INTRODUCTION

The largest organ in the human body, the skin, is exposed to a variety of environmental elements, including ultraviolet (UV), smoking, and pollution. Those factors together with intrinsic factor may accelerated skin aging.1-3 There are two types of skin aging, namely extrinsic aging (photoaging) and intrinsic aging (chronological aging). Chronic UV exposure is major causative factor for photoaging.4,5 It may cause reduced regenerative capacity of epidermal and dermal cells, varied epidermal thickness, disruption of dermal and epidermal junction and degeneration of collagen and elastin.6-8 UV causes reactive oxygen species (ROS), which trigger several downstream signal transduction pathways such as mitogen activated protein kinase (MAPK), nuclear factor-κB (NF-κB) and nuclear-factor-erythroid 2-related factor 2 (Nrf2).9 Activation of the MAPK pathway causes an increase in activator protein (AP-1) thereby increasing the production of matrix metalloproteinase (MMP) which will reduce collagen and cause extracellular matrix (ECM) damage.10 Under normal conditions, there is a balance between tissue metalloproteinase inhibitor (TIMP) and MMP. Exposure to UV will produce ROS which can inactivate TIMP and simultaneously MMP synthesis and activation occurs. Numerous studies demonstrate that three forms of MMP, MMP-1, MMP-3, and MMP-9, are increased by UV light on human skin in vivo.11

Previously treatments for photoaging are less satisfying so that people find better treatment to be more satisfied.12 In our study, we used secretome of adipose stem cell (ASC) as the main active ingredient of our treatment for photoaged skin. Adipose stem cell (ASC) is an adult stem cell which is easily to obtain in large quantities and relatively free from ethical issues. Studies had shown secreted factor of ASC called secretome, contains a lot of extracellular proteins, cytokines and growth factor (GF). Previous study had shown that secretome of ASC had capability to reduce collagen degradation in wound healing.3,4 We investigated the effect of secretome of ASC on photoaged rats skin. The result of this study was expected to be the basic research of the next study about topical secretome of ASC on improvement of photoaging in human subjects.

MATERIAL AND METHODS

Animals
Male Wistar rats (Rattus norwegicus), 10–12 weeks old, weighing an average of 150–200 grams, served as the research subjects for this study, which was conducted in a two-week acclimation period at the Laboratory of the Institute of Tropical Disease at Airlangga University.
Our result showed that epidermal and dermal thickness in UVB irradiated rats were significantly thinner than non-UV irradiated rats. The haematoxylin and eosin-stained skin images of each group are shown in Fig. 1. Our result showed that epidermal thickness in UV irradiated group with topical secretome of ASC (Group P1) was significantly thinner than UV irradiated with topical vehicle alone group (Group P2) and non-UV irradiated with topical secretome of ASC group (Group P3) (p<0.05).

The dermal thickness in UV irradiated group with topical secretome of ASC (Group P1) was significantly thinner than UV irradiated group with topical vehicle alone (Group P2) and non-UV irradiated and no treatment group (Group P0) by 18.84% and 23.17%, respectively (p<0.05). The dermal thickness in non-UV irradiated with topical secretome of ASC group (Group P3) was thicker than non-UV irradiated and no treatment group (Group P0), but it was not significant (p>0.05). These results demonstrate that topical treatment with secretome of ASC, could attenuate the UV-induced dermal thinning and also epidermal thickening in photaged rats.

Effect of topical secretome of ASC on MMP-1 and TIMP-1 expression in UVB irradiated rats

Data analysis using one-sample Shapiro–Wilk test showed that MMP-1 and TIMP-1 expression data were not normally distributed (p>0.05). Our study showed in Fig. 2 that MMP-1 expression in UV irradiated group with topical secretome of ASC (Group P1) was higher than UV irradiated group with topical vehicle alone (Group P2), non-UV irradiated with topical secretome of ASC (Group P3) non-UV irradiated and no treatment (Group P0) but it was not significant difference.

Our study showed in Fig. 3 that TIMP-1 expression UV irradiated group with topical secretome of ASC (Group P1) was significantly higher than UV irradiated group with topical vehicle alone (Group P2), non-UV irradiated with topical secretome of ASC (Group P3) and non-UV irradiated and no treatment (Group P0) (p<0.05).
**Effect of topical secretome of ASC on dermal collagen density in UVB irradiated rats**

The dermal collagen density measurement, used Masson Trichome staining, was measured after 4 weeks treatment with secretome of ASC. The highest value for dermal collagen density was found in UV irradiated with topical secretome of ASC group (Group P1), while the lowest value was found in non-UV irradiated with topical secretome of ASC (Group P3). Dermal collagen density in UV irradiated group with topical secretome of ASC (Group P1) was significantly higher than UV irradiated group with topical vehicle alone (Group P2) and non-UV irradiated with topical secretome of ASC (Group P3) (p<0.05).

**DISCUSSION**

Intrinsic and extrinsic aging are aging processes which occur simultaneously. Extrinsic aging or photoaging is mediated mainly by UV irradiation and more frequent than skin cancer. It is characterized by fine or deep wrinkles, dryness, roughness, skin atrophy, telangiectasia, and also dyspigmentation. Increased stratum compaction, thickened granular cell layer in the epidermis, and thinning epidermis are the histologic characteristics of photoaged skin, which causes the skin to become dry and rough.\(^4,13,14\) Mechanism of photoaging is activation of cell surface receptors which can activate intracellular signaling and synthesis of transcription factors like activation protein-1 (AP-1). This protein will inhibit collagen synthesis also increase collagen degradation.\(^13,14\) Comparing photoaged skin to skin that has been protected from the sun, there is a statistically significant drop in total collagen of 20%.\(^15\) Study of Amirthalingam et al reported that animals in UVB-irradiated group showed an increase in epidermal and dermal thickness by 14.25±1.19 pixels, 99.21±8.73 pixels, respectively.\(^16\)

Secretome of ASC can be used to repair damaged skin such as reduce wrinkle, improve skin barrier, and augment skin thickness.\(^16\) In vitro study by Moon and colleagues (2012) and also Kim and colleagues (2020) reported an increase of production of extracellular matrix.
(ECM) including collagen. Secretome of ASC produced in a hypoxia condition had great benefit for skin especially in dermal regeneration. This study also found that secretome could increase human keratinocyte proliferation and also affect expression of 290 regulated genes which contributed to cell proliferation and cell cycle. Our study showed that secretome of ASC could significantly improve epidermal and dermal thickness in UV irradiated group (p<0.05). Similar with our result, Amirthalingam and colleagues (2019) reported reduced value of epidermal thickness after using secretome of bone marrow stem cell. Secretome of ASC increases regenerative capacity of epidermal and dermal cells by increasing keratinocytes and fibroblasts proliferation.

The UV signal acts directly on the fibroblasts and signals from the damaged keratinocytes, causing the release of MMP-1, which degrades collagen. Other MMPs that are induced and activated rapidly by UV radiation, namely MMP-3, and MMP-9, are strongly regulated by the AP-1 transcription factor. The combined activation of these three MMPs can degrade most of the dermal ECM proteins. An increase in MMP levels is not accompanied by an increase in TIMP. This imbalance causes progressive collagen fragmentation in the dermis, thereby accelerating skin aging. An increase in expression of TIMP-1 will prevent collagen and ECM degradation by suppress the production of tumour necrosis factor (TNF)-α. Yokose and colleagues found that TIMP-1 expression decreased in both intrinsic and extrinsic aging. Our study showed that topical secretome of ASC increased MMP-1 expression, but it was not significant difference (p>0.05) and increased TIMP-1 expression (p<0.05) in UV irradiated group with topical secretome of ASC (Group P1). Ultraviolet irradiation will induce ROS synthesis which will activate MMP transcription and synthesis. From those events, it will increase the collagen degradation. Secretome of ASC will balance this condition with increasing of TIMP-1 which also can reduce the collagen degradation.

The balance of MMP-1 and TIMP-1 will reduce degradation of ECM include
collagen so that the dermal collagen density will increase. Our study showed that dermal collagen density in UV irradiated with topical secretome of ASC group (Group P1) was significantly higher than other groups (p<0.05). Dermal collagen density of UV irradiated group with topical secretome of ASC group (Group P1) was significantly higher than topical vehicle alone (Group P2) and non-UV irradiated with topical secretome of ASC (Group P3) (p<0.05). Similar with our study, Pricillia and colleagues reported that collagen density in secretome of ASC group was significantly increased and denser than control group after injection of secretome of ASC (p<0.05) in aging skin. Similar with ours, their secretome was made in hypoxia condition.

CONCLUSION

We showed that four weeks of treatment of the secretome of ASC improved photoaged skin via a paracrine mechanism by increasing epidermal and dermal layer thickness, MMP-1 and TIMP-1 expression, as well as dermal collagen density. This secretome has potential effect to improve photoaged skin. Therefore, further research is needed with different study designs and larger samples to determine other factors that influence the effect of secretome of adipose stem cells (ASC) in photoaging skin.

AUTHOR CONTRIBUTION

All authors contributed to this study’s conception and design, data analysis and interpretation, article drafting, critical revision of the article, final approval of the article, and data collection.

FUNDING

The authors are responsible for all of the study funding without a grant or any external funding source.

CONFLICT OF INTEREST

There is no conflict of interest for this manuscript.

ETHICAL CONSIDERATION

This study has been declared ethical by the Ethical Commission for Health Research of Universitas Airlangga.

REFERENCES


Figure 7. Effect of topical secretome of ASC on dermal collagen density in UVB irradiated rats.