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The wound-healing and antioxidant effects of adipose-derived stem cells

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Background: The aim of tissue engineering is to repair and regenerate damaged organs using a combination of cells, biomaterials and growth factors. Mesenchymal stem cells within the stromal–vascular fraction of subcutaneous adipose tissue, that is adipose-derived stem cells (ADSCs) have been used in skin repair with satisfactory results. The production and secretion of growth factors has been reported to be an essential function of ADSCs, and diverse regenerative effects of ADSCs in the skin have been demonstrated. *Objective*: Recent research developments concerning the wound-healing and antioxidant effects of ADSCs are briefly described. *Methods*: Various experimental results regarding the wound-healing and antioxidant effect of ADSCs are introduced, and the mechanisms and identification of active proteins involved in these function are further discussed. *Results/conclusion*: Evidence of ADSC differentiation of skin has not been reported *in vivo*, but ADSCs accelerate wound-healing and exhibit antioxidant effects under various conditions. The wound-healing and antioxidant effects of ADSCs are mainly mediated by the activation of dermal fibroblasts and keratinocytes via the paracrine mechanism. Since ADSCs are easily obtained in large quantities and have an advantage over other stem cell sources, ADSCs and their secretory factors show promise for use in skin repair and regeneration.

Keywords: antioxidant, adipose-derived stem cell, paracrine, wound-healing

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1. Introduction

Regenerative medicine that uses the body's own stem cells and growth factors is an alternative therapeutic strategy for repair of damaged tissue, and is becoming a predominant cell-based therapy. The limited availability of human cells capable of self-renewal and differentiation is a barrier to the expansion and development of tissue engineering; adipose-derived stem cells (ADSCs), however, offer the potential for a workable solution to this dilemma and could result in the regeneration of damaged tissue [1,2]. ADSCs can be easily obtained from liposuction of human adipose tissue, can be cultured on a large scale, and can display multi-lineage developmental plasticity [3-5]. ADSCs secrete various growth factors that control and manage damaged neighboring cells, and this has been identified as an essential function of ADSCs [6-8]. Conditioned medium from ADSC cultures (ADSC-CM) activates dermal fibroblasts and keratinocytes, and can repair the skin through a paracrine mechanism (**Figure 1**) [6-10]. For example, ADSC-CM stimulated both collagen synthesis and migration of dermal fibroblasts, which improved wrinkling and accelerated wound-healing in animal models [7,9]. ADSC-CM also inhibited melanogenesis in B16 melanoma cells, and exhibited a skin-whitening effect [8]. ADSC-derived secretory factors protected dermal fibroblasts from oxidative stress induced by chemical and UVB irradiation [6,7]. Recently, it has been demonstrated

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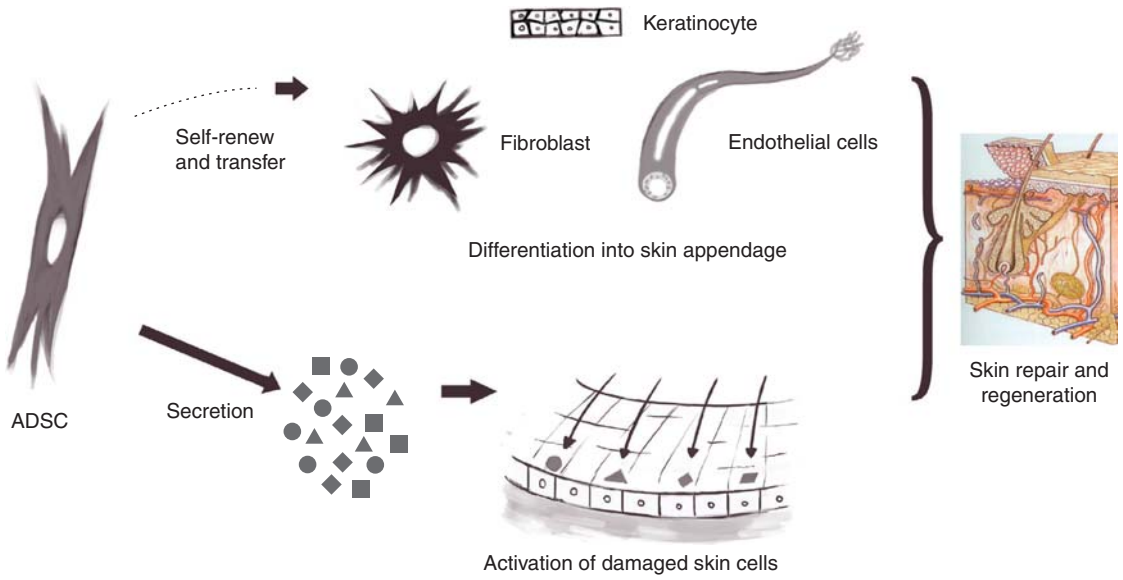

Figure 1. Mechanism of skin repair by adipose-derived stem cells (ADSCs). In response to injury, ADSCs may migrate to the injured area, differentiate into the phenotype of skin appendage, and repopulate the injured skin with healthy cells. In addition, secretary factors from ADSCs activate the dermal fibroblasts and keratinocytes, which exhibit wound-healing and antioxidant effect.

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that ADSC-CM promoted hair growth, which was enhanced by hypoxia (our unpublished data). Based on our previous studies, recent research developments concerning the wound-healing and antioxidant effects of ADSCs and their secretory factors are briefly described in this review.

2. Adipose-derived stem cells

Stem cell therapy is clinically applied as a safe and effective method for repair of several types of tissue damage [10-12]. In the skin, most studies regarding accelerated wound healing by stem cells has been demonstrated in bone marrow-derived mesenchymal stem cells (BMSCs) [13,14]. Recently, ADSCs and their secretory factors have been investigated as a substitute for BMSCs, which offers a potential solution to skin repair and regeneration [2,9].

2.1 Characteristics of ADSCs

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 [15-17]. In bone marrow, adult stem cells have been identified as hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) [18,19]. MSCs have advantages over HSCs, which includes availability from small aspirates of donor bone marrow, ease of expansion in an *in vitro* cell culture, simple isolation via plastic adherence, and an innate ability to evade rejection. MSCs were first characterized in bone marrow, but many studies have reported the existence of MSCs in the connective tissue of several organs [20,21]. The role of these cells is not entirely clear, but they are generally believed to constitute a reserve for tissue maintenance and repair.

The most abundant and accessible source of adult stem cells is adipose tissue and MSCs have been obtained by liposuction of human adipose tissue under physiological and pathological conditions [6,9]. ADSCs displayed fibroblastic characteristics, with an abundant endoplasmic reticulum and a large nucleus relative to the cytoplasmic volume [3,9]. The yield of MSCs from adipose tissue is approximately 40-fold greater than that from bone marrow [22-24]. This abundant and accessible cell population has potential utility for tissue engineering and regeneration.

Several groups have identified highly consistent, although not identical, expression profiles of cell-surface proteins on ADSCs [3,25]. These proteins have included adhesion molecules, receptor molecules, surface enzymes, extracellular matrix proteins and glycoproteins (**Table 1**). Hematopoietic cell markers such as CD14, CD31 and CD45, however, are not expressed [5,25]. Interestingly, the immunophenotype of ADSCs resembles that of other adult stem cells prepared from human bone marrow and skeletal muscle [3]. Differentiation of ADSCs is not restricted to the adipocyte lineage, and they can be differentiated into chondrocytes, osteocytes, cardiomyocytes and neurons [6,26,27]. Although transdifferentiation of BMSCs into multiple skin cell types has been demonstrated [28], it has not yet been reported in the case of ADSCs. Trottier *et al.* have reconstituted skin substitute using ADSCs instead of dermal fibroblasts, which produced a more complete trilayered skin substitute consisting of the epidermis, the dermis, and the adipocyte-containing hypodermis which is the skin's deepest layer [29]. This result suggests that ADSCs could be useful substitutes for dermal fibroblasts in skin reconstruction. Therefore, skin regeneration

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Table 1. Cell surface proteins of ADSCs.

Character	Protein	CD Antigen
Adhesion molecules	Tetraspan protein	CD9
	Integrins α_L (lymphocyte function-associated antigen 1)	CD11a; LFA-1
	Integrins α_M (macrophage antigen-1)	CD11b; Mac-1
	Integrins α_X (complement receptor 4)	CD11c; CR4
	Integrin β1	CD29
	Integrin β2	CD18
	Intercellular adhesion molecule 1	CD54; ICAM-1
	Endoglin	CD105
	Vascular cell adhesion molecule 1	CD106; VCAM-1
	Activated lymphocyte cell adhesion molecule	CD166; ALCAM
	Intercellular adhesion molecule 3	CD50; ICAM-3
	Neural cell adhesion molecule	CD56; NCAM
	Endothelial selectin (Endothelial leukocyte adhesion molecule 1)	CD62E; ELAM-1
Receptor molecules	Hyaluronate (phagocytic glycoprotein I)	CD44; Pgp-1
	Transferrin receptors	CD71; T9
Surface enzymes	Common acute lymphocytic leukemia antigen	CD10; CALLA
	Alanine aminopeptidase	CD13
	Ecto 5′ nucleotidase	CD73
Extracellular matrix proteins and glycoproteins	Type I and Type III collagens	
	Osteopontin	
	Ostenectin	
	T3	CD3
	T4, L3T4	CD4
	T8, Lyte2, 3	CD8
	Thy-1	CD90
	Endoglin	CD105
	MUC-18	CD146
Skeletal proteins	Intracellular a smooth muscle actin	
	Vimentin	
Complement regulatory proteins	Decay accelerating factor	CD55
	Complement protectin	CD59
Histocompatibility antigens	Class I histocompatibility protein	
	Negative for the Class II protein	
Hematopoietic cell markers (negative)	Hematopoietic precursors	CD34
	Integrin α4 (very late activation protein 4)	CD49d; VLA-4
	Platelet endothelial cell adhesion molecule	CD31; PECAM-1
	Leukocyte common antigen	CD45; LCA

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using ADSCs could be promising, particularly when their accessibility and efficacy are considered.

2.2 Mechanism of action for regeneration

Stem cell therapy is clinically applied as a safe and effective method for repair of several types of tissue damage [11,12]. However, the mechanism of action of regeneration is not well characterized. It was initially proposed that immature stem cells migrate to the injured area, differentiate into a phenotype of the injured tissue, and repopulate the diseased organ with healthy cells thereby repairing the tissue (building-block function). However, this theory has drawbacks because the survival of engrafted cells is too low to be therapeutically relevant [30]. Acute functional improvement within days or even hours makes it difficult to fully explain the mechanisms of regeneration [31,32]. Instead, much of the functional improvement and attenuation of injury afforded by stem cells can be repeated by treatment with cell-free conditioned medium from ADSCs [33]. Thus, it can be deduced that ADSCs may exert their beneficial effects via complex paracrine mechanisms in addition to a building-block function.

The paracrine mechanism of stem cells has been well reported in cardiovascular and neurodegenerative diseases [34-36]. Originally, the ability of ADSCs to differentiate into cardiomyocytes and vascular endothelial cells, made them an attractive therapeutic tool for cardiovascular diseases. However, recent studies have revealed that ADSCs exert their role in cardiac repair not only through transdifferentiation but also through paracrine effects via secretion of a variety of angiogenic, antiapoptotic and mitogenic factors. For example, Rehman *et al.* made a hindlimb ischemia model induced by femoral artery ligation in nude mice and injected either ADSCs or ADSC-CM [37] to compare their activity. Although ADSC injection was significantly more effective than ADSC-CM in restoring blood flow to the ischemic hindlimb, regeneration was almost achieved with the conditioned medium. In this study, angiogenic and antiapoptotic factors were detected in ADSC-CM: G-CSF, TGF-β, VEGF, hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF). In addition, secretion of angiogenic antiapoptotic, and anti-inflammatory factors from ADSCs are reportedly increased by exposure to stimuli such as hypoxia, TNF-α and lipopolisachiride, which enhanced the regenerative function of ADSCs [37-40].

2.3 Proteomic analysis of ADSC-CM

Proteomics, which is the large scale study of proteins, can be used to analyze the protein content of stem cells, and some groups have used it to analyze the intracellular and secretory proteins of stem cells, including ADSCs [6,41-43]. Roche *et al.* conducted a two-dimensional electrophoretic gel analysis of intracellular proteins of BMSCs and ADSCs, and confirmed that the proteins were very similar, which is an argument for their interchangeable use in cell therapy [41]. Zvonic *et al.* also analyzed ADSC-CM using two-dimensional gel electrophoresis and detected approximately 300 features from ADSC-CM, including the finding that secreted proteins are upregulated or downregulated by induction of adipogenesis [42]. We analyzed ADSC-CM by direct injection of trypsinized peptide into a liquid chromatography tandem mass spectrometry (LC-MS/MS) system and detected approximately 100 proteins. However, only five proteins (IGF-binding protein 4 (IGFBP4), IGFBP7, metalloproteinase inhibitor 1 precusor, IL-6 and IL-8) were cytokines or growth factors [6]. In addition, we are conducting proteomic analysis by two-dimensional electrophoresis, but only abundant proteins have been detected using our methods. Although the intracellular and secretory proteins of ADSCs have been analyzed either through two-dimensional electrophoresis coupled mass spectrometry or non-gel-based mass spectrometry, these techniques cannot identify the active proteins in ADSCs that are responsible for regeneration. This may be due to the fact that these techniques use a proteomics approach and are limited to analysis of only highly abundant proteins. Instead, we analyzed the growth factors in ADSC-CM using a growth factor antibody array against 41 growth factors, and detected EGF, fibroblast growth factor (FGF) 4, IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, G-CSF, GM-CSF, IGF-3, macrophage colony stimulating factor (MCSF), platelet-derived growth factor (PDGF), TGF-β and VEGF (**Figure 2**, our unpublished data). New proteomic analysis techniques for ADSC-CM are needed in correlation with other state-of-the-art analytical tools, which must be followed up by functional study to clarify the active proteins of skin repair.

3. Wound-healing effect of ADSCs

Although chronic wounds are common, treatment for these disabling conditions remains limited and largely ineffective. Recently, cell therapy using ADSCs was developed to cure skin wounds, and it was concluded that they held promise for wound repair [2,9,10]. In short, the wound-healing effect of ADSCs is mediated by secretory factors and the function is enhanced by hypoxia.

3.1 Wound-healing effect of ADSCs

Wound repair by adult stem cells was originally demonstrated using BMSCs [14]. Wu *et al.* showed that BMSC injection around a wound significantly enhanced wound-healing in normal and diabetic mice compared with that of allergenic neonatal dermal fibroblasts [44]. Sasaki *et al.* demonstrated that BMSCs can differentiate into multiple skin cell types including keratinocytes, pericytes and endothelial cells that contribute to wound repair [28]. Notably, BMSC-CM contains paracrine factors for wound healing [45]. In comparison with dermal fibroblasts, analysis of proteins in BMSC-CM indicated that BMSCs secreted distinctively different cytokines and chemokines, such as greater amounts of VEGF-α, IGF-I, EGF, keratinocyte growth factor (KGF), angiopoietin-1, stromal-derived factor-1, macrophage inflammatory protein-1α and β, and erythropoietin [45].

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A.

	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
2	POS	POS	NEG	NEG	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
3	GCSF	GDNF	GM-CSF	HS-EGF	HGF	IGFSP-1	IGFSP-2	IGFSP-3	IGFSP-4	IGFSP-5	IGF-I	IGF-I SR
4	GCSF	GDNF	GM-CSF	HS-EGF	HGF	IGFSP-1	IGFSP-2	IGFSP-3	IGFSP-4	IGFSP-5	IGF-I	IGF-I SR
5	IGF-III	M-CSF	M-CSF R	NT-3	NT-4	PDGF R α	PDGF R β	PDGF-AA	PDGF-AB	PDGF-BB	FIGF	SCF
6	IGF-III	M-CSF	M-CSF R	NT-3	NT-4	PDGF R α	PDGF R β	PDGF-AA	PDGF-AB	PDGF-BB	FIGF	SCF
7	SCF R	TGF-α	TGF-β	TGF-β 2	TGF-β 3	VEGF	VEGF R2	VEGF R3	VEGF-D	BLANK	BLANK	POS
8	SCF R	TGF-α	TGF-β	TGF-β 2	TGF-β 3	VEGF	VEGF R2	VEGF R3	VEGF-D	BLANK	BLANK	POS

B.

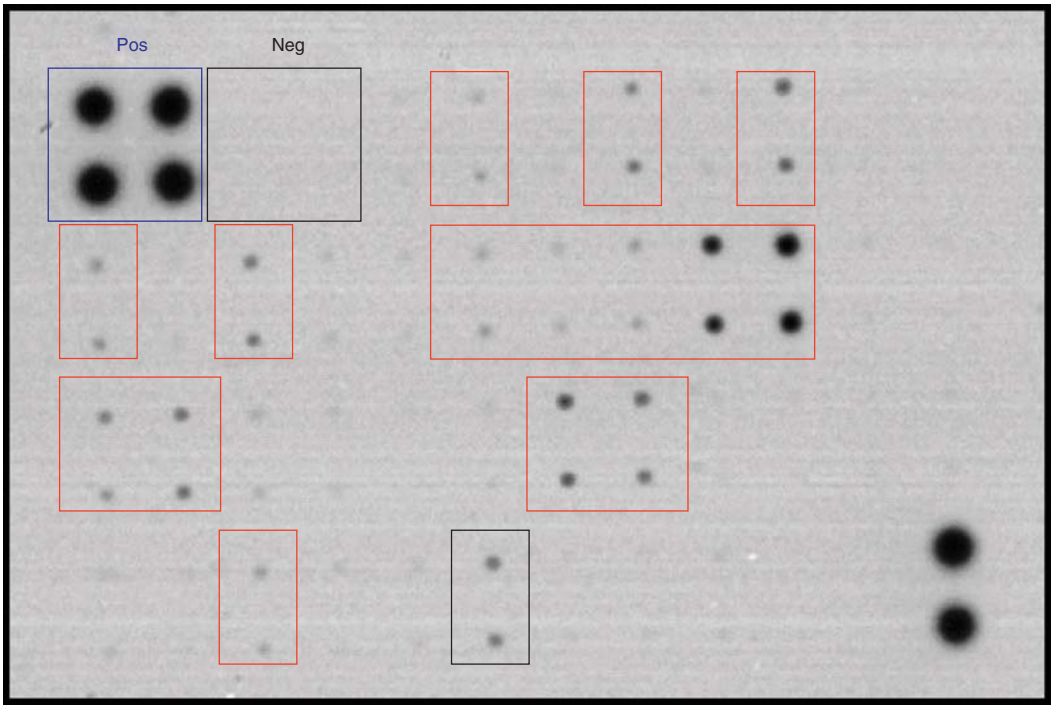

Figure 2. Growth factor antibody array of adipose-derived stem cells-conditioned media (ADSC-CM). List of growth factors in the membrane **(A)** and significantly detected growth factors are marked **(B)**.

AR: Androgen receptor; FGF: Fibroblast growth factor; HB-EGF: Heparin-binding EGF; HGF: Hepatocyte growth factor; IGF-BP: IGF binding protein; M-CSF: Macrophage colony stimulating factor; NEG: Negative control; NGF Nerve growth factor; NT: Neurotrophin; PDGF: Platelet-derived growth factor; PlGF: Placental growth factor; POS: Positive control; SCF: Stem cell factor.

ADSCs have surface markers and gene profiling similar to BMSCs and their soluble factors are not significantly different [3,9,25]. Given their convenient isolation compared with BMSCs and extensive proliferative capacities *ex vivo*, ADSCs hold great promise for use in wound repair and regeneration [24,46]. Recently, evidence has accumulated that demonstrates the wound-healing effects of ADSCs [1,2,9,47,48]. Our group first demonstrated that ADSCs accelerate wound healing in *ex vivo* and *in vivo* experiments [9]. In the present study, ADSC-CM stimulated the migration of dermal fibroblasts after wounds were made in primary cultured fibroblasts. ADSCs secreted a variety of growth factors such as bFGF, KGF, TGF-β, HGF and VEGF into the conditioned medium, which might have mediated 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 and accelerated the re-epithelialization at the wound edge. Like ADSC treatment, ADSC-CM treatment also accelerated wound-healing in laser-induced-burn mouse models (our unpublished data). Nambu *et al.* inflicted full-thickness skin defects on the backs of healing-impaired db/db mice, then applied an atelocollagen matrix with silicon membrane (ACMS) containing freshly isolated autologous ADSCs. Histological sections of the wounds were prepared at 1 and 2 weeks following treatment, which demonstrated significantly advanced granulation tissue formation, capillary formation, and epithelialization [48]. Although differentiation of ADSCs into skin components and

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their contribution to skin repair was not fully investigated, all the results indicate that ADSCs accelerate wound healing and secreted proteins of ADSCs account, at least partially, for their wound-healing effect.

ADSCs are physiologically located beneath dermal fibroblasts, and they may interact with them. However, ADSCs and their secretory factors may reach the epidermis in the case of skin damage because our results suggest that ADSCs may accelerate wound healing of the epidermis. As evidence, ADSC-CM was treated in cultured primary human keratinocytes, which increased the proliferation and migration of keratinocytes (our unpublished data). In addition, Yuan *et al.* demonstrated that rat ADSCs promoted the migration of human epidermal keratinocytes following direct contact [47]. There is *in vivo* evidence that an epidermal wound induced by trichloroacetic acid was significantly reduced by ADSC-CM treatment (our unpublished data). These results indicate that ADSCs activate keratinocytes and accelerate the healing of epidermal wounds in addition to that of dermal wounds.

3.2 Hypoxia-enhanced wound-healing effect of ADSCs

Hypoxia amplifies the paracrine effects of MSCs by enhancing the secretion of certain growth factors [37,38,49]. For example, ADSCs improved perfusion in hindlimb ischemia induced by the ligation of femoral arteries, a function that was enhanced by hypoxic culture conditions [38]. Xu *et al.* also reported that BMSC-CM promoted cardiomyocyte hypertrophy in a paracrine manner, which was enhanced by hypoxia [50]. In the present study, secretion of some growth factors was upregulated by hypoxia, which may enhance the function of ADSCs. Because inflammation and oxidative stress near the wound area evokes an oxygen deficit state and hypoxia reportedly enhanced the function of ADSCs, the wound-healing effect of ADSCs was investigated using a conditioned medium of ADSCs cultured in hypoxia or normoxia (manuscript accepted in *Wound Repair and Regeneration*). As expected, ADSC-CM from cultures in hypoxia significantly reduced the wound area compared with those in normoxia in hairless mice (**Figure 3**). An *in vitro* migration assay using dermal fibroblasts also revealed that hypoxia-cultured CM significantly increased the migration of dermal fibroblasts. Furthermore, mRNA and protein measurements showed that hypoxia upregulated specific growth factors such as VEGF and bFGF. Therefore, an inhibition study using neutralizing antibodies of VEGF and bFGF was carried out in both animal and *in vitro* models, and neutralization resulted in delayed wound healing (manuscript accepted in *Wound Repair and Regeneration*). These results indicate that VEGF and bFGF account, at least partially, for the hypoxia-enhanced wound-healing function of ADSCs.

4. Antioxidant effect of ADSCs

Many environmental stimuli catalyze the production of reactive oxygen species (ROS), which may be involved in the pathogenesis of a number of skin disorders including photosensitivity diseases and some types of cutaneous malignancies. Although there are only a few reports on the antioxidant action of ADSCs *in vivo*, some evidence supports a protective effect of ADSCs and their secretory factors during oxidative injury [51-55].

4.1 Protection from chemicals

In biological systems, the normal processes of oxidation produce highly reactive free radicals, which may continue to damage cells. Antioxidants play a housekeeping role, scavenging free radicals before they get a chance to do harm to the body. Recent evidence has supported the protective role of ADSCs against skin oxidative damage, most of which is mediated by secretory factors [51-55]. For example, IGF reportedly protects fibroblasts and intestinal epithelial cells from free radicals [51,52]. HGF protects the retinal pigment epithelium against oxidative stress induced by glutathione depletion [53]. Pigment epithelium-derived factor (PEDF) is an anti-angiogenic/neurotropic factor and has been shown to have antioxidant effects [54]. Further critical evidence shows that PDGF secreted from the cancer cells protects fibroblasts from oxidative stress via the PI3K pathway [55]. In addition, subtypes of superoxide dismutase (SOD) are expressed and secreted from stem cells [6,56]. All of these reports suggest an antioxidant effect for the secretory factors of ADSCs.

The antioxidant function of ADSCs was first demonstrated in dermal fibroblasts after inducing chemical oxidative stress by tert-butyl hydroperoxide (tbOOH) [6]. We suggested that ADSC-CM protects dermal fibroblasts from oxidative stress. ADSC-CM was analyzed using LC-MS/MS-based proteomic analysis, growth factor antibody array and ELISA methods, which detected various antioxidant proteins such as IGFBPs, G-CSF, GM-CSF, PDGF-AA, SOD2, PEDF, and HGF in ADSC-CM (**Table 2**) [6,9,10]. In addition, morphological change and a cell survival assay revealed that incubation with ADSC-CM aided dermal fibroblasts in their resistance of free radicals induced by tbOOH [6]. The cellular mechanism of protection was the enhancement of the activities of SOD and glutathione peroxidase (GPx) in the dermal fibroblasts after treatment with ADSC-CM. In a cell-cycle analysis, ADSC-CM treatment reversed apoptotic cell death induced by tbOOH, which was demonstrated by a significant decrease in the sub-G1 phase of dermal fibroblasts. A similar anti-apoptotic effect of ADSC-CM was reproduced by caspase-3 activity assay [6]. tbOOH treatment increased the caspase-3 activity in dermal fibroblasts, but this phenomenon was reversed by ADSC-CM pretreatment. Although further *in vivo* study regarding the antioxidant effect of ADSCs was not extended by tbOOH treatment, our preliminary study on the accelerated wound-healing and anti-wrinkling by ADSCs suggested a potent role for ADSCs and their secretory factors in the repair of oxidative skin damage [7,9].

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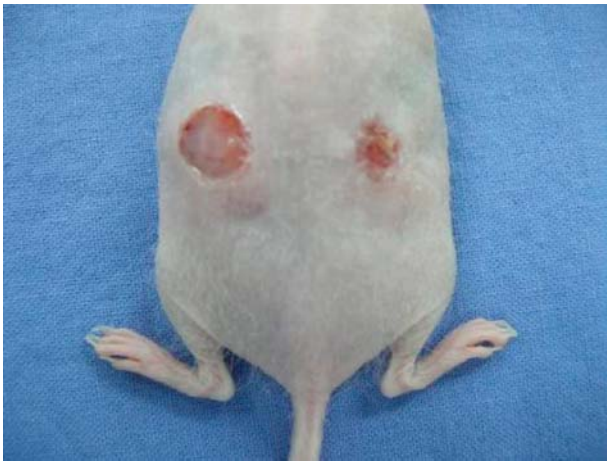

Figure 3. Wound-healing effect of adipose-derived stem cells-conditioned media (ADSC-CM) obtained from cultures grown under normoxia (left side) and hypoxia (right side) 4 days after surgery. Wounds were made by 8 mm punch biopsy and hypoxia enhanced the wound-healing effect in hairless mice.

Table 2. Antioxidant proteins in ADSC-CM.

Secreted proteins	Detection method
HGF	ELISA
G-CSF	Antibody array, ELISA
GM-CSF	Antibody array
IGFBPs	LC-MS/MS, antibody array
IL-12	LC-MS/MS
PDGF-AA	ELISA, antibody array
PEDF	LC-MS/MS, ELISA
SODs	LC-MS/MS, ELISA

LC-MS/MS: Liquid chromatography-tandem mass spectrometry; HGF: Hepatocyte growth factor; PEDF: Pigment epithelium-derived factor; SOD: Superoxide dismutase.

In addition to mesenchymal-originated dermal fibroblasts, survival of epithelial component of skin (i.e., keratinocytes) was investigated after tbOOH treatment; ADSC-CM treatment increased the survival of keratinocytes. Cell cycle analysis revealed that apoptotic cells induced by tbOOH were significantly reduced by ADSC-CM pretreatment. By contrast, SOD and GPx activities of keratinocytes were significantly increased by ADSC-CM (our unpublished data).

4.2 Protection from UVB

UV radiation is believed to be responsible for almost 80% of the skin changes commonly attributed to the aging process. UVB causes redness of the skin, whereas UVA penetrates deeper into the dermis and causes more DNA damage [49,57]. This exposure results in a significant decrease in collagen production and an increase in pigmentation [7]; the accumulation of this exposure is responsible for irregular skin thickening, mottled pigmentation and wrinkling [10,57-59]. The pathological agents responsible for UV-induced changes such as wrinkles and roughness are ROS that deplete and damage the non-enzymatic and enzymatic antioxidative defense mechanism of the skin, leading to oxidative damage of the skin and ultimately to premature aging and cancer [57,59]. Our group investigated the protective effect of ADSCs in UVB-damaged skin using cultured dermal fibroblasts [7]. UVB irradiation induced the cell death of dermal fibroblasts. However, ADSC-CM pretreatment significantly reduced the apoptosis of dermal fibroblasts, which was demonstrated by a significant decrease in the sub-G1 phase of dermal fibroblasts after ADSC-CM pre-treatment. As demonstrated for chemically induced ROS [6], ADSC-CM had a protective effect on UVB-induced apoptosis [7]. In addition, ADSC-CM treatment increased the production of collagen and reduced the expression of matrix metalloproteinase 1 in the dermal fibroblasts [7]. These results indicate that ADSCs play a key role in protecting dermal fibroblasts from UVB-induced oxidative stress.

5. Expert opinion

The wound-healing and antioxidant effect of ADSCs and ADSC-derived secretory factors were reviewed in this paper. To date, application of ADSCs has been investigated mainly *in vitro* and animal studies, and the results are satisfactory. However, clinical application of cultured ADSCs in human skin is in its early stages and can be hazardous considering the risk of inducing cancer. In addition, ADSCs are difficult both to handle and to commercialize from an industrial point of view, so new methods and materials to overcome these limitations are needed. Instead, secreted proteins of ADSCs have numerous advantages over cell-based therapies and have great potential in skin repair, because they can be stored with long-term stability and are relatively devoid of safety issues. The use of stem cell protein also enhances the scalability of production and the potential of developing low-cost therapeutics. Until now, however, characterization of active proteins involved in the wound-healing and antioxidant functions of ADSCs has been in its early stages due to low assay sensitivity of proteomic analysis and relatively low protein concentrations in the ADSC-CM. Therefore, identification of active proteins from ADSC-CM will be the next goal of our research, and drug development using these proteins should reveal many effective strategies for skin repair and regeneration in the future.

Declaration of interest

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