EXPERT OPINION ON BIOLOGICAL THERAPY, 2016
http://dx.doi.org/10.1080/14712598.2017.1259409

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REVIEW

Platelet-rich plasma, a source of autologous growth factors and biomimetic scaffold for peripheral nerve regeneration

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ABSTRACT

Introduction: 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. Evidence is accumulating in both preclinical and clinical settings indicating that Platelet-rich plasma (PRP) and fibrin scaffolds obtained from this technology hold an important adjuvant therapeutic potential.

Areas covered: This review addresses current molecular and cellular data in intrinsic nerve repair processes and describes different strategies to harness and enhance these processes by using biochemical and biomechanical cues. 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.

Expert opinion: PRP is applied as a filler of nerve conduits or vein-muscle grafts across nerve gaps post trauma by infiltrating the nerve stumps perineurally and intraneurally in neuropathies, or as scaffolds to bridge or wrap nerve stumps, with significant neurological recovery and pain reduction. The application of PRP at the injured nerve site might be considered as an 'off the shelf' alternative.

ARTICLE HISTORY
Received 25 July 2016
Accepted 8 November 2016

KEYWORDS
Injured peripheral nerves; Platelet-rich plasma; regeneration; growth factors; scaffold

1. Introduction

Every year, the 350,000 patients are affected by traumatic peripheral nerve injuries, which accounts for $150 billion in annual healthcare costs [1]. Different types of mechanical, thermic, metabolic, or chemical injuries bring about structural nerve disruption, gap, or neuropathy associated with a profound impact on sensory, autonomic, and motor function. Direct tension-free microsurgical repair and/or the transplantation of a nerve autograft to bridge the gap are the gold standard treatment 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 [3,4] in addition to creating, as in the case of autografts, a second iatrogenic injury and morbidity in the donor site [5]. Despite the innate ability of adult mammalian peripheral nervous system (PNS) neurons to regenerate, the functional recovery of nerve injuries is often incomplete or suboptimal leading to pain and disabilities in daily living and work activities [6–8]. Several factors hinder the naturally occurring peripheral nerve regeneration, namely, the type of mechanism, patient age, the proximity of the injury to parent cell body, and the atrophy in both distal Schwann cells (SCs) and denervated muscle tissue, among the most critical factors [9–11].

In recent years, the manufacturing of nerve guidance scaffolds, cell-based therapies, biochemical and electrical cues in the form of growth factors and electrostimulation, or a combination of some of them, have attracted research attention with the aim of synergizing and augmenting the available treatment to improve the functional nerve repair outcomes [3,8,12,13]. Biologic strategies to treat peripheral nerve injury (PNI) combined with *in vivo* tissue engineering approaches through molecular intervention and scaffolding are offering promising outcomes [2,14–17]. These new biomedical engineering strategies might assist conventional surgical and autograft nerve treatment to synergize and enhance functional outcomes [8,18].

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 [19–23] and an enhancer of sensory and motor functional nerve-muscle unit recovery [17,24,25]. Following PNI, these products also act as enhancers of sensory and motor functional nerve-muscle unit recovery [17,24,25] when harnessed by surgeons in the operating room and in the clinical setting [16,18,26–31].

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Article highlights

- Peripheral nervous system possesses a remarkable ability to regenerate, however it might lead to overestimate the fully functional recovery after PNI since even when the gold standard nerve autograft is used to bridge an injured nerve gap, the complete functional recovery is seldom achieved.
- 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.
- The potential therapeutic effects of PRPs are tightly linked to a supportive fibrin matrix containing several neurotrophic and neurotropic factors whose sustained and gradual release appear to be instrumental agents that govern inflammation, angiogenesis, and macrophage polarization.
- PRP speeds up axonal functional recovery which may minimize muscle atrophy and fibrosis, leading to functional recovery of the nerve-muscle unit.
- PRP applied on nerve repair and neuropathies in a combinatorial strategy as a filler and as a perineural and intraneural ultrasound-guided injections, suturable membrane, and scaffold, is a candidate for an adjuvant approach to be harnessed by surgeons in the operating room and in the clinical setting.

This box summarizes key points contained in the article.

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

2. Degeneration and regeneration after PNI: molecular and cellular events

Peripheral nerves are discrete troncular organs made up of bundles of myelinated and unmyelinated sensory, motor, and autonomic axons that are surrounded by myelinating SCs sheaths (Figure 1). Following a PNI, an orchestrated multicellular and pleiotropic molecular response will ensue where the interplay among SCs, resident macrophages, endothelial cells, and fibroblasts, mainly modulated by injured axons, myelin breakdown products, soluble factors, and hypoxia as main signals, 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 (Figure 2) [11,32,33].

At the site of the injury, the noxious agent disrupts the blood–nerve barrier, rendering the nerve gap temporarily bridged by the plasma-fibrinogen leakage and fibrin deposition, as well as by the survival of migrated SCs from both nerve stumps [11,34,38]. In addition, the injury area will be invaded by blood monocytes and by the proliferation of activated resident macrophages. 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 transdifferenciation. 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 [32], influencing SC migration and dedifferentiation [32,39,40]. SCs show a striking chameleonic response to the biological battlefield they are exposed to inside a damaged nerve and are the early detectors of damage (Figure 3). Together with M1 macrophages, the already dedifferentiated SCs play an early and key role in removing nonpermissive myelin debris such as myelin-associated glycoprotein and oligodendrocyte-myelin glycoprotein, that might act as endogenous ligands such as heat-shock proteins, mRNA, and ECM breakdown products (tissue-damage and pathogen molecules DAMPs and PAMPs), although the mechanism that drives SC phagocytosis is poorly understood [41]. 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 [41,42]. In a context- and timing-dependent manner, dedifferentiated SCs perform a variety of cell repair tasks from phagocytizing myelin debris to secreting neurotrophic and neurotropic factors (laminin), to proliferation and migration which results in the formation of SC cords and Bungner Bands in the proximal and distal nerve segment, respectively [11,39,41]. Despite the key role played in the regeneration of PNS, SCs do not operate in a '*cellular vacuum*' [43,44]. Rather, SCs establish a multidirectional partnership with injured axons, macrophages, and fibroblasts thereby orchestrating a coordinated and robust nerve repair response [11,32,35,44]. At distal stump, intrinsic axonal degeneration and myelin breakdown trigger a complex and constructive series of events involving SCs, resident and recruited macrophages, fibroblasts, as well as T lymphocytes, all of which contribute to building an inductive and permissive microenvironment tailored for axonal regrowth [9,39], a process called Wallerian degeneration [11,41]. Although SCs have the reputation of being the engine of peripheral nerve repair, in the nerve repair complex voyage they are fueled by axon growth cones and supportive stromal cells such as macrophages and fibroblasts, the very elements of Wallerian degeneration as a neuroinflammatory process [32–34,39,41]. Macrophages are quite numerous and phenotypically diverse in the immune cell population during nerve injury, whose activation and polarization substantially contribute to nerve regeneration [45]. Emerging evidence suggests that macrophage plasticity contributes to peripheral nerve regeneration via distinct mechanisms: by phagocytizing 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 [33,34,45]. Cattin et al. [33], confirmed an idea suggested by Chen et al. [35] that blood vessels might provide substrate or signaling for axon growth guidance and SC migration by showing that macrophages selectively sense hypoxia in the nerve bridge and drive angiogenesis at the nerve bridge via the VEGF-secretion pathway. Despite the robust repair capacity to regrow peripheral nervous axons shown in the adult mammal [33,41], and meticulous

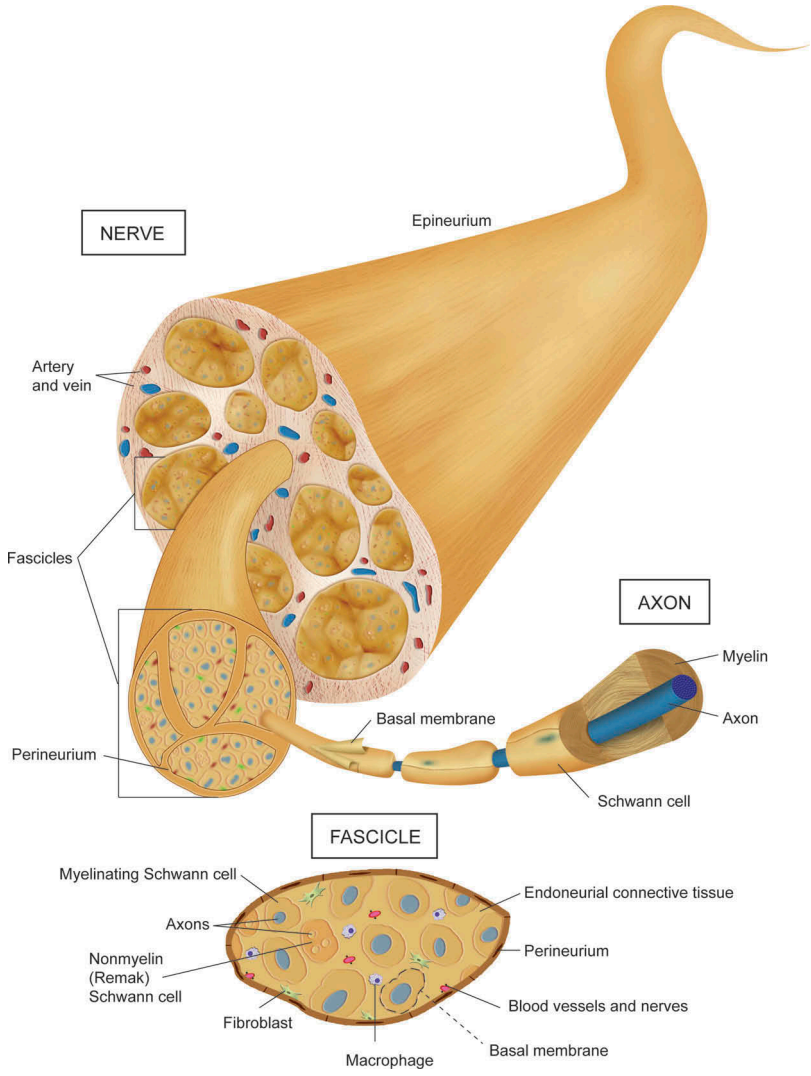

Figure 1. Peripheral nerve structure. Axons are wrapped and in close connection with the basal membrane (lamina), a rich laminin, heparin sulfate proteoglycans (HSPGs) and type IV collagen tube, and constitute the parenchyma of the nerve [29,34] A well vascularized, loose and soft stromal connective tissue, and collagen-rich extracellular matrix containing resident endoneurial macrophages, endothelial cells and fibroblasts called endoneurium surrounds and protects axon-schwann cell basal lamina tubes [30,34–36]. Groups of these axons are enclosed by the perineurium, a multilayered collagenous tube containing vessels and nerves that protect the axon compartment [31,37], forming fascicles. The fascicles are bound by a sheat of loose and elastic fibro-collagenous tissue known as epineurium and constitute a nerve trunk.

microsurgical nerve repair techniques, changes in both chronically denervated SCs and muscle tissue often play havoc with complete functional nerve regeneration [8,11,43]. 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 re-innervate target organs/tissues, as well as their atrophy, and the rate of regeneration, are among the most critical limiting factors [9–11,43]. Furthermore, the prolonged denervation of the distal segment results in an extraneurial and endoneurial accumulation of fat and changes in the distribution of collagen types generating fibrosis, painful dysesthesiae and even a secondary axonal degeneration by compromising the microvascular bed, thereby rendering the target tissue nonreceptive to innervation by new axons [9]. The scarring may even be more severe at the nerve bridge, leading to a misdirectional axonal growth whose prime example is the 'neuroma in continuity' in which the distal and proximal stump are connected but fail to use their bridge [11,43,46].

3. Enhancing and speeding up endogenous mechanisms that support peripheral nerve regeneration

Despite the fact that autologous nerve grafts which are considered the 'gold standard' contain longitudinally aligned and acutely denervated SCs in a natural collagen scaffold, nerve autografting presents some drawbacks, including the need for a secondary surgery and loss of function at the donor site, mismatches in size and internal structure between the harvested nerve and repaired nerve as well as in autograft phenotype, longer surgical times, a limited source of nerve grafts, and the variability of clinical outcomes [5,8,48,49].

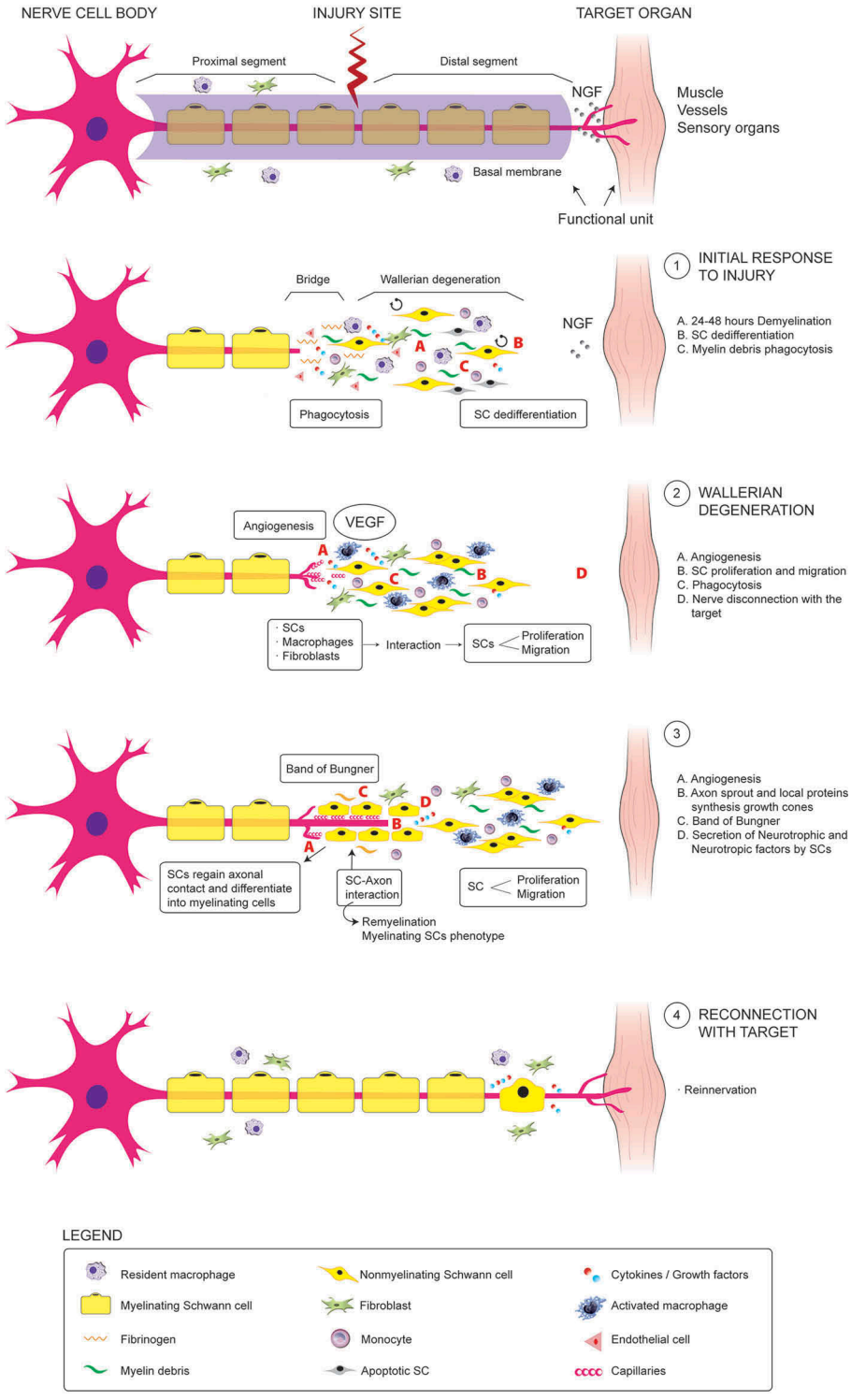

Figure 2. After a crush or transection injury, the proximal and distal nerve stump retracts, generating a gap that will be bridged by a new tissue which will channel new axon cones accompanied by SCs marshalled into cords by cues coming from fibroblasts and guided by neovessels, and these should eventually reach the distal stump and form functional synapsis with their target organs [32,33]. Since the distal portion of the nerve undergoes an intrinsic active process of self-destruction known as Wallerian degeneration [11,33,38], the complete nerve-target functional recovery relies on several factors including a permissive and instructive microenvironment both at the site of injury and at the distal stump, a neuroprotective and trophic stromal cells, and a supportive target [9,11,38].

3.1. Defining targets to devise strategies to aid nerve regeneration

A great many biomedical engineering strategies have been developed to circumvent the neurobiological hurdles that emerge from an inflammatory and hypoxic microenvironment at the site of the injury, among other factors. These two drawbacks are conducive to neuronal death, endoneurial and perineurial fibrosis [3,8,50,51] and to atrophy of chronically denervated SCs and muscle tissue, which are among the most critical roadblocks to achieve a functional nerve repair [9–11]. These strategies include nerve guidance conduits

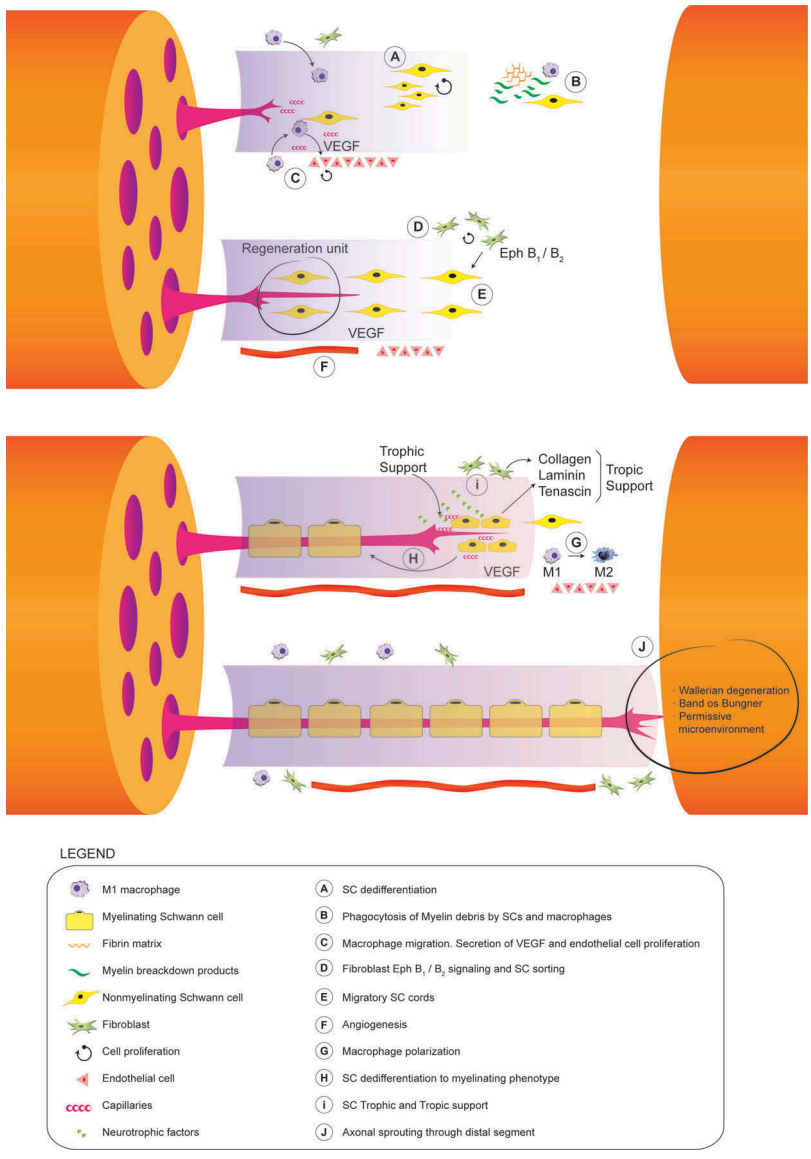

Figure 3. The partnership between the transdifferentiated SCs and macrophages induce the latter to synthesize VEGF [33]. In addition to stimulating the proliferation of endothelial cells, and thereby promoting new vessels that guide the axon growth, thereby serving as tracks for migrating and proliferating 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 [33,47].

(NGCs) and scaffolds [5,8,14,49,52], incorporation of neurotropic factors [35,37,53–56] and delivery of neurotrophic factors [12,36,57–60], incorporation of support cells into NGCs [3,12,61–64], and stimulation of target organs through either neurorehabilitation [65] or intramuscular injections of cells and GFs [66,67].

3.2. Nerve guidance scaffolds

Designing ideal conduits and scaffolds for peripheral nerve repair that reenact physical, chemical, and biological properties of nerve autograft, and thereby fulfilling the whole array of neurobiological roles necessary to nerve repair, is a complex task [14,48]. Tissue-engineered nerve constructs and scaffolds have to support axonal growth, cell migration, and vascularization, allow the delivery of growth factors, contain intraluminal topographical microgroove surfaces and guiding cues such as laminins, collagens, and fibronectin, be porous for oxygen diffusion, have a low antigenicity and be biodegradable in order to integrate with the local ECM [15,49,62,68].

Natural material such as collagen, fibrin, allografts and acellularized allografts alone [69,70] or seeded with autologous SCs [64], bone marrow mesenchymal stem cells (BMSCs) [71], Adipose-derived stem cells (ADSCs) [72] as well as different types of biological tubes including veins, muscles, and tendons, are all conduits with moderate clinical success [46,52]. In addition, there are significant potential side effects of allografts and decellularized allografts arising either from the necessity of 2 years of immunosuppression or from the toxicity of chemical and physical methods applied to remove the immunogenic part as well as the risk of disease transmission [4,52].

As early as 1940 [21], Young et al. devised a procedure called ‘fibrin suture’ to hold the injured nerve stumps together, consisting of applying a coagulated blood plasma

previously enriched with fibrinogen, thereby obtaining as successful a regeneration as with the traditional end-to-end suture in experimental animals. Two years later, Seddon and Medawar, implemented this approach in humans to carry out a median nerve repair which led to sensory recovery eleven month after the nerve suture [28]. Fibrin network is a web of cross-linked fibrils generated from the polymerization of soluble fibrinogen by the enzyme serine protease thrombin, and is an essential part of the healing clot formed between the nerve stumps and within the nerve after damage, supporting angiogenesis and guiding the cell migration and nerve growth dynamic as propelling processes in nerve repair [4,73–76]. This initially acellular matrix attract fibroblasts, SCs, resident macrophages and monocytes [5,48], and modulates cell behavior through the numerous adhesion molecules and heparin sulfate-binding domains for GFs, ECM proteins, and cells [77,78]. Due to its rich bioactivity, its ease of tunability with neurotrophic factors and Schwann and mesenchymal stem cells (MSCs), and the potential as an autologous source of versatile fibrin-based biomaterials such as fibrin gels, fibrin sealants, fibrin scaffolds, polymeric modification through PEGylation, and fibrin filler biomaterial, the fibrin network has been used for a range of therapeutic applications [49,66,78]. These biomaterials have shown to enhance regeneration, nerve conduction velocity, and motor neuron regeneration [13,71,79]. It is noteworthy that injectable forms of fibrin seeded with cells and/or functionalized with GFs such as VEGF, BDNF, PDGF among other, have been shown to support and enhance axonal growth and improve functional nerve repair [12,17,47].

Despite the versatility and safety of fibrin scaffolds, some natural biomaterials present potential drawbacks from their lack of tunability of mechanical properties. The development of material technology and synthetic nerve guides is an ever-expanding field, primarily using conduit materials derived from polyesters such as polyglycol acid, polylactic acid, polyhydroxybutyrate and polycaprolactone [8,56,68]. This new synthetic approach has incorporated the concept of an active scaffold which includes the protective-barrier function by adding physiochemical and biological cues that imbed the conduits in a permissive and active microenvironment [3]. Although they are promising candidates, their potential has to be translated into the neural clinical application, and we are awaiting for more *in vivo* clinical studies [80].

3.3. Cells and signaling factors to promote nerve regeneration

Recent tissue engineering developments have switched the concept of passive NGC which only offered a permissive space for the axon outgrowth to the functionality of scaffolds by adding supportive cells such as SCs, neural stem cells (NSCs), bone marrow-derived MSCs (BM-MSC), macrophages [12,45,81], neurotrophic factors [12,47,57,82], and permissive ECM topographical directional guidance cues such as laminins and fibronectin and other basal membrane components [35,83]. Owing to their central role in nerve regeneration, SCs have been utilized for seeding venous grafts or other tissue-engineered conduits as transplantable cells in nerve repair research with improved repair outcomes However, there exist several problems in the clinical translation of autologous SCs, such as the sacrifice of a functional nerve, which together with the limited expansion capabilities of SCs, have made researchers to look for alternative cell sources [3]. Experimentally, transplantable allogeneic or syngeneic SCs [81,84] as well as SCs, BM-MSC, ADSCs, neural stem cells, and embryonic stem cells combined with natural or synthetic conduits have been used for nerve repair with beneficial outcomes and we suggest two reviews on this topic [8,85,86].

In a recent *in vivo* investigation in adult rat that underwent complete spinal cord, Lu et al. [12] showed that by injecting neural stem cell grafts suspended in a fibrin gel containing a cocktail of growth factors into the injured site, a bridge 2 mm long became available in the reaction area resulting in functional recovery. PRP scaffolds have been used as tissue engineering constructs embedded with several cell phenotypes with promising results [87–91].

3.4. Gene therapy and peripheral nerve repair

Gene therapy entails the introduction of a foreign gene into neural stem cells, MSCs, SCs or fibroblasts prior their transplantation to the damaged area. Likewise, the viral vector conveying the foreign transgene can be delivered through injection into the dorsal root ganglia and motor neurons located in the ventral area of the spinal cord, and in the distal denervated nerve stumps where SCs have lost their regenerative potential [92]. These genetically engineered cells overexpress neurotrophic factors such as BDNF, NGF, VEGF [93], key transcription factors or proteins and ECM compounds that either contribute to axon regrowth or to silence genes encoding inhibitory biomolecules [92,94] biological effects that in some ways partially overlaps with the effects of plasma rich in growth factor therapy since both strategies seek to modify the neurotrophic factor concentration which are conducive to reinforcing the intrinsic robust regenerative nerve process.

To date, gene therapy has not been tested in clinical intervention in human peripheral nerve system injuries. This approach, however, has been a useful tool in ascertaining and revealing the gene expression program which underlies peripheral nerve regeneration [92], and it is close to reaching readiness for clinical translation. For a deeper understanding of this approach, we recommend an excellent review by Hoyng et al. [95].

4. Plasma rich in growth factors: an injectable scaffold to assist in nerve repair

4.1. What is PRP?

PRPs are a blood-derived biological drug delivery products that have emerged as a novel and versatile formulations 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 [17,96,97]. These varied products consist of a pool of GFs, microparticles, and other bioactive mediators stemmed from platelet activation and plasma. 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 [98,99]. As to the PRP preparation and characterization, these have been extensively described in a recent review article published in this journal [100].

4.2. PRP as an emergent coadjuvant treatment to assist PNI repair

Growing *in vitro* and *in vivo* evidence involving neural tissue indicates that PRP and fibrin scaffold obtained from this technology hold an important therapeutic potential not only as a neuroprotective, neurogenic, neuroinflammatory modulator drug delivery system [19,23,25,101,102] but also as enhancer of sensory and motor functional nerve-muscle unit recovery [24,25,27,103,104] after PNI. These products may be harnessed by surgeons in the operating room and in the clinical setting as adjuvant treatment to assist PNI repair and neuropathies [16,18,21,26,27,101,103–105]. (Tables 1 and 2)

4.3. Scientific rationale behind the use of PRPs

Once 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 [17], (Figure 4) tissue fibrinolysis breaks the fibrin down, thereby releasing cell signaling molecules such as neurotrophic (NGF, BDGF, IGF-1, PDGF, VEGF, HGF) and neurotropic factors (fibrin, fibronectin, and vitronectin) [115]. In contrast to a bolus delivery modality of GFs, which has been shown to be less efficacious in the repair process [47], tissue fibrinolysis mediates in PRP gradual and sustained release of several GFs and other biomolecules [66].

These biomolecules have been shown to be instrumental instructive and permissive agents that govern early inflammation, stem cell-like myelinating SC activation, angiogenesis, macrophage polarization, as well as the active resolution of inflammation, angiogenesis, and fibrogenesis, thereby acting as key drivers of full nerve function recovery [25,33,39,69,116]. There are so far six lines of evidence that suggest the therapeutic potential use of PRPs on neural tissue repair and regeneration (Figure 4).

4.3.1. Neuroprotection and prevention of cell apoptosis

Several growth factors including, NGF, BDNF, PDGF, VEGF, IGF-1, TGFB alone or in combination have been shown to exert an antiapoptotic and neuroprotective effect on MSCs, neurons, SCs, and human neural stem cells [47,58,69,117–119]. However, it appears that *in vivo*, rather than a single growth factor application, the combinatorial approach of fibrin matrix containing a growth factor cocktail is offering promising neural tissue repair outcomes [12,47,90]. In this sense, PRPs are an attractive alternative that fulfill these combinatorial strategy requirements. PRP fibrin scaffolds enriched with NGF, BDGF, and retinoic acid and loaded with bone marrow stromal cells (BMSCs) enhance their survival and differentiation into neural phenotype [87]. In addition, they reported that when this PRP scaffold was transplanted into the brain the viability and biologic activity of allogenic BMSC increased [88]. Moreover, in a bilateral cavernous nerve injury rat model and facial nerve guinea pig model, the injection of PRP into the corpus cavernosum and facial nerve suture was assisted with PRP, neuroprotective and antifibrotic beneficial effects [89,106] were reported. In a recent *in vitro* study on neuronal cultures of mouse model of Alzheimer disease [22] showed that the neurotoxicity induced by aggregated B-amyloid added in primary neuronal cultures was significantly reduced the living cell number increased after the co-treatment with PRP [22]. In addition, in the chronic intranasal administration of PRP on Alzheimer's disease mouse model elicits neuroprotection which is likely mediated by the activation of the anti-apoptotic PI3 K/Akt signaling pathway [107].

4.3.2. 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 [66,120–122], there is still a scarcity of studies assessing angiogenesis in nerve repair. Borselli et al. [47] showed in a 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 increased angiogenesis. In this sense, intramuscular infiltrations of PRP enhance angiogenesis and improve reperfusion after a severe-induced skeletal muscle ischemia [66]. 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 [108]. Fibrin is a pivotal element within PRP that provides ECM tissue with a robust and permissive 3-D matrix for angiogenesis [123]. In fact, autologous fibrin matrix is the best tailored transient scaffold for tissue regeneration where complex morphogenetic processes for tissue regeneration take place, as is the case of angiogenesis, cell migration, and proliferation [74,123].

4.3.3. Enhancing axonal outgrowth capacity

In a rat spinal-cord injury model, the infiltration of a liquid scaffold composed of neural stem cells of rat or humans bathed in a cocktail of growth factors mixed into a fibrin gel promoted the repair injury by extending axonal growth and formation of new connections with host axons [12]. Except for the cell transplantation, the repair strategy used by Lu et al. [12] parallels in many respects the PRP infiltrations approach, with a supportive fibrin matrix containing several growth factors. 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 axon growth and numbers as well, a positive effect that was significantly suppressed when antibodies against IGF-1 and VEGF were added [109]. PRP has been used as a cellular carrier, and two studies in acute nerve injury model in guinea pig and rabbits applied PRP seeded with either MSCs or SCs, reporting beneficial effects on axonal counts, myelination, and electrophysiological parameters [89,91]. One example of the use of PRP as a

Table 1. Summary of *in vitro* and *in vivo* effects of platelet-rich plasma.

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. [87]
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. [88]
Sprague-Dawley rats with bilateral cavernous nerve crush	Injection of 200 µL of PRP into the corpus cavernosum immediately after crush injury	Preservation of myelinated axons and prevention of cell apoptosis	Wu et al. [106]
Albino guinea pigs with facial nerve transection	Injection of 5 mL of PRP and perineural microsuture	Improvement in function, increment of neurotrophic factors and preservation of axons and myelin	Cho et al. [89]
Primary cortical and hippocampal neurons from Wistar rat embryos cultured with amyloid-β peptide	Cell incubation with 7% and 10% PRP during 48 h.	Increment of cell survival in primary neurons	Anitua et al. [22]
Double-transgenic APP/PS1 mice (model of Alzheimer disease)	Intranasal administration of 3 µL of PRP, 3 times per week for 4 weeks.	Decrease in brain Aβ deposition, neuroprotection and reduction of inflammation	Anitua et al. [107]
BALB/c mice with hindlimb ischemia	Injection of 6–18 µL of PRP into the adductor and quadriceps region 24 h after surgery	Enhancement of reperfusion and reduction of fibrotic tissue	Anitua et al. [23]
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 sheath.	Kim et al. [108]
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. [20]
Brain cortex and spinal cord cocultures from Sprague-Dawley rats	Cocultures incubated with medium containing 5%-10% of PRP during 14 days	Promotion of axon growth and number	Takeuchi et al. [109]
New Zealand 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. [91]
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. [21]
Isogenic spontaneous hypertensive rats with sciatic nerve gap	Implantation of vein grafts injected with PRP	Increment of sciatic functional index	Sabongi et al. [104]
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. [25]
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. [110]
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. [19]
Sprague-Dawley rats with facial nerve transection	PRP added to perineural sutures	Improvement of functional activity and neurotrophic effect	Farrag et al. [111]
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. [112]
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. [17]
C5tBL/6 J mice lesioned with 1-methyl-4phenyl-1,2,3,6-tetrahydropirydine (Parkinson's disease model)	Intranasal administration of 3 µL 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. [23]
New Zealand 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. [113]
Wistar albino rats with sciatic nerve crush injury	Injections of 15 µg 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. [58]

Table 2. Summary of clinical studies of platelet-rich plasma and nerve.

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. [103]
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. [18]
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. [101]
Double-blind, randomized, control clinical trial *n* = 60	Patients with leprosy peripheral neuropathy	One injection of perineural injection of 1 mL 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. [24]
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. [105]
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 treatment	Malahias et al. [27]
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. [16]
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	Alcaraz et al. [114]

Figure 4. There are so far six lines of evidence that 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.

filler of acellular nerve allografts (ANA PRP) represents the work of Zheng et al. [25] that, having previously shown a dose-dependent effect of PRP on the proliferation, migration and, neurotrophic function in rat SCs cultured with PRP [25], they reported 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 [25]. 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. [108] observed that the number of myelinated axons, the axon diameter and myelin sheath were significantly superior when PRP was used as a filler, results that are in accord with the work of Kaplan et al. [110] 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 [110]. Using platelet-rich fibrin (PRF) as a filler of silicon nerve guidance [124] or nerve grafts [104] in a rat model, the PRP-treated animal improved functional recovery and showed a superior sciatic functional index to non-treated animals; however, the researchers did not find morphometric or structural improvements [104,124]. On the wake of Seddon and Medawar [28] perspective, the application of PRP as a suturable membrane to wrap the neurorrhaphy in an acute injury model of sciatic nerve neurotmesis showed a stronger EMG signal, a significantly larger axonal density, and a lower scarring tissue in treated animal with PRP suturable membranes, and remains of PRP membranes were still present 6 weeks post-surgery [19]. In this sense, two studies reported the positive effects of using PRP as adjuvant in nerve suture. Farrag et al. [111] 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. [112] found no positive effects on axonal size in sutured nerves assisted with PRP, but they showed a better functional outcome associated with improvement in the myelin thickness and the onset latency. Sanchez et al. conducted a study on sheep applying PRP as both filler of the injured nerve and as a scaffold to coat the nerve crush, and reported an earlier electrophysiological response, a higher axonal density, and lower muscle atrophy in treated animals compared with the saline or spontaneous regeneration groups. Therefore, PRPs might significantly contribute to the three key events to axonal outgrowth, namely, the already mentioned angiogenesis, the axon-SC partnership, and a permissive and inductive microenvironment.

4.3.4. Overcoming the inflammatory microenvironment

Though indirect, two important pieces of evidence in neural tissue support the anti-inflammatory effect of PRP. Anitua et al. reported that astrocytes cultured with B-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 NFkB on astrocytes [107]. In a mouse model of Parkinson´s disease, Anitua et al. [23] showed that the neuroinflammatory process, mediated by microglia, was reduced, together with improvement in motor performance, responses that were associated with a robust reduction in nuclear transcription factor-kB (NF-kB) activation, nitric oxide, cyclooxygenase, and tumor necrosis factor expression in the brain [23], results that are in accord with studies reporting that PRP or some bioactive elements within it such as HGF, IGF-1, PDGF, and TGFB inhibit the NFkB [100] on synoviocytes, fibroblasts, tenocytes, chondrocytes, and switch macrophages from M1 to M2 phenotype [125–127]. In a rabbit model of dextrose-induced median nerve injury, the injection of PRP into the carpal tunnel of rabbits injured 4 week before, exerted a significant reduction in nerve swelling compared with the control group [113].

4.3.5. Dampening the denervated target muscle atrophy

One beneficial outcome directly derived from accelerating spontaneous axonal growth is the shortening of time to establish a connection between the axonal sprouting with the target muscle, which can account for the attenuation of the muscle atrophy [128]. Several animal studies have demonstrated that the application of PRP as a filler, as a suturable membrane, or both, induce an earlier axonal regeneration and functional recovery [17,19,58,106,108,111,112]. This is the case reported by Sanchez et al. [17] on sheep, where nerves repaired assisted with PRP was associated with an earlier electrophysiological recovery and a 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 [19]. Anitua et al. [66] showed that intramuscular injection of PRP 24 h after the induction of limb ischemia in mice, mitigates fibrosis and muscle atrophy, results that are in agreement with the reduction of atrophy in denervated muscle reported when muscle was infiltrated with cells [67], effect suggested to be mediated by the IGF-1 [129]. Moreover, TGFB, an important GF within PRP, attenuates the adverse effects of chronically denervated SCs, and reactivated SCs support axon regeneration *in vivo* [130].

4.3.6. The improvement of neurologic parameters in humans (**Table 2**)

In the wake of promising results in animal experimentation, PRP has been applied either as filler of nerve conduits across post-traumatic nerve gaps [18,103], as a liquid dynamic scaffold infiltrated perineurally [24,27,105], intraneurally, or both as in the case of a peroneal nerve palsy [16] (Figure 4) and other damaged nerves (Figure 5) or as scaffold or fibrin membranes [18,101,103] with some beneficial outcomes and better functional recovery. Kuffler applied autologous PRF 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 [103]. In a recent series of cases of surgical nerve repair, Kuffler [18] 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 [24]. 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 pain reduction [105]. 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 8 patients, and a pain alleviation in 3 patients at 3 months of follow-up [27]. Another case report, in this case applying sequential proximal and distal ultrasound-guided PRP injections intraneurally and perineurally in a common peroneal nerve palsy, Sanchez et al. reported a significant functional

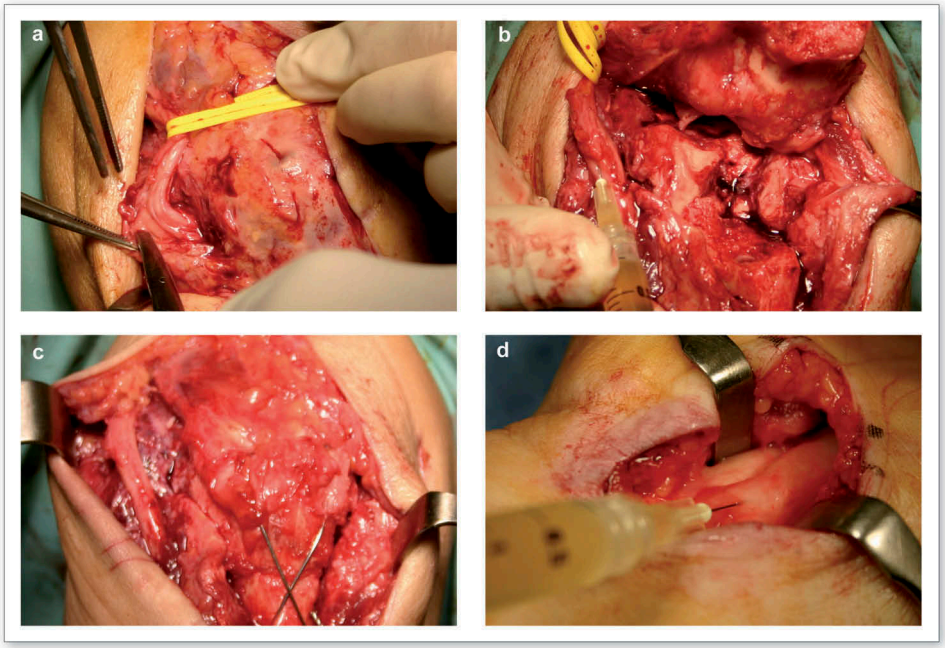

Figure 5. PRP application on peripheral nerve pathologies. (A) Ulnar nerve entrapment after a complex elbow fracture surgically treated . (B) Intraneural infiltration of ulnar nerve with PRP. (C) Appearance of the ulnar nerve after the PRP infiltration. (D) Median nerve infiltration in a chronic carpal tunnel syndrome.

recovery assessed by electromyographic signs of reinnervation for both peroneus longus and tibialis anterior muscles as well as almost full recovery of sensitivity [16]. 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 significantly improved the cognitive and language spheres [114]. However, almost all of the data obtained from humans lack a control group and/or the size of studied groups is small, which diminishes their respective clinical significance.

5. Conclusion

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 help overcome post-traumatic and neuropathic inhibitory microenvironment by combinatorial strategy of delivering neurotrophic and neurotropic factors. They may assist nerve conduit guidance and grafts as a filler, as a liquid in intraneural and perineural injections in nerve entrapments and fibrosis, and as a scaffold to bridge or wrap the injured nerve gap.

6. Expert opinion

The ultimate goal of any peripheral nerve repair strategy is the restoration of nerve-target organ function, while minimizing therapeutic side effects. PRP in its two formulations of liquid and scaffold, fulfills the requirements to be considered as optimum scaffold for nerve repair [49] since they provide the injured site and severed nerve ends with a biodegradable scaffold via normal metabolic pathways, and which will, in 1–3 weeks, be removed [98,131]. Its resorption modulates SC phenotypes [131,132], serves as a mechanical support for cell homing and a guidance for regenerating axons [133], and conveys a plethora of neurotrophic and neurotropic factors [98,134], with nonantigenic and no toxic reactions. In this sense, PRP applied in a combinatorial strategy as a filler, suturable membrane, and scaffold, stands out as a promising candidate for an adjuvant approach which may be harnessed by surgeons in the operating room and in the clinical setting [16,18,21,26,27,43]. Moreover, there are nontraumatic peripheral injuries where the main pathological agent is compression, adhesion and fibrosis, resulting in pain, loss of muscle strength, and dexterity for months, as in the case of carpal tunnel syndrome and fibrotic post-surgical side effects [68]. In these neurological conditions, this novel approach applied as perineural and intraneural injections may additionally avoid or at least diminish undesirable consequences such as fibrotic scars and denervated organ atrophy, thereby speeding up the functional recovery of the nerve-muscle unit [16,91,106,109,112]. The therapeutic potential of PRPs is tightly linked to both the type and number of biologically active mediators as well as the way they are delivered to the tissue ECM, thereby fulfilling two vital criteria: first to produce a prolonged and gradual delivery system of growth-survival-chemotaxis-, and anti-inflammatory supporting cues, and second to function as a transient nerve-guidance scaffold for axonal sprouting [12,17]. In the light of this basic and clinical evidence, it is reasonable to speculate that neurotrophic and neurotropic factors within the PRP [22] might exert their synergistic action in a biphasic manner on SC phenotypes, which are the masters and servers of the nerve regeneration process. At the onset of repair process in the injured area assisted by PRP, release of FGF, PDGF, IGF-1, NGF, BDGF from the fibrin matrix would induce a powerful SC proliferation [36,131], whereas fibrin and fibronectin would simultaneously promote migration and homing while inhibiting the differentiation of proliferating SC [131,133]. As a result of local activation of the tissue plasminogen activator/plasminogen system by PRP molecular intervention, the leaked fibrinogen,

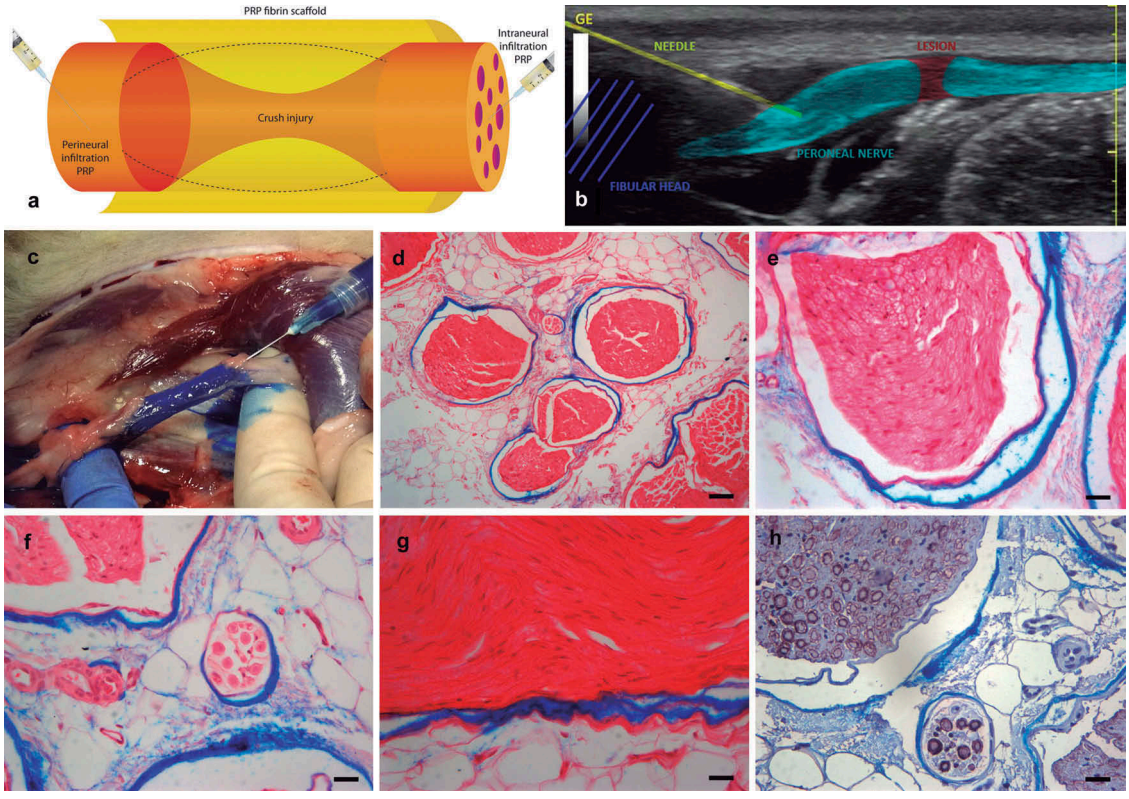

Figure 6. Preclinical investigation in an ovine model of nerve crush injury. (A) Schematic drawing showing the treatment applied in lesions of peripheral nerve. (B) Ultrasound image during the infiltration procedure showing details of anatomical structures during common peroneal nerve (CPN) infiltration. (C) CPN infiltration of activated PRP previously stained with blue indian ink. D to H show histological sections of CPN stained with blue indian ink. D to G preparations are counterstained with eosin and H is a inmunostaining of Schwann cells performed by S100β antibody (brown) and counterstained with hematoxilin. (D) Cross-sectional panoramic view of several fascicles. A detail at high magnification of a large fascicle is provided in E panel, while in F a fascicle of small caliber is shown. (G) Nerve longitudinal section in which a fascicle can be observed at the top of the microphotograph. (H) Schwann cells inmunostaining and blue indian ink location. In all cases, the greatest accumulation of blue stained PRP is observed around the perineurum, but it should be emphasized that the PRP is distributed throughout the endonerium, namely intraneural injections, but not intrafascicular. Apparently the PRP does not pass through the perineurum. Scale bars: 100 µm for D and 400 µm for E to H. Panel B is reprinted from [17] with permission. Full color available online.

stemmed from the disruption of the blood–nerve barrier and deposited in the damaged nerve, would gradually be removed [98,135]. This new SC microenvironment would give way to a nonproliferating, myelin-synthesizing SC phenotype thereby carrying out the re-myelination of the newly sprouted axons [118,131,132].

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 [58,89,91,112] (Table 1) and humans (Table 2), PRP has been applied around the nerve, namely, perineurally [24,27,101,105]. Only our group has applied PRP perineurally and intraneurally both in animal and humans [16,17]. We implemented this combination having already ascertained, in a sheep model, that intraneural injections of PRP previously stained with methylene blue diffused homogenously across the nerve as shown in Figure 6 with no adverse effects. 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 [16,27]. Third, we recommend performing a combination of intraneural and perineural injections, several times every three weeks in the case of nerve palsy or carpal tunnel syndrome [16]. In the case of assisting surgical repair by PRP as in the case of end-to-end neurorrhaphy, nerve compression, 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 Figures 4 and 6. These modifications affecting the way PRP is currently used might in our opinion produce significant functional benefits.

The molecular intervention with PRPs is partially bridging the gap between the basic and clinical application, despite the scarcity and urgent necessity of clinical trials, as is the case of any nascent biologic therapy. Many question and uncertainties persist and efforts to rapidly translate basic to clinical application tend to overtake our basic-science knowledge of the biological roles of this therapy. The cart is said to be ready but the horses are not in the harness yet.

Acknowledgments

The authors thank Fernando Yanguela for assistance with Ultrasound guidance, and Sara Rivas for the elaboration of drawings.

Funding

This manuscript has not been funded.

Declaration of interest

E Anitua is a Scientific Director of BTI Biotechnology Institute, a denal company that investigates the fields of implantology and PRGF-Endoret technology. S Padilla, G Orive and R Prado are also scientists at the BTI Biotechnology Institute. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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