

Muscle repair: platelet-rich plasma derivatives as a bridge from spontaneity to intervention

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ABSTRACT

Muscle injuries account for between 10% and 55% of all sporting injuries. Although the skeletal muscle is a plastic organ capable of responding efficiently to environmental changes, the appropriate treatment of muscle injuries remains a daunting clinical challenge in sports medicine. There is considerable evidence to indicate that growth factors, such as transforming growth factor- β (TGF β), hepatocyte growth factor (HGF) or insulin-like growth factor (IGF), and fibrin matrix are key in cellular events required for muscle repair and regeneration, 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. This autologous, mimetic biomaterial embedded with a pool of growth factors acts as a smart dynamic scaffold, and should be applied taking into account a biological approach. A clinical trial is required to assess the functional repair outcome of PRGF infiltrations in muscle injuries.

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Introduction

Muscle injury is one of the most common traumas in sport, irrespective of the level of sport practiced, and accounts for 10% to 55% of all such injuries [1]. The severity of these types of injuries is measured by the athlete's functional inability to train and compete, and the increased risk of recurrent injury. In many cases, this functional loss or compromise may last 30 to 40 days. The excessive tensile force generated in response to sharp changes in direction and speed, as happens in sprinting and jumping [2], leads to muscle injury and causes tears in the blood vessels of the muscle tissue. In this aseptic injured area, the presence of damage-associated molecular patterns (DAMPs), which stem primarily from necrotic and apoptotic myofibres and extracellular matrix (ECM) host products [3,4], will trigger the biological defence system. This is a complex cascade of mechanisms including haemostasis and clotting, the innate immune system [5,6] and fibrogenesis [2,7], to cope with the two primary life-threatening events, bleeding and microbial invasion [5].

Viewing motility in a wider and deeper context than sports competition will be instructive in treating muscle injury in sports.

When Darwin cited Alfred Lord Tennyson, "Nature, red in tooth and claw", he was referring to the predator-prey relationship, which is made dynamic by movement and enables survival in an ever-changing environment and consequently transformed life on earth [8]. The biological defence system has a role in life preservation and acts in a sequential and intertwined spatio-temporal manner to control multiple lineages of cells, giving rise to myogenesis, angiogenesis, fibrogenesis and reinnervation, processes that will be deployed in many different arrays without a unitary structural outcome. As a byproduct of the mechanisms underlying these modules, this sequential cascade yields muscle repair or regeneration [9–11].

Despite the remarkable plasticity of skeletal muscle, the appropriate treatment of muscle injuries remains a daunting clinical challenge in sports medicine [1,12,13]. The goal of treatment for muscle strain is to improve and accelerate the process of muscle repair, and consequently, to enable the patient to resume daily and sports activities as soon as possible without relapse.

Drawing on the regenerative potential of platelets, thrombin, plasma biomolecules and fibrin matrix [11,14,15], several systems have been developed to produce autologous platelet-rich plasma (PRP) derivatives to trigger and enhance in vivo tissue morphogenesis and regenerative capacity [16] by targeting "the stem cell zone" microenvironment of damaged and healthy tissue [17]. This novel biological approach could be an important option

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to treat muscle tears, considering the knowledge and insight gained in basic science about the role of growth factors and fibrin matrix in muscle tissue repair [18–23], and the promising results with this approach in musculoskeletal pathologies [16,25,25].

Skeletal muscle injury and the toolkit-defence programme

Although myogenesis is often thought to be the pivotal parenchymal cell process in muscle repair and regeneration, there is considerable evidence to show that muscle repair and functional recovery [9,26] also rely on other stromal cell events, such as inflammation involving monocytes and macrophages [27–31], angiogenesis [32], fibrogenesis [2,33], reinnervation [34–36] and physical stress [1,37,38] (Figures 1 and 2).

When muscle tear occurs, there is a massive entry of calcium into the damaged myofibre, which activates complement and proteases, such as calpains, and leads to myofibre necrosis and destruction of the constituents of extracellular matrix (ECM) [22,36,39,40]. The disruption of vessels generates a haematoma and activates platelets and endothelial cells. The haematoma that fills the gap created between the already necrotic and retracted myofibre stumps [2,22,39] turns into a fibrin clot and serves temporarily as provisional ECM for development of stromal and parenchymal cell events, such as angiogenesis, myogenesis, fibrogenesis and innervation of the newly-formed tissue [2,10,33]. Platelets and endothelial cells release cytokines and growth factors that, together with the injured tissue DAMPs [3,29,41], recruit, attract and activate neutrophils, resident macrophages and circulating monocytes to the injured area. Neutrophils appear to play a minor role in the repair process besides exacerbating myofibre damage [28,42,43]. Monocyte-derived cells have the most important role of the innate immune system in the muscle repair process. They adopt a proinflammatory phenotype (M1) in this sterile though necrotic microenvironment. The M1 cells phagocytose tissue debris, clean the necrotic zone and release growth factors, cytokines and cell adhesion molecules that support muscle tissue homeostasis and repair [3,7,29,30]. They also influence the cell fates and behaviour of satellite cells, monocytes, endothelial cells, pericytes and fibroblasts.

Necrotic myofibres are post-mitotic cells that have a poor potential to regenerate themselves [10]; however, new muscle tissue can be formed partially from the activation of satellite cells [36,44,45]. These precursor muscle stem cells [4,21,22] lie sandwiched between the sarcolemma and the basal lamina, which is a highly specialised interstitial connective tissue within the ECM. Despite an impaired basal lamina and the toxic milieu that results primarily from infiltrated neutrophils, inflammatory macrophages [6] and ECM fragments, satellite cells, along with other survivor cells, are activated and migrate to the site of injury within 2 hours after injury, although some of them undergo self-renewal for replenishing the satellite cell pool [44,46]. Once at the site of injury, satellite cells proliferate and differentiate into fusion-competent myoblasts that differentiate and fuse together to form myotubes and new myofibres [10,46], or fuse with existing damaged myofibres to repair them [9,36].

Angiogenesis comprises the activation of quiescent endothelial cells that in mammalian skeletal muscle show a potential to proliferate rapidly after activation by angiogenic stimuli (such as DAMPs and growth factors) in the injured area [32]. Small blood vessels form in this fibrous callus that now joins the ends of the various broken fibres, while the fibrin matrix continues to be infiltrated with macrophages. These new capillaries will later mature and stabilise and generate a structured network of capillaries [9,47]. Moreover, neovascularisation appears to be crucial in functional and structural muscle regeneration,

providing the new tissue with oxygen, other nutrients and blood-derived cells, at the same time as removing carbon dioxide and other tissue-waste products [2,9].

Fibrogenesis is another component of the toolkit-defence system. This process has evolved to fix and replace necrotic areas and the initial fibrin clot with granulation tissue [2], to address the loss of connective tissue, to seal off the injured area, and repair or generate the basal lamina [7,33]. When the aseptic yet toxic microenvironment lingers over time, or when neovascularisation is compromised, an M1 phenotype persists, which leads to a non-resolving inflammation where a myofibroblast profile and fibrogenesis takes over myogenesis; this generates an excessive and persistent deposition of ECM and results in fibrotic scar tissue [10,30,33,40,48]. Myogenesis, angiogenesis and innervations are of paramount importance to the integrity of the basement membrane and cell-cell adhesion [22,49,50]. Repair of the basement membrane is the first key step in reconstruction of the neural canal (space in the fibrillar void): basal lamina not only ensures subsequent compartmentalisation of the repair phenomena [49], but is involved in mechanical support, myogenesis and synaptogenesis, and its molecular composition endows it with adhesive and inductive functions for a variety of cell fates during muscle repair [40,49,51].

Innervation is essential for growth and maturation of newly formed myofibres as well as for the re-expression of myosin heavy chains [10,51]. The newly formed granulation tissue that joins the damaged fibres together should not form a barrier to axon progression from neighbouring nerve endings [2,37]. It should also not surround them with a fibrosis resulting from excessive collagen synthesis or defective synthesis of metalloproteinases (MMPs) [37]. Axon progression leads towards the old synaptic site where the original neuromuscular junction was located or to the basal lamina of new myotubes [22], thereby enabling the restoration of full muscular function, a process that might take months [2].

During the repair process, a mechanical stimulus causes integrins to bind laterally to the edges of muscle cells and to the ECM via laminins, thereby preventing them from retracting: this contributes to the repair process [37]. Controlled physical stress helps to reorientate type I collagen, which enhances the penetration and alignment of myoblasts and stimulates remodelling [1,9,52].

All these biological defence system modules are tightly coordinated through the secretion of growth factors and cytokines primarily, but not exclusively, released by satellite cells, macrophages, platelets, endothelial cells and myofibroblasts [4,7,21,46,53] (Figure 2).

Cellular and molecular mechanisms regulating muscle repair and regeneration

Mammalian muscles are composed of tissues with quite different proliferative cell activity: there are cells that have left the cell cycle and cannot undergo mitotic division in postnatal life, such as neurons and myofibres, and quiescent cells, such as fibroblasts, satellite cells and endothelial cells, which have low level or no proliferation. Although quiescent, the latter types of cells can undergo a boost in mitotic, migratory, and secretory activity in response to environmental cues, such as mechanical injury through DAMPs [4,7,21,22,47]. There is a short inflammatory stage in the first 24 to 48 hours after muscle injury [31,46]: this stems primarily from DAMPs, which are recognised by transmembrane toll-like receptors (TLRs) of platelets, endothelial cells and resident macrophages, and then activated [54]. These activated cells located at the fibrin clot secrete tumour necrosis factor (TNF), interleukin-6 (IL-6) and monocyte

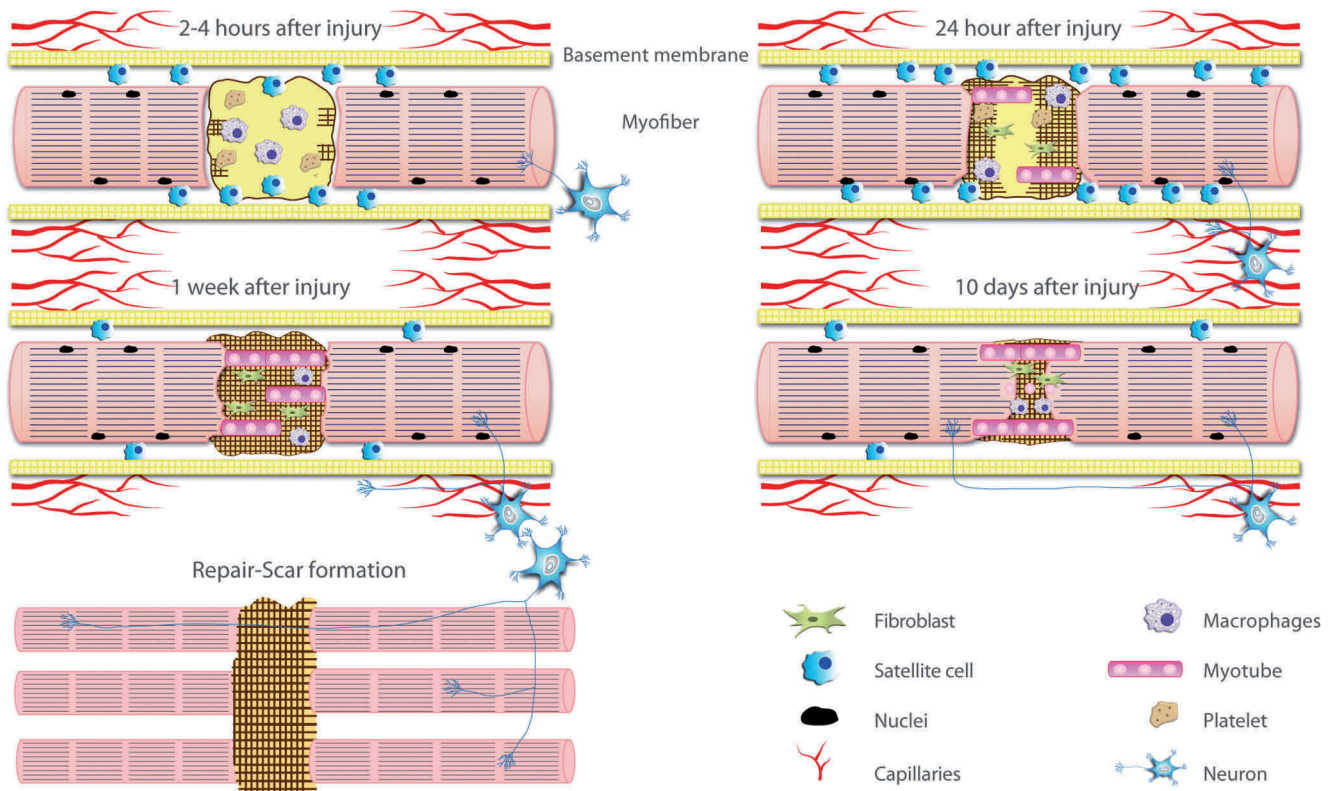


Fig. 1. The most important cell-based phenomena during the repair of muscle injuries [1,4,6,36,46,52]. The importance of the integrity of the basement membrane, which demarcates the neural canal via which signals and cells reach the injury site, should be noted. If the newly formed repair tissue is too fibrotic, it may block or prevent axon growth towards the muscle stumps, thus resulting in non-functional atrophic tissue.

chemoattractant protein 1 (MCP1), which, along with vascular endothelial growth factor (VEGF) [55], attract blood monocytes and more epimysium/perimysium-resident macrophages to the damaged area [29,31,36,42]. Hepatocyte growth factor (HGF) from inflammatory macrophages (M1), activated platelets and endothelial cells simultaneously promote cell cycle re-entry of quiescent satellite cells at the injury site; and fibroblast growth factor (FGF), transforming growth factor β (TGF β), insulin-like growth factor 1 (IGF1), platelet-derived growth factor (PDGF) and VEGF stimulate proliferation and promote migration of activated satellite cells and myoblasts to the repair area and also protect stromal cells and myofibers from an apoptotic fate [10,18,23,26,46]. Moreover, stromal-derived factor 1 (SDF1), released by platelets and fibroblasts, is a mitogenic and motogenic factor for stem and progenitor cells and for circulating monocytes and resident macrophages, which will migrate to the injury sites and modulate their phenotype in a context-sensitive manner [1,26,28,29,56,57]. In addition, SDF1 (also known as CXCL12) plays an important role in angiogenesis by recruiting endothelial progenitor cells from bone marrow through a CXCR4-dependent mechanism [58]. TGF β is a pleiotropic factor that promotes proliferation of satellite cells, activates resident fibroblasts and inhibits myoblast differentiation [10,33,59,60]. The antiapoptotic effect on parenchymal and stromal cells is mainly driven by IGF (I and II), HGF, FGF and VEGF, and it appears to be crucial for myogenesis to be redeployed, as seen in embryo development [46,51].

A pivotal event in muscle repair occurs 48 to 72 hours after injury [29,48] as a result of phagocytic activity of M1 and the new microenvironment created within the callus by the secretory activity of M1, fibroblasts, myoblasts and endothelial cells: this event is the resolution of inflammation. Macrophages have a remarkable ability to reprogramme their gene expression profile

[29,57,61] and switch from a pro-inflammatory macrophage (M1) to a healing, or trophic, macrophage (M2) [28,29,48] and release mainly TGF β and IL-10 [29]. The fibrin matrix generated as a transient ECM, along with other biomolecules of the ECM, may retain several growth factors, such as FGF, HGF, TGF β , VEGF and brain-derived neurotrophic factor (BDNF) that were previously released by platelets, macrophages, endothelial cells and newly activated fibroblasts [9,40,62]. These factors are retained through the cell surface heparan sulphate binding domains [62,63] to be gradually freed up later [62], thereby controlling morphogen gradients at the repair site [64].

IGF1 released by myoblasts, endothelial cells and now trophic macrophages (M2) stimulates the proliferation and differentiation of myoblasts, promotes cell survival [4,21,40,55] and modulates inflammation through the suppression of macrophages (macrophage migration inhibitory factor [MIF]) and transcription factor (nuclear factor kappa B (NF- κ B)), thereby reducing fibrosis and myonecrosis [19]. In addition, the release of platelet factor 4 (PF4) by platelets prevents monocyte apoptosis, promotes trophic macrophages (M2) [56] and may restore cells to a non-inflammatory phenotype. Overall, these stromal and parenchymal events favour a trophic microenvironment, dampen inflammation and may well contribute to the resolution of inflammation, thereby shortening the repair process [12].

Quiescent endothelial cells 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 [47]. The anatomical proximity of endothelial cells, pericytes and satellite cells creates a stem cell zone, mainly through VEGF, HGF, IL-6, and angiopoietin 1 (Ang1). These factors, together with PDGF and FGF, are pivotal in generating, organising, and maintaining the microvasculature [9] and in the development of myogenesis [32,50].

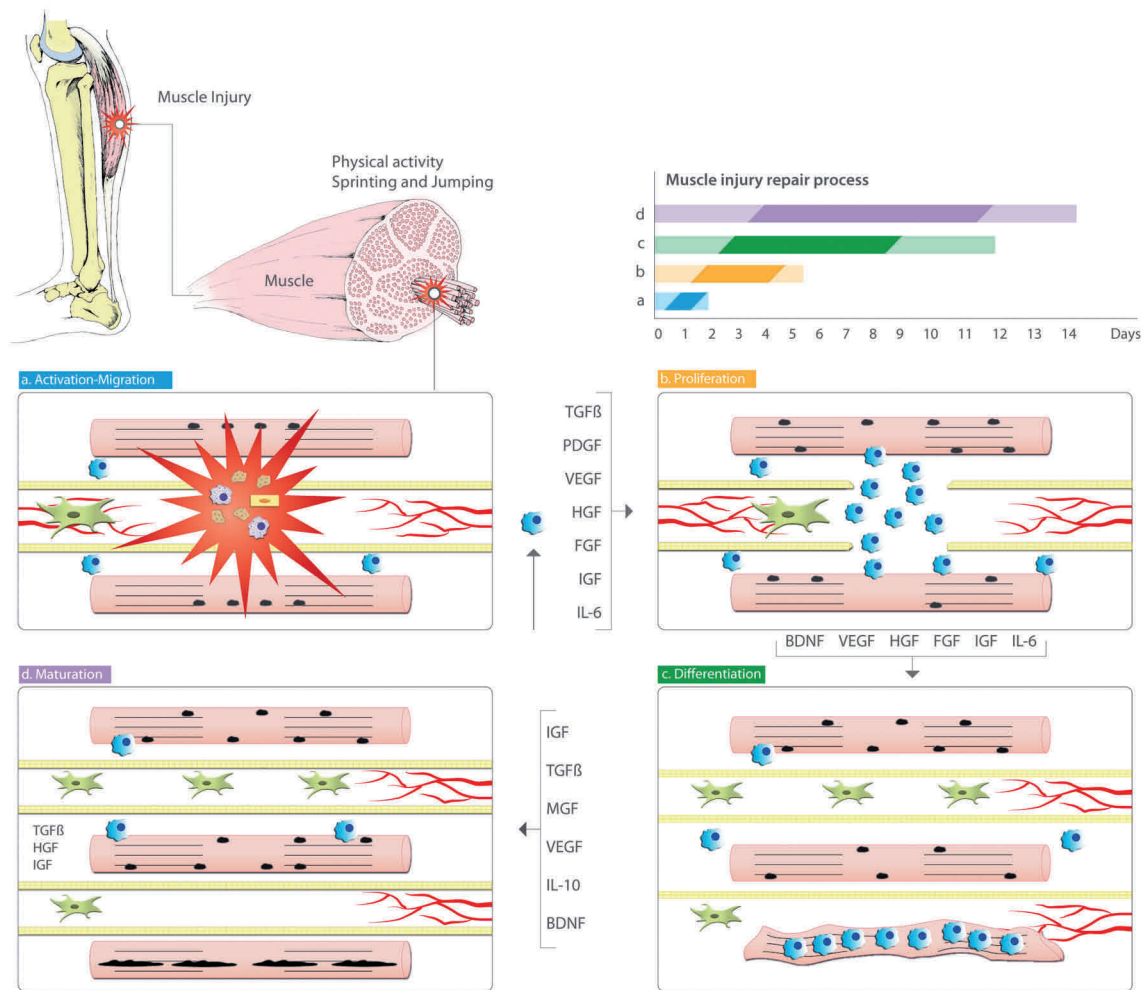


Fig. 2. The key role played by satellite cells (muscle stem cells) in muscle repair under the control of various growth factors [4,10,21,31,36,39,43,53,57]. The participation of IGF and HGF in practically all phases of muscle repair, and the specificity of the niche where these cells are located, should be highlighted. During the initial activation/migration and proliferation phases, cell signalling factors are released by platelets, macrophages, endothelial cells, and the satellite cells themselves. Satellite cells and macrophages tend to govern subsequent phases in this process. The fusion of multiple satellite cells leads to the formation of myotubes, which complete the damaged region and may produce new muscle fibres.

Moreover, the close association of many quiescent cells types (satellite cells, endothelial cells and neural stem cells) with the vasculature enables modulation of cell fate via metabolic cues, circadian rhythms, temperature and mechanical stress, as well as providing feedback with humoral factors and cells from the immune system [17]. Another early event in the stromal cell response to muscle injury is the activation, migration and proliferation of fibroblasts, which take on a myofibroblast phenotype in the presence of TGFβ and PDGF [7,33]. They are also 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 TGFβ [2,7,33,40]. These activated fibroblasts are highly secretory and synthetic cells [2,3,33,37], and infiltrate the fibrin clot that now joins the ends of the various broken fibres. The mechanical and biochemical features of the newly secreted ECM and the provisional fibrin matrix play a role as adhesive cell contacts, and serve as a reservoir of growth factors, including VEGF, PDGF and TGFβ, which are released gradually, thereby modulating macrophages, myofibroblasts, and survival and fate of myotubes [7,26,33,65,66]. The absence or disruption of innervation following muscle injury is the major cause of poor restoration of tissue and compromised function [36,37]. Reinnervation and the creation of a new neuromuscular junction in the repaired or regenerated fibres via the basement

membrane may be driven by growth of new axons from adjacent nerves [4]. These events, often absent in *in vitro* studies, may be mediated by nerve growth factor (NGF) and IGF1, both of which are present in the damaged tissue and are synthesised by muscle cells and fibroblasts under paracrine influence [4]. Biomechanical signalling (BDNF, glial growth factor (GGF)) and ECM stiffness modulate the phenotype and fate of several cells types, including satellite cells, activated resident fibroblasts (myofibroblasts), endothelial cells, macrophages and Schwann cells [9,66,67].

Regulating the gene expression products (TGFβ1, IGF1, IL-10, BDNF, VEGF, collagens, fibronectin and tenascin-C) of satellite cells, activated resident fibroblasts, endothelial cells, macrophages and Schwann cells, appears to be essential in the success of muscle repair [1,9,31,33]. Mechanical and chemical signals, such as mechanogrowth factor (MGF) and IGF1 expressed in an autocrine and paracrine manner, and coming from the cell environment, may complement each other [19,66]. The presence of tenascin C in the ECM is also a prerequisite for muscle reinnervation [40,60]; the synthesis of tenascin C is induced by mechanical stress. In the clinical setting, early gradual mechanical loading stimulates gene expression of trophic factors and signals, such as cyclooxygenase-1 (COX-1), FGFβ, hypoxia-inducible factor (HIF-1), and BDNF, which influences maturation and the correct patterning of myotubes, collagens and tenascin C [36,37]. Both the gradual and controlled mechanical stimulus that induces IGF

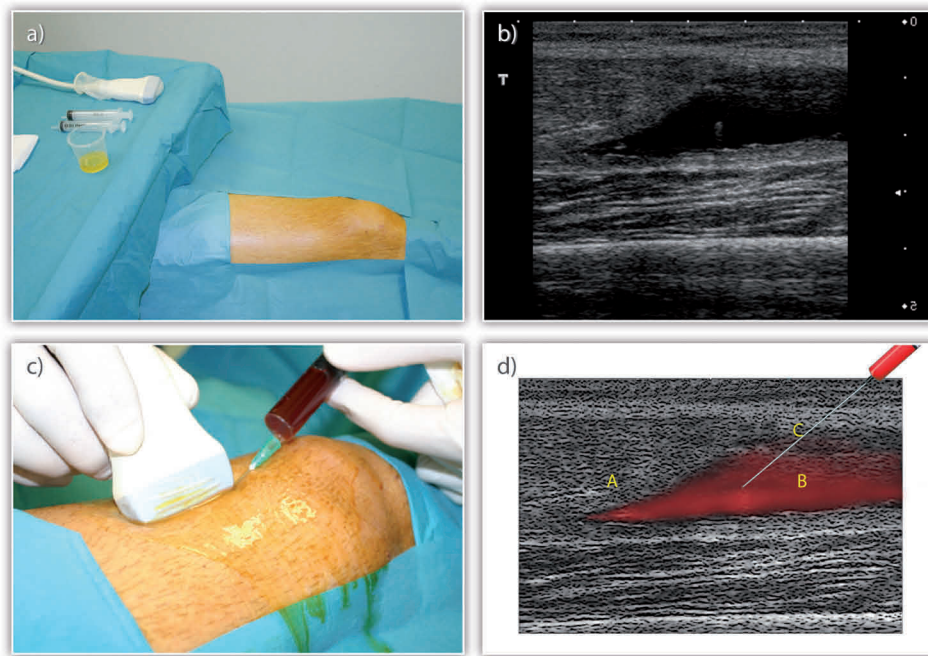


Fig. 3. Preparation of the sterile field (a) required to perform ultrasound control (b) with which we identify the damaged areas, and evacuation of haematoma, if it exists (c and d).

synthesis by muscle cells (by endocrine and paracrine activity) [36,59] and the paracrine and autocrine synthesis of growth factors, such as HGF and TGF β by fibroblasts during the final remodelling phase, appear to be essential, as both these signals may have a synergistic effect on the activity of the fibroblasts that are remodelling the ECM [40,68] and repaired tissue. Moderate sustained mechanical load modulates the fusion and ensuing alignment of myoblasts into myofibres [9] and may minimise or even circumvent the formation of scar tissue by inhibiting the NF- κ B of muscle cells [69], among them fibroblasts, which can promote fibrotic scar [70]. The aforementioned events play a crucial role in balancing tissue remodelling versus fibrotic scar in injured muscle [36,37]; however, what applies for an isolated myofibre does not necessarily apply for the entire muscle [26].

An innovative biological approach to the treatment of muscle injuries: Plasma rich in growth factors

There is considerable evidence to indicate that growth factors and fibrin matrix are instrumental in the muscle repair and regeneration process [9,10,18–23,25,40,53].

An innovative biological approach is the application of Plasma Rich in Growth Factors (PRGF) in intramuscular infiltrations [12]. There is no universally accepted definition of PRGF in terms of platelet concentration and presence or absence of leukocytes; however, PRGF products include plasma and twofold or more increases in platelet concentrations above baseline levels, and the concentration of leukocytes and erythrocytes varies widely from zero to high [16]. Autologous blood-derived products convey growth factors, cytokines, and morphogens contained in platelets, as well as fibrinogen and other plasmatic proteins, in a biologically-balanced aggregate, managed and delivered in a pharmacological manner [16]. These may account for two special features: the resolution of inflammation and the avoidance of fibrosis. In addition to conveying growth factors, PRGF provides the damaged tissue with a transient biological fibrin scaffold that stems from the polymerisation of fibrinogen, which is a pleiotropic blood protein that regulates coagulation, inflammation, and tissue regeneration [15,71].

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: these studies have yielded extremely promising clinical and surgical outcomes in oral and maxillofacial surgery [16], musculoskeletal system pathologies [72] and other medical fields [16]. Although several clinical studies have shown promising functional outcomes with PRGF in repair of muscle damage [12,24,25,73], no clinical trials have so far been conducted to show the improvement of muscle injuries treated with PRGF compared with current treatments, such as physical therapy, ice or corticoid injections. The first results of restoration of muscle function after administration of growth factors and other bioactive molecules using this technology were presented at the 6th EFFORT Congress in Helsinki in June 2003, and the final report at the 2nd World Congress of Regenerative Medicine [12].

PRGF protocol used in muscle injuries

An optimal treatment for repair of muscle injury is a mimetic biomaterial embedded with a pool of growth factors that acts as a smart scaffold [9] at the dysfunctional and deregulated injured site and is a niche therapy [17]. Such a treatment may sustain a gradual delivery of growth factors instead of a bolus delivery modality [23,62,74]. The biomaterial must promote myogenesis, angiogenesis and innervations, and modulate immune response and fibrogenesis to enable functional muscle repair [9,23].

We propose the following general principles in the application of PRGF as a local molecular intervention for muscle injury [75]:

PRGF production

Patients are advised to avoid eating fatty food in the 6 hours prior to blood extraction. Thirty-six mL of peripheral venous blood is withdrawn into 9-mL tubes containing 3.8% (wt/vol) sodium citrate. Occasionally, it may be necessary to extract further amounts of blood because of the size of the lesion. Blood is centrifuged at 580 g for 8 minutes at room temperature. The upper volume of plasma contains a similar number of platelets

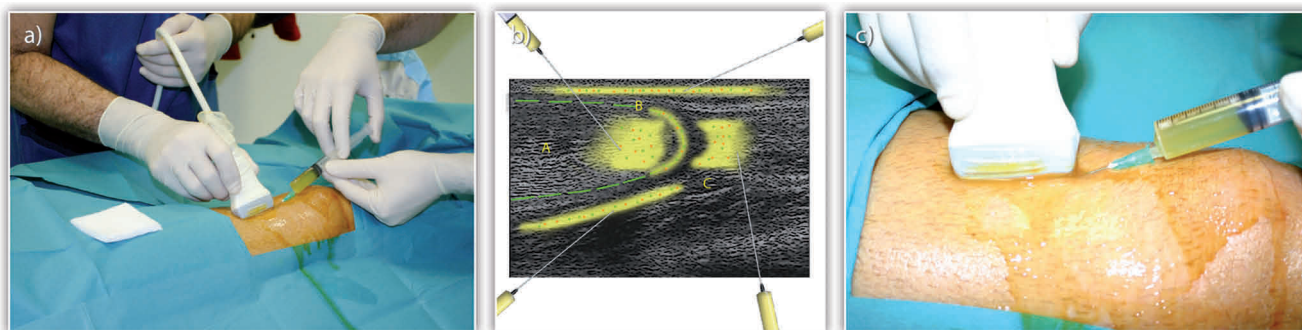


Fig. 4. (a) PRGF Endoret infiltration at the injury site with correct orientation of the needle (always performed with ultrasound guidance). (b) Ultrasound scheme showing the allocation of liquid activated PRGF at the injury site and into surrounding interfascicular and interfibrillar areas.

as peripheral blood (F1) and is collected in a tube. The 2-mL plasma fraction located just above the sedimented red blood cells, is collected in another tube, but without aspirating the buffy coat. This plasma (F2) presents a moderate enrichment in platelets (2–3 fold the platelet count of peripheral blood) with few leukocytes.

Preparation of the injured area

While the nurse is obtaining the PRGF (centrifugation, separation of fractions F1 and F2), the patient is examined clinically to 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 (Figure 3a). An ultrasound longitudinal 8.0–13.0 MHz multi-frequency linear probe wrapped in a sterile cover (Figure 3a) is used to locate the injury and, if applicable, the possible haematoma associated with the muscle tear (seromas, fibrosis and degenerated areas in case of chronic injuries) (Figure 3b). The anatomical location of injection is chosen based on ultrasound and clinical criteria. The use of local anaesthesia must be avoided.

Infiltration procedure at the site of muscle injury

Haematoma, seroma or cysts, if present, are punctured/evacuated using an 18-G needle (Figure 3c and d) under ultrasound guidance (Figure 3c and d). Once the haematoma is evacuated, the F2 of PRGF is activated with calcium chloride (10% wt/vol): this leads to platelet activation, and hydrolysis of prothrombin into thrombin, which simultaneously causes the release of myriad growth factors and the polymerisation of fibrin. In the next 1 to 3 minutes, PRGF-Endoret activated liquid formulation is injected into the injury site under ultrasound guidance (Figure 4a and b). Although the amount of PRGF infiltrated should be the maximum possible and is usually around 8 mL, this volume could reach 10–15 mL depending on the size of the muscle and the extent and severity of the damaged area. Once the injury site has been infiltrated, areas adjacent to the site must also be systematically infiltrated. Therefore, infiltration of PRGF into the peripheral healthy muscle surrounding the injury, including interfascicular and interfibrillar regions, is conducted 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 (Figure 4b) to truly conduct a deregulated area niche therapy [17]. In Figure 5, we summarise 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).

The aim of PRGF muscle infiltrations is to recruit, activate and mobilise satellite cells and resident macrophages [29], which contribute to muscle reparation by cell signalling soluble factors [21,22,46,53], besides the already activated endothelial cells, macrophages, and platelets. Once the activated PRGF is injected, this liquid-to-gel transition 3D injectable scaffold enables 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, it is converted into a matrix-like viscous and malleable structure [16,74]. The fibrin scaffold formed in situ is a provisional ECM and contains binding sites for cell adhesion and proteins, such as thrombospondin-1 (TSP-1), alpha-1-antitrypsin, fibronectin, acute phase proteins and proteins related to lipid metabolism [15,74]. This serves as a highway for mechanical energy to transfer from the environment to the cell, thereby bridging cell-to-cell tissue transition, promoting multicellular assembly, and providing mechanical support and plastic-elastic stiffness; these changes have a drastic impact on the fate of diverse cell types, such as muscle stem cells [76,77], and endow tissues with a suitable mechanical and chemical microenvironment for biological restoration. In addition, fibrin matrix may sequester growth factors such as PDGF, FGF, HGF, BDNF and VEGF [23,62] via heparin-binding domains and gradually release them later, thus exerting a synergistic action on tissue repair [23,62]. As this dynamic, sponge-like fibrin-matrix scaffold is autologous, bio-reabsorbable, biocompatible, and free of leukocytes and red cells, PRGF scaffolds might be considered the best tailored among all the tissue engineering materials [74]. Finally, ice is applied to the infiltration area for about 10 minutes.

Post-infiltration protocol

Patients are advised to apply cold therapy 2–3 times and restrict physical activity during the first 24 hours after infiltration. Clinical and ultrasonographic monitoring is performed weekly during patient follow-up to evaluate the need for further infiltrations. In general, we recommend 2–3 infiltrations on a weekly basis (based on myogenesis and myoblast replication) [10,36,39]. The decision to give further infiltrations is based on ultrasound images and presentation of pain during the treatment period; more than three injections are not normally required. Muscle tissue is a complex mechano-sensitive tissue, therefore, 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 programme must be included in a synergistic manner to help the

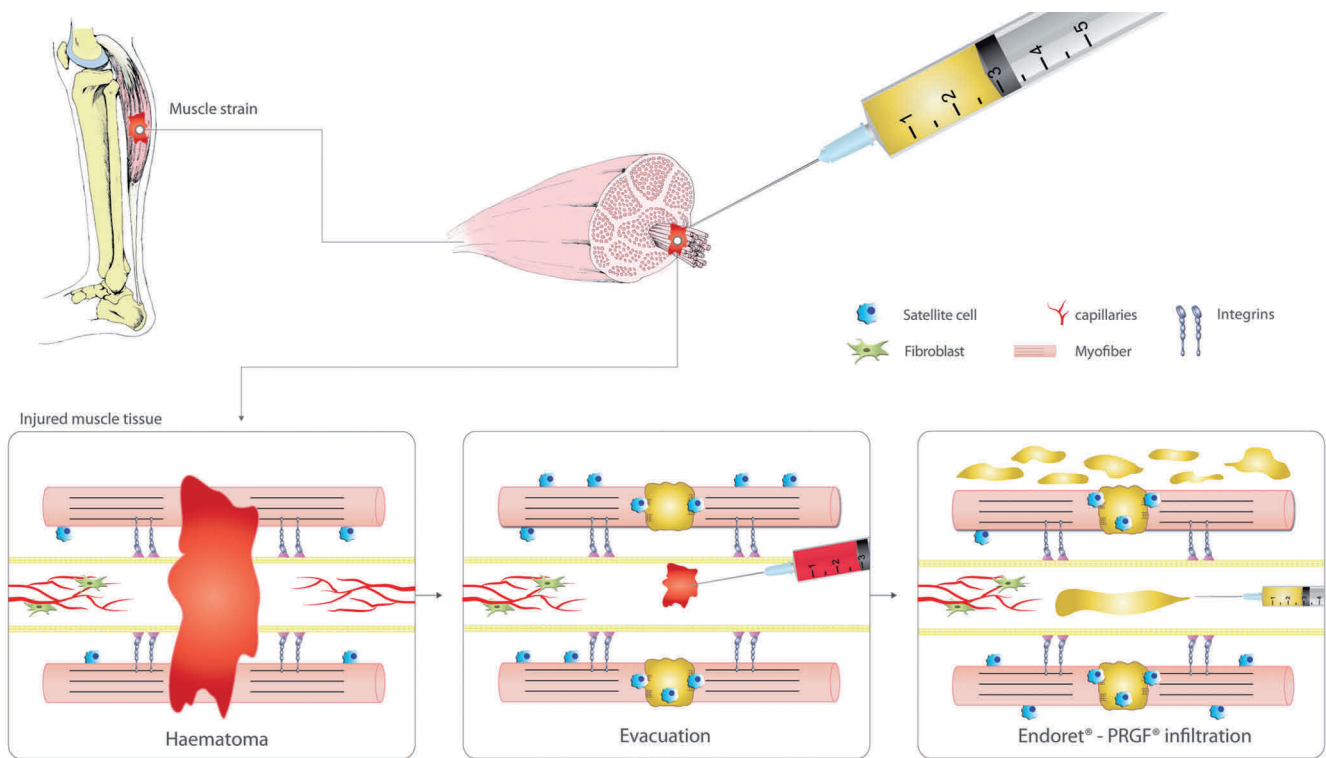


Fig. 5. A summary of the most important phases during percutaneous intramuscular infiltration of liquid activated PRGF-Endoret.

repair and remodelling of injured tissue. Therefore, as the limb has to be mobilised early on in a progressive manner [2,37,52], physiotherapy and rehabilitation treatment are mandatory. The generated mechanical stimulus enables proper recovery of these patients as it acts synergistically with the biological effects of PRGF [52]. Complications, such as seromas, cysts or muscle fibrosis, have to be dealt with using the same principles as in acute ruptures.

Future research

The appropriate treatment of muscle injuries remains a daunting clinical challenge in sports medicine. Harnessing the remarkable plasticity of skeletal muscle by applying PRGF infiltrations, which provide the injured area with an autologous mimetic biomaterial embedded with a pool of growth factors acting as a smart scaffold [9], might sustain a gradual delivery of growth factors at the injured site as a niche therapy. A clinical trial to assess the functional repair outcome of PRGF infiltrations in muscle injuries is needed.

Conflict of Interest Statement

Eduardo Anitua and Sabino Padilla are scientists at BTI Biotechnology Institute, a dental implant company that investigates the potential of plasma rich in growth factors (PRGF). Results of the present study do not constitute endorsement by ACSM.

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