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Muscle repair: platelet-rich plasma derivates 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 (TGFβ), 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.

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) derivates 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 fibrobasts.

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