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REVIEW

Recent Advances in the Development of Nitric Oxide-Releasing Biomaterials and Their Application Potentials in Chronic Wound Healing

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Chronic wounds, such as pressure ulcers, vascular ulcers and diabetic foot ulcers (DFUs), often stays in a state of pathological inflammation and suffers from persistent infection, excess inflammation, and hypoxia, thus they are difficult to be healed. Nitric oxide (NO) plays a critical role in the regulation of various wound healing processes, including inflammatory response, cell proliferation, collagen formation, antimicrobial action and angiogenesis. The important role of NO in wound healing attracts intensive research focusing on NO-based wound healing therapy. However, the application of NO gas therapy needs to resolve the intrinsic shortcomings of gas therapy, such as short storage and release times as well as temporal and spatial uncontrollability on the release mode. So far, various types of NO donors, including organic nitrates ($RONO_2$), nitrites (RONO), S-nitrosothiols (RSNOs), nitrosamines, N-diazeniumdiolates (NONOates), and metal-NO complexes, have been developed to solidify gaseous NO and they were further encapusalted in or conjugated onto a variety of biomaterial vectors to develop NO delivery systems. NO synthetic enzyme mimics to catalyze the production and release of NO from L-arginine had also been developed. This paper reviewed recent advances of NO donors, biomaterial vectors, thus-formed NO delivery systems, as well as recently emerged NO synthetic enzyme mimics. Furthermore, this review also summarized the functions of NO releasing biomaterials that would benefit chronic wound healing, including antibacterial property and the promotion of angiogenesis, as well as the convenient combination of light/thermal induced NO release with light/thermal therapies, and prospected the future developing trends in this area.

1. Introduction

Wound healing is a complex process involving hemostasis, inflammation, proliferation and tissue remodeling.[1-6] However, not all wounds heal in a normal period of time. Some wounds developed into chronic wounds, in which the injured tissue stayed in a state of pathologic inflammation, leading to protracted and incomplete healing, even non-healing.[7-9] The most common chronic wounds include pressure ulcers, vascular ulcers, and DFUs, with DFUs as the representative and most intractable chronic wounds. Diabetes is a main contributor to diagnosed chronic wounds. The population of adult diabetic patients has reached 450 million worldwide, almost 6% of the total population. Diabetics patients are prone to develop DFUs, which are estimated to occur in 15% of all diabetic patients.[10] DFUs have a poor prognosis, bring endless pain and substantial economic burden to the patients, and often lead to amputation.[7, 10-14] DFUs are widely used models for chronic wounds in the development of more effective strategies for chronic wound healing.[10, 15]

Nitric oxide (NO) is an endogenous gasotransmitter, which plays a central role in the regulation of wound healing processes including inflammatory response, antimicrobial action, cell proliferation, collagen formation and angiogenesis.[2, 7, 16-21] NO is endogenously generated from the terminal guanidine moiety of L-arginine catalyzed by three different forms of nitric oxide synthases (NOSs), namely neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS).[16, 22] The constitutive isozymes nNOS and eNOS are expressed in vascular endothelial cells and neurons respectively.[23, 24] The third isozyme, iNOS, generated only in response to acute inflammatory stimuli.[7, 25] It is generally believed that nNOS and eNOS are essential for maintaining normal physiological homeostasis, and iNOS is related to injury healing. In the inflammatory stage, NO involves in the immune response regulation, and possesses a wide-ranging antibacterial activity. In the proliferative stage, keratinocytes proliferation at the wound edge is iNOS-dependent, the re-epithelialization is also NO-dependent.[26] NO can promote the migration and proliferation of fibroblasts, which have an important role in collagen production and deposition for wound healing.[27] Furthermore, NO can stimulate the migration and proliferation of endothelial cells, thus plays a key role in angiogenesis.[28-35] Declined endogenous production

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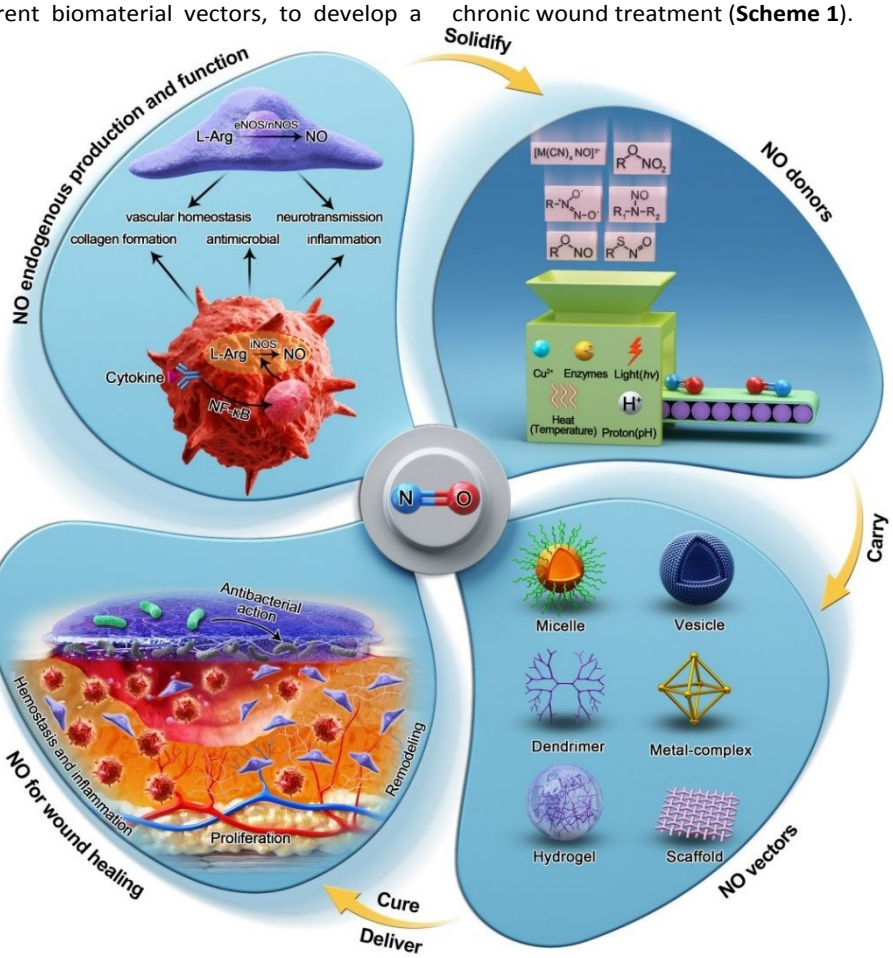
of NO caused by large-area infection and insufficient blood supply, especially in chronic wounds, has been proved being linked to impaired wound healing.[36] Therefore, supplementation of NO at the wound site represents a promising treatment strategy.

Although NO gas therapy is promising in wound healing, the burst release and uncontrollable delivery of NO greatly limited its applications. To solve this problem, researchers have developed different NO donors, which were loaded in or conjugated onto different biomaterial vectors, to develop a series of NO-releasing biomaterials for biomedical applications.[7, 31, 37-47] Recently, mimicking the function of naturally occurring NO synthetic enzymes, various NO synthetic enzyme mimics has also been developed, to catalyze the production and release of NO from L-arginine.[19,31,37] In this review, we will summarize the recent progresses in the development of different NO donors, NO-releasing biomaterials, and NO synthetic enzyme mimics, and highlight their application potentials in wound treatment, especially in chronic wound treatment (**Scheme 1**).



Scheme 1. The endogenous production and functions of nitric oxide (NO), its solidification to give various NO donors/NO prodrugs, the carry of NO donors in organic and inorganic biomaterials, and the application of NO-loading biomaterials for wound healing.

2. NO donors and biomaterial vectors for NO delivery

2.1 NO donors

Various NO donors, also known as NO prodrugs, which can be categorized into organic nitrates ($RONO_2$), nitrites (RONO), S-nitrosothiols (RSNOs), nitrosamines, N-diazeniumdiolates (NONOates), and metal-NO complexes, have been explored to solidify NO for biomedical applications (Figure 1). Among NO donors, RSNOs and NONOates represent the two most widely studied categories due to their ability of spontaneously release NO in physiological media.

Organic nitrates and nitrites, such as amyl nitrite (Figure 1B), isosorbide mononitrate and glyceryl trinitrate, representing the oldest category of NO donors, have been widely used as vasodilators and the treatment of angina pectoris.[2, 48] Amyl nitrite, the first NO donors introduced by T. L. Brunton as early as 1870, was developed for the treatment of patients with coronary artery disease.[49] The main biological effects of nitrates and nitrites are ascribed to the formation of NO. In general, organic nitrates and nitrites can be readily synthesized by reacting alcohols with nitric acid/nitrous acid or other nitrating/nitrosating agents (Figure 1A). The release of NO from organic nitrates and nitrites can be triggered by several enzymatic and non-enzymatic pathways, including xanthine oxidoreductase (XOR), deoxygenated myoglobin (deoxy-Mb), ascorbic acid, polyphenols and protons.[2]

Sulfur and oxygen are congeneric elements, and the reactivity of sulfhydryl/thiol group is much higher than that of hydroxyl group, thus the nitrosyl moiety of nitrites can be easily

transferred to the sulfhydryl group to form S-nitrosothiols (RSNOs).[7, 48] In fact, thiols could also serve as a co-catalyst to trigger the release of NO from furoxans, nitrate, organic nitrite, and other nitro compounds.[48] RSNOs endogenously exist in both tissue and blood, such as S-nitrosocysteine (CysNO), S-nitrosoglutathione (GSNO) and S-nitrosoalbumin (AlbSNO) (Figure 1B).[50] Of note, RSNOs can also be easily synthesized exogenously via the nitrosation of thiol groups on small molecules, peptides or proteins, such as L-cysteine, glutathione or albumin (Figure 1A).[51-53] Nitrosation of inorganic nitrites (NO_2^-) in an acidic medium is probably the most widely used synthesis approach due to the wide availability of inorganic nitrites and the mild reaction condition.[48] The facile synthesis of RSNOs makes them one of the most intensively investigated categories of NO donors. The multifunctionality of peptides or proteins also makes peptide or protein based RSNOs easily modifiable, or could be conveniently conjugated onto biomaterials.[51] The release mechanisms of RSNOs include 1) transition metal (e.g., copper ions)-mediated catalytic decomposition, 2) redox reaction with ascorbate, 3) light or thermal-triggered homolytic cleavage of S-NO bond, and 4) enzyme-mediated release.[29, 52, 54] Due to the low endogenous copper content, photothermal decomposition is the main inducement for RSNO to release NO in biological systems.[55] RSNOs' instability under heat and light may lead to premature release of NO. Although the instability of RSNOs could be circumvented in some extent, this may bring concerns regarding long-term storage of RSNO-based materials and obviously limit the practical application of RSNO-based wound dressings in clinical scenarios.[7, 53]

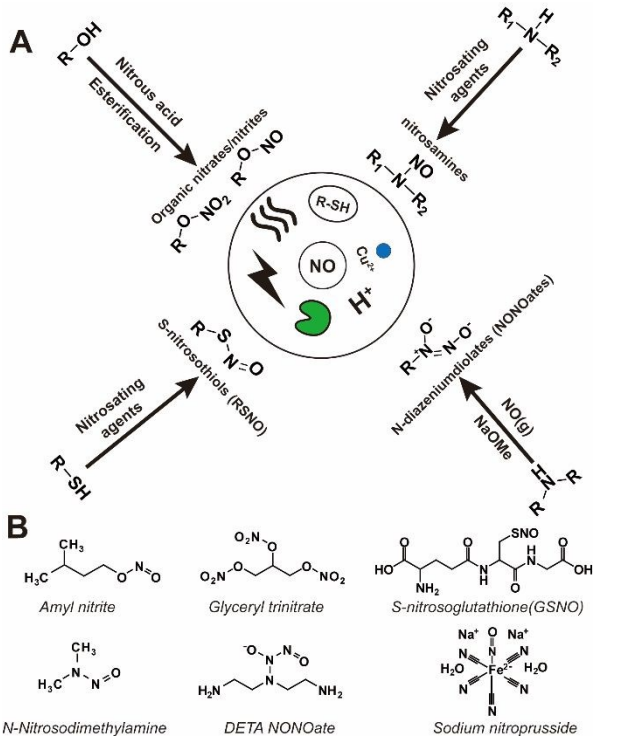


Figure 1. (A) Synthesis of various organic NO donors/prodrugs and their release of NO triggered by different stimuli; (B) Representative NO donors.

Nitrosamines (N-nitroso compounds) are another class of NO donors, their nitroso groups could be homolytically or heterolytically transferred into other species. Nitrosamines are formed by the nitrosation of secondary amines with nitrosation agents (Figure 1A). Although primary amines readily react with nitrosating agents, the formed deamination products are unstable, finally give diazonium ions (RN_2^+). The reactions of secondary amines can be stopped at the nitrosamine stage because there are no necessary R-hydrogen atoms for proton transfer reactions. Most N-nitrosoamines at physiological pH in aqueous solution is relatively stable, but light-sensitive (Figure 1A).[48]

N-diazeniumdiolates (NONOates) are another one type of the most widely studied NO donors, which are synthesized via the reaction of secondary amines with high pressured NO gas.[56-58] NONOates are considered as a class of very useful NO donors due to their ability to release NO triggered by different stimuli and its chemically modification possibility in a highly predictable manner. Efficient NONOates formation needs the help of additional basic residues, such as unreacted amine substrates or metal alkoxide bases, to deprotonate the amine thus promote its nucleophilic attack by NO (Figure 1A). The cation (e.g., protonated amines or metals in alkoxide base) can stabilize the anionic charge of the resulting NONOate.[59-61] NONOates spontaneously decomposed to generate two moles NO per mole of donor with proton-initiation under physiological solution (i.e., 37°C, pH 7.4).[29, 62] The NO-release kinetics of NONOates is also affected by environmental factors (e.g., pH, temperature) and the structure of the NO donor precursor (e.g., polyamines) (Figure 1A).[58, 63]

NO is a powerful ligand of metal ions. The most intensively studied metal-NO coordination compounds is sodium nitroprusside ($Na_2[Fe(CN)_5NO]$, SNP) (Figure 1B), a widely used vasodilator.[64] Although SNP solution is extremely photosensitive, the NO release triggered by photolysis under physiological conditions is not significant. In a recent study, another metal-NO coordination compound, Roussin's Black Salt ($[NH_4]$ $[Fe_4S_3(NO)_7]$), was used as a NO donor.[65] The transition metals (such as cobalt[69] and zinc[45]) on metal-exchanged zeolites could also serve as NO-donors through metal-NO complex formation. NO release from metal-NO compounds requires both light irradiation and single-electron reduction, and the reactions are usually enhanced by thiols.

2.2 Biomaterial vectors for NO delivery

Although NO donors show certain desirable properties in NO solidification, storage and release, their clinical applications are limited by high toxicity, thermal/photo-chemical instability, low NO payload, rapid NO release, and lack of specificity.[42] In contrast, macromolecular nanoparticles or scaffolds are more easily modified to realize targeted and controlled payload release. Therefore, various biomaterial platforms have been developed to carry NO donors and release NO in a sustained and controlled manner. In this section, we will review the NO-releasing biomaterial vectors including organic and inorganic ones (Figure 2).

2.2.1 Organic vectors

Organic NO vectors mainly include micelles, vesicles (including liposomes), dendrimers/branched polymers, polymeric nanoparticles, hydrogels and scaffolds (Scheme 1 and Figure 2A).

Micelles have been extensively used in the delivery of NO. Hubbell *et. al*.[66] designed block copolymer pro-amphiphilic and amphiphilic NO-releasing micelles for long-term release of NO. The hydrophobic core of the micelle can protect the NONOate from the influence of water, thereby protecting it against protons required for NO release, resulting in a significantly prolonged release half-life up to 7 days. Gao *et. al.*[67] grafted an amphiphilic copolymer, methoxy poly(ethylene glycol)-b-poly(lactic acid) (mPEG-PLA) or D-α-tocopheryl polyethylene glycol succinate (TPGS) and nitrate (as a NO donor), on the backbone of poly(2-hydroxyethyl methacrylate) (PHEMA), developed a micelle platform for sustained release of NO.. TGPS-modified PHEMA polymer micelles showed more stable NO release comparing with its counterparts. Kim *et. al*.[68] designed a dual stimuli-responsive polymeric micelle for NO delivery via the coordination between the diol groups of O_2-protection N-diazeniumdiolate (P-NO) and phenylboronic acid (PBA) moieties on the side chain of a diblock copolymer. The NO-containing micelles can efficiently accumulate at the tumor sites and promote the cytosolic NO release in cancer cells. Ding *et. al*.[69] reported the self-assemble of micellar nanoparticles formed by a triblock copolymer of poly(ethylene glycol)-b-PNORM-b-poly(ethylene glycol) (PEG-b-PNORM-b-PEG), in which NO-releasing molecules is a N,N'-dinitroso-p-phenylenediamine (DNP) derivative, capable of releasing NO under light irradiation (Figure 2A1).

Vesicles has been reported as a carrier to encapsulate both gaseous NO and solidified NO donors. Huang *et. al*.[70] reported the encapsulation of NO into echogenic liposomes (ELIP) can be achieved via a freezing under high pressure technique. NO-containing liposomes (NO-ELIP) can protect NO from being scavenged by hemoglobin and realize effective NO delivery. Suchyta and Schoenfisch[71] reported the tune of NO release from N-diazeniumdiolate encapsulated liposomes by changing the molecular structure of NO donor and/or the phospholipid composition (independently or in combination). Katayama's group[72, 73] prepared NONOate-containing PEGylated liposomes (NONOate-LP) that showed protonation induced retarded release of NO. Their study successfully demonstrated for the first time that incorporating an NO donor in the PEGylated liposome can improve the enhanced permeability and retention (EPR) effect (Figure 2A2). Duan *et. al*.[18] fabricated NO-releasing vesicles synthesized by self-assembly of NO-releasing amphiphiles via directly photoresponsive polymerization of N-nitrosoamine-based NO monomers, for corneal wound healing.

So far, various NO releasing dendrimers/branched polymers have been developed.[74-76] Stasko and Schoenfisch reported for the first time the potential of dendrimers as powerful NO storage/release carriers.[77] Compared to their small molecule counterparts, the secondary amine-containing dendrimers showed a unique dendritic effect and had a significantly prolonged NO release time. The Schoenfisch group has conducted a series of research on NO-releasing dendrimers with enhanced anti-biofilm activity.[78-85] (Figure 2A3) Katsumi *et. al.*[84] designed an S-nitrosylated L-serine-modified polyamidoamine dendrimer (SNO-Ser-PAMAM) and their results showed that SNO-Ser-PAMAM is a promising NO donor for kidney, and could be used to efficiently prevent renal ischaemia/reperfusion injury. Frost *et. al.*[85] modified hyperbranched polyamidoamine (HPAMAM) with S-nitroso-N-acetyl-D-penicillamine, which was then nitrosated to form a controlled, high-capacity NO-donating compound SNAP-HPAMAM.

Nanoparticles represent attractive materials for the encapsulation of NO-donors to create NO-release systems.[86-90] Duong *et. al.*[91] reported NO releasing N-diazeniumdiolate moieties crosslinked core nanoparticles, which exhibited slow and controlled release of NO, and showed considerable antibacterial effect. They also used a similar approach to prepare NO and gentamicin co-delivery nanoparticle to reduce the formation of a *Pseudomonas aeruginosa* biofilm.[92] Ghalei *et. al.*[93] described the fabrication of NO-releasing silk fibroin nanoparticles (SF NPs) using S-nitroso-N-acetylpenicillamine (SNAP) as the NO donor. SNAP-SF NPs exhibited strong antibacterial properties against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E.coli*). Wu *et. al.*[94] constructed S-nitrosoglutathione (GSNO) functionalized poly(ethylene glycol)-block-poly(propylene sulfide) (PEG-PPS) nanoparticles to deliver doxorubicin (DOX). Such GSNO functionalized nanoparticles could release DOX in a ROS triggered manner and increase the intracellular accumulation of DOX (Figure 2A4).

Seabra *et. al.*[95] incorporated S-nitrosoglutathione (GSNO) into a thermoresponsive hydrogel consisted of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO, Pluronic F127) and chitosan (CS), which exhibited antibacterial effects against *Pseudomonas aeruginosa*. Recently, they also described the synthesis, cytotoxicity, and antibacterial effects of alginate hydrogel containing S-nitroso-mercaptosuccinic acid (NO donor) and silver nanoparticles (AgNPs).[96] Park *et. al.*[97] incorporated S-nitrosothiolated gelatin (GelSNO) into injectable gelatin-based hydrogels (GHs) crosslinked by horseradish peroxidase (HRP) and H_2O_2 (Figure 2A5). The peroxynitrite ($ONOO^-$) was formed *in situ* to confer effective antibacterial effects. Through a mechanism driven by heat, visible light, or oxidizing agent, NO was released from the GH/GelSNO hydrogels, which showed significant bactericidal effects against both Gram-positive and Gram-negative bacteria. Moreover, Ramadass *et. al*.[98] reported a silk fibroin-polyvinyl alcohol (SF-PVA) nanofibrous scaffold *via* type I collagen peptide (CP), and a NO donor, *S-Nitrosoglutathione* (GSNO), to treat the non-healing diabetic ulcer (Figure 2A6). Electrospun doped with NO donor are presented as widely used macromolecular scaffolds with great potential for tissue engineering applications.[99-101]

2.2.2 Inorganic vectors

Inorganic NO vectors mainly contain metallic nanoparticles, silica particles, zeolites and metal organic frameworks (MOFs) (Figure 2B). Recently, metal and metal oxide nanoparticles serve as intensively researched NO-releasing platforms due to their unique physical, chemical, optical, and electronic

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properties.[45, 102-104] Through the strong coordination between thiol groups of N-diazeniumdiolate-modified thiol containing molecules with gold (Au) nanoparticles, Schoenfisch and co-workers developed NO releasing gold nanoparticles, which can release NO spontaneously in aqueous media (physiological pH).[105] Seabra *et. al.*[106] prepared silver nanoparticles (Ag NPs) and S-nitrosoglutathione (GSNO) separately or simultaneously incorporated polymeric films composed of poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG). The PVA/PEG films containing GSNO and/or AgNPs showed a strong antibacterial activity and cytotoxic effect against tumor cell lines. Singha *et. al.*[107] studied the catalytic and antimicrobial properties of a hybrid SNAP-loading zinc oxide nanoparticle (SNAP-ZnO), which could catalytic release NO under physiological conditions and demonstrate antimicrobial effect.

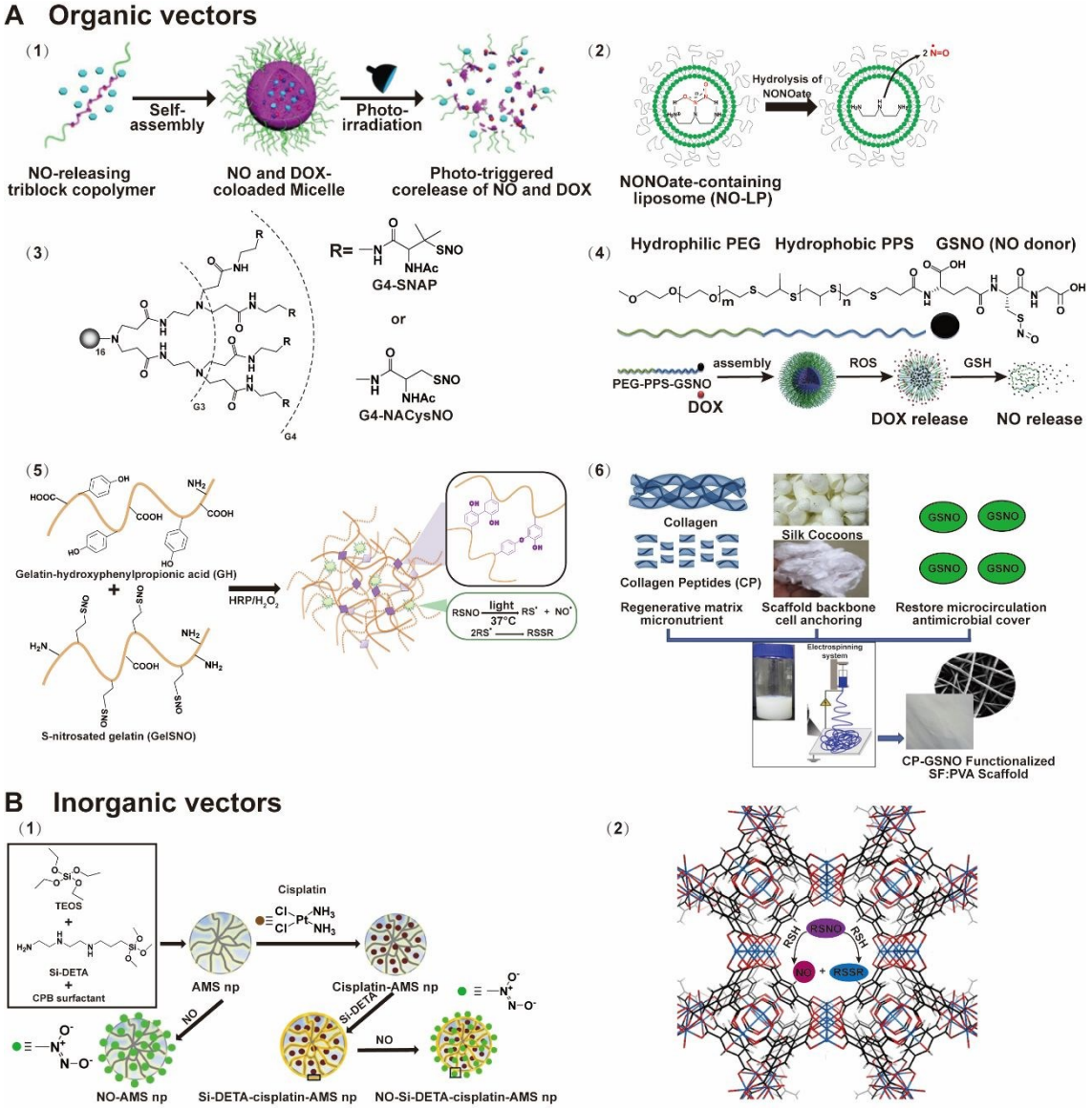

Figure 2. Representative organic (A) and inorganic (B) biomaterial vectors carrying NO donors. (A) Organic vectors: (1) NO-loaded PEG-b-PNORM-b-PEG triblock copolymers micelles and photo-triggered disassembly of the micellar nanoparticles;[69] (2) NO release from NONOate-containing liposome (NO-LP);[73] (3) S-nitroso-N-acetyl-D,L-penicillamine (G4-SNAP) or S-nitroso-N-acetylcysteine (G4-NACysNO) containing generation 4 polyamidoamine (PAMAM);[85] (4) Triblock amphiphilic copolymers nanoparticles containing NO donor;[94] (5) GH/GelSNO hydrogel releases NO by S-N bonds cleavage under thermal or light excitation;[97] (6) CP: GSNO functionalized SF-PVA scaffold.[98] (B) Inorganic vectors: (1) NO- and cisplatin-loaded amine-modified mesoporous silica (AMS);[116] (2) RSNO decomposition to produce NO via a MOF catalyst: $Cu_3(BTC)_2$.[118]

Silica-based materials are widely used in biomedical area due to the customization of size, morphology, and composition benefited from their facile synthesis.[101, 108-115] Munaweera *et. al.*[116] reported a NO and cisplatin releasing wrinkle-structured amine-modified mesoporous silica (AMS) nanoparticles, which were prepared via condensation of tetraethylorthosilicate (TEOS) and 3-triethoxysilylpropyl diethylenetriamine (Si-DETA) and subsequently doped with N-diazeniumdiolate and cisplatin (Figure 2B1). Schoenfisch *et. al.*[117] prepared NO-releasing mesoporous silica nanoparticles (MSNs) using an aminosilane-template surfactant ion exchange reaction.

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To increase the payload of NO and expand the potential applications of NO loading materials in more diverse biomedical areas, porous inorganic materials, such as MOFs and zeolites, have also been used as vectors for NO donors (Figure 2B2).[118-121] The high NO loading capacity of MOFs and zeolites makes them extremely attractive for the use in biological and medical applications. MOFs provide an NO loading substrate with tunable physical and chemical properties. Wheatley *et. al.*[122] used zeolite-A, an alternating alumina/silica network with cobalt (Co) cations, to chemisorb NO, and showed that the NO released from Co-exchanged zeolite-A could inhibit the adhesion and aggregation of human platelets *in vitro*. Fox *et. al.*[43] demonstrated that the NO release capacity of a novel NO-storing Zn^{2+}-exchanged zeolite material and its potent antibacterial properties against both Gram-negative and Gram-positive bacteria. Reynolds *et. al.*[123] showed that copper-based MOF $H_3[(Cu_4Cl)_3\text{-}(BTTri)_8]$ (Cu-BTTri, 1,3,5-tris(1H-1,2,3-triazol-5-yl)benzene) could trigger an elevated level of NO released from GSNO, which was incorporated with naturally derived polysaccharide chitosan to form membranes for would healing applications.

2.3 Enzyme mimics promoting the endogenous and exogenous NO generation

As mentioned above, the NO release from NO donors needs the help of triggers, such as thiols, enzymes and so on, which could serve as catalyzers to promote NO production from NO donors either *in vivo* or *in vitro*. The use of natural enzymes, such as glutathione peroxidase, can realize the biotransformation of endogenous NO donor to produce NO. Nevertheless, low stability and short shelf life are inherent shortcomings of naturally occurring enzymes.[124] Various enzyme mimics, such as polymers and hydrogels, MOFs, and metal or metal oxide nanoparticles, had been developed to simulate naturally occurring enzymes, to catalyze the production of NO from L-arginine.[37, 125, 126]

The Reynolds group considered that incorporation of copper-based MOFs (Cu-BTTri) into hydrophilic poly (vinyl alcohol) (PVA) or hydrophobic polyurethane (PU) can promote the endogenous NO generation (Figure 3A).[127] The obtained Cu-BTTri/PVA films exhibited a higher swelling ratio, thus enhanced the interaction between catalysts and GSNO, resulting in faster NO generation. Zhang *et. al.*[31] reported a one-step metal-catecholamine assembly strategy to prepare a durable *in situ* NO-generating biomimetic dopamine-Cu (II) (DA-Cu(II)) coating. The coating could decompose endogenous S-nitrosothiols (RSNOs) in fresh blood, to generate NO *in situ* (Figure 3B). Zhao *et. al.*[128] reported a copper-based surface-attached metal-organic framework (Cu-SURMOFs) of copper (II) benzene-1,3,5-tricarboxylate (CuBTC) on the surface of alkali-activated titanium using layer-by-layer (LBL) assembly, for NO *in situ* generation from endogenous S-nitrosoglutathoine (GSNO) (Figure 3C). The Chandrawati group[19] discovered that zinc oxide (ZnO) particles could mimic the activities of β-galactosidase and glutathione peroxidase, and catalyzed both exogenous (β-gal-NONOate) and endogenous (GSNO) NO donors to generate NO. The physiological NO levels could be attained by simply modulating the concentrations of GSNO and ZnO. Li *et. al.*[37] developed a natural platelets (PLT) liposome loaded with L-arginine and magnetic γ-Fe_2O_3 nanoparticles (PAMNs) for thrombus-targeted delivery of L-arginine and *in situ* NO generation (Figure 3D). Their rapid targeting to stroke lesions as well as *in situ* NO generation enhanced the expansion of blood vessels and reduced platelet aggregation, thus delayed thrombotic plaques development.

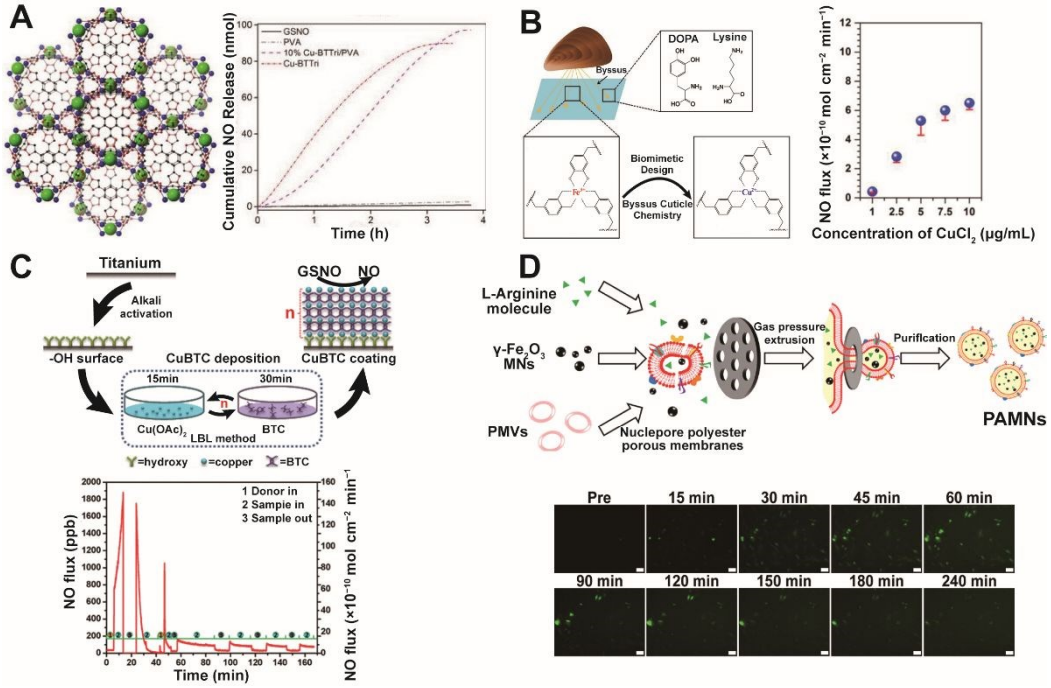

Figure 3. (A) Structure of Cu-BTTri and the cumulative NO release from GSNO loading Cu-BTTri/PVA membranes.[127] (B) Cu (II) crosslinked mussel-inspired adhesive coating and the calculated release rates of NO at different Cu (II) concentrations.[31] (C) The layer-by-layer (LBL) deposition of CuBTC coating on alkali-activated titanium surface and the representative real-time NO

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generation.[128] (D) The fabrication of PAMNs by the extrusion method and the photomicrographs of NO production over time in bEnd.3 cells.[37]

Recently, several redox systems had been employed to *in situ* produce NO from nitrite. L-ascorbic acid could serve as a reducing agent to reduce nitrite into NO. The presence of copper complexes as a catalyst was proved could increase and prolong NO generation when compared to that of nitrite and L-ascorbic acid alone.[129] Amal's group found that Fe (II) ions could also serve as a reducing agent to reduce nitrite into NO.[129-131]

3. Wound healing applications of biomaterial NO-releasing systems

In recent years, the use of NO gas therapy for wound healing has been extensively and intensively studied. Endogenous NO mediates a lot of important biological processes occurring after cutaneous injury, including inflammatory response, cell proliferation and collagen formation. Moreover, NO plays an important role in killing microbes and promoting angiogenesis, to accelerate tissue regeneration and wound healing. Due to the importance of NO in wound healing and the inefficient endogenous NO produce especially in chronic wound where blood supply was mostly destructed, different NO-releasing biomaterials have been designed to deliver NO for promote chronic wound healing.

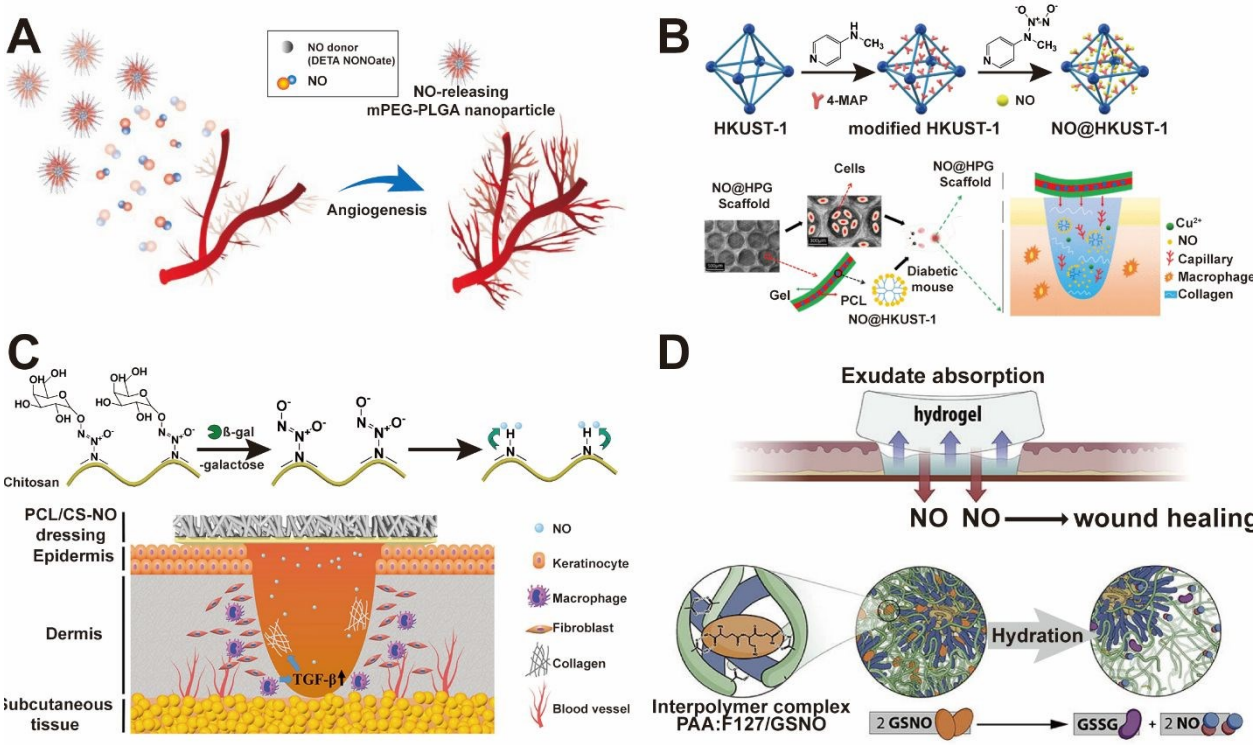

Figure 4. (A) NO-releasing mPEG-PLGA nanoparticles promotes angiogenesis.[137] (B) NO@HKUST-1/PCL/Gel (NO@HPG) scaffold promotes diabetic wound healing.[20] (C) NO released from PCL/CS-NO dressings switching on/off by β-glycosidase improves wound healing.[44] (D) Hydration-controlled NO release from PAA:F127/GSNO topical hydrogels promotes wound healing. [40]

3.1 NO releasing biomaterials promote angiogenesis

NO-releasing materials can promote injured tissue regeneration by enhancing angiogenesis, collagen deposition, and reepithelialization.[132-136] Yang *et. al.*[137] reported the feasibility of methoxy poly(ethylene glycol)-b-poly(lactic-co-glycolic acid) nanoparticles (mPEG-PLGA NPs) as NO-releasing materials could induce enhanced angiogenesis, representing a promising therapeutic for wound healing and treating hind limb ischemia (Figure 4A). Xu *et. al.*[20] developed a copper-based MOF HKUST-1 as a NO-loading vehicle and with core-shell structure through electrospinning (Figure 4B). The obtained NO sustained release system could promote endothelial cell growth and significantly improve angiogenesis and collagen deposition in the wound bed, and also exhibited anti-inflammatory property, thus eventually accelerated diabetic wound healing. Zhao *et. al*.[44, 135] prepared a novel functional wound dressing by combining electrospun poly(ε-caprolactone) (ε-PCL) nonwoven mat with glycosylated NO compound grated chitosan (CS–NO) as NO-releasing biomaterials switched on/off by β-glycosidase (Figure 4C). The PCL/CS-NO dressing exhibited good stability under physiological conditions and could release NO in a controllable and sustainable manner under the catalysis of galactosidase. Results showed that the sustained release of NO accelerated wound healing in comparison with control and PCL/CS groups. Oliveira *et. al*.[40] developed a supramolecular interpolymer complex hydrogels comprising with F127 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol), PEG-PPG-PEG) micelles embedded in a poly (acrylic acid) (PAA) matrix, with GSNO molecules being distributed in the hydrophilic domain (Figure 4D). A preliminary *in vivo* study on full-thickness excisional wounds on mice showed that the topical NO release from the PAA: F127/GSNO hydrogels could

be triggered by the absorbed exudate and led to enhanced angiogenesis and more organized collagen fiber deposition.

3.2 NO releasing biomaterials exhibit antibacterial ability

Wound healing is often accompanied with infection. NO has antimicrobial ability due to the combination of nitrosative and oxidative mechanisms. After reacting with oxygen and superoxide, NO transforms into dinitrogen trioxide and peroxynitrite, respectively. Dinitrogen trioxide causes DNA deamination, while peroxynitrite induces lipid peroxidation and membrane damage.[138] NO was proved exhibiting dose-dependent antibacterial activity against biofilms.[139, 140] As a diatomic free radical, NO gas possesses preferable antimicrobial capability, beneficial for wound healing applications.[141-145] Lee *et al.*[146] developed an *in-situ* hydrogel-forming/NO-releasing powder dressing (NO/GP) possessing the benefits of both powders and hydrogels (Figure 5A). The results of an *in vitro* antibacterial study demonstrated that NO/GP would be a promising alternative to dressings for the treatment of infected wounds. Due to the abuse of antibiotics and the increase of multidrug resistant bacteria, Huang *et. al.*[38] reported a photothermal ingredient loading β-cyclodextrin-functionalized graphene oxide (GO) NIR light responsive nanovehicles combined with the NO donor BNN6 in a GelMA/HA-DA hydrogel for bacteria-infected wound healing. The results revealed that hydrogel was an ideal antibacterial material that could improve collagen deposition and angiogenesis, thus promote wound healing (Figure 5B). Chitosan is also used for wound healing due to its antimicrobial and anti-biofilm effects. Yoo *et. al.*[147, 148] developed NO-releasing films (CS/NO film) composed of chitosan (CS) and GSNO as a NO donor used for the treatment of MRSA biofilm-infected wounds under diabetic condition (Figure 5C). Yu *et. al.*[39] grafted a three generation dendritic poly(amidoamine) (PAMAM-G3) onto polydopamine (PDA) coated iron oxide nanocomposite (Fe_3O_4@PDA), and subsequently loaded NO with the formation of NONOate. Thus-obtained Fe_3O_4@PDA@PAMAM@NONOate exhibited controllable NO release upon 808 nm laser light irradiation, which also showed excellent bacteria-separation efficiency (Figure 5D). A recent study showed an increased and extended NO release from SNAP when combined with cerium oxide nanoparticles (CNP), due to the preservation of the NO donor by CNP, the synergistic effect between NO donor and CNP enhanced the antibacterial effect.[149]

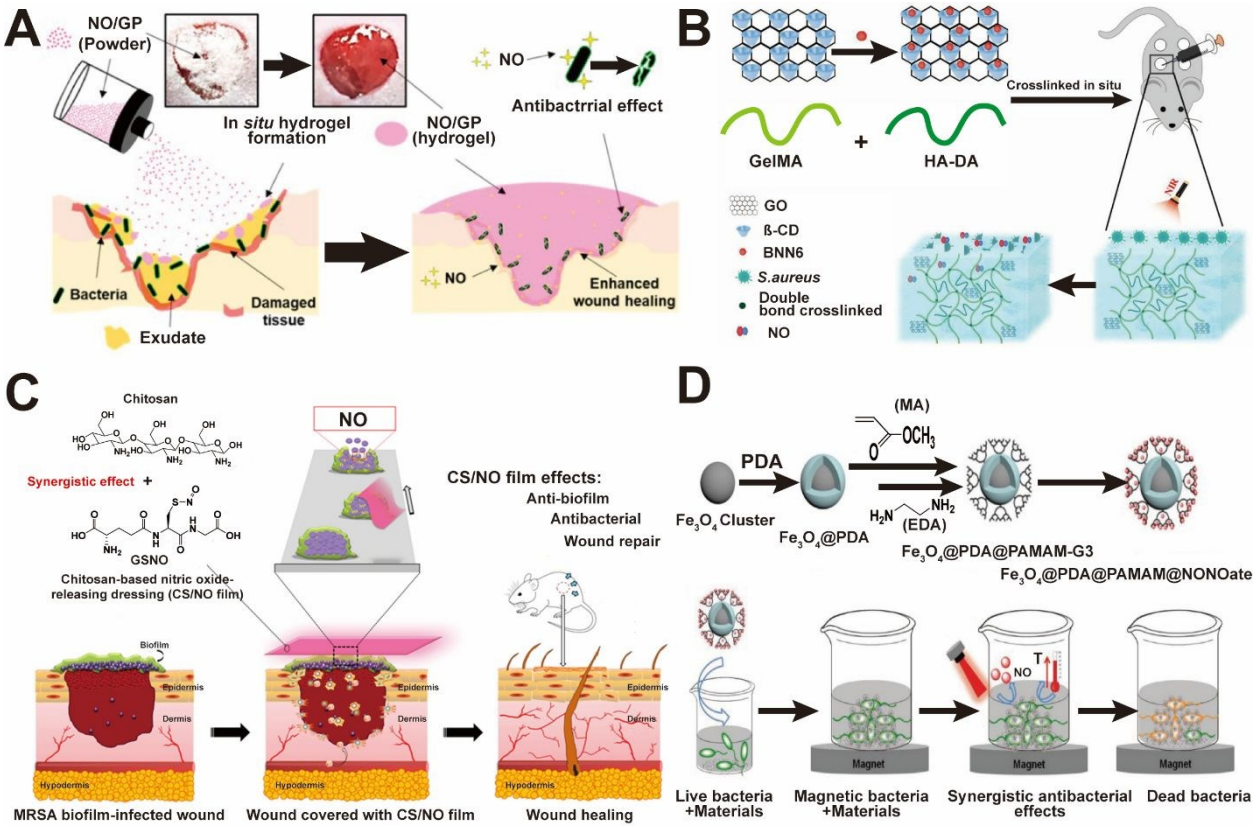

Figure 5. (A) *In-situ* hydrogel-forming/NO-releasing powder dressing (NO/GP) for the treatment of infected wounds.[146] (B) GO-βCD-BNN6 as an NIR laser-mediated NO release nanovehicle for the synergistic elimination of bacteria via NO gas therapy and NIR laser irradiation.[38] (C) CS and GSNO (CS/NO) film developed for the treatment of MRSA biofilm-infected wound healing.[148] (D) Synthesis of Fe_3O_4@PDA@PAMAM@NONOate, and the magnetic separation, synergistic photothermal and NO bacteria killing.[39]

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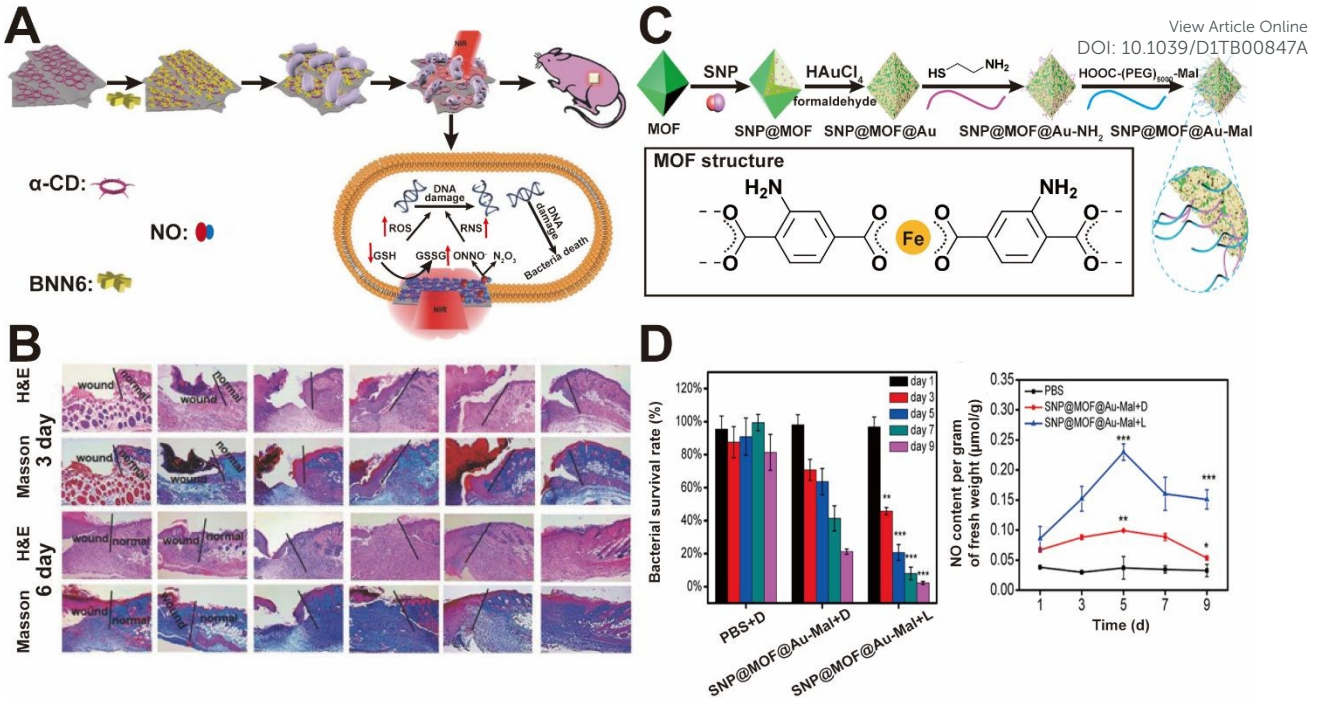

Figure 6. (A) MoS_2-BNN6 as NIR laser-mediated NO release nanovehicle for synergistically eliminating bacteria.[153] (B) Histologic analyses of Ampr *E. coli*-infected wounds treated with PBS, MoS_2-α-CD, BNN6, MoS_2-α-CD + NIR, MoS_2-BNN6, and MoS_2-BNN6 + NIR at the 3rd and 6th day. [153] (C) Synthesis schematic and structure diagram of SNP@MOF@Au-Mal nanogenerators.[154] (D) Bacterial survival rates at the wound (left) and the NO contents in the skin tissues at different treatment time periods (right). [154]

3.3 Synergetic therapy strategies of NO releasing biomaterials

Recently, many near-infrared (NIR) responsive NO release platforms were developed, the NO release from which can be effectively triggered by NIR light.[150-152] Therefore, the photothermal-responsive NO release platform represents a smart gas release strategy with good therapeutic effects. Gao and his team report a new near-infrared 808 nm laser-mediated NO-releasing nanovehicle (MoS_2-BNN6) through simple assembly of α-cyclodextrin (α-CD) modified MoS_2 nanosheets (MoS_2-α-CD) with a heat-sensitive NO donor N,N'-di-sec-butyl-N,N'-dinitroso-1,4-phenylenediamine (BNN6) for safe, rapid, and effective disinfection of inflammatory wounds (Figure 6A).[153] When exposed to 808 nm laser irradiation, hyperthermia from MoS2-BNN6 can precisely control NO delivery and release, exhibited high antibacterial activity against Ampr *E. coli* and *E. faecalis*. The synergetic antibacterial strategy based on NIR photothermally responsive MoS_2-BNN6 can perturb and damage cell membranes structure for rapid and highly effective killing of bacterial (Figure 6B). Han *et. al*. constructed an intelligent NO nanogenerator triggered by NIR light via encapsulated the photothermal sensitive sodium nitroprusside (SNP) inside the MOF that can specifically recognize and adhere to Gram-negative bacteria and depolarize the bacterial membrane to increase permeability (Figure 6C).[154] The synergistic antibacterial effects can achieve precise treatment of bacterial infections and promote wound healing without damaging normal tissues, thus has great clinical application potential (Figure 6D).

4. Conclusion and Outlook

Due to the critical role of nitric oxide (NO) in the regulation of wound healing processes including inflammatory response, antimicrobial action, cell proliferation, collagen formation and angiogenesis, NO therapy has great application potential in wound healing, especially in chronic wound healing. To prolong NO releasing time and improve NO release controllability, NO gas was solidified to give various NO donors/NO prodrugs, including organic nitrates ($RONO_2$), nitrites (RONO), S-nitrosothiols (RSNOs), nitrosamines, N-diazeniumdiolates (NONOates), and metal-NO complexes, and NO donors were further loaded in various organic and inorganic biomaterial vectors to develop NO delivery systems. The developed NO-releasing biomaterials could release NO when explored to different stimuli, such as protons, metal ions, enzymes, heat and light irradiations, and light/thermal induced NO release could be conveniently combined with light/thermal therapies in biomedical applications. To realize more convenient NO delivery, NO synthetic enzyme mimics based on polymers, MOFs, metal or metal oxides, catalysing the *in situ* production of NO from L-arginine were also developed. NO releasing biomaterials are beneficial in killing microbes and promoting angiogenesis, to accelerate tissue regeneration and wound healing, thus have great application potentials in chronic wound healing.

However, the exact role of NO in wound healing is still controversial, the exposure dose and duration of NO effective for wound healing are closely related to microenvironment, cell

type, and animal model. In this respect, more attention should be taken to these factors in the design of NO-delivery systems as a wound healing therapy. In addition, the releasing characteristics of NO-releasing biomaterials should also be carefully designed and evaluated to meet the requirements of clinical applications, especially for chronic wound healing. On the other hand, along with the deepens of the understanding to NO related biology and the progress of material science, we believe that more NO synthetic enzyme mimics and *in situ* NO producing nano-/macro-reactors or scaffold-based NO mini-factories would be developed in the future to realize more convenient NO production and environmental-responsive release for chronic wound healing and smart management.

Author Contributions

Min Wu: Writing, Software; Zhihui Lu: Figures drawing; Keke Wu: Revise; Changwoo Nam: Conception, Revise; Lin Zhang: Revise; Jinshan Guo: Conception, Supervision, Edit.

Conflicts of interest

There are no conflicts to declare.

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