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Improvement of Photoaged Skin Wrinkles with Cultured Human Fibroblasts and Adipose-derived Stem Cells: A Comparative Study

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ABSTRACT

Improvement of Photoaged Skin Wrinkles with Cultured Human Fibroblasts and Adipose-derived Stem Cells: A Comparative Study

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Introduction: We investigated the anti-wrinkle effects of cultured human fibroblasts and adipose-derived stem cells (ADSCs) and the mechanisms underlying the reduction of wrinkles in photoaged skin.

Methods: The fibroblasts and ADSCs were isolated from human tissue and cultured. A total of 28 six-week-old female BALB/c-nude mice were classified into 4 groups, including the normal

control group and 3 groups that were irradiated 6 times a week for 6 weeks using ultraviolet B radiation to induce photoaged wrinkles. ADSCs were injected into the wrinkles in the skin of the second group and fibroblasts were injected into the wrinkles in the skin of the third group. The fourth group was the irradiated negative control group (no therapy). After 4 weeks of injections, wrinkles were compared by replica analysis, biopsies were performed, and the dermal thickness and collagen densities were measured. We determined the amounts of type 1 collagen and matrix metalloproteinases (MMPs) 1, 2, 3, 9, and 13 using real-time polymerase chain reaction and western blot analysis and assessed tropoelastin and fibrillin-1 expression in the dermis by immunohistochemistry.

Results: Replica analysis showed significant wrinkle reduction in the fibroblast group and the ADSC group. ADSCs stimulated collagen expression and decreased MMP expression. Although fibroblasts stimulated more collagen expression than ADSCs, they also increased MMP expression. Overall, the ADSC group showed higher collagen density and had better outcomes in the tropoelastin and fibrillin-1 assessments.

Conclusions: Cultured fibroblasts and ADSCs could both play an important role in wrinkle reduction despite differences in their mechanisms of action.

Keywords: Stem cells, Mesenchymal Stem Cells, Fibroblasts, Photoaging of Skin

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List of Table

List of Figures

CONTENTS

INTRODUCTION

Increasing life expectancy, the phenomenon of population aging, increased income levels, and increased interest in beauty are some of the factors that lead to the high demand for plastic surgery or wrinkle reduction and the increasing interest in anti-aging therapies. This is a global trend that is expected to continue. Aging research is mainly divided into studies of chronological aging and photoaging.[1] Chronological aging refers to the structural, functional, and metabolic changes in the skin that occur, along with degenerative changes in other organs, as a result of growing old.[1] While genetic factors are involved in the formation of wrinkles, the amount of exposure to sunlight is also an influencing factor. Photoaging is a separate process from chronological aging and mainly results in the destruction of the collagen and elastin fibers of the skin.[1,2] It is known that activator protein-1 (AP-1) stimulates matrix metalloproteinases (MMP) transcription in the photoaging process.[2] Increased MMP transcription accelerates the degradation of collagen which induces dermal matrix alterations, and it also leads to decreased collagen synthesis.[2] Several studies have also reported that photoaging decreased collagen synthesis and increased transcription of MMP, which degraded collagen by the same mechanism.[3-9]

Research on photoaging treatments is also important for the field of cell therapy.[5,7-13] The cells that are receiving the most attention for use in cell therapies related to photoaging are adipose-derived stem cells (ADSCs).[5,7-9] Many studies about ADSCs have been conducted recently.[5,7-9] Some researchers have indicated that injected ADSCs were effective for treating photoaged wrinkles, as they significantly increased collagen synthesis, collagen density, and dermal thickness.[5-9] Moreover, the subcutaneous injection of ADSC significantly increased the number of fibroblasts.[9] Similar studies have found that ADSCs lead to an increase in mRNA transcription for procollagen type I protein while decreasing MMP, and as a result, the dermal collagen density increased.[3,5,8,9] The most likely mechanisms underlying wrinkle reduction after injection of ADSCs are fibroblast activation, the creation of collagen, and decreased MMP expression.[5,8,9]

There have been basic research studies and clinical trials related to the injection of fibroblasts; however, the researchers only assessed patient satisfaction, dermis thickness in a biopsy, and the conformation of collagen.[10-17] The exact mechanisms underlying the anti-wrinkle activity of fibroblasts on photoaged skin remain unknown.[16,17] In addition, while there are several reports that provide quantitative correlation between changes in oxytalan fibers and the severity of skin wrinkling,[18] there are no studies that examine changes in elastic fiber with ADSC and fibroblast. We investigated the effects of cultured human ADSCs and fibroblasts

injections on photoaged skin wrinkles in a nude mouse model, and compared the anti-wrinkle effects between fibroblasts and ADSCs. We also examined the changes in elastic fibers, which have not previously been studied.

MATERIALS AND METHODS

Isolation and culture of fibroblasts and ADSCs

Human tissue samples were obtained from a 43-year-old woman undergoing the transverse rectus abdominis myocutaneous (TRAM) flap surgery for breast reconstruction with informed consent. Protocols were approved by the institutional review boards of the Seoul National University Hospital (No. H-1108-098-374) and cultured.[5,7,9] ADSCs were directly isolated from fat tissue, and fibroblasts were isolated from dermis.[5,7,9] Cell isolation and culture were performed as follows. The suctioned fat was washed twice using phosphate-buffered saline (PBS). The samples were digested with 0.1% collagenase type I (Worthington Biochemicals, Lakewood, NJ) under agitation at 37℃ (ADSC for 1 h, fibroblast for 4 h), and centrifuged for 10 minutes at 800 × g. The pellet was washed, and resuspended in PBS.[5,9] After another centrifugation, the supernatant was removed and the cell band buoyant over histopaque was collected.[6] Cells were cultured overnight at 37℃ and 5% CO2 in control medium (10% fetal bovine serum, 100 IU penicillin, 100 mg/ml streptomycin, 5 μg/ml heparin, 2 ng/ml acidic fibroblast factor).[5] The adherent cell population containing ADSCs and fibroblasts was maintained over 3 days and then expanded and cultured.[5]

Animal experiments

Twenty-eight 6-week-old female BALB/c nude mice were provided by ORIENT Inc. (Seongnam, Korea). The mice were fed a standard diet and were rested for 1 week in the animal facility before the experiment. A total of 28 mice were assigned to 4 groups (each group consisting of 7 mice): the normal control group and 3 irradiated groups.[19] We determined the minimal erythema dose (MED) of BALB/c nude mice as the minimal intensity of UVB radiation that produced erythema on a given test area.[20] The recorded MED was 150 mJ/cm^2, which is higher than the MED for the hairless mice that are frequently used as the animal model of photoaging. Wrinkles were induced in the mice in the 3 groups by irradiation with ultraviolet B (UVB) radiation (290–320 nm) 6 times a week for 6 weeks using 4 UVB lamps (TL20W/12RS; Philips, NY, USA) fitted with a Kodacel filter (TA401/407; Kodak, Rochester, NY, USA) to remove the ultraviolet C radiation. The irradiation amounts were slightly increased weekly for a total UVB dosage of 7200 mJ/cm^2.[21]

The 3 groups of irradiated mice were then subjected to different treatments. one group was considered the irradiated negative control group (no therapy).[1,5] ADSCs (1×10^5 cells, 0.5 ml) were injected into the back skin wrinkles of mice in one of the irradiated groups, whereas fibroblasts (1×10^5 cells, 0.5 ml) were injected into the back skin wrinkles of another group. The results were obtained from the experiment using 1×10^4 and 1×10^5 of the cell count according to the previous studies.[5,9] Based on the findings from the previous studies, the 1×10^5 cells were

selected for the experiment. A 30-gauge needle was used for the injection of cells in 0.5 ml HBSS. We injected the cells parallel to deep wrinkles in a retrograde fashion. The cells were injected into intradermal, subdermal and panniculus adiposus layers. Three or 5 injections were administered per mouse. The results were compared after 4 weeks of injection. This animal experiment was approved by the Institutional Animal Care and Use Committee of the Seoul National University (IACUC Protocol No. 12-0352).

Skin replica and image analysis

The replicas of the upper back skin surfaces were prepared using a silicon-based impression material (Courage + Khazaka electronic GmbH, Koln, Germany). The replicas were cut into discs with a diameter of 1 cm, and flipped to achieve oblique illumination. The images of the negative replicas were observed using a high-resolution Visioline® VL 650 camera (Courage + Khazaka electronic GmbH, Koln, Germany) positioned to detect vertical shadows. The software measured the length and depth of the shadows, allowing for quantitative comparisons. We measured total wrinkle area, mean depth, total length, and maximal depth.

Histological analysis

Skin biopsies were performed for investigating the mechanisms of fibroblasts and ADSCs for reducing wrinkles. After 4 weeks of injection, injected skins (1 cm x 1 cm) were taken and fixed with a 10% formalin solution.[5] Then, they were embedded in wax and sectioned at 5 μm. We

carried out hematoxylin & eosin and Masson's trichrome staining. On hematoxylin & eosin-stained tissues, the thickness of dermis was measured at 5 places in 1 slide and the mean value was compared. The density of collagen was analyzed using an image analysis software (Leica Qwin V3 and Leica Microsystems CMS GmbH, Wetzlar, Germany) on tissues stained with Masson's trichrome. This was also measured at five places in one slide and the mean values were compared.

Real-time polymerase chain reaction (PCR) and western blot analysis

Ribonucleic acid (RNA) was isolated from specimens (50 mg) using TRIzol reagent (500 μl) and a homogenizer, resuspended in RNase-free water and quantified with a UV spectrophotometer.[9] Single-strand cDNA was prepared from 1 μg of total RNA in a 20-μl reaction volume using a TOPscript™ RT DryMIX (Enzynomics, Korea).[9] Real-time PCR was performed on an ABI 7500 machine with SYBR Premix Ex Taq™ (TaKaRa, Japan). PCR conditions were as follows: 30 s start at 95℃, followed by 34 cycles of 5 s at 95℃ and 34 s at 60℃.[22] The average threshold cycle for each gene was determined from triplicate reactions.[22] The gene expression levels were normalized to that of the beta-actin gene. The genes used are listed in Table 1.

Tissues lysates were centrifuged for 10 minutes at 14000 *g*, and the supernatants were used to perform western blot analysis.[5,9] Protein concentration was determined by a Bradford assay.[5]

The same amounts of proteins were loaded onto a 10% sodium dodecyl sulfate polyacrylamide gel.[5] The protein samples were transferred electrophoretically to polyvinylidene difluoride (PVDF) membranes.[5] Then, the membranes were blocked with 5% skim milk in Tris-buffered saline and Tween-20 and were incubated with the following antibodies: anti-mouse collagen type 1 (Millipore, Billerica, MA), anti-MMP 1 (Novus Biologicals Inc., Littleton, CO), anti-MMP 2, anti-MMP 3, anti-MMP 9, anti-MMP 13 and anti-Beta Actin (Abcam, Cambridge, MA). Proteins were visualized by enhanced chemiluminescence (Thermo Scientific, Rockford, IL).[9] All real-time PCR and western blots were performed three times per mouse gene or protein.

Immunohistochemical analysis

Sections of the tissue samples were cut and put on microscope slides.[23] Using the Discovery XT automated immunohistochemistry stainer (Ventana Medical Systems, Inc., Tucson, AZ), the slides were stained as described previously.[23] We used the Ventana Chromo Map Kit (Ventana Medical Systems) for detection.[23] The tissue sections were deparaffinized, and CC1 standard (pH 8.4 buffer contained Tris/Borate/EDTA) was used to retrieve the antigen.[23] Inhibitor D (3% H_2O_2, Endogenous peroxidase) was plugged in for 4 min at 37℃.[24] The slides were incubated with anti-Tropoelastin (Abcam, Cambridge, MA) for 32 min at 37℃, and a secondary antibody, Omimap anti-mouse HRP, for 20 min at 37℃.[24] Other slides were incubated with anti-fibrillin-1 (Abcam, Cambridge, MA) for 32 min at 37℃, and a an Omimap anti-mouse HRP secondary

antibody for 20 min at 37°C.[24] The slides were incubated in DAB + H_2O_2 substrate for 8 min at 37°C followed by hematoxylin and bluing reagent counterstaining at 37°C.[24] For washing solution, a Tris buffer at pH 7.6 was used.[24] The stained slides were evaluated by densitometric analysis using an image analysis software (Leica Qwin V3 and Leica Microsystems CMS GmbH, Wetzlar, Germany). This was also measured at five places in one slide, and the mean values were compared.

Statistical analysis

The statistical significance of the results was analyzed using the nonparametric Mann-Whitney tests. SPSS Version 21.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis and a *p*-value of < 0.05 was considered statistically significant.

Table 1. Genes included in the comparison

Gene
Procollagen-sense
Procollagen-antisense
MMP 1-sense
MMP 1-antisense
MMP 2-sense
MMP 2-antisense
MMP 3-sense
MMP 3-antisense
MMP 9-sense
MMP 9-antisense
MMP 13-sense
MMP 13-antisense
beta-actin- sense
beta-actin-antisense

MMP, matrix metalloproteinase.

RESULTS

Characterization of fibroblasts and ADSCs

Human tissue samples were obtained from a 43-year-old woman undergoing transverse rectus abdominus myocutaneous (TRAM) flap surgery for breast reconstruction. ADSCs were cultured from fat tissue and fibroblasts were cultured from dermis without any problems. ADSCs and fibroblasts were identified by comparison with their typical morphology, and we confirmed the molecular phenotype of cells using the fluorescence-activated cell sorting (FACS) scanner.[13] The ADSCs expressed CD13, CD73 and CD 105 but did not express CD14, CD19, CD34, CD45 and HLA-DR (data not shown).

Skin replica and image analysis

We found a significant difference between the 7 mice in the normal control group and the 21 mice in the 3 UVB-irradiated groups ($p < 0.02$, Mann-Whitney tests). After 4 weeks of irradiation, the wrinkles decreased slightly, but remained in the irradiated control group. After 4 weeks of injection of ADSCs or fibroblasts, the wrinkles decreased (Fig. 1). After 4 weeks of cell injection, the ratio of the changes in the total wrinkle area was 0.24 ± 0.12 in the ADSC group and 0.18 ± 0.14 in the fibroblast group. The ratio in the irradiated control group was 0.61 ± 0.15 versus 1.08 ± 0.19 in the normal control group. ADSCs or fibroblasts, the total area of

wrinkles were significantly smaller (p = 0.034 and p = 0.018, respectively) than that in irradiated control group. There was no statistically significant difference in the wrinkle area between the fibroblast and ADSC groups (p = 0.270, Fig. 2). The mean depth levels of wrinkle patterns were similar in all groups.

Histological findings

The average thickness of the dermis in the ADSC group was 248.93 ± 28.62 μm, whereas in the fibroblast group, it was 243.51 ± 29.52 μm. The average thickness in the irradiated control group was 217.58 ± 42.84 μm versus 184.17 ± 31.26 μm in the normal control group. The dermal thickness assessed using hematoxylin & eosin staining was significantly higher in both treatment groups than in the irradiated control group (ADSC group, $p < 0.001$; fibroblast group, p = 0.01). However, there was not a statistically significant difference between the dermal thickness in the ADSC-injected and fibroblast-injected groups (p = 0.49).

The density of collagen was reduced after UVB irradiation. The average density of collagen according to image analysis of Masson's trichrome staining was 86.19 ± 9.11% in the ADSC group, and 68.74 ± 8.37% in the fibroblast group. Collagen density in the irradiated control group was 45.16 ± 9.45% versus 66.31 ± 15.38% in the normal control group. The density of collagen was significantly increased in both groups compared to the irradiated control group (ADSC group, $p < 0.001$; fibroblast group, $p < 0.001$). When compared with the fibroblast

group, the ADSC group had a statistically significant increase in collagen density ($p < 0.001$, Fig. 3).

Real-time PCR and western blot analysis

Real-time PCR revealed that the transcription of type I procollagen decreased significantly after UVB irradiation. The ratio for the normal control group was 0.85 ± 0.12. After injection of cells, the transcription of type I procollagen increased significantly in both groups. The ratio for normal control group was 1.12 ± 0.24 ($p = 0.046$) in ADSC group versus 1.67 ± 0.29 in the fibroblast group ($p = 0.028$). Type I procollagen mRNA level was higher in the fibroblast-injected skin than in the ADSC group ($p = 0.018$, Fig. 4). The collagen type I expression pattern revealed by western blot analysis was consistent with the real-time PCR results. The expression of collagen type I decreased significantly after UVB irradiation. The expression ratio for ß-actin was 0.11 ± 0.02 in the normal controls and 0.07 ± 0.03 in the irradiated controls. After cell injection, the expression increased significantly in both groups. The ratio for ß-actin in the ADSC group was 0.15 ± 0.05 ($p = 0.01$) versus 0.45 ± 0.09 in the fibroblast group ($p < 0.001$). The increase in the fibroblast group was significantly higher than in the ADSC group ($p < 0.001$, Fig. 5). Expression of MMP 13 increased significantly after UVB irradiation. The expression ratio for ß-actin was 0.13 ± 0.07 in the normal control group and 0.61 ± 0.12 in the irradiated controls. After ADSC injection, MMP 13 expression decreased (ratio 0.30 ± 0.15), whereas

after fibroblast injection, it increased (ratio 0.68 ± 0.14, Fig. 6). MMP 1 and MMP 2 expression showed a similar pattern to that of MMP 13, although they were not statistically significant (Fig. 7).

Immunohistochemical analysis

The average density of tropoelastin was 11.68 ± 3.71% in the normal controls versus 20.81 ± 6.27% in the irradiated controls. The amount of tropoelastin was significantly increased after UVB irradiation. The levels decreased after ADSC (17.10 ± 3.78%) or fibroblast (19.25 ± 4.16%) injection. Moreover, the amount of tropoelastin was significantly lower after ADSC injection than in the irradiated control group ($p = 0.011$, Fig. 8). The average density of fibrillin-1 was 15.19 ± 4.32% in the normal controls and 3.64 ± 1.78% in the irradiated controls. Fibrillin-1 was significantly decreased after UVB irradiation and increased after cell injection in both groups (ADSC group, 11.28 ± 4.22%, $p < 0.001$; fibroblast group, 8.75 ± 3.28%, $p < 0.001$). The amount of fibrillin-1 was significantly higher after ADSC injection than after fibroblast injection ($p = 0.004$, Fig. 9).

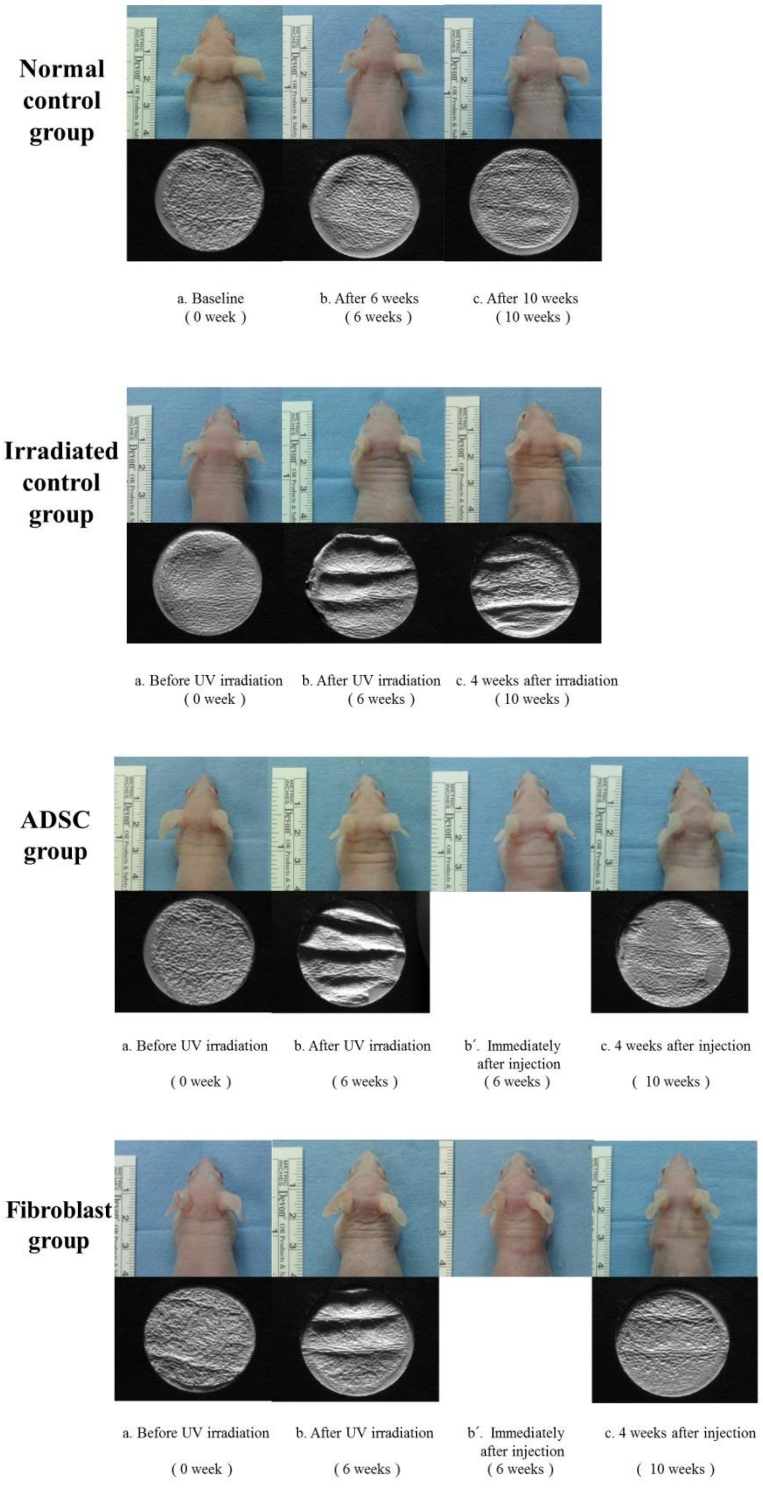

Figure 1. Animal study photograph and an image of the skin replica. (A) Normal control group.

(B) Irradiated control group. After 4 weeks of irradiation, the wrinkles decreased slightly, but

remained in the irradiated control group. (C) ADSC group. We found that there was a significant difference before and after ultraviolet B irradiation. Four weeks after ADSC injection, the wrinkles were decreased. (D) Fibroblast group. The wrinkles also decreased 4 weeks after fibroblast injection. ADSC, adipose-derived stem cell; UV, ultraviolet.

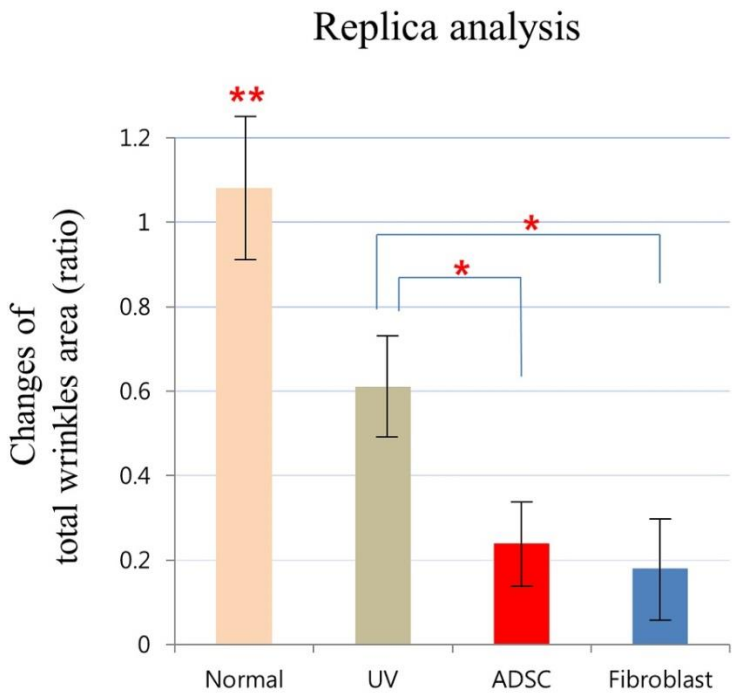

Figure 2. Skin replica analysis. Four weeks after injection, the ratio of changes in total wrinkle area was 0.24 ± 0.12 in the ADSC group and 0.18 ± 0.14 in the fibroblast group. The ratio in the irradiated controls was 0.61 ± 0.15 versus 1.08 ± 0.19 in the normal controls. After 4 weeks of injection of ADSCs, the total area of wrinkles significantly decreased (*p = 0.034). The total area of wrinkles in fibroblast group also statistically significantly decreased after 4 weeks (*p = 0.018). There was no statistically significant difference between the 2 groups (p = 0.270). UV, ultraviolet; ADSC, adipose-derived stem cell. **Statistically significant between all three groups (p < 0.001).

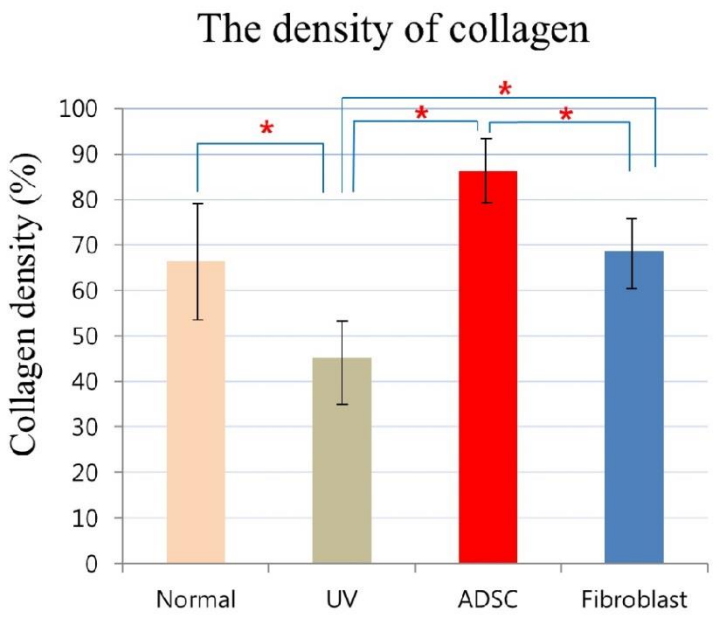

Figure 3. The density of collagen. Average collagen density in the normal control group was 66.31 ± 15.38% versus 45.16 ± 9.45% in the irradiated controls. The density of collagen was reduced after UVB irradiation. After 4 weeks injection, the average density of collagen in the ADSC group was 86.19 ± 9.11% and that of the fibroblasts group was 68.74 ± 8.37%.The density of collagen was significantly increased in both groups compared to the irradiated control group (ADSC group, *$p < 0.001$; fibroblast group, * $p < 0.001$). When compared with the fibroblast group, the ADSC group had a statistically significant increase in collagen density (*$p < 0.001$). UV, ultraviolet; ADSC, adipose-derived stem cell.

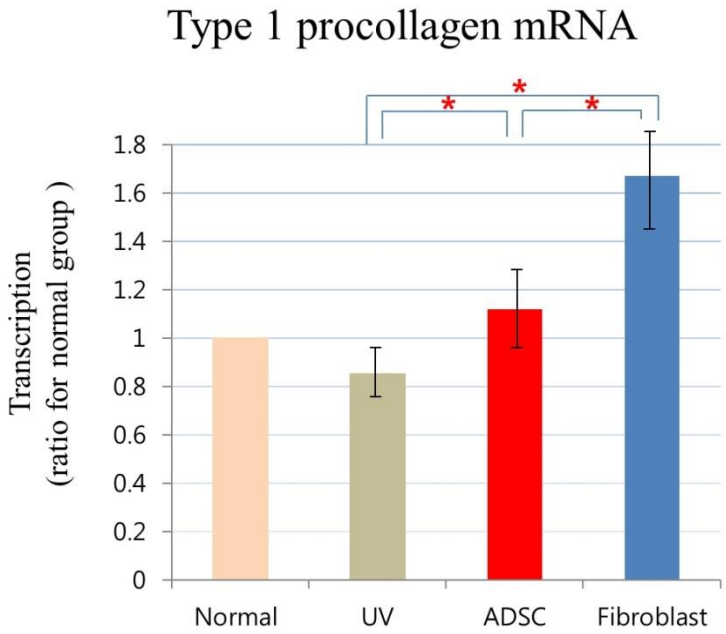

Figure 4. Type I procollagen mRNA assessed using real-time PCR. The transcription of type I procollagen mRNA was significantly decreased after UVB irradiation. The ratio for the normal control group was 0.85 ± 0.12. After cell injections for 4 weeks, type I procollagen mRNA increased significantly in both groups. The ratio for normal control group was 1.12 ± 0.24 (*p = 0.046) in ADSC group versus 1.67 ± 0.29 in the fibroblast group (*p = 0.028). The transcription was higher in the fibroblast-injected group than in the ADSC group (*p = 0.018). RNA, ribonucleic acid; PCR, polymerase chain reaction; UV, ultraviolet; ADSC, adipose-derived stem cell.

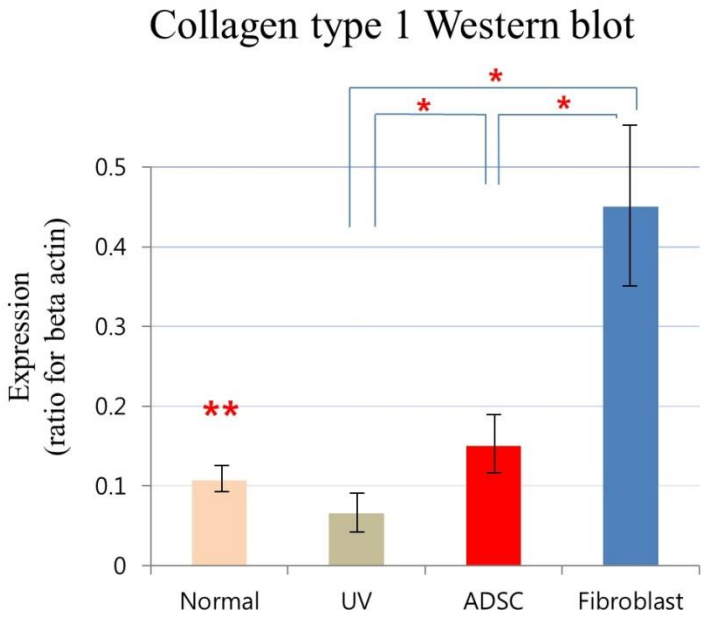

Figure 5. Collagen Type I was visualized on western blots. The expression of collagen type I was significantly decreased after UVB irradiation. The expression ratio for ß-actin in the normal control group was 0.11 ± 0.02 versus 0.07 ± 0.03 in the irradiated controls. Then after injection of cells, collagen type I increased significantly in both groups. The ratio for ß-actin in the ADSC group was 0.15 ± 0.05 (*p = 0.01) versus 0.45 ± 0.09 in the fibroblast group (*p < 0.001). The fibroblast group had a statistically significant increase over the ADSC group (*p < 0.001). UV, ultraviolet; ADSC, adipose-derived stem cell. **Statistically significant versus the UV and fibroblast groups (p =0.028, p < 0.001, respectively).

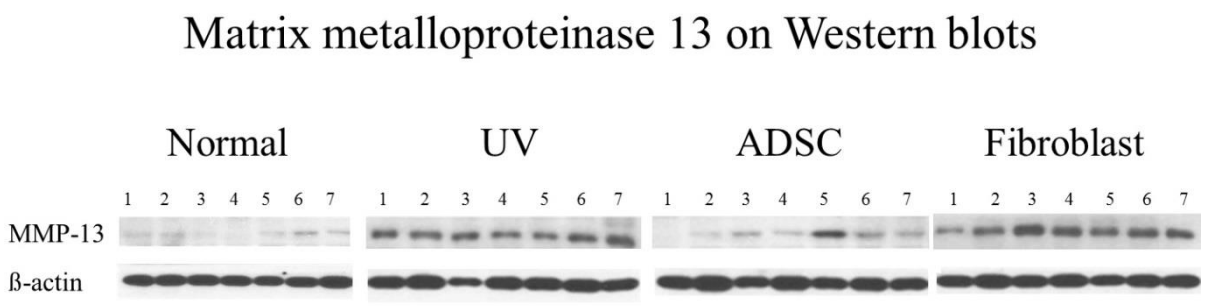

Figure 6. Matrix metalloproteinase (MMP) - 13 on western blots. Expression of MMP 13 was significantly increased after UVB irradiation. The expression ratio for ß-actin was 0.13 ± 0.07 in the normal controls and 0.61 ± 0.12 in the irradiated controls. While expression of MMP 13 was decreased in the ADSC group (ratio 0.30 ± 0.15), MMP expression was increased in the fibroblast injection group (ratio 0.68 ± 0.14). UV, ultraviolet; ADSC, adipose-derived stem cell

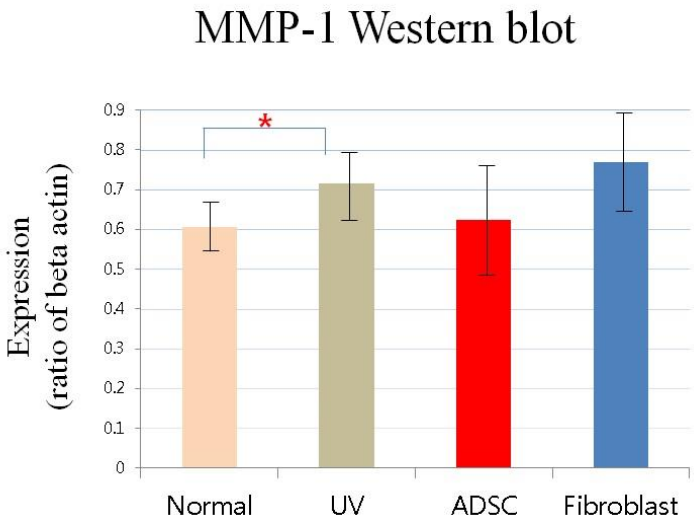

Figure 7. Matrix metalloproteinase (MMP) - 1 was visualized on western blots. Expression of MMP 1 significantly increased after UVB irradiation (*$p < 0.05$). While expression of MMP 1 decreased in the ADSC group, MMP expression increased in the fibroblast injection group. MMP 1 showed similar patterns to MMP 13, although they were not statistically significant. UV, ultraviolet; ADSC, adipose-derived stem cell.

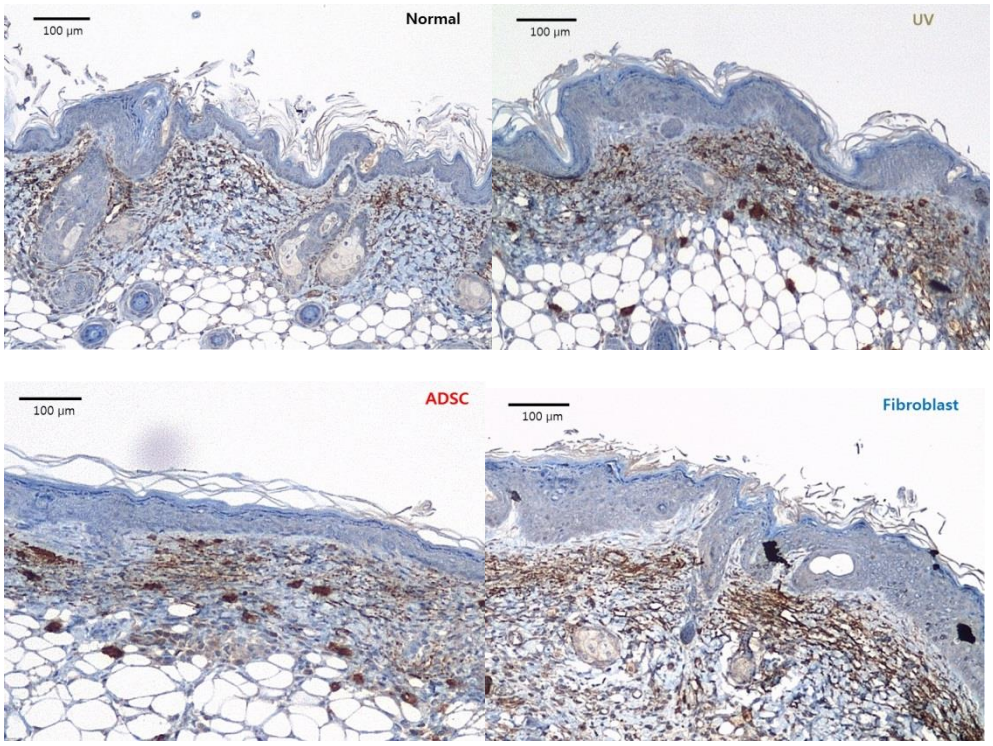

Figure 8. Tropoelastin immunohistochemistry analysis. (*Left, above*) normal control group, (*right, above*) irradiated control group, (*left, below*) ADSC group, (*right, below*) fibroblast group. The average density of tropoelastin was 11.68 ± 3.71% in the normal controls and 20.81 ± 6.27% in the irradiated controls. The levels decreased after ADSC (17.10 ± 3.78%) or fibroblast injection (19.25 ± 4.16%). Moreover, the amount of tropoelastin was significantly lower after ADSC injection than in the irradiated control group (p = 0.011). Scale bars represent 100 μm. Original magnifications: 100 ×. UV, ultraviolet; ADSC, adipose-derived stem cell.

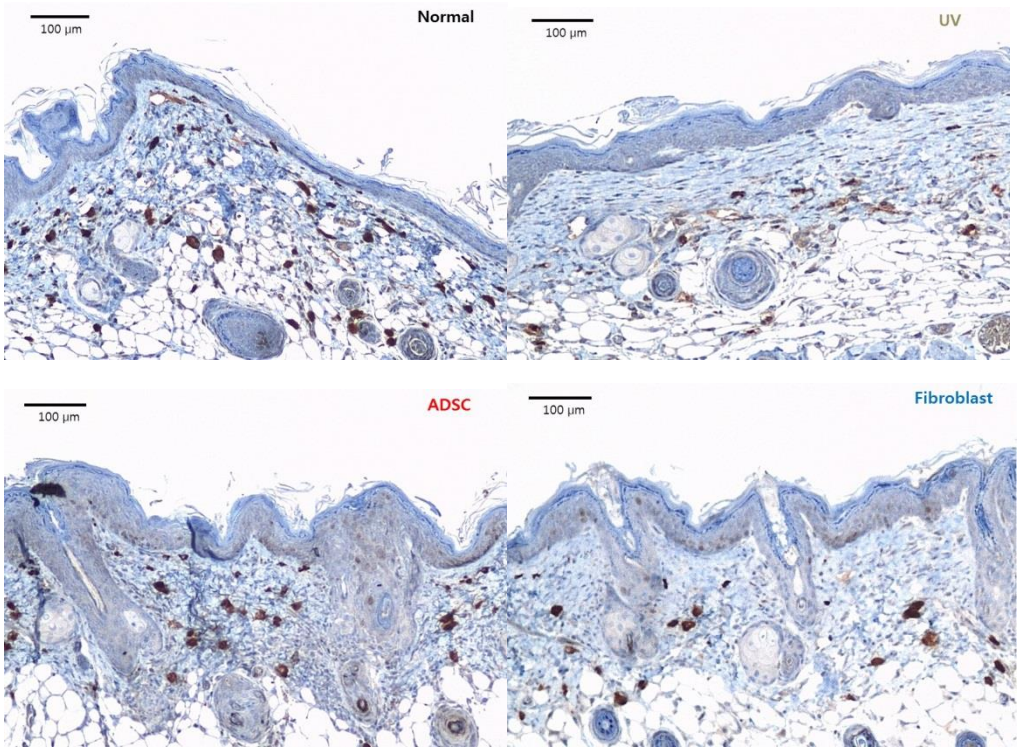

Figure 9. Fibrillin-1 Immunohistochemistry analysis. (*Left, above*) normal control group, (*right, above*) irradiated control group, (*left, below*) ADSC group, (*right, below*) fibroblast group. The average density of fibrillin-1 was 15.19 ± 4.32% in the normal control group and 3.64 ± 1.78% in the irradiated control group. Fibrillin-1 increased after injection of cells in both groups (ADSC group, 11.28 ± 4.22%, $p < 0.001$; fibroblast group, 8.75 ± 3.28%, $p < 0.001$). The amount of fibrillin-1 was significantly higher after ADSC injection than in the fibroblast injection group ($p = 0.004$). Scale bars represent 100 μm. Original magnification: 100 ×. UV, ultraviolet; ADSC, adipose-derived stem cell.

DISCUSSION

Interest in research on cell therapies with ADSCs and fibroblasts for wrinkle reduction and tissue repair has been increasing.[5,7-17,25,26] It is known that in the wrinkles of photoaged skin, ADSCs increase collagen synthesis by secreting a variety of cytokines, which activate fibroblasts.[5,8,27] These experimental studies mostly used mouse models for photoaged wrinkles, replica analysis, and histologic examination.[2,5,8,28] Mice are genetically similar to humans and are easy to manage experimentally. Mice are considered good animal models for the replica analysis known as profilometry, which is used for studying the chronological aging and photoaging of skin.[2] The replica score was better when irregularities were minimized. This may be the reason why wrinkle scores of the fibroblast group were lower than those of the ADSC group, even though dermal thickness and density of collagen were higher in the ADSC group. The thickness of the dermis, as well as the percentage area of collagen, are typically observed by histology only in the photoaging model because no changes in these characteristics have been noted in the skin of chronologically aging animals.[2,29] Type III collagen is replaced by type I collagen during open wound healing process. Because this study does not focus on open wounds, the authors analyzed only type I collagen, which is consistent with how other studies examine this phenomenon.[5,9]

Most photoaging wrinkle models are hairless mouse models, and many analyses have been performed using various filler and cell injections.[2,5,9,26,30,31] However, in order to offer more clinical meaning, it may be appropriate to use nude mice for xenograft research involving human cells.[32] To our knowledge, this is the first application of a photoaging model established using nude mice, and this research provides the basis for future studies on human cells. Since the MED is higher in the nude mouse than in the hairless mouse, high amounts of UV must be used, and unlike the hairless mouse protocol, which requires irradiation 3 times a week, it is important to irradiate more frequently due to faster recovery in nude mice. Of course, we should think about the immunity of nude mouse. The BALB/c nude mouse is a genetic mutant, which has removed thymus gland, resulting in lack of immune system with a reduced number of T cells.[33-35] There were previous studies on the changes of immunity after cell injection in nude mouse.[36-40] Several researches reported that ADSC have low immunogenicity and immunoregulatory action by suppressing the functions of immune cells.[36-38] It is known that the injection of fibroblast had immunomodulatory properties, although it is controversial whether it makes immunosuppression or increases immune response.[39,40]

Although there are studies on ADSCs, no specific studies have analyzed the effects of fibroblast injection on photoaged wrinkles.[16,17] Thus we aimed to observe the effects of directly injecting fibroblasts and to determine if there is a significant difference in injecting ADSCs to

activate fibroblasts indirectly. Moreover, wrinkle reduction and ADSC studies mainly focus on collagen even though the role of elastic fibers cannot be ignored. There have been several reports about the roles of elastic fibers in skin wrinkle formation.[18,41,42] Elastin and fibrillin are 2 major components of elastic fibers. Fibrillins are decreased by proteolysis, and are known to be major components of microfibrils, which are believed to regulate and organize the elastic fibers by making a scaffold for tropoelastin deposits.[18,41-43] In photoaging, conversion of tropoelastin to elastin is impaired, leading to accumulation of precursor tropoelastin.

In ADSC-related studies, fibroblasts have been known to play a major role in cell therapy for the purposes of tissue repair and the reduction of wrinkles.[5-8] There have also been studies regarding the use of fibroblasts in wrinkle reduction and in reducing the depth of depressions.[10-17] Fibroblasts produce proteoglycan, elastin, and other matrix proteins.[44] However, it is equally important that these cells also express the MMPs that negatively affect collagen.[4,45-49] The epidermal and dermal cells break down proteins to create MMP enzymes, which then activate the enzymes that break down the collagen that connects cells in the dermis.[48] In this study, the most important difference in the mechanisms of fibroblasts and ADSCs was the expression pattern of MMPs. Although studies regarding wide varieties of MMPs were conducted, the functional relevance of each MMP is not definitively known and the associations between MMPs are still unclear.[4,5,8,9,44] To study variety of factors related to MMPs, the authors checked

MMPs 1, 3,and 9, which are known as collagenases, and MMPs 2, and 9, which are known as gelatinases.[46] MMP 13 was also checked, because MMP 13 is the primary collagenase found in rodents.[46,47] In this study, the results showed that expression of MMP 13 was statistically significantly decreased in the ADSC group, but significantly increased in the fibroblast injection group. MMP 1 and MMP 2 also showed patterns similar to MMP 13, although they were not statistically significant. Verifying the associations between each MMP and placing significance on this result is not easy, and this is undoubtedly one of the limitations of this study and a potential area of future research.

The ADSC injection resulted in more remarkable results than the fibroblast injection. We believe that the key is MMP. Although the increased expression of collagen was higher in the fibroblast injection group, we found it to be less efficient for wrinkle reduction than ADSCs due to an increased expression of MMP. ADSCs activated collagen production and elastic fiber production while fibroblasts activated collagen remodeling. In general, the collagen remodeling is defined as phenomenon when old collagen is destroyed and a new collagen is produced. However, the pattern seen after fibroblast injection also could be called collagen remodeling. We only investigated the effects of simple cell injections in this study, and we predict that studies examining other types of injections, like growth factors, will have different results. If we are able to reduce the expression of MMPs when we inject fibroblasts, we would expect

fibroblast injection to be a more efficient method for generating collagen than ADSCs. We were able to confirm that wrinkle reduction is influenced by a variety of factors using mechanisms different than just the simple activation of fibroblasts through ADSCs. A variety of additional studies can be conducted based on our study. The injection of cultured ADSCs and fibroblasts could play a vitally important role in anti-aging therapies that are distinct from filler injections.

CONCLUSION

Cultured ADSCs and fibroblasts likely play an important role in wrinkle reduction albeit via different mechanisms. ADSCs activated collagen and elastic fiber production, while fibroblasts activated collagen remodeling. Based on this study, we revealed a new knowledge of the clinical application that it is advantageous to inject ADSCs on depressed scars and to inject the fibroblasts on scars with irregularity. It is recognized as a novel finding that contribute to the development of anti-aging cell therapy.

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국문 초록

배양 인간섬유모세포와 지방줄기세포의 광노화 피부주름 개선효과에 대한 비교

서론: 배양 인간 섬유아세포와 지방유래줄기세포의 광노화 피부주름 개선효과를 비교하고 주름 개선의 작용 기전을 조사하였다.

방법: 사람의 조직으로부터 섬유아세포와 지방유래줄기세포를 분리하여 배양하였다. 6주령 BALB/c 누드마우스 총 28마리를 4개의 그룹으로 분류하였다. 첫 번째 그룹은 정상 대조군으로 하고, 나머지 3개의 그룹은 자외선 B 를 이용하여 한 주에 6회 총 6주를 조사하여 광노화 주름을 만들었다. 이 중 한 그룹에는 지방유래줄기세포를 주입하고, 다른 그룹에는 섬유아세포를 주입하였다. 나머지 한 그룹은 광노화 음성 대조군으로 두었다. 주입 4주후, 주름 복제본 분석(replica analysis)을 하고, 조직검사를 시행하여 진피 두께, 콜라겐 밀도를 측정하였다. 또한 중합효소연쇄반응과 웨스턴블롯검사를 통해 1형 콜라겐과 금속단백분해효소(matrix metalloproteinases)－ 1, 2, 3, 9, 13을 측정하고, 면역조직화학검사를 통해 풋탄력소(tropoelastin)와 피브필(fibrillin)－1을 평가하였다.

결과: 복제본 분석에서는 지방유래줄기세포 그룹과 섬유아세포 그룹 모두에서 주름이 감소하였다. 지방유래줄기세포 그룹에서는 콜라겐 생성이 촉진되고 금속단백분해효소의 발현이 억제되었다. 섬유아세포 그룹에서는 지방유래줄기세포 그룹에 비해 더 많은 콜라겐 생성을 보였지만, 금속단백분해효소의 발현 또한 증가하였다. 전체적으로, 지방유래줄기세포 그룹에서 더 높은 콜라겐 밀도를 보였고, 풋탄력소와 피브릴린-1 에서도 더 좋은 결과를 보였다.

결론: 배양 섬유아세포와 지방유래줄기세포는 작용 기전의 차이가 있지만, 광노화 주름개선효과가 있음을 확인하였다.

주요어 : 줄기세포, 중배엽 줄기세포, 섬유모세포, 광노화

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