Original Research

An application of embryonic skin cells to repair diabetic skin wound: A wound reparation trail

De Jian Qian[1,2], Xiang Kai Guo[1,3], Hui Chuan Duan[4], Zhi Hua Han[5], Fei Meng[1], Ju Liu[6] and Yan Wang[1]

[1]Department of Plastic and Reconstructive Surgery, Shandong Province Qianfoshan Hospital Affiliated to Shandong University, Jinan, Shandong 250014, China; [2]Department of Emergency Surgery, Shandong Province Qianfoshan Hospital Affiliated to Shandong University, Jinan, Shandong 250014, China; [3]School of Medicine and Life Sciences, University of Jinan-Shandong Academy of Medical Sciences, Jinan, Shandong 250014, China; [4]Department of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai 200011, China; [5]Department of Cardiology, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China; [6]Laboratory of Microvascular Medicine, Medical Research Center, Qianfoshan Hospital Affiliated to Shandong University, Jinan, Shandong, 250014, China

Corresponding authors: Yan Wang. Email: wangyandr@126.com and Ju Liu. Email: jliu0228@gmail.com

Abstract

Cell therapy has shown its power to promote diabetic chronic wound healing. However, problems of scar formation and loss of appendages have not yet been solved. Our study aims to explore the potential of using embryonic skin cells (ESkCs) to repair diabetic wounds. Circular wound was created on the back of the diabetic mice, and ESkCs stained with CM-DIL were transplanted into the wound. Wound area was recorded at the day 4, 7, 11, and 14 after transplantation. The tissue samples were obtained at week 1, 2, and 3, and the tissue sections were stained by transforming growth factor β1 (TGF-β1), TGF-β3, vascular endothelial growth factor (VEGF), and CD31. The new skin formed on the wound of the diabetic mice with ESkC treatment at week 1 but not on the wounds of the non-treatment group. The histological scores of diabetic group with ESkC treatment were significantly better than the non-treatment group ($P < 0.05$). The fluorescence examination of CM-DIL and CD31 staining indicated that the ESkCs participated in the tissue regeneration, hair follicles formation, and angiogenesis. The expression of TGF-β1 and VEGF in ESkC-treated groups was noticeable in week 1 but disappeared in week 2. TGF-β3 was not expressed at week 1 but expressed markedly around hair follicles in week 2 in ESkC-treated groups. Our study demonstrated that ESkCs are capable of developing new skin with appendage restoration to repair the diabetic wounds.

Keywords: Embryonic skin cells, skin regeneration, TGF-β, diabetic wound

***Experimental Biology and Medicine* 2014; 239: 1630–1637. DOI: 10.1177/1535370214542067**

Introduction

The healing of a wound needs a well-orchestrated combination of biological events of cell migration, proliferation, and extracellular matrix (ECM) deposition.[1] However, diabetic wounds do not heal at normal stages.[2] About 15% of the 150 million people with diabetes worldwide suffer from foot ulcerations, which often become nonhealing chronic wounds.[3] The healing impairment is caused by lack of normal cellular or molecular signals involved in the steps of wound closure process, such as angiogenesis, granulation tissue formation, and epithelialization.[1] Over the past decades, little improvement has been made in reducing the morbidity and disability from chronic wounds.[3] The most effective treatment for chronic wounds only achieves a 50% healing rate, which is often temporary.[4]

Cell therapy brings the hope to improve the treatment of diabetic coetaneous wounds.[5] The embryonic stem cells (ESCs) have been applied to enhance diabetic wound healing.[6] ESCs decreased the time of wound closure by promoting the expression of the growth factors of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF).[6] However, the ESCs may develop into a teratoma *in vivo*.[7] Several lineage-restricted stem cells, including bone marrow-derived mesenchymal stromal cells (BMSCs) and adipose tissue-derived stromal cells (ATSC), are capable of restoring the wound healing rate via different diabetic models.[8] Systemically or locally utilized BMSCs can promote healing of full-skin wounds in diabetic rats.[9] BMSCs increased the collagen level and the expression of growth factors such as transforming growth factor β

(TGF-β) and keratinocyte growth factor (KGF), which are critical to tissue repair.[9] ATSCs accelerate the healing of ischemic wounds in diabetic nude mice via a paracrine mechanism by the secretion of VEGF, EGF, hepatocyte growth factor (HGF), and basic fibroblast growth factor (FGF-2).[10,11] Though these stem cells are effective in the healing of cutaneous wounds, the results are not optimal due to scar formation and the absence of normal appendages.[4,12]

The skin with hair follicles of mouse embryos is formed at E17.5.[13] The embryonic skin consists of three layers, including of epidermis, dermis, and hypodermis, which develop from different embryonic origins. The epidermis of mouse originated from ectoderm after gastrulation.[13] The stratification of the ectoderm is induced by mesenchymal cells to generate the epidermis and also contribute to commitment of the several appendages.[14,15] The cells in the epidermis include keratinocytes (95%), melanocytes, Langerhans cells, and Merkel cells. The dermis, which has multiple embryonic origins,[16] consists of fibroblasts, dendritic cells, macrophages/monocytes, neutrophils, and lymphocytes.[17] The hypodermis is composed of adipose tissue which is molded to muscles and bones underlying the skin.[14] The growth factors, cytokines, ECM components, and biomechanical stress in fetal regenerative healing are tightly regulated in ECM of embryonic skin.[18] In contrast to wound repair in the adults, the tissues of the mammalian embryos tend to heal scarlessly before the third trimester of pregnancy.[19] In addition, the embryonic epidermal cells and dermal cells have the potential ability to form skin appendages *in vitro*.[20] Therefore, we hypothesize that embryonic skin cells (ESkCs) could form new skin with the hair follicles restoration to repair diabetic wound.

Our study aims to use ESkCs to repair diabetic skin wounds. We developed a mouse model of wound healing to study ESkC therapy for diabetic mice. The ESkCs extracted from the E17.5 embryos and labeled by CM-DIL were transplanted into the wound. The progress of wound healing was examined at different time points. The epidermal regeneration, angiogenesis, and granulation tissue of the wound were evaluated by using the histological scoring system. For detecting the healing mechanism of ESkCs, TGF-β1, TGF-β3, VEGF, and CD31 in the regenerated tissues were examined by immunostaining. Our study demonstrated that ESkCs repair the diabetic wounds by the formation of new skin with appendage restoration.

Materials and methods

Animal model

This study was approved by the local ethics committee (Shanghai Jiao Tong University School of Medicine, China). Eight-week-old male C57BL/6 mice were purchased from SLAC National Rodent Laboratory Animal Resources (Shanghai, China). These mice were divided into four groups: the untreated normal control group (Nor), the untreated diabetic control group (DB), the treated normal group (Nor-T), and the treated diabetic group (DB-T). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ; Sigma, USA; 150 mg/kg). Animal diabetes was confirmed by plasma glucose level >300 mg/dL 1 week after STZ injection. One full-thickness circular wound of 0.9 cm in diameter was created on the back of the mice. The dorsal skin tissues were obtained from E17.5 mouse embryos and digested into single cells suspension. The cells were labeled with fluorescent Cell Tracer CM-DiI (Invitrogen) as previously described.[21] For the Nor-T group and DB-T group, ESkCs were transplanted into wounds with a chamber covered on top of the wound. After planting the cells, antibiotic-gel-tampons were sealed around the chamber to prevent infection (Figure 1). The chamber was removed at week 1 after transplantation.

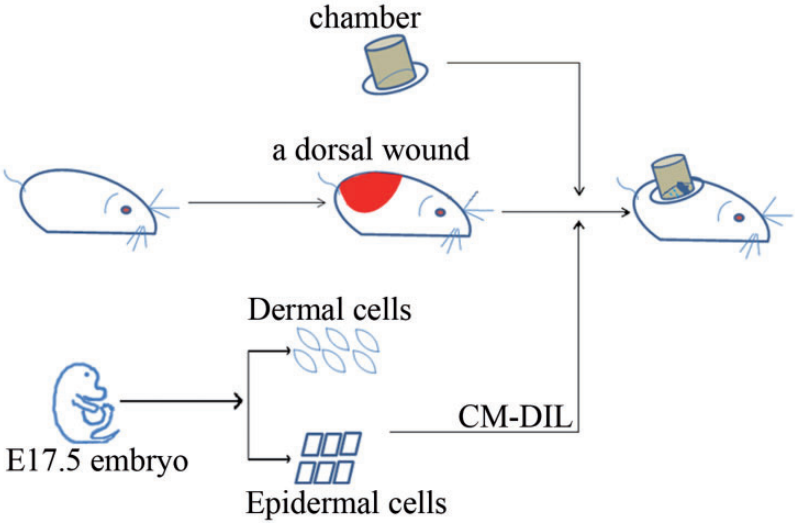

Figure 1 Illustration of the mice model of ESkC treatment. The full-thickness wounds were created by sterile scissors on the back, and a chamber was fixed on the wound. The embryonic skin cells were dissociated from embryos, labeled by CM-DIL, and implanted into the chamber on the wound. (A color version of this figure is available in the online journal)

Wound closure analyses

A digital camera was used to record images of the wound area of the mice at days 0, 4, 7, 11, and 14 post-transplantation. The wound area was measured by Image-Pro Plus Software (version 5.0; Media Cybernetics LP, Silver Spring, MD, USA). Wound healing rates were calculated as: (original wound area − actual wound area)/original wound area × 100%. The Nor-T group and DB-T group were recorded at week 1, 2, and 3 after the operation using the same camera. The wound areas included 2 mm of surrounding normal skin were obtained at 1, 2, and 3 weeks after transplantation.

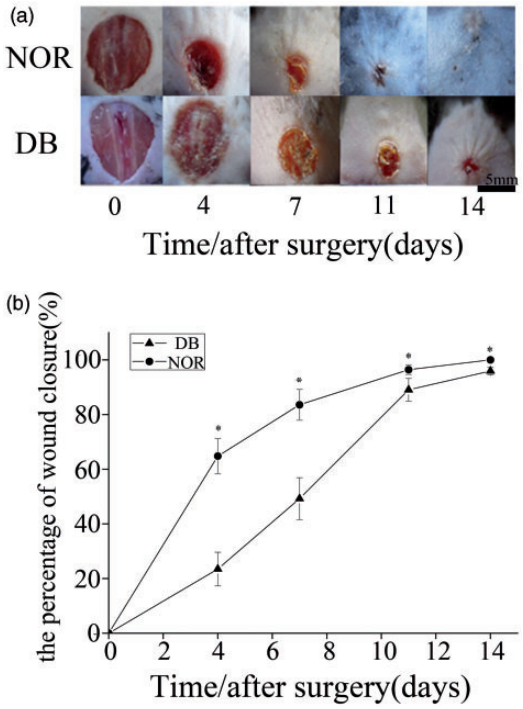

Figure 2 The progress of wound healing of the mice in the Nor and DB groups. (a) Wound area on the mice of the Nor and DB groups at different time points after transplantation. Black bar: 5 mm. (b) The percentage of wound closure of the mice of the Nor and DB groups at different time points after transplantation. $n = 5$; *, $P < 0.05$. (A color version of this figure is available in the online journal)

Immunological staining

For immunohistochemical staining, tissue sections treated with 3% H_2O_2 for 10 min to inactivate endogenous peroxidases. Slides were then washed with PBS (pH 7.2–7.6) twice. Nonspecific binding sites were blocked with 5% BSA in Tris-buffered saline (TBS) for 20 min. Sections were incubated with primary antibodies TGF-β1 (Abcam, 1:200), TGF-β3 (Abcam, 1:200), VEGF (Abcam 1:200), or CD31 (BD, 1:500) at 4°C overnight. After washing, the slides were incubated with HRP-conjugated secondary antibody for 20 min at room temperature. The specific antibody interaction was visualized by incubation with 3,39-diaminobenzidine-H_2O_2 solution, and then the slides were counterstained with hematoxylin for 1 min. The slides stained with CD31 antibody were incubated by FITC-conjugated secondary antibody for 20 min at room temperature and stained with DAPI. The images were photographed by Nikon microscope.

Histological evaluation

The evaluation was performed as described previously.[22] Briefly, all slides were examined by three pathologists without knowledge of the previous treatment, using masked slides under the microscope at 40–200 magnifications. The parameters measured were epidermal and dermal regeneration, granulation tissue thickness, and angiogenesis.

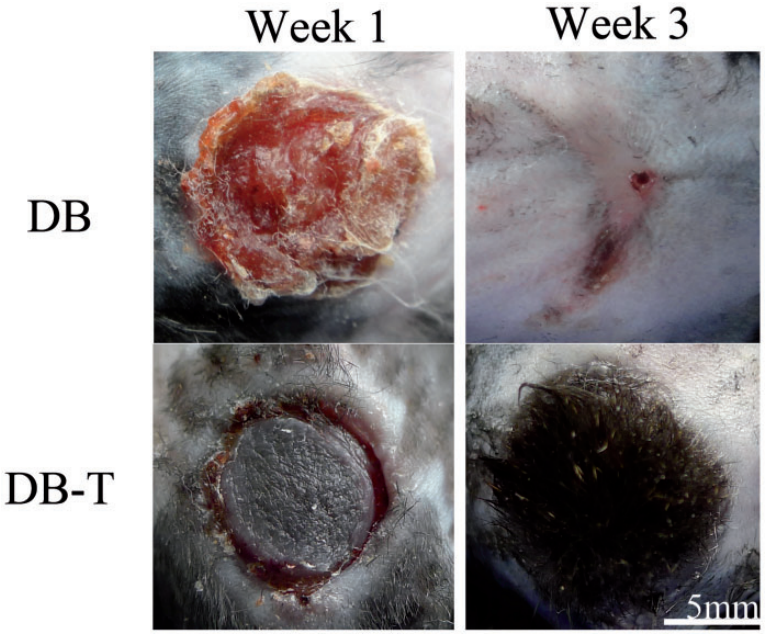

Figure 3 The progress of wound healing of the mice of the DB and DB-T groups. Wound area on the mice of the DB and DB-T groups at week 1 and 3 after transplantation. Black bar: 5 mm. (A color version of this figure is available in the online journal)

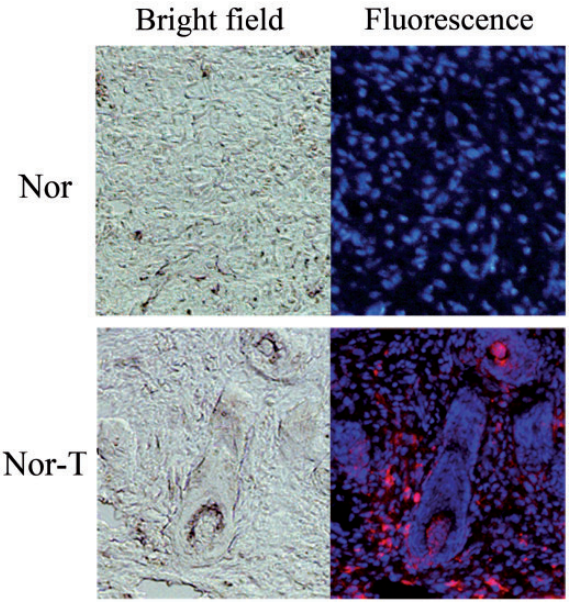

Figure 4 The ESkCs in the regenerated skin tissue. The sections of regenerated skin tissue from treated and non-treated normal mice were observed under bright field and fluorescence. ESkC-derived cells showed red fluorescence. (A color version of this figure is available in the online journal)

The margins of the wound in each of the sections, as well as normal control tissues, were used as comparisons for scoring (Supplementary Table 1). Concerning angiogenesis, only mature vessels containing erythrocytes were counted. To differentiate well formed from poorly formed capillaries, the following parameters were considered: presence or absence of edema, congestion, hemorrhage, thrombosis, and intravascular or intervascular fibrin formation.

Statistical analysis

Data were expressed as mean ± standard deviation. A two-way analysis of variance was used to determine the statistical significance between groups, and a value of $P < 0.05$ was considered statistically significant.

Results

Transplantation of ESkCs-enhanced wound closure and the formation of skin appendages in diabetic mice

At day 4, 7, and 11 after transplantation, the wound closure rate of the Nor group was significantly higher than that of the DB group (day 4:0.65 ± 0.06 vs. 0.23 ± 0.06; day 7: 0.83 ± 0.05 vs. 0.49 ± 0.07; day 11: 0.96 ± 0.017 vs. 0.89 ± 0.04, Nor group vs. DB group, $P < 0.05$) (Figure 2). On day 14, all the wounds in the Nor group had completely healed, but wounds in the DB group had not closed completely (1 vs. 0.96 ± 0.01, Nor group vs. DB group, $P < 0.05$). This confirmed that diabetes caused the delay of wound healing. With ESkC transplantation, new skin covered the wound in DB-T group at week 1 (Figure 3). A small split was present between the regenerative skin and the host skin. At this time point, the wounds from DB group were covered by crusta. At week 3, the hair appeared on the regenerative skin of the DB-T group. The wounds of DB group were closed completely at week 3 with the formation of scar and shrinkage of surrounding tissues (Figure 3). The hair was observed in the regenerative skin of both ESkC-treated groups but not in the two untreated groups (Figure 3). The sections of regenerative skin tissue showed ESkC-derived cells, which labeled by CM-DIL and displayed the red fluorescence, participated in skin regeneration (Figure 4). These cells with red fluorescence were found in the hair papilla and spread over the dermal. Taken together, ESkC transplantation accelerated the diabetic wounds closure with the formation of functional skin.

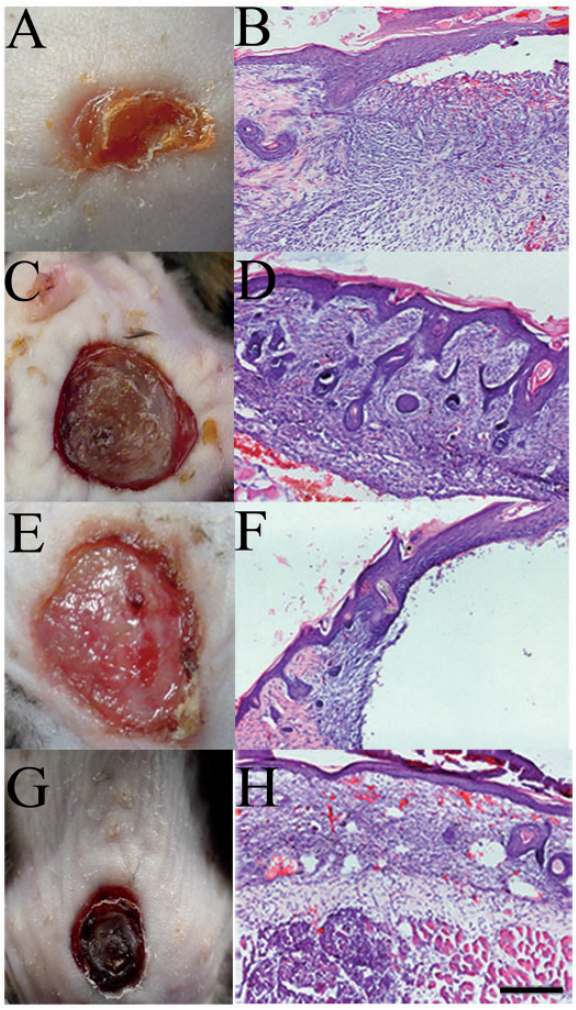

Figure 5 The wound area and histology of the wounds at week 1 after transplantation. The wounds from mice of DB-T group (g and h) and Nor-T (c and d) showed the coverage of the regenerative skin and the histology showed the formation of regenerative skin. Crusta covered on the wounds of both group DB (e and f) and Nor (a and b). The wounds from the mice of DB group showed a slim epidermis and granulation tissue (f). Black bar: 1000 μm. (A color version of this figure is available in the online journal)

Skin regeneration is improved in diabetic mice with transplantation of ESkCs

We evaluated wound healing with histological score system (Supplementary Table 1). The histological results of epidermis and dermis regeneration, granulation tissue thickness,

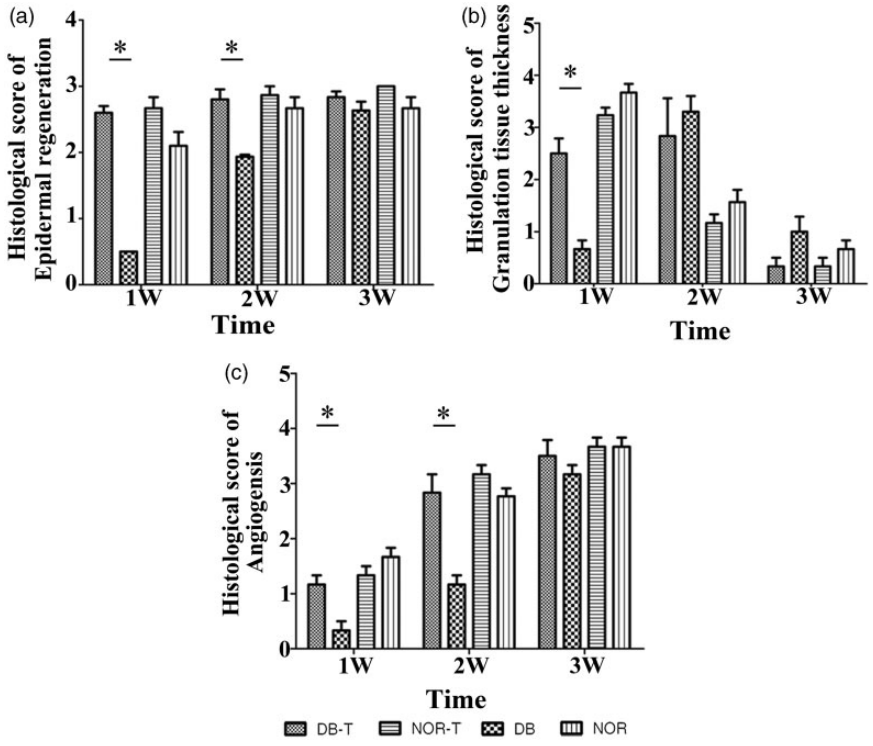

Figure 6 Histological analyses of wound healing. (a) Analysis of histological score of epidermal regeneration. $n = 9$; *, $P < 0.05$. (b) Analysis of histological score of granulation tissue. $n = 9$; *, $P < 0.05$. (c) Analysis of histological score of angiogenesis. $n = 9$; *, $P < 0.05$

and newly formed capillary vessels are shown in Figure 5. The histological scores of epidermis regeneration (Figure 6(a)) of the DB-T group was significantly improved than the DB group at week 1 and 2 ($P < 0.05$). The histological scores of granulation tissue thickness (Figure 6(b)) of the DB-T group was significantly greater than that of the DB group at week 1 ($P < 0.05$). In addition, the histological scores of angiogenesis (Figure 6(c)) parameters of the DB-T group was significantly higher than the DB group at week 1 and 2 ($P < 0.05$). At week 1, new capillaries were observed in the healing wound in the DB-T group and Nor-T group as shown by CD 31 immunofluorescence staining (Figure 7). Some capillaries also showed red fluorescence (Figure 7), suggesting that ESkCs can differentiate into endothelial cells and participate in neovascularization during wound healing.

ESkCs modified the microenvironment of wound healing tissue

The expression and secretion of TGF-β1 and TGF-β3 during the wound healing was examined by immunohistochemistry. A strong TGF-β1 staining was detected in DB-T group and Nor-T group in the tissue at week 1 (Figure 8(a) and (c)). TGF-β1 positive staining was located in the area of regenerative skin tissue of both treatment groups. At week 2, TGF-β1 expression was decreased and almost disappeared in the dermal and epidermal of the both treated groups (Figure 8(b) and (d)). On the sections of regenerative tissues from week 1, no positive TGF-β3 staining was observed in DB-T group and Nor-T group (Figure 8(e) and (g)). However, a strong expression of TGF-β3 was found on the tissue sections of week 2 in both treated groups, especially on the tissues around the hair follicle structures and epidermis (Figure 8(f) and (h)).

VEGF is an important angiogenic cytokine to promote neovascularization in wound healing. A weak expression of VEGF was observed in both of DB-T group and Nor-T group at week 1 after transplantation (Figure 8(i) and (k)). At week 2, VEGF expressed strongly in the epidermal and hair follicles of the sections from the both treated groups (Figure 8(j) and (l)). Thus, VEGF expression is increasing during the wound healing process in ESkC-treated mice.

Discussion

Diabetic skin wound is a serious medical problem which demands for more effective treatment methods. In this study, we explored the potential to use ESkC to repair diabetic wound. The ESkC transplantation accelerated the wound closure and formed new skin with regeneration of hair follicles. The fluorescence examined demonstrated that the ESkCs participates in skin formation and can differentiate into endothelial cells. TGF-β1, TGF-β3, and VEGF were expressed in the repaired skin at different time points. These data support the potential of using ESkC for treatment of diabetic wounds.

The mouse embryo skin starts to develop from an undifferentiated monolayer of epithelial cells at E14.5.[23] On E17.5–18.5, signals regulate epithelial cells to penetrate into hair germ and primary follicles are developed.[24] The embryonic skin wounds could be healed without scars and

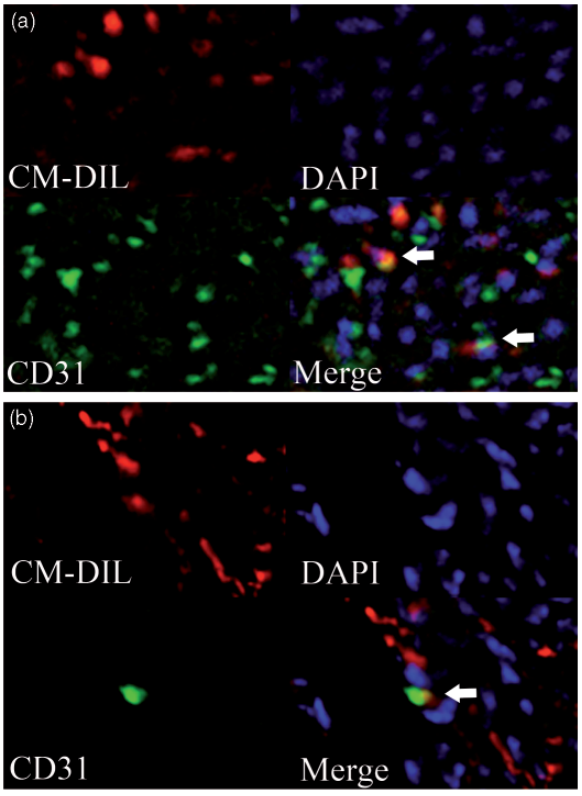

Figure 7 Endothelial cells in the regenerated tissue. CD31 immunostaining on the sections of regenerated tissues of the mice from Nor-T groups (a) and DB-T groups (b) at week 1 after transplantation (blue, DAPI; red, CM-Dil; green, CD31). Arrows refer to the cells with both red and green fluorescences, suggesting that some ESkCs differentiated into endothelial cells. (A color version of this figure is available in the online journal)

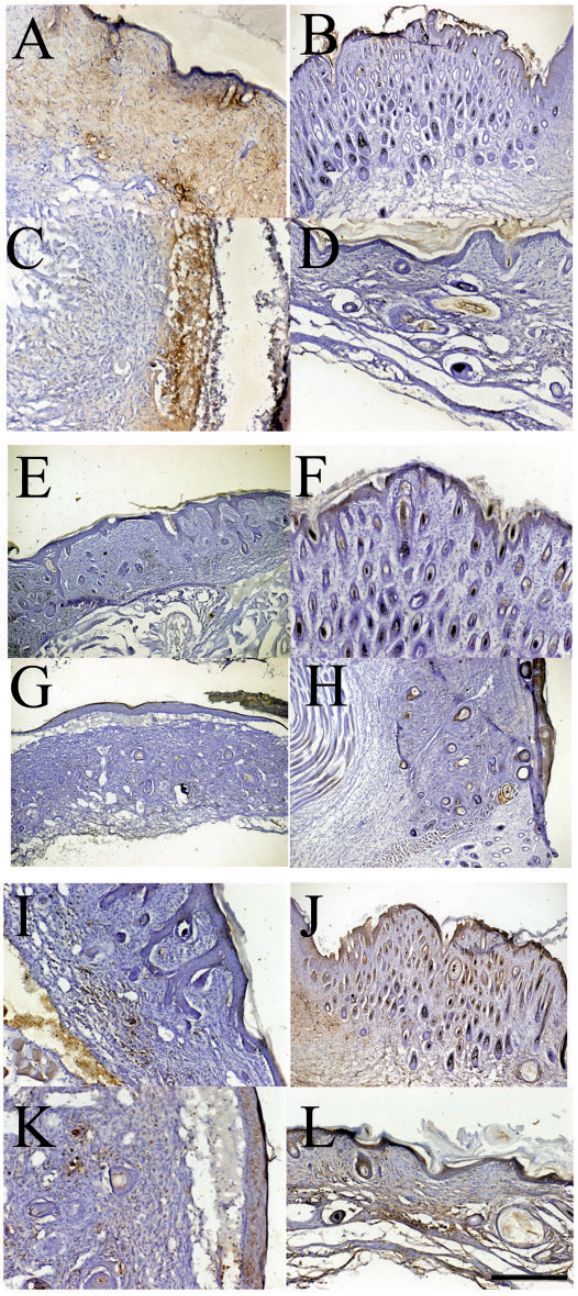

Figure 8 The expression of TGF-β1, TGF-β3, and VEGF in regenerated tissues from ESkC-treated mice. The expression of TGF-β1 was present in tissues from Nor-T (a) and DB-T (c) groups at week 1 after transplantation and was absent in both groups at week 2 (b and d). TGF-β3 is expressed at low level in Nor-T (e) and DB-T (g) groups at week 1, and the expression increased remarkably in both groups at week 2 (f and h). The expression of VEGF in Nor-T (i) and DB-T (k) groups was weak at week 1 and increased significantly in both groups (j and l) at week 2. Black bar: 50 μm. (A color version of this figure is available in the online journal)

with restored appendages.[25,26] The current study used scattered ESkCs to close the diabetic wound and regenerate new skin. In addition to improve the wound closure, ESkC treatment helps to form the scarless skin and the hair follicles. Therefore, ESkC therapy is effective to treat diabetic wound or other chronic wounds. Compared with the skin substitutes and other seed cells, ESkC offers several advantages: first, the ESkC could not develop neoplasia such as teratoma developed from ESCs[7]; second, unlike BSMC or other seed cells, ESkCs do not need extra cytokine induction to form the new skin; and third, in addition to close the wound, ESkCs restore the skin appendages in the new skin.

Angiogenesis is a critical part in the wound healing process. In this study, the quantity of angiogenesis in the ESkC-treated group was significantly higher than that of the non-treatment group. ESkCs may secret pro-angiogenesis factors to stimulate pre-existing vessels to grow into newly formed skin. In this study, the expression of VEGF gradually increased during the first two weeks of the formation of new skin in ESkC-treated normal and diabetic mice. The diabetic wound was lack of the expression of VEGF.[27] The BMSC[28] and ADSC[29] could increase the VEGF level in diabetic wound, so that promote the diabetic wound healing. ATSCs also promote wound healing by paracrine of EGF or HGF.[10,11] Although BMSCs and ADSCs promote wound healing, they do not participate in new skin regeneration.[30] In this study, we found that some ESkCs differentiated into endothelial cells, thus may directly participate in the formation of new blood vessels.

During the diabetic wound healing process, TGF-β1, which plays an important role in cell proliferation, is

reduced in the wounded tissue.[31] Several cytokines, such as VEGF, EGF, PDGF, and IGF which are controlled by TGF-β1, are also reduced in diabetic wounds.[32,33] The wound closure is adjusted by both of TGF-β1 and TGF-β3.[33] The distribution of TGF-β1 and TGF-β3 is associated with progress of the skin wound healing.[34] TGF-β3 is prevalent in nonscarring healing of embryonic skin.[34] Knockdown of TGF-β1 or TGF-β2 with neutralizing antibodies or application of exogenous TGF-β3 promoted scar-free healing.[35] According to our results, the expression of TGF-β1 of both treatment groups peaked at week1; but the expression of TGF-β3 was increased at week 2. This might implicate the coordination of the two TGF-β isoforms to promote wound healing. TGF-β1 promotes cells proliferation, which is necessary at the early stage of the healing process, while TGF-β3 regulates cells differentiation, which is important for tissue remodeling and formation of appendages.[34] The ESkC might maintain the balance of TGF-β isoforms to promote the formation of regenerative skin and wound closure of diabetic mice.

To summarize, ESkC improved diabetic skin wound repair without scars formation and induced generation of hair follicles in diabetic mice. ESkC therapy may provide a new approach to improve the treatment of diabetic skin wounds and other chronic wounds.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data, and review of the manuscript. YW and JL conceived and designed the experiment; XKG, DJQ, and HCD performed the experiment; XKG and MF analyzed the data; XKG, DJQ, HCD, and ZHH reviewed and interpreted the results; XKG and DJQ wrote the paper; and YW and JL integrated and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

This work is supported by Technology Developing Project of Shandong Province (2011GSF11839), Natural Science Funds of Shandong Province (ZR2009CQ001), and Health & Medicine Technology Development Program of Shandong Province (2009QW016). We are grateful for the support from Shandong Taishan Scholarship (JL).

REFERENCES

1. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet* 2005;**366**:1736–43
2. Mustoe TA, O'Shaughnessy K, Kloeters O. Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis. *Plast Reconstr Surg* 2006;**117**:35S–41S
3. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005;**366**:1719–24
4. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 2007;**25**:2648–59
5. Volarevic V, Arsenijevic N, Lukic ML, Stojkovic M. Concise review: mesenchymal stem cell treatment of the complications of diabetes mellitus. *Stem Cells* 2011;**29**:5–10
6. Lee KB, Choi J, Cho SB, Chung JY, Moon ES, Kim NS, Han HJ. Topical embryonic stem cells enhance wound healing in diabetic rats. *J Orthop Res* 2011;**29**:1554–62
7. Aldahmash A, Atteya M, Elsafadi M, Al-Nbaheen M, Al-Mubarak HA, Vishnubalaji R, Al-Roalle A, Al-Harbi S, Manikandan M, Matthaei KI, Mahmood A. Teratoma formation in immunocompetent mice after syngeneic and allogeneic implantation of germline capable mouse embryonic stem cells. *Asian Pac J Cancer Prev* 2013;**14**:5705–11
8. Nambu M, Kishimoto S, Nakamura S, Mizuno H, Yanagibayashi S, Yamamoto N, Azuma R, Nakamura S, Kiyosawa T, Ishihara M, Kanatani Y. Accelerated wound healing in healing-impaired db/db mice by autologous adipose tissue-derived stromal cells combined with atelocollagen matrix. *Ann Plast Surg* 2009;**62**:317–21
9. Kwon DS, Gao X, Liu YB, Dulchavsky DS, Danyluk AL, Bansal M, Chopp M, McIntosh K, Arbab AS, Dulchavsky SA, Gautam SC. Treatment with bone marrow-derived stromal cells accelerates wound healing in diabetic rats. *Int Wound J* 2008;**5**:453–63
10. Nie C, Yang D, Xu J, Si Z, Jin X, Zhang J. Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. *Cell Transplant* 2011;**20**:205–16
11. Kim EK, Li G, Lee TJ, Hong JP. The effect of human adipose-derived stem cells on healing of ischemic wounds in a diabetic nude mouse model. *Plast Reconstr Surg* 2011;**128**:387–94
12. Lee SH, Lee JH, Cho KH. Effects of human adipose-derived stem cells on cutaneous wound healing in nude mice. *Ann Dermatol* 2011;**23**:150–5
13. Forni MF, Trombetta-Lima M, Sogayar MC. Stem cells in embryonic skin development. *Biol Res* 2012;**45**:215–22
14. Koster MI, Roop DR. Mechanisms regulating epithelial stratification. *Annu Rev Cell Dev Biol* 2007;**23**:93–113
15. Millar SE, Willert K, Salinas PC, Roelink H, Nusse R, Sussman DJ, Barsh GS. WNT signaling in the control of hair growth and structure. *Dev Biol* 1999;**207**:133–49
16. Driskell RR, Clavel C, Rendl M, Watt FM. Hair follicle dermal papilla cells at a glance. *J Cell Sci* 2011;**124**:1179–82
17. Descargues P, Deraison C, Prost C, Fraitag S, Mazereeuw-Hautier J, D'Alessio M, Ishida-Yamamoto A, Bodemer C, Zambruno G, Hovnanian A. Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. *J Invest Dermatol* 2006;**126**:1622–32
18. Leung A, Crombleholme TM, Keswani SG. Fetal wound healing: implications for minimal scar formation. *Curr Opin Pediatr* 2012;**24**:371–8
19. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature* 2008;**453**:314–21
20. Toyoshima KE, Asakawa K, Ishibashi N, Toki H, Ogawa M, Hasegawa T, Irie T, Tachikawa T, Sato A, Takeda A, Tsuji T. Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. *Nat Commun* 2012;**3**:784
21. Ferrari A, Hannouche D, Oudina K, Bourguignon M, Meunier A, Sedel L, Petite H. In vivo tracking of bone marrow fibroblasts with fluorescent carbocyanine dye. *J Biomed Mater Res* 2001;**56**:361–7
22. Altavilla D, Saitta A, Cucinotta D, Galeano M, Deodato B, Colonna M, Torre V, Russo G, Sardella A, Urna G, Campo GM, Cavallari V, Squadrito G, Squadrito F. Inhibition of lipid peroxidation restores impaired vascular endothelial growth factor expression and stimulates wound healing and angiogenesis in the genetically diabetic mouse. *Diabetes* 2001;**50**:667–74
23. Fuchs E. Scratching the surface of skin development. *Nature* 2007;**445**:834–42
24. Sennett R, Rendl M. Mesenchymal-epithelial interactions during hair follicle morphogenesis and cycling. *Semin Cell Dev Biol* 2012;**23**:917–27
25. Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. *Plast Reconstr Surg* 2010;**126**:1172–80
26. Namazi MR, Fallahzadeh MK, Schwartz RA. Strategies for prevention of scars: what can we learn from fetal skin? *Int J Dermatol* 2011;**50**:85–93
27. Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, Bunting S, Steinmetz HG, Gurtner GC. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased

angiogenesis and by mobilizing and recruiting bone marrow-derived cells. *Am J Pathol* 2004;**164**:1935–47
28. Kuo YR, Wang CT, Cheng JT, Wang FS, Chiang YC, Wang CJ. Bone marrow-derived mesenchymal stem cells enhanced diabetic wound healing through recruitment of tissue regeneration in a rat model of streptozotocin-induced diabetes. *Plast Reconstr Surg* 2011;**128**:872–80
29. Maharlooei MK, Bagheri M, Solhjou Z, Jahromi BM, Akrami M, Rohani L, Monabati A, Noorafshan A, Omrani GR. Adipose tissue derived mesenchymal stem cell (AD-MSC) promotes skin wound healing in diabetic rats. *Diabetes Res Clin Pract* 2011;**93**:228–34
30. Badillo AT, Redden RA, Zhang L, Doolin EJ, Liechty KW. Treatment of diabetic wounds with fetal murine mesenchymal stromal cells enhances wound closure. *Cell Tissue Res* 2007;**329**:301–11
31. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 1993;**122**:103–11
32. Frank S, Hubner G, Breier G, Longaker MT, Greenhalgh DG, Werner S. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. *J Biol Chem* 1995;**270**:12607–13
33. Frank S, Madlener M, Werner S. Transforming growth factors beta1, beta2, and beta3 and their receptors are differentially regulated during normal and impaired wound healing. *J Biol Chem* 1996;**271**:10188–93
34. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 1995;**108**(Part 3): 985–1002
35. Ferguson MW, O'Kane S. Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. *Philos Trans R Soc Lond B Biol Sci* 2004;**359**:839–50

(Received October 4, 2013, Accepted May 11, 2014)