The Efficacy of Topical Combination Amniotic Membrane Stem Cell Metabolite Product (AMSC-MP) and Vitamin E after Microneedling in Photoaging

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ABSTRACT

Background: Photoaging, also called extrinsic aging, is a premature skin aging mainly resulting from prolonged and extended exposure to ultraviolet (UV) radiation. Amniotic Membrane Stem Cell Metabolite Products (AMSC-MP) are derived from amniotic membranes that contain cytokines and growth factors that have a role in the skin rejuvenation process. Vitamin E is an antioxidant that has a photoprotective effect, so it is expected to reduce the appearance of clinical signs of photoaging. Not only having skin rejuvenation effect, but microneedling is also expected to facilitate the penetration to increase the efficacy of AMSC-MP and vitamin E. This combination is expected to have a better effect on clinical photoaging improvement. **Purpose:** To evaluate the effect of a topical combination of AMSC-MP and vitamin E after microneedling on the clinical improvement of photoaging compared to previous skin conditions. **Methods:** Thirty adult women with photoaging topically administered a combination of AMSC-MP and vitamin E after 3 microneedling sessions at 2 weeks intervals. The evaluation of the improvement of the degree of pores, wrinkles, skin tone and dark spots were performed with the Janus-II Facial Skin Scope System. **Result:** There was a clinical improvement with a statistically significant difference in terms of the degree of pores, wrinkles, polarized black spots, and UV black spots, which were statistically significant differences ($p < 0.05$). **Conclusion:** The administration of a topical combination of AMSC-MP and vitamin E after microneedling provided clinical improvement in photoaging as supported by the results of Janus analysis of pores, wrinkles, skin tones, polarized black spots, and UV black spots.

Keywords: amniotic membrane stem cell metabolite product, vitamin E, microneedling, photoaging.

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BACKGROUND

Skin aging is a complex biological process that affects one's appearance. Two types of skin aging are intrinsic and extrinsic aging or photoaging. Intrinsic aging means skin aging process that is associated with genetic factors, which is a natural process as a person age. Extrinsic aging is caused by environmental factors such as sun exposure/ UV radiation. Ultraviolet light exposure significantly affects the extrinsic skin aging process. The degree of photoaging is correlated to the amount of UV light exposure in a period of time. Clinically, photoaging can cause soft and rough wrinkles, dryness, telangiectasis, loss of suppleness and changes in pigment.[1]

There are many ways to prevent both intrinsic and extrinsic aging, one of them is stem cells treatment. Stem cells are multipotent cells that can renew themselves and differentiate into various cells, promoting wound healing, damaged tissue repair, skin rejuvenation, and skin, hair, nails, and mucous membranes growth. Stem cells are said to be able to renew epidermal keratinocyte cells and dermal fibroblasts.[2,3] Amniotic Membrane Stem Cell (AMSC) is obtained from the amniotic membrane, it is a tissue that has the ability to differentiate and has low immunogenicity. In the culture process, AMSC secretes metabolite products which contain cytokines and growth factors. The function of growth factors has been proven in wound healings and its use for skin rejuvenation is now widely studied.[4]

Ultraviolet radiation which is one of the causes of extrinsic aging as it promotes the formation of free radicals or Reactive Oxygen Species (ROS). Therefore, antioxidants are useful in this case in preventing cell damage and are expected to inhibit the aging process. Vitamin E has a photoprotective, anti-inflammatory, and moisturizing effect. The study of Boelsma et al. found that vitamin E has the greatest photoprotective effect, an antiinflammatory effect, and it can increase hydration of the stratum corneum better than other antioxidants.[5] Research conducted by Packer et al showed no side effects were reported of

topical use of vitamin E in a survey of several women who used topical vitamin E in Slovenia. Compared with vitamin C, some women have reported side effects such as stinging sensations, redness, and dryness.[6]

Microneedle is one of the tools used for skin rejuvenation therapy, which can induce fibroblasts so as to reduce wrinkles. Microneedling can induce percutaneous collagen, have a rejuvenating skin effect, and it is also expected to facilitate the penetration so as to increase the efficacy of a mixture of AMSC-MP and vitamin E for skin rejuvenation. Therefore, microneedling is a minimally invasive procedure, having relatively fast downtime, and lower costs when compared to other treatment such as lasers.[7]

This research is part of large research that examines the effects of AMSC-MP on photoaging. This study used a mixture of AMSC-MP with vitamin E. It is expected that this mixture will provide a better effect on the clinical improvement of photoaging.

METHODS

This was an observational cohort study comparing clinical improvement of photoaging before and after the administration of a topical mixture of AMSC-MP with vitamin E and microneedling. The study samples were 30 eligible photoaging patients of the Dermato-venereology outpatient clinic, Dr. Soetomo General Hospital Surabaya. The acceptance criteria were patients with Glogau II-III photoaging degrees or 40–60 years old 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. The rejection criteria were patients with a history of keloids, active eczema on the face, haemophilia or physiological disorders of blood clotting using anti-platelets, diabetes mellitus, and HIV/AIDS. Thirty eligible research subjects were conveyed of the research purpose and benefits for the health services and scientific development. The subjects were then asked to sign an informed consent voluntarily. The study subjects' skins were examined using Facial Skin Scope JANUS-II for wrinkles, pores, polarized black spots, UV black spots and skin tone as baseline conditions before priming for 2 weeks. After 2 weeks, the research subjects received microneedling treatment and topical application of a mixture of AMSC-MP and vitamin E 3 times at 2 weeks interval. In this study, the microneedling treatment tool was dermapen at level 3 speed (45-50/times/sec), a depth of 0.5 mm, and 2 passes. Evaluation of Facial Skin Scope JANUS-II for wrinkles, pores, polarized black spots, UV black spots, and skin tones was conducted after the treatment at week 0, 4, and 8. The obtained data and Janus-II photo analysis were recorded and analysed. The Ethics Committee has approved this research at Dr. Soetomo General Hospital Surabaya.

RESULT

All research subjects were female (100%). The subjects in this study were aged 40 to 60 years old, and the biggest age group was 45–49 years with 9 subjects (30%), followed by the 50–55 years with 8 subjects (26.7%), the 56–59 years with 7 subjects (23.3%), and the 40–44 years with 6 subjects (20%).

Table 1. The basic characteristics of patients with photoaging at the Cosmetics Division Dermato-venereology Outpatient Clinic, Dr. Soetomo General Hospital Surabaya

Variables	n (%)
Sex	
Male	0(0)
Female	30(100)
Age	
40–44 years	6(20)
45–49 years	9(30)
50–54 years	8(26.7)
55–59 years	7(23.3)
Mean (years) ± SD	49.7 ± 1.29
Glogau	
Type 2	19(63.3)
Type 3	11(36.3)
Fitzpatrick Skin type	
Type 4	14(46.7)
Type 5	16(53.3)
Occupation	
Administrative workers	14(46.7)
Hospital employee	2(6.7)
Private employee	3(10)
Teacher	2(6.7)
Housewife	9(30)

SD = Standard Deviation

There were 19 subjects (63.3%) in the Glogau Category II and 11 subjects (36.3%) in the Glogau Category III. Fitzpatrick skin type assessment showed that there were 14 people (46.7%) with type 4 and 16 people (53.3%) with type 5. A total of 14 research subjects (46.7%) worked as administrative employees, 2 subjects (6.7%) as hospital employees, 3 subjects (10%) as private employees, 2 subjects (6.7%) as teachers, and 9 subjects (30%) as housewives. All of these occupations are mostly carried out indoors. All subjects stated they had direct sun exposure for less than three hours.

Table 2 shows the Janus evaluation for pore values obtained before and after the AMSC-MP topical mixture with vitamin E application and three microneedling treatments. A total of 27 subjects had an improvement in pore values, 1 subject had an unaffected pore value, and 2 subjects had worsened pore values.

Table 2. The results of Janus 1, 2 and 3 analysis for pores

	Range (Minimum-Maximum)	Mean ± SD	P-value
Janus 1 (n=30)	49–53	51.83±5.47	
Janus 2 (n=30)	47–50	49.2±4.72	0.000*
Janus 3 (n=30)	46–51	48.73±6.48	

GLM (generalized linear model) test p=0.00 SD = Standard Deviation

The results of advanced test statistics using pairwise comparison showed that the pores comparison value between Janus 1 and 2 was p = 0.000, between Janus 1 and 3 was p = 0.000, and between Janus 2 and 3 was p = 0.580. The most significant improvements were observed in Janus 1 and 3.

Table 3 shows the Janus evaluation for wrinkle values obtained before and after the AMSC-MP topical mixture with vitamin E application and three microneedling treatments. A total of 23 subjects had improved wrinkle values, 3 subjects had unaffected wrinkle values, and 4 subjects had worsened wrinkle values. Further statistical test using the Wilcoxon test showed that Janus 1 and Janus 3 had significant differences with a value of p = 0.000. The ratio of wrinkles between Janus 1 and 2 was p = 0.227. The comparison value of Janus 2 and 3 was p = 0.120.

Table 4 shows the Janus evaluation for skin tone values obtained before and after the AMSC-MP topical mixture with vitamin E application and three microneedling treatments. A total of 28 subjects had decreased skin tone values, 2 subjects had unaffected skin tone values, and no subject had an increased skin tone value.

Table 3. The results of Janus 1, 2 and 3 analysis for wrinkles

	Range (Minimum-Maximum)	Mean ± SD	P-value
Janus 1 (n=30)	2–17	8.16±4.02	
Janus 2 (n=30)	1–27	7.70±5.70	0.001*
Janus 3 (n=30)	0–15	6.20±3.57	

Friedman test p=0.001
SD = Standard Deviation

Table 4. The results of Janus 1, 2 and 3 analysis for skin tone

	Range (Minimum-Maximum)	Mean ± SD	P-value
Janus 1 (n=30)	31–33	32.87±0.47	
Janus 2 (n=30)	30–32	31.40±0.53	0.000*
Janus 3 (n=30)	30–31	30.96±0.04	

GLM test (generalized linear model) p=0.00
SD = Standard Deviation

The pairwise comparison statistical test result on skin tone comparison between Janus 1 and 2 was p = 0.000. The comparison value between Janus 1 and 3 was p = 0.000. The comparison value between Janus 2 and 3 was p = 0.102. The comparison concluded that it has a significant difference, and the greatest decrease was in the comparison between Janus 1 and 3.

Table 5 shows the polarized black spot values based on Janus evaluations performed before and after topical application of a mixture of AMSC-MP with

vitamin E and three microneedling treatments. A total of 20 subjects had improved polarized black spots values, 6 subjects had unaffected polarized black spots values, and 4 subjects had worsened polarized black spots values.

Table 5. The results of Janus 1, 2 and 3 analysis for polarized black spots

	Mean (Minimum-Maximum)	Mean ± SD	P-Value
Janus 1 (n=30)	30–37	34.10±9.14	
Janus 2 (n=30)	30–36	33.16 ±8.40	0.002*
Janus 3 (n=30)	29–35	32.46 ±8.74	

test GLM (generalized linear model) p=0.00
SD = Standard Deviation

Further pairwise comparison statistical test showed that the comparison result between Janus 1 and Janus 3 which was significantly different was p = 0.002. The comparison of polarized black spots between Janus 1 and 2 was p = 0.194. The comparison value between Janus 2 and 3 was p = 0.269.

Table 6 shows the UV black spot values based on Janus evaluations performed before and after the application of a topical mixture of AMSC-MP and vitamin E after three microneedling treatments. A total of 22 subjects had improved UV black spot values, 5 subjects had unaffected UV dark spot values, and 3 subjects had worsened UV black spot values.

Further Wilcoxon statistical test showed that the comparison value of UV black spots between Janus 1 and 2 was p = 0.032. The comparison value between Janus 1 and 3 was p = 0.002. The comparison value between Janus 2 and 3 was p = 0.191. These results confirmed that the Janus comparison had significantly different results with the greatest improvement was between Janus 1 and 3.

Table 7 shows a comparison of p-values on pores, wrinkles, black spots (polarized), black spots (UV), skin tone between the first, second, and third Janus. There are significant changes in pores, wrinkles, black spots (polarized), dark spots (UV), and skin tones before and after the application of the topical mixture of AMSC-MP and vitamin E after microneedling with the most significant value on Janus 1 and 3.

Table 8 lists the possible side effects of this study, the side effects were assessed regularly after treatments. None of the study subjects showed any side effects after the treatment.

Table 6. The results of Janus 1, 2 and 3 analysis for UV black spot

	Range (Minimum-Maximum)	Mean ± SD	P-value
Janus 1 (n=30)	3–19	8.10 ±3.65	
Janus 2 (n=30)	1–18	7.06 ±3.48	0.001*
Janus 3 (n=30)	2–16	6.36 ±3.17	

Friedman test p=0.001
SD = Standard Deviation

Table 7. Resume p-value comparison of pores, wrinkles, skin tone, black spots (polarized), black spots (UV) between the first, second, and third Janus.

	Pores	Wrinkles	Skin tone	Black spots (polarized)	Black spots (UV)
AMSC-MP+ Vitamin 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

AMSC-MP: Amniotic Membrane Stem Cell Metabolite Product
UV: Ultraviolet

Table 8 Side effects after participating in research

Side Effects	n (%)
Persistent erythema (erythema> 24 hours)	0 (0)
Urticaria	0 (0)
Post-inflammatory hyperpigmentation	0 (0)
Hypopigmentation	0 (0)
Infection	0 (0)
Nothing	30 (100)

DISCUSSION

The study subjects were female with photoaged skins. The uniformity of sex was aimed to minimize the drop out of the study subject considering that women (females) generally concern about their skins more than men (males), suggesting that women are more compliant than men in terms of skin treatment. The sex ratio of patients with photoaged skins at the cosmetics outpatient clinic, Dr. Soetomo General Hospital showed that most patients were female. This was also supported by the patient visitation to the cosmetics outpatient clinic, Dr. Soetomo General Hospital in 2018. There were 711 female patients, and 24 male patients underwent skin rejuvenation treatment.

There were nine subjects with photoaged skins in the 45–49 years age group. The youngest study subject was 40 years and the oldest was 59 years. This was also in line with the research conducted by Lee et al. in 2014 involving 25 subjects with an age range of 41–64 years and the average age of 51.6 years. A total of 19 subjects (63.3%) were classified in category II and 11 subjects (36.3) were in the category III. This was also in line with the research conducted by El Kahky et al. on the rejuvenating effect of mesenchymal stem cells on the skin, 70% of the study subjects had Glogau type II classification and 20% had Glogau type III classification. Eight patients with Glogau II and III photoaging degrees tend to complain more about photoaging problems, while patients with Glogau I photoaging degrees can minimize the appearance of photoaged skins using cosmetics. At the degree of photoaging in Glogau IV, chronological aging is usually more obvious, making it a confounding factor. In this study, 14 subjects (46.7%) had Fitzpatrick skin type 4 and 16 subjects (53.3%) had Fitzpatrick skin type 5. Photoaging was more common in patients with bright skin. Patients with Fitzpatrick skin types 1, 2, and 3 were more likely to suffer from photoaging than skin types 4, 5, and 6.[9] A total of 14 study subjects (46.7%) worked as administrative employees, 2 subjects (6.7%) as hospital employees, 3 subjects (10%) as private employees, 2 subjects (6.7%) as teachers, and 9 subjects (30%) as housewives. Their work activities are mostly carried out indoors. All research subjects claimed to have outdoor activities and were exposed in direct sunlight for less than 3 hours a day. The degree of photoaging also depends on geographical location, exposure to sunlight related to work and lifestyle, and photoprotective activities. The data collected in Sweden by Larko and Swanbeck in 1982 shows that outdoor workers are three times more susceptible to skin cancer than indoor workers.[9] The Korean study by Chung found that more than 5 hours of sun exposure per day was associated with a 4.8 times increased risk of wrinkles compared to those with 1 or 2 hours of sun exposure.[10]

Amniotic membrane stem cell metabolite products contain several types of growth factors that can stimulate the proliferation and migration of dermal fibroblasts, epidermal keratinocytes, and increase collagen synthesis from fibroblasts. The use of metabolite products as therapy is considered safer because they are cellular products that are not carcinogenic and easier to control than products that contain cells. Another benefit is that stem cell culture media can be obtained easier and stem cell metabolite products can be obtained in large quantities at lower costs than the stem cells. The drawback of AMSC-MP is slower regeneration rate than stem cells because stem cells can produce their own growth factors, cytokines, and extracellular matrix.[11]

A similar study conducted at Dr. Soetomo in 2017 by Prakoeswa et al. found a percentage of insignificant clinical improvement on wrinkles, polarized black spots, UV dark spots, and skin tones. However, there were significant improvements in pores in the patients who received microneedling treatment and topical application of AMSC-MP compared to those given microneedling and normal copy treatments.[12] The study conducted by Effendy regarding efficacies comparison between AMSC-MP after microneedling and the AMSC-MP mixture with vitamin C after microneedling resulted in improved degrees of wrinkles, polarized black spots, UV and pore black spots, and faster repair in groups with a mixture of AMSC-MP with vitamin C.[13]

There were significant differences in the results of Janus on all assessments such as pores, wrinkles, polarized black spots, and UV black spots. Clinical signs of photoaged pores significantly improved with p

= 0.000. Improvements were started noticeable on the second Janus and became significant on the third Janus. This was thought to be due to the effects of growth factors and cytokines from AMSC-MP as well as the effects of vitamin E which play a role in collagen synthesis. Pore sizes are influenced by various factors, one of which is photodamage. It is an important component in the assembly of elastic fibres, thereby affecting the elasticity of the skin in the dermis area and pores. Pores are superficial skin structures, so repairs will be usually noticeable before wrinkles, even though they are both affected by collagen.[14] This is evidenced by the results the Janus, which obtained significant improvements starting from the second Janus. This was in line with a previous study conducted by Prakoeswa et al. in 2018, the study found a significant improvement in pores in the group treated with AMSC-MP after microneedling compared to the control group given normal saline alone.[12] One subject had an unaffected pore value and two subjects had worsened pore values. All study subjects were 50 years old. This might affect the subjects' pore conditions. At the age of 50, the collagen production decreases and skin elasticity is much reduced, causing the pore walls to sag and pores to appear large.[14] External factors such as cigarette smoke, pollution, and stress also might contribute.

Sun exposure can cause the formation of wrinkles through reduced stress strength, elasticity, and can cause degradation of structural components that support the dermal extracellular matrix. Radiation from sunlight can increase ROS, which increases Activator Protein (AP)-1 and Nuclear Factor (NF)-kβ, reduce Transforming Growth Factor-β (TGF-β), decrease collagen production, and promote collagen damage resulting in wrinkles. In this study, there was a clinical improvement of wrinkles with a significant difference of p = 0.000 as evidenced in the results of the first and third Janus. This was 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; therefore, improving the skin texture.[15] A research conducted by Lee et al. comparing the stem cell metabolite products application after microneedling and microneedling alone, the research found significant results in wrinkle repair. Their study subjects were treated 5 times at intervals of 2 weeks. The study used a visionometer analysis to assess skin roughness and depth of wrinkle lines.[11] The addition of vitamin E to the topical product was expected to have a synergistic effect on the clinical improvement of photoaged skin. Chung et al. showed that the use of occlusive topical 5% vitamin E for 24 hours provided protection against UV induced metalloelastase *in vivo*. Vitamin E protects cell membranes from lipid peroxidase by free radicals. The research showed that topically applied vitamin E was potentially penetrated into the skin layer where oxidative stress occurs so as to inhibit the photoaging process.[16] Prakoeswa et al. compared the improvement percentages between the subject's first and third visitation data, and they found that there was no significant improvement in terms of wrinkles, spots (polarized), spots (UV) and skin tone between microneedling treatment + AMSC-MP and microneedling treatment + normal saline.[12] A total of 3 subjects had unaffected wrinkle values and 4 subjects experienced a wrinkling value. Their research involved 5 individuals above 50 years old and 2 individuals above 45 years old. Similar to pores, wrinkles are also caused by a reduced production of collagen and elastic fibres, which reduces skin tension, thereby giving a picture of wrinkles on the skin.[14] As we age, the production of collagen decreases and skin elasticity decreases. Pores that are on superficial skin and wrinkles are rather located deeper, making the wrinkle repair slower than visible pores.[17] External factors such as cigarette smoke, pollution, and stress were not possible to consider or controlled yet they are contribute to wrinkles, thus affecting the assessment results of the analysis.

Skin tone values indicate the uniformity of skin colour. Analyst skin tone value is different from other parameters; a lower skin tone value indicates a difference in skin colour uniformity. This study also found a significant difference in skin tone with p-value = 0.000. A total of 28 subjects had decreased skin tone values, 2 subjects had unaffected skin tone values, and no subject had an increased skin tone value. Skin tone is affected by various factors, including skin types, workload, stress, melanin production, sunburn, skin problems, and etc. Each subject has different levels of stress, duration of sun exposure, melanin production, and skin problems. Uniforming these factors was complicated, considering that stress is an intrinsic factor that cannot be measured quantitatively. Another factor is melanin production which correlates with duration of sun exposures and skin type.[18] Uniforming these factors was complicated, considering that each object has different daily activities and different levels of stress.

In this study, there were significant improvements in black spots (polarized) and black spots (UV). Black spots (polarized) repair value was a significant difference with p = 0.002. Black spots (polarized) repair began to be noticeable on the second Janus and became significant after the third Janus. Black spots

(UV) repair also showed a significant improvement with a p-value = 0.001. Improvement of dark spots was observed 2 weeks after the first and third application and became significant after the third Janus. Black spots occur because of increased activity of the tyrosinase enzyme, which is correlated with UV exposure. This can increase melanin production, which eventually colours the keratinocyte layer. Stem cell metabolite products have a brightening effect by inhibiting melanin formation, changing the shape of melanin and affecting the distribution of melanosome transfer. Research conducted by Seo et al. shows that stem cells have a whitening effect by inhibiting the melanin synthesis, tyrosinase activity, and reduce the expression of melanogenic enzymes.[15] 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. The melanin indexes of patients were determined using mexameter. Transforming Growth Factor-β1 found in AMSC-MP can inhibit pigment formation by influencing the tyrosine synthesis.[11] A study by Prakoeswa et al. showed a percentage of clinically insignificant improvement in polarized black spots, UV dark spots, and skin tone.[12] A total of 6 subjects had unaffected polarized black spot values and 4 subjects had worsened polarized black spot values. There were also 5 subjects with unaffected UV black spot values and 3 subjects had worsened UV black spot values. Of all subjects who experienced sedentary and worsening values, 75% of them were aged above 50 years old and 67% of them had Fitzpatrick skin type 5. Similar to skin tone, the appearance of black spots was also affected by various factors, including skin types, workload, stress, melanin production, sunburn, keratin, skin problems, and so on. A darker Fitzpatrick skin type has a higher risk for hyperpigmentation or dark spots.[19] These factors can affect the appearance of hyperpigmentation on the skin.

Possible side effects of the treatment, such as persistent erythema (>24 hours), urticaria, postinflammatory hyperpigmentation, hypopigmentation, or infection were considered. To the end of the research, no side effects were observed or reported. This was because microneedling is a minimally invasive treatment with only 0.5 cm penetration to assist drug delivery into deeper layers of the skin, which was complemented by the effects of AMSC-MP topical mixed therapy with vitamin E. Secretion of Interleukin (IL)-6, IL-8, and Chemokine Ligand (CXL)-1 from AMSC-MP functions as a proinflammatory and antiinflammatory, increasing wound healing by several mechanisms such as recruiting leukocytes, angiogenesis, and collagen production. This is consistent with the research findings of Tamama et al. The research found that AMSC-MP secrete IL-8, CXL1, which can accelerate the healing process.[14] Vitamin E also functions as an antiinflammatory agent and it can affect scars remodelling by interacting with phospholipids on cell membranes, inducing molecular, and maintaining membrane stability. Vitamin E was expected to improve wound healing, prevent hypertrophic scars, and reduce itching. Research conducted by Packer et al. showed that no side effects reported on the use of topical vitamin E in a survey involving women Slovenia who used topical vitamin E.[6] The research by Prakoeswa et al. reported minimal side effects in the form of erythema and transient edema.[12]

The limitations of the study were that this study did not consider or control the external factors such as exposure to cigarette smoke, pollution, and stress that might affect the results of the study and this study did not assess the subjects' satisfaction.

The application of a topical mixture of AMSC-MP and vitamin E after microneedling provided a significant clinical improvement in photoaged skins. This was supported by the results of Janus analysis on pores, skin tones, polarized black spots, and UV black spots. Significant improvements in pores, wrinkles, polarized black spots, and UV black spots were observed in the third Janus. There was no side effect (persistent erythema, urticaria, hyperpigmentation or postinflammation hypopigmentation, and infection) observed or reported. Topical application of a mixture of AMSC-MP and vitamin E can be considered for skin rejuvenation option.

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