Biochemical and Biophysical Research Communications 462 (2015) 171–177

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Adipose-derived stromal cell cluster with light therapy enhance angiogenesis and skin wound healing in mice

In-Su Park [a], Phil-Sang Chung [a, b], Jin Chul Ahn [a, c, d, *]

[a] Beckman Laser Institute Korea, Dankook University, 119 Dandae-ro, Cheonan, Chungnam, 330-714, Republic of Korea
[b] Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Dankook University, 119 Dandae-ro, Cheonan, Chungnam, 330-714, Republic of Korea
[c] Department of Biomedical Science, Dankook University, Cheonan, Chungnam, 330-714, Republic of Korea
[d] Biomedical Translational Research Institute, Dankook University, Cheonan, Chungnam, 330-714, Republic of Korea

ARTICLE INFO

Article history:
Received 7 April 2015
Available online 21 April 2015

Keywords:
Wound healing
Skin regeneration
Adipose-derived mesenchymal stem cells
Low-level light therapy
Cluster culture

ABSTRACT

Human adipose-derived mesenchymal stem cells (hASCs) are attractive cell source for skin tissue engineering. The aim of this study was to investigate the effects of low-level light therapy (LLLT) on transplanted cluster hASC in a skin wound animal model. The hASCs were cultured in monolayer or clusters. The LLLT, hASCs, hASC clusters, and hASC clusters transplantation with LLLT (cluster + LLLT) were applied to the wound bed in athymic mice. Wound healing was assessed by gross evaluation and by hematoxylin and eosin staining, and elastin van gieson histochemistry. The survival, differentiation, and secretion of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) of the cluster ASC were evaluated by immunohistochemistry and Western blotting. The cluster + LLLT group enhanced the wound healing, including neovascularization and regeneration of skin appendages, compared with the cluster group. The secretion of growth factors was stimulated in the cluster + LLLT group compared with the ASCs and cluster group. These data suggest that LLLT is an effective biostimulator of cluster hASCs in wound healing that enhances the survival of hASCs and stimulates the secretion of growth factors in the wound bed.

1. Introduction

Healing of a wound is a complex chain of events with interactions among different cells and tissues, and protracted process of tissue repair [1]. Although various solutions have been recommended for cleansing wounds, the treatment of many chronic wounds remains unsatisfactory and more effective treatment strategies are needed. The ultimate goal for tissue engineers is to regenerate skin such that the complete structural and functional properties of the wounded area are restored to the levels before injury [1]. Angiogenesis, the formation of new blood vessels, is necessary for wound repair since the new vessels provide nutrients to support the active cells [2]. Recent stem cell therapy clinical trials aimed at increasing vascularization to be sufficient for wound perfusion and healing [3].

Human adipose-derived mesenchymal stem cells (hASCs), which are found in adipose tissue, are an attractive cell therapy source for the regeneration of damaged tissues because they are the ability of self-renewal and the ability to differentiate into various cell lineages [4,5]. Transplanting hASCs induces neovascularization and improves blood flow to ischemic tissue in animal models [6,7]. In spite of the angiogenic potential of hASCs for treatment of ischemic wounds, these cell sources have limitations for therapeutic angiogenesis. Although ASCs are favorable with regard to obtaining the number of cells required for transplantation, few transplanted stem cells have been found to differentiate into endothelial cells (ECs) and incorporate into vascular structures in ischemic sites [8]. Most of the applied stem cells die within 1 week of transplantation [9]. Several strategies for improving the survival and engraftment of stem cells in ischemic tissue have been developed [10–12]. In recent studies, it has been reported that hypoxic preconditioning results in improved therapeutic potential of human mesenchymal stem cells. Since cells at the cluster are naturally exposed to mild hypoxia, these cells are naturally preconditioned to an ischemic environment [13]. Therefore, cluster grafting to ischemic tissues may render cells to be resistant to hypoxia.

Low-level light therapy (LLLT) has been used for a long time for various purposes, such as reduce inflammation and improvement

* Corresponding author. Beckman Laser Institute Korea, Dankook University, 119 Dandae-ro, Cheonan, Chungnam 330-714, Republic of Korea. Fax: +82 41 559 7838.
E-mail address: jcahn@dankook.ac.kr (J.C. Ahn).

http://dx.doi.org/10.1016/j.bbrc.2015.04.059
0006-291X/

in the local circulation. Moreover, many studies have demonstrated positive bio-stimulatory effects of LLLT on stem cells [14]. Red Light emitting diode (660 nm) also enhanced tissue healing by stimulating angiogenesis in various animal models of ischemia [15].

This study was performed to determine the effect of LLLT on transplanted cluster hASC in a skin wound animal model. The hASCs were grafted to wound beds of athymic mice as clusters and clusters with LLLT or dissociated cells, and the survival, angiogenic factor secretion, and angiogenic efficacy of the grafted hASCs were examined.

2. Materials and methods

2.1. Culture of ASCs

The hASCs supplied from CEFO (Seoul, Korea) were cultured in low-glucose Dulbecco's modified Eagle's medium F-12 (DMEM/F-12; Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS, Welgene), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37.0 °C in a 5% CO_2 incubator. The hASCs between passage 5 and 8 were used for all experiments.

2.2. Cluster formation

hASCs were split and seeded on 24 well polystyrene plate (low cell binding surface) at a density of 7.5×10^4 cells/cm^2, and allowed to adhere at 37 °C. Within 3 days of culture, hASCs formed clusters [10]. Cluster sizes were measured by counting the area of individual cell clusters by image analysis. The diameters of clusters were presented as median ± SD (n = 10 per group).

2.3. Human angiogenic protein analysis

For analyzing the expression profiles of angiogenesis-related proteins, we used the Human Angiogenesis Array Kit (R&D Systems, Ltd., Abingdon, UK). Cell samples (5×10^6 cells) were harvested and 150 μg of protein was mixed with 15 μl of biotinylated detection antibodies. The signals on membrane film were detected by scanning on an image reader LAS-3000 (Kodak, Rochester, NY) and quantified using MultiGauge 4.0 software (Kodak). The positive signals seen on developed film were identified by placing the transparency overlay on the array image and aligning it with the two pairs of positive control spots in the corners of each array.

2.4. ELISA assay for angiogenic growth factor production

Angiogenic growth factor production in the cluster was assayed with a commercially available ELISA kit (R&D Systems) according to the manufacturer's protocols. Concentrations are expressed as the amount of angiogenic growth factor per 10^4 cells at a given time.

2.5. Histological staining

Samples were harvested 14 days after treatment. Specimens were sliced into 4 μm-thick sections and stained with hematoxylin and eosin (H&E) to examine muscle degeneration and tissue inflammation. Masson's trichrome collagen staining was performed to assess tissue fibrosisin ischemic regions. The criteria used for the histological scores of wound healing were modified from previous reports [16] and are summarized in Supplemental Table 2. The histological parameters were reepithelialization, dermal regeneration, granulation tissue formation, and angiogenesis. The regeneration of skin appendages was assessed by counting the number of hair follicles or sebaceous glands in the wound bed.

2.6. Immunofluorescence staining

Indirect immunofluorescence staining was performed using a standard procedure. In brief, tissues cryosectioned at 4 μm thickness were fixed with 4% paraformaldehyde, blocked with 5% BSA/PBS (1 h, 24 °C), washed twice with PBS, treated with 0.1% Triton X-100/PBS for 1 min, and washed extensively in PBS. The sections were stained with specific primary antibodies and fluorescent-conjugated secondary antibodies (Supplemental Table 1) using a M.O.M kit according to the manufacturer's instructions (Vector Laboratories, Burlingame, CA). The cells were counterstained with DAPI (4,6-diamino-2-phenylindole dihydrochloride; Vector Laboratories). Negative control-mouse IgG (Dako, Carpinteria, CA) and −rabbit IgG (Dako) antibody was used as a negative control. The stained sections were viewed with a fluorescence microscope of model DXM1200F (Nikon, Tokyo, Japan). Processed images were analyzed for fluorescence intensity using ImageJ software (NIH).

2.7. Western blot analysis

Proteins were separated by SDS polyacrylamide gel electrophoresis (PAGE) using 10% or 15% resolving gels followed by transfer to nitrocellulose membranes (Bio-Rad, Hercules, CA). For detection, peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG and enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) were used as described by the manufacturer. Membranes were scanned to create chemiluminescent images and to quantify with an image analyzer (Kodak).

2.8. Preparation of the experimental animal model

The animal studies were approved by the Dankook University Animal Use and Care Committee. Five-week-old male BALB/c nude mice (20 g body weight; Narabio, Seoul, Korea) were anesthetized with ketamine (100 mg/kg). After aseptically preparing the surgical site, two-full thickness skin wounds were created on the dorsal part using a 8 mm biopsy punch. To inhibit wound contraction, 0.5 mm thickness of silicone splint was applied as described previously [17]. The wounds were randomly classified into five groups: control ($n = 9$), LLLT ($n = 9$), ASCs (15×10^5 cells; hASCs group, $n = 9$), cluster (10 masses; clusters group, $n = 9$), and cluster + LLLT (10 masses; clusters group, $n = 9$). In the ASCs, cluster, and cluster + LLLT groups, 15×10^5 cells ASCs in 100 μl of PBS were transplanted intradermally at four injection sites on the border between the wound and the normal skin. The control group received an injection of PBS (PBS group, $n = 9$). The physiological status of ischemic limbs was followed up to 2 weeks after treatment. Tegaderm (3M Health Care, MN, USA) was used for wound protection. An equivalent number of cells were injected in both conditions.

2.9. Low-level light therapy

Light emitting diode (LED; WON Technology, Daejeon, Korea) was applied for 10 min daily from day 1–13. The distance from the LED to the wound was 8 cm. This LED model exhibited an irradiated wavelength of 660 nm and power density of 50 mW/cm^2. The fluence of each wound site was approximately 30 J/cm^2 (1 mW × second = 0.001 J).

2.10. Gross evaluation of the wound area

The wounds were photographed using a digital camera at 3, 7, and 14 days after surgery. The wound area was measured by tracing the wound margin and calculated using an image analysis program (Image J, NIH, MD, USA). The wound area was analyzed as a

percentage of the original wound area. The wound was considered to be completely closed when the wound area was grossly equal to zero.

2.11. Statistical analyses

All the quantitative results were obtained from triplicate samples. Data were expressed as a mean ± SD. Statistical analysis was carried out using two-sample t test for comparing two groups of samples and One-way Analysis of Variance (ANOVA) for three groups. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Characterization of hASCs

Adherent cells obtained from human adipose tissue were expanded *in vitro*. The cells were positive for human MSC markers CD29 (β1 integrin), CD90 (Thy-1), and CD105 (endoglin). However, the cells were negative for human endothelial cell markers CD34, CD31, KDR (VEGF receptor) and hematopoietic cell marker CD45 in immunofluorescence staining (Supplemental Fig. 1). These results indicated that the expanded cells included a large population of hASCs and were not contaminated with endothelial cells.

3.2. Production of angiogenic factors by hASCs in clusters

hASCs were cultured on 24 well polystyrene plate (low cell binding surface) in the presence of FBS and formed a floating cluster 3 days after seeding The diameter of most clusters ranged from 1.2 to 1.5 mm (Fig. 1A). Cluster hASCs showed considerable expression of the angiogenic growth factors, such as HGF, VEGF, and FGF2 (Fig. 1B). The expression of angiogenic growth factors in cluster hASCs was much greater than that of hASCs cultured in monolayer (Fig. 1B and C).

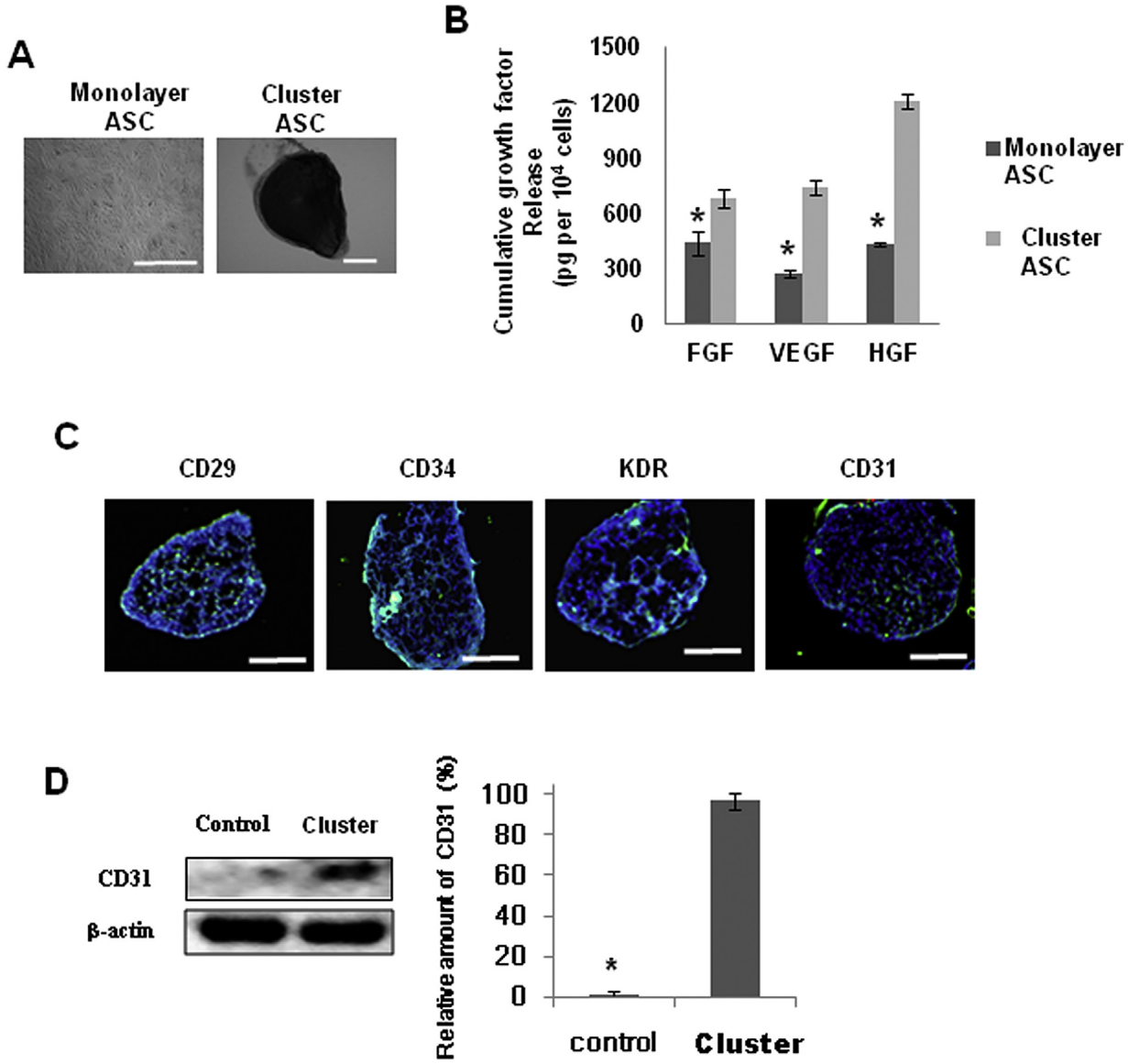

Fig. 1. Endothelial phenotyping of cluster. (A) The hASCs in clusters and monolayer were cultured for 3 days. Scale bars: 500 μm. (B) ELISA measurement of cluster cultured for 3 days. Concentrations of growth factors are presented as pg corrected for 10^4 cells. (*, $p < 0.05$, compared with cluster group, t-test, n = 3 in each group). (C) Monitoring of cell surface markers by immunofluorescence staining. Cluster cultured for 3 days were cryosectioned and stained with anti human CD29, CD34, CD31, and KDR antibodies. Scale bar = 500 μm. (D) Western blotting analysis and quantification of CD31 expressed by hASCs cultured as clusters at 3 days (*$p < 0.01$, compared to the cluster group).

3.3. Endothelial cell differentiation of hASC cluster

The endothelial phenotype of cluster cells was also examined by immunofluorescence staining of a variety of endothelial cell surface markers. Clusters were recognized by human CD34, CD31, and KDR (VEGF receptor) antibodies (Fig. 1C). The endothelial markers (CD34, CD31, and KDR) were expressed in cluster (Fig. 1C and D).

3.4. Survival of ASCs in the wound bed

At 14 days, caspase 3-positive cells and HNA-positive cells were identified using fluorescence microscopy throughout the wound bed to determine whether locally transplanted ASCs were incorporated into the healing wound. In the cluster and cluster + LLLT groups, ASCs were observed in the regenerated skin tissue (Supplemental Fig. 2A). The cluster + LLLT group exhibited significantly increased numbers of HNA-positive cells (ASC group: 6.4%; cluster group: 19.7%; cluster + LLLT group: 40.7% per DAPI-positive cells) (Supplemental Fig. 2B) and decreased proportions of caspase 3-positive ASCs (ASC group: 49.8%; cluster group: 27%; cluster + LLLT group: 12.6% per HNA-positive cells) (Supplemental Fig. 2C). The HNA + cell per DAPI + cell ratio of the cluster + LLLT group was 3.5-times higher than that of the hASC group.

3.5. Enhanced secretion of angiogenic growth factors from grafted hASCs

Transplantation of hASCs into wound bed enhanced paracrine secretion of angiogenic growth factors. Double immunofluorescent staining of HNA and the human angiogenic growth factors bFGF,

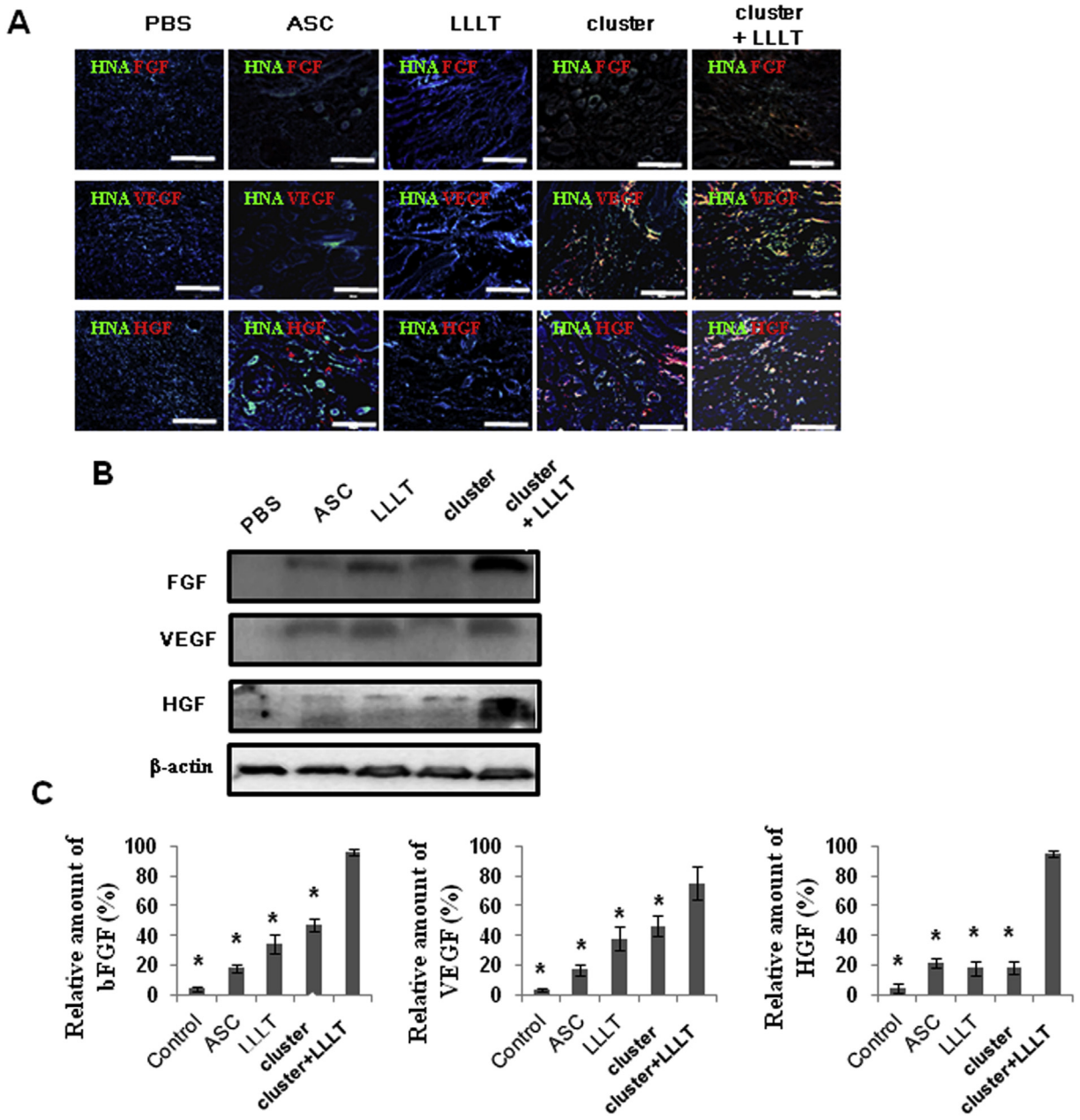

Fig. 2. Enhanced secretion of angiogenic growth factors from hASCs in wound bed. (A) Immunostaining was performed with anti-bFGF and anti-VEGF or anti-HGF antibody (red) at 14 days. The scale bar indicates 100 μm. (B) Western blotting showed the expression of bFGF, VEGF, and HGF at 21 days. (C) The results of Western blotting were analyzed as relative density (*$p < 0.05$, compared to the cluster + LLLT group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

VEGF, and HGF indicated that secretion from transplanted hASCs in the ASC or cluster group (Fig. 2A). Secreted human growth factors were mainly distributed in the vicinity of transplanted hASCs (HNA-positive cells). Compared with the ASC group, more growth factor-positive ASCs were observed in the cluster + LLLT group (Fig. 2A). Western blot assay showed that significantly higher levels of VEGF, bFGF, and HGF were secreted by the cluster and cluster + LLLT groups than by the control group, and greater amount of growth factors were observed in the cluster + LLLT group than in the ASC group (Fig. 2B and C). However, there was no significant difference between ASC-treated tissues and control tissues indicating that the cluster + LLLT group was more effective than the ASC group at increasing transplanted cell retention and angiogenic growth factors expression.

3.6. Angiogenic efficacy

To verify the angiogenic effect of transplanted cells, SMA + cell density and CD31+ vessel number were analyzed ($n = 4$ in each group; Fig. 3A–C). Many of the CD31+ cells in the cluster + LLLT group were double stained for SMA. ECs and perivascular cells differentiated from injected human cells were detected by human αSMA and hCD31 antibodies, respectively (Fig. 3A). The number-per-unit area (1 mm^2) of CD31+ vessel-like structures were compared among groups, as shown in Fig. 4B and C. The area of the cluster + LLLT group increased to 5 times that of the ASCs group. Since we observed increased vessel formation in ischemic tissue, and it is well known that the angiogenic process is stimulated by hypoxia, we further tested the hypothesis that the beneficial effect of LLLT could be due to activation of the transcription factor hypoxia inducible factor-alpha (HIF-1α). Western blot assay demonstrated significantly higher levels of HIF-1α secreted by the cluster and cluster + LLLT groups than by the control group (Fig. 3D and E), and greater amount of growth factors in the cluster + LLLT group than in the ASC group. However, there was no significant difference between ASC-treated tissues and control tissues. HIF-1α could be responsible, at least in part, for some of the beneficial effects of LLLT therapy on ischemic tissues. These findings suggested the greater effectiveness of cluster + LLLT treatment in angiogenesis wound bed.

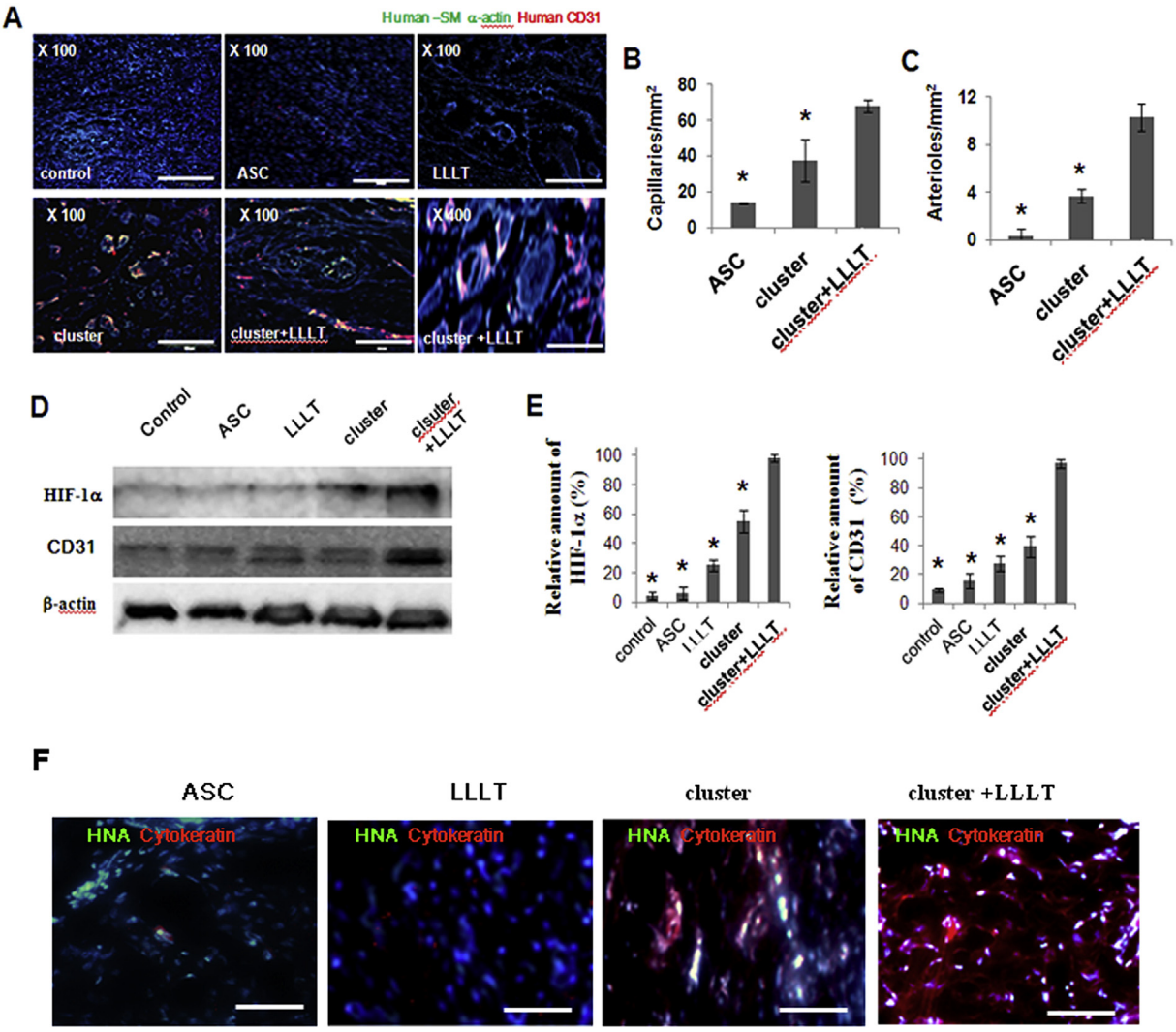

Fig. 3. Endothelial cell and smooth muscle cell differentiation of transplanted cells. (A) The implants were removed on day 14 after transplantation and stained with anti human CD31 and human αSMA antibody. Scale bars indicate 100 μm in × 100 and 50 μm in × 400. All photographs were taken at the same magnification. Quantification of (B) capillary density and (C) arteriole density in the ischemic region (*$p < 0.05$ compared to the cluster + LLLT group). (D) Western blotting shows the expression of HIF-1α and CD31 at 14 days. (E) Western blot analysis quantification (*$p < 0.01$, compared to the cluster + LLLT group). (F) Differentiation of ASCs into epithelial cells. Immunofluorescence images show cytokeratin-positive epithelial cells (red) at 14 days. The scale bar indicates 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

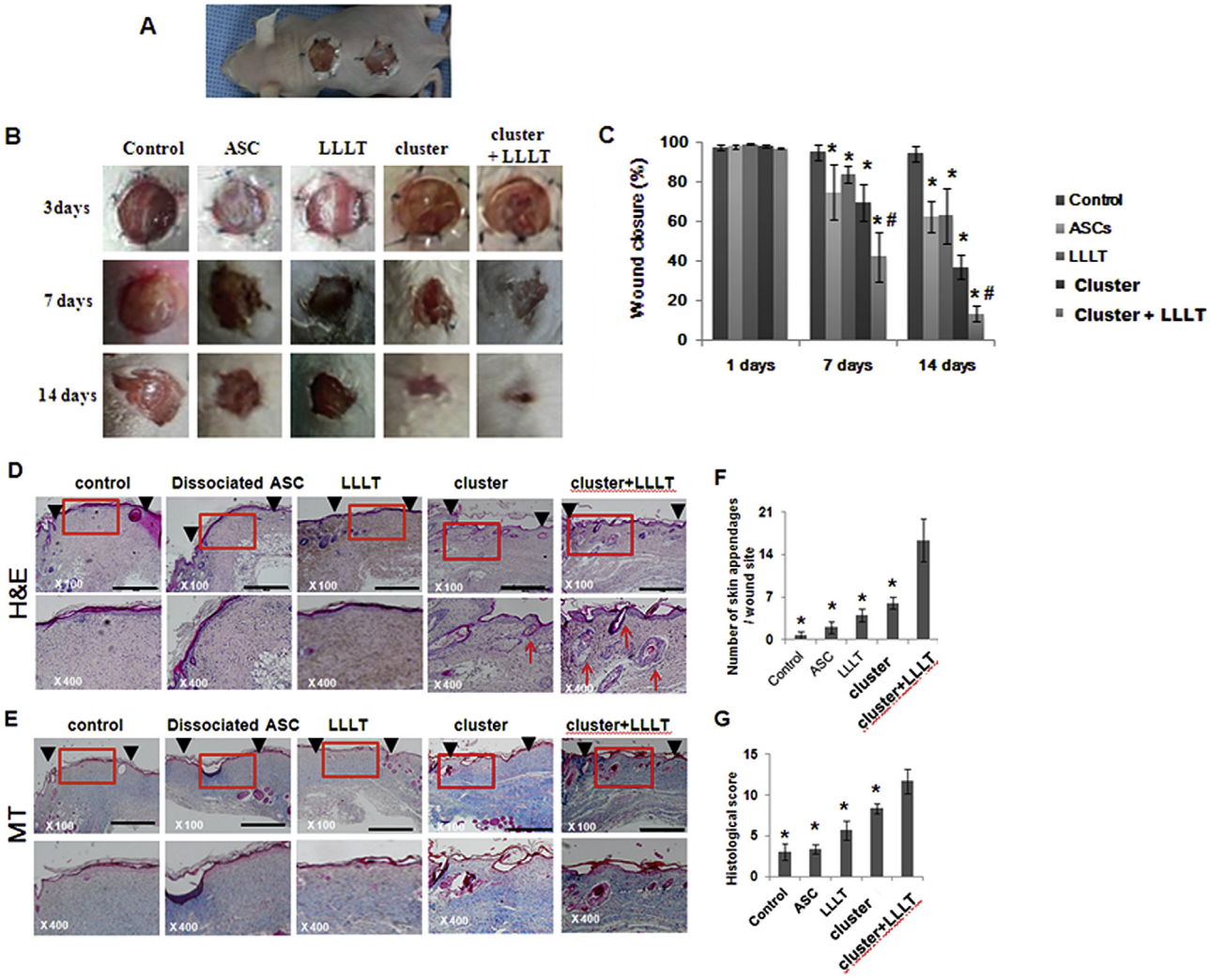

Fig. 4. Evaluation of wound closure. (A) The excisional wound splinting model was prepared. (B) Photographs of the wounds. (C) The percentage of wound area was calculated using photographs of the wounds at 3, 7, and 14 days. $^{*}p < 0.05$ versus the control group; $^{\#}p < 0.05$ versus the cluster group. (D, E) Histological analysis of the wound bed. Wounds were stained with (D) H&E and (E) Masson's trichrome at 14 days. The wound edges are indicated by arrowheads. The closed arrows indicate skin appendages (hair follicles). The scale bar indicates 500 μm. (F) The regeneration of skin appendages was investigated by counting the number of skin appendages per wound section. (G) Histological scoring was performed using the criteria in Supplemental Table 2. $^{*}p < 0.05$, $^{**}p < 0.01$ versus the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus the ASCs group.

3.7. Differentiation of ASCs into epithelial cells

To determine whether the cluster ASCs could contribute to the epidermal structure, immunohistochemistry for pan-cytokeratin was performed at 14 days (Fig. 3F). Some cytokeratin-positive ASCs were found in the epidermis or sebaceous glands in the cluster and cluster + LLLT group. The cluster + LLLT group exhibited significantly increased numbers of cytokeratin -positive cells.

3.8. Wound closure and dermal reaction

The excisional wound splinting model was prepared, and the silicon splints remained tightly adherent to the skin and restricted wound contraction during the experimental period (Fig. 4A). Our data showed that the cluster + LLLT groups accelerated wound closure (Fig. 4B and C). At 7, and 14 days after surgery, the cluster and cluster + LLLT groups exhibited significantly smaller wound areas than did the other groups. At 7 days, the cluster + LLLT group showed a significantly smaller wound area than the ASCs group and cluster group. No significant difference was observed between the control and LLLT group at any time (Fig. 4C). At 14 days, all of the wounds of the cluster and cluster + LLLT groups had achieved complete wound closure, but not all of the wounds of the control and LLLT groups had completely closed. The histological observation showed that the skin regeneration was much greater in the cluster and cluster + LLLT groups compared with the control group. Our data indicated that the cluster and cluster + LLLT treatments enhanced re-epithelialization and granulation at 14 days (Fig. 4D). Furthermore, the cluster and cluster + LLLT groups appeared to have increased numbers of skin appendages (Fig. 4F and G). The cluster + LLLT group displayed significantly increased numbers of hair follicles and sebaceous glands at 14 day. These skin appendages such as hair follicles were found more centrally in the regenerated dermis (Fig. 4D and E).

4. Discussion

In this study, ASCs accelerated wound closure with increased neovascularization, re-epithelialization, and regeneration of skin appendages. In addition to some cytokeratin positive-ASCs were observed in regenerated epidermis. Additionally, some bFGF, VEGF, and HGF positive-ASCs were detected in the wound bed. The bFGF is one of the most important growth factors in wound healing because it

affects on cell proliferation, angiogenesis, and matrix deposition [18]. VEGF is the most specific and effective growth factor that regulates angiogenesis [19]. HGF is another potent proangiogenic factor that induces migration and proliferation and inhibits the apoptotic cell death of ECs [20]. Consistent with our findings, several recent studies reported that ASCs enhanced wound repair by differentiation and paracrine effects. Previous studies reported that the MSCs migrate into the wound area [21] and differentiate into keratinocytes, endothelial cells, sebaceous glands, and hair follicles [16,22]. Additionally, MSCs stimulate the deposition of extracellular matrix [16,23].

Recently, studies reported that cluster cultures are more effective with preconditioning hASC to a hypoxic environment, upregulating hypoxia-adaptive signals, inhibiting apoptosis, and enhancing secretion of both angiogenic and anti-apoptotic factors than monolayer cultures [11,24,25]. Therefore, it is plausible that the environment of cluster can efficiently induce hypoxic conditions and promote the secretion of angiogenic growth factors such as VEGF. Recent studies showed that most of the applied stem cells die in the skin wound bed within the first week [16] [26]. Our data revealed an increased number of ASCs and a decreased percentage of caspase 3-positive ASCs in the cluster + LLLT group compared with the ASCs or cluster groups at 14 days. These data suggest that LLLT enhanced the survival of cluster ASCs by the inhibition of apoptosis. Furthermore, in the cluster + LLLT group, more VEGF-, HGF, or bFGF-positive ASCs were observed in the regenerated dermis, and greater amounts of growth factors were found in the wound bed than in the ASCs or cluster groups. These data suggest that LLLT enhanced not only the survival, but also the functionality of the transplanted ASCs with appropriate energy density and wavelengths in the wound bed [27–29]. It is possible that LLLT enhances cellular responses via increases in the mitochondrial membrane potential and ATP and cAMP levels [28].

In summary, ASC transplantation accelerates wound healing through differentiation and growth factor secretion. Furthermore, our study suggested that the LLLT enhanced the wound-healing effect of the cluster ASCs by enhancing survival of the ASCs and stimulating secretion of growth factors in the wound bed. In this study, the cluster + LLLT group was evaluated to exhibit rapid wound closure and a higher histological score compared with the ASCs or cluster groups. Interestingly, our results showed that the cluster + LLLT group had significantly increased numbers of hair follicles and sebaceous glands in wound bed compared with the ASCs or cluster groups. These data suggest that the cluster + LLLT treatment increased the regeneration of skin appendages due to the enhanced secretion of growth factors.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was supported by a grant of the Ministry of Science, ICT and Future Planning grant funded by the Korea Government (2012K1A4A3053142, NRF-2014R1A1A1038199).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.059.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.059.

References

[1] M. Chen, F. Berthiaume, Stem cells for skin tissue engineering and wound healing, Crit. Rev. Biomed. Eng. 37 (2009) 399–421.
[2] T.R. Kyriakides, The role of thrombospondins in wound healing, ischemia, and the foreign body reaction, J. Cell. Commun. Signal 3 (2009) 215–225.
[3] C. Alev, T. Asahara, Endothelial progenitor cells: a novel tool for the therapy of ischemic diseases, Antioxid. Redox Signal 15 (2011) 949–965.
[4] J.I. Huang, N.F. Jones, M. Zhu, H.P. Lorenz, Chondrogenic potential of multipotential cells from human adipose tissue, Plast. Reconstr. Surg. 113 (2004) 585–594.
[5] J. Gimble, Adipose-derived adult stem cells: isolation, characterization, and differentiation potential, Cytotherapy 5 (2003) 362–369.
[6] M. Sumi, N. Toya, K. Yanaga, T. Ohki, R. Nagai, Transplantation of adipose stromal cells, but not mature adipocytes, augments ischemia-induced angiogenesis, Life Sci. 80 (2007) 559–565.
[7] Y. Harada, S. Tsujimoto, H. Matsugami, A. Yoshida, I. Hisatome, Transplantation of freshly isolated adipose tissue-derived regenerative cells enhances angiogenesis in a murine model of hind limb ischemia, Biomed. Res. 34 (2013) 23–29.
[8] Q.D. Wang, Myocardial regeneration with stem cells: pharmacological possibilities for efficacy enhancement, Pharmacol. Res. 53 (2006) 331–340.
[9] E. Hill, D.J. Mooney, Regulating activation of transplanted cells controls tissue regeneration, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 2494–2499.
[10] I.S. Park, Y. Jung, J.W. Rhie, S.H. Kim, Endothelial differentiation and vasculogenesis induced by three-dimensional adipose-derived stem cells, Anat. Rec. (Hoboken) 296 (2013) 168–177.
[11] S.H. Bhang, W.G. La, T.J. Lee, H.S. Yang, A.Y. Sun, S.H. Baek, J.W. Rhie, B.S. Kim, Angiogenesis in ischemic tissue produced by spheroid grafting of human adipose-derived stromal cells, Biomaterials 32 (2011) 2734–2747.
[12] M. Valcarcel, A. Jaureguibeitia, A. Lopategi, I. Martinez, L. Mendoza, Three-dimensional growth as multicellular spheroid activates the proangiogenic phenotype of colorectal carcinoma cells via LFA-1-dependent VEGF: implications on hepatic micrometastasis, J. Transl. Med. 6 (2008) 57.
[13] I.S. Park, S.H. Kim, A novel three-dimensional adipose-derived stem cell cluster for vascular regeneration in ischemic tissue, Cytotherapy 13 (2013) 00681–00686.
[14] K. Choi, H. Kim, S. Lee, S. Bae, O.K. Kweon, W.H. Kim, Low-level laser therapy promotes the osteogenic potential of adipose-derived mesenchymal stem cells seeded on an acellular dermal matrix, J. Biomed. Mater. Res. B Appl. Biomater. 101 (2013) 919–928.
[15] A.P. de Sousa, N.T. Silveira, J. de Souza, M.C. Cangussú, J.N. dos Santos, A.L. Pinheiro, Laser and LED phototherapies on angiogenesis, Lasers Med. Sci. 28 (2013) 981–987.
[16] Y. Wu, P.G. Scott, E.E. Tredget, Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis, Stem Cells 25 (2007) 2648–2659.
[17] X. Wang, E.E. Tredget, Y. Wu, The mouse excisional wound splinting model, including applications for stem cell transplantation,, Nat. Protoc. 8 (2013) 302–309.
[18] S. Heydarkhan-Hagvall, J.Q. Yang, S. Heydarkhan, Y. Xu, P.A. Zuk, Human adipose stem cells: a potential cell source for cardiovascular tissue engineering, Cells Tissues Organs 187 (2008) 263–274.
[19] C. Nie, S.F. Morris, Local delivery of adipose-derived stem cells via acellular dermal matrix as a scaffold: a new promising strategy to accelerate wound healing, Med. Hypotheses 72 (2009) 679–682.
[20] E.J. Lee, H.J. Jeon, H.S. Kim, M.S. Chang, Potentiated therapeutic angiogenesis by primed human mesenchymal stem cells in a mouse model of hindlimb ischemia, Regen. Med. 8 (2013) 283–293.
[21] A.N. Smith, V.T. Chan, L.A. Muffley, F.F. Isik, N.S. Gibran, Mesenchymal stem cells induce dermal fibroblast responses to injury, Exp. Cell. Res. 316 (2010) 48–54.
[22] C. Nie, J. Xu, Z. Si, X. Jin, J. Zhang, Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis, Cell. Transpl. 20 (2011) 205–216.
[23] W.S. Kim, J.H. Sung, J.M. Yang, S.B. Park, S.J. Kwak, Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts, J. Dermatol Sci. (2007) 15–24.
[24] I. Rosová, B. Capoccia, D. Link, J.A. Nolta, Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells, Stem Cells 26 (2008) 2173–2182.
[25] P.J. Amos, P.C. Stapor, H. Shang, S. Bekiranov, M. Khurgel, G.T. Rodeheaver, S.M. Peirce, A.J. Katz, Human adipose-derived stromal cells accelerate diabetic wound healing: impact of cell formulation and delivery, Tissue Eng. Part A 16 (2010) 1595–1606.
[26] B. Levi, E.R. Nelson, D. Vistnes, B. Wu, M. Lee, Human adipose derived stromal cells heal critical size mouse calvarial defects, PLoS ONE 5 (2010) e11177.
[27] D. Hawkins, Effect of multiple exposures of low-level laser therapy on the cellular responses of wounded human skin fibroblasts, Photomed. Laser Surg. 24 (2006) 705–714.
[28] W.P. Hu, C.L. Yu, C.C. Lan, G.S. Chen, H.S. Yu, Helium-neon laser irradiation stimulates cell proliferation through photostimulatory effects in mitochondria, J. Invest Dermatol 127 (2007) 2048–2057.
[29] K.M. Alghamdi, N.A. Moussa, Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells, Lasers Med. Sci. 27 (2012) 237–249.