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Review

Nanozymes for the Therapeutic Treatment of Diabetic Foot Ulcers

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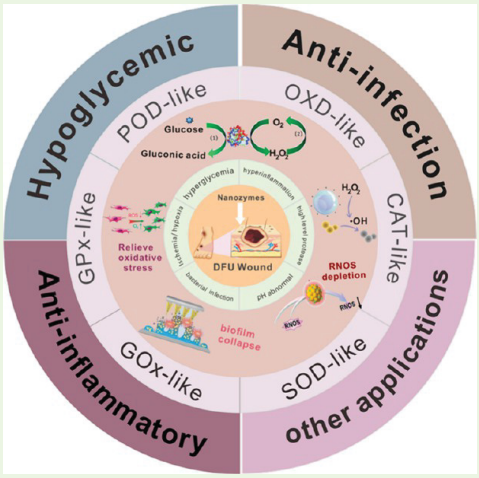
ABSTRACT: Diabetic foot ulcers (DFU) are chronic, refractory wounds caused by diabetic neuropathy, vascular disease, and bacterial infection, and have become one of the most serious and persistent complications of diabetes mellitus because of their high incidence and difficulty in healing. Its malignancy results from a complex microenvironment that includes a series of unfriendly physiological states secondary to hyperglycemia, such as recurrent infections, excessive oxidative stress, persistent inflammation, and ischemia and hypoxia. However, current common clinical treatments, such as antibiotic therapy, insulin therapy, surgical debridement, and conventional wound dressings all have drawbacks, and suboptimal outcomes exacerbate the financial and physical burdens of diabetic patients. Therefore, development of new, effective and affordable treatments for DFU represents a top priority to improve the quality of life of diabetic patients. In recent years, nanozymes-based diabetic wound therapy systems have been attracting extensive interest by integrating the unique advantages of nanomaterials and natural enzymes. Compared with natural enzymes, nanozymes possess more stable catalytic activity, lower production cost and greater maneuverability. Remarkably, many nanozymes possess multienzyme activities that can cascade multiple enzyme-catalyzed reactions simultaneously throughout the recovery process of DFU. Additionally, their favorable photothermal-acoustic properties can be exploited for further enhancement of the therapeutic effects. In this review we first describe the characteristic pathological microenvironment of DFU, then discuss the therapeutic mechanisms and applications of nanozymes in DFU healing, and finally, highlight the challenges and perspectives of nanozyme development for DFU treatment.



KEYWORDS: *nanozyme, diabetic foot ulcer, wound therapy, cascade reaction, multienzyme activity*

1. INTRODUCTION

Diabetes is a lifelong metabolic disease characterized by chronic hyperglycemia caused by multiple etiologies.[1] The danger lies not in the diabetes itself, but in the complications caused by abnormal blood glucose, including multiorgan pathologies, such as renal, cardiovascular, and neurological disorders, as well as chronic wounds that are difficult to heal.[2] Diabetic foot ulcers (DFU) are one of the most persistent complications of diabetes, often increasing morbidity and mortality in diabetic patients.[3] Studies have suggested that approximately 30% of diabetic patients will suffer from DFU, and as much as one-third of the cost of diabetes care is spent on lowering extremity-related problems, which greatly increases the physical and financial burdens on diabetic patients.[4] In addition, the extremely high recurrence rate of DFU, ca. 40% within 1 year after successful healing, poses an even greater challenge for their treatment.[5] It is widely accepted that the microenvironment of DFU is quite complex, characterized by hyperglycemia, hyperinflammation, excessive oxidative stress, recurrent bacterial infection, and ischemia and hypoxia.[6] Specifically, hyperglycemia upregulates the expression of pro-inflammatory factors and triggers inflammation. It also leads to an impaired immune response and provides nutrients for bacteria, resulting in diabetic wounds being more susceptible to bacterial infection.[7] More seriously, it can easily lead to oxidative stress of nerve cells and neuropathy (e.g., nerve damage in the foot, and sensory neuropathy), which can lead to a reduction or loss of basic "sensation" and "pain" in the patient. The damaged nervous system is much less sensitive to wounds, which greatly increases the risk of ulcers and even amputations.[8] In addition, high blood sugar causes glycosylation of hemoglobin, narrowing of blood vessels, and alteration of red blood cell membranes, resulting in inadequate oxygen supply, all of which severely impede wound healing.[9] In

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addition, excessive oxidative stress and disordered immune response around diabetic wounds severely affect the expression of various chemokines and growth factors, hindering epithelial regeneration and angiogenesis.[10] Prolonged ischemia and hypoxia also make it difficult to provide the large amount of energy needed for wound healing, resulting in malignant outcomes, such as collagen damage and localized necrosis.[11] These pernicious effects reinforce each other, forming an endless loop, which severely limits the successful healing of diabetic wounds. However, conventional treatments, such as drugs, surgery, and traditional dressings, have limited efficacy and are accompanied by side effects. Therefore, development of new, effective and affordable treatments for DFU is a top priority to improve the quality of life of people with diabetes.

It is encouraging to note the emergence of nanozymes as an effective solution to the above issue.[12] It is well-known that natural enzymes can exhibit high catalytic efficiencies by dramatically increasing the rate of chemical reactions, reducing reaction time and cutting cost, and have been widely used in chemical, pharmaceutical, and biotechnological applications. For example, natural horseradish peroxidase (HRP) has been used in biotechnology for antibody labeling because of its activity in catalyzing a rapid color change of the substrate, which can be exploited for the detection of antigens that bind specifically to the antibody.[13] However, the structural instability and environmental sensitivity of natural enzymes pose significant challenges in efficient catalysis. Nanozymes are nanomaterials that mimic natural enzymes in catalyzing the conversion of enzyme substrates into products under relevant, but typically milder, physiological conditions.[14] The activity of nanozymes arises from the nanostructures without the assistance of other enzymes or catalysts, implying that the catalytic activity of nanozymes is intrinsic of the materials. Importantly, the activity of the nanozymes can be modulated by the size, morphology, elemental composition and surface properties of the nanomaterials. In addition, nanozymes are more stable and less costly than natural enzymes.[15] In 2007, Yan et al.[16] discovered that Fe_3O_4 nanoparticles possessed peroxidase (POD)-like activity. HRP is known to catalyze reactions by binding one substrate and releasing the first product, then binding another substrate and releasing the second product, in a back-and-forth process known as the "ping-pong reaction" mechanism. The catalytic reaction of Fe_3O_4 nanozymes also follows the "ping-pong reaction" mechanism, mimicking HRP at the molecular level. Since then, research on the enzymology of nanomaterials has progressed rapidly, and a large number of nanozymes have been designed and synthesized for various biomedical applications, such as antitumor[17] and antibacteria.[18] Overall, the enzyme-like activities of nanozymes reported so far mainly include those of POD, oxidase (OXD), catalase (CAT) and superoxide dismutase (SOD), and there have been a variety of hypotheses about the molecular mechanism. As the reactions catalyzed by nanozymes mostly take place on the material surface, the smaller the size, the greater the surface area of the nanozymes, and hence the greater the catalytic activity.[19] Thus, size engineering plays a critical role in the targeted design and preparation of nanozymes for enhanced catalytic activity.[20] Indeed, using nanozymes as a "healer" to eliminate bacterial infections and remodel the DFU microenvironment is a promising strategy to facilitate diabetic wound healing.[21]

There are three general types of mechanisms by which nanozymes promote DFU healing.[22] The first is down-regulation of blood glucose concentration.[23] Since high blood glucose levels are responsible for triggering bacterial infections, inflammation, oxidative stress, and other malignant symptoms, the first priority in treating DFU is to lower the blood glucose concentration in the wound.[24] Some nanozymes exhibit excellent glucose oxidase (GOx)-like activity, which breaks down glucose into gluconic acid and H_2O_2, thereby lowering blood sugar.[25] The H_2O_2 produced also serves as a substrate for the nanozymes to further produce hydroxyl radicals (•OH), enhancing the removal of bacteria.[26] The second is inhibition of bacterial infection.[27] Given the presence of large amounts of H_2O_2 in the DFU microenvironment, nanozymes with POD-like activity have been widely used to kill infectious pathogens by catalyzing the conversion of hydrogen peroxide into •OH, which promotes wound healing.[28] The third is relief of oxidative stress and anti-inflammation.[29] Excessive superoxide anion and hydrogen peroxide, i.e., high levels of reactive oxygen species (ROS), exist in the microenvironment of DFU, which disrupts redox homeostasis and induces oxidative stress.[30] Excessive oxidative stress causes disruption of the immune system response, downregulation of various growth factors, and obstruction of cell proliferation and migration, thereby exacerbating wound inflammation and delaying healing.[31] At this point, the role of antioxidant enzymes becomes particularly important. Typically, nanozymes with SOD- and CAT-like activity can act as excellent antioxidants, scavenging excessive superoxide anion and hydrogen peroxide to alleviate oxidative stress in wounds.[32] At the same time, it also generates oxygen to improve ischemic hypoxia and accelerate wound healing.[33]

There have been various review articles on the application of nanozymes in wound healing. These typically focus on the antimicrobial activity of nanozymes by classifying them according to the substrates (e.g., metal-based, metal oxide-based, carbon-based, sulfide-based, etc.)[34] or elemental compositions,[35] and elaborating on the antimicrobial mechanisms and applications of nanozymes within the context of active centers and structures. With both general wound characteristics and specific microenvironment (e.g., hyperglycemia), the treatments of DFU typically entail similar (e.g., antimicrobial and anti-inflammatory) but not exactly equivalent (e.g., blood glucose lowering) strategies to that of ordinary wounds. The emergence of nanozymes can precisely make up for these deficiencies, which is promising in DFU treatment, as manifested in a number of experimental studies and summarized in relevant reviews. For example, Wang et al.[36] described different enzyme-like materials (beyond nanozymes), with an emphasis on the types of enzymes and the mechanisms of promoting wound healing, the structural design and conformational relationship of enzyme-based biomaterials, as well as the applications in diabetic wounds. Jiang et al.[22] focused on nanozymes and diabetic wounds. They first introduced the basic features of diabetic wounds, then described the catalytic mechanisms and applications of different types of nanozymes in diabetic wounds from three aspects: anti-infection, hypoglycaemia and inflammation alleviation, and finally put forward key problems and outlook. However, most of the examples cited in this review are from the period of 2020−2022, and the classification criteria are not totally precise (e.g., POD-like nanozymes are categorized in the section of "Antimicrobial Therapeutics", as they have other activities, such as anti-inflammation and alleviation of oxidative stress).

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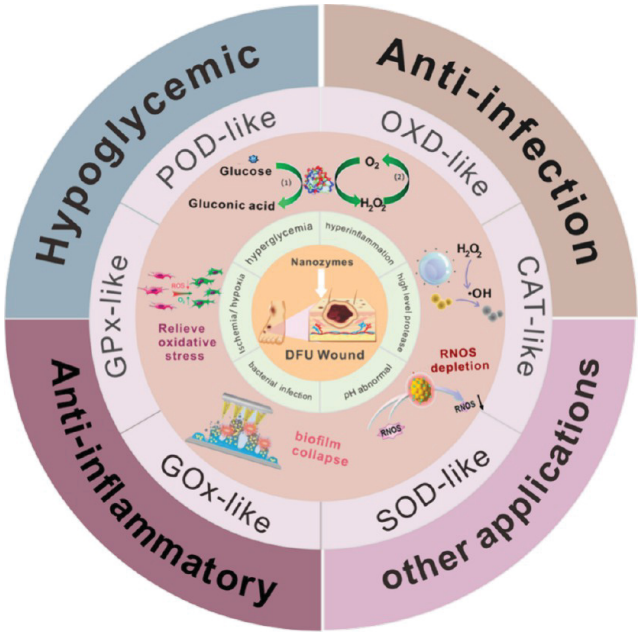
In this review, we first describe in detail the causes and characteristics of the pathological microenvironment of DFU and summarize the conventional treatments as well as their drawbacks, which provide a comprehensive background of DFU. Next, mechanisms by which nanozymes exert their therapeutic effects are described according to the enzyme-like activity (e.g., POD-, OXD-, CAT-, SOD-like, etc.). Then, a detailed overview of the applications of nanozymes in DFU therapy through different pathways (e.g., hypoglycaemic, antimicrobial, anti-inflammatory, etc.) in the last two years is presented. Finally, the challenges and future perspectives of nanozymes in the treatment of DFU are presented (Scheme 1).

Scheme 1. Wound microenvironment and nanozymatic therapeutic strategies for diabetic foot ulcers (DFU)



2. DFU WOUND MICROENVIRONMENT

Skin is the largest organ of the body and is highly susceptible to damage and infection.[37] After injury the skin initiates self-repair and the healing process is generally divided into four distinct phases: hemostasis, inflammation, proliferation and remodeling, which occur chronologically but also overlap due to physiological factors, such as continuous intercellular signaling.[38] Depending on the length of the healing time, wounds can be acute and chronic.[39] Acute wounds heal faster, while chronic wounds are prolonged due to the presence of underlying and persistent pathologic reactions, especially infection.[40] In general, the four distinct phases of wound healing (Figure 1) are closely related to many biological processes, such as reduced M1 macrophage polarization, increased levels of metalloproteinases, bacterial infections and hypoxia, which are detrimental to recovery. In contrast, collagen deposition, increased M2 macrophages, upregulation of angiogenic factor expression and endothelial cell proliferation are favorable factors. These conditions interact with each other to regulate the wound healing process.[41] DFU is a classic example of wounds with chronic persistent inflammatory wound trauma and a complex microenvironment where the clinical healing period typically lasts more than 4 weeks.[42] In this section, several typical features of the DFU microenvironment, such as hyperglycemia, hyperinflammation, and high proteases, will be categorized and discussed so as to gain insights into the causes of DFU and factors affecting healing.[43]

2.1. Hyperglycemia. Hyperglycemia, one of the typical features of the DFU microenvironment, is associated with insulin disorders (deficiency or resistance).[44] Under normal conditions, the body is able to ensure that blood glucose levels remain balanced through hormonal regulation and neuromodulation.[45] Yet, blood glucose rises during pathology, such as when the body does not produce or has difficulty utilizing insulin or when there is abnormal regulation of glucose metabolism by the nervous system.[46] When the organism is in a prolonged state of hyperglycemia, it induces pathologies in various organs. Among them, vascular lesions in the lower limbs cause blood supply impairment and nutrient loss in the distal limbs, resulting in foot-breaking ulcers.[47] Chronic abnormal glucose metabolism in DFU patients inhibits antioxidant expression, induces excessive oxidative stress, and exacerbates wound inflammation.[48] The high-glucose environment of the wound also provides ample nutrients for bacteria, increasing the risk of bacterial infection and impeding the resolution of inflammation.[49] At the same time, localized hyperglycemia inhibits the proliferation and migration of fibroblasts and epithelial cells, limiting the process of cell proliferation and remodeling.[50] More seriously, the neurological lesions reduce the ability to perceive and delay the patient's "pain sensation", delay disease diagnostics, and increase the risk of infection, which may even lead to amputation.

In summary, hyperglycemia brings about a series of severe systemic and local pathologies and is a "trigger" for the symptoms of hyperinflammation and bacterial infections (vide infra). Therefore, hyperglycemia is a major obstacle to DFU wound recovery, and lowering blood glucose is the key therapeutic option and should be treated aggressively.

2.2. Hyperinflammation. Hyperinflammation in DFU is associated with redox homeostatic imbalance caused by hyperglycemia.[51] First, frequent hyperglycemia leads to elevated levels of advanced glycosylation end products (AGEs) in the blood, which contribute to the formation of high concentrations of ROS and reactive nitrogen species (RNS).[52] ROS and RNS are essential for wound healing due to their scavenging capacity for dead tissues and pathogens.[53] However, at high concentrations, it is difficult to maintain the balance of their levels due to the limited antioxidant capacity of the body. Excess ROS/RNS causes tissue damage and also prevents the transition of the wound from the inflammatory phase to the proliferative phase, thus hindering the formation of new healthy tissue.[54] Meanwhile, DFU wound experiences a dramatic increase in inflammatory cytokines (e.g., TNF-α, IL-1, and IL-6) after injury,[55] whereas the expression of chemokines is blocked, thereby delaying the entry of monocytes and macrophages into the wound, preventing the timely clearance of neutrophils, and exacerbating inflammation.[56] Hyperglycemia also hinders the transition from M1 macrophages (pro-inflammatory) to M2 macrophages (anti-inflammatory), further upregulates inflammatory factors, and promotes the senescence of cells involved in tissue regeneration (e.g., fibroblasts, endothelial cells, keratinocytes, and mesenchymal stem cells), which can severely affect granulation tissue regeneration and angiogenesis.[57]

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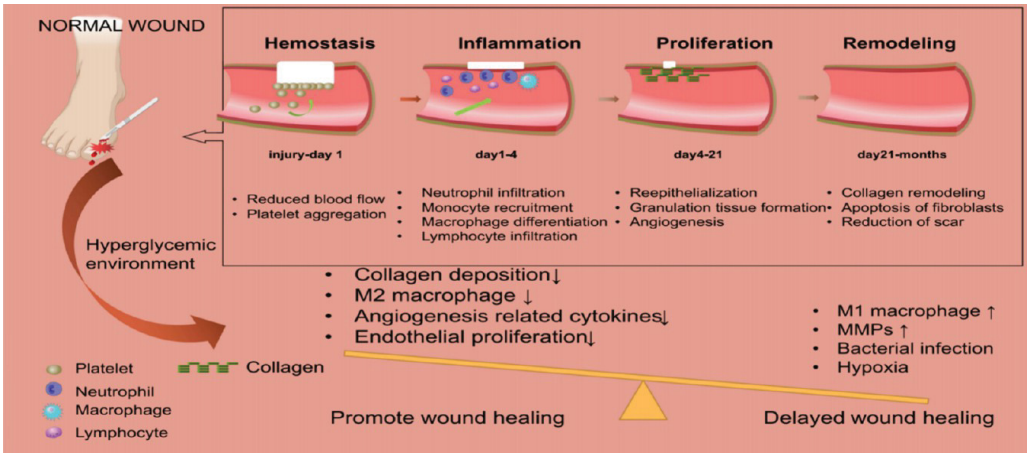

Figure 1. Four stages of wound healing. Comparison of factors that promote and delay wound healing. ref 41. Originally published by and used with permission from Dove Medical Press Ltd., 2024. Copyright Xiaohuan Yuan.

In short, multiple factors, such as sustained release of pro-inflammatory factors, enrichment of M1 macrophages (pro-inflammatory type), and impaired expression of chemokines, collectively result in sustained hyperinflammation and excessive oxidative stress in DFU wounds. The resulting stagnant epithelial cell growth, impaired angiogenesis, and collagen deposition all greatly impede wound healing. Therefore, anti-inflammation and relief of oxidative stress are important measures for DFU healing.

2.3. High level protease. High levels of proteases are another characteristic of DFU, especially matrix metalloproteinases (MMPs), which are involved in every process of wound healing by promoting cell migration and degrading the extracellular matrix (ECM).[58] MMPs are expressed at low levels in normal tissues. However, when the body requires tissue remodeling (e.g., wound healing), the expression of MMPs is upregulated in a variety of cells within the wound (keratinocytes, fibroblasts, endothelial cells, and inflammatory cells), and is also induced in response to signals such as ROS, proinflammatory cytokines, and ECM.[59] Tissue inhibitors of MMPs (TIMPs) can inhibit the overexpression of MMPs, and the two play an important synergetic role in wound healing.[60] DFU exhibits significantly elevated levels of MMPs (mainly collagenase (MMP-1 and MMP-8), gelatinase (MMP-2 and MMP-9), and matrix lysin (MMP-10)) and low-level expression of TIMPs.[61−64] At this point, the balance between the activity of MMPs and the inhibitory effect of TIMPs is disrupted, and growth factors and ECM are excessively degraded, ultimately leading to delayed or arrested wound closure. In addition to endogenous proteases, microorganisms colonizing the periwound also express exogenous proteases to promote their proliferation and exacerbate the infection.[65]

Overall, high levels of endogenous and exogenous proteases in the DFU microenvironment are powerful "catabolizers" that continuously break down the ECM and further degrade already insufficiently supplied growth factors, accompanied by a large amount of proteinous exudates, leading to wound healing failure. High levels of MMPs also decrease VEGF expression and inhibit fibroblast growth. Therefore, MMPs/TIMPs can be a predictor of DFU wound healing, making it possible to treat DFUs by targeting MMP inhibition or increasing the activity of a certain MMP protein.

2.4. Abnormal pH. The normal skin surface pH is weakly acidic due to the secretion of organic acids (pH 4 to 6) by epithelial cells.[66] The pH of the skin increases with depth up to about 7.4. When the skin is damaged, the pH of the wound increases with depth of damage.[67] The pH of acute wounds gradually returns to acidic as the wound closes, while the pH of chronic wounds tends to be weakly alkaline between 7 and 9, which is more favorable for bacterial growth and multiplication as well as the highest activity of MMPs.[68] This suggests that during wound healing, enzyme activity, macromolecule synthesis, metabolite transport, and various cellular activities (inflammation, collagen formation, and angiogenesis) significantly alter the pH of the wound.[69] Therefore, restoration of an acidic environment on the wound surface is significant in reducing microbial colonization on the wound surface and re-establishing the barrier to chronic wounds.[70] Restoration of an acidic environment also helps restore metabolism to adipose tissue, providing nutrients needed for wound healing and accelerating healing.[71] In addition, the pH environment of DFU is a dynamic process, which is related to a variety of factors such as the colonization of different microorganisms, time of onset, severity, and stage of healing. Monitoring pH can capture the dynamic changes in the wound and the healing process. This provides a new idea for the clinical detection of DFU.[72]

2.5. Persistent bacterial infection. Bacterial infections are one of the most common and major problems in wound healing.[73] Hyperglycemia and alkaline pH in the DFU microenvironment are both promoters of bacterial infections. Hyperglycemia affects the morphology and function of immune cells (chemotaxis, phagocytosis, and microbicidal capacity are diminished), while providing nutrients for microorganisms and promoting metabolism.[74] An alkaline environment is closer to the physiological pH of bacteria (ca. 7.4), which facilitates bacterial colonization and reproduction.[75] Induced by various factors, microflora (mainly bacteria) from the external environment colonize DFU wounds, and worse, the accumulation and adhesion of multiple microorganisms stimulate the formation of biofilm, which is more difficult to deal with than microorganisms.[76]

The adverse effects of bacterial infection are manifold. First, infection triggers difficult regeneration of blood vessels and slow healing in DFU wounds.[77] Second, recurrent infections stimulate a sustained release of inflammatory factors (e.g., TNF-α) and proteases that stall wound healing in the inflammatory phase.[78] High levels of proteases degrade

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essential proteins (e.g., growth factors) and disrupt ECM remodeling. In addition, chronically infected wounds are infiltrated with immune cells, such as neutrophils, and unfavorable cell growth and migration, resulting in apoptosis and tissue necrosis.[79] It also alters the pH and lactic acid deposition in the wound, thereby accelerating the formation of an unfriendly microenvironment.[80] At the same time, long unhealed wounds can be infected by multidrug resistant bacteria, which is another thorny issue.[81] Clinically, anti-infective treatment for DFU wounds is mainly based on antibiotic therapy and surgical debridement. However, the emergence of drug-resistant bacteria and the stagnation of the development of new antibiotics have led to a diminishing supply of antibiotics, resulting in a slow process of treatment for infected wounds.[82] In addition, repeated surgical debridement exacerbates patient pain and increases the financial burden.[83] Therefore, there is an urgent need to develop more effective anti-infective treatments for DFU as a way to relieve patients' pain and improve their quality of life.

2.6. Ischemia and hypoxia. Inadequate angiogenesis is an important cause of diabetic wound healing difficulties.[84] Macrophages are the main source of vascular endothelial growth factor (VEGF) and other pro-angiogenic mediators in wounds.[85] During the inflammatory phase of DFU, the macrophage phenotype is altered, failing to promote tissue repair and affecting angiogenesis. Reduced angiogenesis causes prolonged ischemia in the DFU, which prevents neutrophil-mediated sterilization and causes wound hypoxia. In addition, since oxygen plays a key role in wound healing, a hypoxic environment also severely restricts the healing of DFU.[86] The imbalance between low oxygen supply (damaged vascular system) and high oxygen demand (required for cellular repair of wounds) leaves DFU in a chronically hypoxic environment, which is different from that of the transient hypoxia in acute wounds.[87] Hypoxia-inducible factor-1 (HIF-1) mediates the cellular adaptive response to hypoxia by participating in angiogenesis, cell proliferation and migration, and metabolic changes.[88] Under normoxic conditions, HIF-1 is activated and stimulates the expression of downstream genes that promote erythropoiesis and the production of growth factors (e.g., VEGF, PDGF, and basic fibroblast growth factor [bFGF]), which are essential for the regulation of hypoxia.[89] However, in DFU, HIF-1 signaling is inhibited due to hyperglycemia, leading to downregulation of HIF-1/VEGF expression, and hence ischemia and hypoxia.[90] Overall, the combination of impaired vascular regeneration, increased hypoxic conditions, and attenuated cellular responses to hypoxia contribute to delayed wound healing in DFU. The ischemic and hypoxic environment is insufficient to supply the large amount of energy required for wound healing, resulting in a crisis of energy supply. More seriously, ischemia and hypoxia are exacerbated by the large number of inflammatory cells around the wound and the high metabolism of regenerating tissues. Inadequate supply of energy and excessive depletion further impede DFU wound healing.

In response to the above characteristics, there are four strategies in current diabetic wound repair and treatment. The first is hypoglycaemic therapy. There are two kinds of common medicines: biguanide hypoglycaemic drugs and insulin.[91] By downregulating the blood glucose concentration around the wound, the wound microenvironment is gradually restored to normal. In addition, the downregulation of blood glucose is beneficial to the cutting off of nutrient sources of pathogens and the alleviation of oxidative stress. However, long-term use of hypoglycaemic drugs inevitably triggers a series of side effects, such as edema, hypoglycaemia and obesity.[92] The second is antibiotic therapy. There are differences in the types of bacteria that infect the wound depending on the severity of the infection. *Staphylococci* and *streptococci* were most abundant in wounds with mild infections, where penicillins can be the first choice. In more severe cases, bacterial diversity is higher and Gram-negative bacteria are common, aminoglycosides (e.g., amikacin, gentamicin), tertiary cephalosporins (e.g., ceftazidime), and carbapenems (e.g., imipenem) may be chosen.[93] However, frequent use of antibiotics will be more likely to induce the growth of drug-resistant bacteria, reduce the efficacy of antibiotics, and may also trigger adverse reactions in organs such as the liver and kidneys, so the use of antibiotics needs to be strictly controlled and promptly adjusted.[94] The third is traditional dressings, including dry gauze, oil gauze, cotton pads, bandages, etc., that play the role of passive protective dressings and are mainly used to isolate wounds. However, they have no direct effect on wound healing, and their limited ability to absorb exudate increases the frequency of change, leading to secondary wound injury.[95] Therefore, medical wound dressings are constantly being improved toward ideal dressings with good absorbency, no contaminant residue, thermal insulation, antimicrobial properties, avoidance of secondary trauma on removal, low frequency of dressing changes required, pain relief and comfort.[96] The fourth is surgical debridement, which is an important part of the treatment of diabetic wounds that facilitates wound recovery by removing necrotic tissue and reducing the extent of local infection. However, the location of diabetic wounds is usually in the hard-to-reach areas of the arch and toes, which are likely to cause additional tissue trauma and create new wounds during surgery. In addition, the need for repeated debridement is common due to the susceptibility of the wound to recurrence, which greatly aggravates the patient's psychological and financial burden.[97]

3. MECHANISMS OF NANOZYMES FOR DFU TREATMENT

The unfriendly microenvironments of DFU, such as hyperglycemia, hyperinflammation, and persistent infections, are extremely detrimental to wound healing. Nanozymes with multifunctionality, tunable properties and high stability are ideal candidates for modulating the harsh microenvironment and accelerating wound healing. A range of nanozymes exhibit outstanding enzyme-like activities, mimicking POD, OXD, CAT, and SOD, which can directly or indirectly regulate blood glucose levels, inhibit bacterial infections, and thus act as a therapeutic agent for DFU.[36] In this section, the therapeutic mechanisms of nanozymes for DFU are discussed.

3.1. POD-like activity. ROS, mainly hydrogen peroxide, superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (•OH), and singlet oxygen ($^1O_2$), are closely related to various cellular activities, such as physiological regulation and tissue growth.[98] During wound healing, low concentrations of ROS can act as intracellular messengers to regulate relevant pathways and promote wound repair. However, excessive ROS can cause oxidative damage to cells, increase the release of pro-inflammatory factors, and induce inflammation, thereby delaying wound healing.[99] POD is an oxidoreductase enzyme that is essential for the in vivo regulation of ROS. The vast majority of POD are heme-based enzymes (e.g., horseradish

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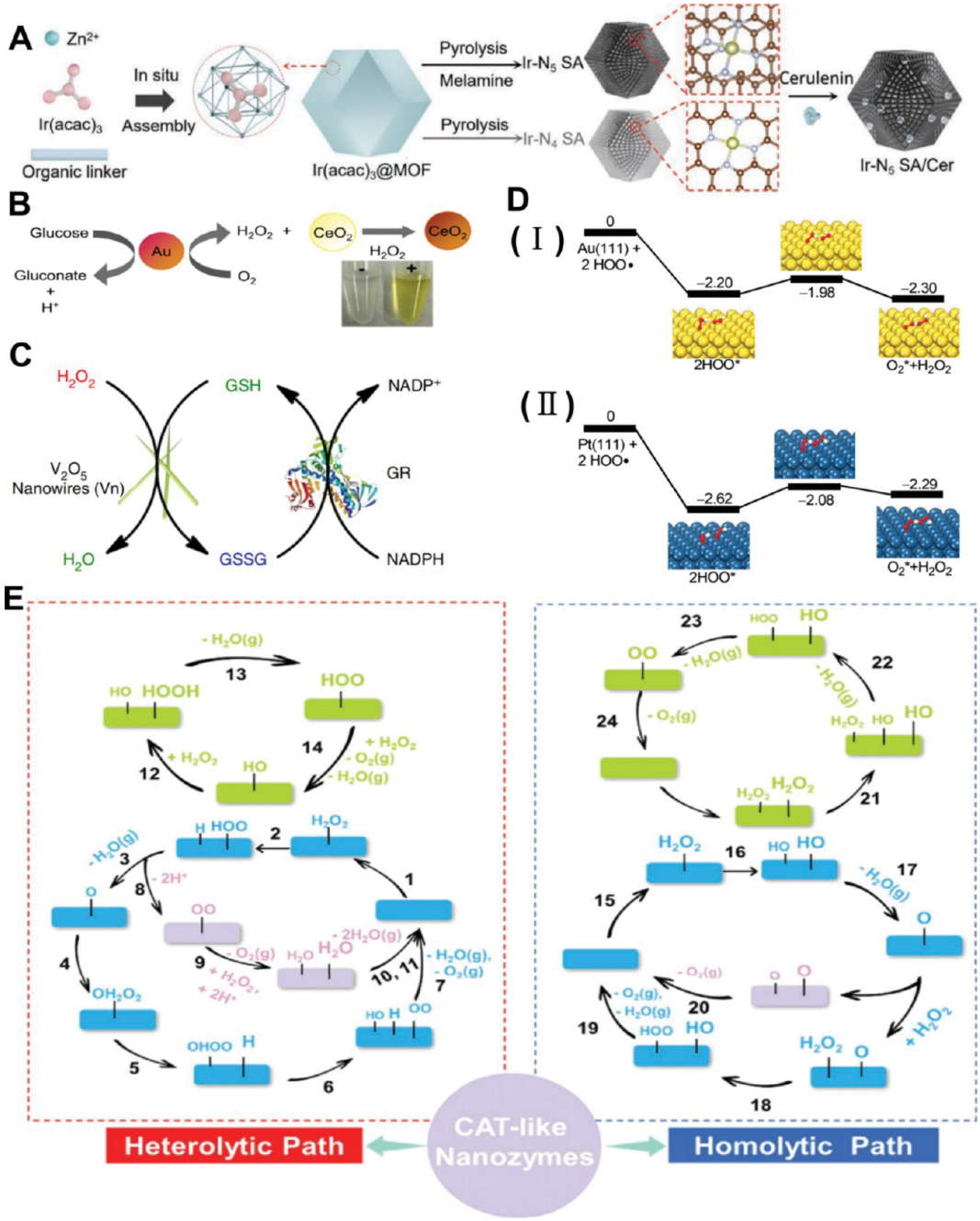

Figure 2. (A) Pyrolytic preparation of single-atom nanozyme (Ir−N_5 SA) with an asymmetric electron distribution using a MOF precursor with the addition of melamine. Reproduced with permission from ref 104, Copyright 2023, Wiley. (B) Schematic illustration of Au NPs reacting with glucose and oxygen to produce gluconic acid and H_2O_2. Reproduced with permission from ref 119, Copyright 2014, Elsevier. (C) Schematic diagram depicting the GPx-like antioxidant activity of V_2O_5 and GSH recycling by glutathione reductase (GR). Reproduced with permission from ref 147, Copyright 2014, Nature. Copyright Amit A. Verneka. (D) Potential energy distributions for the rearrangement of two $HO_2^{\bullet}$ on the (111) faces of (I) Au and (II) Pt. Reproduced with permission from ref 141, Copyright 2015, ACS. (E) Illustration of the mechanism of CAT-like nanozymes. The ring on the left represents the heterogeneous catalytic cleavage pathway and the double ring on the right represents the homogeneous catalytic cleavage pathway. Reproduced with permission from ref 126, Copyright 2022, Wiley.

peroxidase and myeloperoxidase), but natural enzymes are costly and unstable.[100] A variety of nanomaterials have been found to exhibit excellent POD activity and can break down hydrogen peroxide in pathological environments (typically wounds or tumors) to produce cytotoxic hydroxyl radicals. This can be exploited for wound therapy.[101] In general, POD-like nanozymes use hydrogen peroxide as a substrate to initiate the catalytic reaction. There are two reaction pathways: (a) •OH generation pathway, where H_2O_2 is adsorbed on the surface of the nanozymes to form two •OH radicals by splitting the O−O bond, and the •OH will further oxidize other substrates; and (b) electron-transfer pathway, where the nanozymes act as an electron-transfer medium to catalyze the reaction of H_2O_2 directly with the substrate without the generation of •OH.[102] That is, the catalytic mechanism of POD-like nanozymes can be summarized as a Fenton or

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ACS Biomater. Sci. Eng. 2024, 10, 4195−4226

Fenton-like reaction and an electron transfer process.[103] For example, Yang et al.[104] pyrolyzed a metal−organic framework (MOF) precursor along with melamine to construct an Ir−N_5 single-atom nanozyme with an asymmetric electron distribution (Ir−N_5 SA) (Figure 2A). Compared with Ir−N_4 SA, Ir−N_5 SA has an asymmetric electron distribution, and the synergistic effect between the central Ir atom and the axial N coordination structure effectively optimizes the free energies of each transition state on Ir−N_5 SA, leading to enhanced enzyme-like catalytic activity. With a well-defined coordination structure, maximal atom utilization and catalytic activity, Ir−N_5 SA possesses excellent POD- and OXD-like activities, which can catalyze the generation of a large amount of ROS to kill tumor cells, as well as CAT-like activity, which generates O_2 to alleviate hypoxia. In addition, Ir−N_5 SA can mimic NADH oxidase-like (NOX-like) activity and catalyze the generation of H_2O_2 from NADH, while inhibiting mitochondrial electron transport chain (ETC) and aerobic respiration, effectively reducing endogenous O_2 consumption. The synergistic action of the multiple enzymes increases the O_2 and H_2O_2 contents at the tumor sites, greatly increasing the ability of IrN_5 SA to generate ROS via OXD and POD-like activities, and causing irreversible oxidative damage to tumor cells. In fact, POD-like nanozymes show similar mechanisms in antitumor and wound therapy, and are widely used as a mainstream treatment of diabetic wounds.

For instance, Gao et al.[105] synthesized a Fe single-atom nanozyme using erythrocytes as a template. Fe-based nanozymes are one of the most widely used nanozymes, but the limited utilization of Fe atoms often makes their catalytic efficiency lower than that of natural enzymes (e.g., horseradish peroxidase, HRP). Single-atom nanozymes mimic the iron-porphyrin coordination structure of natural enzymes, with improved catalytic efficiency and viability. However, in current synthesis, iron clusters and elemental iron are typically formed in addition to single-atom iron, which negatively affects the enzyme-like activity. HRP, with a heme active center, consists of a single Fe atom coordinating with four N atoms in the same plane. Coincidentally red blood cells in organisms, each of which contains about 260 million hemoglobins containing iron atoms and cyclic heme, make them an ideal source of single-atom iron. Therefore, the erythrocyte templating strategy has been proposed to prepare Fe single-atom nanozyme by combining the processes of cell immobilization, salting out and pyrolytic carbonization using abundant hemoglobin as the source of iron. The obtained erythrocyte template nanozymes exhibited excellent POD-like activity, catalyzed the generation of •OH from H_2O_2, and could be further enhanced by near-infrared (NIR) light irradiation.[105] In addition, its honeycomb-like structure can act as a nanosponge to promote blood coagulation. These properties make it a multifunctional material that can promote healing of MRSA-infected wounds. This suggests that POD-like nanozymes can further shine in biomedicine by combining multidisciplinary technologies.[106]

Repeated bacterial infections and excessive oxidative stress in DFU wounds have a significant impact on tissue healing. For DFU, recurrent bacterial infections and high levels of ROS have a serious impact on tissue healing. High levels of ROS disrupt the activity of biomolecules, leading to excessive oxidative stress. Satisfactorily, nanozymes with POD-like activity have sufficient ability to convert H_2O_2 to •OH and deplete ROS at the wound site.[107] Meanwhile, the large amount of highly toxic •OH can effectively induce the death of infectious pathogens while reducing inflammatory responses, ultimately promoting DFU healing. For example, Wen et al.[108] designed a multifunctional nanozyme based on cerium oxide nanoparticles supported on black phosphorus (BP) nanosheets, where the excellent POD activity was ascribed to the high ratio of Ce^{3+} to Ce^{4+} when the particle diameter was reduced to the nanoscale. With the addition of GOx and DNA aptamers, the obtained GOx-CeO_2@BP/Apt nanocomposites exhibited further enhanced POD-like activity, as hydrogen peroxide generated from the decomposition of glucose by GOx entered a cascade reaction catalyzed by the CeO_2 nanozymes to produce a large amount of cytotoxic •OH; meanwhile the BP nanosheets significantly increased the wound temperature under near-infrared (NIR) irradiation, which also facilitated the production of •OH. These combined contributions were the key to the antimicrobial activity.

However, for most nanozymes, POD activity is optimal in acidic solutions, while the pH of most physiological systems exceeds 7.0 (even >8.0 in chronic wounds),[109] which greatly affects the efficacy of POD-like nanozymes in wound healing. Li et al.[110] constructed a GOx-like and POD-like dual nanozyme catalytic cascade system at room temperature by partial reduction of Fe^{3+} to Fe^{2+} and simultaneous deposition of Au nanoparticles (FeOOH@Fe-Serine@Au NSs). The dual nanozymes first consumed glucose to generate H_2O_2 and gluconic acid via GOx, which provided sufficient H_2O_2 for the subsequent reaction and acidified the wound environment to inhibit bacterial growth. Importantly, the acidic microenvironment generated due to gluconic acid production enhanced the POD activity. The prepared dual nanozymatic system indeed amplified the catalytic cascade activity and showed excellent antimicrobial properties and inflammatory factor modulation for the treatment of diabetic mouse wounds, accelerating angiogenesis and further promoting wound healing. Meanwhile, Li et al.[111] proposed a fiber-based regionalization strategy to construct a multinanozyme system, which was capable of performing incompatible reactions simultaneously. In this system, Fe_3O_4 nanozymes (POD-like) and MnO_2 nanozymes (CAT-like, with optimal activity at neutral or alkaline pH) were immobilized in two regionalized fibers by a one-step electrostatic spinning process to form two reaction domains. Benzoic acid was doped in the fibers encapsulating the POD-like nanozymes to down-regulate the pH. Through a series of experiments, the optimal length of the fibers was determined, and the feasibility of the two enzyme activities to be active independently in different regions was also demonstrated. CaO_2 nanoparticles acted as a H_2O_2 donor, which could be combined with MnO_2 nanozyme to produce O_2 under neutral or alkaline conditions, and with Fe_3O_4 nanozyme under acidic conditions to produce •OH. The cleverly designed fiber nanozyme possessed both POD-like activity and CAT-like activity, which are synergistic for antibacterial, anti-inflammatory and vascular regeneration promotion to accelerate the healing of DFU. Therefore, breaking the pH limitation is the key to highly active POD, and results from these earlier studies may offer insights for the construction of novel and highly efficient POD-like nanozymes to broaden the potential of their application in the diagnosis and treatment of diseases.[112]

In addition, considering the harsh repair microenvironment of DFU, the therapeutic effect of a single POD activity is far from sufficient. Zhu et al.[113] synthesized a nanozyme where MnO_2 was encapsulated with polydopamine (PDA) doped

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with bioactive glasses (BGs), which possessed antioxidant, antimicrobial, and pro-healing abilities. The MnO_2@PDA-BGs nanozymes exhibited significant enzyme-like activity in the early stage of wound healing, and BGs played an important role in the late stage of healing by promoting vascularization and collagen fiber deposition. When MnO_2@PDA-BGs was loaded into a macroporous cryogel prepared by polymerization of gelatin with methacrylate anhydride at −20 °C, the obtained nanocomposite cryogel (MnO_2@PDA-BGs/Gel) displayed multiple enzymatic activities and excellent performance in eliminating ROS. The introduction of MnO_2 endowed the material with POD-like activity for the production of bacteria-killing •OH under acidic conditions, and CAT-like activity for the decomposition of hydrogen peroxide into O_2 and water to reduce cellular damage induced by oxidative stress and to provide a good repair environment for wound healing. The SOD-like activity of PDA decomposed $O_2^{\bullet-}$ into O_2 and hydrogen peroxide to further improve the wound microenvironment. Meanwhile, due to the good photothermal properties of PDA, localized heat was produced to further improve the bactericidal performance under NIR irradiation. Indeed in vivo animal experiments confirmed that multienzyme-active nanozymes promoted processes conducive to diabetic wound healing including killing of bacteria, reduction of inflammatory response, promotion of vascular regeneration, and acceleration of collagen deposition.

3.2. OXD-like activity. Oxidases catalyze the oxidation of substrates by electron transfer in the presence of O_2 to produce H_2O_2 or H_2O.[114] Nanozymes with OXD-like activity can inhibit bacterial growth by generating large amounts of ROS through the above process. Therefore, OXD-like nanozymes are promising for the treatment of DFU. For instance, Li et al.[115] designed a multienzyme-like active nanocomposite (Mo, Fe/Cu, I−Ag@GOx) and immobilized it on a multifunctional fluorescent hydrogel. The nanozyme showed excellent OXD activity in generating cytotoxic ROS and regulating oxidative stress in the wound environment. As the nanozymes possessed other enzyme-like activities, such as POD, CAT, SOD and GOx-like activities, they formed a pH-switchable glucose-initiated cascade reaction system for diabetic wound healing.

Generally, OXD-like nanozymes can be categorized according to the substrates: amino, glucose, polyphenol, sulfur and ferrous groups. Due to a series of adverse consequences triggered by hyperglycemia, lowering blood glucose has become a top priority in DFU clinical treatment. The commonly used drug therapy led by insulin can effectively inhibit glucose absorption, but the side effects triggered by skin allergy, hypoglycaemia and gastrointestinal stress should not be ignored.[116] Therefore, there is an urgent need to find novel methods that can reduce blood glucose levels in DFU wounds while causing few or no side effects. OXD-like nanozymes, especially GOx, which uses glucosyl as a substrate moiety, perfectly fulfill the above needs. In the glucose-lowering mechanism, first, the enzyme strips two protons and electrons from glucose, causing it to hydrolyze to gluconic acid.[117] Then, molecular oxygen is reduced by the enzyme to H_2O_2. Meanwhile, compared with natural GOx, GOx-like nanozymes possess more stable catalytic activity, lower production cost and higher maneuverability, and they can also overcome the disadvantage of decreased activity of natural GOx due to wound hypoxia.[118] Therefore, GOx-like nanozymes are being developed as highly effective "hypoglycaemic agents" for the treatment of DFU.

Au nanoparticles (NPs) are structurally different from the active site of GOx but have efficient GOx-like activity, making them the most widely used GOx mimic[119] (Figure 2B). The approximate steps of glucose oxidation catalyzed by Au NPs are as follows: first, glucose is activated by OH-, which removes the proton to form a hydrated glucose anion. The hydrated glucose anion contacts the Au NPs and reacts with the surface gold atoms to form extremely electron-rich gold species, which then activates molecular oxygen. Next, electrons are transferred from glucose to dioxygen with Au^{+}-O_2^{2-} or Au^{2+}-O_2^{2-} as intermediates. Finally, gluconic acid and H_2O_2 are generated.[120] Due to the constant consumption of glucose from the wound, the bacteria are starved of nutrient supply. In addition, the H_2O_2 produced during glucose oxidation can be converted to •OH by subsequent Fenton or Fenton-like reactions, initiating chemodynamic therapy (CDT) to directly kill the bacteria. For instance, Fan et al.[121] designed molybdenum disulfide nanosheets enriched with bovine serum albumin-modified gold nanoparticles (MoS_2@Au@BSA NSs) and anchored them on an injectable hydrogel to promote healing of diabetic wounds via an O_2 self-feeding cascade reaction. MoS_2 nanosheets showed POD-like, SOD-like and CAT-like activities. However, the application of MoS_2 nanosheets in DFU wound healing was limited by the lack of GOx-like activity, which was compensated by Au NPs. Natural bovine serum albumin (BSA), a stabilizing and protective agent, contributed to the stability and enzymatic activity of Au NPs. The enzymatic reaction began with glucose consumption and H_2O_2 breakdown, and the highly toxic product (•OH) eradicated bacteria from the wound site to accelerate healing. Subsequently, benefiting from the CAT and SOD activities, excessive ROS were scavenged to generate abundant O_2, which alleviated hypoxia and promoted glucose oxidation to ensure a normal cascade reaction. Meanwhile, the nanozyme was mixed into an injectable hydrogel consisting of oxidized dextran and ethylene glycol chitosan cross-linked by Schiff base. The self-healing, shape-adaptive and tissue adhesion activities of the hydrogel improved the physical and mechanical properties of the complex. This nanozymatic hydrogel cleverly utilized the GOx activity of Au NPs to successfully achieve blood glucose downregulation in diabetic wounds and cut off the nutrient source of bacteria. Meanwhile, it combined the enzyme-like activity of 2D materials to produce toxic ROS to kill bacteria. The synergistic effect of the two eradicated biofilm infection and promoted DFU wound healing. In another study, Zhao et al.[122] combined GOx-like nanozymes and photothermal therapy (PTT) to prepare a catalytic microneedle patch (Au-CMS NSs) with dual nanozymatic activity and NIR-II response for the treatment of DFU wound infections. Due to the strong tissue penetration ability of the microneedle patch, Au-CMS NSs could be delivered to deep tissues and fully interact with the wound environment. Based on Au NPs with GOx-like activity and CMS nanozyme with NIR-II photothermal conversion properties, the synthesized catalytic patches achieved in situ glucose consumption, oxygen generation, and bacterial elimination. Notably, their excellent antimicrobial capacity under NIR-II was demonstrated against methicillin-resistant *Staphylococcus aureus* in vitro and in diabetic mice. This boded well for the combination of GOx-like nanozymes and PTT in the treatment and care of DFU wounds.

In addition, many noble metal NPs, such as Pt, Pd, Ru, Rh, and Ir, also catalyze the oxidation of glucose and exhibit GOx

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activity. Ruthenium nanoparticles with GOx-like activity were reported for the first time by Cong et al.[123] In the tumor microenvironment, Ru-based nanozyme with dual POD and GOx activities oxidized glucose to produce H_2O_2, which was then converted to •OH via a Fenton-like reaction. Glucose consumption was similar to "starvation treatment", and the synergistic effect with CDT significantly improved the antitumor efficacy of ruthenium nanozymes. Ju et al.[124] reported a microneedle patch loaded with MnO_2/Cu_2O nanosheets and combined with combretastatin A_4 (MN-MnO_2/Cu_2O-CA_4). In this case, the GOx-mimetic activity of MnO_2/Cu_2O catalyzed the generation of H_2O_2 from glucose, which bound to the released Cu and induced a Fenton-like reaction, effectively generating hydroxyl radicals for CDT. The GOx-like activity of noble metal nanozymes other than Au NPs has been applied in tumor therapy, while little has been reported in DFU, but a similar strategy for the treatment of diabetic wound infections is just around the corner.

3.3. CAT-like activity. To maintain redox homeostasis in the body, antioxidant enzymes play a crucial role. CAT is a representative of these enzymes, which protects cells from the toxicity of ROS by decomposing hydrogen peroxide to H_2O and O_2, and also reduces hypoxia.[125] Therefore, CAT-like nanozymes can be used as ROS scavengers in antibacterial and antitumor applications and as a skin antioxidant to effectively protect against oxidative damage. Hydrogen peroxide consists of two types of chemical bonds, the H−O bond and the O−O bond. Thus, the decomposition process of hydrogen peroxide can be categorized into homogeneous catalytic cleavage and heterogeneous catalytic cleavage. The former refers to the preferential breaking of the O−O bond of adsorbed hydrogen peroxide, and whereas the latter entails cleavage of the H−O bond[126] (Figure 2E). Meanwhile, CAT-like artificial enzymes reduce the production cost, improve the stability, and are recyclable, which makes its application in industrial production or medical field promising.[127] Since citric acid- and pectin-functionalized Au/Pt bimetallic nanoparticles (CP-Au/Pt NPs) have been reported to possess CAT-like and SOD-like activities,[128] an increasing number of CAT-like nanozymes have been used in tumor therapy, wound healing, and other fields.

The DFU healing process begins with a bacterial clearance phase. Killing bacteria and removing biofilms is critical at this time. GOx-like nanozymes can be used to consume glucose to produce gluconic acid and H_2O_2, and the H_2O_2 is catalyzed by POD-like nanozymes to produce a large amount of ROS (mainly •OH). ROS can damage the cell membrane of bacteria, which in turn damages the bacteria RNA or DNA, leading to cell death. Next, a cell proliferation phase is required to generate healthy tissue, where CAT-like nanozymes can play a particularly important role.[129] As efficient "scavengers", they are used to remove excessive H_2O_2 produced in the first phase to protect normal tissues from oxidative stress. In addition, the O_2 produced from the decomposition of H_2O_2 can improve the hypoxic microenvironment.[130] The abundance of O_2 not only promotes glucose consumption, but also provides the energy needed for revascularization, cell growth, and wound healing. For instance, Li et al.[131] derived a CAT-mimicking nanozyme (MnCoO@PLE/HA) from natural polymers (hydrazide-modified hyaluronic acid and aldehyde-modified hyaluronic acid) and MOF. The engineered nanozyme trapped endogenously elevated ROS in diabetic wounds through CAT-driven ability and also synergistically generated O_2 through this process. This resulted in the protection of skin cells (e.g., keratinocytes, fibroblasts, and vascular endothelial cells) from ROS and hypoxia-mediated death and inhibition of proliferation, and promoted DFU wound closure. Similarly, Zhao et al.[132] constructed a medium multicomponent modified CAT-like nanozyme hydrogel (MnCoO@PDA/CPH), where CAT-mimetic MnCoO was modified by polydopamine (PDA) for enhanced stability and activity, and supramolecules of biocompatible polymers (soft and elastic) and conductive polyaniline (PANI)-based derivatives (rigid and strong) were further assembled to recapitulate the cross-linked structure of collagen fibers and elastin fibers in skin. The biomimetic nanozymatic hydrogel not only efficiently scavenged H_2O_2 and generated O_2, but also facilitated cell survival, proliferation and migration. Oxygenation from H_2O_2 decomposition can direct the behavior of the immune system, which in turn enhances re-epithelialization, increases collagen deposition, and increases angiogenesis. Recently, Wang et al.[133] designed a CAT-like nanozyme to improve the DFU microenvironment and promote the healing process. The composite nanozyme (EGAP) consisted of several important components including silver nanoparticles, gallic acid (GA), iron (Fe III) and epidermal growth factor (EGF). The synthesized nanozyme was then doped into hydrogel to obtain thermosensitive hydrogel (EGAP@HG), which displayed broad-spectrum antibacterial activity by releasing GA and silver ions. In addition, under the hypoxic microenvironment, the EGAP nanozyme exhibited excellent CAT-like activity, which enabled the decomposition of H_2O_2 to O_2 and alleviated local hypoxia. Second, the continuous release of EGF promoted cell proliferation. These results suggest that multifunctional nanozymatic hydrogel with significant CAT-like activity can synergize multicomponent advantages to improve the local microenvironment of diabetic wounds for accelerated DFU wound healing through bacterial inhibition, inflammation relief, oxygenation, and angiogenesis.

Nanozymes can also exhibit a combination of CAT and GOx activities. For instance, Li et al.[134] designed a GOx-CAT nanozyme-chitosan (GCNC) hydrogel. Due to the dual enzymatic activity (GOx and CAT), endogenous H_2O_2 and H_2O_2 generated from GOx-catalyzed glucose oxidation were rapidly catalyzed by CAT to generate O_2, which enhanced the catalytic activity of GOx, and thus achieved a sustained and effective regulation of blood glucose. In addition, gluconic acid from glucose oxidation activated the amino group of chitosan in the hydrogel, and improved the antimicrobial activity of chitosan. The depletion of the acidic product downregulated pH, and ensured a neutral/alkaline environment for GOx and CAT enzymes to exhibit optimal activity. The efficient cascade effect of GOx and CAT effectively regulated the glucose concentration in the DFU, improved the oxidative microenvironment of the wound, and continuously generated oxygen in order to promote cell proliferation, migration, and angiogenesis, and facilitated the healing of diabetic wounds.

In addition, pH limitation is a non-negligible challenge for CAT-like nanozymes. In general, CAT and SOD are most active in neutral or alkaline media, whereas POD and OXD, two other common catalases, require acidic conditions. The different optimal pH ranges hinder the crosstalk of multiple enzyme activities. Feng et al.[135] developed a novel pH-responsive composite hydrogel (Alg/CuP) based on copper nanozyme for DFU in an alkaline environment. Alg/CuP dressing exhibited CAT-like activity, where abundant O_2

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derived from hydrogen peroxide conversion and continuous release of copper ions promoted angiogenesis and accelerated wound repair. Subsequently, the catalytic properties of Alg/CuP were transformed into POD due to the drop in wound pH caused by persistent bacterial infection, which together with Cu ions produced toxic •OH to eliminate a variety of bacteria and biofilms. This pH-responsive nanozymatic dressing ensured flexible switching of enzyme activity between CAT and POD based on the change in wound pH, enabling targeted DFU therapy against infection and specific pH.

In short, CAT-like nanozymes play an important role in DFU therapy. However, the issues of insufficient therapeutic efficacy of single enzyme activity and pH limitation need to be urgently resolved, which is a critical direction of future efforts.

3.4. SOD-like activity. Oxidative stress refers to a state of imbalance between oxidative and antioxidant effects in the body caused by the overexpression of superoxide anion, hydroxyl radicals, hydrogen peroxide and other ROS.[136] It has been shown that superoxide levels are the highest during the initial phase of wound healing (the day after injury), and the induced oxidative stress significantly prolongs the wound healing cycle.[137] It is worth mentioning that SOD converts superoxide anions to H_2O_2 and O_2 due to its specific antioxidant properties.[138] Natural SOD is a metalloenzyme widely found in cellular organelles, usually consisting of metal factors and proteins, but the lack of long-term activity and the high cost limit the application. SOD-like nanozymes are the ideal alternatives, which not only possess excellent catalytic activity, but are also stable and controllable.[139] The earliest SOD-like nanomaterials involved C_{60} with O_2 scavenging ability, as reported by Kroto et al. in 1985.[140] Since then, more and more nanomaterials have been shown to possess SOD activity, among which cerium oxide nanoparticles are well-known representatives. Other metals, such as Pt, Au, Cu, Mn, Ni, Co, etc. and their oxides, carbides, nitrides and sulfides also exhibit SOD-like activity. Protonation of $O_2^{\bullet-}$and adsorption and rearrangement of $HO_2^{\bullet}$ on metal surfaces are the main pathways of the SOD-like catalytic mechanism. In general, $O_2^{\bullet-}$ can easily trap protons in water to form $HO_2^{\bullet}$ and OH^-. $HO_2^{\bullet}$ rearrangement on metal surfaces (e.g., Au, Pt, Figure 2D) has a very low potential-energy distribution that allows it to be readily converted to O_2* and H_2O_2* once adsorbed. After that, O_2* and H_2O_2* become $O_2^{\bullet-}$ and H_2O_2.[141] Based on this, metals with SOD activity have been introduced to prepare SOD-like nanoenzymes that reversibly repair the unfriendly microenvironment of wounds through the conversion of $O_2^{\bullet-}$, and become the first line of antioxidant defense in the treatment of DFU.

For example, Huang et al.[142] selected 2,5-dimercaptoterephthalic acid (DCA), a functional small molecule with symmetric carboxyl and sulfhydryl structures, to assist the coassembly of copper ions for the preparation of multifunctional nanozymes (Cu-DCA NZs). The obtained nanozymes effectively mimicked the activities of SOD and CAT and catalyzed the conversion of H_2O_2 into oxygen, thus reducing wound hypoxia and improving inflammation accumulation. More importantly, the incorporation of copper ions accelerated cell proliferation, migration, and angiogenesis, which further promoted diabetic wound healing. Additionally, the dual SOD and CAT activities effectively amplified the ROS scavenging ability, and had a significant effect on the amelioration of hypoxia and inflammation. For instance, Liu et al.[143] developed a nickel-based MOF (Ni-HHTP) with good antioxidant activity and pro-angiogenic function to accelerate the healing process of chronic diabetic wounds. Nickel is the active center of a natural antioxidant enzyme, nickel superoxide dismutase (NiSOD). Electron transfer between Ni(II)/Ni(III) complexes helps catalyze the transformation of superoxide anion ($O_2^{\bullet-}$) to oxygen or water. In vitro experiments demonstrated that Ni-HHTP efficiently removed H_2O_2 in the presence of glutathione (GSH), indicating its glutathione peroxidase (GPx)-like activity. Thus, Ni-HHTP mimicked the catalytic activity of antioxidant enzymes to eliminate multiple types of ROS via electron transfer reactions, thereby protecting cells from oxidative stress-related damage. In addition, this Ni-based MOF could promote cell migration and angiogenesis by activating transforming growth factor-β1 (TGF-β1) and reprogram macrophages to an anti-inflammatory phenotype. With excellent SOD and GPx-like activities, Ni-HHTP effectively promoted the healing process of DFU by inhibiting wound inflammatory responses and enhancing angiogenesis. Similarly, Zhao et al.[144] prepared a Cu-CPNs@EPL nanozyme using guanosine monophosphate (GMP) as a coordination template and copper ions as the center of coordination polymer nanoparticles (CPNs). Based on the key role of copper ions in cellular redox reactions, copper-based nanomaterials containing Cu(I) components were effective in scavenging endogenous ROS and also had a positive effect on promoting angiogenesis and collagen deposition. The nitrogen and oxygen atoms of the bases and phosphate groups in GMP, due to the presence of lone-pair electrons, can serve as potential binding sites for metal ions. The antioxidant properties of GMP allow the Cu-CPN to retain the Cu(I) valence state, resulting in reducing activity and the ability to mimic antioxidant enzymes. Furthermore, with the antimicrobial activity of polylysine (EPL), Cu-CPNs@EPL showed good scavenging ability against superoxide anions (SOD activity) and rapid decomposition of H_2O_2 generated by SOD reaction in the presence of GSSH (GPx activity), as well as strong electropositive and bacterial interaction abilities, resulting in effective anti-inflammatory and antibacterial activities.

Most SOD-like nanozymes use transition metals as active centers, and construct reaction systems similar to natural metal SOD by virtue of the intrinsic properties of the transition metal centers. The synthesized nanozymes have excellent ROS scavenging ability, which can be combined with the functions of other components, such as antimicrobial, pro-angiogenic, etc., and are widely used in DFU wound healing.

3.5. GPx-like activity. Glutathione peroxidase (GPx) is also an important antioxidant enzyme in the body, known for its ability to scavenge H_2O_2. GPx catalyzes the reduction of H_2O_2/organic hydroperoxides to H_2O/alcohols in the presence of reducing GSH.[145] However, it is expensive, unstable, and difficult to produce on a large scale.[146] Therefore, it is crucial to explore artificial GPx. The discovery of V_2O_5 nanowires[147] (Figure 2C) with GPx-like catalytic activity opens up new possibilities. So far, GPx-like nanozymes have been explored and developed for applications including cytoprotection, anti-inflammation, neuroprotection, and also tumor therapy. Due to its scavenging effect on ROS, GPx-like nanozymes show great application in wound healing.

A typical example is the ultrasmall $Cu_{5.4}O$ particle nanozyme prepared by combining Cu_2O and Cu nanocrystals.[148] The nanozyme possesses various enzymatic activities, including SOD, CAT, and GPx, and can realize efficient scavenging of

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ROS, such as $\bullet O_2^-$, H_2O_2, and •OH. The nanozymes showed satisfactory therapeutic performance in three disease models, acute kidney injury (AKI), acute liver injury (AILI) and DFU in mice. The authors then prepared a hydrogel composite ($Cu_{5.4}O$@Hep-PEG)[149] using heparin (Hep) and amine-functionalized star polyethylene glycol (PEG) for chemokine sequestration. The Hep-PEG hydrogel could effectively remove inflammatory factors from wound effusions, thus reducing migration of polymorphonuclear neutrophils and monocytes. $Cu_{5.4}O$ nanozyme with multienzyme activity reduced wound damage by timely removal of ROS, in which the decomposition of H_2O_2 by GPx could not be ignored. In vitro experiments, the composite hydrogel efficiently trapped pro-inflammatory factors through strong electrostatic interactions between heparin in the hydrogel and pro-inflammatory factors, thereby alleviating oxidative stress and inhibiting overinfiltration of immune cells. Similarly, the hydrogel composite showed excellent performance in ROS scavenging which promoted endothelial cell migration and low cytotoxicity. Subsequently, a type I diabetic mouse model was used to study the therapeutic effect of $Cu_{5.4}O$@Hep-PEG on DFU. Three wound dressings, Hep-PEG, $Cu_{5.4}O$, and $Cu_{5.4}O$@Hep-PEG, were applied immediately after wound injury. The results showed that the $Cu_{5.4}O$@Hep-PEG group had the longest length of regenerated epidermis, the most collagen deposition, and the most angiogenesis, resulting in the fastest wound healing. The lowest inflammatory factors and ROS levels were in the $Cu_{5.4}O$@Hep-PEG group due to the capture of pro-inflammatory factors by the hydrogel and the removal of ROS by the nanozymes. This facilitated wound protection from excessive oxidative stress and promotes tissue regeneration in DFU. Therefore, GPx-like nanozymes offer new avenues for DFU therapy. In particular, the enzyme cascade reaction triggered by GPx and other enzyme-like activities for wound healing should be vigorously developed.[150]

3.6. Others. *3.6.1. DNase-like activity.* Biofilms are usually formed during severe bacterial infections, involving bacterial attachment, formation of microbial colonies, maturation and spreading of the biofilm.[151] During the process, bacterial self-synthesis of extracellular polymers (EPS) assists the bacterial adhesion process. Extracellular DNA (eDNA) in the EPS connects the bacteria to other EPS components and further enhances bacterial adhesion, which is essential for biofilm formation. Efficient hydrolysis of biofilm from eDNA can disintegrate the biofilm, which provides a new strategy for wound healing in hyperbacterial infections.[152] Therefore, the development of nanozymes with DNase activity can eliminate biofilms by degrading eDNA in EPS, which is essential for the treatment of bacterial infections in DFU.

Qu et al.[153] developed a DNase-like artificial enzyme in 2016. Au NPs were adsorbed on the surface of magnetic Fe_3O_4/SiO_2 core/shell particles. Subsequently, Ce^{4+} complexes were added onto the surface of Fe_3O_4/SiO_2/Au NPs via Au−S bonds to confer DNase-like activity, which was denoted as DMAE. Study of the enzymatic and antimicrobial activity showed that DMAE could maintain a high activity for a long period of time, and concurrently bind to traditional antibiotics to substantially improve the antibacterial effect of the drug. In another study, Liu et al.[154] constructed an artificial nanozyme ($MOF_{-2.5Au-Ce}$) with synergistic dual enzyme mimetic activity by the combination of a Ce complex (DNase-like activity) and MOF (POD-like activity). Ce^{4+} targeted and hydrolyzed eDNA in biofilm, which led to biofilm decomposition and bacterial exposure. Second, MOF with POD-like catalyzed hydrogen peroxide to produce toxic hydroxyl radicals that killed bacteria. The unique activity of the DNase-like nanozymes to hydrolyze eDNA suggests promising applications in controlling bacterial infections and accelerating wound healing in diabetic wounds by disintegrating bacterial biofilms. Ji et al.[155] developed a nanozymatic platform with graphene oxide-based nitrilotriacetic acid-cerium composites (GO-NTA-Ce). The DNase-like activity of GO-NTA-Ce was found to inhibit biofilm formation through the degradation of eDNA, and effectively eradicated drug-resistant bacterial biofilm infections by the combined triple actions of photothermal therapy and antimicrobial activity of graphene, leading to satisfactory in vitro bacterial inhibition assays with low cytotoxicity. Furthermore, an infected mouse model of MRSA subcutaneous abscess was established and GO-NTA-Ce was utilized for in vivo wound treatment. The results of each experimental group showed that under NIR irradiation the GO-NTA-Ce junction group possessed the lowest microbial survival rate, the lowest inflammatory response, and the fastest wound healing rate, which indicated good bactericidal and biofilm eradication activity of the DNase-like nanozymes have and potential application for wound healing. Recently, motivated by aggregation-induced emission (AIE), Tang et al.[156] designed an AIEzyme (Zr-CPNPs) with DNase-like activity by using AIE luminescent progeny (AIEgens) as the ligand of Zr-based coordination polymer nanoparticles. AIE, as a fluorescence imaging technique, not only facilitates real-time observation of the antimicrobial process, but also circumvents the bursting caused by aggregation of traditional fluorophores, which is suitable for imaging microorganisms, peptides, cancer cells, and so on. However, AIEgens in general lack DNase activity, and their antibiofilm ability is poor. Upon binding of AIEgens to Zr nanozymes, the complexes are endowed with excellent DNase activity and have structurally rigid and stable fluorescence. The efficient DNase-like artificial enzyme not only durably prevents biofilm formation, but also has outstanding bacterial cell imaging ability and self-localization in biofilm due to good durability and strong penetration. Finally, Zr-CPNPs were used to treat back wounds in MRSA-infected mice. Monitoring of the healing process revealed that the wounds in the experimental group of Zr-CPNPs recovered almost completely within 7 days. In contrast, the recovery was delayed without Zr-CPNPs. In addition, the treatment with Zr-CPNPs did not cause organ lesions or inflammatory reactions, and all vital signs of the mice were normal. This suggests that AIEzyme with DNase-like activity not only has excellent catalytic activity, but also exhibits satisfactory biocompatibility, which is promising to be applied in clinical wound healing. Thus, DNase-like nanozymes show great promise for controlling bacterial infection and accelerating wound healing in diabetic wounds.

3.6.2. HPOs-like activity. Haloperoxidases (HPOs) have made important contributions to antimicrobials by catalyzing the reaction of halides with hydrogen peroxide to produce oligohydrochloric acids with broad-spectrum antimicrobial and antimicrobial membrane properties.[157] Natural HPOs are secreted by algae containing vanadium−metal cofactors, but high extraction costs, unstable catalytic activity, and poor environmental suitability have limited their applications.[158] Therefore, HPO-like nanozymes with high activity, high stability, and low cost are of significant interest. For example, Wang et al.[159] fabricated hybrid cerium oxide@zirconium

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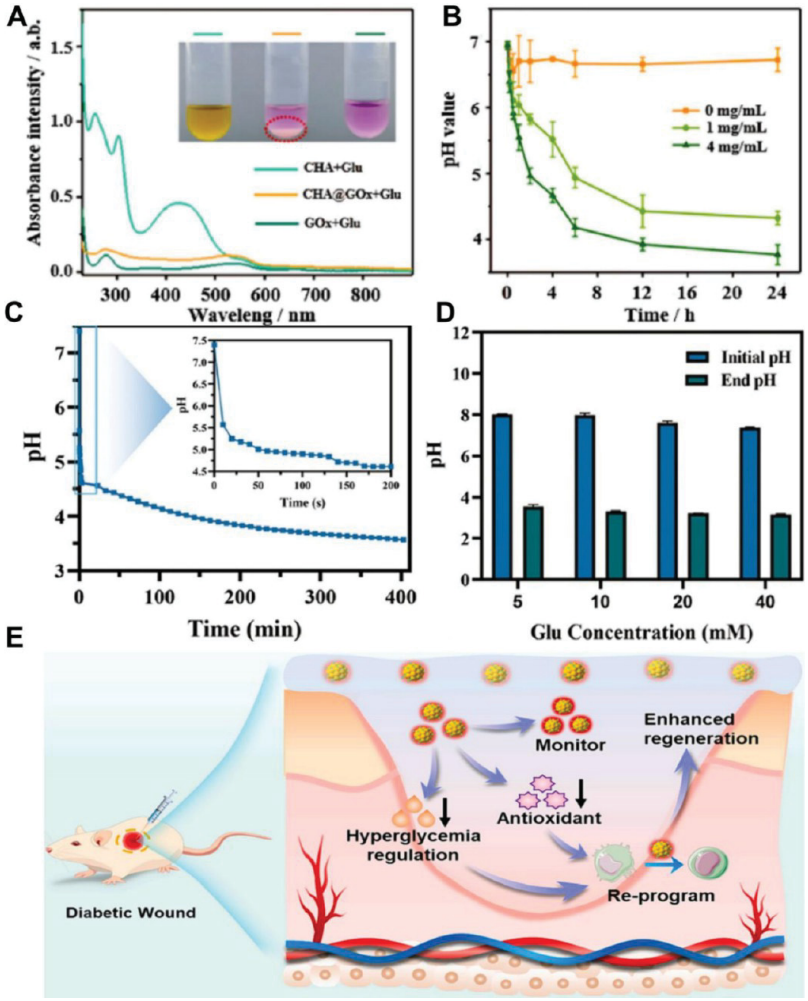

Figure 3. Validation of glucose regulation by CHA and CHA@GOx. (A) GOx activity of CHA+Glu, CHA@GOx+Glu, GOx+Glu by UV−vis and (inset) methyl red titration measurements. (B) pH changes within 24 h. Glucose content: 0, 1, and 4 mg. Reproduced with permission from ref 168, Copyright 2022, Elsevier. (C) Time dependence of GOx-like activity of PFOB@PLGA@Pt. (D) pH-lowering ability of PFOB@PLGA@Pt at different glucose concentrations. Reproduced with permission from ref 171, Copyright 2023, ACS. (E) Diabetic wound treatment application of LC-AuNCs@Sa hydrogel. Reproduced with permission from ref 172, Copyright 2024, Elsevier.

dioxide nanozymes in which high-density ultrasmall cerium clusters (ca. 0.8 nm) were stabilized on zirconium oxide substrates. The heterografted CeO_2@ZrO_2 nanozymes showed apparent and stable haloperoxidase mimicry properties and could catalyze the oxidation of hydrogen peroxide to bromine. The high dispersion of high-density ultrasmall nanoclusters on the carrier circumvented the drawback of low activity due to the natural tendency of nanoparticles to aggregate, resulting in high intrinsic activity and unexpected selectivity. As a result, CeO_2@ZrO_2 exhibited a strong bacteriostatic ability in the presence of Br^- and H_2O_2 substrates, which effectively counteracted the colonization of drug-resistant bacteria and outperformed pristine cerium oxide nanoparticles. Similarly, Wang et al.[160] developed a photothermal nanozyme (Mo SA-N/C) with Mo single atoms as active sites. The results showed that Mo SA-N/C catalyzed the oxidation of Br^- to cytotoxic HOBr with superior broad-spectrum antibacterial activity. The authors prepared another semiconductor nanozyme consisting of chromium monoatom coordinated to carbon nitride (Cr-SA-CN), which generated H_2O_2 from water and O_2 under visible light illumination for self-supply of H_2O_2.[161] The sustained haloperoxidase mimetic reaction induced significant bactericidal ability. The above results indicate that HPOs-like nanozymes have excellent ability to remove biofilm and achieve antimicrobial effect. Although current applications are focused on the treatment of marine environment through the removal of marine microorganisms, it is promising to expand the application to wound healing of DFUs.

In summary, the therapeutic mechanism of nanozymes for diabetic wounds is mainly based on the exertion of enzyme-like activities, combined with the efficient functions of other beneficial components (e.g., Ag ions for antimicrobials,[162] Cu ions to promote the proliferation of epithelial cells,[163] chitosan for bacterial inhibition,[164] curcumin for anti-inflammation and immunomodulation,[165] and so on), which work together to regulate the microenvironment of wounds. Among them, the enzyme-like activities are primarily POD-like, OXD-like, CAT-like, and SOD-like, which can be exploited for scavenging hydrogen peroxide and superoxide anions and generating highly toxic hydroxyl radicals through the effective regulation of ROS species in the wound microenvironment. This is significant in lowering wound glucose, alleviating wound oxidative stress, antimicrobial, anti-inflammatory and remodeling of the wound favorable environment. In addition, DNase-

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like and HPOs-like nanozymes are highly effective in eradicating bacterial biofilms and are irreplaceable in severe bacterial infections and the emergence of drug-resistant strains. Therefore, the development of multifunctional nanozymes with potent catalytic activity through delicate structural design, flexible multicomponent combinations, exploration of optimal action conditions and cascading of multienzyme activities would bring a great gospel for diabetic patients around the world.

4. APPLICATIONS OF NANOZYMES IN DFU HEALING

4.1. Hypoglycaemics. Hyperglycemia in diabetic wounds usually causes a series of malignant events, including bacterial infections, persistent inflammation, vascular damage, neuropathy, and immune system disorders. Therefore, controlling blood glucose at normal levels is particularly important for diabetic wound healing.

Within this context, nanozymes for the treatment of diabetic wounds by lowering blood glucose can be mainly classified into two categories: (a) nanozymes loaded with GOx; and (b) nanozymes with GOx-like activity. For the former, natural GOx is loaded onto the nanozymes by, for instance, physical adsorption, wrapping encapsulation, covalent adsorption, and cross-linking coupling, so that the resulting complexes possess both GOx activity as well as intrinsic nanozymes activity. For instance, Liu et al.[166] chose an ultrathin two-dimensional (2D) MOF (2D Cu-TCPP(Fe)) nanosheet as a POD mimetic model, and successfully prepared a MOF-based hybrid nanozyme by physical adsorption of GOx. GOx can continually convert nontoxic glucose into abundant gluconic acid and hydrogen peroxide. The gluconic acid generated decreases the pH, which significantly activates the POD-like activity of 2D CuTCPP(Fe) nanosheets. The activated nanosheets rapidly catalyze the decomposition of hydrogen peroxide to produce a large amount of toxic •OH, inactivating the bacterial cells. This method not only significantly reduces the blood glucose concentration around the wound via GOx, but also avoids the toxic effects associated with the direct use of high concentrations of hydrogen peroxide. In another study,[167] GOx was coated onto POD-like $Fe_2(MoO_4)_3$ to develop a cascade nanozyme active material ($[Fe_2(MoO_4)_3$@GOx]) for diabetic wound treatment. The consumption of glucose by GOx and the production of cytotoxic •OH by the nanozyme highlighted the synergistic effect of starvation therapy and ROS-mediated treatment. Yu et al.[168] developed a Ce-derived multifunctional nanozymatic system. Alendronic acid (AL) and 2-methylimidazole (HMIM) formed a biligand molecule, and the Ce-driven coassembly formed the nanoparticles CHA. Finally, GOx was embedded to form nanozyme (CHA@GOx) with multiple enzymatic activities. In order to investigate whether the GOx activity was retained after coassembly, the CHA@GOx activity was evaluated in response to the addition of glucose. The GOx-catalyzed oxidation of glucose involved the production of glucuronides, and the change of pH could be indicative of the catalytic activity of GOx. Specifically, glucose was added to a suspension containing CHA nanoparticles and a pH indicator, and after mixing, pH was found to be between 4.4 and 6.2. However, after the reaction of free GOx with glucose, the pH decreased (pH < 4.4). The pH also decreased after the reaction of CHA@GOx and glucose, indicating that the GOx remained active (Figure 3A). In addition, to simulate high and normal glucose concentrations within the wound, two concentrations of glucose (4 and 1 mg/mL) were added to CHA@GOx. The results showed that CHA@GOx was able to catalyze the oxidation of glucose, leading to a gradual decrease of pH over 24 h, which was beneficial for chronic wound healing (Figure 3B). Other experiments also demonstrated favorable SOD and CAT activities of CHA@GOx, which synergistically regulated the ROS and oxygen balance of the wound with the oxidation of glucose and reduced the toxic side effects of GOx. Wu et al.[169] reported excellent multienzymatic activity with a single-molecule cascade multienzymatic conjugate. The cascade enzyme conjugates (CECs) were synthesized by polymerizing the glycan of GOx and ligating it with ferrous ions (Fe^{2+}). The obtained CECs simultaneously confined the multienzyme sites to a nanoscale space, which greatly facilitated uniform, continuous and synergistic cascade reactions. Colorimetric experiments verified the excellent activity and prolonged stability of the loaded GOx. Even at low CEC concentrations, glucose levels decreased significantly, accompanied by a slight decrease of pH (from 6.8 to 6.6). These findings provide evidence for the catalytic activity of CECs in sustained reduction of glucose levels and pH in hypoglycaemic solutions. At the same time, CECs exhibited multiple enzymatic properties of GOx-, SOD- and CAT-like activities. This triple enzyme-polymer coupling promotes hyperglycemic depletion, ROS scavenging, bacteriostatic, anti-inflammatory, and sustained oxygen production, which may independently regulate the microenvironment of diabetic wounds and promote wound healing.

Nanozymes based on gold and platinum nanoparticles are well-known for their super GOx-like activity. For instance, Zhang et al.[170] prepared a multifunctional hydrogel dressing nanozymes for diabetic wound healing (defined as OHCN) by incorporating gold−platinum alloy nanoparticles into an OHC hydrogel formed by Schiff-base reaction between oxidized hyaluronic acid (OHA) and carboxymethyl chitosan (CMCS). The GOx-like activity of Au NPs promoted glucose consumption, and the CAT-like activity converted H_2O_2 to O_2, thus ensuring O_2 release while scavenging ROS. More importantly, by analyzing the GOx-like properties of Au−Pt alloy NPs at different mass ratios, it was found that the Au−Pt alloy NPs (1:1) catalyzed glucose oxidation at the highest efficiency, even better than the monometallic Au NPs, and the activities of the Au−Pt alloy NPs were more stable and superior to those of natural glucose oxidases at high temperatures (60 °C) and at different pH. The GOx- and CAT-like activities of the resultant Au−Pt nanozymes could be maximized by optimizing the Au to Pt ratio, overcoming the limitations of natural enzymes in biomedical applications. It also synergistically promoted wound healing in combination with the restorative ability of hyaluronic acid and the bacteriostatic ability of chitosan in hydrogels. Zhou et al.[171] constructed a nanohybrid dual network hydrogel based on platinum composite nanozymes (PFOB@PLGA@Pt) to manage diabetic wound healing. Perfluorooctyl bromide (PFOB) carried a large amount of oxygen, which ameliorated hypoxia and also promoted the catalytic reaction of Pt NPs with glucose oxidase-like activity. The synthesized PFOB@PLGA@Pt exhibited potent GOx-like activity due to the Pt NPs, which oxidized glucose to gluconic acid and significantly reduced pH in a short period of time (Figure 3C). The ability to downregulate pH was excellent at different glucose concentrations, suggesting good performance of PFOB@PLGA@Pt in lowering the pH of diabetic wounds independent of glucose concentration (Figure 3D). The lowering of pH

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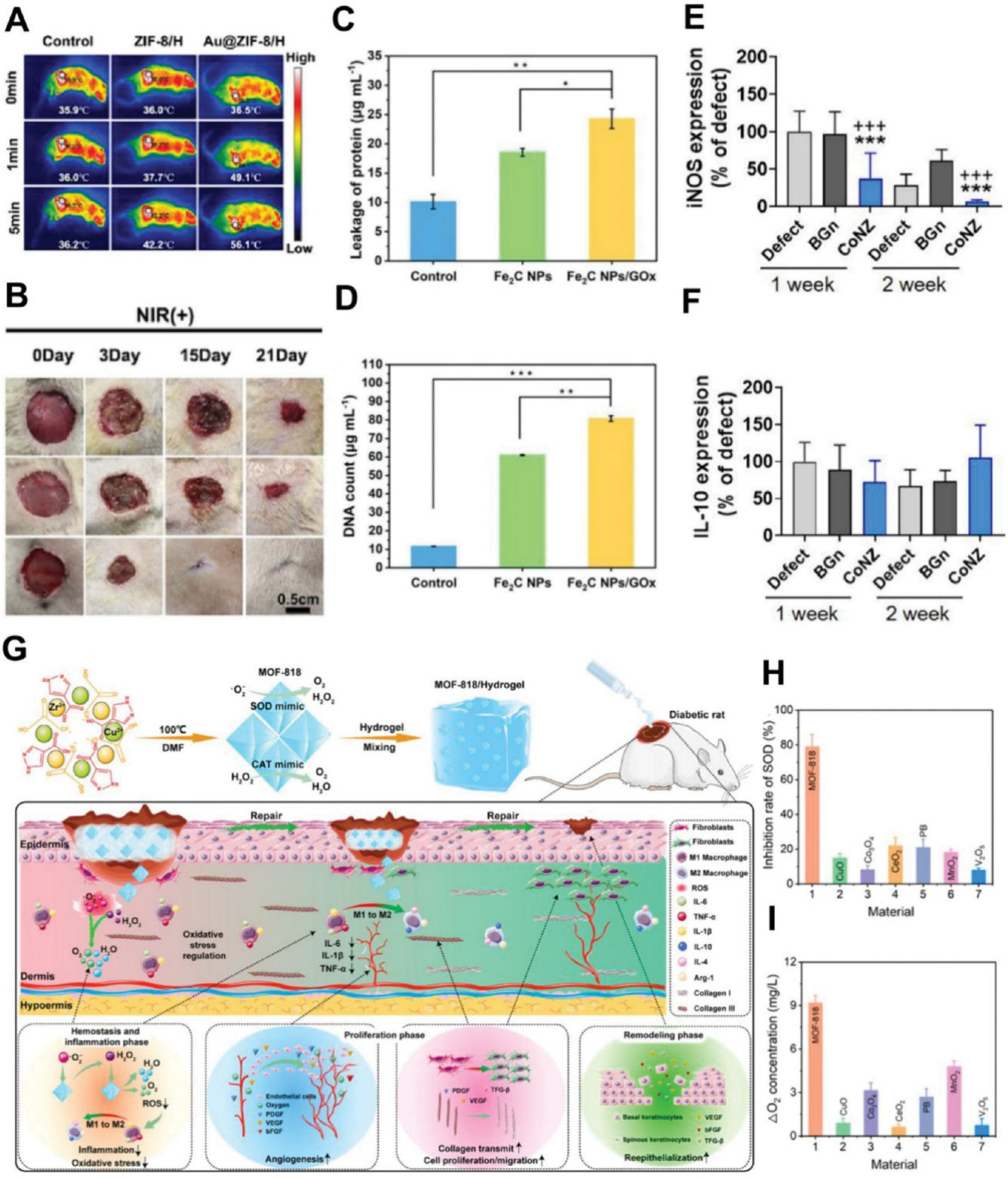

Figure 4. (A) Photothermal images of Au@ZIF-8/H under NIR irradiation in diabetic rats after different treatments and (B) healing images of infected mice at different times. Reproduced with permission from ref 178, Copyright 2023, Elsevier. The content of (C) protein and (D) DNA leakages of MRSA bacteria quantified after different treatments by Control, Fe_2C NPs, Fe_2C NPs/GOx. Reproduced with permission from ref 183, Copyright 2023, Wiley. CoNZ effects on anti-inflammation in diabetic wound. Semiquantitative data of (E) iNOS (pro-inflammatory signaling) and (F) IL-10 (anti-inflammatory signaling) in tissue samples from different treatment groups (based on immunohistochemistry results and ImageJ data analysis) at week 1 and week 2. "Defect" is the blank group and BGn is pure nanoglass without doping. Reproduced with permission from ref 188, Copyright 2024, KeAi. (G) Schematic illustration of the preparation of the MOF-818/Gel antioxidative system and its mechanisms in diabetic chronic wound healing. Comparison of the (H) SOD-like activity and (I) CAT-like activity of MOF-818 with other typical antioxidative materials. Reproduced with permission from ref 189, Copyright 2022, ACS.

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sequentially activated multiple enzyme activities, such as NADH oxidase-like, POD-like, and OXD-like enzymes, which produced synergistic antimicrobial effects through the generation of ROS. After removal of bacteria, the NADH-like properties of Pt were converted to CAT-like and SOD-like activities, which remodeled the redox microenvironment by removing excessive ROS, allowing the wound to transition from the inflammatory phase to the proliferative phase. The microenvironment-adapted nanozymatic hydrogels enabled flexible switching of different enzyme activities by orderly regulation of pH, which breaks the limitation of pH. The resultant hydrogel treatment can cover all stages of wound healing and contribute significantly to the repair of diabetic infected wounds. Recently, Wang et al.[172] designed multifunctional Au NP nanozymes with significant glucose depletion and macrophage reprogramming capabilities. A multifunctional nanozyme hydrogel (LC-AuNCs@Sa) was prepared through coordination engineering of lysozyme, curcumin, and Au NPs and further incorporation of sodium alginate hydrogel. Within the nanozyme, Au NPs possessed GOx-like hypoglycaemic activity, and the concomitant CAT activity effectively eliminated harmful hydrogen peroxide production from glucose oxidation, which avoided the side effects of hydrogen peroxide produced by conventional GOx-like nanozymes. Notably, LC-AuNCs (containing lysozyme and curcumin) showed significantly higher glucose scavenging efficiency as compared to the Lys-AuNCs counterpart (containing only lysozyme), which was attributed to the electron-donating capacity of curcumin (i.e., n-methoxyphenol group) that was essential for facilitating the catalytic process. Curcumin also acted as a bioactive agent for ROS elimination and enhanced immunomodulation, which might synergize the transformation of the high-glucose microenvironment without generating toxic ROS. In addition, LC-AuNCs further induced macrophage polarization from M1-type to M2-type, alleviating inflammation and promoting regeneration. In conclusion, this nanozymatic hydrogel effectively repaired the cell growth microenvironment and accelerated the healing of diabetic wounds by depleting glucose scavenging, scavenging ROS, alleviating inflammation, and promoting vascular regeneration (Figure 3E).

In conclusion, lowering blood glucose by nanozymes is an important measure for the treatment of diabetic wounds. However, lowering blood glucose alone is not sufficient for complete wound healing. Therefore, it is often necessary to combine other enzymatic activities to produce superimposed effects, including antibacterial, anti-inflammatory, and vascular regeneration promotion. However, the inherent drawbacks of natural enzymes and the limited loading efficiency of existing preparation methods reduce the activity of GOx/nanozyme systems. Therefore, vigorous development of nanozymes with both GOx-like and other enzyme-like activities will undoubtedly become the mainstream path.[173]

4.2. Anti-infection. The hyperglycemic environment and weak immune function make diabetic wounds susceptible to a variety of pathogenic bacteria, especially *E. coli* and *Streptococcus pneumoniae*. Also the formation of a refractory biofilm by a large number of bacteria is a serious obstacle to the wound healing process.[174] Therefore, inhibiting bacterial infection and eradicating biofilms have become a great challenge for diabetic foot patients. Although antibiotics provide successful management of bacterial infections, chronic overuse and misuse of antibiotics have led to the emergence of multidrug-resistant bacteria.[175] To address this challenge, nanomaterials with intrinsic antimicrobial properties or as drug carriers have been extensively studied as ideal candidates, and nanozymes have emerged as unique options to sterilize bacteria by increasing the local concentration of harmful substances around the bacteria or by directly targeting the bacteria.[176]

For instance, Shao et al.[177] developed a smart enzyme-like polyphenol copper spray to counteract bacterial infections in diabetic wounds. First, tannic acid (TA) and Cu ions were used together to prepare copper-based nanozymes (TNCNs), followed by the addition of hydrogen peroxide to formulate an acidic spray. In the initial stage, POD-like TCNCs rapidly catalyzed the generation of hydroxyl radicals from H_2O_2 in acidic buffer solution, effectively targeting and eliminating bacteria. As the wound exudate neutralized the acidic buffer solution, the remaining hydrogen peroxide and TCNCs-induced macrophages produced NO and TNF-α, further promoting bacterial death. At a later stage, the increasingly alkaline environment favored CAT and SOD activity, which broke down H_2O_2 and removed superoxide anion, hydroxyl radicals and nitric oxide radicals in vivo, facilitating the transition to the tissue regeneration phase and accelerating wound healing. POD-like activity also induced production of toxic hydroxyl radicals to destroy bacteria and scavenged excessive ROS. Thus, Cu-based nanozymes with multienzymatic activity could drive the entire process of wound healing. Ren et al.[178] synthesized Au NPs on the surface of imidazolate framework-8 (ZIF-8) by an in situ growth method to obtain Au@ZIF-8 nanozymes, which established a multifunctional antibacterial and wound healing platform. Au NPs, with excellent POD-like and antimicrobial activity, could avoid the formation of bacterial resistance. However, the characteristic ease of aggregation affected the performance of Au NPs. MOFs acted as effective carriers for Au NPs, providing ideal confinement to prevent aggregation.

Functionalized gold oxides show enhanced POD-like activity, generating ROS for sterilization. It can also produce good photothermal effect under NIR laser irradiation, which breaks the limitation that most MOFs can only be activated by harmful UV radiation and reduces the damage to normal human tissues. To verify the photothermal properties, the wounds of diabetic rats were treated with Au@ZIF-8/H and photothermal treatment, and the changes in wound temperature were recorded. The photothermal images showed that the temperatures of the wounds in the control and ZIF-8/H groups did not change significantly after 5 min of NIR irradiation, whereas the temperatures of the Au@ZIF-8/H group increased significantly by 19.5 °C (Figure 4A). The increase of temperature promoted the decomposition of Au@ZIF-8, which produced zinc ions and Au NPs and facilitated wound repair by damaging bacteria. In addition, comparing the changes in wound size during repair, the Au@ZIF-8/H+NIR-treated group showed the fastest wound recovery to complete closure (Figure 4B). This suggested that the synthesized Au@ZIF-8 nanozymes possessed enhanced POD-like activity and excellent photothermal response properties, and this dual synergistic function promoted the rupture of bacterial membranes, eliminated bacterial infection, and accelerated wound healing.

In fact, there are many metals with enzyme-like activities, and alloying nanozymes can integrate the properties of multiple metals to obtain nanozymes with enhanced perform-

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ACS Biomater. Sci. Eng. 2024, 10, 4195−4226

ance. Guo et al.[179] observed that PtCuTe nanosheets exhibited an improved catalytic efficiency as compared to PtCu nanozymes. Note that PtCu nanozymes are known to be one of the best catalytic systems, but they show insufficient ROS scavenging capacity and ROS-dependent antimicrobial effects, leading to uncontrolled and poor performance toward diabetic wound healing. The antimicrobial activity of tellurium (Te) and its compounds relied on damage to bacterial cell membranes and inhibition of flagellar motility rather than ROS production and DNA damage, which could effectively mitigate the side effects of uncontrollable ROS content. Based on this, the introduction of Te into the conventional PtCu nanozymes resulted in enhanced POD-, CAT- and SOD-like activities of the PtCuTe nanozymes, which produced toxic •OH and Te synergistically to kill bacteria with good ROS scavenging ability. At the same time, it promoted endothelial cell formation, enhanced vascular regeneration, stimulated the polarization of M1 macrophages to the M2 phenotype, and improved fibroblast mobility. Compared with other current antimicrobial agents that resisted bacteria through external physical stimuli (e.g., light, sound, microwave), PtCuTe nanosheets exhibited a broader prospect for antimicrobial applications.

Biofilms are microbial communities, often composed of bacteria, fungi, yeasts, and other microorganisms, encapsulated by an extracellular polymer matrix. Biofilms tend to form when large numbers of bacteria accumulate and colonize the wound surface, which is more difficult to eradicate than bacteria.[180] PTT directly kills bacteria by raising the temperature under specific laser light. When the temperature exceeds 50 °C, bacterial proteins are denatured and the bacterial membranes are damaged, promoting biofilm eradication.[181] However, PTT, while simple and effective, does not reduce the inflammatory environment of the wound. Therefore, it is imperative to develop a multifunctional photothermal nanomaterial that can simultaneously penetrate biofilms and modulate the microenvironment of inflammatory wounds. Qin et al.[182] developed a multifunctional metal-phenolic nanozyme (TA-Fe/Cu nanoencapsules) by a one-pot method using ZIF-8 as a self-sacrificial template mixed with tannic acid (TA). After effectively disrupting the dense biofilm through PTT, TA-Fe/Cu NPs autonomously capture bacteria through hydrogen bonding interactions with peptidoglycan (a bacterial cell wall component), ultimately enhancing the bactericidal efficacy. In addition, the POD-like enzymatic activity effectively eliminates excessive hydrogen peroxide produced by photothermal reactions in the vicinity of the wound and reduces the inflammatory response. As the wound transitions to the remodeling phase, the presence of Cu^{2+} stimulates vascular migration and regeneration, thereby accelerating the diabetic wounds healing. In addition, Sun et al.[183] designed an integrated therapeutic and prophylactic nanozyme-based microneedle (Fe_2C/GOx@MNs), taking into account the penetration problem during biofilm removal. Soluble microneedles with sufficient mechanical strength could deliver and rapidly release Fe_2C NPs/GOx at the active region of the biofilm, enhancing the permeability of tissues and biofilms to Fe_2C NPs/GOx. The released GOx catalyzed the decomposition of glucose to generate H_2O_2 and gluconic acid, and the higher level of H_2O_2 and lower pH further enhanced the POD activity of Fe_2C NPs to produce •OH. Large amounts of cytotoxic •OH induced the disassembly of bacterial biofilm, which directly exposed the bacteria to unfavorable environment and caused damage to the cell wall and cell membrane. With the disruption of EPS, Fe_2C NPs/GOx could more easily penetrate the biofilm and exert a bactericidal effect, thus completely eliminating the bacterial biofilm and preventing its regeneration. The efflux of intracellular components, such as proteins and DNA, could assess the extent of damage to the bacterial cell membrane and cell wall. The results showed that the extracellular protein and DNA concentrations in the Fe_2C NPs group were significantly higher than those in the control group, while those in the Fe_2C NPs/GOx group were further increased (Figure 4C−D). This was due to the small amount of •OH produced by Fe2C NPs, which damaged the bacterial cell membrane and cell wall while Fe_2C NPs/GOx produced more •OH, which aggravated the damage. Subsequently, the bacterial homeostatic balance was disrupted and the overload of •OH exacerbated bacterial damage, such as genomic DNA fragmentation and metabolic inhibition, ultimately leading to bacterial death. Meanwhile, the chitosan (CS) backing layer not only served as a good physical barrier between the wound and the external environment, but also showed antimicrobial activity that could prevent the reinvasion of bacteria during the wound healing process. Thus, Fe_2C/GOx@MNs proved to be promising in eradicating biofilm and preventing reinfection.

In conclusion, the key to anti-infection in diabetic wounds lies in the removal of infecting bacteria and the eradication of biofilms. Nanozymes can induce bacterial death and disintegrate the biofilm structure by generating ROS with killing power, mediating PTT and delivering antimicrobial active ingredients, thus realizing efficient antimicrobial activity. Meanwhile, it is difficult for bacteria to develop targeted resistance due to the multiple killing effects of nanozymes, which prevents the development of drug-resistant bacteria. However, most of the bactericidal effects of nanozymes are based on the intrinsic properties of metal ions, which puts higher demands on the preparation cost and biocompatibility. The damage to normal tissues caused by excessive ROS generated during treatment and photothermal therapy is also inevitable.[184] Therefore, it is imperative to develop more targeted nanozymes with fewer side effects for the treatment of infections in diabetic wounds.

4.3. Anti-inflammatory. It is well-known that the main difference between diabetic chronic wounds and normal wounds is the prolonged but ineffective inflammatory phase of the former. Despite the widespread use of medications, the persistent hyperinflammatory state and high levels of pro-inflammatory factor expression remain a major challenge in clinical management.[185] To accelerate diabetic wound healing, overcoming inflammation while preventing recurrence is essential to facilitate the transition from the inflammatory to the proliferative phase of the wound. In diabetic wounds, excessive oxidative stress secondary to hyperglycemia is caused by overproduction of ROS and insufficient antioxidants, which disrupts normal redox signaling.[186] Thus, diabetic wounds are characterized by high levels of ROS, especially $O_2^{\bullet-}$ and H_2O_2. The utilization of antioxidant enzymes, mainly SOD, CAT, and GPx, to reduce excessive ROS is considered a viable strategy for treating inflammation. Drug-free therapies based on nanozymes with antioxidant enzyme activity to eliminate inflammation and promote angiogenesis, accompanied by fewer side effects and complications, are undoubtedly ideal options.[187]

Considering the high-inflammatory environment of diabetic wounds and the side effects of anti-inflammatory drugs, Ji et

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ACS Biomater. Sci. Eng. 2024, 10, 4195−4226

al.[188] recently proposed a novel bioactive nanozyme based on cobalt-doped nanoglasses (CoNZ), which exhibited a high enzyme/catalytic activity while releasing therapeutic ions. The ion-doped bioactive glass released ions with anti-inflammatory and pro-angiogenic properties and directed stem cell profiles, thereby accelerating the wound healing process accompanied by few complications and drug resistance. High levels of hydrogen peroxide were present in diabetic wounds, which could react with cobalt ions in CoNZ to produce microcrystals of cobalt oxide (Co_3O_4). This played a key role in the observed enzymatic activity, conferring excellent SOD, POD and CAT properties to CONZ, accomplishing ROS scavenging. To assess the anti-inflammatory efficacy of CoNZ, wound tissue samples were assayed for iNOS (pro-inflammatory signaling) and IL-10 (anti-inflammatory signaling) at week 1 and 2 (Figure 4E-F). The results showed that iNOS was significantly downregulated and IL-10 was significantly upregulated in the CoNZ group, as compared with the other experimental groups, proving the excellent ability of CoNZ to alleviate inflammation. At the same time, the release of cobalt ions promoted cellular angiogenic functions, including cell migration and tubule formation, through activation of HIF-1α and other angiogenic genes. The rapid clearance of ROS and downregulation of pro-inflammatory markers by CoNZ, along with upregulation of signs of tissue healing with proliferating cells and activated angiogenic factors, promoted the healing events of diabetic wounds. Dong et al.[189] prepared an effective antioxidant system (MOF/Gel) for the healing of diabetic chronic wounds by combining MOF nanozymes with antioxidant enzyme activity with hydrogels. The authors reexamined MOF-818 nanozyme for the continuous treatment of diabetic wounds based on a previous report. MOF-818 was synthesized by a simple solvothermal method and integrated with a thermo-sensitive gel to form antioxidant MOF-818/Gel. MOF-818 possessed a higher SOD and CAT-like activities, as compared to other reported antioxidant nanozymes (e.g., CuO, CeO_2, etc.) (Figure 4H−I). The water dispersibility and biocompatibility remained stable for more than 6 months. The treatment of diabetic chronic wounds by MOF-818/Gel started with the scavenging of two ROS (superoxide anion and hydrogen peroxide) predominantly present at the wound site through the excellent SOD- and CAT-like activities, and the oxygen produced alleviated the oxidative stress within the wound microenvironment. Furthermore, MOF-818/Gel with ROS scavenging capacity promoted polarization of the M1 phenotype to the M2 phenotype and downregulated the expression of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α). The oxidative microenvironment was gradually remodeled, and the oxidative stress of the wound was gradually restored to balance, resulting in a natural transition from the inflammatory phase to the proliferative phase. Finally, MOF/Gel upregulated the expression of various factors (VEGF, PDGF, TFG-β), which facilitated a series of processes such as cell proliferation, angiogenesis, collagen synthesis, tissue repair and re-epithelialization (Figure 4G).

Indeed, wound healing, especially refractory wound healing, is a complex, multistep biological process, which is greatly influenced by oxidative stress. Therefore, the development of anti-inflammatory nanozymes in the field of wound repair is needed. In recent years, nanozymes with CAT, SOD, and GPx activities, based on oxides of cerium, manganese and vanadium, Prussian blue, carbon derivatives, and MOFs, have been used as antioxidants to treat a number of common inflammatory diseases. Unfortunately, reports remain scarce on the involvement of antioxidant nanozymes in wound healing by scavenging excessive ROS, although there have been many studies of oxidase and peroxidase nanozymes for antimicrobial applications and for preventing wound infections by ROS production. Therefore, the development of anti-inflammatory nanozymes holds great promise in alleviating oxidative stress in diabetic wounds. The balanced requirements of high activity, sustained effectiveness, good dispersion, multienzyme mimicry and biocompatibility of these novel antioxidant materials should also be taken into account.

4.4. Combination of Light, Heat, and Acoustic Power Therapy. Nanozymes can treat diabetic wounds by catalyzing the generation of ROS and other unique mechanisms. However, the limited targeting and catalytic activity of nanozymes reduces their performance, so combining them with other strategies (e.g., PTT and PDT) is considered an effective approach.[190] PTT can be used to treat wounds by generating localized heat under light irradiation at specific wavelengths.[191] PDT involves the use of photosensitizers to induce a photochemical reaction by light of a specific wavelength and produce cytotoxic ROS, thus achieving therapeutic effects.[192] The combination of these light-, heat-, and sound-based physical therapies and nanozymes has attracted great interest in the biomedical field due to their low drug resistance and good controllability.

For instance, Huang et al.[193] designed a photoswitchable multifunctional nanoplatform (PdMOF@PAzo@SNP) combined with Pd nanozymes and NO. Pd was first encapsulated in situ in the pore space of UiO-66, followed by the assembly of photoresponsive polyazobenzene (PAzo) and β-cyclodextrin-modified hyaluronic acid (β-CD-HA) on the surface. During the self-assembly of β-CD-HA, sodium nitroprusside (SNP), a NO donor, was also adsorbed. Under UV photoirradiation, the interaction between PAzo and β-CD-HA was disrupted, which restored the POD activity of the Pd nanozyme as well as the release of NO. Pd nanozyme catalyzed the production of ROS from H_2O_2, which further reacted with NO supplied by SNP to generate RNS, and the antimicrobial effect was significantly enhanced. Meanwhile, NO, as an endogenous molecule, regulated inflammation and promote angiogenesis. Thus, the synergistic effect stemmed from the enzymatic activity of Pd nanozymes and NO together accelerating the healing of diabetic wounds, overcoming the traditional therapeutic limitations. Tong et al.[194] achieved eradication of drug-resistant bacteria using chlorin e6 (Ce6), citric acid, and Prussian blue nanoparticles (PB NPs) to construct a photosensitive nanozyme (CPB-Ce6). The photodynamic Prussian blue nanozymes were formed by citric acid-modified Prussian blue nanoparticles (PB NPs) and amidated Ce6 via amide bonding. Under visible light (660 nm) irradiation, photoactivated Ce6 produced singlet oxygen ($^1O_2$), leading to the death of pathogenic bacteria at the wound sites. CPB nanoparticles exhibited CAT-like activity to decompose hydrogen peroxide to oxygen, thereby alleviating microenvironmental hypoxia and improving the antimicrobial effect of PDT. This strategy based on PB NPs with nanozymatic activity and PDT could provide an effective treatment for infections associated with drug-resistant microorganisms and tissue repair.

However, short wavelengths of light (e.g., UV and visible) have limited penetration and are harmful to the human body. In contrast, NIR light sources (780−1000 nm) have a higher

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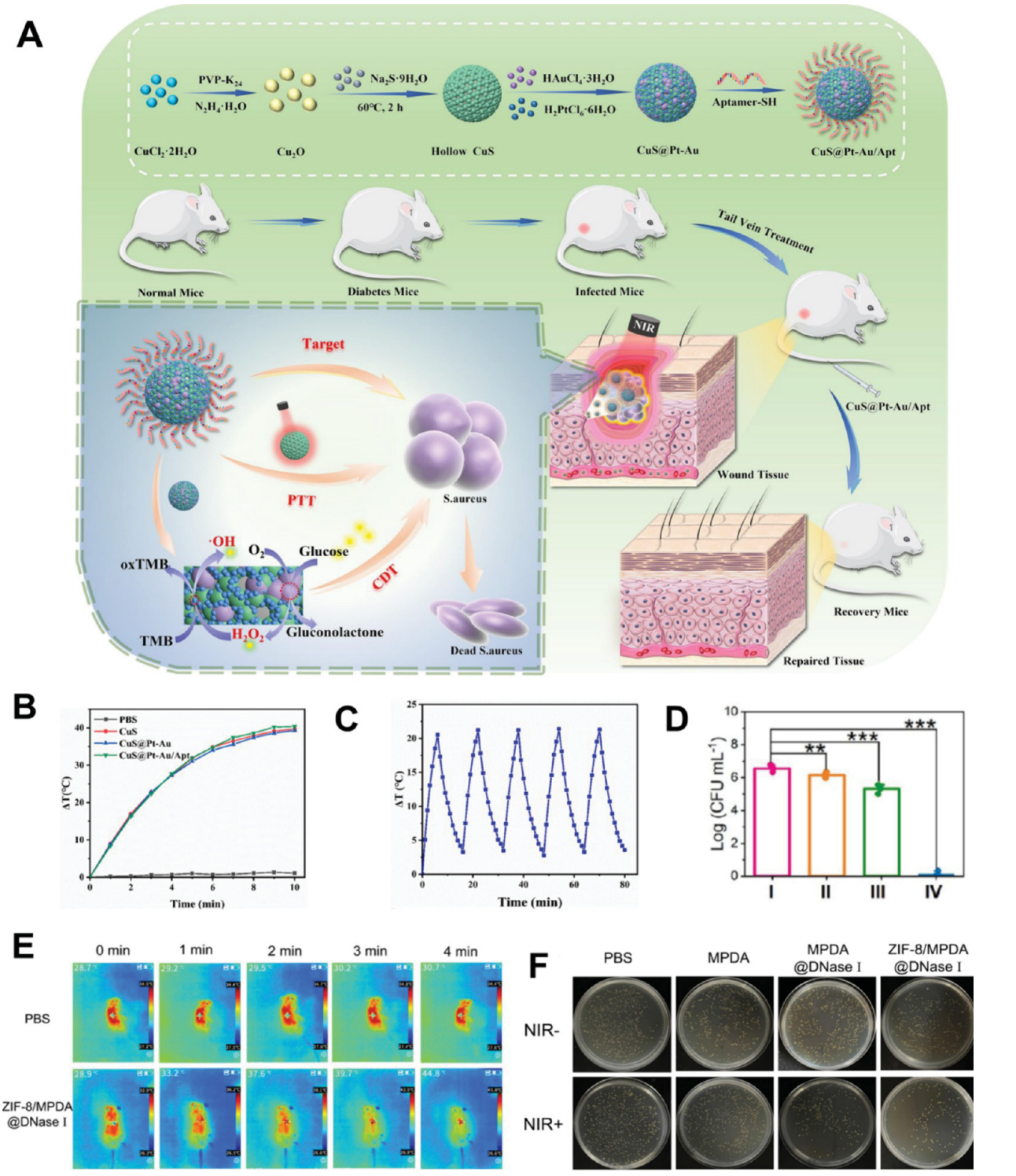

Figure 5. (A) Preparation pathway of CuS@Pt−Au/Apt NPs and multiple synergistic treatment of wound infection in diabetic mice. (B) Photothermal curves of PBS, CuS, CuS@Pt−Au, CuS@Pt−Au/Apt NPs. (C) Heating and cooling profiles of CuS@Pt−Au/Apt NPs over 5 on/off cycles of 808 nm NIR illumination. Reproduced with permission from ref 198. Copyright 2023, Elsevier. (D) Antibacterial activity of Gram-positive bacteria (MRSA) after different treatments. I: Control, II: ultrasound, III: ACPCAH, and IV: ACPCAH+ultrasound. Reproduced with permission from ref 203. Copyright 2023, American Chemical Society. (E) NIR irradiation of *S. aureus* infected wounds of diabetic mice treated with PBS and ZIF-8/MPDA@DNase I NPs in thermograms. (F) Photographs of the growth of *S. aureus* on agar plates after different treatments. Reproduced with permission from ref 201. Copyright 2024, RSC. (F) Illustration of a multifunctional ACPCAH spray for multidrug-resistant bacteria infected diabetic foot wound healing. Antibacterial activity of (G) Gram-positive bacteria (MRSA) and (H) Gram-negative bacteria (*E. coli*) after different treatments. I: Control, II: ultrasound, III: ACPCAH, and IV: ACPCAH+ultrasound. Reproduced with permission from ref 199. Copyright 2023, American Chemical Society.

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depth of penetration in tissues, are less toxic to normal tissues, and have a shorter treatment time (about a few minutes) with significant therapeutic effects, which makes NIR-based PTT preferable.[195] Zhao et al.[196] constructed gold nanocluster-modified zirconium-based porphyrin MOF nanozyme (Au NCs@ PCN) using an in situ growth method to combat bacterial infections in diabetic wounds. Based on the excellent light absorption properties and considerable photothermal conversion efficiency of Au nanoparticles and MOF, the Au NCs@PCN showed an excellent performance in photoexcited ROS generation and photothermal effect. Under NIR laser irradiation, Au NCs@PCN generated high levels of ROS by catalyzing Fenton-like reactions for CDT and enhanced PDT. Meanwhile, NIR irradiation could heat up the wound environment to 56.2 °C, which inhibited bacterial growth by disrupting bacterial membrane structure and inducing protein leakage through localized heat. Yuan et al.[197] synthesized a MXene-based bifunctional nanozyme with POD activity and good photothermal properties. 2D MXenes have attracted much attention due to their high specific surface area, great thermal conductivity, and excellent photothermal conversion efficiency, and the Nb_2C MXenes nanosheets can maintain a high light absorption capacity in the NIR range. As Nb_2C MXenes with a single photothermal capacity could not produce a sustained antimicrobial effect, cerium dioxide nanozymes with POD-like activity were adopted. An ideal and simple strategy was to use Nb_2C nanosheets loaded with sufficient CeO_2 particles to form a CeO_2/Nb_2C nanozyme with photothermal conversion capacity and sustainable enzymatic activity. Under NIR irradiation, the composite generated localized heat through the photothermal effect, which damaged the bacteria membranes. Meanwhile, CeO_2 with POD-like activity rapidly generated ROS within the bacteria, which further increased the bacterial lethality. In addition, the moderate temperature generated by PTT was utilized to enhance the catalytic activity of the nanozyme. The photothermal effect and enzyme activity synergistically cured the bacterial infection of diabetic wounds.

Focusing on the good photothermal properties of CuS and the enzyme-like activity of Pt−Au nanoparticles, Zhang et al.[198] constructed functionalized NIR-responsive nanomaterials (CuS@Pt−Au/Apt NPs) based on cascade nanozymes. Hollow copper sulfide nanoparticles with photothermal properties were first synthesized by hydrazine reduction, followed by in situ deposition of Au and Pt NPs with cascade nanozymatic activity via metal reduction. Finally, thiol-modified nucleic acid aptamers (aptamer-SH) were sequentially adsorbed onto the particles via cryoadsorption method to form CuS@Pt−Au/Apt NPs. The aptamer was protected from nuclease degradation upon binding to the nanomaterials and, at the same time, bound efficiently to bacteria through target recognition, thus improving selectivity. Copper sulfide NPs corroded bacterial membranes and enhanced ROS release under NIR irradiation, while Pt−Au NPs with OXD-like and POD-like activities destroyed bacteria by catalyzing the production of hydrogen peroxide and hydroxyl radicals, which triggered CDT (Figure 5A). Due to the important photothermal properties in antimicrobial therapeutics, a series of experiments were conducted to investigate the photothermal properties of CuS@Pt−Au/Apt NPs. The results showed that the CuS, CuS@Pt−Au, and CuS@Pt−Au/Apt solutions maintained approximately the same temperature change under 808 nm laser irradiation, which was significantly higher than that of the PBS group, demonstrating the excellent photothermal properties of CuS and the negligible effects of loaded nanozymes and aptamers (Figure 5B). In addition, the CuS@Pt−Au/Apt solution showed regular temperature evolution including heating and cooling processes in five consecutive photo/non-photoluminescence cycles, demonstrating its good photothermal stability (Figure 5C). In addition, the POD- and OXD-like activities of the nanozyme could catalyze glucose catabolism and −OH production to regulate blood glucose and kill bacteria. Thus, this aptamer-functionalized nanozyme exhibited a strong synergistic effect between the enzyme-like activity and the photothermal effect, thus synergistically promoting diabetic wound healing.

However, PTT alone can easily cause damage to body tissues, for instance, by high temperatures. Therefore, it is preferable to keep the temperature below 45 °C for in vivo PTT treatment.[199] Shen et al.[200] designed an integrated glucose-responsive photothermal nanozyme (GOX/MPDA/Fe@CDs) consisting of GOx, folded carbon dots (Fe@CDs), and mesoporous polypropylene dopamine (MPDA). Polydopamine (PDA) NPs were formed by auto-oxidative polymerization of dopamine, which showed good biocompatibility and biodegradability, and could effectively convert light energy into heat, making it an ideal photothermal conversion agent. The resultant photothermal nanozyme rapidly increased temperature to damage bacteria under NIR (808 nm) laser irradiation. In addition, the loaded glucose oxidase catalyzed the production of hydrogen peroxide from glucose, followed by the continuous generation of toxic •OH triggered by Fe@CDs nanozyme via a Fenton/Fenton-like reaction. Notably, the temperature of the treatment system only rose to 45 °C, which effectively prevented the heat therapy from damaging normal tissues around the infected wound. Recently, Liu et al.[201] constructed a ternary nanozyme consisting of ZIF-8/(mesoporous polydopamine)MPDA@(deoxyribonuclease I)DNase I. Specifically, DNase I was first encapsulated in MPDA nanoparticles (NPs) and then wrapped by ZIF-8, which was rapidly degraded in an acidic bacterial environment, triggering the release of the antimicrobial drug Zn^{2+} and DNase I. DNase I is a special endonuclease that kills bacteria by cleaving the DNA and inhibits the formation of bacterial biofilm. MPDA has better photothermal conversion efficiency due to higher specific surface area compared to conventional dopamine particles (PDA). The obtained ternary nanozyme was able to achieve effective photothermal antimicrobial therapy at mild temperatures (~45 °C) based on the photothermal bactericidal effect of MPDA and the antimicrobial effects of Zn^{2+} and DNase I. In addition, the nanosystem showed good ROS scavenging ability based on the unique p-hydroxyformic acid structure of MPDA, which effectively alleviated oxidative stress injury and accelerated the repair process of diabetic wounds. The nanosystem was then used for wound treatment in diabetic mice. Under NIR irradiation, the temperature of ZIF-8/MPDA@DNase-treated wounds increased to 45.8 °C within 4 min (Figure 5E). In contrast, there was no significant change in wound temperature in the PBS group. Skin tissues were collected from each group after 7 days of treatment, and the in vivo antimicrobial efficacy of the ternary nanosystems was evaluated using standard plate counting method. Compared with the control group, the survival rate of *S. aureus* gradually decreased in the order of MPDA group < MPDA@DNase I group < ZIF-8/MPDA@DNase I group. Under NIR irradiation, the survival rate of *S.*

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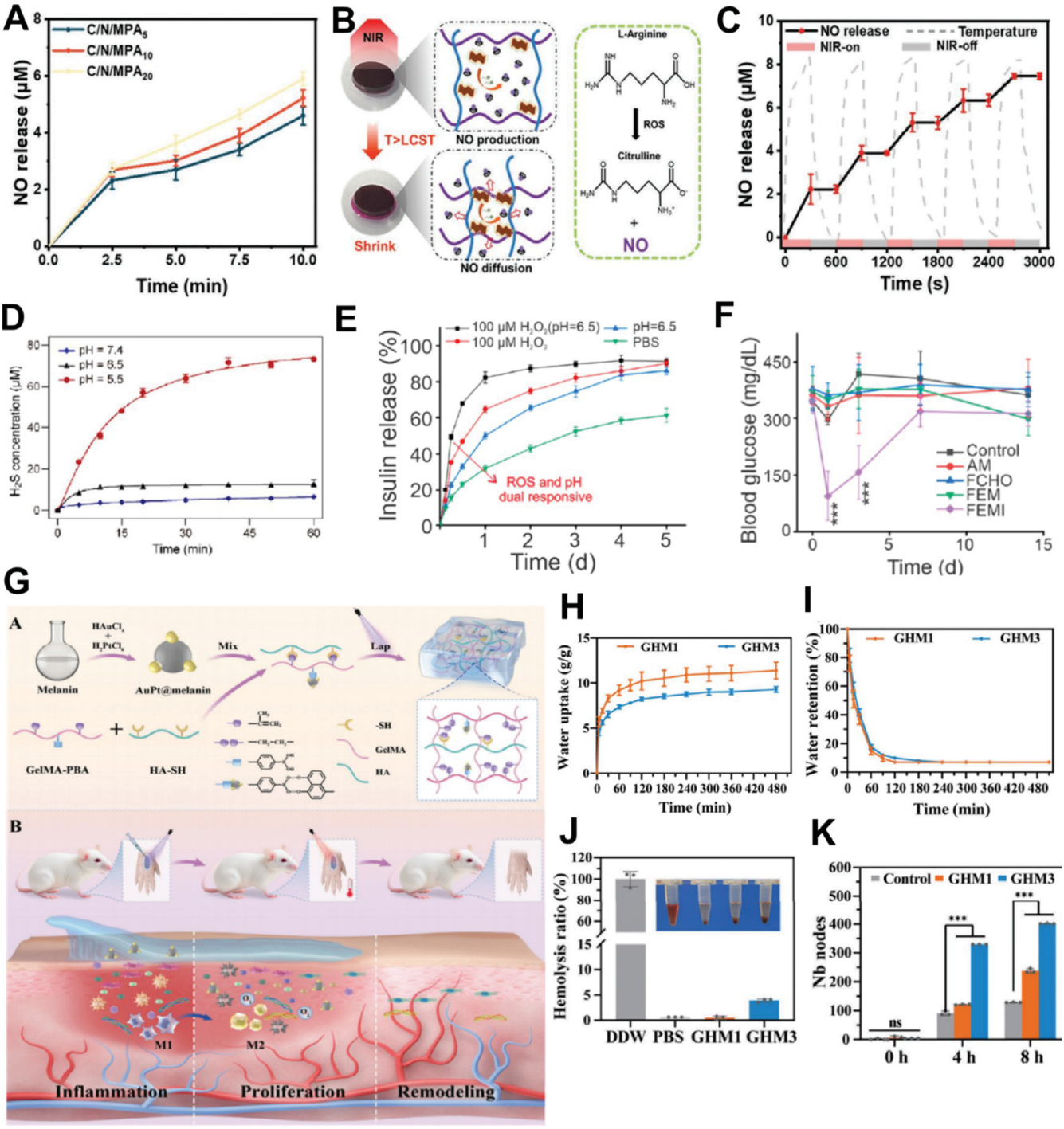

Figure 6. (A) NO release capacity of C/N/MPAs (the subscript numbers represent the different concentrations of polydopamine (MSPA)) under NIR irradiation. (B) Schematic representation of stimuli-responsive NO delivery by C/N/MPA. (C) Controllable NO release ability of C/N/MPA_{10} under NIR for five on/off cycles. Reproduced with permission from ref 207. Copyright 2023, Wiley. (D) H_2S release curves of GOx@MnS NPs in 25 mM acetate buffer (pH = 7.4, 6.5 or 5.5). Reproduced with permission from ref 210. Copyright 2023, American Chemical Society. (E) pH and ROS dual response to insulin release kinetics of FEMI hydrogels. (F) Changes in blood glucose at different time points in diabetic mice of different treatment groups. Reproduced with permission from ref 215. Copyright 2020, American Chemical Society. (G) Preparation of AuPt@melanin-incorporated (GHM_3) hydrogel dressing and therapeutic mechanism in diabetic wounds. Water uptake (H) and water retention (I) performance of GHM hydrogels. (J) Hemolysis test with hydrogel. (K) Statistical outcomes of angiogenesis activity (Nb nodes representing the formation of cellular nodes during the initial process of neoangiogenesis). Reproduced with permission from ref 220. Copyright 2023, Wiley.

aureus in the three experimental groups was further reduced, especially the last group with intact nanosystem components was as low as 21.6% (Figure 5F). This further demonstrated the excellent antimicrobial effect of ZIF-8/MPDA@DNase at mild temperatures, ensuring therapeutic efficacy while protecting normal tissues from high-temperature damage. Meanwhile, NIR laser stimulation accelerated the release of Zn^{2+} with good antibacterial ability. The combined antimicro-

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bial effect of PTT, DNase I, and Zn^{2+}, thereby improved infection treatment and accelerated diabetic wound healing.

In addition, ultrasound is widely used in clinical diagnosis and treatment because of its noninvasiveness, low energy attenuation, and high tissue penetration ability. Recently, there have been some reports of ultrasound-enhanced nanozymes for infection therapy.[202] The enhanced activity of nanozymes originates from carrier separation on the surface and energy band bending by absorbing large amounts of energy released in cavitation bubbles. In addition, ultrasound triggers the formation of transient pores in the biofilm matrix, which increases the diffusion of nanozymes into the biofilm and improves the therapeutic effect. Recently, Zhu et al.[203] developed an ultrasound-enhanced multienzyme-active nanozyme hydrogel spray (ACPCAH) using hyaluronic acid-coated arginine, Au NPs, and $Cu_{1.6}O$ NPs coloaded with phosphorus-doped nitride graphite carbon nanosheets. The ACPCAH possessed five enzymatic activities mimicking SOD, CAT, GOx, POD, and nitric oxide synthase (NOS). This nanozyme utilized the multienzyme activity to establish a cascade catalytic reaction system. Specifically, excess ROS in the DFU microenvironment was first utilized to initiate the SOD-CAT cascade reaction, which converted excess ROS to the intermediate product O_2. A large amount of O_2 solved the problem of insufficient O_2 in the GOx-POD cascade reaction, promoting the oxidation of glucose and the production of H_2O_2, which was subsequently decomposed to produce the highly toxic •OH via POD-like activity. NOS catalyzed the production of NO gas from endogenous L-arginine, which promoted cell proliferation, collagen formation, and angiogenesis, and also eradicates bacterial biofilms via the toxic oxidative byproduct nitrous oxide (N_2O_3). To perform in vitro antimicrobial assay of ACPCAH, representative Gram-positive (methicillin-resistant *S. aureus, MRSA*) and Gram-negative (*E. coli*) strains were cultured in the microenvironmental medium for diabetic patients, and the antimicrobial properties were determined by plate counting method after different treatments. The results showed almost no colony formation in the ACPCAH+ultrasound group, suggesting superior antimicrobial activity to the other three groups. The effect of ultrasound on the enhancement of nanozymatic activity was effectively verified (Figure 5D). Through a highly efficient SOD-CAT-GOX-POD/NOS five-enzyme-like cascade reaction, ACPCAH, with substantially enhanced catalytic efficiency, exhibited extraordinary therapeutic effects in a range of processes including ROS scavenging, alleviation of hypoxia, and lowering of blood glucose to bacterial killing. In addition, the therapeutic effect of ACPCAH could be further enhanced by ultrasound-catalyzed nanozyme coupling. In conclusion, this study not only highlighted an effective multienzyme-like nanozyme but also provided a promising integrated approach for sonodynamic therapy combined with nanozymes for the treatment of DFU.

In summary, nanozyme technology in combination with a range of physical therapies including phototherapy, thermotherapy and acoustic power therapy shows fascinating promise in the treatment of diabetic wounds. However, several key issues remain. (a) Due to the limited tissue penetration of lasers, they are not viable for deeper layers of the organism, and thus lack the means to effectively treat deep lesions. At the same time, normal tissue damage caused by frequent laser irradiation is inevitable. (b) The local high temperature produced by PTT can damage the surrounding tissues. (c) The poor tissue accumulation, low bioavailability and stability, and easy removal and excretion by the body of photosensitizers and acoustic sensitizers have severely limited their further clinical conversion rate. At the same time, in vivo biosafety is also an important issue. (d) In vivo targeting determines whether it is efficient to deal with pathological sites rather than other healthy tissues.

4.5. Combination with Other Components. *4.5.1. Combination with Gases.* It has been shown that gaseous molecules with therapeutic properties, such as oxygen, nitric oxide, carbon monoxide, and hydrogen sulfide, play important roles in all phases of wound repair, including anti-inflammatory and antimicrobial modulation, cell proliferation and migration, pro-angiogenesis, and extracellular matrix remodeling. However, the application of gas therapy (GT) alone is usually limited by its low tissue permeability, poor solubility and bioavailability.[204] Therefore, nanomaterials with gas-producing and gas-carrying functionalities offer great opportunities to significantly improve the efficacy of GT.

NO has been shown to have potent anti-inflammatory, antimicrobial, and vasodilatory effects, modulating cardiovascular function and stimulating skin cell activation leading to wound healing. NO fights inflammation by modulating inflammatory cells and inflammatory factors (e.g., interleukins, monocytes, neutrophils) and achieves antimicrobial activity by being converted to the toxic products of nitrous oxide (N_2O_3) and peroxynitrite ($ONOO^-$), which cause biofilm decomposition.[205] Endogenous NO is produced intracellularly via NOS. However, during hyperglycemia, NOS activity is compromised, so there is a great need for a local supply of NO from the outside world.[206] NO donors can release NO in response to stimuli, such as light and heat, but uncontrollable levels triggering side-effects and a short half-life limits their application. Yang et al.[207] designed a variety of stimulus-responsive nanozyme-based cryogels as controlled NO release carriers for adaptive therapies to cater to different needs at different stages of wound healing. First, double-bond modified carboxymethyl chitosan (CMCSG) and thermosensitive poly(*N*-isopropylacrylamide) (PNIPAM) were copolymerized using ice-based templating technique to form CMCSG/PNIPAM cryogels with macroporous structures. Subsequently, L-arginine loaded MoS_2@polydopamine (MSPA) was added to form the complex CMCSG/PNIPAM/MSPA (abbreviated as C/N/MPA). MoS_2 has been known to generate ROS (e.g., $O_2^{\bullet-}$, $^{\bullet}OH$, and $^1O_2$) via electron and energy transfer, which could excite L-arginine to generate NO via biochemical or catalytic reactions. Under NIR irradiation, MSPA generated a large amount of ROS through energy transfer, which further catalyzed the conversion of L-arginine into L-citrulline and NO. The NO yield showed a C/N/MPA concentration-dependent increase and continued to increase within 10 min (Figure 6A). The specific process of NO release from the cryogel is shown in Figure 6B. To demonstrate the controllability and reproducibility of NO release, the amount of NO release was assayed in an intermittent NIR radiation treatment (Figure 6C). The results showed that NO release occurred in a stepwise-triggered manner, and the amount of NO release increased with irradiation time in the presence of NIR stimulation; when the NIR was turned off, the increase in NO was almost negligible, suggesting that the prepared nanozymatic gels functioned with precisely controllable NO release. In bacterially infected acidic wounds, the amino group of CMCS facilitated the electrostatic trapping of bacteria by

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the cryogel through adaptive protonation, thus enhancing the antibacterial efficiency. MSPA promoted the cascade release of NO by generating ROS under NIR, providing NO-assisted PDT and PTT antibacterial capabilities. After the infection was eliminated and the wound pH increases to ca. 7.4, MSPA exhibited SOD- and CAT-like nanozyme activities to effectively scavenge excess free radicals and alleviate excessive oxidative stress.

Nanozymatic cryogels with controlled NO release capacity and stimulation response have been used together for infected wound treatment through the synergistic effect of PDT, PTT, and NO gas therapy. Li et al.[208] prepared a pH-responsive nanoreactor by encapsulating GOx, iron tetraoxide nanozymes, and L-arginine into a nanocarrier. The nanoreactor was constructed by 1:1:1 ternary complexation of cucur[8]bitridine (CB[8]) with maleimide-modified viologen (MV) and azobenzene-conjugated helical quinoline oligomers (AHQO), which formed a superamphiphile that self-assembled into vesicle-like structures. Conformational changes in pH- and photoresponsiveness of AHQO allowed to modulate the nanoreactor's membranes from nonpermeable to semiosmotic or permeable for selective substance translocation. MV partially contributed to improved bacterial targeting. Under acidic conditions, only small molecules such as glucose could enter the swollen nanoreactor, which was subsequently catalyzed by the encapsulated GOx to generate hydrogen peroxide and gluconic acid, and further altered the pH and increased membrane permeability. The generated hydrogen peroxide was then broken down through the POD-like activity of iron tetraoxide to produce highly toxic $^{\bullet}OH$, which would fight bacterial infections. Hydrogen peroxide also oxidized L-Arg to NO, which acted as a mediator of the inflammatory response and accelerated wound closure. Thus, this nanoreactor provided a versatile and synergistic platform for diabetic wound healing by combining enzyme dynamic therapy with NO gas therapy.

Hydrogen sulfide (H_2S), an important endogenous gaseous molecule, is involved in various important processes associated with wound healing, including inflammation suppression, endothelial cell proliferation, and angiogenesis, in a dose-dependent manner. Notably, H_2S exerts anti-inflammatory effects at physiological concentrations without significant side effects. This is very different from the commonly used clinical anti-inflammatory drugs, steroids, which may lead to endocrine disruption in diabetic patients.[209] Due to these advantages, H_2S could be an ideal drug for the treatment of diabetic wounds through its combined anti-inflammatory and pro-healing effects. However, the therapeutic efficacy of H_2S on diabetic infected wounds is hampered by uncontrolled release behavior. Therefore, a well-designed stimulus-responsive system that ensures on-demand release kinetics of hydrogen sulfide is of great significance to maintain the therapeutic efficacy and avoid potential side effects. Wang et al.[210] reported a H_2S-releasing nanochain for infected diabetic wound treatment. GOx was chosen as the "carrier" of the composite, and the water-insoluble H_2S donor MnS was deposited on the GOx surface via an in situ biomineralization reaction to obtain GOx@MnS nanoparticles (GOx@MnS NPs) without any other stabilizers. Upon encountering high levels of glucose at the ulcer site, GOx catalyzed glucose to lower the local pH and triggered the stable release of manganese ions (Mn^{2+}) and H_2S. The release of H_2S from GOx@MnS NPs was tested in an acetate buffer at pH = 7.4, 6.5, and 5.5. A low H_2S release was observed at pH = 7.4 and 6.5; whereas when the pH was reduced to 5.5, a steady release of H_2S could be sustained for up to 1 h with a significantly higher peak concentration (Figure 6D). The promotion of H_2S release by acidic conditions was consistent with the pH decrease due to glucose catabolism, realizing a synergistic blood glucose regulation-gas therapy.

Notably, H_2S is also critical for the regulation of immune function. Natural RAW264.7 cells are quiescent but readily activate a pro-inflammatory M1 phenotype upon lipopolysaccharide (LPS) stimulation.[211] Therefore, after pretreatment with LPS to obtain M1 macrophages, they were treated with sodium thiocyanate and GOx@MnS NPs, both of which acted as H_2S donors, for 24 h. Detection of various inflammatory factors showed a decrease in the expression of pro-inflammatory factors TNF-α, IL-6, and an upregulation of the expression of the anti-inflammatory factor IL-10 after H_2S treatment. It was demonstrated that H_2S release exerted an effective immunomodulatory effect by inducing polarization of M2 macrophages and regulating inflammatory factors. In addition, the released Mn^{2+} reacted with H_2S to generate cytotoxic $^{\bullet}OH$, which eliminated bacterial infection and accelerated diabetic wound healing. In another study, Zhang et al.[212] designed ultrasmall gold@ferrous sulfide cascade nanozyme (AuNP@FeS) synthesized from Au NPs and FeS NPs with H_2S release capability. Gold nanozymes with GOx-like activity catalyzed the conversion of overexpressed glucose to hydrogen peroxide and gluconic acid, with the potential to promote hydrogen peroxide supply and regulate acidity. The POD activity of FeS nanozymes was accompanied by H_2S releasing properties. H_2S not only inhibited CAT in vivo and reduced endogenous hydrogen peroxide consumption, but also promoted the conversion of glucose to lactate and acidification. Thus, the prepared AuNP@FeS increased hydrogen peroxide production (via GOx of Au NPs) and decreased consumption (released H_2S inhibits CAT), while the synergistic effect of gluconic acid and lactic acid could be utilized to enhance the acidosis effect. Overall, efficient hydrogen peroxide generation and enhanced acidity promoted the POD-like activity of FeS nanozymes, generating abundant ROS that ultimately led to amplified iron death via the Fenton reaction in tumor. Although there are few reports of nanozymes synergizing with H_2S for the treatment of DFU, it is expected that the combination of the two will be widely pursued in the treatment of diabetic wounds due to the similarities between the tumor microenvironment and the diabetic wound microenvironment.

For functional gases like NO, CO, SO_2, H_2S, and H_2, they are essential signaling molecules involved in many important physiological processes within the cell and are crucial for maintaining homeostasis at low concentrations. Yet at high concentrations they can damage biomolecules and even cause cell death. The synergy with nanozymes has shown promising potential in the treatment of diabetic wounds. However, there remain issues that need to be addressed. (a) The intrinsic therapeutic mechanisms and interactions of each gas remain uncertain. Elucidation of the correlation network between the gases, such as NO, H_2S, and CO, is important for unraveling the mechanisms of treating traumatic wounds. Also, the interactions between gases and gas-carrying nanomaterials need to be further explored. (b) For chemically reactive gas molecules, such as NO and H_2S, a strict control of safe therapeutic dosage is essential to prevent cytotoxicity. Overdose is likely to lead to irreversible damage to normal tissues and organs. Therefore, there is an urgent need to

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develop more nanoplatforms that are more sensitive and highly responsive to exogenous/endogenous stimuli to achieve on-demand controlled release of therapeutic gas molecules. (c) Biofilm permeability and tissue utilization of gases need to be further improved.

4.5.2. Combination with Drugs. The wide variety of enzyme-like activities of nanozymes can significantly enhance the effectiveness of wound treatment, but sometimes not as dramatically as the direct action of drugs. Therefore, the targeted efficacy of certain symptoms is substantially improved by skillfully loading various drugs into the nanozyme complex system.[213] Insulin, one of the most important drugs for lowering blood glucose, occupies an irreplaceable position in diabetes treatment. Subcutaneous injection is the main route of insulin administration, with poor patient compliance and many adverse reactions. Oral insulin is safer and more convenient, but it is difficult to achieve the desired bioavailability due to the physicochemical properties of insulin itself and the in vivo absorption barrier.[214] Wang et al.[215] prepared a nanozyme-reinforced multifunctional nanozymatic hydrogel (FEMI) via a Schiff base reaction between ε-polylysine (EPL)-coated MnO_2 nanosheets (EM) and self-assembled aldehyde-polyaldehyde F127 (FCHO) micelles loaded with insulin. This system integrated insulin delivery and MnO_2 nanozymes into an injectable hydrogel at EPL, offering a more comprehensive approach to achieve antimicrobial, hyperglycemic control and to promote diabetic wound healing. MnO_2 nanozymes are known to catalyze the decomposition of the most abundant endogenous ROS (H_2O_2) to O_2, and remodel the harmful oxidative microenvironment. Meanwhile, a more rapid release of insulin was detected under acidic and oxidizing conditions (6 h earlier than the control, Figure 6E), and the pH/redox dual-responsive FEMI hydrogel facilitated a sustained, controlled insulin release to regulate blood glucose. In sustained wound healing experiments, wound closure was significantly faster in diabetic mouse wounds treated with both FEM (insulin-free) and FEMI hydrogels. Wounds dressed with FEMI hydrogel healed faster and better. Blood glucose and insulin levels were further monitored during the experiment. There was no significant change in plasma glucose levels after treatment with other insulin-free hydrogels. In the FEMI experimental group, a significant decrease in glucose levels was observed due to the glucose-lowering effect of insulin, which confirmed that the hydrogel successfully achieved in vivo release of insulin (Figure 6F). Additionally, the decrease of blood glucose helped regulate the inflammatory response in the wound microenvironment and promoted vascularization. With the help of vascularization, cellular metabolism was also accelerated, depositing more collagen fibers and accelerating the formation of new tissue.

Pravastatin sodium is a commonly used drug for the clinical treatment of hyperlipidaemia, and recent studies suggest that it may also be involved in NO synthesis. Tu et al.[216] exploited the drug properties by blending it into a multifunctional hydrogel consisting of a hydrophilic poly(PEGMA-*co*-GMA-*co*-AAm) (PPGA) polymer cross-linked with a hyperbranched poly-L-lysine (HBPL)-modified MnO_2 nanozyme. In addition to utilizing the catalytic ability of MnO_2 nanozymes to scavenge different types of ROS, generate O_2, and protect cells from oxidative stress, the water-soluble drug of pravastatin sodium modulated the inflammatory response and down-regulated inflammatory factors by participating in NO synthesis. Depending on O_2 and NO production, higher concentrations of TGF-β, and higher levels of angiogenesis and collagen deposition, diabetic wounds smoothly transition from the inflammatory phase to the proliferative healing phase. In a previous example, Wang et al.[172] designed novel multifunctional gold cluster enzymes (LC-AuNCs) in which lysozyme and curcumin were incorporated. Lysozyme acted as a natural bacteriostatic agent in the human body, mainly by dissolving the insoluble mucopolysaccharides of the bacterial cell wall resulting in cell wall rupture and subsequent bacterial death. Curcumin, a substance found in turmeric, has medicinal values such as antibacterial, anti-inflammatory and cardiovascular disease prevention. With the addition of the two drugs, the effect of nanozymes in the treatment of diabetic wounds was significantly enhanced.

In conclusion, the incorporation of gases (e.g., NO, H_2S, CO, etc.), drugs (e.g., insulin, antibiotics, antioxidants, antimicrobial actives, etc.) in the nanozymatic system through the delicate conception will greatly improve the therapeutic effect of both sides. Meanwhile, these monofunctional clinical treatments are expensive and ineffective in many cases, but synergistic treatments can effectively improve the utilization efficiency of each component and cut costs. Still, the preparation process should be based on the inherent properties of the dopants, the appropriate processing methods and modulation should be selected, and the interactions with the nanozymes should be carefully considered so as to achieve the best synergistic effect.

4.5.3. Combination with Hydrogel. In recent years, there has been a growing trend in the research of hydrogel-based wound dressings among nanozymatic materials for the treatment of diabetic wounds. Multiple components, including nanozymes, antimicrobial and anti-inflammatory active biomolecules, and drugs can be loaded into hydrogels to simultaneously realize the functions of detection, treatment, and drug delivery. Hydrogel-based wound dressings have the following main advantages.[217] (a) Cold application for analgesia. Hydrogel contains a large amount of water, when the hydrogel is in contact with the skin, due to the temperature difference between the skin and the gel, heat exchange occurs, resulting in a decrease of skin temperature. The temperature of the hydrogel then rises, which in turn accelerates the evaporation of the water inside the gel, causing the skin temperature to drop further. Moisture evaporation is a slow process, which can result in a sustained cooling effect, and have the effect of a cold analgesic. (b) Hemostatic and antibacterial effects. Hydrogel induces vasoconstriction and reduces tissue bleeding. It can also cover the wound and provide a slightly acidic environment favorable for leukocyte reproduction, which enhances bactericidal properties. (c) Promoted healing and inhibition of scarring. Hydrogel provides a wet environment conducive to necrotic tissue and fibrinolysis, maintaining the relative constancy of local wound temperature and humidity, and promoting healing. Hydrogel materials have good compliance and adhesion, which can reduce pigmentation and inhibit the formation of scars. (d) Absorption of exudate. Diabetic wounds often have a large amount of exudate, if not cleaned in time, the wound will be infiltrated by inflammatory cells and pollutants, which is not conducive to wound healing. Hydrogel can absorb a large amount of exudate without adhering to the wound, preventing infection while making dressing changes painless and avoiding secondary injury. (e) Good biocompatibility, nonirritation and non-

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sensitization. It is soft and elastic and can be well adhered to the wound and adapted to different parts of the body.

For instance, Li et al.[218] prepared selenol end-capped polyethylene glycol (PEG) hydrogels with polydopamine nanoparticles (PDA NPs) and Prussian blue nanozyme (PB nanozyme) in the absence of any other chemical additives or organic solvents based on the selenide and selenide bond-directed cross-linking. The hybridized hydrogels (DSeP@PB) were chemically double cross-linked by dynamic diselenide bonds and nondynamic selenides. The cross-linking of PDA NPs improved the injectability and mechanical strength of the hydrogels. Meanwhile, the release of PB nanozyme was induced by light-triggered dynamic exchange of diselenide bonds and photothermal effects. The bioactivity of the released PB nanozyme rendered the hydrogel highly effective in antimicrobial, ROS scavenging, and immunomodulatory applications, thereby protecting the cells from oxidative damage and reducing inflammatory responses. The injection of this hybridized hydrogel into diabetic mice did not cause any changes in body weight, blood biochemical parameters and histological features of the mice, demonstrating its good biocompatibility. Under laser irradiation at 730 nm, it released PB nanozyme on demand, exerting potent anti-inflammatory and antioxidant effects, followed by stimulation of angiogenesis and collagen deposition, showing the most potent wound healing activity. In addition, Zhang et al.[219] prepared high-density platinum nanozymes (PNAs) using dynamic covalently bonded hybrids of metal−organic coordination polymers as synthetic templates. The PNAs not only mimicked antioxidant enzymes (CAT, POD) efficiently, but also replicated the enzyme function of glutathione reductase (GR) to induce the production of GSH under ultrasound via surface plasmon resonance effects enhanced electronic polarization. Subsequently, platinum-based nanozymes were mixed with GelMA hydrogels (PNAs/GelMA) and applied to the treatment of wounds in diabetic mice. The nanorestricting ability of the hydrogel imposed diffusion restriction on the Pt nanoparticles, altering their interparticle distance and preventing aggregation. At the same time, the hydrogel enabled the PNAs to be released continuously and slowly under ultrasound stimulation, which was beneficial to the therapeutic effect. A range of therapeutic effects, including ROS scavenging ability, expression of angiogenic factors, collagen deposition content, and polarization level of M_2 anti-inflammatory macrophages, were superior in the PNAs/GelMA group compared to the single PNAs group. The extraordinary therapeutic properties of hydrogels in wound healing were confirmed. Thus, a comprehensive therapeutic strategy based on nanozymatic hydrogels simultaneously scavenging ROS, alleviating hypoxia, promoting neoangiogenesis, and modulating the immune system was realized. Liang et al.[220] prepared AuPt@melanin-binding (GHM_3) hydrogel dressing via a one-step redox reaction approach designed to promote effective thermotherapy-enhanced local glucose consumption and ROS clearance. Melanin, a classical ROS scavenging material, can also be used for the deposition of Au NPs (GOx-like) and Pt NPs (CAT-like) due to the abundant polyphenol groups on its surface. Phenylboronic acid (glucose-responsive), bis-bonded modified gelatin, and thiol-modified hyaluronic acid were used to encapsulate AuPt@melanin nanoparticles to synthesize a hybrid immune-modulatory hydrogel consisting mainly of thiol−ene cross-links (Figure 6G). A series of experiments verified the properties of the hydrogel. Importantly, the ability of GHM1 (without AuPt@melanin) and GHM3 (with AuPt@melanin) hydrogels to absorb and retain water was assessed by water absorption experiments and water retention experiments. Both GHM1 and GHM3 hydrogels were able to absorb water quickly in a short period of time, with the rate of absorption for GHM3 being slightly lower than that of GHM1 due to the incorporation of AuPt@melanin which enhanced the cross-linking properties of the hydrogels. After the drying process until there was no further change in their respective weights, GHM3 weighed more and exhibited a better ability to retain water (Figure 6H−I). The superior ability to absorb and retain water allowed GHM3 to efficiently absorb wound exudate and maintain optimal moisture levels, which was critical for diabetic wounds. Biocompatibility experiments and hemolysis experiments (Figure 6J) demonstrated that the GHM hydrogel did not cause cytotoxic damage as well as hemolysis, which provided a guarantee for clinical application. Subsequently, the mechanical properties of the hydrogel were further enhanced by UV-induced double-bond free radical polymerization, and the glucose and ROS clearance at the wound was modulated by NIR-triggered hyperthermia (42.5 °C), while promoting angiogenesis (Figure 6K). The synthesized GHM hydrogel significantly promoted the healing of diabetic rat wounds, especially after NIR stimulation, and almost completely closed after 14 d of treatment. The multifunctional GHM3 hydrogel combining enzyme-like activity with photothermal therapy exerted multiple beneficial effects on the healing process of diabetic wounds, including lowering blood glucose, modulating ROS, reducing inflammation, accelerating granulation tissue regeneration and collagen deposition, and promoting neovascularization.

In fact, there are some inspiring hydrogel systems that have recently been applied to the treatment of diabetic wounds. For example, Xiang et al.[221] exploited the potential antioxidant and antimicrobial properties of protocatechuic aldehyde (PA) and trivalent ferric ion (Fe^{3+}) complex (Fe^{3+}@PA) to develop a dual hydrogel network by combining Fe^{3+}@PA complex-modified decellularized dermal matrix (ADM) and light-cured gelatin (GelMA). Subsequently, complementary exosomes were derived from human umbilical vein endothelial cells (HUVEC-Exos) to create an ADM composite hydrogel (ADMFe^{3+}@PA-Exos/EelMA). The decellularized dermal matrix (ADM) was a wound dressing composed of a natural extracellular matrix and enriched bioactive factors with inherent bioactive cues, appropriate biocompatibility and mechanical properties, and nontoxic degradation products. In addition, ADM contained essential components that contributed to wound healing, such as proteoglycans, growth factors, collagen, hyaluronic acid, heparin, primary fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF). The ADMs were digested chemically and physically and then self-assembled to form hydrogels. The resultant ADM hydrogel utilized GelMA to enhance the mechanical properties of the hydrogel, while retaining the inherent 3D structure of ADM and the bioactivity of the cytokines. As a result, this hydrogel displayed commendable mechanical properties, an inherent 3D extracellular matrix structure, and cytokines capable of interacting with cells. ADM composite hydrogel promotes diabetic wound healing by eliminating bacterial infection, reducing ROS levels, protecting cells from oxidative stress damage, and promoting collagen deposition and angiogenesis, providing a promising strategy for optimizing ADM treatment. Liu et al.[222] synthesized CMCS-CEBT

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ACS Biomater. Sci. Eng. 2024, 10, 4195−4226

hydrogels using exosome-coated carboxymethyl chitosan (CMCS), chitosan nanoparticles (CS-NPs), bioactive glass (BG) and titanium dioxide nanoparticles. Mesenchymal stem cell-derived exosomes (MSC-Exos) showed beneficial effects on wound healing through anti-inflammatory and angiogenic properties. Chitosan (CS) had good biocompatibility and accelerates cell migration, adhesion and proliferation. Ions released from bioactive glass (BG) and titanium dioxide (TiO_2) nanoparticles exhibited sustained angiogenic and antimicrobial capabilities. The synergistic effect of several important components combined with the physicomechanical properties of the hydrogel accelerated diabetic wound healing by stimulating angiogenesis, increasing collagen deposition and anti-inflammatory factor expression of anti-inflammatory factors. These design ideas inspired the development of novel nanozyme- hydrogels aimed at combining enzyme-like activity with the functionality of various favorable components.

However, hydrogels are prone to cracking and bacterial invasion due to external forces. Therefore, the mechanical and self-healing properties of hydrogels need to be improved. In addition, considering the complex microenvironment and multipathological characteristics of chronic wounds, the development of a series of injectable, antimicrobial, anti-inflammatory, mucoadhesive, hemostatic, slow-release, antioxidant, multifunctional nanozyme-hydrogels having the ability to respond to environmental stimuli, such as pH, light, and heat, is also an important direction for future development.

5. SUMMARY, CHALLENGES, AND PERSPECTIVES

DFU cause great physical, psychological and economic harms to diabetic patients and their families. The emergence of nanozymes has significantly improved the effective enzymatic ability of nanomaterials, which advances the development of biomedical materials and shows favorable therapeutic activity toward diabetic wound healing. In this paper, we reviewed the typical microenvironmental characteristics of DFUs and the mechanisms of nanozymatic activity in DFU healing, and discussed the specific application of nanozymes in diabetic wound healing. Diabetic wounds develop an adverse microenvironment headed by hyperglycemia, recurrent infections, and persistent inflammation. Regulation by nanozymes mainly involved four different enzyme-like activities (POD-, GOx-, CAT-, and SOD-like). Considering the complex healing process of DFU, one or two substances are not sufficient for complete healing, so the combination of multiple substances released at different stages of the wound accelerates diabetic wound healing. Various multifunctional nanozymes have been developed to drive diabetic wound healing.

Despite substantial progress, a number of critical issues and challenges remain to be addressed.

(a) Biosafety of nanozymes. Good biocompatibility is the key to the successful application of nanozymes. Therefore, the interactions between nanozymes and biological systems should be carefully examined, and efforts should be made to synthesize nanozymes with high safety and high enzyme catalytic activity through structural optimization and surface modification. Synthesis and modification based on biomolecules (e.g., PEG, bovine serum albumin, etc.) are potential strategies to obtain highly biocompatible nanozymes. In recent years cell membrane coating technology has been developed as a novel bioinspired method to modify nanozymes by virtue of their facile functional properties and excellent biological characteristics. In addition, the flexible application of various carriers, such as hydrogels, protein nanocages, liposomes, etc., can avoid the reduction of enzyme-like catalytic activity caused by direct surface modification of nanozymes, and confer better biocompatibility, controlled drug release ability, and targeting ability.

(b) In vivo exploration of the therapeutic mechanism and long-term effects of nanozymes. The catalytic mechanisms of many nanozymes are not yet fully understood. In-depth understanding of the interactions between nanozymes and pathological sites and the specific catalytic mechanisms in wound healing can help develop nanozymes with high specificity and tissue regeneration efficiency. Sometimes, external modifications may alter the physicochemical properties and wound healing pathways of nanozymes. Therefore, the use of computer and machine learning technologies to assist in modeling the kinetics of nanozyme catalysis and examining the energetic changes during reactions is significant in revealing the intrinsic catalytic mechanism and unraveling their constitutive relationships.

(c) Further improvement of the catalytic activity and targeting efficacy. Although the nanozymes have overcome many shortcomings, the catalytic activities remain mostly subpar as compared to those of natural enzymes. To improve the therapeutic effect of wounds, it is necessary to design nanozymes with high catalytic activity through careful engineering of the structure. Utilizing plasma effect and increasing the reaction temperature of nanozymes are considered as emerging strategies to improve the catalytic activity of nanozymes. At the same time, the ability of nanozymes to target wound sites is not yet sufficient, which inevitably causes damage to normal tissues. To mitigate such issues, surface modification of specific molecules is a common solution.

(d) Protocols for efficient nanodrug delivery. There is a need to develop more convenient and controllable routes of drug delivery, to increase the delivery efficiency and self-activity of nanosystems, and to improve the bioavailability of nanodrugs. The use of photothermal stimulation for controlled, on-demand drug release is a promising pathway. Recently nanovesicles are an emerging drug delivery system, and plant-derived vesicles are especially promising for loading nanozymes, nanodrugs due to their low toxicity, good biocompatibility. Meanwhile, the development of more effective and multifunctional nanosystems based on the complex microenvironment of DFU, integrating diagnosis and treatment into one system, is essential for the realization of "monitoring and treatment of diabetic wounds as a whole". For example, it is feasible to monitor wound healing in combination with pH-sensitive substances. Anthocyanins are ideal candidates, not only for monitoring healing by indicating wound pH through color change but also for improving efficacy due to their antioxidant properties. Enzyme catalysis and color rendering reaction can be integrated in nanozymatic systems to monitor glucose concentration.

(e) Currently most research is based on rodent mice or rats for in vivo studies of nanozymes, which is clearly

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different from the human biological systems. To have a more reliable prediction of the efficacy and toxicity of nanozymes, in vivo experiments in large mammals are needed in the future.

(f) Research based on nanozyme technology has remained mostly in the phenotypic stage (e.g., anti-inflammatory, antibacterial, regulating signaling pathways) and limited on gene therapy, cell therapy, and growth factor therapy. Further in-depth studies on precise mechanisms (e.g., exact site of action, molecular interaction targets) are necessary. This is of great significance for the design of nanozymes and the rational guidance of clinical drug delivery.

(g) Considering the susceptibility of diabetic wounds to recurrence, the construction of nanosystems for antirecurrence and good prognostic monitoring has great potential for DFU treatment.

(h) Integration with traditional Chinese medicine (TCM). It has been shown that many TCM ingredients (e.g., curcumin, bitter melon saponin, angelica, astragalus, etc.) have antimicrobial, anti-inflammatory, and hypoglycaemic properties. They can also be processed to the nanoscale to better enhance the efficacy of DFU treatment through synergistic effects with nanozymes.

In summary, multifunctional nanozyme systems based on the convergence of multiple technologies hold unprecedented promise for DFU therapy. They are expected to simultaneously modulate wound healing through multiple pathways (including lowering blood glucose, ameliorating hypoxia, mitigating oxidative stress, and eradicating bacterial infections) at all stages and provide a balanced environment throughout the wound healing process, thereby reducing potential side effects. The practical clinical application of nanozymes still has a long way to go and requires concerted efforts of scientists in various fields.

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Notes

The authors declare no competing financial interest.

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