

The efficacy of fractional erbium:YAG laser (2940 nm) compared to Microneedling of topical amniotic membrane stem cell conditioned medium (AMSC-CM) for photoaging

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Abstract

Background: Photoaging is a complex biologic process that affects various layers of the skin. Amniotic membrane stem cell conditioned medium (AMSC-CM) that contain growth factors and have capability to improve clinical picture of photoaged skin. Microneedling and laser devices are transdermal drug delivery methods which shows promising results to improve photoaging .

Objective: To investigate the efficacy of topical AMSC-CM after YAG erbium fractional laser compare to microneedling in photoaging.

Method :A clinical study, 60 adult women with photoaging. Randomized into two groups A 30 women received AMSC-CM after fractional erbium:YAG laser (2940nm) and group B 30 woman received AMSC-CM after microneedling. Both groups received the treatment 3 times with 2 weeks interval. The improvement of pore, wrinkle, and UV spot as was evaluate before, after and post treatment using computer-stimulated photograph skin analyzer.

Result: The evaluation of pore and UV spot showed greater improvement in the group of AMSC-CM with fractional erbium:YAG laser compare to AMSC-CM with microneedling group (pore p:0,00 and UV spot p:0,00). There was no significant different (p 0.43) of wrinkle improvement between both groups.

Conclusion: The combination of AMSC-CM and fractional erbium:YAG laser have better efficacy in the improvement in photoaged skins.

Keyword: fractional laser, microneedling, photoaging,

Introduction

Skin aging is the cumulative effect of intrinsic (genetics, cellular metabo- lism, hormone and metabolic

processes) and extrinsic factors (chronic light exposure, pollution, ionizing radiation, chemicals, toxins). Among extrinsic factors, the effect of solar radiation on skin health is well characterized, resulting in coarse wrinkles, loss of elasticity, laxity, and rough- textured appearance.^{1,2,3} Exposure to UV radiation is the primary factor of extrinsic skin aging; it accounts for about 80% of facial aging.Chronic photodamage of the skin manifests itself as extrinsic skin aging (photoageing). DNA photodamage and UV-generated reactive oxygen

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species (ROS) are the initial molecular events that lead to most of the typical histological and clinical manifestations of chronic photodamage of the skin.^{2,3}

There has been great interest in understanding the regulation and coordination of the stem cells found within the skin in order to repair aged skin.⁴ 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 fact, AMSCs can be obtained with little donor damage, have multipotency for all three germ layers and low immunogenicity and are ethically acceptable.⁵ Several studies have identified the role of stem cells in improving and increasing dermal thickness and density of collagen fibers in aged mice as well as reducing skin wrinkles.⁶

The use of procedures stimulating the revitalization of skin, including fractional methods: fractional microneedling radiofrequency, fractional microneedling radiofrequency technology combined with LED light (light-emitting diode) and non-ablative, and ablative fractional laser therapy, fractional IR (infrared light) seems to be essential.⁷ The clinical use of lasers in treating dermatological disorders such as skin spots, atrophic acne scars, chronological aging, and cutaneous aging has been growing in recent years.⁸ This type of erbium laser can be found at wavelengths of 1540 or 1550 nm, which are classified as non-ablative erbium glass, and at 2940 nm, classified as ablative erbium YAG. Ablative fractional lasers destroy the superficial layers of the skin, to improve the appearance of the skin after epithelialization.⁷

Dermapens are also used in microneedling mesotherapy. Microneedling therapy is a progressively popular treatment for several dermatologic conditions. This cheap, relatively risk-free office maneuver reverses the clinical as well as histopathological signs of aging and rejuvenates aged skin. Transdermal penetration of the drug was facilitated with the use of a needle length of 0.5 mm. A similar penetration of pharmaceuticals was confirmed also with the use of dermaroller just before the substance application. Moreover, the increase in transepidermal drug delivery is observed for at least 72 hr after the procedure. The microtraumatization creates

micropores, which results in increased transdermal water loss, however, the result of the study has demonstrated that prompt recovery of skin barrier function is achieved within several hours from device usage.^{7,9}

Moreover, combined minimally invasive modalities can improve collagen induction and facilitate penetration of topical agents for skin rejuvenation. We combine the use of fractional erbium: YAG laser (2940 nm) and product (AMSC-MP) in group A compared to microneedling with product (AMSC-MP) in group B. We will evaluate the effectiveness of these two combinations in terms of improving 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 fractional erbium: YAG laser (2940 nm) and product (AMSC-MP) in group A compared to microneedling with product (AMSC-MP) in group B. The participants, who were patients with photoaged skin at the outpatient clinic of the Dermatology and Venereology Department, Dr. Soetomo General Academic Hospital Surabaya, Indonesia, were acquired by consecutive sampling. The inclusion criteria were patients with a Glogau score of II–III who had used tretinoin (0.25%) cream prior to therapy. The exclusion criteria were a history of keloid scars; active eczema; herpes simplex infection; a history of Botox injection, filler injection, chemical peels, or microdermabrasion; the use of cream or oral antioxidants; hemophilia/ physiological disorders of blood clotting using anti-platelets, diabetes mellitus, and HIV / AIDS and uncooperative behaviour. The final number of participants was 30.

Preparation of AMSC-CM and vitamin C mixture: AMSC-CM is a liquid culture product of AMSC that contains cytokines and growth factors that aid in wound healing and skin rejuvenation. In this study, the AMSC-CM was acquired from the Tissue Bank and Regenerative Medicine Department, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

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 and cream sunscreen (sun protection factor 30) for 2 weeks for priming. After 3 days of discontinuation of the tretinoin cream, the treatment procedures were initiated. For group A the AMSC-CM was applied after treatment with a fractional erbium:YAG laser. The therapy was performed three times with an interval of 4 weeks. All participants were provided sunscreen to apply every morning to afternoon and tretinoin cream (0.025%) to apply every night. Tretinoin cream (0.025%) was initiated 1 week after treatment. The progressive changes in wrinkles, spots, and pores were evaluated 4 weeks after the first treatment, before the second treatment (evaluation II), and 4 weeks after the third treatment (evaluation III). For group B A the AMSC-CM was applied after treatment *microneedling*. 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.

Statistical analysis: The accumulated data were analysed using SPSS software version 21 (SPSS, Chicago, IL, USA), with the level of significance set at $P < .05$.

Results

This study was conducted on 60 women with photoaged skin who met the inclusion criteria. All the subjects were woman between 40-60 aged, mean age was 42 ± 4.92 and the mean baseline Glogau score was 2.8 ± 0.4 . All patients work indoors; the most common outdoor activity was motorbike riding (100%), followed by morning exercise (50%; Table 1). 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.

Table 1. Patient characteristics

Variable	Fractional erbium:YAG laser + AMSC-CM n=30	Microneedling + AMSC-CM n=30
Age		
- 20-30	0	0
- 30-50	30	30
- 50-60	0	0
Glogau score		
- II	0	6
- III	30	24
Occupation		
- Indoor	30	30
- Outdoor	0	0

From the group A, the group given topical AMSC-CM after treatment with a fractional erbium:YAG laser obtained significant results in pores, wrinkles, UV spot and polarized spot. In the 1st and 3rd Janus observations there were significant improvements in pore, wrinkle and UV Spots (Table 2).

Table 2. Resume p value of pores, wrinkles, and UV spots between the 1st, 2nd, and 3rd janus in group A

fractional erbium:YAG laser+ AMSC-CM	Pores	Wrinkles	UV spot
Janus 1 vs 2	0,023	0,043	0,04
Janus 1 vs 3	0,023	0,043	0,00
Janus 2 vs 3	0,000	0,000	0,00

Table 3 shows of p values on the value of pores, wrinkles, and UV between the first, second and third janus from the group B microneedling combination of AMSC-CM.

Table 3. Resume p value of pores, wrinkles, and UV spots between the 1st, 2nd, and 3rd janus in group B

Microneedling+ AMSC-CM	Pores	Wrinkles	UV spot
Janus 1 vs 2	0,084	0,216	0,38
Janus 1 vs 3	0,032	0,429	0,258
Janus 2 vs 3	0,029	0,846	0,258

Table 4 shows the results of the comparison of mean value and p value between group A and groups B. The improvement was better in the group A. From the comparison of the two groups showed there were significant different of mean value in pore and UV spot and not significant different in wrinkle. Pore and and UV spots with p-value < 0,005.

Table 4. Comparison of mean value and p value between group A and group B

	P-value
Pore	0,00
Wrinkle	0,428
UV spot	0,000

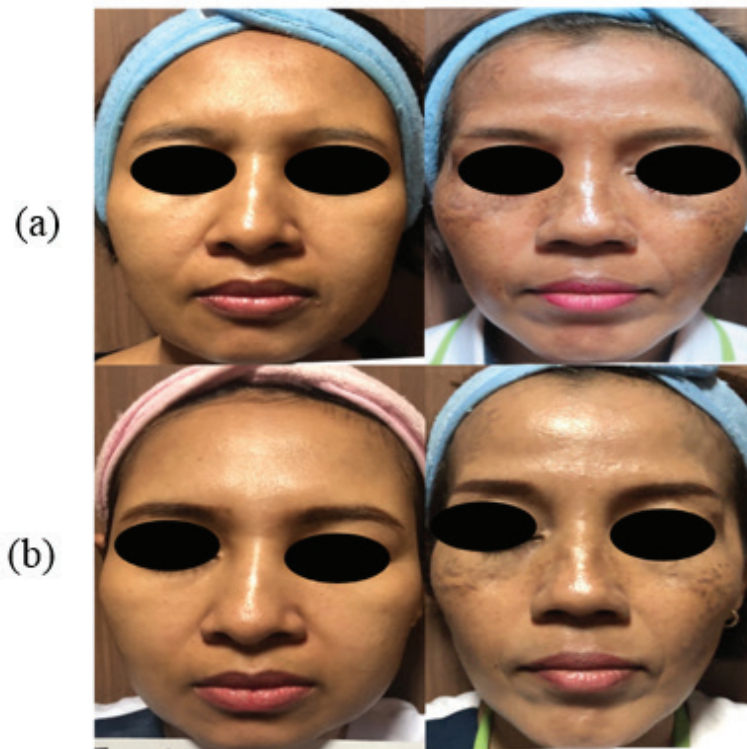


Figure 1. Photograph results (a) before fractional erbium:YAG laser + AMSC-CM (b) after fractional erbium:YAG laser + AMSC-CM last procedure.

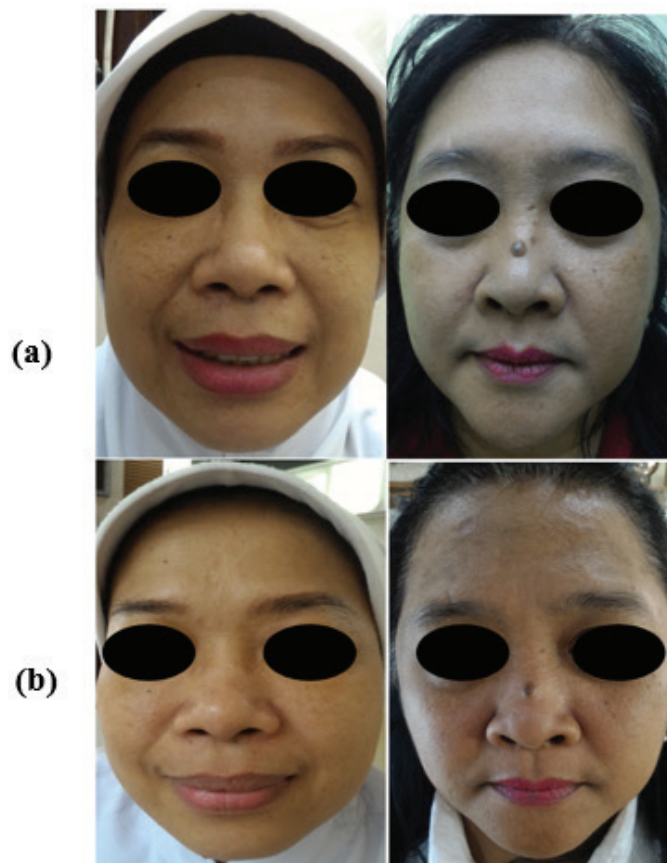


Figure 2. Photograph results (A) before mikroneedling + AMSC-CM (B) after microneedling+ AMSC-CM last procedure.

Figures 1 and 2 show the clinical improvement before and after procedure in group A and group B. Improvement are seen in patient's spot, wrinkle and skin tone.

Discussion

Skin rejuvenation has become the focus of cosmeceuticals, in which various medical treatments and products are used for anti-aging.¹⁰ Aging process in the face area is a complex process that distorts the three-dimensional shape and textures.⁶ The manifestations of photoaging investigated in this study were pore, wrinkle and UV spot. In the comparison of this study, it was found that the use of combination fractional erbium:YAG laser and AMSC-CM is better in terms of reducing photoaging compared to microneedling combination with AMSC-CM.

The skin extracellular matrix (ECM) that consists of glycosaminoglycans, collagen and elastin, is crucial for skin morphology and functions such as growth and elasticity [6]. The degradation of ECM that occurs along with skin aging is related to the increase of the activity of enzymes such as hyaluronidase, elastase and collagenase [7]. Photo-aging causes production of collagenase by Human Dermal Fibroblasts (HDFs), which degenerates collagen production and is exposed on skin as wrinkles. Amniotic membrane stem cell conditioned medium secrete many cytokines and growth factors such as Epidermal growth factor (EGF), basic Fibroblast growth factor (bFGF), Transforming growth factor-beta (TGF- β), which are important in cell growth and maintaining skin tissues.¹⁰ Transforming growth factor-beta can induce a whitening protein, like TGF- β , hepatocyte growth factor, insulin-like growth factor (IGF) induce anti-wrinkle protein.⁵ Recent studies revealed that HAMSCs have important roles in cell differentiation, promoting cell proliferation, enhancing cell viability and function, protecting cells from adverse effects and inhibiting apoptosis *in vivo* or *in vitro*. The present study aimed to determine whether HAMSCs involved in the protection of human dermal fibroblasts (HDFs) from UVA-induced senescence.¹¹ UVA radiation has the effect of reducing the telomere length of fibroblasts in the skin, inhibiting TGF- β 1 secretion inducing G1 phase arrest, reducing superoxide dismutase (SOD), increasing malate dehydrogenase

(MDH) levels and increasing MMP-1.4 expression. On histopathological examination, photoaging skin shows a reduced composition of the extracellular matrix.¹²

Fractional erbium:YAG laser is a technique for the treatment of skin lesions in which an array of microscopic thermal wounds (microscopic treatment zones) is induced into the skin to stimulate a therapeutic response deep in the dermis. In 2008, Lapidot and colleagues reported results from a skin rejuvenation study of a fractionated 2940 nm Er:YAG laser. Pre-treatment biopsies, compared to those taken at 60 days post-treatment, clearly showed the epidermal and upper dermal ablation and healing process with collagen regrowth that is expected with Er:YAG fractionated resurfacing.¹³

Full effect of the series of microneedling procedures involving in the lowering of wrinkles visibility, and an improvement of skin elasticity, is achieved after about 3 months, due to the length of the neocollagenesis process.⁷ El-Domyati et al. have demonstrated the effectiveness of microneedle mesotherapy in the fight against aging signs. Histopathological examinations of skin specimens taken from patients who underwent a series of six microneedling procedures (1 mm in depth) at 2-week intervals showed significant changes within both the epidermis and dermis. The prickle cell layer (stratum spinosum) of the epidermis thickened, which affected the development of rete ridges and the increase of skin layers integrity. The amount of collagen Type I, III, and VII increased significantly. Prakoeswa et al studied compared, microneedle plus AMSC-CM with microneedle plus NS, the result is microneedle plus AMSC-CM was more effective in improving the clinical signs of photoaging.⁵

The two modalities of fractional erbium therapy: YAG laser and microneedling have good effectiveness for photoaging, but because Fractional erbium: YAG laser can affect to the dermis, while the microneedling used in this study is 0.5 mm which does not penetrate to the dermis and the use of microneedling requires a longer evaluation time to see results in terms of reducing wrinkles. So the results fractional erbium: YAG laser is better than microneedling in overcoming photoaging.

Conclusion

The combination of AMSC-MP and fractional erbium:YAG laser (2940 nm) have better efficacy in the improvement of pore and spot in photoaged skins compared to AMSC-MP and microneedling.

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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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