Brief Insight about Skeletal Muscle and Tendons Injury

Basma Salah Abd-Elhay [1], Mohey Elsaid Hulail [2], Manal Mohammad Morsy[3], Mona Abdelatty Ahmed [4]

1 Assistant lecturer of Human Anatomy & Embryology, Faculty of Medicine- Kafr El-sheikh University, Egypt

2 Professor of Human Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt

3 Assistant Professor of Human Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt

4 Assistant Professor of Human Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt

Corresponding author: Basma Salah Abd-Elhay

E-mail: basmaaiad970@gmail.com, basma.ayad2014@med.kfs.edu.eg, Basmasalah20233@gmail.com

Conflict of interest: None declared

Funding: No funding sources

Abstract

Skeletal muscle injuries are the most common sports-related injuries and a major concern in sports medicine. According to the World Health Organization (WHO), musculoskeletal injuries are the most common cause of severe long-term pain and physical disability, and affect hundreds of millions of people worldwide. Muscle trauma is also one of the main reasons for the decline of athlete competition performance.Risk factors for muscle injury are fatigue, weakness, age, medications/drugs, illness, gender, and nutritional status.Tendon healing divides into three overlapping phases: inflammation, proliferation, and remodeling.Inflammation is the first reaction following tendon injury. During this period, cells such as neutrophils and erythrocytes migrate to the injury site, and monocytes and macrophages begin phagocytosis of necrotic material.

Keywords: Skeletal muscles, tendons, injury

TobRegul Sci.™ 2023;9(1):5915 - 5924

DOI: doi.org/10.18001/TRS.9.1.411

Introduction

Skeletal muscle injuries are the most common sports-related injuries and a major concern in sports medicine. According to the World Health Organization (WHO), musculoskeletal injuries are the most common cause of severe long-term pain and physical disability, and affect hundreds

of millions of people worldwide.Muscle trauma is also one of the main reasons for the decline of athlete competition performance *(1)*.

Risk factors

Risk factors for muscle injury are fatigue, weakness, age, medications/drugs, illness, gender, and nutritional status *(2)*.

Mechanism of injury

The cause of muscle injury can be considered indirect or direct. Indirect injury may be of functional cause, due to mechanical overload or neurological injury, or structural, which occurs when there is a partial or complete muscle rupture, such as the lesion in an eccentric concentration. Direct injury occurs at the contact site, which may cause a laceration or contusion. More than 90% of all sports-related injuries are bruises or stretches *(3)*.

The tensile strength exerted on the muscle leads to an excessive stretching of myofibrils and, consequently, to a rupture near the myotendinous junction. Muscle stretches are typically observed in the superficial muscles that work crossing two joints, such as the semitendinous and gastrocnemius muscles *(4)*.

Pathophysiology

Skeletal muscle healing follows a constant order, with no major changes depending on the cause (contusion, stretch or laceration). Three phases were identified in this process: destruction (or inflammation), repair, and remodeling. The last two phases (repair and remodeling) overlap and are closely related *(4)*.

(1) Inflammatory phase

The inflammatory response begins immediately after injury. The induction of the inflammatory response will vary depending on the mechanism of injury. Inflammation is generally considered as resulting from the coordinated response between blood vessels, local tissues, and leukocytes, consisting of a change in blood vessel diameter, structural changes in the vasculature, and leukocyte recruitment to the site of injury. Vasoactive mediators including nitrous oxide (NO), bradykinin , serotonin, and histamine help to produce the predictable signs and symptoms of early inflammation *(5)*.

(2) Repair phase

The repair phase actually begins soon after the injury. Repair involves a balance between two opposing processes; the formation of a connective tissue scar and regeneration of myofibers *(4)*.

While the inflammatory response is working to clear disrupted tissue from wounds, the repair process has begun. Before myocytes begin the repair of damaged myofibers, fibroblasts have begun to stabilize the wound. As fibroblasts arrive, they produce proteins and proteoglycans found in the native ECM. Through the fibroblast production of ECM proteins, the local environment of the wound is altered to resemble the pre-injury state *(6)*.

Because one of the major inducers of the inflammatory cascade is disruption of the basement membrane, early stabilization of this environment may play an important role in limiting the inflammatory response and the transition to the repair phase. Two important proteins synthesized by fibroblasts are fibronectin and tenascin-C. These two proteins form multimeric fibers that add strength and elastic properties to the wound *(2)*.

Following stabilization of the wound by fibroblasts and scar formation, the wound undergoes a process to repair the injured muscle. This requires, first and foremost, the activation of satellite cells. Satellite cells are a quiescent population of cells located between the sarcolemma and the basal lamina, and are the primary cells responsible for myocyte replacement and repair *(7)*.

(3) Remodeling phase

The repair and remodeling phases overlap as the muscle continues to heal. The transition to the remodeling phase is based on the observation of further ECM deposition and initiation of tissue remodeling. Regardless of the point of transition, full remodeling of injuries is a slow process that begins while the repair phase is active and can take weeks to months or even longer depending on the severity of the injury *(4)*.

Each tissue component within the muscle must undergo remodeling before the healing process is considered complete. For example, the connective tissue scar will continue to evolve, secondary to changing collagen structures. The amount of type III collagen continues to decrease and be replaced by type I fibers. Throughout the remodeling phase, the type I collagen also continues to strengthen via formation of cross-links and increased fiber size *(2)*.

Tendon Injury

Phases of tendon healing

Tendon healing divides into three overlapping phases: inflammation, proliferation, and remodeling *(8)*.

(1) Inflammation

Inflammation is the first reaction following tendon injury. During this period, cells such as neutrophils and erythrocytes migrate to the injury site, and monocytes and macrophages begin phagocytosis of necrotic material. Various cells, including neutrophils, monocytes/macrophages

and tendon stem/progenitor cells (TSPCs type I and II), are attached to the site of the injury by pro-inflammatory cytokines, including interleukin-6 and interleukin-18 *(9)*.

Angiogenesis is a crucial component in the healing process during the inflammation phase. The new vascular network nourishes new tissues and removes cell debris. Some studies show that even the slightest decrease in blood flow during this period can have detrimental effects on healing *(10)*.

(2) Proliferative phase

In the proliferative phase, macrophages and endothelial cells release growth factors, in order to direct cell recruitment and form granulation tissue within the injured region, to serve as a provisional matrix during the healing process. Thereafter, fibroblasts and tenocytes are recruited to the injured region to produce collagen type III, fibronectin and proteoglycans in order to initially create an unorganized extra-cellular matrix and bridge the injured region. After that, the collagen type III is replaced by the stronger collagen type I *(11)*.

(3) Remodeling phase

Cellularity also decreases during this stage as the tissue becomes even more fibrous. Type I collagen production increases, replacing type III collagen and GAGs built up during the previous phase. Collagen fibrils start to cross-link in greater numbers, increasing tissue stiffness*(8)*.

Many enzymes play critical roles in the tendon-healing process at the final healing stage, namely matrix metalloproteinases (MMPs). These enzymes play an important role in tendon degradation and remodeling following injury by shaping and influencing the tendon ECM *(12)*.

PRP should refer to the fraction with a platelet concentration 3 to 5 times greater than normal levels. However, the most accepted definition characterizes PRP as a volume of autologous plasma that contains a platelet concentration above basal concentration (150 000-350 000/μL) *(13)*.

Multiple studies have demonstrated a role for platelet-rich plasma (PRP) in accelerating and facilitating response to injury. The cellular response to injury progresses through four general stages: hemostasis, inflammation, proliferation, and finally remodeling. Each phase is characterized by enhanced cellular or molecular activity, all of which involve platelets *(14)*.

Blood plasma and platelets are responsible for hemostasis, while leukocytes and activated platelets mediate inflammation, and growth factors derived from platelet α-granules influence tissue regeneration. Specifically, the leukocyte content of PRP is thought influence the inflammatory phase, while angiogenic and mitogenic growth factor concentrations are believed to aid tissue regeneration *(15)*.

- **Procedure for Obtaining Platelet-Rich Plasma**

First, venous blood is taken and collected in sterile tubes with citrate as anticoagulant. The tubes are then centrifuged in a conventional centrifuge. The duration, speeds, and number of centrifuge steps depend on the method used. To avoid fragmentation of the platelets and the subsequent early release of the secreted proteins, with corresponding negative impact on their bioactivity, low centrifugation speeds are recommended *(16)*.

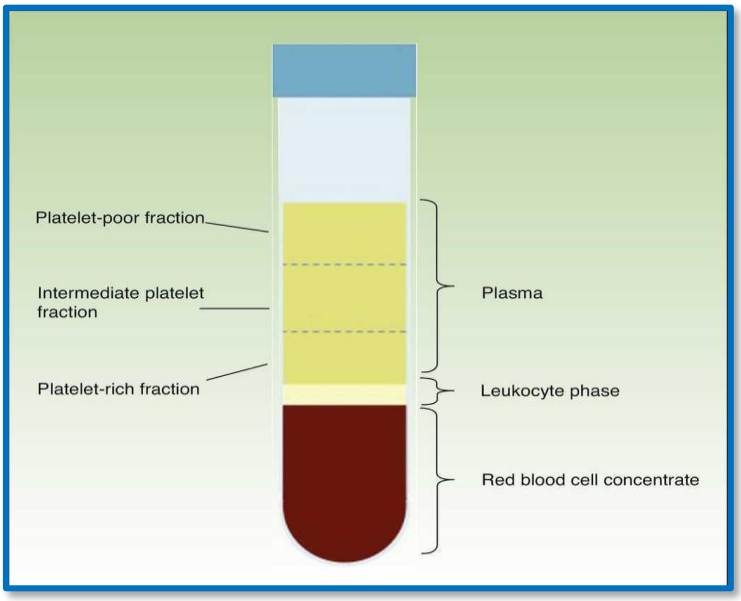

Fig. 1: Different fractions obtained after centrifugation of anticoagulated blood. This division of the plasma phase cannot be detected by eye, so the fraction is simply subdivided into the upper, lower, and middle thirds. Each fraction is separated into different sterile tubes by pipetting (17).

When the anticoagulated blood is centrifuged, 3 layers with different densities separate out: the lower layer, composed of red blood cells; the middle layer, composed of white blood cells and platelets; and the upper layer, composed of plasma. The plasma phase, in turn, can be subdivided into 3 fractions according to the number of platelets present. These fractions are, from most to least abundant: the platelet-poor fraction, the intermediate fraction with a medium concentration of platelets, and the platelet-rich fraction **(17)**.

In order to achieve platelet degranulation and the subsequent release of the growth factors and other bioactive molecules, the lower fraction of the plasma phase has to be activated. The platelet-rich phase can be activated with different agents, with calcium chloride and thrombin being the most widely used. The product can be applied by injection or as a gel. In the former case the activated mix will be injected within 10minutes whereas in the latter case the technician will wait until a gel has formed. This normally requires heating or the addition of bioactive polymers *(18)*.

Bioactive Molecules in Platelet-Rich Plasma &

PRP contains other bioactive molecules with an important role in tissue healing. These include platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet factor 4 (PF4), interleukin (IL) 1, platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), and insulin-like growth factor (IGF) ***(19)***.

These molecules, and other bioactive molecules, have different important functions in the local regeneration environment such as proliferation, migration, and cell differentiation and angiogenesis. It is difficult to define the specific functions of each factor as these overlap to a certain extent. The proteins that act to promote cell adhesion (fibrin, fibronectin, and vitronectin) are another essential component of PRP. These provide the structural support necessary for cell migration and for proliferation and 3-dimensional growth of tissues over these structures. Therefore, PRP has effects not only directly related to the target cells for the various growth factors but also has a broader effect as an extracellular matrix for the stimulation and repair and/or regeneration of the tissue ***(20)***.

Mechanism of beneficial impact of PRP on the Cellular Environment

The cellular environment in which platelet-rich preparations are placed strongly influences the direction and magnitude of tissue response. In orthopedics, the majority of PRP research has focused on its effects on connective tissues such as tendon, ligament and muscle. In addition to their recognized role in hemostasis, platelets have other essential functions in tissue regeneration. After tissue and vascular damage, platelets become activated and aggregate as part of their hemostatic function. This leads to secretion of proteins and other biologically active molecules which, in turn, trigger cascades of secondary messengers implicated in the tissue healing process ***(13)***.

The theoretical basis for the biological benefit of PRP is that concentrations above the physiological one of platelets and plasma proteins may accelerate the repair process. In addition, reinforcement of the fibrin mesh may enable the viability of sustained release of bioactive molecules to be maintained ***(21)***.

Mechanism of beneficial impact of PRP in skeletal muscle injury

Basic science research has demonstrated that muscle regeneration and myogenesis are dependent upon paracrine healing and growth factors, namely insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), fibroblast growth factor 2 (FGF-2), transforming growth factor β 1 (TGFβ-1), tumor necrosis factor-α (TNF-α), platelet-derived growth factor (PDGF), and prostaglandins (PG). In vitro, IGF-1 has been shown to stimulate proliferation and differentiation of myoblasts and improve muscle regeneration in mouse skeletal muscle ***(22)***.

- **Platelet-Derived Growth Factor (PDGF):**

PDGF is naturally synthesized and stored by the body. Its primary role is to encourage the growth of blood vessels within the body. Also known as angiogenesis, the process involves using cells from existing blood vessels to create new blood vessel tissue *(23)*.

- **Fibroblast Growth Factor (FGF):**

Fibroblast growth factors have multiple functions. They can assist PDGFs in the antiangiogenesis process, they can contribute to wound healing, and they even play a role in embryonic development. The key to FGFs in PRP therapy is their ability to encourage the proliferation and differentiation of different kinds of cells for tissue-specific purposes *(24)*.

- **Insulin-Like Growth Factor 1 (IGF-1):**

IGF-1 is both a growth factor and a hormone. Interestingly enough, it is very similar to insulin in terms of its molecular structure. It plays a major role in both childhood growth and adult anabolism *(25)*.

- **Epidermal Growth Factor (EGF):**

EGF is a growth factor that has been discovered in various sorts of human tissue. It is a growth factor that stimulates cell growth along with proliferation and differentiation. As such, the body utilizes it for both wound healing and injury recovery *(26)*.

- **Vascular Endothelial Growth Factor (VEGF):**

This growth factor is actually a protein involved in both angiogenesis and vasculogenesis. Its most important role is to help in the creation of new blood vessels following an injury. However, VEGF is also involved in generating muscle tissue and by passing blocked blood vessels *(27)*.

- **Transforming Growth Factor beta**

TGF-βs (Transforming Growth Factor beta) this superfamily of growth and differentiating factors, including among others, BMP (Bone Morphogenetic Protein), TGF-β1, TGF-β2 (with molecular weights of approximately 25 kDa) are synthesized and found in platelets and macrophages, as well as in some other cell types, and are involved with bone regeneration in mitogenesis of osteoblast precursors, acting as paracrine growth factors *(28)*.

- **Platelet Factor 4**

PF4 (Platelet Factor 4) is a chemokine that functions as a negative regulator of angiogenesis and as a powerful inhibitor of endothelial cell proliferation. It is a chemotactant for neutrophils and fibroblasts and is a potent antiheparin agent *(29)*.

- **Fibronectin and Vitronectin:**

Both of these are proteins called cell adhesion molecules. As part of cellular proliferation and migration particularly seen in bone and cartilage healing, cells move to new positions to lay down their products such as bone or cartilage. Related to bone, this is termed osteoconduction. Fibronectin and vitronectin also seem to be able to provide a foothold or grip for cells as they move. Whether this is through reversible binding to the cell membrane or its surface texture is unknown at this point ***(30)***.

No Conflict of interest.

References:

[1] Timpka, T., Jacobsson, J., Bickenbach, J., Finch, C.F., Ekberg. J., Nordenfelt, L. (2014): What is a sports injury? Sports Med 2014; 44: 423–428.

[2] Swentik, A. (2016): Pathophysiology of Skeletal Muscle Injury. In J. B. Dixon (Ed.), Muscular Injuries in the Posterior Leg: Assessment and Treatment (pp. 35-47). Boston, MA: Springer US.

[3] Mueller-Wohlfahrt, H.W., Haensel, L., Mithoefer, K., Ekstrand, J., English, B., McNally, S., et al. (2013): Terminology and classification of muscle injuries in sport: the Munich consensus statement. British journal of sports medicine, 47(6), 342-350.

[4] SantAnna, J.P.C., Pedrinelli, A., Hernandez, A.J., & Fernandes, T.L. (2022): Muscle Injury: Pathophysiology, Diagnosis, and Treatment. Revista brasileira de ortopedia, 57, 1-13.

[5] Howard, E.E., Pasiakos, S.M., Blesso, C.N., Fussell, M.A., & Rodriguez, N.R. (2020): Divergent roles of inflammation in skeletal muscle recovery from injury. Frontiers in physiology, 11, 87.

[6] Järvinen, T.A.H., Järvinen, M., & Kalimo, H. (2013): Regeneration of injured skeletal muscle after the injury. Muscles, ligaments and tendons journal, 3(4), 337.

[7] Kaczmarek, A., Kaczmarek, M., Ciałowicz, M., Clemente, F.M., Wolański, P., Badicu, G., et al. (2021): The Role of Satellite Cells in Skeletal Muscle Regeneration—The Effect of Exercise and Age. Biology, 10(10), 1056.

[8] Leyden, J., Kaizawa, Y., & Chang, J. (2019): Principles of Tendon Regeneration. In D. Duscher & M. A. Shiffman (Eds.), Regenerative Medicine and Plastic Surgery: Skin and Soft Tissue, Bone, Cartilage, Muscle, Tendon and Nerves (pp. 355-367). Cham: Springer International Publishing.

[9] Docheva, D., Müller, S.A., Majewski, M., & Evans, C.H. (2015): Biologics for tendon repair. Advanced drug delivery reviews, 84, 222-239.

[10] Chang, J., Thunder, R., Most, D., Longaker, M.T., & Lineaweaver, W.C. (2000): Studies in flexor tendon wound healing: neutralizing antibody to TGF-β1 increases postoperative range of motion. Plastic and reconstructive surgery, 105(1), 148-155.

[11] Vasiliadis, A.V., and Katakalos, K. (2020): The role of scaffolds in tendon tissue engineering. Journal of functional biomaterials, 11(4), 78.

[12] Spiesz, E.M., Thorpe, C.T., Chaudhry, S., Riley, G.P., Birch, H.L., Clegg, P.D., et al. (2015): Tendon extracellular matrix damage, degradation and inflammation in response to in vitro overload exercise. Journal of Orthopaedic Research, 33(6), 889-897.

[13] Wroblewski, A.P., Mejia, H.A., & Wright, V.J. (2010): Application of platelet-rich plasma to enhance tissue repair. Operative Techniques in Orthopaedics, 20(2), 98-105.

[14] Rajput, A., Bansal, V., Dubey, P., & Kapoor, A. (2020): A Comparative Analysis of Intra-articular Injection of Platelet-Rich Plasma and Arthrocentesis in Temporomandibular Joint Disorders. Journal of Maxillofacial and Oral Surgery, 1-8.

[15] Lin, S.L., Tsai, C.C., Wu, S.L., Ko, S.Y., Chiang, W.F., & Yang, J.W. (2018): Effect of arthrocentesis plus platelet-rich plasma and platelet-rich plasma alone in the treatment of temporomandibular joint osteoarthritis: A retrospective matched cohort study (A STROBE-compliant article). Medicine, 97(16).

[16] Amin, I., and Gellhorn, A.C. (2019): Platelet-rich plasma use in musculoskeletal disorders: are the factors important in standardization well understood? Physical Medicine and Rehabilitation Clinics, 30(2), 439-449.

[17] Montero, E.C., Santos, M.F., & Fernández, R.S. (2015): Platelet-rich plasma: applications in dermatology. Actas Dermo-Sifiliográficas (English Edition), 106(2), 104-111.

[18] Wasterlain, A.S., Braun, H.J., & Dragoo, J.L. (2016): Contents and Formulations of Platelet Rich Plasma. In Platelet Rich Plasma in Musculoskeletal Practice (pp. 1-29): Springer.

[19] Caruana, A., Savina, D., Macedo, J.P., & Soares, S.C. (2019): From platelet-rich plasma to advanced platelet-rich fibrin: biological achievements and clinical advances in modern surgery. European journal of dentistry, 13(2), 280.

[20] Nolan, G.S., Smith, O.J., & Mosahebi, A. (2021): Enhancing Fat Graft Survival with Autologous Growth Factors: Platelet-Rich Fibrin (PRF) vs Platelet-Rich Plasma (PRP). Aesthetic Surgery Journal, 41(5), NP241-NP241.

[21] Ding, Z.Y., Tan, Y., Peng, Q., Zuo, J., & Li, N. (2021): Novel applications of platelet concentrates in tissue regeneration. Experimental and Therapeutic Medicine, 21(3), 1-1.

[22] Borrione, P., Fossati, C., Pereira, M.T., Giannini, S., Davico, M., Minganti, C., et al. (2018): The use of platelet-rich plasma (PRP) in the treatment of gastrocnemius strains: a retrospective observational study. Platelets, 29(6), 596-601.

[23] Zheng, X., Koh, G.Y., & Jackson, T. (2013): A continuous model of angiogenesis: initiation, extension, and maturation of new blood vessels modulated by vascular endothelial growth factor, angiopoietins, platelet-derived growth factor-B, and pericytes. Discrete and Continuous Dynamical Systems-Series B, 18(4), 1109-1154.

[24] Lieu, C., Heymach, J., Overman, M., Tran, H., & Kopetz, S. (2011): Beyond VEGF: inhibition of the fibroblast growth factor pathway and antiangiogenesis. Clinical Cancer Research, 17(19), 6130-6139.

[25] Ashpole, N.M., Sanders, J.E., Hodges, E.L., Yan, H., & Sonntag, W.E. (2015): Growth hormone, insulin-like growth factor-1 and the aging brain. Experimental gerontology, 68, 76-81.

[26] Wee, P., and Wang, Z. (2017): Epidermal growth factor receptor cell proliferation signaling pathways. Cancers, 9(5), 52.

[27] Giacca, M., and Zacchigna, S. (2012): VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond. Gene therapy, 19(6), 622-629.

[28] Xu, X., and Cao, X. (2020): Transforming growth factor-β and skeletal homeostasis. In Principles of Bone Biology (pp. 1153-1187): Elsevier.

[29] Field, D.J., Aggrey-Amable, A.A., Blick, S.K., Ture, S.K., Johanson, A., Cameron, S.J., et al. (2017): Platelet factor 4 increases bone marrow B cell development and differentiation. Immunologic research, 65(5), 1089-1094.

[30] Arvidson, K., Abdallah, B., Applegate, L., Baldini, N., Cenni, E., Gomez-Barrena, E., et al. (2011): Bone regeneration and stem cells. Journal of cellular and molecular medicine, 15(4), 718-746.