CHAPTER 13

A Novel and Versatile Adjuvant Biologic Therapy in the Management of Neuropathies

AUTHORS

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SUMMARY

In mammals, axons of injured peripheral nerves (PNI) can and do regenerate, but often the functional recovery is incomplete or suboptimal. In recent years in vivo tissue engineering approaches through molecular intervention and scaffolding are offering promising outcomes. In this sense, evidence is accumulating in both preclinical and clinical settings, indicating that platelet rich plasma (PRP) 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.

This chapter addresses current molecular and cellular data in intrinsic nerve repair processes and describes a new strategy to harness and enhance these processes by using biochemical and biomechanical cues. In particular, it focuses on autologous fibrin, plasma and platelet-derived growth factors as filler or scaffolds that can synergize with the gold standard therapy and other nerve guidance conduits.

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1. INTRODUCTION

Every year, 350,000 patients are affected by traumatic peripheral nerve injuries, which accounts for $150 billion in annual health care costs[1]. 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[2]. However, such treatments fail to recreate the suitable cellular and molecular microenvironment of peripheral nerve repair[3] in addition to creating, as in the case of autografts, a second iatrogenic injury and morbidity in the donor site[4]. In recent years, biologic strategies to treat peripheral nerve injury (PNI) combined with in vivo tissue engineering approaches through molecular intervention and scaffolding are offering promising outcomes[2,5,6]. Among them, platelet rich plasma (PRP) products hold an important therapeutic potential as a neuroprotective, neurogenic, and neuroinflammatory therapeutic modulator system[5,7-11] and as enhancer of sensory and motor functional nerve-muscle unit recovery[12-14]. PRPs are emerging as an adjuvant biologic in the treatment of peripheral nerve injuries (PNIs) and neuropathies[12-14]. These autologous products are applied either as a filler of nerve conduits or vein-muscle grafts across nerve gaps post trauma by Ultrasound-guided perineural and intraneural infiltrations , or as scaffolds to bridge or wrap the injured nerve stumps[15-18]. 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)[19], 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[20-24].

Considerable progress has been made in understanding the molecular and cellular events of peripheral nerve regeneration after injury, and this chapter will discuss our current knowledge, and the particular application of plasma rich in growth factors for improving repair and regeneration in PNI.

2. NEUROBIOLOGY OF PERIPHERAL NERVE INJURY AND REPAIR

After injury, approximately 30-40% of sensory neurons die, and these numbers increase as the nerve injury gets closer to the neuron body[25]. Persisting neurons switch their state from a signalling to a growing phenotype through the expression of genes involved in cell survival and axon outgrowth[25,26]. Concomitantly, injured or dying axons signal to Schwann cells (SCs) through their loss of contact, by an as yet poorly understood mechanism, and SCs respond with a radical phenotypic change known as activation or transdifferentiation[27,28]. In addition, supportive stromal cells such as endothelial cells, fibroblasts, and macrophages, will play a key role in the guidance and support of Schwann cell-growing axon regenerative units across first the nerve bridge and then the distal segment to eventually reconnect with their original targets at a rate of about 1mm per day in humans (fig. 1)[25,29].

SCs act as masters and servants in PNI repair, and show a striking chameleonic response to the biological battlefield they are exposed to inside a damaged nerve and are the early detectors of damage (fig. 1). Recent studies have shown that SCs express a variety of Toll-like receptors (TLRs2/3/4) through which SCs recognize these DAMPs, together with resident macrophages also endowed with TLRs, thereby playing a sentinel role to identify nerve injury and hence, activate an inflammatory response known as neuroinflammation[30,31]. In a context-and time-dependent manner, dedifferentiated 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[27,30,32]. 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 (fig. 1)[27,30,33-35]. The macrophages acting as "jack of all trades" 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[27,28,33] (fig. 1). Moreover, emerging evidence suggests that macrophage plasticity contributes to peripheral nerve regeneration via distinct mechanisms: by phagocyitosing myelin debris, synthesizing trophic factors such as 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[34-36]. Cattin et al[34] confirmed an idea suggested by Chen et al[37] 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 (fig. 1). In addition, these SCs come into direct contact with resident fibroblasts that accumulate in large numbers at the site of injury influencing SC migration and dedifferentiation[27,28,33] (fig. 1).

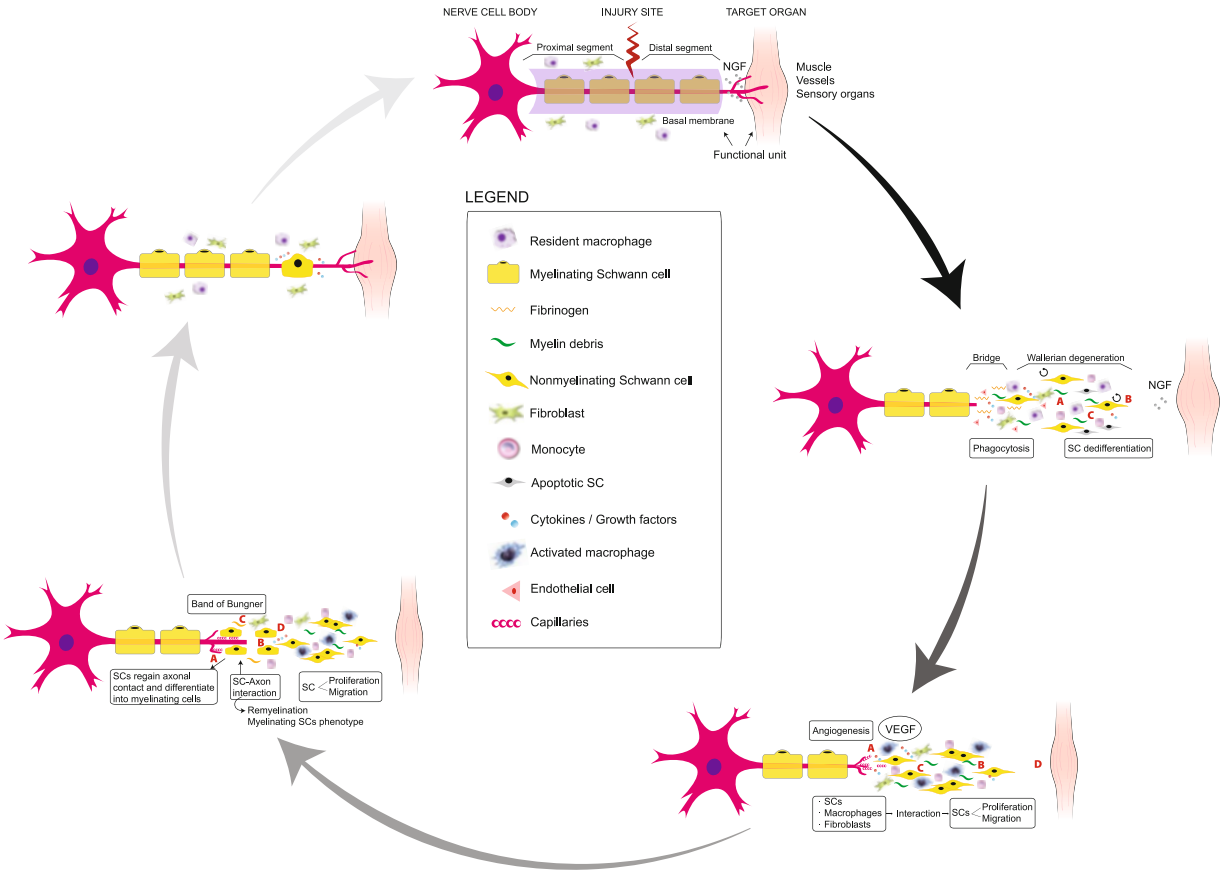

FIG. 1 SPONTANEOUS PERIPHERAL NERVE REGENERATION IS A MULTICELLULAR AND PLEIOTROPIC PROCESS
Schwann cells are the master and servant in peripheral nerve regeneration while macrophages act as "Jack of all trades". The partnership between the transdifferentiated SCs and macrophages induce the latter to synthesize VEGF. In addition to stimulating the proliferation of endothelial cells, and thereby promoting new vessels that guide the axon growth, thereby serving as tracks for migrating and proliferating SCs to from 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. (reprinted with permission from Sanchez, M. et al.[29]

Despite the robust repair capacity to regrow peripheral nervous axons shown in the adult mammal[30,34] 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[25,32,38,39]. Therefore, three key events significantly contribute to axonal outgrowth, namely, angiogenesis, axon-SC partnership, and a permissive and inductive microenvironment where as important as the absence of inhibitory molecules is the presence of nerve guidance, and neurotropic and neurotrophic factors.

3. PLASMA RICH PLASMA: AN INJECTABLE SCAFFOLD TO ASSIST IN NERVE REPAIR

Platelet rich plasma (PRPs) are blood-derived biological drug delivery products that have emerged as a novel and versatile formulation to enhance repair and regeneration in the treatment of musculoskeletal conditions including osteoarthritis and chondral pathologies, non-union fractures, acute and chronic tendinopathies, muscle strains, and peripheral nerve injuries and neuropathies[12,40-42].

These varied products consist of a pool of growth factors (GFs), microparticles, and other bioactive

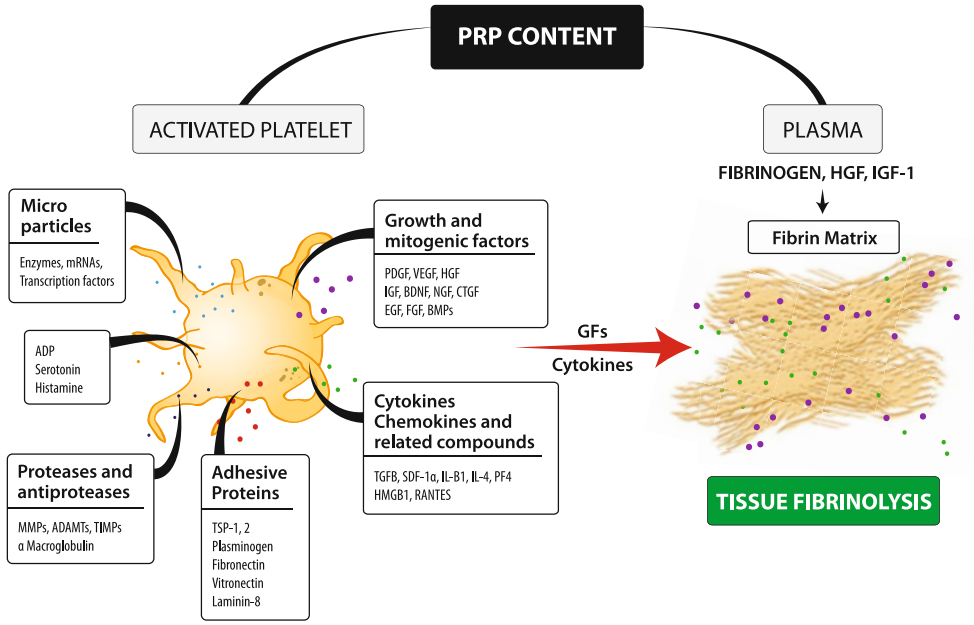

FIG. 2
Illustration of some biological mediators of platelet-rich plasma (PRP) that govern tissue repair by still poorly undestood mechanisms. There are biomolecules and several growth factors which come either from platelet activation and plasma or both. Several of these bioactive mediators and other 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-β1: interleukin-β1; 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-1α: stromal cell-derived factor-1α; TGFB: transforming growth factor beta; TIMPs: tissue inhibitors of metalloproteinases; TSP-1: thrombospondin-1; VEGF: vascular en- dothelial growth factor. (reprinted with permission from Sanchez, M. et al.[29]

mediators stemmed from platelet activation and plasma (fig. 2)[29]. Many of these biomolecules are trapped, through fibrin heparan sulfate-binding domains, in a three-dimensional transient fibrin matrix generated from the polymerization of plasmatic fibrinogen, thereby regulating the tissue concentration of GFs, as is the case in biological repair[43].

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,[12] (fig. 3) tissue fibrinolysis breaks the fibrin down, thereby releasing cell signalling molecules such as neurotrophic (NGF, BDGF, IGF-1, PDGF, VEGF, HGF) and neurotropic factors (fibrin, fibronectin, and vitronectin)[9].

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 Schwann cell activation, macrophage polarization, as well as the active resolution of inflammation, angiogenesis, and fibrogenesis, thereby acting as key drivers of full nerve functional recovery[27,34,44-46] (Table I and II)[6].

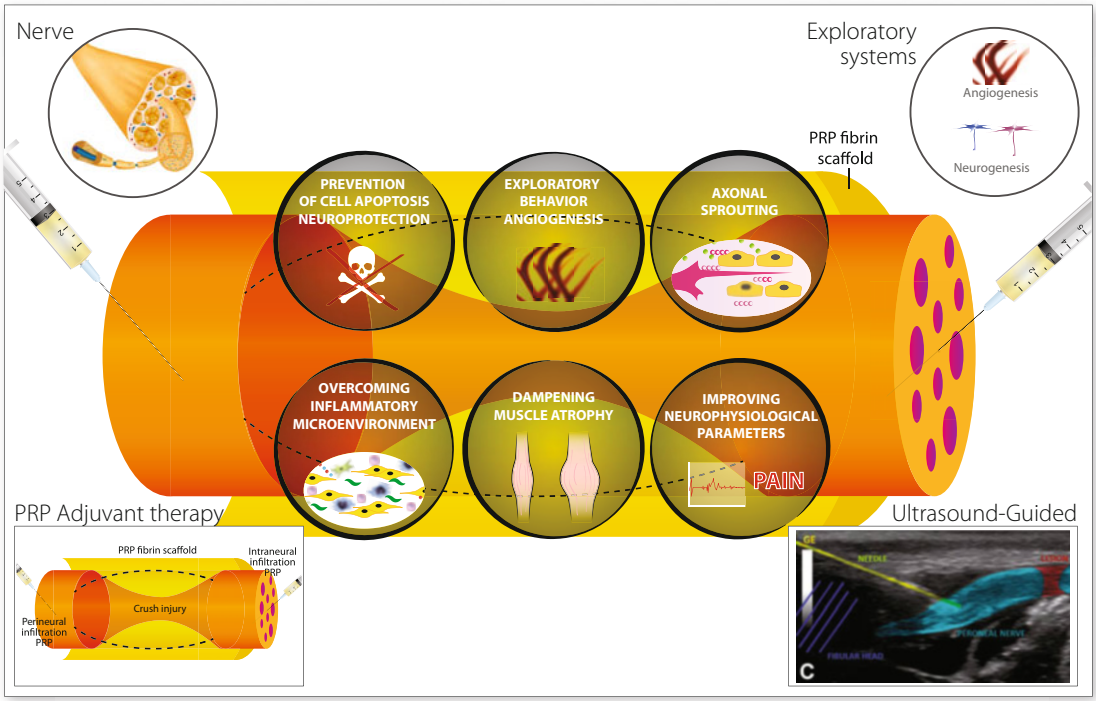

FIG. 3
Six lines of evidence suggest the therapeutic potential use of PRPs on neural tissue repair and regeneration.
These include the prevention of cell apoptosis and neuroprotection, the stimulation of angiogenesis, the modulation of inflammatory microenvironment, the enhancement of axonal outgrowth 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. (reprinted with permission from Sanchez, M. et al.[29]

Cell type/Animal model	Intervention	Outcome	Reference
Human bone marrow stem cells	Cells cultured with PRP scaffolds enriched with NGF, BDGF and retinoic acid.	Prevention of cell apoptosis and differentiation in neural phenotype.	Zurita et al. 2010
Bone marrow stromal cells and Wistar rats with intracerebral hemorrhage	Intracerebral administration of cells embedded in PRP scaffold	Increment of cell viability and biological, neurological and functional activity	Vaquero et al. 2013
Sprague-Dawley rats with bilateral cavernous nerve crush	Injection of 200μL of PRP into the corpus cavernosum immediately after crush injury	Preservation of myelinated axons and prevention of cell apotosis	Wu et al. 2012
Albino guinea pigs with facial nerve transection	Injection of 5mL of PRP and perineural microsuture	Improvement in function, increment of neurotrophic factors and preservation of axons and myelin	Cho et al. 2010
Primary cortical and hippocampal neurons from Wistar rat embryos cultured with amyloid-β peptide	Cell incubation with 7% and 10% PRP during 48 hours.	Increment of cell survival in primary neurons	Anitua et al. 2013
Double-transgenic APP/PS1 mice (model of Alzheimer disease)	Intranasal administration of 3μL of PRP, 3 times per week for 4 weeks.	Decrease in brain Aβ deposition, neuroprotection and reduction of inflammation	Anitua et al. 2014
BALB/c mice with hind limb sichemia	Inyection of 6-18μL of PRP into the adductor and quadriceps region 24 hours after surgery	Enhancement of reperfusion and reduction of fibrotic tissue	Anitua et al. 2015
Sprague-Dawley rats with 10-mm sciatic nerve gap	Inside-out vein graft filled with 0.15-2 mL of PRP	Increment of neoangeogenesis, number of myelinated axons and diameter of axons and myelin sheat.	Kim et al. 2014
Schwann cells from sciatic nerves of Sprague-Dawley rats	Cells cultured with different concentrations of PRP	Stimulation of cell proliferation, migration and neurotrophic function in a dose-dependent manner	Zheng et al. 2016
Brain cortex and spinal cord coculters from Sprague-Dawley rats	Coculters incubated with medium containing 5%-10% of PRP durin 14 days	Promotion of axon growth and number	Takeuchi et al. 2012
New Zeland White rabbits with 10 mm sciatic nerve defect	Implantation of poly (lactic-co-glycolic acid) conduit filled with PRP and Schwann cells in the defect	Increment of the number of regenerating nerve fibers, thickness of the myelin sheath, muscle action potential and nerve conduction velocity	Ye et al. 2012
Rabbit and dog with sciatic nerve cut	"Fibrin suture" with coagulated blood plasma previously enriched with fibrinogen	Growth of new fibers across the junction	Young et al. 1940
Isogenic spontaneous hypertensive rats with sciatic nerve gap	Implantation of vein grafts injected with PRP.	Increment of sciatic functional index	Sabongi et al. 2014
Sprague–Dawley rats 15-mm long sciatic nerve defects	Implantation of acellular nerve allografts loaded with PRP in the nerve gap	Improvement of electrophysiology response for amplitude and conduction velocity , diameter, thickness and numbers of regenerating nerve fiber	Zheng et al. 2014
Wistar rats with 1-cm long sciatic nerve defects	Implantation of collagen nerve conduit with Platelet gel	Improvement of functional and structural outcomes	Kaplan et al. 2011
Wistar albino rats with cross-sectioned sciatic-nerve	Implantation of sutured PRP-membrane sutured around sciatic nerve	Improvement of amplitude and frequency spectrum in the electromyographic data	Giannessi et al. 2014
Sprague-Dawley rats with facial nerve transection	PRP added to perineural sutures	Improvement of functional activity and neurotrophic effect	Farrag et al. 2007
Wistar rats with 1 sciatic nerve transection	PRP added to epineural sutures	Increment of myelin thickness and reduction of latency time in electromyography	Sariguney et al. 2008
Latxa sheep with common peroneal nerve crush injury	PRP membrane placed around the nerve lesion and 3 intraneural injection of 3 mL of PRP, one injection every two weeks.	Earlier electrophysiological response, increment of axonal density and reduction of muscle atrophy	Sánchez et al. 2015
C5tBL/6J mice lesioned with 1-methyl-4phenyl-1,2,3,6-tetrahydropirydine (Parkinson's disease model).	Intranasal administration of 3μL of PRP, 3 times per week for 2 weeks.	Increment of neuroprotection, improvement of motor performance and reduction of inflammatory response, nuclear transcription factor-κβ, nitric oxide, cycloxignase-2 and tumor necrosis factor-alpha.	Anitua et al. 2015
New Zeland white rabbits with carpal tunnel syndrome dextrose-induced median nerve injury	Injection of 0.3 mL of PRP into the carpal tunnel	Improvement of electrophysiological parameters and reduction of nerve swelling	Park et al. 2014
Wistar albino rats with sciatic nerve crush injury	Injections of 15μg of IGF-1 or 0.125 mL of Leukocyte rich-PRP into the crush-injured site	Improvement of functional and sensory recovery of animals treated with IGF-1	Emel et al. 2011

TABLE 1 Summary of in vitro and in vivo effects of Platelet-Rich Plasma (reprinted with permission[6])

Type of study	Clinical cases	Intervention	Outcome	Reference
Case Report n=1	Ulnar nerve trauma at the elbow, with neuropathic pain and nerve dysfunction	12-cm-long nerve gap bridged with a collagen tube filled with autologous platelet-rich fibrin during the surgery at 3.25 years post trauma	Sensory and motor recovery across nerve gap and reduction of neuropathic pain.	Kuffler et al. 2011
Case series n=27	Patients under 58 years with 2-16-cm-long nerve gap in their extremities	Nerve gaps bridged with collagen tubes filled with PRP during the surgery at 0.5-3 years post trauma	Functional recovery	Kuffler et al. 2014
Randomized control study n=20	Patients with benign parotid gland tumor presenting facial muscles and nerve deficit	Superficial parotidectomy using PRP gel	Significant improvements in several clinical parameters	Sacala et al. 2014
Double blind, randomized, control clinical trial n=60	Patients with leprosy peripheral neuropathy	One injection of perineural injection of 1mL of PRP in the posterial tibial and ulnar nerves	Significant two-point discrimination test reduction and significant VAS improvement in week 2.	Anjayani et al. 2014
Retrospective analysis n=10	Patients with persistent pudendal neuralgia after neurolysis and transposition	One injection of PRP around the pudendedal nerve after a transgluteal decompression enclosing the nerve in NeuroWrapNerve Protector	Significant pain reduction	Hibner et al. 2012
Case series n=14	Patients with median nerve injury suffering from carpal tunnel syndrome for over 3 months	One US-guided injection of 1-2 mL of PRP into the region around the median nerve at the proximal edge of carpal tunnel	Pain almost disappeared and upper limb function improved at 1 month after treatmen	Malahias et al. 2015
Case Report n=1	Patients with peroneal nerve palsy with drop foot after multiple ligament injuries of the knee	Serial US-guided intraneural and perineural infiltrations of 3-8 mL of PRP.	Significant pain and function recovery with EMG signs of reinnervation for the peroneus longus and the tibialis anterior.	Sánchez et al. 2014
Case Report n=1	6-year-old boy with perinatal cerebral palsy	On intravenous injection of 25 mL of PRP	Clear improvement in cognitive and language spheres.	Alcazar et al. 2015

TABLE 2 Summary of clinical studies of Platelet-Rich Plasma and nerve (reprinted with permission[6])

4. THE SCIENTIFIC RATIONALE BEHIND THE USE OF PRPS TO ASSIST PNI REPAIR

Six lines of evidence suggest the therapeutic potential of PRPs on neural tissue repair and regeneration (table I and II)[6]. 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 (fig. 3)[29].

Neuroprotection and prevention of cell apoptosis

Several GFs present in PRP including, NGF, BDNF, PDGF, VEGF, IGF-1, TGFB alone or in combination have been shown to exert an antiapoptotic and neuroprotective effect on mesenchymal stem cells (MSCs), neurons, SCs, and human neural stem cells[45,47-51]. PRP fibrin scaffolds enriched with NGF, BDGF, and retinoic acid and loaded with bone marrow stromal cells (BMSCs), enhance their survival and differentiation into the neural phenotype[52]. In addition, when this PRP scaffold was transplanted into the brain the viability and biologic activity of allogenic BMSC increased[53]. Moreover, neuroprotective and antifibrotic beneficial effects[22,54] 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 of mouse model of Alzheimer disease[8], 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, in the chronic intranasal administration of PRP on Alzheimer´s disease mouse model, this treatment elicits neuroprotection which is likely mediated by the activation of the antiapoptotic PIEK/Akt signalling pathway[55].

Stimulation of angiogenesis

Despite the crucial role that blood vessels play as trackers of the axonal growth cones across the injury site, and the meaningful evidence that PRP promotes angiogenesis in bone, tendon, and muscle[11,56-58] there is still a scarcity of studies assessing angiogenesis in nerve repair. Borselli et al 48 showed in an ischemic limb rodent model with a 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[17]. In this regard, it should be taken into account that fibrin is a pivotal element within PRP that provides extracellular matrix tissue with a robust and permissive 3-D matrix for angiogenesis[59].

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[23]. 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[21,54]. One example of the use of PRP as a filler of acellular nerve allografts (ANA PRP) is the work of Zheng et al[7] which, having previously shown a dose-dependent effect of PRP on the proliferation, migration and, neurotrophic function in rat SCs cutured with PRP, subsequently 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[44]. 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.[17] 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., 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[60]. Using platelet-rich fibrin (PRF) as a filler of silicon nerve guidance[61] or nerve grafts[62] 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[61,62]. The application of PRP as a suturable membrane to wrap the neurorraphy in an acute injury model of sciatic nerve neurotmesis showed diverse positive effects. Gianessi et al. observed a stronger EMG signal, a significantly larger axonal density, and a lower scar tissue in animals treated with PRP suturable membranes, and remains of PRP membranes were still present after 6 weeks post-surgery[15]. In this sense, two studies reported the positive effects of using PRP as adjuvant in nerve suture. Farrag et al[18] 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[24] found no positive effects on axonal size in sutured nerves assisted with PRP. However, they showed a better functional out-

come associated with improvement in the myelin thickness and the onset latency. In applying PRP as both filler of the injured nerve and as a scaffold to coat the nerve crush on sheep, Sanchez et al.[12] 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 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-κB (NF-κB) on astrocytes[55]. In a mouse model of Parkinson´s disease, Anitua et al[10] 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-kB activation, nitric oxide, cyclooxygenase, and tumor necrosis factor expression in the brain[10]. 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[63].

Dampening the denervated target muscle atrophy

Several animal studies have demonstrated that the application of PRP as a filler, a suturable membrane, or both, induce an earlier axonal regeneration and functional recovery[12,15,17,18,22,24,47]. This is the case reported by Sanchez et al[12] 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[15]. Anitua et al[11] 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[64], effects suggested to be mediated by the IGF-1[65]. Moreover, TGFB, an important GF within PRP, attenuates the adverse effects of chronically denervated Schwann cells, and reactivated SCs support axon regeneration in vivo[66].

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[5,67], as a liquid dynamic scaffold infiltrated perineurally[13,16,68], intraneurally, or both (as in the case of a peroneal nerve palsy[20] (and other damaged nerves). Furthermore, it has also been applied as scaffold or fibrin membranes[5,67,69] 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[67]. In a recent series of cases of surgical nerve repair, Kuffler[5] reported functional recovery in patients under 58 years whose nerve gaps of 2-16 cm were treated with collagen tube filled with PRP, after 0.5-3 years of 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[13]. 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[68]. In a case series of fourteen 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[16]. Another case report, in this case applying sequential proximal and distal ultrasound-guided PRP injections intraneurally and perineurally (fig. 3) in a common peroneal nerve palsy, Sanchez et al 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[12]. It has been reported that the intravenous injection of 25cc of concentrated platelet-rich plasma in a 6-year-old-boy with perinatal cerebral palsy is safe, and significantly improved the cognitive and language spheres[70].

5. CONCLUDING REMARKS AND FUTURE DIRECTIONS

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 the 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.

There are several areas in which the application of PRPs might be modified to improve the functional outcomes in assisting neuropathies and nerve repair techniques. So far, in the majority of studies on animals (Table I) and humans (Table II), PRP has been applied around the nerve, namely, perineurally[13,16,68,69]. We have applied PRP perineurally and intraneurally both in animal and humans (table I and II). We have implemented this combination after having already ascertained, in a sheep model, that intraneural injections of PRP previously stained with methylene blue diffused homogenously across the nerve with no adverse effects[6]. Second, when we treat nerve palsy or neuropathies such as the common peroneal nerve or carpal tunnel syndrome, the only way to accurately place PRP at the site of injury is by ultrasound-guided injections that confirm accuracy by direct visualization of US imaging. Third, we recommend performing a combination of intraneural and perineural injections, several times depending on the clinical evolution of the patient in case of nerve palsy or carpal tunnel syndrome. In the case of assisting surgical repair by PRP as in the case of end-to-end neurorrhaphy, nerve compression, or nerve entrapment, we recommend combining intraneural and perineural infiltrations of liquid PRP with the application of a PRP membrane as scaffold, which wraps the injured area as indicated in figure 3. These modifications affecting the way PRP is currently used might in our opinion produce significant functional benefits.

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