REVIEW ARTICLE

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SCIENCE

Hair Regeneration Therapy: Application of Adipose-Derived Stem Cells

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Abstract: Background: Adipose-derived stem cells secrete various cytokines that promote hair growth.

Objective: To describe our experience of hair regeneration therapy using adipose-derived stem cell-conditioned medium.

Results: We performed the hair regeneration therapy in numerous Japanese patients and reported good results. We described characteristics of the commercialized conditioned medium, treatment methods, and future directions.

Conclusion: Treatment using adipose-derived stem cell-conditioned medium is highly effective and may represent a new therapy for alopecia.

Keywords: Alopecia, adipose-derived stem cells, conditioned medium, cytokines, trichogram, half-side comparison study.

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1. INTRODUCTION

Various cytokines secreted by adipose-derived stem cells (ADSCs) have been reported to play a key role in angiogenesis and wound healing [1-4], as well as to stimulate hair follicles and induce hair growth [5, 6]. We confirmed that the effects of adipose-derived stem cell-conditioned medium (ADSC-CM) containing multiple growth factors on hair growth using both *in vitro* and *in vivo* experiments [7], developed a hair regeneration therapy that involves local administration of a commercialized ADSC-CM formulation (advanced adipose-derived stem cell protein extract, AAPE[®]; Prostemics Co., Ltd., Seoul, South Korea), and applied this therapeutic technique in clinical settings [8-11]. Currently, 170 facilities provide hair regeneration therapy with AAPE[®] in Japan. In this article, we will describe our experience with this treatment as well as its future directions.

2. CHARACTERISTICS OF AAPE[®]

AAPE[®] is a formulation that contains lyophilized forms of proteins secreted from cultured ADSC collected and isolated from healthy adult women [12]. This product was examined for possible bacterial and viral infections, as described previously [8, 10]. The manufacturing processes are in accordance with Korea Food and Drug Administration (KFDA) guidelines for cell therapy. During production, ADSC are maintained in a low-oxygen environment (2% O₂, 5% CO₂, 93% N₂) for 72 hours to induce cytokine secretion [7, 8, 13]. The product contains numerous cytokines,

including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF) and platelet-derived growth factor (PDGF) [14]. When these cytokines are in powdered form, their activities are not diminished, and consequently, they can be stored for long periods. Cytokines are dissolved in 4 ml of saline per vial immediately prior to usage.

3. TREATMENT SUBJECTS

Regardless of gender, individuals with alopecia of all levels of severity are eligible to receive treatment. According to our experience, in individuals with alopecia affecting nearly the entire head, those who have been bald for a long time, and the elderly, the duration of treatment is often prolonged and the effects may be limited due to atrophy of hair follicles and progression of fibrosis. Such details are explained to patients in advance, and treatments are then administered should they wish to receive them. If inflammatory skin disorders are observed, treatment is given after inflammation has subsided.

4. TREATMENT METHODS

The efficacy of hair regeneration therapy, as well as its side effects and safety, is thoroughly explained to each patient, and treatment is performed upon obtaining written consent. For a single treatment, 3-4 ml of AAPE[®] is administered to the entire scalp using the papule injection (intradermal injection). A 31-gauge needle is used to inject a volume of roughly 0.02 ml into each injection site, which are spaced 1 cm apart (Fig. 1). In most cases, AAPE[®] is administered in combination with vitamin B1 (5 mg), vitamin B6 (2.5-5 mg), vitamin H (1 mg), vitamin C (80-100 mg), vitamin E (5 mg),

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Fig. (1). A 31-gauge needle, whose length is controllable with a screw guide (Feel-Tech Co., Ltd., Busan, South Korea), is used for intradermal injection with an adapter locking to syringe (Medikan Co., Ltd., Seoul, South Korea).

coenzyme Q10 (10 U) and amino acids as nappage mesotherapy, in order to improve the function of mesotherapy with antioxidant effects and induce hair growth [15-19]. We refer to this combined therapy as hair regenerative therapy (HARG[®]) enhanced by hypoxic ADSC-CM [8-11]. Although it is possible to receive treatment without anesthesia, patients who complain of pain are given topical anesthesia with lidocaine or anesthesia that blocks the supraorbital nerve and greater occipital nerve. Typically, injections are given once a month and are repeated 6-8 times. Treatments are performed ≥ 10 times in some patients, depending on the severity of alopecia or the patient's treatment goals. In addition, some men also take finasteride as combination therapy during treatment or as maintenance therapy after treatment completion [8-11].

5. TREATMENT COURSE

Cytokines injected into the scalp are considered to act on local cells immediately after administration. However, our clinical experience suggests that it takes time for these cytokines to promote proliferation and protein synthesis on the scalp, and for such effects to become visible. We describe the general course of our treatment for androgenetic alopecia (AGA) and female pattern hair loss (FPHL), although the degree and speed of improvement vary between individuals.

After the initial 2-3 treatments (2-3 months after first treatment), new growth of thin hair is observed on the scalp by trichogram, while patients rarely notice any changes at this point (Fig. 2). We marked the shaved area with tattoos on the top of the head, and counted number of hairs in the same area over time using a trichogram in 21 patients (16

AGA and 5 FPHL; age range: 27-69 years) who consented to these procedures. The number of hairs at 3 months after the first treatment increased significantly in comparison with the number of hairs before treatment (141.3 ± 31.4 , 109.8 ± 43.5 , respectively; $P < 0.01$) [9].

With an increasing number of treatments, new hair growth and the number of hairs in the anagen phase increases, and such effects become noticeable to patients. We reported that patient satisfaction scores measured by the visual analog scale increased with the number of treatments, which targeted AGA and FPHL [8, 10]. After 4-5 treatments (4-5 months after first treatment), patients are often able to notice changes in hair quality or to confirm reductions of area in the extent of thinning hair through photograph (Figs. 3, 4). In our experience with AGA treatment, hairline improvement follows parietal improvement. We have treated some patients who wanted additional improvement after hair transplantation for frontal baldness at another clinic. Even though they were likely to need repeated treatments, we confirmed the efficacy of our treatment (Fig. 5).

Follow-up is performed after a series of treatment whilst continuing home care, such as taking oral finasteride or massaging the scalp by hand. None of the cases show rapid hair loss after treatment completion. Rather, the effects continue and hair growth gradually increases, often peaking at around 1 year. We believe these results are observed due to the activation of hair follicle cells by various cytokines after treatment, as well as the continuation of this activated state and improvement in the condition of the entire scalp.

6. HALF-SIDE COMPARISON TEST

In order to obtain additional clear evidence regarding the effects of our hair regeneration therapy, after providing a thorough explanation and obtaining consent, we conducted a half-side comparison test in 10 patients (8 AGA and 2 FPHL; age range: 20-73 years) [11]. Without oral finasteride, normal treatment was given 6 times on one side, and the same volume of placebo (saline) was injected on the opposite side. First, the intersection of a line that connects both ears at the top of the head and a line extends from each lateral canthus in the cranial direction are marked with tattoos. Subsequently, the number of hairs within both circles (11

Treatment	1st	2nd	3rd	4th	5th	6th	7th
Month	0	1M	2M	3M	4M	5M	6M
ADSC-CM injection	○	○	○	○	-	-	-
Photograph	○		○		○		○
Trichogram	Before				After 4M		After 6M

Fig. (2). A table showing the time flow of our treatment. Patients undergo multiple courses of ADSC-CM (AAPE) injections once a month. Two or three months after the first treatment, new growth of thin hair is observed on the scalp by trichogram. This case is 4 treatments (injections) of AAPE.

Fig. (3). Photographs of a 53-year-old male patient on 1 mg/day finasteride, starting 1 month before initial treatment. (a) Before treatment. (b) After 7 sessions of treatment with adipose-derived stem cell protein extract (AAPE) (1 month after final treatment). (c) Hairline before treatment. (d) Hairline after 7sessions of AAPE treatment (1 month after final treatment). Marked hair growth was observed after treatment.

mm diameter, 95 mm² area) with the tattooed markings at the center was quantified by trichogram before and after the treatment. Although the total number of hairs increased on both the treatment and placebo sides after 6 months of treatment, the increase over the treatment period was significantly different between the treatment and placebo sides (increase in hair count; 18.4±9.4, 6.5±11.7, respectively; *P* < 0.01). After completion of this test, normal treatment started on the placebo side and none of the patients showed any obvious bilateral differences in the end.

Fig. (4). Photographs of 40-year old female patient without finasteride or minoxidil medication. (a) Before treatment. (b) After 6 sessions of treatment with adipose-derived stem cell protein extract (AAPE). She began to notice improvements in hair quality. (c) After 8 sessions of AAPE treatment (3 months after final treatment). Marked hair growth was observed after treatment.

Fig. (5). Photographs of 35-year old male patient, on two occasions, 500 follicular units had been transplanted at another clinic in the frontal area (at age 25 and 29 years), on 1 mg/day finasteride, starting 5 years before initial treatment. (a) Before treatment. (b) After 8 sessions of AAPE treatment (1 month after final treatment). Marked hair growth was observed after treatment.

7. COMPLICATIONS

Although we have to date performed treatment in over 1,000 patients, we have not experienced complications such as AAPE[®]-induced allergic reactions or infection. None of the patients experienced sustained injection pain, chronic headache or itching sensations in the scalp. Although it has been reported that tissue necrosis and cicatricial baldness [20, 21] can occur after the mesotherapy procedure, such serious complications have not occurred after our treatments.

8. FUTURE DIRECTIONS

Although treatment with the present method accompanies pain at the time of injection, it maintains a high level of safety and is less invasive when compared to treatments that use hair transplantation or autologous stem cells. For these reasons, the present treatment method is becoming more common among individuals who do not show sufficient effects with medications such as oral finasteride and topical minoxidil or among those with female pattern hair loss, and is already in use at many facilities. However, with regard to treatment outcomes, there are no large-scale studies similar to the finasteride study [22, 23] and evidence remains poor. In the future, a high-quality comparison study is needed.

In our experience, most patients exhibited improvements. However, there are individual differences in the degree of improvement. Even if improvement is objectively evident, sufficient satisfaction is not obtained in all patients because treatment expectations increase with improvement. In addition, although ADSC-CM is considered safe because it maintains a physiological ratio, there may be a particular composition of cytokines specific to hair regrowth that also ensures safety. We believe that studies to optimize cytokine composition with ADSC-CM will make our treatments more effective.

CONCLUSION

We have performed hair regeneration therapy by intradermal injection of ADSC-CM in numerous Japanese patients. As this method uses a ready-to-use formulation for intradermal injection, special equipment and tools are not necessary, thus simplifying the procedure. This method maintains a high level of safety, and while its effects are not immediate, it facilitates hair regrowth by improving the scalp with minimal rebound effect and is therefore a useful method.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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