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Short Communication Open Access

Extracellular Vesicles from Platelet-Rich Plasma as Conveyors of Regeneration Potential in Orthopedics

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Short Communication

Treatment with platelet-rich plasma (PRP) for regeneration and healing is recently considered promising in different fields of medicine including orthopaedics. The popularity of this new treatment option has prompted rapid increase in research endeavor; however the recognized effects are not yet decisive [1]. A strong point of the method is its cost effectiveness and low risk as PRP can be applied as an autograft. Moreover, reported adverse effects including scarring, calcification, infection and injury to nerves or blood vessels were found insignificant [2]. To yield 1 ml of PRP for therapeutic procedures, about 10 milliliters of blood is obtained by phlebotomy in a tube with citrate phosphate dextrose solution (CPD) to bind free calcium ions; the sample is processed immediately [3]. The procedure employs physical laws of hydrodynamics according to which larger particles in a fluid are sedimented faster than smaller ones. Centrifugation in a low speed centrifuge is applied to sediment erythrocytes but not platelets. An appropriate fraction of plasma is sampled which can be further sedimented to increase the number of platelets per volume element. For example, the study of Nugraha et al. (2013) suggested that the first step of centrifugation should be made by applying the centrifuge acceleration 1300.g (g=9.81 m/s^2 is the earth's gravitational acceleration) for 5 minutes and the second step by applying the centrifuge acceleration 2300.g for 7 minutes to obtain the highest increase of platelet count in the plasma [3]. Both steps are usually performed in refrigerated centrifuge [4]. Platelets can then be activated in shear stress (by augmentation of the surface of contact with the moving PRP) [5] and injected to the site of the lesion.

PRP use has hitherto been investigated in the treatment of lateral epicondylitis [6,7], knee osteoarthritis [8], hip osteoarthritis [9] patellar and Achilles tendinopathy [10-12], muscle injury [13] and anterior cruciate ligament injury [14]. Some studies concluded with promising outcomes that will hopefully set the new milestones of treatment options. The effects of PRP treatment of lateral epicondylitis were compared with an autologous whole blood [6] and corticosteroid injections [7]: the analysis of 20 patients treated with PRP and 20 patients treated with whole blood for lateral epicondylitis showed that pain assessed by Visual Analog Scale (VAS), Mayo Elbow Performance Index, and pressure pain threshold (PPT) at 0, 4 and 8 weeks improved significantly in both populations. However, the authors indicated that 8 weeks after the treatment only the PRP group showed statistically significant improvement (P<0.05) [6]. Another analysis of lateral epicondylitis treatment considered 51 patients treated with PRP and 49 patients treated with corticosteroid injections [7]. After 1 year, improvement was found in 73% of the PRP-treated patients and approximately 50 % of the corticosteroid-treated patients as regards VAS and Disabilities of the Arm, Shoulder and Hand (DASH) score for pain evaluation. The differences of improvements were statistically significant (VAS: p<0.001, DASH: p=0.005) [7].

In a research by physicians from Hospital for Special Surgery, New York, 15 patients with knee osteoarthritis were treated by injection of PRP. They were evaluated by VAS, Western Ontario and McMaster Universities Arthritis Index (WOMAC Index), functional scores, ability to perform various daily activities (Activity of Daily Living (ADL) score) and magnetic resonance imaging (MRI) [8]. It was found that after 1 year pain measured by VAS was reduced by 56%, function was improved by 24% and ADL score increased for 56%. In approximately three-quarters of patients, the progression of knee osteoarthritis, as assessed by MRI, was suppressed, which was regarded as an improvement over 4-6 % progression suggested by longitudinal studies [8]. A study comparing the effect of PRP and hyaluronic acid for treatment of chronic unilateral symptomatic hip osteoarthritis [9] captured one hundred (100) patients that were randomly distributed into two groups; one group was treated by PRP and the other by hyaluronic acid (HA). Hips were assessed by Harris Hip Score (HHS) and VAS. Between six-month and one-year follow-up the scores slightly worsened, but still the final scores were higher compared to baseline scores (p<0.0005). Analysis showed that PRP and HA were equally effective at one-year follow-up [9].

Studies referring to PRP treatment of Achilles and patellar tendons gave discordant results; pain was evaluated with Victorian Institute of Sport Assessment-Achilles Questionnaire (VISA-A) score in 30 males and 18 females at the mean age of 38 ± 16 years [10]. Analysis of the treatment of the Achilles (n=30) and patellar tendinopathy (n=28) with PRP showed that pain was statistically significantly decreased (p<0.01) in both tendinopathies with respect to untreated subjects at the 6-month follow-up [10]. However, another study of Achilles tendinopathy reported no statistically significant difference of the effect of PRP as compared with placebo [11]; 54 patients were randomized into PRP group (n=27) or placebo group (n=27) [12]. Improvement was determined in both groups after 24 weeks with VISA-A score. The PRP group showed an improvement by 21.7 points (95% confidence interval (CI)=[13.0, 30.5]) and the placebo group by 20.5 points (95% CI=[11.6, 29.4]), but the improvements of the two groups were not statistically significantly different [12].

PRP has proved equally efficient as conventional methods to treat muscle and anterior cruciate ligament injury [13,14]; 80 athletes suffering from acute hamstring muscle injury were treated with intramuscular PRP or placebo injections [13]. The time elapsed until the beginning of sports activities was then measured. The placebo group began with sports activities at approximately the same time as

PRP group. The median time was 42 days in both groups, 95% CI (0.61 to 1.51; p=0.66) [13]. Another study reported no improvement of clinical and radiological parameters in knees treated by PRP after ACL reconstruction [14].

It is yet unclear how PRP constituents act upon the diseased cells. PRP contains molecules and their complexes which play a role in inflammatory response, tissue repair and vascular remodeling, such as growth factors, immunoglobulins, clotting factors and cytokines, depending on the cell content [15]. Activated platelets excrete more than 30 types of proteins from α-granules (e.g. TGF-β, PDGF, bFGF, EGF, VEGF, CTGF, IGF, IL-1, PF4), which were found to promote the formation of extracellular matrix, cellular replication, angiogenesis, and cell differentiation [16]. Furthermore, plasma contains extracellular vesicles (EVs). EVs (microvesicles and exosomes) are very small (down to 30 nm in diameter) membrane-enclosed fragments of cells that are shed from cells of all cell types into the extracellular solution [17]. They are composed of the constituents of the mother cell and are free to move with fluids. Thereby EVs may transfer bio-effective molecules such as mRNA, microRNA, proteins, lipids or small organelles (e.g. mitochondria) from the mother cell to the recipient cell. Their role in spreading of inflammation and infection, in cancer progression and in blood clot formation has already been recognized. EVs are being considered as drug carriers (e.g. interleukin-1 receptor antagonist – doped EVs for osteoarthritis [18] and EVs derived from immature "tolerogenic" dendritic cells for rheumatoid arthritis [19,20]). Also, therapeutic potential of mesenchymal stem cells (MSCs) derived from bone marrow or from blood and their EVs is being evaluated [21].

A possibility should be taken into account that an important or even a major effect of PRP derives from EVs. It was recently found that processing PRP by augmenting shear stress may increase the number of EVs in samples [22]. Also it was found that the identity, size and shape of EVs in isolates are determined by the harvesting process [22]. It is therefore indicated that optimization of the procedure for preparation of PRP is needed for a particular use (Figure 1). Furthermore, PRP could be used in combination with mesenchymal stem cell EVs to give even better results in treatment; it was found that nucleus pulposus cells were significantly activated by combination of coculture system with mesenchymal stem cells and autologous PRP [23].

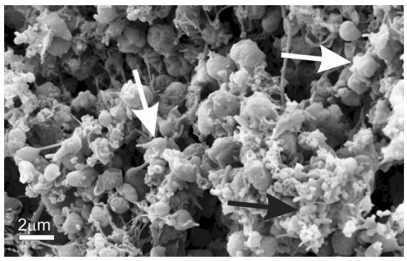

Figure 1: Scanning electron micrograph of activated platelets (white arrows) and extracellular vesicles (black arrow) harvested from platelet rich plasma. Material was prepared as described in [22]. Platelets were activated by shear forces during the harvesting process.

Understanding the mechanisms underlying effects of PRP constituents on cells is necessary to improve the treatment. Increasing interest in EVs promises to render PRP – based methods significantly more effective.

References

1. McCarrel TM, Mall NA, Lee AS, Cole BJ, Butty DC, et al. (2014) Considerations for the use of platelet-rich plasma in orthopedics. Sports Med 44: 1025-1036.
2. Sampson S, Gerhardt M, Mandelbaum B (2008) Platelet rich plasma injection grafts for musculoskeletal injuries: a review. Curr Rev Musculoskelet Med 1: 165-174.
3. Nugraha HK, Muljanti M, Hernanigsih Y, Nugraha J (2012) Platelet rich plasma preparation protocols: a preliminary study. IJTID 3: 104-107.
4. Amable PR, Carias RB, Teixeira MV, da Cruz Pacheco I, Corrêa do Amaral RJ, et al. (2013) Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. Stem Cell Res Ther 4: 67.
5. Mazzocca AD, McCarthy MB, Chowaniec DM, Cote MP, Romeo AA, et al. (2012) Platelet-rich plasma differs according to preparation method and human variability. J Bone Joint Surg Am 94: 308-316.
6. Raeissadat SA, Sedighipour L, Rayegani SM, Bahrami MH, Bayat M, et al. (2014) Effect of Platelet-Rich Plasma (PRP) versus Autologous Whole Blood on Pain and Function Improvement in Tennis Elbow: A Randomized Clinical Trial. Pain Res Treat 2014: 191525.
7. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T (2010) Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. Am J Sports Med 38: 255-262.
8. (2013) Hospital for Special Surgery.
9. Battaglia M, Guaraldi F, Vannini F, Rossi G, Timoncini A, et al. (2013) Efficacy of ultrasound-guided intra-articular injections of platelet-rich plasma versus hyaluronic acid for hip osteoarthritis. Orthopedics 36: e1501-1508.
10. Ferrero G, Fabbro E, Orlandi D, Martini C, Lacelli F, et al. (2012) Ultrasound-guided injection of platelet-rich plasma in chronic Achilles and patellar tendinopathy. J Ultrasound 15: 260-266.
11. Coombes BK, Bisset L, Vicenzino B (2010) Efficacy and safety of corticosteroid injections and other injections for management of tendinopathy: a systematic review of randomised controlled trials. Lancet 376: 1751-1767.
12. de Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, Verhaar JA, et al. (2010) Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA 303: 144-149.
13. Reurink G, Goudswaard GJ, Moen MH, Weir A, Verhaar JA, et al. (2014) Platelet-rich plasma injections in acute muscle injury. N Engl J Med 370: 2546-2547.
14. Nourissat G, Mainard D, Kelberine F; French Arthroscopic Society (SFA) (2013) Current concept for the use of PRP in arthroscopic surgery. Orthop Traumatol Surg Res 99: S407-410.
15. Sundman EA, Cole BJ, Fortier LA (2011) Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med 39: 2135-2140.
16. Blair P, Flaumenhaft R (2009) Platelet alpha-granules: basic biology and clinical correlates. Blood Rev 23: 177-189.
17. Buzas EI, Gyorgy B, Nagy G, Falus A, Gay S (2014) Emerging role of extracellular vesicles in inflammatory diseases. Nat Rev Rheumatol 10: 356-364.
18. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C (2010) Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci 123: 1603-1611.
19. (2014) New treatment of rheumatoid arthritis with anti-inflammatory exosomes.
20. Yang C, Robbins PD (2012) Immunosuppressive exosomes: a new approach for treating arthritis. Int J Rheumatol 2012: 573528.

21. Chong PP, Selvaratnam L, Abbas AA, Kamarul T (2012) Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. J Orthop Res 30: 634-642.

22. Sustar V, Bedina-Zavec A, Stukelj R, Frank M, BobojeviÄ‡ G, et al. (2011) Nanoparticles isolated from blood: a reflection of vesiculability of blood cells during the isolation process. Int J Nanomedicine 6: 2737-2748.

23. Watanabe T, Sakai D, Iwashina T, Hiyama A, Omi H, et al. (2006) Combination of coculture system with mesenchymal stem cells and autologous platelet rich plasma significantly activates nucleus pulposus cells. In: 52nd: Annual Meeting, Orthopaedic Research Society. New Orleans, LA, USA: Orthopaedic Research Society.