pISSN 1738-2696 · eISSN 2212-5469
http://dx.doi.org/10.1007/s13770-015-0055-x
ORIGINAL ARTICLE

TERM

Effect of Mesenchymal Stem Cells and Platelet-Derived Growth Factor on the Healing of Radiation Induced Ulcer in Rats

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Radiation-induced skin ulceration is a frequent complication of radiation therapy. This study investigated the effects of rat mesenchymal stem cells (rMSCs) and platelet-derived growth factor (PDGF) on the healing of radiation-induced soft tissue injury. Sprague-Dawley rats (n=17) were irradiated on the right and left buttocks with a single dose of 50 Gy. The right buttocks were administered with phosphate-buffered solution as a control. The left buttocks were administered with either rMSCs (2×10^6 cells), PDGF (8 μg), or PDGF combined with rMSCs. Administration was done at three weeks after irradiation. Wound healing was analyzed by calculating the percentage of residual ulcerated skin area compared to the total irradiated area during the five week healing period after administration. Modified skin scores were also assessed. Finally, skin lesions were histologically evaluated. More than 40% of the irradiated skin area within the irradiated zone underwent ulceration within 16 days postirradiation, with peak ulceration exceeding 50% around three weeks post-irradiation. Administration of rMSCs or PDGF alone did not confer any significant healing effect. The combined rMSCs+PDGF treatment significantly reduced the wound size compared with the nontreated control up to two weeks postinjection. Regarding the histological examination, lesions administered with PDGF (either alone or mixed with rMSCs) resulted in a greater deposition of highly organized collagen fibers throughout the dermis layer, compared with the control. In conclusion, the combined administration of rMSCs and PDGF efficiently enhanced the healing of radiation-induced skin ulceration.

Tissue Eng Regen Med 2016;13(1):78-90

Key Words: Mesenchymal stem cell; Platelet-derived growth factor; Radiation; Ulceration; Healing

INTRODUCTION

Radiation therapy is often used to treat malignant tumors, either alone or in combination with surgery. To reach the tumor, the radiation waves need to penetrate the skin layer; thus, radiation-induced skin lesions are often unavoidable [1]. The skin response to radiation is complex [2]. The epidermis is damaged in the early stage of radiation, whereas later effects arise from insult to the dermal vasculature. One or two days after radiation exposure, temporary erythema appears, which is then followed by a more intensive erythematous reaction. Afterwards, dermal ischemia and dermal necrosis occur, resulting in skin damage such as dermal atrophy and invasive fibrosis [3].

Conventional treatment for radiation-induced skin ulceration with superficial skin dressings requires a long healing period and frequently leaves residual skin lesions via atrophy, fibrosis, or scar formation. This is especially true for long-term nonhealing ulcers that eventually require surgery [4]. To overcome these disadvantages, a number of alternative treatments have been introduced [5-8]. Among these alternatives, mesenchymal stem cells (MSCs) have shown the capacity to repair hard and soft tissue damage in animal models [9] and human patients [10-12]. With respect to their therapeutic effects on soft tissue damage, efficient results have been reported in animal models of lung injury [13], kidney injury [14], and myocardiac infarction [15]. MSCs produce various cytokines and adhesion molecules for hematopoiesis, and are known to participate in angiogenesis and wound repair [16]. MSCs also express proangiogenic factors that regulate endothelial cell migration and capillary proliferation, and have been reported to be involved in vessel remodeling [17].

Among the numerous identified growth factors, platelet-derived growth factor (PDGF) is considered particularly critical

Received: July 12, 2015
Revised: July 26, 2015
Accepted: August 24, 2015
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for vascular formation. The homodimer PDGF-BB responds to hypoxia, growth factors, and shear stress and is expressed highly at the sprouting tip of forming capillaries, where it contributes to pericyte recruitment [18]. PDGF-BB also stimulates pericyte production of extracellular matrix proteins, including fibronectin, collagen, and proteoglycans, which are necessary for the basement membrane of capillaries. In addition, PDGF-BB upregulates the expression of vascular endothelial growth factor in mural cells and stimulates fibroblasts to produce and secrete collagenases, which are key for cell migration in angiogenesis [19].

The effects and mechanisms of stem cell treatment have been fairly well characterized. In contrast, little is known regarding the interactions of stem cells with other growth factors are poorly characterized on radiation-induced skin ulceration. Therefore, we aimed to analyze the effect of MSCs and/or PDGF alone for treating radiation-induced skin ulceration.

MATERIALS AND METHODS

Animal model of radiation-induced skin ulceration

All animals were treated and handled in accordance with the "Recommendations for Handling of Laboratory Animals for Biomedical Research" compiled by the Committee on the Safety and Ethical Handling Regulations for Laboratory Experiments at the School of Dentistry, Seoul National University.

Adult male Sprague-Dawley rats (n=18; mean mass, 120 g) (Orientbio Inc., Seongnam, Korea) were irradiated on both buttocks at six weeks of age. For each animal, the left buttock was assigned as the experimental side and the right buttock was assigned as the control side. After intraperitoneal injection of Zoletil 50 (0.1 mg/100 g) (Virbac, Paris, France), the buttocks of each rat were shaved and prepared with povidone-iodine solution (MEDICA KOREA, Seoul, Korea). Rats were irradiated with a single electron beam delivered by a Clinac iX linear accelerator (Varian Medical System, Inc., Palo Alto, CA, USA). The total irradiated area for each buttock was 3.5×3.5 cm=12.25 cm^2. A 3-mm lead filter was added to the skin site to remove low energy photons from the beam at a 100 cm source-to-surface distance. Radiation (50 Gy, 6 MeV electron, 5501 MU) was delivered to 3.5×3.5 cm areas on the skin (depth range, 2 mm) of both buttocks. Rats were housed individually to prevent gnawing of wounds and other potentially damaging interactions.

Preparation and culture of rMSCs

Bone marrow aspirate was obtained from rat tibias. Rat MSCs were cultured according to the protocol described by Caterson et al. [20]. Briefly, the marrow suspension was collected in a syringe containing 6000 U/mL heparin (JW Pharmaceutical Co., Seoul, Korea), mixed with an equal volume of phosphate-buffered solution (PBS) (Gibco, Rockville, MD, USA), and spun by centrifugation at 2500 rpm for 10 min. After aspirating the upper PBS layer, the marrow suspension was layered onto Ficoll-Paque (1:5 ratio) (GE Healthcare Life Sciences, Piscataway, NJ, USA) and spun by centrifugation at 2580 rpm for 30 min. Nucleated cells, which concentrated at the interface, were collected and washed with PBS. Adherent cells were plated at a density of 2×10^6 cells/100 mm dish and cultured in expansion medium containing alpha-minimum essential medium (Welgene Inc., Gyeongsan, Korea), 100 units/mL penicillin (Gibco, Rockville, MD, USA), 100 μg/mL streptomycin (Gibco, Rockville, MD, USA), and 10% heat inactivated FBS (Gibco, Rockville, MD, USA) under a humidified atmosphere of 5% CO_2 at 37°C. Culture medium was changed every 3 or 4 days. Rat mesenchymal stem cells (rMSCs) were passaged upon reaching 70% confluence. All experiments were performed using cells in their fifth passage.

Flow cytometry

rMSCs in their fifth passage were resuspended in phosphate-buffered saline (PBS) at 1×10^5 cells/mL and then incubated with fluorescein isothiocyanate-conjugated monoclonal antibodies against CD90 (LifeSpan Biosciences Inc., Seattle, WA, USA) or CD45 (LifeSpan Biosciences Inc., Seattle, WA, USA), each diluted to 1:200 in PBS. Alternatively, cells were incubated with anti-mouse IgG (BD Biosciences, San Jose, CA, USA) as an isotype control. Cells were incubated for 30 or 90 min in the dark after being washed with 1% bovine serum albumin in PBS (BSA/PBS). Cells were washed twice with BSA/PBS and resuspended in BSA/PBS with 0.1% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for fluorescence-activated cell sorting (FACS) analysis. Cells were then fixed in 70% alcohol and sorted using a BD FACS Aria Cell Sorting System (BD Biosciences, San Jose, CA, USA).

Application of rMSCs and PDGF

Throughout the study, identical doses of rMSCs and/or PDGF were used in all groups. Moreover, the cell density also remained constant among the groups (1×10^6 rMSCs/cm^2), which has been shown to be adequate for effective tissue regeneration [5]. rMSCs and PDGF were applied three weeks after irradiation, identical amounts of rMSCs (2×10^6 cells/2 mL) and PDGF (8 μg) were evenly injected subcutaneously over the entire ulcerative skin lesion, which varied in location and size (Fig. 1A). For rMSC delivery, 1 mL of rMSC suspension (1×10^6 cells/ml in PBS) was filled in 1 mL syringe and total 2 syringes of rMSCs were injected subcutaneously at each site. As a control, 1 mL PBS was injected into each site on the control side.

Figure 1. Analysis of ulcer size. (A) Image of a representative wound. (B) Calculation of ulcer size using ImageJ (NIH, Bethesda, MD, USA).

Animal study only included animals for which the radiation-induced ulcers on the experimental side and control side did not exhibit any significant differences in size at three weeks after irradiation. One rat was excluded because its ulceration ratios were significantly different between the control and experimental sides (24.04% vs. 50.24%). Animals (n=17) was subdivided into three groups: Group A: rMSCs (n=6), Group B: PDGF (n=6), and Group C: rMSCs+PDGF (n=5). Skin ulcers were measured over a five-week healing period after the administration of rMSCs and/or PDGF. Five weeks after treatment, skin samples were evaluated histologically by hematoxylin and eosin (H&E) staining and Masson's trichrome (MT) staining. To verify the presence of the delivered rMSCs, two rats were injected with fluorescent dye-labeled rMSCs at three weeks post-irradiation. The rats were sacrificed on the 3rd and 7th days postinjection, respectively, and the presence of the delivered rMSCs was verified. These two rats were excluded from statistical analysis.

Wound model and wound healing assay

Acute skin reactions were assessed by visual scoring 21 days after irradiation. Assessment continued every three days until 35 days postinjection. Skin reactions were scored at each time point; data are presented as means. For scoring, the modified skin score system was used as follows: 0, normal; 0.5, slight epilation; 1.0, epilation in approximately 50% of the irradiated area; 1.5, epilation in more than 50% of the area; 2.0, complete epilation; 2.5, dry desquamation in more than 50% of the area, 3.0, moist desquamation in a small area; and 3.5, moist desquamation in most of the area [21].

For wound size analysis, the irradiated area of each rat was photographed with a digital camera at each time point. From these images, the percentage of the ulcerated area was determined by calculating the ratio of the ulcerated area to the total irradiated area using ImageJ, version 1.47 (NIH, Bethesda, MD, USA) (Fig. 1B). The total irradiated area and ulcerated area were marked three times while blinded to the group assignments, and the average values of these measurements were used for assessment. The irradiated area was defined as the area in which hair loss was observe [21]. The ulcerated area was defined by scabbing, crusting, or desquamation. The numbers of pixels within each zone were calculated using ImageJ, and these values were used to calculate the percentage of skin ulceration within the irradiated zone.

Histological examination

Samples were fixed in 10% formalin-buffered solution (Duksan Pure Chemicals Co., Ansan, Korea) and embedded in paraffin wax (Leica Biosystems Nussloch GmbH, Nußloch, Germany). For histochemical staining, the paraffin sections were cleaned with xylene (Duksan Pure Chemicals Co., Ansan, Korea) for ten minutes. Next, four-μm-thick slices were prepared and stained with both H&E (Sigma-Aldrich, St. Louis, MO, USA) and MT (Sigma-Aldrich, St. Louis, MO, USA). Digital images of the stained sections were captured for histological evaluation using a transmission and polarized light Olympus BX51 Axioskop microscope (Olympus Corp., Tokyo, Japan). Skin damage in the affected areas (epidermal atrophy, number of lymphocytes, dermal degeneration such as edema and collagen fiber loss) is expressed as percentages. Skin damage was scored on a 5-point ordinal scale as previously described [7,22]: Grade 0=normal, Grade 1=minimal, Grade 2=mild, Grade 3=moderate, Grade 4=marked, and Grade 5=severe.

Labeling of rMSCs with diakylcarbocyanine

rMSCs were labeled with the fluorescent dye diakylcarbocyanine (Dil; Molecular Probes®, Thermo Fisher Scientific Inc., Waltham, MA, USA) by incubating cells in medium containing Dil (final concentration 10 ng/mL) for 24 hours. Dil-labeled rMSCs were injected subcutaneously into the left side of the irradiated buttocks of two rats. Histological specimens were prepared after sacrifice at three and seven days postinjection as described above, and the presence of rMSCs was verified using a confocal laser scanning microscope (Carl Zeiss Micro Imaging GmbH, Jena, Germany). To investigate the viability of rMSCs, the expression of proliferating cell nuclear antigen (PCNA) was verified.

Statistical analysis

All wound healing data are reported as means±standard deviations. Data were checked for a normal distribution using the Kolmogorov-Smirnov test. When data were normally distributed, the experimental side was compared with the corresponding control side using a paired t-test. Data with non-normal distributions were compared using the Wilcoxon signed-rank test. All statistical analyses were performed with SPSS (ver. 18.0, SPSS, Chicago, IL, USA). Differences between values were considered statistically significant when the *p*-value was <0.05.

RESULTS

As shown in Figure 2, CD45 and CD90 could be detected by flow cytometric analysis. No systemic or lethal sequelae occurred in any animal. Moreover, no recognizable changes were observed on the irradiated skin until one week postirradiation. The first observable change was hair loss at 7 days postirradiation; ulcerative skin changes were first seen at 10 days postirradiation. By day 14, necrotic changes were observed throughout the entire skin layer of the irradiated area. Peak ulcer size was observed on the 21st day postirradiation. Ulcerative lesions occurred independently of the injection site after irradiation and varied in location, size, and shape.

The modified skin scores were ranged from 3 to 3.5 at the time of injection on both the experimental and control sides of each animal; these scores decreased to 2–2.5 according to the follow-up period (Fig. 3, Table 1). In Groups A and B, the scores decreased to 3.0 after 7–8 days, with no significant difference between the experimental and control sides. In Group C, the scores decreased to 3.0 after only 2 days; however, no significant difference was observed between the experimental and control sides (Fig. 3).

Ulceration size evaluation

rMSCs alone or PDGF alone did not markedly enhance macroscopic healing of the radiation-induced skin wound, while rMSCs+PDGF treatment significantly aided the early stage of wound healing. In Group A, the ulceration ratios at the time of injection were 38.51±10.74% on the experimental side and 41.60±7.78% on the control side. Ulcer size decreased significantly during the first two weeks postinjection on both sides, whereas the reduction rate decreased after this time. However, the ulceration ratio on the experimental side was not significantly different compared with the ratio on the control side. If anything, the ratio tended to be smaller on the control side. At five weeks postinjection, the average ulcerative lesion size on the experimental side was 1.37±0.73%, and two out of the six rats showed no ulcerative skin lesions at all. The ulceration ratio on

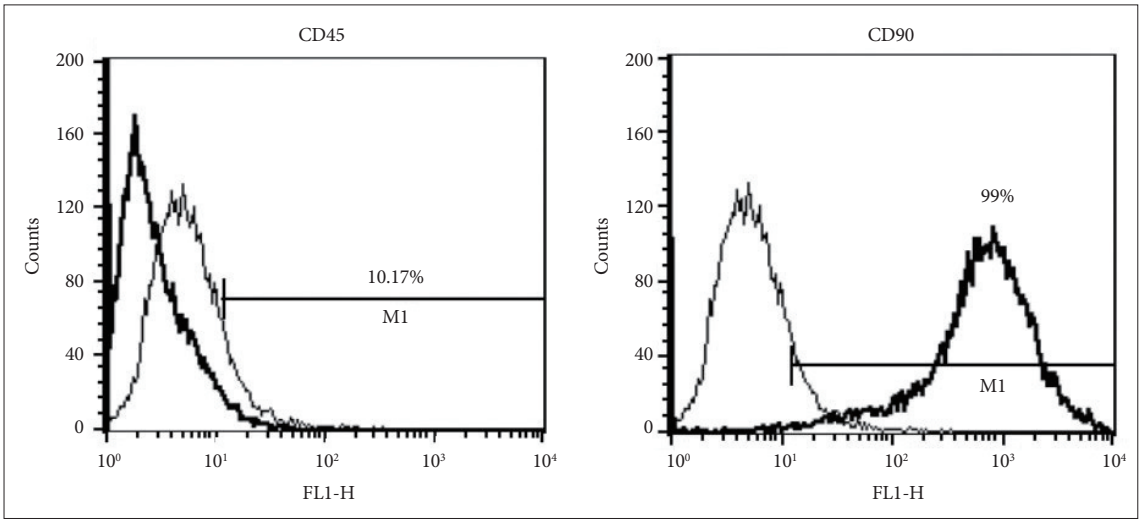

Figure 2. Flow cytometric analysis showing the expression of CD45 and CD90.

Table 1. Modified skin score

Modified skin score (mean±SD)	Days after administration										
	0	3	7	10	14	17	21	24	28	31	35
Group A											
MSCs	3.17±1.22	3.08±0.20	3.08±0.81	2.67±0.26	2.58±0.29	2.58±0.20	2.58±0.24	2.42±0.20	2.42 ±0.63	2.42±0.20	2.33±1.03
Control	3.17±0.26	3.00±0.00	2.92±0.20	2.67±0.26	2.67±0.26	2.50±0.00	2.50±0.00	2.50±0.00	2.50±0.00	2.50±0.00	2.50±0.00
Group B											
PDGF	3.25±0.27	3.17±0.26	3.00 ±0.00	2.75±0.27	2.67±0.26	2.67±0.26	2.67±0.26	2.50±0.00	2.50±0.00	2.50±0.00	2.33±0.26
Control	3.17±0.26	3.00±0.00	2.92±0.20	2.75±0.27	2.67±0.26	2.50±0.32	2.50±0.32	2.42±0.20	2.42±0.20	2.42±0.20	2.17±0.26
Group C											
MSCs+PDGF	3.20±0.27	2.90±0.22	2.90±0.27	2.80±0.27	2.80±0.27	2.80±0.27	2.80±0.27	2.60±0.22	2.60±0.22	2.60±0.22	2.20±0.27
Control	3.10±0.22	3.00±0.35	2.90±0.22	2.70±0.27	2.70±0.27	2.70±0.27	2.70±0.27	2.60±0.22	2.60±0.22	2.50±0.00	2.40±0.22

MSCs: mesenchymal stem cells, PDGF: platelet-derived growth factor

Table 2. Ulceration in percentage

Ulceration in percentage (%) (mean±SD)	Days after administration										
	0	3	7	10	14	17	21	24	28	31	35
Group A											
MSCs	46.81±10.35	40.46±9.98	29.26±12.73	22.96±14.47	19.47±12.58	14.80±15.36	12.05±13.96	8.10±9.92	6.44±8.63	4.36±6.30	0.57±0.71
Control	45.19±10.32	36.93±12.54	24.01±9.02	18.78±8.19	18.89±13.45	11.02±6.49	7.17±5.31	4.98±4.11	3.31±2.31	3.70±2.89	0.92±1.06
Group B											
PDGF	50.22±5.10	41.46±7.70	28.42±10.73	22.96±14.20	19.48±16.02	14.52±13.83	10.84±13.16	8.05±11.08	5.90±8.43	5.05±7.02	2.09±4.16
Control	45.11±6.78	42.62±5.28	27.76±13.80	24.87±13.82	23.43±15.83	14.71±14.08	10.69±12.01	6.92±8.20	4.76±7.13	4.02±7.42	2.80±6.62
Group C											
MSCs+PDGF	44.39±7.54	31.56±14.03*	25.00±10.49*	18.58±11.45**	18.20±13.46	14.09±13.00	12.56±13.80	8.47±10.11	6.30±7.31	6.42±7.22	1.15±2.10
Control	46.39±15.47	35.79±14.07*	26.57±11.18*	20.60±10.42**	17.21±10.86	13.22±9.76	8.06±9.16	6.05±6.71	4.94±5.21	3.29±4.79	1.34±1.34

*p<0.05, paired t-test, **p<0.01, paired t-test. MSCs: mesenchymal stem cells, PDGF: platelet-derived growth factor

the control side was 0.82±0.97%; all six rats exhibited ulcerative lesions on the control side (Fig. 4A, Table 2).

In Group B, the ulceration ratios at the time of injection were not significantly different (50.22±5.10% on the experimental side vs. 45.11±6.78% on the control side). The ulceration ratios decreased over time. As in Group A, ulcer size decreased most dramatically during the first two weeks after the injection, after which the reduction rate decreased. However, no significant

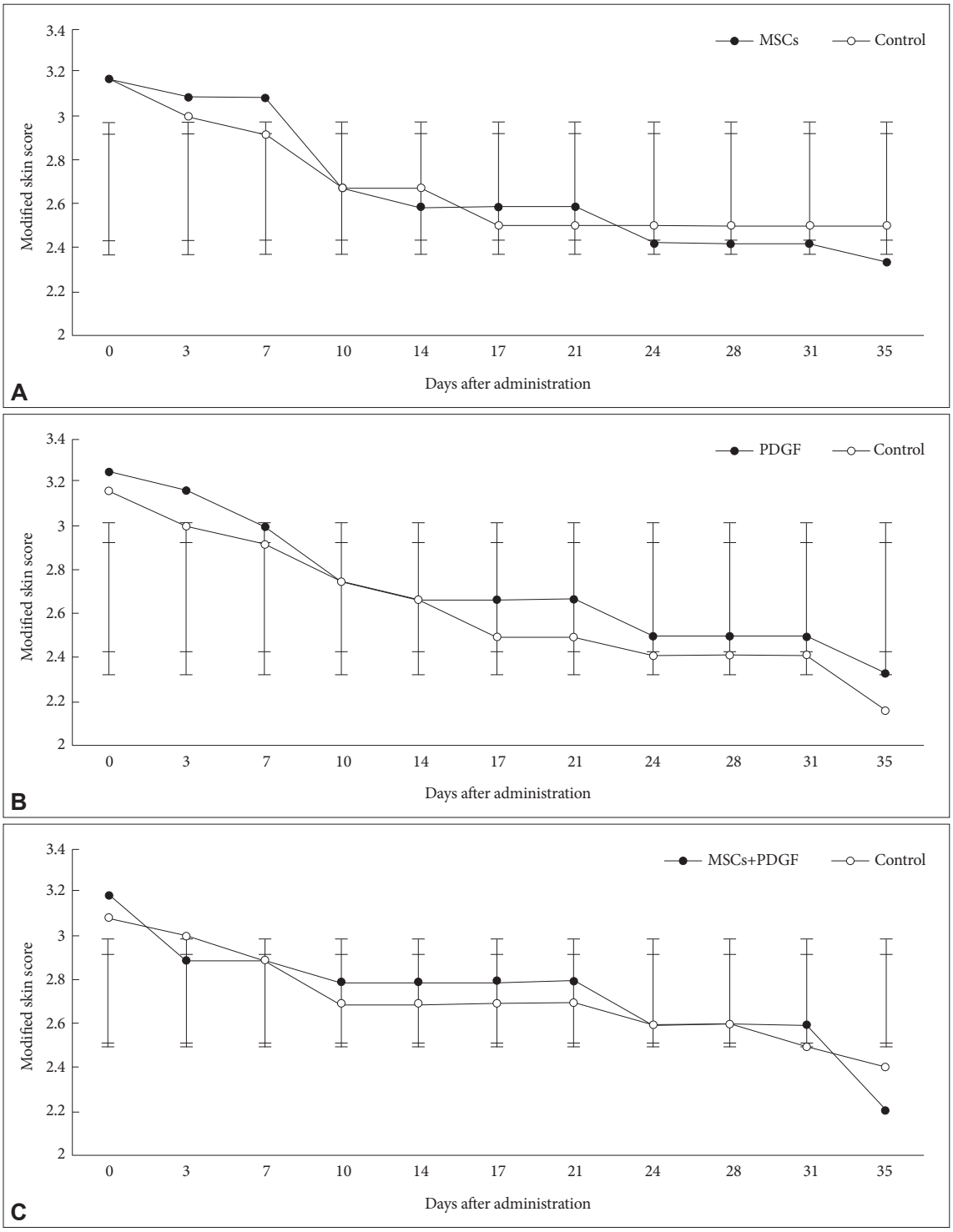

Figure 3. Changes in the modified skin scores after injection of (A) rMSCs (Group A), (B) PDGF (Group B), and (C) rMSCs+PDGF (Group C). PDGF: platelet-derived growth factor, rMSCs: rat mesenchymal stem cells.

difference was observed between the two sides at five weeks postinjection. At this time, four of the six rats showed ulceration on the experimental side, while two of the six rats showed ulceration on the control side (Fig. 4B, Table 2).

In Group C, the ulceration ratios were also not significantly different at the time of injection (44.40±7.54% on the experimental side vs. 46.39±12.54% on the control side) (Fig. 4C, Table 2). However, the two sides did exhibit significant differ-

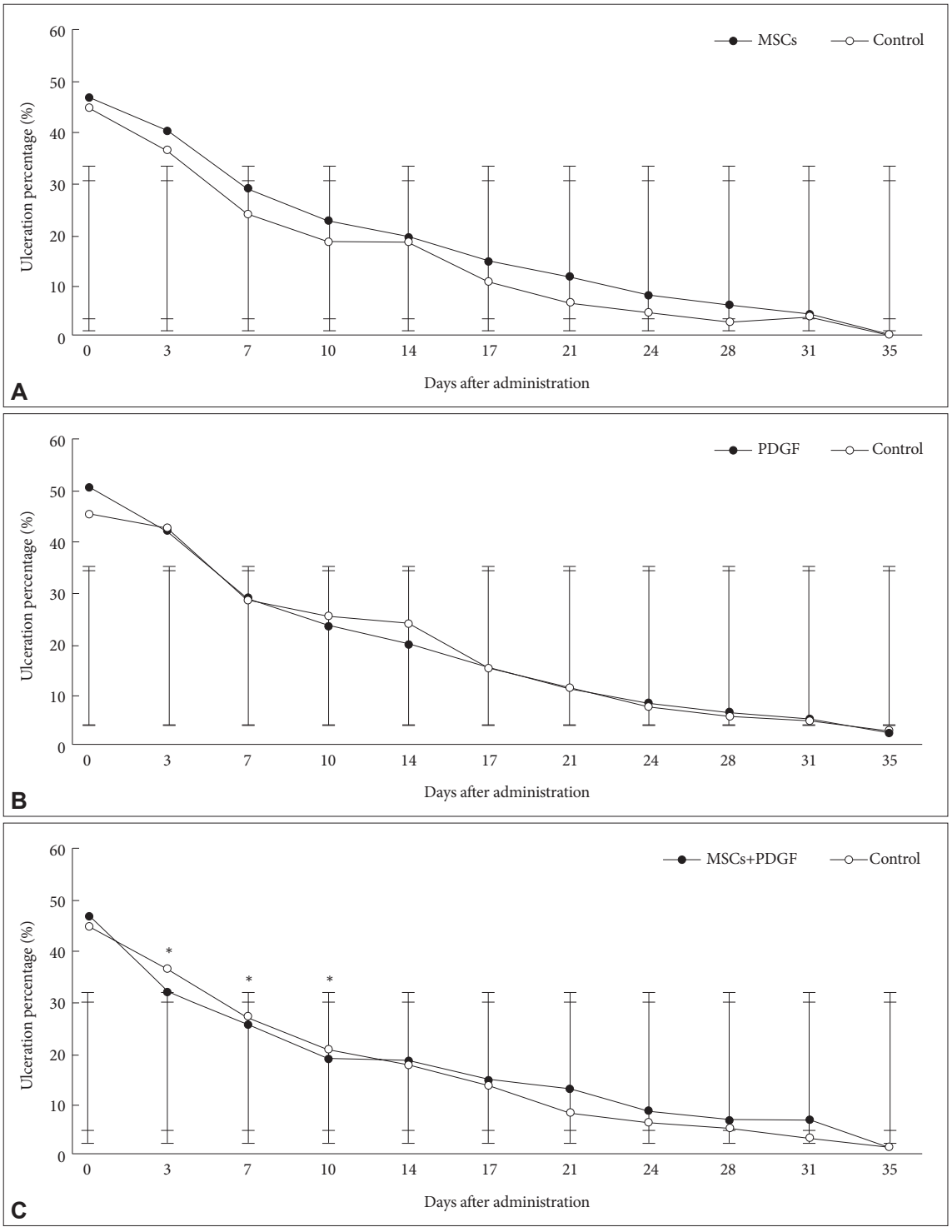

Figure 4. Changes of the skin ulceration ratios (%) in (A) Group A (rMSCs), (B) Group B (PDGF), and (C) Group C (rMSCs+PDGF). *statistically significant differences ($p \leq 0.05$). PDGF: platelet-derived growth factor, rMSCs: rat mesenchymal stem cells.

ences in healing after treatment. Three days after treatment, the ratios were 31.56±14.03% and 35.79±13.11% on the experimental and control sides, respectively (p=0.015). At seven days postinjection, the difference between the two sides had decreased (25.00±10.49% vs. 26.57±10.54% on the experimental and control sides, respectively; p=0.045). At ten days, the ulceration ratio was still significantly smaller on the experimental side (18.58±11.45%) compared with the control side (20.60±10.30%) (p=0.004). Two weeks after the injection, however, no significant difference was observed (18.20±13.46% vs. 17.21±10.94%) (Fig. 5). At five weeks postinjection, two of the five rats exhibited ulceration on the experimental side, while five of the six rats showed ulceration on the control side.

Histological examination

Histological examination of the skin ulcers was performed at five weeks postinjection. All three treatments (rMSCs alone, PDGF alone, and rMSCs+PDGF) resulted in positive microscopic effects on wound healing. In Group A, erosion of the epidermis layer was observed in 43.99±15.20% of the control skin area, and edema (Grade 3–4) was present in all specimens. In contrast, the experimental side of Group A tended to show less erosion of the epidermis layer (25.00±16.42%). Moreover, edema was observed in half of the experimental specimens (3/6) and the edema intensity was less (Grade 2) compared with the control side (Fig. 6A and B). In Group B, the epidermis layer showed erosion in 33.33±22.51% of the control area, and edema (Grade 3) was present in five out of the six rats. In contrast, the experimental side showed less erosion of the epidermis layer (15.00±17.61%), and edema (Grade 2–3) was present in four out of the six rats. In Group C, the control side showed erosion in 48.61±21.05% of the epidermis layer, and edema (Grade 3–4)

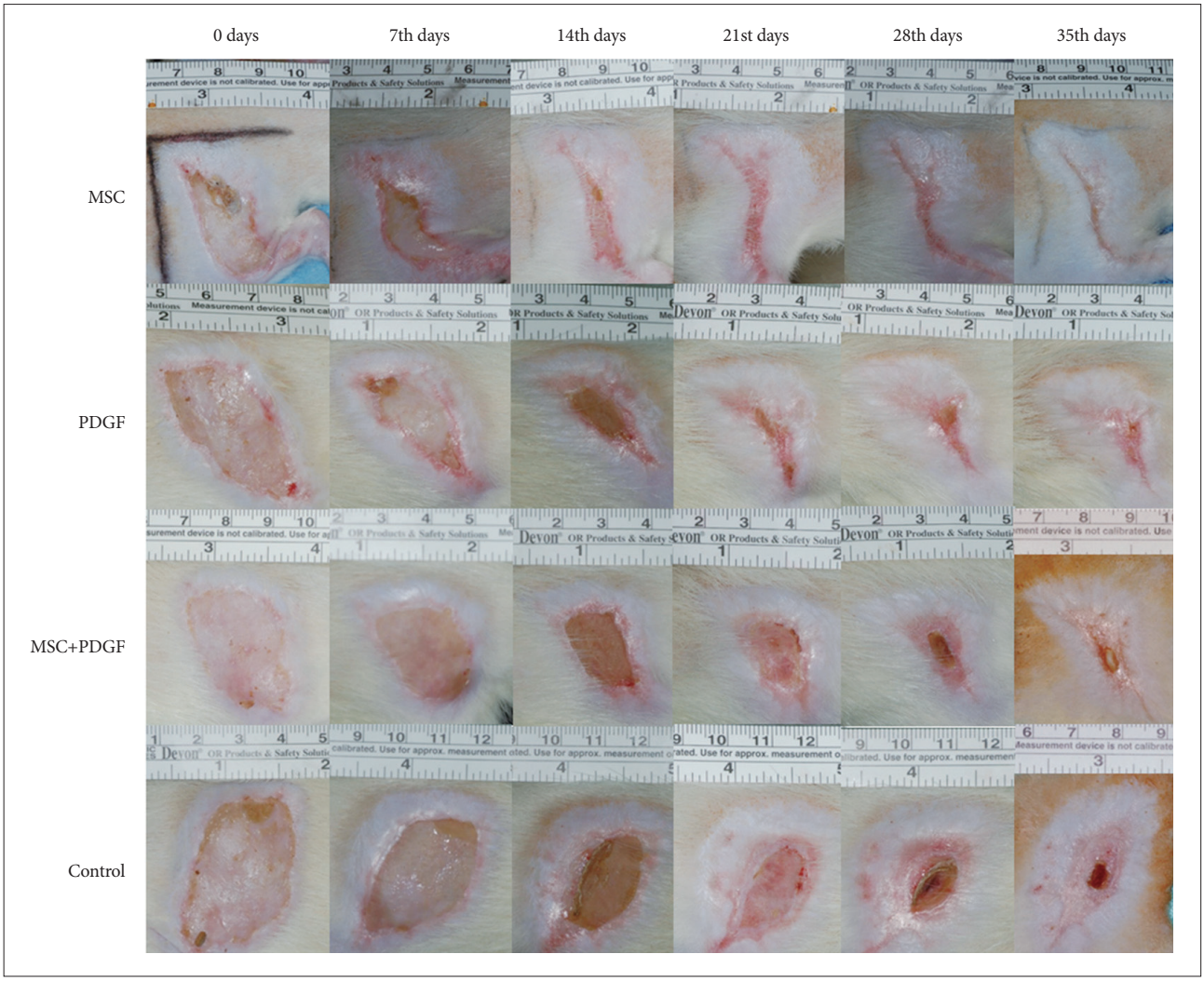

Figure 5. Wound healing progress in Group A (rMSCs), B (PGDF), and C (rMSCs+PDGF) at different stages of healing. PDGF: platelet-derived growth factor, rMSCs: rat mesenchymal stem cells.

was present in all six animals. The experimental side tended to show less erosion of the epidermis layer (37.18±23.83%), and edema (Grade 2–3) was present in four out of the six animals. These differences in epidermal erosion and presence of edema were not significant. MT staining revealed more organized collagen fiber deposition throughout the full thickness of the dermis layer when PDGF was administered (either alone or in combination with rMSCs) compared with the vehicle control (Fig. 6C and D). The wounds were re-epithelialized with regenerated skin appendages in Group C, while the control group exhibited less re-epithelialization and regeneration of skin appendages (Fig. 6E and F).

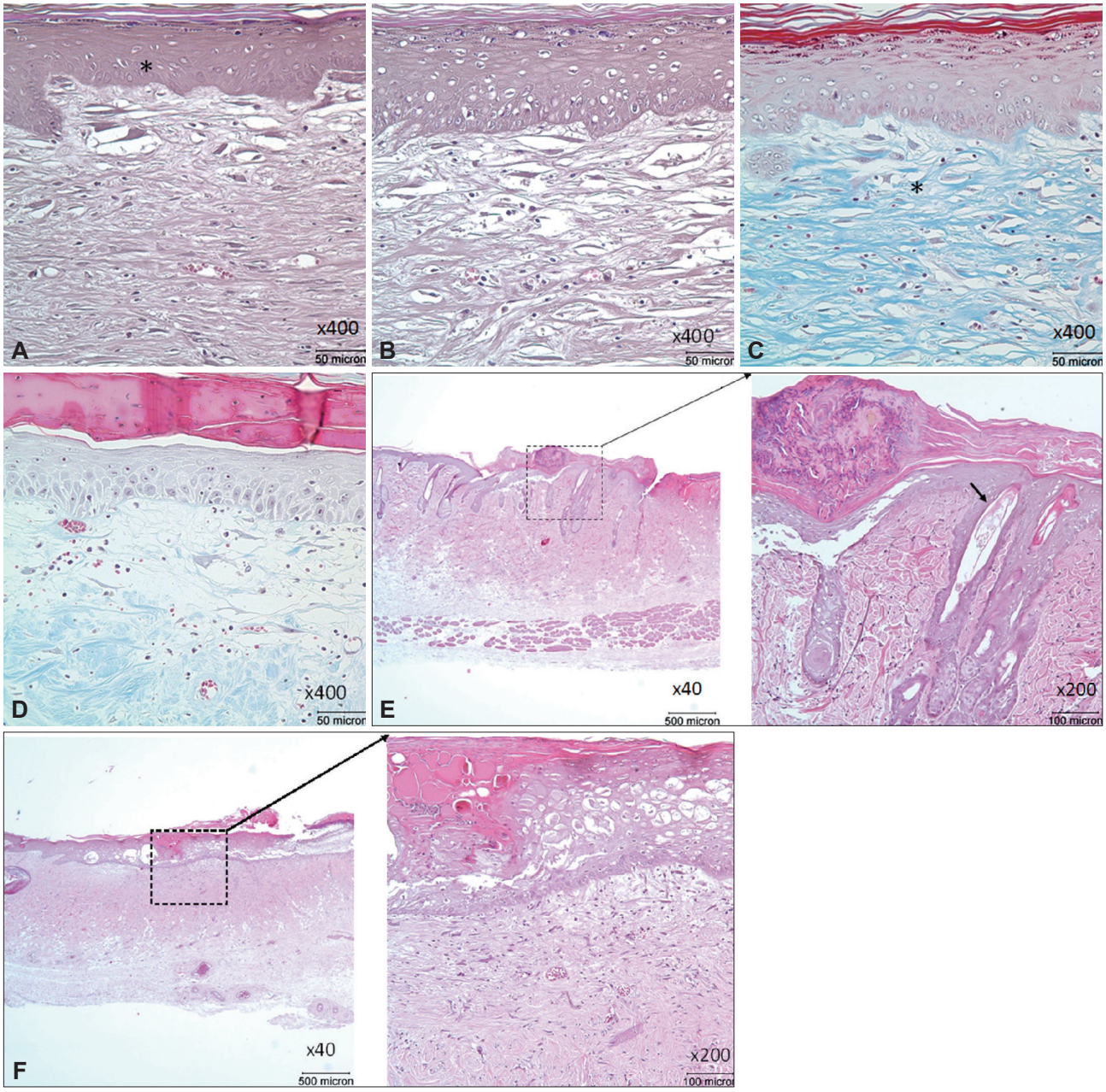

Figure 6. Histological images of radiation-induced skin wounds at 35 days postinjection. (A) HE staining of a representative skin wound from Group A (MSC treatment). Less edema (asterisk) was observed compared with the vehicle control (B). (C) Representative skin wound from Group B (PDGF treatment). More highly organized collagen fiber deposition (asterisk) was observed throughout the entire dermis layer compared with the vehicle control (D). (E) HE staining of a representative skin wound from Group C (rMSCs+PDGF). The wounds were re-epithelialized with regenerated skin appendages (arrow). (F) HE staining of a representative skin wound from the control side in Group C. Reduced re-epithelialization and regeneration of skin appendages were observed compared with experimental side. HE: hematoxylin eosin, PDGF: platelet-derived growth factor, rMSCs: rat mesenchymal stem cells.

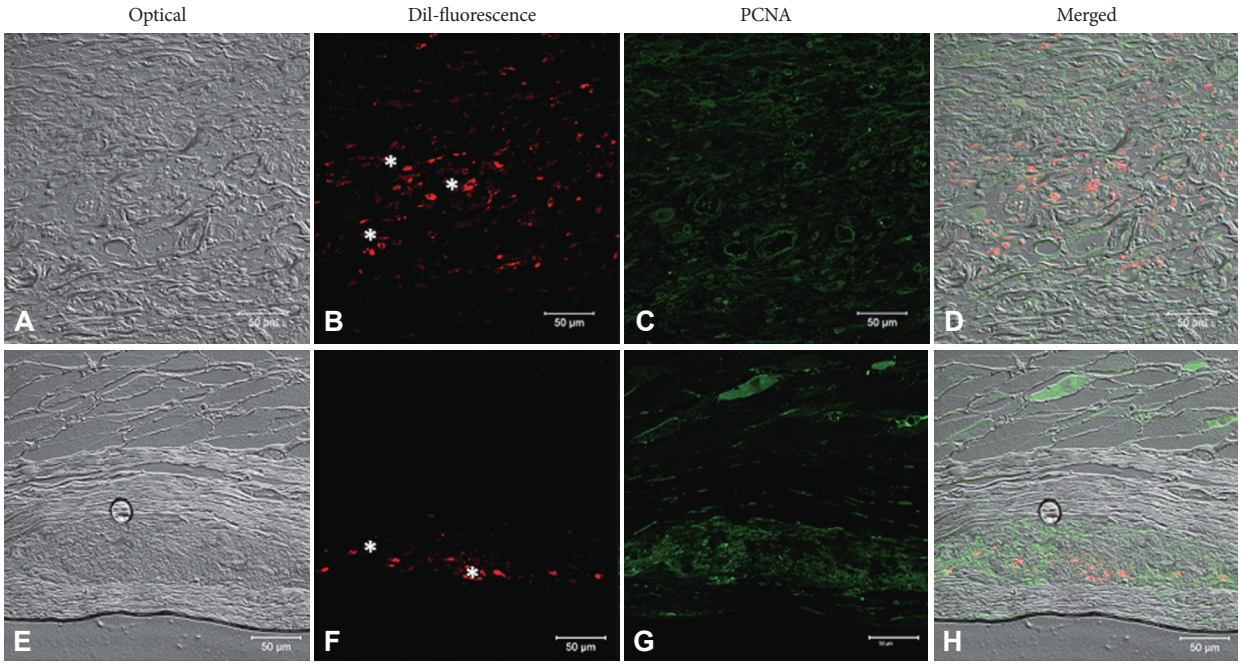

Figure 7. rMSCs tracking. Tracking of injected rMSCs at three (A-D) and seven (E-H) days after injection. Cultured rMSCs were labeled with fluorescent diakylcarbocyanine (Dil) dye (10 ng/mL) and subcutaneously injected into the radiation-induced ulcer area at three weeks postirradiation. Proliferating cell nuclear antigen (PCNA) (green) was used to confirm the viability of the rMSCs. rMSCs appeared near the basal membrane. *Dil-labeled MSCs observed *in vivo*. rMSCs: rat mesenchymal stem cells.

MT staining revealed more organized collagen fiber deposition throughout the full thickness of the dermis layer when PDGF was administered (either alone or in combination with rMSCs) compared with the vehicle control (Fig. 6C and D).

To track the transplanted rMSCs, Dil-labeled rMSCs were traced at three and seven days postinjection. Dil-labeled rMSCs were observed at each time point and were located mainly between the epithelial layer and the dermis layer. Expression of PCNA was verified (Fig. 7).

DISCUSSION

In patients exposed to radiation therapy, radiation-induced skin irritations can cause undesired complications, such as skin ulcers with reduced wound healing ability and prolonged healing progress. Recently, stem cell therapy has been proposed as a complementary therapy to conventional surgical treatment for radiation-induced ulceration [5,22,23]. Bone marrow-derived MSCs can become blood vessel cells and perifollicular cells, which later form hair follicles during the wound healing process [24]. These cells can differentiate into either myofibroblasts or epithelial cells and play an important role in the formation of healthy tissue [25,26]. In an analysis of the human and murine MSC transcriptomes, Tremain et al. [16] found that MSCs express transcripts encoding proteins that regulate a broad range of biological activities, including angiogenesis and wound repair. Some studies have suggested that the trophic capacity of MSCs to alter the tissue microenvironment may play a more prominent role than their transdifferentiation to achieve tissue repair [9,17,27,28].

Although the precise mechanism by which bone marrow-derived MSCs aid wound healing is not yet clear, enhanced healing effects have been reported [29,30]. Several studies in irradiated animal models have reported therapeutic effects of allogenous or autologous human MSCs and rMSCs using a variety of application methods and times [5,31-34]. In the study by Huang et al., [32] adipose-derived stem cells were injected three, four, and five weeks after irradiation and animals were sacrificed at three weeks after the first injection for soft tissue analysis. We also observed therapeutic effects immediately after injection; however, extension of our analysis to five weeks postinjection did not reveal any significant results. Histological examination revealed that the epithelium tended to be less eroded in the rMSC treatment group compared with the control group. However, no significant differences were observed between the two groups. The different results regarding the effects of rMSCs may be due to factors such as the amount of radiation absorbed, the nature of the irradiation site, and the amount/origin of the MSCs.

PDGF has been reported to contribute to wound healing through its proangiogenic effects [35,36]. Moreover, PDGF is a

major regulator of the growth, proliferation, survival, and chemotaxis of MSCs [37-39]. Previous studies have reported that PDGF exerts therapeutic effects on wound healing [40-45]. In this study, no significant difference was observed between the experimental and control sides when PDGF-BB was injected at the irradiated area. Although similar doses of PDGF were used in the previous studies and in our study, the relative amount of PDGF was different in our study since the previous studies used daily topical application while we performed subcutaneous injection. Also, the previous studies examined surgically formed wounds, while we examined radiation-induced wounds. Improvement of wound healing by application of PDGF to radiation-damaged tissue was not observed in this study. Radiation exerts a negative effect on the mitogenic potential of PDGF [46]; thus, the normal function of PDGF could have been impaired in irradiated skin.

Early treatment of radiation-induced skin ulceration can lessen pain and reduce secondary infection and damage of adjacent tissues. Moreover, reducing wound size would also be aesthetically favorable. Several efforts have been made to enhance the therapeutic effect of stem cells in the treatment of chronic wounds by administering them in combination with various growth factors [47,48]. Yan et al. [49] reported that human PDGF A-modified cultured cutaneous substitute cells (porcine bone marrow-derived MSCs and keratinocytes) promoted the healing of radiation-induced skin ulceration. After differentiation, MSCs primarily express PDGF receptor beta, which along with its ligand PDGF-β plays a key role in mediating tropism and differentiation during vascular remodeling [50]. Based on studies of interactions between MSCs and PDGF on the cellular level [48-50], we investigated the clinical effect of applying MSCs in combination with PDGF. PDGF directly affecting differentiation and tropism of rMSCs during angiogenesis [51-54], a major process during skin regeneration, may have contributed to the significant therapeutic results seen in Group C of this study where rMSCs and PDGF were applied in combination.

In conclusion, neither rMSCs alone nor PDGF alone exerted any significant therapeutic effect in a rat model of radiation-induced skin ulceration. However, the early stage of wound healing was significantly enhanced when rMSCs were administered in combination with PDGF onto rat skin that already had radiation-induced skin ulceration. We also observed that the ulcer size tended to be smaller when the rMSC/PDGF treatment was applied immediately after irradiation. These results will contribute to future efforts to successfully prevent and treat skin ulceration that occurs as a complication of radiation therapy. To fully explore the clinical potential of these findings, further studies using different radiation doses, time points, and agent concentrations are needed.

Acknowledgements

This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A120313). The authors thank Mr. BS Lee for the histological staining.

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

The study protocols were approved by Seoul National University Institutional Animal Care and Use Committee. All the animals were treated and handled in accordance with the "Recommendations for Handling of Laboratory Animals for Biomedical Research" compiled by the Committee on the Safety and Ethical Handling Regulation for Laboratory Experiments at the School of Dentistry at Seoul National University.

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