



Diabetic Foot Ulcers Treated with Nanotherapy: A Short Communication

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ABSTRACT

The current study proposes an innovative approach: the development of a nanotherapeutic particles designed to encompass all the desired functionalities outlined by previous researchers. These include: a) bactericidal properties, b) the ability to generate ROS species from lactic acid when the Krebs cycle deviates to the Cori cycle, c) promotion of angiogenesis and oxygenation of wounds, and d) acting as a catalyst for cell regeneration, thereby facilitating the closure of diabetic foot ulcers. These nanotherapeutic particles are synthesized from an organometallic compound integrated into a polymeric amorphous matrix. Crucially, it is superficially coated with a variety of functional groups, exhibiting a particle size ranging from 1 to 5 nanometers as determined by transmission electron microscopy (TEM), with a specific surface area exceeding 700 m²/g. Preliminary preclinical findings have been documented, and we present additional promising outcomes in this report.

Keywords: Bionanocatalyst, Foot wounds, Diabetic foot, Venous ulcers

INTRODUCTION

Obesity and diabetes present significant health challenges in Mexico. According to a 2022 OECD report, Mexico ranks among the countries with the highest prevalence of diabetes mellitus, following the United States and Brazil. This condition is a major cause of morbidity and mortality, particularly affecting those with lower educational and economic resources. Álvaro-Alfonso et al. (2023) recently published a study highlighting the prevalence of diabetic foot

ulcers (DFUs) and their impact on the quality of life, based on a survey of 141 DFU patients [1]. Given the mixed Spanish and Mexican ancestry, the findings are highly relevant to Mexico.

Patients with DFUs typically require hospitalization for two to three months and receive various antibiotics [2]. Studies have shown that diabetic feet host 5 to 8 bacterial species [3], leading to ulcer necrosis and eventual limb amputation [4–6], necessitating 3-6 months of hospitalization. Upon discharge, patients often need home care from family members, resulting in substantial economic and workforce losses. Currently, there is no definitive cure for DFUs [7].

Research by Loera-Valencia et al. [8] suggests that ZnO nanoparticles are effective antimicrobial agents. Ongoing *in vitro* and *in vivo* studies in mice are investigating their potential to regenerate damaged tissues and close wounds. Choudhury et al. [9] synthesized silver nanoparticles (AgNPs), which demonstrated superior results due to their significant bactericidal properties. AgNPs facilitate nucleation with collagen generation around the wound, promoting angiogenesis and immunomodulation. Furthermore, they enhance tissue recovery, stimulate healing with anti-inflammatory and antimicrobial effects, and are widely used for burns and trauma [9–11]. Additionally, they inhibit bacterial biofilm production, proving effective against multidrug-resistant bacteria commonly found in chronic wounds. However, AgNPs have been reported to be toxic [12].

Zheng et al. [13] described an innovative study in the Chemical Engineering Journal involving diabetic mice treated with DMSNs-Pt-LOX@Nisin nanomotors (DPLN) targeting methicillin-resistant *Staphylococcus aureus* (MRSA). These nanomotors convert lactic acid in diabetic wounds to hydrogen peroxide (H_2O_2), which is catalyzed by platinum nanodendrites to produce oxygen, enabling deep penetration into biofilms. Nisin, loaded onto the nanomotors, binds to lipid II in Gram-positive bacteria, attacking MRSA. *In vitro* and *in vivo* experiments confirmed that these nanomotors effectively eradicate MRSA biofilms through a cascade of platinum-catalyzed reactions that dissociate lactic acid into H_2O_2 , generating reactive oxygen species (ROS) that penetrate the biofilm.

Chinese researchers led by L. Wang, Y. Yang, and W. Han [14] proposed *Centella Asiatica* extract-loaded poloxamer/ZnO nanocomposite as a promising adjunct for diabetic foot ulcer (DFU) closure, tested on fibroblasts and mice to form ROS. El-Salamouni et al. [15] developed a nanoparticulated hydrogel incorporating Valsartan, which demonstrated bacterial biofilm inhibition comparable to Levofloxacin [16]. Most studies on diabetic foot focus on controlled-release nanoparticles of silver and other drugs [17].

The current study introduces a novel nanoparticle designed to integrate all desired functionalities identified in previous research. These include bactericidal properties, ROS generation from lactic acid during the Cori cycle, promotion of angiogenesis and oxygenation, and catalysis of cell regeneration to close DFUs. Synthesized from an organometallic compound within a polymeric amorphous matrix, this nanotherapy is functionalized with nitrogen compounds, hydroxyls, and phosphates, with particle sizes ranging from 1 to 5 nanometers, as determined by transmission electron microscopy (TEM), and a specific surface area exceeding $700 \text{ m}^2/\text{g}$. Preliminary preclinical results have been documented, and additional promising outcomes are presented in this report.

MATERIALS AND METHODS

Treatment

Nanotherapy:

The nanotherapeutic coated-nanoparticles were synthesized via oxide precursors. Synthesis parameters were fine-tuned based on previous studies [18] to enhance the surface-to-volume ratio and particle coating, yielding a coated powder (nanotherapeutic nanoparticles). Comprehensive physicochemical characterization was performed as previously detailed [19]. These nanoparticles were incorporated into a mixed-polymeric gel matrix, developed by our team, due to its efficacy in skin applications [20].

Debridement, Nanotherapy Application, and Dressing:

For the treatment protocol, a single surgeon conducted regular local sharp debridement using scalpels, scissors, or forceps once a month, following current standard-of-care guidelines [7]. The goal of this debridement was to remove nonviable tissue. Alternative debridement methods, such as larval, autolytic, hydrosurgery, and ultrasonic therapy, were not employed. Every second day, an adequate amount of nanotherapy containing embedded coated nanoparticles was applied to fully cover the wound surface. No additional medications or therapies were used. After applying the nanotherapy, wounds were covered with standard secondary dressings. This treatment continued until the wounds were completely healed.

Ethics Approval:

The research protocol received approval from the Directorate of Teaching and Research at the Specialized Center for the Care of Diabetic Patients, Dr. Manuel Gonzalez Rivera, under the Ministry of Health of the Federal District, registered under number 101/100/014/13.

CLINICAL CASES

Case 1

A 57-year-old male patient with a history of arterial hypertension, dyslipidemia, and uncontrolled type 2 diabetes of five years' duration, as well as neuropathy of three years' duration, presented to the Family Medicine Centre one year and eight months ago with a malodorous wound on his right lower extremity. Physical examination revealed two ulcers in the plantar region. The first ulcer, measuring approximately 10 cm x 15 cm, was located on the forefoot, involving the five phalanges and exhibiting exudate, fibrin, and necrotic areas. The second ulcer, measuring 5 cm x 5 cm, was located on the calcaneal tuberosity and showed predominantly fibrin and necrotic tissue. Based on these findings, the patient was diagnosed with a Wagner Grade 4 diabetic foot. Due to signs of infection, the patient was referred to Centro Medico Nacional Siglo XXI, where he was treated with broad-spectrum antibiotics for three months. Despite this treatment, there was no improvement, and the necrotic areas continued to expand. Consequently, the medical team informed the patient that the most viable option to save his life was amputation. The patient arrived in very poor condition and was referred to an endocrinologist, where they began treatment with nanotherapy (Figure 1a). One month after starting nanotherapy, significant improvements were observed, including granulation tissue formation, areas of revascularization, and the disappearance of necrotic tissue, indicating optimal wound healing. One year and one month after the initial outpatient therapy, the wound had healed completely (Figure 1b).



Figure 1: Clinical Cases. (a-b) Case 1: Wagner's Diabetic Foot. (a) Wound determined for amputation at $t = 0$ months; (b) Closed wound after treatment nanotherapy at $t = 13$ months. (c-f) Case 2: Venous Ulcer. (c, e) Wounds in the feet determined for amputation. (d, f) Closed wounds after treatment with nanotherapy at $t = 6$ months.

Case 2

A 72-year-old male patient with a history of type 2 diabetes for 20 years, arterial hypertension for 8 years, and venous insufficiency for 3 years, presented with ulcers likely exacerbated by his occupation, which required him to stand for over 10 hours daily. On examination, a venous ulcer measuring 15 cm x 5 cm was observed in the left supramalleolar region, characterized by purulent exudate, fibrin, and myelogenous borders. Additionally, a second ulcer measuring 5 cm x 10 cm was noted on the right ankle, also displaying exudate and fibrin. Surrounding both ulcers, signs of ochre dermatitis were evident, consistent with venous insufficiency (Figure 1c, e). After six months of unsuccessful conventional treatment, the patient was advised that amputation of both legs would be necessary. Before proceeding with amputation, the patient and his family opted to try nanotechnology-based treatment. Six months post-treatment, significant improvements were observed. Within two weeks, there was a notable reduction in edema. The treatment facilitated complete revascularization, removal of infected tissue, and enhanced wound closure through increased fibroblast production. The wound beds became clean, showing granulation tissue and optimal epithelialization. Eventually, both ulcers closed,

leaving typical, fine-lined scars with no inflammatory signs (Figure 1d, f). However, hyperpigmentation remained in the surrounding tissue.

LIMITATIONS

Case series are susceptible to bias, which limits their applicability to broader patient populations. However, the insights gained from the healing processes of the four patients in this study allow researchers to hypothesize about the efficacy and mechanism of action of the bionanocatalysts in chronic wound healing. Additionally, it is important to note that a complementary surgical debridement treatment was also administered, which standard-of-care practices recognize as crucial for treatment effectiveness. In this study, no direct comparison was made against treatment with debridement alone, aside from referencing existing literature. This approach was taken to benefit the patients, given that debridement alone is insufficient for treating chronic wounds. Future work will include such comparisons.

CONCLUSION

Chronic wounds caused by microcirculation issues, such as those associated with diabetes and venous insufficiency, represent a global health crisis requiring urgent resolution. Despite numerous treatment approaches for chronic wounds, no drug has demonstrated wound closure efficacy comparable to that observed with nanoparticles. Traditional treatments often focus on disinfection or wound protection without addressing the underlying molecular processes. This letter introduces a groundbreaking approach using ultra-nanomaterials that not only disinfect the wound area but also promote tissue regeneration. Nanoparticles, due to their nanometric size, can infiltrate microorganisms, eradicate bacteria, and disrupt their genetic structures, effectively eliminating infections. In conditions like diabetic foot ulcers and venous ulcers, impaired healing is often due to insufficient oxygen supply. Nanoparticles address this by delivering oxygen directly to the wound site, thereby accelerating the healing process. Additionally, nanoparticles stimulate tissue regeneration and angiogenesis, ultimately leading to complete wound closure.

Associated Content

Supporting Information. All relevant information is included in the article.

Author Contribution

All authors contributed equally. All authors have given approval to the final version of the manuscript.

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References

- [1] Álvaro-Afonso FJ, García-Álvarez Y, Tardáguila-García A, García-Madrid M, López-Moral M, Lázaro-Martínez JL. Bacterial Diversity and Antibiotic Resistance in Patients with Diabetic Foot Osteomyelitis. *Antibiotics* 2023; 12:212. <https://doi.org/10.3390/antibiotics12020212>.
- [2] Baig MS, Banu A, Zehravi M, Rana R, Burle SS, Khan SL, et al. An Overview of Diabetic Foot Ulcers and Associated Problems with Special Emphasis on Treatments with Antimicrobials. *Life* 2022; 12:1054. <https://doi.org/10.3390/life12071054>.

- [3] Sadeghpour Heravi F, Zakrzewski M, Vickery K, G. Armstrong D, Hu H. Bacterial Diversity of Diabetic Foot Ulcers: Current Status and Future Prospectives. *J Clin Med* 2019; 8:1935. <https://doi.org/10.3390/jcm8111935>.
- [4] Rodrigues BT, Vangaveti VN, Urkude R, Biros E, Malabu UH. Prevalence and risk factors of lower limb amputations in patients with diabetic foot ulcers: A systematic review and meta-analysis. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 2022; 16:102397. <https://doi.org/10.1016/j.dsx.2022.102397>.
- [5] Chamberlain RC, Fleetwood K, Wild SH, Colhoun HM, Lindsay RS, Petrie JR, et al. Foot Ulcer and Risk of Lower Limb Amputation or Death in People with Diabetes: A National Population-Based Retrospective Cohort Study. *Diabetes Care* 2022; 45:83–91. <https://doi.org/10.2337/dc21-1596>.
- [6] Anita H, Manoj A, Arunagirinathan N, Rameshkumar MR, Revathi K, Selvam R, et al. Bacterial etiology, antibiotic resistance profile and foot ulcer associated amputations in individuals with Type II diabetes mellitus. *J King Saud Univ Sci* 2023; 35:102891. <https://doi.org/10.1016/j.jksus.2023.102891>.
- [7] Rayman G, Vas P, Dhatariya K, Driver V, Hartemann A, Londahl M, et al. Guidelines on use of interventions to enhance healing of chronic foot ulcers in diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev* 2020;36. <https://doi.org/10.1002/dmrr.3283>.
- [8] Loera-Valencia R, Neira RE, Urbina BP, Camacho A, Galindo RB. Evaluation of the therapeutic efficacy of dressings with ZnO nanoparticles in the treatment of diabetic foot ulcers. *Biomedicine & Pharmacotherapy* 2022; 155:113708. <https://doi.org/10.1016/j.biopha.2022.113708>.
- [9] Choudhury H, Pandey M, Lim YQ, Low CY, Lee CT, Marilyn TCL, et al. Silver nanoparticles: Advanced and promising technology in diabetic wound therapy. *Materials Science and Engineering: C* 2020; 112:110925. <https://doi.org/10.1016/j.msec.2020.110925>.
- [10] Ahmad SA, Das SS, Khatoon A, Ansari MT, Afzal M, Hasnain MS, et al. Bactericidal activity of silver nanoparticles: A mechanistic review. *Mater Sci Energy Technol* 2020; 3:756–69. <https://doi.org/10.1016/j.mset.2020.09.002>.
- [11] Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. <p>The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry</p>. *Int J Nanomedicine* 2020; Volume 15:2555–62. <https://doi.org/10.2147/IJN.S246764>.
- [12] Hadrup N, Sharma AK, Loeschner K. Toxicity of silver ions, metallic silver, and silver nanoparticle materials after in vivo dermal and mucosal surface exposure: A review. *Regulatory Toxicology and Pharmacology* 2018; 98:257–67. <https://doi.org/10.1016/j.yrtph.2018.08.007>.
- [13] Zheng J, Deng Y, Zhao S, Liu L, Wang W, Li J, et al. Cascade-driven nanomotors promote diabetic wound healing by eradicating MRSA biofilm infection. *Chemical Engineering Journal* 2024; 481:148790. <https://doi.org/10.1016/j.cej.2024.148790>.
- [14] Wang L, Yang Y, Han W, Ding H. Novel design and development of Centella Asiatica extract - loaded poloxamer/ZnO nanocomposite wound closure material to improve anti-bacterial action and enhanced wound healing efficacy in diabetic foot ulcer. *Regen Ther* 2024; 27:92–103. <https://doi.org/10.1016/j.reth.2024.03.006>.
- [15] El-Salamouni NS, Gawayed MA, Younis SE, Abdel-Bary A, Kamel MA, Labib GS. Pentoxifylline/Valsartan co-delivery in liposomal gel alters the inflammatory HMGB-1/ TLR pathway and promotes faster healing in burn wounds: A promising repurposed approach. *Int J Pharm* 2022; 625:122129. <https://doi.org/10.1016/j.ijpharm.2022.122129>.

- [16] She P, Luo Z, Chen L, Wu Y. Efficacy of levofloxacin against biofilms of *Pseudomonas aeruginosa* isolated from patients with respiratory tract infections in vitro. *Microbiologyopen* 2019;8. <https://doi.org/10.1002/mbo3.720>.
- [17] Singh M, Thakur V, Kumar V, Raj M, Gupta S, Devi N, et al. Silver Nanoparticles and Its Mechanistic Insight for Chronic Wound Healing: Review on Recent Progress. *Molecules* 2022; 27:5587. <https://doi.org/10.3390/molecules27175587>.
- [18] López-Goerne TM. Nanotecnología y nanomedicina: la ciencia del futuro... hoy. 1st ed. Mexico City: Arkhé Ediciones; 2011.
- [19] López T, Alvarez M, González RD, Uddin MJ, Bustos J, Arroyo S, et al. Synthesis, characterization and in vitro cytotoxicity of Pt-TiO₂ nanoparticles. *Adsorption* 2011 17:3 2011; 17:573–81. <https://doi.org/10.1007/S10450-011-9330-X>.
- [20] Soni KS, Desale SS, Bronich TK. Nanogels: An overview of properties, biomedical applications and obstacles to clinical translation. *Journal of Controlled Release* 2016; 240:109–26. <https://doi.org/10.1016/j.jconrel.2015.11.009>.