Dear Editor,

Thank you very much for your letter and advice. We have revised the paper and would like to resubmit it for your consideration. We have addressed the comments raised by the reviewers, and the changes are highlighted in red in the revised manuscript. We hope that the revision is acceptable, and we look forward to hearing from you soon.

Yours sincerely, Qinan Wu

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We would like to express our sincere thanks to the reviewers for their constructive and positive comments.

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: Review on manuscript, "Diabetic foot ulcer: Challenges and Future" I think the subject of the study is interesting. However, the following points should be considered by the authors: 1. References 37, 38 and 39 are not mentioned in the text. 2. The number of keywords for the review article is small. 3. The application of laser in diabetic foot ulcer is neglected in this article. I suggest the authors add at least one paragraph about the use of lasers. 4. In some aspects, this manuscript is similar to Manuscript NO: 78915, I reviewed recently.

Author Response:

1.We have updated the reference in the manuscript as follow:

However, the OBSERVE-4D study [34]indicated that although cagelin increases the risk of amputation, the risk islower than that reported in previous CANVAS and CANVAS-R trials[35], especially for patients undergoing timely monitoring, who have a lower risk. The results of a randomized controlled trial conducted by Marfella et al. [36] suggested that the granulation score of wound granulation tissue and the rate of complete wound healing in the Vigliptin group (50 mg/dose, bid) were significantly better than those in the control group, and the incidence of ulcer-related adverse

events (such as ulcer wound infection, osteomyelitis, honeycomb tissue inflammation, etc.) was also significantly reduced, suggesting that venagliptin may improve the healing rate of DFUs possibly by reducing oxidative stress, changing capillary density, increasing angiogenesis and promoting wound healing. Compared with the control group, the healing rate of foot ulcers in the saxagliptin (5 mg/time, qd) group was higher, and the healing time of ulcers was shorter. The main mechanism was that shagliptin directly and indirectly promoted the epithelial-mesenchymal transformation and reduced scarring to improve diabetic wound healing [37]. DDP-IV inhibitors and GLP-1 receptor agonists reduce inflammatory reactions and antioxidant activity, induce angiogenesis and tissue reconstruction, and may promote DFU healing [38]. These treatments represent new directions with potential effects on DFUs. Systemic insulin therapy improves wound healing of diabetic ulcers by increasing angiogenesis and granulation tissue formation and reducing the duration of the inflammatory phase [39].

2.We added the key word: Diabetic peripheral neuropathy.

3.We also added one paragraph to describe the use of laser as follow:

K. Low-level laser therapy (LLLT)

LLLT uses low-energy light to stimulate the wound surface and produce a series of pathophysiological reactions mainly through photobiological regulation. It does not directly induce photothermal injury of the wound tissue and does not damage the normal tissue cells at the wound surface [172]. In the study byKaviani et al., 8 patients in the LLLT group achieved complete healing after 20 weeks, while only 3 patients in the control group achieved complete healing. Although the difference was not statistically significant, the average time of complete healing in patients receiving LLLT (11 weeks) was less than that in the control group (14 weeks), suggesting that LLLT might accelerate the healing process of chronic DFUs and shorten the time of complete healing, but the sample size of this experiment was small. Another analysis of the efficacy of LLLT in the process of chronic wound tissue repair of DFUs showed that the tissue repair index in the LLLT treatment group increased significantly, mainly because LLLT shortened the inflammatory period, promoted angiogenesis and the production of extracellular matrix components, and accelerated the healing process [173, 174]. Steven et al. [175]proposed that LLLT promotes wound healing by inhibiting the microbial membrane of chronic wounds, especially cocci and some gram-negative bacteria. Other studies have shown that LLLT promotes wound healing by improving the blood flow and autonomic nervous system regulation of DFUs [176]. A systematic review and meta-analysis of the efficacy of low-dose laser treatment of DFUs [177] found that the ulcer area in the LLLT treatment group was significantly reduced by 30.90% compared with the control group. Compared with the control group, the ulcer area in the treatment group decreased by 4.2 cm^2. The probability of complete healing of diabetic foot ulcers was 4.65 times higher than that of the control group, indicating that LLLT may accelerate wound healing and reduce the area of DFUs. However, the review did not provide the best laser treatment parameters. However, in another review, LLLT was shown to be safe and effective in treating DFUs. The laser parameters were 632.8–685 nm, 50 mW/cm^2, and 3–6 J/cm^2;

the irradiation time was 30–80 seconds, three times a week, and the duration of one month was beneficial for the prognosis of DFU wounds in patients [178]. Because the pathophysiological mechanism of DFUs is complex and the prognosis of the ulcer surface is different due to the diverse ulcer surfaces and different laser parameters, more rigorous, high-quality and large-sample RCTs are needed to determine the best treatment parameters for different types of ulcers.

Please see the revised manuscript. And we polished our manuscript as required.

4. Verification is not required

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: foot in terms of current therapeutic options. The authors in the title of the work indicate the challenges and future, however, the work focuses on the description of risk factors and methods of treatment of diabetic foot. In the section devoted to risk factors, the authors describe these factors, indicating the extent to which they exacerbate the formation of DFU. In this part of the work, for greater clarity, it would be useful to present the described data in a table. Describing the element of arterial ischemia, the authors point out dryness of the skin, which, admittedly, can be associated with impaired blood supply, but is more typical of autonomic neuropathy, which should be described in the first part of the subsection. In the section on the treatment of DFU, the authors describe very generally the importance of, for example, metabolic compensation of diabetes, pointing more to general recommendations for its compensation than to recommendations for DFU. An example is the listing of groups of antidiabetic agents , where, in addition, they were all named hypoglycemic. Pointing to the recommendations for LDL-cholesterol levels, it would be worthwhile to present the recommendations in force for the situation associated with PAD specialties, which are more restrictive than for high cardiovascular risk. In the section on debridement and "anti-infection", the authors are not consistent in the strategy of describing individual therapies. For example, there is no indication of the directions of antibiotic therapy or modern anti-infective molecules. In contrast, they describe maggot therapy in great detail. Similarly, the description devoted to antiseptics is very laconic. In the section devoted to dressings, it would be worthwhile to provide a table showing their differentiation in terms of composition and properties. Noteworthy is properly described the subsection devoted to cell therapies, which brings the reader closer to the possibility of using these forms of therapies in the treatment of DFU. The description of NPWT, on the other hand, is extremely laconic and gives the impression that this form of therapy is irrelevant to the treatment of DFUs, which is not true. The work, as a whole, is inconsistent in its approach to individual issues and lacks a unified description of them. This gives reason to think that the review made is not a comprehensive overview, but only a compilation of methods with which the authors have become more or less familiar. In many places, the authors do not even use the terminology that is adopted in the description of a particular method i.e. (offloading versus decompression). In conclusion, the paper is worthy of publication after making the indicated corrections.

Author Response: We have modified the manuscript as required as follow：

1. As to **In this part of the work, for greater clarity, it would be useful to present the described data in a table.**

   Response: We have add the Figure 1: Pathogenesis of DFU， please see the end of the manuscript-Figure 1.

2. As to **Describing the element of arterial ischemia, the authors point out dryness of the skin, which, admittedly, can be associated with impaired blood supply, but is more typical of autonomic neuropathy, which should be described in the first part of the subsection.**

   Response: We verified as follow: The autonomic nerves will also be damaged. Dysfunction of the peripheral sympathetic nerves may lead to reduced sweating, dry skin, cracking and an increased risk of calluses complicated with peripheral arterial disease, and the appearance of symptoms increased.

3. As to **In the section on the treatment of DFU, the authors describe very generally the importance of, for example, metabolic compensation of diabetes, pointing more to general recommendations for its compensation than to recommendations for DFU. An example is the listing of groups of antidiabetic agents , where, in addition, they were all named hypoglycemic.**

   Response: We verified as follow:

   2.Treatment of DFU

The ultimate goal of DFU treatment is to achieve healing and prevent wound infection, amputation and reduced quality of life. It mainly includes glycemic control, management of peripheral artery disease(PAD), and wound management, among others.

A. Glycemic control

Glycated hemoglobin may be the best indicator to evaluate average blood glucose control. An HbA1c level ≥ 8% and fasting blood glucose level ≥ 7 mmol/l are associated with an increased risk of lower limb amputation in patients with DFUs[16]. Studies have recommended that the glycated hemoglobin level in patients with DFUs should be controlled at 7% – 8%, which is helpful for ulcer healing and will not increase the mortality of patients[17]. In another study, the glycosylated hemoglobin level was related to the wound healing speed, which was more obvious in patients with neuropathy and lower-limb arterial disease[18]; however, the results were not repeated in another study[19]. However, an appropriate blood glucose level is undoubtedly beneficial to prevent and delay microvascular and macrovascular complications in patients with diabetes[20]. The ideal blood glucose control target is reached when the glycated hemoglobin level is less than 7% and the blood glucose level within 2 hours after a meal is less than 11.1 mmol/l. However, the indicators should be appropriately relaxed for elderly patients and patients prone to hypoglycemia[21]. Regardless of the size of the initial ulcer area, early intensive blood glucose control may improve the prognosis of DFUs in the first 4 weeks of treatment [22]. Intensive blood glucose control reduced the risk of amputation in patients with DFUs and contributed to wound healing [23, 24].However, in another systematic analysis, no evidence was obtained that strict control of blood glucose improved ulcer wound healing [25]. Many factors affect ulcer healing, andan increasing number of large sample RCTs are needed to indicate the effect of intensive blood glucose control

on the prognosis of DFUs. According to the specific conditions of patients and blood glucose control objectives, appropriate hypoglycemic programs are formulated to avoid hypoglycemia. Fifty percent of patients with DFUs may have PAD, suggesting that they have atherosclerotic cardiovascular disease [26-28]. According to the latest recommendations of the American Diabetes Association for patients with type 2 diabetes complicated with cardiovascular disease, if blood sugar cannot be controlled by lifestyle changes and metformin, they should start taking a hypoglycemic drug that has been suggested to reduce adverse cardiovascular events and cardiovascular mortality[29], such as a sodium glucose cotransporter 2 inhibitor and a glucagon-like peptide-1 receptor agonist. Compared with the placebo, liraglutide did not increase the risk of DFUs but reduced the risk of DFU-related amputation in patients with type 2 diabetes mellitus and high-risk cardiovascular events [30]. However, the specific mechanism remains unclear. Studies have suggested that liraglutide may promote diabetic wound healing by inhibiting endothelial dysfunction induced by hyperglycemia [31]. Daggligin significantly reduced the level of inflammation and oxidative stress in diabetic animal models, which may contribute to the improvement of endothelial dysfunction and diabetic vascular complications[32].

Compared with patients with T2DM using insulin and insulin secretion-promoting drugs, patients with T2DM using insulin sensitizers have a lower incidence of PAD, suggesting that insulin sensitizers may reduce the incidence of PAD and its subsequent outcomes [40].

4. **Pointing to the recommendations for LDL-cholesterol levels, it would be worthwhile to present the recommendations in force for the situation associated with PAD specialties, which are more restrictive than for high cardiovascular risk.**

Response: We verified as follow:

Smoking is a risk factor for atherosclerotic plaques. Severe peripheral atherosclerosis may lead to stenosis and occlusion of the vascular lumen with the progression of the disease, which may lead to foot tissue ischemia that causes tissue damage and postpones wound healing [46]. Smoking is also a risk factor affecting the degree of DFU lesions [47] and is an effective predictor of death and amputation in patients with DFUs[48].Smoking cessation is recommended for all patients with PAD. Blood pressure should be controlled within 130/80 mmHg to reduce the risk of cardiovascular and cerebrovascular events[49]. However, another study suggested that the optimal mean systolic blood pressure of patients with PAD was 135–145 mmHg and diastolic blood pressure was 60–90 mmHg. Low blood pressure may increase the risk of cardiovascular events [50]. The use of ACEIs and ARBs bypatients with PAD not only reduces blood pressure but also reduces major cardiovascular adverse events and mortality [51, 52], but it does not reduce major adverse limb events and amputation risk in patients with PAD [52].Treatments regulating lipid levelstarget low-density lipoprotein cholesterol. Researchers have recommended an LDL-C level<1.4 mmol/l (<55 mg/dl) or a decrease in the LDL-C levelby 50% [53]. In patients with PAD, patients who took statins had an 18% reduction in the long-term risk of adverse prognosis of the lower limbs (such as symptom deterioration, peripheral vascular reconstruction and ischemic amputation) and a 17% reduction in the incidence of

cardiovascular events compared with patients who did not take statins, indicating that statins not only reduce the risk of adverse cardiovascular events but also exert a positive effecton the limb prognosis of patients with PAD [54]. Therefore, patients with T2DM and PAD should be prescribed statins. Medications for improving the circulation of patients with PAD include vasodilator drugs, antiplatelet drugs and anticoagulant drugs. Vasodilator drugs include alprostadil injection, beraprost sodium, cilostazol, salgrel hydrochloride, buflomedil and pentoxifylline, which reduce blood viscosity and change hypercoagulability. Aspirin and clopidogrel are considered antiplatelet drugs. Current practice guidelines recommend the use of aspirin or clopidogrel alone as a method for the secondary prevention of cardiovascular events in patients with PAD [55, 56]. Compared with aspirin alone, clopidogrel combined with aspirin significantly reduces all-cause mortality and cardiovascular events, but the risk of severe bleeding is increased[57].The COMPASS study suggested that the absolute benefit of low-dose rivalsaban (2.5 mg bid) combined with aspirin (100 mg qd) in reducing the risk of cardiovascular events and all-cause mortality in patients with stable atherosclerosis seems to be greater than that of nondiabetic patients [58]. Compared with aspirin alone, low-dose rivalsaban combined with aspirin reduced major cardiovascular events and major limb adverse events. Rivalsaban alone only reduced major limb adverse events but did not significantly reduce major cardiovascular adverse events. However, the latter two schemes increased the risk of bleeding, mainly in the gastrointestinal tract, butthe incidence of fatal bleeding or bleeding in key organs did not increase [59]. Routine use of proton pump inhibitors may reduce bleeding from gastroduodenal lesions [60]. Therefore, the combination of low-dose rivalsaban and aspirin provides a new therapeutic direction for patients with diabetes complicated with PAD, but further studies are necessary to determine which subgroups of patients may benefit.

5. **In the section on debridement and "anti-infection", the authors are not consistent in the strategy of describing individual therapies. For example, there is no indication of the directions of antibiotic therapy or modern anti-infective molecules. In contrast, they describe maggot therapy in great detail. Similarly, the description devoted to antiseptics is very laconic.**

Response: We verified as follow:

In the initial stage of superficial DFU infection, gram-positive cocci are mainly detected, among which *Staphylococcus aureus* and *Streptococcus*arethe most common organisms[64, 65]. If chronic infection, extensive necrosis, wet gangrene, deep infection, long-term repeated use of antibiotics and other conditions exist on the wound surface, a mixed bacterial infection is usually present. Currently, the proportion of gram-negative bacterial infections is increasing, and the proportion of fungal infections is also increasing [66]. Due to the autoimmune status of the body, sanitary conditions, repeated hospitalization, frequent use and abuse of antibiotics, multiple microbial infections, insufficient arterial blood supply of the lower limbs and other reasons, the number of multidrug-resistant bacteria is increasing. The most resistant pathogen is methicillin-resistant *Staphylococcus aureus* (MRSA) [67]. Therefore, accurate identification of the pathogen causing thebacterial infection is

essential for anti-infection treatment of DFUs. Once the infection is confirmed, pathogenic bacteria samples should be collected after the necrotic tissues are removed from the infected wound and before the use of antibacterial drugs. Pathogenic bacteria culture samples shall be obtained from deep tissues as far as possible and sent forculture immediately after the samples are collected. In addition, samples should be collected repeatedly during anti-infection treatment to identify pathogenic bacteria and guide the selection of antibiotics. Tissue biopsy is considered the most useful and standard technology, but it may cause the spread of infection and the loss of adjacent tissue structure of limbs; thus, it is not completely feasible. The collection of swab culture samples is easier, and any type of ulcer can be used. However, the cotton swab culture results usually include colonized bacteria, and the test results are not necessarily reliable [68]. Compared with the culture method, the molecular test method is more sensitive and reliable, with high accuracy and a fast test speed. It represents a powerful method or the identification of microbial colonies infecting chronic wounds and has a bright future in the convenient nursing and treatment of diabetic foot ulcers [69]. Molecular microbiological diagnostic techniques improve the prognosis of patients with chronic wounds [70].

The use of antibiotics should follow the principles of selectivity, timeliness, relatively narrow spectrum, shortest course of treatment, safety, minimal adverse reactions, high cost performance, and step-down. At present, IDSA/IWGDF is used to score DFU infection, which is divided into mild (superficial with slight cellulitis), moderate (deeper or more extensive) or severe (with systemic sepsis signs), and the presence of osteomyelitis [71].

The course of antibiotic use is related to the severity of the wound and the presence of bone tissue involvement. The course of treatment ranges from 1 to 12 weeks[61]. However, a comprehensive and individualized analysis is necessary to appropriately adjust the course of antibiotics according to the basic diseases of the whole body, nutritional status, liver and kidney functions, blood supply of the lower limbs, and other parameters. Oral antibiotics and intravenous antibiotics maybe selected, but the narrowest-spectrum antibiotic and the shortest course of treatment for pathogenic bacteria should be selected to prevent drug resistance. The IWGDF recommendations [72] for superficial ulcers with localized soft tissue infection (mild) are to start with empirical oral antibiotic treatment against *Staphylococcus aureus* and *Streptococcus aureus* (unless other pathogens should be considered).For deep or extensive infections (moderate or severe infections),a broad-spectrum antibiotic should initially be intravenously administered that mainly targets common gram-positive and gram-negative bacteria, including specific anaerobic bacteria, and the antibiotic program should be adjusted according to the clinical efficacy of empirical therapy, tissue culture and drug sensitivity results. Biofilms are polysaccharide layers formed by a variety of signal transduction mechanisms that delay the healing of DFUs. Therefore, inhibiting the formation of biofilms is a new direction of modern anti-infection treatment research. Studies have shown that acyl homoserine lactones (AHLs) regulate multiple factors during biofilm formation by *Pseudomonas aeruginosa* and play a fundamental role in regulating different genes

involved in biofilm formation. Therefore, AHL can be used as a therapeutic target to provide a correct path for drug design targeting multidrug-resistant bacteria[73].

6. **In the section devoted to dressings, it would be worthwhile to provide a table showing their differentiation in terms of composition and properties.**

   Response: We have add the Table-1，please see the end of the manuscript-Table-1.

7. **The description of NPWT, on the other hand, is extremely laconic and gives the impression that this form of therapy is irrelevant to the treatment of DFUs, which is not true.**

   Response: We verified as follow:

I. Negative Pressure Wound Therapy(NPWT)

NPWT includes two modes: vacuum-assisted closure (VAC) and vacuum sealing drainage (VSD). The pipeline used by VAC has poor hydrophilicity and a high supporting force. The pipeline is placed on the surface of the dressing to form a device similar to a suction cup. Wound healing is promoted by adjusting the negative pressure level and selecting the intermittent mode [141]. The drainage tube adopted by VSD has high plasticity, good hydrophilicity and contains side holes. It covers the dressings and wound surface with a fully closed and translucent polyurethane film to form a closed space. The necrotic tissues and secretions on the wound surface are drained by negative pressure to promote wound cleaning; it is mainly used for drainage of deep wounds and body cavities. VAC focuses on the treatment of wounds on the body surface and exertsa good effect on treating diabetic foot ulcers, limb soft tissue lacerations, lower limb venous ulcers, deep pressure ulcers, and other wounds [142-144].

NPWT is widely used to treat DFUs as an acute and chronic wound treatment technology. Armstrong et al. [142] suggested that, compared with standard treatment, VAC accelerated wound healing, improved wound healing ratio and reduced the re-amputation rate when treating complicated diabetic foot wounds. In the meta-analysis by LIU S et al. [145], compared with conventional dressing changes, VAC reduced the area and depth of DFU to a greater extent, improved the complete healing rate of ulcer, shortened the healing time of ulcer, reduced the amputation rate of patients, and improved cost-effectiveness.

The mechanism of NPWT is as follows: 1) keep the wound moist and stabilize the wound environment pain [146], and 2) inhibit bacterial growth. Weed et al. [147] indicated that after treatment with negative pressure drainage technology, the number of bacteria in the wound, particularly gram-negative bacteria, was significantly reduced. Additional components of the mechanism include 3) improving wound blood perfusion and promoting wound healing [148]; 4) promoting cell proliferation, angiogenesis and wound tissue repair [149]; and 5) regulating the signaling pathway to modulate cytokine expression [150].

Before using NPWT, the necrotic tissue and dead bone on the wound surface should be completely removed. NPWT also has contraindications, such as deep wound infection, severe ischemia, eschar or necrosis, active bleeding, coagulation dysfunction, exposure of blood vessels or nerves or tendons or ligaments, untreated osteomyelitis, wet gangrene, and malignant tumors. At the same time, the use of

NPWT may lead to tube plugging, poor drainage, residual dressings, wound maceration, residual dressings, and other complications.

Based on the limitations of NPWT, negative pressure wound therapy with installation (NEWTi) emerged at a historic moment. It combines negative pressure therapy with liquid perfusion technology to accelerate the cooperative use of wound water and promote the dissolution and clearance of deep necrotic tissues by intermittently or continuously perfusing solutions to closed wounds. The destruction of biofilms and autolytic debridement are the main factors contributing to the superiority of NPWTi to NPWT. However, a uniform standard for the selection of irrigation solution, irrigation time, irrigation speed, and irrigation frequency is unavailable when NPWTi is used to treat DFUs.

8. **The work, as a whole, is inconsistent in its approach to individual issues and lacks a unified description of them. This gives reason to think that the review made is not a comprehensive overview, but only a compilation of methods with which the authors have become more or less familiar. In many places, the authors do not even use the terminology that is adopted in the description of a particular method i.e. (offloading versus decompression).**

   Response: **We have verified the manuscript as required，please see the manuscript we revised which highlight in red.**

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Always the paper is well-written. Could you collect the several sentences or words? For example page1, anti infection is anti-infection, amongdiabetic is among diabetic.

Author Response: We have modified the anti infection to anti-infection and amongdiabetic to among diabetic. Please see page 1 in the abstract and introduction of the revised manuscript. And we polished our manuscript as required.

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: The authors have considered my suggestion and improved the paper accordingly. I've non further comments on it.

Author Response: Verification is not required and we polished our manuscript as required.

In order to facilitate your review, the revised parts are highlighted in red in the revised manuscript, We also updated references for each verified part and updated the authors as Yang Li, Rong Guichuan, Wu Qinan, we add the support funding as follow: Science and health joint project of Dazu District Science and Technology Bureau(DZKJ2022JSYJ-KWXM1001). If you have any question about this paper, please don’t hesitate to contact us.

Thank you again!

Sincerely yours,
Qinan Wu