Leukocyte-depletion In Platelet-rich Plasma Decreases The Proliferative Effects Of Human Chondrocytes

Morten L. Olesen, BSc1, Helle Lysdahl, MSc, PhD2, Martin Lind, MD, PhD, DMSc3, Casper B. Foldager, MD, PhD1.
1Institute for Clinical Medicine, Aarhus University Hospital, Aarhus C, Denmark, 2Orthopaedic Research Laboratory, Aarhus University Hospital, Aarhus C, Denmark, 3Sports Trauma Clinic, Aarhus University Hospital, Aarhus C, Denmark.


Introduction: The tissues formed by spontaneous articular cartilage repair are often fibrous or fibrocartilaginous, having an abnormal biochemical composition and inferior biochemical function compared with hyaline cartilage.(1,2) Among the various new biological treatments being utilized, platelet-rich plasma (PRP) is a simple, low-cost and minimally invasive technique showing promising preliminary clinical results.(3) Numerous preparation methods are available for PRP generation, but evidence defining the optimum composition is lacking. The purpose of this study was to investigate the effects of PRP containing low and high leukocyte concentrations on both proliferation and the chondrogenicity of human chondrocytes in vitro.

Methods: Two PRP groups, IIIPR and sPRP, were generated from whole blood from 9 healthy donors (5 men and 4 women, mean age 40.7 years) using a simple two-step centrifugation/filtration procedure (fig. 1). The PRP groups had similar platelet concentrations but low and high leukocyte concentrations, respectively. Human chondrocytes were isolated from articular biopsies obtained from 3 patients with healthy cartilage (age 21-41 years) undergoing anterior cruciate ligament reconstruction surgery, and cultured in monolayer for 7 days in either control media alone (DMEM/F12 with 10% fetal calf serum) or control media with IIIPR or sPRP of 1%, 5% or 10% v/v concentrations. Complete blood count analyses for whole blood and the PRP groups were performed with an automated hematology analyzer (Sysmex XE-5000, Sysmex Corp., Kobe, Japan). Proliferation was assessed using an XTT assay. RT-PCR was used to perform quantitative gene expression analyses using primers for collagen type I (Col1a1) and II (Col2a1) and Sox9. Data were collected on day 2 and 7, and evaluated using three-way ANOVA analysis.

Results: IIIPR group showed an average leukocyte concentration of 0.04 x 109 WBC/L while the sPRP group had 10.33 x 109 WBC/L. We observed a positive proliferative effect by both PRP groups compared with the control group (p<0.001). sPRP group showed an increased proliferation compared with the IIIPR group (p<0.05). Presence of leukocytes did not affect the relative mRNA expression of Sox9, Col2a1, or Col1a1 in any of the formulations. Using this method of centrifugation changed the leucocyte composition compared with whole blood (WB). In sPRP the leucocyte pool contained 5.7±2.1% neutrophils (9.7-fold decrease compared with WB), while the lymphocytes represented 81.1±6.7% (2.5-fold increase).

Discussion: The primary objectives of the present study were to determine the effect of platelet-rich plasma white blood cell (WBC; leucocyte) concentration on the cell proliferation and chondrogenic gene expression of human articular chondrocytes. These objectives were accomplished by creating two PRP groups with a set platelet concentration and a low (IIIPR) and high (sPRP) WBC concentration.

The results of the present study demonstrate that both PRP groups increased the cellular viability of human chondrocytes cultured in monolayer. Excluding leucocytes from the PRP formulation (IIIPR) lead to a significant decrease in proliferation compared with the standard formulation (sPRP). This decrease in proliferation might be due to the exclusion of the pool of growth factors contained within WBCs in sPRP. Platelet-derived growth factor, the dominant growth factor in platelet concentrates, has previously been demonstrated to stimulate a decrease in the amount of cartilage proteoglycan, exerting a mitogenic effect.(4) Haematological analysis of our PRP groups proved a shift in PRP leucocyte composition from being dominated by neutrophils to a lymphocyte dominated leucocyte profile in sPRP. Monocyte concentration in sPRP, however, mirrored that in WB (8.4±0.9% vs. 13.2±2.2%). The specific gravity differs among the components of the blood. Red blood cells are the heaviest (spec. gravity = 1.095), followed by WBCs (spec. gravity = 1.063-1.085), whereas platelets (spec. gravity = 1.032) are the lightest. Each component has been isolated by various centrifugation protocols, but cell contamination after separation cannot be avoided because of the components’ slightly overlapping specific gravities,(5) a fact further confirmed by our findings.

One of the limitations of our study is the small sample size of chondrocyte donors (n=3). Our study also only addressed RNA expression and did not investigate quantities of proteins synthesized. We experienced a gelation of the culture media when PRP preparations were added to DMEM/F-12. This might be explained by the interaction between residual anticoagulated/non-activated platelets and calcium chloride (CaCl2) content in DMEM/F-12, as CaCl2 replenishes the binding site previously bound by anticoagulant, initiating growth factor release and polymerization of fibrinogen into fibrin. This could be a possible confounder as different viscosities of PRP clots were observed; giving the possibility of growth factors of various amounts could be trapped inside the fibrin clot.
We conclude that a high absolute leukocyte concentration in PRP increase chondrocyte proliferation. Inclusion of leukocytes in PRP showed no effect on chondrogenic gene expression.

**Significance:** Incomplete reporting of PRP composition in the literature is a known problem, which has recently been described in a review by Smyth et al.(6) Our study emphasizes the importance of correct reporting of key components in PRP, such as platelet and WBC concentrations.

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**References:**
Cell Viability

![Box plot showing cell viability data](image)

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<tr>
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<th>No. of Platelets (x 10^3/μL)</th>
<th>No. of WBCs (x 10^3/μL)</th>
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<tbody>
<tr>
<td></td>
<td>In WB</td>
<td>In PRP</td>
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<tr>
<td>sPRP (n = 9)</td>
<td>246 ± 68</td>
<td>1.482 ± 188</td>
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<tr>
<td>IIPRP (n = 9)</td>
<td>246 ± 68</td>
<td>1.529 ± 195</td>
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