

In Vitro Comparison of Autologous Conditioned Plasma (ACP) to a Buffy Coat-Based Platelet-Rich Plasma (PRP) Product

Arthrex Research and Development

Introduction

ACP is currently being used to extract platelet concentrate from peripheral blood and apply growth factors within the concentrate at an orthopaedic surgical site. However, there are few studies focusing on a side-by-side comparison with buffy coat-based PRP products. The purpose of this study was an *in vitro* comparison of cells treated with a plasma-based PRP system - ACP - and a buffy coat-based PRP system - Biomet's Gravitational Platelet Separation (GPS) system.

Methods and Materials

PRP was prepared from eight donors using each company's own centrifuge and disposables. After PRP samples were prepared according to manufacturer's instructions, platelet and white blood cell counts for whole blood and all PRP samples were performed using a CBC counter.

Human tenocytes, osteoblasts, and myocytes were plated separately at a concentration of 5000 cells/well (2800 cells/cm²) in tissue culture plates. Cells were plated at a low concentration to ensure that they would have enough room to expand. There were five different treatments given to all cells - (1) a negative control group of either 2% or 5% fetal bovine serum (FBS); (2) a positive, proliferative control group of 10% or 15% FBS; (3) whole blood; (4) ACP; and (5) Biomet. After a 5-day culture period, cells were treated with tritiated thymidine and counted with a scintillation counter. Results are reported as average \pm standard deviation in disintegrations per minute (DPM). This measurement is an indicator of cell proliferation.

Data for each separate cell type was analyzed using a one-way ANOVA, and significance was found when $p < 0.05$.

Results

Since none of the experiments passed the normality test, an ANOVA on ranks was performed. A statistically significant difference was found for all experiments ($p < 0.001$); the Dunn's method of multiple comparisons on ranks was then performed to determine differences between groups.

Tenocyte proliferation after five days indicated that ACP performed better than controls and whole blood (Figure 1, $p < 0.05$). Biomet also performed better than controls and whole blood ($p < 0.05$). ACP did have a higher mean than

Biomet, but there was no statistical difference found between the two ($p > 0.05$).

Osteoblast proliferation after five days indicated that ACP again performed better than controls and whole blood (Figure 2, $p < 0.05$), while Biomet performed better than controls, but not whole blood. In addition, ACP was significantly higher than Biomet ($p < 0.05$).

Myocyte proliferation after five days indicated that ACP performed better than controls and whole blood (Figure 3, $p < 0.05$), while Biomet performed similar to controls and whole blood. Again, ACP had a significantly higher proliferation compared to Biomet ($p < 0.05$).

For all cell types, ACP was either statistically better than or approached significance when compared to controls, whole blood, and Biomet. Even though GPS generally has a higher platelet concentration than ACP, GPS has a low ratio of platelets to white blood cells (Figure 4), close to that of whole blood, while ACP has a very high platelet to white blood cell (WBC) ratio (12X greater than that of Biomet). In addition, the percent breakdowns of WBCs in whole blood, ACP, and Biomet (Figures 5-7, respectively) show a difference in the amounts of neutrophils - considered the most harmful of WBCs. ACP contains 11% neutrophils, equal to 60 cells/ μ L. Biomet, on the other hand, contains 42% neutrophils, equal to 8170 cells/ μ L. This demonstrates that ACP not only reduces the total amount of WBCs, but it also reduces the percentage of neutrophils. Biomet instead concentrates WBCs and neutrophils by 3.6X and 2.4X, respectively, over whole blood.

Figure 1.

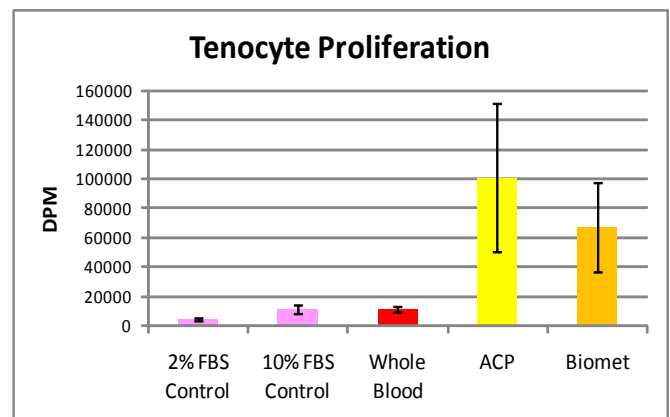


Figure 2.

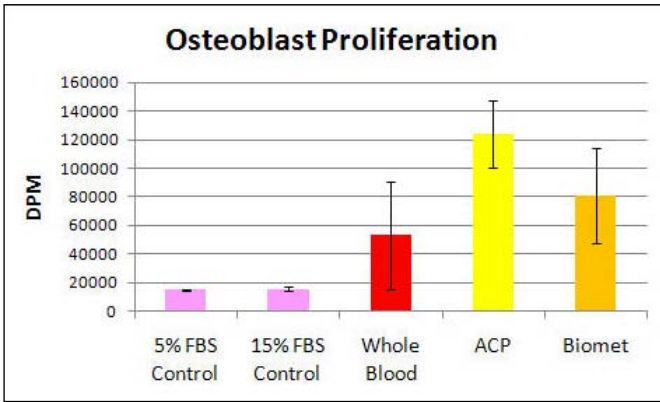


Figure 5.

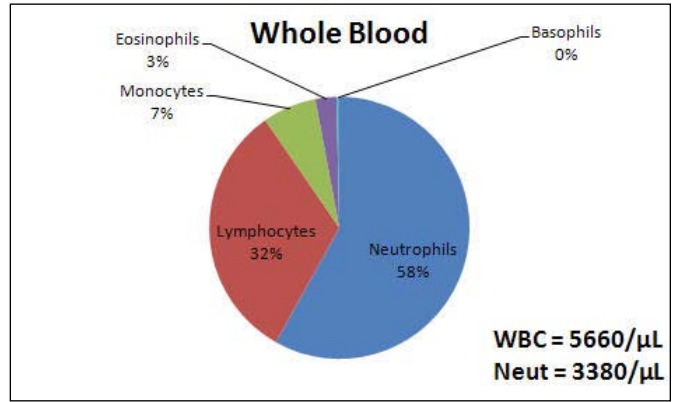


Figure 3.

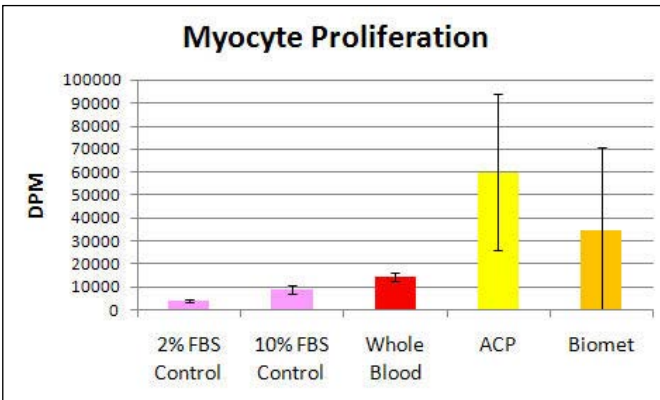


Figure 6.

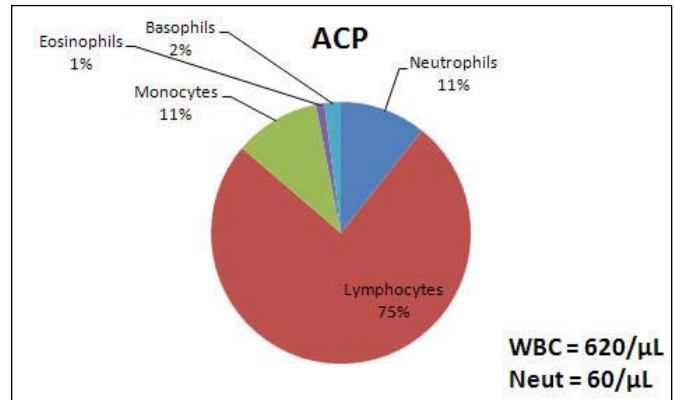


Figure 4.

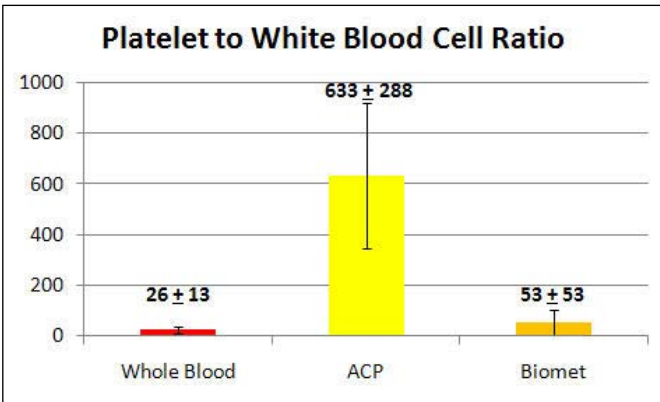
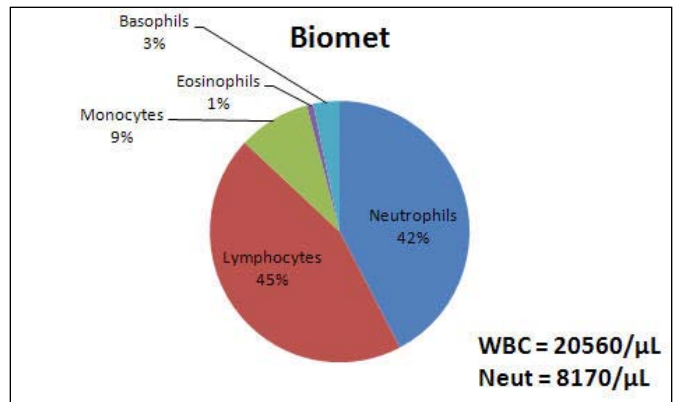


Figure 7.



Discussion

There are three proposed theories that would explain why ACP cell proliferation was either higher or similar to Biomet. One theory hypothesized is that cells have a maximum dose response capability. This would indicate that only a certain level of growth factors released from platelets can initiate a positive proliferative response; the remaining, extra growth factors are not utilized and degrade away over time. Two times concentration may be the maximum amount of platelets needed in order to invoke the greatest cellular response.

The second theory is that a buffy coat-based PRP system may overconcentrate the amount of platelets needed for a maximum physiologic response. Platelet concentrations greater than 4X over baseline has been shown to cause a paradoxical inhibitory effect on cell proliferation^{1,2}. This may indicate that a negative feedback loop could be occurring when platelets and growth factors are overconcentrated. Negative feedback loops are common pathways the body utilizes to compensate for fluctuations in such things as body temperature, hormone production, and protein production³.

The third theory involves the proposed premise that certain WBCs may be detrimental to growth. Activated WBCs release cytokines such as matrix metalloproteinases (MMPs) and Interleukin-1 β , which are inflammatory and can lead to degradation of tissue matrix. In addition, WBCs release reactive oxygen species (free radicals), which also destroy surrounding tissue⁴. Red blood cells (RBCs) will release free radicals similar to WBCs, which are also indiscriminate⁵. Even though Biomet has a high platelet concentration, generally 5-7X over baseline, their system concentrates WBCs and contains RBCs. The WBCs, and even RBCs, may counteract the effects of increased platelet concentration on cell proliferation, as was represented in this study. It is also felt that the release of proteolytic enzymes and free radicals from WBCs and RBCs will have a more significant negative effect on tissue matrix formation rather than cell proliferation. Since this study addresses cell proliferation and not tissue matrix formation, it is possible that the full detrimental effect of WBCs and RBCs is not being revealed. Further studies will be needed to analyze the presumed increase in tissue matrix degradation that occurs with a buffy coat-based PRP compared to a plasma-based PRP.

Even though ACP has a lower overall platelet concentration (2-3X) than Biomet, it has a higher platelet to WBC ratio, which appears to be of greater importance. Due to the basic differences discussed between the two PRP end products, ACP's effect on cell proliferation is either significantly higher or is trending towards a significant increase when compared to the Biomet GPS system.

The Double Syringe (ACP) System is used to facilitate the safe and rapid preparation of autologous platelet-rich plasma (PRP) from a small sample of blood at the patient's point of care. The PRP can be mixed with autograft and allograft bone prior to application to an orthopaedic surgical site, as deemed necessary by the clinical use requirements.

Conclusion

In this model, ACP produced greater amounts of cell proliferation for different cell types compared to a buffy-coat PRP, the Biomet system. These results suggest one of two things: having a higher platelet concentration in PRP does not lead to increased cell proliferation and/or increased WBC presence may impede the maximum growth potential.

References

1. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M, "The *in vitro* effect of different PRP concentrations on osteoblasts and fibroblasts," Clin Oral Implants Res 2006; 17(2): 212-9.
2. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE, "Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration," Bone 2004; 34(4): 665-71.
3. Sherwood L, Human Physiology: From Cells to Systems, Seventh Edition, Belmont, CA: Brooks/Cole, 2007, p. 15.
4. Scott A, Khan KM, Roberts CR, Cook JL, Duronio V, "What do we mean by the term "inflammation"? A contemporary basic science update for sports medicine," Br J Sports Med 2004; 38(3): 372-80.
5. Jiang N, Tan NS, Ho B, Ding JL, "Respiratory protein-generated reactive oxygen species as an antimicrobial strategy." Nat Immunol 2007; 8(10): 1114-22.