Chapter 5

Mesenchymal Stem Cells and Their Conditioned Medium: For Skin Aging

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A. Introduction

Skin is an organ that acts as a barrier to the human body and is one of the most commonly observed indicators of aging. Aging is a complex inevitably process happens to all living things. It occurs naturally but can also be accelerated by various factors both intrinsic and extrinsic. Extrinsic factors like ultraviolet radiation can cause skin damage, which results in the appearance of signs of aging or commonly referred to as photoaging. Photoaging overlaps with intrinsic aging. Some common signs of photoaging include brown spots, wrinkles, broken capillaries, decreased elasticity, and uneven skin texture. The severity of photoaging damage depends on how much the skin is protected from ultraviolet light. Various efforts can

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be made to reduce the signs of photodamage on the skin, such as the use of pharmacotherapy, both systemic and topical, and, recently, the use of stem cells. The application of mesenchymal stem cells and their conditioned medium (CM) provide promising and effective treatment to rejuvenate aged skin.

B. Mesenchymal Stem Cells (MSCs)

Since the discovery of stem cells (SCs) in the medical world, many studies have been conducted to find therapy for disease using stem cells, including in dermatology and plastic surgery, because of their ability to repair and rejuvenate various tissues (Bashir et al., 2019; Ogliari et al., 2014). Stem cells are a unique cell population of undifferentiated cells. Stem cells have three characteristics, including the ability to divide, the ability to regenerate, and plasticity (the ability to produce other types of cells, different from the cell of origin) (Hasegawa & Ikeda, 2017; Rantam et al., 2014). Stem cells can generally be found in various body tissues, from embryonic to adult. Stem cells are classified into embryonic and adult stem cells (Hasegawa & Ikeda, 2017; Ogliari et al., 2014; Prodinger et al., 2017; Rantam et al., 2014). Embryonic SCs are found in the blastocyst, while adult SCs are found in the forming fetus and play a role in repairing more specialized tissue damage. Based on their origin and ability to differentiate, adult SCs are divided into hematopoietic and mesenchymal stem cells (MSCs) (Hasegawa & Ikeda, 2017; Ogliari et al., 2014; Rantam et al., 2014).

Mesenchymal stem cells are progenitor cells of the mesoderm. The name was proposed by Arnold Caplan in 1991 for its ability to differentiate into more than one cell that forms connective tissue. Mesenchymal stem cells are identified based on the minimum criteria given by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) in 2005: (1) adherent cell plasticity with self-renewal ability; (2) expression of cluster of differentiation 90 (CD90), CD73, CD105, and CD36, and no expression of CD11b, CD45, CD31, and CD106; (3) in vitro differentiation potential into osteogenic, adipogenic, and

chondrogenic lineages (Andrzejewska et al., 2019; Fitzsimmons et al., 2018; Hasegawa & Ikeda, 2017; Hu et al., 2018; Lavoie & Rosu-Myles, 2013; Trzyna & Banaś-Ząbczyk, 2021). Mesenchymal stem cells are the most researched adult stem cells for clinical trials in various diseases. These stem cells have immunomodulatory, anti-inflammatory, low immunogenicity, and high immunosuppressive properties that can be effective as therapy in autoimmune and inflammatory diseases (atopic dermatitis, psoriasis and lupus erythematosus, vitiligo and alopecia areata) (Baldari et al., 2017; Hasegawa & Ikeda, 2017). The use of MSCs for tissue regeneration is possible due to the trophic, paracrine, and immunomodulatory properties of these SCs (Damayanti et al., 2021; Pokrovskaya et al., 2020). The ability of MSCs in terms of cell proliferation and differentiation, paracrine signaling, and tissue repair depends on the donor's age, patients with diabetes, obesity, and cardiovascular disease (Baldari et al., 2017).

There are various GFs, such as EGF, b-FGF, TGF-b, and growth differentiation factor (GDF)-11 which play roles in rejuvenation. In addition, MSCs also have antioxidant activity that can increase the levels of endogenous antioxidants, which provides protection to dermal fibroblasts and keratinocytes against oxidative stress (Bashir et al., 2019). Mesenchymal stem cells can produce angiogenesis through VEGF, while the regeneration process can be produced by GFs that have anti-apoptotic effects, including HGF, insulin-like growth factor-1 (IGF-1), VEGF, Cytokine-Induced Neutrophil Chemoattractant-3 (CINC-3), TIMP-1, TIMP-2, osteopontin, growth hormone (GH), bFGF-BP, brain-derived neurotrophic factor (BDNF), TGFα, HGF, EGF, NGF, IGF-binding proteins-1 (IGFBP-1), IGFBP-2, macrophage colony-stimulating factor (M-CSF). Growth factors from MSCs can also reduce tissue fibrosis during regeneration, such as keratinocyte growth factor (KGF), HGF, VEGF, angiopoietin-1, stromal cell-derived factor-1 (SDF-1), IGF-1, EGF, HGF, NGF, and TGFα (Andrzejewska et al., 2019; Harrell et al., 2018). Mesenchymal stem cell sources can be isolated from various tissues such as bone marrow (BM-MSCs), umbilical cord (UC-MSCs), placenta (P-MSCs), umbilical cord

blood (UCB-MSCs), amniotic fluid (AF-MSCs), and adipose (ASCs) (Andrzejewska et al., 2019; Hasegawa & Ikeda, 2017; Hu et al., 2018).

Bone marrow mesenchymal stem cells have been widely used for wound healing (Huynh et al., 2022; Karina et al., 2021; Revilla & Mulyani, 2020; Yonghong et al., 2022). These cells acts to shorten wound healing time, increase VEGF expression, and also increase the number and density of blood vessels in the wound area. (Karina et al., 2021; Yonghong et al., 2022). Epidermal growth factor and epidermal growth factor receptor (EGFR), which play a role in keratinocyte proliferation and migration in wound healing, were reported to increase by Revilla and Mulyani (2020). However, due to their disadvantages of being invasive and associated with complications during BM-MSC source collection, like infection and bleeding, BM-MSC is slowly being replaced by other SCs such as UC-MSC, UCB-MSC and other ASCs (Fitzsimmons et al., 2018; Hasegawa & Ikeda, 2017; Hu et al., 2018).

Umbilical cord-derived MSCs show promising therapeutic effects due to their immunological compatibility, long-term survival, high differentiation potential, and easy manufacturing process. In vitro and in vivo studies for wound healing showed promising results using UC-MSC (Dehkordi et al., 2019). In vivo studies show that UC-MSCs regulate SOD and MDA levels also Col-1 and VEGF expression in aging skin. An in vitro study shows UC-MSCs work by performing cell migration, inhibiting ROS production, and reducing oxidative stress expression through paracrine-mediated autophagy inhibition in aging human fibroblasts (Li et al., 2022). These SCs also increase collagen and fibroblasts in photoaging (Kencanawati et al., 2021).

Adipose stem cells are pluripotent cells that can differentiate into various cell types (Dehkordi et al., 2019; Fitzsimmons et al., 2018; Hasegawa & Ikeda, 2017; Hu et al., 2018). The collection site and type of adipose tissue may affect the proliferation, endocrine function, gene expression, surface antigens, and differentiation potential of ASCs (Trzyna & Banaś-Ząbczyk, 2021). The ASCs are easily obtained, easier to generate, more abundant than other types of MSCs, and have fewer ethical controversies than BM-MSCs (Dehkordi et al., 2019; Hu et

al., 2018). This type of SC is used as a therapy for various diseases due to its paracrine and autocrine properties and by enhancing the recruitment of endogenous precursors (Trzyna & Banaś-Ząbczyk, 2021). Adipose stem cells produce a series of GFs, such as VEGF, bFGF, TGF-β1, TGF-β2, HGF, KGF, platelet-derived growth factor AA (PDGF-AA), and placental growth factor (PGF) (Trzyna & Banaś-Ząbczyk, 2021; Zhang & Duan, 2018).

C. Mesenchymal Stem Cell-Conditioned Medium (MSC-CM)

The development of mesenchymal stem cell-conditioned medium was carried out due to the limitations of MSC in terms of low viability and mode of transport (Andrzejewska et al., 2019; Damayanti et al., 2021; Dehkordi et al., 2019; Harrell et al., 2018; Pokrovskaya et al., 2020). The advantages of MSC-CM are that it is easy to obtain, more economical, and can be produced, packaged, and transported faster (Yang et al., 2021). The primary mechanism of action of MSCs is its paracrine effects of various GFs or cytokines (Damayanti et al., 2021). There are several approaches to optimize MSC-CM, also called preconditioning, including modulation of culture conditions (hypoxia or anoxia), 3D culture, the addition of trophic factors (GFs, cytokines or hormones), lipopolysaccharides and other pharmacological agents. This preconditioning causes the number of MSCs to multiply and produces immunomodulatory or immunosuppressive, anti-apoptotic, pro-angiogenic, and trophic effects (Baldari et al., 2017; Schäfer et al., 2016; Seo et al., 2019).

Mesenchymal stem cell-conditioning medium or secretome consists of GFs, cytokines, chemokines, ECM components, proteins involved in the adhesion process, enzyme activators or inhibitors (Andrzejewska et al., 2019; Harrell et al., 2018; Pokrovskaya et al., 2020). The paracrine activity of MSCs is also associated with their ability to produce extracellular vesicles (EV), including exosomes, microvesicles, and apoptotic bodies. The biological activity of EVs is comparable to that of MSCs (Andrzejewska et al., 2019). Damayanti

et al. (2021) reviewed MSC-CM in the field of dermatology and reported that MSC-CM is used for wound healing, photoprotection, hair growth, and also as an antimicrobial in skin wounds. A systematic review by Putri, Endaryanto, Rantam, et al. (2021) reported that eleven clinical or in vivo studies using MSC-CM showed improvements in clinical outcomes with or without histological examination in skin aging.

1. Bone Marrow Stem Cell-Conditioned Medium (BMSC-CM)

Similar to its SC origin, BMSC-CM has also been widely used for wound healing (Aryan et al., 2018; de Mayo et al., 2017). Balasubramanian et al. (2017) found that most factors in BMSC-CM play a role in fibroblast proliferation (FGF-7/KGF, PDGF), angiogenesis (VEGF, HGF, IGF-1, Ang-1), cell migration (SDF-1α, M-CSF) and epithelialization (TGF-β1, FGF-7/KGF, laminin, TIMP-1, and TIMP-2), and anti-inflammation (PGE-2).

2. Amniotic Fluid Stem Cells-Conditioned Medium (AFSC-CM) and Amniotic Membrane Stem Cell-Conditioned Medium (AMSC-CM)

Previous studies indicated various amniotic fluid stem cells-conditioned medium utilizations such as reduced alzheimer disease-like pathologies in the human neuronal lineage, accelerated cutaneous wound healing, and supressed breast cancer cell (Hasanpour et al., 2022; Jun et al., 2014; Pashaei-Asl et al., 2022). Study by Yoon et al. (2010) showed that AFSC-CM contains high levels of growth factors, cytokines, and chemokines, such as TNF-α, VEGF, TGF-β, Leptin, IL8, and IL-6.

Several clinical studies using AMSC-CM have been conducted. One of them is for acne scars conducted by El-Domyati et al. (2019). They reported that there was an improvement in acne scars using micro-needling and AMSC-CM and an improvement in the shape of collagen and elastin fibers.

3. Chorion-Derived Stem Cell Conditioned Medium (CDSC-CM)

Chorion-derived stem cell has similar functions to ASCs, namely promote human fibroblast growth and secretes growth factors that affect wound healing (Kim et al., 2015). A study by Kim et al. (2015) demonstrated that CDSC secretes various growth factors, such as IL-6, IL-8, MCP-1, and regulated upon activation, normal T cell expressed and presumably secreted (RANTES) growth factors that were measured in higher concentration in CDSC-CM.

4. Umbilical Cord Stem Cell-Conditioned Medium (UCSC-CM) and Umbilical Cord Blood Stem Cell-Conditioned Medium (UCB-CM)

Study on wound healing conducted by Sunarto et al. (2020) using Wistar rats showed that topical UCSC-CM gel from rats could increase VEGF levels from day three of therapy, meanwhile a decrease in VEGF levels was reported on day nine. Collagen density assessed by Masson's trichome staining was reported to increase significantly. Studies on UCSC-CM in skin aging conducted by Liang et al. (2022) reported that the most abundant GF in UCSC-CM was EGF, followed by VEGF-A, HGF, FGF-2, VEGF-D, and PDGF-BB. In addition, a decrease in melanin index, UV spots, brown spots, wrinkles, pores, and increased skin elasticity were also obtained. Kim, Kim, Kim, et al. (2020) reported that the number of areas with micro crusts and erythema was reduced on patients' cheeks post fractional CO_2 laser after using UCSC-CM.

5. Adipose Stem Cell-Conditioned Medium (ASC-CM)

One of the strategies to optimize ASC is to use CM or secretome with a more specific form, namely EV (Trzyna & Banaś-Ząbczyk, 2021). The in vitro study by Guo et al. (2020) reported that the storage life of ASC-CM is less than 13 weeks. The study by Sun et al. (2014) created a hypoxic environment for ASCs by using an oxygen absorber, that caused the oxygen concentration to be less than 1% and the carbon

dioxide concentration to be around 20%. The hypoxic environment exposed to ASCs resulted in CMs containing increased levels of VEGF and/or HGF, as well as GF and other differentiation factors, such as TNF-α, FGF, and EGF (Bertozzi et al., 2018). Studies by Moon et al. (2012) and Son et al. (2015) found that ASC-CM contained HGF, FGF-1, GM-CSF, IL-6, VEGF, and TGF-β3. A subsequent study reported highest concentration of PDGF in ASC-CM (Dubey et al., 2018). PDGF-AA was also reported became a major component of ASC-CM. PDGF-AA, an isoform of PDGF, is bound to the PDGF-α receptor. It increases dermal fibroblast proliferation and elastin expression (Guo et al., 2020). Although not in high amounts, ASCs also secrete GDF-11, which can promote proliferation, cell differentiation, migration, and secretion of ECM, which is important for the repair of skin aging (Mazini et al., 2020).

Studies on wound healing using ASC-CM have been conducted (Alinda et al., 2022; Lee et al., 2014; Moon et al., 2012; Sun et al., 2014). The ability of ASC-CM in cell adhesion and migration plays a role in wound healing and tissue remodeling (Moon et al., 2012; Sun et al., 2014; Xu et al., 2014). These CM also increased the expression of type I collagen, angiogenesis as well as circulating SC capture. Skin graft studies from the skin of C57BL/6 mice transferred to BALB/c mice using ASC and ASC-CM injection showed a significant decrease in proinflammatory cytokines (IL-6). Both cytokine gene expression of interferon-λ (IFN-λ), IL-2, and TNF-α was also decreased (Lee et al., 2014). On histopathologic examination, Lee et al. (2014) also found fewer macrophages and decreased VEGF were seen after ASC or ASC-CM therapy.

D. Application and Risks in Skin Aging

The widely used MSCs and their conditioned medium administration method for skin aging treatment is subcutaneous injection. The use of microneedle as tool to apply MSC-CM was also reported in some studies. Various treatments utilizing stem cell-conditioned medium have resulted in improvements in aging skin. Alhaddad et al. (2019) used

BMSC-CM cream from red deer on photoaging patients and reported improvements in photoaging. An in vitro study by Balasubramanian et al. (2017) reported antiaging potential in UVB-irradiated human fibroblasts using BMSC-CM. These CM improved collagen, elastin, and hyaluronic acid levels and reduced ROS compared to the control. Another study, both in vivo and in vitro, by Amirthalingam et al. (2019) reported the formation of cyclobutene pyrimidine dimers (CPD), which cause primary lesions in UV-irradiated DNA, can be prevented by BMSC-CM. They showed that these CM extensively protected mouse skin from toxic UVB irradiation.

The in vitro study by Huh et al. (2014) used human amniotic fluid stem cells-conditioned medium on UVA-irradiated dermal keratinocytes and fibroblasts. The study reported increased proliferation of dermal keratinocytes and fibroblasts. The study also showed decreased expression of MMP-1 and increased procollagen 1A. This CM can repair cell damage caused by UVA. Parrado et al. (2019) showed that AMSC-CM increased CAT and decreased MDA while also blocking the cell cycle. This may delay premature aging due to oxidative stress.

A study by Li et al. (2016) reported that chorion-derived stem cell conditioned medium (CDSC-CM) contains EGF, TGF-β, IL-6, and IL-8. They also reported that a CDSC-CM could increase proliferation in UVB-irradiated keratinocytes. In addition, this CM increased the number of cells in S and G2/M phases and cell migration and myeloid cell leukemia-1 (MCL-1) protein and modulated the extracellular signal-regulated kinases (ERK) 1/2 signaling pathway. Decreased ROS and DNA damage were also reported in keratinocytes treated with CDSC-CM.

The in vitro study by Kim et al. (2018) using HDF showed that topical UCB-CM exhibited anti-wrinkle effects and significantly increased skin density. The study found that the anti-wrinkle effect was due to increased cell migration and the synthesis of type I and type III collagen. The increase was higher in UCB-CM than in HDF-CM and ASC-CM. They also reported that the secretion of GDF11

(rejuvenation factor) was highest in UCB-CM. Another in vitro study by Dewi and Sandra (2019) used mice fibroblasts that were given UCB-CM and then irradiated with UVB. The use of UCB-CM before UVB irradiation can significantly reduce the percentage of cell apoptosis.

Adipose stem cell conditioned medium is also used as a therapy for skin aging (Kim, Kim, Lee, et al., 2020; Lee et al., 2021). The use of ASC-CM produces a protective effect against skin damage caused by ROS in the form of increased SOD and GPx activity in dermal fibroblasts (Hong et al., 2019; Park & Kim, 2010; Widowati et al., 2022; Xu et al., 2014). This mechanism upregulates Nrf2 expression so that the expression of antioxidant enzymes such as SOD-1 also increases. The ASC-CM can increase the migration and proliferation of keratinocytes and fibroblasts by reducing the number of cells in the S and G2 phases and increasing the number of cells in the G1 phase therefore increasing dermis thickness (Moon et al., 2012; Noverina et al., 2019). Study by Xu et al. (2014) and Putri et al. (2022) reported that dermis thickness increased significantly after the use of ASC-CM. A decrease in epidermal thickness was also found (Putri et al., 2022). Adipose stem cell conditioned medium was given by Kim, Kim, Lee, et al. (2020) to middle-aged women while Putri, Endaryanto, Tinduh, et al. (2021)'s was given to photoaging model Wistar rats. They reported similar improvement in skin moisture and transepidermal water loss (TEWL). Kim, Jung, et al. (2020) also found that melanin, erythema on the cheeks, and eye wrinkles were decreased.

Currently there were no reports on MSC-CM negative effect on its application, but there was adverse effect reported by Prakoeswa et al. (2019) in the form of slight erythema and urticaria due to microneedle used to administer MSC-CM.

E. Conclusion

The use of mesenchymal stem cells and their conditioned medium as a skin aging therapy continues to attract researchers' attention. Recent studies have shown positive results about their use on skin aging.

Based on recent studies, MSCs and MSC-CM proved safe to use. The limitation faced is the absence of standardized procedures in making stem cells conditioned medium and the high cost of production. Further studies are needed on the appropriate manufacture and administration technique of MSCs so that they can provide maximum benefits for treating skin aging.

References

Alhaddad, M., Boen, M., Wu, D. C., & Goldman, M. P. (2019). Red deer umbilical cord lining mesenchymal stem cell extract cream for rejuvenation of the face. *Journal of Drugs in Dermatology: JDD*, *18*(4), 363–366. https://pubmed.ncbi.nlm.nih.gov/31012565/

Alinda, M. D., Christopher, P. M., Listiawan, M. Y., Endaryanto, A., Suroto, H., Rantam, F. A., Hendradi, E., Notobroto, H. B., & Prakoeswa, C. R. S. (2022). The efficacy of topical adipose mesenchymal stem cell-conditioned medium versus framycetin gauze dressing in chronic plantar ulcer of leprosy: A randomized controlled trial. *Indian Journal of Dermatology, Venereology and Leprology*, *89*(5), 656–664. https://doi.org/10.25259/IJDVL_784_2021

Amirthalingam, M., Bhat, S., Dighe, P. A., & Seetharam, R. N. (2019). Human mesenchymal stromal cells-derived conditioned medium based formulation for advanced skin care: In vitro and in vivo evaluation. *Journal of Stem Cells Research, Development & Therapy*, *5*, Article 012. https://doi.org/10.24966/SRDT-2060/100012

Andrzejewska, A., Lukomska, B., & Janowski, M. (2019). Concise review: Mesenchymal stem cells: From roots to boost. *Stem Cells*, *37*(7), 855–864. https://doi.org/10.1002/stem.3016

Aryan, A., Bayat, M., Bonakdar, S., Taheri, S., Haghparast, N., Bagheri, M., Piryaei, A., & Abdollahifar, M.-A. (2018). Human bone marrow mesenchymal stem cell conditioned medium promotes wound healing in deep second-degree burns in male rats. *Cells Tissues Organs*, *206*(6), 317–329. https://doi.org/10.1159/000501651

Balasubramanian, S., Thej, C., Walvekar, A., Swamynathan, P., Gupta, P. K., Seetharam, R. N., & Majumdar, A. S. (2017). Evaluation of the secretome profile and functional characteristics of human bone marrow mesenchymal stromal cells-derived conditioned medium suggest potential for skin rejuvenation. *Journal of Cosmetics, Dermatological*

Sciences and Applications, *7*(1), 99–117. https://doi.org/10.4236/jcdsa.2017.71010

Baldari, S., Di Rocco, G., Piccoli, M., Pozzobon, M., Muraca, M., & Toietta, G. (2017). Challenges and strategies for improving the regenerative effects of mesenchymal stromal cell-based therapies. *International Journal of Molecular Sciences*, *18*(10), Article 2087. https://doi.org/10.3390/ijms18102087

Bashir, B., Butt, G., & Hussain, I. (2019). Stem cells for skin rejuvenation. *Journal of Pakistan Association of Dermatologists*, *29*(2), 142–144.

Bertozzi, N., Simonacci, F., Grieco, M. P., Grignaffini, E., & Raposio, E. (2018). Adipose-derived stem cells as a novel anti-aging therapy in cosmetic surgery: A concise review. *EuroMediterranean Biomedical Journal*, *13*(10), 46–56. https://doi.org/10.3269/1970-5492.2018.13.10

Damayanti, R. H., Rusdiana, T., & Wathoni, N. (2021). Mesenchymal stem cell secretome for dermatology application: A review. *Clinical, Cosmetic and Investigational Dermatology*, *2021*(14), 1401–1412. https://doi.org/10.2147/CCID.S331044

de Mayo, T., Conget, P., Becerra-Bayona, S., Sossa, C. L., Galvis, V., & Arango-Rodríguez, M. L. (2017). The role of bone marrow mesenchymal stromal cell derivatives in skin wound healing in diabetic mice. *PLOS ONE*, *12*(6), Article e0177533. https://doi.org/10.1371/journal.pone.0177533

Dehkordi, A. N., Mirahmadi Babaheydari, F., Chehelgerdi, M., & Raeisi Dehkordi, S. (2019). Skin tissue engineering: Wound healing based on stem-cell-based therapeutic strategies. *Stem Cell Research & Therapy*, *10*, Article 111. https://doi.org/10.1186/s13287-019-1212-2

Dewi, D. A. R., & Sandra, F. (2019). Conditioned media of human umbilical cord blood mesenchymal stem cell inhibits ultraviolet b-induced apoptosis in fibroblasts. *The Indonesian Biomedical Journal*, *11*(1), 85–90. https://doi.org/10.18585/inabj.v11i1.544

Dubey, N., Mishra, V., Dubey, R., Deng, Y.-H., Tsai, F.-C., & Deng, W.-P. (2018). Revisiting the Advances in Isolation, Characterization and Secretome of Adipose-Derived Stromal/Stem Cells. *International Journal of Molecular Sciences*, *19*(8), 2200. https://doi.org/10.3390/ijms19082200

El-Domyati, M., Moftah, N. H., Nasif, G. A., Ragaie, M. H., Ibrahim, M. R., & Ameen, S. W. (2019). Amniotic fluid-derived mesenchymal stem cell products combined with microneedling for acne scars: A split-

face clinical, histological, and histometric study. *Journal of Cosmetic Dermatology*, *18*(5), 1300–1306. https://doi.org/10.1111/jocd.13039

Fitzsimmons, R. E. B., Mazurek, M. S., Soos, A., & Simmons, C. A. (2018). Mesenchymal Stromal/Stem Cells in Regenerative Medicine and Tissue Engineering. *Stem Cells International*. https://doi.org/10.1155/2018/8031718

Guo, S., Wang, T., Zhang, S., Chen, P., Cao, Z., Lian, W., Guo, J., & Kang, Y. (2020). Adipose-derived stem cell-conditioned medium protects fibroblasts at different senescent degrees from UVB irradiation damages. *Molecular and Cellular Biochemistry*, *463*(1–2), 67–78. https://doi.org/10.1007/s11010-019-03630-8

Harrell, C. R., Jankovic, M. G., Fellabaum, C., Volarevic, A., Djonov, V., Arsenijevic, A., & Volarevic, V. (2018). Molecular mechanisms responsible for anti-inflammatory and immunosuppressive effects of mesenchymal stem cell-derived factors. In P. Pham (Ed.), *Tissue engineering and regenerative medicine* (187–206). Springer. https://doi.org/10.1007/5584_2018_306

Hasanpour, M., Rahbarghazi, R., Nourazarian, A., Khaki-Khatibi, F., Avci, Ç. B., Hassanpour, M., Talebi, M., Taghavi, H., & Salimi, L. (2022). Conditioned medium from amniotic fluid mesenchymal stem cells could modulate Alzheimer's disease-like changes in human neuroblastoma cell line SY-SY5Y in a paracrine manner. *Tissue and Cell*, *76*, Article 101808. https://doi.org/10.1016/j.tice.2022.101808

Hasegawa, T., & Ikeda, S. (2017). Mesenchymal stem cells for the treatment of skin diseases. *AIMS Cell and Tissue Engineering*, *1*(2), 104–117. https://doi.org/10.3934/celltissue.2017.2.104

Hong, H.-E., Kim, O.-H., Kwak, B. J., Choi, H. J., im, K.-H., Ahn, J., & Kim, S.-J. (2019). Antioxidant action of hypoxic conditioned media from adipose-derived stem cells in the hepatic injury of expressing higher reactive oxygen species. *Annals of Surgical Treatment and Research*, *97*(4), 159–167. https://doi.org/10.4174/astr.2019.97.4.159

Hu, M. S., Borrelli, M. R., Lorenz, H. P., Longaker, M. T., & Wan, D. C. (2018). Mesenchymal stromal cells and cutaneous wound healing: A comprehensive review of the background, role, and therapeutic potential. *Stem Cells International*. https://doi.org/10.1155/2018/6901983

Huh, M.-I., Kim, M.-S., Kim, H.-K., & Lim, J. O. (2014). Effect of conditioned media collected from human amniotic fluid-derived stem cells (hAFSCs) on skin regeneration and photo-aging. *Tissue*

Engineering and Regenerative Medicine, *11*(2), 171–177. https://doi.org/10.1007/s13770-014-0412-1

Huynh, P. D., Tran, Q. X., Nguyen, S. T., Nguyen, V. Q., & Vu, N. B. (2022). Mesenchymal stem cell therapy for wound healing: An update to 2022. *Biomedical Research and Therapy*, *9*(12), 5437–5449. https://doi.org/10.15419/bmrat.v9i12.782

Jun, E., Zhang, Q., Yoon, B., Moon, J.-H., Lee, G., Park, G., Kang, P., Lee, J., Kim, A., & You, S. (2014). Hypoxic conditioned medium from human amniotic fluid-derived mesenchymal stem cells accelerates skin wound healing through TGF-β/SMAD2 and PI3K/Akt pathways. *International Journal of Molecular Sciences*, *15*(1), 605–628. https://doi.org/10.3390/ijms15010605

Karina, K., Biben, J. A., Ekaputri, K., Rosadi, I., Rosliana, I., Afini, I., Widyastuti, T., Sobariah, S., & Subroto, W. R. (2021). In vivo study of wound healing processes in Sprague-Dawley model using human mesenchymal stem cells and platelet-rich plasma. *Biomedical Research and Therapy*, *8*(4), 4315–4324. https://doi.org/10.15419/bmrat.v8i4.670

Kencanawati, A. C., Pangkahila, W., & Wiraguna, A. A. G. P. (2021). Intradermal injection of umbilical cord mesenchymal stem cell was more effective than platelet rich plasma in increasing amount of collagen and fibroblasts in Wistar rats (*Rattus norvegicus*) back skin exposed to ultraviolet B rays. *Neurologico Spinale Medico Chirurgico*: *Multidisciplinary Journal of Spine Surgery and Medical Sciences*, *4*(1), 42–46. https://doi.org/10.36444/nsmc.v4i1.152

Kim, H. J., Jung, M. S., Hur, Y. K., & Jung, A. H. (2020). A study on clinical effectiveness of cosmetics containing human stem cell conditioned media. *Biomedical Dermatology*, *4*, Article 9. https://doi.org/10.1186/s41702-020-0056-9

Kim, J., Kim, B., Kim, S., Lee, Y. I., Kim, J., & Lee, J. H. (2020). The effect of human umbilical cord blood–derived mesenchymal stem cell media containing serum on recovery after laser treatment: A double-blinded, randomized, split-face controlled study. *Journal of Cosmetic Dermatology*, *19*(3), 651–656. https://doi.org/10.1111/jocd.13063

Kim, K. H., Kim, Y.-S., Lee, S., & An, S. (2020). The effect of three-dimensional cultured adipose tissue-derived mesenchymal stem cell–conditioned medium and the antiaging effect of cosmetic products containing the medium. *Biomedical Dermatology*, *4*, Article, 1. https://doi.org/10.1186/s41702-019-0053-z

Kim, M. K., Seo, B. F., Kim, K. J., Lee, S.-J., Ryu, Y. H., & Rhie, J. W. (2015). Secretory factors of human chorion-derived stem cells enhance activation of human fibroblasts. *Cytotherapy*, *17*(3), 301–309. https://doi.org/10.1016/j.jcyt.2014.10.007

Kim, Y.-J., Seo, D. H., Lee, S. H., Lee, S.-H., An, G.-H., Ahn, H.-J., Kwon, D., Seo, K.-W., & Kang, K.-S. (2018). Conditioned media from human umbilical cord blood-derived mesenchymal stem cells stimulate rejuvenation function in human skin. *Biochemistry and Biophysics Reports*, *16*, 96–102. https://doi.org/10.1016/j.bbrep.2018.10.007

Lavoie, J. R., & Rosu-Myles, M. (2013). Uncovering the secretes of mesenchymal stem cells. *Biochimie*, *95*(12), 2212–2221. https://doi.org/10.1016/j.biochi.2013.06.017

Lee, S. M., Lee, S. C., & Kim, S.-J. (2014). Contribution of human adipose tissue–derived stem cells and the secretome to the skin allograft survival in mice. *Journal of Surgical Research*, *188*(1), 280–289. https://doi.org/10.1016/j.jss.2013.10.063

Lee, Y. I., Kim, S., Kim, J., Kim, J., Chung, K. B., & Lee, J. H. (2021). Randomized controlled study for the anti-aging effect of human adipocyte-derived mesenchymal stem cell media combined with niacinamide after laser therapy. *Journal of Cosmetic Dermatology*, *20*(6), 1774–1781. https://doi.org/10.1111/jocd.13767

Lephart, E. D. (2018). Equol's anti-aging effects protect against environmental assaults by increasing skin antioxidant defense and ecm proteins while decreasing oxidative stress and inflammation. *Cosmetics*, *5*(1), Article 16. https://doi.org/10.3390/cosmetics5010016

Li, Q., Chen, Y., Ma, K., Zhao, A., Zhang, C., & Fu, X. (2016). Regenerative and reparative effects of human chorion-derived stem cell conditioned medium on photo-aged epidermal cells. *Cell Cycle*, *15*(8), 1144–1155. https://doi.org/10.1080/15384101.2016.1158376

Li, T., Zhou, L., Fan, M., Chen, Z., Yan, L., Lu, H., Jia, M., Wu, H., & Shan, L. (2022). Human umbilical cord-derived mesenchymal stem cells ameliorate skin aging of nude mice through autophagy-mediated anti-senescent mechanism. *Stem Cell Reviews and Reports*, *18*(6), 2088–2103. https://doi.org/10.1007/s12015-022-10418-9

Liang, X., Li, J., Yan, Y., Xu, Y., Wang, X., Wu, H., Liu, Y., Li, L., & Zhuo, F. (2022). Efficacy of microneedling combined with local application of human umbilical cord-derived mesenchymal stem cells conditioned media in skin brightness and rejuvenation: A randomized controlled

split-face study. *Frontiers in Medicine*, *9*. https://doi.org/10.3389/fmed.2022.837332

Mazini, L., Rochette, L., & Malka, G. (2020). Adipose-derived stem cells (adscs) and growth differentiation factor 11 (gdf11): Regenerative and antiaging capacity for the skin. In M. S. Choudery, *Regenerative Medicine*. IntechOpen. https://doi.org/10.5772/intechopen.91233

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*(1), 1239–1257. https://doi.org/10.3390/ijms13011239

Noverina, R., Widowati, W., Ayuningtyas, W., Kurniawan, D., Afifah, E., Laksmitawati, D. R., Rinendyaputri, R., Rilianawati, R., Faried, A., Bachtiar, I., & Wirakusumah, F. F. (2019). Growth factors profile in conditioned medium human adipose tissue-derived mesenchymal stem cells (CM-hATMSCs). *Clinical Nutrition Experimental*, *24*, 34–44. https://doi.org/10.1016/j.yclnex.2019.01.002

Ogliari, K. S., Marinowic, D., Brum, D. E., & Loth, F. (2014). Stem cells in dermatology. *Anais Brasileiros de Dermatologia*, *89*(2), 286–291. https://doi.org/10.1590/abd1806-4841.20142530

Park, B.-S., & Kim, W.-S. (2010). Adipose-derived Stem Cells and their Secretory Factors for Skin Aging. In M. A. Farage, K. W. Miller, & H. Howard I. Maibach (Eds.), *Textbook of aging skin* (201–212). Springer. https://doi.org/10.1007/978-3-540-89656-2_20

Parrado, C., Mercado-Saenz, S., Perez-Davo, A., Gilaberte, Y., Gonzalez, S., & Juarranz, A. (2019). Environmental stressors on skin aging. Mechanistic insights. *Frontiers in Pharmacology*, *10*. https://doi.org/10.3389/fphar.2019.00759

Pashaei-Asl, R., Pashaiasl, M., Ebrahimie, E., Lale Ataei, M., & Paknejad, M. (2022). Apoptotic effects of human amniotic fluid mesenchymal stem cells conditioned medium on human MCF-7 breast cancer cell line. *BioImpacts*, *13*(3), 191–206. https://doi.org/10.34172/bi.2022.23813

Pokrovskaya, L. A., Zubareva, E. V., Nadezhdin, S. V., Lysenko, A. S., & Litovkina, T. L. (2020). Biological activity of mesenchymal stem cells secretome as a basis for cell-free therapeutic approach. *Research Results in Pharmacology*, *6*(1), 57–68. https://doi.org/10.3897/rrpharmacology.6.49413

Prakoeswa, C. R. S., Pratiwi, F. D., Herwanto, N., Citrashanty, I., Indramaya, D. M., Murtiastutik, D., Sukanto, H., & Rantam, F. A. (2019). The effects of amniotic membrane stem cell-conditioned medium on photoaging. *Journal of Dermatological Treatment*, *30*(5), 478–482. https://doi.org/10.1080/09546634.2018.1530438

Prodinger, C. M., Reichelt, J., Bauer, J. W., & Laimer, M. (2017). Current and future perspectives of stem cell therapy in dermatology. *Annals of Dermatology*, *29*(6), 667–687. https://doi.org/10.5021/ad.2017.29.6.667

Putri, W. E., Endaryanto, A., Rantam, F. A., & Prakoeswa, C. R. S. (2021). Mesenchymal stem cells-conditioned medium (SECRETOME) in skin aging: A systematic review. *International Journal of Pharmaceutical Research*, *13*(2). https://doi.org/10.31838/ijpr/2021.13.02.020

Putri, W. E., Endaryanto, A., Tinduh, D., Rantam, F., Notobroto, H. B., & Prakoeswa, C. R. S. (2021). Skin barrier before and after topical adipose stem cell-conditioned medium (ASC-CM) treatment in photoaging. *Bali Medical Journal*, *10*(2), 688–691. https://doi.org/10.15562/bmj.v10i2.2589

Putri, W. E., Endaryanto, A., Tinduh, D., Rantam, F., Notobroto, H. B., & Prakoeswa, C. R. S. (2022). Effect of secretome of adipose stem cell (ASC) in photoaging skin. *Bali Medical Journal*, *11*(3), 1212–1217. https://doi.org/10.15562/bmj.v11i3.3543

Rantam, F. A., Ferdiansyah, & Purwati, P. (2014). *Stem cell: Mesenchymal, hematopoetik, dan model aplikasi* (2nd ed.). Airlangga University Press.

Revilla, G., & Mulyani, H. (2020). The effect of human bone marrow mesenchymal stem cells on epidermal growth factor and epidermal growth factor receptor expression in re-epithelialization process in the healing of burns on experimental rats. *Open Access Macedonian Journal of Medical Sciences*, *8*(A), 508–511. https://doi.org/10.3889/oamjms.2020.3959

Schäfer, R., Spohn, G., & Baer, P. C. (2016). Mesenchymal stem/stromal cells in regenerative medicine: Can preconditioning strategies improve therapeutic efficacy. *Transfusion Medicine and Hemotherapy*, *43*(4), 256–267. https://doi.org/10.1159/000447458

Seo, Y., Shin, T.-H., & Kim, H.-S. (2019). Current strategies to enhance adipose stem cell function: An update. *International Journal of Molecular Sciences*, *20*(15), Article 3827. https://doi.org/10.3390/ijms20153827

Son, W.-C., Yun, J.-W., & Kim, B.-H. (2015). Adipose-derived mesenchymal stem cells reduce MMP-1 expression in UV-irradiated human dermal fibroblasts: Therapeutic potential in skin wrinkling. *Bioscience, Biotechnology, and Biochemistry*, *79*(6), 919–925. https://doi.org/10.1080/09168451.2015.1008972

Sun, B., Guo, S., Xu, F., Wang, B., Liu, X., Zhang, Y., & Xu, Y. (2014). Concentrated hypoxia-preconditioned adipose mesenchymal stem cell-conditioned medium improves wounds healing in full-thickness skin defect model. *International Scholarly Research Notices*. https://doi.org/10.1155/2014/652713

Sunarto, H., Trisnadi, S., Putra, A., Sa'dyah, N. A. C., Tjipta, A., & Chodidjah, C. (2020). The role of hypoxic mesenchymal stem cells conditioned medium in increasing vascular endothelial growth factors (vegf) levels and collagen synthesis to accelerate wound healing. *Indonesian Journal of Cancer Chemoprevent*, *11*(3), 134–143. http://dx.doi.org/10.14499/indonesianjcanchemoprev11iss3pp134-143

Trzyna, A., & Banaś-Ząbczyk, A. (2021). Adipose-derived stem cells secretome and its potential application in "stem cell-free therapy." *Biomolecules*, *11*(6), Artikel 878. https://doi.org/10.3390/biom11060878

Widowati, W., Noverina, R., Ayuningtyas, W., Kurniawan, D., Arumwardana, S., Kusuma, H. S. W., Rizal, R., Laksmitawati, D. R., Rinendyaputri, R., Rilianawati, R., & Faried, A. (2022). Potential of conditioned medium of hATMSCs in aging cells model. *HAYATI Journal of Biosciences*, *29*(3), 378–388. https://doi.org/10.4308/hjb.29.3.378-388

Xu, X., Wang, H., Zhang, Y., Liu, Y., Li, Y., Tao, K., Wu, C.-T., Jin, J., & Liu, X. (2014). Adipose-derived stem cells cooperate with fractional carbon dioxide laser in antagonizing photoaging: a potential role of Wnt and β-catenin signaling. *Cell & Bioscience*, *4*, Article 24. https://doi.org/10.1186/2045-3701-4-24

Yang, C.-Y., Chang, P.-Y., Chen, J.-Y., Wu, B.-S., Yang, A.-H., & Lee, O. K.-S. (2021). Adipose-derived mesenchymal stem cells attenuate dialysis-induced peritoneal fibrosis by modulating macrophage polarization via interleukin-6. *Stem Cell Research & Therapy*, *12*, Article 193. https://doi.org/10.1186/s13287-021-02270-4

Yonghong, Q., Aishu, L., Al-Ajam, Y., Yuting, L., Xuanfeng, Z., & Jin, Z. (2022). Topical transplantation of bone marrow mesenchymal stem cells made deeper skin wounds regeneration. *Plastic Surgery*, *30*(1), 76–85. https://doi.org/10.1177/2292550320967404

Yoon, B. S., Moon, J.-H., Jun, E. K., Kim, J., Maeng, I., Kim, J. S., Lee, J. H., Baik, C. S., Kim, A., Cho, K. S., Lee, J. H., Lee, H. H., Whang, K. Y., & You, S. (2010). Secretory profiles and wound healing effects of human amniotic fluid–derived mesenchymal stem cells. *Stem Cells and Development*, *19*(6), 887–902. https://doi.org/10.1089/scd.2009.0138

Zhang, S., & Duan, E. (2018). Fighting against skin aging: The way from bench to bedside. *Cell Transplantation*, *27*(5), 729–738. https://doi.org/10.1177/0963689717725755