Int J Clin Exp Med 2015;8(1):897-903
www.ijcem.com /ISSN:1940-5901/IJCEM0003935

Original Article

Wound bed preparation for ischemic diabetic foot ulcer

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Received November 18, 2014; Accepted January 9, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: Objective: This study is to evaluate the effect of allograft skin on wound angiogenesis and wound bed preparation of ischemic diabetic foot ulcer. Methods: A total of 60 cases of patients with diabetic foot ulcer were randomly divided into the experimental group (n = 30) and the control group (n = 30). After debridement, in the experimental group, allograft skin was used to cover the wound while in the control group, vaseline and gauze was used to cover the wound. The wound was opened and dressed at 3, 5, 7, 14 days after operation and the growth condition of the granulation tissue was observed and recorded. Results: The wound bed preparation time of the experimental group was 14.37 ± 1.06 days, compared with the control group 25.99 ± 4.03 days, there was statistically significant difference (t = 14.78, $P < 0.0001$). The mean cure time of the experimental group was 32 ± 1.93 days and this time was significantly shortened than the control group 39.73 ± 2.55 days (t = 12.521, $P < 0.01$). Conclusions: Allogeneic skin has a protective effect on the wound and could promote vascularization of ischemic diabetic foot ulcer and shorten the wound bed preparation time and treatment cycle.

Keywords: Diabetic foot ulcers, lower limb ischemic disease, wound bed preparation

Introduction

The occurrence rate of diabetic foot in diabetes patients is approximately 1-4% [1, 2]. The amputation rate of diabetes patients with diabetic foot is 25 times higher than that of normal diabetes patients [3]. Diabetic foot is a complication induced by many factors. Among these factors, peripheral vascular disease, peripheral neuropathy and infections are critical factors for the pathogenesis of diabetic foot. These three pathological changes occur in about 15% of the patients with diabetes mellitus [4, 5]. Peripheral neuropathy is the indirect result of peripheral vascular disease, and more than 50% of diabetic foot ulcers are caused by peripheral neuropathy, minor injury and foot deformity [6].

The inadequate vascular perfusion in the lower limb is the main reason causing diabetic foot ulcer hard to heal [7]. And, angiogenesis disorder and loss of function of microvessel in the wound are currently considered to be the main factors causing the ischemic diabetic ulcer wound hard to heal [8-10]. Thus, to improve wound healing of diabetic foot ulcer, it is very important to restore the function and structure of vessel and to improve formation of microvessel. During surgical debridement, not only the necrotic tissues but also the bacterial, necrotic and cellular loads [11, 12] should be removed to maintain the wounds in a relatively closed, moist and healing-favorable environment and to maintain the state of debridement. After surgical debridement, the wound in diabetic foot ulcer is in the ready state for healing [13].

Malign formation of collagen, delay in the epithelial migration and inflammatory response might be the pathological basis of abnormal ulcer wound healing. Because the diabetic foot ulcer does not heal for a long time, many bacteria proliferate in the wound. And, most of these bacteria are multi-drug resistant strains. In addition, the vascular perfusion in the lower limb is inadequate. Therefore, it is difficult for drug to achieve effective antiseptic concentration in the wound, even if the systemic antibiotics are used. How to promote the diabetic foot ulcer wound to grow into the granulation wound (which is appropriate for operation) and how to prevent secondary and progressive necrosis are the major problems to be solved in clinical treatments.

Table 1. Comparison of general data of the experimental group and the control group (d, cm^2, cm, $\bar{x} \pm s$)

Group	Cases	Duration of ulcer (d)	The size of the wound (cm^2)	Depth of the wound (cm)
Experimental group	30	57.86 ± 19.40	14.63 ± 4.62	1.40 ± 0.27
Control group	30	53.96 ± 34.80	17.27 ± 7.29	1.39 ± 0.43

The standard therapy of diabetic foot ulcer is as follows. The vascular status is assessed after measuring ulcer size and depth. The blood glucose should be effectively controlled. After broad and radical debridement, antibiotics should be used under the guidance of bacterial culture and the moist wound dressings which provide a barrier against bacteria and absorb fluid should be used. It is also important to reduce the wound compression. However, even with the standard treatment, many patients still cannot be cured. Especially in diabetic patients combined with lower extremity arterial occlusive disease, the wound is more difficult to heal. In recent years, multiple tissue engineering dressings used to repair burn wound are applied to diabetic foot ulcer wound, such as Integra, Apligraf and Dermagraft. It has been reported that these dressings have good clinical treatment effect on the deep wounds and refractory wounds without obvious toxic side effect [14-16]. However, these products eventually have been eliminated by clinical application because that they just temporarily cover the wounds and can not play a guiding role in epithelial proliferation. They can not completely close the wounds and the price is expensive, as a result, the clinical application is limited [17].

Diabetic foot ulcers and deep burn wounds have some similarities. In the treatment of deep burn, allograft skin after escharectomy is often used to cover the wound for wound bed preparation. Therefore, in this study, we used the method for deep burn ulcer to treat ischemic diabetic foot ulcer. The chronic infectious wound of the ischemic diabetic foot ulcer was changed to acute and relatively clean wound by surgical debridement. Allogeneic skin was used for wound bed preparation and a second procedure was performed for complete wound closure.

Materials and methods

Patients

In total, 60 diabetic patients with diabetic foot ulcers who were admitted in our department during the period of March 2010 to March 2013 were included in this study. Among them, 41 patients were male and 19 patients were female. Their age ranged from 42 to 83 (mean 67.12 ± 2.65 years). The duration of diabetes lasted for 5-20 years (mean 7.52 ± 1.33 years). The duration of ulcers (gangrene) was 10-180 days. The fasting fingertip blood glucose was determined by One-Touch blood glucose meter (Johnson & Johnson, Rochester, NY, USA) and the fasting fingertip blood sugar level was 7.7-28.3 mmol/L. Some patients were complicated with diabetic nephropathy, retinopathy and cardiovascular disease (such as coronary heart disease, hypertension and cerebral infarction). Patients who were diagnosed as diabetic foot according to the diagnostic criteria were included. The exclusion criteria were as follows: patients who died during the treatment; patients those with extensive and complete necrosis in the body and needed immediate amputee at the time they were admitted to hospital; patients who automatically discharged or gave up on therapy; patients who were with serious complications and unfit for surgical treatment. There was no significant differences in the ulcer duration (t = 0.462, $P = 0.642$), the wound size ($t = 1.798$, $P = 0.083$) and the wound depth ($t = 0.046$, $P = 0.964$) in the patients in both groups (**Table 1**).

Grouping

According to the different methods of treatment, 30 cases treated with allogeneic skin for wound bed preparation were considered as the experimental group and 30 cases treated with conventional surgical therapy were considered as control group. Surgical debridement was performed at the black stage of the diabetic foot ulcer wound. The necrotic tissue and eschar was removed and the parabiotic tissue, vessels, nerves and tendons as a support of late repair was retained as much as possible. In the experimental group, allograft skin was used to cover the wound while in the control group, vaseline and gauze was used to cover the wound. Allogeneic skin was from abandoned

Table 2. Growth degree of granulation tissue in the experimental group and the control group (%, $\bar{x} \pm s$)

Group	Cases	Growth degree of granulation tissue (1%)			
		3 d post operation	5 d post operation	7 d post operation	14 d post operation
Experimental group	30	8.20 ± 0.28	13.63 ± 1.33	31.26 ± 1.79	82.06 ± 2.34
Control group	30	7.98 ± 0.18	12.83 ± 0.44	20.15 ± 2.03*	69.74 ± 2.35*

Note: Compared with the control group, *$P < 0.01$.

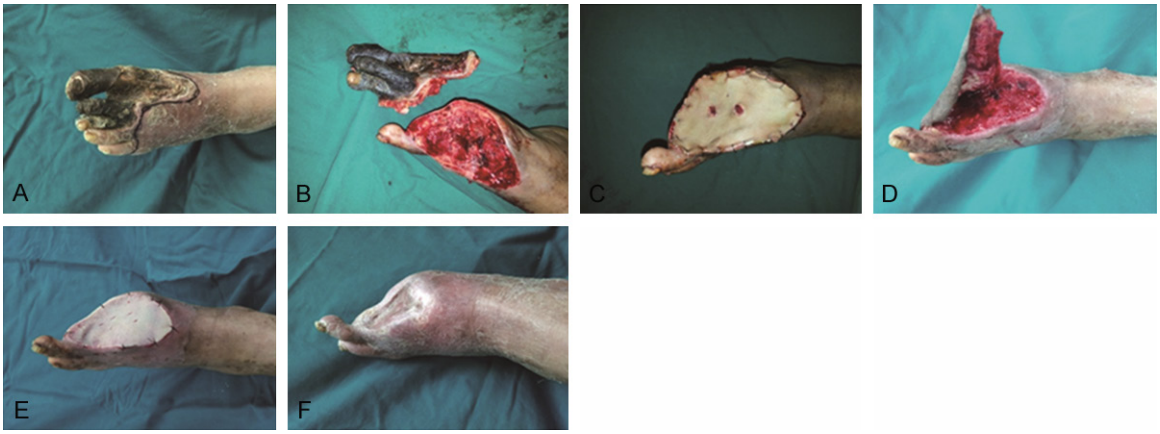

Figure 1. Observation of diabetic foot in the experimental group treated with allograft skin. A. A severe ischemic diabetic foot ulcer with several ischemic necrotic toes. B. After debridement, the necrosis toes were removed. C. Wound coverage with skin allografts. D. After 17 days of allogenic skin covering, the granulation tissue grew well and wound bed was perfectly suited for operation. E. Wound covered by transplantation of autologous skin. F. Following up 1 year after operation.

acute lower extremity traumatic amputation of limbs. The donors had no acute and chronic diseases, no malignant tumors or other diseases. Prior written informed consent was obtained from all patients and the donors before undergoing the examination. The study protocol was approved by the ethical committee of People's Hospital of Xinjiang Uygur Autonomous Region. Split-thickness skin graft was harvested with drum-type hand-operating dermatome from allogeneic full-thickness skin, immediately snap-frozen in liquid nitrogen, and stored at -80°C until use.

Wound treatment

Surgical debridement was performed in diabetic foot ulcer wound at the black stage in the patients of the experimental group. Necrotic tissue and eschar were removed. Drainage was performed effectively. As a support for late repairing, the tendon and nerve those were without obvious liquefactive necrosis were reserved and ecological soft tissue were remained as more as possible. After debridement, the wound was cleaned and covered with allograft skin. Several holes were made on the allograft skin for drainage. The wound were protected by sterile accessories. The body temperature, the hemogram and the local wound changes were observed after surgery. At 3 days, 5 days, 7 days and 14 days after operation, the granulation tissue growth was observed and recorded while changing the dressing. In the control group, the wounds were covered with vaseline gauze after surgical debridement. The dressing was changed 1-2 times daily, and the growth of the granulation tissue was observed and recorded at the same time as the experimental group.

Evaluation of the growth of the granulation tissue

Before treatment, the original ulcer wound size of both groups was recorded with a transparent tracing paper. At 3 days, 5 days, 7 days and 14 days after treatment, the wound size was recorded with the same method. The growth of the granulation tissue was calculated using

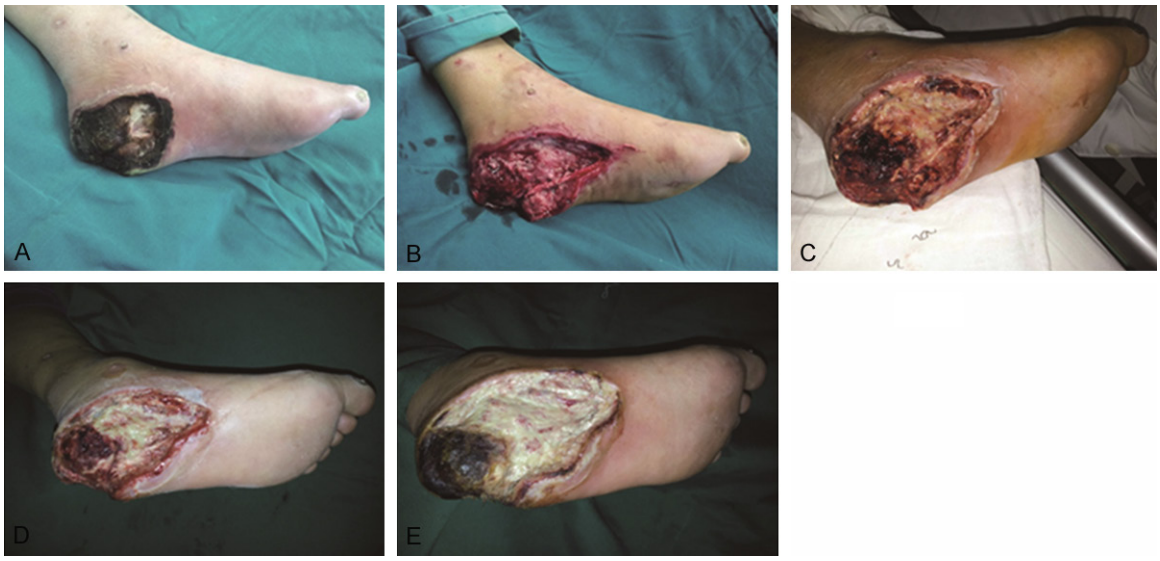

Figure 2. Observation of diabetic foot in the control group treated with vaseline and gauze. A. A severe ischemic diabetic foot ulcer in the heel of the left foot. This ulcer lasted for more than 1 month. Necrotic tissues, purulent secretion, cacosmia and swelling were observed around the ulcer wound. B. After debridement, the necrotic tissues were removed. The parabiotic soft tissues were retained. After washing with 3% H_2O_2 and normal saline, the ulcer wound was covered with vaseline and gauze. And, the vaseline and gauze was changed once a day. C. Seven days after treatment, there were still necrotic tissues in the wound and no obvious granulation tissue was observed. D. Fourteen days after treatment, necrotic tissues did not fall off. Little obvious granulation tissue was observed. E. Twenty-one days after treatment, little obvious granulation tissue was observed. The wound was progressively deepened. The calcaneus was dehydrated, darkened and necrotic. Amputation was performed.

Table 3. Comparison of the healing time after skin allografts covering the ulcer (days, $\bar{x} \pm s$)

Group	Cases	Duration of ulcer (day)	Size of wound (cm^2)
Experimental group	30	14.37 ± 1.06	32.00 ± 1.93
Control group	30	25.99 ± 4.03*	39.73 ± 2.55*

Note: Compared with the control group, *$P < 0.01$.

image analysis software (Olympus Stream, Germany). The formula is as follows: (the original wound size-the wound size that without granulation coverage)/the original wound size × 100% [18]. For the purpose of wound healing, wound closure was achieved by autologous skin grafting under good condition of the wound bed preparation.

CT angiography

CT angiography was performed as previously described [19]. Briefly, the contrast material was IV injected through a 22-gauge antecubital angiocatheter by using a power injector. All patients received a timing run with routine clinical GE Hi-Speed or GE CTI single detector scanners (General Electric, Milwaukee, WI). Image was reconstructed by using a soft-tissue kernel and 180-degree linear interpolation.

Statistical analysis

The data was expressed as $\bar{x} \pm s$. All the statistical analyses were performed using SPSS version 10.0 (SPSS Inc, Chicago, IL, USA) for Windows. The t test was performed and $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of the granulation degree

To determine the effect of allograft transplantation on granulation tissue, we investigated the growth of granulation tissue. The results were shown in **Table 2**. Seven days after allograft transplantation, there was statistically significant difference in the growth of granulation tissue between the experimental group and the control group ($P < 0.05$). The difference was more significant at 14 days after transplantation ($P < 0.05$). The general conditions of granulation tissue in the experimental group and the

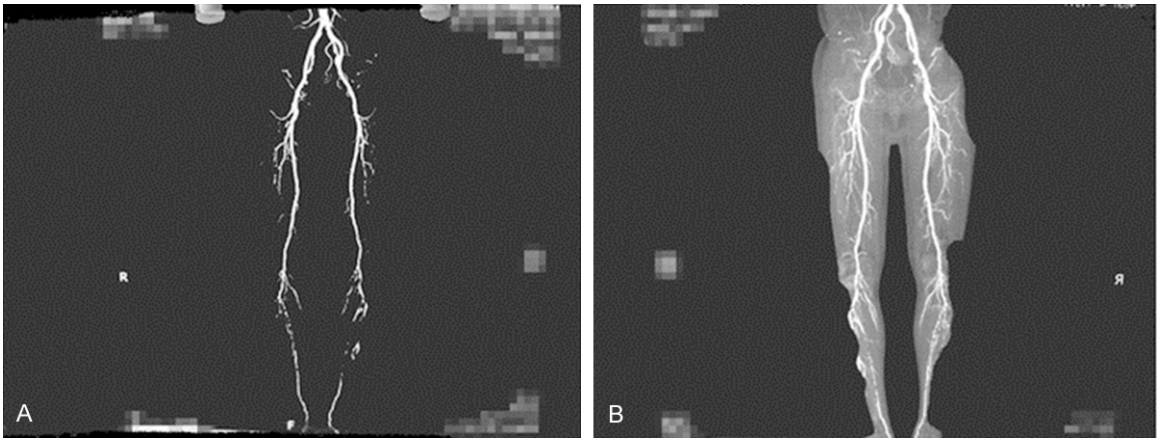

Figure 3. CT angiography imaging of the lower limbs in both groups. A. Representative CT angiography image of the experiment group showing severe stenosis in the distal end of the left popliteal artery and occlusion in the anterior tibial artery, the peroneal artery, and the posterior tibial artery. B. Representative CT angiography image of the control group showing occlusion in the knee artery, the anterior tibial artery, the distal end of the peroneal artery and the posterior tibial artery.

control group were shown in **Figures 1** and **2** respectively. The granulation tissue of the experimental group was in a bright red color, full of particles and easily to bleed when touched, which indicating that the ulcer wound was suitable for transplantation of autologous skin graft. The granulation tissue of the control group, however, was in gray color, accompanied by necrotic tissue and with further expansion of local infection. This result indicates that allogeneic skin could effectively maintain the diabetic foot ulcer wound in a relatively closed and humid environment, which is conducive to the growth of granulation tissue, thus laying the foundation for the later repair.

Comparison of the wound healing time

To compare the treatment effect, we compared the healing time in both groups, and the shorter the healing time the better the treatment effect. In this study, patients those received the transplantation of allogeneic skin showed good safety and tolerability, with normal hemogram and body temperature. Meanwhile, no related discomfort and complications appeared. In the experimental group, the wound bed preparation time after allograft skin was 14.37 ± 1.06 days. There was statistically significant difference ($t = 14.78$, $P < 0.0001$) when compared with the control group (25.99 ± 4.03 days). After the allograft was removed, fresh granulation tissue with intensive bleeding points appeared. The fresh granulation tissue may provide host bed for transplantation of autologous skin and may greatly improve the success rate of the operation. Compared with the control group (39.73 ± 2.55 cm^2), the wound size (32 ± 1.93 cm^2) of the experimental group (32 ± 1.93 cm^2) was significantly reduced. There was significant difference ($t = 12.521$, $P < 0.01$) between the two groups (**Table 3**). The conditions of blood vessels in the lower limbs of both groups were examined by CT angiography. As shown in **Figure 3A**, in the experiment group, there was severe stenosis in the distal end of the left popliteal artery and occlusion in the anterior tibial artery, the peroneal artery, and the posterior tibial artery. As shown in **Figure 3B**, occlusion in the knee artery, the anterior tibial artery, the distal end of the peroneal artery and the posterior tibial artery was also observed in the control group. There was no difference in condition of the blood vessel between the two groups. These results indicated that allogeneic skin covering the ischemic diabetic foot ulcers could prevent wound necrosis, promote granulation tissue growth, shorten the wound bed preparation time and accelerate the ulcer healing time.

Discussion

In the black stage of diabetic foot ulcer, reducing blood glucose, improving microcirculation, anti infection and adequate drainage are the main measures taken to promote the wound healing. Additionally, to maximize the preserva-

tion of the limb function, excessive debridement operation to remove necrotic tissue is avoided. The parabiotic soft tissues are usually kept so as to provide a basis for diabetic foot ulcer wound repair. In this study, allograft skin transplant was implanted instantly after debridement, making the wound temporarily closed and providing a relatively humid and suitable environment for wound repairing. The necrotic tissues that were not completely removed during surgical debridement quickly dissolved and peeled off. Thus, under this environment, the granulation tissue grew rapidly, and the "biological debridement" effect was achieved. Excessive debridement was avoided to provide a support for late repair in case of unnecessary difficulties. The tendon and nerve those without obvious liquefactive necrosis were reserved and parabiotic soft tissues were remained as more as possible. From the results of this study, 7 days after allograft transplantation, there was statistically significant difference in the growth of granulation tissue between the experimental group and the control group ($P < 0.05$). The difference was more significant at 14 days after transplantation ($P < 0.05$).

There are a variety of moist wound repair material [20, 21] and tissue engineering materials such as Integra, Dermagrft and Apligraf used in diabetic foot ulcer wound, however, using allogeneic skin to cover the wound is still currently the most ideal method. With the allogeneic skin directly covering the wound, bacteria are isolated, thus reducing the rate of bacterial infection. On the other hand, the local wound environment is changed, forming a micro environment with increased acidity and temperature. This environment is harmful to the growth and proliferation of the bacteria. And the ability of macrophage cells to prevent bacterial infection is enhanced. Therefore, the necrosis range of the wound is under control and secondary necrosis of the wound is reduced. For large ulcer (> 5% body surface area), more water, electrolyte and nutrients will lose from the wound, causing an adverse effect on the internal environment of the organism. Wound coverage with allogeneic skin can effectively solve this problem.

Acknowledgements

This work was supported by Natural Science Foundation of Xinjiang Uygur Autonomous Region. (NO. 2012211A090).

Disclosure of conflict of interest

None.

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