Cyberspace Chat

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Platelet Rich Plasma (PRP): Pseudoscience or Fact

In a recent online discussion, Bob Leonard stated: "After hearing the lectures in Kuala Lumpur about PRP, I am less sure of using it versus not. I want to add it to my treatment options; however, I'm not sure about it enough to use it on paying patients right now."

This began a discussion with a variety of opinions both positive and negative. Bob True stated that in addition to minoxidil and finasteride, he "would also do two PRP/ACell injections 6 months apart to 'jump-start' medical therapy. I would use 120-160cc whole blood (WB) at 2% hematocrit, getting 3-5cc of PRP." He would then add platelet poor plasma (PPP) to a total volume of 8-10cc and 50mg of ACell MatriStem because he has "been encouraged by the added benefit of this measure for young men on medical therapy with finasteride and minoxidil."

Bob Haber replied: "One of the aspects of PRP that keeps me doubting are the protocols that involve widely spaced out procedures, such as Bob True just mentioned. Hair loss is a chronic condition, and there are virtually no chronic conditions that can be successfully treated with just a few interventions. Certainly there are no other hair loss treatments that do not require indefinite daily or at least weekly interventions. Growth factors are rapidly degraded. What long-term effect could result from 2 interventions spaced out by 6 months?"

Bob True added: "The effect of PRP/ACell is purely temporary UNLESS it is combined with finasteride and minoxidil. The reversal of miniaturization and reactivation that it (PRP/ ACell) stimulates can be maintained for much longer with ongoing finasteride and minoxidil."

Jerry Cooley added: "If PRP/ ACell was like a single shot of minoxidil, I agree it would be useless. But I believe that this treatment stimulates stem cell activation and gene transcription, creating a 'pro-hair' cascade that can then be maintained with finasteride and minoxidil. I don't agree with the assertion that there are no chronic conditions that can be repeated periodically. In fact, it seems to be the way of the future: monthly shots of biologics for psoriasis and yearly shots for osteoporosis. Single series of PRP treatments are being successfully used for chronic arthritis and tendonitis. There is an ever increasing number of reports in the literature that PRP does something and it is up to us in the trenches to figure out the best way to use it. I don't believe there will be a one-size-fits-all protocol because the duration of the PRP (± ACell) will almost certainly depend on 1) whether the patient is on finasteride, and 2) the patient's underlying genetic programming. The results of finasteride wane in most patients after 5 years, and this gives us a good option to regain some of that original thickening."

Paul Rose replied: "In reading the recent comments, I am uncertain as to the science we speak. It seems like a broad statement to say that gene transcription is stimulated and stem cells are stimulated. What genes? Are they all pro hair? Are some pro neoplastic? Which stem cells are activated? Do they go to the dermal papilla, fibroblasts, epidermal cells, neural cells, or neoplasms? We are speaking in such broad terms that it does not seem like a good scientific approach. I still cannot get clear answers about concentrations of platelets to use, depth of the injection, amount per injection, type of PRP, type of delivery system, distance from one injection to another, etc. Until we develop a rational approach to these techniques, it is pseudoscience in my humble opinion."

Bill Rassman responded: "It is difficult for me to accept treatments such as PRP in the situations outlined in these correspondences without evidence of clinical proof. Offering treatments like PRP without proof and making money on each treatment taints those who are doing it. Let's stop this subjective process and apply the science to the test."

Bob Haber then replied: "I think pseudoscience is probably not the best term. That suggests fake science. Rather, there is a scientific vacuum with regard to PRP and hair. Pioneers push the limits of science and often do not follow proper methods. That's important. The next group generally tests the conclusions of the pioneers prior to mainstream acceptance. That step has been skipped for PRP. Highly concerning is the profit motive that clearly motivates many adopters, fueled by the economic projections distributed by various PRP companies. We need pioneers, but we must insist on science or we look like profiteers."

Jerry Cooley responded: "I think some of you are confusing the merits of PRP with the marketing of PRP. You object, and I strongly agree, with the over-selling and deceptive advertising, in many cases by inexperienced docs trying to make a buck. This doesn't make PRP bad; it makes the docs bad. In my practice, patients are fully informed about the variability in results and given realistic expectations. I don't market it at all. I offer it to patients who my experience tells me will benefit, such as those with extensive miniaturization. Substitute low level laser therapy (LLLT) for PRP. How can you recommend LLLT to your patients?"

Bill Parsley answered: "I purchased the Angel PRP machine and some ACell powder. I injected one of my transplant patients for free, injecting the right vertex (with PRP and ACell) but not the left. Even though I tried to make the density of graft quality the same, there was definitely more volume on the injected side. In another patient, who underwent his 5th hair transplant procedure along with injections of PRP and ACell, he noted that his grafts grew 'more quickly and thicker' than any of his other procedures." Since this time, Bill has used PRP and ACell alone or in conjunction with surgery on 15 more patients noting that "so far they (the patients) have been happy and have had outcomes in the higher end of expectations." He goes on to quote a Chinese

proverb: "The person who says it cannot be done should not interrupt the person doing it."

Francisco Jimenez then offered: "I lean on the side of those who consider PRP pseudoscience. I miss some good double blind clinical studies. Carlos Puig presented one in Malaysia that showed no difference between PRP and placebo."

Jerry Cooley replied: "It is especially hard to do a study. There are a multitude of variables with the procedure and an expected bell-shaped variability curve with results requiring both an experienced doctor doing it and a large enough study population to ensure statistical validity. Furthermore, you need a population who are not using any other hair treatment with good follow-up for 1 year. Most of my patients see their results between 8-12 months, so I do not find 6-month results useful."

Paul Rose added: "Having done several assays on platelet concentrations with different devices, I would suggest that anyone doing PRP take some of the fluid and assay it at a local lab for platelet counts. The numbers you get from manufacturers' printed material may not coincide with what you think you have compared to what you really have. This data is important so that we can more accurately compare protocols." Paul also expressed great concern over the "lack of uniformity" in protocols and machines to prepare PRP.

Mario Marzola has experience using PRP to treat osteoarthritis. He added: "PRP's effect in rhytids or hair loss is less certain. Like Bob Haber said, the effect was for 1-2 months with PRP, then arthritis symptoms recurred. A course of 5 to 6 treatments 2 months apart was the average routine."

Brief Introduction to PRP

It was discovered that a number of bioactive substances were discharged from the α-granules of platelets into plasma when platelets were destroyed or activated. Platelets promote stromal stem cell proliferation and angiogenesis. In 1998, PRP was used to accelerate wound healing and tissue repair in dentistry. The role of PRP has expanded to other fields including cardiac surgery, ophthalmology surgery, maxillofacial surgery, plastic surgery, sports medicine, and cosmetic medicine. In 2006, Uebel et al. reported that PRP improved hair growth and density when follicular units were pre-treated with PRP.[1] Rinaldi, Greco, and Cole have shown that PRP is useful to treat alopecia areata.[2,3] Greco showed that PRP can improve coverage in male pattern baldness by increasing hair diameter.[4] Cole has seen an improvement in hair mass in women using a 5× concentration, a 2% hematocrit, injected in all layers from upper adipose to upper dermis using a 25 gauge needle, and activation with Calcium gluconate. In one instance, the HMI increased from 60 to 98 after 1 year.[5]

Platelet rich plasma (PRP) and platelet concentrate (PC) are established terminology for blood components for transfusion. Unfortunately, the continued use of the term PRP for autologous, topical platelet products contributes to the misconception that all therapeutic autologous platelet products are equivalent. Platelet-rich therapy products contain a mixture of bioactive compounds and formed elements, and differ quantitatively in the concentration of platelets, mononuclear leukocytes, granulocytes, and red cells, as well as the potential to provide growth factors, cytokines, chemokines, and other biologic mediators. The differences in PRP products may be a potential cause of conflicting clinical reports on the therapeutic efficacy of PRP. The quantitative and qualitative differences in platelet rich products may influence the biological effects and clinical therapeutic outcome of PRP treatment. Growth factors (GF) in PRP are released from α-granules of thrombocytes. The α-granules of platelets include more than 20 GFs and other bioactive proteins including VEGF, IGF-1, HGF PDGF, TGF, EFG, platelet factor-4, interleukin-1, platelet-derived angiogenesis factor, platelet-derived endothelial growth factor, and epithelial cell growth factor. Current clinical practice targets a platelet concentration of approximately 1,000,000 platelets per ml of PRP or a concentration of 5 times whole blood levels.

PRP Studies

It is understandable that a physician would be confused about the benefits of PRP based on the presentations given in Malaysia.

The most important question to address is the benefit of PRP to induce better hair coverage through an increase in the number of growing hairs and/or an increase in follicle diameter. We already know that increasing hair diameter exponentially increases hair volume, while increasing the number of hair follicles only proportionally increases hair volume.[6] Carlos Puig and Robert Reese studied the Angel System (Arthrex).[7] In their study, they evaluated 26 patients (15 study patients and 11 placebo patients). All patients were Ludwig II FPHL(female pattern hair loss) confirmed by history or biopsy. The investigators were blinded to the treatment and results. They found no statistically significant difference between the study and the placebo populations with regard to the HMI (based on the Cohen Hair Check device) or the hair count (based on a $1cm^2$ study area using a Dermlite Pro and a $1cm^2$ reticle). There was a strong placebo effect as 27.3% reported slight improvement in hair thickness following treatment with placebo, while 33.3% reported slight improvement or substantial improvement in the treatment group. However, a few patients experienced as much as a 24% improvement in the HMI in the study group.

Dr. Puig's study carried some limitations in design including a 2% hematocrit, failure to activate the PRP, and a 1.14-fold increase in platelets with an estimated platelet count of 204.44 (k/µl). Such concentrations are useful for wound healing, but the amount of growth factors will be less than in whole blood as I will point out later. Decreasing the hematocrit reduces proinflammatory neutrophils, but it also reduces the platelet count and fold increase. For example, 60cc of whole blood prepared at a hematocrit of 7% and diluted to 20cc with platelet poor plasma (PPP) would yield a 1.68-fold increase and 301.27 (k/µl) concentration of platelets based on the Angel Application (Arthrex). In most therapeutic studies on platelets, the optimal fold increase is 4-5× and a concentration of greater than 1,000,000 (k/µl). Dr. Puig failed to activate the platelets. In such an instance, the growth factors will only gradually be released as the platelets activate or degrade and the growth factors may become trapped in a fibrous network that forms gradually at the point of injury from the injection. One might expect no benefit. Finally, this study consisted of only a single treatment with a 6-month follow-up. Most therapeutic studies consist of three or more treatments and a 1-year follow-up.

Also in Malaysia, Anil Kumar found no benefit from PRP administered during a hair transplant surgical procedure (neither hair count nor hair diameter) in a small sample size of patients

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with a concentration of 1 million platelets/µl and activation with calcium chloride or thrombin.[8] Dr. Kumar stated that he used TrichoScan to document his results, but he did not present results that documented the use of a TrichoScan. Dr. Kumar did not study the benefit of PRP to improve hair loss alone.

Cerevelli et al. compared the injection of autologous, activated PRP (AA-PRP) to the injection of placebo in 10 patients ranging from a Norwood II to a Norwood IV according to the method of Cascade-Selphyl-Esforax system where he obtained a small volume of whole blood (18cc) using sodium citrate as an anticoagulant.[3] He performed three treatments monthly with AA-PRP. He excluded all patients who had received topical (such as minoxidil, prostaglandin, analogues, retinoids, and corticosteroid) or systemic treatments for male pattern hair loss (MPHL) (such as finasteride, dutasteride, and antiandrogens) in the previous 12 months. He studied the number of platelets in PRP obtained from all participants through microscopic counting. He performed a randomized TrichoScan evaluator blinded study in both the treated and placebo-treated areas. He spun the whole blood at 1100g for 10 minutes to make 9cc of PRP without concern about the white cell count. He activated with Ca^{2+} from the Cascade-Selphyl-Esforax kit to produce AA-PRP. He divided the head into 4 parts: frontal, parietal, vertex, and occipital. In patients with hair loss isolated to the frontal and parietal areas, he injected the frontal area with AA-PRP and the parietal area with placebo using a physiologic solution. In patients with hair loss in the parietal and vertex parts, he injected AA-PRP in the parietal area and placebo in the vertex. He injected AA-PRP at a volume of 0.1cc mL/cm^2. The same numbers of injections were made in both the treated area and the placebo area. Cereveli studied the patients at T0 (beginning), T1 (14 weeks), T2 (6 months), and T3 (12 months).

All patients were studied with global photography, physician's and patient's global assessment scale, and standardized phototrichograms (TrichoScan). TrichoScan evaluated hair density, hair diameter, anagen/telogen ratio, and vellus hair/terminal hair ratio in tattooed regions following hair dying. The evaluator of the TrichoScan was blinded. He performed 3mm punch biopsies at baseline and 2 months after the last AA-PRP treatment. He performed immunohistochemistry using anti-Ki67 and anti-CD31. At baseline, there was no statistical difference in hair count, hair density, and terminal and anagen hair densities between the treatment and control areas of the scalp. There was a statistically significant increase in mean hair count, 18, in the treated areas versus a mean decrease of 2 hairs in the control area (control versus treatment: $P < 0.0001$). In the treatment area, a mean increase in total hair density of 27.7 hairs/cm^2 compared to baseline was observed after 3 months and the control area displayed a mean decrease of 3.0 hairs/cm^2 (control versus treatment: $P < 0.0001$). In addition, terminal hair density improved significantly by 27.0 ± 15.3 hairs/cm^2 in the treatment area compared to baseline, while decreasing by 2.1 ± 12.4 hairs/cm^2 in the control area of the scalp (control versus treatment: $P = 0.0003$). There was no statistically significant difference in vellus hair density between the study and the control area after 3 months. Histomorphometric evaluation showed an increase in epidermis thickness in the PRP-treated hair skin after 3 months, an increase in the number of follicles compared to baseline, an increase in $Ki67^+$ cells in the epidermis and the hair follicular bulge cells, and a slight increase in small blood vessels around hair follicles in the skin treated area compared to baseline.

Takikawa et al. compared the delivery of PRP & dalteparin and protamine microparticles (D/P MPs) to PRP and saline as a control.[9] They injected PRP & D/P MPs on one side of the scalp and saline as a control on the opposite side of the frontal scalp in 13 subjects. They injected PRP on one side of the scalp and saline as a control on the opposite side of the frontal scalp in another 13 subjects. D/P MPs consist of low-molecular weight heparin (dalteparin) and protamine. Injections were carried out at 0, 2, 4, 6, and 9 weeks, and patients were studied at 12 weeks. They studied several growth factors that bind heparin along with those that do not bind heparin (IGF and EGF). The heparin-binding quality of several growth factors allowed for a sustained release of growth factors from the microparticles upon biodegradation. They found that heparin-binding GFs (over 80%) were absorbed by the D/P MPs, while non-heparin-binding GFs (IGF and EGF) were only 19% absorbed. They evaluated hair counts in 1cm^2, mean cross-sections of all hairs in 1cm^2, and took a 4mm punch biopsy in the PRP & D/P MPs or PRP before the first injection and at 12 weeks. They found that the hair counts in the PRP and PRP & D/P MPs treated area all increased with a mean increase of 15 hairs for PRP and a mean increase of 18 hairs for PRP & D/P MPs, but the results were not statistically significant different from the control group even though there was a much greater increase in hair counts in the treated areas versus the control areas. They also found that while PRP increased the mean hair cross-section, PRP & D/P MPs had a more profound effect on an increase in mean hair cross-section and the results were statistically significant (control versus treatment $P < 0.01$). Comparison of biopsies under H&E staining from baseline to 12 weeks showed a thickening of the epidermis, proliferation of collagen fibers and fibroblasts, and greater numbers of blood vessels around hair follicles in both the PRP & D/P MPs and PRP. Hair growth and thickening after administration of PRP & M/Ps and PRP were observed in all participants, but PRP & D/P MPs appeared to provide more substantial changes than PRP alone.

PRP—A More Detailed Look

The specific gravity for the components of blood differs as follows: the heaviest are the red blood cells (RBCs) (1.095), followed by white blood cells (WBCs) (1.063-1.085), and the lightest platelets (1.032). There is also variation in the specific gravity of WB between men and women. These slight differences in specific gravity allow for the separation of the components by centrifugation. However, there is always going to be some cell contamination. There are also differences between the specific gravity of individuals and differences in the number and composition of blood cells. This makes it nearly impossible to standardize any one method for all patients. In reality, the method should optimally be individualized to the patient.

There is white-blood-cell-containing PRP (W-PRP), PRP, platelet-concentrated plasma (PCP), and noncoagulating platelet-derived factor concentrate (PFC). The presence of RBCs and granulocytes may contribute to inflammation, pain at the injection site, and destruction of extracellular matrix proteins. Mononuclear cells, on the other hand, contain stem/progenitor cells and are desirable. PRP is prepared typically by a slow spin to avoid spinning down platelets. Platelet-concentrated plasma is prepared through a second faster spin so the platelets are spun down. There is no standardized protocol for preparing PRP or PCP. Higher volumes of PRP are obtained from higher centrifugal forces. To

help us better understand platelet-processing protocols, Araki et al. studied platelet concentration and growth factors based on a variety of spin rates and concentration methods.[10] Platelet collection rate increases from 190g to 320g and progressively decreases from 320g to 2330g. Although the optimal centrifugal force varies from individual to individual, the optimal centrifugal force may be 230-270g for 10 minutes. This protocol will produce approximately 80% of the platelets in WB. This spin rate should contain low numbers of WBCs (4.1-5.8% of WB) and may be termed WBC-poor PRP. The WBC collection rate decreases as the spin rate increases. The optimal spin rate for W-PRP is a slower 70g for 10 minutes producing 10-35% WBC and 60-80% platelets.

On centrifugation of WB, WBCs are concentrated in a white layer known as the buffy coat that is located above the RBC layer. Platelets are mostly concentrated right on top of the buffy coat. W-PRP contains part of the buffy coat.

If a second spin is performed, a higher spin rate will precipitate a higher percentage of the platelets that were in the initial PRP. At 1010g, there are only 69% ± 10.5% of the platelets in the original PRP versus 91.2% ± 6.05% at 2330g. A higher force is recommended for the second spin. After the second spin, you may make one-third the volume of PCP by removing the upper two-thirds of the PPP, or one-tenth the volume of PCP by removing the upper nine-tenths of the PPP. The concentration of platelets in one-third the volume of PCP is roughly 3 times the concentration of PRP, while one-tenth the volume of PCP is 7.4 times that of PRP.

There are five major anticoagulants used in clinical laboratories: heparin, sodium citrate, sodium fluoride, ethylene diamine tetraacetic acid disodium (EDTA), and acid citrate dextrose solution (ACD). It appears that the anticoagulant can also affect the platelet yield. The collection rate of nonaggregated platelets is significantly higher when EDTA is used as the anticoagulant rather than ACD.[10] EDTA appears to be a better anticoagulant than ACD.

Akari also studied the total PDGF-BB and the concentration of PDGF-BB.[10] As might be expected, the total PDGF-BB is higher in whole blood than in PRP or in PCP because his method of preparation produces 88.7%, 74.8%, and 72.1% of the original number of the platelets for PRP, PCP, and PFC, respectively. Interestingly, PFC results in a remarkably higher total amount of PDGF-BB than whole blood even though the total number of platelets was less. In making PFC, he reconstitutes the one-tenth volume of PRP with phosphate buffered saline (PBS), which removes almost all the fibrinogen. It is believed that a substantial part of the PDGF-BB is trapped in the fibrin glue that forms after thrombin stimulation. While the fibrin glue may be useful as a controlled-release of growth factors, it may also trap much of the growth factors. The concentration of PDGF-BB is also much higher in PFC prepared with EDTA than PFC prepared with ACD.[10]

Platelet Activation

Physicians activate thrombin in a variety of ways. Some feel their incision sites provide enough patient thrombin to activate the platelets. Some use microneedling. Others use bovine thrombin. There are reported cases of developing an immune response to bovine thrombin and factor 5 and subsequent clinical bleeding. In addition to xenogenic immune reactions, there is a remote risk of transmitting prions and other zoonoses from bovine thrombin. The most dangerous prions cause a fatal spongiform encephalopathy, Jakob Creutzfeldt disease, which can have an incubation period up to 50 years.

Robert True injects thrombin droplets 4-6mm below the epidermis.[11] He reconstitutes 5,000U of bovine thrombin in 20cc of calcium chloride or normal saline (manufacturer suggests reconstituting with 10cc of normal saline) and freezes it in 3cc syringes. He uses approximately 2.5cc per case. Recently he has switched to human recombinant thrombin because of a better safety profile. Jerry Cooley injects small amounts using a jackhammer motion at a very acute angle with minimal pressure on the syringe plunger.[12] There is also a human purified thrombin and today there is a recombinant thrombin called Recothrom (https://www.clevelandclinicmeded.com/medicalpubs/pharmacy/pdf/Pharmacotherapy_XII-6.pdf).

Note that there are six distinct haplotypes for factor 8, which is deficient in hemophilia A. Treatment in severe cases includes treatment with plasma-derived or recombinant factor 8. Haplotype differences are thought to play a role in the development of factor 8 inhibitors, which is the most serious complication of replacement therapy with factor 8. If there are haplotype differences for thrombin along with genetic, environmental, or immunologic factors, this too could play a role in the development of thrombin inhibitors, following the administration of plasma-derived or recombinant thrombin, and could potentially lead to a bleeding diathesis perhaps years later.

Still others use calcium choride or calcium gluconate at a 1:10 concentration calcium to PRP; however, it remains unclear how well calcium activates thrombin. What is known is that calcium is required in the activation process. Another way to activate platelets is through sonication.[13] The safest method to activate platelets may be sonication and this should be studied.

Activation does appear to be an essential component to the delivery of enough GF to a desired response in the treatment of hair loss.

Automated Machines

There are many automated devices on the market today for making PRP. Some produce red PRP, which is high in WBCs and contains more RBCs. Others produce a yellow PRP, which is low in WBCs and RBCs. One should aim for a 5× concentration when evaluating the different machines. Note that a high WBC concentration will lead to more swelling.

The Angel system allows us to dial in specific volumes of WB to make PRP with a specific hematocrit, which we can dilute with PPP to make a specific concentration of platelets. However, as Paul Rose points out, the various manufacturers may not create equipment that produces the stated concentrations of platelets. A new product on the market is the Genesis Pure PRP by EmCyte Corporation. This device eliminates 99% of the RBCs, eliminates 90% of the WBCs (with less than 20% granulocytes and over 80% mononuclear cells, recovers 76% of the platelets, produces a 6.7 or more fold increase over baseline, concentrates mononuclear cells, and provides large concentrations of PDGF, TGF-beta1, VEGF, and SDF-1alpha). The pH of this PRP is 7.5, which is more physiologic than the Harvest SmartPrep 2 PRP, which has a pH of 6.8. A lower pH may require buffering to reduce the pain on injection. However, the manufacturer performed these studies so there is a conflict of interest.

Studies show that PRP is capable of improving hair counts and hair diameter. However, the complexities of creating PRP along

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with individual characteristics may alter the response of patients to any one protocol. Optimally, we would individualize the protocol to the patient. However, as Paul Rose points out, we don't even have a standardized protocol for using PRP in the treatment of hair loss. Even papers are a little confusing because they do not always mention the platelet concentration. We need to study a standardized concentration, the depth of the injections, the amount of each injection/cm^2, the method of activation, the hematocrit, the color, the granulocyte count, the mononuclear cell count, the size of needle we inject with, and the method of preparation. From here, the study of PRP only becomes more complex. Do we add ACell and, if so, how much? Should we try adding biologics such as Epi-Fix (MiMedx) and how much? How about platelet liposecretion (PRL) where we combine adipocyte stem cells with PRP? Furthermore, patients should avoid aspirin, NSAIDs, and steroids for two weeks before the surgery and for several weeks following treatment with PRP because these medications may minimize the benefits from PRP. It will probably be many years before we can unravel the mystery behind PRP, but first we all need to get on the same page and begin with a standardized protocol. Only then might we break down the barriers between fact and fiction.

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Editor's note: I want to clarify my comments included in this discussion. When using PRP/ACell as an adjunct to surgery or as a nonsurgical treatment, we need enough volume to allow coverage throughout large areas of the scalp. Small whole blood volumes simply do not produce enough PRP. We do lose some platelets during preparation of PRP usually getting only 70-80% of the platelets found in whole blood (WB).

Based upon the experience of Dr. Jerry Cooley, I usually draw at least 120cc WB, and sometimes when we are covering a very large area, 180cc. With the Angel system, we process to a 2% HCT, and with 120cc WB, an average of 3cc of PRP is produced (with 180-4.5cc). Enough PPP is added to produce a 5× concentration. With 120cc WB, the total volume of PRP plus PPP is usually 10cc, and with 180cc WB, total volume is 13cc. I do use thrombin for activation, but I do not inject thrombin, rather, as demonstrated to me by Dr. Cooley, with very light finger pressure on the syringe plunger and inserting the needle depth to 4-5mm, droplets of thrombin are widely and evenly distributed throughout the field. Recently, I have converted from bovine thrombin to human recombinant thrombin to eliminate the potential of allergic response related to the animal-based product. Activation is important to have a sustained effect from the PRP. —RHT