Expert Opinion

Platelet rich plasma therapies for sports muscle injuries: any evidence behind clinical practice?

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Introduction: At present, no drugs are available to hasten restoration of muscle function after injury. Platelet-rich plasma (PRP) therapies may help athletes by promoting muscle regeneration.

Areas covered: This is a systematic review assessing the evidence base for PRP therapies in the management of muscle injuries. A computerized literature search, citation tracking and hand searching for original studies assessing the effect of PRP therapies on skeletal muscle cell biology, skeletal muscle repair, or regeneration in animals or humans was performed. No randomized trials have studied the merits of PRP injections for muscle healing. Clinical studies indicated that PRP therapies may enhance muscle repair after strain or contusion, and laboratory data indicated that they can enhance diverse aspects of myogenesis. However muscle injuries present a complicated picture that includes many components other than muscle cells, such as blood vessels, connective tissue and neural components.

Expert opinion: The field is relevant but under-researched. No PRP formulation has yet displayed proven solid evidence for the stimulation of healing and recovery after sports muscle injuries. Therefore, major issues, including standardization of formulations and application procedures, need to be addressed to inform clinical studies before recommending best practice guidelines.

Keywords: platelet-rich plasma, regeneration, skeletal muscle, sport injuries

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1. Introduction

Muscle injuries resulting from extrinsic or intrinsic mechanisms are extremely common in sports, accounting for about 35 – 45% of all injuries [1], with contact sports and sports that require the production of large eccentric forces presenting the highest risk [2,3]. The vulnerability of athletes [4] to strains and contusions represents a substantial problem for professional players and their clubs. Such injuries involve significant time lost from training and competition. Given the increasing demands of training and competitions, treatment modalities able to accelerate recovery from muscle injuries without adversely affecting recurrence rate whilst minimizing scarring are of paramount consequence.

At present, no drugs have been proven to hasten the restoration of muscle function after injury. Therefore, in the absence of any available evidence-based treatments, injection therapies may be an important option to help professional athletes [5]. Among the injected agents are Traumeel® (a homeopathic formulation), Actovegin® (an amino acid mixture) [6-8] and autologous serum [9] or platelet-rich plasma (PRP) [10]. PRP involves the use of the patients' own proteins to restore tissue integrity and function. Initially, PRP therapies were developed to treat cutaneous ulcers [11], but an increased understanding of the biological properties

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Article highlights.

- In clinical management of muscle injuries, the current hypothesis is that intramuscular injections of platelet-rich plasma (PRP) deliver supraphysiological concentrations of growth factors and cytokines to the injured site, influencing cell migration, proliferation, differentiation or fusion and ultimately enhancing muscle regeneration.
- Given the biocompatibility of using the patient's own proteins, safety is guaranteed, simplifying translation from the laboratory to the patient. However the rapidity of translation has sparked debate regarding the level of evidence of clinical benefit needed to introduce PRP technologies in the sports medicine setting.
- No randomized trials have tested PRP injections in muscle healing, and our systematic search identified only four clinical reports, all of them level 3 or 4 observational studies. Moreover, although laboratory research typically informs clinical studies in this area, in this field basic science experiments were performed simultaneously or even after clinical applications rather than the other way round. The field is relevant but under-researched.
- Muscle injuries present a complicated picture that includes many components other than muscle cells, such as blood vessels, connective tissue and neural components. PRP therapies are exceptional in that they largely target multiple regenerative processes because of their ability to secrete high levels of the chemokines, cytokines and growth factors, which are required to control activities of different cell types.

This box summarizes key points contained in the article.

of platelets [12,13] and the realization of their healing potential extended the applications to other medical problems [14,15]. Moreover, given the biocompatibility of using the patient's own proteins, safety is guaranteed, simplifying translation from the laboratory to the patient.

PRP therapies may influence muscle regeneration by acting on the satellite cells [16], whose activities are controlled by growth factors and other cytokines, including IGFs, hepatocyte growth factor (HGF), VEGF, basic fibroblastic growth factor (bFGF) or angiopoietin type I (ANGPT-1), plasmin and urokinase plasminogen (uPA) [17-24]. In clinical management of muscle injuries, the current hypothesis is that intramuscular injections of PRPs deliver supraphysiological concentrations of the above-mentioned factors [25-27] at the injured site, influencing cell migration, proliferation, differentiation or fusion and ultimately enhancing muscle regeneration [28]. Assuming this knowledge, PRP therapies hold promise for accelerating muscle healing and returning the elite athlete to competition earlier.

However, the rapidity of translation has sparked debate regarding the level of evidence of clinical benefit needed to introduce PRP technologies in the sports medicine setting. We performed an electronic systematic search using comprehensive sources and focusing on the use of PRP in the management of muscle injuries. To gain a more complete understanding from a scientific and medical point of view, we have covered the entire health research spectrum, and, using pre-specified criteria, we have included all potentially relevant articles from laboratory and clinical research. The field is relevant to orthopaedic sports medicine, but under-researched: we aim to define the current status of our knowledge concerning PRP and muscle healing, a necessary task to guide future research efforts and to identify potential implications.

2. Methods

2.1 Search strategy

The search strategy had two main components. First, in terms of treatment, we searched using all current names that describe this therapy modality, that is, platelet rich plasma (PRP), platelet-rich fibrin matrix (PRFM), autologous fibrin, autologous conditioned serum (ACS), platelet concentrate (PC), platelet gel (PG), autologous growth factors (AGF), plasma or preparation rich in growth factors (PRGF) and platelet releasate or lysate (PL). The applied search strategy (**Table 1**) covers all variants of the treatment in review, including materials containing leukocytes such as leukocyte-platelet rich plasma (L-PRP), platelet-leukocyte-rich plasma (P-LRP) or platelet-leukocyte gels (PLG). Secondly, we searched for the target, combining the following terms: skeletal muscle injury, strain or contusion and skeletal muscle healing, repair or regeneration.

The applied search strategy in Medline and EMBASE using the OVID platform is displayed in **Table 1**. Via the Web of Science, searches combining the above key words were performed in the Science Citation Index Expanded (SCI-EXPANDED) from 1899-present and in the Conference Proceedings Citation Index - Science (CPCI-S) from 1990 to the present (the first week of October, 2010). Google Scholar was also searched. All seemingly relevant articles and reviews were screened for meaningful references and the retrieved article references were further examined for additional publications.

2.2 Criteria for study consideration and data extraction

Studies were eligible if they provided specific information related to the effects of PRP therapies (including ACS) in skeletal muscle and if they were original studies assessing the effect of PRP-therapies on skeletal muscle cell biology, skeletal muscle repair or regeneration in animals or humans. Studies focusing on the repair of non-skeletal muscle were not considered. There were no language or data restrictions. Studies were identified by two authors independently. From the included studies, the following data were extracted: study design (descriptive or controlled, laboratory studies, *in vitro* or *in vivo* or clinical experimentation), sample type (cell line, primary culture, animal species, number of animals, target population, number of patients), type of PRP product,

Table 1. Search strategy in EMBASE 1980 to 2010 Week 41, Ovid MEDLINE® 1959 to October Week 1 2010, Ovid MEDLINE Daily Update October 15, Ovid MEDLINE in process & other non-indexed citations.

No	Search strategy
1	(Plasma adj3 (growth factor* or relasate)).mp
2	((thrombocyte* or platelet*) adj3 (plasma or concentrate* or gel or fibrin* or lysate*)).mp
3	((Autologous or endogenous or autogenous) adj3 (serum or blood)).mp
4	OR/1 – 3 (note: combination of terms related to product)
5	(Musc* adj5 (heal* or injur* or strain* or contus* or regener* or repair*)).mp
6	AND/4 – 5
7	Remove duplicates from 6

*Truncation, adj3: words in either order between 3 words, mp: title, original title, abstract, subject heading word, name of substance word.

anatomical location of the injured muscle, outcome measures and principal conclusions. Articles that focused on satellite cells treated with PRP for tissue engineering were excluded.

3. Results

3.1 Systematic search for PRP and muscle: identified articles

Eligibility of the studies based on titles, abstracts and full-text articles was assessed as shown in **Figure 1**. Numerous reviews and opinion papers highlight the relevance of PRP treatments in orthopedics and sports medicine [28-37]. However, no randomized trials have tested PRP injections in muscle healing, and our search identified only four clinical reports [9,10,38,39], all of them level 3 or 4 observational studies. Three of these were published reports [9,38,39], and the other was an oral presentation [10]. Other relevant articles were three laboratory experimental reports, two *in vivo* [40,41] and one *in vitro* [42] (**Tables 2** and **3**). All papers were published in English.

3.2 Description of the studies

3.2.1 Clinical studies

Wright-Carpenter [9] assessed the effects of ACS injections in a non-blinded, non-randomized case control study. ACS is an autologous liquid serum conditioned by incubation of whole blood with glass beads; it contains signaling proteins that include IL-1b, TNF-α, IL-7, fibroblast growth factor-2 (FGF-2), IL-1 receptor antagonist (IL-1Ra), HGF, platelet derived growth factor (PDGF-AB), TGFβ-1 and IGF-1. The experimental group treated with ACS included 17 patients, while the control group, which was analyzed retrospectively, included 11 patients who had received Traumeel®/Actovegin®(3:2). Traumeel is a homeopathic formulation containing both botanical and mineral ingredients in homeopathic concentrations. It is purported to suppress the release of inflammatory mediators and stimulate the release of anti-inflammatory cytokines. Actovegin is a deproteinized calf blood hemodialysate consisting of a physiological mix of amino acids. The rest ice compression elevation protocol was employed for initial care in both groups. The severity of the tear, which was scored as grade 2 with detection of bleeding on MRI, was similar for all control and experimental groups [43]. Most tears were located in the hamstring and adductor muscles (12 in the experimental group and 9 in the control group). The injected volumes (5 ml) were identical in both groups. The injection technique and post-injury treatment are described well. The mean number of treatments per patient was 5.4 in the ACS group and 8.3 in the reference group. The main outcome measured was the time needed to resume full sporting activities. Return to competition was decided after isokinetic strength assessment. The experimental group returned to competition after 16.6 days, while the control group took 22.3 days; in addition, MRI scans taken at 16 days in both groups confirmed that regression of the edema/bleeding was faster in the ACS group. Both treatments were safe.

At the 2nd World Congress of Regenerative Medicine, Sanchez *et al.* [10] reported the application of leukocyte-free PRP [44] in 21 muscle injuries of different severities and at different anatomical locations; small tears progressed well with a single application, while more severe tears required two or three ultrasound-guided injections. The injected volume depended on tear severity. These athletes, who played in first division teams of the Spanish Soccer League, resumed normal training activities in half the time needed by matched historical controls. Using the same leukocyte-free PRP preparation, Wee *et al.* [38] reported good outcomes (1 week to return to pre-injury activities) after three weekly ultrasound-guided injections to treat adductor longus strain in a professional bodybuilder. Objective measurements, such as swelling or manual muscle testing, were not reported. Pain is always mentioned, but the visual analogue scale or analgesic consumption were not reported as outcome measures. Recently, Hamilton *et al.* [39] reported buffered L-PRP injection in a grade II hamstring strain injury and daily physiotherapy program. Seventeen days after injury, the patient had full range of motion and was pain free in maximal contraction consistent with MRI demonstrating complete resolution.

3.2.2 In vivo controlled laboratory studies

Myogenesis relies upon satellite cell activation, proliferation, migration to the site of injury, differentiation, fusion with existing damaged muscle or other satellite-cell-derived myocytes and maturation (increased myofiber diameter). Thus, to assess the progress of muscle regeneration from a biological perspective, researchers measure the number of activated satellite cells, molecular markers of cell differentiation (i.e., RNA or proteins) or the diameter of regenerating myofibers. Using this strategy, two separate research teams [40,41] used syngeneic

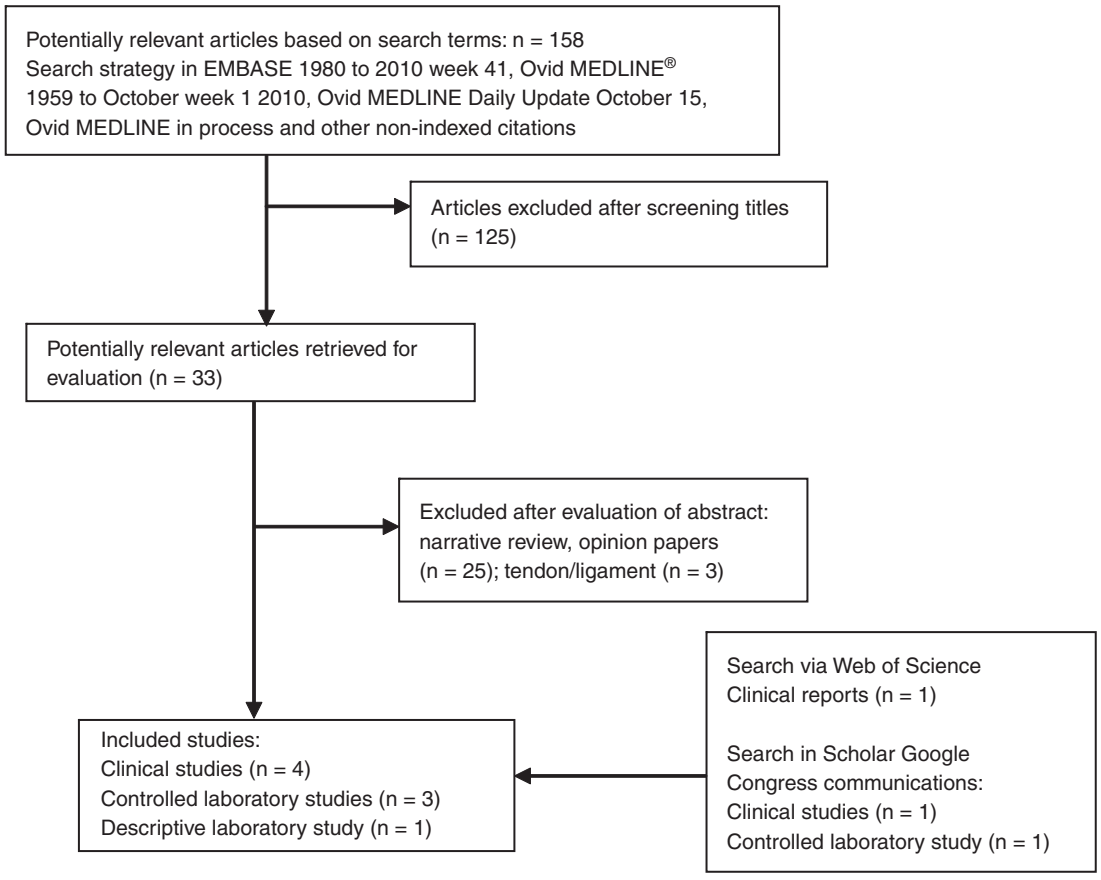

Figure 1. Flow diagram of the systematic literature research. A total of 125 articles were excluded as the title or the abstract clearly indicated that they were not relevant, and 28 articles were further excluded because they were narrative reviews or opinion papers or evaluate tendon/ligament.

animals (mice or rats) to test the therapeutic effects of ACS and L-PRP, respectively.

In 2004, Wright-Carpenter *et al.* [40] applied ACS in a contusion injury model by injecting 10 µl at 2, 24 and 48 h after injury. The control group was treated with the same volume of saline. The number of activated cells was assessed at 30 and 48 h after contusion, and the size of regenerating myofibers was measured in histological samples on days 0, 2, 4, 6, 7, 8, 14, 21, 28 and 35, assessing the progress of regeneration. The number of activated satellite cells was higher in ACS-treated contusions at 30 and 48 h. Moreover, larger regenerating myofibers were observed in the ACS group by days 7 – 8; however, by day 14, there were no differences between groups. These results indicated that ACS hastens muscle regeneration after contusions by promoting earlier initiation of the activation and/or recruitment of satellite cells and by achieving earlier fusion.

Hammond *et al.* [41] described the effects of leukocyte-platelet-rich plasma (L-PRP) in the treatment of strains. The authors induced either weak or severe strains (by single or multiple repetitions) in the tibialis anteriorior of syngeneic rats and injected 100 µl of L-PRP at days 0, 3, 5 and 7. The control group was treated identically but with PPP. Muscle regeneration was assessed by molecular measurements of myogenin and MyoD, measuring both mRNA and protein levels. In addition, myogenesis was assessed by quantification of centrally-nucleated fibers (widely accepted as a marker for muscle regeneration) peaking 2 weeks after injury in the PRP-treated group. Functional recovery was evaluated by torque measurements at days 3, 5, 7, 14 and 21. In weak strains, PRP ameliorated the force loss at day 3, while in more severe strains PRP improved the contractile function at days 7 and 14, and shortened the recovery time from 21 days to 14 days. The authors concluded that L-PRP injections hasten functional recovery and that myogenesis was probably the mechanism underlying this acceleration.

In a poster communication [45], muscle lacerations treated with leukocyte-depleted PRP in a sheep model showed enhanced regeneration when compared to platelet-poor plasma. Thus, three independent *in vivo* studies of different methodological values have assessed the effects of three different autologous preparations injected in three different injury models: contusions, strains and lacerations (**Table 3**).

Table 2. Platelet rich plasma therapies to treat muscle injuries: clinical studies.

Clinical studies (type of article)	Study design/target population	Treatment	Mechanism/ location	Outcome measures	Principal results	Level of evidence
Wright-Carpenter *et al.* 2004 [9] (Original article)	Case-control n = 16 (experimental) n = 11 (control group) /recreational athletes	ACS versus Traumeel/Actovegin ACS:5.4 injections/patient Control group: 8.3 injections/patient	Strain/ Hamstring and adductor(most)	Regression of the oedema Strength (isokinetic test) Return to competition	Faster regression of oedema Faster return to competition Safety	III
Sanchez *et al.* 2005 (Oral presentation) [10]	Case-series n = 20, historical controls/professional athletes,	One to three injections Pure-PRP	Strain or contusion /Different locations	Return to competition No re-injuries	Faster return to competition Safety	IV
Wee *et al.* 2009 (Letter) [38]	Case report /professional athlete	Three injections Pure-PRP	Strain/Adductor longus	Return to competition Pain Safety	Acceleration of healing Reduced pain Safety	IV
Hamilton *et al.* 2010 [39]	Case report /recreational athlete	One injection Buffered L-PRP	Strain/ hamstring	Return to pre-injury activities MRI evaluation Pain Safety	Return to pre-injury activities at 3 weeks Full range of motion and MRI resolution at day 17	IV

ACS: Autologous conditioned serum; L-PRP: Leukocyte platelet rich plasma; PRP: Platelet rich plasma.

3.2.3 In vivo controlled laboratory studies

Ranzato *et al.* [42] evaluated proliferation and motility in C2C12 mouse myoblasts treated with platelet lysates. Due to technical difficulties associated with isolating and maintaining cultures of primary satellite cells, immortalized cell lines are frequently used as satellite cell models. C2C12 is a commonly used cell line, isolated from clonal cultures derived from the thigh muscles of 2-month-old C3H mice 70 h after crush injury. To obtain a platelet lysate, platelet pellets are washed, repeatedly frozen and thawed and finally centrifuged to eliminate debris. A 20% platelet lysate was used in these experiments. The authors used a scratch wound model and chemotaxis assays. In scratch models, the wound healing space is reduced by both migration and proliferation of cells. In the chemotaxis model, on the other hand, the effect of migration does not overlap with proliferation. The results showed increased proliferation and motility, and the latter effect was more evident [42]. This is relevant given the isolation and relatively sparse distribution of satellite cells in uninjured tissues; proliferation and directional motility are both required to reach large populations of activated myoblasts at a site of focal injury. This study also provided a mechanistic explanation by demonstrating that activation of p38 and PI3K was involved in the myogenic program (differentiation) in cell motility.

Taken together, these studies contained too many variables regarding the product (ACS, L-PRP, pure PRP and platelet lysates), the method of application (variable number of injections, volume, frequency), the type of injury (contusion, strain or laceration), anatomical location and severity. Moreover, although laboratory research typically informs clinical studies in this area, in this field basic science experiments were performed simultaneously or even after clinical applications rather than the other way round.

4. Discussion

Although the management of sports injuries with PRP injections has been advocated since 2003 [46], this strategy has not yet been tested in clinical trials dealing with muscle injuries. In reviewing the published work on PRP therapies for muscle injuries, we found only one peer-reviewed clinical study [9] in recreational athletes, and it contained important methodological limitations, such as a lack of blinding, retrospective controls, incomplete reporting and a lack of objective measurements. The absence of studies may impress clinical researchers. This is not so extraordinary for muscle sport injuries, as their management is based largely on experimental studies or empirical evidence. Even when considering the clinical evidence base for the universally-accepted early management of soft tissue injuries, that is ice (also known as cooling or cryotherapy), after meta-analyses [47], conclusions and recommendations were greatly limited and guidelines continue to be formulated on an empirical basis. This presumably reflects not only the importance of key details of the application procedures, such as the interaction of the

Table 3. Platelet rich plasma and muscle repair: laboratory studies.

Animal studies	Study design/animal	Treatment	Injury/anatomical location	Outcome measures	Principal conclusions
Hammond *et al.* 2009 [41]	Controlled laboratory/ syngenic rats, n = 72	L-PRP versus PPP Four injections	Strain Tibialis anterior	Percentage of maximal torque mRNA: MyoD and myogenin Histology: centrally nucleated fibers	Enhanced functional recovery Stimulation of myogenesis
Carda, *et al.* 2005 [45]	Controlled laboratory Sheep, n = 4	Pure-PRP versus PPP One application	Lacerations over the back	Qualitative histology	Enhanced structural outcome with PRP
Wright-Carpenter *et al.* 2004 [40]	Controlled laboratory syngenic mice, n = 108	ACS versus saline Three injections	Contusion gastrocnemius	Histology: number of activated satellite cell, fiber diameter	Enhanced satellite cell activation and larger fiber diameter with ACS
Cell cultures	**Design/Cell type**	**Treatment**	**Assay type**	**Effect measures**	**Principal conclusions**
Ranzato *et al.* 2009 [42]	Controlled laboratory/ mouse myoblasts C2C12	Platelet lysate Inhibitors of MAPK signalling ERK, p38, PI3K	Scratch wound closure Antibody blockade	Percentage wound closure rate Proliferation Chemotaxis	Enhanced proliferation and motility Motility more central than proliferation P38 and PI3K drive cell migration

ACS: Autologous conditioned serum; L-PRP: Leukocyte platelet rich plasma; myoD: Myoblast determination protein; PPP: Platelet-poor plasma; PRP: Platelet-rich plasma.

cooling surface with the tissue, but also the major hurdles for developing adequate clinical trials, which include large variations with regard to injury severity and affected muscle groups, non-specificity of reported symptoms, concomitant treatments, allocation of elite athletes into randomized controlled trials and outcome measurements independent of patient motivation.

PRP injection is a form of management of muscle injuries that can be considered for clinical practice. However, it is hard to recommend it as best practice, first because it is based on scarce level III – IV studies and the recommendations of expert opinion; second because PRP therapies are unclear regarding the best formulation for muscle injuries. Essentially, there are two different liquid formulations gathered under the same PRP terminology. To differentiate and define those, two descriptive terms have been proposed [48]: L-PRP, which contains fivefold to – eightfold more platelets and more leukocytes than peripheral blood; in contrast, P-PRP avoids leukocytes, and has a moderate increase in platelet count (1.5 – 2.5-fold above baseline). It is not known whether muscle injuries treated with L-PRP or P-PRP progress in different ways. Theoretically, L-PRP may mimic the initial phase of inflammation in which a high number of neutrophils infiltrate the injured site; the interactions of neutrophils with platelets can induce a hyperactive leukotactic response of circulating neutrophils toward the injury site. Neutrophils may exacerbate tissue damage via several different mechanisms (i.e., secreting pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6 or IL-1β) that cause matrix destruction through the production of MMP-1, -3 and -13. Moreover, neutrophils secrete high concentrations of a number of cytolytic and cytotoxic chemicals, such as oxygen radicals and hydrochlorous acid [49]. Consequently, interaction of activated neutrophils with the damaged tissue can and does intensify muscle damage, which is known to be the secondary injury related to the inflammatory response [50]. Indeed, research shows that hindering neutrophil infiltration can result in reduced overall muscle damage [51]. Hence, assuming their probable dissimilarities in neutrophil chemotaxis and activation, L-PRP and P-PRP might be critically different in regulating the complex innate immune response and subsequent healing outcome. To gain further information about those critical differences in the early healing phase, both formulations should be compared, preferably using large-animal models and adequate outcome measurements.

Proponents of PRP therapies in muscle applications may offer several arguments in their defense. First, medicine is dynamic, and it is worthwhile to exploit the therapeutic value of an otherwise safe technology that has the potential to benefit patients, as shown in other clinical applications [52,53], even if it will probably be refined as laboratory and clinical research are conducted. Second, while in recreational athletes muscle injuries may recover uneventfully in a matter of weeks, professional athletes need urgent solutions because they must return to higher levels of performance and activities in a

shorter time. Third, knowledge of repair mechanisms supports the biochemical basis of adding supraphysiological concentrations of growth factors to injured tissues [54]. There are important insights arising from research that may help in understanding the clinical potential of PRPs and their suitability as a therapeutic tool in muscle injuries.

Muscle injuries certainly present a complicated picture that includes many components other than muscle cells, such as blood vessels (endothelial cells and pericytes), connective tissue (fibroblasts) and neural components (motor neuron, Schwann cells) [55-57]. In addition, PRP therapies are exceptional in that they largely target multiple regenerative processes because of their ability to secrete high levels of the chemokines, cytokines and growth factors, which are required to control activities of different cell types. It is not known which are the key factors, but several growth factors abundant in PRPs have been extensively studied in muscle regeneration [24]. For example, HGF is the primary component of crushed muscle extract [58], and it is currently the most probable candidate for initiating regeneration by satellite cell activation via c-met receptors. HGF promotes activation, proliferation, differentiation and chemotaxis. IGF-I and -II each increase following muscle injury and promote myoblast proliferation and myofiber differentiation as well as enhancing muscle cell survival and hypertrophy under tissue-specific circumstances. While not as intensively studied as HGF and IGFs, VEGF, bFGF and ANGPT-1 appear to have potential in regeneration by inducing muscle angiogenesis [55]. Also less well-recognized, brain derived neurotrophic factor (BDNF) is a relevant component of PRPs with an important role in regulating satellite cell function and regeneration, as shown *in vivo* [18]. BDNF has been known since the early 1990s in sports research because, of all neurotrophins, it is the most susceptible to systemic regulation by exercise and physical activity; it is also known because of its metabotropic activity [59]. When axonal communication with the muscle cell body is interrupted by injury, Schwann cells produce neurotrophic factors, such as nerve growth factor (NGF) and BDNF [60]. Thus, additional increases in BDNF in the context of PRPs may help in the progressive recovery of neural communication [61-64].

On the other hand, the presence of relatively high concentrations of TGF-β1 prompts the question of whether PRPs may favor healing by fibrosis instead of regeneration. Both platelets and leukocytes secrete TGF-β1, and there is good reason to think that boosting TGF-β1 levels might induce excessive accumulation of fibrotic tissue [16,65]. However, molecular combinations may be antagonistic or even suppressive, and the combined effect of TGF-β1 with other PRP-secreted molecules on collagen synthesis was weaker than that of TGF-β1 in isolation [66]. Then again, with the release of proteases, such as plasmin or thrombin, PRPs may fuel the fibrinolytic activity required for myogenesis [23]. For example, IGF-binding proteins (IGFBPs) still need to be cleaved to deliver bioactive IGF to its receptor and stimulate cell activities. Unraveling the protease-induced activation of IGFs, HGF or TGF-β1 may be the key to understanding some PRP actions needed to optimize PRP formulations. Thus, in complex systems such as PRPs, the major challenge is to disentangle the relative effects of the components and to understand how they influence given cell activities. Indeed, the PRP story has turned out to be immensely more complex that it seemed at first.

5. Conclusion

According to the findings of this review, no PRP formulation has yet displayed proven solid evidence for the stimulation of healing and recovery after sports muscle injuries. Pilot clinical studies along with empirical experience indicate that PRP therapies may enhance muscle repair after strain or contusion. Laboratory data indicate that such treatments can enhance myogenesis. However, the fundamental principles governing when and how PRP therapies can be usefully employed in muscle injuries are emerging at a slow pace. The key to attain standardization and improved formulations will be the identification of crucial elements in these preparations. Given our rudimentary knowledge of the mechanism of action of the PRPs, it remains uncertain how best to use this technology to affect early healing, and produce improved and accelerated functional recovery.

6. Expert opinion

Currently, the use of PRPs in elite athletes and ensuing discussion in the media has fueled clinical demand outpacing basic and clinical research and hindering progress on such therapies. The ease of use and lack of fear of side and adverse effects involved with PRPs is detrimental, as this allows practitioners to use it frequently without guidelines such as timing of treatment, number and technique of injections or volume; frequently, the personal experience of the practitioner is the only source of evidence to substantiate practice. Failure to understand the mechanism of action of PRPs frustrates efforts to develop best formulations regarding the optimal platelet concentration. Earlier, in oral and maxillofacial surgery, a minimum four-fold to fivefold increase in the number of platelets was considered necessary to produce a therapeutic effect [67]. In retrospect, it is obvious that such an assertion was inappropriate, and not supported by basic science [68,69]. The best PRP formulation for muscle injuries will be clearer after research efforts have provided a comprehensive description of the relations between PRP components, healing mechanisms and functional outcome.

In particular, several critical questions about how to optimize PRP therapies should be a high priority for researchers. First, to standardize PRP formulations, research must identify key elements in these preparations. For example, it is relevant to establish differences between pure platelet-rich plasma and leukocyte-platelet concentrates regarding tissue damage

exacerbation [50-57]. In addition, the optimal balance between plasma myogenic factors, such as IGFs and HGF, and platelet-secreted angiogenic or chemotactic factors needs clarification. In fact, platelets are the major source of chemotactic factors such as platelet factor 4 (PF-4) which, in cooperation with PDGF and CXCL7, activates fibroblast migration. Second, to identify the best timing for application, the implications of physicochemical temporal conditions of the tissue (i.e., pH, NO and oxygen) should be evaluated. Indeed, most injured tissues, which are under hypoxic conditions, shift to normoxia after angiogenesis; thus, the biological and clinical effects of PRPs under these circumstances may differ. Moreover, which cells or biological events PRPs target in each temporal phase of repair is unknown [54]. Furthermore, if reduction of scarring is a plausible goal, it would involve identifying the actions of TGFβ-1 in this context.

These questions need to be addressed to standardize the formulations and procedures for application. Because of the safety of these products, basic science, clinical discovery and patient-oriented research should be interdependent rather than successive steps. The substantial challenges of incorporating such research into clinical care must be pursued if the potential of PRPs is to be realized.

Declaration of interest

The authors state no conflict of interest and have received no payment for the preparation of this manuscript.

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