

# Combination therapy of trichloroacetic acid, human autologous fibroblast injection and fibroblast seeded microfibrinous collagen scaffold as a novel treatment for osteomyelitis diabetic foot ulcer

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## Keywords

Autologous fibroblast, Diabetic foot ulcer, Trichloroacetic acid

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## ABSTRACT

A severe complication associated with diabetes is diabetic foot ulcer (DFU). Most patients with DFU require amputation. Although treatment of non-healing diabetic ulcers is challenging, the use of novel therapies can be effective. In this report, we present the case of a woman with type 2 diabetes with DFU-related osteomyelitis, who was treated with a combination therapy of trichloroacetic acid, calcium alginate and foam dressings, human autologous fibroblast injection, and a fibroblast cell-seeded collagen scaffold. The results showed the positive effects of combination therapy on DFU. In the initial treatment, the wound area was measured to be 14 × 7 cm<sup>2</sup>, with a depth of 4 cm. After 6 months, the wound was measured to be 1.5 cm<sup>2</sup>, showing a 90% reduction of the wound area. Overall, this combination therapy was highly effective in the treatment of DFU-related osteomyelitis, and could markedly prevent amputation among DFU patients.

## INTRODUCTION

Diabetic foot ulcer (DFU) is a common chronic complication of diabetes. DFU and diabetic nephropathy are important factors in the development of foot ulcers and lower limb amputations<sup>1</sup>. DFU is considered a major problem for the scientific community, as it affects the patients' quality of life<sup>2</sup>. Amputation of a diabetic foot is associated with the increased risk of recurrent distal sole ulcers. It is known that ulceration can increase infection and osteomyelitis risk, and might cause progressive tissue damage.

Although various treatment strategies have been described for DFU, finding an effective prophylactic treatment is challenging<sup>3,4</sup>. Therefore, it is necessary to develop novel therapeutic

approaches to improve the treatment of DFUs. Here, we describe the successful treatment of a patient with DFU, using trichloroacetic acid (TCA), calcium alginate and foam dressings, human autologous fibroblast injection, and a fibroblast-seeded microfibrinous collagen scaffold as a new effective combination therapy for DFU with osteomyelitis.

## CASE PRESENTATION

The patient was a 43-year-old woman with a 10-year history of type 2 diabetes and insulin dependence. The patient's characteristics are shown in the Table 1. She had first developed a small foot ulcer (0.2 × 0.5 cm) on the lateral side of the right lower foot. After 1 month, the ulcer had progressed, and she presented to another hospital with complaints of a progressively worsening infection of the foot ulcer, swelling, exudation and pus moss on or surrounding the wound site, and tissue pain

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(body temperature 39.5°C). The results of magnetic resonance imaging confirmed osteomyelitis because of a non-healing ulcer. Accordingly, she received adequate antidiabetic treatment. Therapy with intravenous antibiotics (ciprofloxacin and vancomycin), offloading and surgical debridement was carried out on the patient's right foot, and then, wound management was initiated. However, the wound aggravated, and amputation was proposed by the orthopedist.

The patient presented to our Skin and Stem Cell Research Center, Tehran, Iran, after having a non-healing DFU (Wagner grade II) for 2 months<sup>5</sup>. Baseline measurements were carried out for the patient before the intervention (Table 1). However, the results were unremarkable, except for blood glucose (glycated hemoglobin 11%; fasting blood glucose 170 mg/dL). The blood glucose was controlled during the study. No evidence of diabetic nephropathy was observed in the patient laboratory tests (Table 1). Also, no clinical retinopathy and neuropathy were observed (fundus status, Achilles tendon reflex and vibration thresholds were normal). The patient did not have any drug allergies, and did not consume alcohol or smoke tobacco.

To participate in the present study, the patient signed written informed consent, and then her information was recorded.

Combination therapy was initiated for the patient in our center. The wound area was washed with a normal saline solvent, and wound debridement was carried out using a Bisturi No. 12 blade. After disinfecting the ulcer, 70% TCA solution was applied to the ulcer, and 50% TCA was applied around the

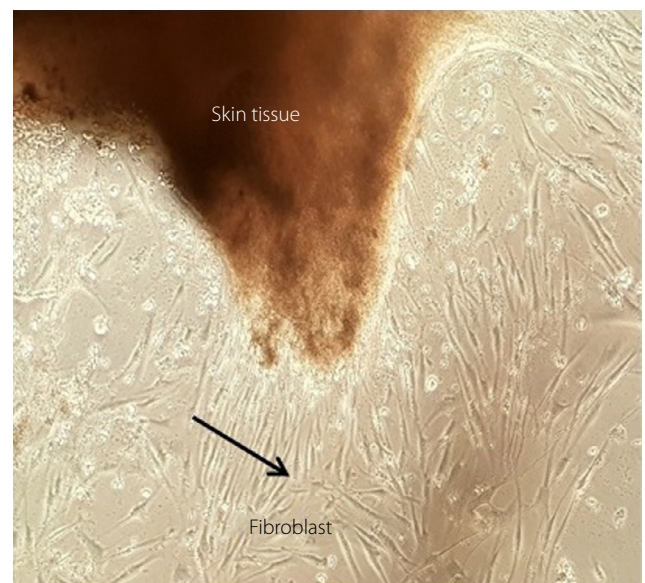
ulcer area with a cotton applicator. Next, the wound area was covered with calcium alginate (Coloplast A/S, Humlebaek, Denmark) and foam (HydroTac; Hartmann, Germany) dressings. The patient received a suitable antibiotic (ciprofloxacin) to control the infection. Also, her blood glucose was controlled, pressure on the ulcer was reduced and preparation programs were applied to control the ulcer; this procedure continued for 80 sessions (three times a week). For preparation of autologous fibroblasts, a punch biopsy (6 mm<sup>2</sup>) of the skin behind the ear was taken and used to obtain skin and blood samples. The samples were transferred to the cell culture laboratory of the Skin and Stem Cell Research Center to culture autologous fibroblasts. In the cell culture laboratory, fibroblasts were cultured after preparation and culturing. After 1 month of skin biopsy explant culture,  $4 \times 10^7$  fibroblasts were extracted (Figure 1).

After 3 weeks of treatment, the wound was prepared for fibroblast cell injection. The ulcer was washed with normal saline and debrided (TCA was not used in this session). Then,  $5 \times 10^5$  cells/cm<sup>2</sup> of wound periphery were injected into the ulcer, and a microfibrillar collagen dressing, seeded with  $1 \times 10^4$  cells/cm<sup>2</sup>, was administered on the hollow parts of the ulcer. Finally, the wound area was covered by calcium alginate and foam dressings. Treatment with autologous fibroblast cells was repeated three times at 3-week intervals. To avoid infection, ciprofloxacin was given. After fibroblast injection, treatment continued, as previously described.

In the final 10 sessions, a collagen scaffold, foam dressing and a honey alginate dressing (Medihoney, USA) were used. After treatment, the length, width and depth of the ulcer were examined monthly (Table 2). Re-epithelialization, as well as

**Table 1** | Baseline characteristics of the patient

Characteristic	
Age (years)	43
Sex	Female
Height (cm)	172
Bodyweight (kg)	92
History of diabetes (years)	10
Treatment method	Insulin
Diabetic complications	Foot deformity
Healthcare utilization	
Hemoglobin A1C (%)	11
Fasting blood glucose (mg/dL)	170
Systolic blood pressure (mmHg)	129.67
Diastolic blood pressure (mmHg)	81.33
Total cholesterol (mg/dL)	163
LDL cholesterol (mg/dL)	89
Thyroid hormone	Normal
Nephropathy status	
Serum creatinine (mg/dL)	1.4
Estimated glomerular filtration rate	93
Albumin-to-creatinine ratio	24
Blood urea nitrogen (mg/dL)	10
Urine protein	Negative
Urine red blood cell	Negative
Retinopathy status	No clinical
Neuropathy status	No clinical



**Figure 1** | Autologous fibroblast cell culture.

**Table 2** | Diabetic foot ulcer size before and after treatment

Patient's ulcer	Before treatment	Month(s) after treatment						
		1	2	3	4	5	6	7
Area (length × width)	14 × 7	12 × 6	10 × 5	7 × 4	4 × 3	3 × 2	1.5 × 1	0 × 0
Depth	5.0	4.0	3.0	2.0	1.5	0.5	0.0	0.0

The values are presented as cm.



**Figure 2** | (a) The patient before treatment. The patient (b) 1 month, (c) 2 months and (d) 3 months after treatment.

reduced ulcer depth and surface, was detected. As shown in Figures 2 and 3, the ulcer was treated after 80 sessions of combination therapy.

## DISCUSSION

DFU, as an acute complication of diabetes, is the main cause of recurrent hospitalization and amputation among diabetes patients. Evidently, amputation imposes a significant burden on patients. However, available treatments for DFU are often inadequate<sup>6</sup>. Therefore, the current study aimed to evaluate the efficacy and safety of a combination therapy with TCA, autologous fibroblast cell injection, a collagen scaffold, and calcium alginate and foam dressings (as a new type of dressing) in DFU treatment.

TCA is a chemical agent, which has recently been used to treat recalcitrant DFU. TCA might accelerate the process of cell regeneration after 2 weeks<sup>7</sup>. Evidence shows that higher TCA concentrations penetrate into the mid-reticular dermis. The skin

stress response system is induced by TCA peel as a result of reconstitution of the epidermis and dermis through wound healing processes. The mechanism of action of TCA involves precipitation of proteins and coagulative necrosis of the epidermis and/or papillary dermis, resulting in the shedding of necrotic layers and reepithelization, followed by dermal collagen remodeling<sup>8</sup>.

In a previous study, the efficacy of TCA in the treatment of recalcitrant DFU was evaluated. All wounds significantly improved in six treated patients after  $7.5 \pm 0.83$  weeks, and the wound areas and infection scores reduced. In that study, 68.6–100% improvements were reported for the efficacy of treatment for patients, without any complications after 3 months of follow-up<sup>3</sup>.

In another study, TCA 80% was used in a chemical debridement technique for chronic venous leg ulcers. It was found that use of an 80% TCA solution is a simple, cost-



**Figure 3** | (a) The patient 4 months, (b) 5 months, (c) 6 months and (d) 7 months after treatment. As the figures show, the diabetic foot ulcer was treated.

effective and noticeably less painful technique for venous leg ulcers<sup>9</sup>.

Cellular therapy is an interesting method in regenerative medicine, which uses self-renewing cells. Researchers have used direct systemic administration, local intramuscular injection and direct application on the wound area to transport cells in cellular therapy<sup>10</sup>. Intramuscular injection into the wound periphery was introduced as a safer and more efficient method, compared with systemic administration in clinical studies<sup>10</sup>. Recently, stem cells, such as fibroblasts, either as scaffolds or suspensions, have been applied to treat ischemic DFUs. Possible mechanisms of stem cell therapy include paracrine secretion of cytokines and growth factors, their direct differentiation into vascular endothelial cells, and formation of new vessels and skin components, which ultimately result in ulcer healing<sup>11</sup>.

In the present study, we carried out peripheral injection of fibroblasts into the ulcer and observed a decrease in the wound size. In this regard, a randomized clinical trial also showed that improvement was significantly higher in the group with fibroblasts, compared with the control group<sup>12</sup>.

Studies on the efficacy of fibroblast injection for DFU have shown that the morphology of ulcers alters during the healing process, and their proliferative ability is reduced. Overall, the use of fibroblasts for the treatment of chronic and acute ulcers releases pain, and accelerates the healing process by reducing scars<sup>4,7</sup>.

The effect of autologous fibroblast suspension was evaluated on refractory DFU treatment<sup>4</sup>. Furthermore, in a study by Nilforoushzhadeh *et al.*<sup>3,7</sup>, the effects of TCA and fibroblasts were investigated on recalcitrant DFU. They reported that the application of fibroblasts is an effective therapeutic approach for recalcitrant DFUs. In another study, topical TCA application, along with autologous fibroblast injection, was used for the treatment of electrical burn ulcers, and satisfactory results were reported<sup>13</sup>. In addition, in a study by Mansbridge *et al.*<sup>14</sup>, three-dimensional fibroblast culture implant was used for DFU treatment. The results showed that the therapeutic properties of dermal implants are majorly dependent on the recovery of growth factor expression, protein synthesis and angiogenesis, based on metabolic activity.

In the present study, we used a fibroblast-seeded collagen scaffold in combination with other treatments. In this regard, Cavallini *et al.*<sup>15</sup> used a mesh of *in vitro* expanded autologous fibroblasts as covering for 10 diabetes patients with profoundly infected leg or foot ulcers with major loss of substance to accelerate the healing of ulcers. They found that this technique is an acceptable option for the treatment of large leg ulcers and can be mainly used for patients with chronic diseases, such as diabetes<sup>15</sup>.

Also, in a randomized controlled trial by You *et al.*<sup>16</sup>, DFU patients were treated with grafting of an autologous fibroblast-hyaluronic acid complex, which was protected with a polyurethane foam dressing (as a secondary dressing); a control group, treated with a non-adherent foam dressing, was also

included in that study. Their results showed that the autologous fibroblast-hyaluronic acid complex could be a safe and effective method for DFU treatment<sup>16</sup>.

Furthermore, in the present study, we used calcium alginate and foam dressings. Alginate and foam dressings can be helpful in the dissolution of fibrin and necrotic tissues, preserving the temperature of the wound surface, increasing the release of various growth factors, and enabling tissue growth without scar formation or mechanical damage to the new granulation tissue. Also, dressings protect the nerve endings in the ulcer and release pain.

In a study by Saco *et al.*, the efficacy of some new dressings, including alginate, foam, hydrofiber, hydrocolloid and hydrogel dressings, was evaluated in the treatment of DFUs. They reported that wound dressings could efficiently enhance wound healing in DFU patients and significantly reduce morbidity. Also, they found that non-adherent dressings were more cost-effective than hydrofiber dressings for DFUs<sup>17</sup>.

Recent research shows that wound dressings can accelerate the wound healing process of DFUs in patients<sup>18</sup>. Therefore, in the present study, we evaluated the effect of a combination therapy with TCA, calcium alginate and foam dressings, human autologous fibroblast injection, and a fibroblast-seeded collagen scaffold on DFUs. The synergistic effects of the combination therapy can be explained as follows. TCA removes the necrotic tissue, and it can induce re-epithelization and collagen reproduction, it can also increase cytokines, thus increasing the feeding of the wound. In the healing presses, fibroblast injection can decrease the wound surface area, and dressing can absorb the exudates. Alginate beads create an excellent immobilization material for human fibroblasts, with physiological activities and viability of the incorporated cells being preserved for times of up to 1 month.

To the best of our knowledge, this is the first study using a combination of TCA, calcium alginate and foam dressings, human autologous fibroblast injection, and a fibroblast-seeded collagen scaffold for the treatment of DFUs. The present results showed that this combination therapy could promote the healing of DFUs and prevent amputation in patients; therefore, it can be considered as a very effective method. We are going to carry out the pilot clinical study of this method for 14 diabetes patients with 6 months' treatment and 1-year follow up.

The present study had some limitations. The combination therapy of TCA, calcium alginate and foam dressing, human autologous fibroblast injection, and fibroblast seeded collagen scaffold is effective on DFU, but preparing autologous fibroblasts is time-consuming, difficult and very expensive because of clinical grade materials are used. Also, on a large scale, a very good cell bank is required. Although the present study was carried out for free, the cost of this therapy is high and the high cost can be a problem for patients. The preparation of the dressing was also difficult.

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## DISCLOSURE

The authors declare no conflict of interest.

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