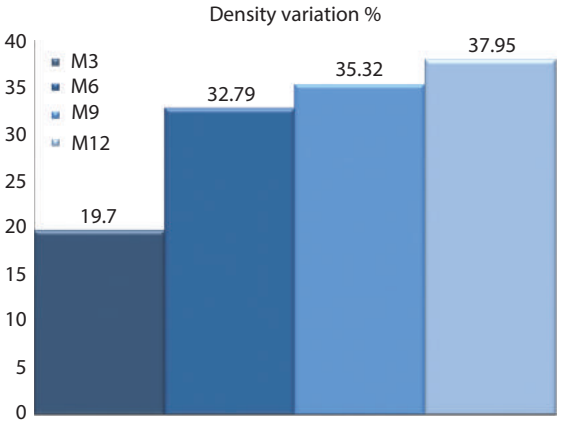

Figure 17.6 **Twelve months density variation.**

density variation reached 32.79%, and it was 38% at 12 months. The other way to evaluate the yield of this method is to report the result to a base 100 (as if every patient had 100 hairs/cm² at day 0). The yield is 23%.The mean caliber did not show variation, but if we separate the male and female population we observe an improvement for women. We can also observe that this curve is not linear but logarithmic. That caused the authors to think that they did not create new follicles but just saved from apoptosis some follicles that were unable to provide a hair. Once the capability is recovered, an annual session can maintain it.

ALOPECIA AREATA

Alopecia areata (AA) is the most common condition to cause inflammation-induced hair loss, having a calculated lifetime risk of 2% (Figures 17.8 and 17.9).[22] It is characterized by well-demarcated patches of hair loss, which can progress to complete loss of hair from the scalp (alopecia totalis) or from the whole body in severe cases (alopecia universalis).[23] Most patients are relatively young, and the disease burden is commonly substantial, leading to overwhelming effects on the patient's quality

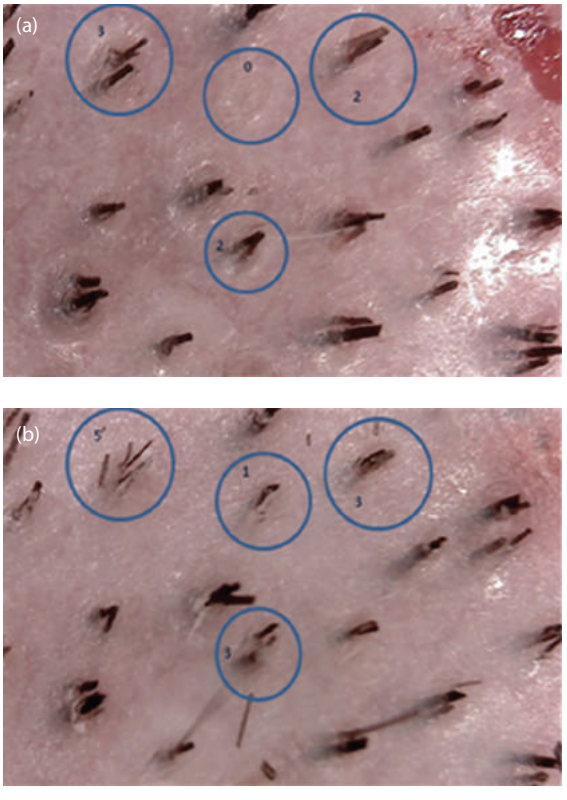

Figure 17.7 **(a) Before and (b) 3 months after PRP session. (Courtesy of Dr. Amgar and Dr. Bouhanna.)**

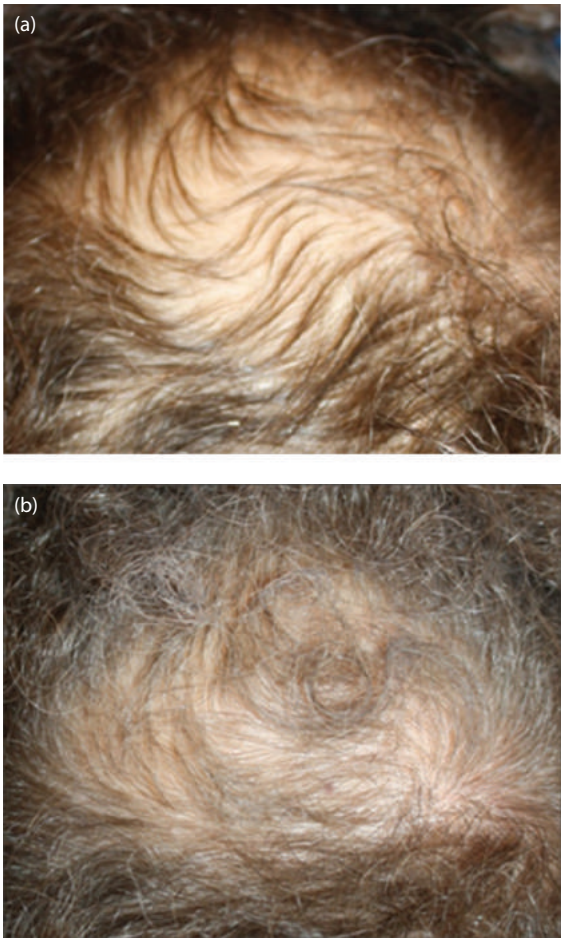

Figure 17.8 **Male androgenic alopecia. (a) Before and (b) 9 months after two PRP sessions. (Courtesy of Dr. Amgar and Dr. Bouhanna.)**

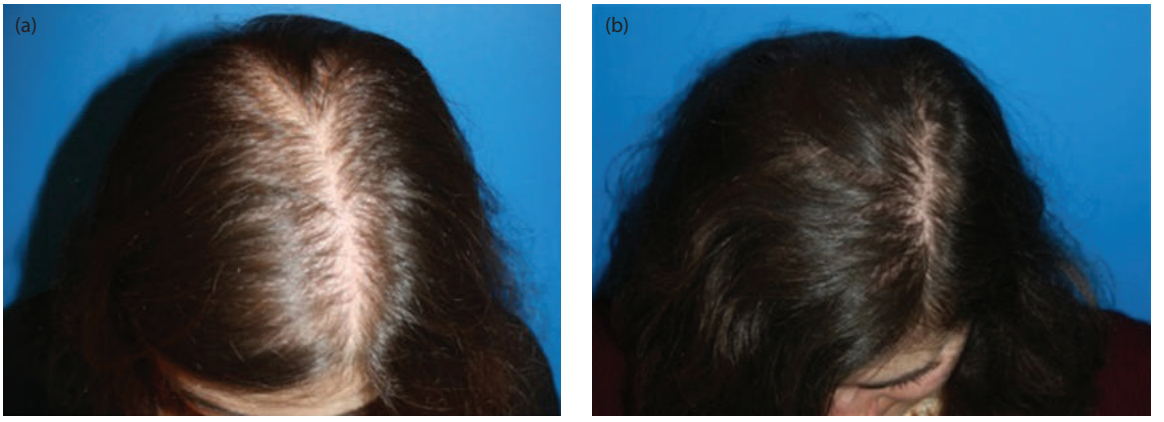

Figure 17.9 **Female androgenic alopecia. (a) Before and (b) 9 months after two PRP sessions. (Courtesy of Dr. Amgar and Dr. Bouhanna.)**

of life and self-esteem. It is considered an organ-specific autoimmune disease, stemming from loss of the hair follicle's (HF) immune privilege; therefore, therapies are mostly immunosuppressive. Nevertheless, treatment is still a challenge in AA, and no treatment is either curative or preventive.[24] Finding new therapies for this condition, and improving effectiveness of existing therapies, are therefore of utmost importance.

Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma.[16] It has been investigated in several disciplines in medicine for its role in wound healing, especially orthopedics and dentistry.[25] Recently, it has also been found to be beneficial in dermatology, for example in acne scarring, wound healing, and fat transplantation. It has also been shown to promote hair survival and growth, both in vitro and in vivo.[16,26]

Rinaldi et al. conducted a randomized, double-blinded, placebo and active-controlled, half-head, parallel group study to evaluate the effects of PRP on AA in vivo and showed that PRP administration leads to major improvements in AA lesions (Table 17.2), with 60% of patients achieving complete remission at study termination. Currently, triamcinolone acetonide[27] is considered as the treatment of choice for patch-stage AA.[28]

The PRP is known to contain more than 20 different growth factors, which are important in promoting cell proliferation and differentiationn.[19] These properties are thought to lead to its beneficial effects on acne scarring[29] and wound healing.[30] More recently, the role of PRP in promoting hair growth has also been investigated. Uebel et al. have shown that storing hair grafts in PRP can enhance graft survival, improve hair density, and stimulate growth of transplanted follicular units.[26] Still, the mechanisms by which PRP exerts its effects on HFs are still obscure. A recent study has shown in vitro that PRP increases the proliferation of dermal papillae cells and activates the signaling pathways' extracellular signal-regulated-kinase and Akt.[25] Additionally, fibroblast growth factor-7 and beta catenin, which are both stimulators of HF growth, were stimulated after PRP administration. Our study gives further support to the growth-promoting effect of PRP in hair, by providing evidence that levels of Ki-67, a marker for cell proliferation, are increased after PRP administration in humans.

In addition to its proliferation-inducing effects, PRP is a potent anti-inflammatory agent, which can suppress cytokine release and thereby limit local tissue inflammation.[31] Since AA is characterized by an extensive inflammatory infiltrate, responsible for secretion of a variety of inflammatory cytokines, it is probable that the anti-inflammatory effects of PRP may be of great benefit in this condition.

Taken together, this study suggests PRP as a new treatment modality for AA, being a safe and a more efficient alternative for TrA, the current treatment of choice for AA. However, further controlled and randomized studies are needed to validate our findings in a larger cohort of patients.

TRANSPLANTATION SURGERY

The primary use for PRP in hair restoration surgery demonstrated an increased follicular yield when utilized as a graft storage medium by Uebel, and similar studies in hair transplant surgery by Perez-Meza demonstrated the positive use of autologous growth factors for wound healing and hair growth.[32–34] In 2007, Greco and Brandt suggested expanding the use of PRP in all aspects of hair restoration surgery.[35]

THE ROLE OF GROWTH FACTORS AND HAIR

Growth factors are present in the bulge area, where stem cells are found, and they interact with cells of the matrix, thus activating the proliferative phase of the hair. Stem

Table 17.2 Evaluation Parameters of AA Patches after Treatment with Placebo, TrA or PRP

	Placebo		TrA		PRP	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
			SALT score			
T1	4.87	4.93	3.27	13.2$^{***\#\#\#}$	4.33	25.6$^{***\#\#\#\dagger\dagger\dagger}$
T2	6.2	5.33	5.8	18.8$^{***\#\#\#}$	5.73	27.6$^{***\#\#\#\dagger\dagger\dagger}$
T3	5.93	7.27	5.07	23.8$^{***\#\#\#}$	6.13	40.73$^{***\#\#\#\dagger\dagger\dagger}$
			Dermoscopic evaluation			
T0	2.67	2.67	2.6	2.6	2.67	2.6
T1	2.67	2.73	2.53	1.53$^{***\#\#\#}$	2.8	1.4$^{***\#\#\#\dagger\dagger\dagger}$
T2	2.13	2.6	2.67	1.27$^{***\#\#\#}$	2.6	0.8$^{***\#\#\#\dagger\dagger\dagger}$
T3	2.6	2.47	2.07	1.2$^{***\#\#}$	2.4	0.53$^{***\#\#\#\dagger\dagger\dagger}$
			Burning/itching sensation			
T0	2.6	2.6	2.67	2.8	2.73	2.73
T1	2.33	2.67	2.47	1.07$^{***\#\#\#}$	2.47	0.53$^{***\#\#\#\dagger\dagger\dagger}$
T2	2.33	2.53	2.53	0.4$^{***\#\#\#}$	2.6	0.07$^{***\#\#\#\dagger\dagger\dagger}$
T3	2.4	2.6	2.67	0.2$^{***\#\#\#}$	2.27	0.07$^{***\#\#\#\dagger\dagger\dagger}$
			Ki-67 levels			
T0	13.15	13.55	13.65	13.72	13.21	12.75
T1	15.53	19.84	13.72	32.37$^{\#\#}$	12.94	50.06$^{***\#\#\#}$
T2	17.61	19.81	13.81	35.59$^{\#\#}$	12.75	55.79$^{***\#\#\#\dagger}$
T3	20.68	21.83	12.23	37.39$^{\#\#}$	12.88	69.09$^{***\#\#\#\dagger}$

Note: Evaluation scores at four time points: T0 = beginning of study, T1 = 2 months, T2 = 6 months, T3 = 1 year. N = 15 for each treatment modality.
Student t test, $^{***}p < 0.001$ when compared to placebo; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ when compared to untreated side of the scalp; $^{\dagger}p < 0.05$, $^{\dagger\dagger\dagger}p < 0.001$ when compared to TrA treatment.

cells are more primitive and of ectodermal origin; they give origin to the epidermal cells and the sebaceous glands. Cells of the dermal papilla, which are found at the capillary base, are of mesenchymal origin. Both cells need each other, and when they interact through the action of various growth factors, they will give rise to the future follicular unit.

The main function of platelet-derived growth factor is to stimulate cell replication (mitogenesis) of healing-capable stem cells. It also stimulates cell replication of endothelial cells. This will cause budding of new capillaries into the wound (angiogenesis), a fundamental part of all wound healing. In addition, PDGF seems to promote the migration of perivascular healing-capable cells into a wound and to modulate the effects of other growth factors.

Numerous studies and practical applications have demonstrated how growth factors are essential for regulating the cellular events involved in wound healing by attracting cells to the wound, stimulating proliferation, and significantly influencing matrix deposition.[36]

The TGF-beta is extremely important because it affects most aspects of tissue wound repair, namely, initiation and termination, and promotes differentiation and proliferation.[37] The PDGF improves dermal regeneration, acts locally to promote protein and collagen synthesis, causes endothelial migration or angiogenesis,[38] and induces the expression of TGF-beta.[39]

METHOD OF USE IN HAIR RESTORATION SURGERY

Phase I: Harvesting

PRP in donor site strip harvesting and follicular unit transplantation FUT

After the subcuticular layer is closed with 3 Monocryl, PRP gel is injected into the wound edges from end to end. The second layer is approximated with a running 3 Prolene suture, and PRP is then injected back into the wound (Figure 17.10).

PRP in follicular unit extraction follicular unit transplantation FUE

Since individual follicular units are extracted in the FUE procedure, PRP is injected into the donor site before extraction to promote hemostasis. After all follicular units are extracted a platelet gel is then covered over the extraction sites and a Sylon dressing is applied for 24

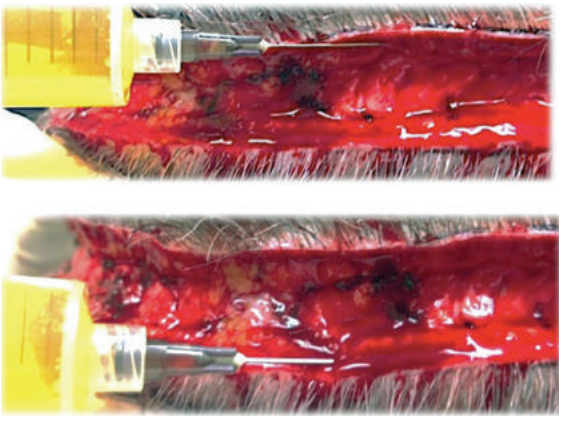

Figure 17.10 **Injections of PRP at both skin edges.**

hours with a bandage to promote more rapid healing of the extraction sites (Figures 17.11 and 17.12).

Phase II: Grafts cultured in PRP

After the follicular units are dissected, they are cultured and bathed in PRP prior to implantation so the growth factors can attach to the stem cells in the bulge region of the dissected follicular units[33] (Figure 17.13).

While dissection is ongoing and the graft design pattern is completed, the PRP is then injected into the recipient scalp area to maximize the effects of the growth factors on hair (Figure 17.14).

The growth factors in PRP provide an enriched environment to promote angiogenesis. In 2001, Yano identified VEGF as a major mediator of hair follicle growth and cycling, providing the first direct evidence that the improved follicle vascularization promotes hair growth and increases follicle and hair size[11] (Figure 17.15).

The latest studies conclude that autologous growth factors demonstrate a considerable effect on the time of hair follicle formation and the yield of hair follicle reconstitution, and higher levels of growth factors were more effective at 15% versus lower levels at 2% or control at 0%.[40]

Injecting PRP into the recipient area (Figure 17.16), may have other advantages for the nontransplanted hairs. This patient had hair transplantation in the frontal half of his head and his crown was injected with 10 mL of 15% PRP and no hair transplantation was done. Notice the miniaturized hair in the crown at 9 months after PRP injections.

In the final analysis, this author's experience utilizing PRP in all phases of hair restoration surgery the past 7 years has been positive. Autologous growth factors in PRP enhance the procedure by (1) decreasing scaring and promote preservation of the donor site, (2) increase the yield of grafts, (3) and promote hair follicle reconstitution.[40]

STEM CELLS

In recent years, adult human hair follicle neogenesis has been an important target in regenerative medicine, both for biological research and clinical interest (Table 17.3). Starting from the studies of the inductive capacity of the intact dermal papilla to produce new follicle formation in recipient human skin, much research has been developed to find other biological sources for hair follicle neogenesis.[41,42]

For this reason, stem cells have been studied for their therapeutic potential in many medical fields, and in hair treatment. Stem cells and regenerative medicine applications evolved rapidly during the last years, especially thanks to the use of adipose tissue-derived stromal cells (SVF and ADSC).

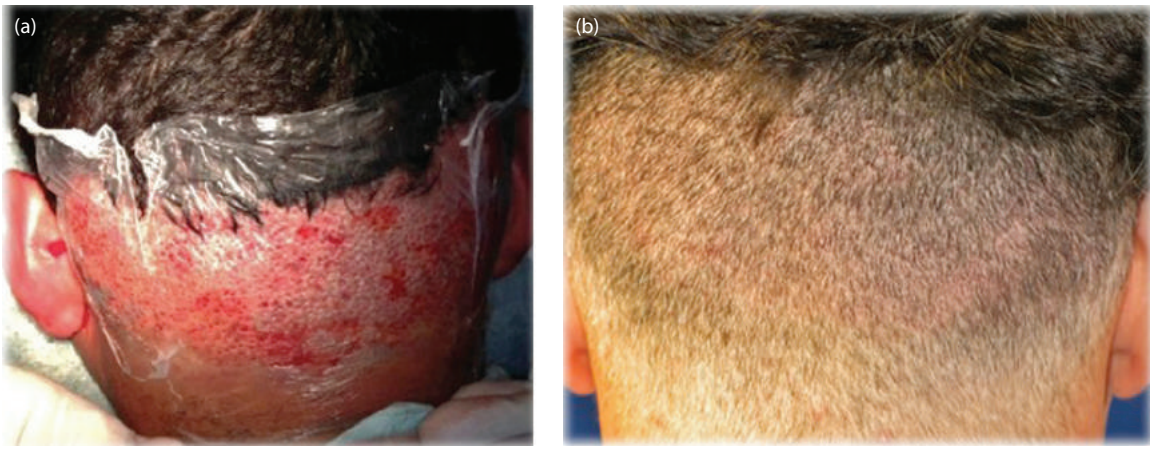

Figure 17.11 **PRP/Sylon dressing applied (a) post-op and at (b) day 7.**

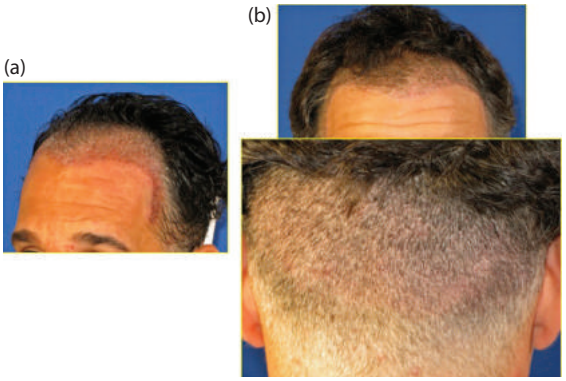

Figure 17.12 **PRP-FUE patient at 1 week. (a) Recipient area and (b) donor area.**

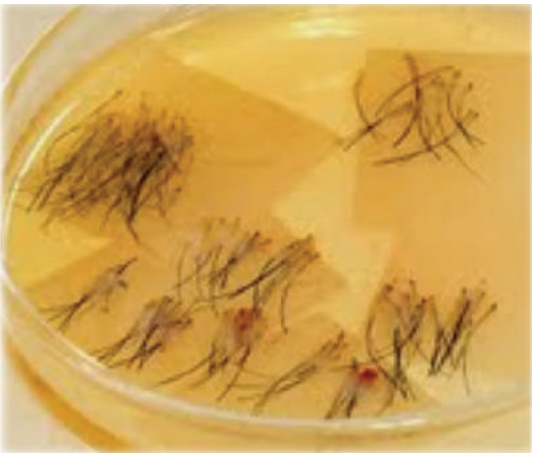

Figure 17.13 **Follicular units cultured in PRP.**

Stem cells are undifferentiated biological cells that can differentiate into specialized cells that can undergo mitosis to produce more stem cells. Stem cells can be divided into two main groups[43]:

- Embryonic stem cells, derived from the epiblast tissue of the inner cell mass of blastocyst (early stage embryo, 4–5 days old in humans, consisting of 50–150 cells).[44]
- Adult stem cells, derived from various tissues of child as well as adult organisms.

Embryonic stem cells are pluripotent and give rise during development to all cell types of the three primary germ layers: ectoderm (nervous system and skin), endoderm, and mesoderm.

At the moment there are no approved medical treatments using embryonic stem cells.

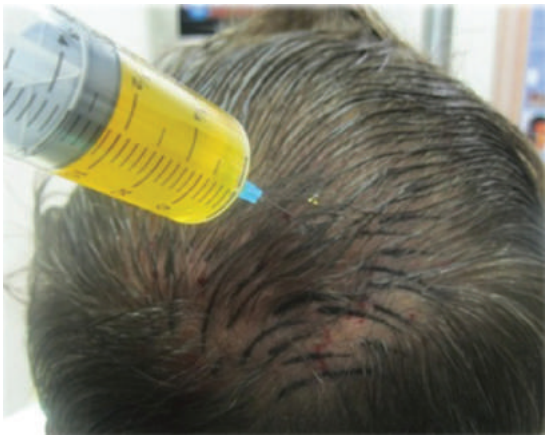

Figure 17.14 **PRP injected into the recipient's scalp.**

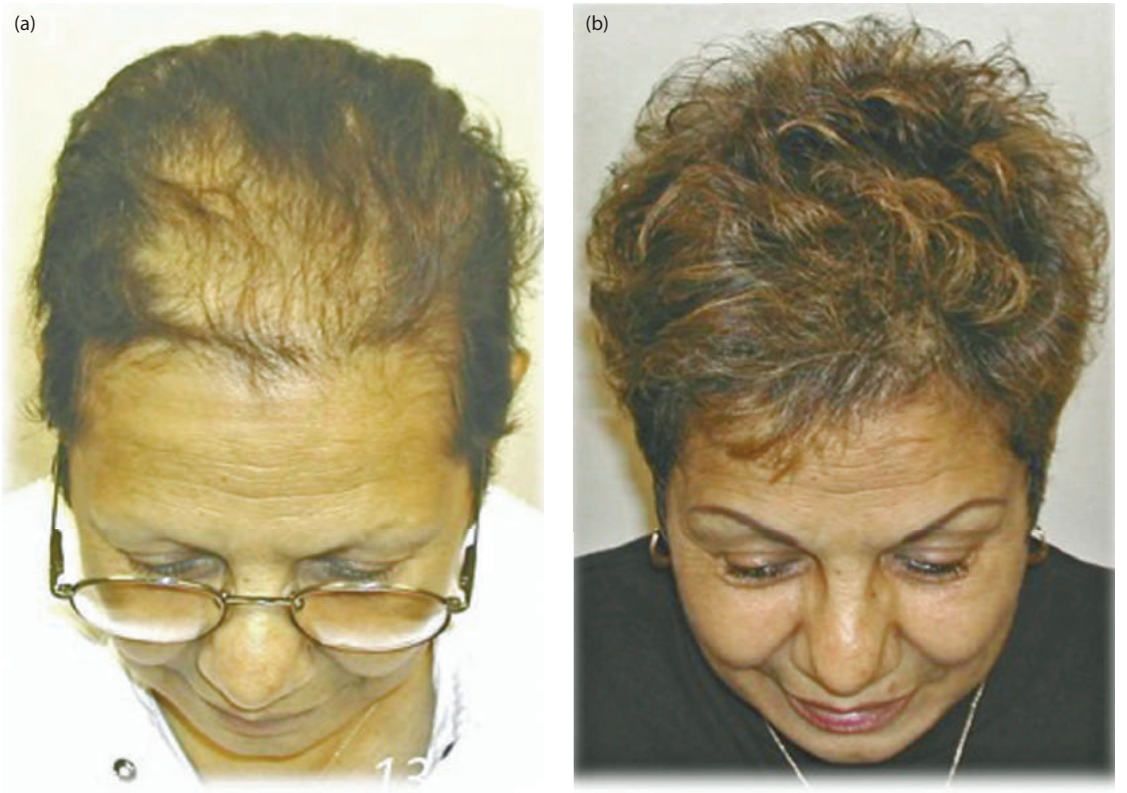

Figure 17.15 **PRP-Surgical combination. Result of PRP hair transplantation at 9 months. This Ludwig III female patient did not have a dense hair character and this is her result at 9 months. (a) Before and (b) after.**

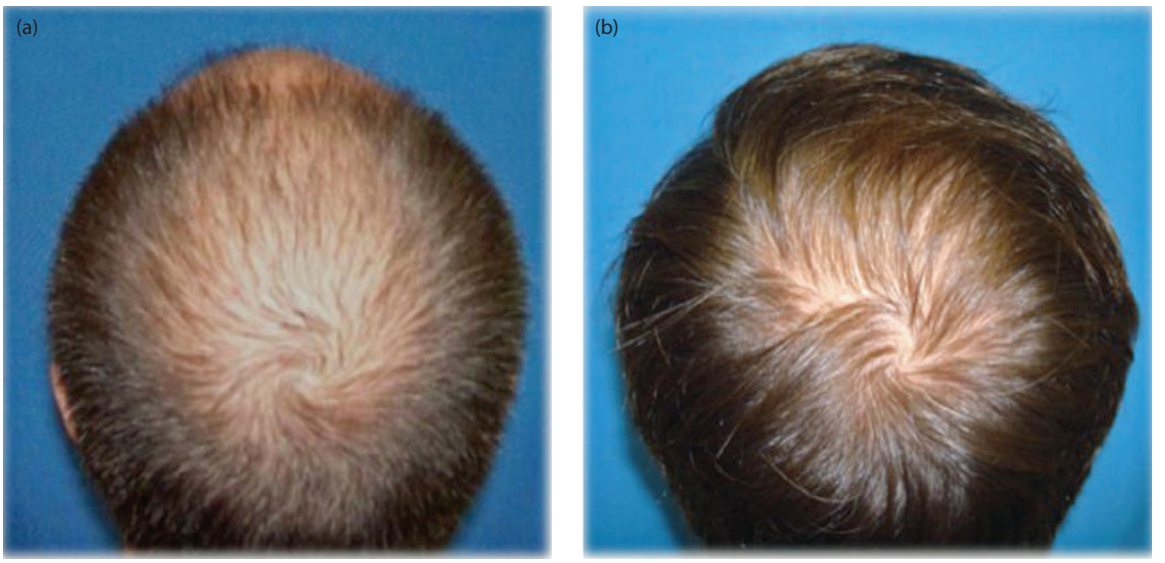

Figure 17.16 **Male androgenic alopecia. (a) Before and (b) after 9 months PRP injections in posterior crown.**

Table 17.3 Stem Cells Properties

Self-Renew: Several Cell Mitosis, Maintaining the Undifferentiated State	Asymmetric Replication
Potency: capacity to differentiate into specialized cell types	Stochastic differentiation
	Totipotent SC
	Pluripotent SC
	Multipotent SC
	Oligopotent SC
	Unipotent SC

Source: Adapted from Pittenger MF et al. *Science*. 1999;284 (5411):143–147.

Fetal stem cells located in the organs of the fetus are referred to as fetal stem cells.

Adult stem cells can be found in children and adults and can be pluripotent and multipotent stem cells[45,46]:

- Pluripotent stem cells in humans are small in number and can be found in umbilical cord blood, amniotic fluid, and bone marrow.
- The typical adult stem cells, present in many tissues, are multipotent (lineage restricted), and they only can originate the same differentiated cell types from the tissue of origin. Many adult stem cells have been identifi d:
  - Hematopoietic stem cells, differentiate into all mature blood cells.
  - Neural stem cells, differentiate into neurons, astrocytes, and oligodendrocytes.
  - Mesenchymal stem cells differentiate into fibroblasts, adipocytes, osteoblasts, chondrocytes, and skeletal muscle cells.[47]

Many other stem cells have been identified, although they are not yet well characterized.

Adult stem cells in research and therapy have been used for many years, especially in the treatment of leukemia and related bone cancers and blood cancers through bone marrow transplants.[48]

Recently, autologous stem cell transplantation has been proven to be effective and to have broad prospects in regenerative medicine.[49]

Many studies have shown that adipose-derived stem cells (ADSCs) have surface markers and gene profiling similar to bone marrow-derived mesenchymal stem cells (BMSCs) (the most effective mesenchymal stem cells). ADSCs have their own capacities of multidirectional differentiation, weak immunogenicity, lack of morbidity at the donor site, contrary to BMSCs.[49]

It has been proven that ADSCs can enhance wound healing by promoting collagen synthesis and the migration of dermal fibroblasts.[50,51] ADSC transplantation induces neovascularization in the ischemic myocardium, and the skin flaps by paracrine vasculogenesis promoting growth factors.[52] Histologic examination of skin in animals has shown that ADSCs transplantation increased the number of blood vessels to support sufficient oxygen and nutriments to the newly formed tissue.[53]

Because of its abundance and its easy sampling, performing a small liposuction from the lower abdomen or thighs, adipose tissue is a rich source of ADSC. It represents a significant alternative to bone marrow mesenchymal stem cells, even though the frequency of ADSC is higher in adipose tissue than that of mesenchymal stem cells in bone marrow.

Therefore, ADSCs have great potential for accelerating skin growth. In adipose tissue, ADSCs are localized in the periendothelial layer of the blood vessel. After collagenase digestion, 1×10^5, ADSCs could be harvested from a human lipoaspiration of 20 mL of fat.[54]

Then, via plastic adhesion, the cells could be expanded to purification. By the later stages of culture, 90% of the adherent cells assume a more homogeneous profile with consistently high levels of stromal markers. It has been shown that ADSCs secrete VEGF, FGFb, EGF, hepatocyte growth factor, transforming growth factor, and KGF. Among these factors, VEGF and FGFb are the most important factors in angiogenesis. After FGFb stimulates angiogenesis, VEGF regulates the proliferation and inhibits the apoptosis of endothelial cells.[55–57]

From these data, it has been rational to try to use ADSCs and SVF in hair treatment.

Adipose-derived stem cells (ADSCs), or sometimes adipose-derived stromal cells, must be distinguished from the crude stromal vascular fraction (SVF). After the extraction of adipose tissue by lipoaspiration from the body, fat must be treated by collagenase digestion to obtain crude SVF.[49]

The SVF is highly heterogenous and contains many cell types, including mature adipocytes, a mixture of endothelial cells, smooth muscle cells, fibroblasts, mast cells, pericytes, a reservoir of immature stromal cells, and hematopoietic cells (up to 20% of total fraction). The SVF is a complex fraction that contains ADSCs (5%–10% more or less) but cannot be defined as a stem cell or mesenchymal stem cell fraction. The ADSCs should be named only the fraction containing the isolated and expanded ADSCs.[49]

Once collected, fat tissue is processed to isolate the SVF using different medical devices able to separate ADSCs from adipose tissue particles (via enzymatic digestion or via mechanical separation). The SVF must be immediately injected in the recipient area[58] (Figure 17.17).

An adipose tissue sample obtained by liposuction is processed to obtain purified ADSCs, and then banked for subsequent cellular expansions in order to obtain a large number of cells stored for further treatments.[59]

Today ADSC can be applied in treatments for which fat transplantations already occur, giving results 10 times

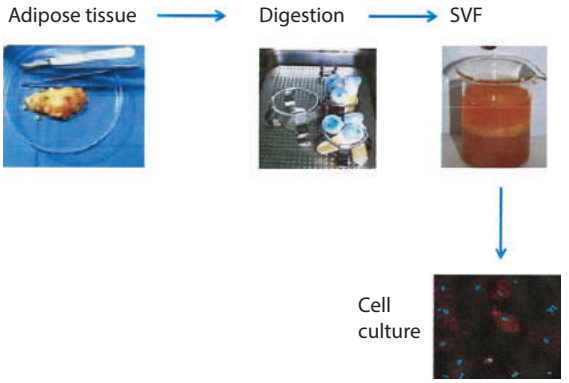

Figure 17.17 **Schema: fat tissue is processed to isolate the SVF.**

higher in aging, burns, scars, breast augmentation, wound healing, wrinkle correction, and breast reconstruction.[60]

The ADSC demonstrated strong angiogenic features in ischemic hind limb, myocardial infarction, and wound healing. In this field they seem to be more efficient than their bone marrow counterpart; they also have the ability to modulate immune and inflammatory functions, probably mediated via paracrine activity.

Research on Crohn disease reported that expanded ADSC are more efficient than the freshly prepared in controlling inflammation and improving healing processes, and the strong immunosuppressive effects of ADSC lead to a planned trial in multiple sclerosis.[49]

There is quite a rich literature on the use of ADSC for soft tissue augmentation in plastic and reconstructive surgery, but no reported experience in the treatment of hair diseases, to our knowledge.

Our personal data (not yet published) include different clinical trials on patients suffering from androgenic alopecia (Table 17.4) and alopecia areata.

In our experience in androgenic alopecia, using SVF we had three very good/good results (improvement of hair shaft diameter, improvement of anagen/telogen ratio, new hair growth) on 15 patients and 12 poor or no results. With this technique we had two patients with important side effects: in one case (Figure 17.18) we had an infectious and necrotic reaction with temporary loss of hair in the area. Infection resolved after antibiotic therapy in 14 days, and original hair regrowth occurred after 3 months.

Table 17.4 Androgenic Alopecia

	SVF	ADSCs
# Patients, men, II–IV Hamilton	15	15
Score efficacy (average)	Very good: 1 patient Good: 2 patients Poor : 3 patients No result: 9 patients	Very good: 4 patients Good: 3 patients Poor : 3 patients No result: 5 patients
Side effects	1 patient: infection	
	1 patient: onset of one patch of alopecia areata in the side of injection	No side effects in all patients

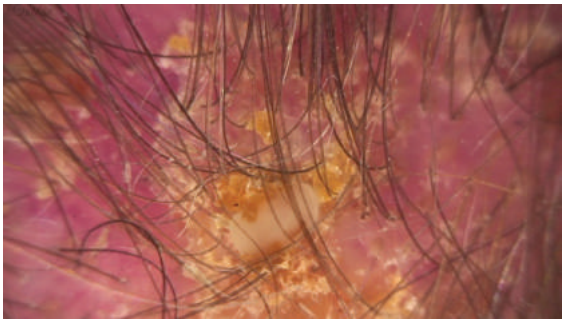

Figure 17.18 **Infectious and necrotic reaction.**

In the other case, the patient had an unexpected patch of alopecia areata (Figure 17.19) after 22 days from the treatment. We treated this patch with topic corticosteroids, and we had resolution of AA after 2 months. This patient never suffered from AA.

Cultured ADSCs gave us a significant result with seven patients with very good/good results (same score, 46.6% improvement) with no side effects reported, after 12 months from the treatment. The hair follicle neogenesis and neangiogenesis could be the rationale of the efficacy of ADSCs in androgenic alopecia (Figure 17.20, Table 17.5).

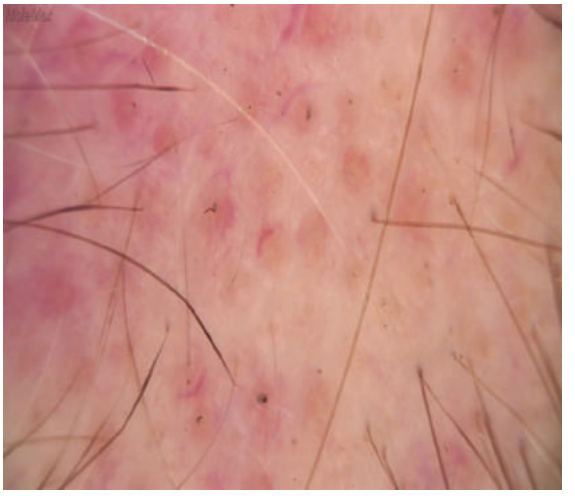

Figure 17.19 **Unexpected patch of alopecia areata.**

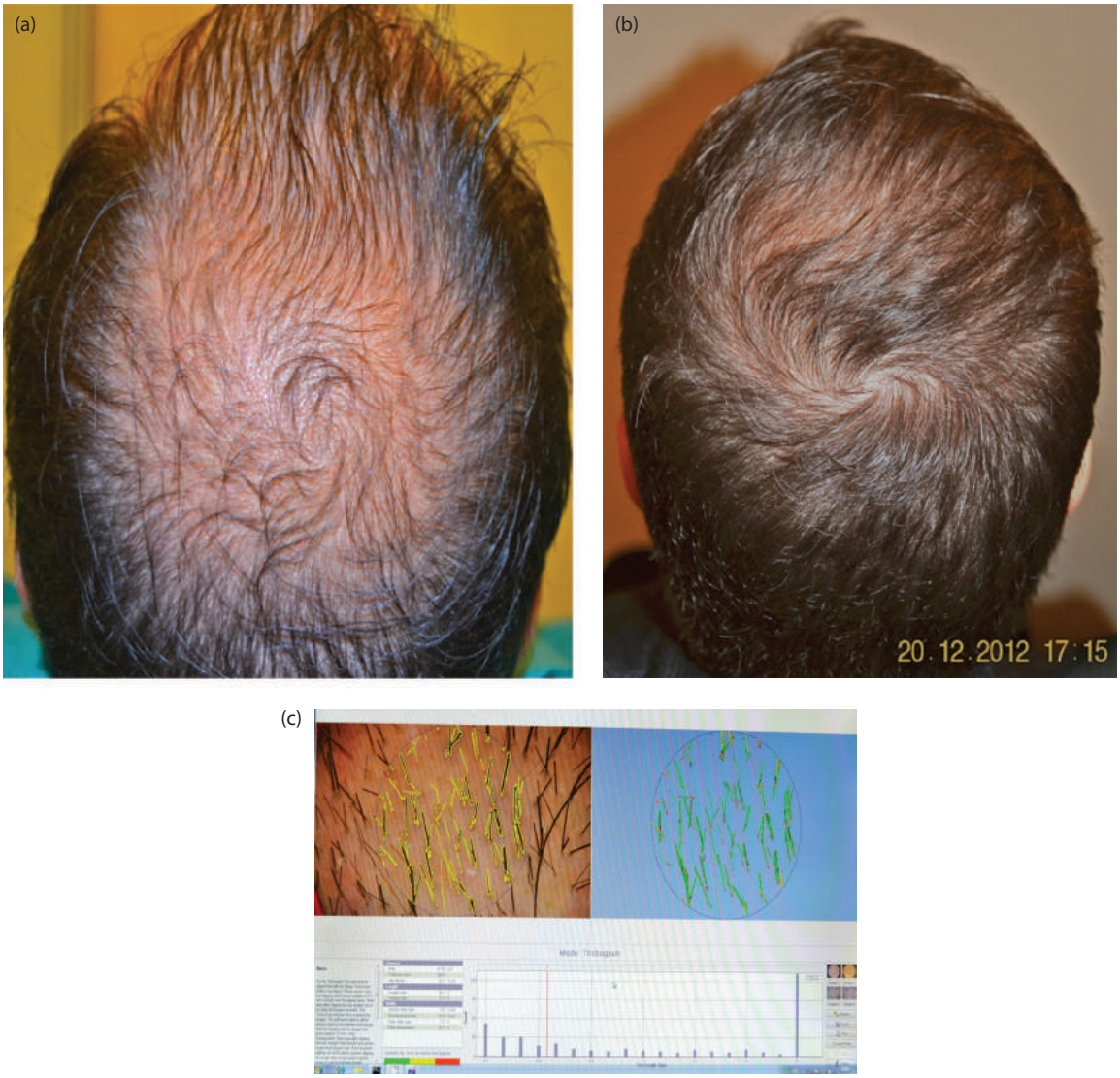

Figure 17.20 **Male androgenic alopecia. (a) Before the treatment. (b) After the treatment. (c) Trichoscan evaluation. Patient with a very good result.**

Table 17.5 Alopecia Areata

	SVF	ADSCs
# Patients, 6 men, 9 women– Chronic AA, multiple patches	15	15
Score efficacy (average)	Very good: 2 patients Good: 2 patients Poor : 1 patient No result: 10 patients	Very good: 2 patients Good: 3 patients Poor : 4 patients No result: 5 patients
Side effects	No side effects	No side effects

We need further controlled studies to verify the proper efficacy of this technique in AGA.

In alopecia areata, using VSF we had four patients with very good/good results (resolution of hair disease, 26,6%) and no side effects in all the patients (Figure 17.21), The immunosuppressive effects of ADSCs and neoangiogenesis could be the therapeutic pathway of this treatment in this autoimmune disease.[47]

The ADSCs, in alopecia areata, gave a better but non-significant result in respect to VSF, with very good/good results in five patients (resolution of hair disease, 33.3%). In this group we had five poor results, more than in the

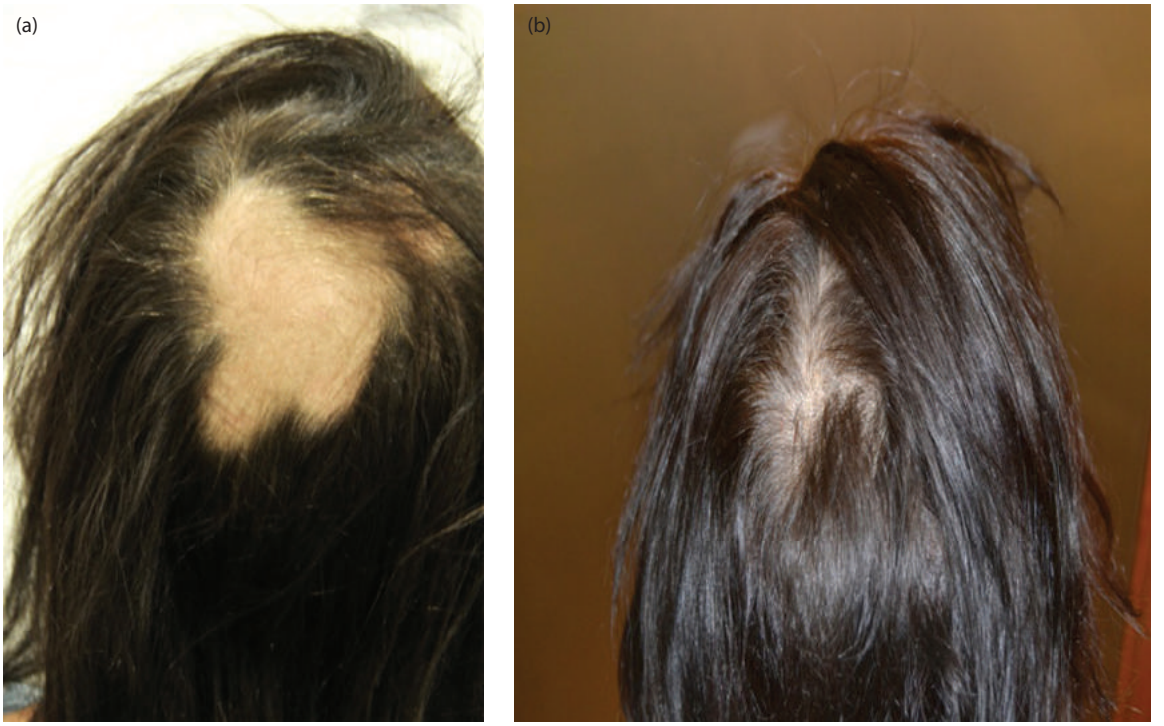

Figure 17.21 **Male androgenic alopecia. (a) Before and (b) result with stem cells treatment ADSC.**

SVF group. In no patients did we note any side effects (Figure 17.22).

We need further controlled studies to verify the proper efficacy of this technique in alopecia areata.

NEW HORIZON: DERMAL PAPILLA CLONING

Hair follicles also contain stem cells, and some researchers predict research on these follicle stem cells may lead to successes in treating baldness through the activation of already existing stem cells in the scalp.

So the second source of stem cells could be the hair follicle itself: we know that the cycling activities of the hair follicle are controlled by a group of specialized mesenchymal cells located in the dermal papilla

It was demonstrated many years ago that adult rodent dermal papilla could be removed from the hair follicle and transplanted into recipient skin, where they were able to induce de novo follicle development and hair growth. Further investigations in humans were unsuccessful because when dermal papilla cells are removed from their hair-follicle microenvironment and grown in culture, they lose key inductive properties. A recent study opens new perspectives demonstrating that in manipulating cell culture conditions in order to grow dermal papilla cells in a 3D spheroid environment, it becomes possible to induce a partial restoration of inductive capability, and that human dermal papilla cells, when grown as spheroids, are capable of inducing de novo hair follicles in human skin. It is the so called "community effect" phenomenon that occurs when groups of cells use close contact interactions to maintain their collective identity. The physical manipulation of dermal papilla is not enough to induce follicles, whose reactivation requires communications from the epidermis, surrounding adipocytes, and the whole follicle environment; each of these cues has the potential to translate to genetic changes in hair follicle and dermal papilla.[61]

SUMMARY

The most researched and publicized 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 (1 mg) works by inhibiting the 5α-reductase from forming DHT. The decreased DHT levels allow some 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