The Effect of Amniotic Membrane Stem Cell Metabolite Product (AMSP-MP) Combine with Vitamin E after Microneedling in Photoaging Progress

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Abstract

Introduction: The photoaging is a cumulative process. Amniotic Membrane Stem Cell Metabolite Products (AMSC-MP) that contain cytokines and growth factors that have a role in the skin's rejuvenation process. Vitamin E is an antioxidant that has a photoprotective effect. Topical combination of AMSC-MP and Vitamin E are expected to have an effect on clinical photoaging improvement.

Objective: To evaluate the effect of topical combination of AMSC-MP with vitamin E after microneedling compared with AMSC metabolite products after microneedling.

Methods: A total 60 photoaging women were included in this experimental analytic, controlled, matching research. Each participant's face was allocated to topical combination of AMSC-MP and vitamin E in intervention group and AMSC-MP only in control group. Microneedling modality was use to enhance epidermal penetration. Three treatment sessions were repeated at two weeks' interval.

Results: From the comparison of the two groups showed there were significant different of mean value in wrinkle, skin tone and UV spots with p-value < 0,005 in the intervention grup. While pore and polarized spot do not show significant differences between two groups.

Conclusion: The administration of a topical combination of MP-AMSC and vitamin E after microneedling provides clinical improvement in photoaging.

Keywords: *vitamin E, microneedling, photoaging.*

Introduction

Extrinsic aging (Photoaging) is caused by environmental factors such as sun exposure/ UV radiation. The effect of UV light exposure on the skin becomes a very significant factor affecting the extrinsic skin aging process. The degree of *photoaging* is directly related to the amount of UV light obtained in a certain period of time. *Photoaging* is clinically can cause soft and rough wrinkles, dryness, telangiectasis, loss of suppleness and changes in pigment.[1]

Amniotic membrane stem cells (AMSCs) are better prospects for cell therapy and regenerative medicine compared with the other adult mesenchymal stem cells because they are abundant and can be acquired easily and inexpensively. In the culture process, AMSC will secrete metabolite products. These metabolite products contain cytokines and growth factors and was reported to significantly improve the proliferation and migration of dermal fibroblasts and epidermal keratinocytes as well as increase collagen synthesis in fibroblasts.[2,3,4] Vitamin E has a photoprotective, anti-inflammatory and moisturizing effect. Hydrophilic molecules larger

than 500 Da have poor penetration through the stratum corneum.[3] Most growth factors are large hydrophilic molecules >20 kDa and are, therefore, unlikely to produce pharmacologic effects. To enhance the skin penetration of AMSCs, microneedle was used.

The aim of this is to examine the effects of Amniotic Membrane Stem Cell Metabolite Products (AMSC-MP) and vitamin E on photoaging.

Method

Research Design and Subject.

This study is an analytical experimental study using controlled clinical trial methods, matching pair selection, and parallel designs that compare topical therapies for AMSC metabolite combine with vitamin E after microneedling (treatment) and microneedling with AMSC metabolites (controls) in patients with photoaging.

The sample of the study were all photoaging patients who met the criteria for receiving samples, who came to the URJ Skin and Sex Hospital Dr. Soetomo Surabaya. Criteria for acceptance of samples are patients with Glogau II-III *photoaging* degrees or aged 40-60 years and patients who have used *priming* with tretinoin 0.025% cream for at least 2 weeks or new patients who are willing to be *primed* before treatment. Criteria for rejection of samples were patients with a history of keloids, active eczema on the face, hemophilia/ physiological disorders of blood clotting using anti-platelets, diabetes mellitus, and HIV / AIDS. All the subjects were randomly divided into two groups. First group was given AMSC-MP only as a control group and second group was given AMSC-MP with vitamin E as an intervention group. To enhance the skin penetration of AMSC-MP, microneedle was used.

Procedure

We analyzed the level of pore, wrinkle, *polarized* spots, UV spots, and skin tone by Janus. Thereafter, the subjects were instructed to apply 0.025% tretinoin cream for 2 weeks for priming. After 3 days of discontinuation of the tretinoin cream, the treatment procedures were initiated. In this study the *microneedling* used was in the form of dermapen, with a level 3 speed (45-50/ times/sec), a depth of 0.5 mm, and 2 *passes*. Evaluation of results using *Facial Skin Scope* JANUS-II which assesses wrinkles, pores, *polarized* spots, UV spots, and *skin tones* was conducted after the action at week 0.4, and 8. The results of the data obtained are entered in the data collection sheet and accompanied by a photo analysis of the face with Janus-II to conduct data analysis. This research has been examined by the Ethics Committee at RSUD Dr. Soetomo Surabaya.

Statistical Analysis

Analysis of variance (ANOVA) test was performed to evaluate the improvement in all parameters on Weeks 4 and 8. Data were analyzed using SPSS version 21 software (SPSS Inc., Chicago, IL). The level of significance was set at $p < .05$.

Result

This study involved 60 photoaging patients. All the subjects were woman between 40-60 aged. All research subjects were willing to take part in the study by signing information of consent, informed consent and medical approval sheets. Sixty patients consisted of 30 patients in the control group who were given AMSC treatment only and 30 patients in the treatment group treated with AMSC and vitamin E. Samples were taken in this study using consecutive sampling which took each photoaging patient at URJ Skin and Gender Cosmetic Division of RSUD Dr. Soetomo Surabaya. Distribution of research subjects using matching pairs between groups based on photoaging criteria. There were no drop-outs in this study and all subjects completed the 8-week protocol. From the control group, the group given topical AMSC metabolites after microneedling obtained significant results in pores, wrinkles, UV spot and polarized spot. In the 1st and 3rd Janus observations there were significant improvements in wrinkle, UV Spots, polarized spots. Significant results are also found in the pore seen in all of Janus's observations. (Table 1).

Table 1. Resume p value comparison of pores, wrinkles, skin tone, polarized spots, UV spots between the 1st, 2nd, and 3rd janus in control group.

	Pores	Wrinkles	Skin tone	Polarized spots	UV spots
AMSC-MP					
Janus 1 vs 2	0,001*	0,148	0,558	0,08	0,094
Janus 1 vs 3	0,001*	0,018*	0,055	0,002*	0,005*
Janus 2 vs 3	0,000*	0,206	0,126	0,081	0,261

Table 2 shows a comparison of p values on the value of pores, wrinkles, polarized spots, UV spots, skin tone between the first, second and third janus from the intervention group combination of AMSC-MP with vitamin E after microneedling. From the table it can be concluded that there are significant changes in pores, wrinkles, polarized spots, UV spots, and skin tones before and after the application of the topical mixture of PM-AMSC and vitamin E after microneedling with the most significant value on Janus 1 and 3.

Table 2. Resume p value comparison of pores, wrinkles, skin tone, polarized spots, UV spots between 1st, 2nd, 3rd janus in intervention group.

	Pores	Wrinkles	Skin tone	Polarized spots	UV spots
AMSC-MP+ Vit E					
Janus 1 vs 2	0,000*	0,227	0,000*	0,194	0,032*
Janus 1 vs 3	0,000*	0,000*	0,000*	0,002*	0,002*
Janus 2 vs 3	0,580	0,120	0,102	0,269	0,191

Table 3 shows the results of the comparison of mean value and p value between control and treatment groups. The improvement was better in the intervention group. From the comparison of the two groups showed there were significant different of mean value in wrinkle, skin tone and UV spots with p-value < 0,005. While pore and polarized spot do not show significant differences between two groups.

Table 3. Comparison of mean value and p value between control and intervention group

	Pore	Wrinkle	Skin Tone	Polarized Spots	UV Spots
AMSC	50,61	16,57	55,85	31,92	12,91
AMSC + Vit E	49,92	11,03	47,62	33,24	7,18
p-value	0,593	0,003*	0,000*	0,533	0,000*

Figure 1. Photograph results (a) before AMSC-MP microneedling procedures (b) after AMSC-MP microneedling last procedure.

Figure 2. Photograph results (a) before AMSC-MP and Vitamin E microneedling procedures (b) after AMSC-MP and Vitamin E microneedling last procedure.

Figures 1 and 2 show the clinical improvement before and after procedure in control group and intervention group. Improvement are seen in patient's spot, wrinkle and skin tone. The intervention group shown a better improvement in clinical and statistic analysis than the control group.

Discussion

The subjects of this study were women who experienced *photoaging*. Gender uniformity in this study aims to minimize the drop out of the study subject, because women pay more attention to the condition of their skin compared to men, so the level of compliance is expected to be higher than men. It can also be seen from *photoaging* patient visits in the cosmetics poly in IRJ Skin and Genital Health Dr. Soetomo is the women in major. This is supported by data on visits to cosmetics at RSUD Dr. Soetomo in 2018, there were 711 female patients and 24 male patients who came for skin rejuvenation treatment. This is also similar to the research conducted by Lee et al, which is all 25 subjects were woman.[5]

The amniotic epithelium and amniotic membrane stroma are sources of epidermal growth factor and keratinocyte growth factor, which promote wound healing. Their low immunogenicity and anti-inflammatory properties make them a suitable alternative material in the field of regenerative medicine. Furthermore, stem cells synthesize and secrete a variety of extracellular matrix proteins, cytokines, growth factors and other bioactive proteins that contribute to the healing process.[6,7]

In this study, the group given topical AMSC metabolite products after microneedling obtained significant results in pores, wrinkles, UV spot and polarized spot. Significant results are also found in the pore seen in all of Janus's observations. Concerning the research conducted by Seo and his colleagues with stem cell metabolite products and microneedling on photoaging showed improvements in skin roughness.[8] The histopathology results in the study also showed an increase in dermal collagen with minimal side effects.[8] Research conducted by Lee and colleagues comparing the use of stem cell metabolites after microneedling and with microneedling alone, showed significant results in wrinkle repair.[5] This is because the effect of AMSC-MP which contains various growth factors that can stimulate the proliferation and migration of dermal fibroblasts, epidermal keratinocytes, as well as increase collagen synthesis from fibroblasts, so there is an improvement in skin texture. Stem cell metabolite products have a brightening effect by inhibiting melanin formation, changing the shape of melanin and affecting the distribution of melanosome transfer.[6,7] Research conducted by Seo et al shows that stem cells have a whitening effect through inhibition of melanin synthesis and tyrosinase activity, and can reduce the expression of melanogenic enzymes.[8] Research conducted by Lee et al also showed a significant improvement in the melanin index of *photoaging* patients who applied *stem cell* metabolite products after *microneedling*.[5]

In the intervention group also showed significant results in wrinkle, UV spot, polarized spot, skin tone and pore. The most significant result showed in 1st and 3rd Janus. In accordance with the theory that topical vitamin E has a photoprotective, anti-inflammatory and moisturizing effect. The addition of vitamin E to this topical product is expected to have a synergistic effect on the clinical improvement of *photoaging*. Chung et al showed that the use of occlusive topical 5% vitamin E for 24 hours provided protection against UV induced metalloelastase *in vivo*.[9] Vitamin E protects cell membranes from lipid peroxidase by free radicals. The research shows that topical vitamin E that is applied topically has the potential to penetrate into the skin layer where oxidative stress occurs so as to protect from *photoaging* process, so the clinical signs of *photoaging* can be reduced.[9,10]

Comparative results of the control and treatment groups showed significant different mean value in wrinkle, skin tone, and UV spot. It means that addition of vitamin E in AMSC-MP give significant different that AMSC-MP alone.

Side effects that may arise from the actions of this study are persistent erythema (> 24 hours), urticaria, *post inflammatory hyperpigmentation*, hypopigmentation, or infection. In this study, the side effects after treatment were minor in 3 subjects, there were erythema in 2 subjects for 2 days and urticaria in 1 subject for 1 days in control group. Both side effects resolved after two days of treatment with topical 1% hydrocortisone cream and

antihistamine. This can occur because of *microneedling* itself is an act with minimal invasion, because the injury that occurs from the *microneedling* needle is only 0.5 cm deep which functions as a drug *delivery* to deeper layers of the skin. This is consistent with what was found in research conducted by Tamama et al, who found that MSC metabolite products secrete IL-8, chemokin ligand 1 (CXL1) which can accelerate the healing process.[11,12] In intervention group there were no side effect reported in all the subjects. It is maybe because vitamin E also functions as an anti-inflammatory agent and can affect scars *remodeling* by interacting with phospholipids on cell membranes, inducing molecular and maintaining membrane stability.[10,13]

Conclusion

In conclusion, compared with the AMSC-PM alone after microneedling, giving topical AMSC metabolite products with vitamin E after miconeedling, was effective in giving a positive impact on photoaging improvement.

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Conflict of Interest : There are no potential conflicts of interest relevant to this article

Ethical Clearance: The study protocol has been approved by the Health Research Committee of Dr Soetomo General Hospital, Surabaya, east Java

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