ALOPECIA

1. ALOPECIA PROTOCOL

A. Clinical response: Clinical response demonstrates a decrease in progression of disease and evidence of an improved repair process. In addition to physical examinations prior to stem cell graft and 6 months post-procedure, laboratory test results serve as evidence of repair process. Internationally recognized lab tests for monitoring Alopecia include:

- Complete Blood Count
- Androgen biomarkers
- Liver enzymes
- Cardiovascular biomarkers
- Adrenal hormone levels
- Thyroid autoantibodies
- Thyroid hormones: thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH)
- Treponema Pallidum hemagglutination (TPHA) test

B. Objective: To provide the patient with a treatment that stimulates his / her immune system, promote cellular regeneration and improve symptoms associated with Alopecia. The endovascular/intravenous Ad-SVF Containing Adult Stem Cell Procedure should serve to compliment the patient's current treatment regimen or to promote healing when current treatment is not responding.

2. PRELIMINARIES

A. Background: Hair undergoes a regular cycle of growth. Each cycle consists of a long growing phase (anagen), a brief transitional apoptotic phase (catagen), and a short resting phase (telogen). At the end of the resting phase, the hair falls out (exogen) and a new hair starts growing in the follicle, beginning the cycle again. Each day, about 100 scalp hairs reach the end of resting phase and fall out. If more than 100 hairs/day go into resting phase, telogen effluvium (clinical hair loss) may occur. A disruption of the growing phase causing abnormal loss of anagen hairs is called an anagen effluvium.

Alopecia or loss of hair has many causes and patterns. Alopecia areata is a non-scarring condition, most commonly appearing on the scalp. This condition presents as sharply defined non-inflammed bald patches, usually on the scalp [1]. During the active stage of hair loss pathognomonic, 'exclamation mark' hairs are seen (broken-off hairs of 3-4 mm long, which taper off towards the scalp). The condition may also affect the eyebrows, eyelashes and beard. Patient's hair usually re-grows spontaneously in small bald patches.

Alopecia Areata is associated with hypothyroidism, autoimmune disorders, atopy and Down's syndrome. Topical or intra-lesional steroids are first line treatments. There are three main types of Alopecia: Alopecia Areata, Alopecia Areata Totalis, and Alopecia Areata Universalis [1].

- ***Alopecia Areta:*** is the most common type of the autoimmune disease [1]
    - Most patients have small, localized patches, and some have widespread involvement. This condition has a risk for chronicity, in which factors include extensive skin involvement, onset before adolescence, and ophiasis (involvement of the peripheral scalp).
    - Androgenic alopecia is the most common type of hair loss. Male-pattern baldness is physiological in men over 20 years old, though rarely it may be extensive and develop at an alarming pace in the late teens. It also occurs in females, usually post-menopause. The well-known distribution (bitemporal recession and then crown involvement) is described as 'male-pattern' but this type of hair loss in females is often diffuse.

- ***Alopecia Areata Totalis:*** is characterized as the total loss of hair on the scalp [1]

- ***Alopecia Areata Universalis:*** is the rarest form and is characterized as the loss of hair over the entire scalp and body [1]

B. Causes of Alopecia**:** Administration of chemotherapeutic agents, medications (valproic acid), endocrine / autoimmune disorders, infection, nutritional deficiencies, mineral deficiencies (Zinc), excessive Vitamin A, heavy metal poisoning, hair shaft abnormalities, and rare dermatologic conditions.

C. Treatment Options: Successful treatment of alopecia is difficult and management of these patients includes support and reassurance. Any underlying condition should be treated.

- ***Triamcinolone (10 mg/ml)***: Alopecia areata sometimes responds to topical or intralesional steroids.

- ***2% Minoxidil solution (topical)***: Is most effective for vertex alopecia in androgenic alopecia. Only about 40% of patients experience significant hair growth and it is generally not effective or indicated for other causes of hair loss except possibly alopecia areata. Hair regrowth can take up to 12 months. Treatment is continued indefinitely, once treatment is stopped hair loss resumes. Frequent adverse effects are mild scalp irritation, allergic contact dermatitis, and increased facial hair.

- ***Finasteride (Propecia)***: Androgenic alopecia (male pattern hair loss) is caused by androgen-dependent miniaturization of scalp hair follicles, with scalp dihydrotestosterone (DHT) implicated as a contributing cause. Finasteride, an inhibitor of type II 5 alpha-reductase, decreases serum and scalp DHT by inhibiting conversion of testosterone to DHT. Treatment for at least 3 to 6 months is necessary to see hair growth or to prevent further hair loss. Continued use is necessary to sustain benefit. Reported side effects include gynecomastia, decreased libido, ejaculation disorders and erectile dysfunction, which resolve in most men who remain on therapy and in all men who discontinue use of Finasteride. When testing prostate specific antigen (PSA) for cancer screening, note use of Finasteride since there may be a decrease in PSA levels in older men. (Finasteride is also used as treatment for benign prostatic hyperplasia.) There are no data to support the use of finasteride in females with androgenic alopecia. Pregnant women should not be exposed to Finasteride either by use or handling of tablets because of teratogenic risk.

- ***Spironolactone***: Used in treating female androgenic hair loss, is a competitive inhibitor of aldosterone also competes with DHT for androgen receptors in target tissues. It also reduces 17-alpha-hydroxylase activity lowering plasma levels of testosterone and androstenedione,

- ***Cyproterone acetate***: Is one of the last resort treatments for treating female androgenic hair loss, because of its toxicity and long term side effects. Cyproterone acetate exerts its effects by blocking the binding of DHT to its receptors.

- ***Other hormone modulators***: such as oral contraceptives may also be useful in treating female androgenic hair loss.

- ***Scalp surgery and autologous hair transplants***: may also be considered by patients with androgenic alopecia.

3. AD-SVF CONTAINING ADULT STEM CELLS TREATMENT OPTION

A. Ad-SVF Containing Adult Stem Cells Procedure

- ***Initial patient evaluation***: A physician reviews the medical information, lab work, and diagnostic imaging provided by the patient in order to determine the stage of the medical condition and any other secondary conditions.

- ***Pre-op Evaluation / post-op medical consultation***: A medical specialist to the specific condition to be treated provides a medical consultation at the location where the procedure will be performed. During pre-op evaluation informed consent is obtained from all patients and medical records are updated, including patient's most recent physical exam, most up-to-date lab results and imaging studies. Physician then performs surgical risk assessment.

- ***Harvesting of adipose tissue***: Adipose tissue acquisition can be summarized as three step process:

    - ***Application of anesthetic / injection of tumescent solution***

    - ***Waiting time***

    - ***Acquisition of adipose tissue:*** An area of the body with sufficient adipose tissue is selected; this is usually the periumbilical area. With the patient supine, the physician infiltrates a small amount of local anesthetic. A tissue sample is then obtained using 60 cc syringe(s) to aspirate 50 to 100 cc of adipose tissue. Immediately following lipo-aspiration, adipose tissue sample is processed (minimally manipulated) to separate stem cells for use as graft.

- ***Preparation of Platelet Rich Plasma (PRP)***: Using a standard phlebotomy technique the patient's own blood sample is obtained. After collection of whole blood, sample is centrifuged to obtain PRP aliquot. The regenerative potential of PRP is based on the release of growth factors / cytokines upon platelet rupture. PRP also enhances stem cell proliferation.

- ***Autologous implant of Ad-SVF***: The stem cells obtained from the adipose tissue sample and the PRP are applied to the patient using appropriate protocol for their condition. Autologous Ad-SVF containing adult stem cells are infused locally by multiple local injections, to affected areas of the scalp.

- ***Procedure for application of local scalp injections*****:** Prepare PRP mixture and sample of Ad-SVF Containing Adult Stem Cells. Using aseptic technique, a 1 cc syringe, and a 27 G needle administer 0.1 cc of cell mixture via subcutaneous injections to affected areas of scalp, each approximately a half inch apart.

B. Risks: There are possibilities for unwanted effects related to the local anesthesia, harvesting procedure, and injection of stem cells. Even with the most established protocol, adequate technique, and careful administration; a medical team may encounter uncontrollable events. Although there is no guarantee of perfect results, excellent results can be attained. The surgeon provides services in the most responsible, professional and diligent manner, always considering that surgeries imply risks. The risks of complications of adipose tissue harvesting and stem cell infusion are very low. Possible risks include but are not limited to:

- Pain at site of injections
- Bleeding at injection site
- Malaise
- Low-grade fever
- Hot flashes
- Itching at injection site
- Vascular spasm or obstruction
- Bruising
- Nerve or muscle injury
- Allergic reaction
- Dizziness
- Nausea
- Vomiting

C. Benefit: Adipose derived stem cell therapy utilize Mesenchymal Stem Cells that express immunomodulatory and anti-inflammatory properties to help mitigate the impairment to the hair follicles. Clinical response showing increased activity of hair growth and a higher number of active hair follicles. Increased activity of follicles and changes to scalp will provide evidence of repair after stem cell graft.

D. Follow-up plan: International standards for follow-up:

- ***Pre-Ad-SVF implant:*** Clinical evaluation of symptoms, use of Ludwig scale for female patients and Norwood scale for male patients. Photograph the area that was treated.
- ***3 months after Ad-SVF implant:*** Clinical evaluation of symptoms, use of Ludwig scale for female patients and Norwood scale for male patients. Photograph the area that was treated.
- ***6 months after Ad-SVF implant:*** Clinical evaluation of symptoms, use of Ludwig scale for female patients and Norwood scale for male patients. Photograph the area that was treated.

ALOPECIA – Adult Stem Cells Schedule of Events

1. **Initial Patient Evaluation:** A physician reviews the medical information, lab work, and diagnostic imaging provided by the patient in order to determine the stage of the medical condition and any other secondary conditions.

   A. Pre-Examination:
   - You will have a physical exam, which will include measuring your blood pressure, temperature and heart rate (vital signs).
   - Your doctor will discuss your medical history and any medications that you are taking.
   - Your doctor will assess how well you can perform your daily activities
   - If needed, you will have a urine or blood pregnancy test.
   - Blood will be taken.

   B. Additional Tests: should be done during or soon after this visit

   - Complete Blood Count
   - Androgen biomarkers
   - Liver enzymes
   - Cardiovascular biomarkers
   - Adrenal hormone levels
   - Thyroid autoantibodies
   - Thyroid hormones: thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH)
   - Treponema Pallidum hemagglutination (TPHA) test

   C. Review Results: After your doctor has reviewed the results of these tests, he or she will assess whether you are a good candidate for stem cell therapy. If you decide to obtain this therapy you will sign a consent form. A medical specialist to the specific condition to be treated provides a medical consultation at the location where the procedure will be performed. During pre-op evaluation informed consent is obtained from all patients and medical records are updated, including patient's most recent physical exam, most up-to-date lab results and imaging studies. Physician then performs surgical risk assessment.

2. **Pre-Operation / Stem Cell Procedure:**

   A. Two Weeks Before Procedure:

   - No Aspirin or medicines that contain aspirin or Ibuprofen since it interferes with normal blood clotting.
   - Discuss with your primary physician to discontinue anticoagulant drugs at least 1 week before the procedure.
   - Please discontinue all herbal medications as many have side effects that could complicate a surgical procedure by inhibiting blood clotting, affecting blood pressure, or interfering with anesthetics.

- Please discontinue all diet pills whether prescription, over-the-counter or herbal.
- NO SMOKING because nicotine reduces blood flow to the skin and can cause significant complications during healing.
- You may take Tylenol or generic forms of this drug.
- Purchase a compressive garment to wear after the lipoaspiration procedure.

B. Morning of the Procedure:

- Have a light breakfast.
- Take your regular prescribed medications
- Wear comfortable, loose-fitting clothes that do not have to be put on over your head.

3. **Stem Cell Procedure:**

A. Preparation & Harvesting of Adipose Tissue:

- ***Application of anesthetic / injection of tumescent solution***
- ***Waiting time (~15 – 20 minutes)***
- ***Acquisition of blood sample***
- ***Acquisition of adipose tissue:*** An area of the body with sufficient adipose tissue is selected; this is usually the periumbilical area. With the patient supine, the physician infiltrates a small amount of local anesthetic. Immediately following lipo-aspiration, adipose tissue sample is processed (minimally manipulated) to separate stem cells for use as graft.

C. Preparation of Platelet Rich Plasma (PRP): Using a standard phlebotomy technique the patient's own blood sample is obtained. After collection of whole blood, sample is centrifuged to obtain PRP aliquot. The regenerative potential of PRP is based on the release of growth factors / cytokines upon platelet rupture. PRP also enhances stem cell proliferation.

D. Autologous Implant of Ad-SVF: The stem cells obtained from the adipose tissue sample and the PRP are applied to the patient using appropriate protocol for their condition. Autologous Ad-SVF containing adult stem cells are infused locally by multiple local injections, to affected areas of the scalp.

E. Procedure for application of local scalp injections: Prepare PRP mixture and sample of Ad-SVF Containing Adult Stem Cells. Using aseptic technique, a 1 cc syringe, and a 27 G needle administer 0.1 cc of cell mixture via subcutaneous injections to affected areas of scalp, each approximately a half inch apart.

B. **Recommended Post-Operation / Stem Cell Therapy Schedule:**

A. Post-Op Medical Instruction - **(Please follow these instructions closely!)**

- ***Post-op medication*** will be given to you the day of your surgery. They will consist of an antibiotic and a painkiller:
  - *Antibiotic*: Cephalexin/Cipro, please take as directed beginning the day after surgery
  - *Painkiller:* Please take as directed and only as needed for pain

    * If you are unable to take any of these medications, please contact your patient coordinator so we can arrange for other medications.
- ***Resume previous medication*** as directed by the physician
- ***Report any symptoms of feeling unwell***: fever, pain, etc. Patients should be seen promptly by an ophthalmologist for full evaluation should any of the above symptoms be encountered.
- It is recommended that the ***patient have a companion stay with him or her*** for at least 24 hours after discharge.
- You should ***expect some of blood-tinged anesthetic solution to drain from the incision sites*** during the first 24 to 48 hours. This will vary from patient to patient. Maxi-pads are recommended for bandages over your incision sites. You may take a shower 24 hours after the procedure.
- ***Compressive garments should be worn*** 24 hours a day for the first week and 12 hours a day for the second week.
- ***Do not shower for the first 24 hours***. ***Do not submerge yourself in any water*** (i.e. taking a bath or swimming) for the 1st week.
- ***If you experience nausea or vomiting it is probably due to the medication***. Please try to take it with food. If it persists, please contact our office.
- ***Diet-meals are not restricted.***
- ***Drink plenty of clear fluids***. We recommend 8 glasses of water or fruit juice every day.
- ***Do not drink any alcohol*** for 48 hours and limit alcohol intake for the first week.

B. Post-Op Medical Consultation Schedule: 3 months & 6 months

- Review of medical history
- Review of medication history
- Review of any adverse events since the previous visit
- Clinical evaluation of symptoms using the Ludwig scale for female patients and the Norwood scale for male patients
- Photograph the area that was treated

Your doctor will contact you by phone within the first week to follow up then future follow up visits will be arranged through your patient coordinator. If you need assistance before do not hesitate to contact us.

ALOPECIA– Ludwig Scale (Female Patients)

Physician (name & clinic): _____

Patient (name & age): _____

Treatment (include date): _____

Please Select a Grade Per Column (Pre-Treatment, Month 3, & Month 6)

Ludwig Scale	Pre Treatment	3 Month	6 Month
 Grade I **Grade I:** Perceptible thinning of the hair on the crown, limited in the front by a line situated 1-3 cm behind the front hair line.			
 Grade II **Grade II:** Pronounced rarefaction of the hair on the crown within the area seen in Grade I.			
 Grade III **Grade III:** Full baldness (total denudation) within the area seen in Grades I and II.			

Dinh, Quan Q, and Sinclair, Rodney. "Female pattern hair loss: Current treatment concepts." Clinical Interventions in Aging (June 2007): Page 2. Web. 12/10/13.

ALOPECIA – Norwood Scale (Male Patients)

Physician (name & clinic): _____

Patient (name & age): _____

Treatment (include date): _____

Please Select a Grade Per Column (Pre-Treatment, Month 3, & Month 6)

Norwood Scale	Pre Treatment	3 Month	6 Month
Norwood 1: Normal head of hair - no visible hair loss.			
2  2A  **Norwood 2**: Hair is receding in a wedge-shaped pattern.			
3  3A  3V  **Norwood 3:** Same receding pattern as Norwood 2, except the hairline has receded deeper into the frontal area and the temporal area.			
4  4A  **Norwood 4:** Hairline has receded more dramatically in the frontal region and temporal area than Norwood 3 and there is the start of a bald spot at the back of the head.			
5  5A  5V  **Norwood 5:** Same pattern as Norwood 4 but much reduced hair density.			
6  **Norwood 6:** The strip of hair connecting the two sides of the head that existed in Norwood 4 & 5 no longer exists in Norwood 6.			
7  **Norwood 7**: Norwood 7 shows hair receding all the way back to the base of the head and the sides just above the ears.			

McAndrews, Paul J., MD. The Norwood Scale & Ludwig Scale. *American Hair Loss Association*. 2010. Web. 12/10/13.

J Invest Dermatol. 2006 Jul;126(7):1459-68.

Epithelial stem cells: a folliculocentric view.

Cotsarelis G.

Source
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Abstract
Putative epithelial stem cells were identified in the hair follicle bulge as quiescent "label retaining cells". The study of these cells was hindered until the identification of bulge cell molecular markers, such as CD34 expression and K15 promoter activity. This allowed for the isolation and characterization of bulge cells from mouse follicles. Bulge cells possess stem cell characteristics, including multipotency, high proliferative potential, and their cardinal feature of quiescence. Lineage analysis demonstrated that all epithelial layers within the adult follicle and hair originated from bulge cells. Bulge cells only contribute to the epidermis during wound healing, but after isolation, when combined with neonatal dermal cells, they regenerate new hair follicles, epidermis, and sebaceous glands. Bulge cells maintain their stem cell characteristics after propagation in vitro, thus ultimately they may be useful for tissue engineering applications. Understanding the signals important for directing movement and differentiation of bulge cells into different lineages will be important for developing treatments based on stem cells as well as clarifying their role in skin disease.

PMID: 16778814 [PubMed - indexed for MEDLINE]

PMID: 17936520 [PubMed - indexed for MEDLINE]

J Dermatol Sci. 2012 Apr;66(1):3-11. doi: 10.1016/j.jdermsci.2012.02.007. Epub 2012 Feb 24.

Multi-layered environmental regulation on the homeostasis of stem cells: the saga of hair growth and alopecia.

Chen CC, Chuong CM.

Source
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Abstract
Stem cells are fascinating because of their potential in regenerative medicine. Stem cell homeostasis has been thought to be mainly regulated by signals from their adjacent micro-environment named the "stem cell niche". However, recent studies reveal that there can be multiple layers of environmental controls. Here we review these environmental controls using the paradigm of hair stem cells, because to observe and analyze the growth of hair is easier due to their characteristic cyclic regeneration pattern. The length of hair fibers is regulated by the duration of the growth period. In the hair follicles, hair stem cells located in the follicle bulge interact with signals from the dermal papilla. Outside of the follicle, activation of hair stem cells has been shown to be modulated by molecules released from the intra-dermal adipose tissue as well as body hormone status, immune function, neural activities, and aging. The general physiological status of an individual is further influenced by circadian rhythms and changing seasons. The interactive networks of these environmental factors provide new understanding on how stem cell homeostasis is regulated, inspiring new insights for regenerative medicine. Therapies do not necessarily have to be achieved by using stem cells themselves which may constitute a higher risk but by modulating stem cell activity through targeting one or multiple layers of their micro- and macro-environments.

PMID: 22391240 [PubMed - indexed for MEDLINE] PMCID: PMC368425

ALOPECIA – Supporting Studies

Med Hypotheses. 2008;70(5):1014-6. Epub 2007 Oct 23.

Treatment of alopecia by transplantation of hair follicle stem cells and dermal papilla cells encapsulated in alginate gels.

Zhao J, Liu LQ, Wang YJ, Yang W, Geng WX, Wei J, Li LW, Chen FL.

Source
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Abstract
The affected individual of hair loss demands help, because hair is viewed as a sign of youth and good health. Nowadays treatment of alopecia includes drug therapy and hair transplantation. Some drugs may promote hair growth, at least temporarily, but the treatment is effective only in milder alopecia, instead of extensive alopecia. Furthermore, the side effect of long period medication could not be avoided. Hair transplantation involves harvesting small pieces of hair-bearing scalp grafts from a donor site and relocating them to a bald area. This method does not increase the number of existing hairs, but only redistributes them. The operation is sophisticated and time-consuming, thus the patient suffers a lot during the process. The discovery of hair follicle stem cells (FSC) brings gospel to the affected individual of hair loss because of its capacity of generating new hair when they interact with mesenchymal dermal papilla cells (DPC). Besides, both FSC and DPC have strong proliferative capacity and the patient's own cells could be expanded considerably in vitro. Thus we hypothesize that the microencapsulation of the two kinds of cells in alginate gels could be implanted into the bald scalp of the patient since alginate gels is effective in cell transplantation. The strategy may provide a more convenient and valid alternative to hair loss if the hypothesis proved to be practical.

ALOPECIA – References

[1] About Alopecia Areata. *National Alopecia Areata Foundation (NAAF)*. 2013. Web. 12/10/13.

Chen CC, Chuong CM. (2012). Multi-layered environmental regulation on the homeostasis of stem cells: the saga of hair growth and alopecia. doi: 10.1016/j.jdermsci.2012.02.007.