

The Effect of Advanced Adipose-derived Stem Cell Protein Extract to Repairment of Collagen Deposition in Cultured Senescent Fibroblast

Erlina Pricilla Sitorus^{1*}, Retno Dwi Utami¹, Gita Hening Bunga¹, Indah Julianto¹, Harijono Karionosentono¹, Chandrani Khoirinaya², Brian Wasita³

¹Dermatology and Venereology Departement of Medical Faculty Sebelas Maret University, Surakarta

²Dermama Biotechnology Laboratories, Surakarta

³Pathology Anatomy Departement of Medical Faculty Sebelas Maret University, Surakarta

Keywords: Advanced adipose-derived stem cell protein extract, aging skin, collagen deposition

Abstract: Aging skin is progressive process in skin that stimulates by environment damage and influences the appearance. There are two factors inducing aging, intrinsic i.e genetic and age, and extrinsic i.e sun expose, air pollutant, smoking, alcoholic and malnutrition. Recently, cell based therapy has been widely reported as the option for aging skin therapy. *Advanced adipose-derived stem cell protein extract* (AAPE) is a conditioned medium cultured under a hypoxia of *Adipose-derived stem cell* (ADSC), which can stimulate collagen synthesis and migration dermal fibroblast, thus induces wound healing and wrinkles repairment. This study samples used cultured fibroblast from amnion, with donor from a 30 year old woman by cesarean section on her first delivery. There were three samples cultured groups, normal fibroblast (control), normal fibroblast with 30 minutes UVB exposure in basal media (study group 1), and normal fibroblast with 30 minutes UVB exposure in basal media added AAPE (study group 2). All groups were evaluated in 48 hours and checked the collagen density by using immunocytochemistry assay with collagen antibody-1. The results were interpreted by *Image-J* software. Study group 2 showed significant increasing of collagen density, with *p* value 0.001. Because the AAPE was derived from ADSC thus it has the same growth factors as those of ADSC. AAPE had been proven in our study that it stimulated collagen deposition.

1 INTRODUCTION

Aging is a progressive degenerative process of all organs in the body, including the skin. Aging in human skin is caused by intrinsic factors such as genetics and age and extrinsic factors like sun exposure, air pollutant, smoking, alcoholic and malnutrition (Yaar and Gilchrest, 2007; Ichihashi and Ando, 2014), which disturb the function and structures of epidermal cells and dermis, also the extracellular matrix. Clinically, skin aging induced by sun exposed or photoaging exhibits as wrinkles, mottled pigmentation, rough skin, loss of skin tone, dryness, sallowness, deep furrows, severe atrophy, teleangiectasis, laxity, leathery appearance, solar elastosis, actinic purpura, precancerous lesions, skin cancer and melanoma (Pandel et al., 2013). The histological features are epidermis and dermis atrophy, elastosis in dermis, collagen changes and elastin fibers fragments. Ultraviolet (UV) exposure induces enhanced reactive oxygen species (ROS) production so that the production of the components

and the oxidative destruction increase as well. This destruction is a significant mechanism in aging process (Stojiljković et al., 2014).

Cell based therapy has become a promising therapy since it can induce repairment or cell regeneration post tissue trauma or organ function failure (Baer et al., 2016). One of cell based therapies which show significant outcome is ADSC. It is an adult stem cells which is also as mesenchymal stem cell from human fat tissue. It has ability to differentiate to be their derivatives and to secrete the various growth factors, which can repair as well as replace the surrounding damaged cells (Kim et al., 2011). The conditioned medium of ADSC known as AAPE containing secreted growth factors, which is beneficial for skin problem i.e face wrinkles as well as wound repairment. AAPE can induce collagen synthesis and fibroblast cell migration into dermis, thus it can be used as rejuvenation therapy and wound healing (Kim et al., 2009; Zhou et al., 2016). Recently, the skin regeneration has become cosmetical and

dermatologist concern as anti aging therapy due to long term exposure to UV, for various non invasive therapies to treat the skin aging symptoms (Moon et al., 2012; Lee et al., 2014).

2 MATERIAL AND METHODS

2.1 Isolation and Culture of Fibroblast

The fibroblast cells were obtained from amnion tissue from a 30 year old woman with history of her first delivery by section cesarean as donor, with informed consent and ethical clearance. After the mechanical procedure, the tissue was cut in 0,5-1 cm² in size then placed to culture flask, immersed with small amount of growth medium consisting of high glucose Dulbecco's Modified Eagle's Medium (DMEM) Gibco®. Then the part of these tissues was incubated in 37°C and 5% CO₂ for 48 hours, until the tissues were attached on the bottom of the well plate. After 48 hours, when the sections had attached the medium was exchange with new medium until the entire of tissue section was submerged. The medium was changed in 2 days until the fibroblast growth with 80% confluences.

2.2 Induction of Cellular Senescence

The cultured fibroblast in DMEM was added with 10% FBS, 100 IU/ml Penicillin and 100 µg/ml Streptomycin with 5% CO₂ in 37°C. After starvation for 24 hours, the cells were washed with PBS and exposed with UVB light dose 100 mJ/cm² in 30 minutes, using applied method by Kim *et al.* (2009). After exposure, the PBS was aspirated and replaced to complete growth medium.

2.3 Preparation of the Advanced Adipose-derived Stem Cell Protein Extract (AAPE)

ADSCs (4 x 10⁵ cells) were cultured in DMEM (Gibco®) serum-free medium. Conditioned medium of ADSCs was collected after 72 h of culture, centrifuged at 400 x g for 5 min and filtered using a 100 mm syringe filter (Kim *et al.*, 2009)

2.4 Experiments

2.4.1 Treatment

Normal fibroblast (in DMEM) group as control

study (2 x 10⁴ cells/400 µl). Second group were 2 x 10⁴ cells/400 µl normal fibroblast + 30 minutes UVB exposed in DMEM (basal media/without AAPE). Third group were 2 x 10⁴ cells/400 µl normal fibroblast + 30 minutes UVB exposed in DMEM added with AAPE 400 µl. Then all groups were evaluated in 48 hours.

2.4.2 Immunocytochemistry with Collagen Antibody-1

The coverslip was taken from the bottom of well plate and pasted to object glass then was gave 1-3 drops of blocking reagen serum for 15 minutes. Then dripped with blocking reagent 1-3 drops for 15 minutes, rinse with wash buffer then dried. After that dripped with HSS-HRP in 30 minutes, rinse with was buffer in every 2 minutes three times, then dripped with 100-200 µL DAB until covered entire tissues section for 20-30 minutes. Rinse in 10 minutes with was buffer for three times. Cover stained tissue with a coverslip of an appropriate size. Place slides vertically on a filter paper or towel to drain excess mounting medium and allow them to dry. Visualize tissue under a light microscope (IHC/ICC Protocol Guide, 2014).

2.5 Measurement

2.5.1 Collagen Deposition

The slides which had been stained with collagen antibody were examined under the microscope, which one of it lenses used Optilab Olympus CX-21® connected to computer installed with *Image-J* software which enabled it to detect collagen deposition and to score it with 0= negative; 1= low positive; 2= positive; 3= high positive (Varghese *et al.*, 2014).

2.5.2 Results Analysis using Statistical Data

Statistical analysis using Kruskal Wallis, the continued with Mann-Whitney assay, with significant p value are < 0.05. The calculation of this data using SPSS software.

2.6 Ethical Clearance

This study had received permission from Dr. Moewardi Hospital's Ethical Comissions after the patient signed the informed consent.

Table 1: Differentiation assay of collagen deposition in each group : control, AAPE (-) and AAPE (+)

Collagen deposition (fibroblast)	Groups			Total (n=95)	P
	Control (n=30)	AAPE(-) (n=35)	AAPE (+) (n=30)		
Negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.001
Low positive	9 (30.0%)	14 (40.0%)	0 (0.0%)	23 (24.2%)	
Positive	21 (70.0%)	21 (60.0%)	30 (100.0%)	72 (75.8%)	

3 RESULT

In the table 1, negative category of collagen deposition was seen in each groups (0%). Low positive score was observed in AAPE (-) or only basal media groups (40%) and followed by control group (30%), but not in AAPE (+) groups. The increase of collagen deposition with the highest proportion in AAPE (+) groups (100%) and the lowest one in AAPE(-) groups (60%).

4 DISCUSSIONS

Changes in mechanical properties of the skin are generally referred to extracellular aspects such as alterations in polymerization and cross-linking of collagen and elastin. In vivo study, the skin fibroblast changes affect the separation of collagen fibers leading to decreased collagen production (Schulze et al., 2012). AAPE are conditioned medium from ADSC had specific ability to organize the protein and secreted growth factors into extracellular environment and had a relevant affect to various organ and human body systems (Li et al., 2015).

Because it is derived from adipose mesenchymal stem cell, this secretome has similar ability to its source, such as promoting the collagen synthesis and fibroblast migration in tissue repairment (Zhou et al., 2016). Both ADSC and AAPE contain various growth factors and they have ability to repaired and renew the surrounding damaged cells. Our study revealed there were significant differences in the increase of collagen density among the control, AAPE (-) and AAPE (+) groups. The highest of collagen deposition was obtained in AAPE (+) groups, as 100% of samples had shown positive category in increasing of collagen deposition.

Kim *et al.* who studied the benefit of ADSC and AAPE as anti aging through the activation of dermal fibroblast by its secreted factors. Animal study demonstrated that adding AAPE and ADSC to skin fibroblast animal study exposed to UVB, resulting in increased dermal layer thickness and collagen amount, as well AAPE can reduced the apoptosis death cell or UVB induced-apoptosis (Kim et al., 2009). Kim *et al* had studied highest concentration of various cytokine concentration of AAPE proved its ability to repair human dermal fibroblast, by stimulating the wound healing in animal study, and the last can play role in anti aging process.

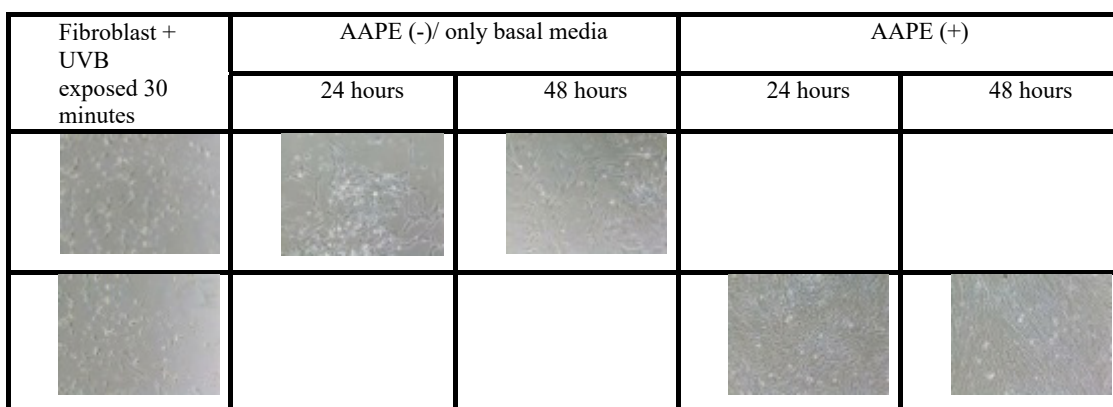


Figure 1. The differentiation of collagen deposition in treatment group, after UVB exposure in 30 minutes. After 30 minutes UVB exposure, many damage fibroblasts were seen. After treatment, in AAPE groups showed repairmen of fibroblasts and had more density of fibroblast than in basal media/ AAPE (-) groups.

Analysis of cell cycle show that adding conditioned medium of ADSC can prevent the apoptotic cell process induced by reactive oxygen species, by significant decreasing of sub-G1 phase of dermal fibroblast cell (Kim et al., 2009).

5 CONCLUSIONS

From all samples (95 photos) there were significant increasing of collagen deposition in control, AAPE (-) and AAPE (+) groups, which the highest deposition showed in AAPE (+) groups, with p value 0.001. Therefore AAPE can repair the damaged fibroblast by increasing the collagen deposition.

ACKNOWLEDGEMENT

There is no conflict of interest while did this study until we had all the results and did not sponsored by any party.

REFERENCES

- Baer, P.C., Overath, J.M., Urbschat, A., Schubert, R.G.H., 2016. Preconditioning of Human Adipose-derived stromal/stem cells: Evaluation of Short-term Preincubation regimens to enhance their regenerative potential. *J Stem Cell Res Ther*;6(3):1–7.
- Ichihashi, M., Ando, H., 2014. The maximal cumulative solar UVB dose allowed to maintain healthy and young skin and prevent premature photoaging. *Experimental dermatology*. doi:10.1111/exd.12393
- IHC / ICC Protocol Guide.
- Kim, J.-H., Jung, M., Kim, H.-S., Kim, Y.-M., Choi, E.-H., 2011. Adipose-derived stem cells as a new therapeutic modality for ageing skin. *Experimental Dermatology* 20, 383–387. doi:10.1111/j.1600-0625.2010.01221.x
- Kim, W.-S., Park, B.-S., Sung, J.-H., 2009. Protective role of adipose-derived stem cells and their soluble factors in photoaging. *Archives of Dermatological Research* 301, 329–336. doi:10.1007/s00403-009-0951-9
- Lee, H.J., Lee, E.G., Kang, S., Sung, J.H., Chung, H.M., Kim, D.H., 2014. Efficacy of microneedling plus human stem cell conditioned medium for skin rejuvenation: A randomized, controlled, blinded split-face study. *Annals of Dermatology* 26, 584–591. doi:10.5021/ad.2014.26.5.584
- Li, Y., Lei, D., Swindell, W.R., Xia, W., Weng, S., Fu, J., Worthen, C.A., Okubo, T., Johnston, A., Gudjonsson, J.E., Voorhees, J.J., Fisher, G.J., 2015. Age-Associated Increase in Skin Fibroblast-Derived Prostaglandin E2 Contributes to Reduced Collagen Levels in Elderly Human Skin. *Journal of Investigative Dermatology* 135, 2181–2188. doi:10.1038/jid.2015.157
- Moon, K.M., Park, Y.-H., Lee, J.S., Chae, Y.-B., Kim, M.-M., Kim, D.-S., Kim, B.-W., Nam, S.-W., Lee, J.-H., 2012. The Effect of Secretory Factors of Adipose-Derived Stem Cells on Human Keratinocytes. *International Journal of Molecular Sciences* 13, 1239–1257. doi:10.3390/ijms13011239
- Pandel, R., Poljšak, B., Godic, A., Dahmane, R., 2013. Skin Photoaging and the Role of Antioxidants in Its Prevention. *ISRN Dermatology* 2013, 1–11. doi:10.1155/2013/930164
- Schulze, C., Wetzel, F., Kueper, T., Malsen, A., Muhr, G., Jaspers, S., Blatt, T., Wittern, K.P., Wenck, H., Käs, J.A., 2012. Stiffening of human skin fibroblasts with age. *Clinics in Plastic Surgery*. doi:10.1016/j.cps.2011.09.008
- Stojiljković, D., Pavlović, D., Arsić, I., Stojiljković@bullet, D., 2014. Oxidative Stress, Skin Aging and Antioxidant Therapy. *Scientific Journal of the Faculty of Medicine in Niš* 31, 207–217. doi:10.2478/afmnai-2014-0026
- Varghese, F., Bukhari, A.B., Malhotra, R., De, A., 2014. IHC profiler: An open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. *PLoS ONE* 9. doi:10.1371/journal.pone.0096801
- Yaar, M., Gilchrest, B.A., 2007. Photoageing: Mechanism, prevention and therapy. *British Journal of Dermatology*. doi:10.1111/j.1365-2133.2007.08108.x
- Zhou, B.R., Zhang, T., Bin Jameel, A.A., Xu, Yang, Xu, Yan, Guo, S.L., Wang, Y., Permatasari, F., Luo, D., 2016. The efficacy of conditioned media of adipose-derived stem cells combined with ablative carbon dioxide fractional resurfacing for atrophic acne scars and skin rejuvenation. *Journal of Cosmetic and Laser Therapy* 18, 138–148. doi:10.3109/14764172.2015.1114638