PLATELET-RICH PLASMA: A THERAPY FOR HAIR GROWTH

Gilbert Amgar and **Pierre Bouhanna** investigate the novel application of platelet-rich plasma preparations as a treatment for hair-loss

GILBERT AMGAR is Aesthetic Practitioner, France, and **PIERRE BOUHANNA** is Dermatologist and Expert in Hair Surgery, Paris, France.

email: gilbert.amgar@orange.fr

KEYWORDS
PRP, hair growth, growth factors, phototrichogram, androgenic alopecia, alopecia areata

ABSTRACT
Medical treatments for hair-loss, such as minoxidil lotion, finasteride 1mg oral, or cyproterone acetate oral, may impede the development of baldness. In recent years, however, it has also been found that platelet-rich plasma (PRP) can also be injected into the scalp to reverse hair miniaturisation. This article will explore three main points: how growth factors interfere with the hair cycle, a review of the literature pertaining to treatments for hair loss (particularly the use of PRP), and a proposal of guidelines for future treatment evaluations.

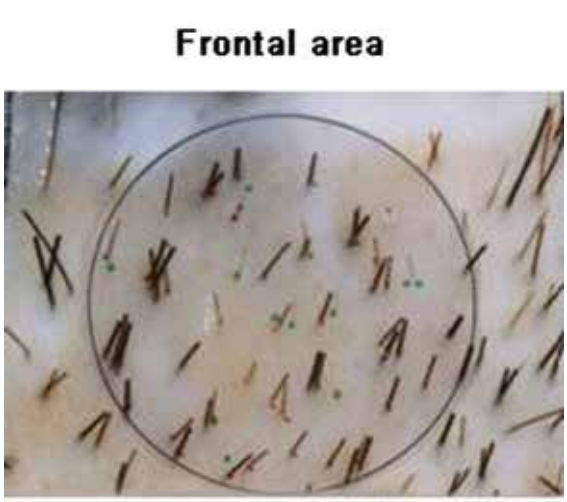
INTEREST IN THE REVERSAL OF baldness has been enhanced by a precise multifactorial classification of each individual to effectively reverse male and female androgenic alopecia (AGA) in selected patients[1]. Dihydrotestosterone (DHT) is the specific hormone responsible for male and female pattern baldness as a result of changes in its metabolism.

In normal hair loss, less than 100 hairs fall each day and are replaced by new, thick hair. In the evolution of male and female pattern baldness, the new hair is fine and thin (intermediate hair or miniaturised hair). Male and female baldness usually progresses in a definitive pattern. Medical treatments for hair loss, such as minoxidil lotion, finasteride 1mg oral, or cyproterone acetate oral, may impede the development of baldness. In recent years, however, it has also been found that platelet-rich plasma (PRP) can also be injected into the scalp to reverse hair miniaturisation.

A phototrichogram using a digital camera enables an objective measurement of hair-growth parameters[2], such as density, number of miniaturised and/or terminal hairs, and hair growth rate on a selected and tattooed area (*Figure 1*).

The most researched and publicised medical treatment available for male pattern baldness is 5% minoxidil lotion, and 2% minoxidil for female baldness. The first signs of improvement generally appear after 3 months of therapy. The side-effects of minoxidil are minimal, but include itching, eczema and hypertrichosis (the latter is more common in female patients).

For male baldness, finasteride taken orally and daily (1mg) works by inhibiting the 5α-reductase from forming DHT. The decreased DHT levels allow some



	0,64 cm²	1 cm²	%
Total hair count	60	94	
Hair < 40 µm	12	19	20
Hair > 40 µm	48	75	80

Figure 1 Phototrichogram evaluation of a frontal bald area before treatment. The goal is to increase the caliber of the miniaturised hair

intermediate follicles to enlarge and regrow normal terminal hairs. Side-effects may include decreased libido.

Cyproterone acetate (in Europe) can effectively block the increased levels of male hormones that cause hair-loss in some women. Spironolactone (in the US) appears to be a competitive inhibitor of DHT-receptor binding.

New interest in preventing hair-loss and baldness has been stimulated by cellular therapy with traumatising and then infusing PRP into the scalp, which normalises hair-loss after the first treatment, and reverses hair miniaturisation of male and female baldness after a second treatment.

Importance of growth factors

For a number of years, the expectations of physicians and patients in medical and surgical procedures were to improve tolerance, obtain satisfying results, and to make the treatment process easier to carry out. As a result, the use of PRP therapy began to take a prominent place in this context. The process consists of using autologous platelet extracts as a cell-repairing product. The revitalising qualities of the platelets are well known and have been used since at least 1970, in a variety of specialist areas, such as plastic surgery, rheumatology, dentistry and orthopaedics. Being an autologous product, tolerance is excellent and no serious adverse effects have been observed to date.

However, for use in hair-loss surgery, the challenge remains to evaluate the results using objective parameters. To assess the effect of PRP treatment on the skin, two studies have been published, one of which used biometric parameters (i.e. anisotropy, hydration, transepidermal water loss)[3] and the other using human histology[4]. Most clinical evaluations are based on subjective evaluations, patient satisfaction, and before and after picture evaluations. Those publications that offer objective parameters are based on biometrology or human histology. For this reason, future studies that investigate the use of PRP and hair-loss must consider objective parameters.

The process begins by taking a blood sample through an anticoagulated tube, with or without separating gel. The tubes are then centrifuged for approximately 5 minutes at 600 G (the speed and time will depend on the specific kit protocol). As a result of their density, the red cells sink in the tube, while at the upper part of the tube the plasma and platelets collect. PRP is usually referred to when a concentration of three-to-five-times normal standards has been collected.

Once injected into the dermal layer, the platelets are activated; they inflate and growth factors are released. The most important growth factors in terms of PRP for hair application are:

- Platelet-derived growth factor (PDGF)
- Vascular endothelial growth factor (VEGF)
- Epidermal growth factor (EGF)
- Insulin-like growth factor 1 (IGF-1)
- Fibroblast growth factor (FGF)
- Nerve growth factor (NGF).

PDGFs stimulate the growth of dermal mesenchyme. PDGF signals are involved in both epidermis-follicle interaction and the dermal mesenchyme interaction required for hair canal formation and the growth of dermal mesenchyme, respectively[5].

VEGF belongs to a family of powerful growth factors with action on mitotic cells and the endothelial cells of blood vessels, and increases vascular permeability. The hair follicle is an avascular structure, the growth of which depends on the vessels and capillaries that form the vascular plexus in the dermal papilla. Yano et al[6] identified VEGF as a significant mediator of hair follicle growth and cycling, providing the first direct evidence that improved follicle vascularisation promotes hair growth, as well as increasing follicle and hair size.

In a trial of 104 patients receiving hair transplants,

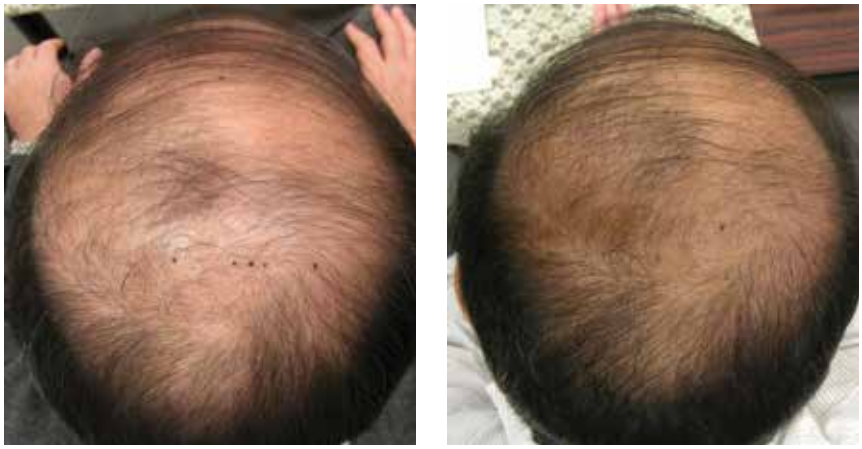


Figure 2 (A) Before and (B) 12 weeks after one session of platelet-rich plasma treatment

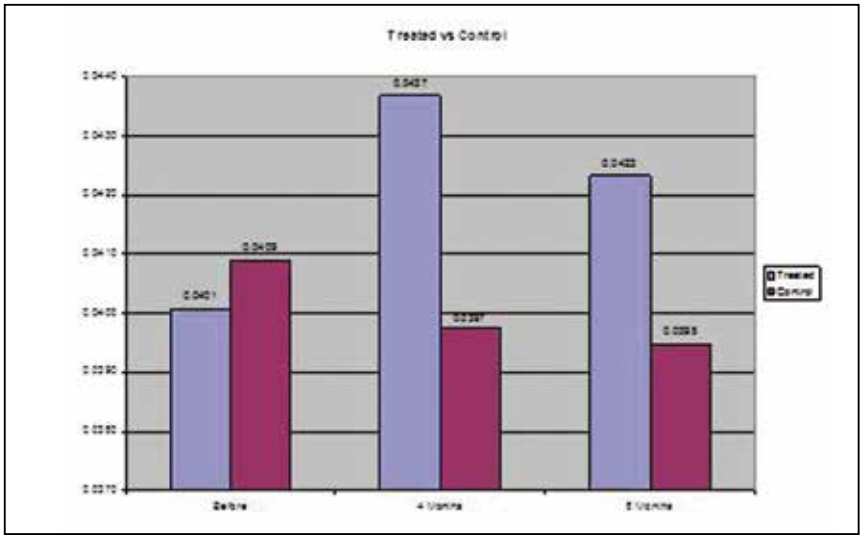


Figure 3 Variation in caliber

Rinaldi et al[7] have shown, by means of confocal microscopy, that the up-stimulation of VEGF through adenosine receptors induces significant changes in the average diameter of the scalp's perifollicular vessels compared with placebo, and an elongation of the transplanted follicles in the anagen phase. Further studies have shown that the up-stimulation of adenosine receptors contributes to the growth of hair through direct stimulation of the production of VEGF by the dermal papilla[8,9].

EGF stimulates mitosis on epithelial cells and fibroblasts, and improves the ratio of anagen. EGF inhibits the entry in the catagen phase, promoting the anagen phase[10]. EGF signals control the orientation and elongation of follicles, probably attributable to its effect on the proliferation of basal keratinocytes and of cells that comprise the outer root sheath of hair. High doses of EGF can induce regression of the follicle. As proposed by Kingston et al, EGF serves as a biologic switch for entering and leaving the anagen phase[11].

IGF slows down apoptosis. IGF-1 acts as a signal in mitotic different cell lines and protects cells apoptosis. IGF-1 regulates the expression of a powerful messenger cellular level, which is anti-apoptotic and capable of preventing the cell death[12].

FGF stimulates the proliferation and differentiation of keratinocytes and endothelial cells. It is over-expressed in then anagen phase and then less so from stage VI of anagen[13].

NGF strongly stimulates hair growth and slows down apoptosis. NGF has a modulating effect on the hair depending on the receptor with which it interacts: at the level of the outer root sheath, the complex NGF TrkA stimulates proliferation keratinocytes, while NGFj p75NTR promotes apoptosis, the regression of the follicle, and inhibits the growth of hair[12].

During anagen-catagen transition, increased neuroimmune communication leads to perifollicular neurogenic inflammation, apoptosis in hair follicles, and premature catagen development. A survey of the literature on key candidates in skin-stress responses reveals that not only noradrenaline, the prominent signaling molecule of the sympathetic stress response, but also NGF, act as stress mediators. This is certainly one of the best ways in which to explain the correlation between stress and hair-loss[14].

Literature review

With regard to evaluations of the use of PRP in androgenic alopecia, there are three main studies currently available. Sorbellini et al[15] carried out an *in vitro* study on 50 patients. Twelve follicles were taken from each patient, of which four follicles were placed in PRP, four in Ringer's solution, and four in a standard solution. The authors then measured mitotic activity. The results showed a significant increase of mitotic activity and a reduction in the apoptotic process in the PRP group.

The purpose of Takikawa et al's study[16] was to see whether there is a difference between simple PRP injection and PRP containing delteparine. Delteparine is a protein carrier for growth factors in PRP. The study was conducted with 26 volunteers with thin hair who received five local treatments—half with PRP plus delteparine and half with PRP alone—and evaluated for a period of 12 weeks (*Figure 2*). Experimental and control areas were photographed, digitally measured and biopsies taken for histology examination. Significant differences were seen in hair cross-sections, but not in hair density. The interesting point for the purposes of the present article is that PRP improved density by 16% at 12 weeks.

Greco and Brandt[17] found that traumatising and infusing growth factors into the scalp reversed miniaturisation over an 8-month period when compared with control. Ten hair samples were taken from each patient; five patients in the Control Group (CG) and five from the Treatment Group (TG). Hair diameter was measured using a Starrett micrometer. In the TG , 60 cc of blood was drawn and 10 cc PRP was processed. The CG also had 60 cc of blood drawn, but this was not processed. The scalp was first traumatised in both groups with a 1mm microneedling roller to initiate keratinocyte

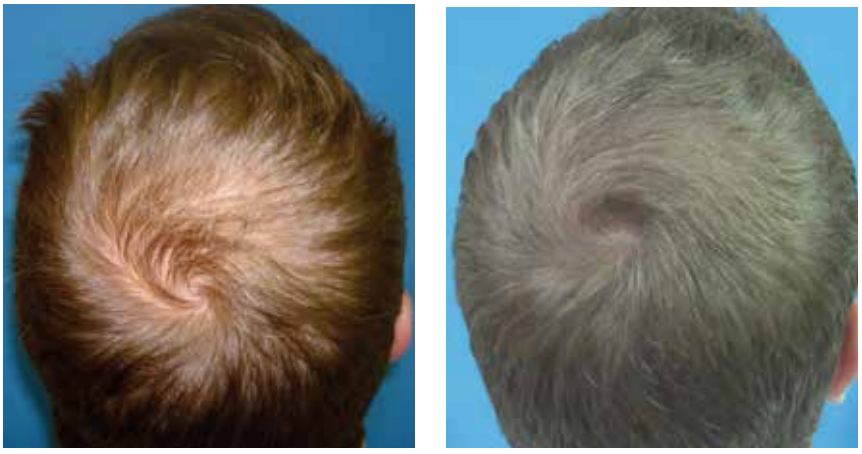


Figure 4 (A) Before and (B) 8 months after one session of platelet-rich plasma treatment

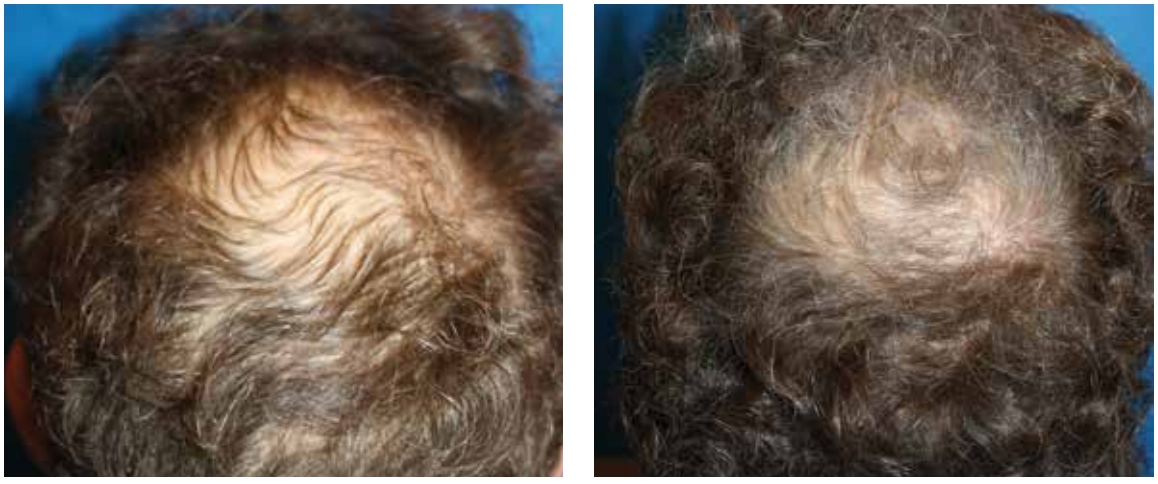


Figure 6 (A) Before and (B) 9 months after two platelet-rich plasma sessions

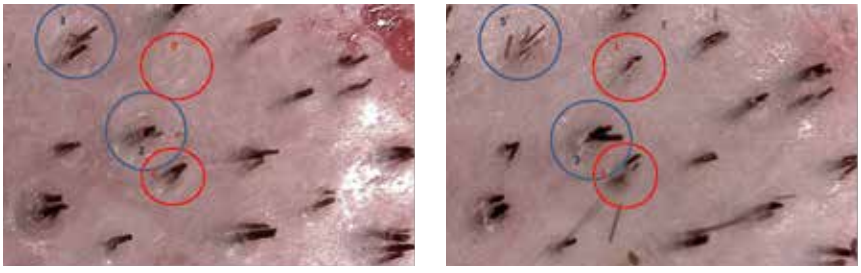


Figure 5 (A) Before and (B) 3 months after one session of platelet-rich plasma treatment

migration and wound healing. The TG was then injected with PRP. Normal saline was injected into the scalps of the CG in a similar fashion. Patients were evaluated and hair diameter measurements were taken using the micrometre at 4 months and 8 months post-treatment.

The CG demonstrated a 2.8% average decrease in hair shaft diameter at 4 months and 3.5% decrease at 8 months (this is the classical evolution of androgenic alopecia). An increase of 9.7% in average hair shaft diameter at 4 months and then 6.1% at 8 months in the TG was very significant (*Figures 3* and *4*).

For the authors' coming evaluation, minimal guidelines should be respected. The number of inclusion must be sufficient (more than 50), digital measurement must be proceeded with a tattoo mark (to be at exactly the same place), short cut and dyeing solution if necessary. The following parameters must also be determined: hair density, caliber and ratio (anagen:telogen). Photography must also be standardised. In the author's study, 60 patients (male and female) were included, who all received digital measurements (tattoo mark, hair density, caliber and ratio) and had standardised photographs taken. As with the Greco and Brandt study[17], 60cc of blood was drawn and 10cc of PRP processed using the MyCells kit. The scalp was first traumatised with a 1.5mm microneedling roller. Patients underwent two treatment sessions at intervals of 3 months. At 3 months an increase in hair density of 18.8% was observed, as well as a decrease in the average of caliber (-2%) as the new hairs are thin (*Figure 5*). At 9 months, this increased to 29% for density and 5% for caliber (*Figure 6*).

In this type of treatment, microneedling initiates the keratinocyte migration and the growth factors improve follicle vascularisation, stimulate mitosis, promote the anagen phase, and slow apoptosis. Compliance with these guidelines will allow the authors of the present article to move forward in the validation and evaluation of the PRP process for hair growth.

Male pattern baldness

The second treatment application of PRP is for improvements in male pattern baldness surgery. PRP can be used at different levels: to strengthen a poor donor area (stimulation will be at least 8–10 months before surgery), improve graft survival, and minimise the postoperative adverse effects in the donor site (reduce donor scarring) and recipient area (less inflammation).

The use of PRP in hair restoration surgery has demonstrated an increased yield when used as a graft storage medium. In this case, it is essential to activate the PRP with calcium chloride or thrombin. When PRP is injected directly in the dermal layer, it is traumatised and influences the production of adrenaline and calcium, which initiate the activation cascade. In this case, however, PRP is used as a storage medium, without any traumatisation, so it is necessary to 'manually' activate the PRP by using calcium chloride or thrombin. When bathed in activated PRP, the growth factors attach to the stem cells in the bulge area of the dissected follicular unit, thus increasing the yield of newly transplanted follicles. Uebel et al[18] observed a significant difference in the yield of follicular units when comparing the experimental with the control areas of the scalp. The areas treated with platelet plasma growth factors demonstrated a yield of 18.7 follicular units per cm^2, while the control areas yielded 16.4 follicular units per cm^2, an increase in follicular density of 15.1%. Among patients who used the experimental protocol, some experienced only 3%, and others experienced a 52% increase in density.

Furthermore, injecting PRP into the recipient site increases vascularisation to the transplanted follicular units, thus increasing yield and the density of non-transplanted hair[19,20].

Alopecia areata

Key points

- The action of different growth factors on the hair follicle is quite complicated, but now well understood
- Many publications confirm the efficacy of platelet-rich plasma (PRP) in hair disorders
- In hair surgery and transplantation, PRP can improve the donor area and increase graft survival
- PRP could be an alternative treatment for androgenic alopecia

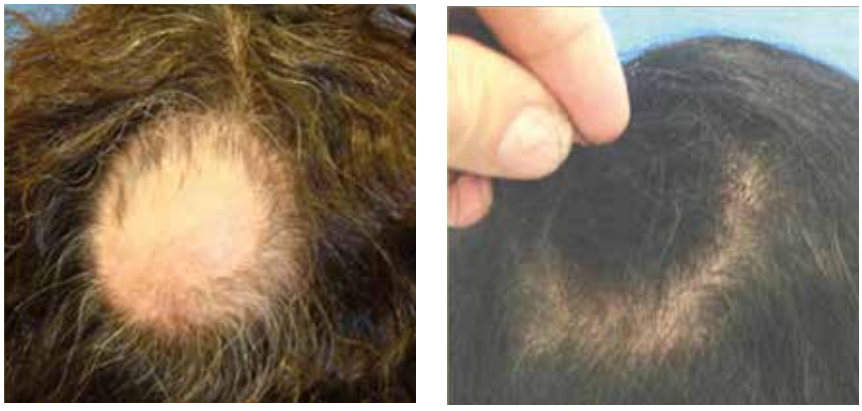


Figure 7 (A) Before and (B) 12 months after one platelet-rich plasma treatment session for alopecia areata

The third application field for PRP concerns alopecia areata. For this indication, evaluation is particularly difficult; indeed, the evolution is naturally capricious. It must therefore be concluded that the case has been developing for more than 1 year without success. Also, digital measurements are delicate as the areas of regrowth are irregular. Macrophotograhy is the best method evaluation in this disease. For now, only case studies rather than standardised evaluations are available. Nevertheless, obvious improvements can be seen (*Figure 7*).

Discussion

In the authors' evaluation, other than objective measurements, the subjective appreciation of patients is evaluated. There is a large chasm between objective measures and patient feelings; for example, the patient may feel satisfied with a small increase (10%), but dissatisfied with a significant result (40%).

With regard to the standardisation of photography, many factors must considered, including colour, length, hairdressing, and lighting. For these reasons, to get suitable results, an objective parameter is essential (i.e. the phototrichogram). Further guidelines for effective analysis are those tools which allow the authors to obtain the best phototrichogram possible (e.g. tattoo points, short cut, and dyeing solution). Most authors also agree that 50 patients is a minimum requirement to obtain efficient statistic results.

Once the efficacy of PRP for hair-loss is demonstrated, it would be interesting to compare the impact of different factors in future studies. For example, with or without dermaroller, with or without activation, or different volumes, as well as other hair pathologies, such as alopecia areata and telogen effluvium.

Those explorations are very important regarding the poor panel of treatments at our disposal almost for some of them which are disputed for long last using. PRP protocol can be an alternative solution.

Conclusions

The use of PRP for applications in hair-loss is far from being minor. In the future, the authors hope to improve the protocol and show that PRP also has a preventive effect on hair disorders. This method will not replace the hair graft, but may be useful to delay such treatment and provide betterresults. The authors hope that this article will encourage clinicians to use objective measurements for their evaluation, respecting the standard guidelines.

Declaration of interest *none*

▶ *Figures 1, 5, and 6 ©Amgar and Bouhanna*
▶ *Figure 2 Reproduced with kind permission of Dr Kiyozawa*
▶ *Figures 3, 4, 7 ©Greco*

▶ *The Hair PRP study tubes used for the authors' study were produced by MyCells.*

References

1. Bouhanna P. Multifactorial classification of male and female androgenetic alopecia. Dermatol Surg 2000; 26(6): 555–61
2. Bouhanna P. The phototrichogram and a macrophotographic study of the scalp. Bioengineering and the Skin 1985; 1(3): 265
3. Amgar G, Bonnet C, Butnaru A, Herault-Bardin F. Using objective criteria to evaluate cosmetic effects of platelet rich plasma. PRIME 2011; 1(6): 31–41
4. Sclafani AP, McCormick SA. Induction of dermal collagenesis, angiogenesis, and adipogenesis in human skin by injection of platelet-rich fibrin matrix. Arch Facial Plast Surg 2012; 14(2): 132–6
5. Takakura N, Yoshida H, Kunisada T, Nishikawa S, Nishikawa SI. Involvement of platelet-derived growth factor receptor-alpha in hair canal formation. J Invest Dermatol 1996; 107(5): 770–7
6. Yano K, Brown LF, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. J Clin Invest 2001; 107(4): 409–17
7. Rinaldi F, Sorbellini E, Bezzola P. The role of up-stimulation of growth factors in hair transplantation: improve the revascularization of transplanted hair growth mediated by angiogenesis. Forum 2007; 2
8. Sharov A, Tobin DJ, Sharova TJ, Atoyon R, Botchkarev VA. Changes in different melanocyte populations during hair follicle involution. J Invest Dermatol 2005; 125(6): 1259–67
9. Li M, Marubayashi A, Nakaya Y, Fukui K, Arase S. Minoxidil-induced hair growth is mediated by adenosine in cultured dermal papilla cells: possible involvement of sulfonylurea receptor 2B as a target of minoxidil. J Invest Dermatol 2001; 117(6): 1594–600
10. Mak KK, Kingston SY. Epidermal growth factor as a biologic switch in hair growth cycle. J Biol Chem 2003; 278(28): 26120–6
11. Rosenquist TA, Martin GR. Fibroblast growth factor signalling in the hair growth cycle: expression of the fibroblast-growth factor receptor and ligand genes in the murine hair follicle. Dev Dyn 1996; 205(4): 379–86
12. Weger N, Schlake T. Igf-I signalling controls the hair growth cycle and the differentiation of hair shafts. J Invest Dermatol 2005; 125(5): 873–82
13. Mak KK, Kingston SY. A transgenic mouse model to study the role of epidermal growthfactor (EGF) in hair and skin development. Hong Kong: University of Hong Kong, 2002. http://tinyurl.com/ncubclj (accessed 22 May 2013)
14. Peters EMJ, Paus R, Klapp BF, Arck PC. L6 neuroimmunological hair growth control is stress-sensitive: an old paradigm revisited. http://tinyurl.com/ncltc3s (accessed 22 May 2013)
15. Sorbellini E. Trink A, Rinaldi F. Experimental clinical assessment of the use of platelet-rich plasma in dermatology and rationale for its use in the treatment of non-scarring alopecia. Presented at the 35th La Medicina Estetica 4 October 2011
16. Takikawa M, Nakamura S, Nakamura S et al. Enhanced effect of platelet-rich plasma containing a new carrier on hair growth. Dermatol Surg 2011; 37(12): 1721–9
17. Greco J, Brandt R. The effects of autologus platelet rich plasma and various growth factors on non-transplanted miniaturized hair. Hair Transplant Forum International 2009; 18(2): 49–50
18. Uebel CO, da Silva JB, Cantarelli D, Martins P. The role of platelet plasma growth factors in male pattern baldness surgery. Plast Reconstr Surg 2006; 118(6): 1458–66
19. Greco J, Brandt R. Our Experience Utilizing Autologous Platelet Rich Plasma in all Phases of Hair Transplant Surgery. Hair Transplant Forum International 2007; 17(4): 131–2
20. Reese R. A single-blinded, randomized controlled study of the use of autologous platelet rich plasma PRP as a medium to reduce scalp hair transplant adverse effects. Hair Transplant Forum International 2008; 18(2): 51–2