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Reactive Oxygen Species-Scavenging Nanosystems in the Treatment of Diabetic Wounds

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Diabetic wounds are characterized by drug-resistant bacterial infections, biofilm formation, impaired angiogenesis and perfusion, and oxidative damage to the microenvironment. Given their complex nature, diabetic wounds remain a major challenge in clinical practice. Reactive oxygen species (ROS), which have been shown to trigger hyperinflammation and excessive cellular apoptosis, play a pivotal role in the pathogenesis of diabetic wounds. ROS-scavenging nanosystems have recently emerged as smart and multifunctional nanomedicines with broad synergistic applicability. The documented anti-inflammatory and pro-angiogenic ability of ROS-scavenging treatments predestines these nanosystems as promising options for the treatment of diabetic wounds. Yet, in this context, the therapeutic applicability and efficacy of ROS-scavenging nanosystems remain to be elucidated. Herein, the role of ROS in diabetic wounds is deciphered, and the properties and strengths of nanosystems with ROS-scavenging capacity for the treatment of diabetic wounds are summarized. In addition, the current challenges of such nanosystems and their potential future directions are discussed through a clinical-translational lens.

1. Introduction

Diabetic wounds are one of the most debilitating sequelae of diabetes mellitus and are characterized by toxic inflammation and excessive oxidative stress that damage healthy tissue and impair the healing process.[1] Diabetic non-healing wounds occur in 15% of people with diabetes and precede 84% of all diabetes-related lower-leg amputations.[1b,2] Physiological wound healing comprises four overlapping phases, namely the blood clotting and hemostasis phase, the neutrophil-macrophage driven inflammatory phase, the fibroblast-centric proliferation phase, and the final phase of connective tissue formation and remodeling.[1a,3] Chronic non-healing diabetic wounds display a prolonged and augmented inflammatory phase as well as a delayed and defective proliferation phase, which is characterized by insufficient angiogenesis, impaired granulation tissue formation, and significantly reduced wound tensile strength.[4] Considerable efforts were made to ameliorate the

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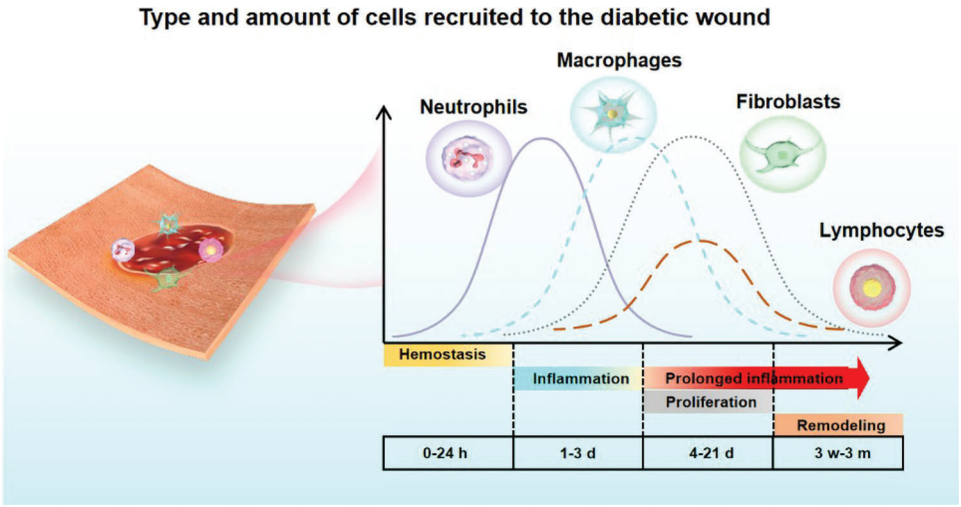

Figure 1. Cell recruitment to the wound site. The healthy wound healing process occurs in four sequential and overlapping stages: hemostasis, inflammation, proliferation, and tissue remodeling. Each stage involves different cell groups secreting a variety of cytokines. Neutrophils accumulated at the wound site occur 1–2 days after injury and play a major role in preventing bacterial infection with the help of macrophages, which also activate keratinocytes, fibroblasts, and immune cells. Lymphocytes begin to migrate to the wound 1 day after injury and gradually increase and reach a peak at 7 days after injury; and then, decrease gradually. Growth factors stimulate the initiation of the proliferative phase, which are characterized by fibrous tissue proliferation, matrix deposition, angiogenesis, and re-epithelialization. In diabetic wounds, dysfunctional immune cells including macrophages and neutrophils promote the healing process to remain in the inflammatory phase, resulting in a local immune microenvironment for difficult-to-heal wounds.

augmented and prolonged inflammatory phase and to facilitate the wounds entering to the proliferation and remodeling phases. Chen and coworkers, for instance, developed a mechanically active adhesive and immunomodulatory dressing for accelerating wound closure in murine and porcine partial-thickness wound models, which provides a promising opportunity for wound healing applications.[5] However, the delayed and/or stagnant healing manifests in persistent physical symptoms including pain and wound maceration and exudation.[6] The high recurrence rate and a dependence on caregivers exacerbates the patient's distress and decreases their quality of life. Accordingly, diabetic wounds represent an urgent challenge, from both a financial–economic and clinical–scientific perspective.[7]

A plethora of intrinsic and extrinsic factors are implicated in the pathogenesis of diabetic wounds, with reactive oxygen species (ROS) playing a key role by augmenting inflammation and resulting in excessive cellular apoptosis.[8] More specifically, ROS is a series of highly reactive molecules and free radicals derived from oxygen metabolism. Although ROS orchestrates normal cellular functions, such as gene transcription, signal transduction, and homeostasis, excess ROS in tissues can overwhelm the physiological antioxidant capacity, and excess ROS activity has emerged as a major cause of refractory diabetic wounds.[4]

In an era of advanced nanomedicine and nanotechnology, a variety of multifunctional nanosystems has been proposed as therapeutic modalities in biomedicine, including for diabetic wounds.[9] Due to their structural versatility, nanosystems can be customized to uniquely address the pathogenesis and therapeutic needs of different diseases. Owing to their physicochemical characteristics (i.e., adjustable size, variable charge, and high surface-to-volume ratio), nanosystems are also particularly suitable as drug-delivery systems, encapsulating functional molecules and agents with favorable pharmacokinetic and pharmacodynamic profiles.[10] In addition, nanosystems can be biochemically modified and upgraded. Accordingly, they may exhibit prolonged active functioning, cell targeting, and controlled release, which ultimately enhances their therapeutic efficacy in vivo.[11] More recently, a wide array of sources for the construction of nanosystems has been reported, ranging from micelles (MICs), through mesoporous silica to metal–organic frameworks. Thus, a broad spectrum of options for the design and synthesis of nanosystems is available, offering operational and engineering flexibility.[12]

This review aims to summarize the role and pathogenic mechanisms of ROS in diabetic wounds, highlight potential therapeutic targets, and provide constructive guidance for the design and development of therapeutic nanosystems. To this end, we outline the biogenesis of ROS and shed light on the arcanum of ROS and ROS-scavenging nanosystems in the treatment of diabetic wounds. Last, we discuss the ongoing challenges and future perspectives of such ROS-scavenging nanosystems, while paving the way for their application in clinical practice and patient care.

2. Pathogenesis of Diabetic Wounds

Normal wound healing occurs in four sequential and overlapping stages: hemostasis, inflammation, proliferation, and tissue remodeling. Each stage involves different cell groups secreting a variety of cytokines (**Figure 1**).[13] Mainly due to the dysregulated blood glucose levels, diabetic patients are particularly vulnerable to wound healing disorders, such as chronic and non-healing diabetic wounds. In fact, the current body of evidence points to an interplay between diabetes-related endogenous impairments and exogenous factors, such as infection, trauma, and pressure as underlying mechanisms for the chronicity of diabetic wounds. Yet, this interaction is complexly intertwined at the cellular and

molecular level, resulting in a pathophysiological mosaic of diabetic wound healing.[14]

Persistent pathologic hyperglycemia in diabetic wounds interferes with the wound healing microenvironment, leading to reduced angiogenesis and microvascular complications. Vascular endothelial cells are considered initial drivers and key players of neovascularization. Long-term exposure to a high glucose environment may decrease the proliferation and migration capacity of endothelial cells and induce apoptosis.[15] Reduced levels of vascular endothelial growth factor (VEGF) are reflected in the decreased angiogenesis, with more friable immature vessels and compromised neovascularization. The secretion of endothelial nitric oxidase synthase (eNOS), a vasoactive factor, is commonly diminished in diabetic wounds, leading to reduced blood flow in the wound area and impaired wound healing.[16] Accordingly, the regulation of eNOS secretion through the phosphatidylinositide 3-kinases (PI3K)/protein kinase B (Akt)/eNOS pathway has emerged as a promising avenue to promote the healing of diabetic wounds.[17] In addition, the non-enzymatic glycosylation of the vascular basement membrane compromises the homing of endothelial progenitor cells (EPCs) and hinders angiogenesis in diabetic wounds. Thus, EPC-based therapies carry translational potential in the treatment of chronic diabetic wounds.[18] The long-term dysfunction of the glucose and lipid metabolism can downregulate the transcription function of hypoxia-inducing factor-1α (HIF-1α) and dampen the reactivity of tissue cells to hypoxia. As a result, cytokines and growth factors, such as VEGF, glucose transporter-1 (GLUT1), and erythropoietin (EPO) are expressed at reduced or pathological levels.[19] Downstream, angiogenic cascades such as HIF-1α/VEGF and HIF-1α/PDGF remain inactivated, with the formation of new blood vessels being delayed or stagnated.[20] Low oxygen and nutrients in the wound milieu lead to a decrease in the migration and chemotaxis of cells that are necessary for wound repair. In the sense of a vicious circle, this cellular deficiency delays the healing of diabetic wounds.

An unrestricted and persistent chronic inflammatory response can also underlie the chronicity of diabetic wounds. More specifically, dysfunctional immune cells cause the healing process to persist in the inflammatory phase. Toll-like receptors recognize signals from necrotic wound tissue and damaged extracellular matrix (ECM) to activate macrophages and further release pro-inflammatory mediators, such as tumor necrosis factor-α (TNF-α), nitric oxide (NO), and interleukin-6 (IL-6). These cytokines chemoattract more immune cells, such as neutrophils and mast cells. Mast cells indirectly activate tissue-bound macrophages, leading to persistent polarization of macrophages toward the pro-inflammatory phenotype (M1). Neutrophils generate antibacterial substances and secrete cytokines, which further mediate the activation of T and B cells and other lymphocytes. In diabetic wounds, this inflammatory response is pathologically intensified and prolonged, without natural standstill signals.

The accumulation of advanced glycated end products (AGEs) is a key feature of the biochemical changes in the diabetic skin environment.[21] AGEs can stimulate the increase of ROS production, leading to cell membrane and endoplasmic reticulum damage, and causing chronic inflammation. Accordingly, blocking the AGEs/ROS/NLRP3 axis has been shown to significantly accelerate epithelial wound healing and nerve regeneration.[22] Meanwhile, ROS can directly damage healthy mesenchymal stem cells (MSC), resulting in decreased proliferation, migration, and adhesive capabilities. In addition, changes in the cytokine secretion profile and induction of apoptosis have been observed.[23] Consequently, MSC and derived products of MSCs, such as exosomes or extravesicles, are used for their therapeutic potential.[24] A persistent hyperglycemic microenvironment, as seen in diabetic patients, can also lead to irreversible depletion of the angiogenic MSC subpopulation, which contributes to wound healing.[25] In addition, AGEs can inhibit proliferation and induce the apoptosis of human dermal fibroblasts by activating the receptor of advanced glycation end products (RAGE). At the same time, the migration and adhesion of fibroblasts, keratinocytes, and endothelial cells, are inhibited, while fibroblast senescence is promoted; thus, impairing the proliferation phase in normal healing.[26]

Diabetic peripheral neuropathy is a significant risk factor for the persistence of diabetic wounds. In both diabetic patients and animal models, reduced skin surface nerve fibers and downregulated secretion of neuropeptides such as substance P (SP), neuropeptide Y (NPY), and calcitonin gene-related peptide (CGRP) have been observed. These changes result in reduced chemotactic and growth factor secretion, hampering the physiological functions of mast cells, endothelial cells, fibroblasts, and keratinocytes. Consequently, vascular regeneration and angiogenesis are impaired, which negatively affects wound healing. Furthermore, the physiological balance between ECM deposition and remodeling is also an important factor for healthy wound healing. Continuous inflammation in diabetic wounds results in increased production of matrix metalloproteinases (MMPs), such as MMP-1, MMP-8, and MMP-9 and decreased secretion of tissue inhibitor of matrix metalloproteinases (TIMP).[27] As MMPs and TIMP are key factors regulating the apoptosis of inflammatory and ECM cells, their imbalance results in abnormal apoptosis of various cell types such as neutrophils, fibroblasts, and vascular smooth muscle cells, leading to impaired wound healing. Accordingly, inhibition of MMP expression[28] and application of the active protein TIMP-1[29] to local wound sites can be used as a therapeutic strategy to promote diabetic wound healing. In addition, epigenetic modifications such as DNA methylation,[30] histone modification,[31] and non-coding RNA expression[32] have been shown to be associated with diabetic wound healing. Specifically, hyperglycemia lowered DNA methyltransferases (DNMT-1 and DMTT-3A) and impaired endothelial function as manifested by diminished eNOS and lower NO production. Males absent on the first (MOF) is a histone acetyltransferase that has been shown to delay diabetic wound repair by influencing macrophage-mediated inflammation. MicroRNAs (miR-129 and miR-335) could downregulate specificity protein-1 mediated MMP-9 expression, which reveals the potential therapeutic benefits in delayed wound healing in diabetes.

Thus, wound healing is a complex biological process involving various cell types, growth factors, and cytokines (**Figure 2**). As a chronic disease, diabetes can affect multiple of these processes, leading to impaired wound healing. This includes abnormal angiogenesis, chronic inflammation, abnormal immune microenvironment, oxidative stress, diabetic peripheral neuropathy, imbalance of ECM deposition and remodeling, and epigenetic modifications. Due to its complexity, further in-depth research

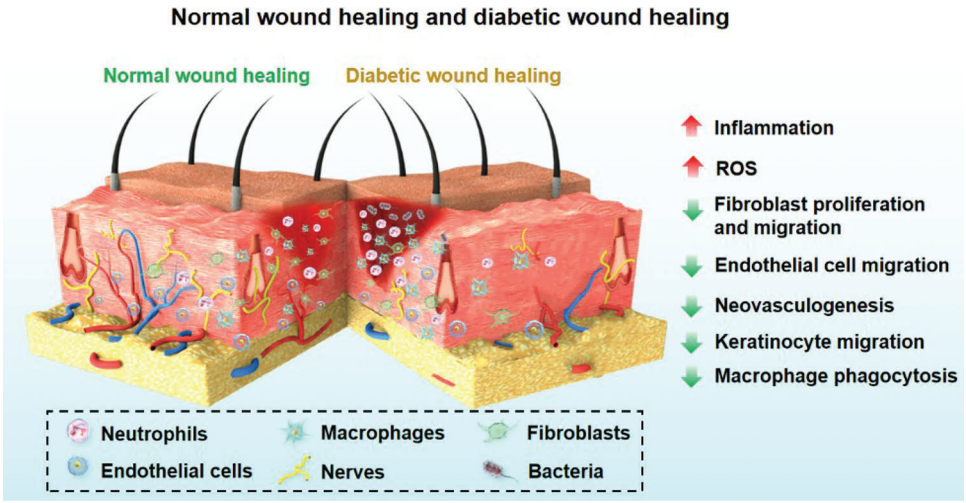

Figure 2. Healing differences between normal and diabetic wounds. Persistent pathologic hyperglycemia in diabetic wounds interferes with the wound microenvironment, leading to reduced angiogenesis and microvascular complications. Furthermore, an unrestricted and persistent chronic inflammatory response can underlie the chronicity of diabetic wounds. Meanwhile, ROS can directly damage healthy mesenchymal stem cells, resulting in decreased proliferation, migration, and adhesive capabilities. Moreover, diabetic peripheral neuropathy is a significant risk factor for the persistence of diabetic wounds.

investigating the mechanisms of diabetic wound healing is needed to advance the development of novel therapeutic agents.

3. ROS Homeostasis and Relevance of ROS in Diabetic Wounds

3.1. Biogenesis of ROS in Diabetic Wounds

Redox processes are necessary for proper wound healing, with ROS playing a central role. Under physiological conditions, low levels of ROS are crucial to combat external damage. Neutrophils and macrophages, for example, produce large amounts of oxide (O_2^-) and hydrogen peroxide (H_2O_2) during the early phase of wound inflammation, thereby eliminating pathogens and preventing wound infection. This is of particular relevance as various studies have underlined the susceptibility of diabetic wounds to bacterial infection.[33] Excessive oxidative stress and reduced antioxidant capacity in infected wounds; however, can lead to redox imbalance, resulting in permanently elevated ROS levels and delayed wound repair. In addition, oxidative stress-induced ischemic injury, disorganized immune microenvironment, and changes in cellular behavior may further affect diabetic wound healing.[34] At the same time, many different types of cells are involved in wound healing, which are closely related to ROS production and clearance. ROS affects the wound healing process by interacting with them. During the inflammatory phase of the wound in diabetic patients, the accumulation of neutrophils and macrophages leads to an elevated baseline expression of inflammatory cytokines and excess ROS production, interfering with the oxidative/antioxidant balance. This imbalance causes cellular damage; inhibits keratinocyte and endothelial cell migration and adhesion; and promotes fibroblast senescence, fibrosis scarring, and uncontrolled inflammation.[35] ROS can enhance the stress state of functional cells, resulting in increased activity of signaling pathways secreting pro-inflammatory cytokines, chemokines, and MMPs in damaged tissues, which are closely associated with difficult-to-heal wounds.[36] Therefore, ROS generation and clearance are necessary conditions to ensure the effectiveness and timeliness of wound healing.

High levels of ROS can lead to various alterations in diabetic wounds (**Figure 3**). First, elevated ROS levels enhance glucose oxidation, resulting in the formation of enediols and dihydroxyl compounds, which in turn stimulate further ROS production. Second, hyperglycemia and hyperlipids in diabetic patients lead to an increase in energy metabolism substrates, making the electron donor in the electron transport chain produce excessive superoxide. As a consequence, the body produces excessive amounts of superoxides, thereby promoting oxidative stress and an increase in corresponding products, which ultimately induces the generation of AGEs.[37] Due to the formation of AGEs, even more ROS are produced by glycosylation of intracellular mitochondrial respiratory chain proteins, consequently increasing NOX activity, which further aggravates oxidative stress.[38] In addition, AGEs can activate RAGE receptors leading to O_2^- and H_2O_2 production, which further intensifies the formation of superoxides in mitochondria. In turn, ROS generated in mitochondria further increase RAGE expression, resulting in a vicious cycle. Other mechanisms contributing to ROS synthesis under hyperglycemia are the polyol metabolic pathway and the protein kinase c (PKC) pathway. Last, hyperglycemia can impair the activity of antioxidant enzymes through glycosylation, thereby hampering the body's antioxidant capacity. In diabetic patients, the redox imbalance caused by reduced expression of antioxidant enzymes and decreased levels of free radical scavengers such as glutathione (GSH) and NO is particularly pronounced. Clinical studies have demonstrated that adjuvant supplementation has beneficial effects on diabetic wounds through increased levels of plasma NO and glutathione, as well as total antioxidant capacity.[39]

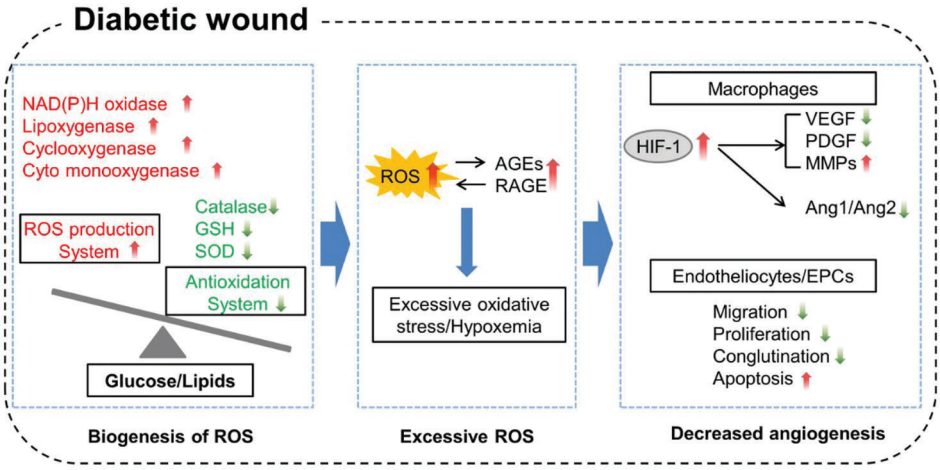

Figure 3. During the inflammatory phase of diabetic wounds, the accumulation of neutrophils and macrophages leads to an elevated baseline expression of inflammatory cytokines and excess ROS production, interfering with the oxidative/antioxidant balance. AGEs can activate RAGE receptors leading to O_2^- and H_2O_2 production, which further intensify the formation of superoxides in mitochondria. In turn, ROS generated in mitochondria increases RAGE expression, resulting in a vicious cycle. Increased levels of ROS downregulate the expression of HIF-1α and its downstream targets. Reduced expression of VEGF and PDGF, for example, further impairs angiogenesis and endothelial progenitor cell recruitment; thus, delaying wound healing.

3.2. ROS and Impaired Angiogenesis in Diabetic Wounds

The excess presence of ROS in diabetic wounds can cause the activation of various signaling pathways that lead to vascular endothelial cell damage and impaired angiogenesis (Figure 3). The HIF-1α/VEGF axis plays an important regulatory role in the angiogenesis in diabetic wounds.[40] Under physiological conditions, local hypoxia leads to stable expression of HIF-1α and promotes angiogenesis, erythropoiesis, cell proliferation, and migration. In diabetic conditions; however, increased levels of ROS down-regulate the expression of HIF-1α and its downstream targets. Reduced expression of VEGF and PDGF, for example, further impair angiogenesis and endothelial progenitor cell recruitment; thus, delaying wound healing. Accordingly, novel therapeutics targeting the HIF-1α/VEGF axis have gained significant interest in the treatment of diabetic wounds.[41] In addition, the Angiogenin 1/angiogenin 2/tyrosine kinase receptor 2 (Ang1/Ang2/Tie2) signaling pathway also plays a pivotal role in vascular maturation.[42] It has been shown that Ang1 and Ang2 expression is reduced in patients with type 2 diabetes, resulting in impaired vascular maturation that impairs wound healing.[43] Moreover, long-term exposure to high glucose levels can reduce the proliferation and migration abilities of endothelial cells and induce apoptosis. At the same time, non-enzymatic glycosylation of the vascular basement membrane impairs homing of EPCs; thus, impeding angiogenesis. Of translational interest, Zhang et al. found that growth differentiation factor 11 (GDF11) can promote diabetic wound healing by inducing EPC mobilization and neovascularization through the HIF-1 alpha/VEGF/SDF-1 alpha axis.[44]

3.3. Immunomodulatory Role of ROS in Diabetic Wounds

Diabetic wounds are characterized by chronic low-grade inflammation that can aggravate the disorder. Mechanistically, excess ROS presence creates an immune microenvironment in which macrophages and neutrophils play a particularly important role. In general, neutrophils are the most prominent leukocytes involved in early wound healing and can release neutrophil extracellular traps (NETs) to eliminate pathogens.[45] The formation and release of NETs, termed NETosis, highly depends on ROS and the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[46] NETosis has been found to comprise three distinct forms: suicidal NETosis, NETosis induced by nuclear DNA, and NETosis induced by mitochondrial DNA. Of these, suicidal NETosis constitutes the main form involved in wound repair. Of interest, neutrophils undergo oxidative stress in diabetic environments; thus, producing cytokines such as IL-6 and TNF-α and forming NETs. The cytotoxic NETs, in turn, can directly damage epithelial and endothelial cells, resulting in slowed keratinocyte proliferation and delayed wound healing.[47]

Macrophages constitute another important immune cell type implicated in wound healing. In the early phase of inflammation, macrophages exhibit a pro-inflammatory phenotype releasing inflammatory mediators, proteases, and ROS to combat pathogens. After the transition to the ECM remodeling phase, M1 cells transform into anti-inflammatory macrophages, which contribute to re-epithelialization, angiogenesis, and wound repair. Under inflammatory conditions; however, excessive levels of ROS can block the conversion of M1 to M2, causing macrophages to remain mainly in a pro-inflammatory state and initiating a vicious cycle leading to chronic wounds.[48] Therefore, the conversion of M1 to M2 represents a potential target for therapeutic strategies.[49] Exploiting this mechanism, Gan et al. developed a konjac glucomannan-modified SiO_2 nanoparticle inducing the aggregation of CD206 nanoclusters on the surface of macrophages; thus, promoting their conversion into M2 macrophages. Interestingly, this experimental approach significantly promoted wound healing when applied to mice with diabetic wounds.[50]

Macrophages are highly plastic. They can undergo morphological and functional changes under the influence of different tissue environments in the body. Their role in immune

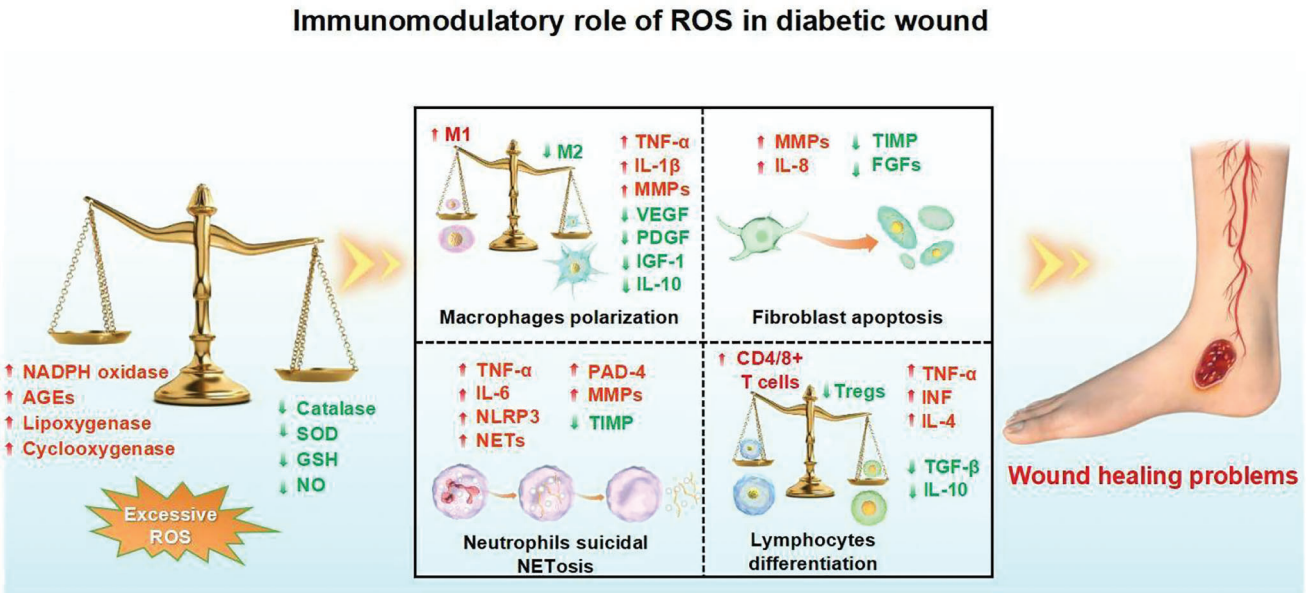

Figure 4. Immunomodulatory role of ROS in diabetic wound healing. Excessive ROS can cause an immune microenvironment in which macrophages and neutrophils play a particularly important role. Generally, neutrophils predominate early wound healing and can release NETs to eliminate pathogens. The formation and release of NETs, termed NETosis, depends on ROS and NADPH oxidase. Neutrophils undergo oxidative stress in diabetic environments; thus, producing cytokines such as IL-6 and TNF-α and forming NETs. Cytotoxic NETs, in turn, directly damage epithelial and endothelial cells, resulting in delayed keratinocyte proliferation and compromised wound healing. Excessive ROS can also block the conversion of M1 to M2, causing macrophages to remain mainly in a pro-inflammatory state and initiating a vicious cycle for the formation of chronic wounds. Pro-inflammatory cytokines and elevated ROS levels in diabetic wounds have been shown to promote Th1 CD4 T cell responses; thus, contributing to a pro-inflammatory microenvironment.

regulation is more complex than that of simple M1/M2 typing. M2 macrophages can be divided into four subtypes: M2a, M2b, M2c, and M2d. These subtypes co-express IL-10 and differ in surface markers, secreted cytokines, and biological functions. In addition to classical M1 and M2, there are tumor-related macrophages (TAM), $CD169^+$ macrophages, and TCR^+ macrophages.[51] TAM, a unique M2 medullary cell population, is associated with specific pathological conditions and has both M1 and M2 signals.[52] The signaling pathways and activation information of $CD169^+$ macrophages and TCR+ macrophages still need to be further studied. They play an important role in immune tolerance, antiviral, and other aspects although the comparison between them and M1/M2 is not clear.[53]

Regulatory T cells (Tregs) are a subset of T cells that regulate autoimmune reactivity. This is achieved by various mechanisms including suppression of the immune response, induction of tolerance to autoantigens, and regulation of anti-inflammatory pathways. The skin of both mice and humans contains large numbers of Tregs. Studies have shown that Tregs can promote skin wound healing and angiogenesis in the ischemic tissues of diabetic mice and patients.[54] These results reveal the role of Tregs in facilitating diabetic wound repair and suggest high translational applications to mediate these effects. Mechanistically, Tregs reduce interferon-γ (IFN-γ) levels and M1-accumulation in wound tissue. In addition, Tregs have been shown to induce the expression of epidermal growth factor receptor (EGFR) in the early posttraumatic phase, thereby attenuating the inflammatory response and promoting wound healing. However, pro-inflammatory cytokines and elevated ROS levels in diabetic wounds have been shown to promote Th1 CD4 T cell responses;[55] thus, contributing to a pro-inflammatory microenvironment. Therefore, regulation of ROS and T-cell differentiation may represent a potential therapeutic target for diabetic wound treatment. Of additional interest, studies have shown that increased amounts of Langerhans cells (LC) in the epidermis of patients with diabetic foot ulcers (DFU) can promote wound healing.[56] Moreover, enhancing efferocytosis of dendritic cells can promote the secretion of growth differentiation factor-15 (GDF15) and accelerate diabetic wound healing.[57] Of note, natural killer T cells (NKT) have the capacity to inhibit wound healing, and depletion of NKT cells in mice has been shown to improve healing rates.[58] In summary, wound healing is a complex process involving a variety of immune cells and molecules (**Figure 4**). A thorough investigation of its mechanisms may serve as the basis for targeted clinical therapeutics promoting diabetic wound repair.

3.4. Role of ROS in Tissue Regeneration

In addition to the multifaceted effects of ROS on immune cells, they play an important role in tissue regeneration. During the proliferation phase of wound healing, low levels of ROS promote fibroblast proliferation, increase ECM production, and enhance angiogenesis and epithelial regeneration. However, high levels of ROS can hinder these processes. As previously described, excessive previous production accompanied by a decrease in antioxidant factors such as glutathione and cysteine can be found in diabetic wounds. As a consequence, the interaction of ROS with proteins, fats, and DNA leads to impaired cell function and can

also entail proteolytic hydrolysis of ECM.[59] These processes can be induced either directly or through the activation of proteases (MMPs and serine proteases) and the inactivation of protease inhibitors.

Tissue repair cells play an important role in the process of diabetic wound healing. The cell viability and adhesive abilities of epidermal keratinocytes, for example, have been found to be decreased in the skin of diabetic patients. In addition, inhibition of keratinocyte proliferation has been observed with altered activities of cell cycle regulators such as cyclin-dependent kinase 4 (CDK4), Ki67, and maturation-promoting factor (MPF). Moreover, the dedicator of cytokinesis protein (DOCK) also plays a key role in regulating keratinocyte function and promoting skin wound healing.[60] Specifically, DOCK5 regulates keratinocyte adhesion, migration, and proliferation and influences ECM deposition by laminin-332/integrin signaling. In addition, fibroblasts showed premature senescence, abnormal morphology, and decreased ability to migrate and proliferate in diabetic wounds. At the same time, aging fibroblasts manifest the senescence-associated secretory phenotype (SASP) with high levels of inflammatory cytokines, immune modulators, growth factors, and proteases, resulting in impaired wound healing. Accordingly, experimental studies have demonstrated that promoting fibroblast proliferation significantly accelerates skin wound healing.[61] Of clinical relevance, targeting connexin activity may improve diabetic wound healing.[62]

3.5. Diagnosis and Monitoring of Local ROS Level

ROS production is closely related to the prognosis of diabetic wounds. Therefore, the diagnosis and monitoring of ROS level in wound area are particularly important. Fluorescence imaging is a powerful tool for monitoring cells and bioactive molecules in vivo with high sensitivity and high spatial and temporal resolution. Therefore, different fluorescence imaging probes were designed to monitor ROS levels in the wound.[63] Quantitative determination of intracellular ROS levels based on changes in fluorescence intensity of fluorescent dye 2″,7″-dichlorofluorescein diacetate (DCFH-DA) is the most commonly used method. The intracellular ROS level can be measured by detecting the fluorescence of 2″,7″-dichlorofluorescein (DCF).[64] Fluorescence microscopy, laser confocal microscopy, fluorescence spectrophotometer, fluorescent enzyme marker, and flow cytometer were used to detect DCF fluorescence; so as to determine the ROS level.[65]

Due to the short half-life of ROS and the high fluorescence intensity of tissues in vivo, it is difficult to determine the changes of ROS by real-time monitoring and tracing the inflammatory process in situ. A near infrared (NIR) ratiometric fluorescence imaging method using a Förster resonance energy transfer (FRET)-based ratiometric fluorescence nanoprobe to monitor ROS production in real time and using in situ tracking of inflammatory processes in vivo was developed. The nanoprobe not only shows rapid response to superoxide anion and hydroxyl radical but also has good biocompatibility, high light stability and signal-to-noise ratio. This design may provide a paradigm for long-term real-time imaging applications that trace inflammatory disease-related pathological processes in vivo.[66] In addition, real-time monitoring of the treatment process of inflammatory diseases under the guidance of image is of great significance for timely adjustment of treatment plan, reduction of unnecessary side effects, and improvement of therapeutic effect. A novel nanotheranostic agent (TMSN@PM) with platelet membrane (PM) coated, tempol-grafted, manganese-doped, mesoporous silica nanoparticles was developed. In an inflammatory environment (mild acidity and excess ROS), TMSN@PM could remove excess ROS, thereby reducing inflammation and releasing manganese ions for enhanced magnetic resonance imaging (MRI). This approach provides an image-based non-invasive strategy for early prediction of treatment outcomes for the treatment of inflammation and may contribute to future precision medicine in prognostic stratification and treatment planning.[67]

4. ROS-Scavenging Nanosystems as Therapeutics for Diabetic Wound Treatment

Nanotherapeutics have been developed to utilize the advantages of engineered nanomaterials for the treatment of medical conditions[12,68] and can be functionalized with salient properties based on the respective pathomechanism.[69] More specifically, the modification of nanotherapeutics with linkers, ligands, or coatings offers various advantages such as targeted drug delivery, controlled and sustained drug release, and improved biocompatibility. This enables both precise and highly effective therapeutic application.[70] Currently, many efforts are underway to develop therapeutic nanosystems for the treatment of super-critical size wound defects which are not able to heal naturally and require therapeutic intervention, predominantly focusing on ROS scavenging. We therefore provide a comprehensive overview of these novel therapeutic agents.

With rapid advances in nanotechnology, a range of biocatalytic or antioxidant nanostructures has been designed with unique ROS scavenging capabilities, demonstrating promising activity to overcome these challenges in clinical anti-ROS and antioxidant efforts. They can achieve anti-inflammatory effects by eliminating a broad spectrum of ROS, rather than targeting specific pathways or molecules, which may provide better therapeutic efficiency than current clinical agents. Thus, the idea of using biocatalytic or antioxidant nanostructures opens up new avenues for ROS scavenging and ROS related biologics, such as cerium oxide, precious metals, metal–organic frameworks (MOFs), nanocarbon, selenium (Se) nanostructures, and polyphenol nanoparticles.[71] Overall, biocatalytic or antioxidant nanostructures exhibit significant advantages compared to natural enzymes, such as remarkable ROS scavenging capacity, broad spectrum ROS elimination activity, robust stability in physiological environments, and satisfactory biocompatibility and biosafety. A simple and efficient one-step preparation of ultra-small $Cu_{5.4}O$ nanoparticles ($Cu_{5.4}O$ USNPs) with multi-enzyme simulation and broad-spectrum ROS scavenging capabilities for the treatment of Ros-related diseases was reported. $Cu_{5.4}O$ USNPs showed cellular protection against ROS-mediated injury at very low doses and significantly improved therapeutic outcomes for acute kidney injury, acute liver injury, and wound healing. The protective effect and good biocompatibility of $Cu_{5.4}O$ USNPs would facilitate the clinical treatment of ROS-related diseases and enable the development of

next-generation nanoenzymes.[72] In this study, a complex hydrogel consisting of amine-functionalized star-shaped polyethylene glycol (starPEG) and heparin for chemokine isolation, as well as $Cu_{5.4}O$ nano-enzyme for ROS removal ($Cu_{5.4}O$@Hep-PEG) was developed. It effectively adsorbed inflammatory chemokines monocyte chemoattractant protein-1 and interleukin-8 and reduced the migration activity of macrophages and neutrophils. In addition, this hydrogel removed ROS from wound fluids to reduce oxidative stress, and the continuous release of $Cu_{5.4}O$ promoted angiogenesis.[73]

4.1. ROS-Scavenging Enzyme-Mimetic Nanosystems

The accumulation of ROS in diabetic wounds derives from various processes,[22] consequently disrupting the delicate ROS balance in vivo.[74] Recently, nanosystems have been reported as promising therapeutic approaches for scavenging ROS, with a particular focus on enzyme-mimetic nanosystems. These nanosystems exhibit several advantages over natural enzymes such as a more efficient catalytic function, better stability under pathological conditions, higher design flexibility, and longer circulation times in the blood.[75] For example, Du et al. recently introduced a pH-switchable and catalytic nanosystem (Fe_3O_4-GO*x*) to modulate ROS levels in diabetic wounds.[76] Biochemically, Fe_3O_4-GO*x* consists of an iron oxide nanoparticle (NP) core and a glucose oxidase (GO*x*) shell. Mechanistically, GO*x* first catalyzes the oxidization of glucose to H_2O_2. Subsequently, the Fe_3O_4 NPs catalyze the decomposition of H_2O_2 into oxygen and hydroxyl radicals (·OH). Thus, Fe_3O_4-GO*x* can release high amounts of ·OH that target biofilms and exert robust antibacterial effects. Moreover, local administration of Fe_3O_4-GO*x* in the wound site can break up the biofilm which allows for more oxygen to reach the wound tissue, thereby further promoting wound healing in the diabetic mouse (**Figure 5**A–C). Moreover, catalase (CAT) and superoxide dismutase (SOD) are two important enzymes in scavenging ROS, which can remove H_2O_2 and superoxide anions, respectively. Thus, a glucose/ROS cascade-responsive nanosystem (CHA@GO*x*) has recently been reported.[77] In detail, Yu et al. demonstrated that local injection of CHA@GO*x* can mimic the activity of SOD and CAT; thus, improving the balance between ROS and oxygen and promoting wound repair in the diabetic mouse.[77]

The combination of hydrogels and catalytic nanosystems has attracted considerable interest due to their biocompatible and biodegradable advantages.[78] Particularly suitable for topical application, these systems can be easily delivered to the wound and exhibit ROS-scavenging activities over an extended period of time. For instance, Wu et al. reported a nanozyme-enhanced self-protective hydrogel (PCN-miR/Col) to alter the oxidative microenvironment of diabetic wounds.[79] Benefiting from the topical application of the collagen-based hydrogel platform, ceria nanocrystals can be sustainably released from PCN-miR/Col and provide excellent ROS-scavenging properties in vivo. Similarly, we developed an injectable nanozyme-enforced hydrogel (HA@MnO_2/FGF-2/Exos) to antagonize excessive ROS production, in which MnO_2/*ε*-PL nanosheets were able to convert H_2O_2 into O_2. This not only mitigated the detrimental effects of H_2O_2 but also provided more O_2 for wound healing.[61b]

Moreover, MOFs have also been applied as ROS-scavenging nanosystems for diabetic wound treatment. MOFs have uniform catalytic centers and porous nanostructures, which increases their catalytic efficiency.[80] More recently, Li et al. reported an MOF-based nanozyme-immobilized hydrogel that attenuates oxidative stress in diabetic wounds.[81] Owing to the natural polymers (hydrazide modified hyaluronic acid and aldehyde-modified hyaluronic acid) and the MOF-derived nanozyme (*ε*-polylysine coated mesoporous manganese cobalt oxide), topical utilization of this composite can effectively remove ROS and provide additional oxygen in the diabetic rats (Figure 5D,E).

4.2. Nanotherapeutics Combined with Antioxidants

As previously described, excessive ROS production can disrupt the balance between cellular oxidants and antioxidants in diabetic wounds, ultimately leading to delayed wound healing and impaired tissue regeneration.[40] Antioxidant-targeting mitochondria have been observed to ameliorate inflammation and enhance tissue regeneration in murine models of diabetic wounds.[82] In recent years, considerable progress has been made in nanomedicine and in multifunctional nanosystems with excellent antioxidant properties developed for the treatment of diabetic wounds.[83] Anti-oxidative NPs composed of lecithin-chitosan and melatonin (MEL-NP), for instance, have been proposed for topical application.[84] Mechanistically, melatonin acts as an antioxidant that neutralizes ROS. Encapsulation in NPs, interestingly, prevents its rapid degradation and increases its biostability, thereby increasing the drug concentration in the tissue.[85] Both in vitro and in vivo studies have documented that MEL-NPs promote angiogenesis and enhance collagen deposition, leading to better healing of diabetic wounds.[85] In addition to synthetic molecules, many plant compounds with antioxidant activities have been used to equip nanomaterials with antioxidant properties.[86] Puerarin (PUE), for example, exhibits well-known antioxidant capacity and can alleviate lipid peroxidation and cell death.[87] However, due to low membrane penetration and poor bioavailability, PUE has rarely been used in clinical practice. To address this problem, Zhang et al. developed an antioxidative nanosystem (PDA/PUE NP) encapsulated in a hydrogel for topical application in diabetic wounds, which can sustainably release PUE into the wound tissue; thus, reducing ROS and accelerating healing in the diabetic rats.[88] Similarly, another research team designed a nanosystem (Ag@Lig NPs) using the natural phenolic compound lignin as a dual reducing and capping agent (**Figure 6**).[89] Experimentally, this nanosystem exhibited excellent cargo-protective capacity, leading to enhanced antioxidant efficacy and biocompatibility. To facilitate sustained release, Ag@Lig NPs were incorporated into a hydrogel, offering antioxidant and antibacterial activity. Both in vitro and in vivo results indicated that topical utilization of this hydrogel played an excellent role in ROS scavenging and promotion of diabetic wound healing.

4.3. ROS-Responsive Nanosystems

It has been delineated that NPs responsive to environmental stimuli can release their cargo more precisely at the desired site

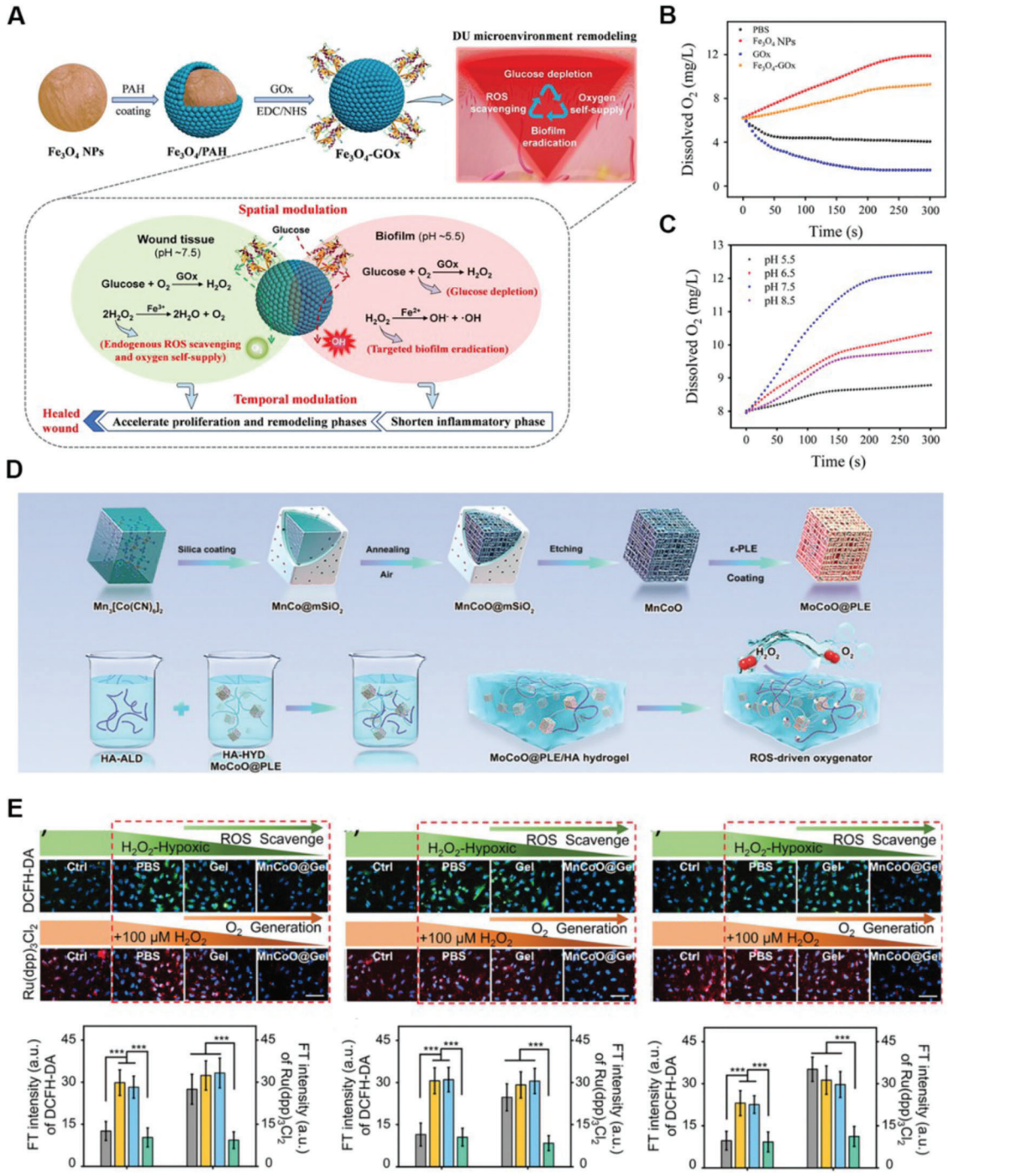

Figure 5. ROS-scavenging enzyme-mimetic nanosystems for diabetic wound treatment. A) Schematic illustration of an Fe_3O_4-GO*x* nanosystem for modulation of the toxic microenvironment in diabetic wounds. B) Dissolved O_2 concentration in a solution of glucose (20 mM) and H_2O_2 (7 mM), pH 7.4 after incubation with different agents for 5 min. C) Dissolved O_2 concentration in a solution of glucose (20 mM) and H_2O_2 (10 mM) with different pH values after incubation with the nanosystem for 5 min. (A–C) Reproduced with permission.[76] Copyright 2022, Springer Nature. D) Conceptual illustration of the synthesis of nanozyme-enhanced hydrogels as ROS-driven oxygenators. E) ROS elimination and oxygen generation by DCFH-DA and $Ru(dpp)_3Cl_2$ after treatment in HaCaT and HAECs cells. (D,E) Reproduced with permission.[81] Copyright 2022, Wiley-VCH GmbH.

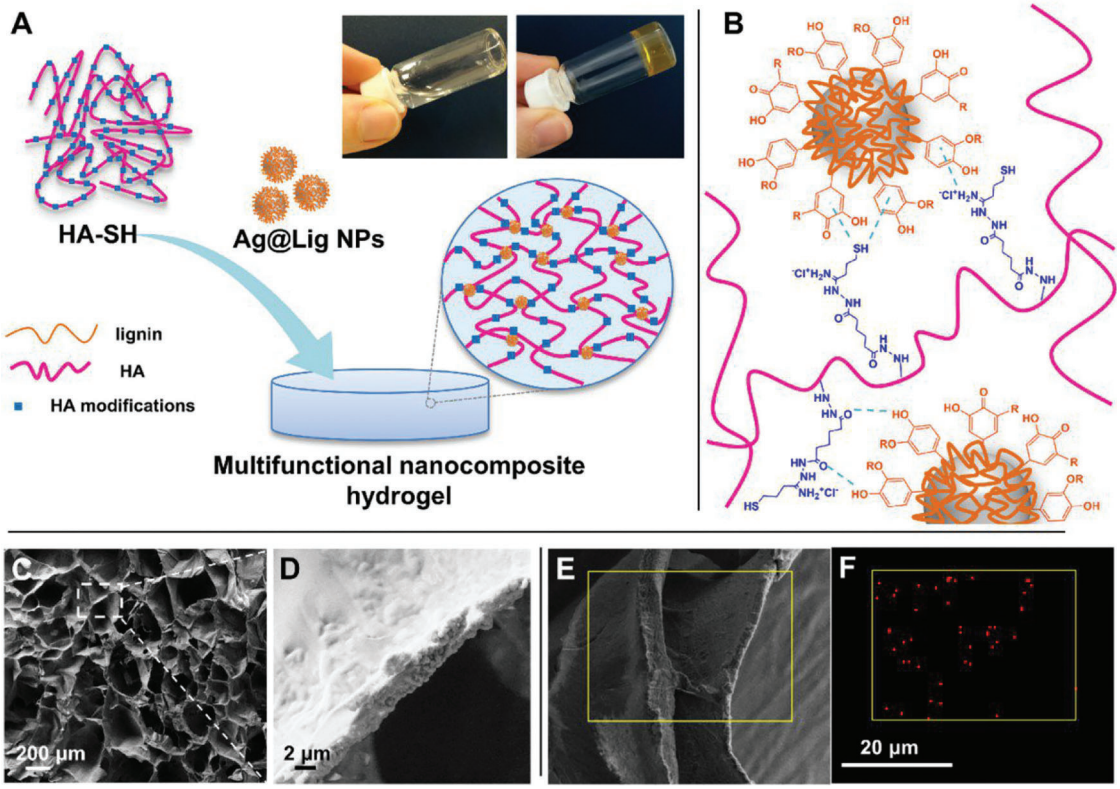

Figure 6. Schematic illustration of A) hydrogel formation and B) suggested polymer-NPs for self-assembling. Non-gelation resulting from mixing pristine HA or HA-ADH with Ag@Lig NPs and gel obtained by mixing HA-SH with Ag@Lig NPs. C) SEM images of 1.5%_0.2 HA-SH/Ag@Lig NPs showing the porous hydrogel structure, D) inclusion of the NPs in the pore's wall, and E) EDX mapping analysis of Ag^0. F) Corresponding to the region labelled in (E). Reproduced with permission.[89] Copyright 2021, Elsevier.

of action.[90] Applying this concept, Fan et al. recently introduced a novel hydrogel loaded with self-assembled nanogels for the depletion of ROS in diabetic wounds.[91] Briefly, ROS-responsive borate ester bonds in the hydrogel are broken up upon contact; thus, releasing the bioactive nanogel (TA-siRNA). TA-siRNA nanogels (tannic acid [TA] and short interfering RNA [siRNA]) can decrease ROS levels and MMP-9 activity;thus, stimulating macrophage polarization toward the M2 phenotype. In the diabetic rats, local management of this hydrogel can significantly promote tissue re-epithelialization and wound repair.

Furthermore, MOFs have also been used to create ROS-responsive nanosystems. MOFs are crystalline structures combined with metal ions or organic ligands and represent promising nanomaterials for biomedical applications.[92] Previously, zeolite imidazolate framework-8 (ZIF-8)-based NPs (SP@ZIF-8-PEG-TK) with ROS-responsive abilities have been reported.[93] In brief, by loading with substance P (SP) and fabricating with polyethylene glycolthioketal (PEG-TK), SP@ZIF-8-PEG-TK NPs were endowed with ROS-responsive abilities. Interestingly, both in vitro and in vivo studies have shown robust pro-regenerative and anti-inflammatory capacities in addition to good biocompatibility.

Deployment mechanisms are another area of interest for ROS-responsive nanosystems to enable even more site-specific drug delivery.[94] For example, nanosystems can be designed to deploy their cargo in response to certain molecular environments. Nanosystems responsive to ROS, for example, release their cargo at the diabetic wound site in response to a high ROS presence; thus, allowing targeted efficacy.[95] Previously, Xia et al. synthesized poly-(1,4-phenyleneacetone dimethylene thioketal) [PPADT], a novel polymer containing ROS-reactive thioketals.[96] More specifically, encapsulated stromal cell derived factor-1α (SDF-1α) was able to increase the in vivo stability, permitting long circulation times in the blood while the ROS-responsive properties allowed for a targeted release of SDF-1α at the wound site. In addition, various dual-responsive nanosystems (e.g., pH/ROS-responsive) have been developed to achieve even more precise delivery.[97] As such, Zhang et al. introduced dual-responsive NPs composed of pH- and ROS-responsive cyclodextrin materials for targeted delivery to sites of acidosis and oxidative stress.[98] Based on the same approach, Duan and coworkers developed silver-based NPs (AgNPs) that are also pH- and ROS-responsive. Of clinical relevance, no adverse effects such as cytotoxicity or hemolysis have been observed.[99] Of note, using tannic acid (TA) as a reductant for AgNP synthesis resulted in a hydrogel with robust antibacterial activities and significant pro-healing effects in vitro. In addition, ROS-responsive nanosystems combined with ROS clearance and other drugs can synergistically promote wound repair and regeneration.[100] In a prior study, Dun et al. introduced a multifunctional ROS-responsive nanosystem encapsulated with P311 peptide MICs, which can effectively reprogram the oxidative microenvironment of diabetic wounds

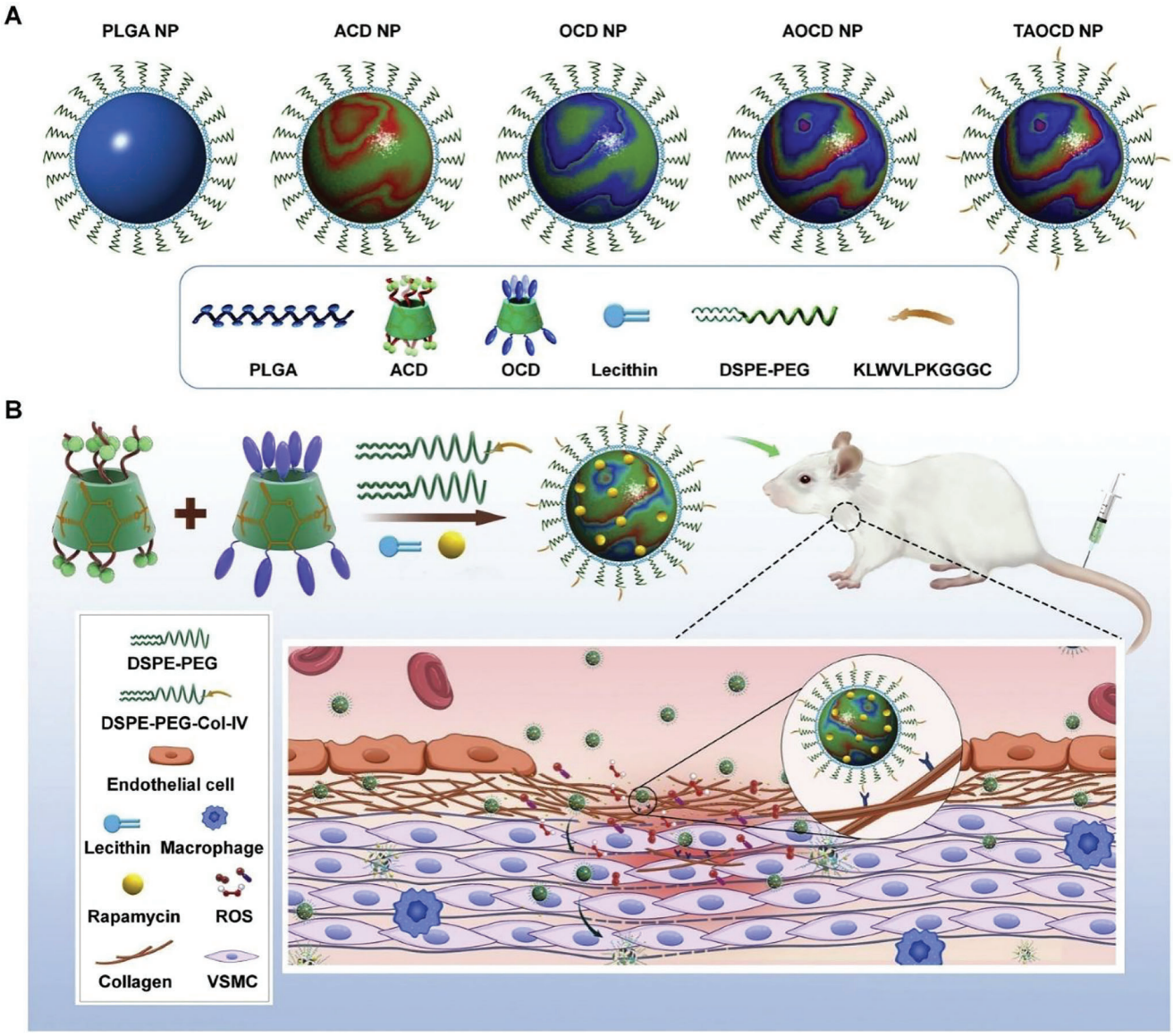

Figure 7. A) Schematic illustration of the different NPs described in this review. B) Engineering of pH/ROS-responsive nanosystems based on pH-sensitive β-CD (ACD) and ROS-responsive β-CD (OCD) materials. Reproduced with permission.[98] Copyright 2020, Elsevier.

to the proregenerative one, while providing beneficial cues for re-epithelialization and wound healing.[101] They demonstrated in vivo that this nanosystem (P311@PEPS) could markedly facilitate granulation tissue formation, collagen deposition, and re-epithelialization in diabetic mouse model, thereby providing a potential therapeutic platform for the treatment of diabetic wounds.[101] Taken together, these studies suggest that ROS-responsive nanosystems have immense potential as a future approach to treat diabetic wounds (**Figure 7**).

4.4. Anti-Inflammatory Nanosystems

Augmented inflammatory responses are a central feature of diabetic wounds. Thus, nanosystems designed to be anti-inflammatory through the combination of polymers and non-steroidal anti-inflammatory drugs (NSAIDs), antioxidant molecules, or enzymes may promote diabetic wound healing. A widely used NSAID, nimesulide (NIM) has been encapsulated in pH-responsive MICs to construct an anti-inflammatory composite (MIC@NIM), which can sustainably deliver NIM to the wound site and alleviate inflammation.[102] Furthermore, a multifunctional composite was created by encapsulation of MIC@NIM and vancomycin-conjugated silver nanoclusters (VAN-AgNCs) in an injectable hydrogel, achieving beneficial effects both in vitro and in vivo (**Figure 8**A).

Beyond NSAIDs, natural compounds have also been encapsulated in NPs to design anti-inflammatory nanosystems.[103] Of interest, Qu et al. incorporated two natural compounds with anti-inflammatory and antioxidant properties, PUE and ferulic acid

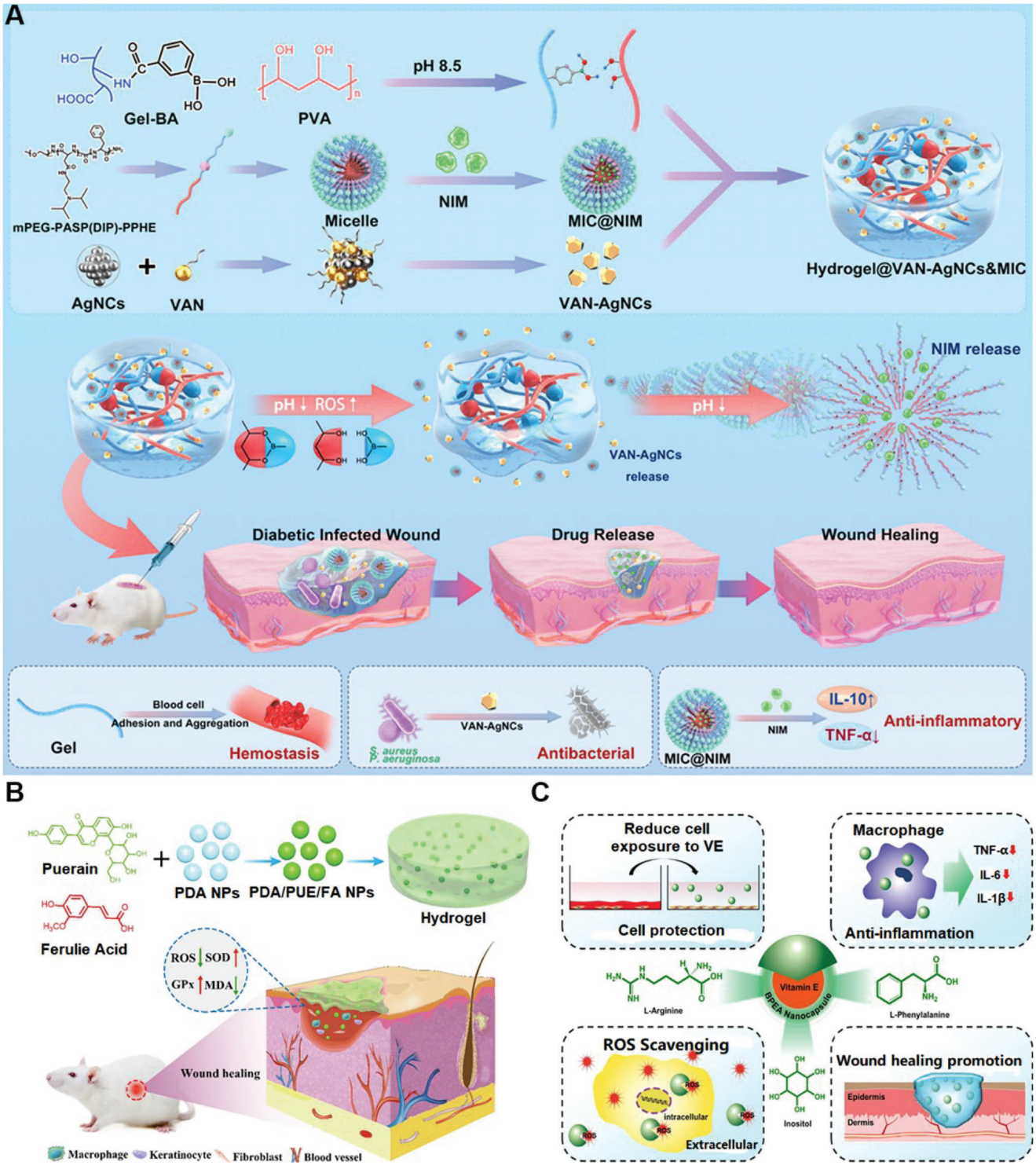

Figure 8. Anti-inflammatory ROS-scavenging nanosystems for diabetic wound treatment. A) Schematic illustration of hydrophobic NSAID-loaded nanosystems. Reproduced with permission.[102] Copyright 2021, American Chemical Society. B) Schematic development of PEG-DA/PDA/PUE/FA hydrogels.[104a] Copyright 2021. Springer Nature. C) Schematic illustration of the application of anti-inflammatory nanosystems (BPEA@VE NCs). Reproduced with permission.[103] Copyright 2021, American Chemical Society.

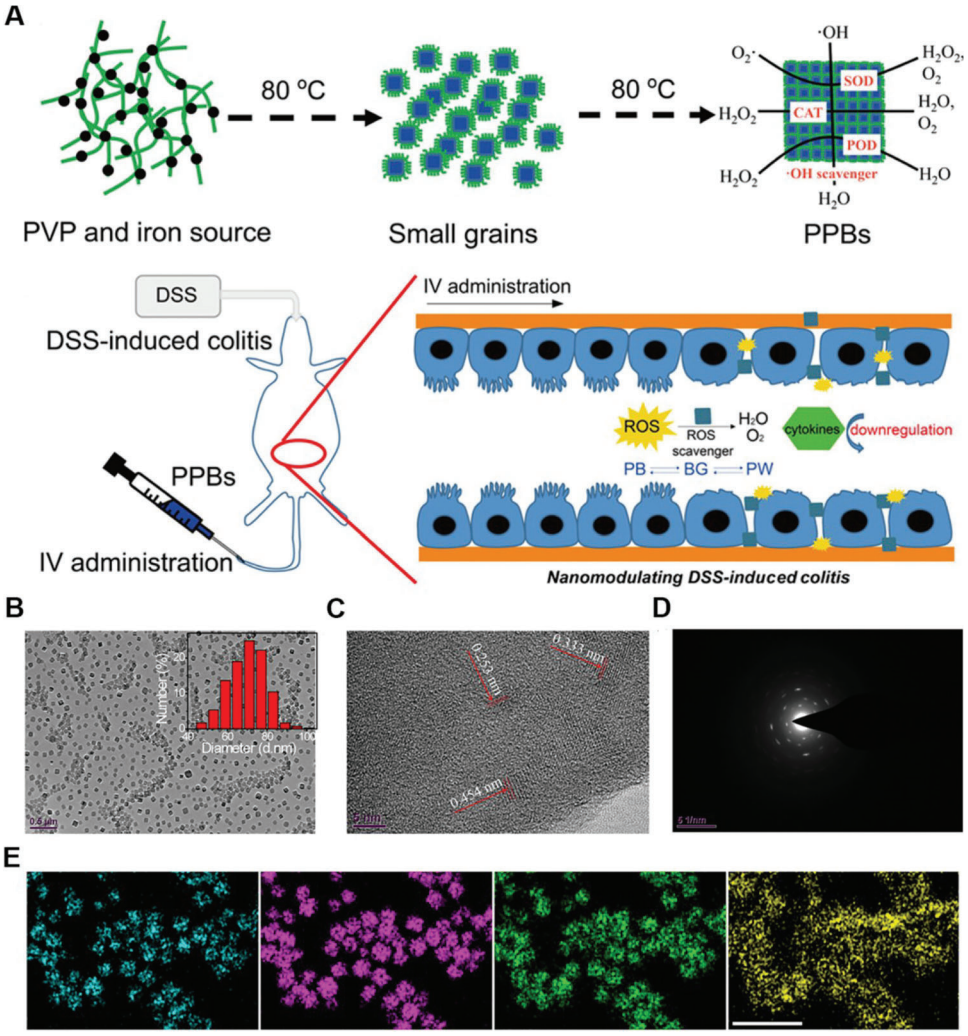

Figure 9. Poly(vinylpyrrolidone)-modified Prussian blue nanosystems (PPBs). A) Illustration of the preparation of PPBs which have capabilities of scavenging ROS and inhibiting proinflammatory cytokines. B) Representative TEM image of PPBs. C) High-resolution TEM image of PPBs. D) X-ray diffraction pattern of PPBs. E) Fourier transform infrared spectrum of PPBs. Reproduced with permission.[107b] Copyright 2018, American Chemical Society.

(FA), into polydopamine (PDA) NPs (PDA/PUE/FA NPs) in a polyethylene glycol diacrylate (PEG-DA) hydrogel to allow longer durations of action.[104] Their in vivo studies demonstrated excellent anti-inflammatory and antioxidative effects in diabetic wounds, indicating huge potential for local application in diabetic wounds treatment (Figure 8B). In a similar fashion, L-arginine and L-phenylalanine-based branched poly (ester amide) (BPEA) nanocapsules (BPEA@VE NCs) have been locally used for vitamin E (VE) delivery to wound sites.[103] This also resulted in robust anti-oxidative and anti-inflammatory effects demonstrated in vivo (Figure 8C). Interestingly, Chen and coworkers introduced an ROS-scavenging suture coating with gallic acid (GA)-based NPs (GANPs) for wound treatment.[105] Both in vitro and in vivo results suggested the fabricated sutures coated with GANPs are able to beneficially facilitate wound closure via scavenging the excessive ROS around the wound sites. Mechanistically, the suture can sustainably release GANPs, significantly induce the secretion of anti-inflammatory factors, and beneficially promote macrophages toward M2 phenotype polarization. Therefore, this ROS-scavenging suture may serve as a promising candidate for surgeons in treating with various refractory wounds. Of additional interest, non-water-soluble drugs have also been utilized to construct anti-inflammatory nanosystems. Resveratrol (Res), for example, has been encapsulated in NPs and functionalized by phenylboric acid to improve solubility and achieve better tissue distribution.[106] Owing to their anti-inflammatory and antioxidative activities, the in vivo results suggested that Res@NPs were capable of enhancing the formation of granulation tissues, re-epithelialization, and collagen deposition, thereby accelerating diabetic wound healing.[106]

Moreover, inorganic compounds have been used in nanosystems with anti-inflammatory functions. Prussian blue (PB), for example, features excellent anti-inflammatory and ROS-scavenging properties (**Figure 9**).[107] Hence, Sahu et al. investigated PB NPs as novel nanosystems for local management of diabetic wound.[108] They found this PB-encapsulated nanosystem not only facilitated the healing of infected wounds but also exhibited robust tissue regeneration properties in vitro and in

vivo owing to the synergistic effect of ROS-scavenging and anti-inflammatory functions.[108] Based on these observations, Oh et al. designed multifunctional nanofibers composed of chitosan-stabilized PB NPs and poly(vinyl alcohol) with anti-inflammatory and ROS scavenging properties.[109] Both in vitro and in vivo results indicated considerable anti-inflammatory and antioxidant properties.[109]

4.5. Small Extracellular Vesicle-Based Therapy

Small extracellular vesicle (sEV)-based therapeutic systems are a class of nanosized membrane particles with a diameter of <200 nm that can be constructed to carry various cargos including proteins, lipids, RNA, and DNA.[110] Accumulating evidence has shown that certain sEV cargos possess considerable antioxidant properties with anti-inflammatory and cytoprotective effects.[111] sEVs with overexpression of miR-223, for example, have been demonstrated to exhibit a beneficial role in diabetic wound healing.[61b] In one study, M2 macrophage-derived sEVs encapsulated with a plasmid inducing miR-223-overexpression allowed enhanced angiogenesis and re-epithelialization; thus, leading to accelerated diabetic wound healing. These observations suggest that sEV combined with bioactive factors may be a promising therapeutic approach for diabetic wounds.[61b]

Derived from MSCs, a cell type widely used in regenerative medicine, MSC-sEVs have attracted increasing attention due to their ROS-scavenging properties.[112] In particular, MSC-sEVs were shown to alleviate H_2O_2-induced apoptosis and inflammation. At the same time, MSC-sEVs lead to a marked decrease in mitochondrial ROS production. Mechanistically, these anti-oxidative effects may be attributed to the activity of the mitochondria-related proteins.[113] In support, MSC-sEVs have also been shown to alleviate irradiation-induced oxidative stress damage in MSCs.[114] Of particular interest, studies have observed anti-oxidative effects of sEV in other cell types.[115] It has been reported that sEVs released under oxidative stress conditions can protect cells from damage by transferring antioxidant enzymes to the targeted cells and acting as ROS scavengers.[116] sEVs derived from human embryonic stem cells, for example, have been demonstrated to enhance the restoration of antioxidants in vascular endothelial cells.[117]

However, after administration into the wound site, free sEVs are usually rapidly degraded. Thus, strategies to optimize the retention and release profiles of sEVs are highly desirable. Most recently, novel ROS-scavenging nanosystems based on the anti-oxidative properties of sEVs have been described. For instance, Li et al. designed novel nanofiber immobilized MSCs-sEVs to act as multifunctional wound dressing for promoting diabetic wound healing in rats by carrying and retaining sEVs and enabling the sustained local release of sEVs.[118] In a similar fashion, we developed an "all-in-one" strategy for diabetic wound treatment based on the topical application of multifunctional hydrogel encapsulated with M2 macrophage-derived sEVs (M2-sEVs) and manganese dioxide (MnO_2) nanosheets.[61b] Mechanistically, MnO_2 nanosheets catalyze the conversion of H_2O_2 into O_2; thus, not only eliminating toxic H_2O_2 but also providing O_2 required for wound healing. Moreover, both in vitro and in vivo results indicated the sustained release of M2-sEVs from the hydrogel could enhance angiogenesis and epithelization. Taken together, these findings suggest that sEV-based ROS-scavenging therapies may be a promising approach for ameliorating oxidative stress in diabetic wounds (**Figure 10**).

4.6. Sustainable and On-Demand Effects of Nanosystems in Promoting Wound Healing

Wound healing is a lengthy healing process that comprises four continuous and overlapping phases. In diabetic wounds, the normal transition of organized phases is disturbed, which leads to the prolonged healing period and impaired wound healing. Versatile heterostructural nanosystems for on-demand procedural distribution to enhance wound healing in the different phases have become a promising strategy.[119]

Recently, we introduced a MnO_2 nanosheets and growth factors enriched hydrogel (HA@MnO_2/FGF-2/Exos) for diabetic wound healing.[61b] In the inflammation phase, HA@MnO_2/FGF-2/Exos could be locally injected in the wound site and effectively scavenge the excessive ROS to attenuate the oxidative damage. In the proliferation phase, the released growth factors and sEVs were capable of enhancing re-epithelialization and angiogenesis for synergistically accelerating wound healing. Furthermore, the enhanced pro-healing effect of this nanosystem-enriched hydrogel was demonstrated in the diabetic mouse model. Of note, emerging attention was also attracted on the metal ion-based nanosystems acting as potential nanoplatforms to facilitate wound healing. In a recent study, Pei et al. introduced a silk fibroin-hyaluronic acid based injectable hydrogel encapsulated with heterostructural NPs (gAu–CuS HSs) for promoting diabetic wound healing.[120] Due to the rough surface of gAu–CuS HSs, local injection of this hydrogel could achieve an excellent hemostatic effect, and during the inflammation phase, the unusual mace-like rough structure of NPs could beneficially induce macrophage toward M2 phenotype polarization and ameliorate the excessive inflammation. In the proliferation and remodeling phase, gAu–CuS HSs could enhance angiogenesis and promote cell migration and differentiation to accelerate wound healing.

Although emerging studies evidenced that strategies enhancing neurogenesis and angiogenesis are a promising direction for diabetic wound treatment, coordination of neurogenesis and angiogenesis simultaneously remains a challengeable issue for facilitating wound healing. Therefore, we constructed a whole-course-repair system by a nanosystem-enriched hydrogel to concurrently gain a mutually supportive cycle of neurogenesis–angiogenesis.[121] This hydrogel can be in situ locally injected to cover wounds long-term for facilitated healing via the synergistic effect of Mg^{2+} and engineered sEVs. During the inflammation phase, this system can effectively induce MSCs toward neurogenic differentiation, meanwhile providing a beneficial immune microenvironment for tissue repair. During the proliferation phase, enhanced angiogenesis occurs by the synergistic effect of the new-borne neural cells and the released Mg^{2+}. Last, the in vitro and in vivo results indicate a promising platform of this whole-course-repair system for diabetic wound therapy (**Figure 11**).

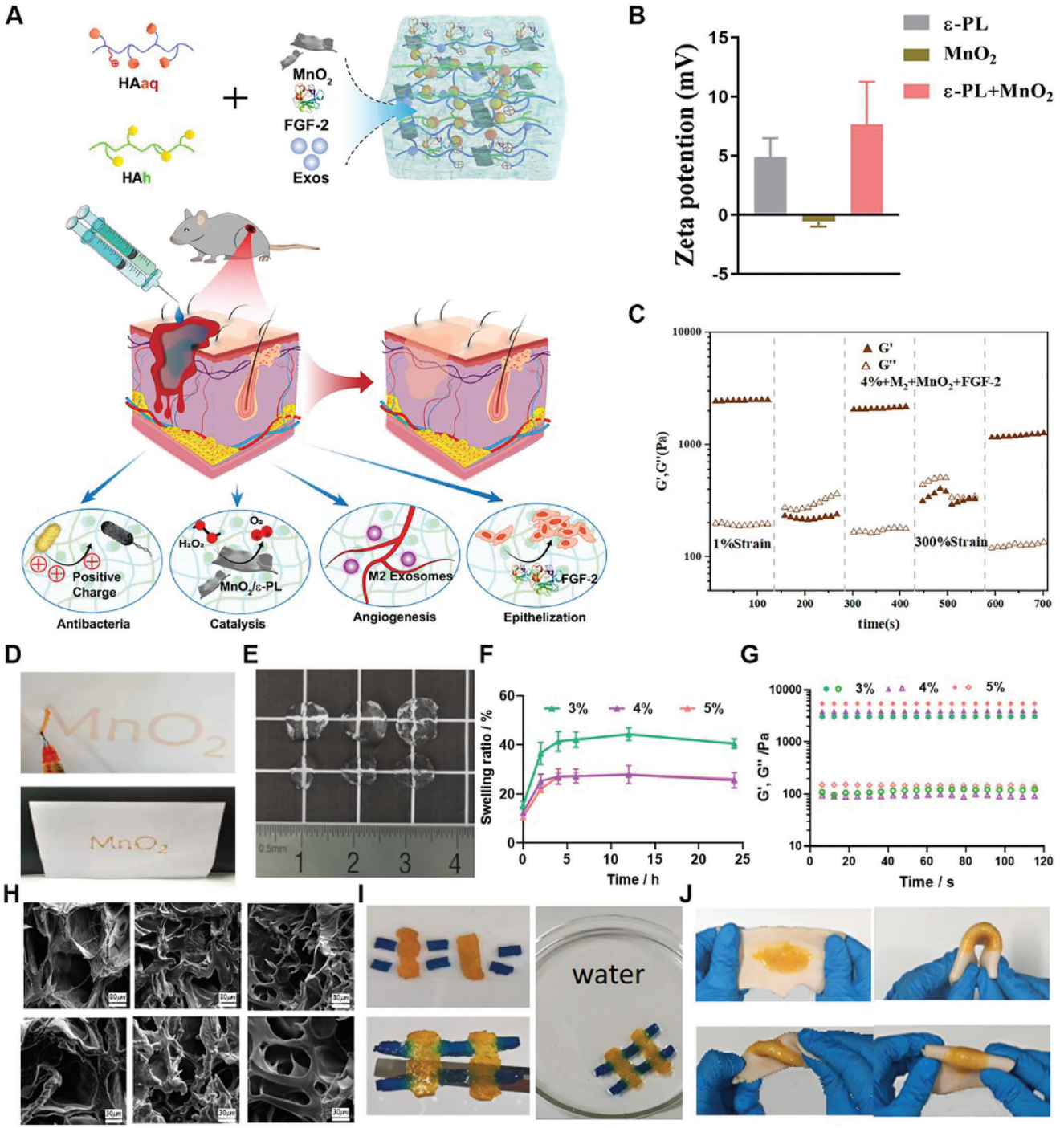

Figure 10. A) Schematic illustration of the preparation and functions of small extracellular vesicle (sEV)-based hydrogels. B) Zeta potential test of ϵ-PL, MnO_2 nanosheet, and MnO_2/ϵ-PL nanosheet. C) Rheological shear-thinning property of sEV-based hydrogels. D) sEV-based hydrogels can easily be re-shaped through a one-step mixed injection. E) Representative images of sEV-based hydrogels with different solid contents before and after swelling equilibrium. F) Quantitative swelling ratio profiles of sEV-based hydrogels. G) Rheological time sweep results of the sEV-based hydrogels. H) Representative SEM images of sEV-based hydrogels. I) sEV-based hydrogels adhered tightly to the pig skin after good gelation. J) Self-healing property of sEV-based hydrogels. Reproduced with permission.[61b] Copyright 2022, Wiley-VCH GmbH.

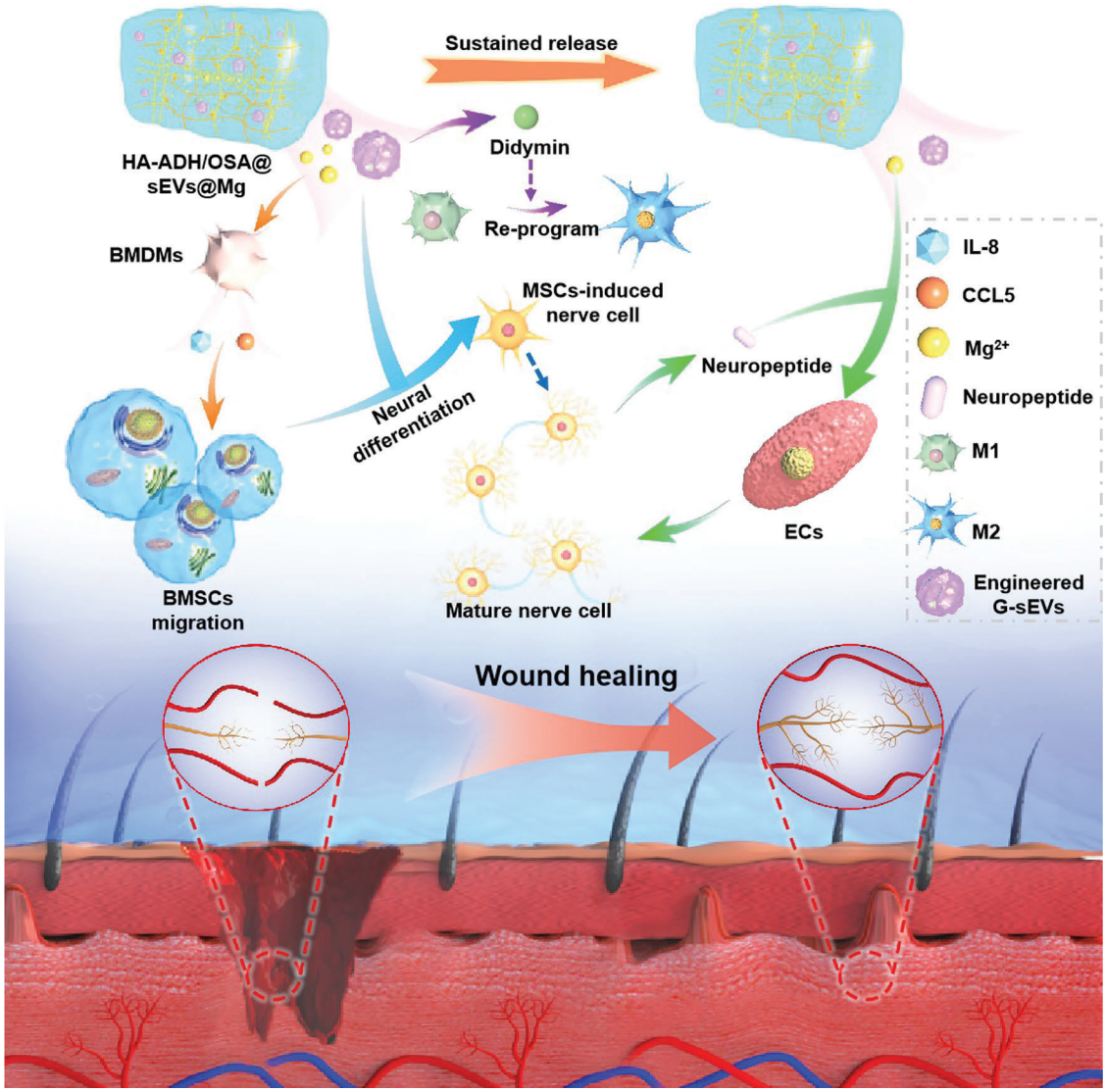

Figure 11. Schematic diagram for the important role of HA-ADH/OSA@Mg@sEVs in promotion of diabetic wound healing. The preparation of this hydrogel needed only one step by mixing the solution of HA-ADH/Mg and the solution of OSA in equal volume. Reproduced with permission.[121] Copyright 2023, Wiley-VCH.

5. Future Trends of Nanosystems for Treatment of Diabetic Wounds

Many of the above-described nanosystems represent promising approaches for the treatment of diabetic wounds. However, due to the pathophysiological complexity of diabetic wounds, mono-functional therapeutic agents have limited efficacy. Therefore, biocompatible scaffolds were used to create multifunctional ROS-scavenging systems. Most recently, Li et al. introduced an injectable hydrogel based on hyaluronic acid-graft-dopamine (HA-DA) and PDA-coated Ti_3C_2 MXene nanosheets.[83a] Briefly, antioxidant MXene nanosheets are capable of eliminating reactive nitrogen species and ROS. Synergistically, the PDA coating further enhances the antioxidant and antibacterial abilities of MXene. Thus, multifaceted benefits of MXene-anchored hydrogels have been described (e.g., tissue adhesion, self-healing, injectability, hemostasis, promotion of human umbilical vein endothelial cell proliferation and migration, and improved diabetic wound healing; **Figure 12**).

Similarly, a multifunctional hydrogel encapsulated with hyperbranched poly-L-lysine (HBPL)-modified MnO_2 nanozymes has been published by Gao and coworkers.[122] HBPL was shown to significantly increase the stability of MnO_2 nanosheets and displayed various functions including scavenging different types of ROS, generating O_2, and killing broad-spectrum reluctant bacteria. Experimentally, this multifunctional hydrogel reduced ROS levels and curtailed the inflammatory response in diabetic wounds (**Figure 13**).

In addition to hydrogels, multifunctional microneedles (MNs) have also been used in combination with ROS-scavenging nanosystems. Most recently, Ma et al. developed a novel core–shell HA MN with ferrum–mesenchymal stem cell-derived

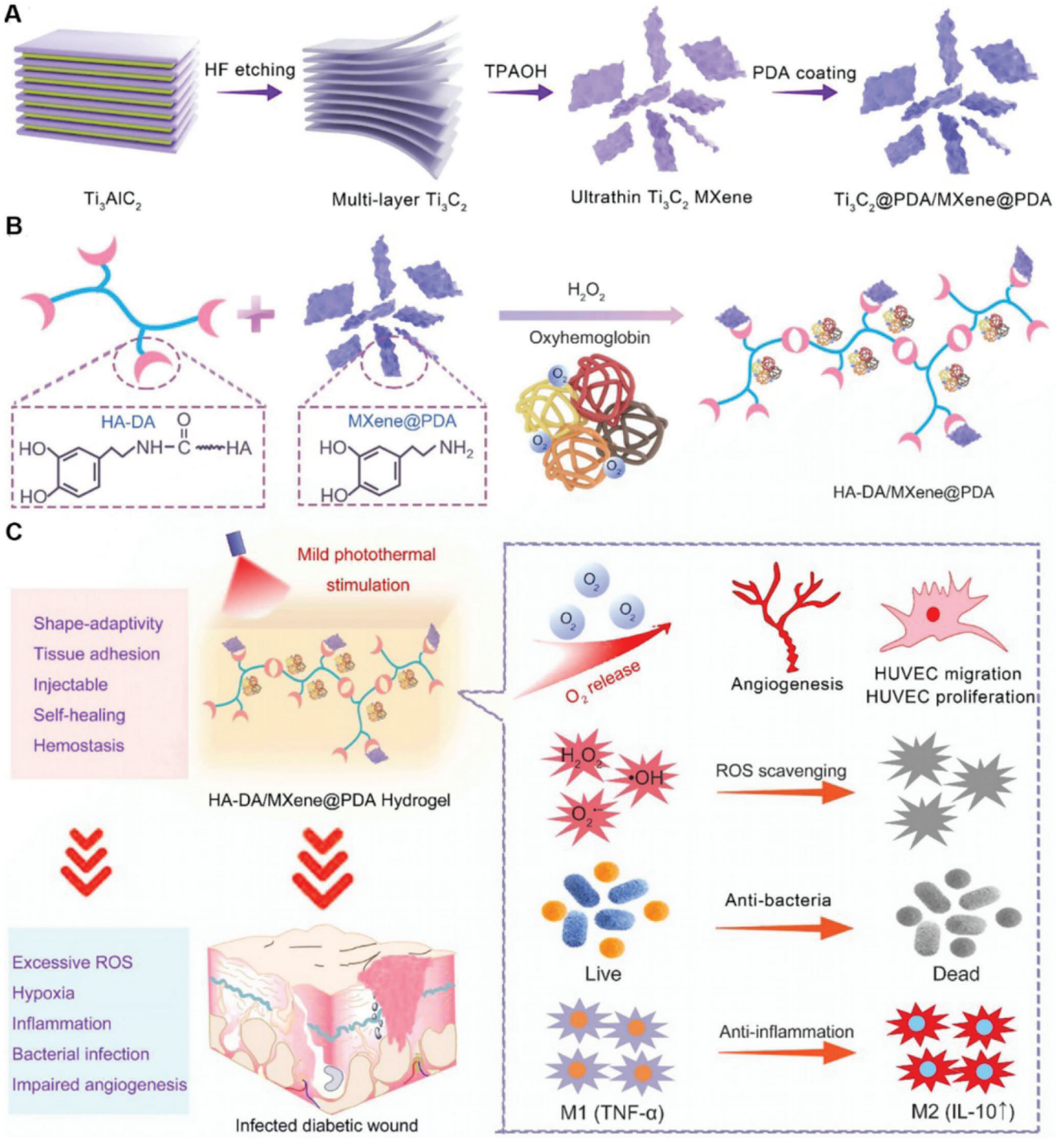

Figure 12. A,B) Schematic diagram for the preparation of MXene@PDA and hyaluronic acid-graft-dopamine (HA-DA)-MXene@PDA hydrogels. C) HA-DA-MXene@PDA hydrogels have multiple functions including scavenging different types of ROS, generating O_2, and killing broad-spectrum bacteria. Experimentally, this multifunctional hydrogel reduced ROS levels and alleviated the inflammatory response in diabetic wounds. Reproduced with permission.[83a] Copyright 2022, American Chemical Society.

artificial nanovesicles (Fe-MSC-NVs) and PDA NPs encapsulated in the needle tips for the treatment of diabetic wounds.[123] Fe-MSC-NVs were loaded in the inner HA core of the MN tips and used to promote angiogenesis. In addition, PDA NPs were embedded in the outer methacrylated hyaluronic acid (HAMA) shell of the tips to attenuate the ROS-induced damage associated with oxidative stress. The gradual degradation of HAMA allowed for sustainable delivery of PDA NPs to the wound site, consequently reducing ROS levels. Moreover, the combination of PDA NPs and Fe-MSC-NVs could further stimulate macrophage phenotype

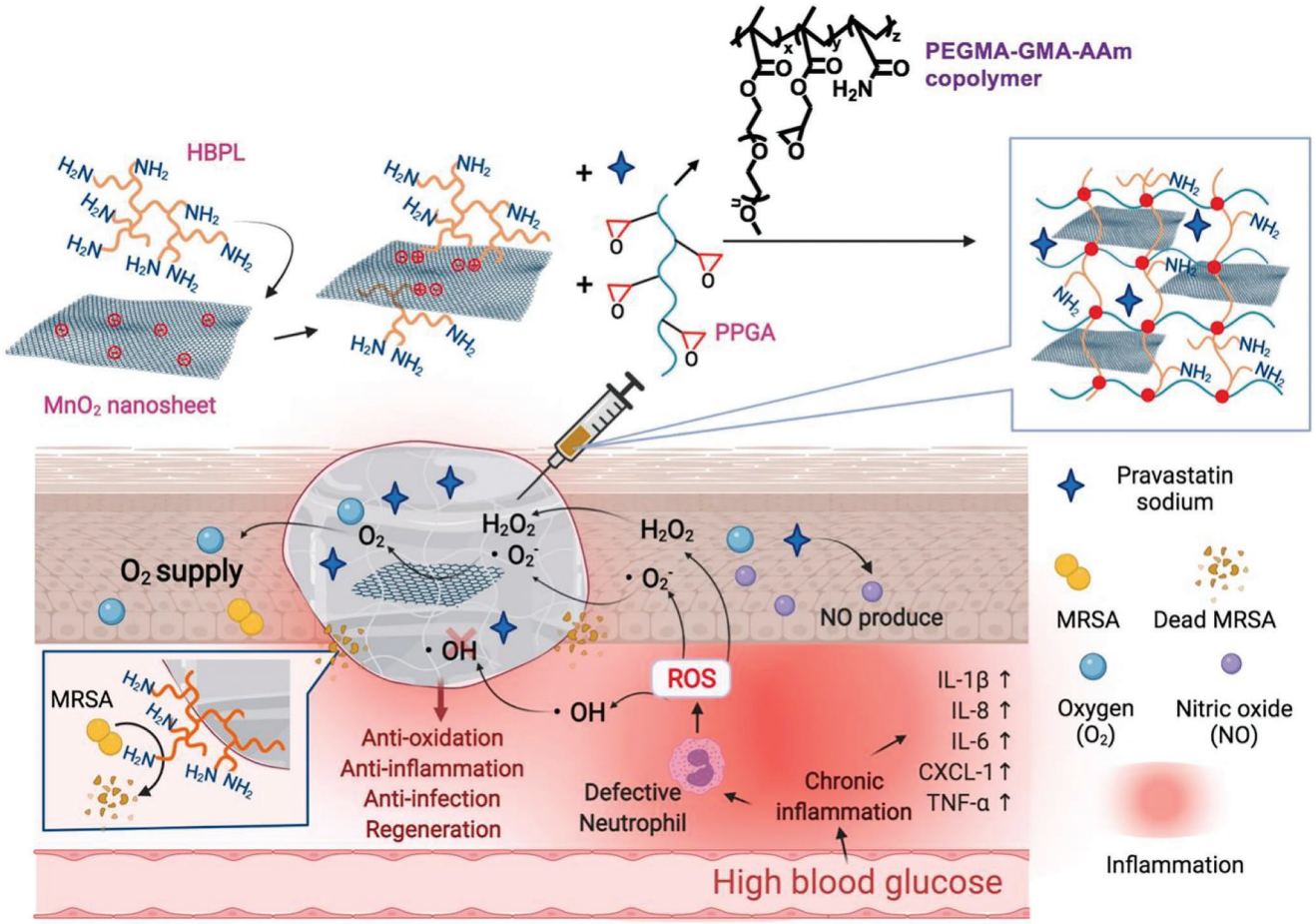

Figure 13. An ROS-scavenging, O_2-generating, and NO-producing anti-bacterial hydrogel encapsulated with hyperbranched poly-L-lysine (HBPL)-modified MnO_2 nanozymes designed for diabetic wound therapy. Reproduced with permission.[122] Copyright 2022, Elsevier.

switching toward M2; thus, ameliorating local inflammation. In addition, in vivo application has shown a significant acceleration of diabetic wound healing. Taken together, the ROS-scavenging, anti-inflammatory, and pro-angiogenic capabilities of this nanosystem-based MN make it highly valuable for future therapeutic applications in diabetic wounds (**Figure 14**).

6. Current Challenges and Future Perspectives

The rapid evolution of nanotechnology as a therapeutic tool underscores its promising potential to greatly impact the future treatment of diabetic wounds. ROS-scavenging nanosystems and their multifunctional composites offer unique advantages in establishing a pro-healing microenvironment in chronic wounds. While potential benefits of ROS-scavenging nanosystems are promising, several issues need to be addressed:

1) Nanosystems are composed of biological and synthetic materials. As the former include cellular components, such as cell membranes and nanovesicles, strict protocols must be followed during production and distribution, including adherence to regulations on the ethical use of biomaterials and their sources. Aside from the biological components, synthetic materials may be toxic to certain tissue or cell types. Thus, extensive preclinical studies are required before widespread clinical use.
2) It is well-described that ROS play a dual role in wound healing in vivo. While a physiological amount of ROS is required for the healing process, excessive ROS production in wound tissues is associated with pathological detrimental effects. For example, ROS release plays a pivotal role in the antibacterial defense during the early inflammatory phase while exerting adverse effects in later stages of the wound healing process. Thus, the appropriate timepoint of the initiation and intensity of ROS-scavenging nanotherapeutics needs to be carefully considered and subject to further research.
3) As diabetic wounds are caused by multiple factors, research and development of multifunctional nanosystems (with antibacterial, pro-angiogenic, pro-repair, and ROS scavenging functions for example) is highly desirable. However, the efficacy of ROS-scavenging nanosystems can be significantly compromised under pathological conditions. Therefore, it is important to develop more biocompatible and multifunctional scaffolds encapsulated with ROS-scavenging nanosystems to improve therapeutic efficacy.

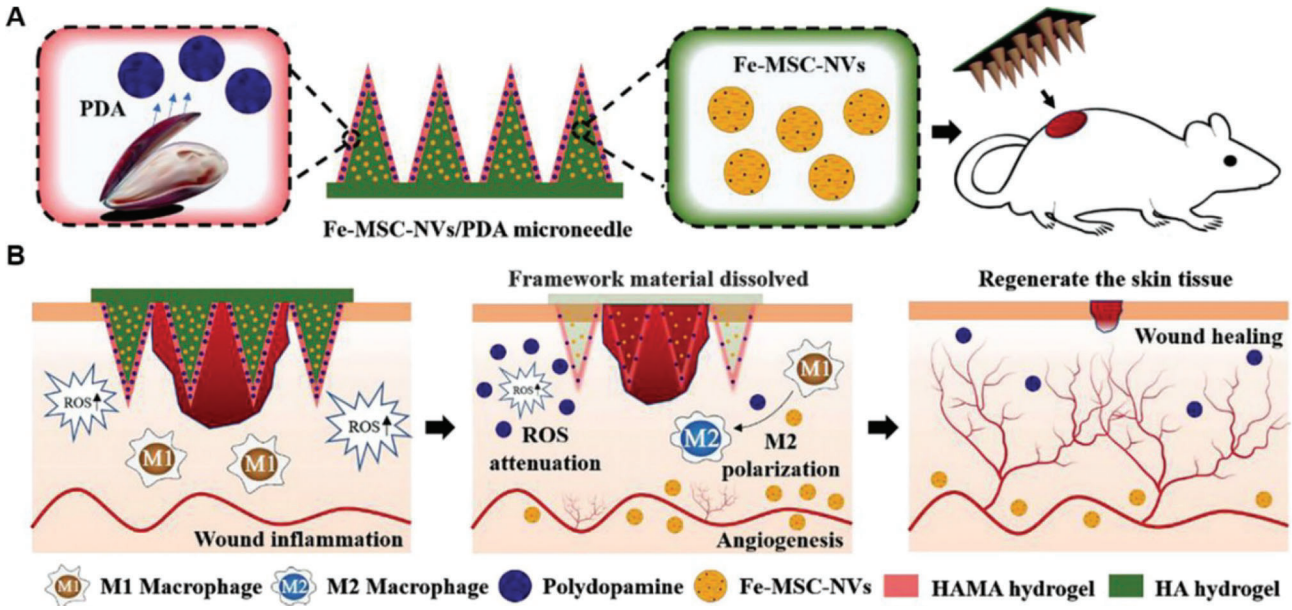

Figure 14. A) Schematic illustration for the preparation of Fe-MSC-NVs/PDA MNs patch. B) Schematic mechanisms for the pro-healing effect of Fe-MSC-NVs/PDA MNs in diabetic wounds. Reproduced with permission under the terms of the CC-BY license.[123] Copyright 2022, the Authors. Published by Wiley-VCH GmbH.

4) Although the efficacy of ROS-scavenging nanosystems has been demonstrated in various small animal models, few studies have been performed in larger animals. This limitation needs to be addressed in order to ensure the safety and efficacy of use, particularly the long-term effects, before clinical implementation.
5) Last, large-scale production is an issue that needs to be addressed. For example, the current production of sEVs can only meet the demands of laboratories, and further technical breakthroughs are required for efficient large-scale commercial production.
6) In the future, more in-depth knowledge of the molecular mechanisms of ROS-scavenging nanosystems and further advances in bioengineering techniques will allow researchers to construct more effective ROS-scavenging systems, eliminate the translational gap between basic studies and clinical studies, and eventually improve the therapeutic efficacy of diabetic wounds.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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