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ORIGINAL ARTICLE

Clinical effect of stem cell transplantation combined with 308-nm excimer laser therapy for 56 cases of vitiligo

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Abstract

Objective: To probe the clinical effect of stem cell transplantation in combination with 308-nm excimer laser therapy for vitiligo and to analyze its value in clinical application.

Methods: A total of 56 patients with stable non-segmental vitiligo in different parts who were not cured by other therapies visiting our hospital from March 2019 to December 2021 were enrolled as study subjects. They were treated by stem cell transplantation combined with 308-nm excimer laser therapy. The treatment efficacy was observed and analyzed.

Results: Among the 56 patients, 38 (67.85%) and 49 (87.5%) patients were cured at 6 and 12 months after treatment, respectively.

Conclusion: Stem cell transplantation combined with 308-nm excimer laser therapy for vitiligo achieves significant efficacy, with the cure rate far superior to that of other therapies for vitiligo. The therapy is worthy of popularization in the clinic.

KEYWORDS
308-nm ultraviolet light therapy device, hair follicle melanocyte stem cells, outer root sheath, transplantation, vitiligo

1 | INTRODUCTION

Vitiligo is a primary, regional, or generalized mucocutaneous depigmentation. Generally, the incidence rate is higher in people with the darker skin. The prevalence rate of vitiligo is approximately 0.1%–2.7%. The major cause is the cessation of function of skin melanocytes.[1] In one study, 80% of patients with vitiligo were not effectively treated, with the ineffective rate of various therapies for vitiligo patients as high as 50%.[2] Vitiligo has no impact on the physical health and physiological function of patients, but the visual effect can impose life stress and mental burden. Based on this, we invented a new treatment method for vitiligo: transplantation of hair follicle melanocytes stem cells to treat vitiligo, and obtained the Patent Cooperation Treaty (PCT): *Technical Method for Treating Leucoderma Based On Hair Follicle Melanocyte Stem Cell Transplantation* (Patent No.: PCT/CN2021/072340).[3] Without drug treatment after surgery, vitiligo can be rapidly cured with the assistance of 308 excimer light therapy. In this study, the clinical efficacy of stem cell transplantation combined with 308-nm excimer laser therapy was determined for 56 patients with vitiligo. The findings of the analyses of the therapy should inform further research and refinement of the therapy.

2 | MATERIALS AND METHODS

2.1 | General data

A total of 56 patients with stable non-segmental vitiligo in different parts who were not cured by other therapies admitted to our hospital from March 2019 to December 2021 were enrolled as study

subjects. The inclusion criteria were: (1) meeting the diagnostic criteria for vitiligo, (2) an age ≥18 years, (3) no contraindications to ultraviolet irradiation and no photosensitivity, (4) no communication or comprehension dysfunctions, (5) delivery of 308-nm excimer laser therapy up to 6 months over 20 times in the month prior to enrollment with no efficacy, with no other treatments performed 1 month prior to surgery, and (6) Exclusion criteria were: (1) malignant skin tumors, (2) mental system diseases, (3) vitiligo site infection, or (4) pregnant or lactating women.

The 56 patients comprised 24 males and 32 females, aged 19–69 years (mean age 30.83 ± 5.34 years). Vitiligo was classified as localized ($n = 27$), generalized ($n = 19$), acrofacial ($n = 6$), and vulgaris ($n = 4$).

2.2 | Approval and informed consent

Provision of signed informed consent. This study was implemented after obtaining approvals from the Ethics Committee of the hospital (Approval No.: 2019 [Clinical Research] RS001).

2.3 | Instrument and minimal erythema dose test

A model XECL-308C 308-nm ultraviolet light system (registration number: YJZZ 202090243; Peninsula Medical Group) with a xenon chloride (XeCl) working medium and 308 nm wavelength was used to deliver the excimer laser therapy. According to 308-nm excimer light laser therapy, the minimal erythema dose was determined for all patients using the device in the operation mode with the abdomen as the test site. The minimal erythema dose of each patient was observed within 24–48 h after irradiation.

2.4 | Methods

All patients underwent stem cell transplantation following local anesthesia in a sterile environment. Outer root sheath containing hair follicle melanocyte stem cells (McSCs) was extracted. Intact hair follicles containing McSCs were acquired by following the utility model patent *Hair Follicle Traceless Extractor* (Patent No.: ZL201921450325.4).[4] The complete outer root sheath containing McSCs was then obtained through the hair follicle separation method described in the PCT patent *Technical Method for Treating Leucoderma Based On Hair Follicle Melanocyte Stem Cell Transplantation*. Hair follicle McSCs were cultured in the special medium described in the invention, *Technical Method for Treating Leucoderma Based On Hair Follicle Melanocyte Stem Cell Transplantation*. The stem cells cultured in vitro were activated to achieve transformation to mature melanocytes. Next, hair follicles containing McSCs were inactivated as described in the utility model patent, *Novel Vitiligo Hair Follicle Inactivation Needle* (Patent No.: ZL201921329885.4),[4] according to the PCT patent, *Technical Method for Treating Leucoderma Based On Hair Follicle Melanocyte Stem Cell Transplantation*. This achieved dark pigmentation in the skin of vitiligo patients without hair growth. Finally, intact hair follicle outer root sheath containing McSCs was transplanted using two utility models: *Planting Needle for Vitiligo Treatment* (patent number: ZL201921450324.X)[4] and *A Plant Pilot Pin for Hair Follicle Transplants* (patent number: ZL201921277579.0)[4] by the method specified in *Technical Method for Treating Leucoderma Based On Hair Follicle Melanocyte Stem Cell Transplantation*.

After surgery, the hair follicle donor site was only bandaged for 24 h. The bandaging could be removed after 24 h. No bandaging was required for the vitiligo operated area. Irradiation was performed once or twice each week using the Peninsula 308-nm ultraviolet light system, with an interval of a week between two irradiations. If the erythema persisted for 24–48 h after treatment, the therapeutic energy was appropriate. If treatment was terminated, given that its efficacy was satisfactory, it was recommended to gradually shorten the treatment time and prolong the interval between irradiations instead of immediately stopping the phototherapy. Treatment for each white patch consisted of 20 irradiations. All patients completed one course of treatment. After treatment, patients were asked to visit the hospital for reexaminations at 1, 3, and 6 months. For patients who had significant treatment efficacy but were not cured, a second course of treatment was required, followed by a subsequent visit to the hospital at 12 months.

2.5 | Criteria for evaluation

Treatment efficacy was assessed based on the efficacy evaluation criteria for vitiligo established by the Pigmentation Disorder Group of the Dermatology and Venereal Disease Committee of the Chinese Society of Integrated Traditional Chinese and Western Medicine. A cure was evident as the complete disappearance of the white patches at the treatment site and the reappearance of normal skin color. Markedly effective treatment was evident as a significantly subsided or shrunken white patch area at the treatment site, with normal skin color returning in ≥50% of the original white patches. Effective treatment was evident as a partially subsided or reduced area of white patches at the treatment site. Ineffective treatment was the absence of melanogenesis or expanded area of a white patch at the treatment site. The response rate = ([number of cured cases + number of markedly effective cases + number of effective cases]/total number of cases) × 100%. The treatment efficacy was compared for the patients.

The incidence rate of adverse reactions in all patients during 308-nm excimer laser therapy, such as blisters, skin itching, burning sensation, and pain, was counted and recorded.

On the last day of follow-up, efficacy satisfaction questionnaires were distributed to all patients, with a total score of 100 points. A score < 90 points suggested unsatisfactory efficacy.

2.6 | Statistical analysis

According to the characteristics of the homologous paired experiment design, the paired chi-squared test was used for statistical inference hypothesis test. The cure rate and effective rate of stem cell transplantation combined with 308 nm excimer light therapy were compared with 308 nm excimer light therapy in patients with vitiligo, using chi-squared test, $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Therapeutic results

In the 56 patients, the treatment was curative in 38 patients (67.85%), markedly effective in 11 (19.64%), effective in 4 (7.14%), and ineffective in 3 (5.35%) within 6 months following one course of treatment. The total treatment response rate was 94.64%. Fifteen patients who completed the second course of treatment were followed up for 1 year. Eleven of them were cured. After two course of treatment a total of 49 (87.5%) patients were cured. The 49 cured patients were treated with 308 nm excimer light for an average of 18.9 times, and the average healing time was 135 days. Of the seven patients who were not cured after two courses of treatment, one had hyperthyroidism, two had hypothyroidism, three were over 50 years of age and had acrofacial vitiligo, and one was lost to follow-up and the treatment was considered ineffective.

3.2 | Adverse reactions

During the 6-month follow-up after treatment, skin itching, and folliculitis occurred in one and 14 of the 56 patients, respectively. The incidence rate of adverse reactions was 26.7%.

3.3 | Satisfaction degree

On the last day of follow-up, efficacy satisfaction questionnaires were distributed to all patients. There were 28 cases of 100 points, 21 cases of 95 points, and seven cases of <90 points. The majority (87.5%) patients indicated satisfaction with their treatment (Tables 1 and 2).

3.4 | Statistical analysis

By chi-squared test $\chi^2 = 36.03 (p < 0.01)$, there was a significant difference in the cure rate between stem cell transplantation combined with 308 nm excimer light therapy and 308 nm excimer light therapy in vitiligo (Table 3).

By chi-squared test $\chi^2 = 51.02 (p < 0.01)$, the effective rate of stem cell transplantation combined with 308 nm excimer light therapy was significantly different from that of 308 nm excimer light therapy (Figure 1).

4 | DISCUSSION

4.1 | Current status of melanocyte transplantation in the treatment of vitiligo

Melanocyte apoptosis at sites of white patches on the skin is the major pathological change of vitiligo. Stable vitiligo is commonly clinically treated by epidermal grafting and melanocyte transplantation.[5] Melanocyte transplantation is one of the effective approaches to treat stable vitiligo. Epidermal grafting for vitiligo treatment is not applicable to extensive areas of skin lesions as few melanocytes are obtained through the method. The successful in vitro culture of melanocytes has created a new therapeutic regimen for vitiligo patients. However, prior to melanocyte transplantation,

TABLE 1 Clinical treatment of stem cell transplantation combined with 308 nm excimer light (*n*,%).

Treatment condition	Combination treatment group ($n = 56$)	Excimer light treatment group ($n = 56$)	Total
Effectivity			
Overall effectiveness	53 (94.64)	0 (0)	53 (47.32)
Cure	38 (67.85)	0 (0)	38 (33.93)
Positive effect	11 (19.64)	0 (0)	11 (9.82)
Effectivity	4 (7.14)	0 (0)	4 (3.57)
Inefficient	3 (5.36)	56 (100)	59 (52.68)
Skin pruritus			
Yes	1 (1.79)	0 (0)	1 (0.89)
No	55 (98.21)	56 (0)	55 (99.10)
Folliculitis			
Yes	14 (25)	0 (0)	14 (12.50)
No	42 (75)	56 (100)	42 (87.50)

TABLE 2 Comparison of cure rates between stem cell transplantation combined therapy and clinical treatment with 308 nm excimer light (*n*).

Combination treatment group	Excimer light treatment group				
	Cure	Uncured	Total	χ^2	*p*
Cure	0	38	38	36.03	<0.001
Uncured	0	18	18		

TABLE 3 Comparison of overall effectiveness between stem cell transplantation combined therapy and clinical treatment with 308 nm excimer light (*n*).

Combination treatment group	Excimer light treatment group				
	Overall Effectiveness	Inefficient	Total	χ^2	*p*
Overall effectiveness	0	53	53	51.02	<0.001
Inefficient	0	3	3		

preparation of a melanocyte suspension should be completed under the action of pancreatic enzymes to facilitate adhesion of the melanocytes in the transplanted wound.[6,7] During melanocyte transplantation, melanocyte loss is easily triggered due to the flow of the melanocyte suspension. The survival rate and function of melanocytes in the transplanted wound are affected by changes in the cellular environment. Autologous tissue-engineered skin has gradually become widely applied clinically in recent years.

Clinical reports have described single hair follicle transplantation for the treatment of vitiligo. Thirty years ago, Na et al.[8] treated 21 vitiligo patients with single hair follicle transplantation. Disadvantages of this approach include the slow treatment period and limited source of donor hair follicles. The approach is not applicable to large areas of vitiligo, especially in hairless areas. Recent clinical reports described the use of outer root sheath suspension for the treatment of vitiligo. Vinay et al.[9] obtained occipital anagen hair via the follicular unit extraction method, and used non-cultured outer root sheath suspension transplantation to treat 30 patients with stable vitiligo. The outer root sheath suspension transplantation was not accepted by patients, as the efficacy was uncertain or because of the many side effects. Outer root sheath suspension transplantation has not been widely applied in clinical practice.

4.2 | Basic research and innovation of hair follicle intact outer root sheath transplantation in the treatment of vitiligo

A recent study described immune privilege in hair follicular bulge cells.[10] Accordingly, McSCs stored in the bulge[11] can be protected against destruction during disease development. Vitiligo lesions with pigmented vellus hairs are more likely to induce repigmentation in contrast to lesions with depigmented vellus hairs (white hairs).[12] The affected skin in hairless areas are more resistant to repigmentation induction therapy than the skin in hairy areas.[13] Therefore, implanting exogenous pigmented hairs to induce the repigmentation of the affected skin of vitiligo patients is a novel and potentially effective approach for the treatment of vitiligo.

Amelanotic melanocytes (AMMCs) in the outer root sheath.[14] McSCs and pre-melanocytes are collectively referred to as AMMCs, and pre-melanocytes are transitional cells between McSCs and melanocytes. Hair follicle McSCs[15] are located in the bulge region at the bottom of the constant part of the hair follicle (the upper one-third of the hair follicle), mostly in a resting state, with slow periodicity and the ability to maintain self-renewal. They are one of the typical representatives of regenerative stem cells. A 2002 study published by Nishimura et al.[16] on the proliferation of melanoblasts documented that stem cell factors expressed in the epidermis can form a channel between the outer root sheath and the epidermis, along which melanocytes migrate from the hair follicle to the epidermis. Hence, activating follicular McSCs makes their transport along the outer root sheath become the processing of melanocytes. Based on this, Liu et al. proposed a new theory of "treating vitiligo from hair", involving transplanting a complete outer root sheath of hair follicles containing hair follicle McSCs. The proposal was the core of an approved patent by the PCT: *Technical Method for Treating Leucoderma Based On Hair Follicle Melanocyte Stem Cell Transplantation* (Patent No.: PCT/CN2021/072340).[3] Further patents have been granted in the United States, European Union, Japan, South Korea, and Thailand.

Immune regulation is one of the mechanisms of action of 308-nm excimer laser therapy for vitiligo. Immune regulation can promote the apoptosis and participate in the cleaning of pathological T lymphocytes infiltrated in vitiligo and psoriasis lesions and reduce the destruction rate of melanin during treatment.[17] Among all laser wavelengths, the 308 nm wavelength delivered by laser is strongly absorbed by human DNA and proteins. The absorbed light contributes to the production of pyrimidine dimers, purine dimers, and other molecular structures, triggering the corresponding biological photoimmune response and repigmentation.[18] The 308-nm laser irradiation can change the microenvironment of hair follicles, facilitate the maturation and differentiation of McSCs, and stimulate the migration of melanocytes to the epidermis.[19]

In this study, the overall cure rate after 1 year was as high as 87.5% in 56 patients with vitiligo. This cure rate was significantly higher compared with that of various previous therapies, and the effect was more marked. This method is markedly effective for vitiligo

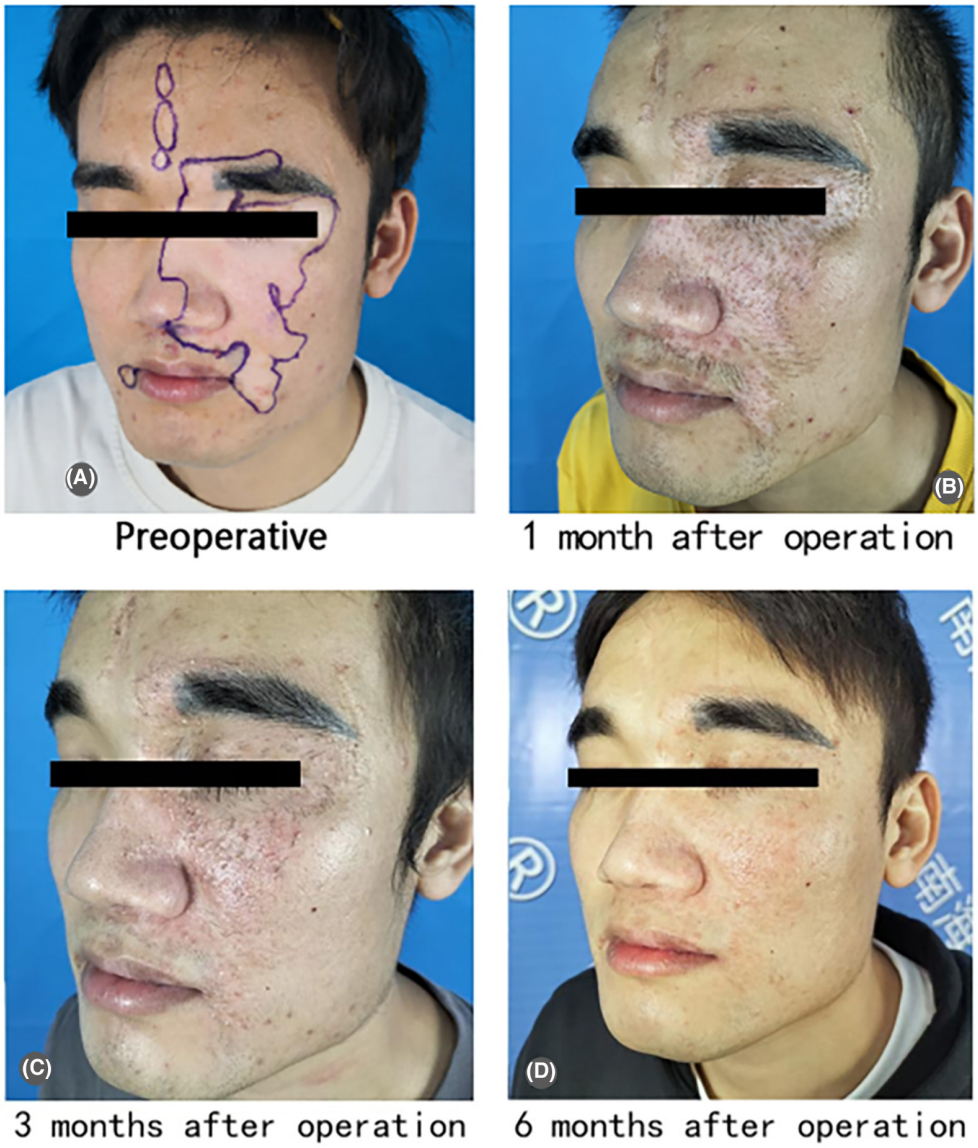

FIGURE 1 Features of a typical case. Patchy depigmentation had been present on the left side of the face of a 27-year-old man for 8 years. Panel 1a is the preoperative appearance. Others display the appearance 1, 3, 6 months, respectively, after therapy.

on the head, face, trunk, and limbs as well as vitiligo on fingertips, eyelids, alae nasi, lips, vulva, and anal mucosa that had not responded to various therapies. White patches could still be repigmented, even in the case of vitiligo with the hair turning white. In this study, the single maximum surgical area was 351 cm^2, only three hair follicles per square centimeter were required during surgery, and the theoretical single surgical treatment area could exceed 2000 cm.[2] In addition, the surgery did not require dermabrasion at the white patch site. As a result, epidermal injury was slight, there was no need to bandage the white patch area after surgery, normal bathing could be resumed 48 h later, and there was no color difference in repigmentation. However, acrofacial vitiligo patients over 50-years-of-age displayed slow repigmentation, and the efficacy for patients with thyroid disease was poor. This challenges remain to be solved.

In conclusion, stem cell transplantation for vitiligo treatment is a process of implanting a melanocyte processing plant under the epidermis of vitiligo patients. Irradiation with 308-nm excimer laser light at this time delivered in vitro activates non-functional McSCs in the outer root sheath to achieve transformation into mature melanocytes. However, for the treatment of vitiligo patients with more than moderate area, due to the effective number of autologous stem cells, allogeneic or heterologous hair follicle melanocytes stem cell transplantation should be explored to solve the treatment needs of vitiligo patients with more than moderate area.

CONFLICT OF INTEREST STATEMENT

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review

of, the manuscript entitled, "Clinical effect of stem cell transplantation combined with 308-nm excimer laser therapy for 56 cases of vitiligo".

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

1) this material has not been published in whole or in part elsewhere; 2) the manuscript is not currently being considered for publication in another journal; 3) all authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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