HAIR RESEARCH AND THE NEWEST MICROGRAFT TECHNIQUES
ABSTRACT
Both male and female hair-loss and baldness can be treated using a range of methods. The indications consider a range of parameters included in multifactorial classifications. Minoxidil and finasteride have shown some efficacy as treatment methodologies. The newest molecules and cellular therapies are currently under investigation. However, the newest follicular unit transplantation, carried out via extraction (FUE) or with long hair (FUL), provides a definitively aesthetic and natural looking hair transplantation for the majority of scalp alopecia, or for a better correction of the eyebrows, the eyelashes, the pubis, the beard and the moustache.

MEN AND WOMEN HAVE SOUGHT remedies for baldness for more than 5000 years. With today’s emphasis on youth, virility or femininity exemplified in large part by a full head of hair, the prevention or reversal of baldness has become more important than ever before.

Indeed, interest in the reversal of baldness as a specialty has been enhanced by a precise multifactorial classification of each individual to effectively reverse male and female androgenetic alopecia (AGA) in selected patients.

The diversity of hair transplant techniques allows a definitive aesthetic solution for most instances of vellus or hair alopecia. The indications consider a range of parameters of the thinning hair—aetiology, location, widthness or evolution—and the specificity of each patient, such as age, sex, ethnic origin, self-motivation for hair transplant of the scalp, beard, moustache, eyebrows, pubic vellus and eyelashes.

The ‘follicular unit long hair’ (FUL) allows for an immediate visualisation of the result. With this procedure, the surgeon has an easier approach for the best choice and the orientation of each graft, and the patient can immediately imagine the future result. The FUL and conventional segmentation (FUS) techniques provide a natural aspect as a result of the fine implantation of 1–4 hairs at a time, during one session of 1000–4000 hairs. The follicular unit extraction (FUE) sometimes has pertinent indications for those patients who routinely shave their scalps.

Whichever method is used by the surgeon, simple postoperative care is followed by a definitive hair regrowth. Combining hair transplantation and hair medical treatments (minoxidil, finasteride) improves the result and postoperative evolution.

The most recent research with regard to cellular therapy with follicular cell implantation, platelet rich plasma (PRP) infusion, or adipose-derived stem cells are still currently under investigation.

Background
In societies in which a full head of hair is considered beautiful, feminine or virile, balding or the sudden loss of hair can be very distressing. Two out of every three men experience some degree of balding (1). A number of medical treatments have been shown to be at least partially effective in retarding or reversing hair loss.
Follicular unit micrografting procedures allow for a definitive aesthetic and natural-looking hair restoration for most male and female baldness (Figure 1). The amount and thickness of scalp hair varies tremendously between different ages, sexes and ethnic origins. The hereditary incidence is notable in both men and women who have a strong family history of baldness.

The male and female hair loss can begin as early as the age of 18 years. It usually evolves in spurts. 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.

Dihydrotestosterone (DHT) is the specific hormone responsible for male and female pattern baldness. The number of testosterone receptors of the follicular cells and the activity of the 5-alpha-reductase enzyme in different areas of the scalp are increased.

Objective measurement of alopecia using a digital camera
A digitalised phototrichogram (2) or videotrichogram (3) (Figure 2) enables an objective measurement of hair growth parameters, such as density, calibre, number of miniaturised, terminal hairs, and the hair growth rate. It is possible to determine the precise hair quantity available for the transplantation session (e.g. a strip 30 cm long and 1 cm wide, with a density of 150 cm\(^2\) will provide approximately 4500 hairs).

The Bouhanna multifactorial classification
A range of hair and scalp parameters are evaluated and computerised:
- Bald and hairy areas
- Scalp thickness and laxity
- Hair emergence obliquity
- Hair density
- Hair length
- Hair colour
- Hair growth rate.

The Bouhanna multifactorial classification (Figure 3) (4) provides a better assessment of each male and female baldness for an easiest and precise medical and surgical indications.

Medical treatments for male and female baldness
Minoxidil
By far the most publicised medical treatment available for male pattern baldness is 5% minoxidil lotion and 2% minoxidil for female baldness (Figure 4—overleaf) (5, 6). The exact mechanism of action is still debated, but it is able to increase the duration of the anagen growth phase, leading to the production of progressively thicker and longer hair.

It is important to point out that the bioavailability of sulfotransferase differs among individuals, and this could explain the different response to treatment. The first signs of improvement generally appear after 4-6 months of therapy.

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Minoxidil lotions, the concentrations of which ranged from 2-5%, were studied in more than 3000 patients, who all displayed an AGA. The number of hairs was assessed by counting on a round surface of 2.5 cm in diameter. Results indicate an aesthetically satisfactory and statistically significant regrowth for 24-42% of all patients, over a 12-month period (6). A personal study of the author's revealed an average density of 118 hairs/cm² for 66 male patients displaying an androgenetic alopecia of types II to IV on the Hamilton scale (3, 5).

The efficacy and the safety of 2% minoxidil lotion as a form of treatment of female AGA was proved in a 32-week double-blind study conducted versus placebo (7). This study evaluated 550 female subjects displaying an AGA type I or II, according to Ludwig's classification (Figure 4).

The study encompassed the counting of the hairs on a 1 cm² tattooed surface using a Quantimet analyser. New hair growth was indicated in 54% of all patients who had been taking minoxidil (vs 34% for those taking placebo). A computerised counting method confirmed an average density of 211 hairs per square centimetre, which had been observed on the frontoparietal area of 62 women. There was no increase in density for the remaining 488 women. From these results, it is possible to deduce that a 38% loss of hair regrowth (i.e. approximately one third of all hair lost) was obtained with minoxidil compared with only 16% in those treated with placebo. Side-effects of minoxidil are minimal, but include itching, eczema, and hypertrichosis in females.

**Finasteride**

Finasteride taken orally and daily (1 mg) works by inhibiting the 5α-reductase from forming DHT (8). The decreased DHT levels allow some intermediate follicles to enlarge and regrow normal terminal hairs (Figure 5).

Studies carried out on 1879 men showed a variety of improvements after photographic assessment: 5% great improvement; 31% moderate improvement; and 30% slight improvement. Finasteride's side-effects were limited, with only 1.8% of the study group experiencing decreased libido (erectile and ejaculation disorder), which is reversible.

Persistant sexual side-effects have also been recently mentioned, but this requires further studies for confirmation (9). Recently, a warning with regard to a small risk of male breast cancer was included in the product information (10). Finasteride is not indicated for use in women, and dutasteride is not yet approved for the treatment of hair loss.

**Anti-androgens**

Cyproterone acetate (11) (in Europe) can effectively block increased levels of male hormones that cause hair loss in some women. A 12-month randomised controlled trial compared the effects of 2% minoxidil versus cyproterone acetate (50 mg/day) in female AGA (11). After 6 months of treatment minoxidil was found to be more effective. The side-effects of cyproterone acetate are hepatotoxicity, nausea, headache and increased weight.

Spironolactone (12) (in the US) is an aldosterone antagonist which appears to be a competitive inhibitor of DHT-receptor binding. No controlled clinical trials have been published. With regard to the use of spironolactone in humans, there are no publications on teratogenicity as yet, but animal studies have revealed a feminisation of the male foetus after spironolactone treatment during the second trimester of pregnancy.

It is of the author's opinion, therefore, that spironolactone should not be used during pregnancy, and that prescriptions of oral contraceptives are recommended while administering this treatment.

**New treatments**

**Platelet rich plasma**

In a study by Greco and Brandt (13), traumatising and infusing platelet rich plasma (PRP) into the scalp
was found to reverse miniaturisation over an 8-month period when compared to a control. This procedure is still under investigation.

**Adipose-derived stem cells and hair restoration**

Stem cells have been sought for the treatment of a range of medical conditions, such as facial rejuvenation and for a variety of anti-ageing techniques. Adipose tissue has been shown to contain adult mesenchymal stem cells that have a therapeutic application in regenerative medicine (14). For scalp treatment, the focus is on isolation of the stromal vascular fraction (SVF). Abdominal wall fat cells extracted using liposuction have been used to treat diabetes, coronary heart disease, and stroke for example. Success has been reported in the majority of cases (15). A standard benchtop centrifuge is required to perform the fat processing. It takes only 2 months to see the growth of new hair compared with 3–6 months after FUT.

Stem cells of the hair follicle are found in the bulge area of the pilosebaceous unit. Intradermal injection of activated adipose tissue-derived autologous stem cells may stimulate these stem cells to restore the normal hair cycle, changing the vellus hair back into terminal hair, or halting the process of miniaturisation in areas programmed for AGA.

Even if adipose-derived stem cells are effective, would the effect on the hair cycle be short lived, ultimately overruled by the genetic dominance toward AGA? This procedure is currently under investigation.

**Prospective molecules in research**

Extensive research indicates that minoxidil or finasteride will not significantly help the majority of balding men and women (16), although they will help a specific population of minimally balding young men or women aged 18–21 years. This research has led to greater insight into research for other possible medications to treat male and/or female pattern baldness.

- Prostaglandins. Hair growth can be stimulated by prostaglandins. Latanoprost (PGF2α), currently used as a treatment for glaucoma, also initiates the growth of eyelashes. It might be considered a future treatment for male or female AGA.
- TRPV1 (transient vanilloid receptor 1) is a receptor activated by the capsaicin contained in black pepper, which is an inhibitor of hair growth. The development of antagonists might stimulate hair growth and decrease the evolution to catagen.
- Cannabinoids (CB) increase the evolution through the catagen phase. CB1 antagonists might be a future treatment of alopecia.
- L-carnitine, made from amino acids, is a good hair growth stimulator in vitro with apoptosis decrease and stimulation of the hair matrix keratinocyte proliferation. Local or general treatment with L-carnitine might be a treatment for alopecia.
- Interferon-gamma. In vitro, interferon-gamma stimulates a catagen evolution.

**Anti-sense oligonucleotide (AO).** Hair growth is regulated by many growth factors, such as insulin, fibroblast, vascular endothelial growth factors. Some of these growth factors accelerate the transition from the anagen to catagen phase. The block of the synthesis of this molecule with AO is a potential treatment for AGA.

**Tacrolimus** has not only an immunomodulation effect, but it can also, similarly to minoxidil, stimulate entry into the anagen phase, possibly owing to hair growth factor synthesis (vascular endothelial growth factor, hepatocyte growth factor)

**Zinc.** In a murine model, high doses of zinc initiated delaying the anagen phase and the extension of the catagen phase.

**Research in cellular therapy**

**Follicular cell implantation**

Follicular cell implantation for hair restoration (17) is based on the hair inductive power of the dermal papilla (DP) cell, a property that the DP retains throughout adult life. DP cells can be expanded in culture, which makes it possible to take cells from a few dozen donor follicles and generate enough cells to induce many thousands of new follicles. The research has suggested a number of strategies for hair regeneration (17):
The simplest approach is to implant DP cells by themselves. The DP cells would be placed in or near existing miniaturised follicles. These DP-only approaches are under study in a phase II clinical trial conducted by Intercytem.

Another strategy under investigation is to co-implant cultured keratinocytes together with DP cells. In one version, cells would be implanted as a mixed suspension that contains both cell types. After implantation, hair neogenesis would occur within the implant site. In another form of mixed-cell implants, keratinocytes and DP cells would be cultured together. These culture-grown hairs would then be implanted into the patient. This phase II clinical trial is being conducted by Aderans.

An alternative that is under development is to implant cells together with a matrix composed of collagen. A matrix might be used with DP cells alone or in combination with cultured keratinocytes. Clinical trials must be performed.

Androgenetic alopecia and hair follicle stem cells

AGA is characterised by a marked decrease in hair follicle size, which could be related to the loss of hair follicle stem or progenitor cells (18). These findings support the notion that a defect in conversion of hair follicle stem cells to progenitor cells plays a role in the pathogenesis of AGA. The loss of CD200hiITGA6hi cells and CD34hi cells in AGA provides insight into possible mechanisms leading to follicular miniaturisation, the hallmark of AGA.

Decreased matrix cell numbers would result in diminutive hair shafts. CD200hi hair follicle stem cells are preserved in chronologic ageing of the scalp.

Hair cloning

The success of hair transplantation is sometimes limited by the availability of donor hair. Hair cloning is based on the inductive capacity of the dermal papilla cell to form a new hair follicle (19). A few hair follicles are first excised from the occipital scalp by biopsy punch. These follicles are then dissected to isolate the dermal papilla. Each DP contains approximately 200–400 cells. These cells can be cultured in the laboratory to make millions of offspring cells. On reinjection into the bald scalp of the same individuals, the cultured DP cells will induce the development of new hair follicles. This process is basically hair multiplication, or follicular neogenesis, rather than true ‘cloning’.

Although the principle of hair cloning is proven (15), turning it into a practical technique for clinical use is full of problems, for instance:

- The new hair follicles induced in studies using rodents are usually disorientated and grow at a variety of different angles.
- Hair follicles induced by hair cloning do not have an even distribution over the skin as seen in natural hair follicles. They appear as clumps of growing hair.
■ Once cloned hair was shed in the normal hair cycle, it did not follow this cycle and regrow.
■ There are no published studies to prove that hair cloning would work in humans. A significant technical problem to hair cloning in humans is that the cells in culture lose the ability to produce new hair, which appears to be the single greatest obstacle to this form of therapy. Unfortunately, there are also no published studies to prove that follicular cell implantation would work in humans.

**Surgical treatment of baldness**
Surgical procedures are currently used daily to provide a definitive aesthetic correction of alopecia. All the methods described in this article are not only for the treatment of male and female pattern baldness, but also for alopecia caused by traumatic injury, second and third degree burns, post-radiotherapy for brain tumour, African-American traction alopecia, post-lifting scars, eyebrows, eyelashes, beard, moustache and pubis.

The choice of follicular unit transplantation technique will depend on the degree and location of the alopecia, as well as the age and ethnic considerations of the patient.

Most hair transplant procedures are performed on an outpatient basis, using local anaesthesia (anaesthetising cream and nerve blocks). Follicular unit micrografting is a procedure during which 1000–4000 hairs are redistributed into the thinning or balding areas.

Once harvested from the ‘donor’ area (Figure 6), hair follicles are microscopically dissected into ‘grafts’ (FUL) or extracted with a hand or a power micropunch (FUE). Each micrograft contains a single ‘follicular unit’ consisting of between one and four hair follicles. FUE or FUL are then carefully implanted into the bald or thinning ‘recipient’ area. Meticulous artistic and technical skill is required to design an appropriate hairline, as well as ensure the appropriate angle, orientation and position of each transplanted hair. FUL or FUE create a result that defies detection and is never ‘pluggy’. The hair transplant session is usually completed in approximately 2–5 hours.

The almost painless surgery and simple postoperative care allows few discomforts for the patient. Some itching and swelling can occur, but this is generally mild. Normal physical activities can be resumed immediately and the first shampoo 1 day after the procedure. Crusting and scabbing of the transplanted areas are hidden by the long hair and last for approximately 10-12 days. The transplanted hair falls out in 2 weeks and generally begins to grow after 3 months. Transplanted hair lives and grows indefinitely. The hair can be styled in any way the person desires.

**The techniques**

**Follicular unit long hair technique**
It has been many years since the author developed the follicular unit long hair (FUL) technique (20) (Figure 7). Hairs are not shaved like in the conventional FUT technique, but the progress is almost the same. For the patient, the advantages of this procedure are numerous.

The ‘wait and see’ is replaced by the ‘see and wait’—the patient will see the result immediately, although most of the transplanted hairs have the transient loss 2–3 weeks later (the use of minoxidil lotion and finasteride can decrease this hair loss). Furthermore, the donor area is not shaved and patient scabs of the recipient area are hidden by the long hair.

For the surgeon, the benefits of this procedure include a better evaluation of the orientation and the obliquity of transplanted hairs, a better choice for the refinements of the frontal hairline with thinner or lighter hairs, and a greater amount of hair can be transplanted during one treatment session. In the author’s opinion, the only disadvantage is for the patients who routinely shave their scalp.
A strip with long hair is harvested (10–30 cm long and 1.5 cm wide) and the donor area is closed with running suture or staples. The scar will be very fine, linear, horizontal and almost undetectable. Sometimes a trichophytic closure is used, which will require the surgeon to carry out a desepidermisation of the inferior border of the excision. The strip with long hair is then segmented under stereomicroscope into follicular units (1–4 hairs).

The newest implantation techniques allow for a natural and aesthetic appearance with a fine implantation using microsurgical needles, blades and forceps, and a good choice of orientations and obliquities, an irregular and fine frontal hairline with hair done ‘one-by-one’ with 1000–4000 hairs per session. These refinements can be carried out using a final ‘stick and place’ technique.

The follicular unit extraction

In the follicular unit extraction (FUE) technique (21) (Figure 8), the patient’s hairs are shaved throughout the donor area. The aim of FUE is to harvest intact follicular units from the donor area by hand or using power micropunches (0.7–1.25 mm), introduced at a depth of 2–5 mm. Follicular units are then gently extracted from the surrounding tissue with the help of microsurgical forceps; 500–1000 hairs can be transplanted in one session.

The indications of the FUE techniques are for patients who:

- Routinely shave their scalp
- Have no laxity of the scalp
- Are afraid of getting a fine linear scar (which can be eventually corrected with FUE a second time)
- Want a short treatment session
- Have no sign of wide baldness evolution

The contraindications of this technique are for patients who have a very low hair density in the donor area, or those who want a great amount of transplanted hair.

The conventional follicular unit technique

The conventional follicular unit technique (FUs-FUT) is a procedure similar to that of the FUL technique. The
The only difference is that the hairs are previously shaved. Specifically, this treatment method can combine the use of minoxidil, finasteride and FUT for treatment efficacy (Figure 9). The use of a 2% minoxidil solution before and after hair transplantation will reduce the postoperative hair-loss and improve the transplanted hair regrowth (22). The prescription of 5% minoxidil and finasteride 1 mg 3 months before and during the 6 months after the FUT, and especially after the FUL technique, has shown a number of benefits (23), including a decrease of the hair-loss of the transplanted and remaining hair, which usually occurs 2-3 weeks later, and a quicker regrowth of the remaining hairs and the transplanted hairs, which often occur 2-3 months after treatment.

Other techniques
There are a number of other techniques that can be used in the treatment of male and female pattern baldness and AGA, but that have fewer indications and efficacy, including:

- 2 mm punch-transplants harvested with a power punch. This treatment has few indications
- Automatic hair implanters may have a limited diffusion
- Lasers—the positive trichogenic effect of lasers needs a more objective evaluation
- Dermo-pigmentation or tattooing is sometimes indicated for scars or eyebrows.

Indications
For men
Male baldness, particularly with regard to AGA, has a specific topography and evolution. In 1976, a simplified classification was proposed (24), which borrowed from the Hamilton classification (Figure 10):

- Stage 1: a = lateral frontotemporal recession; b = with crown alopecia
- Stage 2: a = complete frontotemporal recession; b = with crown alopecia
- Stage 3: complete baldness of the fronto-vertex area.

The FUT indications for these types of male baldness are:

- For stage 1: a = 1000 hairs (Figure 11); b = 2000-3000 hairs in 1-2 sessions (Figure 12)
- For stage 2: a = 2000-3000 hairs in 1-2 sessions; b = 4000 hairs in 1-2 sessions
- For stage 3: 6000 hairs in 2-3 sessions (Figures 13 and 14).

"Beard and moustache alopecia can be the result of scarring after injuries such as burn, car accident, acne, or cleft lip. More frequently it is apparent that an aesthetic correction for ethnic or religious reasons is needed for beards and moustaches."
The youngest patient the author has performed a transplant on was 19 years of age, and the oldest patient was 87 years of age.

FUT can be combined with 5% minoxidil lotion and the oral 1mg finasteride, the goals of which are to decrease the loss of pre-existing hairs, to decrease the transient hair-loss on transplants, and to initiate a quicker regrowth of both the transplanted and non-transplanted hairs.

Beard and moustache alopecia (Figure 15) can be the result of scarring after injuries such as burn, car accident, acne, or cleft lip (25). More frequently is it apparent that an aesthetic correction for ethnic or religious reasons is needed for beards and moustaches. Eyelash and eyebrow reconstruction are carried out for scars, usually for aesthetic reasons.

For women
The evolution of female baldness and AGA (26) is evaluated according to the Ludwig classification (Figure 16), or can be done more precisely with the Bouhanna multifactorial classification. The three Ludwig stages are:
- Stage 1 = moderate thinning of the vertex
- Stage 2 = more significant thinning with a persistence of the frontal anterior fringe
- Stage 3 = nearly complete baldness of the fronto-occipital area.

Hair thinning can occur at a young age, and even before 18 years of age. A combined treatment with 2% minoxidil, cyproterone acetate and FUT will sometimes be indicated. The combined treatment with 2% minoxidil lotion, hormonal treatment and FUT are increasingly indicated for post-menopausal AGA (Figure 18). The oldest patient to receive this treatment in the authors’ experience was 83 years old.

African–American definitive traction alopecia
Repeated tractions of hair with brushing, straightening and braids for example, are more frequently seen in African-American patients (26) (Figure 19). They determine a p-
Definitive frontotemporal occipital alopecia. FUT is indicated 6 months after stopping the traction and stimulation of the regrowth is done using 2% minoxidil lotion.

Frontal fibrosing post-menopausal alopecia
It seems possible to do a follicular unit reconstruction of the definitive fronto-temporal and eyebrow alopecia if the evolution of the disease is completely stopped (25). A prior implant test is recommended.

Post-lifting alopecia
Sometimes a post-lifting alopecia needs a follicular unit reconstruction of the pre-temporal and the anterior frontal hairline. Transplantation should be fine, with the surgeon having complete respect for the orientation and natural obliquity of hair.

Definitive eyebrow alopecia
Definitive eyebrow alopecia is mostly the result of trauma or repeated traction (25, 28) (Figure 20). The very fine implantation of hair ‘one-by-one’ allows a complete reconstruction of the eyebrows. Obliquity and orientations must be well respected.

Eyelash alopecia
It is now possible to carry out a complete reconstruction or increase the density and the length of the eyelashes with the fine implantation of hair ‘one by one’ (Figure 21). The local application of a new molecule (Lanaprost—off-label usage) increases the growth of the eyelashes.

Pubis alopecia
The aesthetic reconstruction of the pubis is guided by a number of parameters, such as aetiology, age, ethnic origin, and shape and colour of the hair (25). It is very important to get a natural aesthetic result, to choose the follicular unit in the donor area, and to respect the obliquity and the inward orientation of the transplanted hairs (Figure 22).

Conclusions
A new interest in preventing baldness has been stimulated by the publicity given to certain products (minoxidil, finasteride, cyproterone) that have shown an ability to retard or reverse male and female pattern baldness in certain individuals.

Newest molecules and cellular therapy with stem cells may offer new possibilities in the future. We can imagine in the future the development of cell hair implantation and the possible help of robotics, but the artistry and final result will always be in the hands of the surgeon.
cells and PRP are still currently under investigation. The newest follicular unit transplantation techniques, such as FUE (follicular unit extraction) and FUL (follicular unit long hair) provide a definitively aesthetic and natural looking hair restoration for the majority scalp alopecia incidences in patients from the ages of 19 to 87 years. With regard to vellus alopecia, the refinements of the FUL technique allows for a better correction of the eyebrows, the eyelashes, the pubis, the beard and the moustache.

We can imagine in the future the development of cell hair implantation and the possible help of robotics, but the artistry and final result will always be in the hands of the surgeon.

Declaration of interest None

References


Figure 20 Eyebrows alopecia (a) one day after one session of follicular unit long hair (b).

Figure 21 Eyelashes before (a) and immediately after (b) one session of 1-hair follicular unit long hair

Figure 22 Pubis alopecia (a) after one session of follicular unit long hair (b)