International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 7,
2020 ISSN: 1475-7192

A Study on the Physiological Activity of Stem Cell-Derived Conditioned Medium and Its Application for Cosmetic Materials

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Abstract

Background*: According to a study on the efficacy of fat derived stem cell culture on skin regeneration, an increase in collagen synthesis was observed when stem cell culture was applied to fibroblasts and keratinocytes. In this study, the authors tried to identify the anti-aging effect of fat derived stem cell cultures through intracellular mechanism of action on skin cells.*

Methods*: In order to confirm the physiological activity potential of stem cell culture, skin cell activity test was performed to determine the toxicity, collagen production inhibitory activity, and MMP-1 expression.*

Findings*: Cytotoxicity was not identified at all concentrations, it was confirmed that the collagen production increased by about 20% compared to the control group without the sample treatment, MMP-1 expression through JNK and ERK signaling pathway even at low concentration.*

Improvements*: In this study suggest that fat-derived stem cell cultures are not toxic when used and may be useful as functional cosmetic materials with anti-aging effects.*

Keywords: *Anti-aging, Collagen**,** Cosmetic materials, Physiological Activities, Stem Cell-derived Conditioned Medium*

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1. INTRODUCTION

Fat-derived stem cells refer to those that have the property of growing by adhering to collagen degrading enzymes of fat tissue. Stem cells are primitive cells with self-renewal ability to produce cells of their own capacity and differentiation into specific cells when placed in an appropriate environment [1]. Stem cells are divided into two types, embryonic stem cells and adult stem cells depending on their cytological origin [2]. Adult stem cells are being actively studied as a means of treating diseases such as stem cells obtained from bone marrow, stem cells of umbilical cord blood, and fat tissue, which are already in our bodies. Because of the yield, fat-derived stem cells are expected to be widely applied to clinical treatment in the future. According to US media, stem cells are being used in clinical trials for ten selected incurable diseases. Cosmetics containing plant stem cells, cosmetics

containing marine stem cells, and cosmetics containing stem cell activators have been developed as the stem cell applying cosmetics. In addition, cosmetics containing human-derived stem cell culture are being developed, and technology development is gradually accelerating along with securing intellectual property rights at home and abroad. Methods to apply stem cells to cosmetics include, first, applying stem cells directly to cosmetics, second, applying stem cell culture medium to cosmetics, third, applying specific components extracted from stem cell cultures to cosmetics, and direct screening of stem cell activators and the application of the extracted components to cosmetics. Stem cell cosmetics means cosmetics using stem cell cultures or extracts as raw materials, rather than using live stem cells as raw materials for cosmetics. It is reported that stem cell cultures allowed as a composition of cosmetics can be used as a useful composition for the development of various cosmetics aimed at promoting whitening, wrinkle improvement and skin soothing effect by promoting skin regeneration and enhancing moisturizing function [3]. In addition, recently the optical aging prevention [4], skin regeneration [5], androgenetic alopecia [6], promotion of cell proliferation and infiltration [7] and the research through the beauty industry and cosmetics approach have been steadily reported. According to a study on the efficacy of fat derived stem cell culture on skin regeneration, an increase in collagen synthesis was observed when stem cell culture was applied to fibroblasts and keratinocytes, which are human cells that play a crucial role in skin regeneration. In addition, it was confirmed from the DNA microarray results that the expression of 133 genes involved in cell proliferation, cell migration, cell adhesion, and wound reaction is regulated [8]. These results indicate that fat derived stem cell cultures can activate cell biological functions and can be clinically applied to skin regeneration. Therefore, in this study, the authors tried to identify the anti-aging effect of fat derived stem cell cultures through intracellular mechanism of action on skin cells.

2. Materials And Methods

2.1. Sample preparation

The human adipose stem cell (ASCs) used in this experiment was cultured in the α-MEM (α-modification of Eagle's minimum essential medium, Hyclone, USA) medium added with 10% FBS(fetal bovine serum) (FBS, Sigma) and 1% streptomycin/penicillin (100 IU/50 mg/mL) in the 5% CO_2 wet incubator at 37℃, and for the stem cells, after 3~4 times of subculture when the confluence reached to 70 ~80%, α-MEM culture medium was removed and replaced with DMEM/F12 low glucose and serum-free medium. On third day under the incubation condition, the culture medium was recovered and used for the experiment after filtering with 0.2 mm syringe filter (13 mm, 100 units/Whatman Cat No. 6779 1302). With the abovementioned method, the stem cell culture medium was recovered and filtered with syringe filter (0.2 mm) and used after obtaining freeze dried stem cell conditioned media (powder): SCMF)

2.2. Cell culture

HDF fibroblast, the strain used in this experiment, was purchased from Korean Cell Line Bank. For the medium, the high glucose Dulbecco's modified Eagle’s medium (DMEM; Sigma-Aldrich) was used by adding 10% FBS(fetal bovine serum) (FBS, Sigma) and 1% streptomycin/penicillin (100 IU/mL; GE Healthcare Life Sciences) and was cultured under the condition of 37℃, 95% relative humidity and 5% CO_2. Human Adipose Stem Cells (ASCs) from 25 to 45 years of age were purchased from MCTT and cultured in α-MEM medium (Hyclone, USA) supplemented with 10% FBS(fetal bovine serum). The cells were cultured in a 5% CO_2 humidified incubator

maintained at 37 ° C with serum (FBS, Sigma) and 1% penicillin / streptomysin (100 IU / 50 mg / mL).

2.3. Measurement of collagen production promoting ability

In order to measure the collagen production promotion, HDF cells were injected into 96 well plates at a concentration of 1×10^4 cells/well and incubated for 24 hours to allow the cells to adhere to the bottom. After confirming cell adhesion, samples were incubated for 48 hours by diluting and adding the concentrations. 50mL of the culture supernatant was transferred to a maxisorb 96 well plate, and then 100 mL of carbonate coating buffer ($Na_2 CO_3$ + $NaHCO_3$ + 10% NaN_3, pH 9.5) was added thereto and fixed at 4 °C for 24 hours. After fixing, washing was performed three times with 200 mL of PBS-T (PBS, 0.05% Tween-20) and blocking with 100 mL of blocking solution (PBS, 0.1% BSA) at 37 °C for 1 hour. After blocking, washed with PBS-T 200 mL three times and 100 mL of the primary antibody (anti-collagen type I-Ab mouse IgG) diluted 1000-fold with a blocking solution to each well and left for 1 hour at 37 °C. After washing three times with 200 mL of PBS-T, diluted 4000 times with a blocking solution of alkaline phosphatase conjugated secondary antibody (anti-mouse IgG-antibody), and then treated with 100 mL of each well and was left at 37 °C for 1 hour. Finally, washing with PBS-T 200 mL 3 times, adding 200 mL of substrate p-nitrophenyl phosphate (in 9.7% diethanolamine buffer, 0.5 mM MgCl2, pH 9.8) to each well, wrapped the plate with aluminum foil, and the reaction was carried out at 1 °C for 1 hour and the absorbance was measured at 405 nm.

2.4. Measurement of Suppression of MMP-1 expression

In order to measure the effect on MMP-1 expression, HDF cells were split into 96 well plates at 3×10^4 cell/well concentrations and incubated for 24 hours to allow cells to adhere to the bottom and was treated by concentration and irradiated with UVB at 100 mJ/cm^2 for 20 minutes and incubated for 24 hours. 100 mL of the culture supernatant was transferred to 96 wells, and then 100 mL of coating buffer was added and allowed to stand overnight. After removing the culture supernatant was washed three times with 200 mL of PBS-T (PBS, 0.05% Tween-20), and treated with 100 mL of blocking buffer (PBS, 0.1% BSA) and left for 1 hour at 37 °C. After blocking, washed three times with 200 mL PBS-T, and the primary antibody (anti-MMP-1 mouse antibody) diluted 1000-fold with a blocking solution 50 mL each and left for 1 hour at 37 °C. After washing three times with 200 mL of PBS-T, diluted 4000 times with a blocking solution of alkaline phosphatase conjugated secondary antibody (anti-mouse IgG antibody), and then treated with 50 mL in each well and was left at 37 °C for 1 hour. Finally, washing with PBS-T 200 mL 3 times, adding 200 mL of substrate p-nitrophenyl phosphate (in 9.7% diethanolamine buffer, 0.5 mM MgCl2, pH 9.8) to each well, wrapped the plate with aluminum foil and the reaction was carried out at 1 °C for 1 hour and the absorbance was measured at 405 nm.

2.5. Statistical processing

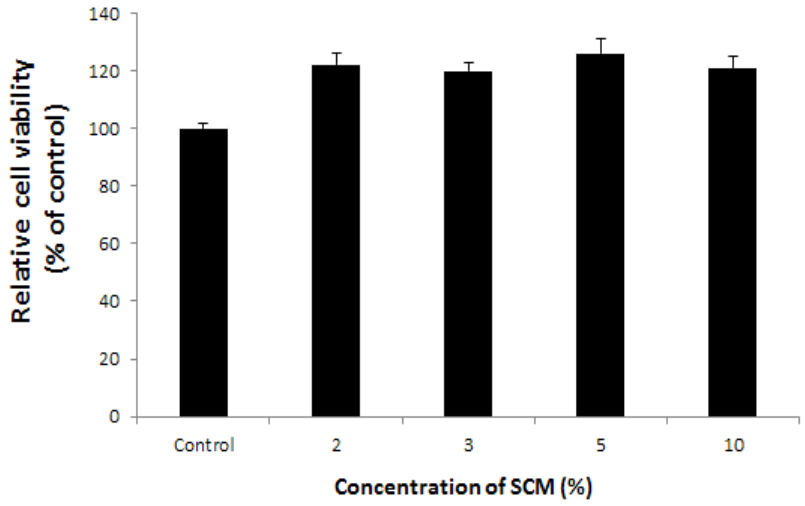
All experiments in this study were repeated three times, expressed as mean ± standard deviation (Mean ± SD), and statistical analysis was performed using SPSS Window Version 17.0 (SPSS Inc., Illinois, USA). Student's t-test was used to demonstrate the significance of the differences between the conditions. As a result of Student 's t-test, $p < .05$ value was considered to be significant, and a significant value was given an asterisk (*).

3. RESULTS AND DISCUSSION

3.1. Cell viability

Nutral red assay is a method to determine the toxicity of cell organelles to lysosome [9]. The US National Cancer Institute uses toxicant assays such as anticancer agents. In order to examine the effect of SCM on the cell proliferation and survival of HDF cells, the cell survival rate was changed after SCM, SCMF treatment of HDFs cells at concentrations of 2, 3, 5, and 10% by NR assay (Figure 1 (A, B)). Survival was high at all sample treatment concentrations, and SCM showed more than 20% growth effect than control, so it would not appear skin damage in the development and application of cosmetic raw materials when applied to skin. The cell viability was maintained above 100% at the SCMF 5, 10, 20, 50, 100 mg/mL treatment concentration, and 10% even at the 50, 100 mg/mL concentration range.

(A)



(B)

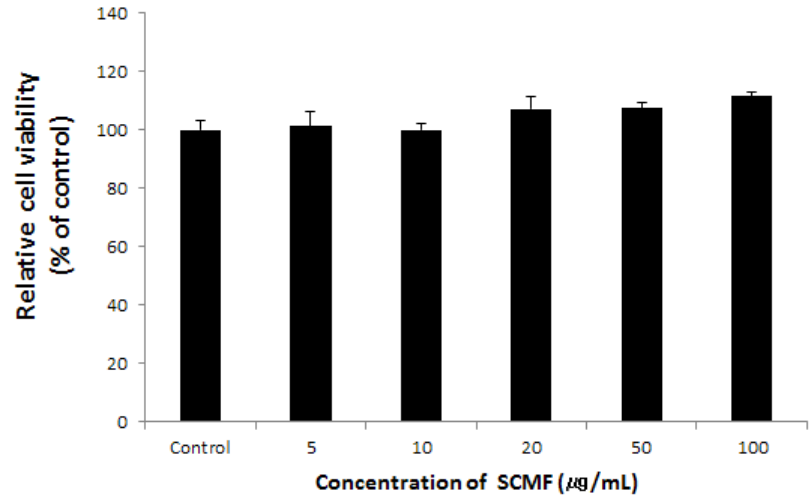


Figure 1. Effects of SCM and SCMF on cell viability in HDF cells. (A) SCM: stem cell conditioned media (liquid), (B) SCMF: freeze dried stem cell conditioned media (powder)

3.2. Changes in Growth Factor Gene Expression in HDF Cells

In order to identify the promotion of various growth factor secretion of HDF cells, the expression change of mRNA was measured using RT-PCR assay (Figure 2). When the fat stem cell culture medium was added to HDF cells, growth factors were secreted in a large amount, and basic fibroblast growth factor (bFGF) increased SCM 4 times compared to control, and VEGF (vascular endothelial growth factor) increased 5 times. KGF (keratinocyte growth factor) was increased 1.5 times and PDGF (platelet derived growth factor) was confirmed to increase the SCM 1 times compared to the control.

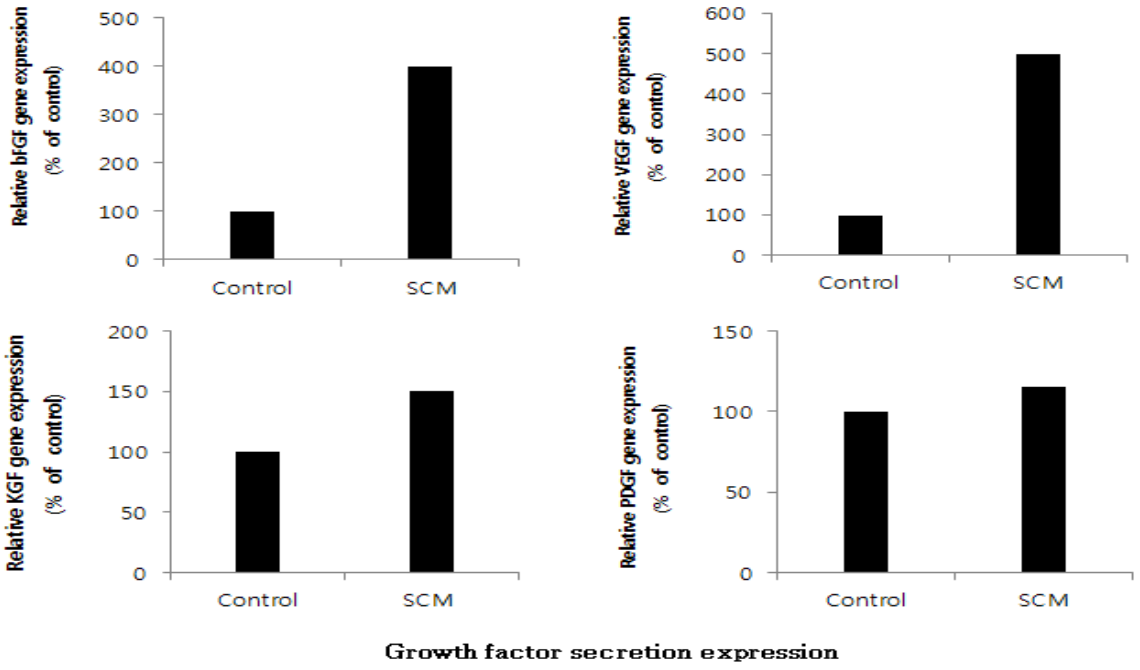
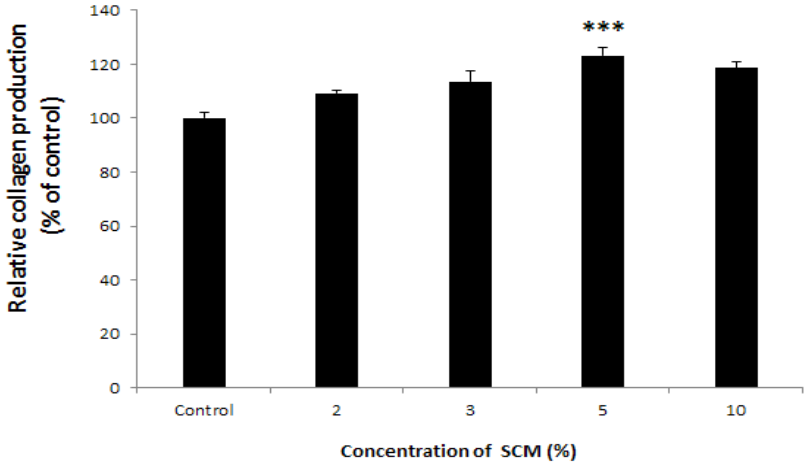


Figure 2. Effect of SCM on the expression of growth factor genes in HDF cells. SCM: stem cell conditioned media (liquid), bFGF: basis fibroblast growth factor, VEGF: vascular endothelial growth factor, KGF: keratinocyte growth factor, PDGF: platelet derived growth factor

3.3. Measurement of Collagen production promoting ability

The skin consists of the epidermis, the dermis and the subcutaneous tissue. The dermis is composed of fibrous and matrix components, and the collagen that exists as fibrous component is the major protein that accounts for 90% of the dermis. Stem cell cultures were treated with HDF cells for 48 hours to observe changes in collagen production promoting ability of the stem cell cultures against HDF cells of the samples. Herein, the culture supernatant was taken to measure the amount of collagen secreted by ELISA (Enzyme-Linked Immunosorbent Assay) method, and the results are shown in Figure 3 A, B. It was confirmed that collagen production was promoted in both SCM and SCMF compared to the negative control without sample treatment. SCM showed the highest increase in collagen production of 20% at 5% concentration, and SCMF increased collagen production with concentration at 1, 5, 20, 50 and 100 mg/mL. This result shows that the cytokine is secreted in large amounts, which shows the effect of cell proliferation and collagen production promoting ability. Therefore, this result confirmed the collagen production promoting effect of the fat-derived stem cell culture medium and suggest that it can be applied as a functional cosmetic material.

(A)



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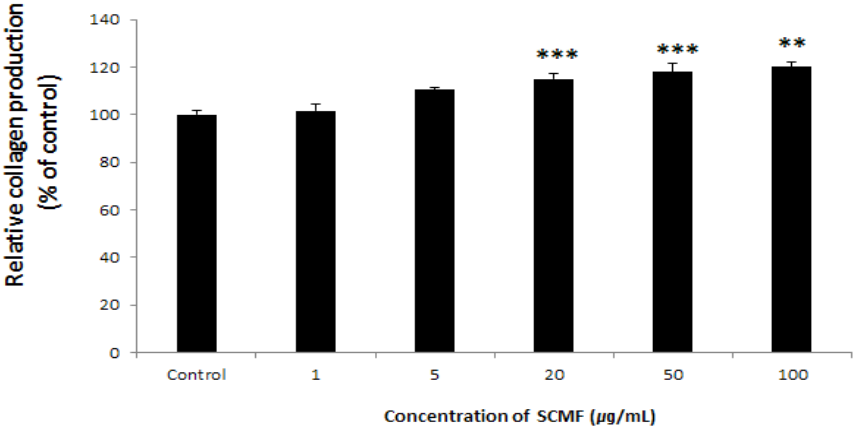


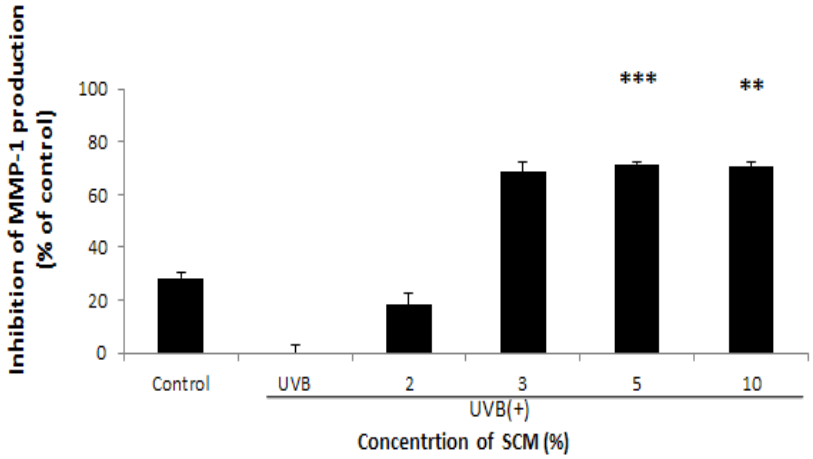
Figure 3. Effects of SCM and SCMF on the collagen secretion by HDF cells. Result is represented as mean ± SD. **$p<0.01$, ***$p<0.001$ (A) SCM: stem cell conditioned media (liquid), (B) SCMF: freeze dried stem cell conditioned media (powder)

3.4. Suppression of MMP-1 expression

Matrix metalloproteases (MMPs) are important proteins that degrade the extracellular matrix, and when excessively activated, TIMPs (tissue inhibitor of MMPs), which inhibit the activity of MMPs, are timely expressed and their activation is controlled by interaction [10]. In terms of dermatology, the increased expression and activity of these MMPs promotes collagen breakdown and is an important cause of skin aging. Samples were treated with HDF cells for 24 hours to determine the change in MMP-1 protein secretion of SCM and SCMF on HDF cells. Herein, the culture supernatant was taken to measure the amount of secreted MMP-1 by ELISA (Enzyme-Linked Immunosornent Assay) method. HDF cells were treated with SCM 2, 3, 5, 10% and SCMF 5, 10, 20, 50 and 100 mg/mL concentrations, respectively, and irradiated with UVB lamps at 100 mJ/cm^2 intensity. As a result (Figure 4 A, B.), it was confirmed that MMP-1 expression was suppressed in both SCM and SCMF, and in the case of SCM, MMP-1 expression was suppressed from 3% to 70%, and SMCF at 10 mg/mL concentration. It was confirmed that MMP-1 protein secretion is significantly suppressed by more than 80%. In addition, the

Suppression of MMP-1 protein secretion by stem cell culture has not been reported yet.

(A)



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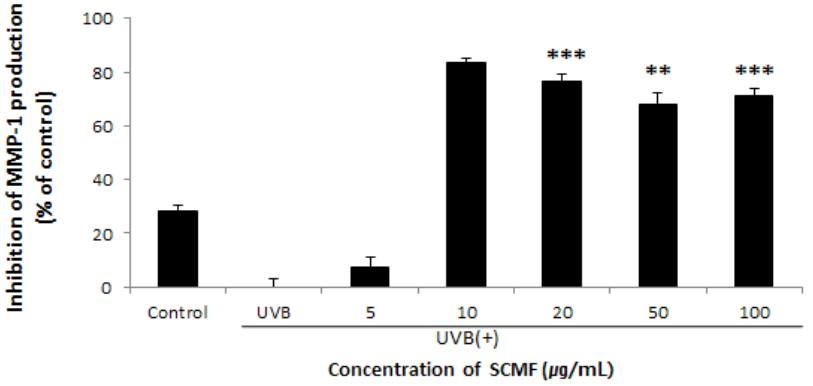


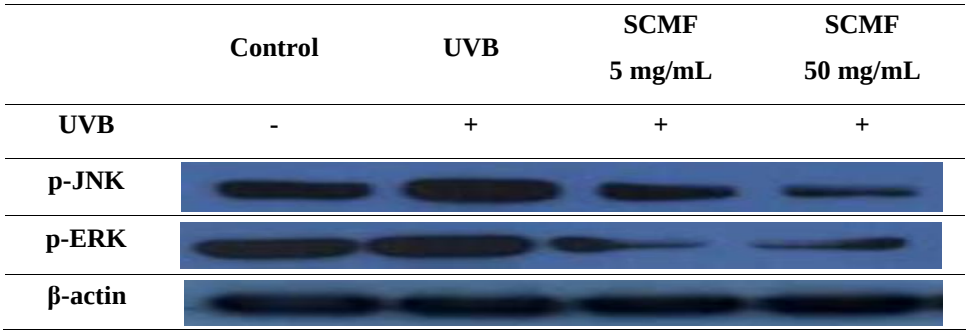
Figure 4. Inhibitory effects of SCM and SCMF on UVB-induced secretion of MMP-1 by HDF cells. Result is represented as mean ± SD. **$p<0.01$, ***$p<0.001$,(A) SCM: stem cell conditioned media (liquid), (B) SCMF: freeze dried stem cell conditioned media (powder)

3.5 Cell signaling pathways involved in MMP-1 gene expression

UVB irradiation in HDF cells results in not only intracellular DNA damage but also optical aging, and optical senescence in HDF cells suppresses the growth and promotes the expression of MMPs, thereby promoting the breakdown of collagen present in the dermal layer [11]. MMP expression is promoted by NF-κB, a transcription factor activated by UVB. UVB irradiated to HDF cells increases ROS to activate NF-κB, an inflammatory transcription factor, and activated NF-κB migrates into the nucleus to promote MMP expression. In this study, the degree of phosphorylation of p-JNK and p-ERK known to be related to MMP-1 gene expression by UVB was measured by Western blotting. After treatment with SCMF 5, 50 mg/mL concentration was irradiated with an intensity of 100 mJ/cm^2 using a UVB lamp. As shown in Figure 5 A, B, it was confirmed that phosphorylation of p-JNK and p-ERK increased after UVB irradiation, and that phosphorylation of p-JNK and p-ERK was suppressed by treatment with low concentration of SCMF 5 mg/mL. It can be inferred that it suppresses the expression. This

suggests that SCMF can suppresses the expression of MMP-1 gene by suppressing the phosphorylation of p-JNK and p-ERK induced by UVB in HDF cells.

(A)



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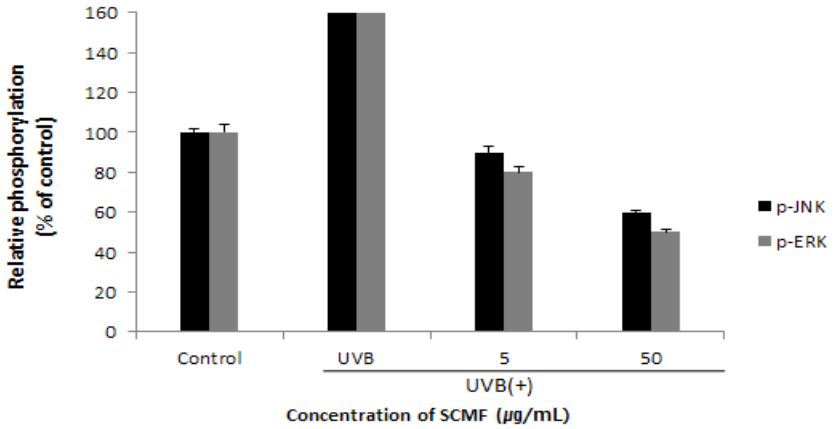

Figure 5. Inhibitory effects of SCMF on UVB-induced activations of p-JNK and p-ERK in HDF cells. In order to check the anti-aging effects of SCMF, HDF cells were treated with UVB, and inhibitory effects on p-JNK and p-ERK activity were measured by using western blotting. From results p-ERK significant inhibitory effects were checked. (A) A variation of p- JNK and p- ERK protein expression due to SCMF was shown. (B) p- JNK and p- ERK protein expression SCMF was graphed. The results are presented as the M±SD of three independent experiments. SCMF: freeze dried stem cell conditioned media (powder), UVB: ultraviolet B, p-JNK: phosphoc-Jun N-terminal kinase, p-ERK: phospho-extracellular signal regulated kinase, HDF: human dermal fibroblast

4. CONCLUSION

In this study, in order to confirm the physiological activity potential of stem cell culture, skin cell activity test was performed to determine the toxicity, collagen production inhibitory activity, and MMP-1 expression. As a result of measuring cytotoxicity in HDF, cytotoxicity was not identified at all concentrations. Such results may be regarded as a cell proliferation effect by a growth factor derived from fat derived stem cell culture. As a result of examining the change in the ability to promote collagen production, it was confirmed that the collagen production increased by about 20% compared to the control group without the sample treatment. As a result of experiments on the effects of MMP-1 expression, it was confirmed that MMP-1 expression was suppressed in both the stem cell culture medium and lyophilization of stem cell culture medium. As a result of confirming the phosphorylation

change, it was confirmed that lyophilization of stem cell culture medium suppressed MMP-1 expression through JNK and ERK signaling pathway even at low concentration. Results of all experiments in this study suggest that fat-derived stem cell cultures are not toxic when used and may be useful as functional cosmetic materials with anti-aging effects.

REFERENCES

[1] Thomson JA, Sander SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jeffrey MJ. Embryonis stem cell lines derived from human blastocysts. Science. 1998. Nov;282(6):1145-1147.

[2] Flores I, Canela A, Vera E, Tejera A, Cotsarelis G, Maria A. The longist telomeres: a general signature of adult stem cells compartments. Genes. Dev., 2008. Feb;22(18):654-667.

[3] Gonzalez-Rey E, Gonzalez MA, Varela N, Valle FO, Hernandez P, Rico L, Büscher D, Delgado M. Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. Ann. Rheum. Dis., 2010.Jan;69(1):241-248.

[4] Lei W, Jixian D, Weichen T, Bo X, Tonghua Y, Gang L, Jian W, Marco G, et el. Adipose-derived stem cells are an effective cell candidate for treatment of heart failure: an MR imaging study of rat hearts. AM. J. Physiol. Heat. Circ. Physiol., 2009. **Sep**;297(3): 1020-1031.

[5] Seo KY, Kim DH, Lee SE, Yoon MS, Lee HJ. Skin rejuvenation by microneedle fractional radiofrequency and a human stem cell conditioned medium in Asian skin: a randomized controlled investigator blinded split-face study. J. Cosmet. Laser. Ther., 2013. Jan;15(1):25-33.

[6] Daniela B, Vincenzo N, Angela M, Fulvio M. Clinical Efficacy of a Cosmetic Treatment by Crescina Human Follicle Stem Cell on Healthy Males with Androgenetic Alopecia. Dermatol. Ther., 2013. Feb;3(1):53-62.

[7] Joshua M. Hare, Jay H. Traverse, Timothy D. Henry, Nabil Dib. A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) After Acute Myocardial Infarction. J. Am. Coll. Cardiol., 2009. Dec;54(24):2277-2286.

[8] Jeffrey MG, Adam JK, Bruce AB. Adipose-Derived Stem Cells for Regenerative Medicine. Circ. Res., 2007. May;100(9):1249-1260.

[9] Borenfreund E, Puerner JA. Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicol. Lett., 1985. Feb-Mar;24(2-3):119-124.

[10] Homebeck W. Down regulation of tissue inhibitor of matrix metallo- protease-1(TIMP-1) in aged human skin contributes to matrix degradation and impaired cell growth and survival. Pathol. Biol., 2003. Dec;51(10): 569-579.

[11] Kim JM, Lee ST, Chu K, Jung KH. Systemic transplantation of human adipose stem cells attenuated cerebral inflammation and degeneration in a hemorrhagic stroke model. Brain Res., 2007. Dec;1183(5): 43-50.