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Therapeutic effect of continuous diffusion of oxygen therapy combined with traditional moist wound dressing therapy in the treatment of diabetic foot ulcers

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ABSTRACT

Aims: To investigate the therapeutic effect of continuous diffusion of oxygen (CDO) combined with traditional moist wound dressing (MWD) on the diabetic foot ulcers (DFUs) inpatients.

Methods: The inpatients from May 2016 to April 2018 were enrolled and randomly divided into MWD, CDO and combination treatment groups (n = 40 each group). The moist dressing was used in the MWD group while a micro-oxygen supply device was used in the CDO group. The combination treatment group was given both MWD and CDO. All patients were treated for 8 weeks. The wound healing, amputation rate, and inflammatory control were evaluated.

Results: Compared with MWD and CDO groups, the combination group showed a higher wound healing rate ($P < 0.05$), lower white blood cell count ($P < 0.05$) and lower high-sensitivity C-reactive protein level ($P < 0.05$). During 1-year follow-up, the amputation rate was 0% in combination group, which was significantly lower than that in other two groups ($P < 0.05$).

Conclusions: The combination of MWD and CDO was effective in promoting healing and preventing infection of DFUs, which holds a potential to be a new strategy for the treatment of this critical clinical condition.

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1. Introduction

Type 2 diabetes mellitus (T2DM) has emerged as a global health issue with a high prevalence around the world. A national survey in China found an estimated incidence of T2DM in adults of 9.7%, which corresponds to 92.4 million

patients [1]. Diabetic foot ulcers (DFUs) are common chronic, refractory complications of diabetes, which are also intractable healthcare concerns with features including long course, high medical cost, and potential risk of disability. Approximately, 12–25% of diabetes patients will develop DFUs during their lifetime, which affects patients' quality of life seriously

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and can even become life-threatening [2]. Therefore, therapeutic approaches for this critical condition have been continually sought in clinical practice.

The healthy clean wound heals under a 21% oxygen concentration, while the infectious wound heals best under 50% oxygen. Apart from systemic or local hyperbaric oxygen treatment, direct topical oxygen application may also create an oxygen-rich environment in the wound site. The physiological basis for this convenient procedure lies in the fact that the oxygen penetrates up to 700- μ m into the skin surface [3]. Studies have revealed that direct topical oxygen therapy for DFUs mitigates infection of wound, promotes the growth of granulation tissue, and accelerates epithelialization, which facilitates the early healing of wound, even for chronic, refractory ulcers [4,5]. Continuous diffusion of oxygen (CDO), a new form of topical oxygen therapy, uses a portable machine to extract oxygen from the air. Through an oxygen supply tubing, this technique pressurizes the target region of the wound continuously, delivers oxygen 24 h a day, and enables a moist and oxygen-rich environment for wound healing without limiting the patient's activities. Multiple studies have demonstrated that CDO therapy helps promote wound closure [6–8]. In the present study, we investigated the therapeutic effect of CDO therapy in combination with traditional moist wound dressing (MWD) for the treatment of DFUs.

2. Materials and methods

2.1. Patients and study design

This was a prospective, randomized, controlled, single-center study. The patients were enrolled according to the flowchart shown in Fig. 1. From May 2016 to April 2018, 122 diabetic patients with foot ulcers admitted to the First Affiliated Hospital of Guangdong Pharmaceutical University were consecutively recruited. Patients were eligible when they met the following criteria: type 2 diabetes mellitus; aged 50–85 years; Wagner ulcer grade 2–4; ulcer persisting for >1 month without any sign of healing; peripheral nerve sensory loss of lower limbs; local foot infection including redness, heat and pain; palpable pulses at either the dorsalis pedis artery or posterior tibial artery of the ankle; and willing to participate in the study and sign the consent form [9]. Patients with the following conditions were excluded: pregnancy or lactation; serious cardiovascular, liver, kidney, or neurological diseases or complications; abnormal coagulation function; advanced tumor; multisystem organ dysfunction or failure; and refusal to provide informed consent. The study protocol was reviewed and approved by the local ethics committee of the First Affiliated Hospital of Guangdong Pharmaceutical University.

Eligible inpatients were identified based on physical examination and review of medical records at a screening visit, and eligibility was further assessed based on general laboratory tests. Using the numerical table method, all patients were randomly divided into three groups: traditional MWD group (42 cases), CDO group (40 cases), and combination treatment group (40 cases). Two patients in the MWD group were lost

to follow-up, and therefore, the clinical information of 40 patients in each of the three groups was analyzed and compared.

2.2. Baseline processing

All patients with DFUs were managed according to a unified treatment protocol, including control of blood pressure, blood glucose, and lipid levels with intravenous infusion of alprostadil (10 μ g, Bid), treatment of peripheral neuropathy, nutritional support, and correction of hypoproteinemia and electrolyte imbalance. The off-loading therapy was carried out according to the guidelines developed by the International Working Group on the Diabetic Foot (IWGDF). The patients wore therapeutic footwear or custom-made footwear, insoles, or toe orthoses to reduce plantar pressure and mitigate or stop the development of foot ulcers [10]. Secretions from DFUs were collected for bacterial culture and antibiotic sensitivity tests [11]. Patients were empirically treated with antibiotics before the susceptibility test results were available. For instance, third-generation cephalosporins were used on the wounds of moderately or severely infected diabetic feet, and anti-anaerobic antibiotics were employed on malodorous diabetic foot wounds. The antibiotic choice was then refined based on the results of susceptibility tests.

The antibiotic treatments were maintained until 1–2 weeks after clinical signs and symptoms of infection being resolved. All diabetic foot wounds were managed according to a standard protocol by a team composed of trained physicians and specialist nurses. The wounds were debrided as early as possible to remove necrotic tissue, spare deep structures, and eliminate dead space, and then left open with adequate drainage. After debridement, the wounds were dressed with sterile antibacterial silver ion dressing and physiological saline-soaked dressing, with adequate drainage for 1 week.

2.3. DFUs' evaluation and wound care procedures

Prior to wound assessment and before each treatment application, DFUs were thoroughly cleaned with saline solution to remove debris and necrotic tissue and expose healthy tissue. Initial DFU evaluation included ulcer size measurement, microbial culture, DFU description, and clinical photography. Patients in the MWD group were treated with alginate dressing (Alginate, Coloplast, UK). In the CDO group, a micro-oxygen supply device (Greens O-4-3, developed by the Institute of Fuel Cells, Shanghai Jiao Tong University; Wuxi Guoying Technology Co., Ltd., China) was used to generate high-purity oxygen, which was continuously delivered to the center surface of the wound at a constant flow rate through oxygen connection tubing. The wound surface was covered with sterile gauze and coated with biological semi-permeable membrane (3 M Company, USA). Patients in the combination treatment group were treated with alginate dressing, continuous high-purity oxygen supplied by the micro-oxygen supply device, and its surface was covered with sterile gauze and coated with biological semi-permeable membrane.

Dressing changes were performed two or three times per week. All patients were ambulatory, and offloading devices used were preapproved by the clinical research coordinators

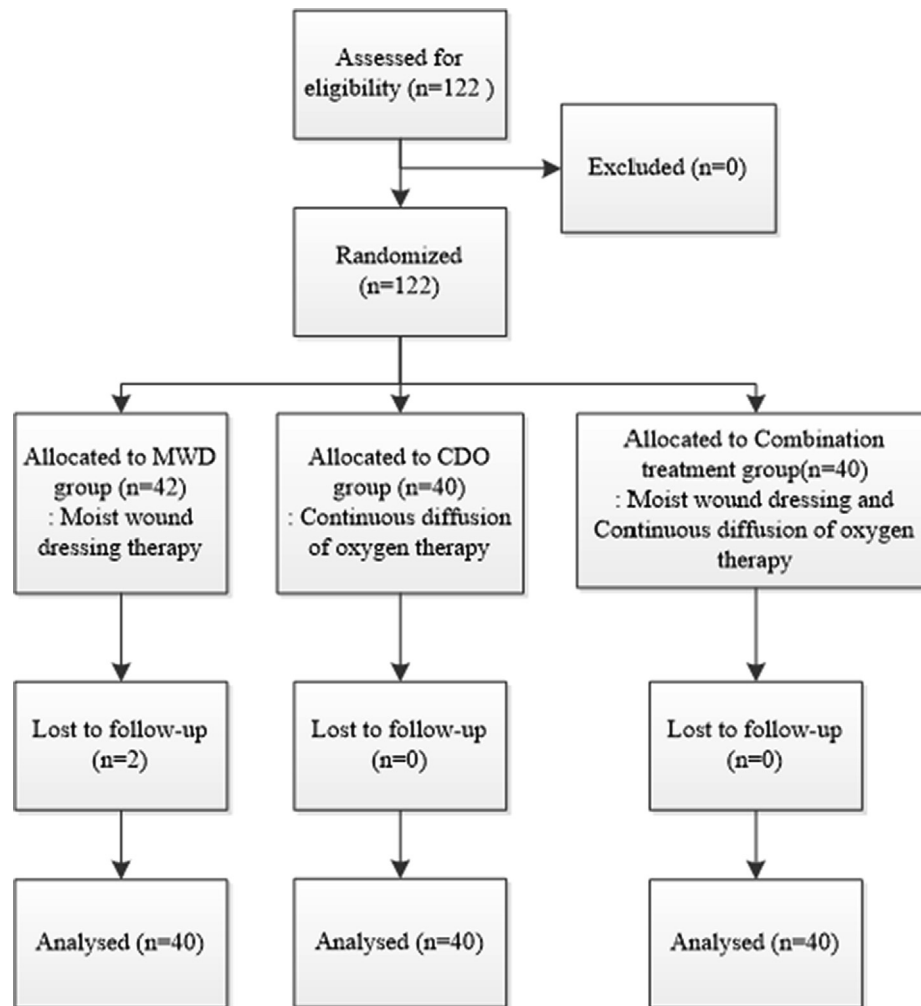


Fig. 1 – CONSORT flowchart for patient enrollment in the study. Abbreviations: CONSORT, consolidated standards of reporting trials.

and selected by the investigators at patient enrollment. Patients were informed the importance of wearing the offloading device.

2.4. Observation indicators and efficacy evaluation

The typical course of treatment for all groups was 8 weeks, unless a defined end point (complete wound healing, amputation, withdrawal from treatment, etc.) occurred sooner. Patients' data were recorded at admission (0 week) and 1, 2, 3, 4, 5, 6, 7, and 8 weeks after treatment. All patients were followed up for 1 year, with their wounds assessed and dressings changed every 7 days in our outpatient clinic.

Photographs of the wounds were taken at the initial diagnosis for three-dimensional measurements (length, width and depth). The subcutaneous sinuses were recorded using a clock method, and the wound reduction rate was calculated with the formula of $(W_0 - W_i)/W_0 \times 100\%$, where W_0 is the original area of the wound and W_i denotes wound area after treatment [12].

The wound exudate was evaluated and scored as follows: dry (0 point); moist (1 point); very moist/wet (2 points);

saturated wet dressing (3 points); and copious exudate extending beyond dressing (4 points). The appearance of tissue in the wound bed was assessed by trained physicians and specialist nurses and classified/scored as follows: black necrosis tissue (4 points), yellow tissue (3 points), red granulation tissue (2 points), and pink epithelial tissue (1 point). Inflammation control was evaluated by determining the white blood cell count and high-sensitivity C-reactive protein (hs-CRP) concentration in peripheral blood samples within 1 week after treatment [10].

The wound healing time was defined as the duration from the start of treatment to complete wound closure. The amputation rate was the proportion of amputees among all patients in the study during follow-up period. Therapeutic efficacy was evaluated 8 weeks after treatment based on the following clinical end points: 1) complete wound healing: the wounds in the foot area healed completely and the symptoms disappeared; 2) partial wound healing: the wound area of the treated foot was reduced by >75%; 3) improved: the wound area was reduced by 25%–75%; or 4) failure: the wound area was reduced by less than 25%. Any case that required amputation due to infection or recurrence of ulcer or that experienced

aggravation of pain indicated worsening infection. Increased wound area, treatment refusal or abandonment was classified as failure [13,14].

The wound healing rate was calculated 8 weeks after treatment, using the formula: wound healing rate = (number of wounds healed/total number of wounds) $\times$ 100%.

2.5. Statistical analysis

The SPSS for Windows version 22.0 software package (SPSS Inc., USA) was used for statistical data analysis. Quantitative data are expressed as mean $\pm$ standard deviation (SD) values. Differences among groups were analyzed by one-way analysis of variance (ANOVA), and the least significant difference (LSD) method was used for the comparison between two individual groups. Categorical variables were analyzed using the chi-squared test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Basic characteristics of the patients

A total of 122 individuals met the inclusion criteria and were randomly assigned to MWD ($n = 42$), CDO ($n = 40$) or the combination treatment group ($n = 40$). Overall, 120 (98.36%) patients completed the trial and 2 patients were lost to follow (Fig. 1).

Among the 120 enrolled patients, 24 males and 16 females were in the MWD group with an average age of 63.1 years, 25 males and 15 females in the CDO group with an average age of 62.7 years and 23 males and 17 females in the combination treatment group with an average age of 63.5 years. There were no statistically significant differences in age, gender distribu-

tion, Wagner's grade, disease duration, ulcer wound area, hemoglobin, serum albumin, and blood glucose among the three groups ($P > 0.05$). The demographic data of the patients are presented in Table 1.

3.2. Area, depth and area reduction rate of DFUs in the three groups

No significant differences were observed among the three groups in the baseline wound area and depth. After 8 weeks of treatment, the area and depth of wounds were smaller in the combination group as compared to those in the MWD and CDO groups ($P < 0.05$). Meanwhile, the area reduction rate after combination treatment was higher than that after single MWD or CDO treatment ($P < 0.05$) (see Table 2).

3.3. Wound inflammation control after 1 week of treatment

There was no statistical difference in the baseline white blood cell count and hs-CRP concentration among the three groups. The white blood cell count in the combined group was $(6.42 \pm 2.01) \times 10^9/L$ after 1 week of treatment, which was significantly lower than those in the MWD and CDO groups $[(11.78 \pm 4.53) \times 10^9/L$ and $(11.52 \pm 3.62) \times 10^9/L$, respectively, both $P < 0.05$; Table 3]. However, there was no significant difference in white blood cell count between the MWD and CDO groups ($t = 0.328$, $P = 1.0$).

After 1 week of treatment, the hs-CRP concentration was significantly lower in the combination treatment group (12.39 ± 4.32 mg/L), as compared to that in the MWD and CDO groups (66.42 ± 32.78 mg/L and 67.52 ± 25.62 mg/L, respectively, both $P < 0.05$; Table 3). No significant difference

Table 1 – Demographic and clinical data of T2DM patients in three groups.

	MWD group ($n = 40$)	CDO group ($n = 40$)	Combination treatment group ($n = 40$)	F or χ^2 [†]	P
Age, years	63.1 $\pm$ 9.3	62.7 $\pm$ 7.4	63.5 $\pm$ 10.1	0.079	0.924
Male, n (%)	24 (60.0)	25 (62.5)	23 (57.5)	0.208	0.901
Duration of diabetic foot ulcers, months	4.00 (3.03, 4.68)	4.00 (3.00, 5.00)	3.00 (2.00, 5.80)	1.210	0.546
Duration of diabetes, years	3.95 $\pm$ 0.88	4.17 $\pm$ 1.74	3.90 $\pm$ 2.09	0.317	0.729
Ulcer wound area, cm ²	42.07 $\pm$ 38.55	39.59 $\pm$ 23.36	35.35 $\pm$ 20.83	0.561	0.572
Hemoglobin, g/L	113.00 $\pm$ 12.89	110.00 $\pm$ 10.45	116.00 $\pm$ 11.23	2.690	0.072
Serum albumin, g/L	68.00 $\pm$ 9.06	70.00 $\pm$ 8.62	72.00 $\pm$ 8.67	2.073	0.130
Glycated hemoglobin, %	7.85 $\pm$ 1.23	8.03 $\pm$ 1.02	7.91 $\pm$ 1.17	0.257	0.774
Wagner's grade, n(%)				1.340	0.512
II	15 (37.5)	18 (45.0)	13 (32.5)		
III	25 (62.5)	22 (55.0)	27 (67.5)		
Area				1.566	0.815
Dorsalis pedis	17 (42.5)	16 (40.0)	16 (40.0)		
Heel	6 (15.0)	3 (7.5)	4 (10.0)		
Plantar	17 (42.5)	21 (52.5)	20 (50.0)		
Bacterial culture of wound secretions				0.800	0.670
(+)	22 (55.0)	18 (45.0)	20 (50.0)		
(-)	18 (45.0)	22 (55.0)	20 (50.0)		

[†] F values for quantitative variables, and χ^2 values for categorical variables.

was observed in the hs-CRP concentration between the MWD and CDO groups ($t = 0.204$, $P = 1.0$).

3.4. Comparison of wound healing time and amputation rate among groups

The average wound healing times were 76.42 ± 32.78 days in the MWD group, 75.52 ± 25.62 days in the CDO group, and 48.39 ± 13.32 days in the combination treatment group. Thus, combination treatment facilitated wound healing, reducing the duration significantly (both $P < 0.05$; Table 4).

During the 1-year follow-up, 6 (15%) and 5 (12.5%) patients in the MWD and CDO groups, respectively, underwent amputation. In contrast, no patients were treated by amputation in the combination treatment group (Table 4).

3.5. Comparison of clinical outcomes among the three groups after 12 weeks of treatment

Eight weeks of treatment did not lead to complete healing of DFU wounds. However, the combination treatment group exhibited a better clinical outcome than the other two groups, with a higher percentage of partially healing and no failure to heal (Table 5). After treatment for 12 weeks, DFU wounds were completely healed in 72.5% of patients ($n = 29$) in the combination treatment group (Table 5). In contrast, only 42.5% ($n = 17$) and 47.5% ($n = 19$) of patients in the MWD and CDO groups, respectively, had healed diabetic foot wounds. The difference was statistically significant between the combination treatment group and the other two groups ($P < 0.05$).

Table 2 – Area, depth and area reduction rate of diabetic foot ulcer wounds in the three groups.

Group	Area		Depth		Area reduction rate
	Baseline	8 weeks	Baseline	8 weeks	
MWD group ($n = 40$)	42.07 ± 38.55	19.25 ± 14.66	1.42 ± 0.91	0.68 ± 0.72	45.79 ± 33.21
CDO group ($n = 40$)	39.59 ± 23.36	19.96 ± 17.10	1.40 ± 0.89	0.67 ± 0.57	52.20 ± 26.60
Combination treatment group ($n = 40$)	35.35 ± 20.83	$12.91 \pm 8.05^{* \#}$	1.31 ± 0.81	$0.40 \pm 0.28^{* \#}$	$63.13 \pm 14.61^{* \#}$
F	0.561	3.159	0.164	3.354	4.561
P	0.572	0.046	0.849	0.038	0.012

* $P < 0.05$ vs. MWD group;

$P < 0.05$ vs. CDO group.

Table 3 – Comparison of wound inflammation control after 1 week of treatment (mean $\pm$ SD)

Group	Blood white cell count ($10^9/L$)		Serum hs-CRP (mg/L)	
	Baseline	1 week	Baseline	1 week
MWD group ($n = 40$)	12.58 ± 2.38	11.78 ± 4.53	28.29 ± 1.06	26.42 ± 2.78
CDO group ($n = 40$)	13.01 ± 1.62	11.52 ± 3.62	28.68 ± 0.89	17.52 ± 5.62
Combination treatment group ($n = 40$)	12.78 ± 1.58	$6.42 \pm 2.01^{* \#}$	27.87 ± 2.13	$12.39 \pm 4.32^{* \#}$
F	0.515	29.102	3.052	104.309
P	0.599	<0.01	0.051	<0.001

* $P < 0.05$ vs. MWD group.

$P < 0.05$ vs. CDO group.

Table 4 – Wound healing time and amputation rate among the three groups

Group	Wound healing time (days)	Amputation rate (%)
MWD group ($n = 40$)	76.42 ± 32.78	6 (15.0)
CDO group ($n = 40$)	75.52 ± 25.62	5 (12.5)
Combination treatment group ($n = 40$)	$48.39 \pm 13.32^{* \#}$	0 (0) $^{* \#}$
F or χ^2 [†]	15.957	6.205
P	<0.001	0.045

* $P < 0.05$ vs. MWD group.

$P < 0.05$ vs. CDO group.

[†] F values for quantitative variables, and χ^2 values for categorical variables.

Table 5 – Clinical outcomes of diabetic foot ulcer wounds among the three groups after 8 and 12 weeks of treatment [n (%)]

Time point and group	Completely healed	Partially healed	Improved	Failed
8 weeks				
MWD group (n = 40)	0	7 (17.5)	23 (57.5)	10 (25)
CDO group (n = 40)	0	7 (17.5)	24 (60.0)	9 (22.5)
Combination treatment group (n = 40)	0	9 (22.5)	31 (77.5)	0 (0)
χ^2		11.381 ^{*,#}	–	–
P		0.03	–	–
12 weeks				
MWD group (n = 40)	17 (42.5)	8 (20)	2 (5)	13 (32.5)
CDO group (n = 40)	19 (47.5)	7 (17.5)	1 (2.5)	13 (32.5)
Combination treatment group (n = 40)	29 (72.5)	9 (22.5)	2 (5)	0
χ^2	8.324 ^{*,#}	–	–	–
P	0.016	–	–	–

* P < 0.05 vs. MWD group.

P < 0.05 vs. CDO group.

4. Discussion

In the present study, as compared with the single MWD and CDO treatments, the combination treatment resulted in a higher wound healing rate, faster control of inflammation and a lower amputation rate, indicating that the application of both MWD and CDO could be an effective clinical approach for DFUs.

Patients with DFUs are prone to experience clinical manifestations such as hyperglycemia, insulin resistance, lipid metabolism disorder, vascular endothelial dysfunction, and infection. The pathogenesis and pathological features are still unclear and complex. DFUs are usually slow to heal, do not heal well, or never heal. In some severe cases, the wounds are even limb- and life-threatening [15]. Therefore, there is an urgent need to develop a new and effective wound care strategy for DFUs.

Adequate oxygen is vital for wound healing [16,17], and oxygen therapy serves as an effective adjuvant therapy for chronic wound treatment [18]. Combined with traditional moist wound therapy, it can facilitate the formation of “pus” in wounds of diabetic foot and promote the growth of granulation tissue and epithelialization [19]. At present, there are three types of oxygen-based therapies for chronic refractory wounds, including hyperbaric oxygen therapy, topical oxygen therapy, and CDO therapy [20,21]. Continuous diffusion of oxygen therapy is characterized by a 24-h/day continuous supply of high-purity oxygen, at a stable and suitable oxygen flow rate of 3–10 ml/h, which can reach the bottom of the wound. As compared with hyperbaric oxygen therapy, CDO technology holds the advantages of easy operation, safety, and a low risk of oxygen toxicity [22]. Also, its portable and compact design does not limit the daily activities of patients, thus improving patients' quality of life.

At present, there are different opinions on the optimal topical oxygen concentration for wound healing [23,24]. In the present study, a high concentration of micro-oxygen, generated by a micro-oxygen supply device through the electrochemical method, was delivered to the wound continuously for 24 h per day, to provide an accurate, stable, and sufficient oxygen supply. The results showed that the area, depth and

area reduction rate of the wounds were improved significantly by combination treatment as compared to single CDO or MWD treatment. The improvement achieved in the present study (63.13%) appears higher than that reported in a previous study (51%), which may be related to the difference in oxygen supply mode [25]. In addition, the white blood cell count and hs-CRP level in the combination treatment group were significantly lower than those in the MWD or CDO group ($P < 0.05$), indicating that CDO therapy combined with MWD prevents infection and facilitates the development of granulation tissue [26]. On the other hand, no significant differences were found in the white blood cell counts and hs-CRP levels between the MWD and CDO groups, which is probably due to the short intervention time. CDO therapy has been reported promoting the growth of granulation tissue and shortening the healing time, and has established as a safe and effective treatment for DFUs [8,27]. Our result was in accordance with these previous studies.

Clinical outcome assessment showed the wound healing time in the combination group was 28 and 27 days shorter, while the wound healing rate was 30% and 25% higher than MWD and CDO groups respectively ($P < 0.05$), suggesting the therapeutic benefits of combination approach. This effect may be related to the pressure induced by continuous delivered oxygen, which compresses the surface of wound, leading to the reduction of wound area and increase of vascular endothelial growth factor expression [23].

This study has several limitations, including a small sample size and the lack of molecular mechanism exploration. To demonstrate the full therapeutic value of this approach, a large-scale, randomized, double-blinded study would be warranted.

5. Conclusion

In conclusion, the results of this study showed that CDO therapy in combination with traditional MWD therapy could effectively shorten the wound size, mitigate the inflammation and facilitate the wound closure. Thus, this combination therapy holds potential as a new clinical strategy for the treatment of DFUs.

6. Funding sources

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7. Contributors

Shumin He and Miaoli Wu designed the research. Shumin He, Cailan Yi and Caiyi Liang completed the main experiments. Caiyi Liang assisted with data processing. Shumin He drafted the paper and Miaoli Wu modified the paper. Shumin He was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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