Generation of Skin Tissue Using Adipose Tissue-Derived Stem Cells

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Background: The aim of this study was to generate skin tissue using adipose tissue-derived mesenchymal stem cells.
Methods: Thirty Wistar albino rats were used. A 2-cm-diameter full-thickness skin defect on the back of each rat was formed. A secondary wound healing model was constituted in group 1, fibrin matrix only was applied in group 2, a keratinocyte-coated fibrin matrix was applied in group 3, an adipose tissue-derived mesenchymal stem cell–seeded fibrin matrix was applied in group 4, and a keratinocyte-coated and adipose tissue-derived mesenchymal stem cell–seeded fibrin matrix was applied to the defects in group 5.
Results: A similar form of wound healing, with contraction from the edges and ulceration at the center, was observed in groups 1, 2, and 3. The wound contraction was reduced in group 4, and the epidermis was creeping from the surrounding tissue but with some ulcerations in the central part of the wounds. In group 5, the defect area was almost totally epithelialized, with minimal wound contraction. By microscopic analysis, significant increases in the collagen volume ratios and vascular volume ratios were determined in groups 4 and 5. From the fluorescent micrographs, fibroblastic differentiation and extracellular matrix synthesis, endothelial differentiation of stem cells, and neoangiogenesis and epithelium derived from marked keratinocytes were observed in group 5.
Conclusion: Formation of the adipose tissue-derived mesenchymal stem cell–seeded and keratinocyte-coated autologous fibrin scaffold leads to significant skin replacement. (*Plast. Reconstr. Surg.* 137: 134, 2016.)

Autologous skin grafting is the main reconstructive procedure for full-thickness skin defects. Donor-site shortage, donor-site morbidity, and suboptimal results remain both aesthetically and functionally challenging. Although various types of skin substitute are used clinically, satisfactory results have not been achieved to date. The limitations of current skin substitutes are the tendency toward microbial contamination, lack of mechanical strength, blistering, lack of skin appendages, and poor cosmetic effects. To develop a regenerative scaffold, in which cellular elements reproduce and generate dermal matrix proteins, collagen and skin substitutes using mesenchymal stem cells are favorable. Stem cells may overcome the limitations of skin substitutes because of their ability to differentiate into various cell lineages and may affect adjacent cells in a paracrine manner.[1–8]

The aim of our study was to regenerate skin tissue using cultured adipose tissue-derived mesenchymal stem cells, cultured keratinocytes, and fibrin scaffold. No similar previous study was identified through a literature review.

MATERIALS AND METHODS

The Animal Research Ethics Committee of Bezmialem Vakif University approved the present study (November 23, 2011; n0: 17). Thirty Wistar

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Received for publication September 24, 2014; accepted August 28, 2015.
Presented at the 35th Congress of the Turkish Plastic, Reconstructive and Aesthetic Surgery Association, in Istanbul, Turkey, October 28 through 31, 2013, where it was honored with first prize in the research contest category.

DOI: 10.1097/PRS.0000000000001927

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

albino rats weighing 200 to 300 g were used in the current study. Diet and water were provided ad libitum. All of the rats were placed in single cages after surgery.

Tissue samples were harvested under ketamine hydrochloride (35 mg/kg, intramuscularly) and xylazine hydrochloride (5 mg/kg, intramuscularly) anesthesia. Next, 1 × 1-cm skin and 1-cm^3 fat tissues from the inguinal region, and 3 ml of tail blood, were taken. Gentamicin 3 mg/kg (Genta; I.E. Ulagay, Istanbul, Turkey) was administered intraperitoneally 15 minutes before and 1 day after surgery for antibiotic prophylaxis.

Keratinocyte Culture

The skin was washed in fetal bovine serum, and any residual fat was trimmed away. Skin samples were placed into petri dishes with the dermal layer side down and treated with collagenase solution at 4°C for 24 hours. Thereafter, the epidermis was scraped from the dermis and incubated in trypsin solution for 30 minutes. The solution was then centrifuged, and cells were taken and cultured in keratinocyte serum-free medium containing 1% penicillin and 10 ng/ml epidermal growth factor at 37°C in 5% carbon dioxide. The culture medium was exchanged for fresh medium once every 3 days, and the cells were grown until 70% confluence.

Passage 1

The medium was removed from the flasks when the cells reached 70% confluence. Next, 10 ml of trypsin/ethylenediaminetetraacetic acid solution was added to the flasks, followed by incubation in an incubator for 5 minutes. After neutralization of the trypsin reaction with 1 ml of fetal bovine serum, the cells were collected in a tube and centrifuged with fetal bovine serum at 400 *g*. Cells were resuspended in fresh complete medium and seeded in flasks.

Passage 2

The medium was removed from the flasks when the cells reached 70% confluence. Cells were trypsinized and resuspended in fresh complete medium. Next, a 1-ml sample was removed for analysis.

Adipose Tissue-Derived Mesenchymal Stem Cell Culture

The fat tissue was washed in fetal bovine serum. The washing step was repeated until all of the blood and connective tissue particles were removed. To facilitate subsequent tissue digestion, the adipose tissue was minced into small pieces and then incubated at 37°C in digest solution (Trypsin EDTA Solution C; Biological Industries, Kibbutz Beit Haemek, Israel). Adipocytes and free oil were separated from the stromovascular components by centrifugation of the resultant digested material at 800 *g*. Cells were suspended and plated into 150-cm^2 culture flasks in Dulbecco's Modified Eagle Medium, Low Glucose (SH3002101; HyClone Laboratories, Inc., Logan, Utah) containing 10% fetal bovine serum (SV3016003; HyClone) and 1% penicillin, and cultured at 37°C in 5% carbon dioxide. The culture medium was exchanged for fresh medium once every 3 days, and the cells were grown to 70% confluence. Passage 1 and passage 2 processes were performed using the procedure described above for keratinocytes.

Cell Labeling

Stem cells were labeled with Qtracker (Thermo Fisher Scientific, Grand Island, N.Y.) cell labeling kits, which designed for loading cells grown in culture with highly fluorescent Qdot nanocrystals. Qdot nanocrystals target the cytoplasm of live cells. Component A and component B were premixed in a tube and incubated for 5 minutes at room temperature. Fresh complete medium was added to the tube and centrifuged for 30 seconds. Cells were then added, incubated at 37°C for 45 to 60 minutes, and washed twice with complete growth medium. Labeled live cells were visualized by flow cytometry using appropriate filters.

Production of Fibrin Scaffold

Peripheral blood in tubes containing citrate was centrifuged at 3000 rpm for 5 minutes, and the plasma was collected. Next, 10 ml of plasma were placed in each well of a six-well plate, followed by 10 mmol of calcium and thrombin (thrombin was generated in silica petri dishes with plasma activation) to generate an inert fibrin matrix, which is hereafter termed Dermoplast (Prestige Brands, Tarrytown, N.Y.).

Preparation of Fibrin Scaffold Containing Keratinocytes

For keratinocyte culture on fibrin scaffold, a 4-cm, 2 × 2-mm fibrin matrix was generated in each well of a six-well plate. Next, 4×10^6 keratinocytes were seeded onto the fibrin matrix and cultured in complete keratinocyte serum-free medium containing 100 IU epidermal growth factor for 24 hours for cell implantation in the matrix. During the 14-day culture, the medium was exchanged for fresh medium every 3 days. On day 14, a biopsy specimen was taken, and the keratinocyte layer was subjected to histopathologic evaluation.

Preparation of Fibrin Scaffold Containing Adipose Tissue-Derived Mesenchymal Stem Cells

A 4-cm, 2 × 2-mm fibrin matrix was generated in each well of a six-well plate. Adipose tissue-derived mesenchymal stem cells were seeded inside and outside of the fibrin matrix with an injector and cultured in complete medium. During the 7 days of culture, the medium was exchanged for fresh medium, and cells were added once every 3 days.

Preparation of Fibrin Scaffold Containing Adipose Tissue-Derived Mesenchymal Stem Cells and Keratinocytes

Adipose tissue-derived mesenchymal stem cells were seeded inside and outside of the fibrin matrix, and the keratinocytes were seeded only outside of the fibrin matrix. The fibrin matrix was cultured in complete medium containing 10% fetal bovine serum and 1% penicillin. On day 3, the culture medium was exchanged for fresh medium containing epidermal growth factor 100 IU and cell seeding was performed again. The culture was terminated on day 7.

Cell Counts and Viability

One hundred microliters of cell suspension was combined with 100 μl of trypan blue working solution and transferred to a hemacytometer chamber. Cells were counted under a microscope at 10 × 40 magnification. Both blue-stained dead cells and total cells were enumerated, and the cell viability rate was calculated.

Characterization of Mesenchymal Stem Cells and Keratinocytes

After passage 2, the cells were incubated with 1 μg of R-phycoerythrin–, fluorescein isothiocyanate–, and allophycocyanin-conjugated antibodies or isotype-matched control immunoglobulin Gs at room temperature for 45 minutes. The samples were analyzed after the incubation and washing steps using a FACSCalibur (BD Biosciences, San Jose, Calif.) flow cytometer with CD45, HLA-DR, CD34, CD90, CD105, and CD73 antibodies for mesenchymal stem cells and with CD44, CD90, CD105, CD200, and K15 antibodies for keratinocytes.

Flow Cytometric Analysis of Labeled Adipose Tissue-Derived Mesenchymal Stem Cells and Keratinocytes

Labeled cells and control unlabeled cells were analyzed using flow cytometry with the appropriate filter (FL2) for red emission. Of the adipose tissue-derived mesenchymal stem cells, 98.7 percent were labeled and 0 percent were not. Of keratinocytes, 98.5 percent were labeled, and 0 percent were not.

Groups

The animals were divided into the following five groups ($n = 6$ each): group 1, no-graft, secondary healing; group 2, fibrin scaffold; group 3, cultured keratinocyte-coated fibrin scaffold; group 4, adipose tissue-derived mesenchymal stem cell–implanted fibrin scaffold; and group 5, adipose tissue-derived mesenchymal stem cell–implanted and keratinocyte-coated fibrin scaffolds.

Surgical Procedures

The dorsum was prepared and scrubbed with povidone-iodine (Batticon 10%; Adeka, Istanbul, Turkey). A 20-mm diameter circular full-thickness skin sample was excised at the center of the dorsal region. Grafts were secured in place with 4-0 polypropylene (Prolene; Ethicon, Inc., Somerville, N.J.) interrupted sutures. The surgical sites were covered with Bactigras (Smith & Nephew, Mississauga, Ontario, Canada). Perioperative views are shown in Figure 1. Animals were observed postoperatively and placed in individual cages. At the end of day 21, the engrafted areas were excised, including the surrounding native skin margins and deep fascia.

Evaluation

Specimens were fixed in 10% formalin solution, dehydrated by irrigation with increased concentrations of alcohol solution, and embedded in paraffin. Next, 5-μm slices were obtained and stained with hematoxylin and eosin, Masson trichrome, pancytokeratin, and CD31. Unstained slices were preserved for fluorescence microscopic evaluation. Next, the slices were deparaffinized and stained with 4′,6-diamidino-2-phenylindole to visualize the nuclei, and visualized using a Nikon ECLIPSE Ni florescence microscope (Nikon Corp., Tokyo, Japan). Photographs were obtained using a Nikon DS-Fi1c. Tissue-vessel ratios and tissue collagen ratios were assessed under a stereomicroscope (Stereoinvestigator 9.0; MicroBrieldField, Colchester, Conn.). The tissue collagen ratios were calculated using the Cavelieri method.

Statistical Analysis

A post hoc Dunn test was used for double comparisons, the Kruskal-Wallis test was used for comparisons of multiple groups, and the Mann-Whitney *U* test was used for comparisons of vessel

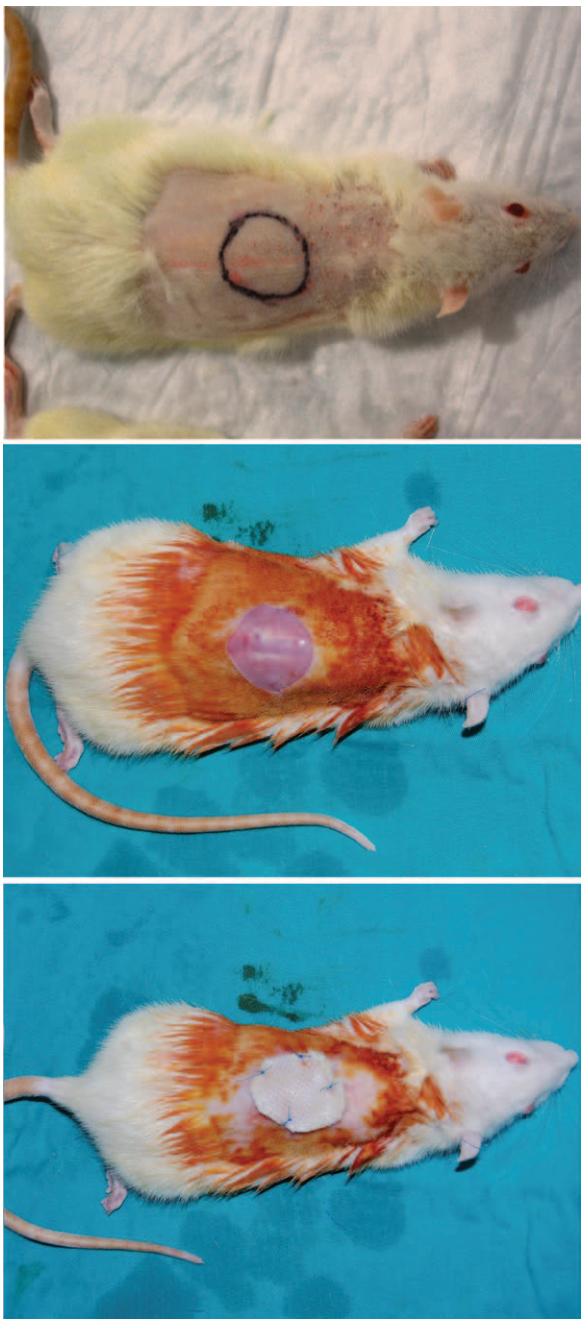

Fig. 1. Perioperative figures. (Above) Marking of the desired defect; (center) the product is applied; and (below) a tie-over dressing was performed.

and collagen ratios. All of the findings were evaluated using IBM SPSS Version 19.0 (IBM Corp., Armonk, N.Y.). A value of $p < 0.05$ was deemed to indicate statistical significance.

RESULTS

All of the 25 rats survived throughout the study, with no infection or tissue reaction. According to the flow cytometric analysis, the percentages of adipose tissue-derived mesenchymal stem cells positive for the following markers were as follows: CD90, 99.3 percent; CD105, 99.8 percent; CD73, 99.7 percent; HLA-DR, 0.8 percent; CD45, 0.2 percent; and CD34, 0.1 percent. In addition, the percentages of keratinocytes positive for the following markers were as follows: CD40, 99.5 percent; CD90, 99.5 percent; CD271, 23.9 percent; CD34, 0.1 percent; CD200, 0.1 percent; cytokeratin, 4.1 percent; nestin, 91 percent; and CD29, 99 percent.

Ulcerated areas were present in the center of the lesions, and severe contracture and less epithelialization were observed in groups 1, 2, and 3. Less contracture, more epithelialization, and minimal ulceration were observed in group 4. Little decrease in size caused by wound contracture and complete epithelialization was observed in group 5. There were no hair follicles on the implants in any of the groups. In groups 4 and 5, the implants were pink and red; the adjacent normal skin was pink and white. Postoperative views are shown in Figure 2.

There was no foreign body reaction or inflammation indicative of tissue rejection detected by light microscopic evaluation of the hematoxylin and eosin–stained specimens; mononuclear cell infiltration was seen only around the suture materials. In all of the experimental groups (2, 3, 4, and 5), the fibrin scaffolds were infiltrated by host inflammatory cells and fibroblasts. In groups 3 and 5, cuboid-formed keratinocytes at the basal layer were transformed into the squamous form at the superficial layer. In addition, keratin deposits were observed at the outermost layer.

No epidermal rete formations at the dermoepidermal junction, hair follicles, or skin appendices (such as apocrine sweat glands and sebaceous glands) were observed, and the epithelium was placed in a flat plane in all specimens. Some spindle cells resembling dense fibroblasts oriented parallel to each other were observed in almost all of the layers of the specimens in groups 4 and 5. These cells were covered with extracellular matrix, which stained positively with trichrome. In groups 1, 2 and 3, epithelialization and keratin formation were detected by cytokeratin staining in areas close to normal skin. Some ulcerated areas were observed in the center of the lesions. Epithelial progression was greater in group 4, and nearly complete epithelialization was found in group 5. Some sorts of keratinization were also present in all layers of the group 4 specimens, and a greater extent of keratinization was evident in group 5. As a conclusion, no epithelization in group 1 and almost full epithelization in group 5 were observed, as shown in Figure 3.

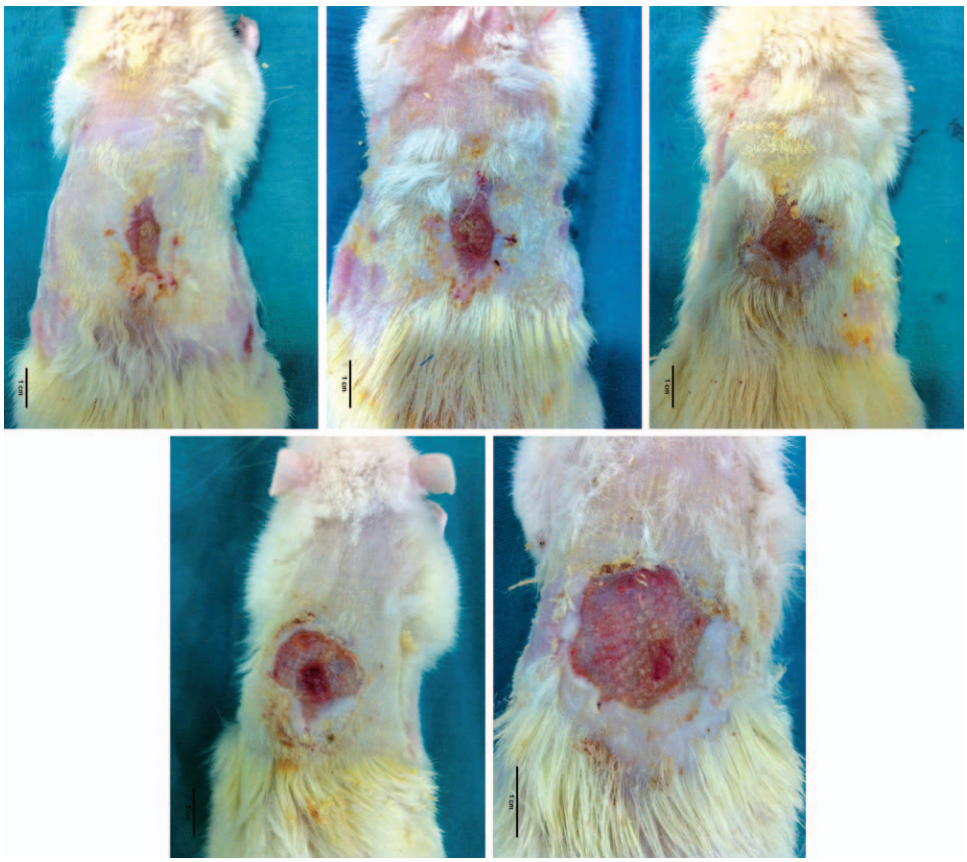

Fig. 2. Postoperative photographs of group 1 (*above, left*), group 2 (*above, center*), group 3 (*above, right*), group 4 (*below, left*), and group 5 (*below, right*).

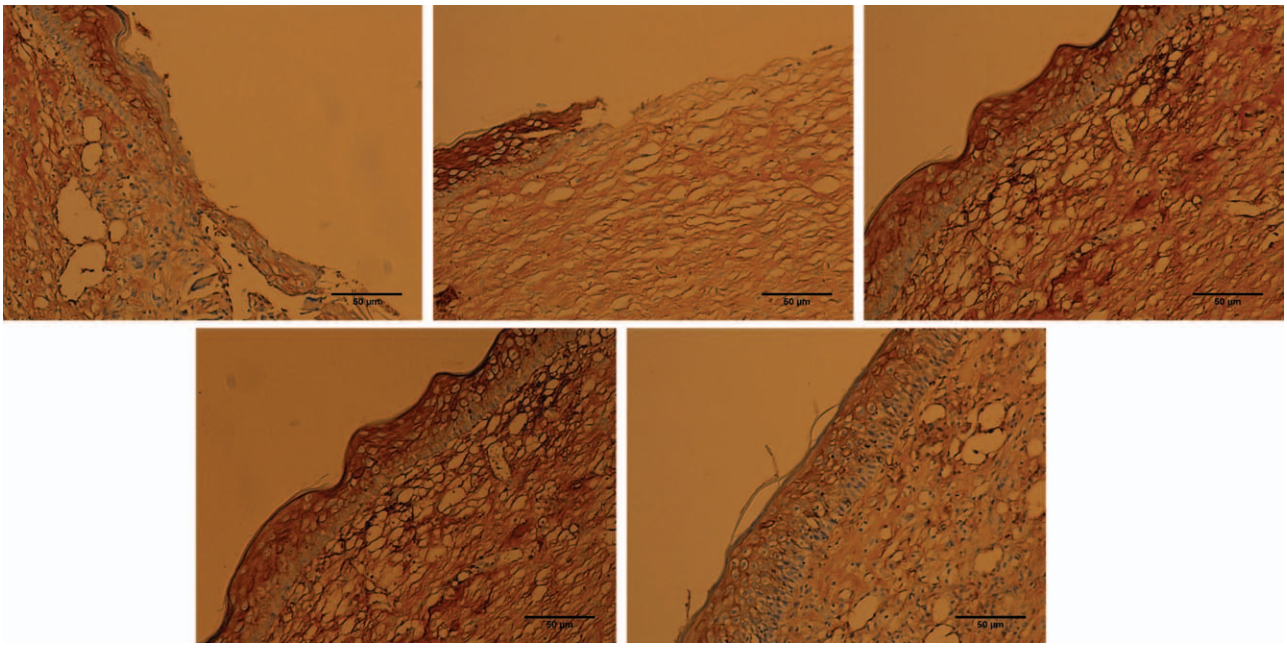

Fig. 3. Pan-cytokeratin staining images of group 1 (*above, left*), group 2 (*above, center*), group 3 (*above, right*), group 4 (*below, left*), and group 5 (*below, right*).

Neovascularization without plexus formation was suggested by CD31 beneath the epithelium and above the fascial layer. The concentration and size of the vascular structure was advanced in groups 4 and 5. Rough, irregularly shaped, simple collagen fibers of a single thin form were found in the specimens, as shown in Figure 4.

Fluorescence Microscopic Evaluation

Nuclei were 4′,6-diamidino-2-phenylindole–positive and cytoplasm was 4′,6-diamidino-2-phenylindole–negative in groups 1, 2, and 3. Nuclei were stained blue with 4′,6-diamidino-2-phenylindole and the cytoplasm was stained red by Qdots in groups 4 and 5. Cells had transformed into endothelial cells and formed vascular structures. Lumen formation occurred also and erythrocytes were found in these lumens. Marked cells were parallel to each other, resembling fibroblasts and spindle cells, and were surrounded by a large extracellular matrix. No skin appendages or cells, such as hair follicles or secretory cells, were observed. These findings are shown in Figures 5 and 6.

Stereoscopic Evaluation

Vascular size differed significantly between the control group and all implanted groups (post hoc Dunn test, $p = 0.007$). Vascular size did not differ significantly between groups 2 and 4 (post hoc Dunn test, $p = 0.223$) or groups 3 and 4 (post hoc Dunn's test, $p = 0.062$). The vascular size measurements of group 5 were significantly greater than those of groups 3 and 2 (post hoc Dunn test, $p = 0.007$ and $p = 0.033$, respectively). The vascular size measurements did not differ significantly between groups 4 and 5 (post hoc Dunn test, $p = 1.0$). The vascular size measurements of groups 4 and 5 were significantly greater than those of groups 2 and 3 (Mann-Whitney test, $p < 0.0001$).

Collagen Volume

The stereoscopic collagen volume measurements did not differ significantly between groups 2 and 4 (post hoc Dunn test, $p = 0.084$) or between groups 3 and 4 (post hoc Dunn test, $p = 0.727$). The stereoscopic collagen volume measurements of group 5 were significantly greater than those of group 3 (post hoc Dunn test, $p = 0.023$). The stereoscopic collagen volume measurements did not differ significantly between groups 4 and 5 (post hoc Dunn test, $p = 1.0$). The stereoscopic collagen volume measurements of groups 4 and 5 were significantly greater than those of groups 2 and 3 (Mann-Whitney test, $p < 0.0001$).

DISCUSSION

Adipose tissue-derived mesenchymal stem cells were first defined in 2001 by Zuk et al.[9] Adipose tissue-derived mesenchymal stem cells are preferable compared with bone marrow–derived

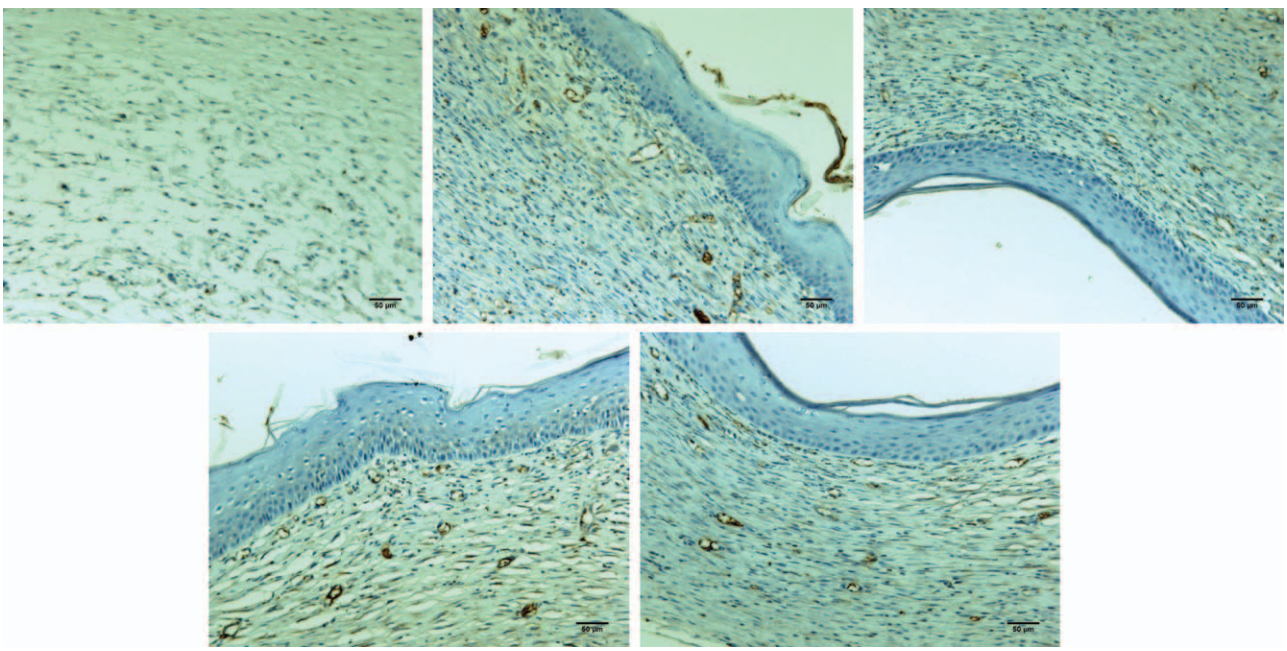

Fig. 4. CD31 staining figures of group 1 (*above, left*), group 2 (*above, center*), group 3 (*above, right*), group 4 (*below, left*), and group 5 (*below, right*).

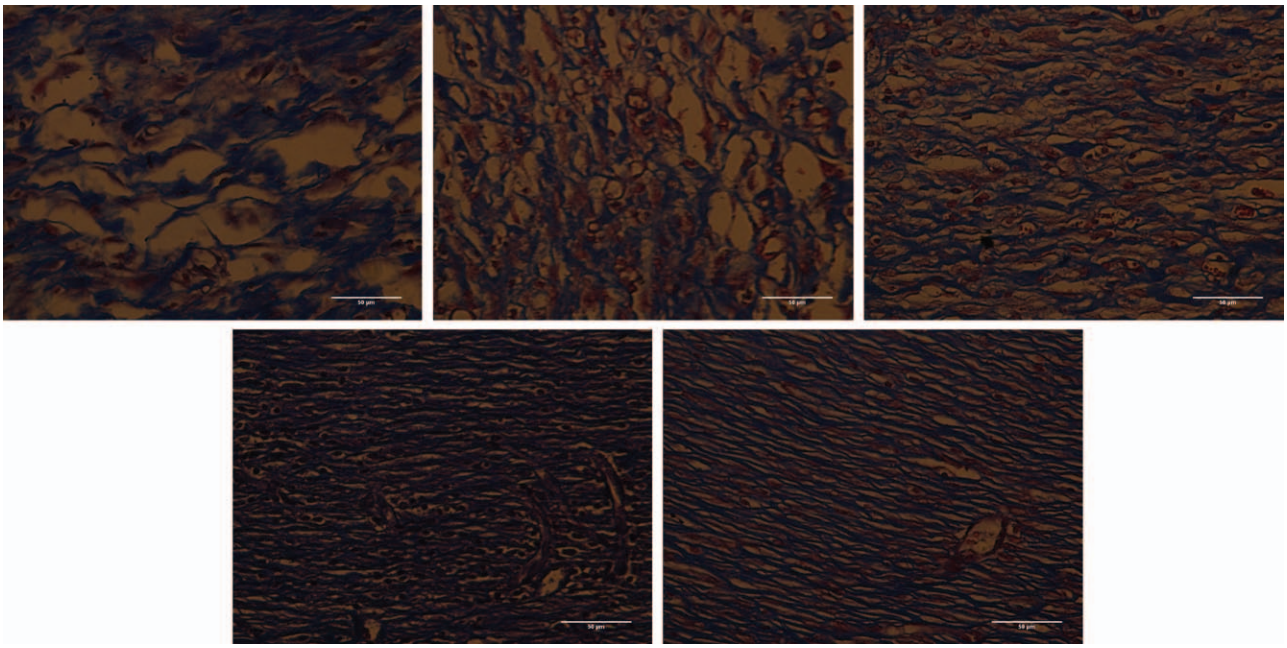

Fig. 5. Masson trichrome staining indicates cell nuclei (*blue*) and cytoplasm (*pink-red*) in group 1 (*above, left*), group 2 (*above, center*), group 3 (*above, right*), group 4 (*below, left*), and group 5 (*below, right*).

stem cells because of their multipotential advantages (i.e., ease of procurement and isolation, and their applicability in the clinic).[4] Adipose tissue-derived mesenchymal stem cells show promise for vein and skin engineering because of their dermal differentiation, vascular regeneration, and angiogenesis capacity.[5–7] Adipose tissue-derived mesenchymal stem cell incorporation into acellular dermal matrixes has been achieved using various tissue scaffolds.[8] Altman et al. demonstrated that implanted stem cells adhere to fibroin-chitosan tissue scaffolds and remain permanent, providing support for regeneration.[10] Similarly, in 2012, Meruane et al. reported that adipose tissue-derived mesenchymal stem cells adhere to the dermal matrix and integrate following implantation in Integra (Integra LifeSciences, Plainsboro, N.J.), a dermal equivalent. The stem cells provided increased microvascular density and collagen synthesis, thus enhancing skin regeneration and tissue integration.[11] The greatest issue with dermis equivalents, which are frequently used in skin tissue engineering, is the necessity of waiting for 2 weeks for production of fibroblasts and vascularization in clinical applications. Within this period, the tissue may become infected or may be destroyed, resulting in an increased bacterial density. Proliferation, migration, and spontaneous differentiation of stem cells within the fibrin tissue scaffold is caused by angiogenesis, which is facilitated by the use of a culture environment appropriate for stem cells and endothelial cells.[12] The three-dimensional fibrin matrix provides sufficient physical support and surface area.[13] The fibrin increases the activities of adipose tissue-derived mesenchymal stem cells in both in vivo and in vitro media.[14,15] Fibrin is a biodegradable autogenous material to which no tissue reaction has been reported. In addition, stem cells are dispersed in three dimensions within the fibrin tissue scaffold, exhibit a fusiform morphology similar to fibroblasts, and form a surrounding extracellular matrix. For tissue regeneration, rapid angiogenesis and preparation of the recipient site are of particular importance. Because fibrin tissue scaffolds enable rapid angiogenesis, they are preferred in skin tissue engineering studies. However, some features of fibrin (e.g., relatively rapid degradation and limited mechanical support) make its use difficult. Some authors have used chemical modification to overcome these disadvantages.[16,17]

Adipose tissue-derived mesenchymal stem cells increase tissue regeneration by strengthening the local microvascular network. This vascular support is mediated by either direct vascular differentiation of the stem cells or by stimulating neoangiogenesis in a paracrine manner.[18,19] The size of the angiogenic effect is directly related to the synergic interaction between tissue scaffold molecules and adipose tissue-derived mesenchymal stem cells.[20] Use of adipose tissue-derived mesenchymal stem cells and acellular dermal matrixes

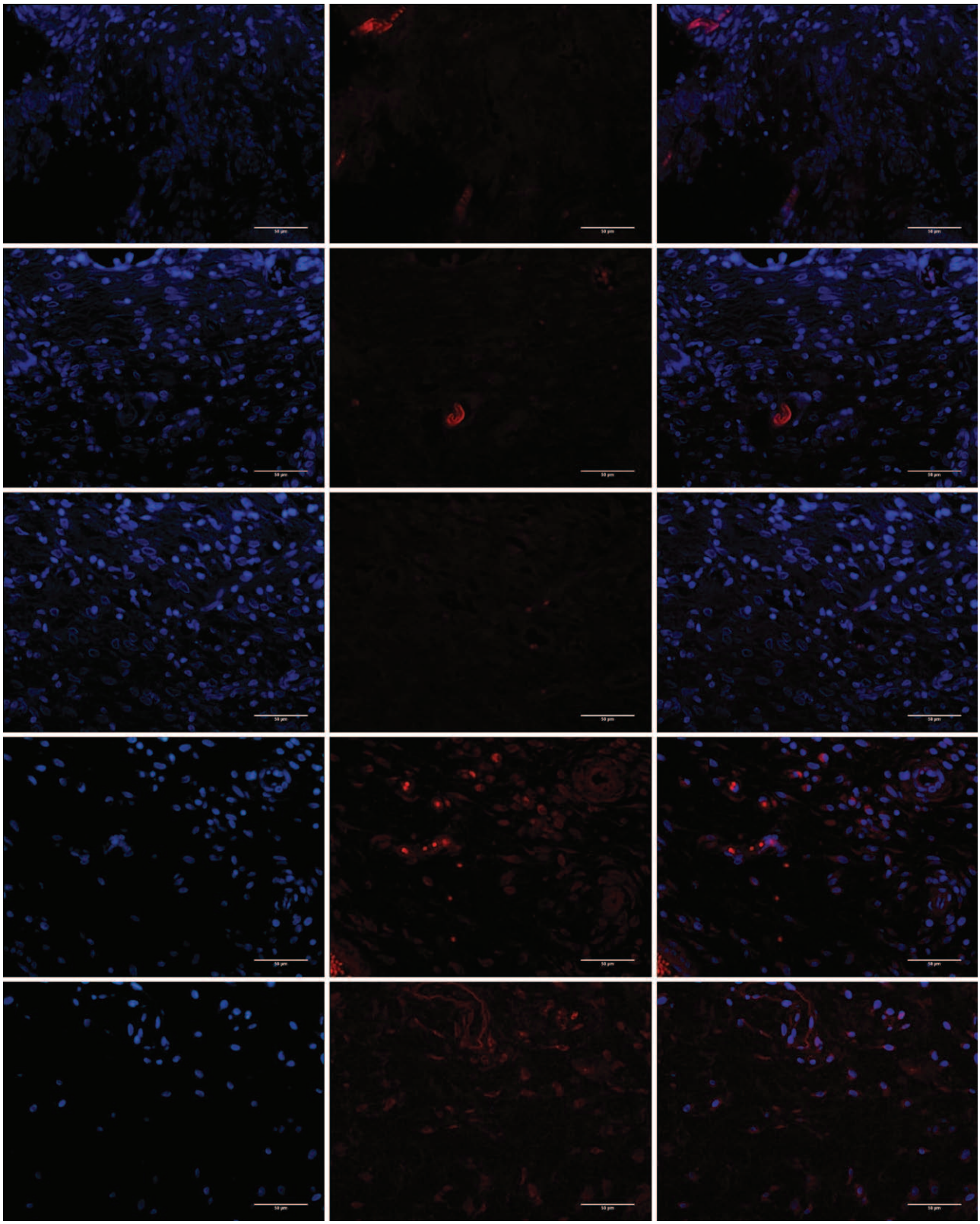

Fig. 6. Blue 4′,6-diamidino-2-phenylindole staining indicates cell nuclei (*left*), and Qdot staining indicates cytoplasm (*center*) and merged views (*right*) of group 1 (*above*), group 2 (*second row*), group 3 (*third row*), group 4 (*fourth row*), and group 5 (*below*).

resulted in increased wound healing significantly after the seventh day postoperatively, and microvascular endothelial proliferation increased significantly after the second week postoperatively. The effects of adipose tissue-derived mesenchymal stem cells on wound healing have been reported to be attributable primarily to paracrine effects rather than differentiation.[9,10] Maharlooei et al. demonstrated that adipose tissue-derived mesenchymal stem cells increase collagen accumulation on scars and contribute to wound healing.[21] In our study, the increase in collagen was greater in the implanted groups compared with the other groups. We believe that this increase was caused by fibroblastic differentiation of adipose tissue-derived mesenchymal stem cells and their paracrine effects.

Adipose tissue-derived mesenchymal stem cells enhance antioxidant effects and scar recovery by stimulating dermal fibroblasts and keratinocytes.[22] Epithelialization from scar edges occurred more rapidly in the adipose tissue-derived mesenchymal stem cell groups, because of the proliferation and migration of keratinocytes.[23–25] Studies of rat bone marrow–derived stem cells reported that these cells can differentiate in vivo into epidermal keratinocytes, sebaceous gland cells, epithelial cells, dendritic cells, and endothelial cells.[26]

Adipose tissue-derived mesenchymal stem cells differentiate into epithelial cells in the presence of *trans*-retinoic acid. Their epithelial cell morphology and expression of cytokeratin-18, an epithelial cell marker, confirm the differentiation of the cells.[27] In our study, the epithelial area in group 5 was shown by fluorescence microscopy. They were likely derived from either keratinocytes marked during culture or epithelial differentiated forms of marked adipose tissue-derived mesenchymal stem cells. No marked keratinocytes were detected by fluorescence microscopy after 21 days of implantation in group 3. The survival ratio of keratinocyte cell cultures has been reported to differ markedly. The ischemia-sensitive keratinocytes may not have survived because of the 2- to 3-mm thickness of the fibrin tissue scaffold. Survival and proliferation of adipose tissue-derived mesenchymal stem cells are enhanced under ischemic conditions. Adipose tissue-derived mesenchymal stem cells accelerate neoangiogenesis and exert an antioxidant effect.[28,29] The different survival rate of, and epidermis formation by, keratinocytes in group 5 compared to those in group 3 was likely attributable to them being less exposed to rapid neoangiogenesis and ischemia. Green fluorescent protein–expressing mesenchymal stem cells originating from fat tissue displayed epithelial differentiation 4 weeks after application, as determined by staining with cytokeratin-19. Staining of green fluorescent protein–expressing stem cells with HSP47, a marker of fibroblastic differentiation, demonstrated their transformation into fibroblasts. In addition, cells positive for smooth muscle actin and von Willebrand factor revealed a neovascular structure and vascular differentiation.[9,13] In the present study, in the dermal parts of the groups 4 and 5, adipose tissue-derived mesenchymal stem cells differentiated into endothelial cells and formed vessels, as determined by their morphologies and formation of blood vessels, the lumen of which contained erythrocytes. Endothelial differentiation occurs spontaneously. This differentiation likely explains the significant difference in vascular volume in the adipose tissue-derived mesenchymal stem cell groups, as determined by CD31 staining. We believe that, in the adipose tissue-derived mesenchymal stem cell groups 4 and 5, the cells of fusiform morphology covered with extracellular matrix, and positioned parallel to each other in the dermal part, formed through fibroblastic differentiation.

Altman et al. reported that adipose tissue-derived mesenchymal stem cell differentiation into endothelial cells and fibroblasts, which are mesodermal-originating cells, occurred 2 weeks later, whereas differentiation into ectodermal structures occurred only after 4 weeks.[10] Our study was terminated on day 21, based on the assumption that collagen synthesis peaked in week 3. At the end of the study, vascular and fibroblastic differentiation, but not differentiation into epidermal and skin appendages, had occurred. The lack of differentiation into skin appendages was possibly caused by termination of the study on day 21. The use of rats is a limitation of the present study. Wound healing and cell proliferation occur more rapidly in rats than in humans,[30] leading to faster contraction of the wound; therefore, acquisition of long-term results is difficult. The improvements in wound healing and stem cell differentiation demonstrated in this work are promising for clinical applications.

CONCLUSIONS

Keratinocyte-coated adipose tissue-derived mesenchymal stem cell–seeded fibrin tissue scaffolds showed promise for skin tissue replacement. Adipose tissue-derived mesenchymal stem cells support skin regeneration with spontaneous differentiation and paracrine effects. The

autogenous nature of the formed skin significantly decreases the risk of a harmful immune response and microbiological contamination. The engineered artificial biological skin shows promise for the treatment of loss of skin tissue.

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ACKNOWLEDGMENT

This study was supported by a grant from the Turkish Ministry of Science and Technology (SanTez Code; 01101.STZ.2011–2).

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