Autologous Cell-Based Therapy for Male and Female Pattern Hair Loss using Dermal Sheath Cup Cells: A Randomized Placebo-Controlled Double-Blinded Dose Finding Clinical Study

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Capsule summary

- **Injection of autologous dermal cup sheath cells on the scalps of male and female patients with pattern hair loss resulted in temporary increases in total hair density and cumulative hair diameter.**

- **Autologous cell-based therapy may become an alternative hair loss treatment that is useful both for men and women.**

Article type: Original article

Title: Autologous Cell-Based Therapy for Male and Female Pattern Hair Loss using Dermal Sheath Cup Cells: A Randomized Placebo-Controlled Double-Blinded Dose Finding Clinical Study

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Abbreviations

MPHL ….. Male Pattern Hair Loss

FPHL ….. Female Pattern Hair Loss

DSC ….. Dermal Sheath Cup

PHL ….. Patterned Hair Loss

FDA ….. Food and Drug Administration

DP ….. Dermal Papillae

SPEC ….. Shiseido Cell Processing and Expansion Center

ALP ….. Alkaline Phosphatase

ANCOVA ….. Analysis of Covariance

FAS ….. Full Analysis Set

PPS ….. Per Protocol Set

Abstract

***Background*:** Few effective treatments are available for male pattern hair loss (MPHL) and especially for female pattern hair loss (FPHL). Recently, cell-based therapies using autologous or allogeneic cells have been used clinically.

***Objective*:** We examined the safety and efficacy of autologous cell–based therapy using dermal sheath cup (DSC) cells to treat MPHL and FPHL.

***Methods*:** DSCs dissected from occipital hair follicles were cultured to manufacture DSC cells. Subjects with MPHL or FPHL received single injections of $7.5x10^6$, $1.5x10^6$ or $3.0x10^5$ DSC cells or a placebo in 4 randomized separate regions on their scalp, and hair densities and diameters were measured until 12 months later.

***Results*:** Fifty males and 15 females aged 33 to 64 were injected with DSC cells. Total hair density and cumulative hair diameter at the $3.0x10^5$DSC cells injection site was significantly increased compared with the placebo after 6 and 9 months. Men and women showed similar improvements and there were no serious adverse events.

***Limitations*:** No lower cell numbers were tested, and the positive effect was temporary until 9 months.

***Conclusion*:** The results suggest that cell therapy with autologous DSC cells may be useful as a new therapeutic method for treating MPHL and FPHL.

Capsule summary

- Injection of autologous dermal cup sheath cells on the scalps of male and female patients with pattern hair loss resulted in temporary increases in total hair density and cumulative hair diameter.
- Autologous cell-based therapy may become an alternative hair loss treatment that is useful both for men and women.

INTRODUCTION

Patterned hair loss (PHL) occurs with genetic and physiological predispositions as the background. PHL is the most frequent type of alopecia where hair loss progresses gradually according to a specific pattern. In men, due to the influence of male hormones, hair loss often starts after adolescence and is termed male pattern baldness or androgenetic alopecia. In the case of male pattern hair loss (MPHL), the anagen phase of each hair cycle becomes shorter and the hair follicles do not grow sufficiently and enter the next hair cycle while still miniaturized, so that the hair becomes progressively thinner and shorter, and the hair density is reduced.[1] While MPHL in men progresses under the influence of androgens, this is not clear for women.[2] Also, the pattern of the progression of hair loss in women is different from that in men and is characterized by thinning typically on the crown and around it, while the hairline is maintained,[3] and therefore it is termed female pattern hair loss (FPHL).

Two types of drugs that promote hair growth have been approved by the Food & Drug Administration (FDA), one topically (Minoxidil) and the other orally (Finasteride) though the efficacy of Finasteride in women has not been recognized.[4] Hair transplantation may be considered as an alternative treatment mainly for men by changing hair distribution. Currently there are limited treatment options for PHL, especially for women, and these treatments are not always satisfactory.

Dermal papillae (DP) are an essential mesenchymal part of hair follicles that promote and control hair growth and elongation. Dermal sheath cup (DSC) cells surround the DP and are also thought to play a pivotal role as progenitors of DP cells.[5] DSC cells grafted in mouse ear skin elicited relatively “more ordered hair follicle distribution” compared to DP cells.[6] Moreover, Reynolds et al. isolated and transplanted DP cells and DSC cells in humans, and reported that hair growth was observed when DSC cells were transplanted, but not with DP cells.[7]

A phase I/IIa study for cell-based therapy of hair loss using autologous DSC cells was conducted with 19 male and female subjects in Europe[8], and showed no serious adverse events with some improvement in total hair density at 6months interim analysis (unpublished preliminary data) .

Here, we performed a randomized, placebo-controlled double-blinded dose finding clinical study with autologous DSC cells to treat PHL in 66 male and female subjects, to examine the efficacy and safety of injecting autologous DSC cells into bald areas.

METHODS

Detailed descriptions of exclusion criteria, injections, DSC dissection procedures and the culture of DSC cells are available online at Mendeley Data Sets as http://dx.doi.org/10.17632/jhpj54ycmt.

Study participants. Eligible male and female subjects were aged over 20 years with MPHL in males classified as type III-vertex, IV, V and VI using the Norwood-Hamilton classification[9], and FPHL in females classified as grades 3-6 using the Shiseido classification presented as supplementary data at Mendeley Data Sets.[10] Characteristics of the study subjects are listed in Table 1. All subjects signed informed consent forms approved by the Institutional Review Board at each center and the Certified Committee for Regenerative Medicine at the Tokyo Medical University under the Act on the Safety of Regenerative Medicine.

Study design. This study was a randomized, double-blinded, placebo-controlled, dose finding, 12 months clinical study conducted at two centers in Japan. A schematic overview of the study is shown in Fig.1. After informed consent, subjects eligible for the study were screened and DSCs from each subject were dissected as previously described [11] (from July 2016 to April 2018) and cultured as detailed in the next section and supplementary data at Mendelely Data Sets . Four circular injection sites (each approximately 2 cm^2) for each subject were fixed inside the hair loss areas. Three concentrations of DSC cell suspensions ($7.5x10^6$, $1.5x10^6$ and $3.0x10^5$ cells) and a placebo (each in a volume of 1 ml) were injected separately into 4 randomly allocated injection sites. The efficacy was evaluated by taking images of phototrichograms, before the injections and at 3, 6, 9 and 12 months later (from July 2016 to April 2019), and the hair densities and hair diameters were measured using image analysis system software as described in detail in the efficacy evaluation section. Safety evaluations assessed the local safety at the injection sites, the extent of systemic adverse events, and their relevance to the injections, and this clinical study was periodically monitored by an independent research contract organization agency (SRD

Co., Ltd., Tokyo, Japan). After the end of the clinical study period 12 months after the injections, follow-ups will be conducted for another 2 years.

DSC dissection and culture of DSC cells. DSC dissection and preparation of DSC cell suspensions are described in the online supplementary data at Mendelely Data Sets.

Efficacy evaluation (Assessments). Before the injections and at 3, 6, 9 and 12 months later, the hairs at the four injection sites of each subject were clipped to 1 mm length. A tattoo ink was used to identify each target region. Phototrichogram images were taken with an EOS 600D digital camera (Canon Inc, Japan) equipped with a Cutiscope (Ennoblement Hohlrieder Martin Dr. Co, Austria). These phototrichogram images were given random codes, and hair characteristics were measured by three trained technicians using image analysis system software (Hybrid Measure: Inotech Corp, Japan). Characteristics measured included total hair density (hairs/cm^2), cumulative hair diameter (sum of hair diameters per square centimeter, mm/cm^2) and mean hair diameter (average of the diameters of all measured hairs, μm).

Safety evaluation. Each subject underwent a physical examination and a physician's consultation before the injections and at 1, 3, 6, 9 and 12 months later. A physician's consultation was also performed 2 and 7 days after the injections.

Statistical analysis. The difference between the baseline and 3, 6, 9 and 12 months after the injections was calculated for each parameter. These data were compared by analysis of covariance (ANCOVA) using the baseline as a covariate, and subject, dose level, injection site and technician as factors. Estimations of the difference between each dose level and the placebo were performed using a 95% Wald confidence interval.

Safety assessments were performed using McNemar's test on paired contingency tables of the placebo site and each dose site, that counted the presence or absence of adverse events.

RESULTS

Subject characteristics. A total of 67 subjects were selected and biopsied, and 65 subjects (50 males and 15 females with a mean age of 51.1 ± 7.0 years) were injected with autologous DSC cells (FAS; Full Analysis Set). Table I shows the baseline characteristics of those subjects. The average number of DSC cells derived from each subject after passaging was $7.1 \times 10^7 \pm 3.5$ cells, and their viability was stable and high at 97.2 ± 2.2%. ALP activity was positive (low to medium range) in all DSC cell cultures. A total of 62 subjects completed the 12-month observation period (PPS; Per Protocol Set).

Efficacy. Differences from the baseline to 3, 6, 9 and 12 months after the injections were calculated, and the means of those differences were compared with the placebo for each dose level. Total hair density (Fig 2a) and cumulative hair diameter (Fig 2b) increased significantly at 6 and 9 months at the low-dose DSC cell injection site compared to the placebo. There was no significant change in mean hair diameter (Fig 2c) in any group over the course of the study.

Both males and females showed similar results at the low-dose injection site (Fig 3a). Stratified analysis by age and hair loss progression showed that the treatment was more successful in older subjects (51 years or older) (Fig 3b) and in subjects with moderate severity (Hamilton grade III, IV and Shiseido grade 3, 4) (Fig 3c). The treatment was more successful in older subjects with moderate severity (Fig 3d). Representative phototrichogram images of effective cases showed increases in hair density and diameter (Fig 4).

Safety. Mild adverse events, such as erythema, swelling, purpura and small hemorrhages, at the injection sites were observed in 14 cases (45 by sites). There was no indication suggesting there was a difference in the occurrence of adverse events between the DSC cells and the placebo injection sites (McNemar's test). Three mild vagal reflexes were seen at the time of injection as systemic adverse

188 events. These local and systemic adverse events were mild and occurred during the injection or within 2
189 days, after which their disappearance was confirmed.
190

DISCUSSION

This is a clinical study reporting a cell-based treatment for hair loss using autologous DSC cells that shows a significant result. In the present study, $3.0x10^5$ DSC cells, the lowest dose among the three doses of DSC cells tested, elicited a statistically significant increase in total hair density and cumulative hair diameter compared with the placebo. The increase of hair density is thought to be due to the induction of anagen transition in existing resting hair follicles according to a human hair follicle model.[12] Within the $3.0x10^5$ dose injection, subjects stratified by moderate severity (Hamilton grade III, IV and Shiseido grade 3, 4) and older (≥51 years) were significantly improved compared with the placebo, indicating the existence of a subpopulation of higher responders to the treatment. In other words, older patients may possess higher numbers of resting inactive hair follicles (telogen hairs) so that injected DSC cells showed more prominent improvement in the induction of hair growth. Since the same result was found for female subjects as well as males, this cell therapy treatment is expected to be useful for female subjects whose options are limited compared with male subjects.

Regarding adverse events, although mild adverse events resulting from the injections occurred in 14 cases, there was no significant difference in the incidence of adverse events between the DSC cells and the placebo injection sites. This clinical study was well tolerated.

Regarding the proof of concept of this treatment, a reasonable explanation is that due to the migration of injected DSC cells into pre-existing miniaturized hair follicles and their incorporation into DP and DSC regions, they presumably differentiated into DP cells. As support for this hypothesis, it has been recently shown that human DSC cells injected in reconstituted human hair follicles in the dorsal skin of nude mice migrate and are taken into hair follicles.[12] To determine whether cultured DSC cells retain their hair inductive property, ALP is the only candidate potency marker for DP,[13] although that has not yet been proven. The DSC cells used in this study had moderate to weak ALP activity, however

215 we did not perform any histological analysis after DSC cell injection. Further study is needed to trace
216 injected DSC cells and to identify additional new markers for the hair inductive potency of DSC cells.
217 The fact that mean hair diameter was unchanged suggested that the improvement was not
218 limited to an increase in vellus hair, but also that the number of other thicker or thinner hairs were also
219 increased at the same time.
220 The highest dose used in this study was chosen according to a phase I/IIa trial in Europe.[8] The
221 medium dose was set as a 1:5 dilution of the highest dose, and a further 1:5 dilution of the medium dose
222 was set as the lowest dose. The improvement by the lowest dose of DSC cells used in this study was
223 demonstrated, and the reason why a dose dependency was not observed in the medium and higher
224 doses of DSC cells is presumed to be tissue damage or a poorer environment of the tissue caused by the
225 injection of higher numbers of DSC cells. A preclinical study of mice confirmed that the number of viable
226 cells remaining in the skin after injection is immediately reduced by a certain amount when higher cell
227 numbers (> $1.5x10^6$ cells/mL) are injected, indicating that there may be an upper limit to the number of
228 viable cells that can be retained in the skin (unpublished observation). Further, the debris of dead cells
229 may cause inflammatory reactions such as immune cell migration and cause a poorer environment for
230 remaining viable DSC cells. Another possibility is that there is certain range in the number of DSC cells
231 per injected skin area that activates resting hairs to enter an active hair cycle (Anagen hair). Although
232 $3.0x10^5$ DSC cells was the lowest dose tested, this does not imply that this number of DSC cells is
233 insufficient, and rather it is advantageous both in terms of manufacturing and clinical viewpoints in
234 which larger bald areas could be treated with relatively small cell numbers per area and further non-
235 invasive, safer treatments would be useful.
236 Careful examination is needed to determine if the reduced hair growth at 12 months is due to
237 the lifetime of the injected cells and/or to another factor. Recently we have shown that injected DSC
238 cells are retained for at least four months in hair follicles in a human hair follicle model established with

a 6 month-lifespan in nude mouse dorsal skin.[12] This time we used treatment with a single injection of DSC cells at each site, but preclinical studies using human hair follicle models have shown the effectiveness of repeated injections (unpublished data). For an improved clinical protocol, the effectiveness of sequential injections of multiple doses of DSC cells after specific periods of time is also an issue to be examined.

The phototrichogram method used to evaluate hair growth is an objective and relatively accurate method, however, it has a limited quantitative detection range, and therefore, an additional global asset evaluation method by clinical doctors that assesses the overall appearance is also necessary in future studies.

In conclusion, this clinical study of autologous cell therapy using DSC cells to treat male and female PHL has shown positive, although temporary, responses at the lowest cell concentration injected, and further studies are warranted to determine the best concentration of cells and treatment regimen. In order to determine if this cell-based treatment provides a significant clinical change noticeable to patients and practicing physicians, additional clinical studies injecting DSC cells in larger hair shedding areas should be performed to demonstrate a visible effect by global photo-assessment.

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296

297

FIGURE LEGENDS

Fig 1. Overview of the clinical study. Scheme showing the sequence of skin biopsies, cell injections and phototrichogram measurements performed at the Medical Centers and the microdissection of DSC cells and their production performed at the Cell Processing Center. Hair follicles were isolated from each scalp skin biopsy and DSCs were dissected from those follicles. Isolated DSCs were incubated in culture flasks for cell expansion. After completion of the expansion cultures, the concentrations of DSC cells were adjusted to 7.5×10^6/mL (High-dose), 1.5×10^6/mL (Med.-dose) and 3.0×10^5/mL (Low-dose) along with a placebo (without cells). They were then blinded by randomized codes for injection, frozen in vial tubes and stored in liquid nitrogen until shipped to the hospital.

Fig 2. ANCOVA analysis of each parameter. a) Total hair density, b) Cumulative hair diameter, and c) Mean hair diameter. The difference of the 3 doses of DSC cells (Low, Med. and High) from the baseline are shown as a difference from the placebo. Low: Low-dose (3.0×10^5/mL), Med.; Medium-dose (1.5×10^6/mL) and High; High-dose (7.5×10^6/mL).

Fig 3. ANCOVA analysis of gender, age and severity of hair loss. **a)** Both males and females show similar results with the low concentration DSC cell injection. **b)** Older subjects (51 years old and over), and **c)** Moderate hair loss subjects (III, IV, 3 and 4) demonstrated significant response compared to the placebo. **d)** Total hair density of stratified older subjects with moderate severity showed significant increase compared with the placebo.

Fig 4. Representative phototrichogram images of male and female subjects before the injection and 9 months later. DSC cells ($3.0\text{x}10^5$ cells) were injected from the center marked with a red tattoo. The

measurement area was in a circle with a diameter of 15 mm. a) Male, 53 years old subject; total hair density increased by 2.5/cm^2 and cumulative hair diameter increased by 0.30 mm/cm^2 (vs. placebo). b) Female, 43 years old subject; total hair density increased by 3.0/cm^2 and cumulative hair diameter increased by 0.60 mm/cm^2 (vs. placebo).

Table I

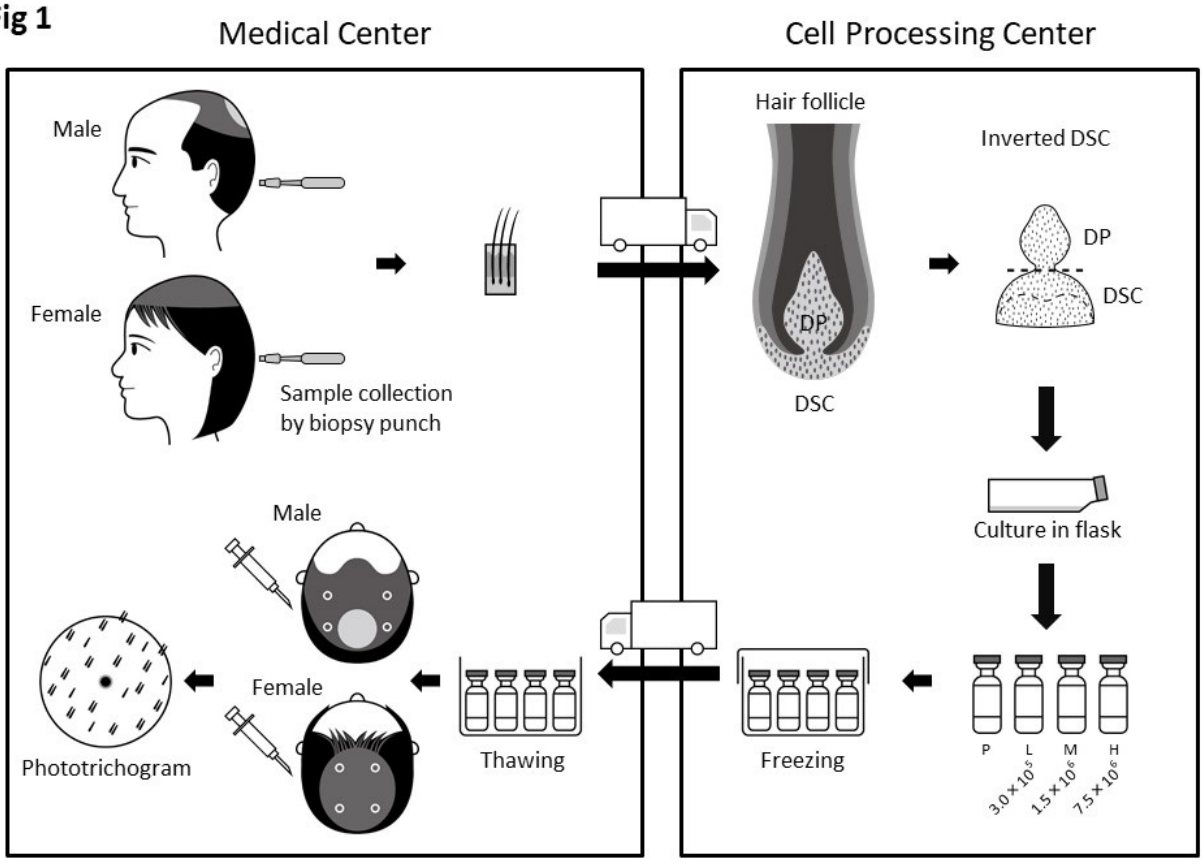
	All N=65	Male N=50	Female N=15
Age, y			
Mean ± SD	51.1 ± 7.0	52.0 ± 6.7	48.0 ± 7.3
Minimum-maximum	33 - 64	35 - 64	33 - 57
Stage of MPHL, n (%) (Norwood-Hamilton)			
III-vertex		5 (10.0)	
IV		16 (32.0)	
V		15 (30.0)	
VI		14 (28.0)	
Stage of FPHL, n (%) (Shiseido grade)			
3			3 (20.0)
4			3 (20.0)
5			4 (26.7)
6			5 (33.3)

TABLE LEGEND

Table I. Baseline characteristics of subjects injected with DSC cells.

The 65 subjects in the FAS population receiving DSC cell injections included 50 males and 15 females, with an average age of 51.1 years (52.0 years for men, 48.0 years for women). For men, subjects with Norwood-Hamilton scale [9] type III-vertex to type VI and for women with Shiseido scale [10] 3 to 6 hair loss were selected.

Fig 1


Medical Center
Cell Processing Center
Male
Female
Sample collection
by biopsy punch
Hair follicle
DP
DSC
Inverted DSC
DP
DSC
Culture in flask
P
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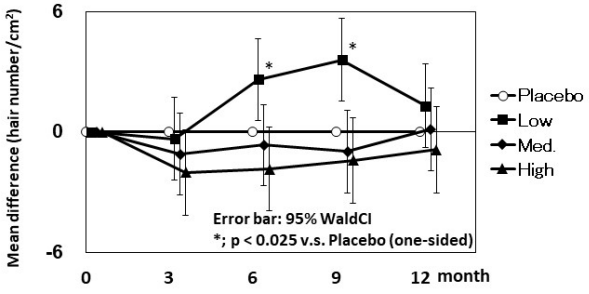
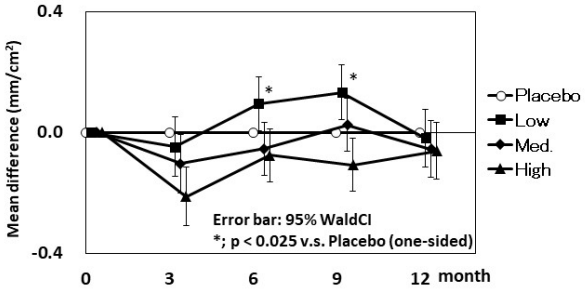
H
3.0×10^5
1.5×10^6
7.5×10^6
Freezing
Thawing
Male
Female
Phototrichogram

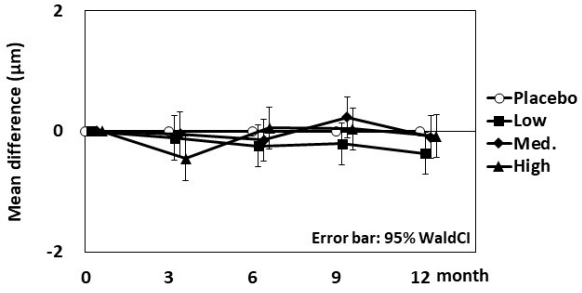
a) Total hair density



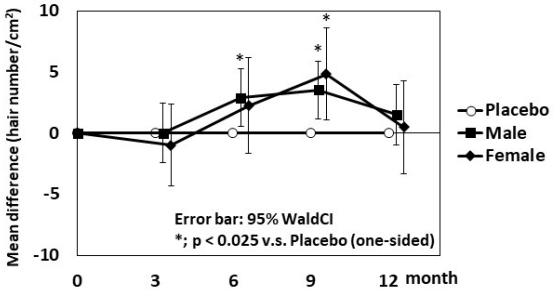
b) Cumulative hair diameter



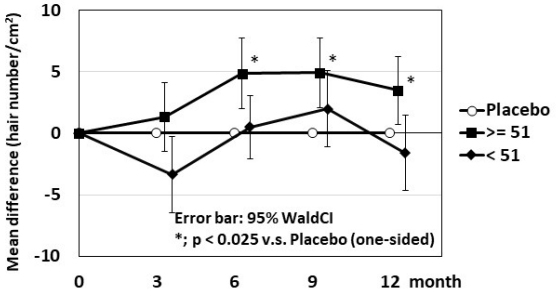
c) Mean hair diameter



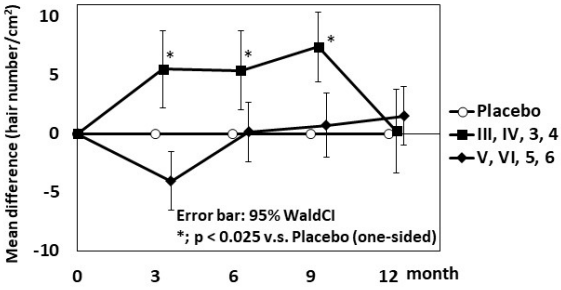
a) Total hair density
: Male (N=50) / Female (N=15)



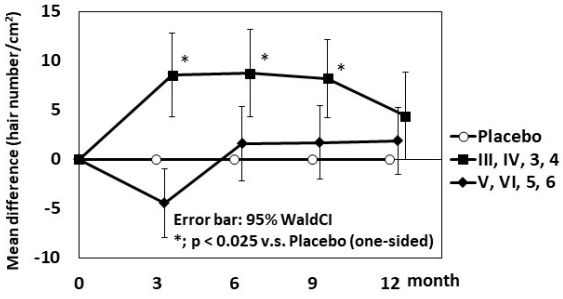
b) Total hair density
: >= 51 years old (N=39) / < 51 years old (N=26)



c) Total hair density
: III, IV, 3, 4 (N=27) / V, VI, 5, 6 (N=38)

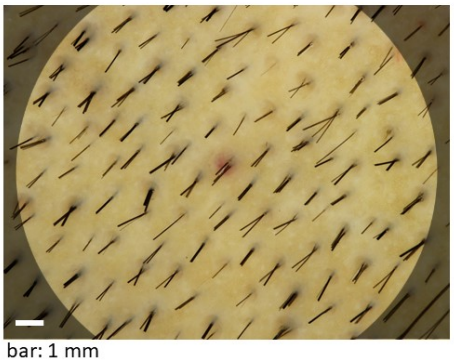


d) Total hair density of 51 years or older subjects
: III, IV, 3, 4 (N=22) / V, VI, 5, 6 (N=17)



a) Male, 53 years old

Before



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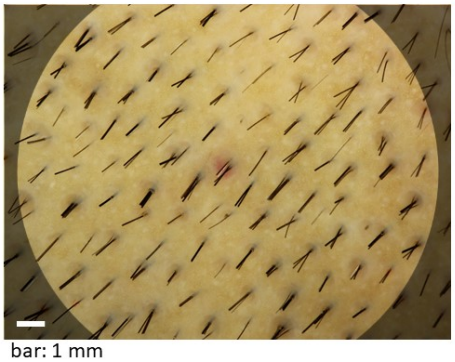
9M



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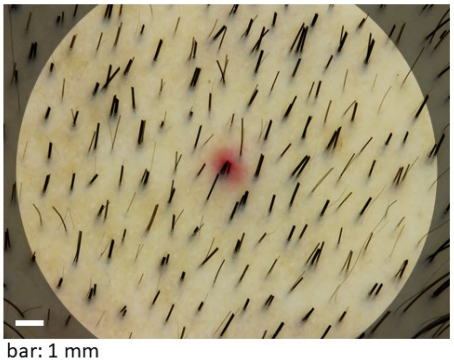
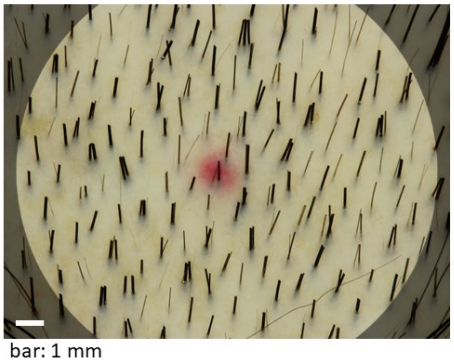
b) Female, 43 years old

Before



bar: 1 mm

9M



bar: 1 mm