# Enhancement of Mesenchymal Stem Cell Proliferation by Platelet Rich Plasma

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**Introduction:** Platelet rich plasma (PRP) has been used clinically in the wound healing arena since the 1980's and has recently been adapted into use in the field of bone regeneration[1,2]. The clinical use of PRP in bone regeneration as a graft medium, and as a source of growth factors, reflect the well established role of platelets and plasma proteins such as fibrinogen in hemostasis and wound repair. In addition, the variety of platelet-derived growth factors such as PDGF, TGF-β and VEGF are believed to contribute to the healing response[1,2,3]. While encouraging data has been established regarding the clinical use of PRP, the basic mechanisms for these effects have not been elucidated. This study was designed to evaluate the effect of PRP on mesenchymal stem cells (MSCs) that play an essential role in the bone regeneration process.

**Methods:** Platelet rich plasma (PRP) was isolated from approximately 55 ml of fresh human blood (IRB-approved protocol) using the Symphony™ Platelet Concentration System (DePuy AcroMed, Raynham, MA), designed to be used at the point-of-care for obtaining a platelet concentrate from a small amount of blood. Samples of the starting material and platelet concentrates were analyzed to determine the absolute concentrations of platelets. PRP, PPP and unfractionated blood were clotted with thrombin (1000 U/ml in 10% $CaCl_2$) by adding 1 part thrombin stock solution to nine parts PRP, PPP or blood to yield a final thrombin concentration of 100 U per ml. The soluble platelet releasates from the clotted preparations were isolated by centrifugation and cleared by ultrafiltration. PRP and PPP releasates were diluted in serum-free DMEM to the appropriate final dilutions.

Human MSCs (hMSCs) were isolated and culture-expanded from bone marrow (IRB-approved protocol) as described previously[4]. In order to evaluate the mitogenic activity of PRP, second passage hMSCs were replated at a density of $3x10^3$ cells per $cm^2$ in serum-free DMEM. Cells were allowed to attach and incubate for 48 h, at which time culture medium was replaced with test media. Test media consisted of DMEM supplemented to 10% and 20% (v/v) with undiluted PRP releasate. In addition, DMEM was supplemented to 10% (v/v) with PRP releasate that was diluted in PPP such that the final concentration of PRP releasate ranged from 0.625- to 2.5-fold of that in native peripheral blood. For comparison, control media consisted of serum free medium, or DMEM supplemented to 10% (v/v) with the following preparations: fetal bone serum selected for optimal growth and retention of the hMSC phenotype (hMSC Growth medium), PPP releasate alone, or serum from clotted human blood. At the end of the 7 day time course, cells were released with trypsin and counted with a hemocytometer.

The cells culture-expanded in the PRP-supplemented media or in control media were then subjected to osteogenic differentiation conditions that consisted of serum-supplemented media containing dexamethasone, ascorbic acid & β-glycerophosphate. The differentiation of the cells was assessed by morphology, alkaline phosphatase enzyme activity and mineral deposition.

**Results:** The efficiency of platelet recovery was ~66%, thus creating a ~5-fold concentration following removal of platelet-poor plasma (PPP) from the disposable concentration device. For example, this produced a PRP concentrate of 1600 x $10^3$ platelet per μl, which is a 5-fold enrichment compared to the 315 x $10^3$ platelets per μl in the same patient's peripheral blood. Exposure of hMSCs to PRP releasate caused a dramatic mitogenic effect in a dose-dependent manner (Figure 1). Cultures that received media supplemented with 5-fold and 10-fold PRP releasate generated 848% and 720% more hMSCs by day 7 than cultures incubated in hMSC Growth medium ($p < 0.01$). Cultures exposed to 2.5- or 1.25-fold PRP concentrations similarly responded with increases of 325% ( $p < 0.05$) and 356% ($p < 0.01$), respectively. Interestingly, the 1.25-fold releasate was more mitogenic than the serum from a fresh blood clot, which stimulated proliferation by 208% ($p < 0.05$). And lastly, it is useful to note that the PPP supported proliferation comparable to the selected lot of fetal bovine serum in hMSC Growth Medium. This is important since the data regarding the mitogenic action of PPP is a critical control in this study, against which the effects of PRP must be measured.

No significant differences were observed in the differentiation potential of MSCs that were culture-expanded in PRP when compared to those cells culture-expanded in control media. Quantitative and qualitative aspects of osteogenesis were not different along the cellular cascade of bone formation.

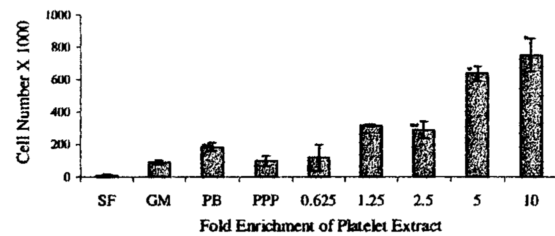


*Figure 1. Mitogenic response of hMSCs to culture media supplemented with various concentrations of platelet releasate. Numbers on x-axis represent fold-concentrations of platelets used to generate releasate relative to peripheral blood platelet concentration. For controls, hMSCs were incubated in serum-free medium (SF), hMSC Growth medium (GM), peripheral blood clot releasate (PB), or PPP releasate (PPP). Statistical differences (two-tailed paired T-test) are shown relative to hMSC Growth medium control, *$p < 0.01$ and **$p < 0.05$.*

**Discussion:** PRP releasate possesses the ability to stimulate proliferation of hMSCs in a dose-dependent manner without adversely affecting their developmental potential. While these experiments demonstrate that serum from a fresh human blood clot, and even PPP, can stimulate cell proliferation, approximately 90% of the mitogenic activity in PRP releasate is derived from the platelet concentrate. In the setting of wound healing or tissue repair, concentrated PRP could therefore stimulate proliferation of mesenchymal progenitor cells that give rise to musculoskeletal tissues such as cartilage and bone

In the set of in vitro experiments described here, continuous exposure to high levels of PRP releasate was shown to cause robust proliferation of MSCs in the absence of specific osteoinductive molecules. In tissue repair sites, however, where diffusion will eventually reduce the local concentration of such a releasate, cells of the repair blastema will have the opportunity to respond to other bone or cartilage differentiation signals in the local environment. Slater et. al.[5] have previously shown that that platelet concentrate exerts a stimulatory effect on human fetal osteoblast-like cells and maintains their differentiated function. However, Slater's experiments were performed with osteoblast-like cells and not with uncommitted MSCs. The data from the current study comparing the differentiation of MSCs culture-expanded in PRP-supplemented media or control media clearly shows that MSCs maintain their differentiation potential even after rapid proliferation induced by PRP. Thus in the tissue regeneration setting, application of PRP may lead to robust proliferation of osteoprogenitor cells, and the formation of a repair blastema competent to respond to the endogenous downstream signals that trigger osteogenesis and bone regeneration.

Experiments underway in the laboratory now are aimed at further elucidating the early events of tissue repair mediated by platelets.

**References:**

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