Comparison Of PRP And Platelet Lysate Used For Stimulation Of Human Tenocyte Cultures

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Disclosures:

Introduction: Over the last 10 years different platelet preparations are under investigation regarding their stimulating impact on wound healing [1]. This possible positive effect is attributed to the high content of growth factors (GF) like PDGF, IGF, VEGF and others in the alpha granula of the platelets, which are known to trigger or even be involved in angiogenesis and tissue regeneration. However, the efficacy of platelet rich plasma (PRP) for clinical applications especially for tendon repair remains unproven and clinical studies showed variable outcome results [2]. This may be due to the high individual differences of each patient PRP composition regarding platelet number and therefore concentration of growth factors. Platelet lysate (PL) is another platelet based product, which contains bioactive molecules (especially GFs) released by the cells after freeze-thawing destruction. It has proved capable of promoting the healing of cornea lesions [3]. One advantage of PL compared to PRP is that it can be stored frozen and therefore easily be used for consecutive applications without further blood sampling. Furthermore, it could be analyzed for growth factor content before its use to standardize the product. Because no study investigated the effect of PL on tendon healing, it was the aim of this pilot study to compare the effect of PL and PRP on human tenocytes regarding cell activity and gene expression in vitro.

Methods: PL or PRP from one male and one female healthy donor was prepared following standard protocols [4] or the instructions of the manufacturer. PL was produced by apheresis and three circles of freezing, thawing and centrifugation. PRP was obtained using the double syringe system from Arthrex (autologous conditioned plasma-ACP). Platelet concentration was determined in both preparations. Tenocytes from 4 male and 4 female donors were isolated from supraspinatus tendon biopsies by collagenase digestion. A total of 1x10⁴ cells were seeded into 24-well plates. After 24 hours an Alamar blue test was performed to analyze cell activity. Afterwards cells were stimulated with 10% PRP or 10% PL in cell culture medium with 2% FCS. The PRP or PL was applied to the cells in cell culture inserts with 0.4µm pore size. Cells cultured in medium with 2% FCS served as negative control, cells incubated in medium with 10% FCS served as positive control. After 5 days cell activity was again analyzed by Alamar Blue test. Furthermore, RNA was isolated and gene expression analysis of collagen I, III, decorin, tumor necrosis factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β) was performed by Real-Time PCR analysis. For statistics Mann-Whitney U test followed by Bonferroni-Holm correction was performed.

Results: Platelet content was 1.27x10¹¹/ml in male PL and 1.22 x10¹¹/ml in female PL. In PRP 6.6x10⁸/ml in male and 3.0x10⁹/ml in female PL were counted. Cell activity (Fig 1A) was significantly increased in all groups compared to negative control (p≤0.004). Stimulation with male PRP resulted in a significant higher increase of cell activity compared to the stimulation with male PL (p=0.015). Collagen I expression was significantly decreased compared to negative control after stimulation with male PRP (p=0.03). The male and female PL showed significantly increased collagen I expression compared to the respective PRPs (p=0.02; p=0.03). However, collagen I expression was decreased in all groups compared to positive control (Fig 1B). Collagen III expression was significantly increased after stimulation with both PRP (male p=0.028; female p=0.0001) and female PL (p=0.0001) compared to negative control (Fig 1B). Therefore, collagen I to III ratio was significantly decreased in both PRP groups (female and male PRP p=0.0001) and female PL (p=0.007) compared to negative control. Additionally, male PRP had a significantly stronger effect on the collagen I/III ratio than male PL (p=0.042). The expression of the pericellular matrix proteoglycan decorin (Fig 1B) and the inflammatory marker TNF-α showed no significant differences between the groups. Interestingly, IL-1β gene expression was affected by the stimulation with male and female PRP. In 6 of 8 tenocyte cultures stimulated with female PRP, the expression of IL-1β was highly increased (median: 6.03) compared to negative control, while in two cultures the IL-1β expression was decreased (median: 0.60). In cells treated with male PRP IL-1β expression was increased in two donors (median: 10.34) and decreased in 6 donors (median: 0.61). In contrast, stimulation with male or female PL did not increase expression of IL-1β. Because values showed high variances in both PRP groups no statistical analysis was performed.

Discussion: Tendon healing is limited due to poor vascularity and intrinsic healing capacity. PRP or other blood preparations might offer the possibility to promote tendon healing without any negative side effects and low costs. However, new clinical studies showed a positive effect on pain relief but only few effects on tendon healing [2]. This decreased the first enthusiasm
regarding PRP and related products. Platelet lysate offers a controlled preparation of thrombocyte concentrates, which can be shelved and analyzed for their growth factor content. This could improve healing outcomes. Furthermore, consecutive treatments are possible. This pilot study showed comparable increase of cell activity after stimulation with PRP and PL. Collagen I/III ratio showed a shift to collagen III in both treatment groups. However, collagen I expression was significantly higher in PL stimulated cells compared to PRP stimulated cells. This could indicate a higher regenerative capacity of PL, because collagen I is the most important matrix protein in tendons and responsible for mechanical competence. Our results of increased IL-1β expression, most pronounced with female PRP could indicate a potential pro-inflammatory effect, which was not observed with either PL preparation. However this could be due to the allogenic use of the PRP which does not correspond to the clinical situation. In our opinion PL seems to be a promising new biological for regenerative approaches especially tendon regeneration. However, further studies are necessary to verify our results with a higher case number and different PL’s. Furthermore growth factor content has to be analyzed and compared between PRP vs. PL.

**Significance:** The use of platelet preparations for tendon repair is a promising approach because it is a safe and easy method to use the body’s own growth factors. However, clinical results remain controversial. Therefore, further developments to standardize the preparation would offer new therapeutic possibilities and may help to improve therapy outcome.

**Acknowledgments:**

**References:** [1] Halpern et al 2012, Hospital for Special Surgery
Figure 1: A: Cell activity was significantly increased after stimulation with both PRP and PL compared to negative control. Male PRP showed the strongest effect. B: Collagen expression analysis showed a decrease in all groups compared to positive control which was more pronounced in PRP compared to PL. Decorin expression was decreased in all groups compared to negative control and showed no differences between the groups.

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