Impact of Injection Frequency of Adipose-Derived Stem Cells on Allogeneic Skin Graft Survival Outcomes in Mice

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Abstract

Previous studies indicated that mesenchymal stem cells (MSCs) exhibit immunomodulatory properties in composite tissue allotransplantation. However, due to the high immunogenicity of skin, although the single administration of MSCs improves survival of the skin allotransplant, immune rejection is still inevitable. The aim of our study was to evaluate whether multiple administrations of MSCs would improve immune tolerance in the allogeneic skin graft, compared to that with a single administration in a mouse model. After full-thickness skin allotransplantation on the backs of the mice, the recipient mice were infused with phosphate-buffered saline and isogenic 1.5×10^5/mL adipose-derived stem cells (ADSCs). ADSCs were transplanted into different mice according to the different injection frequencies such as single, once a week, and twice a week. Skin sections were taken on days 7 and 21 post-transplantation in all groups for gene expression and histological studies. ADSCs increased skin allograft survival compared to that in control mice ($P < 0.05$). Interleukin-6 and tumor necrosis factor-alpha messenger RNA levels were decreased, and the abundance of lymphocytes, based on immunohistochemistry, was also decreased in ADSC-infused mice ($P < 0.05$). However, among the different ADSC injection frequency groups, multiple ADSC infusion did not improve the survival rate and decreased proinflammatory cytokines and lymphocytes, compared to those with the single administration of ADSCs ($P > 0.05$). Conversely, the results with single administration were slightly better than those with multiple administrations. Our study demonstrated that ADSCs have the potential for immunomodulation in vivo. However, the results with multiple ADSC administration were not as good as those with single administration, which indicates the complexity of ADSCs in vivo and implying the need for adequate preclinical experimentation.

Keywords

allogenic skin graft, adipose-derived stem cells (ADSCs), immunomodulation, anti-inflammation

Introduction

Composite tissue allotransplantation (CTA) has been introduced, with considerable potential for the treatment of extensive traumatic injuries of the face, dysfunctional organs, and limb replacement[1]. However, due to skin is the most antigenic tissue, which is the major obstacle for grafted tissue survival, resulting in rejection approximately 10 days after transplantation without immunosuppressive therapy, thus, it is necessary to take immunosuppressive agents for a long time after CTA[2,3]. The long-term use of immunosuppressive drugs will result in certain side effects, such as liver and kidney toxicity and infection[1,2]. Therefore, seeking a new type of immunosuppressant that exerts a better-desired effect and produces minimal side effects is an urgent problem.

Mesenchymal stem cells (MSCs) are cells derived from various fetal and adult organs and have the capacity to self-renew and differentiate into several tissues including bone, cartilage, stroma, fat, muscle, and tendon[4,5]. MSCs have been extensively studied in regenerative medicine and

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Table 1. Summary of animal grouping.

Group	Routes of administration	Dosage	Injection time
Group A (control)	Injected PBS via intraperitoneal injection, IP	0.1 mL PBS	Immediately
Group B (once)	Injected ADSCs via intraperitoneal injection, IP	1.5×10^5/ 0.1 mL PBS	Immediately
Group C (once/week)	Injected ADSCs via intraperitoneal injection, IP	1.5×10^5/ 0.1 mL PBS	Immediately and days 3 and 7 post-operatively
Group D (twice/week)	Injected ADSCs via intraperitoneal injection, IP	1.5×10^5/ 0.1 mL PBS	Immediately and days 3, 5, 7, and 10 post-operatively

ADSCs, adipose-derived stem cells, IP: intraperitoneal injection, PBS: phosphate buffered saline.

tissue engineering, and reports have indicated that they can reduce inflammatory reactions and promote angiogenesis, thereby promoting wound healing or improving the survival rate of autologous grafts[6–8]. Moreover, numerous in vitro experiments have demonstrated that MSCs have potent immunosuppressive properties in which they can suppress T cell proliferation by cellular or nonspecific mitogenic stimuli and inhibit T cell functions through the induction of cellular stress[9–12]. Although in vitro results support the immunosuppressive properties of MSCs, their effects in vivo remain controversial. Previous studies have demonstrated that MSC infusion can obviously improve the survival of skin allografts in the early period by inducing immunologic tolerance and reducing the expression of pro-inflammatory cytokines[10,13]. However, these in vivo studies used a single infusion of MSCs, with which it is difficult to induce tolerance to tissue allografts for a long time; therefore, immune rejection is inevitable. In general, the inflammatory phase starts from 1-3 days after the injury[8], and immune rejection starts from 3-5 days after the skin allograft[14]. Moreover, 7-10 days after the graft, the skin begins to show signs of immune rejection[3]. Thus, we hypothesized that multiple administrations of MSCs based on these time points could improve immune tolerance for a long period. Based on the aforementioned findings, the purpose of this study was to investigate the effect of multiple administrations of MSCs on tolerance induction in mouse allogeneic skin graft, compared to that with a single administration. We also evaluated whether MSC infusion could affect the gene and immune cell expression of pro-inflammatory cytokines.

Materials and Methods

Animals

Six-to-eight-week-old male BALB/c mice and C57BL/6 mice were used in this study. BALB/c mice ($n = 18$) weighing 19–24 g were used as skin graft donors and C57BL/6 mice ($n = 36$) weighing 18–23 g were used as skin graft recipients. Other C57BL/6 mice were used as ADSC donors. The mice were kept under specific pathogen-free conditions for more than 1 week to adapt to the new environment. This experimental protocol was approved by the Animal Care and Experiment Committee (IACUC NO.: 19-0165-S1A0).

Study Design

According to the ADSC injection intervals, this experiment was divided into four groups (Table 1). Group A was the control group that was injected with phosphate-buffered saline (PBS) via intraperitoneal (IP) injection immediately after the skin graft. Group B was the one-time injection group, which received an injection of ADSCs one time immediately after the skin graft. Group C was the once/week group, and ADSCs were injected immediately after the skin graft, as well as on days 3 and 7 via IP injection. Group D was the twice/week group in which the ADSCs were injected immediately and then again on days 3, 5, 7, and 10 after the skin graft (Fig. 1).

ADSC Isolation and Culture from C57BL/6 Mouse fat Tissue

ADSCs were obtained from subcutaneous adipose tissue of C57BL/6 mice and isolated using previously established methods[15]. Fat tissues were washed by PBS (Biosesang, Seongnam, Korea) and then finely minced and digested with collagenase type I (Sigma-Aldrich, Albuch, Germany) in an incubator for 40 minutes at 37°C. Then, the cell suspension was mixed with low-glucose Dulbecco's modified Eagle's medium (Invitrogen, Seoul, Korea) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD, USA) and 1% penicillin/streptomycin (Invitrogen, Waltham, Massachusetts, USA) and centrifuged at 420 g for 5 min. Subsequently, the cell pellet was washed twice with PBS and re-suspended in the same medium. Cells were plated and incubated at 37°C in 5% CO2. Adherent cells were maintained in culture for four passages. At passage four, ADSCs presented spindle-shaped marphology, and when these cells were approximately 90% confluent, they were digested with 0.25% trypsin/EDTA (Gibco, Gaithersburg, MD, USA) at 37°C, washed with PBS, and pelleted by centrifugation. The number of cells was adjusted to 1.5×10^5/mL in 0.1 mL PBS for injection.

PKH26 and 4'-6-Diamidino-2-Phenylindole (DAPI) Labeling

To detect the ADSCs that survived in the transplanted skin, after preparing passage-four cells, we used PKH26 red
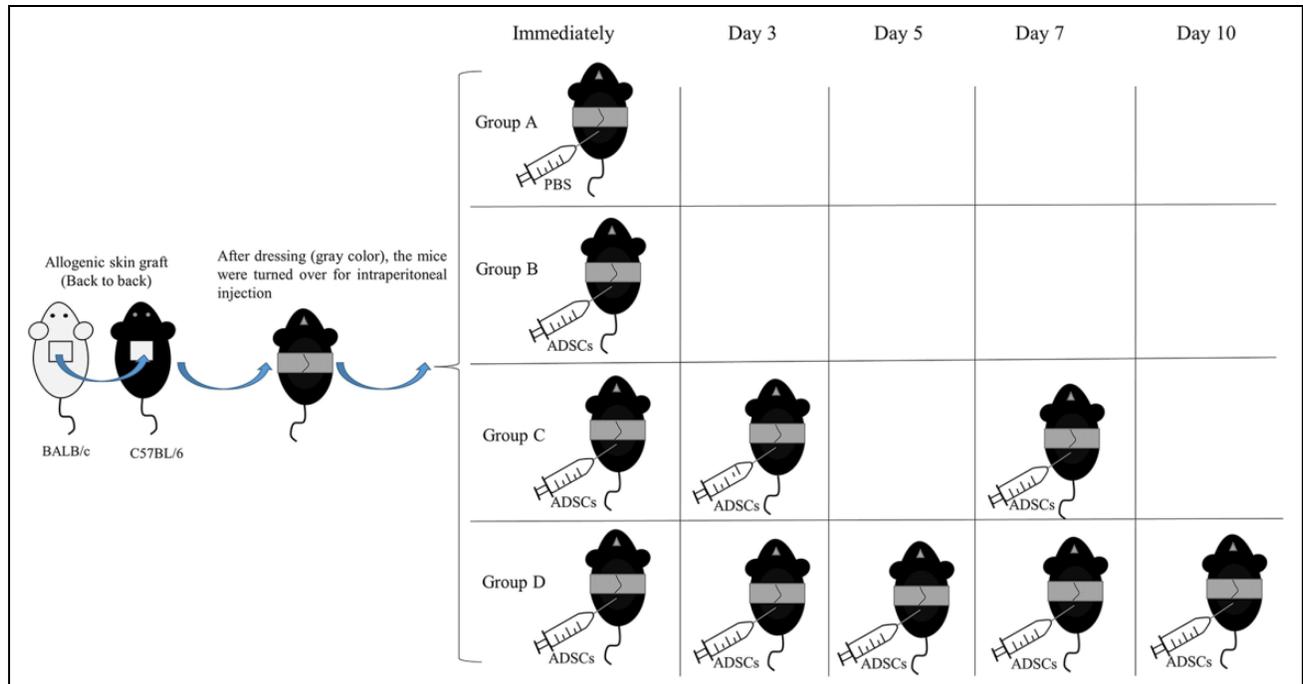
Figure 1. Mouse skin allotransplantation, and PBS or adipose-derived stem cell (ADSC) infusion in the four groups.

fluorescent cell linker kits (Sigma-Aldrich, Germany) to mark the cells. On postoperative day 21, histological sections were prepared according to the manufacturer' s instructions and counterstained with DAPI (Sigma-Aldrich, USA).

Skin Graft and Evaluation of Rejection

After mice were sedated with 2% isoflurane, the back hair of donor mice was removed with depilatory cream, and then, 1.5×1.5 cm full-thickness skin was harvested from the mouse back, and the panniculus layer was meticulously removed. The skin was placed on the midline of the back of the recipient mouse and fixed with simple interrupted sutures using 6-0 silk (Ailee, Busan, Korea). After the skin graft was completed, PBS or ADSCs were injected as described. Finally, mice were dressed with Bactigras® (Smith & Nephew, Seoul, Korea), Medifoam® (Mundipharma, Seoul, Korea), and Hypafix® (BSN Medical, Seoul, Korea) for 7 days. From post-transplantation day 7, the skin grafts were monitored daily and recorded by photography until post-operative day 21. Following wound evaluation, the dressing was changed every day to prevent mice from scratching the skin. The graft survival areas were roughly estimated based on photographs, and six (from to 0-5) different score levels were defined according to the estimated graft necrotic area (Table 2)[16].

RNA extraction and real-time quantitative RT-PCR

Total RNA was prepared from mouse skin using an RNA isolation reagent (QIAzol Lysis Reagent Cat No./ID: 79306), and complementary DNA (cDNA) was prepared from total

Table 2. Scores to characterize skin graft rejection, described as the percentage of the total graft area.

Score	Graft rejection
5	0–20%
4	20–40%
3	40–60%
2	60–80%
1	80–100%
0	Graft removal

RNA using a power cDNA Synthesis Kit (iNtRON Biotechnology, Sungnam, Korea). Polymerase chain reactions (PCRs) was performed in a 20 μL mixture containing 10 μL SYBR Premix Ex Taq (Takara Bio, Otsu, Japan), 0.4 μL ROX Reference Dye (Takara Bio, Otsu, Japan), cDNA, and primers, using an ABI PRISM 7500 (Applied Biosystem, Carlsbad, CA, USA). The sequences of the primers used are as follows: Interleukin 6 (IL-6), forward primer 5′-ATAGTCCTTCCTACCCCAATTTCC-3′, reverse primer 5′-GATGAATTGGATGGTCTTGGTCC-3′; Tumor necrosis factor (TNF-α), forward primer 5′-GGCAGGTCTACTTTG-GAGT CATTGC-3′, reverse primer 5′-ACATTCGAGC-CAGT GAAT TCGG-3′. Gyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward primer: 5′-TGTGTCCGTCGTGGA TCTGA-3′, reverse primer: 5′-CCTGCTTCACCACCT TCTTGAT-3′. Thermo-cycling conditions were 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. All targeted cytokine expression levels were calculated based on their threshold cycle (CT) values, and were expressed as

relative mRNA expression ratios normalized to a reference gene (GAPDH).

Immunohistochemical Staining and histologic Examination

Skin samples were harvested on days 7 and 21 post-transplantation and fixed with formalin for embedding in paraffin. Paraffin-embedded skin was sectioned to 4 mm thick. All sections were stained with hematoxylin and eosin (HE) and immunohistochemistry (IHC). Immunohistochemical staining was used for CD4+ T cells, vasoactive endothelial growth factor (VEGF), and platelet endothelial cell adhesion molecule with a recombinant anti-cluster differentiation 4 (CD-4) antibody (ab183685, Abcam, Cambrige, MA, USA), anti-VEGF monoclonal antibody (ab1316, Abcam, Cambrige, MA, USA), and cluster differentiation 31 (CD 31) antibody (GTX130274, GeneTex Co., USA), respectively. Standard procedures for these staining processes were performed as recommended by the manufacturers. For anti-CD 4, quantitative analysis was performed with Image-Pro Plus (Media Cybernetics, Rockville, MD, USA) by measuring the integrated optical densities of the positively stained area in the epidermis and dermis layers in five randomly selected fields (200× magnification); moreover for anti-VEGF, quantitative analysis was performed with Image J (Media Cybernetics, Rockville, MD, USA) by measuring the propotion of the positively stained area in the epidermis and dermis layers in five random fields (200× magnification) in each section. Angiogenesis was estimated with CD-31 staining by measuring the number of CD-31-positive vessels from five random fields (400× magnification) in each section.

Statistical Analysis

The results are expressed as mean values ± SDs. SPSS (SPSS, Chicago, I11, USA) software was used for statistical analysis. The survival rate in the four groups was evaluated by the Kaplan-Meier method using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA) and the efficacy of treatment was assessed by the log-rank test. Differences in the expression of CD-4, VEGF, and CD-31 among the different groups were tested using the mixed model test, and pairwise comparisons between the four groups were tested by post-hoc analysis with Bonferroni correction. A P-value <0.05 was considered statistically significant.

Results

Skin graft survival

Seven days after transplantation, the dressing was removed, and necrotic areas of the skin graft were monitored daily and recorded by photography up to 21 days. On day 9, necrotic spots were observed in the control group, which occurred at approximately day 14 in the three experimental groups

(Fig. 2A). Statistical analysis indicated that the necrotic levels of skin grafts were lower in the three ADSC injection groups than in the control group from day 9 to day 21 ($P < 0.05$). Among the three experimental groups, although the scores in group B were slightly higher than those in groups C and D, there were no significant differences ($P > 0.05$; Fig. 2B). The survival rate was significantly increased after ADSC treatment compared to that in the no-treatment group ($P < 0.05$). In the comparison of groups B, C, and D, there were no significant survival differences, even though the survival rate was slightly higher in groups B and C than in group D ($P > 0.05$; Fig. 2C).

### Assessment of PKH26-Labeled ADSCs

The presence of PKH26-labeled cells in tissue sections from the ADSCs groups was assessed by fluorescence microscopy (Fig. 3). PKH26-labeled cells were detected in the graft tissue in the three ADSC injection groups at day 21 post-transplantation. More cells showed positive staining (red color) in groups C and D than in group B (Fig. 3).

Cytokine Expression Analysis

Using the quantitative real-time PCR method, mRNA expression of different genes in mouse allografts were investigated at day 7 and 21 post-transplantation. Specifically, we studied expression of the pro-inflammatory cytokine genes *IL-6* and *TNF-a*. The mRNA levels were expressed as fold-changes related to those in the control group (PBS; Fig. 4). On day 7, the *IL-6* mRNA levels were significantly lower in the ADSC injection groups than in the control group ($P < 0.0001$). Group B showed the lowest *IL-6* mRNA levels in comparison with those in groups C and D ($P < 0.05$). Between groups C and D, there was also a significant difference ($P < 0.05$; Fig. 4A). On day 21, the reduction in *IL-6* mRNA levels was still lower in the three experimental groups compared to levels in the control group. Group B still showed significantly lower *IL-6* mRNA levels in comparison with those in groups A and D ($P < 0.05$; Fig. 4B). The *TNF-a* mRNA levels were significantly lower in the three ADSC injection groups than in the control group on day 7 ($P < 0.0001$; Fig. 4C). Moreover, on day 21, there were still significant differences between three experimental groups and control group ($P < 0.05$; Fig. 4D). However, although the *TNF-a* mRNA levels increased sequentially among the three ADSC injection groups, there were no statistically significant differences on days 7 and 21 ($P > 0.05$; Fig. 4D).

ADSCs Reduce Lymphocyte Infiltration in Allogeneic Skin Grafts

Immunohistochemistry for CD-4 was performed on days 7 and 21 after transplantation. On day 7, more lymphocytes seemed to infiltrate the epidermis and dermis in the control group than in the three ADSC injection groups. Among the three experimental groups, group B showed lower lymphocyte infiltration
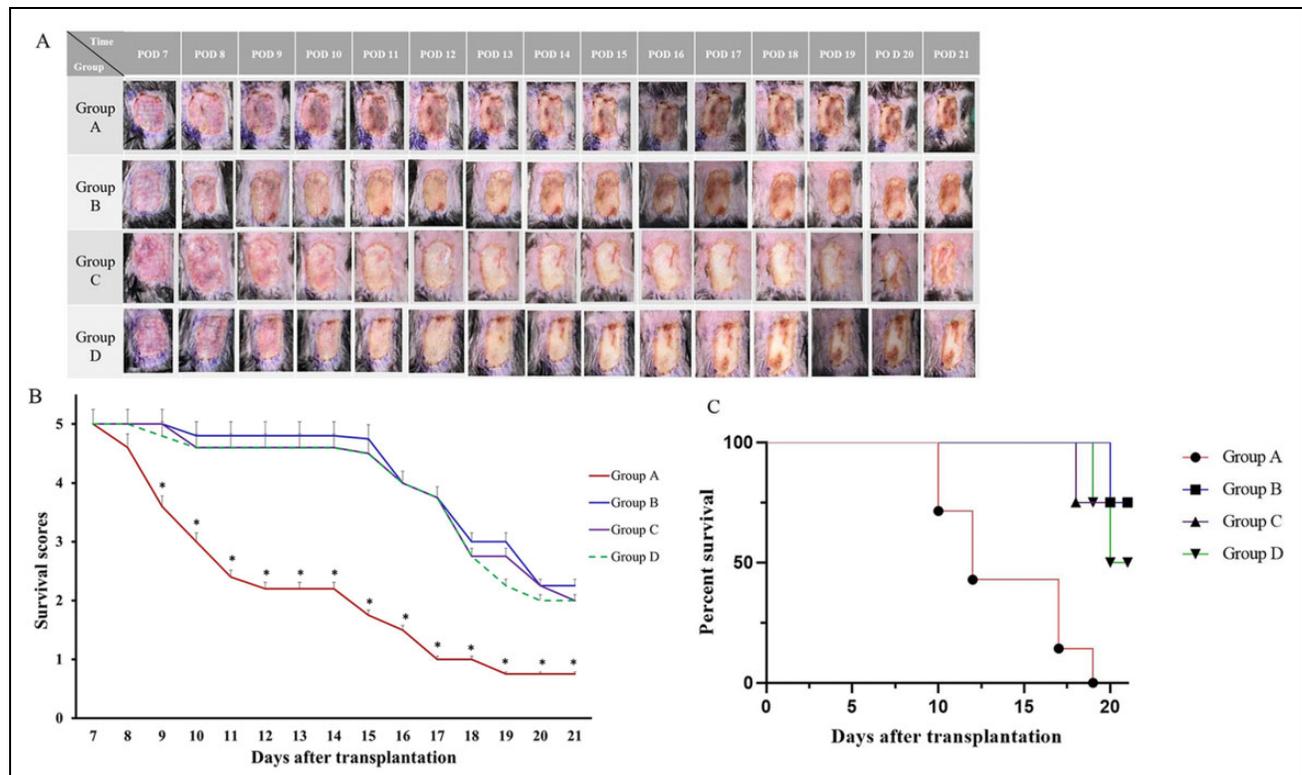
Figure 2. Allograft rejection in the four groups. (A) Skin allograft appearance of the four groups from day 7 to 21 post-transplantation. (B) Different score levels indicate different necrotic areas of the mouse skin allograft (*: *P* < 0.05 comparison between the control group and adipose-derived stem cell [ADSC] injection groups). (C) The graft survival rates in four groups, which were evaluated by the Kaplan-Meier method. ADSC infusion markedly improved the skin allograft compared to that in the control group (*P* < 0.05). However, among the three ADSCs injection groups, there were no significant differences (*P* > 0.05).
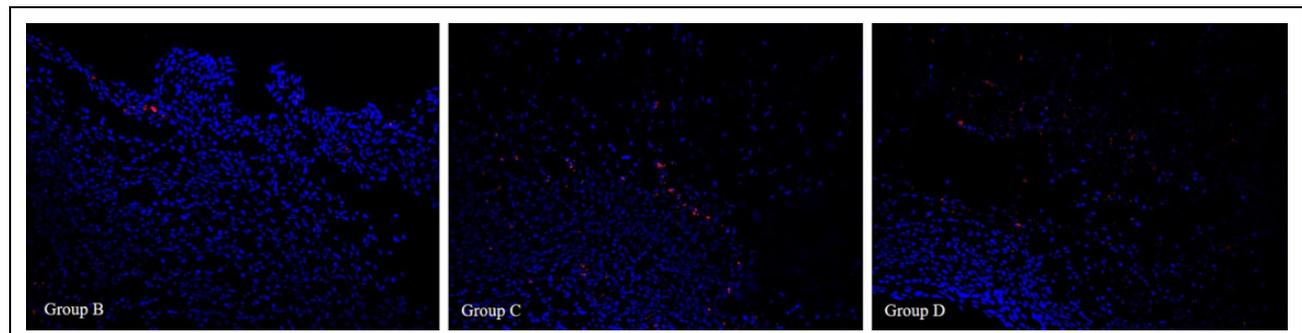
Figure 3. Fluorescence microscopy evaluation of skin samples containing adipose-derived stem cells (ADSCs) in experimental groups were on day 21 after transplantation. The red marks show the PKH26-labeled ADSCs. In groups C and D, more cells could be detected compared to numbers in the group B (×200).

than groups C and D (Fig. 5A). On day 21, the results were similar, with fewer lymphocytes infiltrating the tissues in three ADSC injection groups thanin the control group (Fig. 5B). The results of the quantitative analysis showed that ADSCs injection reduced lymphocyte infiltration very significantly relative to that in the control group (*P* < 0.0001) on days 7 and 21 (Fig.

5C). Among the three ADSC-injection groups, the reduction in lymphocyte infiltration was more prominent in group B than in groups C and D (*P* < 0.05), whereas there was no statistical difference between groups C and D (*P* > 0.05) on day 7. Moreover, there were no statistically significant differences among the three groups (*P* > 0.05) on day 21.
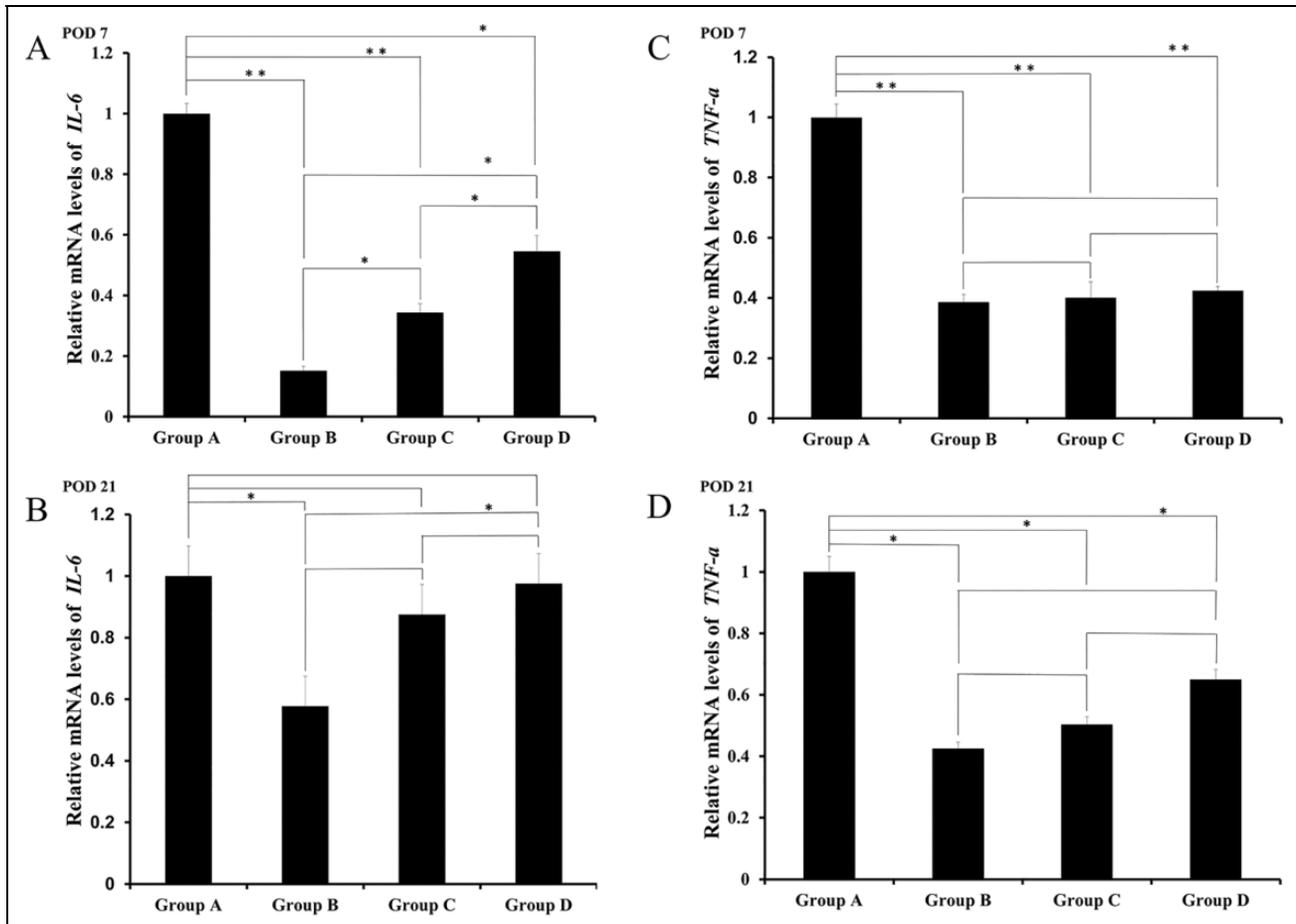
Figure 4. mRNA expression of *IL-6* (A, B) and *IFN-a* (C, D) in skin graft. Data are expressed as fold-changes in relation to levels in the control group. Statistically significant differences among groups are reported above the columns (**: *P* < 0.0001, *: *P* < 0.05).

VEGF and CD31- Positive Neovascularization in the graft

VEGF expression was higher in ADSCs injection groups than in the control group on day 7. There were no significant differences among the three ADSC injection groups (Fig. 6A). On day 21, although less VEGF was expressed than on day 7 in all groups, the control group had the lowest expression (Fig. 6B). The quantitative analysis is shown in Fig. 6C, and the differences between the control group and the three experimental groups were statistically significant (*P* < 0.0001); however, comparing the three groups with each other, there was no obvious difference on days 7 and 21. Based on CD-31 immunohistochemistry, the average number of newly formed blood vessels, observed using ×400 magnification, was 4 (±1.4) neo-vessels in group A, 7.8 (±1.3) in group B, 7.6 (±1.7) in group C, and 7.2 (±0.4) in group D on day 7 (Fig. 7A). On day 21, the average number of blood vessels was lower in the four groups, which was 1.6 (±0.5) in group A, 3.8 (±1.3) in group B, 3.6 (±0.5) in group C, and 3.4 (±0.9) in group D (Fig. 7B). The differences between the control group and the three experimental groups were statistically significant

(*P* < 0.0001) on days 7 and 21. However, the number of vessels among the three ADSC-injection groups was not significantly different (*P* > 0.05; Fig. 7C).

Histologic Evaluation

Histopathologic changes were evaluated on days 7 and 21 after transplantation. The histopathology was assessed as the degree of inflammatory cell infiltration based on H&E staining. More inflammatory cells infiltrated the epidermis and dermis in the control group on day 7, which was lower than that in the three experimental groups (Fig. 8A). On day 21, the epidermis was obviously degenerated, and more apoptotic keratinocytes infiltrated into the dermis in the control group, compared to that in the three experimental groups (Fig. 8B).

Discussion

Despite considerable evidence showing the immunosuppressive properties of MSCs in vitro[9,17], their immunomodulatory characteristics have not been fully determined in vivo. In our study, we compared the effects of injection frequency
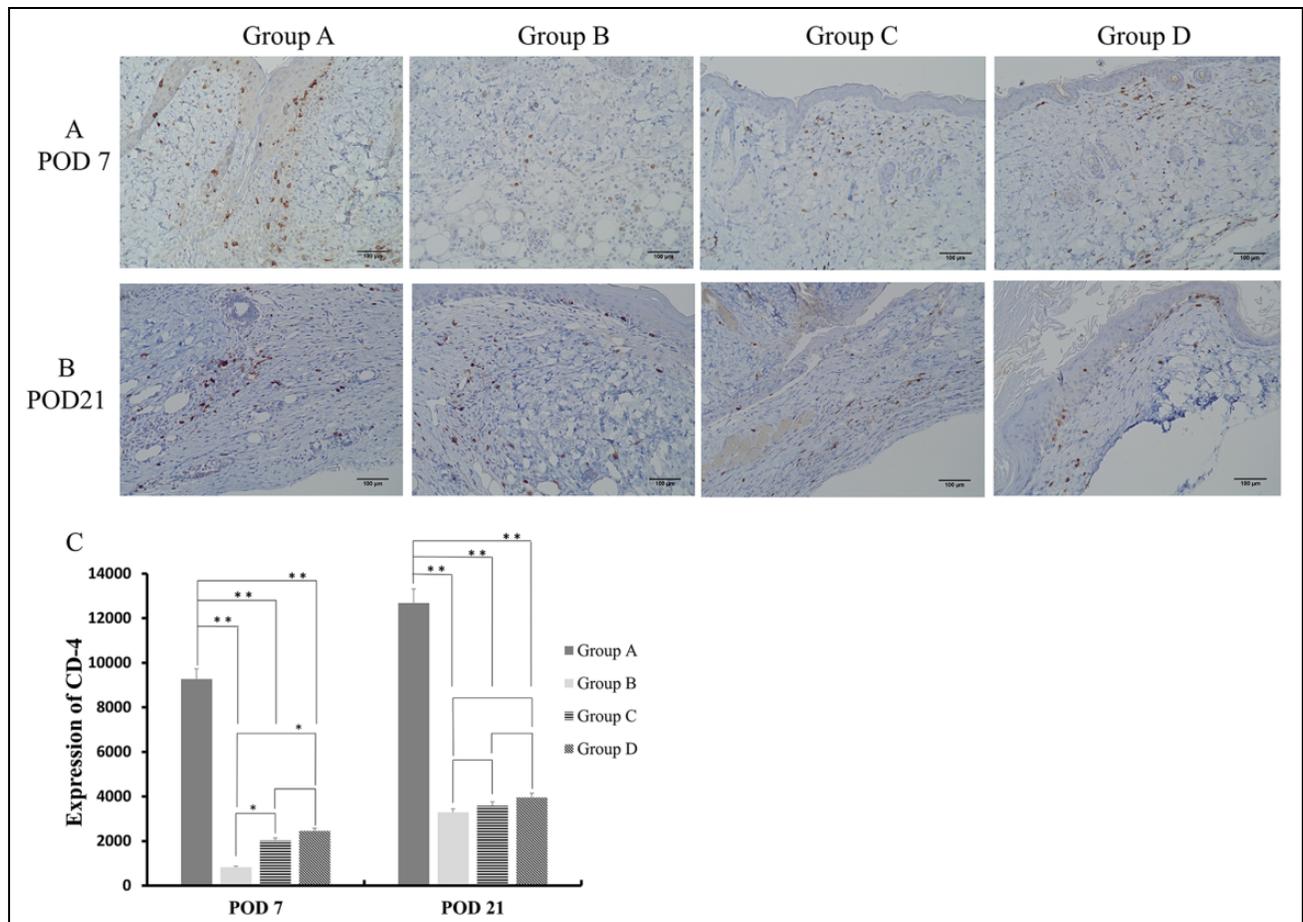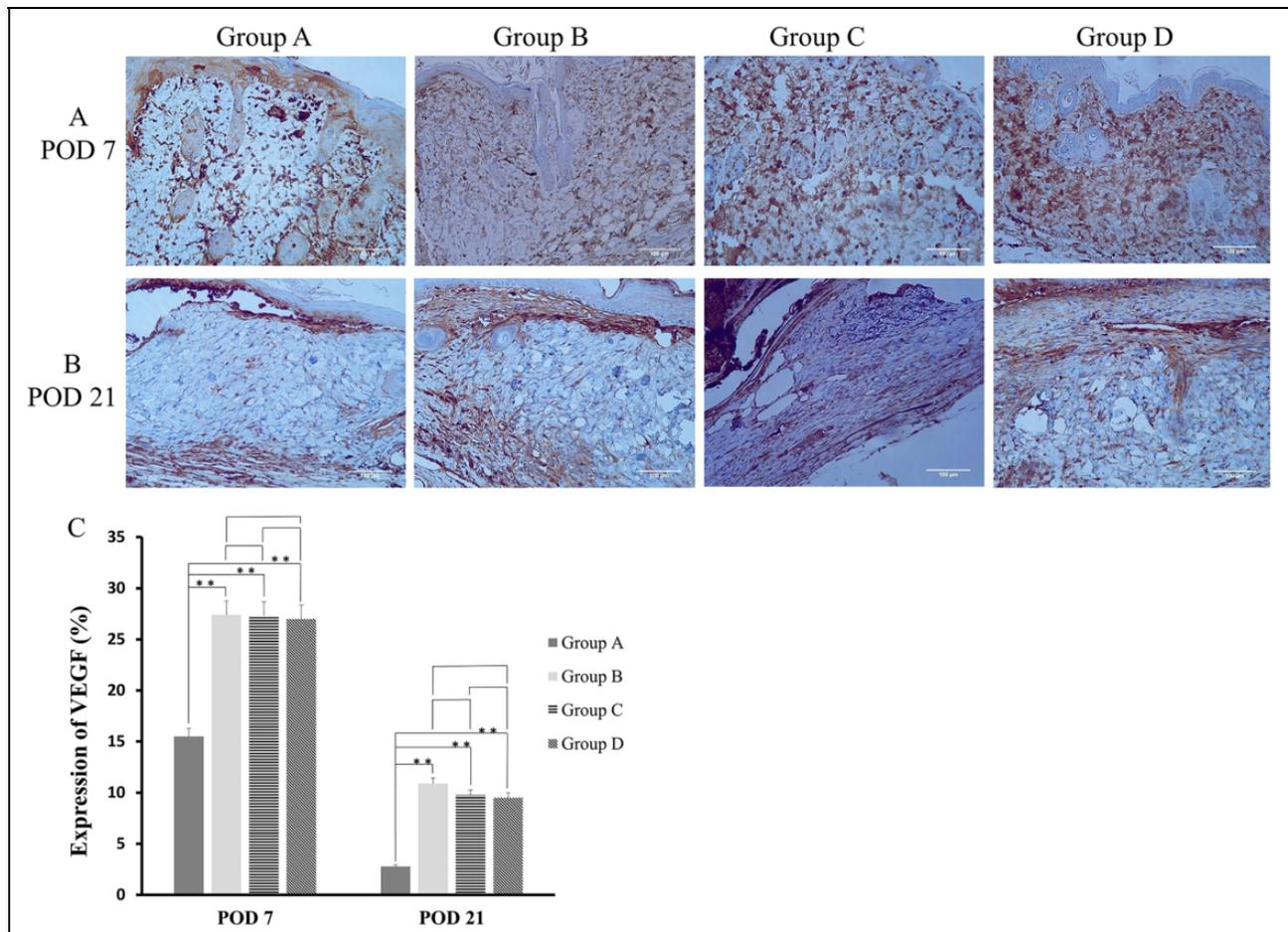
Figure 5. Immunohistochemistry analysis of CD-4 expressions in the four skin transplantation groups. (A) On day 7, in the control group, more lymphocytes infiltrated the epidermis and dermis than in the adipose-derived stem cell (ADSC) experimental groups, and the fewest lymphocytes could be detected in the group B. (B) On day 21, in the control group, there were still more lymphocytes infiltrated to the tissue than in the experimental groups. (C) The integrated optical densities of CD-4 were measured from at least five random fields per section at 200-fold magnification (**: $P < 0.0001$, *: $P < 0.05$).

of ADSCs on allogeneic skin transplantation outcomes in mice. Compared with those in the untreated group, the administration of ADSCs significantly reduced the rejection necrosis level and improved skin graft survival. However, compared to those with single administration, the necrotic level was slightly higher and the survival rate was slightly lower in the multiple administration groups although it was not statistically significant. One study on allogeneic skin graft in mice from SM Lee et al.[13] indicated that a single administration of ADSCs (1×10^6/mice) improved allograft survival. The opinion in this study was that MSCs have immune privilege, which allows them to evade host immune surveillance in vivo. The previous studies have revealed that the immune privilege of MSCs is attributed to their expression of major histocompatibility complex (MHC) class I molecules and the lack of expression of MHC class II molecules[18–20]. However, Sbano et al.[21] demonstrated that MSC infusion twice (5.6×10^6/rat) could stimulate the rat skin allograft rejection. This research considered that MSCs have an immunogenic role that accelerates allograft rejection.

Based on our results, we suggest that both of these properties existed when MSCs were administered in vivo, which is related to the frequency of MSC infusion.

The best dosage of ADSCs for skin allotransplantation is still unknown. In a study where intraperitoneal administration was performed, a single injection of 5×10^5 ADSCs was used for mouse skin allotransplantation, improving their survival[22]. Considering that our study involved multiple intraperitoneal administrations of large numbers of ADSCs, which might distress the mice, we finally decided to reduce the dosage to 1.5×10^5 cells/administration. Even though this dosage exerted a positive effect in our study, we believe that the optimal administration dose of MSCs still needs further research to provide a strong basis for future clinical research.

The transplantation of allogeneic skin grafts is associated with a potent inflammatory immune response. After transplantation, immune cells of the recipient will become activated, which leads to the destruction of donor cells and rejection of the graft. Meanwhile, some pro-inflammatory
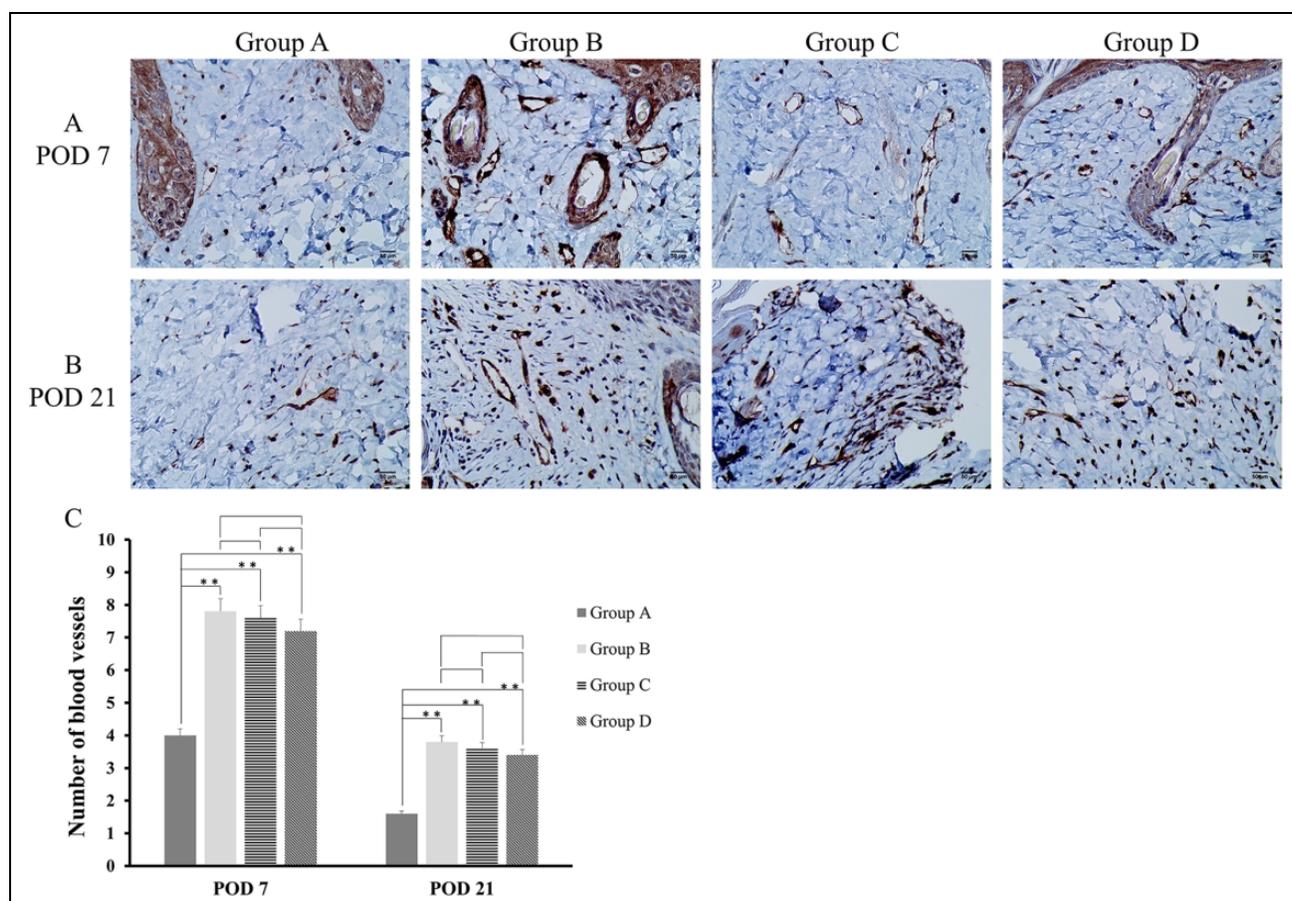
Figure 6. Immunohistochemistry analysis of VEGF expression in four skin transplantation groups. (A) On day 7, more VEGF was expressed in the three adipose-derived stem cell (ADSC) experimental groups than in the control group. (B) On day 21, in the experimental groups, there was still more VEGF expressed than in the control group ($\times$ 200). (C) Quantification of the expression of VEGF in the four groups (**: *P* < 0.0001).

cytokines such as IL-6 and TNF-a will be produced[14,23–25]. Previous studies demonstrated that MSCs could suppress T cell proliferation in vitro and reduce the expression of pro-inflammatory cytokines in vivo[10,13,16,22,26]. However, some in vitro studies indicated that MSCs could induce T cell proliferation and inhibit the apoptosis of lymphocytes, with the anti-apoptotic effect being proportional to the number of MSCs added and occurring via the secretion of IL-6[27,28]. Based on our results, we speculated that after a single administration of ADSCs in vivo, these cells could inhibit the proliferation of lymphocytes and the secretion of pro-inflammatory factors involved in immune rejection, whereas with multiple ADSC infusion, the cells secreted more pro-inflammatory cytokines and promoted the proliferation of lymphocytes. However, multiple injections of ADSCs still exerted a certain immunosuppressive effect in our study. Moreover, the histological sections of our study also revealed that ADSC infusion inhibited the infiltration of inflammatory cells into the donor skin.

In the literature, the survival time of transplanted stem cells had been reported to be 3 months, and PKH 26-labeled cells were found to migrate to the skin, subcutaneous tissue, and even the spleen and brain[29] after infusion. In our study, PKH 26-labeled ADSCs could be detected on the skin sections on day 21 post-transplantation after intraperitoneal injection which may indicate that the high regenerative potential of ADSCs[30].

In addition, some studies also showed that MSCs promote tissue repair and immunomodulation through paracrine actions, which was determined to be highly regulated by Rap1/NF-κB signaling[31–33]. The paracrine actions of MSCs do not only induce local neovascularization but also enhance platelet recovery following irradiation injury, and this reduces the apoptosis of megakaryocytes via the secretion of some angiogenetic factors such as VEGF and PDGF[34–36]. Moreover, enhanced neovascularization has an active effect on would healing, which could improve skin survival[37–38]. ADSCs also increase the expression of CD-31 in vivo[39]. Our results of VEGF and CD-31 also show the ability of ADSCs to promote angiogenesis; however, the administration of multiple ADSC treatment did not increase the expression of VEGF and CD-31 more than that with single

Figure 7. Immunohistochemistry analysis of CD-31 expression in the four skin transplantation groups. (A) On day 7, more blood vessels were formed in the three adipose-derived stem cell (ADSC) injection groups than in the control group. (B) On day 21, the number of blood vessels was larger in the three experimental groups than in the control group (×400). (C) Quantification of the expression of CD-31 in the four groups (**: $P < 0.0001$).
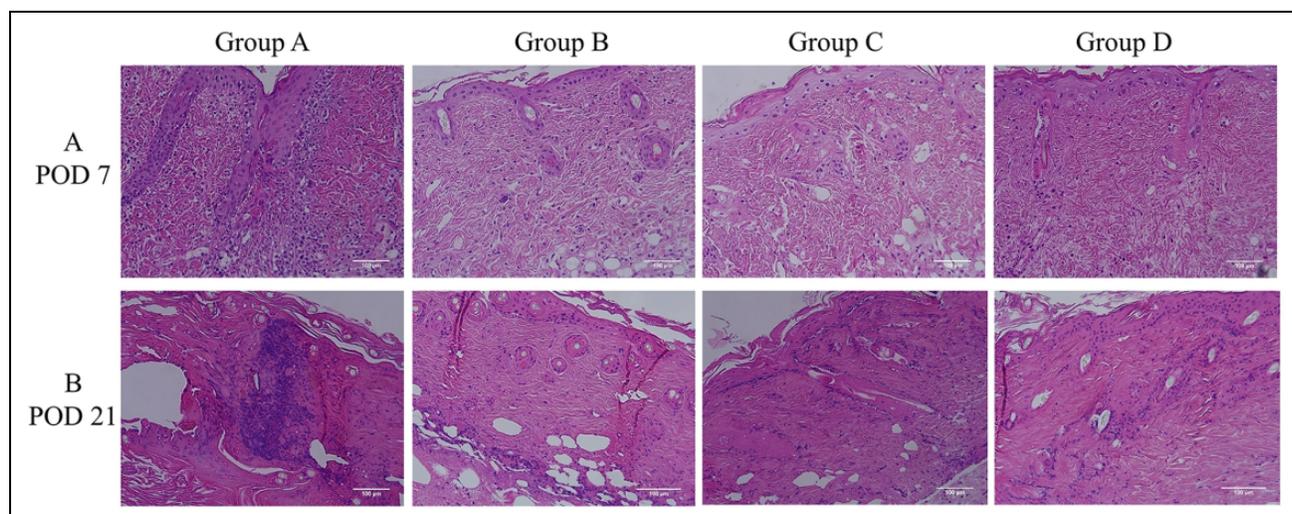
Figure 8. Comparison of histologic changes among the four skin transplantation groups. (A) Inflammatory reactions were more prominent in the control group than the adipose-derived stem cell (ADSC) injection groups on day 7 post-transplantation. (B) Inflammatory reactions were still more prominent in the control group than in the three experimental groups on day 21 (×200).

administration. Thus, it seems that there was enough neo-vascularization in the transplanted skin.

Nevertheless, there were some limitations in this study. The underlying immunomodulatory mechanism of multiple MSC infusions was not investigated. The in vivo dynamic effects of multiple MSC infusions on lymphocytes needs further study. Furthermore, although compared to other tissues or organs, adipose tissue is considered to be rich in cell that easily supply high numbers of stem cells[40], there are also some disadvantages of using adult MSCs such as vulnerability to stem cell senescence, batch-to-batch variations in MSC quality, and limited proliferative potency[41]. Recently, parental pluripotent stem cells (PSC)-derived MSCs were proved to possess better cell quality than adult MSCs. In the near future, PSC-MSCs may be another putative cellular source that overcomes many limitations of adult MSCs used in immune modulation[42,43].

At present, MSC therapy has a huge potential as an immunosuppressant treatment in composite tissue allotransplantation due to its potential for tissue repair, immunomodulation, and regeneration[44]. However, our study indicated that multiple infusions of stem cells cannot improve immune tolerance for a long period. Perhaps the combined use of MSCs and traditional immunosuppressants can not only reduce the side effects of traditional immunosuppressants but also improve the therapeutic effect. This will be the direction of our further research.

In conclusion, we demonstrated that ADSCs have anti-inflammatory effects, suppress T cell proliferation potential, and promote angiogenesis in a mouse model. Multiple administrations of ADSCs did not improve the skin survival rate compared to that with a single administration. Conversely, the results of single administration were slightly better than those with multiple administrations. Thus, further in-depth research is required to fully investigate the safety of multiple ADSC administration.

Ethical Approval

This experimental protocol was approved by the Animal Care and Experiment Committee (IACUC NO.: 19-0165-S1A0), Seoul, Republic of Korea.

Statement of Human and Animal Rights

All of the experimental procedures involving animals were conducted in accordance with the Animal Care guidelines of Seoul National University College of Medicine and approved by the Animal Care and Experiment Committee (IACUC NO.: 19-0165-S1A0), Seoul, Republic of Korea.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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