Platelet-rich Blood Derivatives for Tendon Regeneration

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Abstract

Tendon injuries constitute a significant healthcare problem with variable clinical outcomes. The complex interplay of tissue homeostasis, degeneration, repair, and regeneration makes the development of successful delivery therapeutic strategies challenging. Platelet-rich hemoderivatives, a source of supra-physiologic concentrations of human therapeutic factors, are a promising application to treat tendon injuries from the perspective of tendon tissue engineering, although the outcomes remain controversial.

Tendon injuries are common debilitating musculoskeletal problems. More than 30 million tendon surgical procedures take place annually worldwide, with an associated expenditure of more than €150 billion in the United States and European Union.[1-3] Healing tendons display high re-rupture risk during the first weeks after surgical repair.[4] Hence, augmentation strategies hold interesting potential to shift the injury environment toward enhanced healing and ultimately to achieve tendon repair and regeneration, particularly for chronic tendinopathies.[5,6] The interplay between different biomolecules makes tissue homeostasis, degeneration, repair, and regeneration complex processes. The orchestration of tendon healing is differentially regulated when comparing fetal and adult tissues,[7-10] and it involves numerous cytokines (eg, interleukin [IL]-6, IL-1β) and growth factors (GFs, eg, basic fibroblast GF, transforming GF-β, insulin-like GF [IGF]-1, platelet-derived GF, and bone morphogenetic proteins-12, -13, and -14) which are released in a temporally and spatially controlled manner.[11-13] Different strategies have been pursued for delivering these biomolecules, including gene therapy and local delivery of GFs through injection or biomaterial vehicles.[11,14] Recently, the recognition that several GFs come into play during tendon healing contributed to the investigation of concentrated collections of various GFs.

Platelet-rich Hemoderivatives

The rationale for using platelet-rich hemoderivatives (PRHds) stems from the regulatory role of the cytokines and GFs released from platelets on activation during the healing process. PRHds have been explored as a source of human therapeutic factors in both autologous and allogeneic settings.[15] Platelet-derived GF, transforming GF-β, vascular endothelial GF, basic fibroblast GF, epithelial GF, bone morphogenetic proteins, platelet-secreted GFs with relevance to tissue regeneration are present in high levels in PRHds.[16] Other molecules with therapeutic interest can be found in high concentrations in PRHds, namely, fibrinogen, a precursor of fibrin clot,[17] and immunomodulatory cytokines (eg, IL-1, IL-4, IL-6, and

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None of the following authors or any immediate family member has received anything of value from or has stock or stock options held in a commercial company or institution related directly or indirectly to the subject of this article: Dr. Costa-Almeida, Dr. Babo, Dr. Reis, and Dr. Gomes.

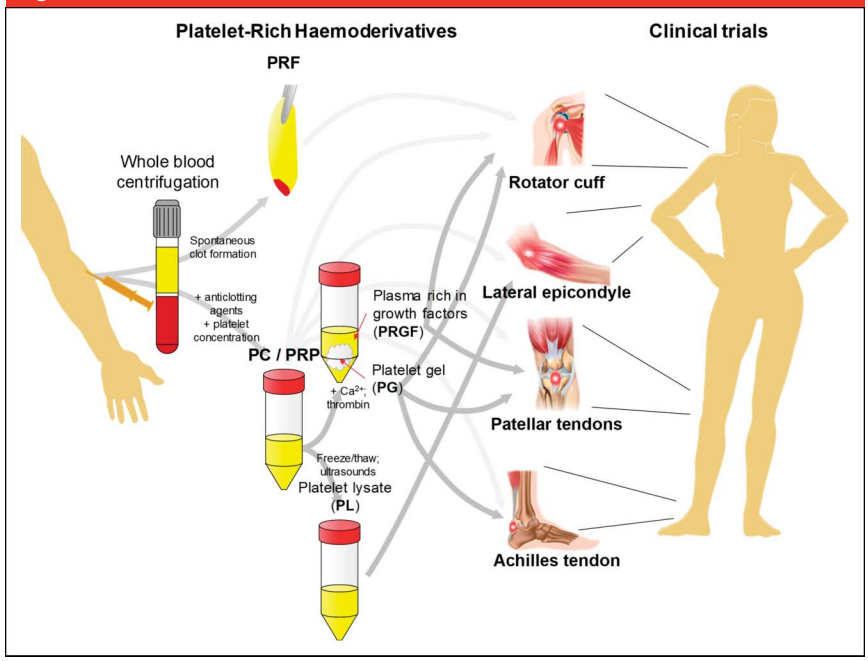
J Am Acad Orthop Surg 2020;28: e202-e205

DOI: 10.5435/JAAOS-D-18-00638

tumor necrosis factor-α).[16] Moreover, molecules with antimicrobial properties or those involved in humoral immunity can be found in PRHds, namely, β-lysin, platelet factor-4, or complement proteins.[18,19]

The composition of different PRHds, and thus their therapeutic effect, greatly depends on the preparation protocol, activation method,[20] and donor variability.[21] PRHds are usually obtained by centrifugation of the peripheral whole blood (WB) (Figure 1). On centrifugation, the WB separates into two phases, which clot spontaneously. The upper phase, a yellowish clot rich in fibrin, platelets, and leucocytes, Choukroun and coworkers called platelet-rich fibrin,[23] has been proposed as an autologous patch for regenerative applications. However, if an adequate anticlotting agent (usually citrate or heparin) is added to the WB, the upper phase can be collected and submitted to further platelet concentration protocols, such as *buffy coat* or the *PRP* methods, commercial kits,[24] or apheresis,[25,26] yielding platelet concentrate, also termed platelet-rich plasma (PRP). The platelet concentrate (or PRP) can be used directly or as activated (eg, with thrombin and/or calcium salts[27,28]), producing a solid fibrin clot, termed platelet gel, which releases a solution of plasma rich in GFs. The PRP produced from different protocols present significant differences in platelet, leukocyte, and fibrin content that translate in variable clinical outcomes of the PRP-based treatments[24] and have raised the need for standardization of PRP preparation and nomenclature (revised elsewhere[20]). Finally, the platelet lysate (PL) is a liquid suspension of fibrin and GFs in plasma obtained by cryogenic[25,26] or ultrasonic[29] disruption of the platelets' membranes contained in the PRP.

Figure 1



Schematic representation of the generic protocols for the production of platelet-rich hemoderivatives and the targets of the clinical trials proposing their applications in the management of tendinopathies. PC = platelet concentrate, PRF = platelet-rich fibrin. (Adapted with permission from Mendes BB, Gómez-Florit M, Babo PS, Domingues R, Reis RL, Gomes ME: Blood derivatives awaken in regenerative medicine strategies to modulate wound healing. *Adv Drug Deliv Rev* 2018;129:376-393 and Babo PS, Reis RL, Gomes ME: Periodontal tissue engineering: Current strategies and the role of platelet rich hemoderivatives. *J Mater Chem* B 2017;5:3617-3628.[16])

Platelet-rich Hemoderivatives for Tendon Tissue Regeneration

Outlook on Clinical Studies

PRP injection has been the most explored application of PRHds for treating tendinopathies reported in in vivo studies.[30-33] Local PRP injection in rat models of rotator cuff injury prevented the acute inflammatory response and scar tissue formation, resulting in improved tendon healing, through the formation of reparative fibrous and granulation tissue, and biomechanical performance.[30,31] The combination of PRP and biomaterials has also been explored. Collagen sponges impregnated with PRP and harboring tendon stem cells delivery to acute Achilles tendon repair rat model resulted in increased collagen type I gene expression as soon as 3 days after treatment under loading conditions, but no other differences were observed regarding tissue morphology.[32]

Clinical studies have shown midterm positive outcomes (improved activity level, reduced pain) on multiple PRP injections for the treatment of Achilles[34] and rotator cuff[35] tendinopathy but without differences in tissue integrity comparatively with the controls. A meta-analysis of eight studies comparing clinical outcomes of rotator cuff repair with or without PRP using the most commonly used clinical score system (patient self-reported outcome measures: Constant Score, American Shoulder and Elbow Society, University of California-Los

Angeles, Simple Shoulder Test, Visual Analog Scale scores[36]) showed differences in Constant Scores, Simple Shoulder Test, and Visual Analog Scale but no effect on the American Shoulder and Elbow Society, University of California-Los Angeles, and re-tear rates.[37] A meta-analysis of eight randomized clinical trials of PRP compared with steroid on lateral epicondylitis treatment reported that PRP seems to be more effective in reducing pain and improving elbow function in the intermediate term (12 weeks) and long term (half year and 1 year).[38] PRP administration to the patellar tendon donor site in anterior cruciate ligament reconstruction procedures resulted in improved donor site healing and reduced pain after 6 months.[39] The clinical outcomes of the PRHds might depend on several factors not included in most scientific reports. For instance, the leukocyte in PRP correlates with the inflammatory response in the recipient tissue, as observed after injection of leukocyte-rich or -poor PRP in healthy patellar tendon rabbit models.[33] PRP treatment efficacy of Achilles tendinopathy has also been reported to depend on patient's age,[40] indicating that these preparations do not overcome the limited tendon healing capacity in elderly patients. Hence, patient's age could be a determinant factor to unravel the efficacy of PRHds in managing tendinopathies.

Platelet-rich Hemoderivatives for Tendon Tissue Engineering

Despite the inconsistent results in terms of clinical translation,[6] the use of PRHds other than PRP in in vitro settings has been gaining attention to modulate cell behavior, particularly for tendon tissue engineering. Plasma rich in GF has been shown to induce rabbit patellar tendon stem cells differentiation toward a tenocytic phenotype and to stimulate tenocytes derived from chronic rotator cuff tendon tears, leading to increased expression of tenogenic genes and proteins in vitro.[41,42] Additionally, PL-based patches have also been reported to induce a proregenerative state on tendon cells through an upregulation of tenogenic genes and matrix deposition.[43]

Conclusions and Perspectives

Overall, a direct injection of PRP or the activation of the coagulation cascade pre-/postinjection (eg, use of thrombin and/or calcium) is still the preferred method in clinical scenarios of the management of tendinopathies. Recently, other PRHds, namely, platelet-rich fibrin and PL, have also been proposed for clinical trials aiming at the management of rotator cuff tear[44] (Clinical Trials no. NCT01612845 and no. NCT02256891) and lateral epicondylitis (Clinical Trial no. NCT01668862), respectively (Figure 1). The contradictory results of therapeutic studies, likely reflecting the lack of consensus on PRHds preparation protocols and nomenclature, as well as donor/patient variability, limit PRHds translation to clinic. A concerted effort is needed to develop more predictable PRHds-based therapies, by not only addressing the above-listed issues but also combining refined tendon tissue engineering strategies, like PRHds-based biomaterials or composites,[43,45,46] ultimately allowing the fine modulation of the spatial, temporal, and dosage release of the adequate biochemical stimuli.[46,47] This could ultimately shift applications from the injury environment to enhance healing and accelerate regeneration of difficult-to-heal tendinopathies.

Acknowledgments

The authors acknowledge the financial support from the ERC Grant CoG MagTendon nr 772817, the Project NORTE-01-0145-FEDER-000021, the Project FOOD4CELLS (Pedro Babo post-doc grant) (PTDC/CTM-BIO/4706/2014-POCI-01-0145-FEDER 016716), and HORIZON 2020 under the TEAMING Grant agreement no 739572—The Discoveries CTR.

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