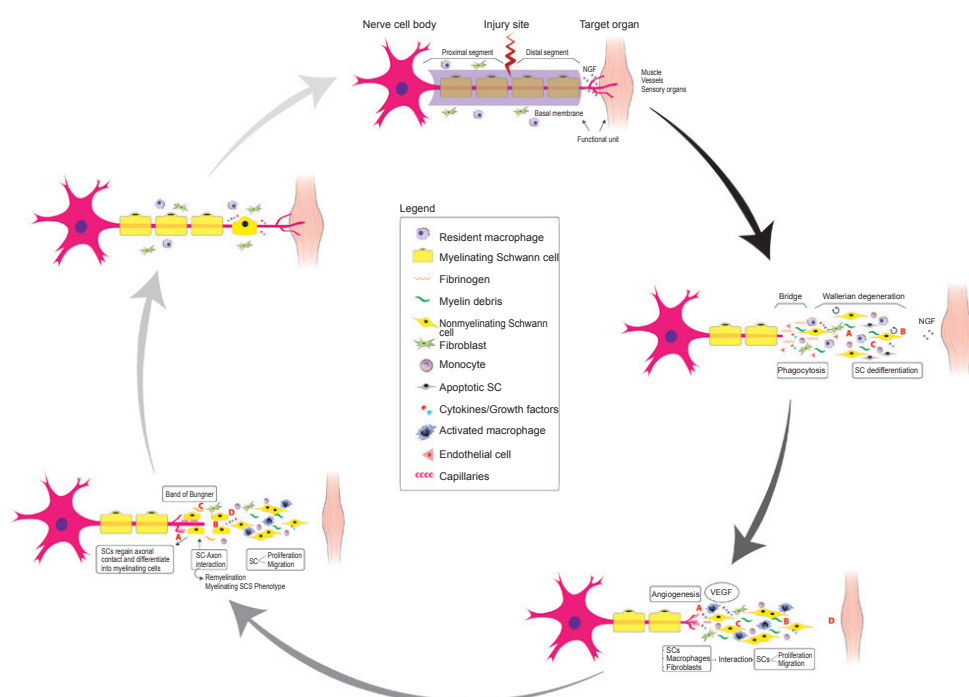


Figure 1 Spontaneous peripheral nerve regeneration is a multicellular and pleiotropic process.

Schwann cells are the master and servant in peripheral nerve regeneration while macrophages act as “Jack of all trades”. The partnership between the transdifferentiated SCs and macrophages induce the latter to synthesize VEGF. In addition to stimulating the proliferation of endothelial cells, promoting new vessels that guide the axon growth, thereby serving as tracks for migrating and proliferating SCs to form a Band of Bungner, VEGF enhances the survival, migration and proliferation of SCs, all of which contribute to the outgrowth of axons, restoration of basal lamina and facilitation of the formation of Band of Bungner at both nerve stumps. NGF: Nerve growth factor; SCs: Schwann cells; VEGF: vascular endothelial growth factor.

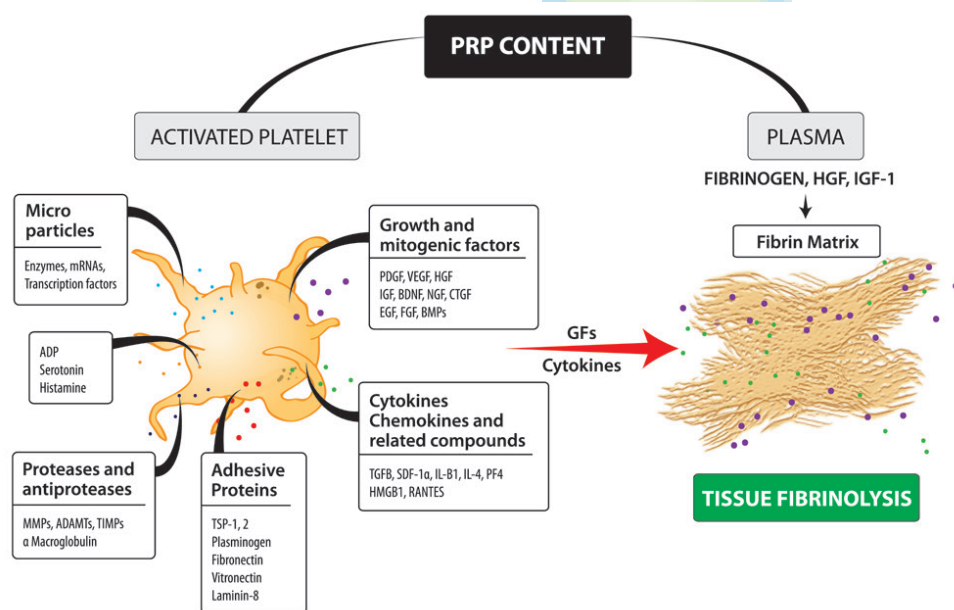


Figure 2 Illustration of some biological mediators of platelet-rich plasma (PRP) that govern tissue repair by still poorly understood mechanisms.

There are biomolecules and several growth factors which come either from platelet activation and plasma or both. Several of these bioactive mediators and other growth factors or proteins remain trapped through fibrin heparan sulfate-binding domains, in a three-dimensional transient fibrin matrix to be released later by tissue fibrinolysis. ADAMTs: A disintegrin and metalloprotease with thrombospondin motifs; ADP: adenosine diphosphate; BDNF: brain-derived neurotrophic factor; BMPs: bone morphogenetic proteins; CTGF: connective tissue growth factor; EGF: epidermal growth factor; FGF: fibroblast growth factor; GFs: growth factors; HGF: hepatocyte growth factor; HMGB1: high mobility group box 1; IGF: insulin-like growth factor; IL- β 1: interleukin- β 1; MMPs: matrix metalloproteinases; NGF: nerve growth factor; PDGF: platelet-derived growth factor; PF4: platelet factor 4; RANTES: regulated upon activation, normal T cell expressed and presumably secreted; SDF-1 α : stromal cell-derived factor-1 α ; TGFB: transforming growth factor beta; TIMPs: tissue inhibitors of metalloproteinases; TSP-1: thrombospondin-1; VEGF: vascular endothelial growth factor.

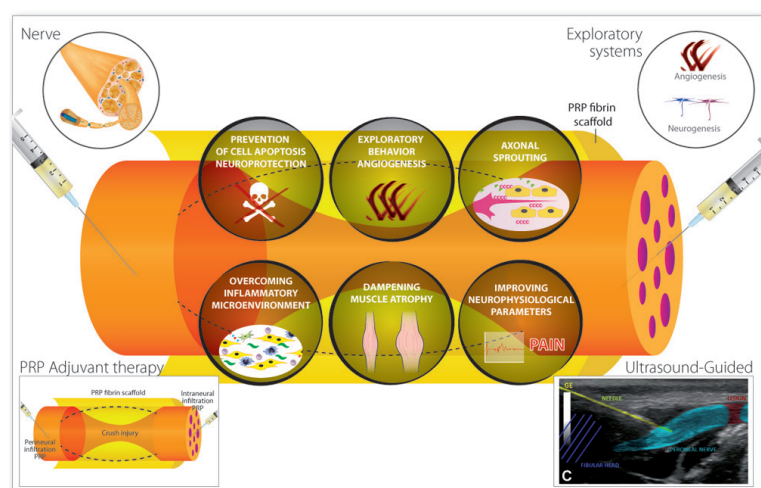


Figure 3 Six lines of evidence suggest the therapeutic potential use of PRPs on neural tissue repair and regeneration.

These include the prevention of cell apoptosis and neuroprotection, the stimulation of angiogenesis, the modulation of inflammatory microenvironment, the enhancement of axonal outgrowth and nerve guidance, the dampening of both denervated muscle atrophy and scarring that follow peripheral nerve trauma and damage, and the improvement of neurologic parameters in humans. PRP: Platelet-rich plasma.

(Malahias et al., 2015). Another case report, in this case applying sequential proximal and distal ultrasound-guided PRP injections intraneurally and perineurally (**Figure 3**) in a common peroneal nerve palsy, Sanchez et al. (2014) reported a significant functional recovery assessed by electromyographic signs of reinnervation for both peroneus longus and tibialis anterior muscles as well as almost full recovery of sensitivity. It has been reported that the intravenous injection of 25 cc of concentrated PRP in a 6-year-old-boy with perinatal cerebral palsy is safe, and can significantly improve the cognitive and language functions (Alcaraz et al., 2015).

Conclusion

The ultimate goal of any peripheral nerve repair strategy is the restoration of nerve-target organ function, while minimizing therapeutic side effects. PRPs are versatile and safe biological products to be harnessed by surgeons and clinicians as an adjuvant therapeutic tool to enhance the robust intrinsic nerve repair processes and overcome post-traumatic and neuropathic inhibitory microenvironment by combinatorial strategy of delivering neurotrophic and neurotropic factors. They may assist nerve conduit guidances and grafts as a filler, as a liquid in intraneural and perineural ultrasound-guided injections in nerve entrapments and fibrosis, and as a scaffold to bridge or wrap the injured nerve gap.

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Conflicts of interest: SP is a researcher at BTI (Biotechnology Institute) a dental implant company that investigates in the fields of oral implantology and PRGF-Endoret technology.

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