Introduction: Platelets are known to perform multiple functions during injury and tissue repair. While their role in hemostasis is well understood, their mechanism of action in promoting wound healing requires further characterization. As a repository of multiple growth factors such as PDGF, EGF, VEGF, and TGF-Beta (1), degranulation of platelets at wound sites serves to initiate or enhance the healing cascade. Armed with this knowledge, clinicians have used platelet concentrates in conjunction with bone graft materials to enhance osseous repair (2, 3). In addition, experimental evidence has shown that when PDGF or platelet concentrates are combined with demineralized bone or certain other materials, augmentation of bone formation ensues (4, 5). The purpose of this study was to begin elucidating the cellular mechanisms that underlie these observations. Since mesenchymal stem cells (MSCs) are known to be an important component of the tissue repair process, we sought to characterize elements of their response to platelet concentrates in the controlled in vitro environment.

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, platelet poor plasma (PPP) and unfractonated blood were clotted with thrombin (1000 U/ml in 100 mg/ml CaCl₂) by adding 1 part thrombin stock solution to nine parts PRP, PPP or blood to yield a final thrombin concentration of 100 U/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 generate appropriate final dilutions of PRP.

Human MSCs (hMSCs) were isolated and culture-expanded from bone marrow (IRB-approved protocol) as described previously. In order to evaluate the mitogenic activity of PRP, second passage hMSCs were replated at a density of 3x10^3 cells per cm² 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 (hMSCs) were allowed to incubate in test and control media for 7 days with complete media changes taking place on day 4. At the end of the 7 day time course, cells were released with trypsin and counted with a hemocytometer.

Results: The efficiency of platelet recovery was ~66%, thus creating a ~5-fold concentration following removal of PPP from the disposable concentration device. For example, this produced a PRP concentrate of 1600 x 10^3 platelets 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.

**References:**