Platelet-Rich Plasma in Androgenic Alopecia: Indications, Technique, and Potential Benefits

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Purpose: The purpose of this study was to provide an overview of platelet-rich plasma (PRP) injected into the scalp for the management of androgenic alopecia.

Materials and Methods: A literature review was performed to evaluate the benefits of PRP in androgenic alopecia.

Results: Hair restoration has been increasing. PRP's main components of platelet-derived growth factor, transforming growth factor, and vascular endothelial growth factor have the potential to stimulate hard and soft tissue wound healing. In general, PRP showed a benefit on patients with androgenic alopecia, including increased hair density and quality. Currently, different PRP preparations are being used with no standard technique.

Conclusion: This review found beneficial effects of PRP on androgenic alopecia. However, more rigorous study designs, including larger samples, quantitative measurements of effect, and longer follow-up periods, are needed to solidify the utility of PRP for treating patients with androgenic alopecia.

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Androgenic alopecia, also known as androgenetic alopecia or male pattern baldness, is a common disorder that affects men and women. Prevalent in approximately 70% of men, androgenic alopecia is the loss of hair in the crown combined with an "M"-shaped hairline recession. It also is seen in approximately 40% of women as diffuse thinning of hair.[1] Hair loss can have a considerable influence on psychological stress and quality of life. Common associations with hair loss include feelings of low self-esteem, depression, feeling unattractive, neuroticism, and introversion.[2] Therefore, the development of a safe and effective treatment modality can be of great benefit to patients in an oral and maxillofacial surgery practice setting.

In 2010, more than 100,000 hair restoration procedures were performed in the United States, and almost 300,000 were performed worldwide.[3] Common therapies currently include finasteride and minoxidil, laser therapy, and scalp surgery.[4-6] Finasteride and minoxidil, although approved by the Food and Drug Administration for the treatment of androgenic alopecia, have several drawbacks. The undesirable side effects of finasteride and daily treatment regimen necessary for minoxidil give many patients reasons to seek alternative treatments.[4-7] A treatment modality that has become popular in regenerative plastic surgery is platelet-rich plasma (PRP) technology. This type of therapy has been shown to increase hard and soft tissue

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wound healing across multiple fields and more recently has been studied in hair rejuvenation applications as a result of its minimal invasiveness, lower costs compared with traditional therapy for hair loss, and absence of major side effects.[8-11]

The utility of PRP in the treatment of androgenic alopecia is rooted in the presence of growth factors in plasma. These include 3 main components: platelet-derived growth factor (PDGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF). Acting in concert, these protein molecules play a role in angiogenesis, which stimulates the healing and growth of tissue structures.[12,13] Furthermore, there is a dose-to-response relation between platelet concentration and the proliferation of human adult mesenchymal stem cells, the proliferation of fibroblasts, and the production of type I collagen.[14] Researchers have discovered through in vitro studies that PRP induces a considerable initiation and prolongation of the anagen phase of the hair growth cycle.[8,15] This article reviews the available literature on the use of PRP therapy for treating androgenic alopecia.

Review of Literature

Numerous investigators have conducted randomized controlled trials aimed at studying the effects of PRP with different treatment parameters. Alves and Grimalt[16] designed a randomized placebo-controlled double-blinded study that evaluated the efficacy of androgenic alopecia treatment with PRP. Twelve men 18 to 65 years old with Hamilton-Norwood patterns II to V[17] and 13 women 18 to 65 years old with stage I to III patterns of hair loss according to the classification of Ludwig[18] were studied. Patients were randomized to receive a half-head treatment with PRP and the other half-head with saline placebo. The prepared PRP solution was injected into 4 selected areas of the scalp. Patients received a total of 3 treatments administered at 1-month intervals and were evaluated at each session. The evaluation criteria were assessed in all patients by global photography and phototrichogram. The mean total hair density for the treatment areas after 3 months showed a mean increase of 14.8 $\pm$ 32.1 hairs/cm^2 compared with baseline, whereas the control area showed a mean decrease of 0.7 $\pm$ 32.7 hairs/cm^2 ($P < .05$). After 6 months, the PRP-treated area showed a mean increase of 12.8 $\pm$ 32.6 hairs/cm^2 and the control area showed a decrease of 2.1 $\pm$ 31.3 hairs/cm^2 ($P < .05$). Furthermore, they found statistically relevant differences in the mean number of anagen hairs and telogen hairs after 6 months. For the total hair count, there were no relevant differences between the PRP-treated area and the placebo-treated area, and no differences in the vellus hair density between the PRP and placebo areas were observed. Although the investigators found statistically relevant differences in hair density, the small sample and short follow-up duration presented weaknesses to the study design. They concluded that PRP can be used as a safe and complementary treatment option for androgenic alopecia.

A randomized controlled trial conducted by Gentile et al[19] studied 23 men 19 to 63 years old with stage IIa to IV hair loss. The primary outcome of the study was residual hair count and hair density based on computerized trichogram. Secondary outcomes were microscopic evaluation of the epidermis thickness in PRP-treated skin and increase in the number of follicles compared with the baseline value and an evaluation of safety and feasibility. PRP was injected 3 times in each patient at intervals of 30 days in select areas of the scalp. All patients were evaluated in 6 stages for up to 24 months after the initial injections. Phototrichograms were taken of all scalps by a trained evaluator. Incisional punch biopsy specimens also were obtained at baseline and after 2 months from the last PRP treatment to evaluate the thickness of the epidermis and number of follicles per square millimeter. The investigators used mouse monoclonal anti-Ki67 to quantify the percentage of Ki67$^+$ cells in the basal layer of the epidermis and in the outer root sheath of hair follicles. The number of vessels per square millimeters was calculated using morphometric criteria. Results showed a marked increase in the mean hair count for the treatment area after 3 months, with a mean increase of 33.6 hairs in the target area compared with baseline, whereas the control area showed a mean decrease of 3.2 hairs. A mean increase in total hair density of 45.9 hairs/cm^2 compared with baseline was observed after 3 months, and the control areas displayed a mean decrease of 3.8 hairs/cm^2. Terminal hair density improved markedly by 40.1 hairs/cm^2 and decreased by 5.6 hairs/cm^2 in the control areas. No relevant differences in vellus hair density between the study and control areas were seen after 3 months. Microscopically, there was an increase of epidermis thickness and an increase in the number of follicles. There also was an increase of Ki67$^+$ basal keratinocytes of the epidermis and of hair follicular bulge cells compared with baseline and an increase in small blood vessels around hair follicles in the treated skin compared with baseline. Although the sample was small, the investigators found that their use of PRP presents a viable option for androgenic alopecia treatment because of the positive clinical results obtained and lack of serious adverse effects.

Cervelli et al[20] studied 10 men in a randomized controlled trial. They also used biopsy specimens for a histomorphometric evaluation of growth activity in the epidermis. Each patient's scalp was divided in

4 parts. Half the sites were treated with autologous activated PRP and sites of the other half were treated with a placebo. Patients were evaluated at the beginning of the study, at 14 weeks, at 6 months, and at 12 months. Global photography, physician's and patient's global assessment scales, and standardized phototrichograms were used. Incisional punch biopsy specimens (diameter, 3 mm) of the hair were obtained at baseline and after 2 months from the last PRP treatment. Immunohistochemistry was performed using mouse monoclonal anti-Ki67 and anti-CD31, with positive and negative controls. Results showed a marked increase in the mean hair count for the treatment area after 3 months, with a mean increase of 18.0 hairs in the target area compared with baseline, whereas the control area showed a mean decrease of 2.0 hairs. The mean hair density also increased by 27.7 hairs/cm^2 after 3 months. Terminal hair density improved considerably by 27.0 $\pm$ 15.3 hairs/cm^2 and decreased by 2.1 $\pm$ 12.4 hairs/cm^2 in the control area. There was no relevant difference in vellus hair density after 3 months. Microscopic evaluation showed an increase of epidermis thickness, increase in the number of follicles, increase in number of Ki67$^+$ basal keratinocytes of epidermis, and increase of hair follicular bulge cells. PRP treatment also was associated with a slight increase of small blood vessels around hair follicles in the skin treated compared with baseline. Despite the small sample, the data suggest that the injection of PRP has a positive therapeutic effect on pattern hair loss without major side effects.

Uebel et al[21] conducted a study using implanted follicular units embedded with platelet plasma growth factors. Twenty patients with male pattern baldness in the frontal, parietal, or occipital area were studied. The investigators obtained a hair-bearing flap from the occipital area of each patient's scalp above the neck, from which follicular units were harvested. The units were divided into 2 groups: the first was imbibed with plasma growth factors and the second was soaked with saline. On the right side of each patient's head, follicular units embedded with platelet plasma growth factors were implanted; on the left side, the untreated follicular units were implanted as controls. After 7 months, the investigators observed a marked difference in the yield of follicular units when comparing the experimental with the control areas of the scalp. The experimental group with the platelet plasma growth factors showed a difference of 2.4 follicular units/cm^2 compared with the control area, which was an increase in follicular density of 15.1%. The investigators found a range of increases in follicular density from 3 to 52% compared with control areas. This study provides preliminary evidence for a promising viable option for combining hair transplantation and PRP therapy.

Kang et al[22] studied the effects of CD34$^+$ cell-containing PRP with concomitant finasteride treatment on pattern hair loss. They set out to study the angiogenic effects of mobilized CD34$^+$ cells compared with the bioactive properties of placental extract. Placental extracts are enriched in bioactive molecules, including growth factors, amino acids, nucleic acids, vitamins, fatty acids, and minerals, and have been used for various purposes.[22] This randomized controlled trial evaluated 15 men and 11 women with pattern hair loss. The patients were divided into 2 groups. The investigators prepared CD34$^+$ cell-containing PRP and injected the solution into scalp areas affected by hair loss in patients in the first group. This was performed twice at a 3-month interval. The second group of patients was treated with interfollicular placental extract injection into affected scalp areas. Men also were treated with oral finasteride therapy. Measurement was performed using a computerized handheld USB camera PT system at baseline and 3 and 6 months after the first treatment. At 3 months after the first treatment, the patients treated with CD34$^+$ cell-containing PRP presented clinical improvement of the mean number of hairs, mean hair thickness, and mean 2-point scores compared with baseline values. These parameters also were improved in the controls treated with placental extract. Hair thickness and hair count were greater in the placental extract group. At 6 months, the continued trend of increases above baseline were seen in all parameters in the 2 groups, with hair thickness and 2-point score measurements being greater in the CD34$^+$ cell-containing PRP treatment group compared with the placental extract group. The investigators found that concomitant finasteride therapy did not markedly affect hair count, hair thickness, or degree of disease progression in either group. These results should be confirmed through the implementation of optimized prospective studies with a controlled or split-scalp design to confirm the clinical efficacies of CD34$^+$ cells in patients with pattern hair loss.

Another study examined the effects of a variation on a PRP formulation by using dalteparin and protamine microparticles (D/P MPs). The material consists of a mixture of low-molecular-weight heparin (dalteparin) with protamine, resulting in water-insoluble MPs. These MPs are a carrier for controlled release of growth factors such as fibroblast growth factor-2 (FGF-2).[23] FGF-2–containing D/P MPs have shown a substantial ability to induce vascularization and fibrous tissue formation.[24] Takikawa et al[25] examined 26 volunteers with thin hair in frontal or parietal areas. One group of patients received 5 local treatments of PRP-D/P 3 mL and the other received PRP and saline injections at 2- to 3-week intervals. The groups were evaluated at 12 weeks. Experimental

and control areas were photographed. Marked increases in hair cross-section, but not in hair numbers, were seen in the 2 groups at the end of the 12-week period, with greater increases seen in the PRP-D/P group. Microscopic findings showed thickened epithelium, proliferation of collagen fibers and fibroblasts, and increased vessels around follicles in the 2 groups. The investigators concluded that the addition of D/P MPs enhanced the effects of PRP in their patients and could be useful to explore further in future controlled studies.

Additional studies without control groups or split-head protocols have shown the clinical effects of PRP on treating androgenic alopecia. Of note, Gkini et al[26] performed a prospective cohort study with 20 patients. The men in the study exhibited type II to V androgenic alopecia and the 2 women exhibited type I and III alopecia. PRP was prepared and injected into the androgen-related areas of the scalp in men and into the problematic areas in women. Three treatment sessions were performed with an interval of 3 weeks. At 6 months from the beginning of the treatment, a booster session was performed. Outcome measurements included hair loss, hair density, and patient satisfaction, measured through hair-pull tests, photomicrographs, photographs, and patient questionnaire. Results showed that hair density markedly increased throughout the study, with the greatest density at 3 months. However, the rate of increase began to slow after the third assessment. Macroscopic photographs showed an overall improvement in hair density and quality, because laguno-like hair became thicker, normal hair. Patients reported a mean satisfaction rating of 7.1 on a linear analog scale of 1 to 10. Patients reported an improvement in hair density and thickness, although at the fifth assessment (at 6 months), 100% of patients indicated a need or want of a booster session. Minimal side effects, including mild transient pain and scalp sensitivity, were reported. Although the study did not have a control group and used nonobjective forms of evaluation, their findings suggested positive results and invite further study into PRP treatments. The investigators found that patients with grade II to III androgenic alopecia have more favorable results than those with more advanced alopecia.

Discussion

To reap the benefits of PRP, it must first be isolated from whole blood. The process begins with drawing a sample of a patient's venous blood, followed by anticoagulation with an acid citrate dextrose type A anticoagulant such as sodium citrate. The anticoagulated blood must be centrifuged into 3 separate layers: a bottom layer of erythrocytes, a middle layer of platelets and white blood cells (also known as PRP or the buffy coat layer), and a top layer of plasma with a small number of platelets (also known as platelet-poor plasma). There are many commercial centrifugation devices available that can achieve the goal of separating the 3 blood layers to obtain a concentrated platelet layer. Platelet concentrations higher than 1,000,000 platelets/μL have been shown to be required for optimal results (Table 1, Fig 1).[8,27] Platelet membranes are kept intact during the centrifugation process by the action of the anticoagulant and low gravity forces.[27,28] Secondary ultrafiltration also can be performed to further concentrate plasma proteins involved in the coagulation cascade.[28] Once isolated, the platelets within the plasma must be activated to release their α-granule contents. This can be achieved by adding topical bovine thrombin 1,000 U per milliliter of 10% calcium chloride to the PRP.[29,30] Once clotted, the activated PRP should be used immediately, because α-granules release their contents rapidly.

Although most investigators cited in this review used a standard protocol for centrifugation, several sought to discover different formulations to produce better results. Borhan et al[31] prepared PRP by mixing the PRP layer with the platelet-poor plasma layer and studied its effects without prior activation. The results showed increases in hair number, although there were mixed results on hair density. They concluded that the use of nonactivated PRP as opposed to activated PRP might have limited the production of growth factors, although positive clinical results were still obtained. Kang et al[22] sought to use the angiogenic and vasculogenic effects of autologous $CD34^+$ hematopoietic stem cells by incorporating extracts into PRP preparations. They found marked improvements in hair thickness and hair counts compared with baseline. However, a non-$CD34^+$ PRP control group was not used, so the magnitude of any potential synergistic effect was not

Table 1. COMPARISON OF PHYSIOLOGIC AND PRP CLOT

	Physiologic Clot	PRP Clot
Red blood cells	30-50% hematocrit	<1% hematocrit
Platelets	Native level	2-6 times native level
Growth factors	Native level	3-8 times native level
Fibrinogen	Native level	Native level

Note: The concentration of platelets and growth factors is higher in the PRP clot.

Abbreviation: PRP, platelet-rich plasma.

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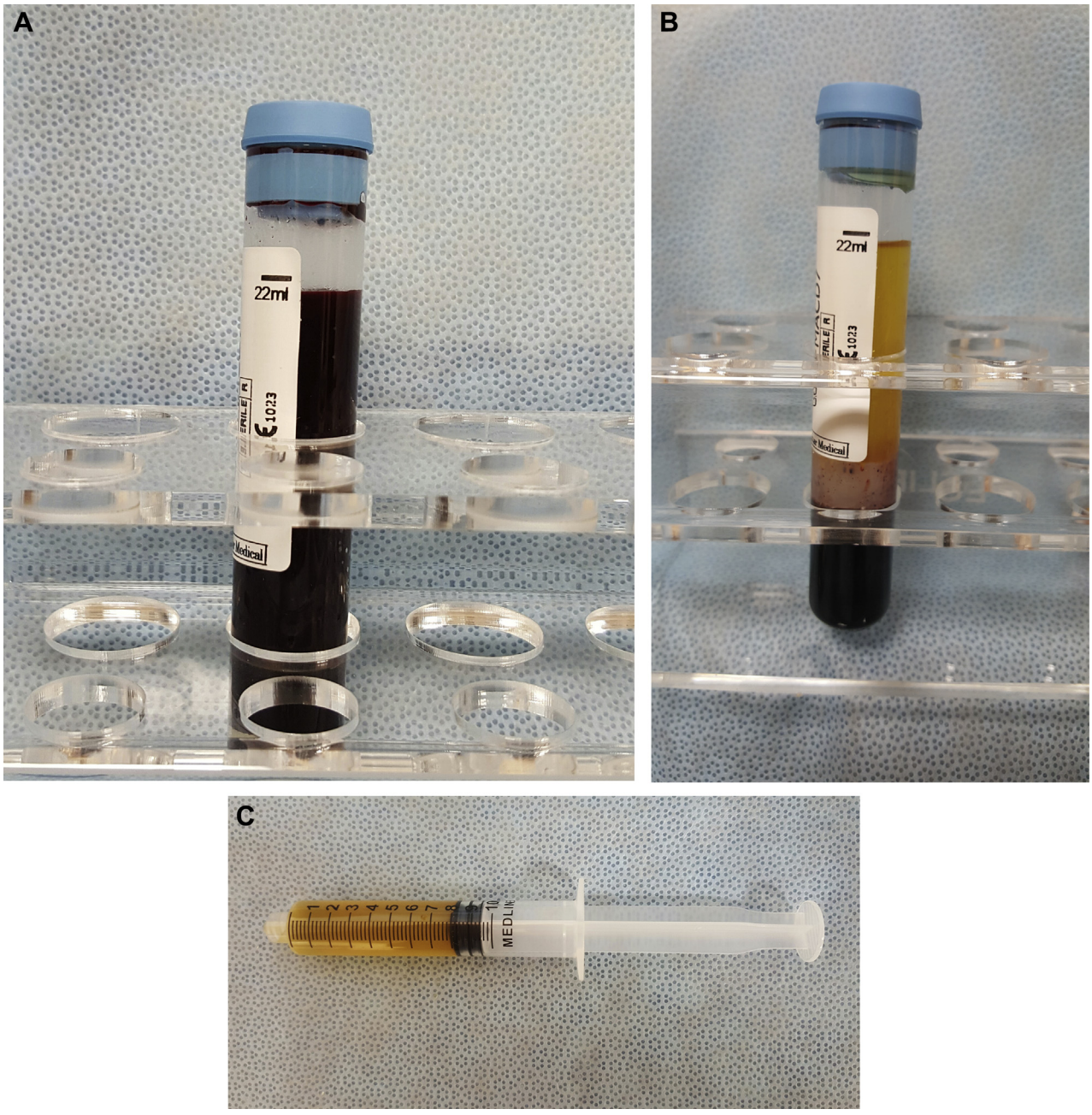

FIGURE 1. *A*, A 22-mL sample of a patient's venous blood. *B*, Sample after centrifugation. *C*, Harvested platelet-rich plasma.

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elucidated. Nonetheless, the results showed that the use of $CD34^+$ hematopoietic stem cells for hair loss therapy should be further studied for a measurement of effect. Takikawa et al[25] studied the use of D/P MPs in a PRP preparation and reported promising results in a controlled trial. They prepared a D/P solution and added it to PRP, which was prepared through a standard centrifugation protocol. The effects of the combined solution (PRP-D/P) and PRP alone were compared. The PRP-D/P solution markedly increased hair diameter compared with the PRP treatment. The PRP-D/P and PRP treatment groups showed similar increases in proliferation of collagen fibers, fibroblasts, angiogenesis around hair follicles, and thicker epithelium compared with controls. The investigators posited that D/P MPs might be able to absorb various heparin-binding substances involved in cell proliferation, migration, and angiogenesis, such as the growth factors and cytokines in PRP. Thus, the use of these MPs could serve as an effective agent to immobilize, retain, and release various growth factors in PRP for induction of hair follicle growth.

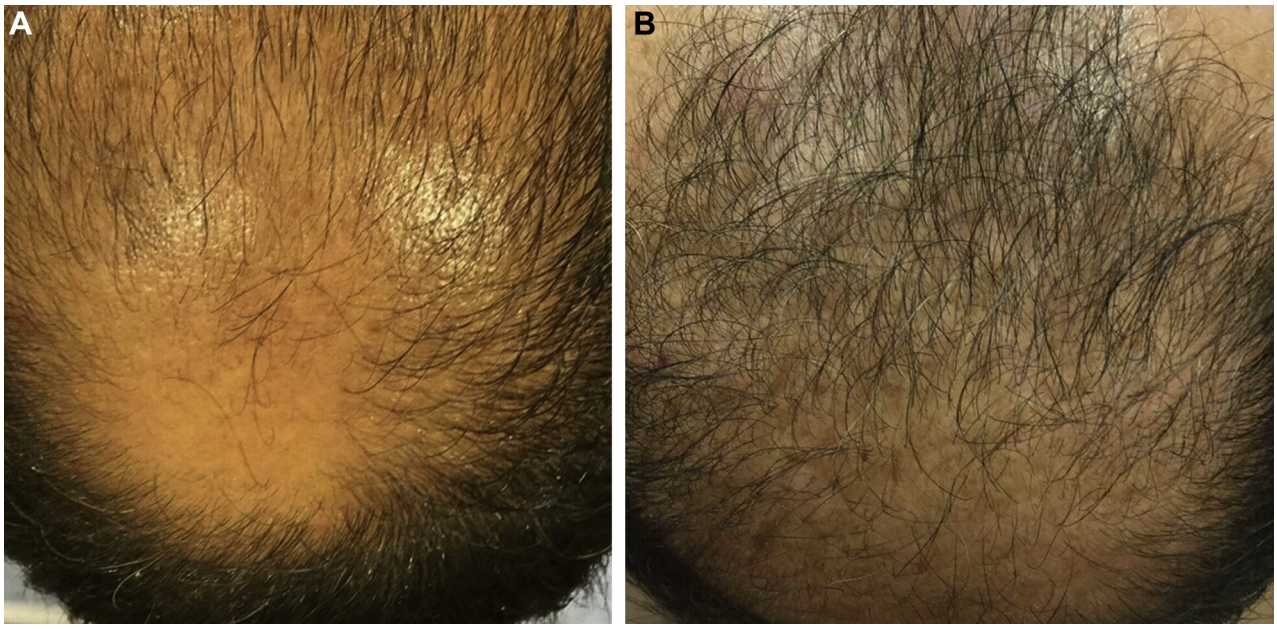

FIGURE 2. *A*, Patient presenting with androgenic alopecia. *B*, Patient after 3 platelet-rich plasma treatments at 4-week intervals. Note the marked increase in hair density and quality.

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After PRP is isolated, it is injected into the scalp in areas that have been affected by androgenic alopecia. Injection methods typically involve cleansing the area with an alcohol-based product and then injecting approximately 0.1 mL/cm^2 in selected scalp areas.[16,19,20,22,26] Some investigators elected to use local anesthesia before injection, although this was not found to be a necessary component of treatment because of otherwise mild and transient intra- and postoperative pain.[22,32] The investigators recommended using topical local anesthetics, such as EMLA cream, to minimize intraoperative pain. An additional strategy that was used was the induction of a cutaneous inflammatory response through gentle pressure 1.0 mm deep using a Scalproller (Nanogen Pangaea Laboratories, Elstree, UK) on the thinned scalp areas to be treated, although the efficacy of such a practice has not been shown through clinical trials.[32] Micro-needling also has been advocated.

The action of PRP is dependent on the composition of growth factors retained in α-granules. Once activated by compounds such as thrombin and calcium chloride,[33] α-granules release their contents into the extracellular environment. These growth factors include PDGF-α, PDGF-β, and PDGF-$\alpha\beta$, TGF-β1 and TGF-β2, epithelial growth factor, and VEGF.[33,34] Application of these growth factors to dermal papilla cells has been shown to lead to the initiation and prolongation of the anagen growth phase in hair follicles.[15] It also has been suggested that the antiapoptotic effects of activated PRP are one of the major contributing factors stimulating hair growth. PRP-induced activation of antiapoptotic regulators such as Bcl-2 and Akt signaling prolongs dermal papilla cell survival during the hair cycle.[15] These findings show the biological plausibility of using PRP in treating androgenic alopecia.

The evidence available on the efficacy of PRP in treating androgenic alopecia suggests promising results for its use as an alternative to traditional treatments for hair loss (Figs 2, 3). With the undesirable side effects of finasteride and long-term commitment necessary for minoxidil application, a therapy that has minimal side effects and requires only periodic treatment sessions offers patients a sound alternative. Investigators have determined that PRP is primarily effective for mild to moderate hair loss (Hamilton-Norwood stage IV or lower),[26,31] although there is a need for more controlled and well-designed clinical trials with longer follow-up periods and larger samples. In addition, studies that evaluate the optimal dosing interval schedule would allow for oral and maxillofacial surgeons to use PRP more efficiently and effectively. However, given the preliminary results, investigators have begun to study different formulations for improved patient outcomes in the future. Such methods include the addition of CD34$^+$ cells, D/P MPs, or changing the composition of the PRP extract.[22,25,32] The continued refinement of protocols and discovery of novel formulations will continue the forward progress in this field.

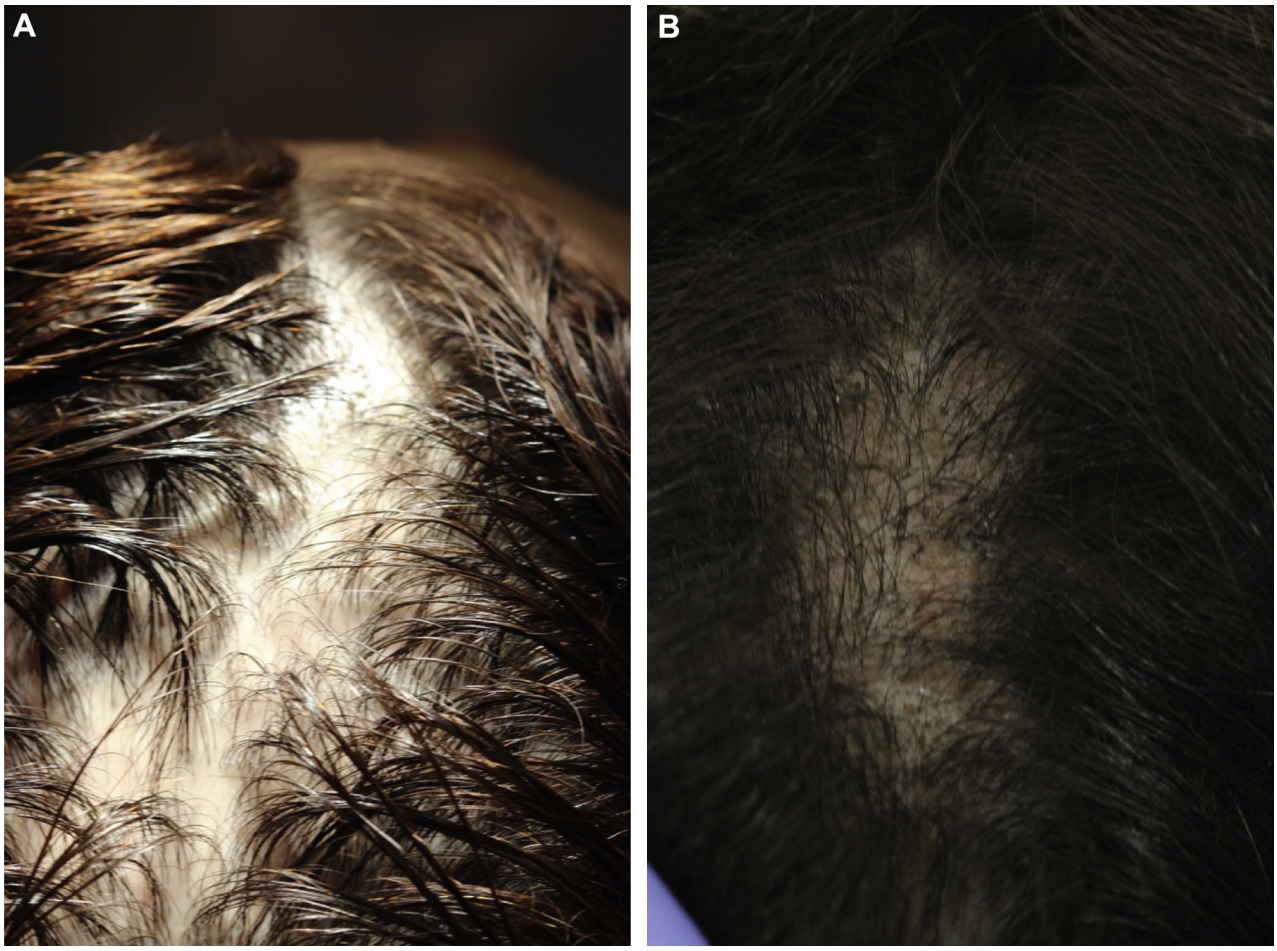

FIGURE 3. *A*, Patient presenting with androgenic alopecia. *B*, Patient after 4 platelet-rich plasma treatments at 4-week intervals. Note the marked increase in hair density and quality.

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The studies conducted thus far on the effects of PRP on androgenic alopecia overall have shown beneficial results. Given the low level of invasiveness, minimal side effects, and biological plausibility, the future investigation of PRP as a therapeutic agent will benefit men and women with androgenic alopecia and allow for oral and maxillofacial surgeons to adopt a uniform treatment protocol. More rigorous study designs, including larger samples, quantitative measurements of effect, and longer follow-up periods, are needed to solidify the utility of PRP for treating androgenic alopecia.

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