molecular pharmaceutics

pubs.acs.org/molecularpharmaceutics

Review

Oxygenated Wound Dressings for Hypoxia Mitigation and Enhanced Wound Healing

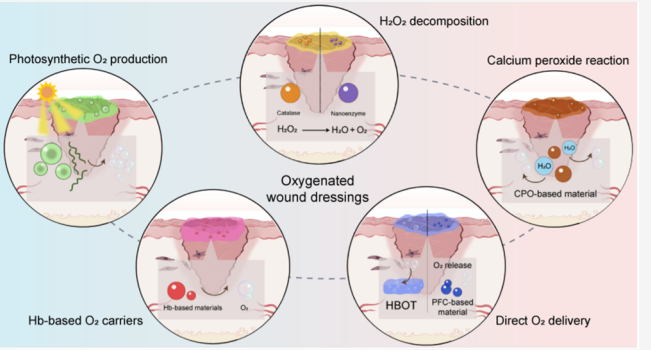
Xiaoxue Han, Leah Suyeon Ju, and Joseph Irudayaraj*

Cite This: *Mol. Pharmaceutics* 2023, 20, 3338−3355

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Oxygen is a critical factor that can regulate the wound healing processes such as skin cell proliferation, granulation, re-epithelialization, angiogenesis, and tissue regeneration. However, hypoxia, a common occurrence in the wound bed, can impede normal healing processes. To enhance wound healing, oxygenation strategies that could effectively increase wound oxygen levels are effective. The present review summarizes wound healing stages and the role of hypoxia in wound healing and overviews current strategies to incorporate various oxygen delivery or generating materials for wound dressing, including catalase, nanoenzyme, hemoglobin, calcium peroxide, or perfluorocarbon-based materials, in addition to photosynthetic bacteria and hyperbaric oxygen therapy. Mechanism of action, oxygenation efficacy, and potential benefits and drawbacks of these dressings are also discussed. We conclude by highlighting the importance of design optimization in wound dressings to address the clinical needs to improve clinical outcomes.



KEYWORDS: *hypoxia, oxygenated wound dressing, wound healing, bioactive materials, skin repair*

1. INTRODUCTION

Impaired wound healing is a significant public health concern affecting millions of people worldwide. In the United States, the treatment for acute wounds (surgical and traumatic wounds, abrasions, or superficial burns) and chronic wounds (usually caused by ischemia, secondary to diabetes mellitus, venous stasis, aging, and pressure) costs several billion dollars annually, accounting for 4% of the overall healthcare expenses.[1] The high cost and the accompanying chronic pain impact the quality of life of patients, possibly causing serious psychosocial stress and increased social burden.[2] Due to rising incidences of chronic wounds and an increasing elderly population, it is projected that the advanced wound care market, which focuses on surgical wounds and chronic ulcers, will surpass $22 billion by 2024.[2] Hence, research efforts on developing functional wound dressings have recently gained significant importance.

Wound healing is a complex process that can be impaired by various factors such as chronic inflammation, bacterial infection, diseases, and hypoxia.[3] Hypoxia, which is characterized by a deficiency in oxygen supply to the wound bed, is a key factor in wound healing since acute hypoxia can stimulate angiogenesis by increasing the expression of hypoxia inducible factor-1 (HIF-1) and its target gene vascular endothelial growth factor (VEGF), while chronic hypoxia not only impairs angiogenesis but also downregulates cell signaling, which can contribute to delayed wound healing.[4] The oxygen level in human tissue 3−4 mm below the wound is around 50 mmHg pressure of oxygen (pO_2), which is much lower than atmospheric oxygen levels (21% O_2, pO_2 = 159 mmHg).[5] Hypoxia can occur as a result of various factors such as a poor blood supply, tissue damage, and inflammation. Hypoxia can hamper tissue regeneration, delay wound healing, and increase the risk of infections by impeding multiple stages of healing including cell proliferation, angiogenesis, and re-epithelialization.[6] Thus, effective strategies to overcome hypoxia and enhance wound healing are urgently needed. Topical oxygen chambers have been approved by the US Food and Drug Administration (FDA) for extreme use to aid in the healing of chronic skin ulcers such as bedsores. Various topical oxygen therapy (TOT) devices and dressings have been applied to clinical wound management such as Natrox (Inotec), OxyBand (OxyBand Technologies), and O_2Boot (GWR Medical Inc.).[7] However, the TOT efficiency is limited by (1) the insufficient penetration of external gas to the tissues and (2) uncontrollable oxygen delivery. To enhance the efficacy of oxygen

Received: April 22, 2023
Revised: June 1, 2023
Accepted: June 2, 2023
Published: June 20, 2023

delivery and to precisely control release, several strategies that deliver oxygen to the wound bed have been recently developed including catalase-based hydrogen peroxide decomposition, nanoenzyme-mediated oxygen generation, metal peroxide decomposition, hemoglobin-based oxygen carriers, hyperbaric oxygen wound dressing, perfluorocarbon-based oxygen carriers, and photosynthetic oxygen production.

In this paper, we summarize the different stages of the healing process and the role of oxygen in wound healing. In addition, we provide an overview of current oxygenation strategies in wound dressings and their potential applications in wound healing. We discuss the mechanisms underlying the effects of oxygenated wound dressings on hypoxia alleviation, angiogenesis, and anti-inflammation. Finally, we highlight the challenges and future directions in the development and translation of oxygenated wound dressings for clinical use.

2. PHYSIOLOGY IN WOUND HEALING

Skin is the largest organ of the human body, comprising approximately 15% of the total adult body weight.[8,9] It is made up of three distinct layers, epidermis, dermis, and subcutaneous tissue, which collaborate to fulfill several critical physiological functions including protecting against external factors, aiding in the retention of body fluids, and regulating body temperature.[8]

The formation of a wound, resulting from the disruption of skin, mucosal surfaces, or organ tissue, can be due to a disease process, accidental injury, or intentional injury.[10] Despite the different etiologies, the repair processes of wounds are similar. At the time of injury, multiple cellular and extracellular pathways are activated in a tightly regulated and coordinated fashion to restore tissue integrity. The wound healing cascade is a complex process, and its successful occurrence is remarkable. However, several factors can interfere with this process, leading to delayed healing, increased patient morbidity and mortality, and a poor cosmetic outcome. The classic wound healing process is categorized into four phases: hemostasis, inflammation, proliferation, and tissue remodeling[11] (Figure 1).

2.1. Hemostasis. Hemostasis is the initial phase of wound healing that begins immediately following injury and involves the formation of a blood clot to prevent further bleeding.[12] It is essential for the subsequent stages of wound healing as it provides a temporary barrier to protect the underlying tissues from further injury and infection. Hemostasis involves a complex interplay of platelets, coagulation factors, and endothelial cells.

Upon injury, blood vessels undergo vasoconstriction to reduce blood flow and limit blood loss, resulting in tissue hypoxia and acidosis. The exposed subendothelial matrix of the vessel wall triggers platelet activation, resulting in the formation of a platelet plug at the site of injury. Activated platelets secrete various mediators, such as thromboxane A2 and serotonin, which promote further platelet activation and vasoconstriction.[13] The platelet plug served as a provisional matrix for the subsequent stages of wound healing. Simultaneously, activated platelets secrete various cytokines and growth factors that persist in controlling the sequence of events involved in the healing process: transforming growth factor α (TGF-α) and platelet-derived growth factor (PDGF) enhance fibroblast proliferation. Transforming growth factor β (TGF-β) promotes collagen matrix construction. VEGF stimulates angiogenesis.[14] Additionally, arachidonic acid, a platelet-derived molecule, is degraded into multiple influential

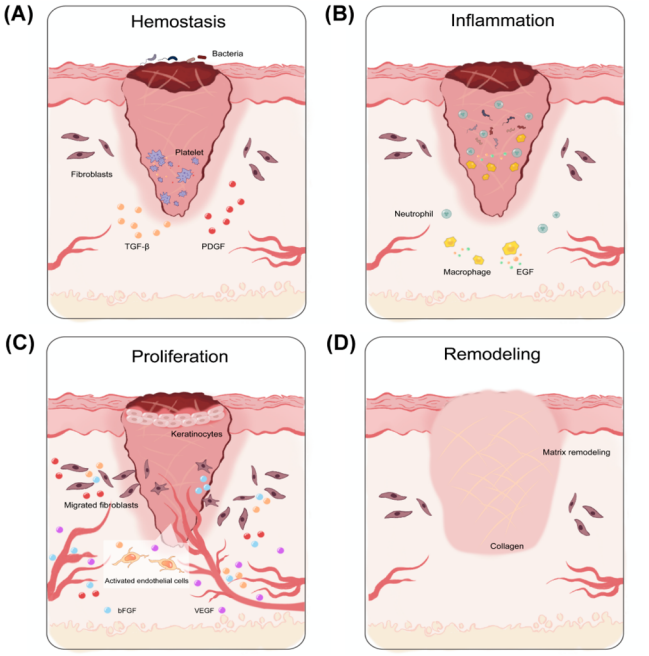


Figure 1. Wound healing phases: (a) hemostasis; (b) inflammation; (c) proliferation; (d) remodeling.

signaling molecules, including prostaglandins and leukotrienes, which actively participate in provoking the subsequent inflammatory phase.

2.2. Inflammation. The inflammation phase is an essential step in the wound healing process, aimed at eliminating invading microorganisms and removing cellular debris, as the physical barrier that once served as the primary defense against pathogenic microorganisms has been compromised.

At the inflammation phase, immune cells such as neutrophils and monocytes are recruited to the wound site and release a variety of inflammatory mediators, including cytokines, chemokines, and growth factors, which play a vital role in activating immune cells, further attracting cells, and promoting healing.[15] Neutrophils, which reach peak concentration in the wound after 24 h after injury, are responsible for phagocytosis, bacteria elimination, and cellular debris clearance from the wound bed. The influx of neutrophils is followed by the recruitment of macrophages, which can clear debris and produce growth factors. TGF-β and epidermal growth factor (EGF) secreted by macrophages can modulate the immune response, stimulate neovascularization, and enhance the formation of granulation tissue.[12] 72 h after injury, lymphocytes are attracted to the wound and modulate healing through extracellular matrix scaffold production and collagen remodeling, thereby directing the healing process into the proliferation stage. The inflammation phase is tightly regulated, and an imbalance in the inflammatory response can result in delayed wound healing or chronic wounds.

2.3. Proliferation. Once the inflammation subsides, the proliferation phase of the healing cascade can initiate to restore the vascular network, form granulation tissue, deposit collagen, and re-epithelialize. The re-establishment of the vascular network is crucial in providing nutrients and oxygen during wound healing. Angiogenesis, the process of new blood vessel formation, is triggered by the growth factors produced from platelets such as VEGF, PDGF, basic fibroblast growth factor (bFGF), and the serine protease thrombin in the wound site,

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

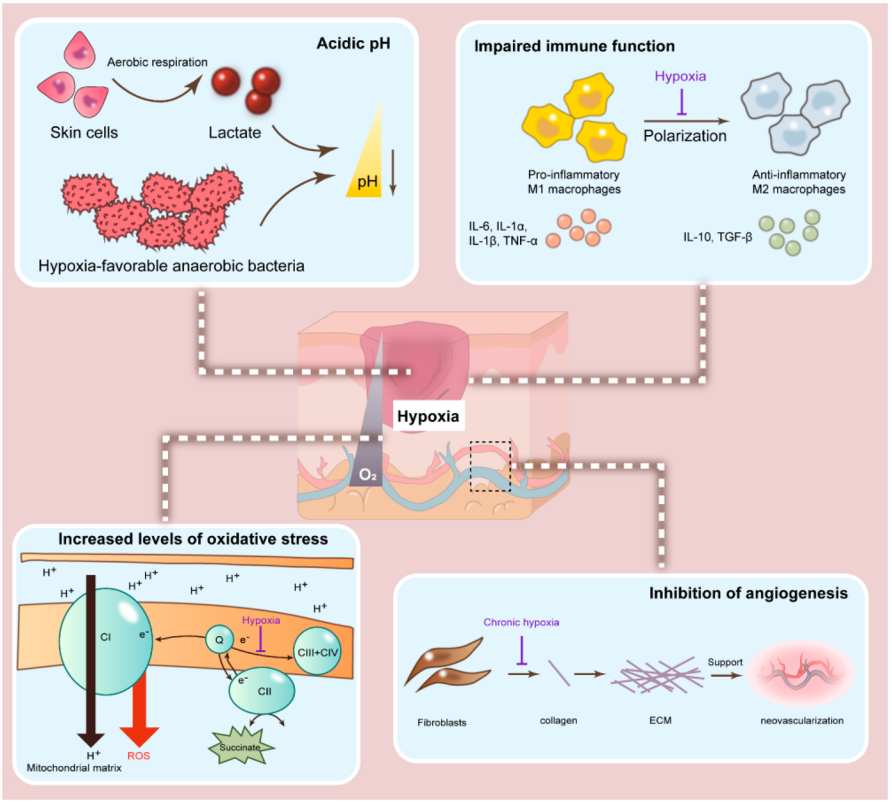


Figure 2. Hypoxia and its role in wound healing.

which can activate endothelial cells and induce neovascularization.[16] During this stage, fibroblasts are activated to proliferate due to the growth factors released from the hemostatic clot and then migrate from the nearby dermis to the wound through the stimulation of TGF-β and PDGF.[12] Abundant fibroblasts lay down extracellular matrix proteins, such as hyaluronan, fibronectins, and proteoglycans, followed by the production of collagen and fibronectin. This results in the formation of granulation tissue, which fills the wound gap and provides a scaffold for cell adhesion, migration, growth, and differentiation during wound repair.

2.4. Remodeling. The remodeling phase is the final stage of wound healing initiated at the end of granulation tissue development, during which fibroblasts undergo apoptosis and the newly formed tissue optimizes its mechanical strength and function.[17] In this phase, collagen synthesis and degradation reach a gradual balance.[18] The rapid deposition of collagen III in the extracellular matrix is eventually substituted by the deposition of collagen I, which has greater tensile strength but takes longer to produce. This phase can commence from day 8 up to 2 years after injury.

Overall, wound healing is a complex process that involves a series of overlapping phases including hemostasis, inflammation, proliferation, and remodeling. In addition, wound healing is regulated by a variety of factors such as cytokines, growth factors, nutrients, immune response, and oxygen level. Any disruption in these phases and factors can lead to delayed healing and creates "non-healing" wounds, which increases the psychological and physiological burden on patients.

3. HYPOXIA AND ITS ROLE IN WOUND HEALING

Oxygen is essential for the survival of cells involved in the wound healing process such as fibroblasts and endothelial cells.[19] In various key cellular processes (aerobic glycolysis, oxidation of fatty acids, citric acid cycle, etc.), oxygen is required to produce biological energy equivalents such as adenosine triphosphate (ATP). Adequate oxygen supply ensures a panel of cellular processes, and maintains the normal cell functions, including the activation and migration of immune cells, collagen synthesis, skin tissue generation, and the formation of new blood vessels, which are crucial for wound healing.[19] Additionally, oxygen can be favorable to control infection in the wound since several bacteria require anaerobic conditions to thrive.

Hypoxia, or a lack of oxygen, refers to a deficiency in the amount of oxygen reaching tissues (usually 1% to 2% of oxygen tension), which can lead to cellular damage and impaired function. During the initial stages of wound healing, the wound site is hypoxic due to disrupted blood flow and increased demand for oxygen by the influx of inflammatory cells involved in the healing process.[20] These cells accumulate in areas with low oxygen levels and play a crucial role in processes such as new skin cell proliferation and granulation as part of the healing process. However, if the wounds remain hypoxic for an extended period, then it can obstruct tissue repair and recovery, which can slow down the healing process in both acute and chronic wound healing. In acute wound healing, hypoxia can hinder cell function and cell proliferation, leading to delayed wound healing and an increased risk of infection. In chronic wound healing, hypoxia is an important factor and can have a negative impact as well, but it may play a more complex role.

Chronic wound refers to a wound that has failed to heal in an orderly and timely manner following proper physiological stepwise phases, usually lasting for more than 6 weeks. Chronic wounds can result from diverse factors including infections,

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

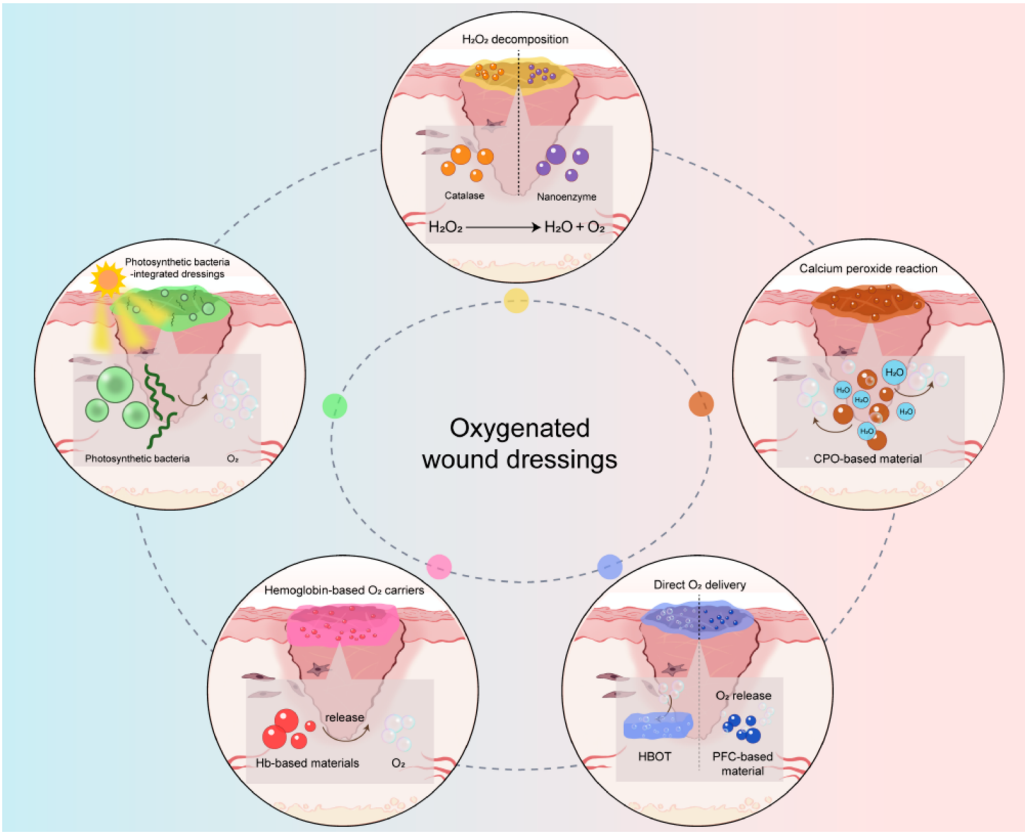


Figure 3. Different oxygenation strategies in wound dressing.

poor blood circulation, and underlying medical conditions such as obesity or diabetes mellitus. Although the cause of chronic wounds may vary, they often exhibit similar characteristics and develop in a similar manner. The common features of chronic wounds include hypoxia, infection, and scarring. Among these, hypoxia is important since it has a negative impact in all stages of the healing process. Hypoxia is also associated with multiple key features of microenvironment in chronic wounds as shown in Figure 2.

3.1. Acidic pH. Normal skin surface is an acidic environment.[21] The pH environment in wounds varies depending on the stage of the wound and the length of time it has been present. Acute wounds and chronic wounds in their healing process often have an acidic pH, typically ranging from 5.5 to 7.5.[22,23] A slightly acidic pH in the wound bed is favorable for wound healing as it can prevent bacteria growth and stimulate fibroblast migration. However, an excessively acidic pH level in the wound can negatively affect the healing process by harming the cells and reducing the amount of nutrients and growth factors needed for healing.[24] Furthermore, the natural healing process of several wounds is inadequate, necessitating the utilization of skin grafts. A significant body of evidence indicates that a wound bed with an alkaline pH is more favorable for the healing of chronic wounds that involve skin grafts.[24] Therefore, it will be beneficial for wound therapy by controlling the pH of the wound bed.

The relationship between hypoxia and acidic pH in chronic wounds is complex, with both factors contributing to and perpetuating each other. On one hand, hypoxia can lead to an acidic pH in chronic wounds through several mechanisms. In the presence of hypoxia, cells utilize anaerobic respiration, which does not require oxygen but produces much less energy than aerobic respiration. This switch of respiration may result in the production of metabolic waste products, such as lactate, which can accumulate and contribute to an acidic pH in the wound environment.[25] Bacteria overgrowth is another hypoxia-associated factor that contributes to low pH in chronic wounds since hypoxia-favorable anaerobic bacteria produce acidic metabolic byproduct. On the other hand, an acidic environment in chronic wounds can lead to further hypoxia through its negative effects on hemoglobin's ability to carry oxygen and the growth of new blood vessels, creating a vicious cycle that prolongs the persistence of chronic wounds.

3.2. Increased Levels of Oxidative Stress. Normally, cells produce a limited number of reactive oxygen species (ROS) as metabolic byproducts. Under low oxygen level, in response to hypoxia, cells increase the production of ROS to compensate for the lack of oxygen, which is intended to maintain energy production through anaerobic glycolysis as a survival mechanism.[26] In hypoxic conditions, the increased production of ROS is primarily attributed to the mitochondria. The process of oxidative phosphorylation requires mitochondria complex IV to utilize oxygen to receive electrons from complex III through cytochrome c.[27] However, in the absence of hypoxia, the electron transfer system (ETS) is reversed, which leads to a decreased production of ROS by complex III, while complex I activity slows down and produces more ROS due to a decreased amount of coenzyme Q available.[27] During hypoxia, complex II significantly contributes to ROS production through the reverse enzyme reaction (fumarate reductase) and the reduction of the coenzyme Q pool during reverse electron transport.[28] The excessive ROS can surpass the cellular antioxidant defense, leading to oxidative stress,

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

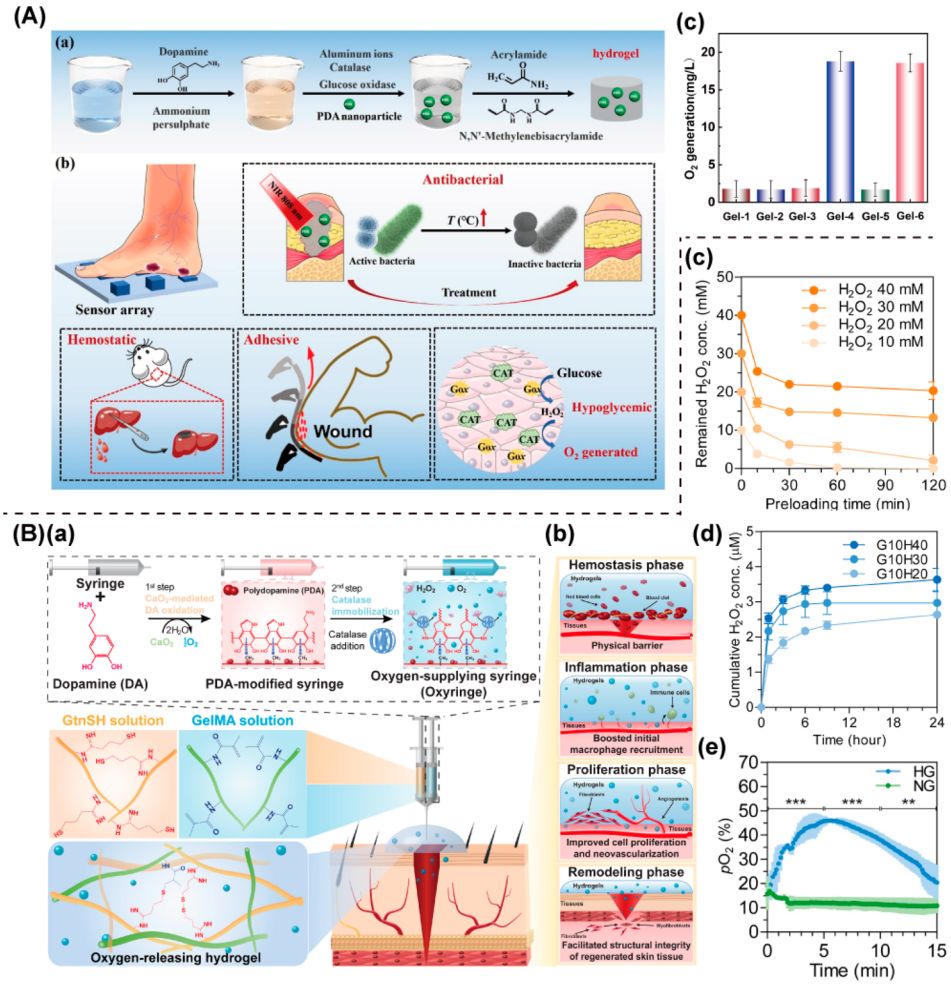


Figure 4. (A) (a) Synthesis of PDA/AM/GOx/CAT/Al^{3+} hydrogel; (b) multifunctional hydrogel with antibacterial, hemostatic, adhesive, and oxygen supplying properties; (c) O_2 generation through cascade enzymatic reaction. Reprinted from ref 44 with permission. Copyright 2022 Elsevier. (B) (a) Synthesis of Oxyringe and hyperoxia-inducible hydrogels; (b) Oxyringe enhances wound healing through effecting different phases; (c) H_2O_2 elimination; (d) effective H_2O_2 release from hydrogel. Reprinted from ref 45 with permission. Copyright 2023 Elsevier.

cellular damage, and inflammation, all of which impede wound healing.

3.3. Inhibition of Angiogenesis. Although acute hypoxia results in a transient increase in vascular endothelial growth factor (VEGF) expression that stimulates the proliferation of human dermal fibroblasts, the persistence of chronic hypoxia in chronic wounds has been shown to hinder angiogenetic process via multiple mechanisms.[29] New blood vessels require a 3D structure in the form of an extracellular matrix (ECM) to support their growth. However, the insufficient production of collagen by fibroblasts, a process that depends on oxygen, contributes to the lack of ECM synthesis in chronic wounds. In addition, oxygen is necessary for the hydroxylation of proline and lysine during collagen synthesis and for the transformation of protocollagen into the strong, triple-helical form of collagen, all of which hinder the vascularization.

3.4. Impaired Immune Function. Hypoxia has proven to play a key role in regulating macrophage polarization.[30] After an injury, monocytes accumulate in the wound and differentiate into proinflammatory macrophages known as the M1 type, which manage the production of ROS and inflammatory cytokines, such as IL-6 and TNF-α, that eliminate infection. During the subsequent stages of healing process, the phenotype evolves into anti-inflammatory/wound healing macrophages (M2 type) and promote tissue growth and angiogenesis by secreting anti-inflammatory cytokines including IL-10 and TGF-β.[31,32] However, in the presence of pathological chronic hypoxia, macrophages are stuck in the M1 phase, leading to chronic inflammation and impeded angiogenesis.[33] It is also reported that wound hypoxia can impair neutrophil bacterial killing capacity by limiting neutrophil respiratory burst metabolism, which may exacerbate wound infection and hinder the healing process.[34]

Overall, hypoxia affects all sequential stages of physiological wound healing including granulation, re-epithelialization, infection clearance, angiogenesis, and tissue regeneration. Hence, it is important to manage hypoxia to enhance wound healing.

Since we understand the significance of oxygen in different stages of wound healing, significant strides were taken to develop novel oxygenated wound dressings (Figure 3). In this section, we discuss advances in various oxygenation strategies in wound healing.

4. H_2O_2 DECOMPOSITION STRATEGY IN WOUND DRESSING

4.1. Catalase-Based Nanoparticles Laden Wound Dressing. Catalase is an essential endogenous antioxidant

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

Table 1. Summary of Nanoenzyme-Integrated Wound Dressing[a]

Nanoenzyme	Nanoenzyme synthesis	Co-loaded compounds	Enzyme mimics	Hydrogel scaffold	Cell models	Animal models	ref
MoS_2	React in solution, collect sediment	TA/Fe	CAT, POD	PVA, dextran, borax	L929s	*S. aureus* infected, NIR treated, H_2O_2 added rat model	48
MoS_2	Hydrothermal method, collect sediment	CNTs	CAT, POD, SOD	SA, PVA, borax	L929s	*S. aureus* infected full thickness skin defect rat model	49
MoS_2	Hydrothermal method, $NaBH_4$ reduction	Au@BSA	GOx, POD, CAT, SOD	ODex, gC	HUVECs	Diabetic, *S. aureus* infected full thickness rat model	50
MnO_2	React in solution, lyophilize sediment	EPL, insulin loaded FCHO micelles	CAT		L929s, C2C12s	Diabetic, MRSA infected full thickness cutaneous wound mouse model	51
MnO_2	React in solution, ultrasonication	HBPL, pravastatin sodium	CAT, POD	PPGA	L929s	Diabetic, MRSA infected full thickness rat model	52
MnCoO	React in solution, collect sediment	EPL	CAT	HA-HYD, HA-ALD	HaCaTs, HDFs, HAECs	Diabetic full thickness rat model	53

[a]TA: tannic acid, POD: peroxidase, CAT: catalase, PVA: poly(vinyl alcohol), L929: mouse fibroblast cell line, NIR: near-infrared radiation, CNT: carbon nanotubes, SOD: superoxide dismutase, SA: sodium alginate, BSA: bovine serum albumin, GOx: glucose oxidase, ODex: oxidized dextran, gC: glycol chitosan, HUVEC: human umbilical vein endothelial cell, EPL: ε-polylysine, FCHO: aldehyde Pluronic F127, C2C12: mouse myoblast cell line, HBPL: hyperbranched poly-L-lysine, PPGA: poly(PEGMA-*co*-GMA-*co*-AAm), HA-HYD: hydrazide-modified hyaluronic acid, HA-ALD: aldehyde-modified hyaluronic acid, HaCaT: human keratinocyte, HDF: human dermal fibroblast, HAEC: human arterial endothelial cell.

enzyme that exists in the mammalian blood and liver that can oxidize diverse electron donating substrates, resulting in H_2O_2 breakdown and O_2 production.[35] Given its high catalytic specificity and activity, catalase-induced O_2 generation is considered as a key strategy to increase O_2 levels in various biomedical applications. Several catalase-incorporated nanosystems have recently been developed to scavenge ROS and combat hypoxia in different diseases such as tumor, stroke, Alzheimer's disease, and Parkinson's disease.[36−38]

In the wound healing process, catalase can act not only as ROS scavenging antioxidants but also as O_2 generators that oxygenate wound tissue to stimulate re-epithelialization, fibroblast proliferation and migration, and angiogenesis to aid in tissue regeneration and wound healing.[39,40] However, catalase, crucial for endogenous antioxidant defense, is often dysfunctional in chronic wounds due to the pathological factors such as diseases and aging, which can lead to increased levels of H_2O_2 in the wound microenvironment.[41] Excessive H_2O_2 accumulated in the initial stages of wound healing results in chronic inflammation, cellular damage, and oxidative stress, which delays the healing process.[41,42] Therefore, strategies to deliver exogenous catalase have recently been studied, and attempts to immobilize catalase directly into scaffolds as wound dressings are a promising option in chronic wound healing. Guan et al. reported a core−shell oxygen release microsphere (ORM) embedded-injectable, thermosensitive hydrogel for sustained oxygen release in diabetic wound healing.[43] ORMs were composed of a polyvinylpyrrolidone (PVP)/H_2O_2 complex core and poly(*N*-isopropylacrylamide-*co*-2-hydroxyethyl methacrylate-*co*-acrylate-oligolactide-*co*-*N*-acryloxysuccinimide) shell conjugated with catalase in the outer layer. The first released PVP/H_2O_2 could be catalyzed and converted into oxygen before it is released into wound beds and avoids potential cell apoptosis caused by H_2O_2. *In vitro* studies demonstrated up to 14 days of continuous oxygen released from ORMs, which was long enough for essential process in wound healing including granulation, angiogenesis, and re-epithelialization. After 24 h of hypoxia (1% oxygen), 2.5-times greater intracellular oxygen content was detected in ORMs-treated HaCaT cells compared to the nontreated cells, suggesting the oxygenating capacity of ORMs on cells. In diabetic wound mice model, the continuous oxygenation of ORMs was proven to promote cell proliferation and angiogenesis and mitigate oxidative stress and inflammation. The ORMs that codelivered H_2O_2 and catalase demonstrate the capacity of long-term oxygen production, which is favorable in distinct phases of the healing process.

The concentration of H_2O_2 in the wound is quite limited, and the exogenous delivery of H_2O_2 is extremely difficult to control, which may aggravate oxidative stress and cause potential toxicity. Thus, some studies have proposed an oxygen production strategy based on the multienzymatic cascade reactions. Wang and colleagues fabricated a polydopamine (PDA) nanoparticles, glucose oxidase (GoX), and catalase embedded polydopamine/acrylamide (PDA/AM) hydrogel with antibacterial, hypoglycemic, and hypoxia reversing functions for diabetic wound healing[44] (Figure 4A a). GoX and catalase induced the cascade enzymatic reactions: (1) excessive glucose in diabetic wounds was catalyzed and converted into H_2O_2, and subsequently (2) H_2O_2 as the substrate of the catalase reaction decomposed to generate O_2, which was up to 18 mg/mL (Figure 4A b, c). Meanwhile, glucose was effectively consumed via the cascade reaction and was reduced to the normal blood glucose levels. The embedded PDA nanoparticles were able to sanitize bacteria under the irradiation of 808 nm near-infrared (NIR) laser. Thus, the PDA/AM hydrogel was validated to lower blood glucose, provide oxygen, and kill bacteria in diabetic wounds.

Even though catalase delivery has proved to be an efficient strategy for increasing oxygen contents in wound beds, catalase concentration greater than 500 U/mL can eliminate crucial cell signaling molecules, resulting in potential cell toxicity.[45] Therefore, it is important to minimize catalase incorporation while ensuring the efficacy of oxygen generation in wound healing. Utilizing this approach, Kang and Park developed an oxygen-supplying syringe (Oxyringe) where catalase was immobilized to the PDA-conjugated syringe via Michael-type addition and Schiff's base reaction[45] (Figure 4B a). Thiolated gelatin and methacrylated gelatin solutions were injected through an Oxyringe carrying high oxygen to in situ from the hyperoxia-inducible hydrogel, which showed positive effects in different stages of the healing process (Figure 4B b). The Oxyringe can effectively eliminate H_2O_2 released from hydrogel and was able to control oxygen release kinetics by

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

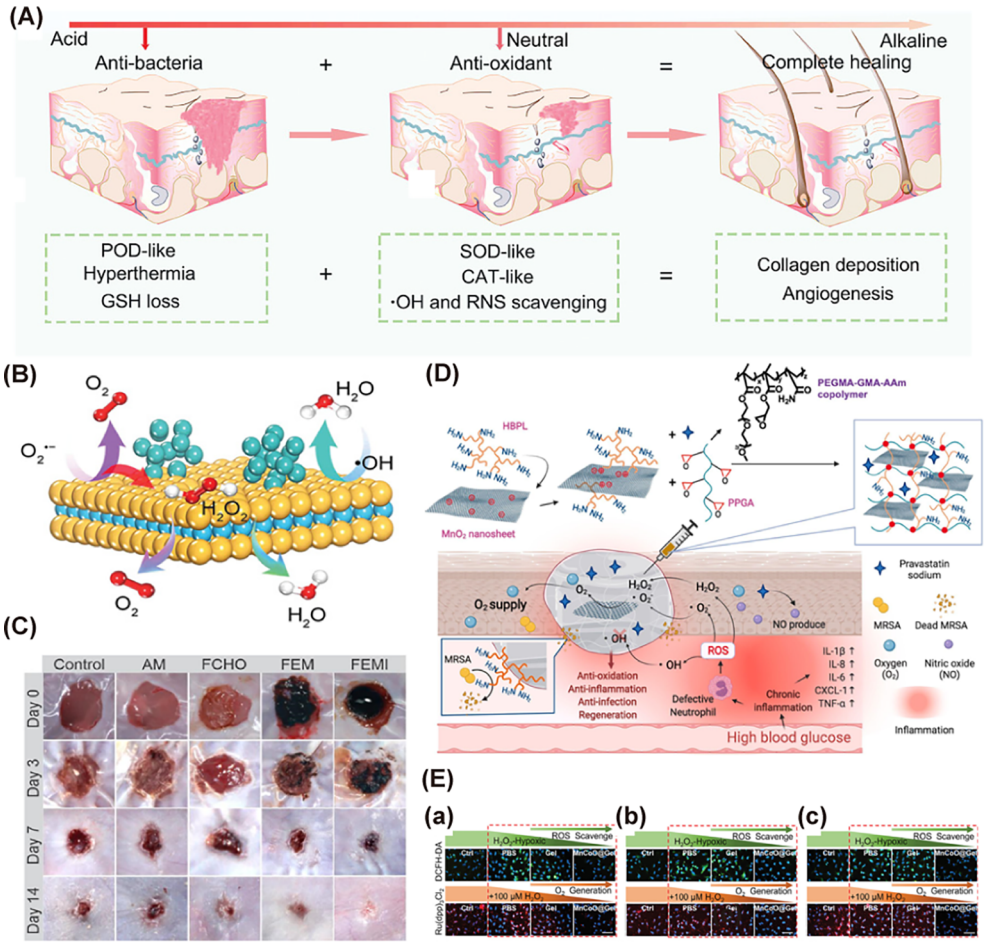


Figure 5. Hydrogel wound dressings functionalized with catalase-mimicking nanoenzymes. (A) pH-dependent dual action of the CNT@MoS_2 hydrogel. Reprinted from ref 49. Copyright 2021 Wiley-VCH. (B) MoS_2@Au@BSA hydrogel scavenges various types of ROS in the wound bed. Reprinted from ref 50. Copyright 2022 Wiley-VCH. (C) FEMI hydrogel increases the rate of wound closure in vivo. Reprinted from ref 51. Copyright 2020 American Chemical Society. (D) HMP gel destroys bacteria, decomposes ROS, and generates oxygen and nitric oxide to support wound healing. Reprinted from ref 52 with permission. Copyright 2022 Elsevier. (E) MnCoO gel exhibits excellent H_2O_2 scavenging and subsequent oxygen generation in (a) HaCaTs, scale bar 50 μm; (b) HDFs, scale bar 50 μm; (c) HAECs, scale bar 50 μm. Reprinted from ref 53. Copyright 2022 Wiley-VCH.

adjusting the immobilization conditions and H_2O_2 concentration (Figure 4B c, d). The hyperoxia-inducible hydrogel was planted subcutaneously and validated to induce transient hyperoxia (up to 46% pO_2) in vivo (Figure 4B e). Notably, the hyperoxia-inducible hydrogel can be a physical barrier and play a hemostatic effect in the initial stages of the healing process. In a mouse liver bleeding model, the mass of blood loss in the hydrogel group was significantly lower than that in the control group. Furthermore, the hyperoxia-inducible hydrogel showed enhanced early macrophage recruitment, cell proliferation, neovascularization, expedited skin regeneration, and wound remodeling, suggesting the positive effects of hydrogel in all stages of wound healing. The Oxyringe has promising clinical implications given the flexibility of various gel materials in the fabrication of different hyperoxia-inducible hydrogels for the clinical needs.

4.2. Nanoenzyme-Laden Wound Dressings. One strategy to produce oxygen is through the decomposition of H_2O_2 utilizing nanoenzymes. Nanoenzymes are a class of nanomaterials with a variety of catalytic capabilities that can serve as artificial enzymes. Because biological enzymes require very specific conditions to function, nanoenzymes are being used as a low-cost, stable, and mass-producible alternative[46] due to two reasons. (1) Nanoenzymes can be designed from various metals including iron, copper, and gold and have many applications in therapeutics and detection technology. Several nanoenzymes have been formulated to mimic the activity of catalase, a biological enzyme that breaks down hydrogen peroxide into oxygen and water.[47] (2) Elevated concentrations of ROS and oxidative stress have been observed in the environments of chronic wounds, and the slow revascularization in the wound reduces access to oxygen vital for healing. Incorporating catalase-like nanoenzymes into wound dressings can eradicate ROS and generate oxygen at the wound site, counteracting these effects and expediting the wound healing process. The recent strategies based on various nanoenzymes for wound healing are summarized in Table 1.

Molybdenum disulfide (MoS_2) nanosheets have been incorporated into several wound dressing designs. Their many applications in biomedicine are evidence of their biocompatibility, in addition to catalase-like activity.[54] In 2021, Li et al. incorporated tannic acid-chelated Fe-decorated (TA/Fe) MoS_2 nanosheets into a poly(vinyl alcohol) (PVA)/dextran blended hydrogel.[48] TA/Fe possesses antioxidant and anti-inflammatory properties and was loaded onto MoS_2 nanosheets with antimicrobial and catalase-like properties.

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

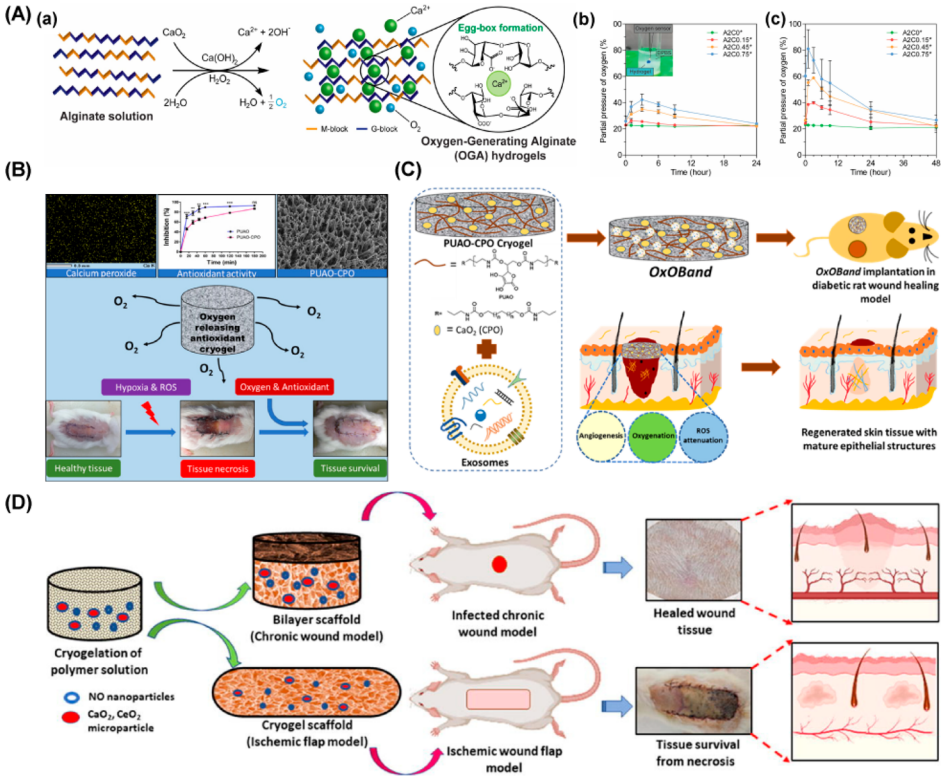


Figure 6. CPO decomposition strategy in wound dressings. (A) (a) Structure of the OGA hydrogel; (b,c) controllable generation of O_2 from the OGA hydrogel with different CPO concentration. Reprinted from ref 67 with permission. Copyright 2019 Elsevier. (B) PUAO−CPO scaffolds and their properties including O_2 releasing, antioxidant, and enhanced tissue survival. Reprinted from ref 68. Copyright 2018 American Chemical Society. (C) Structure of OxOBand. Reprinted from ref 69 with permission. Copyright 2020 Elsevier. (D) The cryogel with NO nanoparticles and CPO and CeO_2 particles for infected chronic wound healing and tissue survival. Reprinted from ref 70 with permission. Copyright 2023 Elsevier.

The resulting hydrogel scavenged ROS and RNS, down-regulated inflammatory cytokines, and destroyed bacteria under photothermal therapy (PTT). The compatibility of the dressing with PTT, used in advanced wound care treatment, is a strong prospect for its synergistic therapeutic effects. In order to increase the nanoenzymatic activity of MoS_2, they then loaded MoS_2 nanosheets onto carbon nanotubes,[49] which is known to allow faster electron transfer and electrical signal transmission, and treated them with near-infrared light. The CNT@MoS_2 was incorporated into a PVA/sodium alginate/borax blended hydrogel. As shown in Figure 5A, in acidic environments, the nanoenzymes converted H_2O_2 to hydroxyl radicals, producing an antibacterial effect. At a neutral pH, catalase-like activity was observed, and the hydrogel scavenged ~90% of the H_2O_2 in its environment resulting in an oxygen content of 26.3 mg/L. The conversion of cytotoxic endogenous hydrogen peroxide into byproducts that can aid in healing is a promising multiaction mechanism for such wound dressings. In 2022, the group developed a hydrogel wound dressing combining the antibacterial and oxygen-supplying properties of MoS_2 nanoenzymes with the glucose oxidative properties of Au nanoparticles[55] to address barriers to healing, specifically in diabetic wounds.[50] MoS_2@Au@BSA was networked into an oxidized dextran and glycol chitosan cross-linked hydrogel. The increased oxygen content due to the decomposition of ROS by MoS_2 (Figure 5B) led to improved glucose oxidation, which was further enhanced by gold nanoparticles. Histological analyses revealed elevated collagen deposition, vascularization, and anti-inflammatory cytokine levels, demonstrating the ability of the dressing to facilitate the healing process despite diabetic conditions. These studies validate the catalase-like properties of MoS_2 nanoenzymes and their benefits in wound dressing design.

Manganese-based nanoenzymes have established catalase-like activity.[56] Several studies have reported the success of Mn-based nanoenzymes in wound dressings in breaking down ROS and producing oxygen in diabetic wounds. Wang et al. developed a hydrogel consisting of EPL-coated MnO_2 nanosheets and insulin-loaded micelles to elicit antibacterial, antioxidant, and glucose oxidative activity.[51] This FEMI hydrogel was able to lower the concentration of H_2O_2 by over 75% in fibroblasts and produce oxygen up to 20 ppm. In vivo tissue studies demonstrated consistently lower concentrations of H_2O_2 and a greater degree of wound closure 14 days postoperation in groups treated with the gel (Figure 5C). The results highlight the potential to specifically address complications due to diabetes in chronic wound healing and are a promising prospect for the utilization of this technology in diabetic wound care. Another hydrogel was fabricated with MnO_2 nanosheets coated in hyperbranched poly-L-lysine cross-linked with PEGMA-GMA-AAm copolymer and encapsulating pravastatin sodium, called the HMP gel, shown in Figure 5D.[52] The antioxidant activity of MnO_2, antimicrobial activity of HBPL, and involvement in nitric oxide synthesis of pravastatin sodium resulted in lower counts of M1 macrophages and higher counts of M2 macrophages after 14 days, indicating more rapid progress in wound healing. The gel directly targets oxidative stress and microbial contamination, two known barriers to wound healing, by eliminating various ROS and destroying MRSA bacteria, to accelerate wound healing. A

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

MnCoO nanozyme-laden hydrogel was also engineered as a dual action ROS-scavenging and oxygen-producing wound dressing.[53] HaCaT, HDF, and HAEC cells cultured with the complete hydrogel in hypoxic environments saw significantly lower internal ROS concentrations and greater oxygen contents (Figure 5E). Diabetic rat models treated with the hydrogel experienced faster wound closure, better tissue granulation, and decreased HIF-1α expression. As demonstrated in these findings, Mn-based nanoenzyme-laden hydrogels have promising applications for wound healing.

5. METAL PEROXIDES DECOMPOSITION STRATEGY IN WOUND DRESSING

Metal peroxides is a group of chemical compounds composed of metal ions and peroxo groups including calcium peroxide (CPO), magnesium peroxide (MgO_2), copper peroxide (CuO_2), zinc peroxide (ZnO_2), barium peroxide (BaO_2), and titanium peroxide (TiOx).[57] Metal peroxide can produce oxygen when it encounters moisture, yielding a broad range of applications in various disease characterized by hypoxia such as tumor therapy and tissue regeneration.[57−59] In wound healing, CPO is the most used metal peroxide due to its desirable oxygen-generation potential, outstanding thermal stability, and eco-friendly end products.[60,61]

The oxygen generation mechanism of CPO works through a decomposition reaction, which occurs when CPO is exposed to moisture and undergoes an exothermic reaction.[62] This results in the release of oxygen and the formation of calcium hydroxide ($Ca(OH)_2$) and hydrogen peroxide. The two-step reaction can be represented as follows:

$$CaO_2 + 2H_2O \rightarrow Ca(OH)_2 + H_2O_2$$

$$2H_2O_2 \rightarrow O_2 + 2H_2O$$

The oxygen generated by CPO can be utilized for various purposes including oxygen generation in wound dressings, water treatment, and soil aeration.[62,63] The oxygen release rate from CPO depends on several factors such as the particle size, surface area, and the surrounding temperature and humidity, leading to controllable oxygen release through adjusting these parameters.[61,64] CPO is considered an effective oxygen source due to its high solubility and stability in water, making it a promising candidate for multiple applications requiring oxygen.

CPO has been recently investigated for its use in wound healing.[65] It releases oxygen, which can be beneficial for promoting the growth of skin cells and angiogenesis in wounds. In addition, CPO has been shown to have antibacterial and anti-inflammatory properties, reducing the risk of infection to accelerate the healing process.[66] Kang et al. described an oxygen-generating alginate (OGA) hydrogel for wound healing.[67] The OGA gel was fabricated through a CPO-mediated ionotropic interaction, allowing for controllable oxygen release (Figure 6A a). Ca^{2+} provided by CPO can not only control the mechanical properties of alginate hydrogel but can also serve to dynamically cross-link the alginate hydrogel to sustain its stability for a longer duration. The OGA hydrogels were found to accelerate wound healing in mice in a full thickness wound model with ability to reoxygenate for up to 80% pO_2, promoting tissue infiltration, wound closure, and wound restoration (Figure 6A b, c). The OGA hydrogels have exhibited excellent potential as bioactive acellular matrices in wound management and tissue regeneration. However, the decomposition of CPO leads to H_2O_2 accumulation, which may cause severe oxidative stress. In this work, the authors introduced additional catalase in the OGA hydrogel for H_2O_2 elimination; however, this makes fabrication challenging as well as contributing to lower storage stability.

To further address the risk of oxidative stress caused by the production of H_2O_2 during CPO reaction, Shiekh and colleagues reported an oxygen-releasing scaffold that incorporates antioxidant material scaffolds for a range of tissue engineering applications[68] (Figure 6B). In this work, they showed that by incorporating CPO as an oxygen-generating material into an antioxidant polyurethane polymer (PUAO), the scaffold was able to generate oxygen for over 10 days and reduce the production of free radicals, which can attenuate hypoxia and increase cell viability in vitro. In the mice skin flap model, the PUAO−CPO cryogel showed a 20% approximate decrease of necrosis at day 3, and this gap even expanded to around 50% by day 9. The PUAO−CPO cryogel can effectively delay the onset of necrosis and enhance skin flap survival. Hence, CPO-embedded scaffolds have shown promise for improving regeneration in multiple tissue engineering applications including chronic wound healing.

Based on the encouraging results of sustained oxygen release and promoted skin flap regeneration from PUAO−CPO scaffolds, they further proposed a novel wound dressing (OxOBand) incorporating both CPO and adipose-derived stem cells (ADSCs) secreted exosomes for the treatment of nonhealing chronic diabetic wounds[69] (Figure 6C). Exosomes from ADSCs contain multiple therapeutic miRNAs, and have been shown to promote cell migration efficiency, essential for wound closure. CPO can continuously generate O_2 under wet wounds for an extended period (over 10 days), which is long enough for the tissue regeneration process. OxOBand may have been suggested as a promising new therapeutic approach for the oxygenated treatment of diabetic ulcers. The authors also suggested that future studies will evaluate the potential of OxOBand in larger animal models. Due to the antioxidant properties of the PUAO material, it is unnecessary to add extra antioxidant reagents in the scaffolds, which simplifies the fabrication process and favors clinical translation.

Infectious chronic and ischemic wounds are highly refractory due to chronic inflammation, oxidative stress, infections, and hypoxic conditions. CPO was demonstrated to help reoxygenation in wound healing. However, H_2O_2 as a byproduct in the reaction of CPO may further aggravate cellular oxidative stress. To address these multifactorial complications, Singh and colleagues proposed a novel bilayer cryogel scaffold to alleviate the hypoxic conditions, persistent inflammation, and infections in chronic wounds.[70] The bioactive scaffold is a bilayer structure with a chitosan-gelatin base layer incorporated nitric oxide nanoparticles, cerium oxide (CeO_2) microparticles, and CPO microparticles for signaling, antioxidant, and oxygen releasing properties, respectively, while the top layer encapsulated iodine in a polyvinylpyrrolidone (PVP) matrix for antibacterial action (Figure 6D). Sustained oxygen release was observed in 10 days with a ratio of 5−10% approximate increase per day, which was favorable to the whole tissue regeneration process. *In vivo* studies of a rat full-thickness infectious wound model demonstrated that the bioactive scaffold expedited wound closure and tissue regeneration compared to control groups. The study provides valuable insights for the development of clinical dressing materials for the treatment of chronic wounds.

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

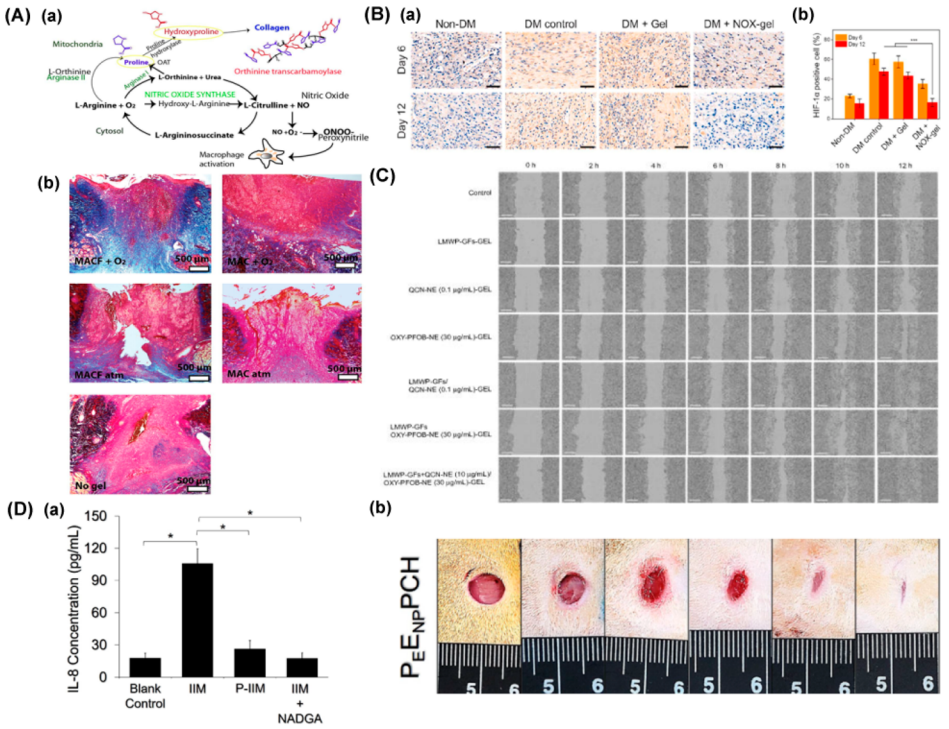


Figure 7. Wound dressings directly deliver oxygen to mitigate hypoxia and encourage wound healing. (A) (a) Arginine and proline pathway stimulates collagen synthesis. (b) MACF+O_2 resulted in increased collagen production due to enhanced wound oxygenation. Reprinted from ref 73 with permission. Copyright 2016 Elsevier. (B) (a) HIF-1α staining postoperation at 6 and 12 days. Brown cells are HIF-1α positive. (b) Quantification of the HIF-1α positive cells. Reprinted from ref 74 with permission from Elsevier. (C) In vitro scratch assay for cell migration and proliferation. *International Journal of Nanomedicine* **2019** *14*, 5449−5475 (ref 75). Originally published by and used with permission from Dove Medical Press Ltd. (D) (a) IL-8 concentration measured after 12 h in an inflammatory environment. (b) Wound closure progression at 0, 3, 6, 9, 12, and 15 days with hydrogel treatment. Reprinted with permission under a Creative Commons Attribution 4.0 International License from ref 76. Copyright 2022 Yu-Hsiang Lee and Sheng-Jhe Lin.

However, it is important to note that the presence of excessive H_2O_2 as a byproduct may induce oxidative stress, which results in cellular damage. Further research should focus on solutions that can effectively eliminate the potential adverse effects of excessive H_2O_2. In addition, there is limited scientific evidence to support the use of calcium peroxide for wound healing, and the use of calcium peroxide in wound care is not approved by FDA. Additional research is needed to determine its safety and effectiveness.

6. OXYGEN DIRECT DELIVERY METHODS

Oxygen is a vital component of several mechanisms involved in wound healing including re-epithelialization, angiogenesis, and infection resistance.[71] There has been growing interest in providing additional oxygen to expedite wound healing. Hyperbaric oxygen therapy (HBOT) is one such approach. Patients are placed in an elevated pressure and high oxygen environment for a prescribed period of time to uptake extra oxygen through respiration[72] to treat specific conditions resulting from oxygen deficit conditions. However, because of the dangers of concentrating highly combustible gas, HBOT requires expensive facilities and large machinery, which makes the logistics complex, in addition to posing a financial constraint for some patients. To circumvent these concerns, several groups have developed oxygen-carrying wound dressings that can directly deliver oxygen to the wound site over a period. These findings are reviewed in this section.

Multiple studies have utilized perfluorocarbons (PFCs) as oxygen carriers in wound dressings. PFCs can dissolve high quantities of oxygen gas, and their strong C−F bonds render them biologically inert.[77−79] These properties are favorable as nontoxic oxygen carriers and for delivery to the wound site. In 2016, Patil et al. modified methacrylamide chitosan with pentadecafluorooctanoic chains in an oxygenating wound dressing, called MACF+O_2.[73] The hydrogels were saturated with 100% O_2 gas for 10 min prior to experimentation and could maintain an oxygen partial pressure higher than atmospheric oxygen tension in a closed environment for 48 h. Metabolomics studies elucidated changes in metabolic pathways for arginine and proline (Figure 7A a), which were down-regulated in the presence of oxygen, leading to increased collagen production. These findings were verified further with histological analysis in Figure 7A b. Similarly, Yang et al. reported lyophilized perfluorodecalin-encapsulated albumin nanoparticles immersed in a hyaluronate gel capable of administering supplemental oxygen and improving wound healing.[74] The dressing significantly reduced HIF-1α expression in a hypoxic environment compared to the hypoxia control in vitro, and immunohistochemical analysis showed about 30% fewer HIF-1α positive cells in diabetic wounds treated with the gel than in the diabetic controls (Figure 7B a,b). Complete wound healing in the diabetic wound model treated with the gel was achieved in nearly half of the time for complete healing of the diabetic control. PFCs have emerged as an effective means of oxygen delivery from a functionalized wound dressing and demonstrate great potential for wound care.

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

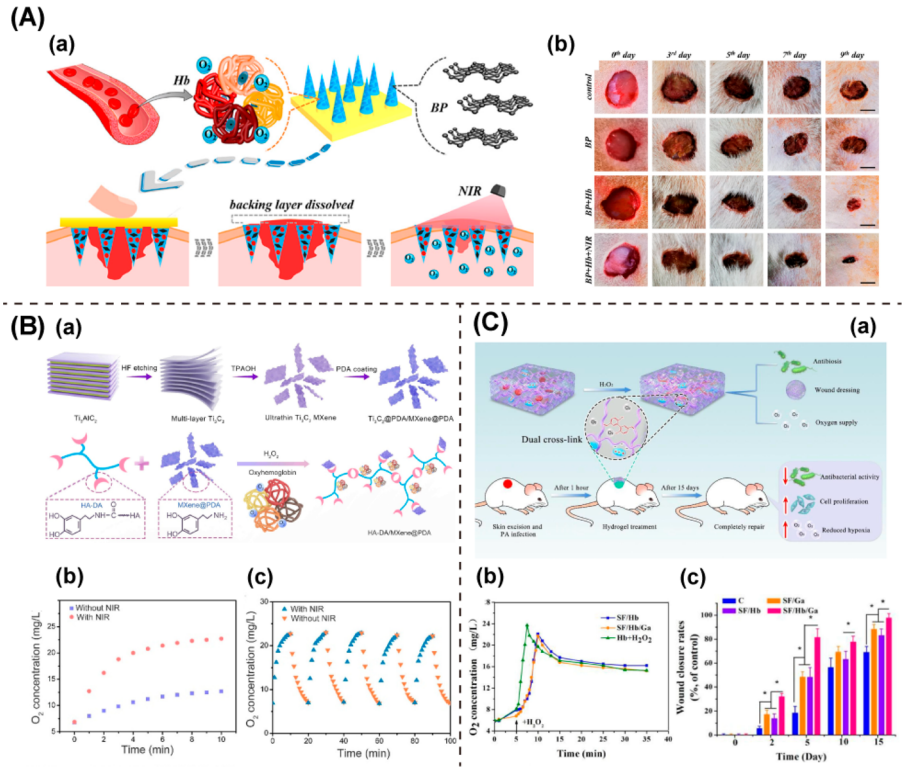


Figure 8. Hemoglobin-based oxygen carrier embedded dressings. (A) (a) The structure of separable microneedle wound dressing; (b) Wound repairing photos during treatment. Reprinted from ref 83. Copyright 2020 American Chemical Society. (B) (a) Illustrations of MXene@PDA nanosheets and injectable HA-DA/MXene@PDA hydrogel synthesis; (b) O_2 release under NIR irradiation; (c) repetitive O_2 generation during on/off NIR. Reprinted from ref 84. Copyright 2022 American Chemical Society. (C) (a) SF/Hb/Ga hybrid hydrogel promotes wound healing by hypoxia mitigation and antibacterial function; (b) O_2 production through Hb-mediated H_2O_2 decomposition; (c) wound closure rate within 15 days. Reprinted from ref 85 with permission. Copyright 2021 Elsevier.

PFCs have also been utilized alongside growth factors incorporated into hydrogel wound dressings to create synergistic benefits for wound healing. Jee and colleagues loaded four different growth factors, quercetin antioxidant, and 1-bromoperfluorooctane into a Carbopol and polyethylene glycol gel matrix.[75] Treatment with the complete hydrogel resulted in almost 50% greater wound recovery than the control group during in vitro scratch experiments, as shown in Figure 7C. In full thickness diabetic wound models, more rapid maturation of follicles and glands in the healed skin was attributed to the addition of growth factors, and improved organization of collagen fibers was attributed to increased wound oxygen from the PFCs. Lee and Lin also included PFC nanoemulsions into a hydrogel dressing along with epidermal growth factor (EGF)-loaded nanoparticles and polyhexamethylene biguanide to supplement oxygen, facilitate growth, and provide antimicrobial effects, respectively.[76] Groups treated with the complete gel saw increased cell proliferation and reduced IL-8 concentration in vitro and yielded the greatest degree of wound closure in vivo after 15 days (Figure 7D a,b). The success of these studies supports the combination of PFCs and growth factors in future approaches to wound healing.

However, PFCs have not been authorized by the FDA for use in wound management. One of the main concerns with PFCs includes their potential side effects. Studies have shown that PFCs entrapment can be years, which causes long-term adverse events and chronic tissue reactions.[80,81] Additionally, the long-term effects of PFCs on the environment are not well understood. PFCs are known to persist in the environment and may contribute to the depletion of the ozone layer and global warming.[82] The indiscriminate and improper disposal of wound dressings containing PFCs may present environmental challenges of uncertain nature and magnitude.

7. HEMOGLOBIN-BASED OXYGEN CARRIERS EMBEDDED DRESSINGS

Hemoglobin (Hb) is the primary protein in red blood cells that is responsible for oxygen delivery to tissues. It is composed of 4 subunits, each of which contains a heme group that contains a porphyrin ring structure with the iron atom in the center, which can bind to O_2 molecules. Hemoglobin-based oxygen carriers (HBOCs) have been developed for the potential treatment of multiple ischemic conditions/diseases that arise due to oxygen deficit such as cancer, anemia, ischemia, and wound healing. HBOCs are engineered to mimic the oxygen-carrying capacity of red blood cells and can be used as a substitute for red blood cells.

In wound healing, hemoglobin-embedded wound dressings have been shown to improve the healing process by delivering O_2 to the wound bed, promoting cell proliferation, reducing inflammation, and preventing infection. Due to the high oxygen-carrying capacity of hemoglobin, large bursts of oxygen may lead to increased oxidative stress and cellular damage; therefore, controllable oxygen release is important for wound healing. Hence, Zhang et al. proposed a separable microneedle with a polyvinyl acetate (PVA) backing layer and gelatin methacryloyl (GelMA) tips loading with black phosphorus

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

Table 2. Summary of Photosynthetic Bacteria-Integrated Wound Dressing[a]

Photosynthetic bacteria	Loading methods	Dressing material	Co-loaded drug	Cell model studies	Animal model studies	Mechanism	ref
C. reinhardtii	Direct adhesion on the matrix	Integra matrix		3T3		Oxygen supply to decrease HIF-1α expression	95
C. reinhardtii	Dispersed in fibrinogen	Integra matrix			Full-skin defect model in nu/nu mice	Oxygen supply to increase vascularization levels	96
S. elongatus	Dispersed in sodium alginate solution	Polyurethane film, 0.22 μm porous polytetrafluoroethylene membrane		HUVECs, HaCaTs, HSFs	STZ-induced diabetic BALB/c mice model	Oxygen supply to promote cell proliferation, angiogenesis, and cell migration	97
S. elongatus	Dispersed in agarose/CMCS solution		PCN-224	L-929, HCECs	Full-thickness wound Sprague−Dawley rats model; STZ-induced, MRSA infected diabetic BALB/c mice model	ROS production to enhance PDT against bacterial infection; oxygen supply to improve angiogenesis and eliminate inflammation	102
S. platensis	Dispersed in chitosan solution			HaCaTs, Epithelial cells	*S. aureus* infected wound mice model	Enhanced PDT to sterilize bacteria; hypoxia mitigation	98
S. platensis	Dispersed in SA/CMCS solution		Berberine	HFF-1	STZ-induced diabetic BALB/c mice model	Inhibited quorum sensing to kill bacteria; hypoxia mitigation	101
C. vulgaris	Dispersed in GelMa solution	PVA substrate with the GelMa microneedles		3T3, HUVECs	DB/DB mice model	Oxygen supply to promote cell proliferation, angiogenesis, and cell migration	100
Chlorella sp.	Dispersed in HA solution			HUVECs, HaCaTs, L-929	Diabetic BALB/c mice model	Oxygen supply to decrease ROS, promote angiogenesis and anti-inflammation	99
Chlorella pyrenoidosa	Dispersed in 3D-printable GelMA/SA	In situ bioprinting		HSFs, HUVECs	STZ-induced diabetic C57BL/6 mice model	Oxygen supply to increase angiogenesis and promote collagen synthesis	103

[a]STZ: streptozotocin, 3T3: mouse embryonic fibroblasts; HUVECs: human umbilical cord derived endothelial cells; HaCaTs: human immortalized keratinocytes; HSFs: human skin fibroblasts; L-929: mouse skin fibroblast; HCECs: human corneal epithelial cells; HFF-1: human foreskin fibroblasts-1.

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

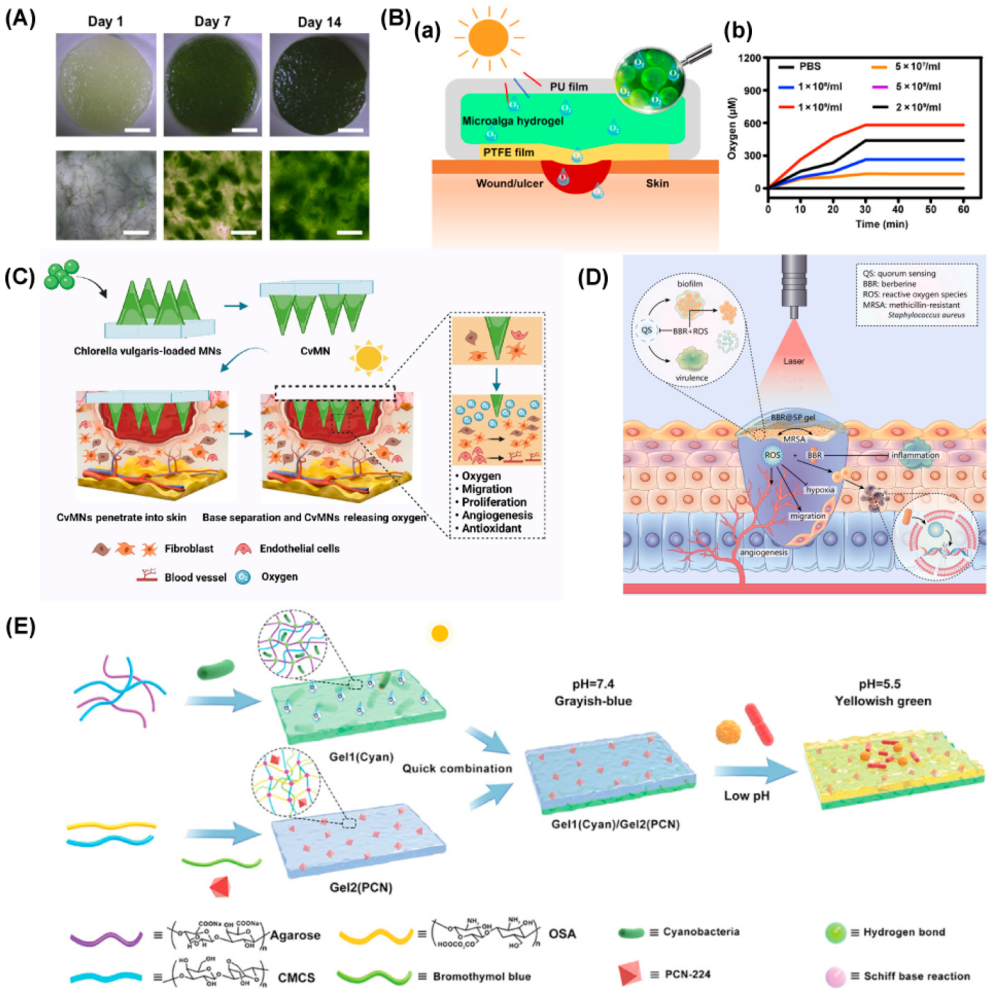


Figure 9. Photosynthetic bacteria impregnated wound dressings. (A) *C. reinhardtii* dispersed collagen-based scaffold; algae grow within 14 days. Reprinted from ref 95 with permission. Copyright 2014 Elsevier. (B) (a) Illustration of *S. elongates* alginate hydrogel embedded patch dressing to produce oxygen under light irradiation; (b) O_2 production of different algae concentration. Reprinted from ref 97 with permission. Copyright 2020 American Association for the Advancement of Science. (C) CvMN microneedles release O_2 and enhance wound healing. Reprinted from ref 100. Copyright 2023 American Chemical Society. (D) Illustration of BBR@SP gel that can destroy bacteria biofilms and mitigate hypoxia via combined chemo-photodynamic therapy. Reprinted from ref 101 with permission. Copyright 2022 Elsevier. (E) The structures of Gel1 and Gel2. Reprinted and modified from ref 102. Copyright 2022 Wiley-VCH.

(BP) and hemoglobin[83] (Figure 8A). The microneedle wound dressing has a responsive oxygen release ability, attributed to the photothermal effect of BP and reversible oxygen binding property of Hb. The results suggested that through near-infrared (NIR) irradiation, oxygen release was accelerated and the amount of oxygen released can be significantly increased. In a full-thickness cutaneous wound type I diabetes rat model, the microneedle treated wounds with NIR irradiation exhibited a better recovery compared with other groups. All of these indicated the potential healing ability of microneedles.

In order to further explore the reversible oxygen release property in Hb, Li and colleagues have reported a photothermal-controlled oxygen releasing hydrogel incorporated with an antioxidant nanosheet and oxygenated-hemoglobin for diabetic wound healing.[84] As shown in Figure 8B, the injectable hydrogel fabrication is based on hyaluronic acid-*graft*-dopamine (HA-DA) and polydopamine (PDA) coated Ti_3C_2 MXene nanosheets, which are catalytically cross-linked by an oxyhemoglobin/hydrogen (HbO_2/H_2O_2) system. Under NIR irradiation, the production of a mild heat stimulated HbO_2 releases oxygen in a controllable manner, where an increase in temperature can reduce the oxygen binding ability of hemoglobin (Hb) and therefore promote oxygen release. Here, HbO_2 can recurrently bind to oxygen when the NIR is off. Additionally, MXene nanosheets have been proven to function as a nonenzymatic antioxidant to remove excessive reactive nitrogen species (RNS) and ROS, alleviating oxidative stress, and eliminating bacteria, thereby preventing infection. The present study investigated the ability of the hydrogel to regulate oxygen release using NIR irradiation. The results indicated that without NIR irradiation, the oxygen release from the hydrogel was limited, with a maximum oxygen concentration of only 12.7 mg/mL. However, when NIR interference was applied, there was a burst release of oxygen, with the oxygen content reaching as high as 22.7 mg/mL (Figure 8B b). To promote wound healing, it is beneficial that the oxygen release is reversible, allowing oxygen to be released under NIR-triggered heat and rebound when the temperature cools. The study found that the oxygen release behavior of the hydrogel was repeatable and could be activated by heat generated via NIR, as demonstrated by five consecutive on/off cycles of NIR (Figure 8B c). These findings suggest that the hydrogel with

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

NIR-triggered oxygen release could be a promising approach for wound oxygenation to promote wound healing.

In addition to directly carrying oxygen, Hb has also been reported to act as a peroxidase to catalyze the decomposition of hydrogen peroxide to produce oxygen in wound healing. Qian et al. utilized this property and proposed a silk fibroin (SF)-based hybrid hydrogel loaded with hemoglobin (Hb) and gallium (Ga).[85] Hb was used as a substitute for peroxidase to create a dual cross-linking network within the hydrogel by cross-linking the tyrosine groups of SF. Furthermore, as the mimic peroxidase, Hb could decompose H_2O_2 and produce O_2. Ga is recently proposed as an antimicrobial metal with a broad antibacterial spectrum. The resulting SF/Hb/Ga hybrid hydrogel was validated to have multifunctional properties of antibacteria and hypoxia alleviation for infectious diabetic wound healing (Figure 8C). The results showed that after the addition of H_2O_2, both SF/Hb/Ga and SF/Hb hydrogels can substantially generate a large amount of O_2 (~20 mg/L) (Figure 8C b). In a *P. aeruginosa* infected, full-thickness diabetic wound rat model, the SF/Hb/Ga hybrid hydrogel indicated the most efficient therapeutic effect, with a closure rate of 95 ± 3.1% (Figure 8C c). This work proposed a new view that Hb can act as a mimic peroxidase, favoring not only hydrogel cross-linking but also O_2 production due to H_2O_2 decomposition.

8. PHOTOSYNTHETIC BACTERIA-INTEGRATED WOUND DRESSING

Photosynthetic biomaterials incorporate living microorganisms, such as algae or cyanobacteria, into the structure.[86] These materials have the ability for carbon dioxide fixation, which leads to oxygenic photosynthesis to produce oxygen, nutrients, and other byproducts under visible light irradiation.[87] In this process, oxygen can be produced sustainably, and it is well-known that the primary source of atmospheric oxygen for living organisms can be produced by oxygenic photosynthesis.[88] Photosynthetic microalgae have recently gained much importance for their potential application in wound healing since microalgae produce not only oxygen but also compounds with anti-inflammatory and antimicrobial properties, which can favor infection prevention and promote the healing process through oxygenation.[89] In addition, some species of microalgae have been shown to produce extracellular polysaccharides that can stimulate the growth of skin cells and enhance wound closure.[90] Furthermore, chlorophyll as a natural photosensitizer can photodynamically generate reactive oxygen species (ROS) against bacterial infection,[91−93] which is beneficial for wound healing. The recent strategies based on various microalgae for wound healing are summarized in Table 2.

Microalgae administered orally or topically has been previously reported to accelerate wound healing via hypoxia mitigation and increased angiogenesis.[94] Subsequently, research on microalgae-embedded scaffolds as wound healing dressings was initially conducted by Hopfner and colleagues.[95,96] They developed an integrated unicellular alga *Chlamydomonas reinhardtii* (*C. reinhardtii*) collagen-based scaffold (Integra matrix), as shown in Figure 9A. The *C. reinhardtii* distributed in the inner cavities of the matrix and proliferated in ~14 days. Meanwhile, the incorporated scaffold showed superior time- and microalgae-concentration-dependent photosynthetic oxygen production ability under light illumination. *In vitro* fibroblasts coculture studies under hypoxic conditions revealed that microalgae did significantly increase oxygen concentration by 26.1%, and decrease the hypoxia-inducible factor-1α (HIF-1α) expression.[95] In another work, they designed the algae−fibrinogen incorporated scaffold, which was demonstrated to result in high vascularization levels in full-skin defects athymic nude mice model, suggesting the potential of promoting wound healing diven by microalgae.[96]

Chen et al. developed a *Synechococcus elongates* (*S. elongates*) alginate hydrogel embedded patch dressing to produce oxygen for diabetic chronic wound healing[97] (Figure 9B a). The oxygen concentration was shown to increase to 600 μM in 30 min of red-light irradiation (Figure 9B b). In vivo diabetic mouse studies revealed the enhanced skin flap regeneration and angiogenesis of the alga-gel dressing. This study provided an advanced perspective of photosynthetic microalgae−hydrogel. Accordingly, there are several algae-loaded hydrogels designed for wound oxygen supply. Li and colleagues fabricated a novel *Spirulina platensis* containing chitosan hydrogel, which was verified to not only relieve wound hypoxia but also eliminate *Staphylococcus aureus* (*S. aureus*) infection in a mice wound model, suggesting that microalgae has excellent potential to combat bacteria-infected chronic wounds.[98] Another *Chlorella* sp. (Chlorella)-based bioactive hydrogel was created for diabetic wound healing via circadian regulation mode, which allowed oxygenation and ROS depletion during daytime, while inactivated algae at night can provide nutrition and inflammation relief.[99] The in vitro and in vivo results demonstrated the ameliorative diabetic wound microenvironment rendered by microalgae, including hypoxia, extra ROS, and inflammation, which confirmed the potential of microalgae as an effective strategy for diabetic chronic wound healing. Zhao and colleagues reported a separable microneedle, called CvMN, consisting of a PVA backing layer and GelMA tips loaded with active *Chlorella vulgaris* (Cv).[100] The CvMN microneedles are applied to the diabetic wound, where the PVA substrate dissolves and the Cv-encapsulated GelMA hydrogel tips remain in the skin (Figure 9C). The system provides a continuous, controlled supply of oxygen, promoting cell proliferation, migration, and angiogenesis in hypoxic wounds, while the Cv also yielded antioxidant properties that reduce inflammation. The CvMN system exhibited an effective therapeutic effect in a diabetic mice model. The studies of active microalgae-embedded wound dressings present a promising new strategy for treating chronic diabetic wounds and addresses the limitations of the current oxygen delivery systems.

Multifunctional wound dressings coloaded with microalgae and other therapeutics were widely explored for combined wound healing therapies. Hu and colleagues reported a carboxymethyl chitosan (CMCS)/sodium alginate (SA) hybrid hydrogel loaded with *Spirulina platensis* (*S. platensis*) and quorum sensing inhibitor, berberine (BBR@SP gel).[101] As illustrated in Figure 9D, berberine blocked the pathway by which bacterial species interact, also known as quorum sensing, inhibiting the formation of biofilms and killing methicillin-resistant *Staphylococcus aureus* (MRSA). Meanwhile, the coloaded *S. platensis* swiftly produced oxygen under 650 nm laser irradiation, which induced ROS generation and enhanced activity against MRSA. The BBR@SP gel was confirmed not only to destroy bacteria biofilms but also to mitigate hypoxia via combined chemo-photodynamic therapy. Their work proposed an efficacious multifunctional strategy comprising of antibacterial, reoxygenation, and anti-inflammation, benefi-

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

cial for MRSA-infected diabetic wound healing. In another study as shown in Figure 9E, Zhu et al. described a multidrug loaded, double-layered hydrogel, which incorporated *S. elongates* in the outer layer (Gel1) and a photosensitizer PCN-224 with a pH indicator in the inner layer (Gel2).[102] The Gel1 and Gel1/Gel2 performed high-level photosynthetic oxygen production and continuous generation for up to 21 days. Meanwhile, due to the acidification resulting from bacteria growth, the color of Gel1/Gel2 changed during bacterial infection, which is favorable for real-time monitoring of chronic wounds. These studies provided a new perspective for a potential combination between microalgae and photodynamic therapy in bacteria-infected wound healing.

Current efforts have examined the development of microalgae in 3D-printed scaffolds to promote wound healing. Wang et al. employed a hollow fibrous scaffold containing live microalgae (*Chlorella pyrenoidosa*) via a microfluidic chip-based 3D printing strategy.[103] The microalgae exhibited high viability and increased cell numbers within 7 days. The resulting scaffolds showed effective oxygen production under light irradiation. In the diabetic C57BL/6 mice model, in situ bioprinting of scaffolds was performed to accelerate collagen deposition, improve angiogenesis, and relieve tissue hypoxia, which are essential features in wound healing. Since 3D printed wound dressing provides significant flexibility in the treatment by allowing for the deposition of scaffolds onto irregular-shaped defects, microalgae-laden 3D printing hydrogels can be a promising strategy in wound management.

Despite demonstrating beneficial photosynthetic oxygen generating properties, as well as promoting wound healing in various wound models, the integration of microalgae into wound dressings poses the potential risk of inducing immunogenic responses that could compromise clinical trials. The negative outcomes associated with microalgae-induced immunogenicity include allergic reactions and reduced drug half-life,[104] which should be considered in the development and implementation of microalgae-based wound dressings. It is possible that in the future engineered microalgae could be designed to minimize immunogenic responses while optimizing the production of oxygen, thereby mitigating the potential clinical issues.

9. PERSPECTIVES AND CONCLUSIONS

Wound healing is a complex process that involves cell proliferation, angiogenesis, and matrix remodeling. Oxygen plays a critical role in the different stages of the healing process. The importance of oxygen in wound healing has been well documented since the late 1960s. Hypoxia can induce an acidic pH environment, increased ROS production, inhibited angiogenesis, and impaired immune function. Oxygenated wound dressings are a promising strategy that can accelerate wound healing in clinical wound management. This review provides an overview of currently developed oxygenated-wound dressing materials and their mechanisms. While several oxygen-releasing materials have been recently developed, including catalase-based material, nanoenzyme, metal peroxides, hemoglobin, PFC-based material, and photosynthetic biomaterials, challenges exist that need to be addressed.

From a basic science perspective, development of new materials for prolonged oxygen release in the wounded area is critical. It is important to ensure that oxygen is released in a controlled manner from dressings since either hypoxic or hyperoxic conditions may result in cellular damage. The mechanism of interaction between oxygen encapsulation, dressing materials, and tissue properties needs to be further explored. In the future, multifunctional sensors could be included in advanced oxygenated wound dressings to monitor a panel of parameters such as oxygen levels, pH, and bacterial infections in the wound area. While progress has been made in the development of such technologies, an approach that offers all of these features in a single design is still technically challenging.

From clinical perspectives, the next generation of oxygenated wound dressings should ensure minimal side effects and low cytotoxicity. For HBOCs and peroxides, it is important to develop strategies to prevent excessive ROS generation. For PFCs material, it is imperative to gain a comprehensive understanding of the adverse effects in humans and the mechanisms of tissue interaction. Additionally, it is crucial to elucidate environmental repercussions resulting from the utilization of PFC-containing wound dressings. For photosynthetic bacteria material, the potential immunogenic response must be avoided. The favorable biocompatibility of oxygen nanobubbles (ONBs) may render it a potential alternative oxygen-delivery material for incorporation into wound dressings in the future.[105] However, strategies to efficiently control the delivery of oxygen to avoid side effects should be a priority. Finally, a standardized method for the measurement of oxygen concentration and release rates will help to determine the optimal oxygen delivery rates for specific wounds (acute vs chronic wounds) to maximize healing and increase safety.

AUTHOR INFORMATION

Corresponding Author

Joseph Irudayaraj − *Department of Bioengineering, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States; Cancer Center at Illinois, Urbana, Illinois 61801, United States; Biomedical Research Center, Mills Breast Cancer Institute, Carle Foundation Hospital, Urbana, Illinois 61801, United States; Beckman Institute, Holonyak Micro and Nanotechnology Laboratory, Carle R. Woese Institute for Genomic Biology, Urbana, Illinois 61801, United States;* orcid.org/0000-0002-0630-1520; Phone: (217) 300-0525; Email: jirudaya@illinois.edu

Authors

Xiaoxue Han − *Department of Bioengineering, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States; Cancer Center at Illinois, Urbana, Illinois 61801, United States; Biomedical Research Center, Mills Breast Cancer Institute, Carle Foundation Hospital, Urbana, Illinois 61801, United States; Beckman Institute, Holonyak Micro and Nanotechnology Laboratory, Carle R. Woese Institute for Genomic Biology, Urbana, Illinois 61801, United States*

Leah Suyeon Ju − *Department of Bioengineering, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States; Biomedical Research Center, Mills Breast Cancer Institute, Carle Foundation Hospital, Urbana, Illinois 61801, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.molpharmaceut.3c00352

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

Funding

We acknowledge partial funding from the CSTAR fellowship for XH.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Saghazadeh, S.; Rinoldi, C.; Schot, M.; Kashaf, S. S.; Sharifi, F.; Jalilian, E.; Nuutila, K.; Giatsidis, G.; Mostafalu, P.; Derakhshandeh, H.; et al. Drug delivery systems and materials for wound healing applications. *Adv. Drug Delivery Rev.* **2018**, *127*, 138−166.
(2) Sen, C. K. Human Wounds and Its Burden: An Updated Compendium of Estimates. *Adv. Wound Care* **2019**, *8* (2), 39−48.
(3) Guo, S. a.; DiPietro, L. A. Factors affecting wound healing. *J. Dent. Res.* **2010**, *89* (3), 219−229.
(4) Ahluwalia, A.; arnawski, A. S. Critical Role of Hypoxia Sensor - HIF-1o in VEGF Gene Activation. Implications for Angiogenesis and Tissue Injury Healing. *Curr. Med. Chem.* **2012**, *19* (1), 90−97.
(5) Hunt, T. K. The physiology of wound healing. *Ann. Emerg. Med.* **1988**, *17* (12), 1265−1273.
(6) Han, G.; Ceilley, R. Chronic Wound Healing: A Review of Current Management and Treatments. *Adv. Ther.* **2017**, *34* (3), 599−610.
(7) Frykberg, R. G. Topical Wound Oxygen Therapy in the Treatment of Chronic Diabetic Foot Ulcers. *Medicina* **2021**, *57* (9), 917.
(8) Li, R.; Liu, K.; Huang, X.; Li, D.; Ding, J.; Liu, B.; Chen, X. Bioactive Materials Promote Wound Healing through Modulation of Cell Behaviors. *Advanced Science* **2022**, *9* (10), 2105152.
(9) Kolarsick, P. A. J.; Kolarsick, M. A.; Goodwin, C. Anatomy and Physiology of the Skin. *Journal of the Dermatology Nurses' Association* **2011**, *3* (4), 203.
(10) Wilkinson, H. N.; Hardman, M. J. Wound healing: cellular mechanisms and pathological outcomes. *Open Biology* **2020**, *10* (9), 200223.
(11) Broughton, G.; Janis, J. E.; Attinger, C. E. The Basic Science of Wound Healing. *Plast. Reconstr. Surg.* **2006**, *117* (7S), 12S.
(12) Velnar, T.; Bailey, T.; Smrkolj, V. The wound healing process: an overview of the cellular and molecular mechanisms. *J. Int. Med. Res.* **2009**, *37* (5), 1528−1542.
(13) Singh, S.; Young, A.; McNaught, C.-E. The physiology of wound healing. *Surgery (Oxford)* **2017**, *35* (9), 473−477.
(14) Strodtbeck, F. Physiology of wound healing. *Newborn Infant. Nurs. Rev.* **2001**, *1* (1), 43−52.
(15) Stadelmann, W. K.; Digenis, A. G.; Tobin, G. R. Physiology and healing dynamics of chronic cutaneous wounds. *American journal of surgery* **1998**, *176* (2), 26S−38S.
(16) Landén, N. X.; Li, D.; Ståhle, M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell. Mol. Life Sci.* **2016**, *73* (20), 3861−3885.
(17) Pastar, I.; Stojadinovic, O.; Yin, N. C.; Ramirez, H.; Nusbaum, A. G.; Sawaya, A.; Patel, S. B.; Khalid, L.; Isseroff, R. R.; Tomic-Canic, M. Epithelialization in Wound Healing: A Comprehensive Review. *Adv. Wound Care* **2014**, *3* (7), 445−464.
(18) Liang, Y.; He, J.; Guo, B. Functional Hydrogels as Wound Dressing to Enhance Wound Healing. *ACS Nano* **2021**, *15* (8), 12687−12722.
(19) Castilla, D. M.; Liu, Z.-J.; Velazquez, O. C. Oxygen: Implications for Wound Healing. *Adv. Wound Care* **2012**, *1* (6), 225−230.
(20) Schreml, S.; Szeimies, R. M.; Prantl, L.; Karrer, S.; Landthaler, M.; Babilas, P. Oxygen in acute and chronic wound healing. *Br. J. Dermatol.* **2010**, *163* (2), 257−268.
(21) Schade, H.; Marchionini, A. Der Säuremantel der Haut (Nach Gaskettenmessungen). *Klin. Wochenschr.* **1928**, *7* (1), 12−14.
(22) Rodriguez, P. G.; Felix, F. N.; Woodley, D. T.; Shim, E. K. The Role of Oxygen in Wound Healing: A Review of the Literature. *Dermatol. Surg.* **2008**, *34* (9), 1159−1169.
(23) Raju, R.; Weiner, M.; Enquist, I. F. Quantitation of local acidosis and hypoxia produced by infection. *American Journal of Surgery* **1976**, *132* (1), 64−66.
(24) Schneider, L. A.; Korber, A.; Grabbe, S.; Dissemond, J. Influence of pH on wound-healing: a new perspective for wound-therapy? *Archives of Dermatological Research* **2007**, *298* (9), 413−420.
(25) Hoenig, M. R.; Bianchi, C.; Sellke, F. W. Hypoxia inducible factor-1α, endothelial progenitor cells, monocytes, cardiovascular risk, wound healing, cobalt and hydralazine: a unifying hypothesis. *Curr. Drug Targets* **2008**, *9* (5), 422−435.
(26) Eaton, L.; Pamenter, M. E. What to do with low O2: Redox adaptations in vertebrates native to hypoxic environments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **2022**, *271*, 111259.
(27) Fuhrmann, D. C.; Brüne, B. Mitochondrial composition and function under the control of hypoxia. *Redox Biology* **2017**, *12*, 208−215.
(28) Sen, C. K. Wound healing essentials: Let there be oxygen. *Wound Repair Regen.* **2009**, *17* (1), 1−18.
(29) Thackham, J. A.; McElwain, D. L. S.; Long, R. J. The use of hyperbaric oxygen therapy to treat chronic wounds: A review. *Wound Repair Regen.* **2008**, *16* (3), 321−330.
(30) Lewis, C.; Murdoch, C. Macrophage Responses to Hypoxia: Implications for Tumor Progression and Anti-Cancer Therapies. *American Journal of Pathology* **2005**, *167* (3), 627−635.
(31) Mantovani, A.; Biswas, S. K.; Galdiero, M. R.; Sica, A.; Locati, M. Macrophage plasticity and polarization in tissue repair and remodelling. *Journal of Pathology* **2013**, *229* (2), 176−185.
(32) Louiselle, A. E.; Niemiec, S. M.; Zgheib, C.; Liechty, K. W. Macrophage polarization and diabetic wound healing. *Translational Research* **2021**, *236*, 109−116.
(33) Yunna, C.; Mengru, H.; Lei, W.; Weidong, C. Macrophage M1/M2 polarization. *Eur. J. Pharmacol.* **2020**, *877*, 173090.
(34) Allen, D. B.; Maguire, J. J.; Mahdavian, M.; Wicke, C.; Marcocci, L.; Scheuenstuhl, H.; Chang, M.; Le, A. X.; Hopf, H. W.; Hunt, T. K. Wound Hypoxia and Acidosis Limit Neutrophil Bacterial Killing Mechanisms. *Arch. Surg.* **1997**, *132* (9), 991−996.
(35) Nishikawa, M.; Hashida, M.; Takakura, Y. Catalase delivery for inhibiting ROS-mediated tissue injury and tumor metastasis. *Adv. Drug Delivery Rev.* **2009**, *61* (4), 319−326.
(36) Han, X.; Jeong, Y.; Irudayaraj, J. Nanocatalase-Based Oxygen-Generating Nanocarriers for Active Oxygen Delivery to Relieve Hypoxia in Pancreatic Cancer. *ACS Applied Nano Materials* **2022**, *5* (11), 17248−17257.
(37) Haney, M. J.; Klyachko, N. L.; Zhao, Y.; Gupta, R.; Plotnikova, E. G.; He, Z.; Patel, T.; Piroyan, A.; Sokolsky, M.; Kabanov, A. V.; et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J. Controlled Release* **2015**, *207*, 18−30.
(38) Singhal, A.; Morris, V. B.; Labhasetwar, V.; Ghorpade, A. Nanoparticle-mediated catalase delivery protects human neurons from oxidative stress. *Cell Death Dis.* **2013**, *4* (11), No. e903-e903.
(39) Bryan, N.; Ahswin, H.; Smart, N.; Bayon, Y.; Wohlert, S.; Hunt, J. A. Reactive oxygen species (ROS)−a family of fate deciding molecules pivotal in constructive inflammation and wound healing. *Eur. Cell Mater.* **2012**, *24* (249), No. e65.
(40) Kurahashi, T.; Fujii, J. Roles of Antioxidative Enzymes in Wound Healing. *Journal of Developmental Biology* **2015**, *3* (2), 57−70.
(41) Arya, A. K.; Pokharia, D.; Tripathi, K. Relationship between oxidative stress and apoptotic markers in lymphocytes of diabetic patients with chronic non healing wound. *Diabetes Res. Clin. Pract.* **2011**, *94* (3), 377−384.
(42) Cano Sanchez, M.; Lancel, S.; Boulanger, E.; Neviere, R. Targeting Oxidative Stress and Mitochondrial Dysfunction in the Treatment of Impaired Wound Healing: A Systematic Review. *Antioxidants* **2018**, *7*, 98.
(43) Guan, Y.; Niu, H.; Liu, Z.; Dang, Y.; Shen, J.; Zayed, M.; Ma, L.; Guan, J. Sustained oxygenation accelerates diabetic wound healing by promoting epithelialization and angiogenesis and decreasing inflammation. *Science Advances* **2021**, *7* (35), No. eabj0153.

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355

(44) Wang, Q.; Qiu, W.; Liu, H.; Li, X.; Qin, X.; Wang, X.; Yu, J.; Li, B.; Li, F.; Huang, L.; et al. Conductive hydrogel dressings based on cascade reactions with photothermal effect for monitoring and treatment of diabetic wounds. *Composites Part B: Engineering* **2022**, *242*, 110098.
(45) Kang, J. I.; Park, K. M. Oxygen-supplying syringe to create hyperoxia-inducible hydrogels for in situ tissue regeneration. *Biomaterials* **2023**, *293*, 121943.
(46) Yu, Z.; Lou, R.; Pan, W.; Li, N.; Tang, B. Nanoenzymes in disease diagnosis and therapy. *Chem. Commun.* **2020**, *56* (99), 15513−15524.
(47) Nandi, A.; Yan, L.-J.; Jana, C. K.; Das, N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 9613090.
(48) Li, Y.; Fu, R.; Duan, Z.; Zhu, C.; Fan, D. Construction of multifunctional hydrogel based on the tannic acid-metal coating decorated MoS2 dual nanozyme for bacteria-infected wound healing. *Bioactive Materials* **2022**, *9*, 461−474.
(49) Li, Y.; Fu, R.; Duan, Z.; Zhu, C.; Fan, D. Adaptive Hydrogels Based on Nanozyme with Dual-Enhanced Triple Enzyme-Like Activities for Wound Disinfection and Mimicking Antioxidant Defense System. *Adv. Healthcare Mater.* **2022**, *11* (2), 2101849.
(50) Li, Y.; Fu, R.; Duan, Z.; Zhu, C.; Fan, D. Injectable Hydrogel Based on Defect-Rich Multi-Nanozymes for Diabetic Wound Healing via an Oxygen Self-Supplying Cascade Reaction. *Small* **2022**, *18* (18), 2200165.
(51) Wang, S.; Zheng, H.; Zhou, L.; Cheng, F.; Liu, Z.; Zhang, H.; Wang, L.; Zhang, Q. Nanoenzyme-Reinforced Injectable Hydrogel for Healing Diabetic Wounds Infected with Multidrug Resistant Bacteria. *Nano Lett.* **2020**, *20* (7), 5149−5158.
(52) Tu, C.; Lu, H.; Zhou, T.; Zhang, W.; Deng, L.; Cao, W.; Yang, Z.; Wang, Z.; Wu, X.; Ding, J.; et al. Promoting the healing of infected diabetic wound by an anti-bacterial and nano-enzyme-containing hydrogel with inflammation-suppressing, ROS-scavenging, oxygen and nitric oxide-generating properties. *Biomaterials* **2022**, *286*, 121597.
(53) Li, Z.; Zhao, Y.; Huang, H.; Zhang, C.; Liu, H.; Wang, Z.; Yi, M.; Xie, N.; Shen, Y.; Ren, X.; et al. A Nanozyme-Immobilized Hydrogel with Endogenous ROS-Scavenging and Oxygen Generation Abilities for Significantly Promoting Oxidative Diabetic Wound Healing. *Adv. Healthcare Mater.* **2022**, *11* (22), 2201524.
(54) Ren, C.; Li, D.; Zhou, Q.; Hu, X. Mitochondria-targeted TPP-MoS2 with dual enzyme activity provides efficient neuroprotection through M1/M2 microglial polarization in an Alzheimer's disease model. *Biomaterials* **2020**, *232*, 119752.
(55) Sharifi, M.; Faryabi, K.; Talaei, A. J.; Shekha, M. S.; Ale-Ebrahim, M.; Salihi, A.; Nanakali, N. M. Q.; Aziz, F. M.; Rasti, B.; Hasan, A.; et al. Antioxidant properties of gold nanozyme: A review. *J. Mol. Liq.* **2020**, *297*, 112004.
(56) Tang, M.; Zhang, Z.; Sun, T.; Li, B.; Wu, Z. Manganese-Based Nanozymes: Preparation, Catalytic Mechanisms, and Biomedical Applications. *Adv. Healthcare Mater.* **2022**, *11* (21), 2201733.
(57) Hu, H.; Yu, L.; Qian, X.; Chen, Y.; Chen, B.; Li, Y. Chemoreactive Nanotherapeutics by Metal Peroxide Based Nanomedicine. *Advanced Science* **2021**, *8* (1), 2000494.
(58) dos Santos, D. M.; Dias, L. M.; Surur, A. K.; de Moraes, D. A.; Pavarina, A. C.; Fontana, C. R.; Correa, D. S. Electrospun Composite Bead-on-String Nanofibers Containing CaO2 Nanoparticles and MnO2 Nanosheets as Oxygen-Release Systems for Biomedical Applications. *ACS Applied Nano Materials* **2022**, *5* (10), 14425−14436.
(59) He, J.; Fu, L.-H.; Qi, C.; Lin, J.; Huang, P. Metal peroxides for cancer treatment. *Bioactive Materials* **2021**, *6* (9), 2698−2710.
(60) Balaji, S.; Short, W. D.; Padon, B. W.; Belgodere, J. A.; Jimenez, S. E.; Deoli, N. T.; Guidry, A. C.; Green, J. C.; Prajapati, T. J.; Farouk, F.; et al. Engineering Antioxidant and Oxygen-Releasing Lignin Composites to Promote Wound Healing. *bioRxiv* **2022**, DOI: 10.1101/2022.03.18.484913.
(61) Camci-Unal, G.; Alemdar, N.; Annabi, N.; Khademhosseini, A. Oxygen-releasing biomaterials for tissue engineering. *Polym. Int.* **2013**, *62* (6), 843−848.
(62) Farris, A. L.; Rindone, A. N.; Grayson, W. L. Oxygen delivering biomaterials for tissue engineering. *J. Mater. Chem. B* **2016**, *4* (20), 3422−3432.
(63) Lu, S.; Zhang, X.; Xue, Y. Application of calcium peroxide in water and soil treatment: A review. *J. Hazard. Mater.* **2017**, *337*, 163−177.
(64) Khorshidi, S.; Karkhaneh, A.; Bonakdar, S. Fabrication of amine-decorated nonspherical microparticles with calcium peroxide cargo for controlled release of oxygen. *J. Biomed. Mater. Res., Part A* **2020**, *108* (1), 136−147.
(65) Garima; Agarwal, T.; Costantini, M.; Pal, S.; Kumar, A. Oxygenation therapies for improved wound healing: current trends and technologies. *J. Mater. Chem. B* **2022**, *10* (39), 7905−7923.
(66) He, Y.; Li, K.; Yang, X.; Leng, J.; Xu, K.; Yuan, Z.; Lin, C.; Tao, B.; Li, X.; Hu, J.; et al. Calcium Peroxide Nanoparticles-Embedded Coatings on Anti-Inflammatory TiO2 Nanotubes for Bacteria Elimination and Inflammatory Environment Amelioration. *Small* **2021**, *17* (47), 2102907.
(67) Kang, J. I.; Park, K. M.; Park, K. D. Oxygen-generating alginate hydrogels as a bioactive acellular matrix for facilitating wound healing. *Journal of Industrial and Engineering Chemistry* **2019**, *69*, 397−404.
(68) Shiekh, P. A.; Singh, A.; Kumar, A. Oxygen-Releasing Antioxidant Cryogel Scaffolds with Sustained Oxygen Delivery for Tissue Engineering Applications. *ACS Appl. Mater. Interfaces* **2018**, *10* (22), 18458−18469.
(69) Shiekh, P. A.; Singh, A.; Kumar, A. Exosome laden oxygen releasing antioxidant and antibacterial cryogel wound dressing OxOBand alleviate diabetic and infectious wound healing. *Biomaterials* **2020**, *249*, 120020.
(70) Singh, P.; Andrabi, S. M.; Tariq, U.; Gupta, S.; Shaikh, S.; Kumar, A. Functionally multifaceted scaffolds delivering bioactive compounds for treatment of infectious chronic and ischemic wounds. *Chemical Engineering Journal* **2023**, *457*, 141359.
(71) Yip, W. L. Influence of oxygen on wound healing. *International Wound Journal* **2015**, *12* (6), 620−624.
(72) Kirby, J. P.; Snyder, J.; Schuerer, D. J. E.; Peters, J. S.; Bochicchio, G. V. Essentials of Hyperbaric Oxygen Therapy: 2019 Review. *Mo. Med.* **2019**, *116* (3), 176−179.
(73) Patil, P. S.; Fountas-Davis, N.; Huang, H.; Michelle Evancho-Chapman, M.; Fulton, J. A.; Shriver, L. P.; Leipzig, N. D. Fluorinated methacrylamide chitosan hydrogels enhance collagen synthesis in wound healing through increased oxygen availability. *Acta Biomaterialia* **2016**, *36*, 164−174.
(74) Yang, Z.; Chen, H.; Yang, P.; Shen, X.; Hu, Y.; Cheng, Y.; Yao, H.; Zhang, Z. Nano-oxygenated hydrogels for locally and permeably hypoxia relieving to heal chronic wounds. *Biomaterials* **2022**, *282*, 121401.
(75) Jee, J.-P.; Pangeni, R.; Jha, S. K.; Byun, Y.; Park, J. W. Preparation and in vivo evaluation of a topical hydrogel system incorporating highly skin-permeable growth factors, quercetin, and oxygen carriers for enhanced diabetic wound-healing therapy. *Int. J. Nanomed.* **2019**, *14*, 5449−5475.
(76) Lee, Y.-H.; Lin, S.-J. Chitosan/PVA Hetero-Composite Hydrogel Containing Antimicrobials, Perfluorocarbon Nanoemulsions, and Growth Factor-Loaded Nanoparticles as a Multifunctional Dressing for Diabetic Wound Healing: Synthesis, Characterization, and In Vitro/In Vivo Evaluation. *Pharmaceutics* **2022**, *14* (3), 537.
(77) Riess, J. G. Perfluorocarbon-based Oxygen Delivery. *Artificial Cells, Blood Substitutes, and Biotechnology* **2006**, *34* (6), 567−580.
(78) Wang, W.; Wang, X.; Tao, F.; Hu, K.; Zhang, J.; Wu, J.; You, L.; Zhao, W. Fluorinated Hyaluronic Acid Encapsulated Perfluorocarbon Nanoparticles as Tumor-Targeted Oxygen Carriers to Enhance Radiotherapy. *Mol. Pharmaceutics* **2022**, *19* (11), 3948−3958.
(79) Wang, W.; Cheng, Y.; Yu, P.; Wang, H.; Zhang, Y.; Xu, H.; Ye, Q.; Yuan, A.; Hu, Y.; Wu, J. Perfluorocarbon regulates the

intratumoural environment to enhance hypoxia-based agent efficacy. *Nat. Commun.* **2019**, *10* (1), 1580.
(80) Castro, C. I.; Briceno, J. C. Perfluorocarbon-Based Oxygen Carriers: Review of Products and Trials. *Artif. Organs* **2010**, *34* (8), 622−634.
(81) Zhang, X.; Flaws, J. A.; Spinella, M. J.; Irudayaraj, J. The Relationship between Typical Environmental Endocrine Disruptors and Kidney Disease. *Toxics* **2023**, *11* (1), 32.
(82) Tsai, W.-T.; Chen, H.-P.; Hsien, W.-Y. A review of uses, environmental hazards and recovery/recycle technologies of perfluorocarbons (PFCs) emissions from the semiconductor manufacturing processes. *Journal of Loss Prevention in the Process Industries* **2002**, *15* (2), 65−75.
(83) Zhang, X.; Chen, G.; Liu, Y.; Sun, L.; Sun, L.; Zhao, Y. Black Phosphorus-Loaded Separable Microneedles as Responsive Oxygen Delivery Carriers for Wound Healing. *ACS Nano* **2020**, *14* (5), 5901−5908.
(84) Li, Y.; Fu, R.; Duan, Z.; Zhu, C.; Fan, D. Artificial Nonenzymatic Antioxidant MXene Nanosheet-Anchored Injectable Hydrogel as a Mild Photothermal-Controlled Oxygen Release Platform for Diabetic Wound Healing. *ACS Nano* **2022**, *16* (5), 7486−7502.
(85) Qian, Y.; Xu, C.; Xiong, W.; Jiang, N.; Zheng, Y.; He, X.; Ding, F.; Lu, X.; Shen, J. Dual cross-linked organic-inorganic hybrid hydrogels accelerate diabetic skin wound healing. *Chemical Engineering Journal* **2021**, *417*, 129335.
(86) Wang, Y.; Xue, Y.; Zhang, T.; Fang, Q.; Jin, M.; Wang, X.; Wang, Z.; Hu, Y.; Zhao, W.; Lou, D.; et al. Photosynthetic biomaterials: applications of photosynthesis in algae as oxygenerator in biomedical therapies. *Bio-Design and Manufacturing* **2021**, *4* (3), 596−611.
(87) Dawiec-Lisniewska, A.; Podstawczyk, D.; Bastrzyk, A.; Czuba, K.; Pacyna-Iwanicka, K.; Okoro, O. V.; Shavandi, A. New trends in biotechnological applications of photosynthetic microorganisms. *Biotechnology Advances* **2022**, *59*, 107988.
(88) Lyons, T. W.; Reinhard, C. T.; Planavsky, N. J. The rise of oxygen in Earth's early ocean and atmosphere. *Nature* **2014**, *506* (7488), 307−315.
(89) Zhong, D.; Du, Z.; Zhou, M. Algae: A natural active material for biomedical applications. *VIEW* **2021**, *2* (4), 20200189.
(90) Miguel, S. P.; Ribeiro, M. P.; Otero, A.; Coutinho, P. Application of microalgae and microalgal bioactive compounds in skin regeneration. *Algal Research* **2021**, *58*, 102395.
(91) Suvorov, N.; Pogorilyy, V.; Diachkova, E.; Vasil'ev, Y.; Mironov, A.; Grin, M. Derivatives of Natural Chlorophylls as Agents for Antimicrobial Photodynamic Therapy. *International Journal of Molecular Sciences* **2021**, *22* (12), 6392.
(92) Rastogi, R. P.; Singh, S. P.; Häder, D.-P.; Sinha, R. P. Detection of reactive oxygen species (ROS) by the oxidant-sensing probe 2′,7′-dichlorodihydrofluorescein diacetate in the cyanobacterium Anabaena variabilis PCC 7937. *Biochem. Biophys. Res. Commun.* **2010**, *397* (3), 603−607.
(93) Qiao, Y.; Yang, F.; Xie, T.; Du, Z.; Zhong, D.; Qi, Y.; Li, Y.; Li, W.; Lu, Z.; Rao, J. Engineered algae: A novel oxygen-generating system for effective treatment of hypoxic cancer. *Science Advances* **2020**, *6* (21), No. eaba5996.
(94) Hidalgo-Lucas, S.; Bisson, J.-F.; Duffaud, A.; Nejdi, A.; Guerin-Deremaux, L.; Baert, B.; Saniez-Degrave, M.-H.; Rozan, P. Benefits of Oral and Topical Administration of ROQUETTE Chlorella sp. on Skin Inflammation and Wound Healing in Mice. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistryrrent Medicinal Chemistry - Anti-Inflammatory and Anti-Allergy Agents)* **2014**, *13* (2), 93−102.
(95) Hopfner, U.; Schenck, T.-L.; Chávez, M.-N.; Machens, H.-G.; Bohne, A.-V.; Nickelsen, J.; Giunta, R.-E.; Egaña, J.-T. Development of photosynthetic biomaterials for in vitro tissue engineering. *Acta Biomaterialia* **2014**, *10* (6), 2712−2717.
(96) Schenck, T. L.; Hopfner, U.; Chávez, M. N.; Machens, H.-G.; Somlai-Schweiger, I.; Giunta, R. E.; Bohne, A. V.; Nickelsen, J.; Allende, M. L.; Egaña, J. T. Photosynthetic biomaterials: A pathway towards autotrophic tissue engineering. *Acta Biomaterialia* **2015**, *15*, 39−47.
(97) Chen, H.; Cheng, Y.; Tian, J.; Yang, P.; Zhang, X.; Chen, Y.; Hu, Y.; Wu, J. Dissolved oxygen from microalgae-gel patch promotes chronic wound healing in diabetes. *Science Advances* **2020**, *6* (20), No. eaba4311.
(98) Li, W.; Wang, S.; Zhong, D.; Du, Z.; Zhou, M. A Bioactive Living Hydrogel: Photosynthetic Bacteria Mediated Hypoxia Elimination and Bacteria-Killing to Promote Infected Wound Healing. *Advanced Therapeutics* **2021**, *4* (1), 2000107.
(99) Wu, H.; Yang, P.; Li, A.; Jin, X.; Zhang, Z.; Lv, H. Chlorella sp.-ameliorated undesirable microenvironment promotes diabetic wound healing. *Acta Pharmaceutica Sinica B* **2023**, *13* (1), 410−424.
(100) Zhao, E.; Xiao, T.; Tan, Y.; Zhou, X.; Li, Y.; Wang, X.; Zhang, K.; Ou, C.; Zhang, J.; Li, Z.; et al. Separable Microneedles with Photosynthesis-Driven Oxygen Manufactory for Diabetic Wound Healing. *ACS Appl. Mater. Interfaces* **2023**, *15* (6), 7725−7734.
(101) Hu, H.; Zhong, D.; Li, W.; Lin, X.; He, J.; Sun, Y.; Wu, Y.; Shi, M.; Chen, X.; Xu, F.; et al. Microalgae-based bioactive hydrogel loaded with quorum sensing inhibitor promotes infected wound healing. *Nano Today* **2022**, *42*, 101368.
(102) Zhu, Z.; Wang, L.; Peng, Y.; Chu, X.; Zhou, L.; Jin, Y.; Guo, H.; Gao, Q.; Yang, J.; Wang, X.; et al. Continuous Self-Oxygenated Double-Layered Hydrogel under Natural Light for Real-Time Infection Monitoring, Enhanced Photodynamic Therapy, and Hypoxia Relief in Refractory Diabetic Wounds Healing. *Adv. Funct. Mater.* **2022**, *32* (32), 2201875.
(103) Wang, X.; Yang, C.; Yu, Y.; Zhao, Y. In Situ 3D Bioprinting Living Photosynthetic Scaffolds for Autotrophic Wound Healing. *Research* **2022**, 9794745 DOI: 10.34133/2022/9794745.
(104) van Beers, M. M. C.; Bardor, M. Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. *Biotechnology Journal* **2012**, *7* (12), 1473−1484.
(105) Afshari, R.; Akhavan, O.; Hamblin, M. R.; Varma, R. S. Review of Oxygenation with Nanobubbles: Possible Treatment for Hypoxic COVID-19 Patients. *ACS Applied Nano Materials* **2021**, *4* (11), 11386−11412.

https://doi.org/10.1021/acs.molpharmaceut.3c00352
Mol. Pharmaceutics 2023, 20, 3338−3355