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A Clinical Trial of Treating Androgenic Alopecia with Mesenchymal Stem Cell Suspension Derived from Autologous Hair Follicle

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Background: Androgenic alopecia (AGA) is characterized by progressive hair follicle miniaturization. Novel treatments are needed to intervene in the miniaturization process. The authors aimed to evaluate the efficacy, safety, effectiveness, and effective population of autologous hair follicle mesenchymal stem cell therapy for the treatment of advanced AGA in Chinese individuals.
Methods: Fifty patients ranging in age from 25 to 45 years (average, 32 ± 1.24 years) were included. None of them had ever used minoxidil, finasteride, or other drugs to promote hair growth. Healthy hair follicles were extracted from the occipital area and treated to obtain hair follicle mesenchymal stem cell suspensions. The recipient sites were divided into 2 groups. Nine points were injected in a 1-cm^2 area, and 100 μL of solution containing either 1×10^5 cells or normal saline was injected at each point. The follow-up duration was 9 months. Observers were blinded to patient groupings and measurements.
Results: An increased proportion of terminal hair and hair shaft diameter was observed in the experimental group at 1 month. The effect lasted for 3 months. The hair-thickening effect of advanced miniaturized hair follicles with hair shaft diameter less than 60 μm was more notable than that for above 60 μm. No patient experienced any obvious side effects.
Conclusions: Hair follicle mesenchymal stem cells were effective in the treatment of advanced AGA in Chinese individuals. A hair shaft diameter of 60 μm can be used as a key index to predict the effectiveness of the therapy. *(Plast. Reconstr. Surg.* 154: 444e, 2024.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, II.

Androgenic alopecia (AGA), the most common form of hair loss, starts in adolescence, and is mainly characterized by progressive hair follicle miniaturization.[1] In the continuous hair cycle process, the pathologic hair follicle mesenchymal niche in the hair loss area is continuously damaged. The mesenchymal cells are continuously consumed, resulting in functional disorder, gradually losing the ability to induce the proliferation and differentiation of hair follicle epidermal cells to form hair follicles. This process eventually leads to the complete miniaturization and loss of hair follicles.[2] Nonsurgical treatments for AGA, such as minoxidil or finasteride, vary widely in effectiveness in different populations, and may not achieve the expected effect

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in patients with advanced AGA, although they are recommended as first-line drugs for androgenic alopecia.[3–5]

Hair follicle mesenchymal stem cells (HF-MSCs) include dermal papilla (DP) and dermal sheath (DS) cells. Similar to other types of MSCs, HF-MSCs possess stem cell characteristics such as multidirectional differentiation potential, the ability of integrated homing to a specific niche, and secretion of exosomes and cytokines, which have great application prospects.[6,7] HF-MSCs are considered an important therapeutic agent to promote hair growth. A previous study has indicated that injection of DPC spheres can promote hair growth on the back of mice.[8] A double-blind clinical trial in Japan confirmed that treatment of expanded DS cup cells of healthy human hair follicles could promote the growth of hair follicles in the hair loss area of the forehead.[9] However, the population and stage of AGA for which HF-MSC therapy is effective have not been reported.

We conducted a randomized clinical trial in which we extracted hair follicles from the human occipital area, prepared an HF-MSC suspension, and injected it locally into the forehead hair loss area. Through follow-up observations, we evaluated the safety and effectiveness of autologous HF-MSC therapy in the treatment of AGA and considered possible effective populations and stages.

PATIENTS AND METHODS

Patient Information

Fifty Chinese patients (25 female, 25 male), 25 to 45 years of age, with an average age of 32 ± 1.24 years, were included. The Norwood grade for male hair loss was II through V, and the Ludwig grade for female hair loss was I through III. Exclusion criteria included the following: (1) using medications or supplements, including finasteride, dutasteride, ketoconazole, minoxidil, or any other hormonal products that can affect hair growth; (2) severe systemic, immune, endocrine, or nervous system diseases; or (3) head skin infection, allergic disease, or malignant tumor. Written informed consent was obtained from all the patients. The ethics committee of Nanfang Hospital approved this study.

Experimental Design

A diagram of the clinical trial is shown in Figure 1. Two hair loss areas on the left and right of the midline of each patient's head were designated as the experimental and control groups, respectively. The size of the selected area was 1 cm^2, and a dot was made using a scalp tattoo tool to mark the center of the selected area. Each patient received a multiple-point injection pattern in a 1 cm^2 box area centered on the marking dot. The volume of each injection was 100 μL, with an interval of approximately 0.5 cm between each injected point, and the cell volume was 1×10^5 per point. The control group was injected with the same volume of normal saline. All patients received the cell suspension and normal saline injection at the same time. Which solution was injected on each side was randomly assigned, and the people who performed the injection and the observers were blinded to patient groupings and measurements.

After the injection, dermoscopy was performed using the marked points as a reference at 0 days and 1, 3, 6, and 9 months. The diameter of each hair within the treatment area was analyzed and divided into 6 subgroups: less than 30, 30 to 40, 40 to 50, 50 to 60, 60 to 80, and greater than 80 μm. Hair diameter thickening to the next highest group was considered an effective change. Hair diameter and terminal hair proportion were measured using the phototrichogram dermoscopy system.

Preparation of HF-MSC Suspension

The obtained healthy human occipital hair follicles were placed under a stereoscope, excess adipose tissue was removed, and hair bulbs were isolated by microdissection. The treated hair follicle tissue was digested with 0.1% Dispase for 15 minutes, and the dermal and epidermal components were separated by blowing. Next, 0.2% collagenase was added, digested at 37°C, and blown every 10 minutes. After observing that the DS was completely digested into single cells and DP spheres were separated from the hair bulb, digestion was stopped with an equal volume of normal saline. The enzyme solution was removed by centrifugation. After resuspension in normal saline, the suspensions were allowed to stand until the hair shaft sank to the bottom of the centrifuge tube. The supernatant containing DS single cells and DP spheres was obtained and repeated 2 or 3 times. After centrifugation, the cells were resuspended in normal saline to obtain the final HF-MSC suspension.

Safety Assessment

Each patient underwent a physical examination before injection and at 1 week and 1, 3, 6, and 9 months after injection. During follow-up,

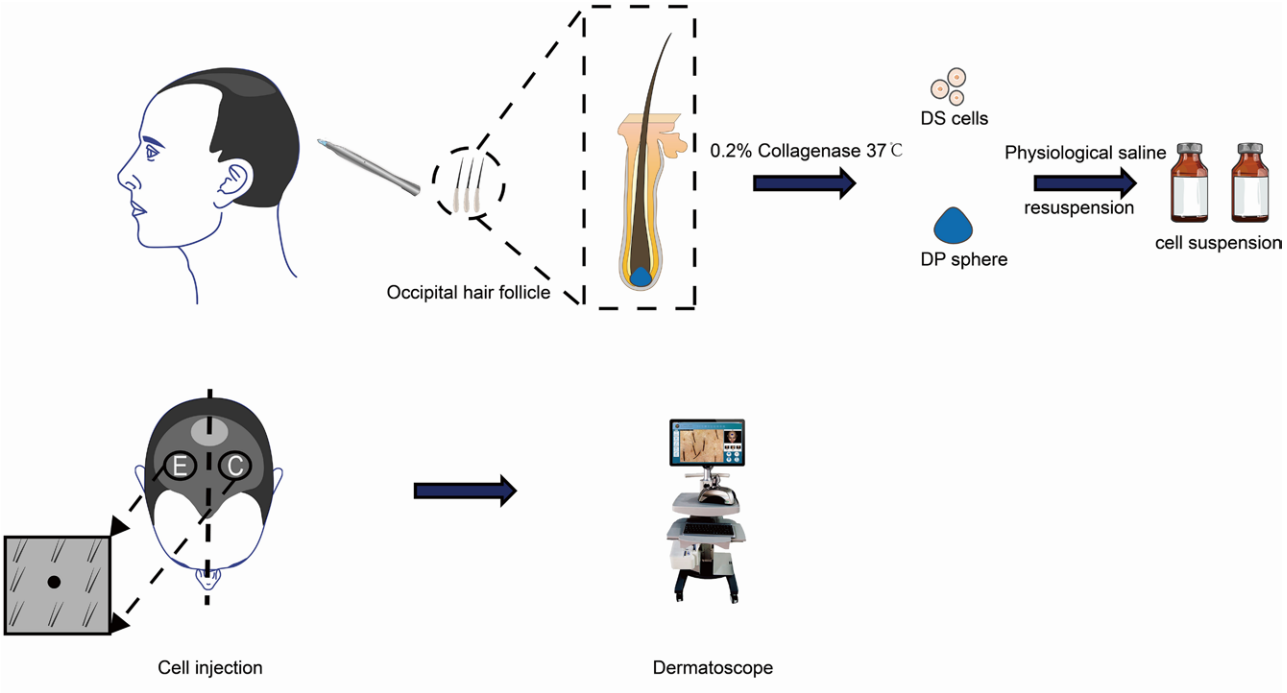

Fig. 1. Overview of the clinical trial. Healthy autologous occipital hair follicles were extracted. After microdissection and enzyme digestion, dermal sheath (*DS*) cells and dermal papillae (*DP*) spheres were resuspended with physiologic saline to prepare HF-MSC suspensions and ready for injection. Experimental (*E*) and control (*C*) sides were randomly selected on the same hair loss areas. Each selected area was marked by scalp tattoo tools. Each area was 1 cm^2 with 9 injected points. After injection of HF-MSC suspensions, patients were followed up regularly by dermoscopy.

Table 1. Baseline Data

Data	Experimental Side	Control Side	*P*[a]
Mean hair diameter, μm	62.56 ± 8.17	62.20 ± 7.64	0.76
Proportion of terminal hair, %	44.36 ± 13.16	45.61 ± 12.34	0.40
Cumulative hair diameter, μm	13,040 ± 3707	13,749 ± 4619	0.26
Follicular unit/cm^2	91.12 ± 25.90	88.82 ± 24.20	0.80

[a]No differences were significant (paired-samples Wilcoxon signed rank test; all $P > 0.05$).

any discomfort or side effects were reported to the registration department.

Statistical Analysis

Differences between measurements at baseline and at 3, 6, 9, and 12 months after the injections were calculated. These data were compared by paired-samples Wilcoxon signed rank test. All results of our study were analyzed using GraphPad Prism 8.0.1. Data were expressed as mean ± SEM; $P < 0.05$ was deemed significant.

RESULTS

Participant Characteristics

Fifty patients received autologous healthy hair follicle MSC suspension injections. The average cell volume of each patient was 1×10^6, and the cell activity was 96.3 ± 1.7%. All patients completed a 9-month follow-up period (Fig. 1). The baseline data are shown in Table 1.

Regarding the efficacy assessment, taking the day of injection as the baseline, the proportion of terminal hair and hair diameter at 1, 3, 6, and 9 months was compared with the control side (see Table 1 for baseline data).

As for the proportion of terminal hair, through dermoscopy follow-up, we found that at the first month after injection, the proportion of terminal hair in the experimental group was significantly higher than in the control group; the effect lasted for 3 months, and then began to subside (Fig. 2). Meanwhile, the proportion of terminal hair in the control group decreased continuously for 9 months without any treatment. There

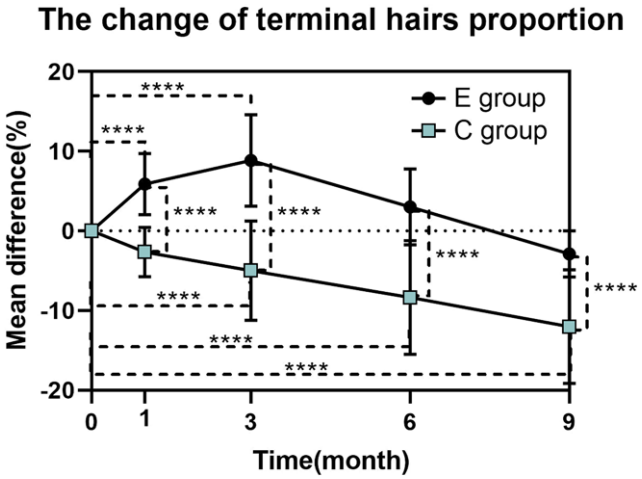

Fig. 2. The proportion of terminal hair changes after injection. The proportion of terminal hair started to increase at the first month after injection, lasted for 3 months, and then the effect began to decrease. The control group had a continuous decrease. *C*, control side; *E*, experimental side. Paired-samples Wilcoxon signed rank test; ****$P < 0.0001$.

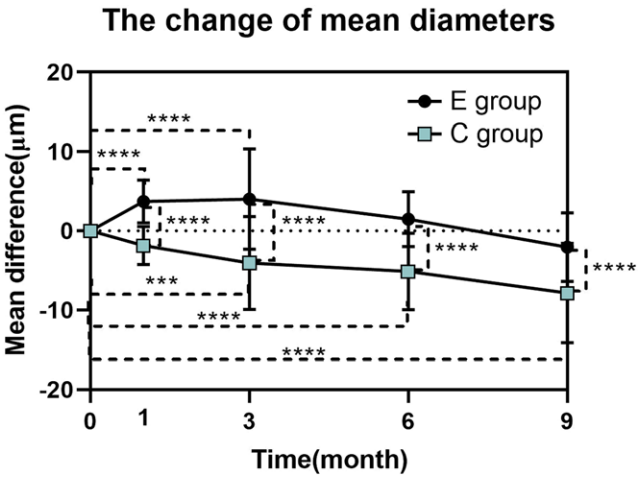

Fig. 3. Mean diameter changes after injection. The mean hair diameters of the selected experiment area started to increase at the first month after injection, lasted for 3 months, and then the effect began to decrease. The control group had a continuous decrease. *C*, control side; *E*, experimental side. Paired-samples Wilcoxon signed rank test; ***$P < 0.001$, ****$P < 0.0001$.

was no significant difference between the male and female patients. (**See Figure, Supplemental Digital Content 1**, which shows a comparison of terminal hair proportion changes between male and female patients. No significant difference in the effect of terminal hair proportion was noted, ***http://links.lww.com/PRS/G914***.)

The cumulative and mean diameters in the experimental group were higher than in the control group. The effect began to appear in the first month after treatment and lasted for 3 months. Consistent with the change in the terminal hair proportion in the control group, the cumulative and mean diameters of hair follicles in the control group decreased continuously within 9 months (Fig. 3). (**See Figure, Supplemental Digital Content 2**, which shows changes in cumulative diameters in the experimental and control groups. The cumulative hair diameters of the selected experiment area started to increase at the first month after injection, lasted for 3 months, and then the effect began to decrease. The control group had a continuous decrease. Paired-samples Wilcoxon signed rank test ***$P < 0.001$, ****$P < 0.0001$, ***http://links.lww.com/PRS/G915***.) The mean follicular unit did not change significantly before and after treatment. (**See Figure, Supplemental Digital Content 3**, which shows changes in mean follicular unit in the experimental and control groups. After injection of the suspension, the mean follicular unit did not change significantly in either group, ***http://links.lww.com/PRS/G916***.)

To further explore which population and stage of AGA may have a better response to HF-MSC therapy, we set 6 groups—less than 30, 30 to 40, 40 to 50, 50 to 60, 60 to 80, and greater than 80 μm—according to our previous research on AGA stage divided by hair diameter. Hair follicle thickening to a higher-level group was defined as an effective change. Compared with the baseline, the effective thickening proportion of hair follicles with a diameter less than 60 μm was the largest; the thinner the original hair diameter, the better the thickening effect, whereas the effect decreased as the diameter of primary hair follicles increased (Fig. 4). When the initial hair diameter was less than 60 μm, after treatment, the hair diameter in the experimental group was significantly higher than in the control group (Fig. 5). When the initial hair diameter was greater than 60 μm, the thickening effect of the HF-MSC treatment was not remarkable, but showed the effect of preventing hair follicles from further miniaturization. (**See Figure, Supplemental Digital Content 4**, which shows 2 representative dermoscopy images of autologous MSC suspension therapy. Before treatment, the average diameter of the hair follicles was greater than 60 μm. (*Left*) Before injection. (*Right*) Three months after injection, ***http://links.lww.com/PRS/G917***. **See Table, Supplemental Digital Content 5**, which shows statistical information for the dermoscopic images in Fig. 5 and Supplemental Digital Content 4. Diameters are in micrometers, ***http://links.lww.com/PRS/G918***.)

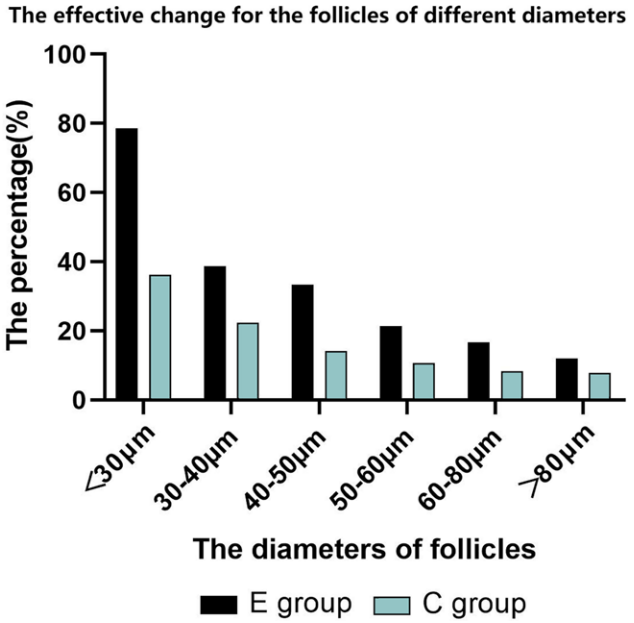

Fig. 4. Effective change for follicles of different diameters. In all groups, the change in the diameter of the experiment group was larger than in the control group. The effective change was more substantial when the diameter of the hair follicle was less than 60 μm.

With regard to the safety assessment, some patients had slight redness, erythrosis, or mild swelling at the injection site within 24 hours after injection, which disappeared after 24 hours (Table 2). None of the patients experienced obvious infection, erythrosis, or other adverse reactions. There were no serious adverse reactions in either the experimental or control groups.

DISCUSSION

In this randomized controlled clinical trial of autologous healthy hair follicle–derived MSC therapy to treat AGA, the results showed a good effect with no obvious side effects.

For the substances contained in the suspension, we acquired primary HF-MSCs by microdissection and enzyme digestion, prepared cell suspensions, and injected them without in vitro expansion. The patients completed hair extraction and injection on the same day. Using primary cell suspensions for injection can prevent the loss of stem cell properties, cell contamination, and cell differentiation caused by in vitro expansion

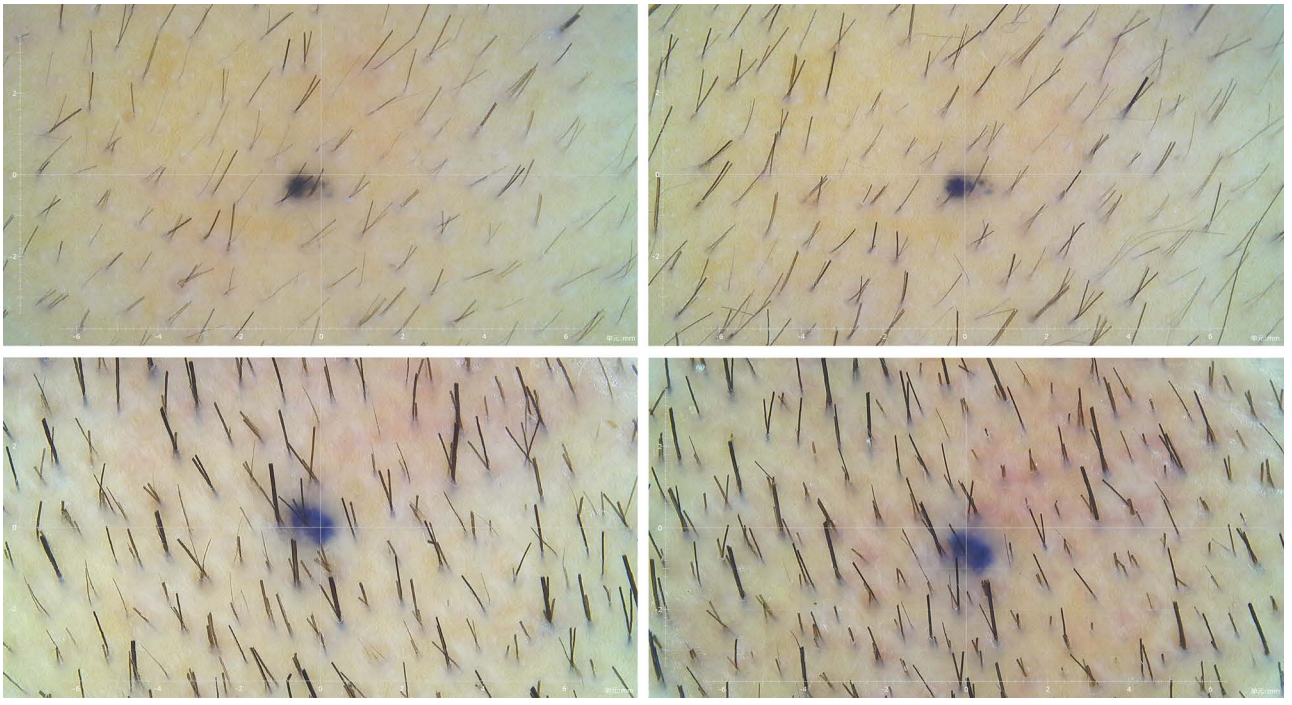

Fig. 5. Two representative dermoscopy images of the hair-thickening effect of autologous MSC suspension therapy. Nine injection points occupied 1 cm^2 selected areas; each point was injected with 100-μL solution containing 1×10^5 cells. (*Left*) Before injection. (*Right*) Three months after injection. See Supplemental Digital Content 5 for associated data.

Table 2. Complications after the HF-MSC Suspension Injection[a]

	Redness	Swelling	Erythema	Pruritus	Erythrosis	Infection	Caking	Pigmentation
Occurrence ratio	2%	4%	0%	2%	2%	0%	0%	0%

[a]No serious complications occurred in any patients. Four patients had minor complications, but their symptoms disappeared 24 hours after treatment.

and culture. Furthermore, according to our research in mice (unpublished data), the mechanism of local injection of HF-MSCs to promote hair growth may include homing and paracrine effects. DP and DS cells functioned as HF-MSCs, which can be integrated into the host hair follicle mesenchymal niche after intradermal injection to supplement the consumption of the mesenchymal niche. The longer the culture time in vitro, the worse the integration efficiency. However, we did not collect samples for further research in this trial. Follow-up experiments are needed to clarify the therapeutic mechanism of HF-MSC injections.

The injection pattern we used was selected to avoid excessive amounts of cells at the same injection point, which may result in skin necrosis and the release of various inflammatory factors, causing a local immune response and hindering the desired effect. This was consistent with previous research by a Japanese team in 2020.[9] In addition, multipoint injection in a certain area may improve the paracrine effect. Further research is needed to explore the scope of paracrine signaling, using the lowest number of injection points to achieve the maximum effect, in future applications.

The proportion of terminal hair and hair diameter in the experimental group was higher than in the control group 1 month after injection. The effect lasted up to 3 months, and then began to subside. There were no significant differences between men and women. Correspondingly, the proportion of terminal hair in the control group continued to decline, indicating that hair loss was progressing.

We also analyzed changes in hair diameter with this treatment using a dermoscope. We found that further miniaturization can be avoided for hair follicles with diameters larger than 60 μm after injection of HF-MSC suspension without hair-promoting drugs. However, the effect of promoting growth was not significant. For hair follicles with a diameter less than 60 μm before treatment, especially hair follicles with diameters less than 30 μm, the effect of promoting growth was more obvious, and the response to this cell therapy was better.

A hair follicle diameter of 60 μm was found to be a significant index for evaluating the progress of AGA. Rushton et al.,[10] in 1983, and Ishino et al.,[11] in 2014, reported that 80 μm, 60 μm, and 40 μm were significant indices of AGA. According to our previously published data,[12] the progress of AGA can be divided into three stages according to hair follicle diameter: early miniaturized hair follicles of 80 to 100 μm, mediate miniaturized hair follicles of 60 to 80 μm, and advanced miniaturized hair follicles (AMHFs) of 0 to 60 μm. Our results showed that autologous HF-MSC therapy could prevent further miniaturization, as well as the progression of AGA, without using any hair growth–promoting drugs. For AMHFs, HF-MSCs can prevent the progress of miniaturization and also reverse it, promoting hair growth to a certain extent. AMHFs have a high loss of MSCs and need an increased supply of exogenous stem cells; therefore, injecting HF-MSCs has better curative effect. However, for hair follicles with a lower miniaturization level (>60 μm), the stock of MSCs is more sufficient than advanced miniaturized hair follicles, and fewer exogenous stem cells were required for replenishment, so the effect of cell injection is not as good as that of advanced miniaturized hair follicles; however, the paracrine effect of HF-MSC can prevent further miniaturization of these hair follicles.

Previous literature has reported the treatment of AGA with stem cell therapy.[13] Clinical trials in Japan using DS cup cells[9] and 2 clinical trials in 2017 and 2019 using adipose MSCs[14,15] have been reported. Both teams used autologous MSC for treating AGA; Tsuboi et al.[9] used HF-MSCs. The results of these studies indicated the hair diameter thickening effect, which was consistent with our results. However, our results indicated that the effect of a single injection of the cell suspensions appeared at 1 month after injection, and only lasted up to 3 months after injection, which revealed limited duration of effect. This limitation may be related to the survival and differentiation of HF-MSCs in vivo. The local environment may influence the exogenous stem cells, because injected HF-MSCs were not in their original stem cell niche, and the changes in the microenvironment may affect the function and final outcome of the cells. Therefore, stem cell therapy for treating androgenic alopecia may be limited by the survival time and further prognosis of cells in vivo. Further applications in clinic may require appropriate treatment intervals and longer follow-up to determine whether the reverse miniaturization effect will disappear. Furthermore, Tsuboi et al.[9] reported that HF-MSC therapy works better for patients with a higher degree of AGA. We agree with this statement and quantify a specific indicator: 60 μm hair shaft diameter. Briefly, for patients with hair loss, the stage of hair follicle miniaturization can be evaluated by dermoscopy before treatment to estimate the effectiveness of HF-MSC cell therapy. This study provides a simple and convenient method to estimate the effect so that doctors can select the effective population

for cell therapy and achieve the best therapeutic effect. More importantly, HF-MSC therapy in this study showed a significant effect on reversing the miniaturization process of AMHFs. Further improvement of therapy may provide new treatment options for patients with advanced AGA.

Side effects, all of which were minor, disappeared completely after 24 hours, and there were no serious adverse reactions during follow-up. Autologous hair follicle–derived MSC therapy is safe. It will be necessary, however, to refine the injection time interval and observe its long-term effectiveness in the future.

Overall, this study demonstrated the efficacy and safety of autologous healthy hair follicle–derived MSC therapy in Chinese patients with advanced AGA. Although the effect lasted only 3 months, the treatment is still worth exploring. Future research needs to clarify the best treatment scheme for AGA.

CONCLUSIONS

HF-MSCs were effective in the treatment of advanced AGA in Chinese individuals. A hair shaft diameter of 60 μm can be used as a key index to predict the effectiveness of the therapy.

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DISCLOSURE

The authors declare they have no financial relationships or conflict of interest to disclose.

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