Adipose-Derived Stem Cells and Their Secretory Factors for Skin Aging and Hair Loss

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Contents

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Abstract

Human mesenchymal stem cells, by virtue of its capability to self-renew and differentiate into a variety of cell types, represent the first pluripotent stem cells to be used in clinical settings related to damage or degeneration. Therefore, there is an urgent need to understand how mesenchymal stem cells and their secretory factors contribute to regenerative medicine. Recent studies on the role of stem cells for skin and hair regeneration by many researchers including the authors have been remarkable. These scientific data enabled us to achieve the cost-effective treatment of skin aging using the legally acceptable cell therapeutic agents and their secretory factors. Objective data on the improvement of diverse aspects of skin aging including wound healing, wrinkle, and melasma due to photoaging have been available. Another progress has been made using the protein extract of the mesenchymal stem cells from the adipose tissue to promote hair growth in vitro, ex vivo, and in vivo by modulating the follicular cell cycles and hair cycle and protecting the follicular cells from androgens and reactive oxygen species. These approaches might mark the first practical application of stem cells among various trials in the field of skin and hair regeneration.

Introduction

The term "stem cell" has attracted increasing attention of the scientific community as well as of the general public. Overcoming confusion and difficulty to understand and interpret information about stem cells, much effort has been made in the field of skin and hair regeneration. They are vital to humans for numerous reasons. Groups of stem cells in some adult tissues give rise to replacement cells for the tissues that are destroyed through injury, disease, or aging [1]. Knowledge relating to how healthy cells replace diseased or otherwise damaged cells would allow development of medical therapies focusing on creation of compatible cell lines to replace aged or diseased cells in the body. The concept of regenerative medicine using the body's own stem cells and growth factors to repair tissue may be realizable as science and clinical experience converge to develop alternative therapeutic strategies to treat the damaged or diseased tissue. Stem cell-based therapies are also being tried in tissue engineering: the aim of tissue engineering is to repair and regenerate damaged organs or tissues using a combination of cells, biomaterials, and cytokines [1–4].

This chapter addresses the human subcutaneous adipose tissue as a promising source of adult mesenchymal stem cells. Adipose-derived stem cells (ADSCs) may offer a solution for the problem of limited availability of human cells that are capable of self-renewal and differentiation. ADSCs can be easily obtained from liposuction of human adipose tissue, cultured in a large scale, and display multi-lineage developmental plasticity. In addition, ADSCs secrete various cytokines and growth factors, which control and manage the damaged neighboring cells, and this has been identified as essential functions of ADSCs [5–7]. As reviewed elsewhere in this book, aging and photoaging are complex processes involving the wound-healing cascade and/or repetitive oxidative stress. Conventional antiaging skin treatments such as light-based or radiofrequency devices and/or peelings have been less than satisfactory because their primary mechanism is mainly inducing new collagen synthesis via activation of dermal fibroblasts. On the basis of previous studies that demonstrated wound healing, antioxidant, anti-wrinkle, and antimelanogenic effects of ADSCs and their secretory factors, they may be good candidates for the treatment of aging [5–9]. Another progress has been made in the field of hair growth using the ADSC protein extract [10, 11]. It was revealed that the protein extract promotes hair growth by modulating the follicular cell cycles and hair cycle and protecting the follicular cells from androgens and reactive oxygen species (ROS) [12, 13]. This chapter compiles the authors' recent research and clinical developments on skin and hair regeneration using ADSCs and their secretory factors.

Stem Cells and ADSCs

Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of multi-lineage differentiable cells for tissues. Although embryonic stem cell has multipotency, there are many limitations such as difficulties in control of differentiation and issues relating to ethics. As a result, use of adult stem cells with fewer implicating issues is becoming an area of increased interest in stem cell medicine. Given the vast potential of treatments utilizing stem cells, validation and evaluation regarding safety and efficacy will result in greater benefits.

ADSCs and Regeneration

Due to the lack of a specific and universal molecular marker for adult stem cells, functional assays for multiple differentiations must be used to identify stem cells in a tissue. Mesenchymal stem cells (MSCs) were first characterized in bone marrow, but many studies have reported the existence of MSCs in the connective tissue of several organs [14, 15]. The role of these cells is not entirely clear, but they are generally believed to constitute a reserve for tissue maintenance and repair. It was recently demonstrated that the most abundant and accessible source of adult stem cells is adipose tissue. The yield of MSCs from adipose tissue is approximately 40-fold greater than that from bone marrow [16–18].
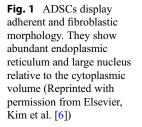
The following are the highly consistent, although not identical, expression profiles of cell-surface proteins on ADSCs [2, 19]: adhesion molecules, receptor molecules, surface enzymes, extracellular matrix (ECM) proteins, and glycoproteins. However, hematopoietic cell markers such as CD14, CD31, and CD45 are not expressed. Interestingly, the immunophenotype of ADSCs resembles that reported for other adult stem cells prepared from human bone marrow (bone marrow stromal cell [BMSC]) and skeletal muscle [2]. Differentiation of ADSCs is not restricted to the adipocyte lineage, but they can be differentiated into chondrocyte, osteocyte, cardiomyocyte, neuron, etc. [20, 21]. In addition, activity comparison with BMSC revealed a similar regenerative capacity. Therefore, this abundant and accessible cell population has potential clinical utility for regenerating damaged or aged tissue and tissue engineering.
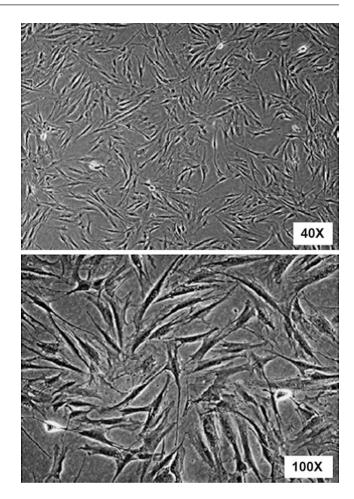
As with many rapidly developing fields, diverse names have been proposed to describe the plastic-adherent cell population isolated from collagenase digests of adipose tissue: adipose-derived stem/stromal cells, adipose-derived adult stem cells, adipose-derived adult stromal cells, adipose stromal cells (ASCs), adipose mesenchymal stem cells (AdMSCs), lipoblast, pericyte, preadipocyte, processed lipoaspirate (PLA) cells, and stromal vascular fraction (SVF) cells. To address the confusion due to diverse nomenclature, the International Fat Applied Technology Society reached a consensus to adopt the term "adipose-derived stem cells" to identify the isolated, plastic-adherent, multipotent cell population. Questioning the validity of the term "stem cell" led to the use of the acronym to mean "adipose-derived stromal cells" [22].

Although studies are limited, the quality and quantity of the ADSCs varies according to interperson differences, the harvest site, harvesting method, and culture conditions. Age and sex are the most obvious of the interperson differences. Stem cell recovery varies between subcutaneous white adipose tissue depots [23, 24]. Yield and growth characteristics of ADSC (Fig. 1) are also affected by the type of surgical procedure used for adipose tissue harvesting. Resection and tumescent liposuction seem to be preferable above ultrasound-assisted liposuction [25].

Mechanism of Action for Regeneration

Stem cell therapy is a safe, practical, and effective source for repair of damaged tissue [26, 27]. Despite rapid translation to the bedside, the mechanism of action for regeneration is not well characterized. It was initially hypothesized that immature stem cells migrate to the injured area, differentiate into the phenotype of injured tissue,

Fig. 1 ADSCs display adherent and fibroblastic morphology. They show abundant endoplasmic reticulum and large nucleus relative to the cytoplasmic volume (Reprinted with permission from Elsevier, Kim et al. [6])

repopulate the diseased organ with healthy cells, and subsequently repair the tissue (building-block function). However, this theory has some drawbacks because the levels of engraftment and survival of engrafted cells are too low to be therapeutically relevant [28]. In addition, acute stem cell-mediated improvement within days or even hours makes it difficult to fully explain the mechanisms by which regeneration occurs [29, 30]. Instead, much of the functional improvement and attenuation of injury afforded by stem cells can be repeated by treatment with cell-free conditioned media derived from ADSCs (ADSC-CM) [31]. Thus, it can be deduced that ADSCs may exert their beneficial effects via complex paracrine actions (manager function) in addition to building-block function.

Proteomic Analysis of ADSCs and Their Secretomes

Proteomics, large-scale studies of proteins, can be used to analyze the intracellular and secretory proteins of ADSCs. For example, Roche et al. conducted a 2-DE gel analysis of BMSCs and ADSCs and confirmed the similarity [32]. Zvonic et al. also analyzed the ADSC-CM by 2-DE gel electrophoresis, detected approximately 300 features from ADSC-CM, and found that secretomes are up-/downregulated by induction of adipogenesis [33]. Although the intracellular and secretory proteins of ADSCs have been analyzed through 2-DE-coupled mass spectrometry or non-gel-based mass spectrometry, the active proteins of ADSCs responsible for the tissue regeneration are

not fully identified. This may be due to the fact that studies using proteomics has limitations as this approach is capable of analyzing highly abundant proteins only. Therefore, new mass spectrometry-based proteomic analysis techniques for stem cell proteins in correlation with other state-of-the-art analytical tools and functional study by neutralizing the candidate proteins are needed to clearly characterize the active proteins of regeneration.

Diverse Pharmacologic Actions

Wound-Healing Effect of ADSCs

Several studies of the pathophysiology of photoaging have detected similarities with certain aspects of acute and/or chronic wounds. Histologically, photoaged skin shows marked alterations in ECM composition. Skin wound repair by adult stem cells was originally demonstrated using BMSC. Wu et al. showed that BMSC injection around the wound significantly enhanced wound healing in normal and diabetic mice compared with that of allogeneic neonatal dermal fibroblasts [34]. Sasaki et al. demonstrated that BMSCs can differentiate into multiple skin cell types including keratinocytes, pericytes, and endothelial cells, which contribute to wound repair [35]. Notably, analyses of proteins in conditioned medium of BMSC (BMSC-CM) indicated that BMSCs secret distinctively different cytokines and chemokines compared to dermal fibroblasts [36]. ADSCs have surface markers and gene profiling similar to BMSCs, and their soluble factors are not significantly different [6, 14]. Given their convenient isolation compared with BMSCs and extensive proliferative capacities ex vivo, ADSCs hold great promise for use in wound repair and regeneration. However, there is little evidence demonstrating the wound-healing effects of ADSCs. It was also demonstrated that ADSCs accelerate wound healing, especially with regard to fibroblast activation [6]. They promote proliferation of dermal fibroblasts, not only by direct cell-to-cell contact but also by paracrine activation
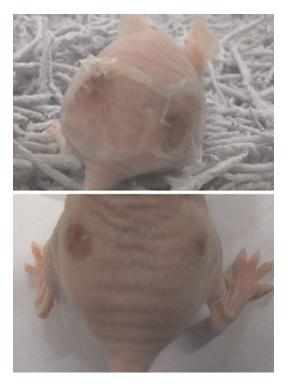

Fig. 2 Wound-healing effect of ADSCs in nude mice. Artificial wounds were made using a 6-mm punch biopsy and ADSCs were topically applied. The wound size was reduced significantly in the ADSC-treated side (*right* side of the back) 7 days after surgery (Reprinted with permission from Elsevier, Kim et al. [6])

through secretory factors. This fibroblast-stimulating effect of ADSCs was superior to that of the fibroblasts. Furthermore, ADSC-CM enhanced secretion of type I collagen from dermal fibroblasts and stimulated fibroblast migration in in vitro wound-healing models. ADSCs secreted a variety of growth factors such as basic fibroblast growth factor (bFGF), KGF, TGF-b, hepatocyte growth factor (HGF), and VEGF into the conditioned medium, which might mediate the wound-healing effect of ADSCs. In addition to the in vitro evidence, the wound-healing effect of ADSCs was also verified in an animal study, which showed that topical administration of ADSCs significantly reduced the wound size (34 % reduction) and accelerated the re-epithelialization at the wound edge (Fig. 2). Similar to ADSC treatment,

ADSC-CM treatment also accelerated wound healing in laser-induced burn mouse models (authors' unpublished data). In this experiment, burn wounds were made by laser surgery in the epidermis, and they were significantly reduced by single and multiple administration of ADSC-CM. As ADSCs are physiologically located beneath dermal fibroblasts, they may interact with dermal fibroblasts. However, ADSCs and secretomes of ADSCs may reach the epidermis in wounded area and may affect the recovery of this layer. As such, ADSC-CM was treated in cultured primary human keratinocytes and shown to increase the proliferation and migration of keratinocytes [37]. This result suggests that secretomes of ADSCs also accelerate the healing of epidermal layer.

Antioxidant and Antimelanogenic Effects of ADSCs

ROS produced in the catalytic reactions by many environmental stimuli may be involved in the pathogenesis of a number of skin disorders including photoaging, photosensitivity diseases, and some types of cutaneous malignancy. Antioxidants, as a popular term in drug and cosmetics, take the form of enzymes, hormones, vitamins, and minerals. In biological systems, the normal processes of oxidation produce highly reactive free radicals, which may continue to damage even the body's own cells. Antioxidants scavenge free radicals before they get a chance to harm the body. As of now, there are few reports on the antioxidant action of stem cells. However, some evidences support the protective role of secretomes of ADSCs against the skin damage induced by reactive oxygen species. For example, IGF reportedly protects fibroblasts and intestinal epithelial cells from free radicals [38, 39]. HGF protects the retinal pigment epithelium against oxidative stress induced by glutathione depletion [40]. Pigment epithelium-derived factor (PEDF) is an anti-angiogenic/neurotropic factor and has been shown to have antioxidant effects [41]. Interleukin-6 (IL-6) reduces the epithelial cell death induced by hydrogen peroxide [42]. In addition, subtypes of superoxide dismutase (SOD) are expressed and secreted from ADSCs [43]. Therefore, antioxidant function of ADSC was investigated in dermal fibroblasts after inducing chemical oxidative stress by the tert-butyl hydroperoxide (tbOOH). Morphological change and cell survival assay revealed that incubation with ADSC-CM aided dermal fibroblasts to resist free radicals induced by tbOOH. In addition, activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were enhanced in the dermal fibroblasts treated with ADSC-CM. In a cell cycle analysis, ADSC-CM treatment reversed the apoptotic cell death induced by ROS, which was demonstrated by a significant decrease of sub-G1 phase of dermal fibroblasts [8].
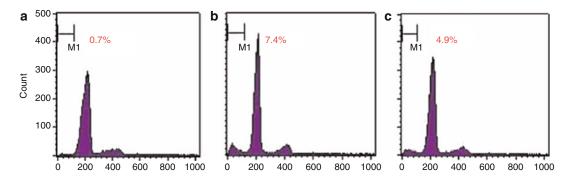

Fig. 3 Antioxidant effect of ADSCs in UVB-irradiated fibroblasts as shown by cell cycle analysis of DNA contents. Untreated fibroblasts showed little or no sub-G1 phases (**a**). However, UVB irradiation significantly increased sub-G1 (apoptotic) cells (**b**), which were reversed by ADSC-CM pretreatment (**c**) (Reprinted with permission from Elsevier, Kim et al. [5])

Photoaging is believed to be responsible for up to almost 80 % of the skin changes commonly attributed to the aging process. The study further investigated the antioxidant and protective effects of ADSCs in the photodamage of the primarily cultured dermal fibroblasts (Fig. 3). In this experiment, ADSC-CM pretreatment significantly reduced the apoptosis of dermal fibroblasts from UVB-induced damage, which was demonstrated by a significant decrease of sub-G1 phase of dermal fibroblasts after ADSC-CM pretreatment. In addition, ADSC-CM treatment increased the production of collagen and reduced the expression of matrix metalloproteinase-1 in the dermal fibroblasts. These results indicated that ADSCs can play a key role in protecting dermal fibroblast from UVB-induced oxidative stress [5].
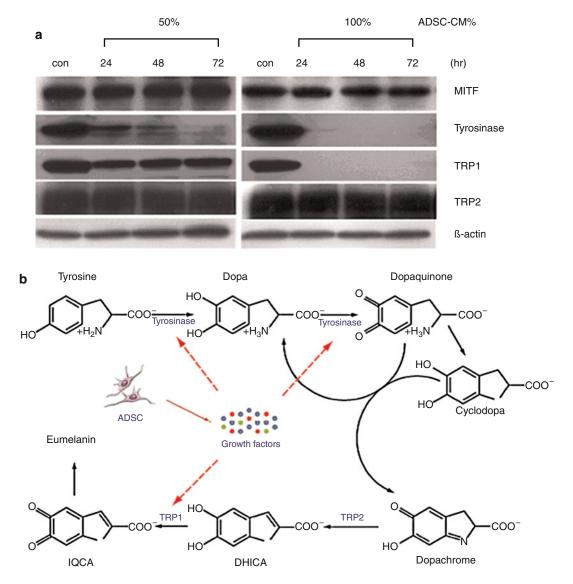

Fig. 4 (**a**) Antimelanogenic effect of ADSC-CM. Expression of MITF and TRP2 remained unchanged, but expressions of tyrosinase and TRP1 were downregulated by ADSC-CM treatment in B16 melanoma cells. (**b**) The inhibitory effect of ADSC on melanin synthesis is schematically represented (Reproduced with permission from Pharmaceutical Society of Japan, Kim et al. [9])

As antioxidants inhibit the chemical reactions leading to melanin formation, change the type of melanin formed, and interfere with the distribution of pigment and melanosome transfer, they are good candidates for skin whitening resources. As ADSC-CM is a free radical scavenger and has potent antioxidant activity, antimelanogenic effect of ADSC was investigated. ADSC-CM treatment inhibited the synthesis of melanin and the activity of tyrosinase in melanoma B16 cells. In addition, expressions of tyrosinase and tyrosinase-relating protein 1 were downregulated by ADSC-CM treatment, which indicated the mechanism of action for antimelanogenic effect of ADSCs and their soluble factors (Fig. 4) [9].

Animal Studies for Skin Aging

To study the effects on in vivo skin, ADSCs (1×10^6 cells) and ADSC-CM were intradermally injected on the back of a micropig, twice in a 14-day interval ($n = 3$). One month after the second injection, skin samples were obtained at the treatment and the control sites of adjacent normal skin. Although the increase in the dermal thickness was not significant, increased collagen expression was noted by Western blot in the ADSC- and ADSC-CM-treated skin samples (Fig. 5) [7].

In another experiment, photodamage was induced by an 8-week UVB irradiation in hairless mice. The irradiation dose was one MED (minimal erythema dose; 60 mJ/cm^2) in the first 2 weeks, two MED in the third week, three MED in the fourth week, and four MED in the fifth through eighth weeks. After wrinkle induction, varying numbers of ADSCs (A group: control; B group: 1×10^3 cells; C group: 1×10^4 cells; and D group: 1×10^5 cells) were subcutaneously injected into the mice ($n = 8$ for each group). In a replica analysis, parameters involving skin roughness were improved with mid-level and higher dose groups of ADSCs (C and D group) (Fig. 6). Dermal thickness was increased in the ADSC-injected groups (16 % and 28 % in C and D groups, respectively) and ECM contents in the dermis were also increased by Masson's trichrome staining results of collagen (blue) in the ADSC-treated groups (Fig. 7). As cell transplantation between species mediates immune rejection, the survival of ADSC from humans was investigated after injection of ADSCs labeled with PKH26
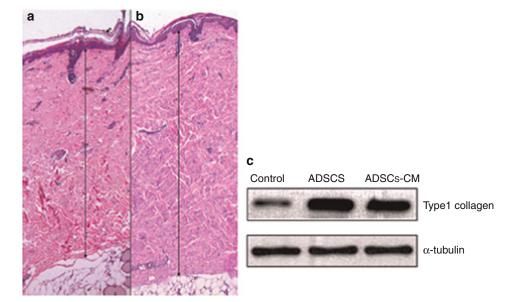

Fig. 5 Micropig experiment shows the change of dermal thickness without (**a**) and with (**b**) intradermal injections of ADSCs. Increased collagen expression was noted by Western blot in the ADSCs- and ADSC-CM-treated skin (**c**) (Reproduced with permission from Wiley-Blackwell, Park et al. [7])
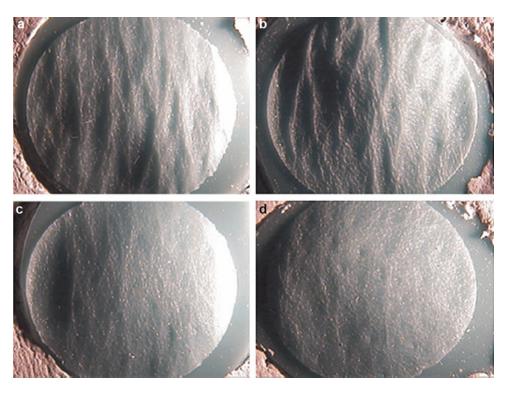
Fig. 6 Anti-wrinkle effects of ADSCs. Photodamage was induced by 8-week UVB irradiation in hairless mice, and ADSCs were intradermally and subcutaneously injected three times. Wrinkles were evaluated by replica analysis. (**a**) Control; (**b**) 1×10^3 cells; (**c**) 1×10^4 cells; (**d**) 1×10^5 cells (Reprinted with permission from Elsevier, Kim et al. [5])

(red color, Fig. 8 inset). As shown in Fig. 8, survival of the ADSCs was clearly demonstrated [5].

Clinical Application for Skin Aging

ADSCs and the ADSC Protein Extract for Skin Aging

As a pilot study, intradermal injections of purified autologous SVF cells (1×10^6 cells), which contain approximately 20–30 % ADSCs, were tried with photoaged skin of one patient [7] after informed consent. The female patient had two successive injections at 2-week intervals. Two months after the second injection, the patient showed improvements in general skin texture and wrinkling as evidenced by medical photographs of periorbital wrinkles. Measurements of dermal thickness by a 20 MHz high-frequency ultrasonographs (Dermascan-C, Cortex,

Hadsund, Denmark) also indicated increased thickness (2.054 vs. 2.317 mm) (Fig. 9).

In a large-scale pilot study, the effects of the ADSC protein extract applied transdermally in the treatment of the various signs of skin aging were evaluated: (1) wrinkles, (2) acquired pigmentary lesions, and (3) dilated pores [44]. Korean patients visiting for the treatment of skin aging were recruited during September 2006–August 2007. The population ($n = 235$) aged 28–71 years (mean 41 years) had skin phototypes III and IV with mild to moderate photodamage. The advanced ADSC protein extract (AAPE®; Prostemics Inc., Seoul, Korea) was applied 3–12 times at 2-week intervals. The changes were evaluated objectively by photographic documentation and Robo Skin Analyzer CS100/VA100 (Inforward Inc., Tokyo, Japan) and subjectively by patient questionnaire. The evaluation score was based upon the following scales: 0 = poor/worsened; 1 = no change/no change; 2 = fair/
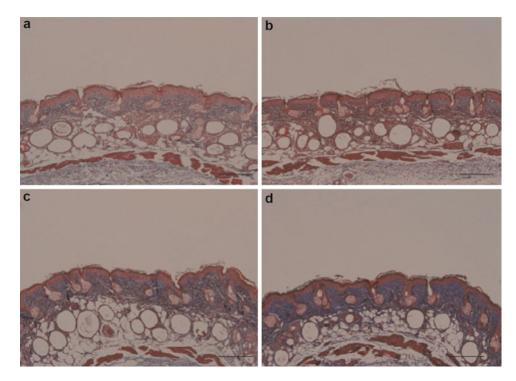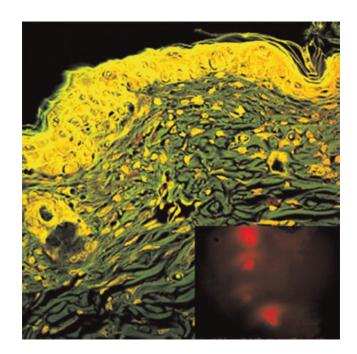
Fig. 7 Massson's trichrome staining shows that collagen contents (*blue*) are significantly increased in the mid-level (**c**) and higher dose (**d**) groups of ADSCs compared with control (**a**) and lower dose group (**b**) in photodamaged hairless mice experiment. (Reprined with permission from Elsevier, Kim et al. [5])

Fig. 8 Survival of ADSCs labeled with PKH26 *(insert)* injected in the skin of hairless mice. Two weeks after injection, mouse skin block was cryosectioned and counterstained with green-fluorescent nucleic acid stain. ADSCs are stained *red* (Reprinted with permission from Elsevier, Kim et al. [5])
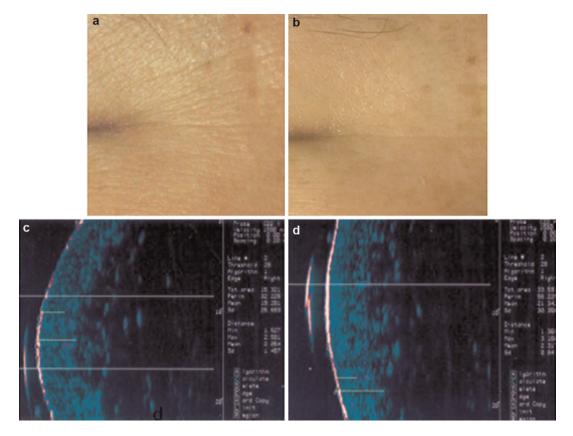
Fig. 9 Clinical study using intradermal injections of purified autologous SVF cells. Medical photographs of periorbital wrinkles were taken before (**a**) and after (**b**) treatment, and dermal thickness was measured by ultrasonographs before (**c**) and after (**d**) treatment. Improved general skin texture and increase thickness (2.054 vs. 2.317 mm) were evident 2 months after two injections (**b**, **d**) (Reprinted with permission from Wiley-Blackwell, Park et al. [7])

mild improvement; 3 = good/moderate improvement; and 4 = excellent/marked improvement. As compared to 47.4 % showing good to excellent improvement in wrinkle, 63 % of the patients were judged to have good to excellent improvement in acquired pigmentary lesions and dilated pores (Fig. 10).

Objective measurement of the periorbital wrinkles was performed with skin surface topography using PRIMOS (Phase Rapid In vivo Measurement of Skin) 3D in vivo optical skin measuring device. Twenty-three healthy females (range, 39–47; ages, mean 43 ± 5 years) applied AAPE® twice daily for 8 weeks. The evaluation was performed before and 4 and 8 weeks after treatment (data not published). There was statistically significant improvement ($p < 0.05$) in the crow's feet (Fig. 11).

Melasma is a multifactorial disorder caused by sun exposure, hormonal imbalance, and genetic predisposition. In many countries including Asia, melasma ranks among the top ten most common skin conditions. Ethnic differences between Asian and other skin types may influence the efficacy and tolerability of melasma treatments. Recent clinicopathologic studies on melasma show that lesional skin showed more prominent solar elastosis when compared to the normal skin [45]. Moreover, it has been suggested that interactions between the cutaneous vasculature and melanocytes might have an influence on the development of pigmentation [46]. The coexistence of telangiectasia and/or solar elastosis with melasma points out that photodamage is closely linked to the pathogenesis of melasma. The actions of ADSCs
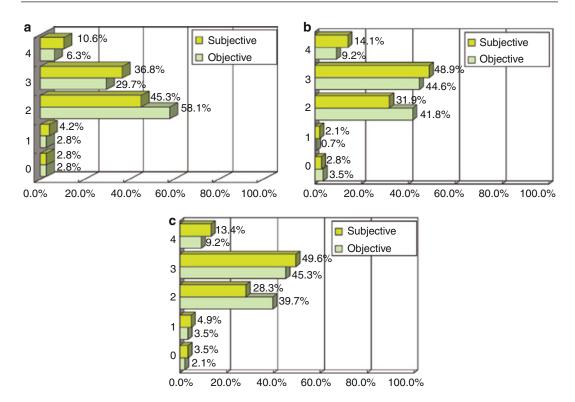
Fig. 10 Objective and subjective evaluation of the ADSC protein extract in a large-scale ($n = 235$) pilot study in terms of: (**a**) wrinkles, (**b**) acquired pigmentary lesions, and (**c**) dilated pores. The evaluation score is based upon the following scales: 0 poor/worsened; 1 no change/no change; 2 fair/mild improvement; 3 good/moderate improvement; and 4 excellent/marked improvement. As compared to 47.4 % showing good to excellent improvement in wrinkle (**a**), 63 % of the patients were judged to have good to excellent improvement in acquired pigmentary lesions (**b**) and dilated pores (**c**)

in wound healing, antioxidation, antimelanogenic effects and the reversal of photodamage in vitro, and in animal models prompted the clinicians to bring these biologic actions to bedside. The representative cases with marked response in melasma were shown in Fig. 12. These clinical results for the past 10 years suggest that the ADSCs and the protein extract are promising rational strategies for melasma and photodamage.

Combination with Other Procedures and Active Transdermal Delivery

Various light source and radiofrequency devices have been used for the treatment of skin aging by selectively heating up the collagen in the dermis to stimulate collagen remodeling. In general, both ablative and nonablative techniques lead to new collagen formation. As ADSCs and their secretory factors promote the wound healing by activating dermal fibroblasts, it can be speculated that when combined, ADSCs and the protein extract might augment the clinical effects beyond the intrinsic fibroblast-stimulatory effect of the various devices.

Based upon the previous documentation of wound-healing and antimelanogenic effects of ADSCs, the efficacy of the ADSC protein extract in reducing healing time and PIH or erythema was investigated after fractional CO_2 laser treatment (MiXto SX[®], Lasering, Italy) in a pilot study as prospective, randomized, placebo-controlled, double-blinded, and split-face setting [47]. CO_2 fractional treatments have emerged as one of the new technologies in skin rejuvenation. However, comparatively increased incidence of PIH is problematic especially in dark-skinned patients. In this study, Korean patients of Fitzpatrick skin types III
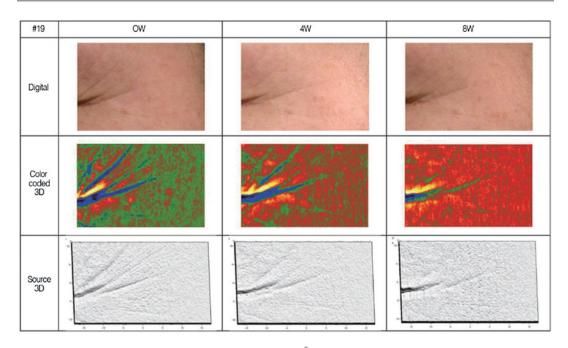
Fig. 11 Images of crow's feet before and after applying AAPE® cream 4 weeks and 8 weeks later (*upper*, fluorescence image; *middle*, color coded 3D image; *lower*, source 3D image)

Fig. 12 The representative cases with marked response in whitening of melasma before (**a**, **c**) and after (**b**, **d**) treatment with the ADSC protein extract

and IV (mean age 45.7 years) with facial wrinkles were treated with full-face fractional CO_2 laser (parameter: 8 W, index level 8). All subjects were randomly allocated to split-face application of either the ADSC protein extract or emollient only. Serial photographs were taken at each visit during the treatment and 3-month follow-up period. Marked difference in the duration of erythema and healing was observed (Fig. 13). The quality of wound healing was noted to be improved. This therapy was well tolerated by majority of patients with minimal adverse effects.

Fig. 13 A split-face comparison shows that the application of the ADSC protein extract results in less intense erythema and microcrusting 2 days after fractional CO_2 laser resurfacing

It was concluded that the ADSC protein extract can be safely and effectively used to prevent PIH and to accelerate wound healing after fractional CO_2 laser treatment in dark skin.

As the secretory factors of ADSCs generally contain ingredients of large molecular weights, various new "active" enhancement technologies designed to transiently circumvent the barrier function of the stratum corneum would be required for transdermal delivery: e.g., iontophoresis, sonophoresis, electroporation, or microneedle arrays or skinstamp.

Basic Mechanism of Hair Regeneration

It was revealed that treatment of the conditioned media of ADSCs (ADSC-CM) enhanced the proliferation of cultured human dermal papilla cells (DPCs) in vitro up to 130 % by activation of both Erk and Akt signaling pathways, which is crucial in enhancing the survival and proliferation of DPCs [12]. In addition, ADSC-CM increased cyclin D1 and CDK2, key cell cycle-related molecules [12]. These results reflect the beneficial efficacy on hair growth because the size and the number of dermal papilla correlate with the hair growth cycle [48]. DPCs are regarded as a key element for regenerating hair cycle [49]. It was revealed that ADSC-CM enhances the elongation

of hair shafts by 40 % in ex vivo human hair organ cultures. The length of the cultured human hair follicles in an ADSC-CM-treated group significantly increased, as much as that seen in the follicles treated with 1 mM of minoxidil [12]. In in vivo study, the phase transition from telogen to anagen was found with the topical application of ADSC-CM on C3H/HeN nude mice. It was also revealed that ADSC-CM therapy can protect stressed follicular DPCs against ROS and DHT (Fig. 14) [13]. DHT directly inhibits the proliferation of androgen-sensitive DPCs, and this inhibition can be rescued by ADSC-CM [50]. The proposed mechanism of hair growth with the ADSC-CM treatment is summarized in Table 1.

Furthermore, the efficacy of ADSC-CM in hair growth promotion is potentiated through hypoxic preconditioning. When ADSCs are cultured under hypoxic conditions in vitro, their proliferative and self-renewal capacities are markedly increased [51]. ADSCs in hypoxic conditions produce increased amount of growth factors such as VEGF, PDGF, and insulin-like growth factor-binding protein (IGFBP) [52] which are related to hair growth. It was confirmed that ADSC-CM produced in hypoxic conditions induced the anagen phase of mouse more rapidly than that produced in normoxic conditions [52]. Based on those results of studies, AAPE® is produced under hypoxic conditions by providing 2 % O_2.
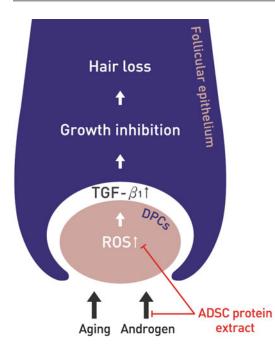
Fig. 14 Currently suggested potential mechanism of action in hair growth by ADSC-CM or the ADSC protein extract. *ADSC* adipose-derived stem cell, *DPCs* dermal papilla cells, *ROS* reactive oxygen species, *TGF* transforming growth factor

Table 1 The proposed mechanism of hair regeneration in the treatment with the ADSC protein extract

Increase the proliferation of hair follicular cells (DPCs, hair follicular epithelium) through modulation of cell cycle
Stimulate the phase transition from telogen to anagen
Protect DPCs from cytotoxic injury by androgen and ROS

Clinical Application for Hair Regeneration

The ADSC Protein Extract for Female Pattern Hair Loss

An observational pilot study was performed to evaluate the efficacy of the ADSC protein extract for the treatment of female pattern hair loss (FPHL) comparing with the baseline status without control [10]. Twenty-seven patients (aged 22–69 years, mean 42 ± 13 years) with FPHL received applications of AAPE® (now termed as NGAL™) for 12 weeks (Fig. 15). Hair density increased from 105.4 to 122.7 counts/cm^2 ($P < 0.001$). Mean hair thickness increased from 57.5 to 64.0 μm ($P < 0.001$). None of the patients reported severe adverse reactions.

The microneedling is widely used to enhance the absorption of topical therapeutics. There has been no report that the use of the microneedle itself ameliorates hair loss [53]. Nine patients among 27 were followed up to 6 months, and one up to 1 year. The clinical improvement was maintained, and they did not complain aggravation of hair loss.

The ADSC Protein Extract for Male Pattern Hair Loss

Another pilot study was performed to evaluate the efficacy of ADSC protein extract for the treatment of male pattern hair loss (MPHL) [11]. This pilot study compared the efficacy with the baseline status without the control group. Twenty-five patients (aged 28–60 years, mean 49 ± 9 years) with MPHL received weekly applications of AAPE® (now termed as NGAL™) without any other treatments such as oral and topical agents. As in the study for FPHL, a microneedle or a mesotherapy gun was used to deliver AAPE® to the scalp. After 12 weeks of therapy, hair density increased from 97.7 to 108.1 counts/cm^2 ($P < 0.001$). Mean hair thickness increased from 65.4 to 71.8 μm ($P < 0.001$). None of the patients reported severe adverse reactions.

These results reveal that the application of the ADSC protein extract could be also effective for MPHL (Fig. 16). The current gold standard treatment for MPHL consists of finasteride medication and topical minoxidil alone or in combination. It remains to be further elucidated whether the ADSC protein extract could completely substitute for the current medical treatment or could be an excellent synergistic tool for patients who feel that the current therapeutic regimen is not fully satisfactory.
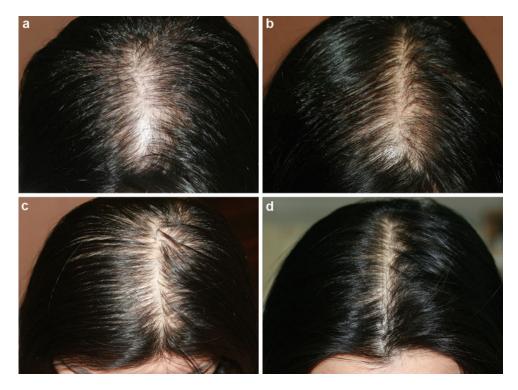
Fig. 15 The representative cases with marked response in hair growth before (**a**, **c**) and after (**b**, **d**) treatment of FPHL with the ADSC protein extract

Split-Scalp Comparison Study Using the ADSC Protein Extract in Patients with MPHL

Split-scalp study was designed to objectively evaluate the effect of the ADSC protein extract on hair loss [11]. Six male healthy patients (aged 20–52 years, mean 36 ± 12 years) with MPHL (Hamilton-Norwood classification type II–IV) were recruited. The scalp was split into the right and left sides: each reference point was shaved to create a circle of 1.0 cm in diameter and then tattooed. AAPE® (now termed as NGAL™) and the vehicle placebo were applied to one half of the scalp weekly, respectively. They had not used any products or taken any drugs that might have affected hair growth for more than 6 months prior to the study.

Close-contact photographic images were taken using a phototrichogram (Folliscope®: LeadM, Seoul, Korea) at a magnification of $\times 30$ at first and 12 weeks after treatment: after 12 weeks, the previously tattooed reference area was shaved again for the follow-up phototrichogram to evaluate the efficacy of the treatment. This phototrichogram comparison study allowed the observer to trace the changes follicle by follicle as each follicle was designated by serial numbers (Fig. 17). This study was more accurate than the previously described studies in which the density and the hair thickness were measured. After 12 weeks of therapy, the total hair count was significantly higher on the AAPE®-treated side of the reference circle than on the vehicle-treated side ($p = 0.002$, paired t-test). The incidence of adverse effects such as pruritus and local irritation was negligible.

Conclusion

The current topics of increasing interest in the dermatological field are anatomical-functional damage to the skin and every possible means to counteract the injurious effects. In the beginning,

Fig. 16 The representative cases with marked response in hair growth before (**a**, **c**) and after (**b**, **d**) treatment of MPHL with the ADSC protein extract

ADSCs were shown to increase the survival rate in fat transplantation [54]. This chapter explains that ADSCs and their secretory factors have diverse pharmacologic effects for skin aging and hair regeneration. There were some safety concerns on the clinical application of cultured ADSCs and their secretory factors for human skin: the threat of passing on viruses, passing on diseases from other animal source nutrients to cultured stem cells in the laboratory. However, in 2010, Korean Ministry of Food and Drug Safety issued a safety guideline on manufacturing conditioned media from human cells and tissues. The safety guideline includes donor eligibility determination, sanitation and maintenance of cell culture facilities, manufacture of human origin cell/tissue culture media, safety evaluation of human origin cell/tissue culture media, and analytical procedure of human origin cell/tissue culture media [55].

In addition, ADSCs have to overcome the obstacles in that they are difficult both to handle and to commercialize in an industrial point of view: how to store the ADSCs, the containers to store them, how to transport them to the point, and the shelf life in various environments. Therefore, new methods and materials to overcome these limitations are needed. Secretomes of ADSCs have some advantages over cell-based therapies and might have greater potential in skin regeneration, because they can be manufactured in a large scale with long-term stability and they are relatively devoid of safety issues. As such, the study demonstrated that photodamage can be reversed by utilizing the ADSCs/their secretory factors alone [5, 7, 44] or in combination with other devices minimizing unwanted effects [47]. Identification of active proteins will be the next goal, and drug development using these proteins will suggest better strategies for skin aging in the future.
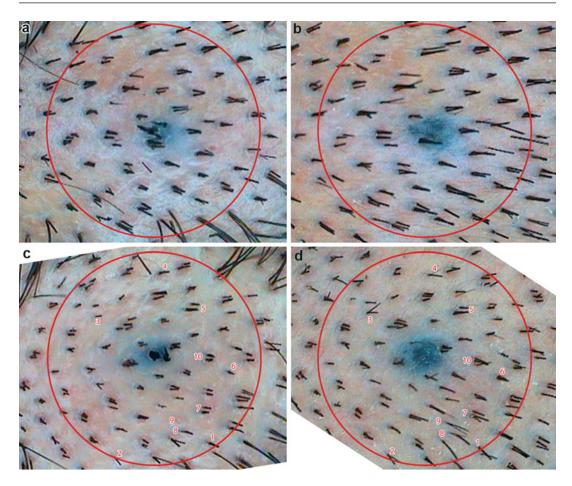
Fig. 17 Phototrichogram analysis of a patient with male pattern hair loss who received split-scalp treatment. Total hair count was measured in the circle of 1 cm diameter. The total hair count remained almost unchanged on the vehicle-treated side before (**a**) and after 12 week-treatment (**b**). By contrast, the total hair count on the AAPE®-treated side increased by 12 after 12 weeks of treatment (**d**) compared with that before treatment (**c**). Split-scalp comparison allowed the observer to trace the changes follicle by follicle as each follicle was designated by serial numbers. *Red* figures indicate the follicle numbers that showed increase in hair counts

References

1. Barry FP, et al. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol. 2004;36:568–84.
2. Gimble J, et al. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy. 2003;5:362–9.
3. Kinnaird T, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004;94:678–85.
4. Zuk PA, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13:4279–95.
5. Kim WS, et al. Antiwrinkle effect of adipose derived stell cell: activation of dermal fibroblast by secretory factors. J Dermatol Sci. 2009;53:96–102.
6. Kim WS, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci. 2007;48:15–24.
7. Park BS, et al. Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol Surg. 2008;34:1323–6.
8. Kim WS, et al. Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. J Dermatol Sci. 2008;49:133–42.
9. Kim WS, et al. Whitening effect of adipose-derived stem cells: a critical role of TGF-beta 1. Biol Pharm Bull. 2008;31:606–10.

10. Shin H, et al. Clinical use of conditioned media of adipose tissue-derived stem cells in female pattern hair loss: a retrospective case series study. Int J Dermatol. 2015;54(6):730–5.

11. Shin H, et al. Up-to-date clinical trials of hair regeneration using conditioned media of adipose-derived stem cells in male and female pattern hair loss. Curr Stem Cell Res Ther. (accepted for publication).

12. Won CH, et al. Hair growth promoting effects of adipose tissue-derived stem cells. J Dermatol Sci. 2010;57(2):134–7.

13. Won CH, et al. The basic mechanism of hair growth stimulation by mesenchymal stem cells and their secretory factors for the treatment of hair loss. Curr Stem Cell Res Ther. (accepted for publication).

14. Izadpanah R, et al. Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. J Cell Biochem. 2006;99:1285–97.

15. Porada CD, et al. Adult mesenchymal stem cells: a pluripotent population with multiple applications. Curr Stem Cell Res Ther. 2006;1:365–9.

16. Boquest AC, et al. Epigenetic programming of mesenchymal stem cells from human adipose tissue. Stem Cell Rev. 2006;2:319–29.

17. Huang T, et al. Neuron-like differentiation of adipose derived stem cells from infant piglets in vitro. J Spinal Cord Med. 2007;30:35–40.

18. Kern S, et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells. 2006;24:1294–301.

19. Katz AJ, et al. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells. 2005;23:412–23.

20. Anghileri E, et al. Neuronal differentiation potential of human adipose-derived mesenchymal stem cells. Stem Cells Dev. 2008;17:909–16.

21. Bunnell BA, et al. Differentiation of adipose stem cells. Methods Mol Biol. 2008;456:155–71.

22. Gimble JM, et al. Adipose-derived stem cells for regenerative medicine. Circ Res. 2007;100:1249–60.

23. Schipper BM, et al. Regional anatomic and age effects on cell function of human adipose-derived stem cells. Ann Plast Surg. 2008;60:538–44.

24. Jurgens WJ, et al. Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: implications for cell-based therapies. Cell Tissue Res. 2008;332:415–26.

25. Oedayrajsingh-Varma MJ, et al. Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy. 2006;8:166–77.

26. Schachinger V, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med. 2006;355:1210–21.

27. Schachinger V, et al. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J. 2006;27:2775–83.

28. Uemura R, et al. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. Circ Res. 2006;98:1414–21.

29. Wang M, et al. Pretreatment with adult progenitor cells improves recovery and decreases native myocardial proinflammatory signaling after ischemia. Shock. 2006;25:454–9.

30. Crisostomo PR, et al. In the adult mesenchymal stem cell population, source gender is a biologically relevant aspect of protective power. Surgery. 2007;142:215–21.

31. Patel KM, et al. Mesenchymal stem cells attenuate hypoxic pulmonary vasoconstriction by a paracrine mechanism. J Surg Res. 2007;143:281–5.

32. Roche S, et al. Comparative proteomic analysis of human mesenchymal and embryonic stem cells: towards the definition of a mesenchymal stem cell proteomic signature. Proteomics. 2009;9:223–32.

33. Zvonic S, et al. Secretome of primary cultures of human adipose-derived stem cells. Mol Cell Proteomics. 2007;6:18–28.

34. Wu Y, et al. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007;25:2648–59.

35. Sasaki M, et al. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol. 2008;180:2581–7.

36. Chen L, et al. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One. 2008;3:e1886.

37. Moon KM, et al. The effect of secretory factors of adipose-derived stem cells on human keratinocytes. Int J Mol Sci. 2012;13(1):1239–57. doi:10.3390/ijms13011239. Epub 2012 Jan 23.

38. Baregamian N, et al. IGF-1 protects intestinal epithelial cells from oxidative stress induced apoptosis. Surg Res. 2006;136:31–7.

39. Rahman ZA, et al. Antioxidant effects of glutathione and IGF in a hyperglycaemic cell culture model of fibroblasts: some actions of advanced glycaemic end products (AGE) and nicotine. Endocr Metab Immune Disord Drug Targets. 2006;6:279–86.

40. Shibuki H, et al. Expression and neuroprotective effect of hepatocyte growth factor in retinal ischemia-reperfusion injury. Invest Ophthalmol Vis Sci. 2002;43:528–36.

41. Tsao YP, et al. Pigment epithelium derived factor inhibits oxidative stress-induced cell death by activation of extracellular signal-regulated kinases in cultured retinal pigment epithelial cells. Life Sci. 2006;79:545–50.

42. Kida H, et al. Protective effect of IL-6 on alveolar epithelial cell death induced by hydrogen peroxide. Am J Physiol. 2005;288:342–9.

43. Liochev SI, et al. How does superoxide dismutase protect against tumor necrosis factor: a hypothesis informed by effect of superoxide on "free" iron. Free Radic Biol Med. 1997;23:668–71.

44. Kang SH, et al. Improvement of melasma and scars with the secretory factors from ADSCs. Korean J Dermatol. 2007;45 Suppl 2:136.

45. Kang WH, et al. Melasma: histopathological characteristics in 56 Korean patients. Br J Dermatol. 2002;146:228–37.

46. Kim EH, et al. The vascular characteristics of melasma. J Dermatol Sci. 2007;46:111–6.

47. Park BS, et al. Rejuvenation of aging skin using fractional CO_2 laser resurfacing followed by topical application of ADSC protein extract. Korean J Dermatol. 2008;46 Suppl 1:266–7.

48. Elliott K, et al. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: implications for the control of hair follicle size and androgen responses. J Invest Dermatol. 1999;113(6):873–7.

49. Yoon SY, et al. A role of placental growth factor in hair growth. J Dermatol Sci. 2014;74(2):125–34.

50. Shin H, et al. Induction of transforming growth factor-beta 1 by androgen is mediated by reactive oxygen species in hair follicle dermal papilla cells. BMB Rep. 2013;46(9):460–4.

51. Kim JH, et al. The pivotal role of reactive oxygen species generation in the hypoxia-induced stimulation of adipose-derived stem cells. Stem Cells Dev. 2011;20(10):1753–61.

52. Park BS, et al. Hair growth stimulated by conditioned medium of adipose-derived stem cells is enhanced by hypoxia: evidence of increased growth factor secretion. Biomed Res. 2010;31(1):27–34.

53. Lee YB, et al. Effects of topical application of growth factors followed by microneedle therapy in women with female pattern hair loss: a pilot study. J Dermatol. 2013;40(1):81–3.

54. Matsumoto D, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng. 2006;12:3375–82.

55. Park BS. Stem cell cosmetics: development, safety regulation, mechanism and practical application. J Korean Med Soc Cosmetics. 2014;3(2):66–72.