Biomedical & Pharmacology Journal, March 2025. Vol. **18**(1), p. 713-720

The Combination of Mesenchymal Stem Cell (MSC) Secretome and Vitamin C has a Promising Effect in Treating Skin Aging, as it is Not Significantly Different From Secretome or Vitamin C Alone, Compared to the Untreated Group in a Rat Model of Skin Aging

Komang Ardi Wahyuningsih*, Veronika Maria Sidharta, Ecie Budiyanti and Retnaningtyas Siska Dianty

Department of Histology, School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia, South Jakarta, Jakarta, Indonesia.
*Corresponding Author E-mail: komang.wahyuningsih@atmajaya.ac.id

https://dx.doi.org/10.13005/bpj/3122

(Received: 16 December 2024; accepted: 11 March 2025)

Skin aging occurs due to gene mutation or hormonal factors called intrinsic factors. Mechanisms of glycation, free radicals, and other cellular and molecular mechanisms can cause dermal atrophy and decreased collagen. The secretome of stem cells, which consists of many growth factors and ascorbic acid, can stimulate cell proliferation and increase the production of intracellular matrixes. This property is well-known for its antioxidant activity in eradicating the accumulation of free radicals contributing to skin aging. This study will observe the role of secretome and ascorbic acid combination in increasing the antioxidant levels and type 1 collagen production in the intrinsic skin aging rat model. Thirty male Sprague Dawley rats were divided into five groups consisting of the nonaging control group; the intrinsic aging control group, which was injected with 1000 mg/kg BW of 15% D-Galactose; three intrinsic aging groups treated with secretome only, ascorbic acid only, or a combination of both. After 4 weeks of treatment, all skin tissue was collected and divided to examine dermal thickness, Super Oxide Dismutase (SOD), dan type 1 collagen using Enzyme-Linked Immunosorbent Assay (ELISA). Data were analyzed statistically. All treatment groups show a significant difference compared to the control group across all parameters. An enhancement with secretome-only injection was observed in all examinations, showing a significant difference ($p<0.05$) compared to the intrinsic aging in dermal thickness and also non-aging control groups for SOD and type 1 collagen concentration parameters. Both secretome and ascorbic acid or one another can be used for skin aging treatment. Even though the secretome only gave better results, this combination's dose, application method, optimization, and time need further study.

Keywords: Antioxidant; Ascorbic acid; Proliferation; Secretome; Skin aging; Stem cell.

Skin is a vital organ that serves as a barrier from the outside and is involved in the synthesis, production, and secretion of biomolecules such as lipids, proteins, and glucans, as well as the production and secretion of hormones.[1] Aging is a complex biological process characterized by changes in the structure and physiological function of the skin. Factors contributing to this process, intrinsic factors, can come from within. These include a decrease in hormone production and the formation of free radicals due to aging.[2] The signs of skin aging can include changes in pigmentation, wrinkles, and loss of elasticity. Moreover, while recent therapies to address the signs of skin aging

are available, they still need more satisfactory outcomes.[3]

Mesenchymal stem cells (MSC) have been used in various medical therapies due to their characteristics, such as being easy to isolate, expand, and culture in vitro and their ability to differentiate into several types of cells.[4,5] Mesenchymal stem cells can be isolated from various adult tissues such as umbilical cord tissue (especially from Wharton's jelly), adipose tissue, and bone marrow. Mesenchymal stem cells (MSCs) derived from Wharton's jelly are easily obtainable, pose no harm to donors, involve no ethical controversies, and have low contamination rates.[6,7] The proliferation ability of Wharton's jelly-derived MSCs is superior to that of bone marrow, making it one of the best sources for clinical tissue regeneration.[8]

The primary mechanism of stem cell therapy is the secretion of secretome products, which have been chosen for cell-free therapy in regenerative medicine.[10] The secretome of MSCs can stimulate angiogenesis, induce collagen and fibronectin production, and thus serve as a potential modality for skin aging therapy.[11] The secretome consists of VEGF, PDGF, IGF, HGF, b-FGF, SDF-1, TGF-â and GDF11, which stimulate angiogenesis through the differentiation of stem cell and fibroblast into endothelial cells, promoting collagen synthesis.[10,11]

It has been reported that the secretome of MSCs can inhibit melanin synthesis in vitro. However, research on molecular mechanisms and clinical studies has yet to be extensively reported.[12] Ascorbic acid, or vitamin C, is a water-soluble antioxidant that stimulates collagen production and has an anti-aging effect. Ascorbic acid is capable of inhibiting tyrosinase activity and can help address pigmentation issues in the skin.[13]

The utilization of secretome offers many advantages, including a reduced risk of immune incompatibility, tumorigenicity, embolism, and the transmission of infection. Furthermore, the safety evaluation, dosage determination, and therapeutic potential of secretome products can be assessed similarly to other drugs, making them more economical and practical for tailoring specific therapy needs.[14] Combination therapy could be expected to yield a better effect and provide an efficient preparation of products.

MATERIALS AND METHODS

Research Design

The experimental research followed a Completely Randomized Design (CRD) using male Sprague-Dawley rats from the Food and Drug Surveillance Centre Ministry of Health Indonesia (Balai Pengawasan Obat dan Makanan Kementerian Kesehatan Republik Indonesia), Jakarta, Indonesia, for animal treatment. Forty rats, aged 2 months, weighing 200-250 g[15] on average, were housed at a temperature of 25°C under a 12-hour light/dark cycle with access to food and water *ad libitum*. The rats were acclimatized for 1-2 weeks and treated with care.

Animals and treatment

All rats were randomized into experimental groups by assigning them to a draw. The Spraque-Dawley rats were divided into five groups (n=8 per group) as follows: 1. NAC (Non-Aging Control): non-aging skin model, injected intradermally with 2 ml PBS (Gibco); 2. IAC (Intrinsic-Aging Control): intrinsic aging model, injected intradermally with 2 ml PBS; 3. ISC (Intrinsic Secretome + Ascorbic Acid): intrinsic aging model, injected intradermally with secretome + ascorbic acid (Mesoesthethic) @ 1 ml; 4. IC (Intrinsic Ascorbic Acid): intrinsic aging model, injected intradermally with 2 ml ascorbic acid; 5. IS (Intrinsic Secretome): intrinsic aging model, injected intradermally with 2 ml secretome. The secretome used in this study was obtained from the Stem Cell and Cancer Institute (SCI) and administered using an injector.

All treatments for each group were conducted once a week for four weeks, following the establishment after the rat aging model was established. The intrinsic factor for the rat aging model was administered as a subcutaneous injection of 1000 mg/kgBW D-Galactose (Sigma) every day for 8 weeks. In the end, all rats were euthanized with ketamine (KET-A-100 Agrovet) at a dose of 100 mg/kgBW and xylazine (Xyla Holland) at 10 mg/kgBW. Researchers ensured they understood the treatment codes and maintained correspondence between groups before the end of the study.

After all treatment and termination of rats, the dorsal skin of each rat around the injection site was collected in a 1x1 cm sample for every group, which was then divided into

tissue samples for ELISA and for staining with H&E (in 4% formaldehyde). This study was conducted following all international guidelines and regulations.

Measurement of Dermal Thickness

Before tissue processing, all collected skin tissues were fixed in a 4% paraformaldehyde. After gradual dehydration and cleaning with ethanol and xylene, the tissues were embedded in paraffin wax, cut into 6-micron thickness, mounted on slides, and stained with H&E. The tissue slides were observed under a binocular microscope.

The dermal thickness was measured using a microscope camera (Olympus) at 40x magnification, and the images captured were analyzed using Image J software. The dermis layer was measured from the top of the dermal papilla to the adipose layer and blood vessels. Measurements were conducted from the three randomly selected views for each slide, and the average result was obtained.

Measurement of SOD Concentration

Skin aging occurs due to the accumulation of free radicals, which become reactive oxygen species (ROS). One of the enzymes that eradicate free radicals is SOD, which is found at lower levels in aging skin. Moreover, the level of SOD is vital to observe after treatment with secretome and/or ascorbic acid.

Skin tissue that has been collected must be washed first in a mixture of cold PBS 1X (pH 7.4) and protease inhibitor to remove blood residual. After that, the skin sample is cut into pieces, mixed in PBS 1X (pH 7.4), and homogenized using a glass homogenizer on ice. The suspension of skin tissue is sonicated with an ultrasonic cell disrupter to break down the cells. Next, the homogenate is centrifuged for 5 minutes at 5000 g, and the supernatant is collected. The total concentration of SOD from each sample in the supernatant was measured according to the instruction from the Rat SOD ELISA kit (MyBioSource). Each sample was measured in duplicates.

Measurement of Collagen-type-1 Concentration

The alteration of skin elasticity is one sign of aged skin. Collagen fibers compose the matrix of the dermis layer, which is built on collagen proteins. The secretomes of MSCs have mechanisms for angiogenesis and induce collagen and fibronectin production, as does ascorbic acid, contributing to the pathological development of skin aging.[11,13] Moreover, the level of collagen-type-1 protein is essential to observe after treatment with secretome and/or ascorbic acid.

Skin tissue that has been collected must be washed first in a mixture of cold PBS 1X pH 7.4 and protease inhibitor to remove blood residual. After that, the skin sample must be cut into pieces, mixed in PBS 1X pH 7.4, and homogenized by glass homogenizer on ice. The suspension of skin tissue is then sonicated with an ultrasonic cell disrupter to break down the cells. Next, the homogenate is centrifuged for 5 minutes at 5000 g speed, and the supernatant is collected. The total concentration of collagen-type-1 from each sample in supernatant was measured according to the instructions from the Mouse Collagen Type 1 (COL1) ELISA kit (Mybiosource). Each sample was measured in duplicates.

Statistical Analysis

The resulting data were checked for normal distribution and homogeneity with Shapiro-Wilk and Levene tests. If the data were normally distributed and homogenous, a one-way ANOVA followed by Bonferroni post hoc analysis was used to compare each group. If data were not normally distributed and homogenous, Kruskal-Wallis's and Mann-Whitney tests were used for group comparison. A p-value of < 0.05 indicated statistical significance.

RESULTS

The treatment effect on dermis thickness

The dermal thickness of skin tissue samples is shown in Figures 1. There was an increase in dermal thickness after administering secretome and/or ascorbic acid. ANOVA results showed that all secretome and/or ascorbic acid treatment groups significantly increased dermal layer thickness compared to the control aging group ($p < 0.05$), especially in normal group. However, figure 1 shows that the dermal layer of aging skin had appear more collagen fibers and became thicker after treatments, especially in the IS group (1D), compared to others. The thickness of the dermis layer made skin more elastic and helped prevent wrinkles, although it did not fully restore it to normal skin yet.

The collagen-type-1 concentration in skin tissue samples from each group is shown in Figure 2A. There was an increase in collagen-type-1 concentration after administering secretome and/or ascorbic acid compared to the control groups. ANOVA results showed that all secretome and/

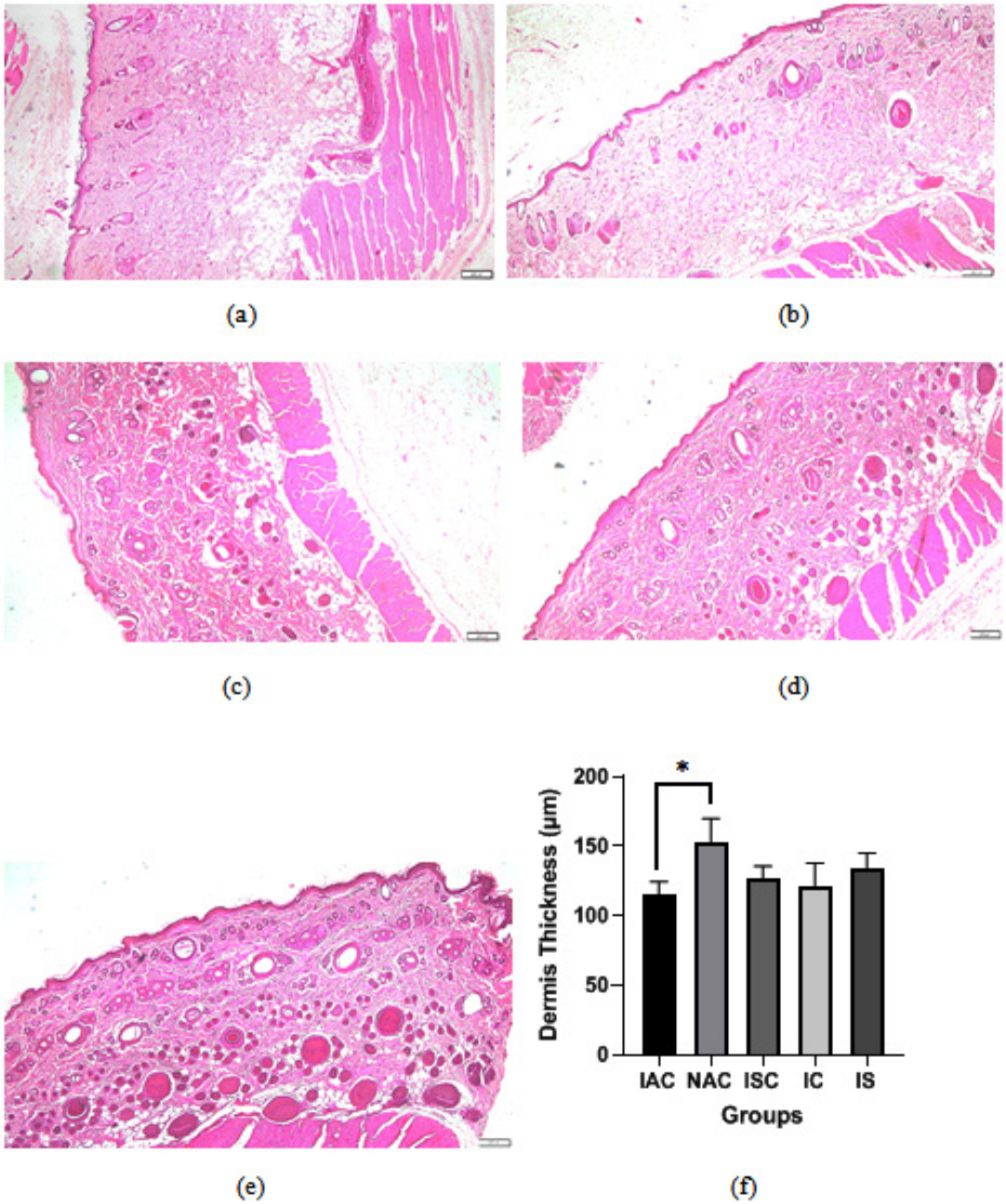

Fig. 1. Histological appearance of skin tissue from D-gal induced aging model of rats. Representative images of sections stained with H&E were presented at 40x magnification; scale bar = 200µm. (a) Intrinsic aging control group; (b) non-aging control group; (c) Intrinsic aging group had injection of secretome and ascorbic acid combined; (d) Intrinsic aging group had injection of secretome; (e) Intrinsic aging group had injection of ascorbic acid; (f) Different Dermal Thickness among experiment group;
*: p value was less than 0.05 compared to IAC and NAC

or ascorbic acid treatment groups significantly increased collagen-type-1 concentration compared to the control groups ($p<0.05$; $p<0.01$; $p<0.001$; $p<0.0001$). Post hoc Bonferroni analysis of collagen-type-1 concentration showed that the IS group had a significantly higher increase than the controls and other treatment groups.

The treatments effect on SOD concentration

The SOD concentrations in skin tissue samples are shown in Figure 2B. There was an increase in SOD concentration after the administration of secretome and/or ascorbic acid compared to the control groups. ANOVA results showed that all secretome and/or ascorbic acid treatment groups significantly increased SOD concentration compared to the control groups ($p<0.05$; $p<0.01$; $p<0.001$; $p>0.0001$). Post hoc Bonferroni analysis of SOD concentration showed that the IS group had a significantly higher increase compared to controls and other treatment groups.

DISCUSSION

The secretome, as a bioactive molecule of MSC, is known to contain several growth factors, cytokines, and extracellular vesicles that modulate the formation of new skin.[16] The ability of secretome to regenerate and repair damaged tissue involves various physiological prosses, including signal transduction to provide biological responses.[17] This makes secretome one of the alternatives for therapeutic approaches in treating various skin conditions, especially skin aging. Age-related skin changes are inevitable and include thinning, wrinkling, loss of elasticity, and reduced synthesis of collagen type I.[18] This study found that the treatment of secretome in the IS group (Figure 2B) is the most promising approach to regenerating skin by enhancing collagen type 1 formation. The histological feature remained thicker compared to the skin aging rat model (Figure 1). The histological

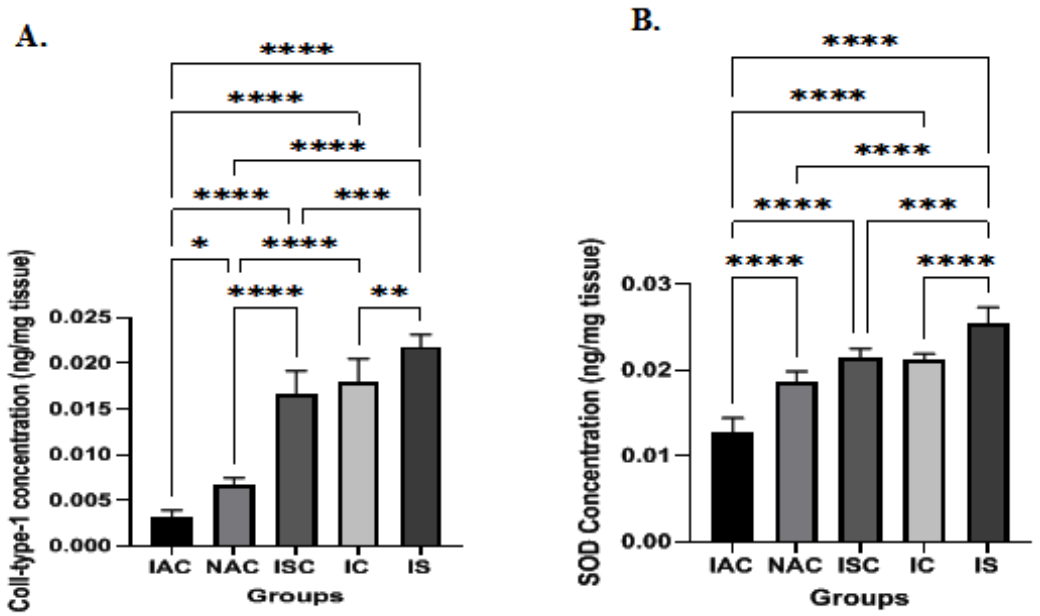

Fig. 2. Measurement of an injection secretome and or ascorbic acid to intrinsic aging skin. (A Different Collagen Type 1 concentration among experiment group.); (B) Different SOD concentration among experiment group IAC: intrinsic aging control group; NAC: non-aging control group; IC: intrinsic aging group had ascorbic acid injection; ISC: intrinsic aging group had injection of ascorbic acid and secretome combined; IS: intrinsic aging group had secretome injection

*: p value was less than 0.05 compared to IAC and NAC; **: p value was less than 0.01 among groups; ***: p value was less than 0.001 among groups; ***: p value was less than 0.0001 among groups. Test was done using ANOVA one way, with Bonferroni post-hoc test.

changes in the d-galactose-induced skin aging rat model showed a thinner epidermis, a reduced cell layer, and broken collagen fibers in the dermal layer.[19] The increased dermal thickness in the IS group (Figure 2A) suggests that secretome becomes a potent therapy for restoring the elasticity of aging skin.

The ISC group exhibited lower dermal thickness than the IS group, reflecting the effect of the different half-dose therapy. Although ascorbic acid supplementation could optimize the secretome's ability,[20] this treatment was applied in a half-dose regimen, which was not superior to the IS group alone. However, the dermal thickness in the ISC and IC groups increased compared to the aging skin group (Figure 2A). Histologically, the dermal layer appeared thicker in the ISC and IS groups, but the collagen fiber (collagen type I) concentration showed the opposite trend (Figure 2B). This discrepancy may be attributed to the half-dose therapy in the ISC group, which resulted in less collagen fiber production than the IS and IC groups, which received full-dose therapy. The total protein content in the secretome from stem cells used for treating leprosy ulcers was 5,05 mg/ml, and the ulcers showed better improvement than those of other study groups.[21] At this point, the secretome used by the experiment was less than 1 mg, and it appeared that the production of collagen fibers was not maximum.

Ascorbic acid is known to regulate collagen expression and appears to enhance collagen biosynthesis. An *in vivo* study reported that ascorbic acid simultaneously induced type I and type III collagen synthesis.[22] Even though *in vitro* studies have shown that ascorbic acid-treated stem cells increase the production of growth factors and contribute to extracellular matrix repair[23], the optimal dose for *in vivo* and clinical studies still needs further investigation.

Production of ROS in cells causes an increase in oxidative stress that consequently leads to various problems, such as accelerated skin aging.[24] The d-galactose-induced skin aging rat model was used in this study, where d-galactose reacts with free amines of amino acids through non-enzymatic glycation, forming advanced glycation end products (AGE). This reaction generates ROS as a side-product. This reaction generates ROS as a byproduct.[25] Superoxide dismutase (SOD) functions as an antioxidant enzyme that scavenges oxygen radicals through the unbalanced of superoxide free radicals both inside and outside cells.[26] A study on skin tissues from aging mice and humans found that the expression levels of SOD3 were significantly lower in aged skin compared to young skin.[27] In this study, the IS group showed an increased SOD level compared to the aging skin rat model, demonstrating better protection of skin tissue from free radicals than the non-aging group (Figure 3C). The antioxidant activity of the secretome has been reported to be associated with its cytokines.[28] Another study found that when secretome was incubated with human skin fibroblasts under oxidative conditions, it helped balance antioxidant and oxidant levels, including nuclear factor erythroid-2 related factor (Nrf2) and hypoxia-inducible factor (HIF).[29]

Ascorbic acid, also known as an antioxidant[30,] has been shown in *in vitro* studies to reduce ROS production when used in stem cell cultures.[31] In this study, the IC group exhibited an increased SOD concentration level, although the addition of secretome resulted in better outcomes in overcoming ROS. The d-galactose-induced skin aging rat model was used to stimulate ROS production, and the addition of ascorbic acid to the stem cell culture could reduce the expression of p16 and SA-â-gal, markers of cellular senescence.[32]

CONCLUSION

From the experiment on the skin aging rat model, we found that both secretome and ascorbic acid, either alone or in combination, can be used for skin aging treatment. However, while ascorbic acid is already known as an effective agent for treating skin aging, its potential toxicity to cells and stability remains uncertain. On the other hand, MSC secretome contains various bioactive molecules, depending on factors such as the source, culture media, and preconditioning agents, leading to variations in its effectiveness for skin aging treatment. The combined use of both agents needs further exploration, including optimization of the dosage, application method, treatment frequency, and the potential role of ascorbic acid supplementation in MSC culture media to enhance the secretome's effectiveness against skin aging.

ACKNOWLEDGEMENT

We thank the Animal Laboratory Unit and Histology, Faculty of Medicine, Atma Jaya Catholic University of Indonesia for providing facilities to perform animal care, experimental treatment, and data collecting.

Funding Sources

This study was supported by Institute for Research and Community Outreach from Faculty of Medicine Atma Jaya Catholic University of Indonesia for supporting and funding this research. Grant number is 0699/III/LPPM-PM.10.01/05/2021

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The experimental protocols that used in this study comply with international guidelines for humane animal treatment and were checked by the Ethics Committee on the Use of Animals of Faculty of Medicine Atma Jaya Catholic University of Indonesia number 07/09/KEP-FKIKUAJ/2021.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not Applicable

Author Contributions

Conception, methodology and writing: Komang Ardi Wahyuningsih, Veronika Maria Sidharta; Acquisition of data: Komang Ardi Wahyuningsih; Analysis and/or interpretation of data: Komang Ardi Wahyuningsih, Retnaningtyas Siska Dianty; Drafting manuscript, review/ revise, and editing: Komang Ardi Wahyuningsih, Retnaningtyas Siska Dianty; Supervision, Funding acquisition, Resources: Veronika Maria Sidharta, Ecie Budiyanti. All authors have read and approved the version of the manuscript to be published.

REFERENCES

1. Gaur M, Dobke M, Lunyak V. V. Mesenchymal stem cells from adipose tissue in clinical applications for dermatological indications and skin aging. *Int J Mol Sci*. 2017;18(208)1-29.
2. Zhang S, Dong Z, Peng Z, Lu F. Anti-Aging Effect of Adipose-Derived Stem Cells in a Mouse Model of Skin Aging Induced by D-Galactose. *Plus one*. 2014;9(5):1-7.
3. Lee H. J., Lee E. G., Sangjin K, Sung J. H., Chung H. M., Kim D. H. Efficacy of microneedling plus human stem cell conditioned medium for skin rejuvenation: A randomized, controlled, blinded split-face study. *Ann Dermatol*. 2014;26(5):584-591.
4. Choudhery M. S., Mahmood R, Harris D. T., Ahmad F. J. Minimum criteria for defining induced mesenchymal stem cells. *Cell Biol Int*. 2022;46(6):986-989.
5. Son W. C., Yun J. W., Kim B. H. Adipose-derived mesenchymal stem cells reduce MMP 1 expression in UV-irradiated human dermal fibroblasts: therapeutic potential in skin wrinkling. *Biosci, Biotech, and Biochem*. 2015;79(6):919-925.
6. Lafolla M. A. J., Tay J, Allan D. S. Transplantation of umbilical cord blood-derived cells for novel indications in regenerative therapy or immune modulation: A scoping review of clinical studies. *Biol Blood Marrow Transplant*. 2014;20:20-25
7. Xie Q, Liu R, Jiang J, Peng J, Yang C, Zhang W, Wang S, Song J. What is the impact of human umbilical cord mesenchymal stem cell transplantation on clinical treatment?. *Stem Cell Res Ther*. 2020;11:519.
8. Li X, Bai J, Ji X, Li R, Xuan Y, Wang Y. Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. *Int J Mol Med*. 2014;34(3):695–704.
9. Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, Hua D, Shao C, Shi Y. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. *Sig Transduct Target Ther*. 2022;**7**:92.
10. Mazini L, Rochette L, Admou B, Amal S, Malka G. Hopes and limits of adipose derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) in wound healing. *Int J Mol Sci*. 2020; 21(1306):1-19.
11. Suzdaltseva Y, Zhidkih S, Kiselev S. L., Stupin V. Locally delivered umbilical cord mesenchymal stromal cells reduce chronic inflammation in long-term nonhealing wounds: A randomized

study. Stem Cells Int.. 2020; 2020:1-11.
12. Kim E. S., Jeon H. B., Lim H, Shin J. H., Park S. J., Jo Y. K., Oh W, Yang Y. S., Cho D. H., Kim J. Y. Conditioned media from human umbilical cord blood-derived mesenchymal stem cells inhibits melanogenesis by promoting proteasomal degradation of MITF. *Plos one*. 2015;10(5):1-11.
13. Miao F, Su M. Y., Jiang S, Luo L. F., Shi Y, Lei T. C. Intramelanocytic acidification plays a role in the antimelanogenic and antioxidative properties of vitamin C and its derivatives. *Oxid Med Cell Longev*. 2019;2019:1-14.
14. Vizoso F. J., Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. *Int J Mol Sci*. 2017;18(9):1852.
15. Syofyan S, Almahdy A, Wulandari A. Alen Y, Diliarosta S, Kurniawan H, Noverial N, Putra P. P., Dillasamola D. Effect of Ethanol Extract of Sungkai (Peronema canescens Jack.) on Fertility of Female Wistar Mice (Mus musculus L.). *Trop J Nat Prod Res*. 2023;7(5):2863-2866.
16. Sagaradze G, Grigorieva O, Nimiritsky P. Basalova N, Klinina N, Akopyan Z. Efimenko A. Conditioned medium from human mesenchymal stromal cells: towards the clinical translation. *Int J Mol Sci*. 2019;20(7):1656.
17. Wei W, Riley N. M., Yang A. C., Kim J. T., Terrell S. M., Li V. L., Contreras-Garcia M, Bertozzi C. R., Long J. Z. Cell type-selective secretome profiling in vivo. *Nat Chem Biol*. 2021;17(3):326–334.
18. Damayanti R. H., Rusdiana T, Wathoni N. Mesenchymal stem cell secretome for dermatology application: a review. *Clin Cosmet Investig Dermatol*. 2021;14: 401-1412.
19. Fan Y, Ru W, Su N, Zheng H, Liu J, Zhao H, Liao F. Effects of dietary intake of *Colla corii asini* on skin parameters and appearance: Results of a 12-week randomized, controlled, open-labelled trial. *Pharmaco Res -Modern Chinese Med*. 2022;2:1000065.
20. Wahyuningsih K. A., Karina K, Rosadi I, Rosliana I, Subroto W. R. Effect of ascorbic acid on morphology of post-thawed human adipose-derived stem cells. *Stem Cell Investig*. 2020;7:16.
21. Prakoeswa C. R. S., Natallya F. R., Harnindya D, Thohiroh A, Oktaviyanti R. N., Pratiwi K. D., Rubianti M. A., Yogatri B, Primasari P. I., Herwanto N, Alinda M. D., Kusumaputra B. H., Astari L, Listiawan M.Y., Agusni I, Rantam F. A. The efficacy of topical human amniotic membrane-mesenchymal stem cell-conditioned medium (hMSC-CM) and a mixture of topical hAMMSC-CM + vitamin C and hAMMSC-CM + vitamin E on chronic plantar ulcers in leprosy: A randomized control trial. *J Dermatolog Treat*. 2018;29(8):835-840.
22. Boo YC. Ascorbic Acid (Vitamin C) as a Cosmeceutical to Increase Dermal Collagen for Skin Antiaging Purposes: Emerging Combination Therapies. *Antioxidants*. 2022;11(9):1663.
23. Marconi G. D., Fonticoli L., Guarnieri S., Cavalcanti M. F. X. B., Franchi S, Gatta V, Trubiani O. Pizzicannella J. Diomede F. Ascorbic acid: a new player of epigenetic regulation in LPS-gingivalis treated human periodontal ligament stem cells. *Oxid Med Cell Longev*. 2021;2021:6679708.
24. Papaccio F, D2 Arino A, Caputo S, Bellei B. Focus on the Contribution of Oxidative Stress in Skin Aging. *Antioxidants*. 2022;11(6):1121.
25. Han H., Liu Z., Yin J., Gao J., He L., Wang C, Hou R, He X, Wang G, Li T, Yin Y. D-galactose in duces chronic oxidative stress and alters gut microbiota in weaned piglets. *Front Physiol*. 2021;12:634283.
26. Tikhonov I.V., Pliss E.M., Borodin L.I., Sen V.D. Effect of superoxide dismutase on the oxidation of methyl linoleate in micelles inhibited by nitroxyl radicals. *Russ Chem Bull*. 2016;65:2985–2987.
27. Kim J.H., Jeong H.D., Song M.J., Lee D. H., Chung J. H., Lee S. T., SOD3 suppresses the expression of MMP-1 and increases the integrity of extracellular matrix in fibroblasts. *Antioxidants (Basel)*. 2022;11:928.
28. Rahimi B., Panahi M., Saraygord-Afshari N., Taheri N., Bilici M., Jafari D., Alizadeh E. The secretome of mesenchymal stem cells and oxidative stress: challenges and opportunities in cell-free regenerative medicine. *Mol Biol Rep*. 2021;48(7):5607-5619.
29. Jiang J., Wang K., Nice E. C., Zhang T., Huang C. High-throughput screening of cellular redox sensors using modern redox proteomics approaches. *Expert Rev Proteom*. 2015;12(5):543–555
30. Wahyuningsih K. A. Evaluation of Stem Cells Secretome and Their Correlation with Aging: A Review. *Teikyo Med J*. 2021;44(3):757-762.
31. Diomede F., Marconi G.D., Guarnieri S., D'Attilio M., Cavalcanti M. F. X. B., Mariggio M. A., Pizzicannella J. Trubiani O. A Novel role of ascorbic acid in anti-in flammatory pathway and ROS generation in HEMA treated dental pulp stem cells. *Materials (Basel)*. 2019; 13(1):130.
32. Yang M., Teng S., Ma C., Yu Y., Wang P., Yi C. Ascorbic acid inhibits senescence in mesenchymal stem cells through ROS and AKT/ mTOR signaling. *Cytotechnology*. 2018; 70(5): 1301-1313.