Understanding Hair Transplants and Hair Loss

Robert Michael Elliott, MD, FAACS, ABHRS

with Marc S. Dauer. MD, ABHRS

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Foreword

Hair loss affects over 50% of men over 50 years old. This book is intended as the standard of hair restoration for you or someone you know. With so much confusion surrounding hair loss, I was glad to hear that Dr. Elliott was tackling this task. Dr. Elliott has an uncanny way of reducing complex issues to their simplest parts. He started with the first hair restoration techniques and has been a major part of every innovation since. I am honored to write this foreword for my friend and hair restoration mentor.

For those of you who know me from the media, I am a cosmetic surgeon, specializing in hair restoration and NONsurgical cosmetic and reconstructive facial restoration. In 2002, while on vacation in Thailand, a motorcycle accident left me with a fractured nose and multiple facial lacerations. From this point, I embarked on a journey to NONsurgically restore my face. This journey led to the field of medicine now known as Facial Cosmetic Derma Surgery. I have since performed over 15,000 NONsurgical facial procedures. The Nettles NONsurgical nose job was featured on Bravo channel for an audience of over 10 million viewers.

Not only is hair loss intriguing, but I also have a personal interest. Hair loss is prevalent in my family and, at the age of 30, I began to notice “miniaturization” and hair thinning. While completing my retina eye surgery training, I noticed an interesting side effect of a glaucoma medicine. The medicine augmented the anagen phase of the growth cycle of the follicle of the lash. Its utilization in hair loss had not been research ed.

Upon landing in Los Angeles as a newly-trained surgeon with this concept for a formula that grows eyelashes to be used in medical hair restoration, I started searching for the most advanced and respected hair restoration practice. The one name that continued to surface was Pacific Hair Institute, founded by Dr. Elliott. A phrase that was often uttered in the same sentence was “consummate professional,” so I

contacted Dr. Elliott, discussed my research and asked to learn the follicular transplant technique from him. He graciously opened his office to me and exposed me to every facet of his technique. His patience and willingness to take the extra effort to ensure my grasp of the technique is something that I remember very well. Not only did he understand every aspect of hair loss, its underlying cause, and the differential diagnosis, but his communication skills with the frustrated hair loss patient was superb. Aside from mastering the technique, I also learned how to directly, yet compassionately, deal with the anxiety of the hair loss patient. Oftentimes, they had tried various creams, gels, laser combs and other snake oil potions to no avail. Dr. Elliott had the experience, the knowledge base and the patience to advise the patient of his/her options with integrity and compassion, thereby alleviating the anxiety of the patient. The surgical aspect of the procedure was effortless for one of the pioneers in the field.

As once a student learning the hair transplant technique from whom most of us in the field would refer to as the hair transplant surgeon's surgeon, I am forever grateful for those lessons. I have trained under many talented surgeons, and Dr. Elliott was more than just an educator, he was a compassionate physician and a gifted surgeon. He took pride in his work as well as pride in teaching his craft. Although a motorcycle accident led me down a slightly different path, I am pleased to join Dr. Elliott as we continue to mold and shape this artistic field of cosmetic hair restoration.

Robert Nettles, MD, ISHRS, AACS

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Introduction

I have put together this book in order for the non-physician public to understand the process of hair loss and hair transplants. Many people think that hair loss generally is the common male and female pattern baldness. In reality, they are close to being right, as almost all cases are either male or female pattern baldness.

Nevertheless, there are some rare endocrine abnormalities, scarring alopecias from a variety of causes, and other reasons why a person may have hair loss. That is why it is very important to consult an experienced physician for proper diagnosis before deciding what you should do.

In this book, we first walk you through the diagnostic process for each type of hair loss, and then describe the treatments available for the most common types of hair loss, the pattern baldnesses.

Following that, we discuss in detail the process of hair transplantation and, finally, bring you a brief synopsis of some of the latest research regarding hair loss.

I hope you enjoy it.

ROBERT MICHAEL ELLIOTT, M.D.

What is a Hair Transplant?

Hair transplants are minor dermatologic surgical procedure in which hair follicles are transferred from the permanent and thick donor area around the sides and back of the head to areas of thinning or balding generally found on the front, top, and crown of the head, as well as eyebrows, beard areas, and sometimes even chest. In rare cases, even body hair can be used as donor, if it is very thick and luxurious in areas such as the chest.

Proper Diagnosis of Hair Loss

However, before one can decide if they might need a hair transplant, a proper diagnosis of their hair loss condition should be made by a dermatologist or hair loss specialist. Hair loss may be pattern hair loss (male or female) or non-pattern hair loss.

Most hair loss is male or female pattern hair loss.

In your consultation, your doctor must consider:

- Actual hair loss versus hair breakage
- Focal hair loss versus diffuse hair loss
- Hair thinning versus hair shedding
- Scarring hair loss versus non-scarring hair loss
- Hair shaft miniaturization versus reduced density

Pattern Hair Loss

Miniaturized or missing hairs in a distinct male or female pattern occurs in the pattern illustrated below.

Norwood: Male Pattern Hair Loss

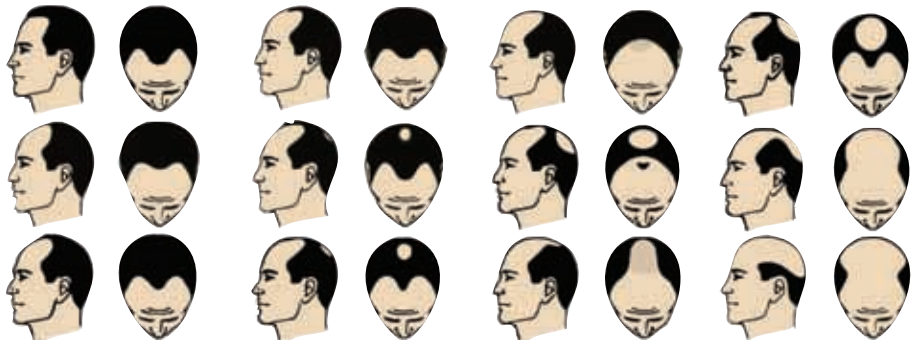


Figure #1

Nugget

The great majority of all hair loss is male or female pattern baldness. The treatment for these is chemical and/or surgical therapy.

Ludwig: Female Pattern Hair Loss

1 2 3 4

1 Christmas Tree Pattern
2 Ludwig Pattern I
3 Ludwig Pattern II
4 Ludwig Pattern III

Non-Pattern Hair Loss

Hair loss (or alopecia) that is **not** in a genetic male or female pattern is divided into:

1) Hair shedding.
2) Scarring alopecia.
3) Focal non-scarring alopecia.
4) Telogen effluvium.
5) Hair breakage problems.
6) Diffuse thinning.

A discussion of each follows.

Hair Shedding

Sometimes generalized hair thinning is caused by hair shedding. More than 100 hairs per day are significant – it usually is a **telogen** effluvium (hairs which have entered the resting or telogen phase of the growth cycle – and are thus falling out). When hair follicles enter the telogen phase, the hairs held firmly in those follicles become loose and fall out. Certain severe toxins can cause **anagen** effluvium – where hairs are shed during the anagen (growth) phase of the cycle – as the follicles are destroyed by a toxin. A telogen effluvium usually occurs about three months after the precipitating event, whereas anagen effluvium occurs closer to the toxic event. ***(See fig, 5 on page 16.)***

Causes of Hair Shedding (telogen or anagen effluvium)

Telogen Effluvium Common Precipitating Events	**Common Drugs That Can Cause Telogen Effluvium**
Childbirth	ACE inhibitors
Drug-induced	Androgens
General anesthesia	Anticholesterol agents
High fever	Beta blockers
Hormonal changes	Cimetidine
Protein-deficient diet	Coumadin, Heparin

Starting or stopping OCAs
Stress
Sudden weight loss
Systemic disease
Lithium
Oral contraceptives (OCAs)
Vitamin A

Anagen Effluvium –Common Precipitating Events

Chemotherapy
Early alopecia areata
Loose anagen syndrome
Radiation
Toxins

Focal Non-Scarring Alopecia

Entity	Distinguishing features
Patchy alopecia areata	History, exclamation point hairs, hair pull test, depigmented hairs
Secondary syphilis	Serology for syphilis (contagious)
Tinea capitis (ringworm)	Broken hairs, scaling, erythema, positive smear and culture
	(contagious)
Traction alopecia	Typical pattern from traction
Triangular alopecia	Pattern, configuration and history on temple
Trichoterlomania	Shaved hairs
Trichotillomania	Broken hairs present from manipulation, hairs of various lengths

Scarring Alopecias

Generally, scarring alopecias present with a smooth, shiny scalp without pores, because the hair follicles have been destroyed by the scarring process. Usually a biopsy is required in these cases, as well as lab tests. There may be redness and scaling at the active borders. They may usually be transplanted after they have been “burned out” (inactive) for one year

Scarring Alopecia Entities

Discoid lupus	Lichen planopilaris	Folliculitis decalvans
Morphea	Pseudopelade	Infection – Pseudofolliculitis barbae
Sarcoidosis	Fibrosing alopecia in a pattern distribution	

Follicular degeneration syndrome (hot-comb alopecia) (CCCA)

Hair Breakage, Causes:

1) Chemical or Physical Damage
2) Trichotillomonia
3) Anagen Effluvium
4) Hair Shaft Anomalies:
    - Monilethrix (beaded hair)
    - Pili torti (twisted hair)
    - Trichorrhexis invaginata (bamboo hair)
    - Pili annulati (ringed hair)
    - Bubble hair (damage from heat of hair dryers, curling irons, etc.)
    - Trichorrhexis nodosa (nodes on hair)
    - Trichonodosis (knotted hair)
    - Trichoptilosis (split ends)
    - Trichoschisis

Pattern Baldness Versus Generalized Diffuse Hair Loss

Note that male and female pattern baldness are just that, hair loss in a pattern area, generally on the top, sides, and back of the head, but sparing a thick donor area. Other types of systemic problems such as low thyroid, iron deficiency, collogen disorder, growth or sex hormone deficiency, secondary syphillus all may cause diffuse hair thinning. If you have generalized hair thinning, you need a complete medical workup for the various causes. Also note that some people have both a pattern hair loss as well as a diffuse or generalized decrease in density. These people may well have both conditions simultaneously but still require a complete medical workup, normally with lab tests and biopsy.

Comprehensive Medical Workup for Diffuse Thinning and Hair Loss

A) **LAB:** CBC, Free T3 and T4 (thyroid), Ferritin, Total and Free Testosterone, SHGB, and Estradiol, DHEAS, Prolactin, RPR, TSH, IGF-1, DHT, Progesterone, ANA

B) **SCALP BIOPSIES:** Vertical and horizontal sections

C) **OFFICE TESTS:** Hair-pull test, hair window, KOH prep, bacterial and fungal culture and sensitivity

Medical Treatment – Male Pattern Hair Loss

For optimal chemical therapy for male pattern hair loss, the combination of Rogaine Foam and Propecia (1 mg per day) is the place to start for men with early thinning and miniaturization of their hair (in a pattern as described in the chart). When you see your dermatologist or hair restoration specialist, he will prescribe these items for you. These are generally tried for several months, following which a second set of detailed photographs are compared with the ones taken at your initial evaluation. If you have either stayed the same or improved, that is a win for the medical therapy.

You may very well need hair transplants in addition (to restore your hair), but you may have stopped the progression of the balding process with the medical therapy. For example, sometimes younger men have their hairline transplanted where it has receded in the front, but the medical therapy keeps the back from falling out for many years. The nuances of this should be discussed with your physician. Remember that Rogaine must be used twice a day to be effective.

Propecia's generic name is finasteride, and it blocks conversion of testosterone to dihydrotestosterone in the hair follicle to the extent of about 70%. Another prescription drug which has not been fully studied in hair loss is Dutasteride, and it may well block the levels of DHT in hair

follicles down about 90%. However, with Dutasteride, you are blocking both isoenzymes of 5-alpha reductase, and the systemic side effects have not been worked out. As men get older, side effects from these medications may present that were not present in their younger years. You need to speak to your physician about the details of this.

Men may suffer generalized hair thinning (sometimes with pattern loss also) from low thyroid or iron, as do women. In older men, low testosterone can cause thinning of the donor area on the sides and back of the head. The donor, like beard and body hair, needs testosterone. See the discussion in the appendix.

Medical Treatment – Female Pattern Hair Loss

For female pattern baldness, the true causes have not been worked out well. We believe most commonly that it is caused by the small amount of male hormone, or testosterone, in women, but there probably are other causes. After menopause, it is common for women to show generalized hair thinning due to lower levels of female hormones, as well as the loss of the ability to convert T4 to T3 in the tissues, which results in a low thyroid-type of thinning hair, as well as thinning of the outer third of the eyebrows.

T4 is the principal type of thyroid hormone produced in the thyroid gland. T3 is the more active form, which is converted from T4 in the tissues. As we age, the ability to convert T4 to T3 in the tissues diminishes so that many people that are in excess of 50 years old have a normal T4 level in their blood, but have diminished T3 in the tissues. This is determined by measuring the free T3 hormone blood level. Even if you are at the lower end of the normal range, your hair may well benefit from additional thyroid supplement, which will bring you up to the high end of the normal range. This also results in greater energy, higher metabolism, and usually some degree of weight loss. Blood workup is required, as well as a thorough evaluation by your physician. Other

causes of diffuse thinning are iron deficiency, collagen disease, infectious disease, and other hormone deficiencies.

The principal medical treatment for female pattern hair loss (the three Ludwig patterns as well as the Christmas tree pattern in Fig. 1) is female Rogaine used twice a day, with some improvement in about 30% of cases. Generally, assuming there is a good and thick area of donor hair in the back of the head, women can be transplanted one to three times in the area of thinning to recover decent density. Sometimes the thinning goes over the sides and back of the head so that there is insufficient donor hair to do all of it. Generally, in these cases, if one starts at the hairline and transplants back to the apex (or highest point of the head), a drastic improvement in appearance will be had by adding several thousand hairs to the top of the head. It is not necessary or possible to transplant the entire thinning area in many women.

Laser Treatment of Hair Loss

In the last few years, “so-called” lasers (actually a group of red light diodes) have been introduced to the hair loss treatment market as a means of encouraging hair growth, much like minoxidil solution might. Our offices have had the large type of cold laser for three years and we offer it to our patients without charge.

Another type of laser is a hand-held, “comb” style. There is no scientific evidence that these facilitate hair growth.

There is evidence of physiologic changes in the hair follicle. Many people have anecdotal stories that these may help. Our experience has not proven any benefit, but also no detriment. They are used by some doctors after transplant to supposedly encourage hair growth.

History of Hair Transplantation

The history of hair transplantation began in 1931 in France, where French surgeon Passot moved some hair from a thicker area to a bald area, creating what is thought to be the first hair transplant.

In 1939, Japanese hair researcher, Dr. Okuda published his results in a Japanese medical journal regarding his technique of inserting small hair-bearing grafts into needle-stick recipients to fill in defects in eyebrows. This publication was lost during the Second World War and later discovered in the 1970s.

Dr. Norman Orentrich, a prominent New York dermatologist and researcher, reinvented the process of hair transplantation in 1956. At that time, he was doing some experimental work on skin grafts, and noticed that the hair in the grafts grew after the grafts had been transplanted. He developed a theory of donor dominance, which states that the thicker hair from the donor area will remain thick once it has been moved to the formerly bald area. In the 1970s, many physicians began doing punch graft hair transplants using 4-mm round plugs taken from the thicker donor area in the back of the head and transplanting it to the balding area on the top of the head. The author began this in 1971, while a dermatology resident. At that time, a great deal of skill was required to get these plugs to look natural. Unfortunately, many physicians tended to plant them more or less like trees so that you could easily see the plugs sticking up on the top of the head and, of course, this produced an unnatural appearance. The correct way to place plugs was to have them exit the

Micro-Plug Hair Transplant 1985 - Dr. Elliott

Before

1/2 done

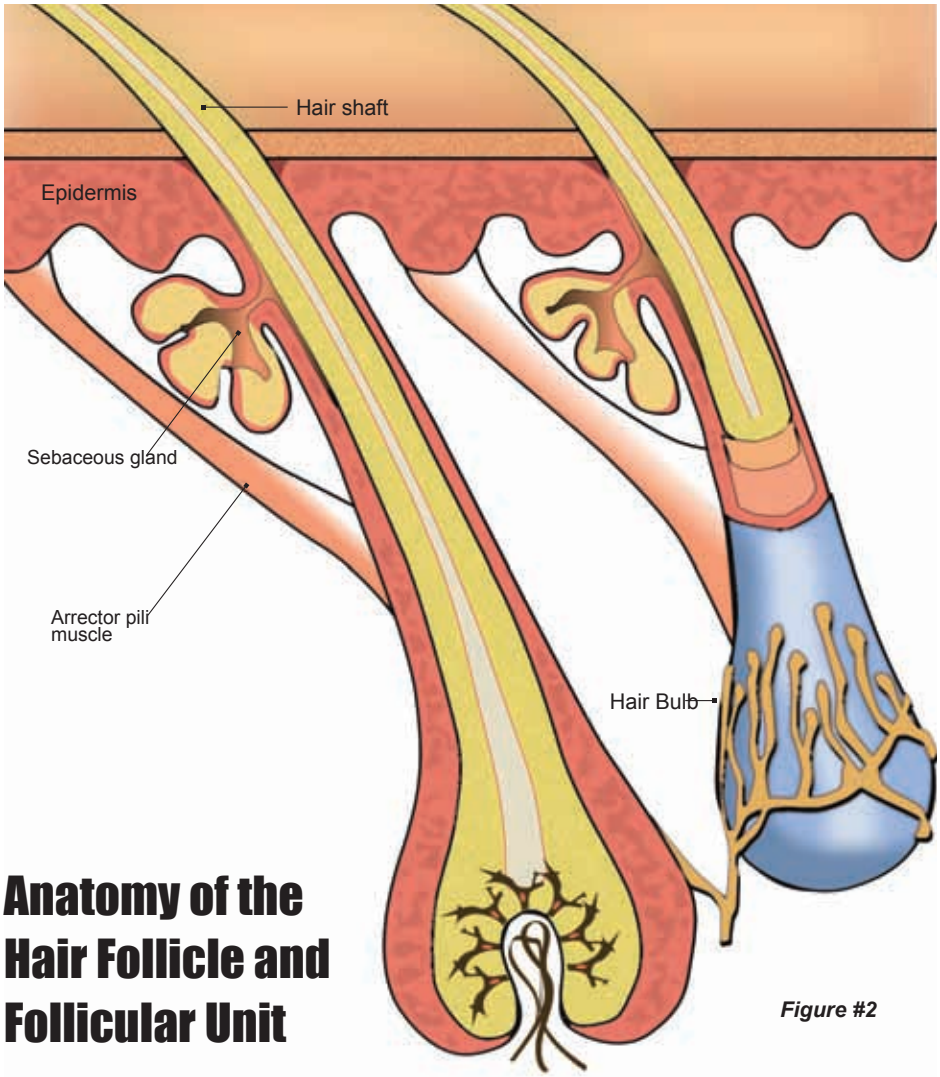
1/2 done

After

After

skin at about a 30-degree angle so the hairs overlapped each other like the shingles on a roof. In this way, when the hair was combed, it looked good, but not as good as using much smaller plugs for the hairline. In 1981, the author custom ordered a number of sizes of very small punches down to 1.0 mm. These punches could harvest down to one or two individual hairs and, at that point, it was possible to create a natural-looking head of hair using the plug technique, properly angled, and properly designed. Photographs on previous page are from of a case in 1985, showing this technique used on one of the author's patients.

In the 1990s, the method of donor harvesting (various sized, round plug grafts) evolved into using a strip, which was closed in a thin line. The donor strip was then carefully dissected under microscopes and/or high-power magnification to create small follicular unit grafts containing one to four hairs in one follicular unit.



Anatomy of the Hair Follicle and Follicular Unit

Figure #2

The hair follicle is a complex but small organ, which contains nerve fibers and blood vessels around the actual hair follicle. (fig. 2) About 80% of hair follicles are paired (or come in clusters of two). The rest are either singles, triples or an occasional quad. These clusters are called follicular units because they share a common blood and nerve supply. When doing hair transplantation and dissecting the donor area, it is important not to cut these follicular units apart because this generally results in miniaturized transplanted hairs rather than the full-sized hairs that are desired.

Figure #3 **Scalp Hair : Types**

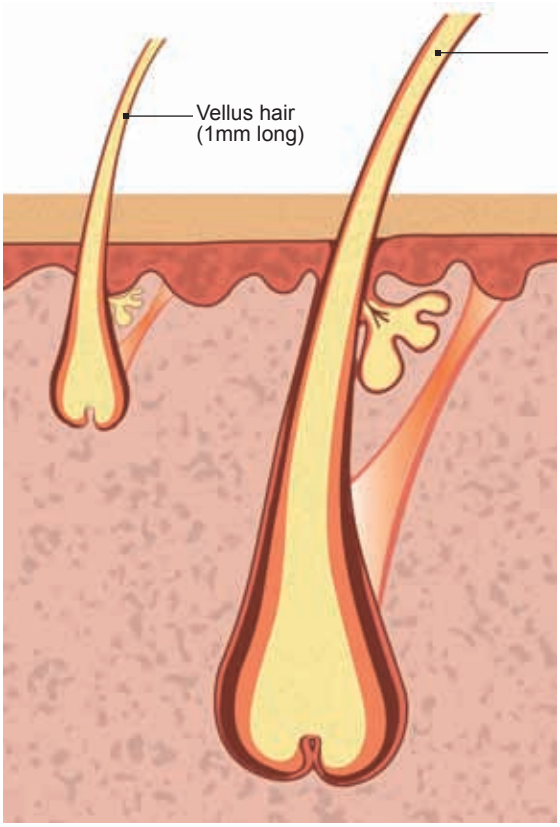


Figure #3 shows the two general types of scalp hair, the full-growing, long terminal hair, which is what is desired in hair transplantation, and the velus (or miniaturized) hair, which is not long enough to really be of any benefit in solving a hair loss issue.

Figure #4 **Time-lapse : Miniturization of hair follicles in baldness**

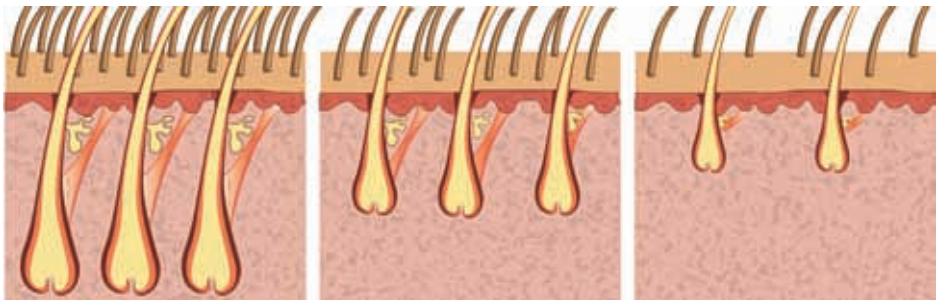


Figure #4 shows the gradual miniaturization of hair follicles in male or female pattern baldness. Note that this process takes a few to several years.

Human Hair : Growth Cycle

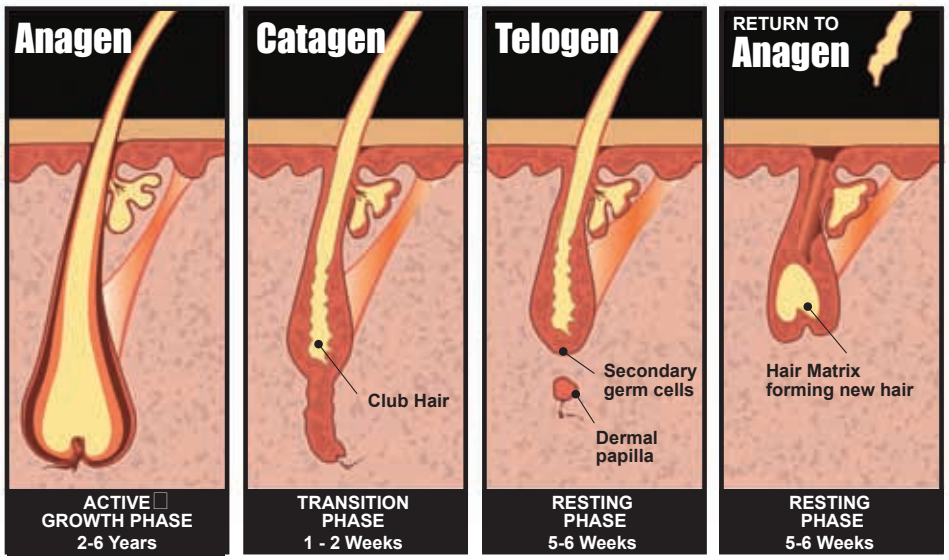


Figure #5

Figure #5 illustrates the four growth cycles of a human hair. Note that human hairs generally grow for two to six years in the anagen phase, and then shift into the catagen and subsequently telogen phase. The telogen phase usually lasts about three months. During telogen, the hair becomes loose and falls out, and the hair follicle withers up and virtually disappears. This is shown in the illustration of return to anagen. Miraculously, after about three months, the hair follicle regenerates (from the interaction of two types of stem cells, those from the bulge area around the sebaceous gland, and others from the epithelial layer.) Following this, the regenerated hair follicle generates a new hair, which then grows for two to six years.

Figure #6 illustrates the differences in hair shaft characteristics between straight hair, curly hair, and very curly to wooly hair. Note it is simply a difference in the shape of the cross-sectional area of the actual hair shaft. In other words, round hairs generally grow straight, and oval hairs grow in various degrees of curliness.

Hair Fiber : Characteristics

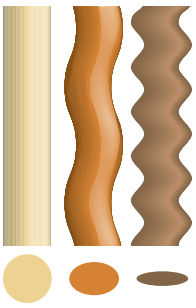
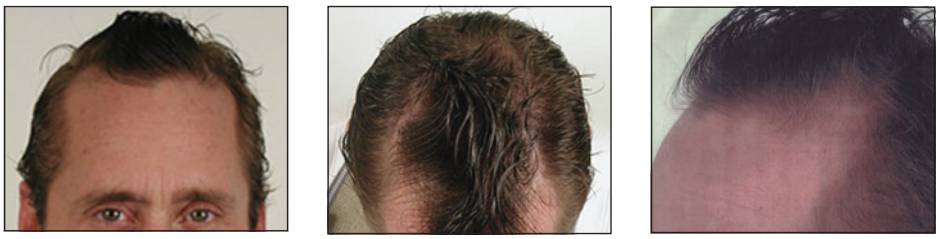


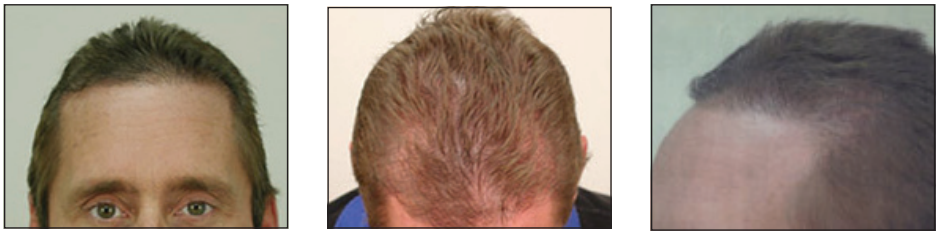
Figure #6

Why Transplanted Hairs Grow at Three Months

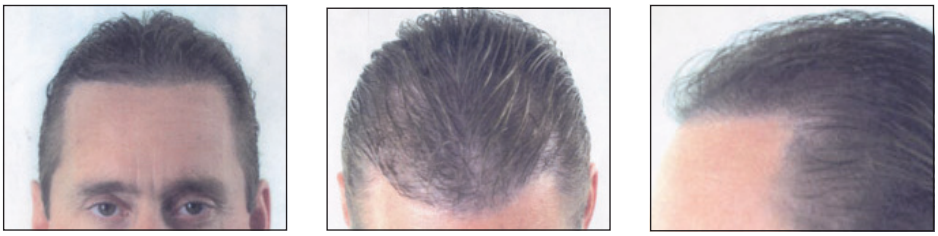
Now you have learned from Figure #5 above the details of the hair growth cycle. You have learned that the resting or telogen phase generally lasts about three months. When hairs are transplanted in a hair transplantation procedure, the small hairs in the hair grafts (which are about 1-2 mm long), will generally fall out within the first two weeks following the transplant. Those hair follicles then go into the resting or telogen phase for about three months. At the end of three months, the hairs will grow out.



Before



After 5 months – Two months of growth



After two procedures - Two years out

How to Encourage Grafts to Grow Before Three Months

Currently our offices are in experimental trial with a thyroid (T-3) spray-on solution. This solution is applied after transplant surgery. Several cases have begun to grow at one month post-op. These are preliminary results. Some physicians recommend the cold laser for this.

Follicular Unit Transplantation (FUT) Versus Follicular Unit Extraction (FUE)

In general, hair transplants in 2010 are done in either follicular unit transplantation or follicular unit extraction, otherwise known as FUT and FUE. Follicular unit transplantation is the method whereby a strip of donor hair is removed from the back and/or sides of the head, usually about the width of your little finger, with the length determining the number of grafts. For example, a 1-cm wide strip will usually generate about 100 grafts per running centimeter. If 1000 grafts are needed, the strip would need to be 1 cm wide and 10 cm long, etc. The donor strips are taken from the nuchal ridge, around the sides and over the ears in the very thickest and best permanent donor hair area. The experience of the physician determines where this should be, neither too high nor too low, so that the fine line scar is hidden regardless of future progression of pattern hair loss. Typically 3000-5000 grafts are harvested in one session.

FUE is a method where 1mm plugs are extracted and used as transplant grafts. Some physicians claim that there is less donor scarring with FUE versus FUT. This is incorrect.

FUT	2500 grafts taken twice tesulting in a donor scar 2mm wide x 350mm long = 700 mm^2 for 4000 grafts	**FUE**	400 1mm round scars resulting in donor scars .5 x .5 x 3.14 x 4000 = 3(40mm^2) for 4000 grafts

The scar area is much larger with FUE than FUT.

Evaluation for Follicular Unit Transplantation (FUT)

When you are seen by your hair restoration physician, he will evaluate the size and progress of your hair loss. You may be in an early stage in which you will require several treatments over many years, or in a late stage where you are nearly fully bald and require one or two treatments over one to two years. Generally, the number of follicular units that can be transplanted per session vary from about 20 to 40 follicular units per square centimeter of baldness. A practical method which allows for transplanting most of the head in most cases is one in which the physician's objective is to install about 20 follicular units per square centimeter of baldness on the first session, with a second session of similar density about one year later. The one year delay is necessary to allow the donor area to relax and loosen up so that a similar strip can be taken in the same place as the previous one, thus removing the old scar and leaving only one fine-line scar. With good surgical technique, it is frequently possible to even do a third procedure in the same area, still leaving only one fine-line scar.

Evaluation for Follicular Unit Extraction (FUE)

Follicular unit extraction is a method whereby small individual 1-mm plugs containing one hair follicle are extracted from the scalp in a scattered area throughout the donor area. This is the advanced version of the original plug technique. These individual rounded single-hair or single-follicular unit grafts are then inserted into very small incisions on the top of the head. This process is much slower than follicular unit transplantation, and generally physicians charge at least 50 to 100% more due to the excessive time involved. The limitation is around 500 to 800 grafts per session, so several sessions would be required to do a substantial area in transplantation. Initially, the proponents of this method thought that the small extracted round grafts would heal with no scarring. Unfortunately, that turned out not to be correct, and thus the result is a donor area peppered with small 1-mm round scars. The sum

of the area of the scars with this method is far more than the sum of the area of a fine-line strip scar, so most authors believe that follicular unit transplantation using the strip donor method is the first choice, with the FUE method as a backup selection in cases where people have had substantial old scarring in their donor area in years gone by, or for other reasons in which the donor might not be suitable for strip excision.

In very extreme cases where there is insufficient good terminal donor hair on the head and it is necessary to resort to using body hair as the donor, the FUE method is the best choice. Note that these cases are extremely rare and not optimal.

Treatment of Various Patterns

Generally, there are two main groups of hair loss by age. One group is more severe hair loss which usually begins in the twenties and frequently progresses to a 6 or 7. The second group generally begins in the forties or fifties, and progresses to a 5 to 6 by the time life ends. Dr. Otar Norwood, an early researcher and hair transplant specialist/dermatologist from Oklahoma City, believes that all men would eventually go to a Class 6 if they lived long enough. In between the two main groups, there are other individuals who fall into the other age groups. A particularly difficult group is boys who begin to lose their hair in their mid-teenage years and become bald by their early twenties. These individuals are a different genetic makeup and frequently have early heart disease as well. Persons presenting with this picture should be evaluated for the various cardiac risk factors to help them in their future life.

Progression of Hair Loss

Generally, a young man who is a Class 4 by age 21, will probably be a Class 6 by age 30. Sometimes there is not enough donor hair to transplant the entire head once one projects the size of the future balding area. Nevertheless, there is usually enough donor hair to do at least the

hairline and the entire top of the head, leaving only a round spot in the back if there is not sufficient donor hair. If years go by and it turns out there is sufficient donor hair and the pattern is not as rapidly advancing as was suspected, at least a modest coating of hair can be put in the crown or vertex of the head, which will present a very nice appearance.

Younger Men

When beginning transplanting on young men, it is best to decide where to put the hairline based on previous pictures, family pictures, and with the sense of what will generally make the person look the best. It is not necessary to create a receded hairline look in young men. There are plenty of men who have non-receded hairlines well into their sixties, the author being one. Note the pictures of the author's grandfather, Dr. J.T. Waggener, from age 28 to age 100. Dr. Waggener's hair was quite thin at age 100, but with very little hairline recession present. Note that this thinning of old age is what is termed **senile alopecia** and probably results from the falloff in thyroid hormones and growth hormones in later life. It is **not** male pattern hair loss. If a young man has lost his hairline, it is not too early to put him on medical therapy and rebuild his hairline with a hair transplant.

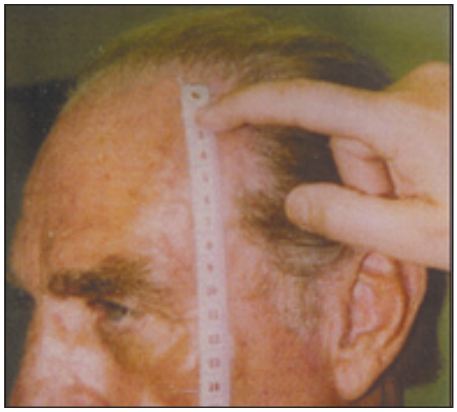
John Todd Waggener, MD

Age 28

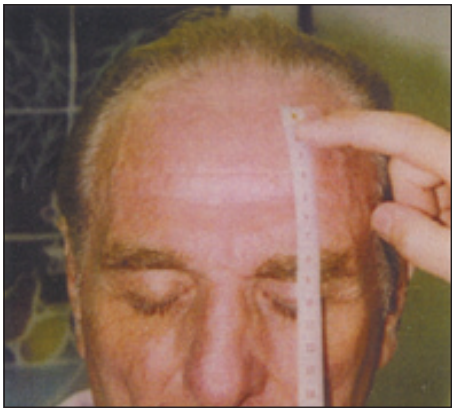
Age 62

Age 85

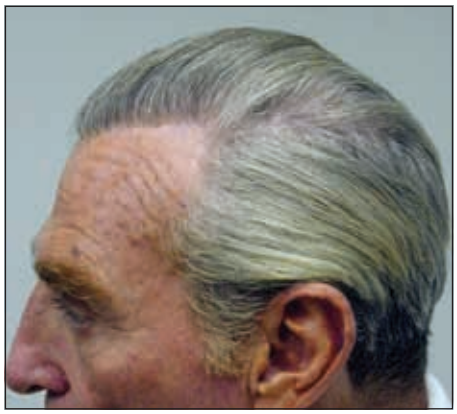
Case Presentations



Before



Before



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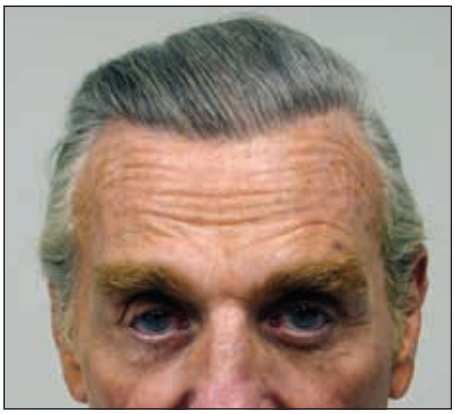
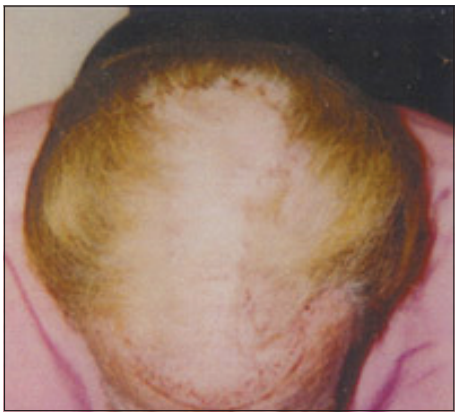


Case Study: Pattern 5
Patient : J.D.

This patient has had two full hair transplant sessions plus one touch up procedure totalling 3,488 grafts

Case Presentations



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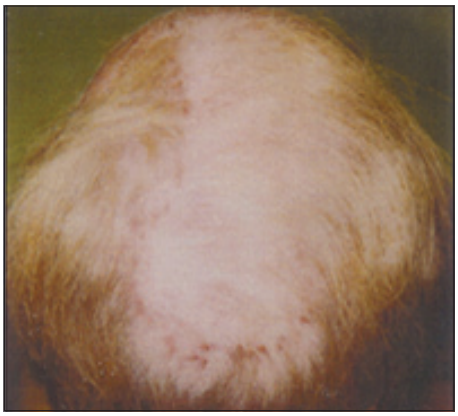

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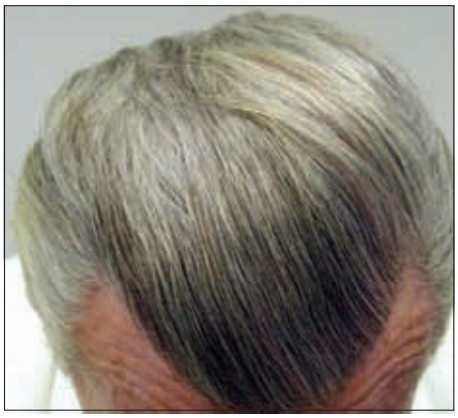

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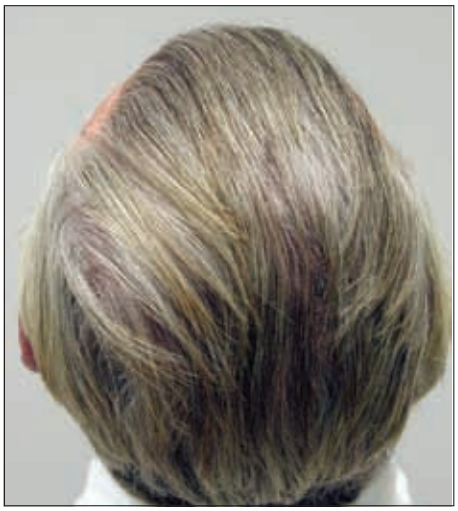
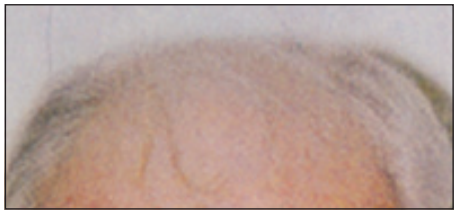


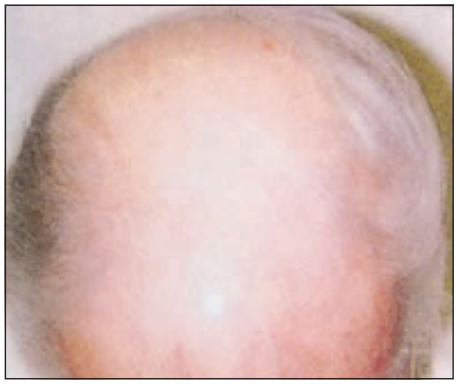
After

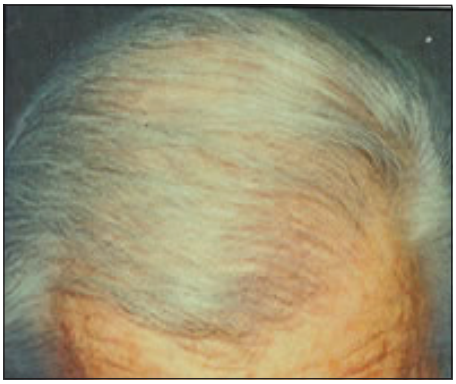
Case Study: Pattern 5 (cont'd)
Patient : J.D.

This patient has had two full hair transplant sessions plus one touch up procedure totalling 3,488 grafts

Case Presentations

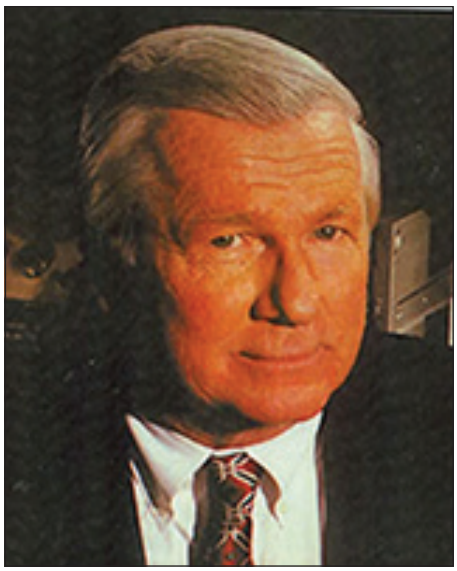






Before (top & bottom)

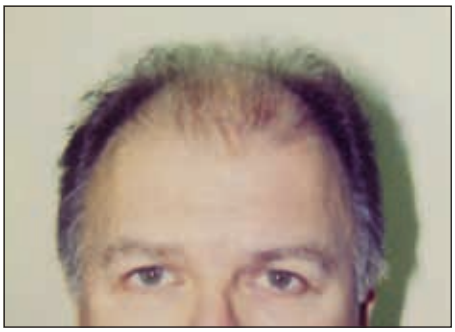
After (top & bottom) one hair transplant of 2,405 grafts



Case Study: Density
Patient : J.S.

The patient had two hair transplants of 4,894 grafts. The photo (right) was taken 14 months after second surgery; hair has reached maximum density.

Case Presentations



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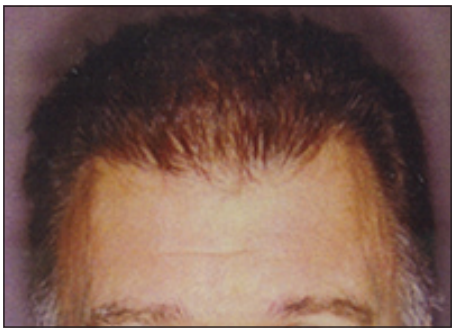
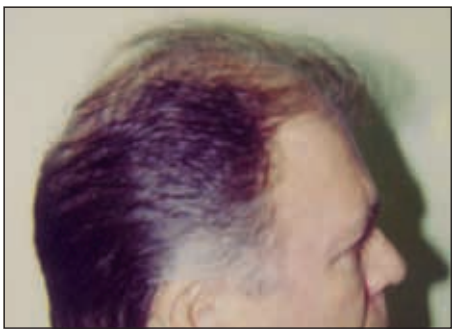
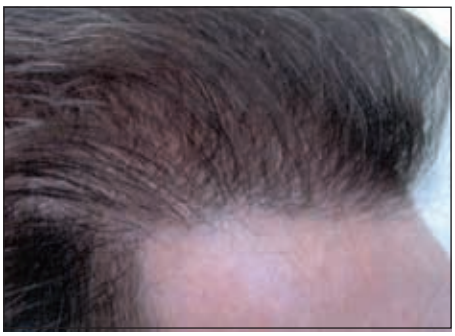


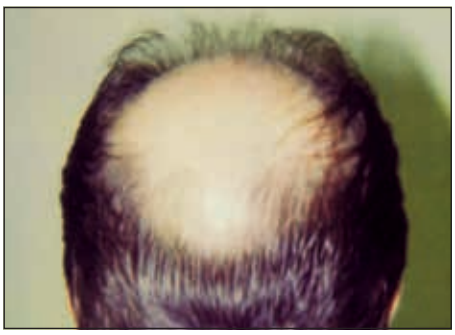
After 2100 grafts



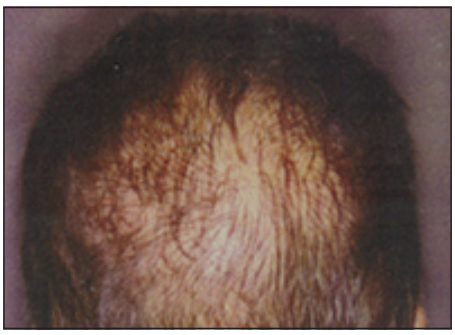
Before



After 2100 grafts



Before

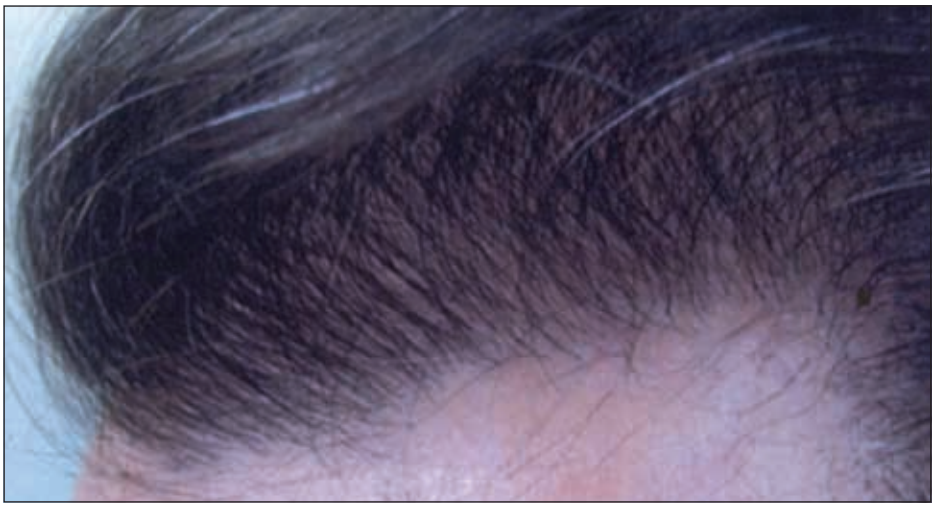


After 2100 grafts

Case Study: Pattern 5-1/2
Patient : R.A.

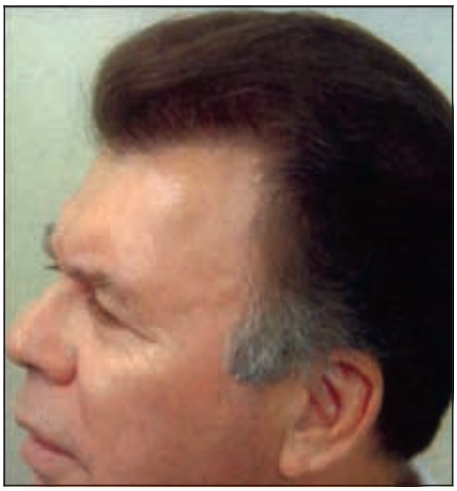
Patient before (left) and after (right) one hair transplant at six months. This patient had one session of approximately 2,100 grafts.

Case Presentations



After 4200 grafts

After 4200 grafts



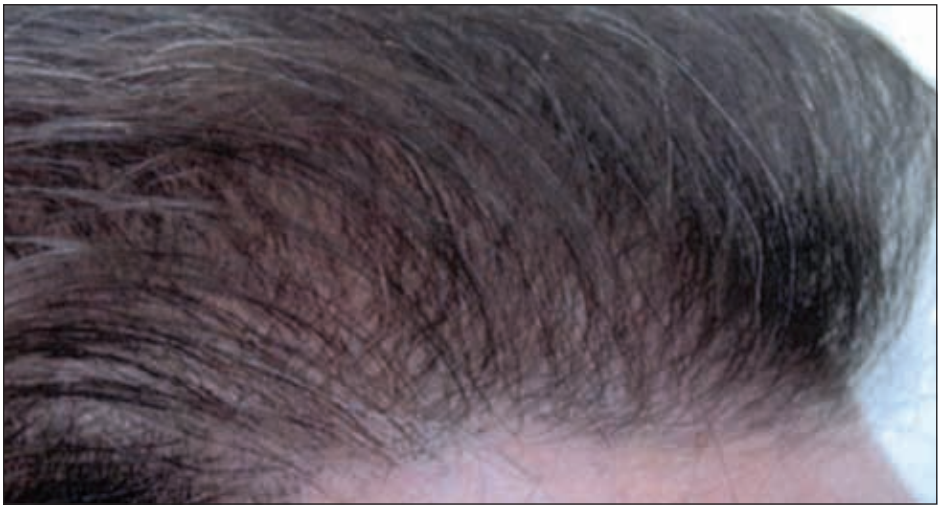
After 4200 grafts

Case Study: Pattern 5-1/2

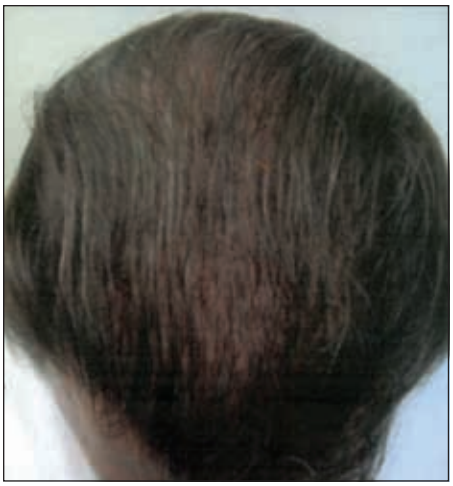
Patient : R.A.

Patient after 4200 grafts

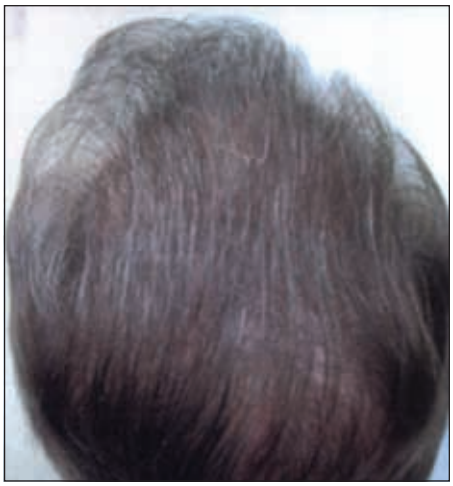
Case Presentations



After 4200 grafts



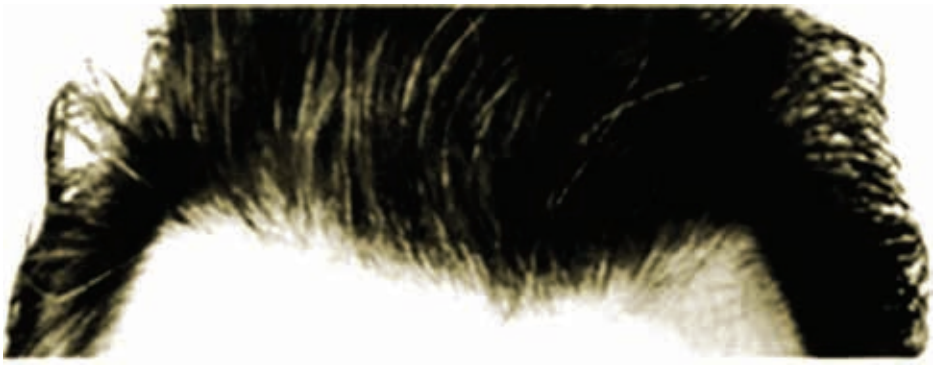
After 4200 grafts



After 4200 grafts

Case Study: Pattern 5-1/2
Patient : R.A.

Patient after 4200 grafts



Patient R.A.

Hairline Design

Probably the most important part of the hair transplantation process is the design of the hairline. The objective is to make the person look as good and as natural as possible, whether male or female. A hairline that is slightly lower in the center of the forehead and slightly rises towards the corners will give the most attractive appearance for most men with oval heads. For men with wider brows and a more flat forehead, a more straight-across hairline will generally look the best and is commonly found in some Asian and Hispanic head shapes. This is frequently seen in Japanese and Korean persons, but note that Chinese and Vietnamese head shapes are more oval and similar to the typical Caucasian head shape. African head shapes can approach either the oval or the more flat brow appearance. Generally a female blush with rounded corners of the hairline is created when replacing a female hairline. This is especially true in women who have lost hair in the typical male 3 pattern, frequently occurring after menopause.

Process of Hair Transplantation at Pacific Hair Institute

The process and methods used for hair transplantation vary amongst physicians. Pacific Hair Institute Physicians, (including the author for 38 years), have developed certain techniques which they feel to be the best. The following are the steps in the author's hair transplant process:

1) The first thing to do when you present to the office for a hair transplantation is to go through the paperwork with your physician, including the postop instructions so that you have a thorough understanding of what is going to happen. This includes deciding what prescriptions you would like after the procedure, sending a copy of your medical insurance card to the drugstore along with the prescriptions for delivery later in the day and, of course, reviewing the consent form and settling your account.

2) The second step is to review what old photographs you have brought with you, and design the hairline. The doctor will take considerable time working with you to come up with the perfect hairline design that will please you the most. This is done by drawing with eyebrow pencil on your forehead where you would like to see the hairline, and then reviewing with a mirror. Adjustments are then made until perfect.

3) Since the hair transplant process is done in a comfortable chair similar to an airplane first-class chair, in a reclining position, you will need to pick out a couple of movies from the selection at the office (or decide you will read a book, listen to music, watch satellite TV, or bring your own movies). The procedure rooms have large plasma screens for your entertainment.

4) The next step is to take a small amount of preop medication such an antibiotic and Valium, which will relax you and counteract the agitative effects of the anesthetic you will receive. Time is then allowed for the preop medications to begin working.

5) The next step is for the doctor to select the proper donor area, which will provide a sufficient number of grafts for your proposed transplant. Note that the number of grafts required have already been worked out at your previous consultation. Please remember that it is important to have a thorough consultation with your hair transplantation physician at least one week prior to the actual surgery. In this way, a complete history can be taken, you can be taken off whatever medicines you might be on which might cause difficulties in the transplants (such as bleeding from aspirin), and the design and measurements of the area of balding can be taken so that the number of grafts can be projected. Do not expect to have a consultation and a hair transplant on the same day, as this is not a practical or prudent idea.

6) Once the selected donor area for the strip harvest has been shaved and painted with Betadine or other antiseptic, your physician will anesthetize the area. In the author's offices, this is done with four to six nerve blocks along the length of the donor area where the principal nerves lie. A vibrator is used to block the feeling while a very small injection is placed. After that, several minutes elapse while the injections soak in to the nerve areas, thus anesthetizing the donor area. Once the donor area is asleep, a little additional anesthesia is put in, another 10 minutes or so are allowed to elapse, and the donor area is ready for harvesting.

7) The next step is to place the patient in a prone position (on stomach) on the operating chair which has now been made flat, with the headrest to hold the forehead. Once you are in a comfortable position, the donor process can begin. If the donor strip is just on the back of the head, the harvesting will be done with your head centered, but if back and both sides are necessary, the doctor will turn the head to one side and harvest the right side and back first. Once this has been harvested and sewn up with a careful plastic closure (which will result in a very small scar), the head is rotated

to the other side and the left side is harvested. All of this takes about half an hour. There is absolutely no discomfort or feeling as the process goes on because the area has already been 100% anesthetized. There is also not any significant bleeding because the infrared cautery is used to eliminate any bleeders that are encountered. Once all bleeders have been eliminated, the dry field is sutured. If there was a previous donor scar, it is removed. In cases of severe widened old scarring from transplants done in other offices, your doctor can remove those widened older scars as part of the new strip, thus resulting in much less scarring when done. Of course, when removing old scars, it is not possible to harvest as many grafts. The secret to success for small scars in donor harvesting is a long but narrow strip being removed, and closed under no tension. A short but wide strip will almost always result in a widened scar, thus the design of the actual strip layout is, as in the hairline, extremely important.

8) Once the donor has been harvested, the strip will go to the cutting area where it will be dissected into follicular unit grafts under microscopic and high-power magnification. Two to six well-trained surgical technicians will perform this cutting task, depending on how large the case is. For example, a case of about 2000 grafts would probably require three technicians. The dissection process will take one to two hours. During this time, lunch will be ordered in for you and the staff. You will select what you wish to eat from a variety of menus, and have about a one and one-half hour hiatus before additional procedures are done. During this time, you can walk around, watch television, go to the restroom, or whatever you wish to do.

9) After your hiatus, your physician will place anesthesia in the hairline design (which you have previously drawn out working with the physician). Again, the vibrator will be used to block the feeling while the anesthesia is put in, in about four spots to block the

front of the head. Note that once the donor area and the hairline and front of the head are blocked, you have a ring block going all the way around the head. This ring block will completely block all the feeling in the top of the head. Once in a great while there is a small perforator nerve that comes through the skull to the center or top of the head. This is taken care of by the anti-swelling solution.

10) Once the anesthesia is fully instilled and the recipient area is anesthetized, the physician will instill the anti-swelling solution. This consists of an anti-inflammatory steroid, a small amount of local anesthetic, and in some cases, a small amount of epinephrine. This will puff up the scalp for a few minutes. After the anti-swelling solution has been absorbed, it is time to make the recipient sites.

11) Using high-power magnification, your hair transplant surgeon will next make the recipient sites using custom-cut blades of 0.6 mm to 1.2 mm in size. While he does this, an assistant will count using a special electronic counter. Obviously one must have the same number of recipient sites as grafts. The final graft count will be determined at the end of the dissection process, and if there are extra grafts, as there usually are, the physician will make extra recipient sites to receive them. The recipient sites are made by the physician in a precise pattern, which will determine the direction that the hair grows out (once it begins growing). This is an extremely important part of the process, as the hair direction is constantly turning on the top of the head. The author has seen some hair transplants where inexperienced physicians placed all of the hairs pointing forward, and this will result in an unnatural appearance. Hair direction must match up in one corner of the hairline with the existing hairs on the side of the head and gradually rotate to match up with the hair direction on the other corner on the other side of the head. Similarly, the hair in the whorl (back of the head)

Patient ready for donor harvesting

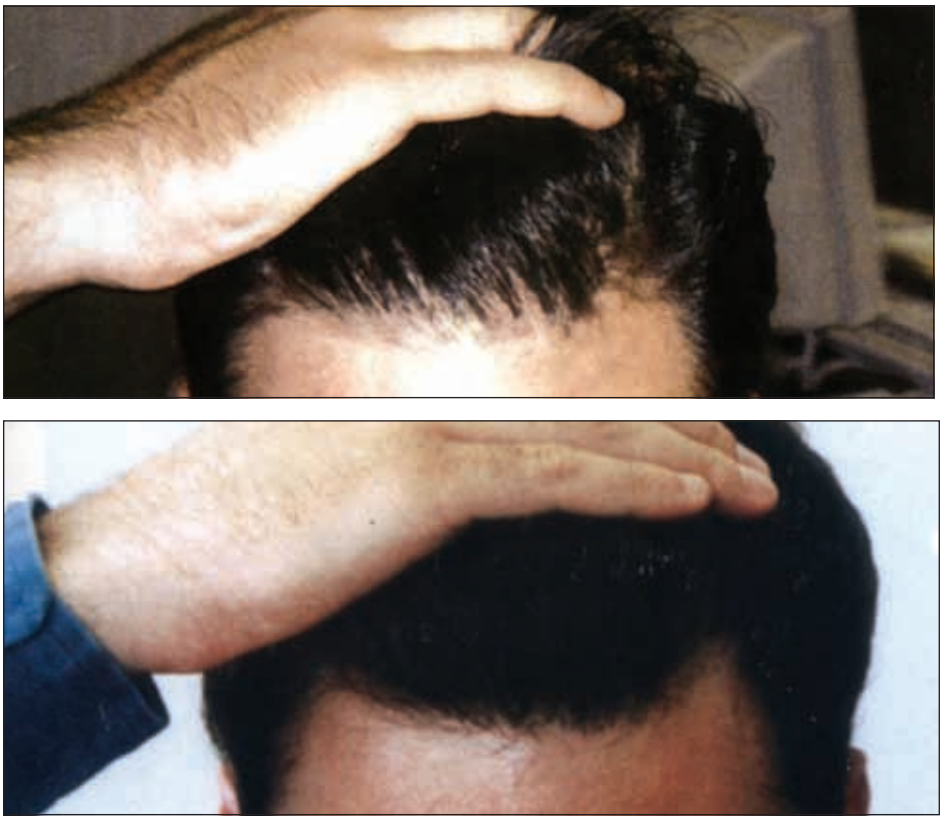
must follow the original radial pattern, or a newly-created radial pattern in cases of severe and complete baldness. It usually takes about an hour for the hair transplant physician to make all of the recipient sites in a typical case of 2000-3000 grafts. As the recipient sites are being made with one hand, the surgeon is placing pressure on the other areas previously done to control bleeding. It is not necessary to cut the hair short, as the left hand can move the hairs out of the way as the right hand makes the recipient sites. It is important that the surgeon be ambidextrous for this process.

12) Following the surgeon's making of the recipient incisions, it is time for the installation of the grafts. The surgical techs have been cutting the grafts for about two to three hours now, and they should be about finished. Having placed hundreds of thousands of grafts each, they are now ready to install the grafts with one person working in the back of the head, and two others working on the right and left sides. Simultaneously working as described, the

surgical techs, under the supervision of the surgeon, can install 2000-3000 grafts in about 2-3 hours. Of note is that a soft scalp can have its grafts installed more quickly than a firm scalp, and there are other variables.

13) Once all the grafts are in place and adjusted to the proper position, (which is slightly puffy and not completely flat), the doctor will review the overall placement and review the postop instructions. The postop instructions will also be put on the plasma screen in front of you for your review. If you want to view them now, you may go to the Pacific Hair Skin & Laser Institute website and click on “Postop Instructions” on the video selections.

Revising Old Plugs



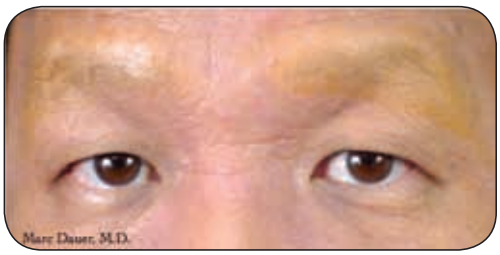
Patient J.A. after five hair transplants of 200 round "plug" grafts done elsewhere (top) and after four transplants of 558 grafts by Dr. Elliott to refine the "plug" look (bottom).

If you have had old transplant plug grafts which are not angled well and are readily apparent, and assuming you want more density because you may have lost more hair over the years, and you would like a properly designed natural hairline, this usually can be done (with sufficient donor hair). Most of the time, it is possible to harvest a strip through the little white scars of the old plug donor areas. We simply dissect the white scars out of the strip once we have it removed. It is usually most important to build a new hairline slightly in front of the old plugs so they are hidden and no longer seen. Similarly, it is frequently necessary to fill in along the part lines or in the crown of the head with the new smaller grafts to hide the appearance of old plugs from the back.

Eyebrow Hair Restoration

Marc Dauer, M.D., ABHRS

Eyebrows are one of the most important defining characteristics of the face. Often you don't even realize the full impact that eyebrows make until you see a person without them. With the Follicular Unit Transplantation, it is now possible to restore natural looking eyebrows that will last a lifetime.



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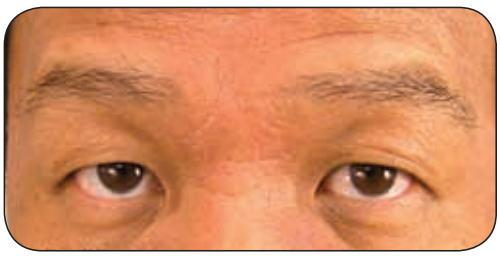
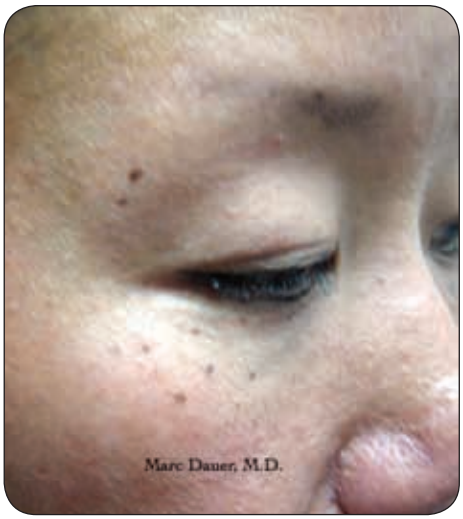
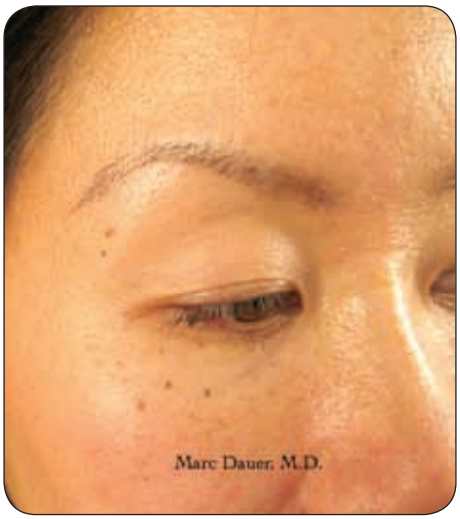


After

Eyebrow hair loss can occur for several reasons. Physical trauma (such as burns or lacerations), medical treatments (such as chemotherapy or radiation therapy), excessive plucking, and even menopause, can all contribute to eyebrow hair loss. In the past some people opted for eyebrow tattoos to recreate lost eyebrow hair. Eyebrow transplants can be implanted over eyebrow tattoos to recreate natural looking eyebrows.

The hair to be transplanted into the eyebrows is usually harvested from either the the back of the head in the middle of the scalp or just behind the ear. In both cases the hair in these areas is of finer quality, thus more accurately resembling natural eyebrow hair. The hair may be harvested with the traditional "Strip Method" or by Follicular Unit Extraction, which involves removing the donor hairs individually one at a time. With an artistic eye, and keen attention paid to the individuals facial characteristics, the boundaries of the new eyebrows are drawn in so that the patient can see the shape of their new eyebrows. Once the design is completed, the area to be transplanted is anesthetized with lo-





Before ***After***

cal anesthetic. Small recipient sites are then made with custom blades measuring approximately .5mm. Only single hair grafts are placed in the eyebrows and special care is taken to angle the eyebrow hairs as flat as possible to the skin surface and in the proper direction based on the location of the eyebrow hair. Since eyebrow hairs change directions acutely depending on where in the eyebrow you are located, this process requires a strong attention to detail to be performed correctly.

After the eyebrow hair grafts are placed, within 24 hours they form small scabs that appear like tiny grains of sand. Within one week most of these scabs fall off. Rarely there is a small amount of swelling around the eyes which usually resolves in 2-3 days. Some of the grafts may remain and start to grow, but usually most of the grafts fall out within 4 weeks and then begin to grow again in 8-12 weeks. Since the donor hair comes from the scalp, the hair will grow longer than standard eyebrow hair and will require periodic trimming. For most people this is a minor inconvenience given the prospect of having new eyebrows.

How to Select a Hair Transplant Surgeon

The first step is to go to the website of the American Board of Hair Restoration Surgery and select from the many diplomates of the board. Once you have decided on one or two surgeons, schedule a consultation and let the doctor evaluate your case and make recommendations.

Generally you will find that a Class 2 requires about 2000 grafts, a Class 4 about 5000, and a Class 6 around 8000 total grafts. Obviously the numbers depend on the size of the balding area and the availability of donor hair. In some severe cases of baldness, it may be necessary to leave a small bald spot or a thinly covered bald spot in the back of the head.

The doctor will be able to design something that will make you look good and fulfill most of your wishes for having a head of hair. Fees generally run in the range of $3.00 to $8.00 per graft, depending on the number of grafts and the location and individual charging practices of the medical group. Remember, however, the best physicians are not necessarily the ones that charge the most.

Do not be afraid to seek a thorough evaluation with the best physicians you can find. This is true not just in hair transplantation but in almost every field of cosmetic surgery or cosmetic medicine.

Today there is also long-term financing available through three or four different financial institutions for almost every type of cosmetic surgical procedure. Your doctor will be able to point you in the right direction for that. Otherwise, you can simply pay with cash, check, or credit card.

Lastly, remember that it will take six to ten months for your hair to grow out and look good. As you remember, the hair will not grow for the first three months, so at six months, you have three months of hair growth. Hair grows on the average at about one-half inch per month, so expect about 1 ½" of hair after six months. You can do the math on the growth and, of course, there is variation depending on individuals. When transplanting into areas of old scar, it may take six or eight months for the hair to begin growing due to the limited blood supply.

Signal Molecules and Mineral Ascorbate Containing Media for Human Hair Follicle Cloning

R.C. Dana, D. Kong, M. Elliott, J. Yang, and E. Suponeva-Dana Committee for World Health, Foothill Ranch, CA 92610 and **Elliott and True, Newport Beach, CA rdana@cworldhealth.org**

Abstract

Hair follicle cells continuously cycle in the skin and are rapidly renewed from plastic stem cells. The mechanism for the formation of a hair follicle is not understood, however, signaling molecules and growth conditions which include mineral ascorbates have recently been identified which together control stem cell migration, follicle development, and maintenance. We are developing an in vitro system to better understand how the different signaling molecules work synergistically to produce a hair follicle. We recently found that we can increase the life-span of hair follicle cells by adding repeated telomeric DNA sequences to chromosome endings and are starting to determine how the signal molecules regulate cell proliferation and differentiation. Using both surgically-removed follicles and anagen hair follicle sheath, papilla, fibroblast, and bulge regions, we are transfecting cells with different constructs, including hTERT (Geron) which has been engineered with a TET on switch (Clonetech). The goal of these studies is to have a sufficient number of cells for regulation studies and to better define conditions for gene delivery and gene regulation, and produce cells which can be used therapeutically to restore hair follicles.

Introduction

We have been introducing inducible plasmid constructs (Geron Corp;Clonetech) into hair follicle cells which can be turned on with tetracycline (tet). The induction of telomerase synthesis following the administration of a tet inducer increases the length of the ends of the chromo-

somes and then the tet inducer is removed, by replacing the media with tetracycline-free media, to stop the process. Without this ability to shut down the telomerase gene, there will be no control over the cells and cancerous growth cycles would proceed. Therefore, we walk a fine line between creating immortality and starting a process which leads to cancer. It has been reported by Shay (1998), that high levels of telomerase activity is found in 90% of malignant tumors and absent in all normal body tissues.

Increasing the length of the telomere restores the cells to an earlier state of development, at least from the standpoint of chromosome length, as young cells have longer chromosomes. This now permits us to expand the number of different types of stem cells, since there appears to be a heterogeneous population (Ghazizadeh and Taichman, 2001). Furthermore, we can increase our understanding of the signaling factors which are required to grow cells for hair follicle replacement and possible also for replacing the heart, brain, liver, pancreas, and essentially all organs of the body.

The New Revolution: Plastic Cells

A major advance in biology has just occurred; dynamic Plastic Cells throughout the body have been discovered. And as we work on creating immortal stem cells, we must integrate the plastic cell concept into our research programs. Plastic cells can be anything that they want to be. The view of the adult stem cell is rapidly changing. Adult bone marrow cells are one form of plastic cells that can not only restore the immune cells of the blood but can also repopulate brain, blood vessels, heart, liver and muscle (Blau et al., 2001). Muscle cells and brain cells may also be a source of blood cells.

It appears that cells can move from one organ to another and differentiate in an environment-dependent manner. Therefore, a cell can change its morphology as required and start performing the function

as its neighbors dictate. Human hair follicle stem cells appear to move around to different parts of the follicle (Akiyama, et al., 2000), although it is possible that these cells provide evidence for the properties of mobile Plastic Cells. The signal molecules which determine the fate of these hair follicle Plastic Cells appear to be beta-1 integrin and epidermal growth factor and the cells have keratin 19 markers. These findings are very unsettling because it can set the entire practice of medicine upside down. New therapeutic procedures which use a patient's own stem cells have already been performed. In fact, Modex, a Swiss firm established in June 2000, has a produced called EpiDex, which is a skin product that the company makes from a patient's own outer root sheath hair follicle cells. Repair of damaged heart muscles has been accomplished after intravascular injection of bone-marrow-derived stem cells (Jackson et al., 2001)

Mineral Ascorbates and the Growth of Plastic Cells

One view that has not been turned upside down is the fundamental need of all cell types to have the proper amount of mineral ascorbates within their cells. A plastic cell requires mineral ascorbates for survival and may indeed respond better with higher amounts of ascorbate present. The brain has as much as 10 mM ascorbate inside of the cells and has been found to have plastic cells throughout life and even after death (Gage, 2000). Therefore, mineral ascorbates surely play a role to ensure that these plastic cells can be mobilized to act either locally in the organs where they normally are found, or move to distant tissues of another type to play a role in regeneration.

It is amazing that cells may change from a complex differentiated cell like a neuron or a striated muscle cell, that is multinucleated with actin and myosin proteins, to an undifferentiated plastic cell. The factors which induce these changes in cells are starting to be determined.

Control of Differentiation

Adults have 5 million hair follicles, 1 million on the head, and 100,000 in the scalp (Szabo 1958). A human hair follicle will survive for only three years before they atrophy and a new follicle develops. Only the palms of the hands and soles of the feet are devoid of hair follicles. Akiyama et al., (2000) reported that putative stem cells in human hair follicles had high levels of beta-1 integrin and low levels of beta and gamma-catenin.

There are about 100 other factors which direct the differentiation of the plastic hair follicle stem cells, including: axin, beta-catenin, beta-integrins, Cerberus, Dickkopf-1, Dishevelled 2 (DVL2) protein, Frizzled-related proteins, Lef1, Sonic Hedgehog, Stem cell factor, TCf3, and Wnt signaling proteins. The Wnt signaling protein family induce both embryonic and adult skin cells to make hair follicles (Kishimoto et al., 2000) and work synergistically with Lef1, Tcf3, and Sonic Hedgehog proteins. In addition to Wnt signaling, Lef1 requires stabilized beta-catenin to express the hair specific keratin genes and control hair differentiation (Merrill et al., 2001). Tcf3 regulates the formation of the outer sheath and also the bulge region of the hair follicle which is made up of multipotent Plastic Cells.

Millar et al., (1999), has been working on the characterization of the molecular pathways controlling differentiation and proliferation in mammalian hair follicles. The proto-oncogene Wnt3, which encodes a paracrine signaling molecule, is expressed in developing and mature hair follicles. The report that a short-hair phenotype is due to altered differentiation of hair shaft precursor cells, and cyclical balding resulting from hair shaft structural defects and associated with an abnormal profile of protein expression in the hair shaft. Dishevelled 2 (DVL2) protein is a candidate cystoplasmic effector molecule for WNT3 signaling, that is normally present at high levels in a subset of cells in the outer root sheath and in precursor cells of the hair shaft cortex and cuticle which lie immediately adjacent to Wnt3-expressing cells.

Millar found that overexpression of Dvl2 in the outer root sheath mimics the short-hair phenotype produced by overexpression of Wnt3. This supports the hypothesis that Wnt3 and Dvl2 have the potential to act in the same pathway in the regulation of hair growth.

These experiments demonstrate a previously unrecognized role for WNT signaling in the control of hair growth and structure, as well as presenting the first example of a mammalian phenotype resulting from overexpression of a Dvl gene and providing an accessible in vivo system for analysis of mammalian WNT signaling pathways.

Bafico et al., (2001), report that Wnt signaling has an important role in hair follicle fate determination, tissue patterning and tumorigenesis. Secreted antagonists of Wnt include Frizzled (Fz)-related proteins (FRPs), Cerberus, Wnt inhibitory factor (WIF) and Dickkopf (Dkk). FRPs, Cerberus and WIF have all been shown to act by binding and sequestering Wnt. We report a novel mechanism of Wnt-signaling inhibition by human Dkk-1. Dkk-1 demonstrated no interaction with Wnt but bound a single cell surface site with high affinity (KD=0.39 nM). Its receptor was detectable in a complex with a relative molecular mass of 240,000 (Mr 240K) with [125I] Dkk-1 by covalent affinity cross-linking. Wnt signaling through beta-catenin is mediated by the Fz receptor and a recently identified low-density-lipoprotein-receptor-related co-receptor, LRP6/Arrow. Overproduction of the 200K LRP6 protein, but not of Fz, strikingly increased Dkk-1 binding as well as the amount of the 240K cross-linked complex, which was shown to be composed of Dkk-1 and LRP6. Moreover, Dkk-1 function was completely independent of Fz but LRP6 dramatically interfered with the Dkk-1 inhibition of Wnt signaling. Thus, unlike Wnt antagonists, which exert their effects by molecular mimicry of Fz or Wnt sequestration through other mechanisms, Dkk-1 specifically inhibits canonical Wnt signaling by binding to the LRP6 component of the receptor complex.

S100A6 calcium binding protein is expressed in the epithelial sac of the hair follicle during regenerative processes (Ito and Kizawa, 2001). This

protein is present in the bulge area where the stem cells are located during the catagen-telogen-anagen transition periods.

Lu et al., report that cytokeratin K19 is expressed in epithelial cells, basal cells of non-keratinized stratified squamous epithelium, epidermal cells during the embryonic stage, and squamous carcinoma cells, but it is not expressed in adult epidermis. Interestingly, when epidermal cells are cultured in vitro, K19 is re-expressed in the supra-basal layer. K19 expression was used as a marker for epidermal cell growth and differentiation. In order to clarify the temporal and spatial sequential expression in cultured keratinocyte, two-stage human keratinocyte culture systems were used to examine K19 expression in keratinocytes in a proliferation and differentiation stages through immunoblotting and immunohistochemistry assay. According to their results, K19 was not expressed in cultured human keratinocytes in the proliferation stage but was re-expressed in keratinocytes three days after the cultured medium was changed to a differentiation medium. Immunohistochemical observation revealed that K19 was persistently expressed in the supra-basal layer of cultured keratinocytes during the first three weeks of culturing, but none was detectable in the basal cell layer. When keratinocytes were cultured with an “inserted cultured dish”, K19 was persistently expressed in all layers of keratinocytes nourished by medium both from an inner chamber and an outer chamber. The different expression of K19 in these two different culture systems seemed to indicate that down regulation of K19 expression in keratinocyte was related to the direction of medium supply.

Randall et al., (2001), reported that androgens regulate many aspects of human hair growth in both sexes. After puberty, they transformed tiny vellus follicles in many areas, e.g., the face, to terminal ones producing long, thick, pigmented hairs. In genetically predisposed individuals, androgens also caused the reverse transformation of terminal scalp follicles into vellus ones, causing balding. In the current hypothesis for androgen action, androgens control most follicular cells indirectly act-

ing via the mesenchyme-derived dermal papilla which regulates many aspects of follicular activity. In this model, androgens binding to androgen receptors in dermal papilla cells alter their production of regulatory molecules which influence other follicular components; these molecules may be soluble paracrine factors and/or extracellular matrix proteins. This hypothesis is supported by immunohistochemical localization of androgen receptors in dermal papilla cell nuclei and the demonstrations that androgen receptor content and testosterone metabolism patterns of cultured dermal papilla cells from various body sites reflect hair growth in androgen-insensitivity syndromes. The next question is whether androgens alter the paracrine factors secreted by dermal papilla cells. Cultured dermal papilla cells do release soluble, proteinaceous factors into their media which stimulate the growth of keratinocytes and other dermal papilla cells. Importantly, testosterone in vitro stimulates the mitogenic potential of beard cells, but in contrast inhibits production by balding scalp cells reflecting their in vivo androgenic responses. Since androgens in vitro do alter the secretion of paracrine factors, the current focus lies in identifying specific factors produced, e.g. IGF-I and stem cell factor (SCF), using ELISA and RT-PCR, and comparing their expression in cells from follicles to androgens.

Hair follicles have a life cycle which is broken down into three main stages: catagen, telogen, and anagen. During catagen cells cycling segment, epithelial cells die, and their dermal papilla moves up to the bulge. The hair falls out during telogen. In the anagen stage, signals from the dermal papilla, including the Wnt proteins, stimulate the growth of new hair sheath cells and hair growth.

Detmar et al., (1993), reported on a variety of in vitro models for the cultivation of hair follicles and their constituents. Outer root sheath (ORS) keratinocytes (KC) have been mainly studied in explants cultures, planted on bovine eye lens capsules, collagen substrata, 3T3 cell feeder layers, or dermal equivalents, yielding outgrowth if a multilayered stratified epithelium with some biochemical and ultrastructural characteristics of

keratinocytic differentiation. More recently, ORS KC cultures have also been initiated from single cell suspensions, and organotypic cultures have been obtained by recombination with dermal cells, inducing a higher degree of epidermal differentiation. Hair matrix cells have been isolated from plucked anagen hair follicles and have been successfully propagated on 3T3 cell or normal human fibroblast feeder layers, giving rise to multilayered stratified KC cultures. In contrast, only preliminary data exist concerning the cultivation of bulge cells that have been suggested to represent follicular stem cells. ORS KC and hair matrix cell cultures that have increased our knowledge on the regulation of the human hair cycle by soluble factors and dermal-epidermal interactions.

Moll (1995), reported that he "examined colony-forming ability, localization of colony-forming cells, and in vitro life spans of outer root sheath keratinocytes of different fragments of adult human plucked hair follicles. These were shown by immunohistochemical staining for cytokeratins and integrins to contain a preserved basal cell layer. By microdissection, five fragments of the outer root sheath (B1, B2, B3-1, B3-2, B4) were separated, dispersed by trypsin into single cell suspensions, and grown on human feeder fibroblasts. All fragments gave rise to at least some colonies, but colony-forming ability was mostly marked in the intermediate part (B2) and the lower half of the central part (B3-1); approximately 60% of colony-forming cells of a hair follicle localized to the fragment B3-1 and 28% to the fragment B3-2 (upper half of the central part, including bulge). To compare the in vitro life spans of cells from the various fragments, we subcultured isolated keratinocytes under identical conditions. The longest was found in the fragment B3-2 and the shortest in the fragment B1 (bulb). Moreover, the differentiation state of the native cells and the cells of all cultures were studied during their whole life spans by immunocytochemical analysis of various proliferation and differentiation markers. Surprisingly, keratinocytes of all fragments, as shown by expression of high-molecular-weight cytokeratins and filaggrin, were capable of terminal differentiation. These

data indicate that cells with long life spans are localized in central parts of the outer root sheath close to the bulge area and that cells with high colony-forming ability are localized in the lower central parts. The latter are usually removed by plucking and may therefore not represent stem cells but rather cells important for hair growth during a single cycle. Cells with long life spans—also included in plucked hair follicles—may be immediate progeny of stem cells that will be segregated in the bulge area. Finally, our results are important for gene transfer and stem cell gene therapy in genodermatoses, because plucked hair follicles are easily available and keratinocytes close to the bulge area should be used selectively.

Yang (1993), reported that corneal keratinocyte stem cells can proliferate in vitro better than their progeny cells. In this paper, we applied this approach to the identification of hair follicular stem cells. When human scalp hair follicles were placed in explant culture, the bulge area yielded best outgrowths. In another experiment, we isolated different subpopulations of human follicular keratinocytes by micro-dissection, dispersed them by trypsin/EDTA into single cells, and grew them in the presence of 3T3 feeder cells. The keratinocytes were then subcultured under identical conditions to compare their in vitro life span. Our results indicate that the life span of keratinocytes of the upper follicle (containing mainly the isthmus area)>sebaceous gland> lower follicle (between the bulge and bulb) > bulb (containing the matrix cells). The cultured upper follicular keratinocytes tend to be small and relatively uniform in size. The poor in vitro growth of matrix cells may reflect their non-stem cell nature and/or special growth requirement(s) satisfied in vivo by the neighboring dermal papilla cells. Unexpectedly, we found that the upper follicular keratinocytes grow even better than epidermal keratinocytes. The existence of a subpopulation of keratinocytes with an in vitro growth potential superior than other known keratinocytes of the skin supports the hypothesis that follicular stem cells reside in the upper follicle. Our data also raise the possibility that putative follicular stem cells

are involved not only in forming the follicle, but also in the long-term maintenance of the epidermis. Finally, we discuss the possibility that keratinocyte stem cells, as defined by their in vivo slow-cycling nature, are absent in culture.

Hung et al., (2001), reported that human Frizzled-3 is important to developing hair follicle and identified the mRNA for human Frizzled-3 in epidermal keratinocytes and in the HaCaT keratinocyte cell line. Human Frizzled-3 mRNA encodes a 666 amino acid protein maps to the short arm of chromosome 8 between the markers WI-1172 and WI-8496 near the loci for the Hypotrichosis of Marie Unna and Hairless genes.

Signal molecules that control hair growth

SUBSTANCE	SITE OF ACTION	EFFECT ON HAIR GROWTH
Basic fibroblast growth factor (bFGF)	Dermal papilla cells	increase (H)
Platelet-derived growth factor (PDGF)	Dermal papilla cells	increase (H)
Transforming growth factor beta (TGF-$)	Dermal papilla cells	decrease (H)
Interleukin 1-alpha (IL-l-")	Hair matrix cells	decrease (H)
Fibroblast growth factor type 5 (FGF5)	Hair matrix cells	decrease (H)
Epidermal growth factor (EGF)	Hair matrix cells	decrease (H)
Keratinocyte growth factor (KGF)	Hair matrix cells	increase (R)
Insulin-like growth factor I (IFG-I)	Hair matrix cells	increase (H)
Substance P	Unknown	increase (M)
Parathyroid hormone (PTH)	Unknown	decrease (M)
S100A4	Epithelial sac	increase (M)

Table 1. Signal molecules that control hair growth. The species studied is noted in parentheses adjacent to the effect: H = human, R = rat, M = mouse. It should be noted that there are vast differences between animal models and human hair follicles.

New Stem Cell Differentiation Concepts: Sonic Hedgehog

Hooper is currently doing research at the University of Colorado on two secreted proteins, Wingless and Hedgehog. Wingless (Wg) is expressed by the most posterior cells in each parasegment; Hedgehog (Hh) is expressed in the most anterior cells of the next parasegment. Immediately after gastrulation, the two cell types are mutually dependent. Local Wg signaling stabilized Hh expression and local Hh signaling stabilizes Wg expression. Direct Wg autoregulation (autocrine signaling) is masked by its paracrine role in maintaining Hh, which in turn maintains Wg. I have used zeste-white 3 (zw3) and patched (ptc) mutant backgrounds to uncouple genetically this positive-feedback loop and to study autocrine Wg signaling. I report here that direct Wg autoregulation differs from Wg signaling to adjacent cells in the importance of fused (fu), smoothened (smo) and cubitus interruptus (ci) relative to zw3 and armadillo (arm). I also find that Wg autoregulation during this early Hh-dependent phase differs from later Wg autoregulation by lack of gooseberry (gsb) participation.

Zhu et al., (1999), has stated that although Sonic Hedgehog (Shh) plays a critical role in brain development, its actions on neural progenitor cell proliferation and differentiation have not been clearly defined. Transcripts for the putative Shh-receptor genes patched (Ptc) and smoothened (Smo) are expressed by embryonic, postnatal, and adult progenitor cells, suggesting that Shh can act directly on these cells. The recombinant human amino-terminal fragment of Shh protein (Shh-N) alone did not support the survival of cultured progenitor cells, but treatment with Shh-N in the presence of bFGF increased progenitor cell proliferation. Furthermore, treatment of embryonic rat progenitor cells propagated either in primary culture or after mitogen expansion significantly increased the proportions of both beta-tubulin- (neuronal marker) and O4- (oligodendroglial marker) immunoreactive cells and reduced the proportion of nestin- (uncommitted neural progenitor cell marker) immunoreactive cells. By contrast, Shh-N had no effect on the elabora-

tion of GFAP – (astroglial marker) immunoreactive cells. Cotreatment with Shh-N and bone morphogenetic protein-2 (BMP2) inhibited the anti-proliferative, astroglial-inductive, and oligodendroglial-suppressive effects of BMP2. Our observations suggest that Shh-N selectively promotes the elaboration of both neuronal and oligodendroglial lineage species and inhibits the effects of BMP2 on progenitor cell proliferation and astroglial differentiation.

In a report entitled “Hedgehog signal transduction: from flies to vertebrates,” by Murone et al., it was recently shown that the patterning and morphogenesis of multicellular organisms require a complex interplay of inductive signals which control proliferation, growth arrest, and differentiation of different cell types. A number of such signaling molecules have been identified in vertebrates and invertebrates. The molecular dissection of these pathways demonstrated that in vertebrates, mutations or abnormal function of these signaling pathways were often associated with developmental disorders and cancer formation. The Hedgehog (Hh) family of secreted proteins provides a perfect example of such signaling proteins. In the following review, we will not discuss in detail the role of Hh as a morphogen, but rather focus on its signal transduction pathway and its role in various human disorders.

The Hedgehog induces opposite changes in turnover and subcellular localization of patched and smoothened. It was demonstrated by Denef et al., (2001), from Heidelberg, Germany, that secreted signaling proteins of the Hedgehog family organize spatial pattern during animal development. Two integral membrane proteins have been identified with distinct roles in Hedgehog signaling. Patched functions in Hedgehog binding, and Smoothened functions in transducing the signal. Current models view Patched and Smoothened as a preformed receptor complex that is activated by Hedgehog binding. Here we present evidence that Patched destabilizes Smoothened in the absence of Hedgehog. Hedgehog binding causes removal of Patched from the cell surface. In contrast, Hedgehog causes phosphorylation, stabilization, and accu-

mulation of Smoothened at the cell surface. These findings raise the possibility that Patched acts indirectly to regulate Smoothened activity.

Kalderon has reported that the sonic Hedgehog receptor patched associates with caveolin-1 in cholesterol-rich microdomains of the plasma membrane and Karpen et al., (2001), reported that the Hedgehog signaling pathway is involved in early embryonic patterning as well as in cancer; however, little is known about the subcellular localization of the Hedgehog receptor complex of Patched and Smoothened. Since Hh has been found in lipid rafts in Drosophila, we hypothesized that Patched and Smoothened might also be found in these cholesterol-rich microdomains. In this study, we demonstrate that both Smoothened and Patched are in caveolin-1-enriched/raft microdomains. Immunoprecipitation studies show that Patched specifically interacts with caveoloin-1, whereas Smoothened does not. Fractionation studies show that Patched and caveolin-1 can be co-isolated from buoyant density fractions that represent caveolae/raft microdomains and that Patched and caveolin-1 co-localize by confocal microscopy. Glutathione S-transferase fusion protein experiments show that the interaction between Patched and caveolin-1 involves the caveolin-1 scaffolding domain and a Patched consensus binding site. Immunochemistry data and fractionation studies also show that Patched seems to be required for transport of Smoothened to the membrane. Depletion of plasmalemmal cholesterol influences the distribution of the Hh receptor complex in the caveolin-enriched/raft microdomains. These data suggest that caveolin-1 may be integral for sequestering the Hh receptor complex in these caveolin-enriched microdomains, which act as a scaffold for the interactions with the Hh protein.

Martin from Madrid has the following report, “The Hedgehog (Hh) family of signaling molecules function as organizers in many morphogenetic processes. Hh signaling requires cholesterol in both signal-generating and –receiving cells, and it requires the tumor suppressor Patched (Ptc) in receiving cells in which it plays a negative role. Ptc both blocks the

Hh pathway and limits the spread of Hh. Sequence analysis suggests that it has 12 transmembrane segments, 5 of which are homologous to a conserved region that has been identified in several proteins involved in cholesterol homeostasis and has been designated the sterol-sensing domain (SSD). In the present study, we show that a Ptc mutant with a single amino acid substitution in the SSD induces target gene activation in a ligand-independent manner. This mutant Ptc(SSD) protein shows dominant-negative activity in blocking Hh signaling by preventing the downregulation of Smoothened (Smo), a positive effector of the Hh pathway. Despite its dominant-negative activity, the mutant Ptc protein functioned like the wild-type protein in sequestering and internalizing Hh. In addition, we show that Ptc(SSD) preferentially accumulates in endosomes of the endocytic compartment. All these results suggest a role of the SSD of Ptc in mediating the vesicular trafficking of Ptc to regulate Smo activity." Strutt et al., MRC Intercellular Signaling Group, Centre for Developmental Genetics, Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom, "The tumor suppressor gene patched (ptc) encodes an approximately 140 kDa polytopic transmembrane protein that binds members of the Hedgehog (Hh) family of signaling proteins and regulates the activity of Smoothened (Smo), a G protein-coupled receptor-like protein essential for Hh signal transduction. Ptc contains a sterol-sensing domain (SSD), a motif found in proteins implicated in the intracellular trafficking of cholesterol, and/or other cargoes. Cholesterol plays a critical role in Hedgehog (Hh) signaling by facilitating the regulated secretion and sequestration of the Hh protein, to which it is covalently coupled. In addition, cholesterol synthesis inhibitors block the ability of cells to respond to Hh, and this finding points to an additional requirement for the lipid in regulating downstream components of the Hh signaling pathway. Although the SSD of Ptc has been linked to both the sequestration of, an the cellular response to Hh, definitive evidence for its function has so far been lacking. Here we describe the identification and characterization of two missense mutations in the SSD of Drosophila Ptc; strikingly,

while both mutations abolish Smo repression, neither affects the ability of Ptc to interact with Hh. We speculate that Ptc may control Smo activity by regulating an intracellular trafficking process dependent upon the integrity of the SSD.

In Vitro Growth of Hair Follicle Cells

Mass-Szabowski et al., (2001), reported that organotypic cocultures of keratinocytes and fibroblasts generate normal epithelial cells. These results were obtained with fibroblasts from any species of any tissue type. Further, they report that the "use of mouse fibroblasts and human keratinocytes facilitates the identification of the origin of compounds involved in epidermal tissue reconstitution and growth regulation. Moreover, the functional significance for the keratinocyte phenotype of genetically modified fibroblasts from transgenic or knockout mice, even those exhibiting an embryonic lethal phenotype, can be studied in such heterologous in vitro tissue equivalents. Here we communicate results of such studies revealing the antagonistic function of mouse fibroblasts defective in the AP-1 constituents c-June and JunB, respectively, on human keratinocyte growth and differentiation. Furthermore, the hematopoietic growth factor granulocyte macrophage-colony stimulating factor has been identified as a novel regulator of keratinocyte growth and differentiation. As will be reported in detail elsewhere, both granulocyte macrophage-colony stimulating factor and keratinocyte growth factor have been identified as major mediators of fibroblast-keratinocyte interactions and their expression is induced via AP-1 by interleukin-1 released by the epithelial cells. Thus, these heterologous cocultures provide a novel promising tool for elucidating molecular mechanisms of epithelial-mesenchymal interactions and their consequences on epithelial cell proliferation and differentiation."

Identification of Plastic Cells

The tissue source or site of Plastic Cells which form the human hair follicle may not be known, but there is some evidence supporting the hair follicle bulge cells as being part of the cascade of development and having properties which make them biochemically distinct. Lyle et al., (1999) reported that the C8/144B monoclonal antibody, raised against a CD8 peptide sequence, immunostains the human hair follicle bulge and recognizes cytokeratins 15 (K15) in keratinocytes, and that K15-positive bulge cells possess a slowly cycling nature, proliferate the onset of new hair follicle growth, and have a high level of beta1 integrin expression. These results suggest that human hair follicle stem cells are localized to the bulge and that K15 is preferentially expressed in epithelial stem cells. A line of experiments which uses the c8/144B monoclonal antibody as a marker for Plastic Cell isolation might therefore prove fruitful. A recent observation that keratinocyte stem cells have p63 transcriptions factors should also be considered during the characterization of plastic stem cells (Pellegrini et al., 2001). P63 is a transcription factor which is essential for regenerative proliferation of keratinocytes. The marker may have clinical applications because of its usefulness in identifying cells with proliferative potential and then separating them by monoclonal antibodies attached to magnetic beads.

Transfecting of Human Hair Follicle Cells

Gupta el al., (2001), reported that the human hair follicle consists of plastic cells for hair follicle cycling and for epidermal keratinocytes, melanocytes, and Langerhans cells. They found that a combination of liposomes and vector DNA can be used to target hair follicle cells in human scalp xenografts. The liposome composition and stage of the hair cycle were found to be important parameters influencing transfection of human hair follicles. Transfection was only successful during anagen onset. Hoffman (1998) has reported that liposomes may also be used topically to deliver genes to hair follicles.

Conclusion

It is important to have cells which survive long enough to permit us to study their properties and which signal molecules control their differentiation. Adult human hair follicle cells do not proliferate and die in culture after several weeks. After transfection with hTERT, we now have the ability to increase their lifespan and study their differentiation under the control of signal peptides.

It is not clear how hair follicle morphogenesis is controlled, but it appears that many signal proteins are involved. Mesenchymal and epithelial cells use fibroblast growth factor and bone morphogenic proteins to provide the critical signals to the stem cells.

The use of mineral ascorbates in modern medicine has the ability to improve many of the above reviewed processes. It is imperative the researchers work together and use mineral ascorbates in every procedure to validate their beneficial effects. Every cell process requires mineral ascorbates in one way or another. It is the job of the research community to serve the consumer and devote substantial research efforts to the mineral ascorbates which the public has already started to consume in great quantities.

Contact Dr. Elliott for a list of references.

Appendix

RECENT SCIENTIFIC WORK ON HAIR GROWTH, WORLD WIDE

1) Hormone replacement therapy is frequently used as a technique for anti-aging in Southern California and other locations. People are usually prescribed various vitamins, supplements, thyroid hormone (in the form of Armour thyroid, which is T3 and T4), growth hormone, and sex hormones such as Testosterone or Estradiol. The theory goes that as we age, the ability to convert T4 into T3 (which is done in the tissues) diminishes so that persons over 55 are frequently deficient in free T3, even though they may have sufficient T4.
2) It has been observed for several years that grey hair tends to darken up on these therapies. It was previously speculated that this was largely due to the growth hormone. The article by Redondo, et al., in the *Forum*, March/April 2008, shows two mice whose hair darkens up on thyroid hormone. More interestingly, the follicles of the "thyroid solution stimulated mouse" entered Anagen phase at day 6 compared to day 10 or 16 for the mouse without thyroid solution applied. Similar results were found with follicular units in a thyroid solution. The thyroid solution is tri iodothyronine (T3).
3) About five years ago, the author transplanted a 50-year-old man, who postoperatively had relatively sparse results from his hair transplant. After his hair transplant, his donor hair began to thin, so that some sort of diffuse thinning process was suspected. A hormonal workup was advised, but he declined at that time. A year or two later, he had a hormonal workup from his internist, who then put him on AndroGel (testosterone). Within a few months of being on AndroGel, the patient reported that all of his hair, both transplanted and original donor hair, thickened up and developed a luxurious quality that he had had when he was much younger. He is now ready for a second hair transplant.

We have known for a long time that body hair and beard hair requires the support of testosterone. We have also all assumed that scalp hair is negatively affected by DHT.

Based on the above observations in this patient, it would appear that donor hair requires testosterone to support it, as well as body hair and beard hair. This may explain why some late-middle-aged men have diffuse thinning of their hair. Perhaps all should have an endocrine panel and then be treated with testosterone, as well as T3, and other hormones as indicated by lab evaluation.

4) Another observation by Dr. Neal Rouzier in Palm Springs, CA, who has a large anti-aging practice, relates the two following facts. 1) A very common cause of depression in postmenopausal women is low free T3. Women who are at the low end of the normal range are frequently treated by Dr. Rouzier to bring them up to the high end of the normal range of free T3 or even a little higher. By titrating free T3, he is able to reverse the depression, reverse the loss of energy, observe darkening of white hair, and thickening of hair overall. He also has communicated to me that generally postmenopausal women have immeasurable levels of androgens. That being the case, perhaps some of the diffuse hair thinning commonly seen in postmenopausal women is related to loss of androgen support for their donor hair. Perhaps treatment with testosterone for both postmenopausal women and for aging men will be a principal factor, along with thyroid hormone, in reversing the DPA or diffuse thinning situations that are commonly seen.

5) In looking at the article on gene expression by Drs. Kim and Kim of Korea in the July/August *Forum*, they observed that DHT in frontal cells of the scalp induced DKK-1, which inhibits hair growth. Contrarily, in beard dermal papilla cells, DHT stimulated the EDA pathway which caused hair growth of the beard cells. Is

it likely that occipital and parietal donor area cells are stimulated by DHT (and/or testosterone) to activate the EDA pathway, rather than the DKK-1 pathway? It would seem that this is probable, based on the observations.

In general, activation of the Wnt pathway in hair follicles and insulin-like growth factors (IGFs) are involved in androgen induction, and TGF-β pathways are related to catagen progression. Frontal DP cells also more predominantly produced many stress proteins including heat shock proteins. Thus, frontal DP cells are, before androgen stimulation, more severely stressed cells.

DKK-1 is induced by DHT in frontal DP cells and inhibits hair growth.

Ectodysplasin A (EDA) pathway is activated in beard DP cells by DHT stimulation and induces anagen hair growth.

In beard DP cells, DKK-1 was not induced by DHT, as it was in frontal balding DP cells. DHT rapidly increased the EDA2R pathway stimulating hair growth in beard DP cells.

6) Nilofer Farjo writes in her “Hair Sciences” column in the March/April issue of the *Forum* that the addition of IGF-1 or 2 (insulin growth factor) maintains antigen growth in vitro in dermal papilla cells. IGF-1 is essentially growth hormone, and so the question presents itself whether or not using perhaps a double dose of growth hormone on patients for two or three months after a hair transplant would get the hairs growing a lot sooner. Perhaps it would eliminate the hair shock and three months of telogen, which is commonly experienced. That therapy could be systemic or topical. Research will need to be done with this.

7) Similarly, topical T3 solution postop may also contribute to a shortening of the telogen phase or its elimination. Another recent report has suggested that there may be little or no type 2 5-alpha

reductase located in occipital (donor) dermal papilla cells. Of course this would explain, if true, why occipital dermal papilla cells are relatively impervious to the effects of DHT, since most DHT is located intracellular and not in the circulation. It may be that there are very low levels of DHT in these cells due to the lack of 5-alpha reductase type 2. It may also be that testosterone, not DHT, is the stimulator for the growth factors in these cells.

8) Dr. Perez- Mesa, et al. found that growth factor activity appeared as follows following hair transplantation:

TGFA appeared on day 1, others appeared on day 3. Growth factors increased on days 7 through 21. By day 28, EGF and BEGF were the only ones present.

Three phases of revascularization of hair grafts were seen:

1) Plasmatic imbibition at days 1 to 3.

2) Primary inosculation and growth of blood vessels.

3) Secondary inosculation/neovascularization with changes in the vessels including the lymphatics. These changes took place simultaneously with the increase in growth factors.

The study showed that after the hair graft takes and survives, the process may continue with one of the following scenarios:

a) The hair will continue to grow in anagen phase from day one with no hair loss.

b) The hair will fall out from one to six weeks postop (anagen effluvium) with new hairs beginning to grow two to four weeks later.

c) The hair will fall out from seven to twelve weeks

postop (telogen effluvium) with new hairs beginning to grow two to four weeks later. Hairs with individual follicular units do not grow at the same rate and may take any of the above three alternatives. They conclude that maintaining a healthy blood supply to the scalp and minimizing hypoxia and ischemia of the hair graft during transplant surgery and for five days postop should increase survival rate.

9) Dr. Cooley has found that ischemia reperfusion injury results in 600% increase in free radicals within the hair follicles. This can be decreased by:

    1) Giving the patient anti-oxidants such as vitamin E or melatonin.

    2) Corticosteroids.

3) Additives to graft holding solutions. Hypothermasol contains two potent anti-oxidants, glutathione and a synthetic analog of vitamin E.

Dr. Cooley's current practice is to keep a mini-fridge in the O.R. and placed unslivered strips in Hypothermasol there. Slivers are also kept in Hypothermasol on coolers. It is important to keep Hypothermasol cold, and they switch out coolers at lunch. In this way, we keep the solution at 10°C or less. Thus all tissue has been soaked in antioxidants for theoretical protection against ischemia reperfusion injury.

The dissected grafts are then kept on DMEM/HEPES culture media. DMEM storage media stands for Dullbecco's Modified Eagles Medium. This culture media contains glucose, vitamins, and a buffer to keep the pH steady. He keeps the grafts on DMEM-soaked Reston foam at the air liquid interface as opposed

to immersion. He does not chill them. Both the Hypothermasol and DMEM have the HEPES buffer in them which keeps the pH steady.

10) Dr. Cotsarelis has found that stem cells in hair follicles present as keratinocytes, which maintain a high proliferative potential and are long lived, slowly cycling, and apparently immune privileged cells. Topical treatment with RU486 to these bulged stem cells resulted in hair follicles entering the anagen phase after only five days. These cells have the potential to regenerate new hair follicles at about four weeks after implantation into mouse skin.

11) The regular spacing of hair follicles throughout the scalp and elsewhere is controlled by an Edar/BMP activation-inhibition mechanism, which operates along side a label pre-pattern, suggesting that Edar-mediated stabilization of beta-catenin active foci is a key event in determining definitive follicle locations. The BMPs repress epidermal Edar and hence, follicle rate. Edar activation also induces connective tissue growth factor, an inhibitor of BMP signaling, allowing BMP action only at a distance from their site of synthesis. Consistent with this model, transgenic hyperactivation of Edar signaling leads to widespread overproduction of hair follicles.

12) Plasma-lyte A has a pH of 7.4 and uses an acetate buffer. DMEM used in hair studies normally contains the more expensive HEPES buffer, which works well in open-air situations.

13) Dr. Klugluger of Vienna with the Mosier Clinic has found that a tissue culture medium containing inhibitors of nitric oxide has resulted in grafts with no transient hair loss that begin growing immediately after hair transplantation. A variety of additives have been included in storage solutions, most commonly energy substrates and anti-oxidants. DMEM-containing inhibitors of inducible nitric oxide synthase (iNOS) prevented post-transplant hair shedding

in grafts in six or six patients. The primary inhibitor of nitric oxide synthase was amino guanidine. DMEM containing arachidonic acid inhibitors prevented graft hair shedding in five of six patients versus zero of six in controls. Both additives also demonstrated significant improvement in hair shaft elongation studies.

14) Work is currently being conducted to determine the effectiveness of an inexpensive ATP supplementation preparation called Lipo-Tripolyphosphate, which would be applied topically postoperatively in hair transplantation.

15) Postoperative anti-oxidants which could be administrated orally include vitamin C and vitamin K.

16) Platelet-rich plasma contains growth factors such as PDGF, TGF Beta 1, and VEGF. Using this for grafts and donor healing may result in a 15% increase in growth.

17) Grafts will grow better if left slightly chubby with a little tissue beyond the dermal sheath and papilla.

18) Dr. Rinaldi of Milan studied the twice-daily topical application of a postop solution containing adenosine sulfate 0.1%, taurine 1.0%, and ornithine chloride 1.0% (called 1-3 Atodine). Adenosine sulfate regulates vascular endothelial growth factor (VEGF) and follicular growth factor-7 (FGF-7), while taurine and ornithine stimulate outer root sheath growth. At one month, vessel diameter and hair shaft diameter were both larger than placebo. Revascularization was quicker by nearly threefold, and the follicle growth was improved.

    Since the outer root sheath is more accessible to topical therapy than the dermal papilla, it may be that topical 1-3 Atodine solution might be effective.

19) According to Dr. Carlos O. Uebel OF Brazil, the way to obtain platelet-derived growth factors is as follows:

"The flasks are centrifuged at 1000 rpm for 10 minutes. The slow speed is important so that the platelets remain moved and redistributed into 4 other flasks for a second centrifugation of 5000 rpm for 10 minutes. The plasma supernatant is then removed, leaving only 2 cc of the concentrate, which is the platelet-rich plasma (PRP). The PRPR contains four to six times more platelets than normal plasma and includes a high concentration of growth factors. This concentrate is then added to the FUs prior to their implantation. The PRP is kept in contact with the hair follicles for 15 minutes to allow the growth factors to attach to the stem cells located in the bulge area. Next, we add ten drops of 10% calcium chloride with the intent of transforming fibrinogen into fibrin in order to produce a plasmatic gel that will seal the micrografts with the growth factors around them."

20) According to Drs. Kim and Kim of Korea, frontal scalp and beard dermal papilla cells, respond differently to DHT. Beard dermal papilla cells respond positively through the Wnt pathway and produce hair growth stimulating signals such as EDA2R. Frontal scalp dermal papilla cells respond with the production of Wnt inhibiting signals such as dickhoff (DKK)-1, which inhibit hair growth.

Circulating androgens enter the dermal papilla through its capillaries and, after binding to androgen receptors, activate or repress target genes. These target genes produce paracrine regulatory factors. Dermal papilla cells in the balding frontal scalp secrete transforming growth factor (TGF)-β, which inhibits the epithelial cell growth in response to androgens. These frontal DP cells also contain more AR and type II 5-alpha reductase than non-balding occipital DP cells. They are known to secrete inhibitory autocrine factors that affect the growth of DP cells. The data suggests that androgen-driven alteration of the autocrine and paracrine factors may be the key to the pathogenesis of androgenetic alopecia.

21) Dr. Nilofer Farjo of Britain writes that local autocrine/paracrine factors are involved in the regulation of hair growth. She noticed that the addition of IGF-I or II (insulin growth factor) maintains anagen growth rates. When these factors are removed, the follicles go into premature catagen phase. The implication is that human growth hormone through its IGF-I factor is obviously necessary for good hair growth. The old observation that anti-aging therapies with growth hormone caused the hair to thicken up is thus validated.

22) Dr. Francisco Jimenez-Acosta, M.D. of Spain writes that caffeine alone led to significant stimulation of hair follicle growth at low concentrations of .001% and .005% in vitro. Paradoxically, high concentrations of caffeine showed inhibitory effects.

23) Dr. Reese of Minnesota notes that, through the use of platelet-rich plasma, there was more rapid normalization of the scalp with respect to both crusting and erythema in those cases in which grafts were bathed in PRP.

24) Dr. Sinclair of Australia writes that the aromatase gene is also a candidate for female pattern hair loss. The female balding scalp is characterized by low levels of aromatase and high levels of dihydrotestosterone. The implication of this is that the low aromatase does not produce sufficient estrogen to supply the female balding scalp. In other words, the intracellular estrogen may be low, even though the circulating estrogen could be normal. This is consistent with the loss of hair following drop in estrogens postpartum. Naturally, if there is low aromatase in female balding scalps, the testosterone levels and DHT levels would rise since testosterone is not getting converted to estrogen.

25) Dr. Acosta of Spain also writes that iron deficiency has long been associated with telogen effluvium, but recently Dr. Kantor in the JID in 2003, noted that ferritin levels in female patients with

androgenetic alopecia and focal alopecia areata was significantly lower than in normals without hair loss.

26) The prostaglandins Bimatoprost and Latanoprost, which are used as eye drops to treat glaucoma, are noted by an ophthalmologist to increase the length and thickness of eyelashes.

27) Hair Dx Test - androgen receptor allele hypothesis that females with this defect may be responsive to finasteride under investigation.

28) Hair or follicle cloning: Dr. Beeson Farjo and Dr. Nilofer Farjo in England are working with a research company to develop the ability to clone hair folicle stem cells. Once this is developed, the next step is to inject these cells onto the scalp of human subjects (from whom the cells were derived) to see if they will generate hair follicles. After that, the challenge is to control hair direction as is currently done with hair transplants.

    This tecnology will be expensive and will probably be used for those with little donor hair.

    Please see our research paper (page 43) which was presented to the Hair and Wool Society in 2002

"Dr. Elliott has an uncanny way of reducing complex issues to their simplest parts. He started with the first hair restoration techniques and has been a major part of every innovation since."

Robert Nettles, MD, AACS

About the Author

Dr. Elliott grew up in Omaha, Nebraska, the fourth generation in medicine. He attended John Hopkins University Undergraduate, University of Nebraska College of Medicine for medical school, and University of Iowa Hospitals, Department of Dermatology for Residency. He was trained in hair transplantation in 1971, while a Dermatology Resident. Over the years, he invented many things which advanced the process of hair transplantation. He has always been at the forefront of the latest technology. You can rest assured that the techniques used at the Pacific Hair Institute have been and will continue to be the latest technology available. He is a Diplomate of the American Board of Hair Restoration Surgery. He lives in Newport Beach and is founder of Pacific Hair Skin & Laser Institute, APMC. (800-990-HAIR). If you wish a complimentary video, call John Peters at 949-263-0800

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