Autologous platelet rich plasma (PRP): what do we know? Important concepts relevant to hair restoration surgery

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Introduction

The alpha and dense granules of platelets are a rich source of growth factors, cytokines (messenger molecules that allow cells to communicate and alter one another's functions), chemokines (a family of cytokines that mobilize and activate white blood cells), and other biomolecules important for tissue repair and regeneration.[1] It is therefore not surprising that products derived from platelets and platelet rich plasma (PRP) have shown promise in dentistry and oral-maxillofacial surgery, wound healing, orthopaedic surgery and eye surgery.[2] Historically, the use of products derived from PRP has been limited within specific indications (e.g., orthopaedic and dental procedures), but more recently there has been a surge in interest around broader clinical applications of autologous PRP. Not surprisingly, although clinical benefit of PRP in hair restoration has been considered since the early 1990s[3], renewed excitement and interest in the use of autologous PRP to foster rapid healing and robust hair growth is also present in the hair restoration community. In a presentation given at the 2009 ISHRS Annual Meeting in Amsterdam, Dr. Carlos Uebel indicated that the use of PRP adds little time to the procedure, provides hair growth benefit that outweighs risk to the patient, and is cost effective. In addition, published studies by a number of investigators including Drs. Uebel, Perez-Meza, Greco, Rinaldi, and this author support benefit of incorporating PRP into hair restoration techniques for improving hair growth, improving transplanted follicle revascularization, and speeding the healing of donor and recipient incision sites.[4-10] Considering increasing evidence and interest by ever more hair restoration practices to explore the benefits of PRP for the patient, it is the author's belief that as we move towards evaluating different treatment strategies, an understanding of the science behind the use of PRP is warranted.

The purpose of this review, therefore, is to provide background on the methods used for producing PRP and underlying science that is relevant for a deeper understanding of the procedures we as a collective professional Society evaluate on patients and implement as strategy within regimens for hair restoration surgery.

The Efficacy of PRP Relates to Biological Activity within Platelet Releasate

The influx of platelets is an early event in the process of wound healing and contributes signals that are critical for tissue regeneration. Upon activation by collagen fragments, thromboxane A2, ADP, and/or thrombin, biomolecules (growth factors, cytokines, and chemokines, among others) are released from the alpha and dense granules of platelets.[11,12] It is known, through efforts such as the platelet proteome project, that more than 300 proteins are released by human platelets in response to thrombin activation.[13] Considerable effort has gone into the characterization of proteins released by platelets and much is known about their specific roles as regulators of biological activity. For example, platelet releasates or active platelet gel generated by the addition of thrombin to PRP, contains a broad range of growth factors, cytokines, and chemokines, such as vascular endothelial cell growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EFG), fibroblast growth factor (FGF), transforming growth factor-beta (TGF-B), insulin growth factor (IGF), Interleukin-8 (IL-8), macrophage inflammatory protein-1α (MIP-1α), and platelet factor-4 (PF-4) among others.[1] These protein ligands (a molecule or a molecular group that binds to another chemical entity to form a larger complex) are known to regulate cell migration, vascularization, cell proliferation, and deposition of new extracellular matrix.[14] These activities contribute to organized tissue regeneration and the biological activities of protein ligands in PRP likely underlie the efficacy associated with PRP for many indications. Specific to hair restoration surgery, it has been demonstrated that the application of active platelet gel to surgical sites reduces erythema, crusting, and swelling.[9] Furthermore the VEGF8 and PDGF4 found in PRP are known to facilitate angiogenesis around the hair follicle, and this may be one mechanism underlying observations that PRP can foster robust hair growth. Considering the early clinical evidence and basic science that supports the application of PRP in hair restoration surgery, it is reasonable to evaluate PRP with intent of improving outcomes and speeding recovery of patients. As a first step in this process and to avoid conflicting results associated with the use of PRP in other indications, it is important to match available technology for producing PRP with the intended end-use.

All PRPs Are Not Equal

A number of devices for preparing autologous PRP have become commercially available in the United States, and while the availability of different PRP systems provides clinicians with flexibility, it is critical to appreciate the significant difference associated with end products produced by various systems. Although all of the devices used to produce PRP rely on centrifugation to separate platelets from other blood components, the different PRP systems utilize different centrifugation chambers and spin parameters that dramatically impact the product they produce.[15] The different systems produce PRPs that can vary in platelet concentration, as well as content of erythrocytes and leukocyte populations. Furthermore, PRP systems can be used to produce either PRP containing platelets in suspension, or PRP gels. PRP systems also can be distinguished further based on their FDA cleared indication for use. Thus, PRP systems are not equal and thoughtful consideration is important for selecting a PRP system that is appropriate to the intended application.

Technology	Increase in Platelet Concentration	Time to Process	Final Product	FDA-Cleared Indications for Use
Cytomedix AutoloGel	1 × (Physiologic)	10 minutes	Active PRP GEL	The AutoloGel System is intended to be used at the point of care for the safe and rapid preparation of PRP gel from a small sample of a patient's own blood. Under the supervision of a healthcare professional, the PRP gel produced by the AutoloGel™ System is suitable for exuding wounds, such as leg ulcers, pressure ulcers, and diabetic ulcers and for the management of mechanically or surgically-debrided wounds.
Harvest Smart-PRep 2 APC +	4.6 × to 7.6 ×	16 minutes	Platelet Concentrate	The SmartPReP is designed to be used for the safe and rapid preparation of autologous PRP from a small sample of blood at the patient's point of care. The PRP can be mixed with autograft or allograft bone grafting material prior to application to an orthopedic surgical site as deemed necessary by the clinical use requirements.
Sorin Angel	3.0 × to 4.3 ×	25 minutes	Platelet Concentrate	Intended to be used at the patient's point of care for the safe and rapid preparation of platelet poor plasma and PRP from a sample of whole blood. The plasma and concentrated platelets produced can be used for diagnostic tests. The PRP from the COBE® Angel Whole Blood Separation System can also be mixed with autograft and/or allograft bone prior to application to an orthopedic site as deemed necessary by the clinical use requirements.
ArterioCyte-Medtronic Magellan	3.6 × to 5.1 ×	33 minutes	Platelet Concentrate	The Magellan Autologous Platelet Separator System is designed to be used in the clinical laboratory or intraoperatively at the point of care for the safe and rapid preparation of platelet poor plasma and PRP from a sample of a mixture of blood and bone marrow. The plasma and concentrated platelets produced can be used for diagnostic tests. Additionally, the PRP can be mixed with autograft and/or allograft bone prior to application to an orthopedic site.
Biomet GPS II	2.3 × to 8 ×	27 minutes	Platelet Concentrate	For the safe and rapid preparation of autologous 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 orthopedic surgical site as deemed necessary by clinical use requirements.
De Puy Symphony II	4 × to 6 ×	16 minutes	Platelet Concentrate	Designed to be used for the safe and rapid preparation of autologous PRP from a small sample of blood at the patient's point of care. The PRP can be mixed with autograft or allograft bone grafting material prior to application to an orthopedic surgical site as deemed necessary by the clinical use requirements.

Table 1. Data in table is reviewed in Kevy, et al.[32] and Roukis, et al.[15]

The Sorin Angel, Harvest SmartPrep® 2, Arteriocyte Magellan™, BioMet GPSII, and DePuy Symphony™ are cleared by the FDA for producing autologous PRP that is intended for use in diagnostic and orthopaedic indications. The devices typically rely on centrifugation to separate platelets and plasma from other blood components to yield PRP containing platelet concentrations ranging from physiological to 7.6 × physiological and also containing varying levels of erythrocytes and leukocytes. The PRP derived from these devices traditionally is diluted by mixing with different amounts of bone graft material prior to application to a surgical site. While most of the PRP systems have been developed for orthopaedic indications, only the Cytomedix AutoloGel™ system is cleared by the FDA for wound healing applications. The AutoloGel system also relies on centrifugation to separate platelets from other blood components but is used to produce an activated PRP gel that is applied directly to a wound. (See Table 1 for comparison of systems.)

An important consideration of the AutoloGel system is that it yields plasma containing physiologic concentrations of platelets that is subsequently activated by thrombin to produce fibrin gel abundant in growth factor activity. Considering that the many commercially available PRP systems produce significantly different end products, it is not surprising that it is hard to find a consistent body of evidence that supports clinical applications of PRP. Outcomes can vary depending on the PRP systems used, and well-informed consideration is advised when selecting a PRP system. When considering commercially available PRP systems, it is important to understand differences in the PRP end products that are obtained. Important differences include the platelet concentration, the presence of red cells and leukocyte populations, and clinical requirements for either non-activated PRP or activated PRP gel.

Relating to the platelet concentration, it initially might seem logical to conclude that higher platelet concentrations are desirable, but as suggested by Anuita et al., the "more is better" approach is not always the case.[2] For example, while physiological levels of platelet releasate are well-established to induce wound healing responses, recent data by Han, et al., Choi, et al., Krasna,et al., and Rughetti, et al. indicate support that bell-shaped response curves are associated with PRP.[16-19] PRP releasate from concentrated PRP actually inhibited cell growth and chemotaxis activities associated with regeneration. Clausen, et al. also reported that intermediate concentrations of platelet releasate representing physiological numbers of platelets are more proliferative when incubated with primary bone cells as compared to lower or higher concentrations of platelet releasate. Furthermore, high concentrations of platelet releasate actually caused apoptotic cell death (programmed cell death, a pattern of cell death affecting single cells).[20] Although intriguing, these results are not surprising with an explanation rooted in basic biology. Dating from the early 1980s, volumes of published research on individual growth factors including many found in PRP (PDGF, FGF, VEGF, and TGF-ß) has established that many cellular receptors respond to their ligands with bell-shaped dose curves.[21-24] Several different receptor desensitization and downregulation mechanisms have been defined for the bell-shaped dose response curves associated with chemokine and growth factor receptors, and this is reviewed in Lin and Bucher, Bohm, et al., and Ali, et al.[25-27]

Understanding the aforementioned, PRP concentrates may be most useful in applications wherein PRP is to be

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diluted; for example, with bone graft material in orthopaedic applications. In contrast, PRP containing physiologic concentrations of platelets would be most appropriate for applications such as wound healing where PRP is not diluted. It therefore is appropriate that multiple PRP systems are available to serve the two FDA cleared indications for use.

Relating to hair restoration, the application of PRP has been evaluated as a treatment to both speed wound healing and foster hair growth. Both of these endpoints relate to the delivery of growth factor, cytokine, and chemokine activities, and based upon available past and present data, this author suggests that PRP containing physiologic concentrations of platelets is most appropriate. Further supporting this opinion are reports that high concentrations of growth factors TGF-ß, EGF, and PDGF may contribute to impaired wound healing and increased scarring.[28] Whether or not this observation is related to bell-shaped dose response curves is unknown, however, the use of PRP containing physiological levels of platelets is recommended to avoid a possible detriment to patients.

The Cytomedix AutoloGel system is the only system that is FDA cleared for wound healing indications. The Cytomedix AutoloGel system produces an end product derived from physiologic concentrations of platelets that is activated with thrombin to produce active PRP gel. It is also noteworthy to point out that the Cytomedix AutoloGel system produces a PRP that has no or minimal contaminating red cells or leukocytes. The presence of red cells is an obvious aesthetic consideration, whereas recent data supports that contaminating leukocytes may detrimentally impact performance. Frechette, et al. have demonstrated that contaminating leukocytes present in PRP can lead to high levels of Interleukin-1ß (IL-1ß).[29] Although platelets release low levels of IL-1ß in response to activation, contaminating leukocytes can actively synthesize significantly elevated levels of this pro-inflammatory cytokine. In the context of hair restoration, the presence of elevated IL-1ß is not desirable. It is well established that IL-1ß induces cells to express inflammatory cytokines, such as tumor necrosis factor α (TNF-α), and increase production of proteases (any enzyme that catalyzes the hydrolytic breakdown of proteins into peptides) that degrade collagen and other matrix proteins.[30,31]

Conclusion

All of the above considered, this author recommends that for purposes of safety, efficacy, and standardization across the hair restoration community, investigators consider the Cytomedix AutoloGel system, which produces activated PRP gel from physiological concentrations of platelets with little or no contaminating red cells or leukocytes.

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There has been much progress in the field of plasma and platelet-derived growth factors. One pivotal discovery that has fueled the research in regenerative medicine and tissue engineering has been the role that cytokines and growth factors play in the process of tissue repair.[1] These molecules provide signals at local injury sites, regulating the mechanisms and pathways that govern wound healing and tissue regeneration.[2] The discovery that platelets are endogenous reservoirs of hundreds of biologically active proteins and that plasma-derived fibrinogen can easily be transformed into a three-dimensional fibrin scaffold has opened the door to the use of plasma and platelet-derived formulations for tissue repair and regeneration purposes in many different fields of medicine including dentistry, oral implantology, orthopaedics, ulcer treatment, sports medicine, and hair restoration surgery among others.

As a consequence, many different products, generally termed "platelet rich plasma," have appeared, especially in the last few years. Many of these products vary significantly both in the composition and in the elaboration process, leading to different and in some cases opposed biological effects. I agree with Dr. Reese that not all the PRPs are equal and that, to avoid general misunderstandings, it is totally necessary to define some critical requirements for these types of products. The latter will help to identify more clearly the specific properties of each formulation and more importantly to define their biosafety and repair potential.

One important consideration is the platelet concentration of the PRP, which should be maintained between 1.5 and 3 above the physiological concentration of platelets in blood. Lower levels may lead to inefficacy, whereas higher platelet levels may lead to the absence of biological effects or even to side-effects. However, this is not the only parameter to be considered. Others such as the anticoagulant used, the type of platelet activator, and the application methodology may dramatically affect the biosafety of the product and the final therapeutic effects. These were probably some of my major challenges when I started to work in this field more than 20 years ago. Developing a platelet and plasma-based biotechnology that only needs small volumes of blood to create the different plasma-formulations, avoiding the use of bovine thrombin (a major biosafety concern) as activator, and defining precise and predictable protocols to apply to these products in surgery were some of my initial challenges. Our research group was able for the first time in the literature to define a 100% autologous and biocompatible plasma rich in growth factors.[3] Some of the distinguishing properties of this biocompatible technology include the need of small volumes of the patient's blood (< 100mL), the use of sodium citrate as an anticoagulant, but more importantly, calcium chloride as activator.[4] The latter reduced the initial burst effect in the release of growth factors from platelets and at the same time that eliminated all the biosafety risks and hurdles related to the use of thrombin. The future may see novel applications of plasma and platelet-derived products but to progress in the right direction it is necessary that the scientific community clearly define the advantages and risks of these types of products.

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Dr. Anitua is a world-renonwed pioneer of the bio-implantology and bio-regeneration techniques. He holds several patents related to the method of extraction and use of plasma rich in growth factors (PRGF) and its application in sports medicine. He has authored and co-authored more than 10 books and book chapters and more than 100 research and review articles.

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