Platelet-Rich Plasma

Be Confident in What You're Injecting

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1. Introduction

The use of autologous platelet-rich plasma (PRP) as a means to promote healing of soft and hard tissue injury is well documented[1]. Although it was initially developed for use as a hemostatic agent[2], it was soon discovered that PRP also possessed growth-promoting properties that could be leveraged to accelerate the repair of aged and damaged tissues[3]. It is now known that PRP contains many growth factors, hormones, nutrients, protein stabilizers (such as albumin), and other important bioactive compounds[4,5,6,7] important for cellular and tissue regeneration.

Its use in the field of cosmetic surgery has also become popular in the past ten years, owing in part to its ease of isolation and use, its long history as a safe, low-risk autologous therapy (because it is derived from the patient's own blood), and its natural effect on cosmetic outcomes following injection for facial rejuvenation[8], sexual wellness[9], fat transfer[10] and androgenetic alopecia[11,12,13,14,15] – the latter application being one of great scientific interest and high unmet need.

In its most basic form, PRP can be defined as a volume of blood plasma that has a platelet count above normal physiologic levels. However, due to the increasing interest in the use of PRP to treat a variety of tissue injuries, as well as the need to administer it in different forms or states (e.g. liquid or gel), various methods of preparing PRP and PRP-related products have been developed over the years, such that the generic definition is no longer adequate to describe all these products[16].

2. PRP Products and Methods of Isolation

Over the years, different protocols and commercial kits for preparing PRP have been developed to address different clinical applications and/or to drive different clinical outcomes. It's therefore important for the clinical practitioner to be aware that variation in purity, yield, and final composition can and will result both across and within the use of these different methods[17] and that such variation will likely affect the performance of the PRP produced.

Many of these techniques vary significantly with respect to procedure time, starting volume of whole blood required, final concentration of platelets obtained, as well as the amount of contaminating red and white blood cells in the final PRP solution. Finally, some of these techniques also employ the addition of exogenous activators, such as calcium chloride, calcium gluconate or animal-derived thrombin, to initiate formation of a fibrin scaffold or to activate platelets.

While much has been made of the ultra-high increase in platelet concentration over baseline, our view is that this parameter, while important, is inadequate by itself and must be presented alongside the total characteristics of the PRP. Our thesis is that the maximum benefit of increasing platelet concentration is likely found somewhere on a bell curve as opposed to being linear. In other words, there is likely a sweet spot concentration for each application. However, we need more randomized clinical trials comparing platelet concentrations to determine the optimal concentration for various applications.

Total platelets (as opposed to platelet concentration) is a more accurate way to quantify the dose of platelets injected. Additionally, PRP containing significant levels of contaminating white and red blood cells may be equally important to predicting successful patient outcomes. Ease-of-use and reproducibility between various staff should also be considered significant features.

3. Starting volumes and platelet concentration

Castillo et al. examined three different commercial methods for PRP isolation and showed that the GPS III system (Biomet) required a significantly greater starting volume of whole blood than that of Fibrinet (Cascade Medical) and Megellan (Anteriocyte) (55ml vs. 18ml and 26ml, respectively) to produce similar final volumes of PRP[18]. Total centrifugation time was also significantly longer for the Megellan and GPS III methods (17 and 15 minutes, respectively), whereas the Fibrinet method required a single spin of 6 minutes.

Similar to the GPS III product, Harvest StartPrep APC requires as much as 60mL of whole blood to isolate the same volume of PRP. Such practical applications should be considered in clinical practice since they will impact procedure/visit time as well as the patient's experience with the product.

Rose (unpublished data) examined three different commercial methods for PRP isolation: EclipsePRP, Selphyl and Arthrex. Five healthy volunteers were studied, and platelet count were performed using an independent laboratory. While Arthrex demonstrated the highest increase in platelet count (6.0X over baseline), this system produces the greatest level of contaminating red blood cells and white blood cells (notably inflammatory granulocytes). EclipsePRP removes the most contaminating cells and resulted in a platelet increase of 3.1X. Selphyl peformed the poorest in this study, showing a platelet increase of 1.1X[19].

4. Platelet Concentrations, Number and Dosing

Several factors can impact the final number of platelets recovered in any preparation of PRP including, but not limited to, natural physiologic variation in platelet count between and among patients, the starting volume of whole blood taken, platelet recovery efficiency associated with different methods of isolation, removal of platelet-poor plasma and the proficiency of the clinical practitioner performing the isolation.

These results should give the clinician some pause, as the performance of the final PRP product will certainly be less predictable with increasing variation in platelet recovery efficiency between preparations. Our review of the literature suggests that moderately supraphysiologic level of platelets (defined here as 2.5 – 4X baseline) might be more ideal than higher levels (5X or higher)[20]. While platelet concentration depends on the volume of plasma that the platelets are suspended in, what is likely more relevant is the total or absolute number of platelets that are injected. The total number of platelets is likely to show greater correlation to the quantity of growth factors and cytokines that may be released at the site of injection. Additional studies are required to further understand optimal platelet concentration, total platelet numbers and the importance relative to clinical outcomes.

5. Erythrocyte contamination and the risk of hemosiderin staining

Ideally, PRP and PRP-related products should contain as few contaminating erythrocytes as possible. However, given that most, if not all, of the methods for isolating PRP employ plasmapharesis as their core technology, it's not feasible to produce PRP completely free of erythrocytes. Differences in centrifugation times, number of physical manipulations involved in the protocol, starting volume of whole blood used, and choice of cell separation media all impact the purity of the final PRP product. Differences in the amount of erythrocyte contamination have been demonstrated between some of the various protocols for isolating PRP.

Why should an aesthetic physician be concerned with erythrocyte contamination in the final PRP product? When erythrocytes are lysed, extracellular hemoglobin becomes oxidized, which in turn results in the release of heme[21,22]. The hydrophobicity of free heme allows it to integrate into cell membranes where it can

generate other ROS, thereby initiating a cascade of redox events leading to cellular senescence and cell death[23,24,25]. Much in the way UV exposure contributes to photoaging of the skin vis-a-vis generation of ROS, the lysis of erythrocytes could potentiate a similar response through the release of reactive ferric oxide and heme.

Systems such as Arthrex, EmCyte, Harvest and Magellan (Arteriocyte), while useful for concentrating platelets, result in very high amounts of red blood cells. Results show that EclipsePRP removes almost all red blood cells and this should be taken into consideration by the practicing physician.

6. White blood cells, why take the chance?

Circulating white blood cells (WBC) are known to play a pivotal role in initiating the early phases of wound repair, however, there is some debate as to whether any additional clinical benefit is gained by including WBCs in the final PRP Product[26]. While it is possible that such PRP products may be useful in promoting the repair of specific types of tissue injury, well controlled studies to comparatively examine the risk/benefit profile of PRP plus or minus WBCs have not yet been conducted. Moreover, some investigators have shown that PRP containing WBCs may inhibit healing in certain situations and induce more local pain than with pure PRP alone[27,28.] As a result, the scientific merit of using PRP rich in WBCs in the clinical setting is not well established, particularly in the field of aesthetic medicine.

There is growing evidence that cross-talk between platelets and white blood cells exist in certain pathophysiologic processes, such as thrombosis and inflammation[29,30]. Activated platelets have been shown, for example, to induce superoxide release by monocytes and neutrophils[31]. This interaction is facilitated in part via the formation of platelet-leukocyte aggregates or conjugates (PLAs)[32,33]. Upon activation, mononuclear cells degranulate, releasing free radicals and inflammatory cytokines.

It is therefore possible that PRP preparations containing high numbers of both platelets and WCBs could lead to similar unwanted events in vivo following subcutaneous injection into the skin or other tissues. The concentrations of WBCs found in PRP prepared by several commercial kits are not insignificant. The clinical practitioner should be cognizant of these differences when evaluating which product to use.

Systems such as Arthrex, EmCyte, Harvest and Magellan (Arteriocyte), while useful for concentrating platelets, result in relatively high amounts of white blood cells. Eclipse PRP removes the vast majority of white blood cells and this should be taken into consideration by the practicing physician.

7. Reproducibility, the standard or the exception?

Several studies have shown that significant variation in the final composition of PRP can result when different methods of isolation are employed[34,35,36]. What does this mean then for the clinician and how does this impact the patient? It means that the effective dose of platelets administered to the patient could vary as much as 0.5 to 1.5 X per injection, thereby making the clinical outcome less predictable.

It's our view that gel separation technologies, like those see in EclipsePRP, are ideal for all applications. The method is reproducible, operator independent and does not require the operator to visually separate the PRP from the contaminating red and white cells.

8. Safety & FDA view on Platelet-rich plasma

FDA cleared products will carry an Instruction for Use identical, or similar to the following:

The [insert name] system is designed to be used for the safe and rapid preparation of autologous platelet-rich plasma (PRP) from a small sample of blood at the patient point of care. The PRP is mixed with autograft and/or allograft bone prior to application to a bony defect for improving handling characteristics.

Therefore, manufacturers are only able to promote this use to their customers in their marketing materials, website etc. The use of PRP in aesthetics is considered off-label. While a physician may practice medicine as he or she sees fit, we caution against working with manufacturers to promote off-label uses, unless done in the appropriate educational forum, such as Continuing Medical Education workshops.

However, of much greater concern, and liability to physicians is the use of PRP "products" that have no FDA clearance whatsoever. Section 201(h) of the Federal Food, Drug, and Cosmetic Act (the Act) requires that manufacturers of medical devices obtain marketing approval or clearance for their products from the FDA before they may offer them for sale in the United States. This is to protect public health by ensuring that medical devices are safe and effective or substantially equivalent to other devices already legally marketed in the United States.

Unfortunately, many companies appear to have disregarded the Act and not obtained premarket approval or clearance. As medical practitioners we highly caution against using products that are in violation of the FDA. Physicians should not use products from companies such as Dr. PRP America, YESPRP and ezPRF. These products are either not listed with FDA or are cleared only as blood collection tubes, and not for the preparation and injection of PRP at the patient point-of-care.

Manufacturers of In Vitro Diagnostic Tubes (IVD) like BD (Becton, Dickinson and Company, Franklin Lakes, NJ) go out of their way in their precautions and warnings inserts to prevent medical providers from collecting blood with their products for the purpose of reinjection into patients. Chemical additives contained in some of their tubes could cause severe toxicity if injected into a patient. Additionally, these tubes are not manufactured to be pyrogen-free. Physicians must be educated on the difference between sterile and pyrogen-free. Dr. Cathy Miller has written extensively on sterility issues.[37] "Sterility ensures the absence of viable living bacteria. However, the act of sterilizing a contaminated vial can actually result in the release and deposit of pyrogens, as sterilization destroys bacteria, leading to bacterial cell lysis and release of LPS or other endotoxins and exotoxins. Pyrogens are not destroyed by autoclaving, not filterable, and when injected intrathecally they are 1000 times more potent and dangerous."

In other words, sterile does not mean "safe to inject" or pyrogen-free. FDA-cleared products are required to be pyrogen-free. Eclipse PRP is FDA-Cleared and manufactured in a pyrogen-free environment which should be taken into consideration when selecting a PRP system.

9. Summary

There are a number of platelet-rich plasma systems available to medical practitioners in the United States. Only FDA cleared Class II medical devices should be used in any circumstance.

Systems that are cleared by the US FDA can be divided into technologies that either use an "automated" gel separation or that require some level of operator skill. The gel separation systems remove the most contaminating red and white blood cells and are preferred in our view, given the ease of use and reproducibility. Based on our experience, patient satisfaction, and ease of use, gel separation systems seem to be best suited to the aesthetic practice. EclipsePRP performs well on all hematologic parameters, shows favorable clinical results, and importantly, allows a physician to be confident in what they are injecting.

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