CHAPTER 14

PRGF Molecular Intervention: a Bridge from Spontaneity to Muscle Repair

AUTHORS

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SUMMARY

Muscle injuries are some of the most common sport injuries, accounting for between 10% and 55% of all such lesions. Although the skeletal muscle is a plastic organ capable of efficiently responding to environmental changes, the appropriate treatment of muscle injuries remains a daunting clinical challenge in sports medicine. There is a great deal of evidence in basic science pointing towards growth factors such as TGFB, HGF or IGF and fibrin matrix as the key players in cellular events required for muscle repair and regeneration process, namely, myogenesis, angiogenesis, and fibrogenesis. An innovative biological approach to the treatment of muscle injuries is the application of Plasma Rich in Growth Factors (PRGF) in intramuscular infiltrations. PRGF delivers growth factors, cytokines and adhesive proteins present in platelets and plasma, as well as other biologically active proteins conveyed by the plasma such as fibrinogen, prothrombin, and fibronectin among others. But the application of this autologous mimetic biomaterial embedded with a pool of growth factors, acting as a smart dynamic scaffold, must be carried out [taking into account a] using a particular biological approach.

E. Anitua et al. (eds.), *Platelet Rich Plasma in Orthopaedics and Sports Medicine*,
https://doi.org/10.1007/978-3-319-63730-3_14

1. INTRODUCTION

Muscle injury is one of the most common traumas in sports irrespective of the level of sport practiced, accounting for 10% to 55% of all such injuries[1] and encompasses contusions, strains, and lacerations. The most common mechanism of skeletal muscle strain in elite sportsmen is the concentric/eccentric muscle movements associated with high levels of explosive force in response to sharp changes in direction and speed. This is the case in sprinting and jumping, where the excessive tensile force generated in response to sharp changes in direction and speed[2] leads to muscle injury causing tears in the blood vessels of the muscle tissue. The severity of these types of injuries is measured by the athlete´s functional inability to train and compete, in addition to the increased risk of recurrent injury. In many cases this functional loss or compromise may last 30–40 days.

In spite of the fact that skeletal muscle may be seen as the paradigm of tissue plasticity which conserves and shares modules of regulatory pathways and transcription factors of embryonic myogenesis and development, to be redeployed for tissue repair after muscle injury[3,4], the appropriate treatment of muscle injuries remains a daunting clinical challenge in sports medicine[1,5,6].

There is a great deal of evidence in basic science pointing towards growth factors such as TGFB, HGF or IGF and fibrin matrix as the key players in cellular events required for muscle repair and regeneration process, namely, myogenesis, angiogenesis, and fibrogenesis[7]. Drawing on the regenerative potential of platelets, thrombin, plasma biomolecules and fibrin matrix[8-10], several systems of producing autologous Platelet Rich Plasma (PRP) derivates have been developed and aimed at triggering and enhancing the natural in vivo tissue morphogenesis and regenerative capacity[11] by targeting "the stem cell zone" microenvironment of damaged and healthy tissue-areas[12].

This novel biological approach could be an important option to treat muscle tears in light of the knowledge and insight gained in both basic science about the role of growth factors and fibrin matrix in muscle tissue repair process[13-18] and in the promising results yielded by this approach in musculoskeletal system pathologies[11,19,20].

2. TISSUE-BASED PHENOMENA IN SKELETAL MUSCLE INJURY AND REPAIR

Although it is tempting to refer almost exclusively to myogenesis as the pivotal parenchymal cell process in muscle repair and regenerations, there is a great deal of evidence showing that muscle repair and functional recovery[21,22] also rely on other stromal cell events such as inflammation involving monocytes and macrophages[23-27], angiogenesis[28], fibrogenesis[2,29], reinnervation[30-32] and physical stress[1,33,34] (figs. 1-2).

Immediately following muscle tear, the massive entry of calcium into the damaged myofiber and subsequent activation of complement and proteases such as calpains, will lead to myofiber necrosis and destructuring of the constituents of ECM[17,32,35,36]. Moreover, the disruption of vessels and acute injury will both generate a haematoma and activate satellite cells (SCs), platelets, endothelial cells (ECs), fibro/adipogenic progenitor (FAPs) and muscle connective tissue (MCT) fibroblasts[37-39]. The haematoma that fills the gap created between the already necrotic and retracted myofiber stumps[2,17,35] will turn into a fibrin clot and will temporarily serve as provisional extracellular matrix (ECM) that will house the development of stromal and parenchymal cell events such as angiogenesis, myogenesis, fibrogenesis and innervation of the new-formed tissue[2,29,40]. Platelets and endothelial cells release cytokines and growth factors that, together with the injured tissue damage-associated molecular patterns (DAMPs)[25,41,42], recruit, attract, and activate neutrophils, resident

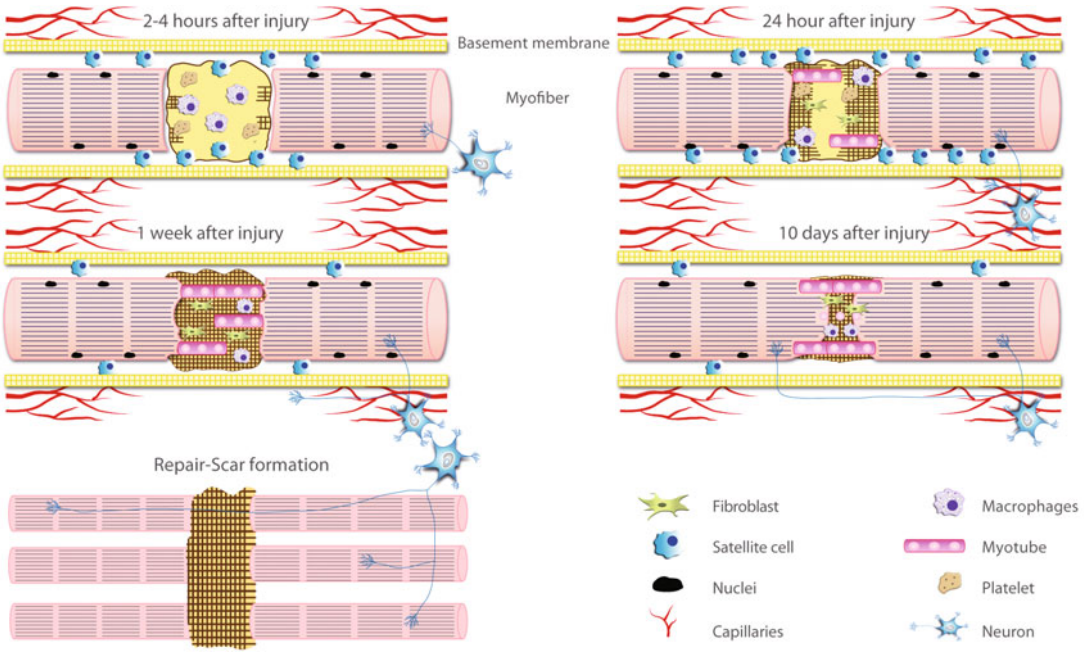

FIG. 1
The most important cell-based phenomena during the repair of muscle injuries. (Reprinted with permission from Sanchez et al[7]).

macrophages, and circulating monocytes to the injured area. Whereas neutrophils appear to play a minor role in the repair process besides exacerbating myofiber damage[24,43,44], monocyte-derived cells are the main players of the innate immune system in muscle repair process, and adopt, in this sterile though necrotic microenvironment, a proinflammatory phenotype M1 (macrophage type 1)[39]. These M1 phagocytose tissue debris, clean the necrotic zone and release growth factors, cytokines and cell adhesion molecules that, together with those coming from the degranulation of activated platelets and through influencing the cell fates and behavior of SCs, monocytes, ECs, pericytes and fibrobasts, will support muscle tissue homeostasis and repair[25,26,38,39,41].

Once they have undergone necrosis myofibers have a poor potential to regenerate themselves given that they are postmitotic cells[40]. However, new muscle tissue can be formed partially from the activation of SCs[32,45,46]. These precursor muscle stem cells[16,17,47] lie sandwiched between sarcolemma and the basal lamina (BL), which is a highly specialized interstitial connective tissue within the ECM. In spite of an impaired basal lamina and the toxic milieu brought about primarily by infiltrated neutrophil, inflammatory macrophages[48], and ECM fragments , satellite cells along with other survivor cells, are activated and migrate to the site of injury within 2h after injury, though some of them undergo self-renewal for replenishing the SC pool[45,49]. Once at the site of injury, SCs proliferate and differentiate into fusion-competent myo-

blasts which will differentiate and fuse with one another to form myotubes and new myofibers by about 7 days in injured mouse muscle[40,49], or with existing damaged myofiber to repair them[21,32].

Angiogenesis is carried out by the activation of quiescent ECs that in mammalian skeletal muscle show a potential to rapidly proliferate after being activated by angiogenic stimuli coming from the injured area such as DAMPs and growth factors, namely, VEGF and PDGF[28]. The sprouting of small blood vessels takes place in this fibrous callus that now joins the ends of the various broken fibers while the fibrin matrix continues to be infiltrated with macrophages. These new capillaries will later undergo a maturation and stabilization process which involve pericytes, to eventually end up generating a structured network of capillaries[21,50]. Moreover, neovascularization appears to be crucial in functional and structural muscle regeneration, furnishing the new tissue with oxygen and other nutrients as well as with blood-derived cells, at the same time as removing carbon dioxide and other tissue-waste products[2,21].

Fibrogenesis is another key component of toolkit-defense system that (see chapter 3) has evolved to rapidly fix and replace the necrotic areas and the initial formed fibrin clot with new ECM and connective tissue[2,38,39] in order to address the loss of connective tissue, to seal off the injured area, and repair or generate the BL[29,38]. When the aseptic yet hypoxic and necrotic microenvironment lingers over time, or when neovascularization is compromised, the M1 phenotype persists, which leads to a non-resolving fibrogenesis where a myofibroblast profile and fibrogenesis will take over myogenesis thereby generating an excessive and persistent deposition of ECM which results in a fibrotic scar tissue[26,29,36,40,51-53]. For myogenesis, angiogenesis and innervation to develop functionally, the integrity of the BL and the 3D structure provided by the fibrin adhesive protein matrix that support ECM and cell-cell adhesion is of paramount importance[17,54,55]. Repair of the BL is the first key step in reconstruction of the neural canal since BL not only ensures subsequent compartmentalization of the repair phenomena[54], it is also involved in mechanical support, myogenesis, and synaptogenesis. Moreover, the molecular composition of the muscle BL endows it with adhesive and inductive functions for a variety of cell fates during muscle repair[4,36,54].

Innervation is essential for growth and maturation of newly formed myofibres as well as for the re-expression of myosin heavy chains[4,40]. It is important that the newly formed granulation tissue which joins the damaged fibers together does not form a barrier to the axon´s progression from neighboring nerve endings[2,33] nor surround them with fibrotic tissue resulting from an excess collagen synthesis or defective metalloproteinase (MMPs) synthesis[33,51]. This progression of axons leads toward the old synaptic site where the original neuromuscular junction (NMJ) was located or to the basal lamina of new myotubes[17], thereby allowing the restoration of full muscular function, a process which might take months[2].

During the repair process, the existence of a mechanical stimulus causes integrins to laterally bind the edges of muscle cells to the extracellular matrix via laminins, thereby preventing them from retracting and thus contributing to the repair process[33]. Controlled physical stress helps to reorient type I collagen, thereby enhancing the penetration and alignment of myoblasts and stimulating remodeling[1,21,56].

All these biological defense system modules are tightly coordinated through the secretion of growth factors and cytokines primarily but not exclusively released by SCs, macrophages, platelets, ECs , and myofibroblasts[16,38,47,49,57] (fig. 2).

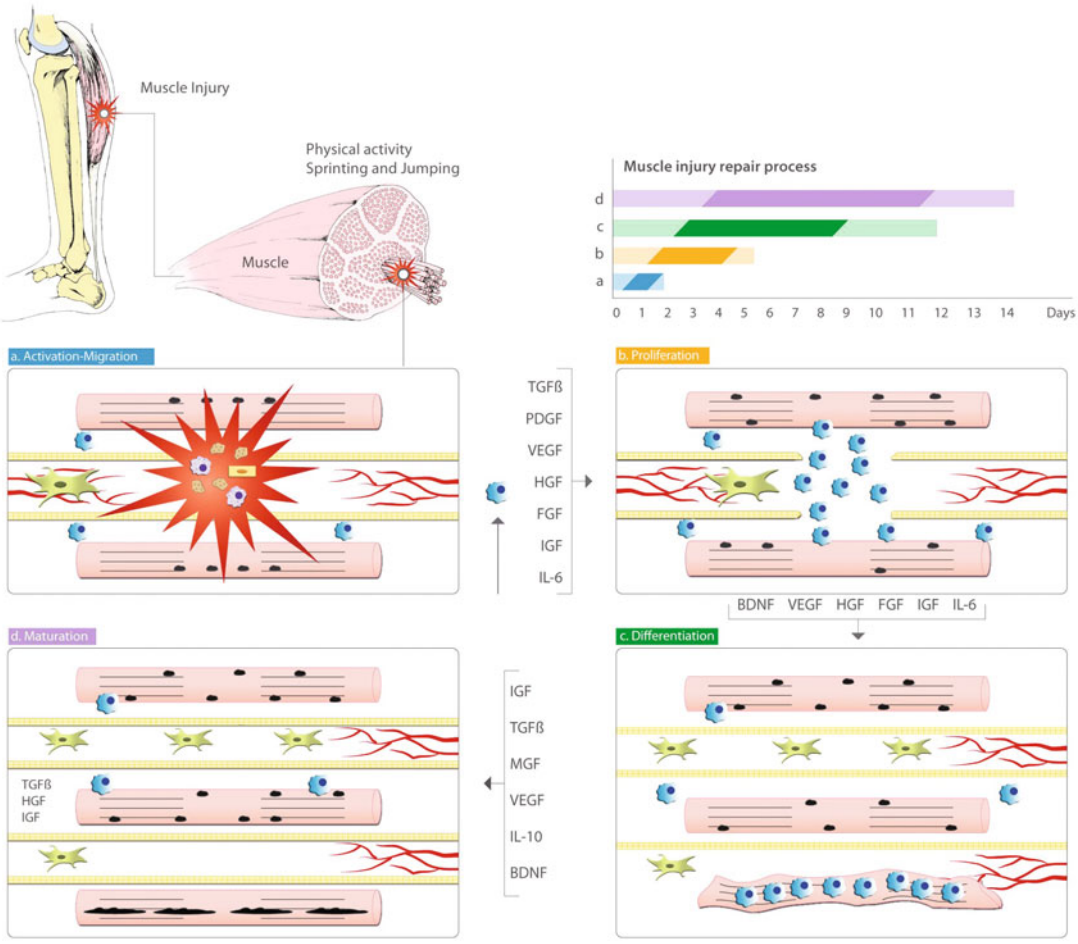

FIG. 2
The key role played by satellite cells (muscle stem cells) in muscle repair under the control of various growth factors. (Reprinted with permission from Sanchez et al[7]).

3. CELLULAR AND MOLECULAR MECHANISMS REGULATING MUSCLE REPAIR AND REGENERATION

Mammalian muscles are made up of tissues with quite different proliferative activity of their cell; cells that have left the cell cycle and do not undergo mitotic division in postnatal life such as neurons or myofibers and quiescent cells such as fibroblasts, SCs, and ECs with low or no level of proliferation. But SCs and ECs can, in response to environmental cues such as mechanical injury through DAMPs[16,17,38,47,50], undergo a boost in mitotic, migratory, and secretory activity. Following muscle injury, a short inflammatory stage ensues (in the first 24-48 hours after injury)[27,49], primarily stemming from the presence of DAMPs that are first recognized by transmembrane toll-like receptors (TLRs) of platelets, ECs, and resident macrophages, and then activated[58]. These activated cells located at the fibrin clot will secrete TNF, IL-6, MCP1 (monocyte chemoattractan protein

1) which along with VEGF[59], attract blood monocytes and more epimysium/perimysium resident macrophages to the damaged area[25,27,32,43]. Simultaneously, at the injured site, the presence of HGF coming from inflammatory macrophages (M1), and activated platelets and ECs, promote cell cycle reentry of quiescent SCs, and FGF, TGFB, IGF1, PDGF and VEGF will stimulate their proliferation and promote the migration of activated SCs and myoblasts to the repair area as well as protect stromal cells and myofibres from an apoptotic fate[13,18,22,40,49]. Moreover, stromal-derived factor 1 (SDF1) released by platelets and fibroblasts, is a mitogenic and motogenic factor for stem and progenitor cells as well as for circulant monocyte and resident macrophages which will migrate to the injury sites and modulate their phenotype in a context sensitive manner[10,22,24,25,60,61]. This SDF1 (known as CXCL12) also plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from bone marrow through a CXCR4 dependent mechanism[62]. TGFB with pleiotrophic effects will promote SCs proliferation and FAP activation, proliferation and differentiation towards fibroblasts, at the same time inhibiting myoblast differentiation[29,40,63-65]. Moreover, skeletal muscle, like many other musculoskeletal tissues, contain multipotent mesenchymal progenitor cells, termed fibro/adipogenic progenitors (FAPs) and muscle connective tissue (MCT) fibroblasts[37,38,66], and they rapidly enter the cell cycle in response to acute muscle damage, and might be at the origin of fat accumulation and fibrosis in skeletal muscle[66]. The antiapoptotic effect on parenchymal and stromal cells is mainly driven by IGF-I and II, HGF, FGF, VEGF, and it appears to be crucial for myogenesis to be redeployed as in embryo development[4,49] although doing so in a hostile and necrotic tissue-injured microenvironment, and sometimes with impaired or with no basal lamina as an instructive scaffold that in embryonic myogenesis acts as template[27,32].

In the wake of phagocitic activity of M1 and the new microenvironment created within this callus by the secretory activity of M1, fibroblasts, myoblasts, and ECs, a pivotal event in the muscle repair will occur 48-72 hours after injury[25,53], namely, the resolution of inflammation, and macrophages, that exhibit a remarkable ability to reprogram their gene expression profile[25,61,67], will switch from a pro-inflammatory macrophage (M1) to a healing or trophic macrophage profile (M2)[24,25,53] releasing mainly TGF-β and IL-10[25,52]. The fibrin matrix generated as a transient ECM along with other biomolecules of the ECM may retain several growth factors such as FGF, HGF, TGFB, VEGF, BDNF previously released by platelets, macrophages, ECs and newly activated fibroblasts[21,36,68] through the cell surface heparan sulphate binding domains of heparin sulfate proteoglycans (HSPGs)[68,69] to be gradually freed up later 68 thereby controlling morphogen gradients at the repair scenario[70,71]. IGF-I released by myoblasts, endothelial cells and now trophic M2, will stimulate the proliferation and differentiation of myoblasts, promote cell survival[16,36,47,59] and modulate inflammation through the suppression of macropage MIF and transcription factor NF-kB, thereby reducing fibrosis and myonecrosis 14. In addition, the release of platelet factor 4 (PF4) released by platelets prevents monocyte apoptosis, promotes trophic M2[60] and may restore cells to a noninflammatory phenotype. Overall, these stromal and parenchymal events will favour a trophic microenvironment as well as dampen inflammation, and may well contribute to the resolution of inflammation, and thereby shorten the repair process[5].

Quiescent endothelial cells will enter the cell cycle in the presence of microenvironmental stimuli such as DAMPs and VEGF, the latter as a hierarchically superior master switch of the angiogenic cascade[50.] The anatomical proximity among endothelial cells (ECs), pericytes and satellite cells make this area work as a stem cell zone, mainly through the cross-talking through VEGF, HGF, IL-6, and Angiopoietin-1(Ang1)[29] which are, together with PDGF and FGF, pivotal in generating, organizing, and maintaining the microvasculature[21] as well as in the development of myogenesis[28,55]. Moreover, this close association of many quiescent cell types (SCs, ECs and Neural stem cells (NSCs)) with the vasculature allows the modulation of these cell-fate decisions via metabolic cues, circadian rhythms, temperature, mechanical stress as well

as providing a feedback with humoral factors and cells from the immune system[12]. Another early event in the stromal cell response to muscle injury is the activation, migration and proliferation of fibroblasts that, in presence of TGFB and PDGF, take on a myofibroblast phenotype[29,38] and are, in cooperation with myofibers, responsible for tissue homeostasis, and synthesis and secretion of ECM components such as collagens, laminins, tenascin C, metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and TGFB[2,29,36,38] among others. These activated fibroblasts, under the stimulation of TGFB, are highly secretory and synthetic cells[2,29,33,41], and infiltrate the fibrin clot that now joins the ends of the various broken fibers. The mechanical and biochemical features of the newly secreted ECM along with the provisional fibrin matrix play a role as adhesive cell contacts, and serve as reservoir of growth factors such as VEGF, PDGF, TGFB that will be release gradually, thereby modulating macrophages, myofibroblasts, and survival and fate of myotubes[22,29,38,72,73]. After muscle injury, the absence or disruption of innervation is the major cause of poor restoration of tissue, leading to compromised function[32,33]. Reinnervation and the creation of a new neuromuscular junction (NMJ) in the repaired or regenerated fibers, via the basement membrane, may be driven by growth of new axons from adjacent nerves[47]. These events, often absent from in vitro studies, might well be mediated by nerve growth factor (NGF) and IGF-1, both present in the damaged tissue and synthesized by muscle cells and fibroblasts under paracrine influence[47] in addition to biomechanical signaling (Brain-derived neurotrophic factor BDNF, Glial growth factor GGF) and ECM stiffness which modulates the phenotype and fate of several types of cells such as SCs, activated resident fibroblasts (myofibroblasts), ECs, macrophages and Schwann cells[21,73,74].

Regulating the gene expression products (TGF-β1, IGF-1, IL-10, BDGF, VEGF, collagens, fibronectin, tenascin-C) of SCs, activated resident fibroblasts, ECs, macrophages and Schwann cells appears to be essential in the success of muscle repair[1,21,27,29] and mechanical and chemical signals such as mechanogrowth factor (MGF or CTGF) and insulin-like growth factor-1 expressed in autocrine and paracrine manner, and coming from the cell environment, might well complement each other[14,73]. It should also be noted that the presence of tenascin C in the extracellular matrix, the synthesis of which is induced by mechanical stress, is a prerequisite for muscle reinnervation[36,64]. In the clinical setting, early gradual mechanical loading stimulates gene expression of trophic factors and signals such as cyclooxygenase-1 (COX-1), FGFb, hypoxia-inducible factor (1HIF-1), and BDNF influencing maturation and the correct patterning of myotubes, collagens and tenascin-C[32,33]. Both the gradual and controlled mechanical stimulus that induces IGF synthesis by muscle cells (by endocrine and paracrine activity)[32,63] and the paracrine and autocrine synthesis of growth factors such as HGF and TGF-β by fibroblasts during the final remodeling phase, appear to be essential, since both these signals may have a synergistic effect on the activity of the fibroblasts that are remodeling the ECM[36,75] and repaired tissue. Moderate sustained mechanical load modulates the fusion and ensuing alignment of myoblasts into myofibres[21] and may minimize or even avoid the formation of scar tissue by inhibiting the NF-kB of muscle cells[76] among them fibroblasts which can promote fibrotic scar[77]. The aforementioned events, which play a crucial role in balancing tissue remodeling versus fibrotic scar in injured muscle[32,33] imply that what is true for an isolated myofiber is not necessarily true for the entire muscle[22].

4. AN INNOVATIVE BIOLOGICAL APPROACH TO THE TREATMENT OF MUSCLE INJURIES: PLASMA RICH IN GROWTH FACTORS

There is an increasing body of evidence in basic science pointing out that growth factors and fibrin matrix are instrumental in the muscle repair and regeneration process[13,18,20,21,36,40,57,78].

One innovative biological approach is the application of Platelet Rich Plasma (PRP) in intra-muscular infiltrations[5]. Autologous blood-derived products convey growth factors, cytokines, and morphogens contained in the platelets, as well as fibrinogen and other plasmatic proteins in a biologically balanced aggregate, managed and delivered in a pharmacological manner[11] which may account for two special features: the resolution of inflammation and avoidance of fibrosis. In addition to conveying GFs, PRGF provides the damaged tissue with a transient biological fibrin scaffold which stems from the polymerization of fibrinogen, a pleiotropic blood protein that regulates coagulation, inflammation, and tissue regeneration[10,79].

Our group has been designing rigorous and well-defined protocols for the application of different PRGF-based formulations in several acute and chronic-degenerative pathologies, yielding extremely promising clinical and surgical outcomes in oral and maxilofacial surgery[11], in musculoskeletal system pathologies[78,80] as well as in other medical fields[11]. A wealth of preclinical works suggest that PRP early intervention in muscle injury significantly improves several molecular and cellular events involved in muscle regeneration[15,51,81-83]. Although several clinical studies seem to shed light on the effect of PRP on muscle damage repair with promising functional outcomes[5,6,19,20,84-86], it is fair to say that so far the only clinical trials conducted so far have shown no improvement of muscle injuries treated with PRP 87. However, it is worth highlighting that the delayed administration, and the low dosage of GF conveyed by 3 ml of PRP injection in the Reurink clinical trial may well have rendered PRP treatment ineffective[88]. In an elegant study conducted by Dimauro et al[81] on animal model of muscle injury, the authors showed that the early PRP application, namely, immediately after the injury, exerted a multi-directional effect on myogenic regulators and growth factors involved in inflammation and myogenesis.

Two main challenges remain unresolved when it comes to muscle injury treatment: the slow down of the functional recovery and the relapses, both linked to fibrotic scar after the muscle tear. These concerns have prompted some researchers to conduct intense research to customize PRPs using antibodies to neutralize TGFB and myostatin in order to reduce fibrosis and optimize myogenesis[51,82,89]. However, and due both to the dynamic nature of fibroblast-satellite cell interaction and to the pleiotrophism of growth factors, therapeutic interventions aimed at minimizing fibrosis after muscle injury will need to be carefully controlled in order to avoid interfering with the early pro-regenerative crosstalk among FAPs, MCT fibroblasts, and satellite cells[37,38].

5. PRGF PROTOCOL USED IN MUSCLE INJURIES

An optimal treatment for muscle injury repair should convey a mimetic biomaterial embedded with a pool of growth factors acting as a smart scaffold[21] which might sustain a gradual delivery of growth factors as a niche therapy[12] at the dysfunctional and deregulated injured site instead of a bolus delivery modality[18,68,90]. This biomaterial must promote myogenesis, angiogenesis and innervation as well as modulate immune response and fibrogenesis in order to generate a functional muscle repair[18,21,38,39,51].

We propose the following general principles in the application of PRGF as a local molecular intervention to the muscle injury treatment[91].

5.1. The process to produce PRGF

The patients are advised not to eat fatty food in the 6 hours prior to the blood extraction. Thirty-six mL of peripheral venous blood is withdrawn into 9-mL tubes containing 3.8% (wt/vol) sodium citrate. Occasionally, due to the size of the lesion, it may be necessary to extract further amounts of blood. Once the blood is centrifuged (BTI technology, Vitoria, Spain), the upper volume of plasma F1 is collected in a tube. The 2-mL plasma fraction located just above the sedimented white and red blood cells (buffy coat), is collected in another tube but without aspirating the buffy coat. This plasma fraction F2 presents a moderate enrichment in platelets (2-3 fold the platelet count of peripheral blood) with scarce leukocytes.

5.2. Injured area preparation

While the nurse is obtaining the PRGF (centrifugation, separation of fractions F1 and F2), the patient is clinically examined to correctly assess and mark the area of maximum tenderness and /or swelling. After pinpointing the injured site, the area of skin to be infiltrated is prepared and demarcated with disposable cloths as a sterile field and antiseptic solution is applied (fig. 3a). By using an ultrasound longitudinal 8.0-13.0 MHz multi-frequency linear probe which is wrapped with a sterile cover (fig. 3a), the injury and, if applicable, the possible hematoma associated with the muscle tear (seromas, fibrosis and degenerated areas in case of chronic injuries), are located (fig. 3b). We choose the anatomical location of injection based on utrasound and clinical criteria. The use of local anesthesia must be avoided.

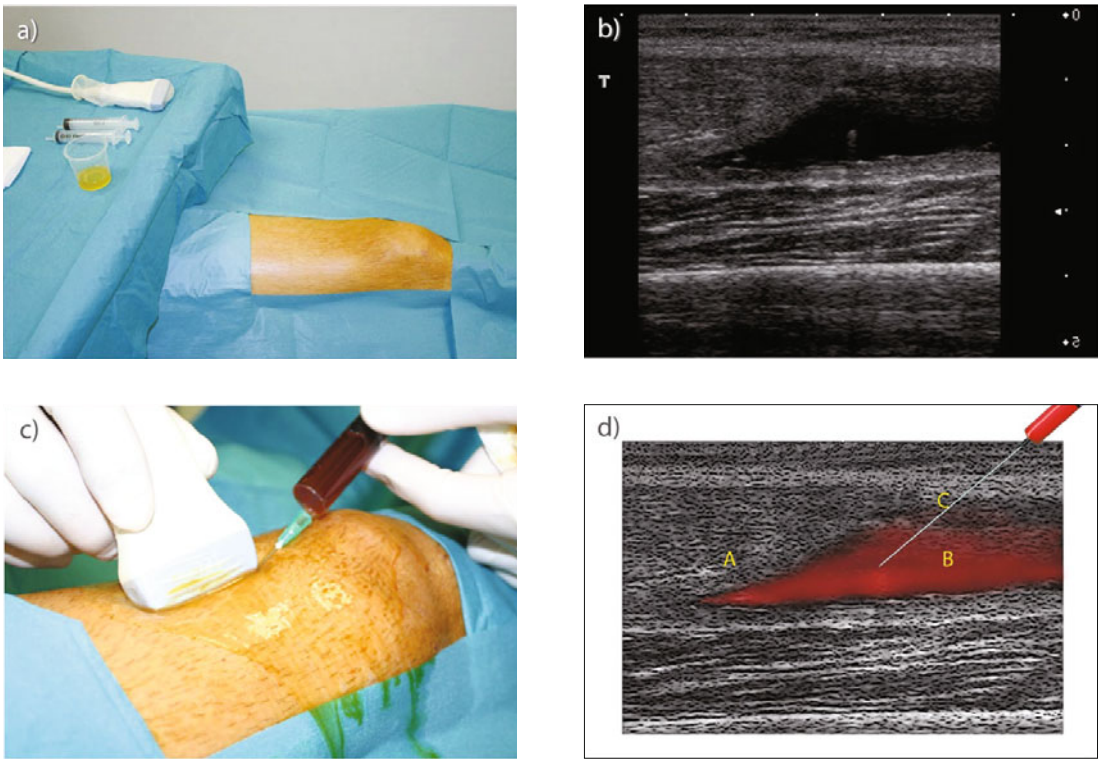

FIG. 3
Preparation of the sterile field (A) required to perform ultrasound control (B) with which we identify the damaged areas, and evacuation of hematoma if exists (C and D). (Reprinted with permission from Sanchez et al[7]).

5.3. Muscle injury site infiltration procedure

Hematoma, seroma, or cysts, if present, are punctured/evacuated using a 18-G needle (figs 3c and d) always under ultrasound guidance (figs 3c and d). Once the hematoma is evacuated, the F2 of PRGF is activated with calcium chloride (10% wt/vol). PRGF-Endoret activated liquid formulation is, in the ensuing 1-3 minutes, injected into the injury site under ultrasound guidance (figs. 4a and b). Although the amount of PRGF infiltrated should be the maximum possible and is usually around 8 mL, this volume could reach 10–12mL depending on the size of the muscle and extent and severity of the damaged area. Once the injury site has been infiltrated, areas adjacent to the site must be systematically infiltrated as well. Therefore, we allocate PRGF infiltration into the peripheral healthy muscle surrounding the injury, including interfascicular and interfibrillar regions (a source of resident macrophages and FAPs), by redirecting the needle in all directions— ventral, lateral, medial, and dorsal, thereby reaching the injury/stump, proximal-stump, distal-fascia, or deep and proximal interfascicular zone (fig. 4b) in order to truly conduct a deregulated area niche therapy[17]. In figure 5, we summarize the most important steps of our procedure. We primarily make use of the F2 fraction, and only when high volumes of PRGF are required, do we draw on the F1 to infiltrate the peripheral areas applying the same activation procedure as with the F2.

PRGF muscle infiltrations are aimed at recruiting, activating and mobilizing satellite cells and resident macrophages[25] which contribute to muscle reparation processes by cell signaling soluble factors[16,17,49,57] besides the already activated ECs, macrophages, and platelets in the injured area. Once the activated PRGF is injected, this liquid-to-gel transition 3D injectable scaffold allows a successful filling of the muscle gaps and defects. With a local and gradual activation ("in vitro" and "in vivo") and a homogeneous distribution through and interaction with the ECM of tissue, it is converted into a matrix-like viscous and malleable structure[11,90]. This fibrin-scaffold formed "in situ" as a provisional extracellular matrix and containing binding sites for cell adhesion as well as proteins such as thrombospondin-1 (TSP-1), alpha-1-antitrypsin fibronectin, acute phase proteins or proteins related to lipid metabolisms[10,90], serves as a highway for mechanical energy to transit from the environment to the cell, thereby bridging cell-to-cell tissue transition, promoting multi-cellular assembly, providing mechanical support and plastic-elastic stiffness which has a drastic impact on fates of diverse cell types such as muscle stem cells[92,93], and endowing tissues with a suitable mechanical and chemical microenvironment for biological restoration. In addition, fibrin matrix, by heparin-binding domains, may sequester growth factors such as PDGF, FGF, HGF, BDNF, and VEGF[18,68,70] and gradually release them later, exerting a synergistic action on tissue repair [18,68]. Finally, ice is applied to the infiltration area for about 10 minutes.

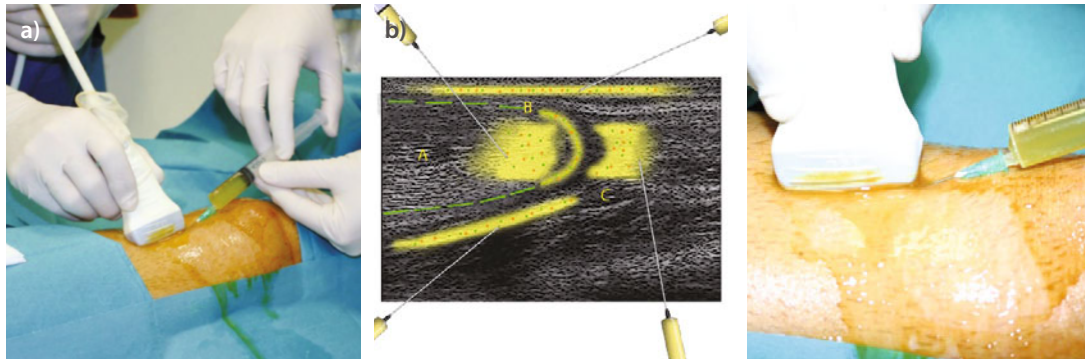

FIG. 4
A)PRGF infiltration at the site injury with correct orientation of the needle, always performed with ultrasound guidance. B)Ultrasound scheme showing the allocation of liquid activated PRGF at the injury site and into surrounding interfascicular and interfibrillar areas. (reprinted with permission from Sanchez et al[7]).

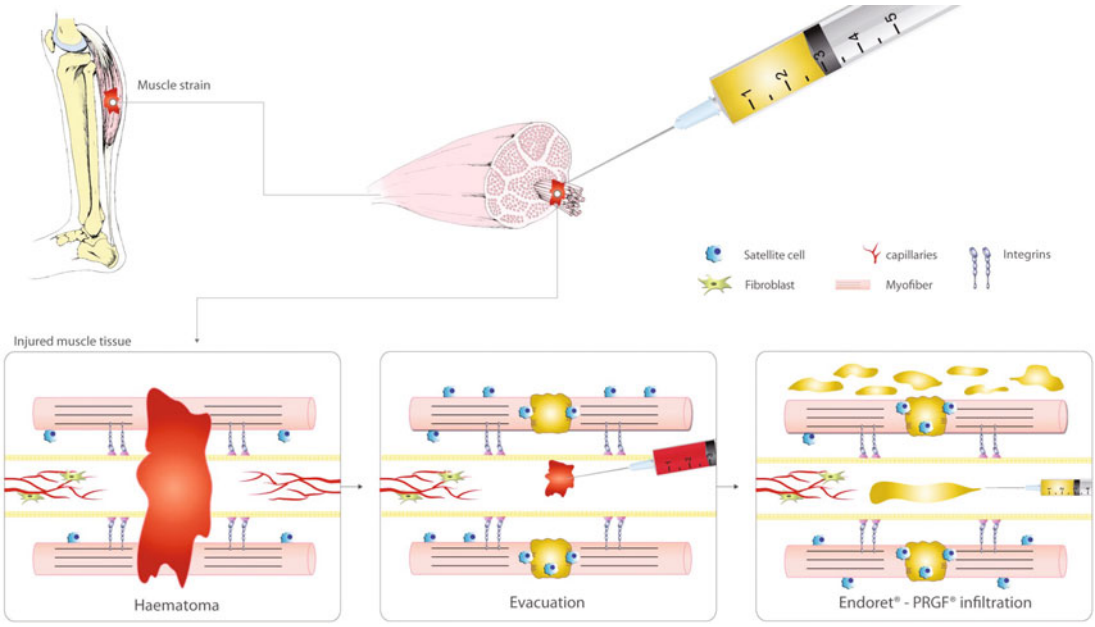

FIG. 5
A summary of the most important phases during percutaneous intramuscular infiltration of liquid activated PRGF. (Reprinted with permission from Sanchez et al[7]).

5.4. Post-infiltration protocol

The patient is advised to apply cold therapy 2-3 times and restrict physical activities during the first 24 hours. Both clinical and ultrasonographic monitoring are performed weekly during patient follow-up in order to evaluate the potential need for more infiltrations. In general, we recommend 2-3 infiltrations, on a weekly basis (relying on the myogenesis and myoblast replication process)[32,35,40]. This decision is based on ultrasound images and pain that the patient presents during the period of treatment, and more than three injections are not normally required. Since muscle tissue is a complex mechano-sensitive tissue, every pharmacological and surgical therapy should be assisted by mechanotherapy. In this respect, and as a clinical application of cell mechanotransduction, a post-infiltration rehabilitation program must be included in a synergistic manner which would play a crucial role in promoting the repair and remodeling of injured tissue. Therefore, since the limb has to be mobilized in an early and progressive manner[2,33,56], physiotherapy and rehabilitation treatment are mandatory. The generated mechanical stimulus achieves a proper recovery of these patients, since it acts in synergistic concert with the biological effects of PRGF[56]. Complications such as seromas, cysts, or muscle fibrosis, have to be approached based on the same principles used in acute ruptures.

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