Adipose-Derived Stem Cells and Their Secretory Factors as a Promising Therapy for Skin Aging

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BACKGROUND Adipose-derived stem cells (ADSCs) and their secretory factors can stimulate collagen synthesis and migration of fibroblasts during the wound healing process. Conventional treatments for skin aging, such as lasers and topical regimens, induce new collagen synthesis via activation of dermal fibroblasts or growth factors. Considering the results of our previous study, ADSCs can also be used for the treatment of skin aging.

OBJECTIVE The aim was to verify the effectiveness of ADSCs for the treatment of skin aging.

METHODS We analyzed secretory factors of ADSCs and intradermally injected ADSCs (1×10^6 cells in 1 mL of Hanks' buffered salt solution) and conditioned media of ADSCs on the back of a micropig. In addition, as a pilot study, intradermal injections of purified autologous processed lipoaspirate (PLA) cells were tried with the photoaged skin of one patient.

RESULTS We demonstrated that ADSCs produce many useful growth factors, increase collagen production in animal study, and reverse skin aging in human trial.

CONCLUSION ADSCs and their secretory factors show promise for application in cosmetic dermatology, especially in the treatment of skin aging.

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The concept of regenerative medicine, using the body's own stem cells and growth factors to repair tissue, is an alternative therapeutic strategy for damaged tissue repair and is becoming a major candidate for cell therapy.[1] Adipose-derived stem cells (ADSCs) are pluripotent mesenchymal stem cells, which have characteristics similar to bone marrow–derived mesenchymal stem cells. In addition, compared with bone marrow–derived mesenchymal stem cells, ADSCs can be acquired in large quantities with a simple surgical procedure.[2] There have been many clinical applications for mesenchymal stem cells, including ADSCs for compensation of skin defects and wound healing.[3–5] In a previous study, our group demonstrated that ADSCs, and conditioned media of ADSCs (ADSC-CM), stimulated collagen synthesis and migration of fibroblasts during the wound healing process. These results may be associated with numerous other factors produced by ADSCs (accepted by the *Journal of Dermatologic Science*). Conventional treatments for skin aging, such as laser and topical regimens, induce new collagen synthesis via activation of dermal fibroblasts or growth factors.[6] Considering the results of our previous study, ADSCs can also be used for the treatment of skin aging.[5] To verify this, we conducted in vitro and in vivo animal studies, including a pilot study using volunteer and fresh autologous ADSCs.

For the in vitro study, adipose tissue samples were obtained through elective liposuction with informed consent. After isolation of the ADSCs, as mentioned in many previous reports, we depicted ADSCs by

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flow cytometric characterization. For the analysis of the secretory factors of ADSCs, ADSCs (4×10^5 cells) were plated on a 100-mm dish overnight with a control medium and were cultured in a Dulbecco's modified Eagle's medium/F12 (Invitrogen-Gibco-BRL, Grand Island, NY) serum-free medium. CM was collected after 72 hours of culture, centrifuged at 300 rpm for 5 minutes, and filtered through a 0.22-μm syringe filter. The concentration of cytokines and extracellular matrix proteins were measured using sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN); vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor (TGF)-β1, TGF-β2, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF)-AA, placenta growth factor, type I collagen, and fibronectin, as shown in (Table 1). In addition, superoxide dismutase (SOD) was observed in our proteomic analysis (data not shown).

TABLE 1. Concentration of Cytokines and Extracellular Matrix Proteins

Proteins	*Concentration*
PDGF	44.41 ± 2.56 pg/mL
bFGF	131.35 ± 30.31 pg/mL
KGF	86.28 ± 20.33 pg/mL
TGF-β1	103.33 ± 1.70 pg/mL
HGF	670.94 ± 86.92 pg/mL
VEGF	809.53 ± 95.98 pg/mL
Type I collagen	921.47 ± 49.65 ng/mL
Fibronectin	1466.48 ± 460.21 ng/mL

To study the effects of ADSCs on in vivo skin, we intradermally injected ADSCs (1×10^6 cells in 1 mL Hanks' buffered salt solution [HBSS]) and ADSCs-CM on the back of a micropig, twice in a 14-day interval ($n = 3$). One month after the second injection, we obtained skin samples at the injection site and used normal skin as a control. Histologic evaluation showed a small, but not definite, increase of dermal thickness. A distinctive increase in collagen expression, however, was observed in the injected skin samples by ADSCs and ADSCs-CM in the Western blot study (Figure 1). In addition, as a pilot study, intradermal injections of purified autologous processed lipoaspirate (PLA) cells (1×10^6 cells in 1 mL of HBSS solution), which contains approximately 20% to 30% ADSCs, were tried with the photoaged skin of one patient. She initially visited our clinic for liposuction and gave consent for the trial after understanding the expected effects of using the ADSCs and the low risk of using autologous PLA

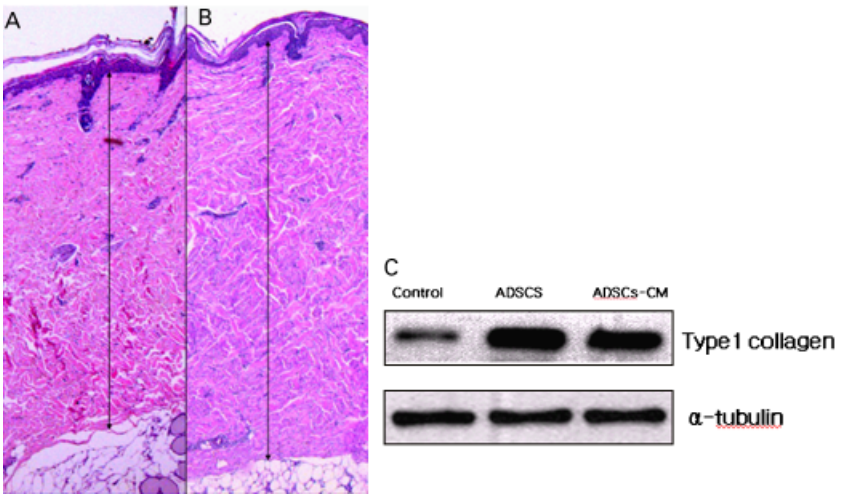

Figure 1. Micropig experiment. Histopathologic findings for the back of micropig without (A) and with (B) the treatment of ADSCs (1×10^6 cells) in a 14-day interval. Original magnification, × 40. Increased collagen expression was determined by Western blot in the ADSC- and ADSC-CM–treated groups (C).

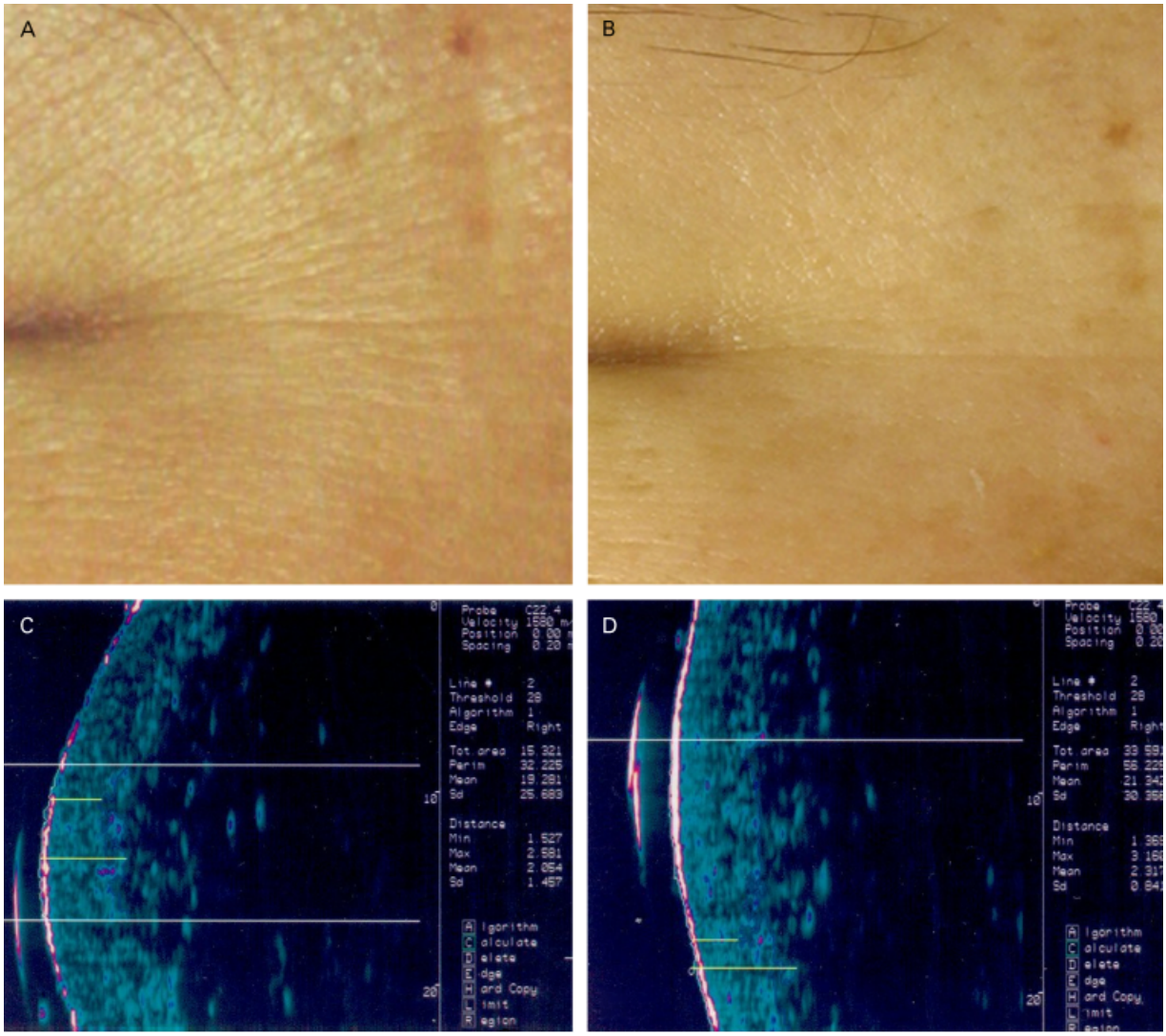

Figure 2. Clinical study using intradermal injections of autologous PLA cells. Medical photographs of periorbital wrinkle were taken (A, before treatment; B, after treatment) and dermal thickness was measured by an ultrasonograph (C and D). Improved skin texture and dermal thickness (2.054 mm vs. 2.317 mm) were clearly observed 2 months after injection of PLA cells (B and D).

cells. She had two successive injections at 2-week intervals and showed improvement of general skin texture and wrinkles 2 months after the second injection. These positive results are clearly demonstrated through a comparison of medical photographs and dermal thickness using a 20 MHz high frequency ultrasonograph (Dermascan-C, Cortex, Hadsund, Denmark; Figure 2).

For the proper application of ADSCs or secretory factors of ADSCs for skin aging, more studies about the action mechanisms of ADSCs on skin and standard application method of ADSCs are necessary. This study does not clarify the exact mechanism of the regenerative effect of the ADSCs. These results, however, may be accomplished by neocollagen synthesis of the ADSCs themselves and the protective effect of SOD and the growth factors on skin fibroblast.

Based on this pilot study, we are planning supplemental in vitro experiments and human studies.

In conclusion, although preliminary, the results from this study suggest that ADSCs and their secretory factors show promise for application in cosmetic dermatology, especially in the treatment of skin aging.

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