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Review Article

The role of keratinocyte function on the defected diabetic wound healing

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Abstract: Non-healing wounds are a major complication of diabetes that can lead to limb amputation and disability in patients. The normal process of wound repair progresses through well-defined stages including hemostasis, inflammation, proliferative, and remodeling, which may be impaired in diabetic wounds. In recent years, it has been reported that keratinocytes, a major cell type in human skin, play a key role in the healing process of wounds. In this overview, firstly, a summary of the wound healing process is provided and the role of keratinocytes in wound healing is briefly reviewed. Then, a set of evidence about the impaired keratinocytes activities in diabetic wounds and clinical trials focused mainly on improving keratinocytes in the context of diabetic wound therapeutics are summarized. Keratinocytes can produce signaling molecules that act in a paracrine and autocrine way, causing pleiotropic effects on various cell types. The affected cells respond to keratinocytes by creating several signaling molecules, which also adjust keratinocyte activation through wound healing. In diabetic wounds, disruption of various biological mechanisms leads to dysfunction of keratinocytes including impaired migration, adhesion, and proliferation. The function of abnormal keratinocytes can lead to poor diabetic wound healing. Taken together, clarification of molecular and functional disturbances of keratinocyte cells and applying them in diabetic wounds can contribute to enhanced treatment of diabetic wounds. Based on the location of keratinocytes in the epidermis and the central role of keratinocytes in the diabetic wound healing process, applying keratinocytes has great potential for the treatment of diabetic burn wounds.

Keywords: Diabetic wound healing, keratinocytes, diabetes, inflammation

Introduction

Diabetes mellitus is one of the most common endocrine disorders with several organ damages that decrease life span [1]. The prevalence of diabetes is continuing to rise, thereby contributing to increasingly high healthcare costs [2]. Diabetes arises through two etiologically distinct routes. Type I diabetes is mediated through immunological destruction of the pancreatic insulin-producing cells [3] and Type II is specified by a combination of alterations in insulin sensitivity and insulin secretion [4].

Individuals afflicted with diabetes are prone to complications such as impaired wound healing, which is a common and potential complication in diabetes [5, 6]. Diabetic wounds are a major health concern affecting 15% of diabetic individuals. One of the most common impaired wound healing in patients who have uncontrolled diabetes is diabetic foot ulcers (DFU) which have adverse effects on functional ability and quality of patients' life [7, 8]. Researches to date determined that various factors have been associated with wound healing defects in diabetic patients including hypoxia, infection status, chronic inflammation, impaired neuropeptide signaling, and peripheral arterial disease [8]. Recently, the disturbed activity of keratinocytes has been reported to be a key underlying cause in the pathology of the impaired wound healing in diabetic individuals. Multiple studies have been proposed several therapeutic approaches to heal diabetic wounds [9, 10]. However, despite advances in treatment strategies, diabetic wounds are still a major clinical challenge.

Keratinocytes are the predominant cells in the epidermis, which form a protective barrier consisting of a stratified multi-layered epithelium

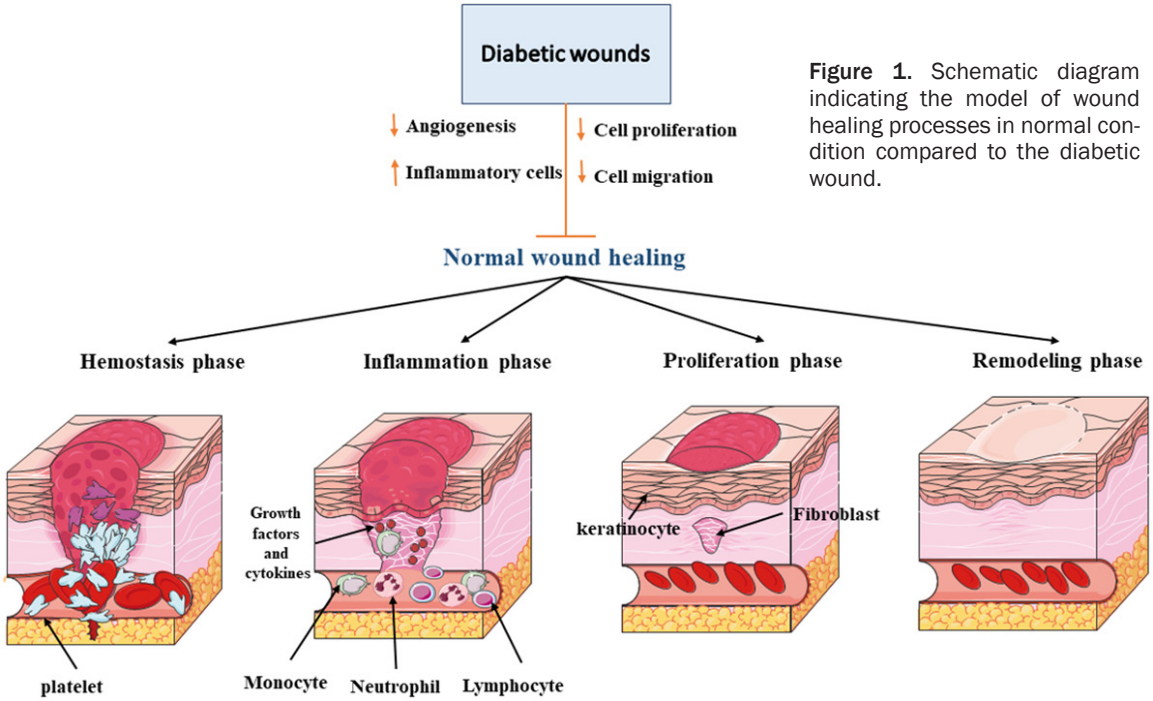

Figure 1. Schematic diagram indicating the model of wound healing processes in normal condition compared to the diabetic wound.

Table 1. Summary of normal wound healing events

Phase	Key events
Hemostasis	• platelet aggregation • vascular constriction
Inflammation	• entering of inflammatory cells
Proliferation	• Secretion of anti-inflammatory cytokines • Secretion of several growth factors • synthetization of collagen and ECM • re-epithelialization by proliferation and migration of keratinocytes
Remodeling	• reducing vessel density • remodeling collagen

that plays a crucial role in the wound healing process. Keratinocytes proliferate, differentiate, migrate and promote angiogenesis to regenerate the epidermal barrier, leading to a re-epithelialization in the wound healing process [11-13]. Keratinocytes release growth factors, cytokines, chemokines, and matrix metalloproteinase (MMPs) that regulate biologic wound healing [14]. Concerning the role of keratinocytes in wound healing, various studies have focused on finding different aspects of the role of keratinocytes in diabetic wound healing [15, 16]. On the other hand, it is now well known that abnormal keratinocyte function is known to be associated with poor healing ability of diabetic wounds [17]. Overall, in this review study, we provided an overview of the wound healing process and briefly reviewed the role of keratinocytes in wound healing. We also summarized a set of evidence about the impaired keratinocyte activities in diabetic wounds.

Basic mechanisms of wound healing

Generally, the processes of wound healing involve several complex biological and molecular pathways. The wound healing process is classified into four general stages: homeostasis, inflammation, proliferation, and remodeling phase (**Figure 1** and **Table 1**) [5, 18].

Hemostasis is known as the first step of the wound healing process, in which contact of platelets to collagen of tissue and vascular constriction has the main role in the coagulation process in this stage [19, 20]. Inflammation phase initiates immediately following the hemostasis phase. The inflammation phase is characterized by the entering of inflammatory cells including neutrophils, monocytes, and lymphocytes into the wound place [8, 21]. Next, in the proliferation phase, macrophages release anti-inflammatory cytokines and multiple

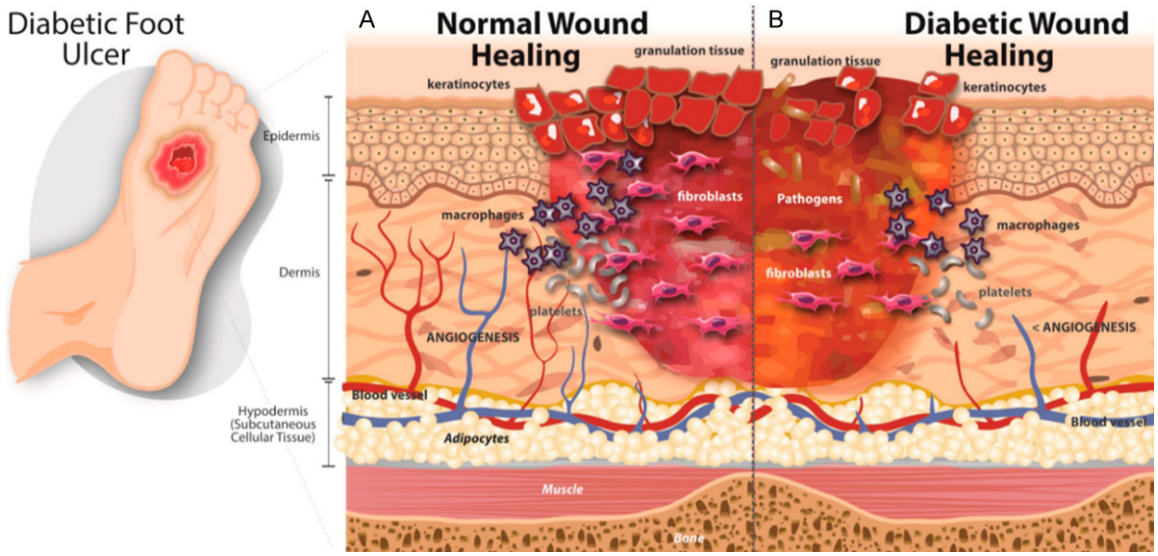

Figure 2. The healing process in normal and diabetic wounds. (A) Events in normal wound healing process include: platelet aggregation (hemostasis phase); release of pro-inflammatory cytokines by neutrophils, macrophages, and mast cells (inflammation phase); healing response is initiated by decreasing inflammation, increasing angiogenesis, migration of fibroblasts and keratinocytes, and formation of ECM (remodeling phase) (B) Healing process in diabetic wounds is affected with a decrease in fibrinolysis and an imbalance of released cytokines, a decrease in angiogenesis, impaired proliferation and migration of keratinocytes, which resulted in delayed re-epithelialization in wounds, impaired collagen deposition and the poor synthesis of collagen and the ECM contributes to deficient wound closure (Figure adapted from Hesketh and others [18].

growth factors such as vascular endothelial growth factor (VEGF) and transforming growth factor (TGFβ) that enhance cell proliferation and protein synthesis [22]. Afterward, wound size is decreased by the contracting of mesenchymal cells. Besides, collagen and extracellular matrix (ECM) are synthesized through migration and proliferation of fibroblasts in the wound site [8]. It is also well established that keratinocytes proliferate and migrate from the wound site during the proliferative phase, which has a critical role in wound re-epithelialization [5]. Additionally, various growth factors including endothelial growth factor (EGF), keratinocyte growth factor (KGF), and fibroblast growth factor-2 (FGF-2) induce keratinocyte proliferation and migration [8]. The remodeling phase as the last stage of wound healing starts at 2-3 weeks after tissue injury (**Figure 2**). This phase is characterized by reducing vessel density and remodeling collagen that is regulated by MMPs [19, 20].

Keratinocytes play an essential role in epidermal repair after injury through proliferation and re-epithelialization [23]. Differentiated keratinocytes perform their barrier function by creating a keratinized layer and responding to pro-inflammatory mediators released at the wound site [24]. Keratinocytes produce multiple differentiation proteins, such as keratins, known as an intermediate filament in epithelial cells. Keratins have several crucial functions, including structural support, protecting epithelial cells from stress, and regulating apoptosis and protein synthesis [25]. There is also evidence that interaction of keratinocytes and fibroblasts induces fibroblasts to release growth factors and several cytokines including KGF, fibroblast growth factor, GM-CSF, and IL6, which subsequently stimulate the proliferation of keratinocytes. Besides, fibroblasts play an important role in wound contraction by acquiring a keratinocyte-controlled myofibroblast phenotype [26]. Furthermore, migration of keratinocytes leads to closure of the wound after 6-24 h wounding. For migration and proliferation of keratinocytes, released growth factors by macrophages, MMPs, components of the ECM, integrin, and structural proteins in the wound site are essential. After repairing the injury, the keratinocytes eventually inactivate and return to their normal differentiation pathway [23].

The biology of impaired wound healing in diabetes

The diabetic wound healing process may be different from non-diabetics (**Figure 1**) and is

associated with poor healing, resulting in chronic wounds that do not heal after 12 weeks [17]. Recent investigations have reported that poor wound healing has various causes including artery disease, neuropathy, and altered immune function [27].

Intravenous stasis and incurable chronic wounds are always associated with hypoxia [28]. Prolonged hypoxia, which may result from both angiogenesis and insufficient perfusion, is detrimental to wound healing [29]. The hypoxic condition can enhance the early inflammation, thus prolonging damage by an accumulation of the reactive oxygen species (ROS) [30]. When ROS generation overwhelms the antioxidant capacity, hyperglycemia can also lead to oxidative stress [31]. A study also reported that the formation of advanced glycation end products (AGEs) and interaction with their receptors are related to delayed wound healing in diabetic animal models [32]. Moreover, diabetic foot ulcers are characterized by high levels of matrix metalloproteinases (MMPs), which cause tissue damage and inhibit the normal healing process [33, 34]. Several studies that have examined the mechanisms involved in reducing vascular repair in diabetic wounds have found that endothelial progenitor cell mobilization is disrupted, the permeability of capillaries is increased, and VEGF levels are reduced in diabetic patients [13, 35-37]. Furthermore, the production of nitric oxide (NO) is reduced and subsequently leads to impaired angiogenesis and recruitment of endothelial cells in diabetic patients [35]. Also, it is well known that neuropathy occurs in diabetic patients, which may also be involved in impaired wound healing. Neuropeptides, which are small proteins produced by neurons, such as nerve growth factor, substance P, and calcitonin gene-related peptide are essential for wound healing, causing cell chemotaxis, growth factor production, and cell proliferation [6, 38]. Decreased neuropeptides have been reported in diabetic patients associated with impaired wound healing [33, 38].

However, the most important effect on wound healing is due to functional changes in cells activated by immune response. For example, leukocyte function is impaired in diabetic patients, and inadequate migration of neutrophils and macrophages to the wound site increases the risk of wound infection [11, 39-41]. Furthermore, it is well known that abnormal keratinocyte function including impaired migration and proliferation of keratinocytes can contribute to poor diabetic wound healing [8]. Accordingly, abnormal keratinocyte function is one of the most crucial reasons for the poor healing ability of diabetic wounds that recent studies have focused on.

Various researches to date have shown that there are various factors in diabetic patients that affect keratinocyte function and the healing process of diabetic wounds including impaired keratinocyte proliferation and migration, chronic inflammation, and infections, decreased angiogenesis, and unusual MMPs expression [5, 8, 11, 15].

It is widely indicated that the proliferation and migration of keratinocytes have a key role in the re-epithelialization of wound healing events. However, finding from several sources have recently found that keratinocyte proliferation and migration are reduced in diabetic patients. Besides, various hypotheses have recently been proposed that several mechanisms are involved in reducing keratinocyte proliferation and migration in diabetic wound healing [15]. Lan and colleagues reported that hyperglycemic conditions in diabetic patients significantly reduced the keratinocyte proliferation and migration in the wound healing process [42, 43]. In diabetic animal models, keratinocyte proliferation status was decreased in diabetic animals as compared to normal [44, 45]. Although the exact mechanism of impaired keratinocyte proliferation in diabetic patients has not been elucidated, various hypotheses have been suggested, including an increased level of apoptotic-related proteins (bcl2 and caspase 3), suppressor of cytokine signaling (SOCS)-3, and reduced keratin expression in diabetic patients [46-48]. On the other hand, phosphorylated focal adhesion kinase (p125FAK) was proposed as an important mechanism that affects keratinocyte migration. In line with this, it has been shown that hyperglycemia leads to a decrease in the expression of phosphorylated p125FAK in diabetic patients [42]. Accordingly, to further support the impaired keratinocyte migration in diabetic patients, several studies confirmed the results of the previous study. Furthermore, a set of evidence found

that the hyperglycemic condition in diabetic wounds causes non-enzymatic glycation of type I collagen. They also demonstrated that keratinocyte migration on glycated collagen is reduced [49]. In another study, Zhang and colleagues reported that forkhead box O1 (FOXO1) improved keratinocyte migration by stimulating TGFβ1 in a normal glucose environment, but they also found that FOXO1 couldn't interact with TGFβ1 promoter in high glucose [50]. Therefore, hyperglycemic condition can alter various mechanisms that subsequently reduce the proliferation and mobility of keratinocytes.

Chronic and long-term inflammation and increased prevalence of wound infections are the hallmarks of diabetic wounds that lead to an impaired wound healing [17, 51]. In support of this hypothesis, recent studies have found that the number of immune cells such as macrophages and neutrophils increased in diabetic wounds [8, 21]. Elevated neutrophils in diabetic wounds produce a high rate of ROS and proteases that can injure normal tissue and contribute to the healing of diabetic wounds [21]. Moreover, many researchers have indicated that hyperglycemia is associated with a variety of immune disorders including impaired leukocyte adhesion, phagocytosis, and chemotaxis [52]. Increased production of pro-inflammatory mediators including interleukins (IL-1, IL-6), and tumour necrosis factor α (TNFα) in diabetic wounds has also been reported, which could be one of the long-term inflammatory causes following wound injury in diabetic patients [8, 51]. To date, keratinocyte cells in diabetic wounds have been revealed to play a key role in promoting chronic inflammation. Together with this end, keratinocytes secrete several chemokines and cytokines such as IL-8, which are involved in recruiting neutrophils and increasing ROS production [53]. Recently, an *in vivo* study revealed that high-glucose condition results in increased expression of IL-8 through the regulation of epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK) axis and subsequently enhanced ROS production in keratinocytes. Hence, increased IL-8 expression in keratinocytes contributes to elevated ROS levels and poor wound healing in diabetic rats compared with control rats [54]. As mentioned, the process of angiogenesis is one of the essential processes in wound healing, but various pieces of evidence have reported that the process of angiogenesis in diabetic wounds has been disrupted [27, 55]. Galiano and colleagues found that administration of an angiogenic agent such as VEGF to diabetic mice induces angiogenesis and wound healing [56]. Additionally, keratinocytes in diabetic wounds are involved in the process of impaired angiogenesis. In this regard, thrombospondin-1 (TSP-1) has been identified as the physiological inhibitor of angiogenesis, which is overexpressed in the keratinocytes of diabetic wounds and leads to inhibit the angiogenesis of diabetic wounds. Moreover, elevated oxidative stress stimulates DNA hypomethylation of TSP1 promoter in keratinocytes of diabetic wounds. Therefore, the administration of antioxidants can contribute to normalizing keratinocyte-derived TSP1 in diabetic patients [57]. Besides, VEGF produced by keratinocytes plays an effective role in angiogenesis in the wound healing process. One study found that VEGF levels in chronic wounds in diabetic mice were lower than in normal rats. This study also found that VEGF protein biosynthesis in keratinocytes was mediated by protein kinase B (Akt) [58].

MMPs, as a class of endopeptidase enzymes, can destroy components of the ECM, including collagen, fibronectin, and laminin [57]. During the healing process of the wound, several remodeling and migratory events occur that require the function of MMPs and their natural inhibitors (tissue inhibitors of MMPs or TIMPs). TIMPs are secretory proteins that play a key role in the regulation of ECM metabolism by inhibiting the proteolytic activity of MMPs [5, 19, 20]. In non-repairable wounds, such as diabetic wounds, the normal balance between MMPs and TIMPs is disturbed, causing the wound to delay or not close [59]. However, the expression of MMPs and TIMP levels are controversial in diabetic wounds. For example, several studies found that enhanced expression of MMPs and reduced TIMPs levels are hallmarks of the diabetic wound index, and targeting the MMPs may prove helpful in the management of wound healing [27, 60, 61]. Toward this end, it has been suggested that high levels of MMPs in diabetic wounds disrupt ECM components including fibronectin, cytokines, and growth factors, resulting in impairing the wound healing process [62]. On the other hand, a previous study claimed that keratinocytes require proteolysis of ECM proteins by MMPs to migrate

to wounds. They also found that in diabetic wounds, the level of MMPs expression decreased, resulting in reduced migration of keratinocytes to wound healing [42]. Besides, the expression of a2b1 integrin and MMP-1 is essential for keratinocyte migration following skin injury. In this regard, Lan and colleagues showed that keratinocyte motility was significantly reduced as a result of decreased a2b1 integrin and MMP-1 expression in high glucose conditions [47]. To further investigate the role of MMPs in keratinocyte migration, recent evidence has shown that cytokines produced by mononuclear cells such as IL-22 play an important role in the secretion of MMPs by keratinocytes [57]. However, they found that IL-22 expression is suppressed from blood mononuclear cells of diabetic rats, leading to decreased keratinocyte expression of MMP-3 and impaired keratinocyte migration [63].

The role of keratinocytes in the treatment of diabetic wounds

Previous studies have suggested that the interplay between keratinocytes and multiple cell types such as fibroblasts play a crucial role in the wound healing process. It has been shown that keratinocytes stimulate fibroblasts to produce growth factors and signaling mediators which may, in turn, lead to keratinocyte proliferation and migration resulting in wound contraction.

Due to the crucial role keratinocytes play in wound healing, it was suggested as adjunctive treatment options for diabetic wounds. For example, Mansoub and colleagues investigated the application of keratinocytes and platelet-rich-plasma (PRP) on wound healing in diabetic animal models. The results showed that wound closure was initiated earlier in the keratinocytes and PRP-treated groups in comparison to the control group. Macroscopic evaluation of wound closure indicated that the combinational treatment causes more accelerated healing when compared to a single treatment at the end of the second week (**Figure 3**) [64].

Accordingly, two products including Apligraf® and Dermagraft® (Organogenesis, Inc., Canton, Massachusetts, USA) were introduced as therapeutic procedures for diabetic wounds [8]. Apligraf® is a graft made from a cultured living dermis consisting of four components, including an ECM, skin fibroblasts, stratum corneum, and epidermal keratinocytes. In this product, epidermal keratinocytes release growth factors to induce biologic wound healing and subsequently stimulate fibrosis, chemotaxis, angiogenesis, and other cellular activities [65].

Dermagraft® is another allogeneic skin fibroblast product that is derived from human neonatal seeded on bio-absorbable polyglactin scaffold. Moreover, Dermagraft® is a rich source of fibroblasts and various growth factors such as platelet-derived growth factors A (PDGF-A), KGF, heparin-bound epidermal growth factor (HBEGF), insulin-like growth factor (IGF) that enhances the proliferation and migration of keratinocytes [66]. However, neither of these two products has been approved by the FDA for the treatment of diabetic wounds because there is insufficient clinical data to prove the benefits of these products [8].

Cell-based therapy

Despite of the improvement of various forms of skin substitutes for the treatment, using of skin substitutes face some trouble including incapacity of reconstitution of skin appendages, excessive cost and low efficiency [67]. In addition, there are some limitations of cultured epithelial autograft (CEA) sheet for the treatment of disorders in skin, such as keeping and management of fragile CEA. In addition, in the clinical application cases of CEA, taking a long time to achieve multilayered complete CEA sheet is another limitation [68]. Application of Stem cells (SCs) and differentiated cells such as keratinocytes is a promising treatment for the development of cell therapeutics. Treatment with these cells faces some limitations Such as Possibility of tumorigenicity immunogenicity and short survivability of SCs after treatment are a key obstacle to the treatment of SC-based therapeutic approaches [69]. A variety of clinical trials focused on cell-based products such as cultured allogeneic keratinocytes in the context of diabetic wound therapeutics (**Figure 4**) (as summarized in **Table 2**) [70].

Recently, a pilot clinical trial involving 59 patients with DFUs was performed by You and colleagues, to assess the efficacy of the cultured allogeneic keratinocyte sheets in treatment of diabetic wound healing. After 12-weeks

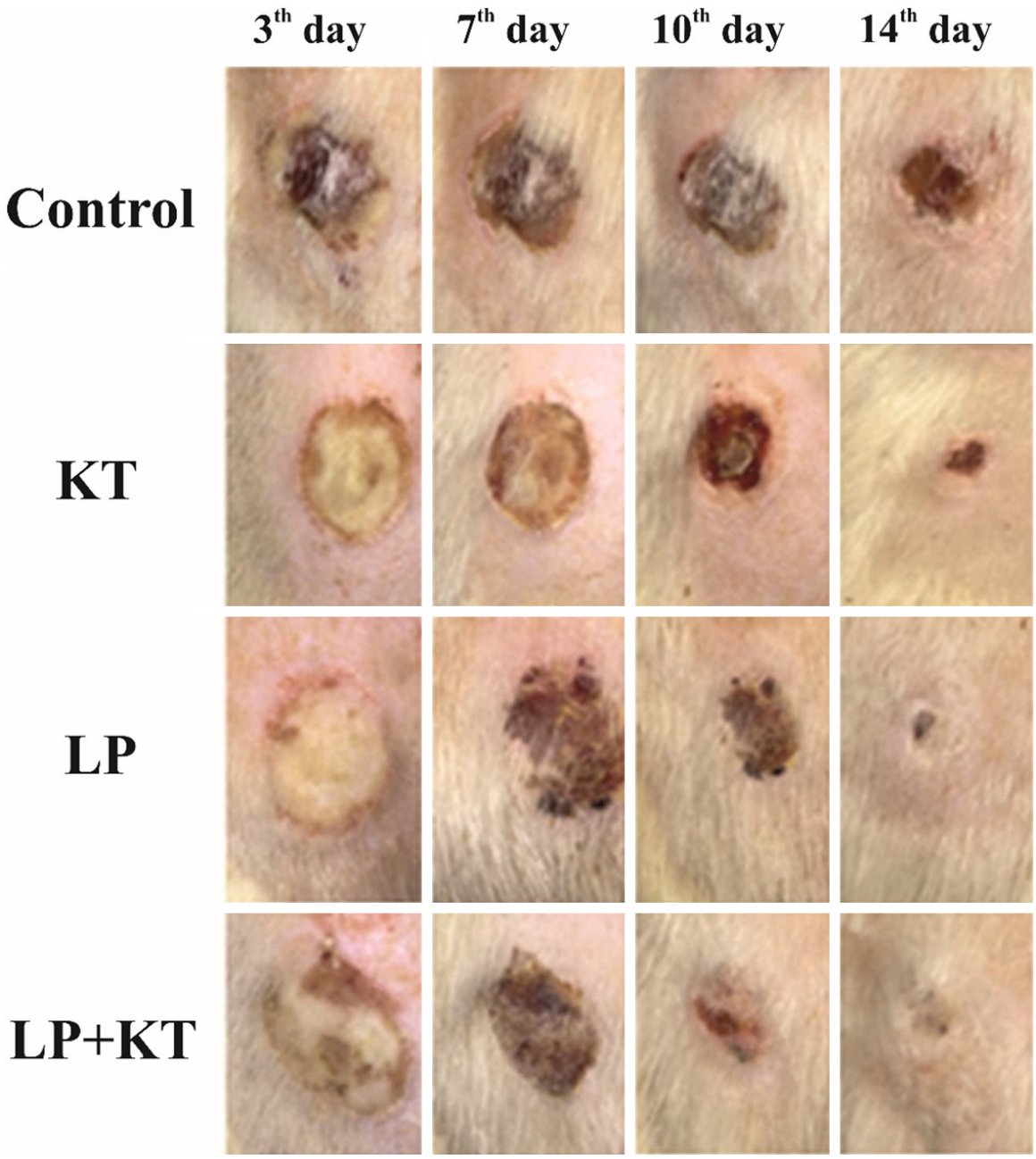

Figure 3. Macroscopic evaluation of wound in diabetic rat models. The day when the wounds were created was designated as 1th day. On 3rd, 7th, 10th and 14th days after treatment, wound areas were measured. The four treatment groups include control (Non-treated diabetic rats), KT (keratinocytes-treated groups), LP (PRP-treated groups), LP + KT (Local PRP + keratinocytes-treated groups). Figure adapted from Mansoub and others [64].

of treatment, all patients (100%) in the keratinocyte-treated group displayed complete wound healing and 69% in the control group ($P< 0.05$). The Kaplan-Meier median time to complete closure was 35 days significantly lower when compared to 57 days observed in the control group. In this randomized clinical trial, allogeneic keratinocytes indicated promising results in diabetic foot ulcers (DFUs) healing. They suggested that cultured allogeneic keratinocytes may provide a safe and effective treatment for DFUs [71]. Moreover, another clinical trial examined the efficiency of autologous cell therapy using skin cells including fibroblasts and keratinocytes on the treatment of chronic diabetic wounds (five severe patients

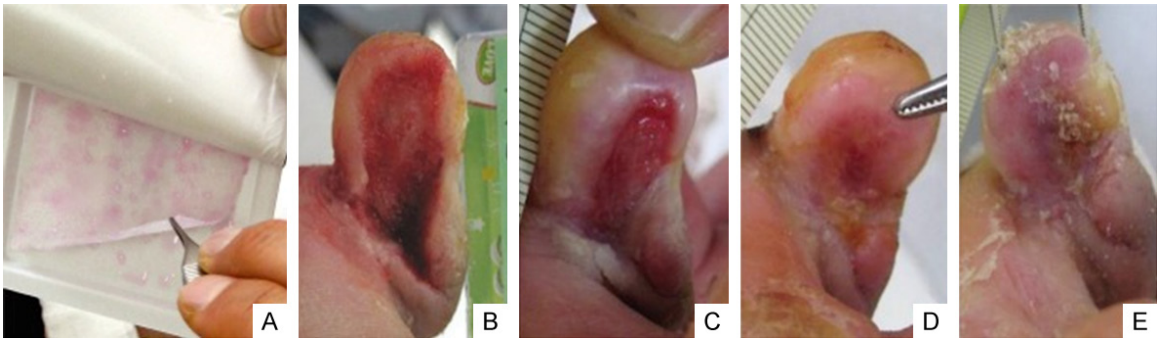

Figure 4. Treatment of a diabetic foot ulcer using an allogeneic keratinocyte product. A 63 years old woman with a chronic diabetic wound on the right fifth toe. (A) Cultured allogeneic keratinocyte sheet. (B-E) The ulcer was completely healed after 3 weeks of treatment. (Figure adapted from You and Han [70]).

Table 2. Summary of clinical trials reported on the role of keratinocytes in the treatment of diabetic ulcers

Type of treatment	No. of enrollment	Intervention	Ref.
Allogeneic keratinocyte sheets	57 (n = 27 in Group 1, n = 32 in Group 2)	Group 1: keratinocyte treatment group for a maximum of 11 weeks. Group 2: control group treated with vaseline gauze	[71]
Autologous skin cell (fibroblasts and keratinocytes) implants	5	10×10^6 cells were applied with 2 ml of autologous fibrin glue to the ulcers	[72]
Autologous skin fibroblast and keratinocyte grafts	7	Hyalograft 3D and Laserskin Autograft	[73]
living allogeneic keratinocytes	40 (n = 20 in Group 1, n = 20 in Group 2)	Group 1: received cell-based dressing, Group 2: microcarriers kept in culture medium overnight	[74]
Autologous keratinocytes	21	Group 1: cultured autologous keratinocytes (active treatment), Group 2: cell-free discs (placebo)	[75]
Cellular versus acellular matrix treatment	120	Group 1: standard of care (SOC), Group 2: SOC plus Dermagraft® (bioengineered ECM containing living fibroblasts), Group 3: SOC plus Oasis® (ECM devoid of living cells)	[81]

with chronic ulcers and unresponsive to classic medical treatments). The investigators concluded that this cell therapy method is a potential treatment strategy for diabetic wounds that can heal faster. Besides, the important advantage of autologous skin cells is a minimally invasive method and can be performed in an outpatient clinic [72]. Moustafa and colleagues in a randomized controlled trial (RCT) with 21 patients, showed that using cultured autologous keratinocytes delivered on the carrier dressing improved wound healing in non-healing diabetic ulcers (with 18 out of 21 ulcers responding). Similarly, recent clinical studies have confirmed that fibroblast and keratinocyte graft transplantation is effective in the treatment of diabetic lower extremity ulcers [73-75].

Limitations in transplantation and keratinocyte cell culture techniques have impeded the widespread use of this method in the clinical trials. Using single-cell suspension was shown mainly to shorten the culture time [76, 77]. Hwang and colleagues evaluated the efficacy of allogeneic keratinocyte application for Chronic diabetic foot ulcers DFUs. They performed weekly keratinocyte treatment for up to 12 weeks in patients with DFUs. They analyzed wound healing velocity, time to 50% wound size healing rate and analyzed affecting wound healing. Their results showed that cultured allogeneic keratinocyte, which are safe, advanced and easy to apply, are an effective protocol for the treatment of DFUs [78].

Uluer and colleagues studded on the role of keratinocyte which are differentiated from embryonic on wound healing. In the experimental group wound healing was faster than control groups. Keratinocyte increased the expression of FGF-2, IL-8 and MCP-1 during early stages of wound healing and during late stages of wound healing the expression of collagen-1 and EGF

was increased. Keratinocytes influenced the wound healing process and improved wound healing [79]. Mansoub and colleagues evaluated the effect of keratinocyte on diabetic burn wound model for their contribution in wound healing process. They showed that applying keratinocyte increased the level of gene expression and evaluated growth factors and COL1α2, while MCP-1 levels decreased when compared to the untreated diabetic group [64].

However, for better understanding and optimal application, more insight into the mechanism of action of cell-based therapies is needed. A clinical trial to evaluate the advantage of living cells in bioengineered structures over matrix alone in the treatment of diabetic wounds is currently ongoing [80].

Conclusion

Patients afflicted with diabetes experience more wound healing failures than those without. The causes of non-healing diabetic wounds are very complex, which involves many cellular and biochemical events. Increasing research on diabetic wound pathogenesis has resulted in the discovery of treatment strategies to promote wound repair, but there remain many challenges and complexities regarding the events leading to impaired diabetic wound healing. In recent years, the disturbing activity of keratinocytes has been reported as a key factor in impaired wound healing in diabetic patients. A high glucose environment may disrupt keratinocyte physiology and activity resulting in reduced epithelialization of the wound site. Keratinocytes-related factors that may affect the impaired diabetic wound healing include impaired keratinocyte proliferation and migration, gap junction abnormalities, chronic infection and inflammation associated with perturbed innate immunity, increased ROS generation, impaired angiogenesis, changing FOXO1 activity and abnormal expression of MMPs. Given the central role of keratinocytes in the wound healing process and their location in the epidermis, it can be nominated as a targeted non-invasive treatment option for diabetic wound healing. Keratinocytes were successfully cultured in the presence of fibroblast feeder cells. However, further investigation is required to improve keratinocyte cell expansion as an effective treatment strategy for treating non-healing diabetic wounds for usage in the clinical setting.

Disclosure of conflict of interest

None.

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References

[1] Shaw JE, Sicree RA and Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87: 4-14.
[2] den Dekker A, Davis FM, Kunkel SL and Gallagher KA. Targeting epigenetic mechanisms in diabetic wound healing. Transl Res 2019; 204: 39-50.
[3] Eisenbarth GS. Type I diabetes mellitus. N Engl J Med 1986; 314: 1360-1368.
[4] Padhi S, Nayak AK and Behera A. Type II diabetes mellitus: a review on recent drug based therapeutics. Biomed Pharmacother 2020; 131: 110708.
[5] Falanga V. Wound healing and its impairment in the diabetic foot. Lancet 2005; 366: 1736-1743.
[6] Moura LI, Cruz MT and Carvalho E. The effect of neurotensin in human keratinocytes--implication on impaired wound healing in diabetes. Exp Biol Med (Maywood) 2014; 239: 6-12.
[7] Boulton AJ. The pathway to foot ulceration in diabetes. Med Clin North Am 2013; 97: 775-790.
[8] Baltzis D, Eleftheriadou I and Veves A. Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights. Adv Ther 2014; 31: 817-836.
[9] Moura LI, Dias AM, Carvalho E and de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment--a review. Acta Biomater 2013; 9: 7093-7114.
[10] Dumville JC, Deshpande S, O'Meara S and Speak K. Hydrocolloid dressings for healing diabetic foot ulcers. Cochrane Database Syst Rev 2013; 2013: Cd009099.
[11] Kasuya A and Tokura Y. Attempts to accelerate wound healing. J Dermatol Sci 2014; 76: 169-172.
[12] Usui ML, Mansbridge JN, Carter WG, Fujita M and Olerud JE. Keratinocyte migration, proliferation, and differentiation in chronic ulcers

from patients with diabetes and normal wounds. J Histochem Cytochem 2008; 56: 687-696.
[13] Brem H and Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J Clin Invest 2007; 117: 1219-1222.
[14] Kawai K, Kageyama A, Tsumano T, Nishimoto S, Fukuda K, Yokoyama S, Oguma T, Fujita K, Yoshimoto S, Yanai A and Kakibuchi M. Effects of adiponectin on growth and differentiation of human keratinocytes--implication of impaired wound healing in diabetes. Biochem Biophys Res Commun 2008; 374: 269-273.
[15] Huang SM, Wu CS, Chiu MH, Wu CH, Chang YT, Chen GS and Lan CE. High glucose environment induces M1 macrophage polarization that impairs keratinocyte migration via TNF-α: an important mechanism to delay the diabetic wound healing. J Dermatol Sci 2019; 96: 159-167.
[16] Liang Y, Yang C, Lin Y, Parviz Y, Sun K, Wang W, Ren M and Yan L. Matrix metalloproteinase 9 induces keratinocyte apoptosis through FasL/Fas pathway in diabetic wound. Apoptosis 2019; 24: 542-551.
[17] Hu SC and Lan CE. High-glucose environment disturbs the physiologic functions of keratinocytes: focusing on diabetic wound healing. J Dermatol Sci 2016; 84: 121-127.
[18] Hesketh M, Sahin KB, West ZE and Murray RZ. Macrophage phenotypes regulate scar formation and chronic wound healing. Int J Mol Sci 2017; 18: 1545.
[19] Pradhan L, Nabzdyk C, Andersen ND, LoGerfo FW and Veves A. Inflammation and neuropeptides: the connection in diabetic wound healing. Expert Rev Mol Med 2009; 11: e2.
[20] Blakytny R and Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med 2006; 23: 594-608.
[21] Dovi JV, Szpaderska AM and DiPietro LA. Neutrophil function in the healing wound: adding insult to injury? Thromb Haemost 2004; 92: 275-280.
[22] Koh TJ and DiPietro LA. Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med 2011; 13: e23.
[23] Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff RR and Tomic-Canic M. Epithelialization in wound healing: a comprehensive review. Adv Wound Care (New Rochelle) 2014; 3: 445-464.
[24] Ter Horst B, Chouhan G, Moiemen NS and Grover LM. Advances in keratinocyte delivery in burn wound care. Adv Drug Deliv Rev 2018; 123: 18-32.
[25] Gu LH and Coulombe PA. Keratin function in skin epithelia: a broadening palette with surprising shades. Curr Opin Cell Biol 2007; 19: 13-23.
[26] Werner S, Krieg T and Smola H. Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol 2007; 127: 998-1008.
[27] Dinh T, Tecilazich F, Kafanas A, Doupis J, Gnardellis C, Leal E, Tellechea A, Pradhan L, Lyons TE, Giurini JM and Veves A. Mechanisms involved in the development and healing of diabetic foot ulceration. Diabetes 2012; 61: 2937-2947.
[28] Tandara AA and Mustoe TA. Oxygen in wound healing--more than a nutrient. World J Surg 2004; 28: 294-300.
[29] Eskandani M, Vandghanooni S, Barar J, Nazemiyeh H and Omidi Y. Cell physiology regulation by hypoxia inducible factor-1: targeting oxygen-related nanomachineries of hypoxic cells. Int J Biol Macromol 2017; 99: 46-62.
[30] Guo S and Dipietro LA. Factors affecting wound healing. J Dent Res 2010; 89: 219-229.
[31] Vincent AM, Russell JW, Low P and Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev 2004; 25: 612-628.
[32] Huijberts MS, Schaper NC and Schalkwijk CG. Advanced glycation end products and diabetic foot disease. Diabetes Metab Res Rev 2008; 24 Suppl 1: S19-24.
[33] Gary Sibbald R and Woo KY. The biology of chronic foot ulcers in persons with diabetes. Diabetes Metab Res Rev 2008; 24 Suppl 1: S25-30.
[34] Woo K, Ayello EA and Sibbald RG. The edge effect: current therapeutic options to advance the wound edge. Adv Skin Wound Care 2007; 20: 99-117; quiz 118-119.
[35] Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Buerk DG, Nedeau A, Thom SR and Velazquez OC. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. J Clin Invest 2007; 117: 1249-1259.
[36] Quattrini C, Jeziorska M, Boulton AJ and Malik RA. Reduced vascular endothelial growth factor expression and intra-epidermal nerve fiber loss in human diabetic neuropathy. Diabetes Care 2008; 31: 140-145.
[37] Okonkwo UA, Chen L, Ma D, Haywood VA, Barakat M, Urao N and DiPietro LA. Compromised angiogenesis and vascular Integrity in impaired diabetic wound healing. PLoS One 2020; 15: e0231962.
[38] Galkowska H, Olszewski WL, Wojewodzka U, Rosinski G and Karnafel W. Neurogenic factors in the impaired healing of diabetic foot ulcers. J Surg Res 2006; 134: 252-258.

[39] Wysocki J, Wierusz-Wysocka B, Wykretowicz A and Wysocki H. The influence of thymus extracts on the chemotaxis of polymorphonuclear neutrophils (PMN) from patients with insulin-dependent diabetes mellitus (IDD). Thymus 1992; 20: 63-67.
[40] Ganesh GV and Ramkumar KM. Macrophage mediation in normal and diabetic wound healing responses. Inflamm Res 2020; 69: 347-363.
[41] Seraphim PM, Leal EC, Moura J, Gonçalves P, Gonçalves JP and Carvalho E. Lack of lymphocytes impairs macrophage polarization and angiogenesis in diabetic wound healing. Life Sci 2020; 254: 117813.
[42] Lan CC, Liu IH, Fang AH, Wen CH and Wu CS. Hyperglycaemic conditions decrease cultured keratinocyte mobility: implications for impaired wound healing in patients with diabetes. Br J Dermatol 2008; 159: 1103-1115.
[43] Spravchikov N, Sizyakov G, Gartsbein M, Accili D, Tennenbaum T and Wertheimer E. Glucose effects on skin keratinocytes: implications for diabetes skin complications. Diabetes 2001; 50: 1627-1635.
[44] Sakai S, Endo Y, Ozawa N, Sugawara T, Kusaka A, Sayo T, Tagami H and Inoue S. Characteristics of the epidermis and stratum corneum of hairless mice with experimentally induced diabetes mellitus. J Invest Dermatol 2003; 120: 79-85.
[45] Werner S, Breeden M, Hübner G, Greenhalgh DG and Longaker MT. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. J Invest Dermatol 1994; 103: 469-473.
[46] Galkowska H, Olszewsk WL, Wojewodzka U, Mijal J and Filipiuk E. Expression of apoptosis- and cell cycle-related proteins in epidermis of venous leg and diabetic foot ulcers. Surgery 2003; 134: 213-220.
[47] Lan CC, Wu CS, Kuo HY, Huang SM and Chen GS. Hyperglycaemic conditions hamper keratinocyte locomotion via sequential inhibition of distinct pathways: new insights on poor wound closure in patients with diabetes. Br J Dermatol 2009; 160: 1206-1214.
[48] Goren I, Linke A, Müller E, Pfeilschifter J and Frank S. The suppressor of cytokine signaling-3 is upregulated in impaired skin repair: implications for keratinocyte proliferation. J Invest Dermatol 2006; 126: 477-485.
[49] Morita K, Urabe K, Moroi Y, Koga T, Nagai R, Horiuchi S and Furue M. Migration of keratinocytes is impaired on glycated collagen I. Wound Repair Regen 2005; 13: 93-101.
[50] Zhang C, Ponugoti B, Tian C, Xu F, Tarapore R, Batres A, Alsadun S, Lim J, Dong G and Graves DT. FOXO1 differentially regulates both normal and diabetic wound healing. J Cell Biol 2015; 209: 289-303.
[51] Yang L, Zhang L, Hu J, Wang W and Liu X. Promote anti-inflammatory and angiogenesis using a hyaluronic acid-based hydrogel with miRNA-laden nanoparticles for chronic diabetic wound treatment. Int J Biol Macromol 2021; 166: 166-178.
[52] Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H and Genetet B. Impaired leucocyte functions in diabetic patients. Diabet Med 1997; 14: 29-34.
[53] Baggiolini M, Walz A and Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 1989; 84: 1045-1049.
[54] Lan CC, Wu CS, Huang SM, Wu IH and Chen GS. High-glucose environment enhanced oxidative stress and increased interleukin-8 secretion from keratinocytes: new insights into impaired diabetic wound healing. Diabetes 2013; 62: 2530-2538.
[55] Drela E, Stankowska K, Kulwas A and Rość D. Endothelial progenitor cells in diabetic foot syndrome. Adv Clin Exp Med 2012; 21: 249-254.
[56] Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, Bunting S, Steinmetz HG and 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: 1935-1947.
[57] Lan CC, Huang SM, Wu CS, Wu CH and Chen GS. High-glucose environment increased thrombospondin-1 expression in keratinocytes via DNA hypomethylation. Transl Res 2016; 169: 91-101, e1-3.
[58] Goren I, Müller E, Schiefelbein D, Gutwein P, Seitz O, Pfeilschifter J and Frank S. Akt1 controls insulin-driven VEGF biosynthesis from keratinocytes: implications for normal and diabetes-impaired skin repair in mice. J Invest Dermatol 2009; 129: 752-764.
[59] Martins VL, Caley M and OToole EA. Matrix metalloproteinases and epidermal wound repair. Cell Tissue Res 2013; 351: 255-268.
[60] Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S and Lehnert H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. Diabetologia 2002; 45: 1011-1016.
[61] Menghini R, Uccioli L, Vainieri E, Pecchioli C, Casagrande V, Stoehr R, Cardellini M, Porzio O, Rizza S and Federici M. Expression of tissue inhibitor of metalloprotease 3 is reduced in ischemic but not neuropathic ulcers from pa-

tients with type 2 diabetes mellitus. Acta Diabetol 2013; 50: 907-910.
[62] Lobmann R, Schultz G and Lehnert H. Proteases and the diabetic foot syndrome: mechanisms and therapeutic implications. Diabetes Care 2005; 28: 461-471.
[63] Huang SM, Wu CS, Chao D, Wu CH, Li CC, Chen GS and Lan CC. High-glucose-cultivated peripheral blood mononuclear cells impaired keratinocyte function via reduced IL-22 expression: implications on impaired diabetic wound healing. Exp Dermatol 2015; 24: 639-641.
[64] Hosseini Mansoub N, Gürdal M, Karadadaş E, Kabadayi H, Vatansever S and Ercan G. The role of PRP and adipose tissue-derived keratinocytes on burn wound healing in diabetic rats. Bioimpacts 2018; 8: 5-12.
[65] Shen JT and Falanga V. Innovative therapies in wound healing. J Cutan Med Surg 2003; 7: 217-224.
[66] Marston WA. Dermagraft, a bioengineered human dermal equivalent for the treatment of chronic nonhealing diabetic foot ulcer. Expert Rev Med Devices 2004; 1: 21-31.
[67] Lenihan C, Rogers C, Metcalfe AD and Martin YH. The effect of isolation and culture methods on epithelial stem cell populations and their progeny-toward an improved cell expansion protocol for clinical application. Cytotherapy 2014; 16: 1750-1759.
[68] Hu MS, Leavitt T, Malhotra S, Duscher D, Pollhammer MS, Walmsley GG, Maan ZN, Cheung AT, Schmidt M and Huemer GM. Stem cell-based therapeutics to improve wound healing. Plast Surg Int 2015; 2015: 383581.
[69] Herberts CA, Kwa MS and Hermsen HP. Risk factors in the development of stem cell therapy. J Transl Med 2011; 9: 1-14.
[70] Kosaric N, Kiwanuka H and Gurtner GC. Stem cell therapies for wound healing. Expert Opin Biol Ther 2019; 19: 575-585.
[71] You HJ, Han SK, Lee JW and Chang H. Treatment of diabetic foot ulcers using cultured allogeneic keratinocytes--a pilot study. Wound Repair Regen 2012; 20: 491-499.
[72] Marcelo D, Beatriz PM, Jussara R and Fabiana B. Tissue therapy with autologous dermal and epidermal culture cells for diabetic foot ulcers. Cell Tissue Bank 2012; 13: 241-249.
[73] Monami M, Vivarelli M, Desideri CM, Ippolito G, Marchionni N and Mannucci E. Autologous skin fibroblast and keratinocyte grafts in the treatment of chronic foot ulcers in aging type 2 diabetic patients. J Am Podiatr Med Assoc 2011; 101: 55-58.
[74] Bayram Y, Deveci M, Imirzalioglu N, Soysal Y and Sengezer M. The cell based dressing with living allogenic keratinocytes in the treatment of foot ulcers: a case study. Br J Plast Surg 2005; 58: 988-996.
[75] Moustafa M, Bullock AJ, Creagh FM, Heller S, Jeffcoate W, Game F, Amery C, Tesfaye S, Ince Z, Haddow DB and MacNeil S. Randomized, controlled, single-blind study on use of autologous keratinocytes on a transfer dressing to treat nonhealing diabetic ulcers. Regen Med 2007; 2: 887-902.
[76] Ojeh N, Pastar I, Tomic-Canic M and Stojadinovic O. Stem cells in skin regeneration, wound healing, and their clinical applications. Int J Mol Sci 2015; 16: 25476-25501.
[77] Mcheik JN, Barrault C, Levard G, Morel F, Bernard FX and Lecron JC. Epidermal healing in burns: autologous keratinocyte transplantation as a standard procedure: update and perspective. Plast Reconstr Surg Glob Open 2014; 2: e218.
[78] Hwang YG, Lee JW, Park KH and Han SH. Allogeneic keratinocyte for intractable chronic diabetic foot ulcers: a prospective observational study. Int Wound J 2019; 16: 486-491.
[79] Uluer E, Vatansever H, Aydede H and Ozbilgin M. Keratinocytes derived from embryonic stem cells induce wound healing in mice. Biotech Histochem 2019; 94: 189-198.
[80] Lev-Tov H, Li CS, Dahle S and Isseroff RR. Cellular versus acellular matrix devices in treatment of diabetic foot ulcers: study protocol for a comparative efficacy randomized controlled trial. Trials 2013; 14: 8.