Ann Dermatol Vol. 33, No. 4, 2021
pISSN 1013-9087 · eISSN 2005-3894
https://doi.org/10.5021/ad.2021.33.4.324

ORIGINAL ARTICLE

Keratinocyte-Like Cells Trans-Differentiated from Human Adipose-Derived Stem Cells, Facilitate Skin Wound Healing in Mice

Jonghun Kim[1], Toshio Hasegawa[1], Akino Wada[1], Yuichiro Maeda[1], Shigaku Ikeda[1,2]

[1]Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, [2]Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Background: Mesenchymal stem cells (MSCs) have been reported to promote wound healing in both animal models and human studies. Among MSCs, adipose-derived stem cells (ADSCs) can be easily harvested in large quantities. **Objective:** We investigated whether skin wound healing in mice can be facilitated by keratinocyte-like cells differentiated from ADSCs (KC-ADSCs). **Methods:** For the wound contraction and epithelialization model, a 20 mm×20 mm full-thickness skin wound was made on the dorsum. For the wound epithelialization model, a 6 mm×6 mm full-thickness skin wound was made on the dorsum. A nitrile rubber stent with an inner diameter of 8 mm was sutured around the wounds to minimize wound contraction. Undifferentiated ADSCs (uADSCs) or KC-ADSCs was injected around the wound base in both models. To evaluate whether the injected ADSCs could enhance wound contraction in a skin wound, the contractile activity of ADSCs was assessed by an *in vitro* type I collagen gel contraction assay. Alpha-smooth muscle actin (α SMA) expressions in uADSCs and KC-ADSCs were also evaluated by flow cytometry and real-time polymerase chain reaction. **Results:** In a wound contraction and epithelialization model, KC-ADSCs further facilitated wound healing compared with uADSCs. In a wound epithelialization model, KC-ADSCs also further facilitated wound epithelialization compared with uADSCs. The contractile activity of KC-ADSCs was lower than that of uADSCs. The uADSCs expressed high levels of α SMA, which decreased after the differentiation into keratinocyte-like cells. **Conclusion:** Our results suggest that the wound healing effect of KC-ADSCs depends primarily on re-epithelialization rather than wound contraction. **(Ann Dermatol 33(4) 324~332, 2021)**

-Keywords-
Keratinocytes, Mesenchymal stem cells, Wound healing

INTRODUCTION

Mesenchymal stem cells (MSCs) can be isolated from various tissues such as bone marrow, umbilical cord, placenta, fetal tissues, skin, and adipose tissues[1-3]. Adipose-derived stem cells (ADSCs) are MSCs that can be easily harvested in large quantities and produce 500-times more colony-forming units than bone marrow-derived MSCs, which are the major source of MSCs for clinical use[4]. Although the dermis can also be easily harvested, a previous study showed that dermal stem cells represent only 0.3% of the human dermal foreskin fibroblasts[2,5]. Therefore, ADSCs have the potential to be a practical and promising tool for regenerative medicine[6].

MSCs are immune-evasive cells because they do not express major histocompatibility complex (MHC) class II antigens and minimally express MHC class I antigens[7]. This lack of immunogenicity enables the use of MSCs in clinical applications such as allogeneic cell transplantation[8].

Received September 16, 2020, Revised November 5, 2020, Accepted for publication November 19, 2020

Corresponding author: Shigaku Ikeda, Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Tel: 81-3-5802-1226, Fax: 81-3-3813-9443, E-mail: ikeda@juntendo.ac.jp
ORCID: https://orcid.org/0000-0002-7520-0707

MSCs secrete immunomodulatory, anti-inflammatory, pro-angiogenic, pro-mitogenic, and antibacterial factors such as transforming growth factor β1 (TGF-β1), hepatocyte growth factor, haemoxgenase-1, prostaglandin E2, interleukin (IL)-10, and HLA-G5[9-12]. Of the different MSCs available, ADSCs show low immunogenicity and high immunosuppressive potential[13-19].

MSCs have been reported to promote wound healing in both animal models and human studies[20-27]. The clinical utility of MSCs in wound healing is based on repairing and replacing cellular substrates, attenuating inflammation, increasing angiogenesis, and enhancing migration of reparative cells by cytokine/chemokine production. The differentiation potential of MSCs into keratinocytes is also required for re-epithelialization of wounds. It has previously been reported that keratinocyte progenitor cells reside in ADSCs, and ADSCs can further differentiate into keratinocyte-like cells[28,29]. ADSCs may be utilized in wound repairs, including those for deep wounds and burns. In this study, we investigated whether keratinocyte-like cells differentiated from ADSCs (KC-ADSCs) can facilitate skin wound healing in mice.

MATERIALS AND METHODS

Transdifferentiation of ADSCs into keratinocyte-like cells

ADSCs (Lonza Group AG, Basel, Switzerland) were co-cultured with normal human dermal fibroblasts (NHDFs) as previously reported[28,30]. Briefly, NHDFs were seeded in six-well plates (IWAKI, Shizuoka, Japan) and cultured in Dulbecco's modified Eagle's Medium (DMEM) (Gibco, Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA) for 24 hours, while ADSCs were seeded on 0.4-μm Millicell® hanging cell culture inserts (Merck Millipore, Darmstadt, Germany) coated with type IV collagen (Nitta Gelatin, Osaka, Japan) and placed onto the plates. All-trans retinoic acid (Sigma-Aldrich) was added at 1 μM to the upper chamber. After culturing for 3 days, 25 ng/ml of bone morphogenetic protein 4 (R&D Systems, Minneapolis, MN, USA) was also added to the upper chamber. After 4 days, the media were replaced with keratinocyte serum-free medium (KSFM) (Thermo Fisher Scientific, Waltham, MA, USA). After 7 days of culture in KSFM, ADSCs were removed from the co-culture system and cultured on a dish coated with type IV collagen in KSFM for an additional 14 days.

Green fluorescent protein transfection into ADSCs

ADSCs were cultured at 60% to 80% confluence for transfection of green fluorescent protein (GFP). EmGFP Vector (Invitrogen, Carlsbad, CA, USA) was diluted with Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific) (1:1 ratio) before being added to the cells. Cells were incubated at 37°C for 48 hours and then the transfected cells were analyzed.

Mice

All animal experiments were performed according to the guidelines of the Ethical Committee of Juntendo University. All experimental mice were housed in cages with a 12-hour light–dark cycle at 22°C to 25°C with 55% to 60% humidity. Solid food and water were supplied ad libitum. Eight-week-old ICR-nu mice (Crlj:CD1-Foxn1^{nu}) were purchased from Charles River Laboratories Japan (Yokohama, Japan). We used the ARRIVE checklists when writing our report[31].

Wound healing model

For the wound contraction and epithelialization model, a 20 mm×20 mm full-thickness skin wound was made on the dorsum under systemic anesthesia with isoflurane (Phoenix Pharmaceuticals, Burlingame, CA, USA). Five hundred microliters of phosphate buffered saline (PBS) containing 1×10^6 undifferentiated ADSCs (uADSCs) (n=6) or KC-ADSCs (n=6) was injected intradermally around the wound base at four sites. The wound was then covered by Tegaderm™ (3M, Saint Paul, MN, USA) to prevent licking and drying of the wound. In the control group (n=6), the same volume of PBS without cells was injected intradermally around the wound base at four sites.

Detection of ADSCs in the wound healing model

Wound specimens for histological analysis were harvested from the euthanized animals 2 days after cell injection. The specimens were frozen in optimal cutting temperature compound for cryosectioning. Three-micrometer thick sections were made by cutting from the central region of the wound. Frozen sections were embedded in 4% paraformaldehyde and nuclei were counterstained with 4,6-diamino-2-phenylindole (DAPI). Sections were imaged and digitized in their entirely at 40-fold magnification (40×) with a BZ-700 microscope (Keyence, Osaka, Japan).

Wound epithelialization model

For the wound epithelialization model, a full-thickness skin wound was produced by 6-mm biopsy punches lateral to the midline of the back under systemic anesthesia with isoflurane (Phoenix Pharmaceuticals). A nitrile rubber stent with an inner diameter of 8 mm was sutured with 5-0 nylon (Ethicon, Somerville, NJ, USA) around the wounds to minimize wound contraction and to ensure healing by epithelialization. Two hundred and fifty microliters of PBS

containing 1×10^6 uADSCs (n=6) or KC-ADSCs (n=6) was injected intradermally around the wound base at four sites. The wound was then covered by Tegaderm™ (3M) to prevent licking and drying of the wound. In the control group (n=6), the same volume of PBS without cells was injected intradermally around the wound base at four sites.

Detection of α-smooth muscle actin-positive cells in wound epithelialization model by immunohistochemistry

Alpha-smooth muscle actin (αSMA), an actin isoform found in vascular smooth muscle cells, has been observed to be one of the most useful markers for myofibroblast differentiation[32,33]. αSMA-positive cells in the wound healing model were detected by immunohistochemistry. Wound specimens were harvested from the euthanized animals 3, 5, and 7 days after cell injection for immunohistochemistry. The specimens were fixed with 4% paraformaldehyde/PBS and embedded in paraffin. The sample tissues were cut into 3-μm thick sections and stained with an anti-αSMA rabbit monoclonal antibody (Abcam, Cambridge, MA, USA) (1:1,000). Each section was automatically stained using Benchmark (Ventana Medical Systems, Oro Valley, AZ, USA) and was imaged and digitized in its entirety at the 40 times magnification (40×) with a BZ-700 Analyzer (Keyence).

Wound healing analyses

The wounds of individual mice were photographed and the wound area was measured on days 1, 4, and 7 (wound healing model) and on days 0, 3, 5, and 7 (wound epithelialization model) after cell transplantation. Photographs were taken with a 20-megapixel digital camera (SX 720HS; Canon, Tokyo, Japan) from a distance of 3 cm, with the lens facing the wound vertically.

The wound area was measured by tracing the wound margin and calculated using an image analysis program (Photoshop CC; Adobe Systems, San Jose, CA, USA) and Image J (National Institute of Health, Bethesda, MD, USA). The wound healing rate was calculated as follows: {1-[(wound area/original wound area]}×100 (%).

Collagen gel contraction assay

To evaluate whether the injected ADSCs could enhance wound contraction in a full-thickness skin wound, the contractile activity of ADSCs was assessed by an *in vitro* type I collagen gel contraction assay, which is used for evaluating contractile activity of fibroblasts[34-36]. Briefly, a collagen solution was prepared by mixing acid-soluble porcine type I collagen (3 mg/ml), a five-fold concentration of DMEM, and buffer solution (0.05 M NaOH, 2.2% $NaHCO_3$, 200 mM HEPES) in the ratio of 7:2:1 (all purchased from Nitta Gelatin). A 2.0-ml mixture of cell suspension in serum-free adipose-derived stem cell basal medium (ADSC-BM) (Lonza Group AG) and collagen solution was added to each well of a 12-well plate (IWAKI) and then gelled at 37°C for 30 to 40 minutes (final concentration 1.0×10^5 cells/ml and 2.1 mg/ml collagen). A further 1 ml of serum-free ADSC-BM was then poured on to the gel to prevent the surface from dehydrating. After incubation of 1 hour, each gel was separated from the well, floated, and 10 ng/ml of recombinant human TGF-β1 or vehicle control was applied. At 48 hours after the application of TGF-β1 or vehicle control, the major and minor axes of each gel samples were measured, and the surface area was calculated. The contraction of the gel was expressed as a percentage of the surface area of the non-contracted state (defined as 100%). Both uADSCs and KC-ADSCs were assessed in this manner, and NHDF-embedded collagen gel was also assessed as a control. Twelve-well culture plates were used for each group (n=6).

Detection of α-smooth muscle actin in ADSCs by immunofluorescence and flow cytometry

ADSCs and NHDFs were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and immunostained for αSMA using a rabbit monoclonal anti-αSMA antibody (Abcam). The nuclei were counterstained with DAPI, and samples were mounted in Mounting Medium with DAPI H-1200 (Vector Laboratories, Burlingame, CA, USA) to detect fluorescence and visualized under a BZ-X700 microscope (Keyence).

ADSCs and NHDFs were also incubated with anti-αSMA rabbit monoclonal antibody (Abcam) (1:1,000), then incubated with goat anti-rabbit immunoglobulin G H&L FITC (Abcam), and analyzed by flow cytometry.

Expression analysis of α-smooth muscle actin mRNA in ADSCs by real-time polymerase chain reaction

αSMA mRNA expressions in uADSCs, KC-ADSCs, and NHDFs were evaluated by real-time polymerase chain reaction (PCR). Total RNA extracted from each cells was converted into cDNA using the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA) was used to amplify cDNA for 50 cycles on a StepOnePlus system (Applied Biosystems). αSMA expression (using primer Hs00426835_g1; Applied Biosystems) was normalized to GAPDH levels, and the comparative cycle threshold (Ct) method using the formula $2^{-\Delta\Delta Ct}$ was used to calculate the relative mRNA levels.

Statistical analysis

All experimental values were expressed as mean±standard deviation. Groups were compared using one-way ANOVA with Bonferroni post hoc analysis. Statistical significance was defined as a p-value <0.05, p-value <0.01.

Ethical approval

The study protocol was approved by the Ethics Committee of the Juntendo University Graduate School of Medicine (approval no. 2012020). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Data were analyzed in a blinded fashion, and procedures were carried out according to

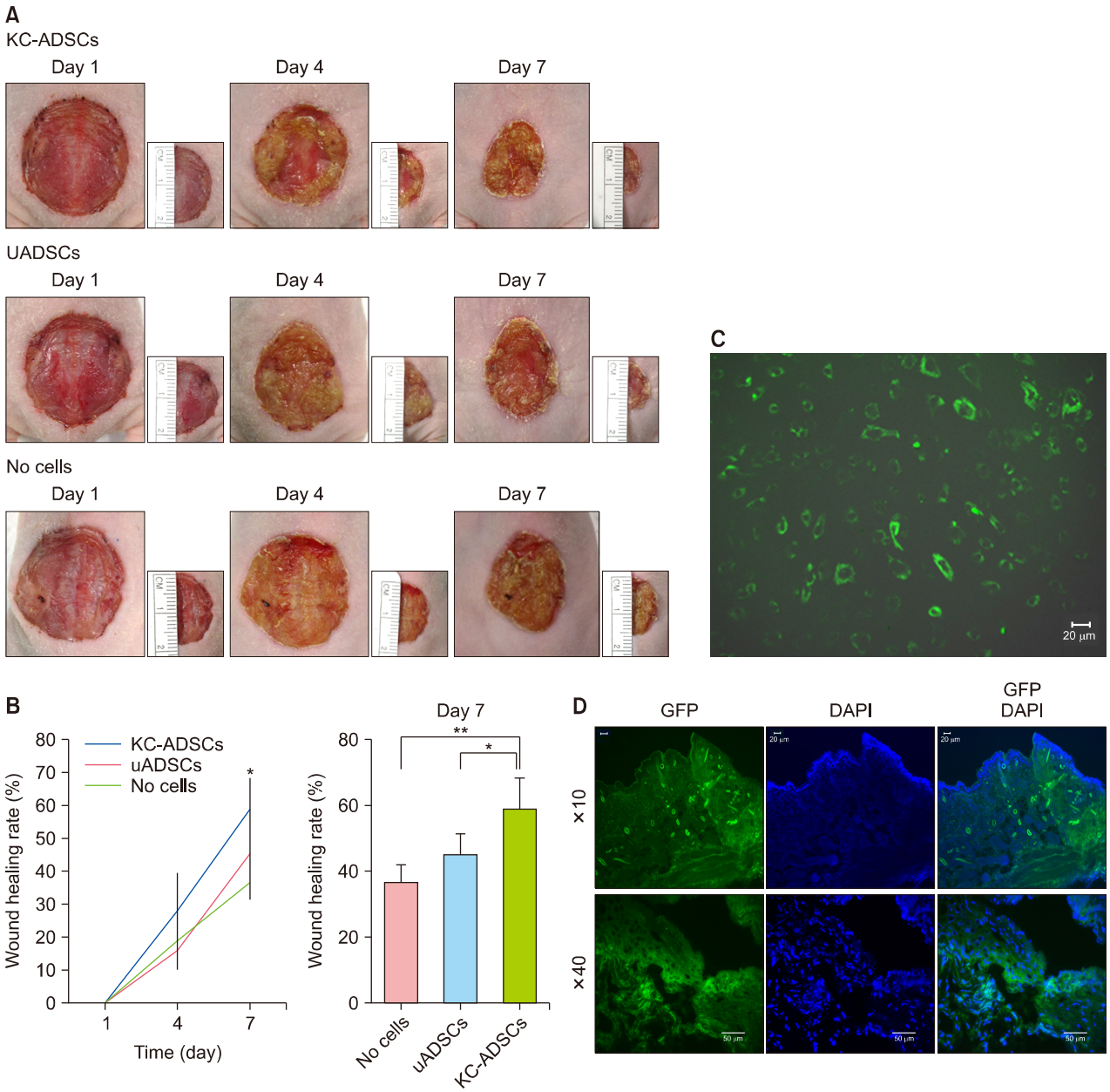

Fig. 1. (A) Photographs of the wounds on days 1, 4, and 7. (B) The average wound healing rate of the uADSCs-injected group was significantly higher than that of the control group on day 7. The average wound healing rate of the KC-ADSCs-injected group was also significantly higher than that of the uADSCs-injected group. (C) Before transplantation, it was confirmed that 80% to 90% of total ADSCs expressed GFP. (D) GFP+ADSCs were sporadically identified in the dermis of the wound. uADSCs: undifferentiated adipose-derived stem cells, KC-ADSCs: keratinocyte-like cells differentiated from ADSCs, No cells: phosphate buffered saline (control), GFP: green fluorescent protein. Statistically significant (*$p<0.05$, **$p<0.01$).

the principles of the Declaration of Helsinki.

RESULTS

ADSCs facilitated wound healing in mice

Digital photographs of the wounds were obtained on days 1, 4, and 7 (Fig. 1A). The average wound healing rate of the mice injected with uADSCs was significantly higher than that of the control group on day 7 (Fig. 1B). The average wound healing rate of mice injected with KC-ADSCs was also significantly higher than that of the uADSCs-injected group on day 7 (Fig. 1B).

Before transplantation, it was confirmed that 80% to 90% of total ADSCs expressed GFP (Fig. 1C). After transplantation, GFP+ADSCs were sporadically identified in the dermis of the wound of both uADSCs-injected group and (Fig. 1D) and KC-ADSCs-injected group, while no GFP+ ADSCs were detected in the wounds of controls (data not shown).

ADSCs facilitated wound epithelialization in mice

Digital photographs of the wounds were obtained on days 0, 3, 5, and 7 (Fig. 2A). As in the wound healing model, the average wound healing rate of the mice injected with uADSCs was significantly higher than that of the control group on day 7 (Fig. 2B). The average wound healing rate of mice injected with KC-ADSCs was also significantly higher than that of the uADSCs-injected group on day 7 (Fig. 2B). These results suggested that in this wound healing model, KC-ADSCs further promoted wound healing compared with uADSCs not only by epithelialization but also by granulation tissue contraction. Samples from the uADSCs-injected group showed an increase of α SMA-positive cells in the upper dermis compared with controls (Fig. 2C). There was no significant difference in the appearance of α SMA-positive cells between the uADSCs-injected group and KC-ADSCs-injected group.

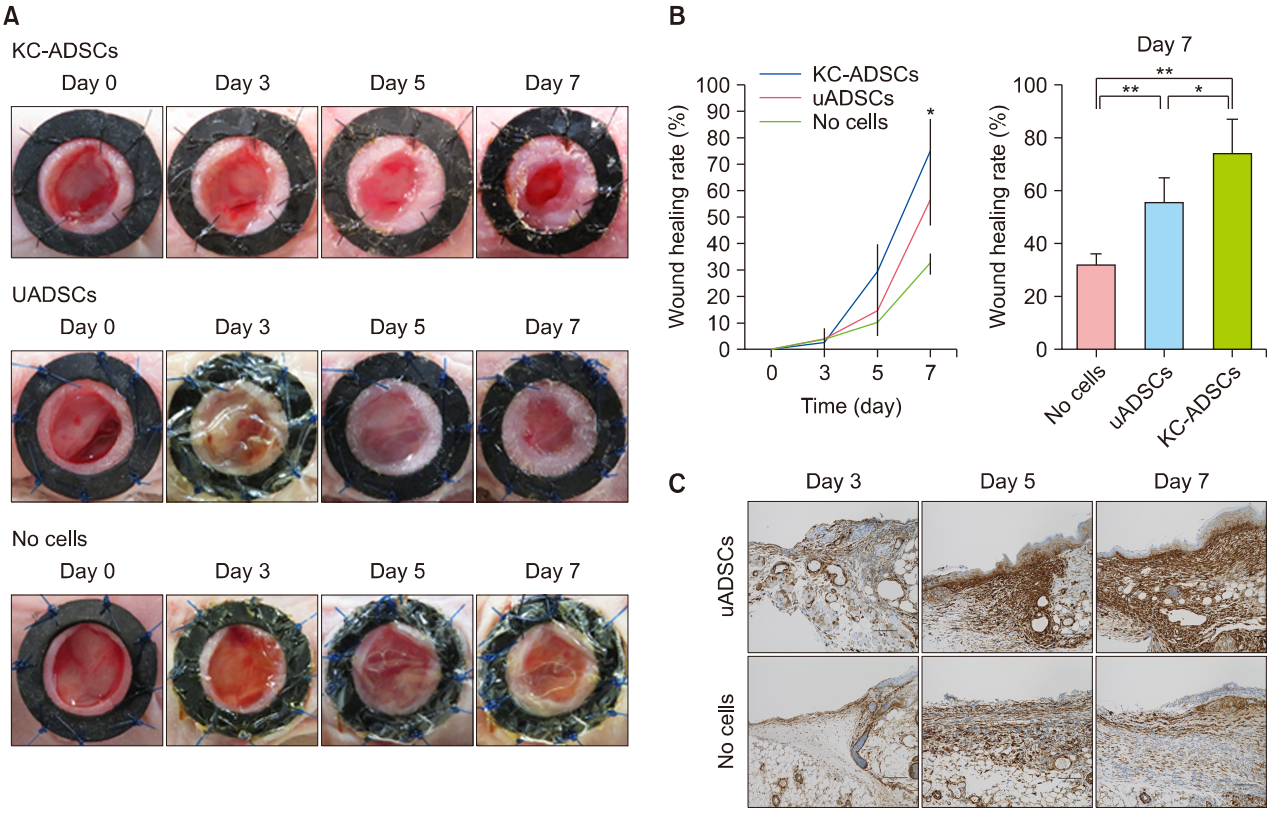

Fig. 2. (A) Photographs of the wounds on days 0, 3, 5, and 7. (B) The average wound healing rate of the uADSCs-injected group was significantly higher than that of the control group on day 7. The average wound healing rate of the KC-ADSCs-injected group was also significantly higher than that of the uADSCs-injected group. (C) Samples from the uADSCs-injected group showed an increment of α-smooth muscle actin (α SMA)-positive cells in the upper dermis compared with controls by immunohistochemistry (original magnification ×20). There was no significant difference in the appearance of α SMA-positive cells between the uADSCs-injected group and KC-ADSCs-injected group. uADSCs: undifferentiated adipose-derived stem cells, KC-ADSCs: keratinocyte-like cells differentiated from ADSCs, No cells: phosphate buffered saline (control), GFP: green fluorescent protein. Statistically significant (*$p<0.05$, **$p<0.01$).

KC-ADSCs have less contractile activity than uADSCs

The uADSCs-embedded collagen gels showed contraction rate of 79.6%±5.0% as compared with control NHDF-embedded collagen gels whose contraction rate of 98.0%±1.7%. TGF-β1 further promoted contraction of uADSCs-embedded collagen gels to 61.6%±4.2%, while NHDF-embedded collagen gels with added TGF-β1 showed enhanced contraction to 96.2%±0% (Fig. 3A, B).

KC-ADSCs-embedded collagen gels showed contraction rate of 91.7%±4.7%, which was promoted to 74.6%±6.2% by the addition of TGF-β1 (Fig. 3A, B). The contractile activity of KC-ADSCs was lower than that of uADSCs. This result suggests that the effect of KC-ADSCs does not primarily depend on contractile activity but rather on promoting epithelialization.

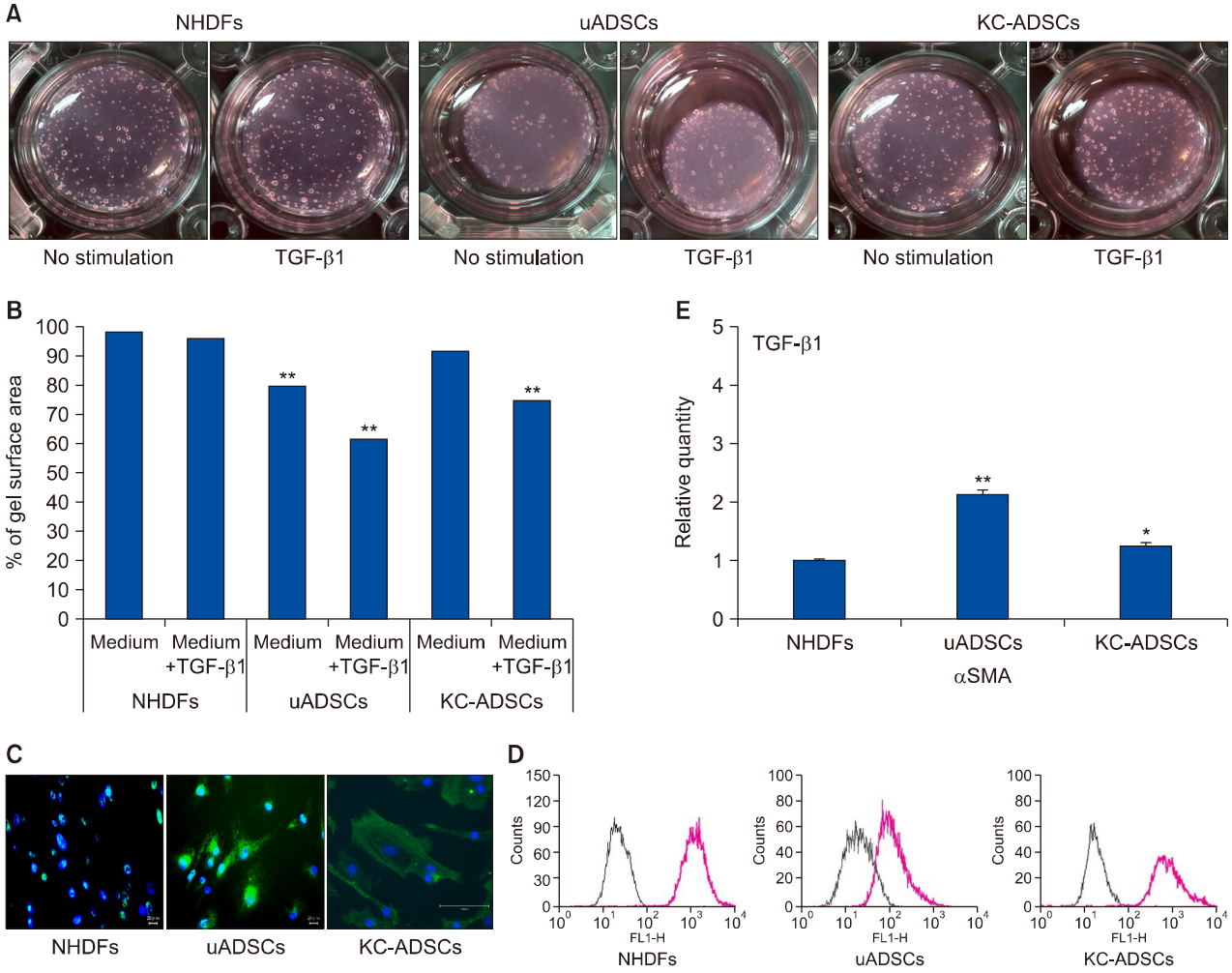

Fig. 3. (A) The uADSC-embedded collagen gels showed enhanced contraction compared with NHDFs. However, KC-ADSCs-embedded collagen gels showed lower contraction than uADSCs-embedded collagen gels. TGF-β1 further promoted contraction of all collagen gels. (B) The uADSC-embedded collagen gels showed higher contraction rate of 79.6%±5.0%, while NHDF-embedded collagen gels showed contraction rate of 98.0%±1.7%. TGF-β1 further promoted contraction of uADSC-embedded collagen gels of 61.6%±4.2%, while NHDF-embedded collagen gels showed less contraction rate of 96.2%±0% by TGF-β1. The KC-ADSC-embedded collagen gels showed contraction rate of 91.7%±4.7%, which was promoted to that of 74.6%±6.2% by TGF-β1. The contractile activity by KC-ADSCs was lower than that of uADSCs. (C) The uADSCs expressed higher levels of α-smooth muscle actin (αSMA) than NHDFs as determined by immunofluorescence microscopy. KC-ADSCs also expressed αSMA. (D) The uADSCs and KC-ADSCs expressed αSMA as determined by flow cytometry. (E) The expression level of αSMA mRNA in the uADSCs decreased after the differentiation into keratinocyte-like cells, although uADSCs expressed higher levels of αSMA mRNA than NHDF as determined by real-time polymerase chain reaction. NHDFs: normal human dermal fibroblasts, TGF: transforming growth factor, uADSCs: undifferentiated adipose-derived stem cells, KC-ADSCs: keratinocyte-like cells differentiated from ADSCs, αSMA: α-smooth muscle actin. Statistically significant (*$p<0.05$, **$p<0.01$).

The uADSCs expressed high levels of α-smooth muscle actin before differentiation into keratinocyte-like cells

The uADSCs expressed higher levels of α SMA than NHDF as determined by immunofluorescence microscopy (Fig. 3C). The expression of α SMA in ADSCs was also detected by flow cytometry (Fig. 3D). The levels of α SMA mRNA in uADSCs as determined by real-time PCR were also higher than those in NHDFs, but they decreased after the differentiation into keratinocyte-like cells (Fig. 3E). These observations were consistent across three independent experiments performed using three different lots of ADSCs.

DISCUSSION

In general, the human skin consists of two layers: the epidermis and dermis. The epidermis acts as a barrier against envelopment factors such as pathogens and allergens, and regulates inside-out water loss or outside-in stimuli. On the other hand, the dermis is composed of connective tissue, nerves, vessels and appendages, and provides strength and flexibility to the skin. The epidermis is a rapidly regenerating tissue that is maintained by the continuous transformation of transient amplifying cells and epidermal stem cells located in the basal layer of the interfollicular epidermis, the bulge region of hair follicles, and sebaceous glands. Epidermal stem cells differentiate into keratinocytes that are directed to the upper layers of the epidermis and terminally form stratum corneum.

Wound healing is divided into three sequential phases: the inflammatory phase, the proliferative phase, and the remodeling phase[37]. Fibroblasts and myofibroblasts play critical roles in both early and late phases, where they contribute to wound contraction, collagen deposition, and fibrosis[38,39]. Because no epidermal stem cells remain in full-thickness skin defects, re-epithelialization from the inside of an ulcer hardly occurs. Therefore, the ulcer is supposed to be closed by cell migration of epidermal stem cells surrounding the ulcer, and by wound contraction. Conventional autologous split-thickness skin grafting is the gold standard for the treatment of a large skin defect. However, for patients with extensive skin defects, split-thickness skin grafting is limited due to donor skin availability. Various biological dressings, including autologous and allogeneic cultured skin substitutes, have been used for intractable ulcers. However, allogeneic cultured skin substitutes cannot be permanently adopted. Autologous cultured skin can serve as a permanent covering; however, it is cumbersome and time-consuming to create a cultured epidermis from autologous keratinocytes.

Stem cell therapy involving autologous and allogeneic ADSCs may be used for intractable skin ulcers as ADSCs promote wound healing and can differentiate into multiple cell lineages including keratinocytes. In this study, the effectiveness of KC-ADSCs in full-thickness wound healing was experimentally studied by allogeneic transplantation of ADSCs into a mouse skin ulcer model. The experimental groups included: no cells (control), uADSCs-injected group, and the KC-ADSCs-injected group. The wound healing in all groups was examined and compared clinically and histopathologically on days 1 to 7 after cell transplantation. Our results showed that uADSCs promoted wound contraction and healing compared with controls, and that KC-ADSCs further promoted wound reepithelization, rather than would contraction.

We also determined the effectiveness of ADSCs using an animal model in which wound contraction was suppressed by attaching a stent, enabling easy evaluation of re-epithelialization. In this model, uADSCs promoted wound healing, and KC-ADSCs further promoted wound healing. This may indicate that KC-ADSCs promote not only granulation tissue contraction but also re-epithelialization of the wound.

In wound healing model, the healing rate of uADSCs was lower than that of the control group on day 4. Similarly, the healing rate of uADSCs was lower than that of the control group on day 3 in wound epithelialization model. The transplanted ADSCs may not be effective before engraftment. In immunohistochemistry, there was no significant difference in hepatocyte growth factor, IL-10, matrix metalloproteinases-10, acellular dermal matrix, and VGEF staining between the ADSCs treated and control groups (data not shown). Although the collagen gel contraction assay revealed that the contractile activity of KC-ADSCs was lower than that of uADSCs, KC-ADSCs further promoted wound healing compared with uADSCs. Although uADSCs and KC-ADSCs showed a wound healing effect, and administration of ADSCs induced an increased number of α SMA-positive cells, our results suggested that the wound healing effect of KC-ADSCs primarily depends on their re-epithelialization effect.

The use of ADSCs has several advantages, including their abundance in donors and their potential for easy isolation by minimally invasive methods such as liposuction. In addition, ADSCs can be used for the treatment of congenital or acquired large skin defects that cannot be treated with autologous split-thickness skin grafting, including epidermolysis bullosa and burns, due to not only their wound healing properties but also their potential for differentiation into keratinocytes. The method for the administration of MSCs is still debatable, and also establishment of an opti-

mal delivery procedure of ADSC by later-coming preclinical and clinical studies will be definitely required. The use of serum-free medium should be considered for clinical application to prevent infection.

ACKNOWLEDGMENT

The authors thank Yuko Kojima, Tamami Sakanishi, Hiroshi Koide, Tomomi Ikeda, Katsumi Miyahara, and Reiko Mineki for their helpful discussions and technical assistance.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

FUNDING SOURCE

None.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jonghun Kim, https://orcid.org/0000-0001-8462-5195
Toshio Hasegawa, https://orcid.org/0000-0001-9047-8427
Akino Wada, https://orcid.org/0000-0001-7585-6194
Yuichiro Maeda, https://orcid.org/0000-0001-6960-3684
Shigaku Ikeda, https://orcid.org/0000-0002-7520-0707

REFERENCES

1. Charbord P. Bone marrow mesenchymal stem cells: historical overview and concepts. Hum Gene Ther 2010;21:1045-1056.
2. Sellheyer K, Krahl D. Skin mesenchymal stem cells: prospects for clinical dermatology. J Am Acad Dermatol 2010;63:859-865.
3. Shi C, Zhu Y, Su Y, Cheng T. Stem cells and their applications in skin-cell therapy. Trends Biotechnol 2006; 24:48-52.
4. Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol 2006;24:150-154.
5. Zouboulis CC, Adjaye J, Akamatsu H, Moe-Behrens G, Niemann C. Human skin stem cells and the ageing process. Exp Gerontol 2008;43:986-997.
6. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211-228.
7. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-317.
8. Barry FP, Murphy JM, English K, Mahon BP. Immunogenicity of adult mesenchymal stem cells: lessons from the fetal allograft. Stem Cells Dev 2005;14:252-265.
9. Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell 2011;9:11-15.
10. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 2008; 3:e1886.
11. Wang J, Liao L, Tan J. Mesenchymal-stem-cell-based experimental and clinical trials: current status and open questions. Expert Opin Biol Ther 2011;11:893-909.
12. Hoogduijn MJ. Are mesenchymal stromal cells immune cells? Arthritis Res Ther 2015;17:88.
13. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 2002;30:42-48.
14. McIntosh K, Zvonic S, Garrett S, Mitchell JB, Floyd ZE, Hammill L, et al. The immunogenicity of human adipose-derived cells: temporal changes in vitro. Stem Cells 2006; 24:1246-1253.
15. Lombardo E, DelaRosa O, Mancheño-Corvo P, Menta R, Ramírez C, Büscher D. Toll-like receptor-mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential. Tissue Eng Part A 2009;15:1579-1589.
16. Puissant B, Barreau C, Bourin P, Clavel C, Corre J, Bousquet C, et al. Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. Br J Haematol 2005;129:118-129.
17. González MA, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 2009;60:1006-1019.
18. Fang B, Song Y, Liao L, Zhang Y, Zhao RC. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. Transplant Proc 2007;39:3358-3362.
19. Cho KS, Roh HJ. Immunomodulatory effects of adipose-derived stem cells in airway allergic diseases. Curr Stem Cell Res Ther 2010;5:111-115.
20. Badillo AT, Redden RA, Zhang L, Doolin EJ, Liechty KW. Treatment of diabetic wounds with fetal murine mesenchymal stromal cells enhances wound closure. Cell Tissue Res 2007;329:301-311.
21. Li H, Fu X, Ouyang Y, Cai C, Wang J, Sun T. Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. Cell Tissue Res 2006;326:725-736.
22. Ma K, Liao S, He L, Lu J, Ramakrishna S, Chan CK. Effects

of nanofiber/stem cell composite on wound healing in acute full-thickness skin wounds. Tissue Eng Part A 2011; 17:1413-1424.
23. Schneider RK, Püllen A, Kramann R, Bornemann J, Knüchel R, Neuss S, et al. Long-term survival and characterisation of human umbilical cord-derived mesenchymal stem cells on dermal equivalents. Differentiation 2010;79:182-193.
24. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007;25:2648-2659.
25. Kwon DS, Gao X, Liu YB, Dulchavsky DS, Danyluk AL, Bansal M, et al. Treatment with bone marrow-derived stromal cells accelerates wound healing in diabetic rats. Int Wound J 2008;5:453-463.
26. Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, et al. Wound therapy by marrow mesenchymal cell transplantation. Plast Reconstr Surg 2008;121:860-877.
27. Falanga V, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, et al. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng 2007;13:1299-1312.
28. Hasegawa T, Sakamoto A, Wada A, Fukai T, Iida H, Ikeda S. Keratinocyte progenitor cells reside in human subcutaneous adipose tissue. PLoS One 2015;10:e0118402.
29. Chavez-Munoz C, Nguyen KT, Xu W, Hong SJ, Mustoe TA, Galiano RD. Transdifferentiation of adipose-derived stem cells into keratinocyte-like cells: engineering a stratified epidermis. PLoS One 2013;8:e80587.
30. Maeda Y, Hasegawa T, Wada A, Fukai T, Iida H, Sakamoto A, et al. Adipose-derived stem cells express higher levels of type VII collagen under specific culture conditions. Arch Dermatol Res 2017;309:843-849.
31. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 2010; 8:e1000412.
32. Desmoulière A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;122:103-111.
33. Desmoulière A, Rubbia-Brandt L, Abdiu A, Walz T, Macieira-Coelho A, Gabbiani G. Alpha-smooth muscle actin is expressed in a subpopulation of cultured and cloned fibroblasts and is modulated by gamma-interferon. Exp Cell Res 1992;201:64-73.
34. Lee YR, Oshita Y, Tsuboi R, Ogawa H. Combination of insulin-like growth factor (IGF)-I and IGF-binding protein-1 promotes fibroblast-embedded collagen gel contraction. Endocrinology 1996;137:5278-5283.
35. Suhr KB, Tsuboi R, Ogawa H. Sphingosylphosphorylcholine stimulates contraction of fibroblast-embedded collagen gel. Br J Dermatol 2000;143:66-71.
36. Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 1994;124:401-404.
37. Nassar D, Letavernier E, Baud L, Aractingi S, Khosrotehrani K. Calpain activity is essential in skin wound healing and contributes to scar formation. PLoS One 2012;7:e37084.
38. Kopecki Z, Luchetti MM, Adams DH, Strudwick X, Mantamadiotis T, Stoppacciaro A, et al. Collagen loss and impaired wound healing is associated with c-Myb deficiency. J Pathol 2007;211:351-361.
39. Urich K. Extracellular structural and secretory proteins. In: Urich K, editor. Comparative animal biochemistry. Berlin: Springer, 1994:376-402.