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Original Article

Efficacy of topical application of a mixture of amniotic membrane stem cell metabolic products and vitamin C after microneedling treatment in patients with photoaging

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Abstract

Background Extrinsic factor-related aging is associated with a decrease in growth factors that results in degradation of the skin structure. Amniotic membranes are currently promising candidates for use in cellular therapy and regenerative medicine. When amniotic membrane stem cells (AMSCs) are cultured, several bioactive materials are secreted into the medium as metabolic products. Vitamin C is a water-soluble vitamin that is widely used in dermatology as an antioxidant and to promote depigmentation and collagen synthesis. Microneedling facilitates the penetration of these molecules and thus improves the efficacy of the mixture of AMSC-metabolic products (AMSC-MPs) and vitamin C in skin rejuvenation. Topical combinations of metabolic products of AMSCs (AMSC-MPs) and vitamin C are expected to have an effect on improvement of clinical photoaging.

Methods A total of 60 photoaging women were included in this analytic, controlled, matched-pair experimental study. They were allocated to receive a topical facial combination of AMSC-MP and vitamin C in the intervention group or AMSC-MPs alone in the control group. A Dermapen® was used to enhance epidermal penetration. Three treatment sessions were given at intervals of 4 weeks.

Results Compared with the control group, subjects in the treatment group showed significant improvement in wrinkles ($p = 0.008$), ultraviolet spots ($p = 0.046$) and pores ($p = 0.046$).

Conclusion The combination of AMSC-MPs and vitamin C is an effective treatment for photoaging.

Key words
Collagen, stem cells, vitamin C.

Introduction

The ageing process involves decline in function and reserve capacity of all organs, including the skin. The ageing process in the skin is affected by intrinsic and extrinsic factors. Facial skin is constantly exposed to daylight, which results in the accumulation of damage and changes in the skin. Skin changes include alterations in skin pigment and vascular homogeneity, loss of

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elasticity and degradation of skin texture. Among extrinsic factors related to skin aging, decrease in growth factors causes degradation of the structure of the skin, causing the skin to look older than it should.[1]

The amniotic membranes and the amnion contain stem cells that have differentiation abilities and low immunogenicity. Amniotic membranes, which are obtained from the placenta, are promising candidates for use in cellular therapy and regenerative medicine. When amniotic membrane stem cells (AMSCs) are cultured, several bioactive metabolic products, such as cytokines and growth factors, are secreted into the culture medium. Growth factors are beneficial for wound healing, and the use of growth factors for skin rejuvenation is being widely investigated.[2-4]

With advances in medicine and biotechnology, there are more ways to prevent aging, including lifestyle changes, nutrition, hormone administration, environmental changes and stem cell therapy. These methods of preventing aging also help maintain optimal body function.[5]

Vitamin C is a water-soluble vitamin that is widely used in dermatology as an antioxidant and to promote depigmentation and collagen synthesis. Vitamin C as an antioxidant neutralises reactive oxygen species (ROS), which contribute to the pathological mechanisms of photoaging.[6]

Microneedling is a percutaneous therapy that induces collagen proliferation. Microdamage from microneedling stimulates collagen remodelling and induces synthesis of new collagen and elastin and new vascularisation, thus reducing wrinkles. Growth factors and cytokines from AMSC metabolites have difficulty in penetrating intact skin because of their large molecular size. In addition to rejuvenating the skin, microneedling facilitates the penetration of these molecules and thus improves the efficacy of the mixture of AMSC-metabolic products (AMSC-MPs) and vitamin C in skin rejuvenation.[2,7]

The aim of this study was to determine the efficacy of topical application of a mixture of AMSC-MPs and vitamin C after microneedling treatment compared with AMSC-MPs without vitamin C.

Methods

Research design and subjects This was an analytical experimental study using controlled clinical trial methods, matching pair selection and parallel design that compared topical application of a mixture of AMSC-MPs and vitamin C (treatment) after microneedling treatment with topical application of AMSC-MPs without vitamin C (control) in patients with photoaging.

The participants were selected using consecutive sampling that included all female photoaging patients who met the criteria and were outpatients at the Dermatology and Venereology Outpatient Clinic, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Inclusion criterias such as female with photoaging II-III who have been or willing to use tretinoin 0.25% cream before the procedure. Exclusion criterias are history of keloid, active eczema, hemophilia, herpes simplex infection, and diabetes mellitus. Sixty participants were randomly divided using the matching pair technique into treatment and control groups.

Preparation of mixture of AMSC-MPs and Vitamin C AMSC-MPs are the liquid by-product from culture of AMSCs, containing cytokines and growth factors that have a role in wound healing and skin rejuvenation. Each

application contained 3 mL of AMSC-MPs and 0.09 g of vitamin C as sodium ascorbyl phosphate for the entire face. The AMSC-MPs and the mixture with vitamin C were prepared at the Stem Cell Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

Procedure This study has been approved by the Ethical Committee of Universitas Airlangga, Surabaya, Indonesia. The participants were informed about the research procedure prior to treatment. All participants signed information of consent, informed consent and medical approval sheets. Before treatment with AMSC-MPs and vitamin C, the participants were given facial skin care (priming) for 2 weeks. We used 0.025% tretinoin cream to prime the skin, minimise side effects, and accelerate wound healing. To obtain objective baseline data, the participants were examined with Janus 3D Facial Analysis System (Janus) before treatment. Five features were measured: wrinkles, polarised spot, ultraviolet (UV) spots, pores and skin tone. Application of the mixture of AMSC-MPs and vitamin C or AMSC-MPs alone was carried out after the patient underwent microneedling with a Dermapen®. Microneedling is a collagen-inducing therapeutic modality that uses a microsized needle to create small invisible holes. We used Dermapen®, an automated microneedling device with adjustable speed and depth.[7-9] The application was performed three times at 4 week intervals. Evaluation of clinical improvement using Janus was performed two times at weeks 4 and 8. All the procedures were performed by one of the researchers, a dermatologist.

The participants were scheduled for visits every 2 weeks unless there were side effects such as itchiness, redness or other conditions that needed medical treatment. The study was planned to be discontinued if severe side effects, such as purulent infection or other complications related to the application of either the mixture of AMSC-MPs and vitamin C or AMSC-MPs alone, occurred. For best results, the participants were asked to minimise sun exposure during the study.

Statistical Analysis The data were analysed by SPSS software, version 21 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to indicate statistical significance.

Results

The study included 60 female photoaging patients who met the inclusion and exclusion criteria. **Table 1** compares the results for the control and treatment groups. The control and treatment groups showed significant outcomes in wrinkles, UV spots and pores. When compared with baseline (week 0), UV spots showed significant improvement at the end of the study (week 8). Wrinkles and pores consistently showed significant improvement starting from the second observation (week 4) until the end of the study (week 8). Polarised spots and skin tone showed improvement, although it was not significant.

Discussion

This study aimed to determine the efficacy of topical application of a mixture of AMSC-MPs and vitamin C compared with topical application of only AMSC-MPs in photoaging patients after microneedling treatment.

All the participants in this study were women who experienced photoaging. Gender uniformity was intended to minimise dropouts, since women pay more attention to their skin appearance than do men. This can be seen from the number of women patients, compared with men, who came to the Cosmetic Division of

Table 1. Experimental results in the control and treatment groups

		Week		
		0	4	8
Wrinkles	AMSC-MPs	11.90 (7.35)	10.40 (4.79)	10.83 (7.12)
	AMSC-MPs+vit C	13.37 (6.95)	8.13 (3.79)	6.77 (3.79)
	p	0.430	0.047	0.008
UV spots	AMSC-MPs	14.87 (8.57)	11.73 (6.21)	12.5 (7.71)
	AMSC-MPs+vit C	11.93 (7.05)	8.97 (4.69)	9.17 (4.54)
	p	0.153	0.096	0.046
Polarised spots	AMSC-MPs	32.63 (8.02)	31.57 (7.99)	31.57 (9.15)
	AMSC-MPs+vit C	32.83 (7.63)	30.57 (6.24)	30.40 (6.68)
	p	0.922	0.591	0.575
Skin tone	AMSC-MPs	39.33 (7.12)	37.97 (7.35)	34.4 (4.44)
	AMSC-MPs+vit C	38.97 (7.38)	36.8 (6.70)	36.9 (7.48)
	p	0.835	0.716	0.587
Pores	AMSC-MPs	51.2 (6.72)	50.97 (4.76)	49.67 (5.47)
	AMSC-MPs+vit C	49.03 (4.30)	46.83 (5.20)	47.07 (4.34)
	p	0.142	0.002	0.046

UV, ultraviolet; AMSC-MPs, metabolic products of amniotic membrane stem cells; vit, vitamin.

Dermatology, Venereology Outpatient Clinic, Dr. Soetomo Teaching Hospital, Surabaya, for skin rejuvenation in 2016; registration data for new patients showed that the proportion of women to men was 200:9.

UV radiation causes wrinkles through decrease in skin tension and elasticity and degradation of structural components of the skin that support the dermal extracellular matrix. UV radiation also increases ROS, which increases activator protein 1 and NF-κB and decreases transforming growth factor beta 1, resulting in decreased collagen production and increased collagen damage.[10] AMSC-MPs contain various factors that can stimulate dermal fibroblast proliferation and migration as well as increasing collagen synthesis from fibroblasts.[11,12]

In this study, the control group given only AMSC-MPs showed significant results for wrinkles, UV spots and pores at 8th week. Observations of UV spots did not show immediate progress, but the overall results at the end of the study were still significant. Our results are in line with those with a study by Seo *et al.* of AMSC-MPs after microneedling treatment. The study showed improvement in skin coarseness with histopathological proof of increasing amount of collagen with minimal side effects.[11] A study by Lee *et al.* comparing the use of AMSC-MPs after microneedling treatment with microneedling alone showed a significant improvement in wrinkles for AMSC-MPs group. In that study, each participant received five treatments at 2 week intervals and was evaluated for skin coarseness and depth of wrinkles by visiometer analysis.[2]

The treatment group in the present study, which received AMSC-MPs with vitamin C after microneedling treatment, also showed significant improvement in wrinkles, UV spots and pores. Contrary to the results for the control group, improvement in pores was not as immediate as improvement in wrinkles and UV spots. These results are in accordance with the theory that topical vitamin C plays a role in collagen synthesis as the cofactor for lysyl and prolyl hydroxylase, thus promoting improvement in wrinkles.[13-15] In a study by Crisan *et al.*, evaluation by ultrasound after topical application of vitamin C in 60 faces showed a significant increase in collagen synthesis.[16]

Comparison between the control and treatment groups showed significant differences in improvement of wrinkles, UV spots and pores in favour of the treatment group.

Conclusion

The addition of vitamin C to AMSC-MPs improves the efficacy of AMSC-MPs in preventing photoaging.

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