Chapter 28
Plasma Rich in Growth Factors for the Treatment of Skeletal Muscle Injury

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Abstract Skeletal muscle tissue represents between 35 and 50 % of an adult's body weight, and it responds efficiently to changes in homeostasis. Muscle injuries are some of the most common sporting injuries and cause alterations that disrupt the force-transmission chain and result in functional impotence. Current treatments for muscle injuries have not undergone any major changes in recent years irrespective of the level of sport practiced, and their appropriate treatment remains a daunting clinical challenge. One innovative biological approach is intra-muscular injection of platelet rich plasma (PRP), which creates a suitable microenvironment to accelerate repair processes. Appropriate treatment requires an adequate diagnosis of the injury, which must include clinical history, physical examination and complementary tests. Ultrasound and magnetic resonance imaging (MRI) techniques are required for muscle injures, not only for diagnosis but also in the application of PRP that will be carried out once the type and level of injury have been defined. The chapter ends with a description of the protocol we have developed including PRP elaboration, patient preparation and PRP infiltration, indispensable factors for a speedy and successful recovery of the athlete.

28.1 Introduction

Skeletal muscle tissue in humans represents between 35 and 50 % of adult body weight, depending on age, sex, diet, and level of physical activity. Skeletal muscles are extremely plastic and dynamic organs capable of responding efficiently to

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changes in homeostasis. Protein synthesis and degradation are coordinated and regulated by cellular signaling pathways that are influenced by mechanical stress, physical activity, growth factors and the availability of nutrients. By converting chemical energy into mechanical force, thereby generating movement, muscles allow the body to survive and adapt to its surroundings. They are the connection between the environment and gene expression of myofibers, acting like an interface between the environment and the central nervous system. Moreover, muscles are mechanical brakes and buffers or springs, and besides their purely mechanical roles, perform other functions, such as metabolic, temperature-regulating, or endocrine roles.

Long overlooked as a non-genetic input in tissue patterning and remodeling, mechanical load or stress plays a crucial role both in the diversity of cell phenotypes and their functions found in the skeleton [36]. Mammalian muscles are made up of a purposeful-looking array of ingredients such as the cells known as muscle fibers or myofibers, which can reach up to 30 cm in length. In addition, there is a highly organized complex extracellular matrix (ECM) made up mainly of water and collagens, perlecan, laminins, tenascin C, fibronectin, entactin, several glycoproteins and proteoglycans as well as metalloproteases which account for about 1–10 % of the muscle tissue [16]. The ECM adopts a net-support configuration that confers elasticity and encompasses nerve endings, blood vessels, fibroblasts as well as adipocytes and macrophages, and forms a scaffold (endomysium) that intimately surrounds each myofibers. Included in this ECM and intimately related to the sarcolemma, there is a highly specialized interstitial connective tissue organized in a layer known as the basement membrane which is made up of laminins, collagen IV, nidogens and glycosaminoglycans. The basement membrane is closely bound to the sarcolemma by integrins and dystrophins, and is involved mainly in compartmentalization, although its molecular composition endows it with adhesive and inductive functions for a variety of cell fates [28].

The mechanical energy generated in the actin/myosin myofilaments of sarcomeres is transferred from them to the sarcolemma and then through the basement membrane to the ECM and finally to the tendon. These structures operate as the highway of mechanical energy known as mechano-transduction. In addition to the structural and mechanical support for muscle tissue, the molecular composition of the ECM is crucial for a wide range of cellular behavior such as adhesion, migration, growth and differentiation, since laminins, collagens and other proteins located in the ECM play a vital role as signaling molecules and binding sites for many cells [10], thereby inducing and organizing muscle tissue in development and repair processes. Since virtually all the cells that make up the musculoskeletal tissues are mechano-sensitive and experience mechanical stress through the distortion of the ECM complex and respond to them by changing their cellular biochemistry and physiology (mechano-transduction and plastic adaptation), mechanical forces are required to maintain the physical integrity of anatomical structures and homeostasis of the tissues by regulating cell functions, including gene induction, protein synthesis, and cell proliferation, differentiation, growth, survival and apoptosis [20, 33]. It

has in fact been understood that whereas growth factors or soluble factors drive tissue development, mechanical factors govern tissue pattern [19].

Adult skeletal muscles are made up of multinucleated myofibers (fibers) which are established during embryogenesis by the fusion of myogenic cells (myoblasts). Adult myofibers, on the other hand, show their plasticity, namely, muscle growth and repair processes (in the absence of myofibers necrosis and not involving an inflammatory response) by changing either fiber size, in response to physical activity (disuse atrophy/hypertrophy), nutrition status, inflammation, denervation and age, or fiber type (fast-to-slow or slow-to-fast switch), in response to the type of exercise and denervation, or even by forming new myofibers (or segments of them) as a result of injury or damage [9, 29]. This myofiber plasticity is influenced by the balance of protein synthesis and degradation which can cause the loss or increase of organelles and cytoplasm, and/or by the natural cell-cell fusion of myoblasts, to generate multinucleated adult skeletal myofibers which are mainly fueled by the addition of new myonuclei and myofibers involving proliferation of satellite cells [29]. However, the muscle regeneration process, defined as the formation of new myofibers (or segments of them) after necrosis has become established, deploys different patterns of tissue remodeling after injury, illustrating how muscle regeneration is an open condition-sensitive process ruled by microenvironmental cues (mainly mechanical and physical-chemical). As a paradigm for regenerative biology, skeletal muscle may be seen as tissue that conserves and shares modules of regulatory pathways and transcription factors of embryonic myogenesis and development which are redeployed for tissue repair and regeneration after muscle injury [13, 14].

28.2 Skeletal Muscle Injury and Repair Process

Muscle injuries are some of the most common sporting injuries, irrespective of the level of sport practiced, accounting for between 10 and 55 % of all such injuries and encompassing contusions, strain, and lacerations [21]. In football, for example, muscle injuries are responsible for the largest number of both training and competitive days lost. The severity of this type of injury depends on the functional inability to train and, obviously, compete; in many cases this functional loss lasts for 30–40 days.

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 type of muscle exercise injury is one of the physiological injury models. Biarticular muscles such as the hamstrings, rectus femoris, calf muscles, or femoral biceps are most commonly affected by muscle ruptures or tears, although the bruising and trauma resulting from direct impact of the muscle mass during activation should also be taken into account. Muscle damage, whether extrinsic (bruising) or intrinsic (strain-rupture), brings about necrosis and destruction of the constituents of muscle tissue such as

sarcomeres, the sarcolemma, capillaries, or other extracellular matrix elements such as integrins and dystrophins, depending on the intensity of the force and myofibers, although the impairment of their basal lamina might be diverse in degrees. This alteration disrupts the force-transmission chain and results in a functional impotence. Furthermore, it generates a tissue necrosis area, mainly due to the mass entry of calcium into the muscle cells and subsequent activation of proteases such as calpains that break down myofibrils and other cell constituents. The resulting hematoma from the torn blood vessels fills the gap created between the already retracted myofiber stumps. The processes of defense, proliferation, regeneration, maturation and remodeling of the different cells and structures in muscle tissue slowly take over in a spatial and temporal interaction [7].

As part of the endomysium, cells, basal lamina and peripheral capillaries may present a disruption in muscle tears or bruising. Such ruptures often affect the neuromuscular junction itself, resulting in stumps of "non-innervated" muscle fibers. The most common site for muscle ruptures is the region known as the myotendinous junction (MTJ). Subsequent to a muscle rupture and regardless of the mechanisms that bring about the injury or impairment, muscle regeneration as a spontaneous event involves the launch of a series of biological programs such as local defense, myogenesis, angiogenesis, reinnervation, and remodeling, and spans several hierarchical levels from genetic through molecular and cellular levels to tissue and organ levels. From the beginning of the process, angiogenesis and neovascularization appear to be crucial in functional muscle regeneration, furnishing the new tissue with oxygen and other blood-derived cells and nutrients, at the same time removing carbon dioxide and other tissue-waste products. Innervation, another tissue process that shows an exploratory behavior, is essential for growth and maturation of newly formed myofibers. In general, reinnervation occurs at the original junctional basement membrane which is endowed with a specific memory [15] provided that the fibrotic scar tissue does not impinge on and infiltrate the basement membrane and thereby preventing the progression of axons.

These events are regulated by multiple soluble molecules including cytokines and growth factors released by several cell processes, and by biochemical and cell signaling pathways, such as Notch-1, PI3K/Akt or NF-kB [32] or those arising due to mechanical stress [9]. It is worth recalling that damaged muscle cells (myofibers) in mammals have a poor potential to repair themselves once they have undergone necrosis, and end up generating a granulation tissue which will be replaced by a fibrotic scar tissue. Nevertheless, new muscle might form from the proliferation (regeneration) of the satellite cells. These cells are considered to be muscle stem cells, located between the surface of myofibers and the basal lamina, which can somehow redeploy as in embryo development. When the mechanical damage is less severe and the tissue impairment is reversible, in the absence of necrosis and cell death, there is merely a disruption of subcellular architectural organization, giving tissue the potential to inaugurate the repair or restoration process [15]. Under physiological conditions, muscles generate and respond very differently in terms of duration, intensity, type of muscle action, and frequency, meaning that this tissue remodels and repairs "small damage foci" by activating various basic biological programs.

For example, damage to the cell membrane or sarcolemma as a result of eccentric muscle actions (simultaneous braking and stabilization, such as walking downhill for example) is repaired by the muscle cell by the gluing/fusion of vesicles obtained from the sub-sarcolemma. This type of repair occurs under the action of dysferlin [9].

28.3 Cellular and Molecular Mechanisms Regulating Muscle Repair and Regeneration

Skeletal muscle tissue responds to sports-related muscle injuries on three timescales. First, the biological defense programs are initiated immediately after the injury (0 to 2–3 days) by activation of hemostasis and the innate immune system, thereby preventing possible hemorrhage and infection as well as sealing the injured area. The pain episode entails a natural period of immobilization, hence mechanical stress should be avoided. Both platelets and macrophages in the tissue itself synthesize and release bioactive substances and growth factors such as PDGF, VEGF, HGF, TGFB, TNF and IL-6 mainly with chemotactic, migratory and satellite cell activation effects, attracting monocytes and more macrophages to the damaged area [7, 9, 31]. The contribution of cells from the innate immune system is important in this repair process as the macrophages adopt a proinflammatory phenotype in this microenvironment, thereby phagocytosing tissue debris and cleaning the necrotic zone. This phagocytosis and proteolysis is essential to ensure that subsequent repair processes commence in the rupture zone, which is currently occupied by a fibrin clot. The other organic cell defense line, namely the platelet, belongs to the hemostasis and clotting system. The hematoma organizes itself into a clot while the previous process is under way. Platelets come into contact with fibronectin and collagen from the extracellular matrix, thereby triggering the release of growth factors which, together with those released by macrophages, endothelial cells, and pericytes, stimulate tissue repair [7, 9].

By 48 h, fibrin and fibronectin have created a matrix/clot that serves as a bridge between the edges of the myofibers affected, thereby demarcating the repair site [22]. Platelets, endothelial cells, macrophages, and proliferating satellite cells all express growth factors, which in turn, stimulate other cells such as fibroblasts that initially synthesize type III collagen (type I collagen after day 5 or 6), tenascin, and fibronectin, as well as integrins that will laterally affix the broken edges to adjacent fibers. This new tissue re-establishes the bond between the ends, although the tissue formed is very fragile and somewhat elastic [21]. Angiogenesis is involved in the synthesis of new capillaries and starts with endothelial cells and pericytes induced by VEGF in this fibrous callus that now joins the ends of the various broken fibers while the matrix continues to be infiltrated with macrophages. As a result of the new microenvironment created within this callus by the activity of fibroblasts, myoblasts, and endothelial cells, these macrophages express an anti-inflammatory phenotype, releasing TGF-β and IL-10 [8].

On a longer timescale (4–15 days), and through appropriate environmental influences, adaptive plasticity enables the emergence of cell phenotypes which are apt to generate an ECM that will face external cues (mechanical load). Angiogenesis, myogenesis and reinnervation overlap with the ECM regeneration process. In other words, these events run in parallel with the synthesis of the interstitial tissue (synthesis of tenascin C, fibronectin, collagen, and other proteins) that make up what is traditionally known as the fibrous callus. Around 6 or 7 days after the injury, myogenesis is already generating differentiated myoblasts which are integrated into the fibrin matrix that penetrates and infiltrates the damaged stumps. Within the fibrin scaffold, myoblasts differentiate and fuse together to form myotubes which then evolve into the synthesis of a new cell, a process that may be somewhat slower. Moderate sustained mechanical load modulates the fusion and ensuing alignment of myoblasts into myofibers [11] and minimizes or even avoids the formation of scar tissue by inhibiting the NF-kB of fibroblasts which might promote fibrotic scar. If an early and controlled mobilization of the damaged zone containing the callus/bridge or fibrotic zone is initiated at this stage, the maturation-remodeling process can be triggered, thus resulting in a reciprocal activity among:

- The orientation of the myoblasts inside the matrix, a diversion of tenascin and fibronectin synthesis towards the synthesis of more type I collagen and its reorientation, while not blocking the presence or fusion of either myoblasts or nerve endings [23];
- The creation of lateral connections via integrins;
- Contact with new capillaries.

This is the ideal three-dimensional microenvironment to ensure that the capillary buds join together and do not collapse. Once the clot has formed (from the initial hematoma), the integrity of the basement membrane and the 3D structure provided by the fibrin callus or matrix are essential for myogenesis and reinnervation. As a result of the growth of new axons from adjacent nerves, reinnervation and the creation of a new neuromuscular junction in the repaired or regenerated fibers may be driven by NGF and IGF-1, both of which are present in the damaged tissue and synthesized by muscle cells and fibroblasts under paracrine influence [7]. Repair of the basement membrane is the first key step in reconstruction of the neural canal (space in the fibrillar void) that ensures subsequent compartmentalization of the repair phenomena. 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. The path used by the axons (myelin synthesis), whose viability is enhanced by the TGF-βand fibrin network to reach the new fiber and establish a neuromuscular connection, is via the basement membrane. As a result, it is important that the newly formed granulation tissue which joins the damaged fibers together does not form a barrier to progress of the axon from neighboring nerve endings [22, 23] and does not surround them with fibrosis resulting from excess collagen synthesis or defective MMP synthesis.

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 [23] and contributing to the repair process. Both the gradual and controlled mechanical stimulus that induces IGF synthesis by muscle cells (by endocrine and paracrine activity) 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 synergic effect on the activity of the fibroblasts that are remodeling the ECM [25] and repaired tissue. Controlled exercise helps to reorient type I collagen, thereby enhancing the penetration and alignment of myoblasts and stimulating remodeling [11, 21, 23].

In the wake of these biological processes, about 15 days after sustaining the injury the new tissue begins to acquire the order and configuration of its components, thereby recovering its function, the goal of the whole repair process. The correct coordination and spatial and time sequence of the different steps in this biological muscle repair program will be key to re-establishing the structural integrity and functional properties of the repaired tissue. However, the fibers also need an electrical stimulus during the repair process. This means that a new neuro-muscular junction containing this repaired fiber or, if applicable, the new fiber formed, must be established beforehand in the corresponding motor end-plate [9]. It is important to note that each fiber or skeletal muscle cell connects to a motor neuron at a single point known as the motor end-plate or neuromuscular junction, and this connection is essential for cell trophism and function (in contrast to cardiac muscle cells). As such, a rupture of the fiber may directly affect the neuromuscular junction or leave one of the stumps with no neural connection [22, 23]. As with the vascular system, the nervous system also exhibits an exploratory behavior by searching for, and establishing connections with muscle cells, probably while angiogenesis is underway [14].

28.4 An Innovative Biological Approach to the Treatment of Muscle Injuries: Plasma Rich in Growth Factors (PRGF-Endoret)

The appropriate treatment of muscle injuries remains a daunting clinical challenge. Since muscle tissue is a complex mechano-sensitive tissue, every pharmacological and surgical therapy should be assisted by mechano-therapy [24]. In this respect, and as a clinical application of cell mechano-transduction, a rehabilitation program which included the employment of PRGF in a synergistic manner would play a crucial role in both promoting the repair and remodeling of injured tissue.

One innovative biological approach is the application of platelet rich plasma (PRP) in intra-muscular injections. Although a universally accepted definition of PRPs in terms of platelet concentration and presence or absence of leukocytes is lacking, PRP products include plasma and twofold or more increases in platelet concentrations above baseline levels, while the concentration of leukocytes and erythrocytes varies widely [2, 12] from a complete absence to a high concentration.

Plasma rich in growth factors (PRGF-Endoret) is an endogenous blood-derived product which conveys growth factors, cytokines, and morphogens contained in the platelets as well as fibrinogen and other plasmatic proteins in a biologically balanced aggregate that does not contain leukocytes. It is managed and delivered pharmacologically [2, 3]. The process of platelet activation and hydrolysis of prothrombin into thrombin is driven by the addition of calcium chloride, simultaneously causing the release of a plethora of growth factors and the polymerization of fibrin [5]. Once activated, the liquid formulation is quickly injected as a solution into muscle, and due to its local "in vitro" and gradual "in vivo" activation and homogeneous distribution through and interaction with the ECM of muscles, it is converted into a matrix-like viscous and malleable structure [5]. After the intramuscular infiltration over the injured areas, afibrin-scaffolding formed in situ as an extracellular matrix 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 and providing mechanical support as well as endowing tissues with a suitable microenvironment for biological restoration [27]. Since they are autologous, bioreabsorbable, bio-compatible, and free of leukocytes and red cells, PRGF scaffolds are the best tailored among all the tissue engineering materials.

Current treatments indicated for muscle injuries have not undergone any great change in recent years. Only recently, some novel techniques seem to have emerged, but none of these have yet been supported by a body of evidence in basic science or in clinical research [18]. It is fair to say that none of the other currently available treatments has demonstrated experimental efficacy when administered either in a general or local manner, such as percutaneous infiltrations into a damaged area. These infiltrations involve a large number of molecules and substances that range from homeopathic preparations to autologous serum, including vitamin B and its derivatives. The goal of all these treatments is to improve and accelerate the process of muscle repair, and consequently, to achieve a speedy recovery of the patient to both daily and sports activities as soon as possible. A pilot study conducted in athletes who presented muscle strains showed that the patients treated with autologous serum recovered faster [35].

Concerning PRP, clinical research has not yet clarified the therapeutic effect of this treatment in muscular injuries [1]. Furthermore, the large number of PRP systems on the market and the poor standardization of its applications make difficult its understanding and hamper its correct use. So far, no clinical trials have been conducted showing the improvement of these injuries treated with PRP compared to current treatments such as physical therapy, ice or corticoid injections, and only a few clinical reports seem to shed light on the effect of PRP in muscle damage [17, 26, 34]. However, these pathologies present a high prevalence in athletes and entail lost days from competition and training, making it necessary to search for alternatives to traditional treatments. Our group has designed a rigorous and well defined protocol for the application of different PRGF-Endoret-based formulations in several pathologies, among them muscular injuries. The first results of muscle recovery after administration of growth factors and other bioactive molecules using this technology were presented at the 6th EFFORT Congress in Helsinki in June

2003 [4]. Thereafter, the application of PRGF-Endoret in this field of musculoskeletal injuries has become a standard practice in our clinic [6]. The protocol that we will describe was used by Jaadouni, who discussed in his doctoral thesis the use of PRP (PRGF-Endoret) in acute muscle injuries in athletes. A single PRGF infiltration was performed in a group of 50 patients, most of them rugby players, within the first ten days after injury. Among the results, it highlighted an average recovery of 35 days and a low rate of relapses, finding only one case (2 %), while in the literature the rate of relapse in these muscle injuries is around 30 % [30].

28.5 PRGF-Endoret Protocol Used in Muscle Injuries

Sports-related muscle injuries can be classified as acute such as muscle tears, or chronic like fibrosis and muscle cysts or seromas. To perform an appropriate medical treatment it is essential to conduct an adequate diagnosis of the injury, which has to include clinical history, physical examination and complementary tests. Ultrasound and magnetic resonance imaging (MRI) techniques are specially required in muscle injures, not only in the diagnosis but also in the application of PRGF-Endoret that will be carried out once type and level of injury have been defined [3].

1. Thirty-six mL of peripheral venous blood is withdrawn into 9-mL tubes containing 3.8 % (wt/vol) sodium. Occasionally, due to the size of the lesion, it may be necessary to extract further amounts of blood. Blood is centrifuged at 580 g for 8 min at room temperature (PRGF-Endoret, Vitoria, Spain). The upper volume of plasma contains a similar number of platelets as peripheral blood, and it is drawn off (F1). The 2-mL plasma fraction, located just above the sedimented red blood cells, is collected in another tube without aspirating the buffy coat. This plasma contains a moderate enrichment in platelets (two to threefold the platelet count of peripheral blood) with scarce leukocytes (F2) (Fig. 28.1).
2. With ultrasound guidance, the injury and, if applicable, the possible hematoma associated with the muscle tear (seromas and fibrosis in the case of chronic injuries), are located.
3. After locating the lesion, the area of skin to be infiltrated is prepared with a sterile field and antiseptic is applied; the area is demarcated with disposable cloths to perform ultrasound control in a comfortable way (the probe should be covered with a sterile cover) (Fig. 28.2). Next, hematoma, seroma, or cysts, if present, are then punctured/evacuated using a syringe (Fig. 28.3).
4. Once the hematoma is evacuated, F2 is activated with calcium chloride (10 % wt/vol).
5. PRGF-Endoret activated liquid formulation is injected into the injury site under ultrasound guidance (Fig. 28.4). The volume injected should be the maximum possible, depending on the size of the muscle, injury and severity. Although the amount of PRGF-Endoret infiltrated is usually 6–8 mL, volume can reach 10–15 mL.

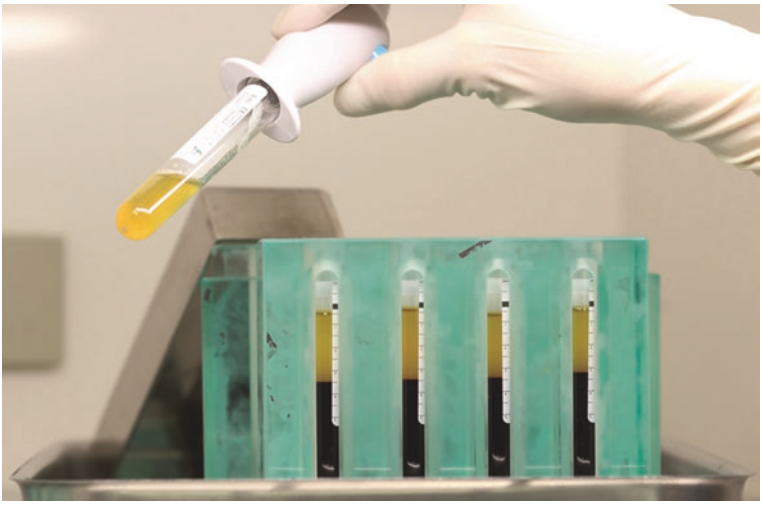

Fig. 28.1. Preparation of liquid PRGF®-Endoret®

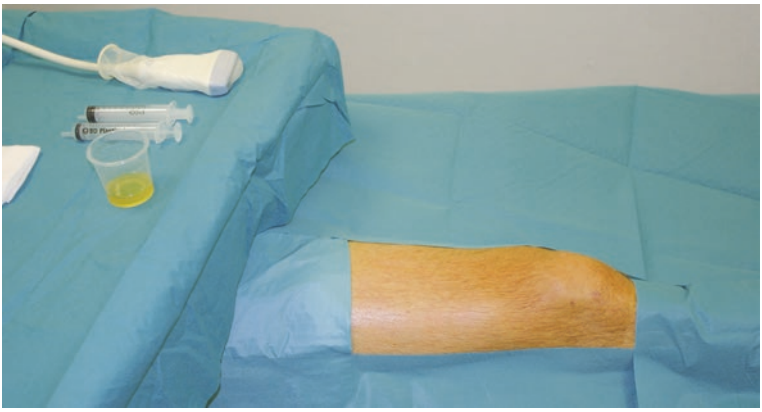

Fig. 28.2 Preparation of the sterile field required to perform ultrasound control, evacuation of hematoma if it exists, and infiltration of PRGF®-Endoret®

6. When the injury site has been infiltrated, it is necessary to conduct PRGF-Endoret infiltrations into the peripheral healthy muscle surrounding the injury, including interfascicular and interfibrillar regions. With these infiltrations, satellite cells are activated and mobilized, triggering muscle reparation processes and cell signaling pathways by activating endothelial cells, macrophages, and platelets. Infiltrations adjacent to the site of injury must be carried out systematically, for instance injury/stump, proximal-stump, distal-fascia, or deep and proximal interfascicular zone (Fig. 28.5).

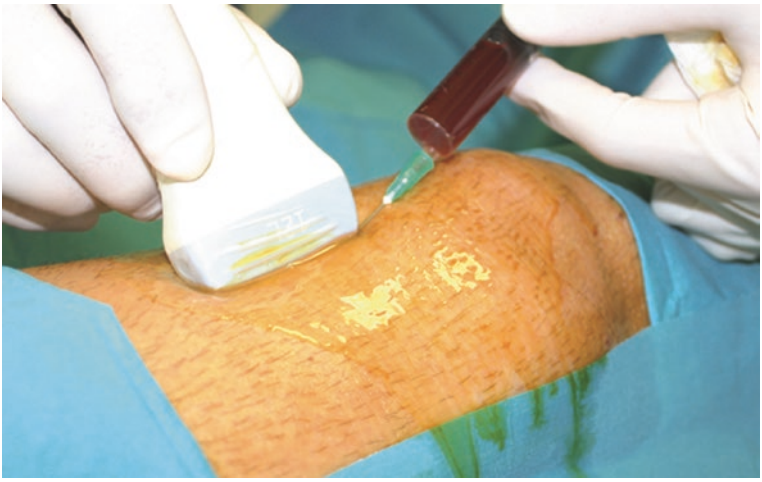

Fig. 28.3 The damaged regions are identified by ultrasound and the hematoma, if present, is then punctured and evacuated

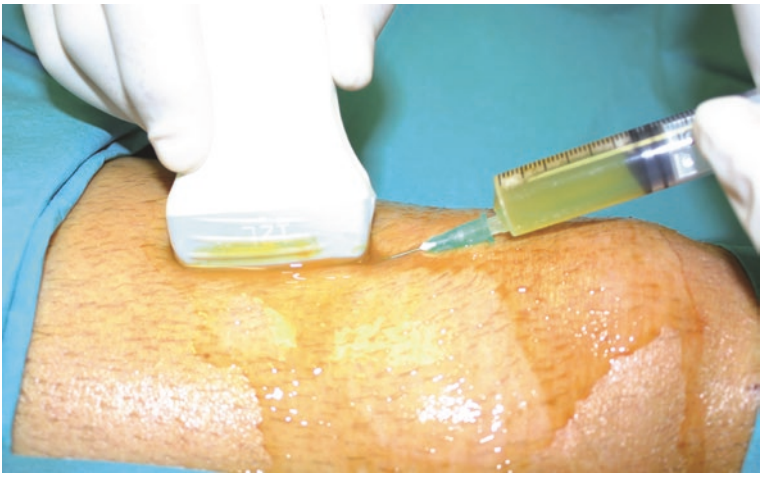

Fig. 28.4 PRGF Endoret infiltration at the site injury and into surrounding (interfascicular and interfibrillar) areas with correct orientation of the needle

7. Finally, ice is applied to the infiltration area for about 10 min.

Both clinical and ultrasound monitoring are performed weekly during patient follow-up to evaluate the potential need for more infiltrations. This decision is based on ultrasound images and any pain that the patient presents during this period. One or two sequential infiltrations (on a weekly basis) are usually sufficient, and more

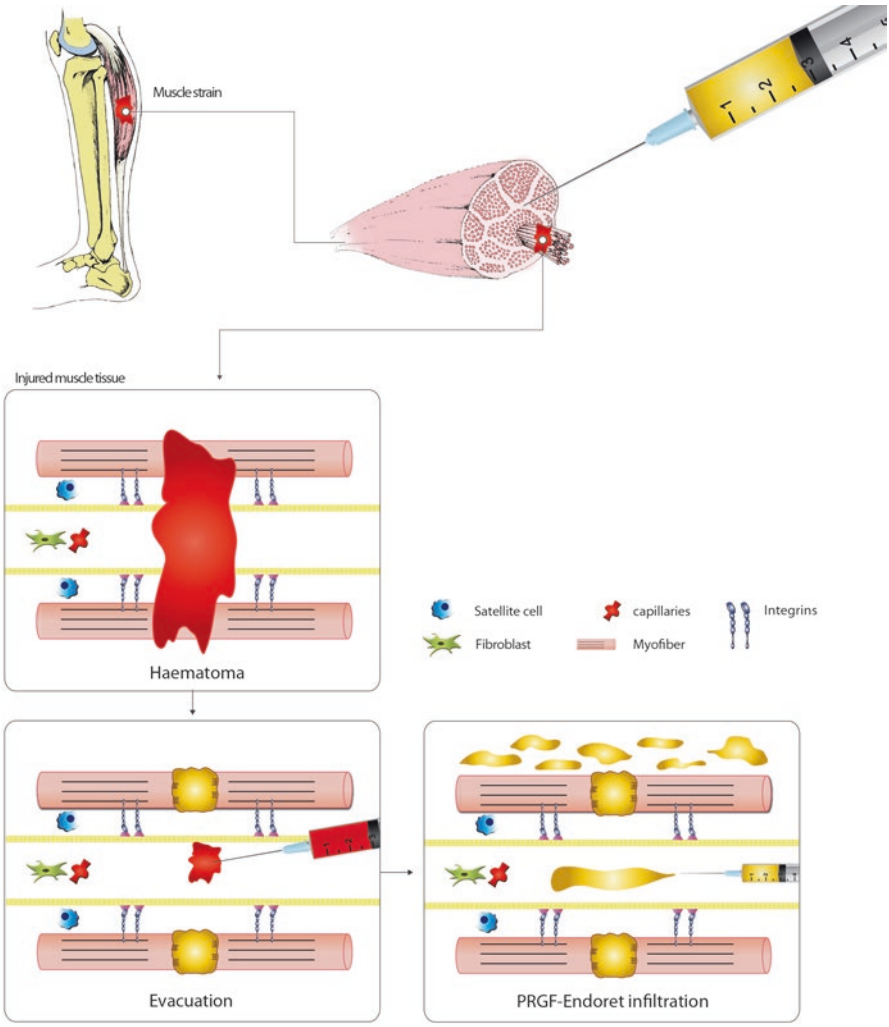

Fig. 28.5 A summary of the most important phases during percutaneous intramuscular infiltration of liquid activated PRGF®-Endoret®

than three injections are not normally required. In addition, physiotherapy and rehabilitation treatment are mandatory since the limb has to be mobilized in an early and progressive manner. The mechanical stimulus leads to proper recovery of these patients, since it acts in a synergic ways with the biological effects of PRGF-Endoret [23, 24]. It does not, however, replace continued rehabilitation, but simply shortens the functional recovery times and stages. Complications such as seromas, cysts, or muscle fibrosis, have to be approached based on the same principles used in acute ruptures.

28.6 Conclusion

Despite the considerable evidence indicating that growth factors and fibrin matrix are instrumental in the muscle repair and regeneration process, there is a gap between basic science and clinical assessment in the treatment of muscle injuries. This gap should be bridged by performing clinical trials to evaluate the efficacy of PRGF for shortening the healing process and avoiding relapse.

References

1. Andia I, Sánchez M. Maffulli. Platelet rich plasma therapies for sports muscle injuries: any evidence behind clinical practice? Expert Opin Biol Ther. 2011;11:509–18.
2. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004;91:4–15.
3. Anitua E, Prado R, Sanchez M, Orive G. Platelet-rich plasma: preparation and formulation. Oper Tech Orthop. 2012;22:25–32.
4. Anitua E, Sánchez M, Andia I. Application of plasma rich in growth factors in skeletal muscle injuries. Communication at the 6th EFFORT Conference, 4–10 June, Helsinki; 2003.
5. Anitua E, Sánchez M, Orive G. Potential of endogenous regenerative technology for in situ regenerative medicine. Adv Drug Deliv Rev. 2010;62:741–52.
6. Anitua E, Sánchez M. A new biological approach to orthopedic surgery and sports medicine. 1st ed. Team work Media España; 2012.
7. Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev. 2004;84:209–38.
8. Chazaud B, Brigitte M, Yacoub-Youssef H, Arnold L, Gherardi R, Sonnet C, et al. Dual and beneficial roles of macrophages during skeletal muscle regeneration. Exerc Sport Sci Rev. 2009;37:18–22.
9. Ciciliot S, Schiaffino S. Regeneration of mammalian skeletal muscle: basic mechanisms and clinical implications. Curr Pharm Des. 2010;16:906–14.
10. Conboy I, Freimer J, Weisenstein L. 5.526 - Tissue Engineering of Muscle Tissue. In: Ducheyne P, editor. Comprehensive Biomaterials. Oxford: Elsevier; 2011. p. 345–59.
11. Conboy I, Freimer J, Weisenstein L. Tissue engineering of muscle tissue. In: Ducheyne P, editor. Comprehensive biomaterials. Oxford: Elsevier; 2011. p. 345–59.
12. DeLong JM, Russell RF, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy. 2012;28(7):998–1009.
13. Gayraud-Morel B, Chretien F, Tajbakhsh S. Skeletal muscle as a paradigm for regenerative biology and medicine. Regen Med. 2009;4:293–319.
14. Gerhart MW, Kirschner W. Cells, embryos, and evolution: toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability. 1st ed. Boston: Blackwell Science; 1997.
15. Grounds MD. Regeneration of muscle. In: Wiley: Chichester; 2011. doi:10.1002/9780470015902.a0001106.pub2 (http://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0001106.pub2/abstract).
16. Grounds MD. Complexity of extracellular matrix and skeletal muscle regeneration. In: Schiaffino S, Partridge T, editors. Skeletal muscle repair and regeneration. Dordrecht: Springer; 2008. p. 269–301.
17. Hamilton B, Knez W, Eirale C, Chalabi H. Platelet enriched plasma for acute muscle injury. Acta Orthop Belg. 2010;76:443–8.

18. Hamilton B. Hamstring muscle strain injuries: what can we learn from history? Br J Sports Med. 2012;46(13):900–3.
19. Huang S, Ingber DE. The structural and mechanical complexity of cell-growth control. Nature Cell Biology. 1999;1:E131–8.
20. Ingber DE. Cellular mechanotransduction: putting all the pieces together again. FASEB J. 2006;20:811–27.
21. Jarvinen TAH, Kaariainen M, Aarimaa V, Jarvinen M, Kalimo H. Skeletal muscle repair after exercise-induced injury. In: Schiaffino S, Partridge T, editors. Skeletal muscle repair and regeneration. Dordrecht: Springer; 2008. p. 217–42.
22. Kaariainen M, Jarvinen T, Jarvinen M, Rantanen J, Kalimo H. Relation between myofibers and connective tissue during muscle injury repair. Scand J Med Sci Sports. 2000;10:332–7.
23. Kannus P, Parkkari J, Jarvinen TLN, Jarvinen TAH, Jarvinen J. Basic science and clinical studies coincide: active treatment approach is needed after a sport injury. Scand J Med Sci Sports. 2003;13:150–4.
24. Khan KM, Scott A. Mechanotherapy: how physical therapists prescription of exercise promotes tissue repair. Br J Sports Med. 2009;43:247–51.
25. Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev. 2004;84:649–98.
26. Loo WL, Lee DY, Soon MY. Plasma rich in growth factors to treat adductor longus tear. Ann Acad Med Singapore. 2009;38:733–4.
27. Nurden AT, Nurden P, Sánchez M, Andia I, Anitua E. Platelets and wound healing. Front Biosci. 2008;13:3525–48.
28. Sanes JR. The basement membrane/Basal lamina of skeletal muscle. JBC. 2003;278:12601–4.
29. Shavlakadze T, Grounds M. Of bears, frogs, meat, mice and men: complexity of factors affecting skeletal muscle mass and fat. BioEssay. 2006;28:994–1009.
30. Sofian J. Apport des plasmes enrichis en plaquettes dans le traitement des lésions musculaires traumatiques – à propos de 50 cas. Thèse pour l´obtention du diplome d´ Etat de Docteur en Medicine. 2012
31. Tidball JG. Inflammation in skeletal muscle regeneration. In: Schiaffino S, Partridge T, editors. Skeletal muscle repair and regeneration. Dordrecht: Springer; 2008. p. 243–68.
32. Wagers AJ, Conboy IM. Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis. Cell. 2005;122:659–67.
33. Wang JHC, Thampatty BP. An introductory review of cell mechanobiology. Biomech Model Mechanobiol. 2006;5:1–16.
34. Wetzel RJ, Patel RM, Terry MA. Platelet-rich plasma as an effective treatment for proximal hamstring injuries. Orthopedics. 2013;36:e64–70.
35. Wright-Carpenter T, Klein P, Schaferhoff P, Appell HJ, Mir LM, Wehling P. Treatment of muscle injuries by local administration of autologous conditioned serum: a pilot study on sportsmen with muscle strains. Int J Sports Med. 2004;25:588–93.
36. Young RL, Badyaev AV. Evolution of ontogeny: linking epigenetic remodeling and genetic adaptation in skeletal structures. Integr Comparative Biol. 2007;47:234–44.