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Randomized Trial of Electrodynamic Microneedle combined with 5% Minoxidil Topical Solution for the Treatment of Chinese Male Androgenetic Alopecia

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Running title: Treating AGA by Microneedle and Minoxidil

Abstract

Background: In treating androgenetic alopecia, 5% minoxidil is a commonly used topical drug. By using electrodynamic microneedle at the same time may increase absorption of minoxidil and further stimulate hair growth. Objective: A 24-week,

randomized, evaluator blinded, comparative study was performed to evaluate the efficacy of treating Chinese male androgenetic alopecia using microneedle combined with 5% minoxidil topical solution. Methods: Randomized subjects received topical 5% minoxidil (group 1, n = 20), local electrodynamic microneedle treatments (group 2, n = 20), or local electrodynamic microneedle treatments plus topical 5% minoxidil (group 3, n = 20). A total of 12 microneedle treatments were performed every 2 weeks with 2ml 5% minoxidil delivery in group 3 during each microneedle treatment. Patient receiving topical 5% minoxidil applied 1 ml of the solution twice daily over the course of the study. A total of 60 Chinese male subjects with Norwood-Hamilton type III-VI androgenetic alopecia were treated. Results: The mean improvement in total hair density from baseline to 24 weeks was 18.8/cm^2 in group 1, 23.4/cm^2 in group 2, and 38.3/cm^2 in group 3. The hair growth in the 3 groups was significantly different (P = 0.002), but there were no significant differences in toxicity found between the 3 groups. Conclusions: Treatment with microneedle plus topical 5% minoxidil was associated with the best hair growth.

Key words: Androgenetic alopecia, microneedle, Chinese, minoxidil

Introduction

Androgenetic alopecia (AGA), also known as male pattern baldness, mainly occurs in men 22 to 55 years of age in China (1). AGA is characterized by miniaturization of the hair follicles in the frontal and parietal scalp (2) and a decrease in the proportion of hair follicles in the growing and resting stages from the normal ratio of 12:1 to 5:1. The average follicle density decreases from 325/cm^2 in a normal adult to 278/cm^2 (3). Serum androgen levels and a family history of AGA are the most commonly reported risk factors for developing the condition.

Treatments used to promote hair growth include oral traditional Chinese medicine, 5α-reductase inhibitors, anti-androgenic drugs, and topically applied minoxidil (3-9). Minoxidil is the only topical US Food and Drug Administration-approved drug for the treatment of AGA; other current medical treatments are often not successful and hair transplantation can be an expensive, painful procedure.

Microneedle is a kind of micro-needle array device, which makes holes on the skin to damage the barrier of stratum comeum, so that drugs can enter into skin through microneedle holes and make effects locally or enter into the capillary networks of dermis. In treating AGA, using an electrodynamic microneedle may significantly improve the percutaneous absorption rate of minoxidil. The intense stimulation caused by microneedle at the local alopecia site may also give rise to hair follicle regrowth in the process of wound-repair (10-12). However, the relative researches were rare.

We conducted a 24-week, randomized, evaluator blinded, comparative study to evaluate treatment safety and efficacy of treating Chinese male AGA using microneedle combined with 5% minoxidil topical solution.

Methods

Subjects

Male subjects aged 20–50 years with Norwood-Hamilton type III-VI AGA were recruited for treatment in the Department of Dermatology at The First Affiliated Hospital of China Medical University, Shenyang, China from December 2015 to January 2016. Subjects were asked to cut their hair regularly in order to keep the same hair length and hairstyle each follow-up visit. Hair coloring was discouraged unless patients were already doing so. Patients taking any medications within the previous 6 months or with an abnormal physical examination or laboratory test results were excluded. Subjects were not allowed to use any treatments—other than the study drugs—that promote hair growth. Informed consent was obtained from each subject prior to the study. The trial protocol was reviewed and approved by the Institutional Review Board at The First Affiliated Hospital of China Medical University and the study conformed to the principles of the Declaration of Helsinki.

Study design

Subjects were randomly allocated into 3 groups using the random number table method: topical 5% minoxidil (group 1, n = 20), electrodynamic microneedle treatment (group 2, n = 20), and electrodynamic microneedle treatment with topical 5% minoxidil (group 3, n = 20). Patients received microneedle treatments every 2 weeks, for a total of 12 times.

Patients were asked to wash their hair on the nights before the treatments and have their hair cut every 4 weeks. Local sites were swabbed with alcohol wipes 2-3 times before treatment and anesthetic ointment was placed on the head 1 h prior to the injections. The depth of electrodynamic microneedle (Yuanxiang Biological

Technology Company, Guangzhou, China) treatments was between 1.5 to 2.5 mm. The treatments were repeated 3–4 times, until the scalp became reddish, and local hemorrhaging occurred. For group 3, 2ml of 5% minoxidil (Wanma Pharmaceutical Company, Hangzhou, China) was delivered into the scalp at the mean time of microneedle treatments. The scalp was then massaged to promote the absorption of the drug. But for group 2, only microneedle treatments were conducted without delivery of minoxidil. Patients were instructed not to wash their hair for 24 h after the microneedle treatment. Oral antibiotics (azithromycin, erythromycin, or cephalosporin) were administered for 3 days to prevent infection. Patient receiving topical 5% minoxidil applied 1 ml of the solution twice daily over the course of the study, but for group 3, the topical minoxidil was stopped using on the microneedle treatments days because the same amount of drug had already been delivered into the scalp during microneedle treatments. Patients were evaluated every 4 weeks at which time treatment-related toxicity of all groups was recorded. Subjects' self-reported treatment efficacy was also recorded.

Efficacy evaluation

A high-pixel digital camera (D610, Nikon, Japan) was used to photograph subjects' bald area at each 4-week follow-up visit. Patient posture, patient positioning, camera angle, camera position, and lighting conditions were reproduced during each photo session. We took microscopic photographs for analysis every 4 weeks from the baseline to Week 24. We photographed the same 1 cm^2 areas that were the most severely damaged by AGA with the contact dome of the camera applied to the head. Hair in that area was clipped to a length of 1–2 mm before taking photographs. Areas of AGA that were followed for response were marked by keeping the hair 1–2 mm in length over the course of the study because tattoo marking is not widely accepted in

China. We applied plastic belts to the subjects' heads to check monitored areas for a response. The location on the belt, the angle of the belt, and prior photographs were reviewed to ensure the same area was evaluated each 4-week follow-up visit.

A hair microscope system (CBS-1717, Taiwan, China) that included a computer and a camera was used to evaluate the hair density and thickness; 70X magnification was used for all microscopic photographs. Hair density was calculated as the number of hairs per cm^2. Hairs less than 40 μm in diameter were counted as vellus hairs while hairs greater than 40 μm in diameter were counted as non-vellus hairs. Three board-certified dermatologists blinded to the treatment (they did not participate in the treatment process of the clinical trial) counted the number and diameter of hairs present. Only non-vellus hairs were used to characterize the hair thickness.

Investigator assessment of hair growth

Hair growth was evaluated using a 7-point scale: obvious improvement (3 points), moderate improvement (2 points), mild improvement (1 point), no change (0 points), mild exacerbation (−1 points), moderate exacerbation (−2 points), and obvious exacerbation (−3 points). A fourth blinded board-certified dermatologist (who did not participate in the treatment process of the clinical trial) assessed the hair growth of the monitored area at the baseline and at 12 and 24 weeks after starting the treatment by examining photos of the bald area.

Patient assessment of hair growth

Patients evaluated their own hair growth in the bald areas at the baseline and 12 and 24 weeks after starting the treatment. A 5-point scale was used: no change (0 points), 1–25% improvement (1 point), 26–50% improvement (2 points), 51–75% improvement (3 points), and 76–100% improvement (4 points).

Compliance and safety evaluation

Patients treated with minoxidil were provided with a new 60-ml spray bottle at every 4-week follow-up visit and the volume that remained in the older bottles was measured. Patients who used less than 45 ml from any of the bottles were not evaluated for efficacy.

Patients receiving 1 or more doses of the treatment were evaluated for toxicity at 1, 12, and 24 weeks after starting the treatment. Patients were evaluated for local toxicity, including erythema, infection, erosion, edema, seborrheic dermatitis, dryness, itching, increased dandruff, and broken hairs. The pain experienced by each patient treated via microneedle treatments was measured using a visual analogue scale (VAS), where 0 indicated no pain and 10 indicated intolerable pain.

Statistical analysis

A random number table was used to assign subjects to the 3 treatment groups. The primary endpoint of the study was the change in hair density from the baseline to 24 weeks. The secondary endpoint of the study was the change in hair thickness from the baseline to 24 weeks.

Data are presented as the mean ± standard deviation. A Student's one-sample and two-sample *t* test were used for analysis of hair thickness and density. The Wilcoxon rank sum test was used for the global assessment of hair growth. Differences between the 3 groups were analyzed using one-way ANOVA. Adherence to treatment and toxicity were analyzed using a χ^2-test. $P < 0.05$ was considered to be statistically significant. All P values were 2-sided. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Study population

Twenty patients were randomly placed into 1 of the 3 treatment arms (n = 60, Figure 1). The 3 treatment groups had similar age, duration of hair loss, and hair density at the start of the study (Table 1). Eighteen patients in group 1 and 2, all 20 patients in group 3 completed the study and whose data were available for an analysis of efficacy. All of the 60 patients were available to assess toxicity.

Efficacy

The mean increase in the non-vellus hair density from the baseline to 24 weeks was 24.1/cm^2 in group 1, 20.5/cm^2 in group 2, and 34.7/cm^2 in group 3 (Table 2, $P < 0.001$ for each group from the baseline, $P = 0.014$ between groups). The increase in the hair density of group 3 was greater than that of group 2 ($P < 0.05$, two-sample *t* test). There was no difference in the increase in the hair density found in groups 1 and 2 or in groups 1 and 3.

The mean change of the vellus hair density from the baseline to 24 weeks of treatment was −5.4/cm^2 in group 1, 3.4/cm^2 in group 2, and 4.2/cm^2 in group 3 (Table 2, $P < 0.05$ for each group from the baseline, $P < 0.001$ between groups). The change in the hair density of group 1 was greater than that of group 2 and the change in the hair density of group 3 was greater than that of group 1 ($P < 0.001$, two-sample *t* test for both groups). There was no difference in the change in the hair density of groups 2 and 3.

The mean change in the total hair density from the baseline to 24 weeks of treatment was 18.8/cm^2 in group 1, 23.4/cm^2 in group 2, and 38.3/cm^2 in group 3 (Table 2, $P < 0.001$ for each group from the baseline, $P = 0.002$ between groups). The change in the hair density of group 3 was greater than that of group 1 and the change

in the hair density of group 3 was greater than that of group 2 ($P < 0.05$, two-sample *t* test for both groups). There was no difference in the change in the hair density of groups 1 and 2.

The mean increase of the non-vellus hair thickness from the baseline to 24 weeks of treatment was 10.7 μm in group 1; 3.2 μm in group 2; and 11.8 μm in group 3 (Table 3, $P < 0.001$ for group 1 and 3 from the baseline, $P = 0.005$ between groups). The thickness of the hairs in group 3 was greater than that of group 2($P = 0.001$, two-sample *t* test). There was no difference in the hair thickness of groups 1 and 2 or of groups 1 and 3.

Investigator assessment of hair growth

We noted that 5.6% (1/18) of the patients in group 1, 5.6%(1/18) of the patients in group 2, and 35% (7/20) of the patients in group 3 showed an obvious improvement (Table 4) at Week 24. We discovered that 44.4% (8/18) of the patients in group 1, 27.8% (5/18) of the patients in group 2, and 60% (12/20) of the patients in group 3 had moderate improvement while 16.1% (9/56) of the patients in all of the groups showed obvious improvement, 44.6% (25/56) had moderate improvement, 33.9% (19/56) had mild improvement, and 5.4% (3/56) showed no improvement after treatment at Week 24. No patient experienced worse AGA after the treatments. Group 3 had a better outcome than group 1 ($P = 0.005$, one-way ANOVA) and 2 ($P < 0.001$, one-way ANOVA). Group 1 and 2 had similar outcomes.

Patient assessment of hair growth

In Group 3, 25% (5/20) of the patients showed 76-100% improvement at Week 24 (Table 5, Figure 2). No patients had 76–100% improvement in group 1 or 2 while 11.1% (2/18) of patients in group 1, 5.6% (1/18) of patients in group 2, and 55% (11/20) of patients in group 3 showed 51–75% improvement at Week 24. Of all of the

patients, only 2 in group 2 thought their scalps showed no improvement after the treatment. Group 3 had a better outcome than group 1 ($P < 0.001$, one-way ANOVA) and 2 ($P < 0.001$, one-way ANOVA). Group 1 and 2 had similar outcomes.

Toxicity

There were 3 patients each in group 1 and 2 and 4 patients in group 3 who experienced toxicity (Table 6). There were no significant differences in the frequency of toxicity between the groups, although different toxic symptoms were seen in the different groups. Increased scury, infection, and enlarged lymph nodes were only seen in patients undergoing microneedle treatments with the elderly and immunocompromised patients more commonly affected. Enlargement of lymph nodes was self-limiting, resolving without treatment within 3–4 days. There were 3 patients who withdrew from the study: 2 patients after microneedle treatment and enlargement of lymph nodes occurred and 1 patient after eczema developed. All treatment-related toxicity was mild to moderate in severity and did not require treatment. The mean VAS pain score for all patients treated via microneedle treatments was 4.52 ± 3.7, a moderate score.

Discussion

The best therapeutic effect was observed in group 3, which was treated via microneedle treatment plus topical 5% minoxidil. These results are supported by the improvements seen in the non-vellus and the total hair counts, the hair thickness, investigator assessment, and patient self-assessment. Patients in group 3 had the best subjective cosmetic result (Figure 2c); 80% of these patients showed greater than 50% improvement of hair growth, but only 11.1% of the patients in group 1 and 5.6% in group 2 had the same results.

Minoxidil has been reported to promote non-vellus hair thickness (6). Use of microneedles was associated with a similar non-vellus hair thickness as minoxidil alone. This finding may be due to the small number of patients treated, as the means appeared different, but the standard deviations were large.

Microneedles delivering 5% minoxidil were associated with the largest growth of new vellus hairs in patients we treated; in contrast, the topical use of 5% minoxidil was associated with a decrease in vellus hair density.

There was no significant difference in toxicity seen across the 3 treatment groups. There were, however, patterns of toxic symptoms that were suggestive and will need to be confirmed in a larger study as scury, infection, and enlarged lymph nodes were only seen in patients undergoing microneedle treatments.

Minoxidil is the only topical drug approved by the US Food and Drug Administration for the treatment of both male and female AGA. It prolongs the active growth phase of hair follicles by promoting proliferation and differentiation of hair follicle cells (7). Minoxidil increases both the local blood supply with its direct vasodilator effects and the local expression of vascular endothelial cell growth factor (7,8); together these factors promote angiogenesis of the dermal papilla.

Minoxidil is widely used in China for the treatment of hair growth. Hu R et al. treated 130 Chinese AGA patients with a topical 1-ml application of 5% minoxidil daily for 12 months and saw a 59% overall response rate (6). Similar outcomes have been reported in the treatment of Japanese men with AGA (9).

Hair follicles can form during wound repair (10,11); trauma seems to activate hair bulb stem cells, increasing the expression of VEGF and Wnt10b, which are genes associated with hair growth (12-14). Epidermal growth factors are also released during skin regeneration and repair (12). Use of microneedles could simulate this process.

The epidermis is formed by layers of cells without blood vessels and nerves, and is the rate-limiting barrier to the absorption of topically applied drugs (15). The most superficial layer of the epidermis, the stratum corneum, consists of dead cells and is the greatest barrier to drug diffusion across the epidermis (15,16). Application of the microneedle perforates the epidermis at multiple sites, allowing drug passage through the stratum corneum and into the capillaries of the dermis (17,18).

Microneedles have been previously used to increase transdermal drug absorption (18). The absorption of calcein (with a molecular radius of 6 A°) was increased 1,000 times after application of a microneedle (17). An electrodynamic microneedle contains a disposable head with 9 needles that are 0.25 mm in diameter and have an adjustable length. When activated, the needles oscillate at a high frequency, diminishing needle pain and increasing drug delivery. An electrodynamic microneedle was used in this study. Topical minoxidil entering the skin by diffusion or through pores made by a microneedle injection is converted into its active metabolite, minoxidil sulphate, by sulphotransferase enzymes located in the human scalp and epidermal keratinocytes.

Only two studies have reported the use of microneedles in treating male AGA. Dhurat R administered topical 5% minoxidil twice daily plus the application of microneedles once a week to treat 50 men with AGA (19) while a control group received topical 5% minoxidil twice daily. The microneedle treatment group had the best outcome after 12 weeks of treatment. Four additional men with AGA that showed no benefit from the treatment of finasteride and topical 5% minoxidil did show an increase in hair growth after the microneedle treatments (20). However, the prior studies were lacking of complete research processes, the data were general and the treatment period was short.

Adverse events associated with the use of microneedle in treating AGA have not been previously reported; however, the use of microneedles may not be suitable in patients with altered immunity or cardiovascular disease because of the increase risk of infection and stress associated with the microneedle application. It is important to note that scalp infection and enlarged cervical lymph nodes were only seen in patients treated with microneedles.

In this study, we performed a systematic research about treating Chinese male AGA using microneedle and 5% minoxidil. The combined treatment method was proved to be better than either treatment alone; however, there was a limitation to our study that the small number of patients limited our ability to determine differences in their response rates, hair thickness, and the toxicity of different treatments. So a larger study is needed to better define the risks and benefits of this treatment. Moreover, further researches about the mechanisms of how microneedle treatments can promote hair growth or increase topical minoxidil absorption need to be explored.

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Conflicts of interest

The authors declare that there are no competing financial interests.

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Figure 1. Randomized controlled trial design.

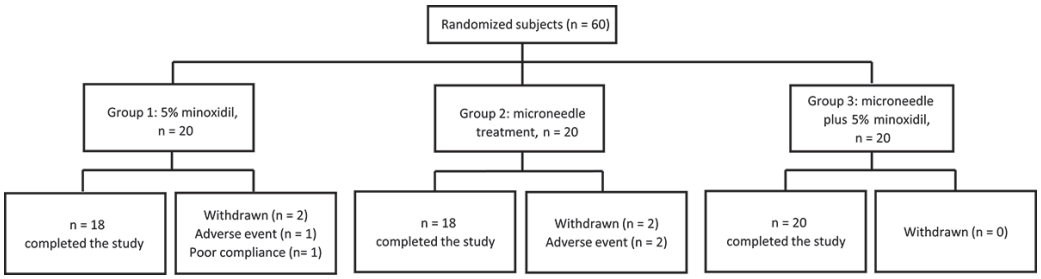

Figure 2. Photographs of patients at the baseline and after 24 weeks of treatment. (a) Group 1, (b) Group 2, and (c) Group 3. Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil.

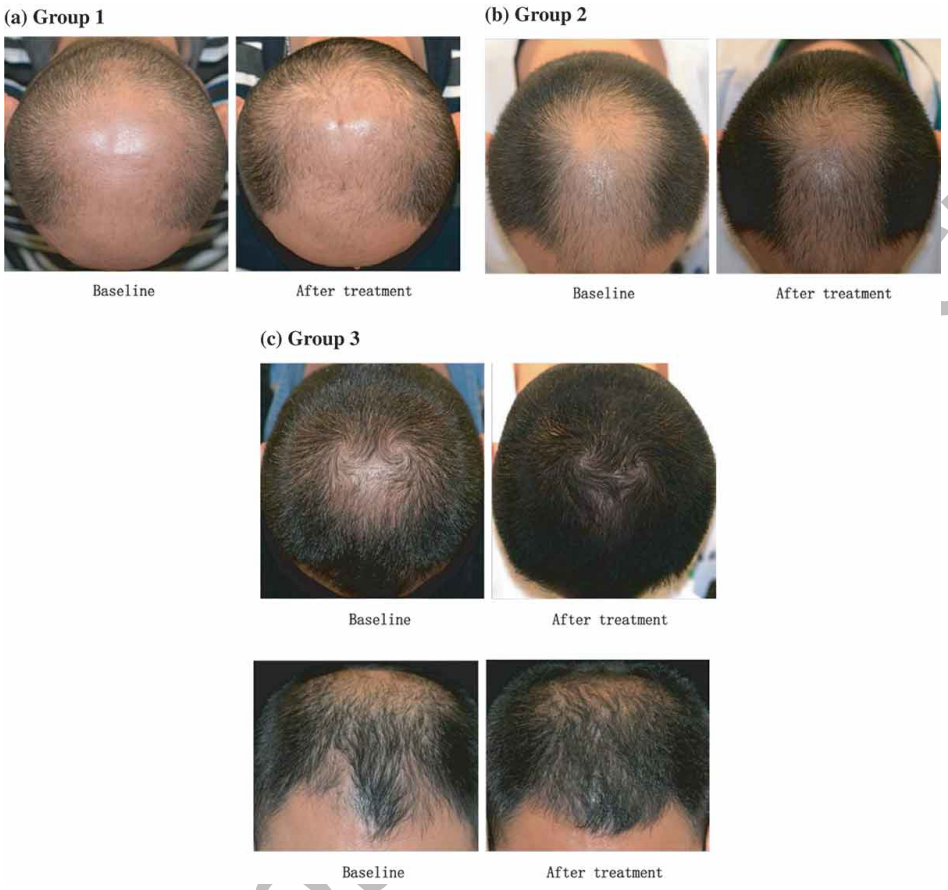

Table 1. Patient characteristics

	Group 1 (n = 18)	Group 2 (n = 18)	Group 3 (n = 20)
Age (years)	34.7 ± 6.9	34.1 ± 3.1	35.2 ± 3.3
Duration of hair loss (years)	6.4 ± 1.5	6.6 ± 4.3	6.92 ± 3.8
Non-vellus hair count/cm^2	91.9 ± 10.7	89.5 ± 7.3	88.9 ± 11.9
Vellus hair count/cm^2	60.5 ± 5.5	62.1 ± 3.7	62.38 ± 8.2
Total hair count/cm^2	152.5 ± 17.1	151.6 ± 23.2	151.3 ± 16.6
Mean hair thickness (μm)	65.3 ± 3.5	64.4 ± 5.1	64.71 ± 12.9
Hamilton-Norwood classification, n			
III	5	4	4
IV	6	6	7
V	5	6	6
VI	2	2	3
Previous treatments, n			
Never treated	15	14	14
Minoxidil	1	3	2
Propecia	2	0	2
Traditional Chinese medicine	0	1	2

Data is presented as mean ± standard deviation. Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil.

Table 2. Changes in hair density after 24 weeks of treatment

Hair count/cm^2	One-sample *t* test			One-way ANOVA	P value Two-sample *t* test		
	Group 1 (n = 18)	Group 2 (n = 18)	Group 3 (n = 20)	P value between the three groups	Group 1 vs Group 2	Group 1 vs Group 3	Group 2 vs Group 3
Non-vellus hair count	24.1 ± 7.8 P < 0.001	20.5 ± 7.3 P < 0.001	34.7 ± 13.2 P < 0.001	P = 0.014	P = 0.99	P = 0.065	P = 0.01
Vellus hair count	−5.4 ± 3.8 P < 0.001	3.4 ± 3.5 P = 0.002	4.2 ± 2.1 P < 0.001	P < 0.001	P < 0.001	P < 0.001	P = 1.000
Total hair count	18.8 ± 9.6 P < 0.001	23.4 ± 5.1 P < 0.001	38.3 ± 11.1 P < 0.001	P = 0.002	P = 0.098	P = 0.001	P = 0.01

Data is presented as mean ± standard deviation. Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil.

Table 3. Mean change in hair thickness from baseline to Week 24

	One-sample *t* test			One-way ANOVA	P value Two-sample *t* test		
	Group 1 (n = 18)	Group 2 (n = 18)	Group 3 (n = 20)	P value between the three groups	Group 1 vs Group 2	Group 1 vs Group 3	Group 2 vs Group 3
Change in Non-vellus hair thickness (μm)	10.7 ± 5.5 P < 0.001	3.2 ± 6.2 P = 0.09	11.8 ± 3.7 P < 0.001	0.005	P = 0.102	P = 0.1	P = 0.001

Data is presented as mean ± standard deviation. Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil.

Table 4. Investigator assessment of hair growth at 24 weeks

Assessment	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Total n (%)
Obvious improvement (3 points)	1 (5.6)	1 (5.6)	7 (35.0)	9 (16.1)
Moderate improvement (2 points)	8 (44.4)	5 (27.8)	12 (60.0)	25 (44.6)
Mild improvement (1 point)	8 (44.4)	10 (55.5)	1 (5.0)	19 (33.9)
No change (0 points)	1 (5.6)	2 (11.1)	0 (0.0)	3 (5.4)
Mild exacerbation (−1 point)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Moderate exacerbation (−2 points)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Obvious exacerbation (−3 points)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil. $P < 0.001$ between the 3 treatment groups (one-way ANOVA). Group 1 vs Group 2, $P = 0.24$; Group 1 vs Group 3, $P = 0.005$; and Group 2 vs Group 3, $P < 0.001$.

Table 5. Patient assessment of hair growth at 24 weeks

Assessment	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Total n (%)
76–100% improvement (4 points)	0 (0.0)	0 (0.0)	5 (25.0)	5 (8.9)
51–75% improvement (3 points)	2 (11.1)	1 (5.6)	11 (55.0)	14 (25.0)
26–50% improvement (2 points)	10 (55.6)	8 (44.4)	2 (10.0)	20 (35.7)
1–25% improvement (1 point)	6 (33.3)	7 (38.9)	2 (10.0)	15 (26.8)
No change (0 points)	0 (0.0)	2 (11.1)	0 (0.0)	2 (3.6)

Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil. $P < 0.001$ between the 3 treatment groups (one-way ANOVA). Group 1 vs Group 2, $P = 0.09$; Group 1 vs Group 3, $P < 0.001$; and Group 2 vs Group 3, $P < 0.001$.

Table 6. Adverse events

Adverse events	Group 1 n = 20	Group 2 n = 20	Group 3 n = 20	Total n = 60
Total number of adverse events	3	3	4	10
Seborrheic dermatitis	1	-	1	2
Itching	1	-	-	1
Increased scurf	-	1	1	2
Infection	-	1	-	1
Enlargement of cervical or posterior auricular lymph nodes	-	1	2	3
Eczema	1	-	-	1

Group 1: 5% minoxidil; Group 2: microneedle treatment; and Group 3: microneedle combined 5% minoxidil. Comparison of the 3 treatment groups, P = 1.0 (χ^2-test).