Prayogik Rasayan

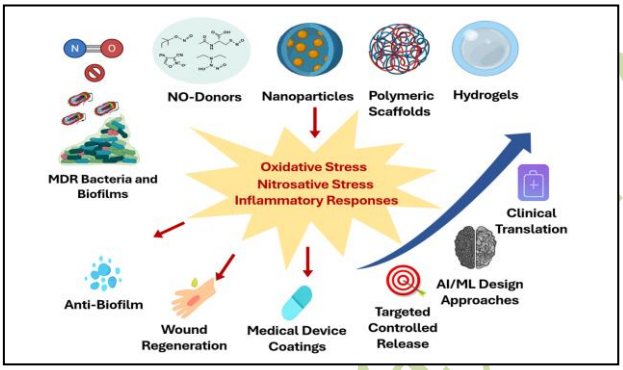
Nitric Oxide in Antibacterial Therapy: Advances in Delivery Systems and Future Prospects

Grace Kaul, Asmita Shrestha, Sandeep Verma*

Department of Chemistry & Gangwal School of Medical Sciences and Technology, Indian Institute of Technology Kanpur, Kanpur, Uttar Pradesh-208016, India
Email: sverma@iitk.ac.in

Received: September 25, 2025 | Accepted: October 28, 2025 | Published online: November 14, 2025

Abstract



Nitric oxide is a crucial gasotransmitter with a diverse range of physiological functions, notably including its significant antimicrobial properties. This review succinctly summarizes its antibacterial mechanisms and current advancements in NO delivery systems pertinent to infection-associated biomedical applications. Furthermore, it explores the challenges associated with NO delivery, emphasizing how computational and machine learning-based approaches can aid in overcoming these limitations by optimizing design and predicting efficacy.

Keywords: Nitric Oxide (NO), Antibacterial Therapy, NO Delivery Systems, Biomedical Applications

1. Introduction

The escalating challenge of antimicrobial resistance (AMR) represents a profound global public health crisis, projected to cause over 10 million deaths annually by 2050, surpassing mortality rates from diseases like malaria, HIV, and tuberculosis combined.[1] Multidrug-resistant (MDR) bacteria like carbapenem-resistant *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* are the major contributors to fatal healthcare-associated infections worldwide.[2] A significant compounding factor is the formation of bacterial biofilms, particularly on biomedical devices, which drastically increases resistance to conventional antibiotics and necessitates up to 1000 times higher dosages for treatment.[3] At present, the development of innovative antibacterial strategies that can circumvent traditional resistance mechanisms is a pressing demand.

Nitric oxide (NO) was recognized as the "Molecule of the Year" by *Science* in 1992 for its diverse physiological functions as a gasotransmitter, including vasodilation, neurotransmission, and immune regulation.[4] Besides these roles, NO possesses potent, broad-spectrum antimicrobial activity against a wide range of pathogens comprising MDR bacteria, fungi, and viruses.[5–9] Its antibacterial efficacy stems from inducing severe oxidative and nitrosative stress within bacterial cells, culminating in critical cellular damage such as DNA lesions, protein modifications, and lipid peroxidation-mediated membrane disruption.[5] The multifaceted modes of action make bacterial resistance development unlikely, providing NO with the critical advantage of circumventing typical drug resistance mechanisms, a persistent problem with other antibacterial agents.[10] Crucially, NO also inhibits bacterial biofilm formation and disperses established biofilms by modulating cyclic di-GMP signaling in bacteria, enhancing bacterial susceptibility to host immune responses and other antibacterials.[11–14] These unique properties position exogenous NO as a promising therapeutic candidate against MDR infections in the ongoing efforts to tackle antimicrobial resistance (AMR).

Despite its immense therapeutic potential, NO's inherent high reactivity, gaseous nature, and extremely short biological half-life have historically limited its direct clinical application.[15,16] Significant advancements, however, have led to the development of sophisticated NO delivery systems designed to enable controlled, sustained, and targeted release of the molecule at infection sites.[15,17–26] These systems are engineered to respond to specific triggers such as light, pH changes, or enzymatic activity present at infection sites.[27–31]

This mini review discusses the antibacterial properties of NO, the latest developments in various NO-releasing platforms and their diverse biomedical applications in persistent infections, medical device coatings, and wound healing. Current challenges and prospects are addressed, emphasizing the potential role of computational and artificial intelligence (AI)-based machine learning (ML) approaches in overcoming the limitations.[32–36] ML-based approaches can aid in designing platforms with optimized NO release, stability, precise targeted delivery, and predict integration into combination therapies for clinical translation.[36–42]

2. NO Biosynthesis and Its Role as an Antibacterial Agent

This section discusses the pathways of endogenous NO production in humans and bacteria and elaborates on the molecular mechanisms behind its broad-spectrum antibacterial activity.

2.1. Pathways for NO Biosynthesis

NO is endogenously produced in the human body for regulating vital physiological processes such as cardiovascular control, neurotransmission, host immune response, and the maintenance of cellular homeostasis.[5] Intriguingly, some bacteria also reportedly produce NO for modulating their metabolic processes.[43] Further, we discuss the major pathways of endogenous NO production in mammals and bacteria.

2.1.1. Endogenous NO production in Humans

The primary source of endogenous NO in humans is enzymatic, involving the conversion of L-arginine by the Nitric Oxide Synthase (NOS) family of enzymes (Figure 1A).[5] These are homodimeric oxidoreductase enzymes that cleave L-arginine, producing NO and L-citrulline, and require cofactors such as NADPH and oxygen. The availability of intracellular L-arginine is a rate-limiting step in NOS-dependent NO release.[5] Three main isoforms of NOS regulate human NO production. **Constitutive NOS (cNOS)** includes **neuronal NOS (nNOS or NOS1)**, involved in neurotransmission, and **endothelial NOS (eNOS or NOS3)**, which is crucial for regulating physiological processes like vasodilation and angiogenesis. These isoforms typically produce NO at low, basal concentrations.[15] Primarily, **Inducible NOS (iNOS or NOS2)** is expressed in immune cells, including macrophages and neutrophils, and in a subset of non-immune cells. This expression is typically induced in response to inflammatory stimuli, including bacterial lipopolysaccharides and certain cytokines. iNOS produces significantly higher concentrations of NO over a longer duration compared to constitutive NOS, playing a vital role in host defense against pathogens and in inflammatory responses.[15]

2.1.2. Bacterial NO generation

Interestingly, bacteria themselves can also produce NO through various mechanisms. Some bacteria possess their own nitric oxide synthases (**bNOSs**) that can generate NO from L-arginine.[43] However, these bNOSs typically lack an essential reductase domain, meaning that for NO generation, they often require the assistance of eukaryotic reductases *in vivo*.[24] Additionally, bacteria can produce NO through NOS-independent pathways. A notable example is during denitrification, an anaerobic respiration process in bacteria, where nitrate undergoes stepwise reduction, producing NO as an intermediate product.[44] Bacteria such as *Pseudomonas aeruginosa* and *P. stutzeri* are notable opportunistic pathogens that survive through denitrification in hypoxic environments, effectively decomposing NO as part of this process, aiding their survival.[44] Nitrite reduction to NO by **nitrite reductases (NIR)** is another pathway, particularly observed in denitrifying bacteria such as *Pseudomonas thiobacillus* (Figure 1A).[25] Species like *Legionella pneumophila*, *Nitrosomonas europaea*, and *Neisseria gonorrhoeae* are also noted in contexts related to NO-based dispersal in biofilms, suggesting their production of and interaction with NO.[45]

2.2. NO as an Antibacterial Agent

The endogenous production of NO, often compromised in chronic infections, can be mimicked. Exogenous production of NO can be achieved via various NO donors directly or by incorporating them into smart nanomaterials or polymeric delivery systems for controlled and sustained antibacterial activity.[21] NO and its derivatives exert potent antibacterial effects, and their efficacy is often dependent on the NO concentration, with low levels (<1 µM) involved in signal transduction and high levels (>1 µM) associated with cytotoxicity. Typically, low concentrations (pM–µM) can affect the formation or dispersal of biofilms, while concentrations higher than 1 µM are bactericidal.[25] Exogenous NO demonstrates broad-spectrum activity against various pathogens, including Gram-negative bacteria such as *P. aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *Burkholderia cepacia*, *Serratia marcescens*, *Vibrio cholerae*, *Fusobacterium nucleatum*, and *Burkholderia multivorans*. It is also effective against Gram-positive bacteria like *Staphylococcus aureus*, *methicillin-resistant Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Bacillus licheniformis*, *Bacillus subtilis*, and *Group B Streptococcus*, and even *Mycobacterium abscessus* and *Streptococcus pneumoniae*.[5,15,23,25] The activity can be dose-dependent and varies with bacterial species, with Gram-negative bacteria often being more susceptible due to their thinner peptidoglycan layer compared to Gram-positive bacteria's thicker peptidoglycan layer.[5] Studies with *S. pneumoniae* showed varying effects of NO depending on the infection model. Also, mucoid and non-mucoid strains of *P. aeruginosa* showed different susceptibilities to NO-releasing chitosan under varying aerobic and anaerobic conditions.[46] This underscores the complexity of NO's interaction with different bacterial species and their environments and needs to be explored in detail.

2.2.1. Mechanisms of Action and Propensity for Resistance Development

NO exerts its antibacterial effects primarily through its capacity to generate reactive nitrogen and oxygen species (RNS and ROS), which consequently induce cellular stress and damage to vital bacterial components. (Figure 1B). Alternatively, NO also exerts indirect effects on microbes via host immune response modulation.[5] At bactericidal concentrations, NO reacts with oxygen or reactive oxygen intermediates like superoxide (O_2^-), forming highly oxidizing species such as peroxynitrite ($ONOO^-$) and dinitrogen trioxide (N_2O_3).[5,47] These reactive products lead to oxidative and nitrosative stress, causing significant damage to bacterial membrane, DNA, lipids and proteins.[48] NO and its congeners, particularly N_2O_3, can deaminate DNA bases and cause oxidative damage to DNA, leading to abasic sites and strand breaks.[5] Studies with *S. typhimurium* have shown mutagenicity consistent with a DNA deaminating mechanism upon exposure to NO donor compounds.[5] Importantly, NO has also been found to inhibit DNA alkyl transferases involved in DNA repair by reacting with their sulfhydryl groups, thereby exacerbating DNA damage.[15] Peroxynitrite, in particular, can initiate lipid peroxidation in bacterial membranes, leading to membrane destruction and compromised cell integrity.[47] Elevated levels of NO and O_2^- within lipid membranes enhance the generation of nitrosative and oxidative species like N_2O_3 and NO_2, contributing to the membrane destruction and compromised cell integrity.[48] NO interacts with proteins through heme groups, iron-sulfur clusters, reactive thiols, and aromatic amino acid residues.[5] Nitrosative species like S-nitrosothiols and N_2O_3 nitrosate thiols on both cell surface and intracellular proteins, altering vital protein functions, leading to their inactivation.[15] The modification of surface thiols is reported to be responsible for S-nitrosothiol–mediated *Bacillus cereus* spore outgrowth inhibition.[5] It has been reported that peroxynitrite and NO_2 can also nonspecifically cause oxidation of proteins at various cellular sites. This includes the inhibition of key metalloproteins in bacterial respiratory reactions and the destruction of adhesion proteins, particularly in prokaryotic cells that are highly sensitive to NO due to their reliance on iron-sulfur clusters.[15] NO and RNS degrade these clusters, releasing iron. This free iron then catalyzes the formation of more free radicals, which in turn damage DNA and cell membranes.[15]

A significant advantage provided by NO's multiple and diverse antimicrobial pathways is its ability to bypass typical antibiotic resistance mechanisms. They make it unlikely for bacteria to develop resistance, as observed in studies where no significant increase in the minimum inhibitory concentration was found for *S. aureus*, MRSA, *S. epidermidis*, *E. coli*, and *P. aeruginosa*.[10] However, studies have shown that bacteria have developed certain mechanisms to resist NO toxicity. *P. aeruginosa* and *Salmonella enterica* detoxify NO by employing enzymes like nitric oxide reductase (NOR) and flavohemoglobin, which convert NO into less harmful compounds such as nitrous oxide or nitrate.[44,49] Enterohemorrhagic *E. coli* also relies on NOR for survival within macrophages.[44] Additionally, Gram-positive bacteria such as *Staphylococcus aureus* can produce their own NO via bNOS, which aids in resisting immune responses and enhances antibiotic tolerance.[50–52] This endogenous NO production in species like *S. aureus* can significantly increase their ability to survive antibiotic treatments.[50] These diverse strategies underscore the complex interplay between bacterial defense and NO-based antibacterials, highlighting the need for developing innovative NO donor systems capable of circumventing these sophisticated defense mechanisms.

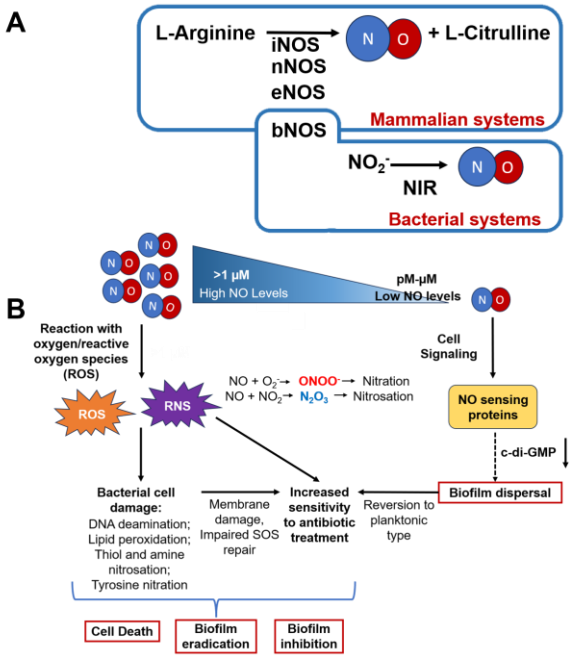


Figure 1. A. Schematic of endogenous nitric oxide (NO) production in mammalian and bacterial systems; B. Proposed antibacterial mechanisms of NO against planktonic bacteria and biofilms. Adapted with permission under Creative Commons Attribution License (CC-BY) from ref.[25] Copyright 2022, The Authors. Published by MDPI, Basel, Switzerland.

2.2.2. Biofilm Inhibition and Dispersion

NO plays a crucial role in regulating bacterial biofilms by acting as an important inhibitor and dispersant.[11,12] The biofilm life cycle, including attachment, colonization, maturation, and dispersal, is governed by the intracellular levels of second messenger cyclic-diguanylate-guanosine monophosphate (c-di-GMP).[53,54] Elevated intracellular levels of c-di-GMP promote biofilm formation, whereas lower concentrations induce bacteria to adopt a free-living planktonic mode of existence.[54] NO mediates biofilm dispersal by reducing intracellular c-di-GMP concentrations, thereby triggering the synthesis and release of hydrolytic enzymes that degrade the biofilm matrix.[11,55] The impact of

NO on biofilm inhibition and dispersal has been observed across a range of various Gram-negative bacteria, such as *S. marcescens*, *V. cholerae*, *E. coli*, *F. nucleatum*, *P. aeruginosa* and *L. monocytogenes*. Biofilms of Gram-positive bacteria like *B. licheniformis* and *S. epidermidis*, as well as clinical and MDR isolates and even mixed species biofilms from water distribution and treatment systems have been reported to be inhibited by NO.[13,25,56,57] Low levels of exogenous NO, such as 0.025–0.50 × 10^{-9} M, have been demonstrated to disperse mature *P. aeruginosa* biofilms over a period of 24 hours, initiating bacterial detachment while preserving the viability of planktonic bacteria. This dispersal makes bacteria more susceptible to antimicrobial treatments, as demonstrated by the increased efficacy of chlorine disinfection against multi-species biofilms pretreated with NO.[58] Additionally, sustained release of NO via nanoparticles has shown considerable promise in the prevention and disruption of *S. aureus* adhesion and biofilm formation inhibition in a preclinical rat model of central venous catheter.[59] NO can also modulate bacterial communication pathways such as quorum sensing, thereby contributing to its anti-biofilm properties.[60]

3. Advancements in NO Delivery Systems for Antibacterial Applications

The inherent reactivity and short half-life of NO necessitate advanced delivery strategies to harness its therapeutic potential effectively.[15,16] There is a persistent requirement for systems capable of spatiotemporally regulating NO concentrations in intended applications, particularly for exploring its utility in translational clinical research. These systems aim to provide controlled, sustained, and targeted release of NO to overcome its gaseous nature and high reactivity in biological environments.[18,25,61–64]

3.1. NO Donors

NO donors can generate and release exogenous NO, and their design focuses on tuning the release rate and kinetics to match desired therapeutic applications.[21,65] These donors are crucial for leveraging NO's therapeutic potential, particularly in antibacterial applications. NO donors can be broadly classified based on their chemical nature and mechanisms of NO release. The most studied and utilized types include N-Diazeniumdiolates (NONOate), S-Nitrosothiols (RSNOs), Furoxans, Metal Nitrosyl Complexes and Organic Nitrates (Figure 2).[21]

3.1.1. N-Diazeniumdiolates (NONOates)

NONOates are a well-studied class of NO donors recognized for their capacity to spontaneously release NO.[17] Under physiological conditions, a single NONOate molecule can spontaneously release two molecules of NO. This spontaneous release makes them valuable for mimicking transient, low-level NO release. In antibacterial therapy, NONOates have shown efficacy against various pathogens. Diethylenetriamine (DETA) NONOate and spermine NONOate have demonstrated time- and concentration-dependent antibacterial activity against *B. pseudomallei*.[66,67] DETA NONOate has also been utilized against uropathogenic *E. coli*.[66,68] To effectively control their spontaneous NO release and achieve therapeutic concentrations, NONOates are frequently incorporated into various delivery systems, such as polyamidoamine (PAMAM) conjugates, pluronic F68-branched polyethyleneimine-NONOate (F68-BPEI-NONOate), β-cyclodextrin derivatives, alginates, and polymeric nanoparticles.[69–76] These systems help in modulating the NO release kinetics to provide sustained low concentrations for biofilm dispersal or higher concentrations for bactericidal effects.[45]

3.1.2. S-Nitrosothiols (RSNOs)

S-Nitrosothiols (RSNOs) represent naturally occurring NO reservoirs and carriers within biological systems, generally exhibiting greater stability than NONOates. They release a single molecule of NO under specific conditions, including exposure to UV light, elevated temperatures, certain metal ions, acids, or enzymatic activity.[19] This conditional release mechanism offers a way to control NO concentrations. The conversion of RSNOs to NO is a primary mechanism for their antimicrobial effects, particularly through inducing DNA damage at cytotoxic NO concentrations.[15] A novel bifocal antimicrobial agent, SNAPicillin, was developed using SNAP (S-nitroso-N-acetylpenicillamine), an RSNO donor, to initially release NO gas to disperse biofilm matrices. This was followed by the combined action of NO and ampicillin, showing enhanced lethality against *P. aeruginosa* and MRSA biofilms.[77] Studies on RSNO donors like S-nitrosoglutathione (GSNO), S-nitrosocysteine (CySNO), S-nitroso-N-acetylcysteine (SNAC), and (2-(2-S-nitroso propionamide) acetic acid (GAS) against *E. coli* and *S. aureus* have shown potent antibacterial activity.[25,78] RSNOs can also be integrated into medical-grade polymeric devices for prolonged and controlled NO release, enabling the maintenance of effective antibacterial concentrations over time.[79,80]

3.1.3. Furoxans (1,2,5-oxadiazole N-oxides)

This class of NO donors is characterized by its requirement for a thiol attack to initiate the release of NO. This mechanism allows for a degree of control over NO release, potentially enabling the achievement of a specific antibacterial concentration.[25] Certain furoxan compounds have demonstrated notable antibacterial capabilities. Specifically, 3-{[2-(dimethylamino)ethyl]oxy}-4-phenylfuroxan and 3-nitro-4-phenylfuroxan have proven effective in eliminating *P. aeruginosa* biofilms as well as exhibiting strong bactericidal activity against it.[81,82]

3.1.4. Metal Nitrosyl Complexes

These complexes contain at least one NO functional group directly bonded to a central metal atom. NO release from these complexes can be triggered by light or single-electron reduction, providing another avenue for controlled delivery and concentration.[25,31] Sodium nitroprusside (SNP) is a

prominent example within this category, shown to disperse *P. aeruginosa* biofilms, with studies indicating that 250 µM SNP after 24 hours can achieve 63.5% dispersal.[65] However, a significant limitation of SNPs is the cytotoxicity arising from the dissociation of cyanide during its administration, which necessitates careful monitoring and minimal dosing to avoid harmful high concentrations.[45]

3.1.5. Organic Nitrates

Organic nitrates are nitrite esters featuring the nitroxide functional group (-ONO_2). Nitroglycerin (glyceryl nitrate, GTN) is a historic example. While isosorbide mononitrate (ISMN) has functioned as an effective NO donor in nanoparticle and gel-based delivery systems against *S. aureus*, its clinical safety in this application requires further research.[83,84] The challenge with organic nitrates is often in achieving sustained, non-toxic NO concentrations.[25,66]

3.1.6. Other NO Donors

Beyond the primary classes of NO donors, various other compounds and strategies are being explored for their NO-generating capabilities in antibacterial therapy. These include **nitroaromatic compounds**, such as specifically designed nitrobenzene derivatives, which release NO via metabolic processes and have shown promising antibacterial effects.[85,86] Another class includes **Inorganic nitrites**, such as $NaNO_2$, which serve as specific NO donors, particularly in hypoxic or acidic conditions, with nitrite being reduced to NO.[23,66,87] In addition, **L-arginine-based approaches** leverage the natural precursor for NO production, with exogenous L-arginine enhancing NO synthesis.[87,88] Innovative approaches also include **NO-donating antimicrobial peptides**, where peptides are functionalized with NO-donor moieties or delivery systems for controlled release that can be utilized to enhance bactericidal activity and biofilm dispersion.[89,90] Photolabile NO donors such as **N-Nitrosamines** are also an emerging notable class offering precise spatial and temporal control over NO release upon photoirradiation.[91] Furthermore, **electrochemical methods** offer a controlled way to generate and deliver NO, especially for localized applications on medical devices. These methods typically involve the electrochemical reduction of nitrite ions, often catalyzed by metal complexes such as copper-ligand complexes or iron sulfide nanoclusters, by applying a specific voltage to release gaseous NO [92–95]

While the primary focus of this review remains on discussing the role of NO and its donors in antibacterial therapy, it is important to mention that our laboratory has also contributed to understanding NO's broader therapeutic potential through innovative chemical scaffolds for NO release.[17,96] This includes studies on peptide-based self-assembling soft structures for releasing intracellular NO and promoting neurite outgrowth, as well as for NO-induced differentiation of neuroblastoma cells.[97,98] Furthermore, we have also investigated the anti-proliferative effects of purine-based ligands with sustained nitric oxide release in the HepG2 cancer cell line.[99] These diverse NO-releasing scaffolds, although initially developed for neuroprotection or anti-cancer applications, could potentially be modified through altered NO release kinetics or surface functionalization to target bacterial cells and biofilms for antibacterial therapies.

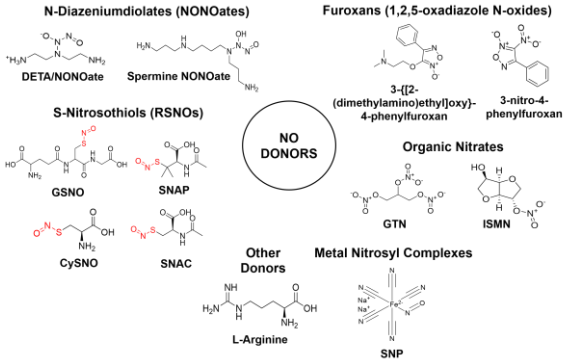


Figure 2. Major classes of NO donors used in antibacterial studies.[100] (DETA) NONOate: Diethylenetriamine N-diazeniumdiolates; GSNO: S-nitrosoglutathione; SNAP: S-nitroso-N-acetylpenicillamine; CySNO: S-nitrosocysteine; SNAC: S-nitroso-N-acetylcysteine; GTN: Glyceryl Nitrate (Nitroglycerin); ISMN: Isosorbide mononitrate; SNP: Sodium nitroprusside.

3.2. Stimuli-Responsive Release (pH, light, enzymes, ROS)

To achieve precise control and localized delivery, NO donors are often designed to be stimuli-responsive, releasing NO in response to specific physiological or external triggers such as pH, light, intracellular enzymes and ROS, temperature and metal ions (Figure 3A).[101] Many infection sites, such as areas of inflammation or bacterial growth, exhibit lower pH.[101] NO donors have been engineered to release NO preferentially in these acidic conditions, enabling targeted delivery.[29,102] A study by Choi *et al.* reported a pH-jump reagent 2-nitrobenzaldehyde (o-NBA), encapsulated within mesoporous silica nanoparticles (MSNs) and further coated with NONOates and calcium phosphate (pH@MSN-CaP-NO), for targeted NO delivery. The composite led to corneal wound healing, activated by light exposure at 365 nm. (Figure 3B).[103] Researchers have also developed small-molecule photosensitized NO donors triggered by various wavelengths, including visible light (e.g., 390 nm, 405 nm, 500 nm) and near-infrared light (e.g., 800 nm, 980 nm), which can penetrate deeper into tissues than UV light.[27,104–106] Manganese-nitrosyl sol-gel coatings have been shown to release NO upon visible and NIR light exposure, leading to significant reduction of *S. aureus*, *E. coli*, and *A. baumannii* bacterial loads.[107] Some NO delivery systems leverage specific enzymatic activity to trigger NO release.[30,86,108–110] Bacterial nitroreductase, an enzyme almost exclusively present in bacteria, activated nitroaromatic-protected diazeniumdiolate prodrugs, leading to site-specific NO release and bacterial killing.[86] Systems designed to release NO in the presence of elevated ROS, often found in inflammatory and infectious environments, have allowed for targeted NO generation.[111] The catalytic release of NO from S-nitrosothiols has also been observed with various metallic

ions, notably Cu^{2+}, Au^{3+}, Pd^{2+}, Pt^{2+}, V^{3+}, In, Hf^{4+}, Fe^{2+}, Sn^{2+}, and Zr^{4+}. Copper ions stand out as the most thoroughly examined catalysts, driving the breakdown of RSNOs to generate NO.[35,78,112] While substantial progress has been made in leveraging various triggers, the future of these intelligent systems in antibacterial applications is poised for further refinement, with computational and artificial intelligence-based machine learning approaches expected to play a crucial role.

3.3. NO Delivery Systems for Controlled Release

Various materials serve as scaffolds or encapsulants for NO donors, enabling controlled release kinetics and targeted delivery to maximize NO's therapeutic efficacy upon exposure to different stimuli. Major delivery systems that have been utilized in biomedical applications pertinent to infections include nanoparticle-based systems, polymeric materials and hydrogels (Table 1).[15,18,26,64,113]

3.3.1. Nanoparticle-Based Systems

Nanoparticles are widely investigated for NO delivery due to their ability to encapsulate NO donors, protect them from premature degradation, and facilitate targeted and localized sustained release of NO under various stimuli and triggers in multicomponent delivery systems. These can improve bioavailability, reduce systemic toxicity to host tissues, and enhance penetration into bacterial biofilms.[63,83,102,105,114–119]

3.3.1.1. Polymeric Nanoparticles

These biodegradable and biocompatible nanoparticles, often made from polymers like polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA) and chitosan, can encapsulate NO donors for sustained release.[71,83,116,119–123] Nanoparticles formulated with PLGA loaded with ISMN have demonstrated potent antibacterial effects against *S. aureus* biofilms.[63,83] Liu *et al.* reported co-assembled NO-releasing nanoparticles combined with Pluronic F127 exhibiting potent antimicrobial efficacy against MRSA strains.[124,125] Furthermore, NO-releasing nanoparticles incorporated into a chitosan hydrogel-glass composite have been reported with augmented antimicrobial activity, preventing biofilm formation on medical catheters.[59] Nonoate-loaded chitosan oligosaccharides (COS-EA/NO) have also been reported to exhibit bactericidal activity against *S. aureus* and *P. aeruginosa* strains.[126] Polyethylenimine (PEI) NONOates doped PLGA nanoparticles have been designed for extended NO release over 4 days to effectively bind to and diffuse into MRSA biofilms.[122] Dendrimers, a class of synthetic polymers, have been modified with NO donors to effectively deliver NO in high concentrations and control its release kinetics.[127,128] Some bifunctional dendrimers co-deliver NO and ursodeoxycholic acid for anti-inflammatory synergy.[129] Another dual-action approach involved NONOate-functionalized PAMAM dendrimer and low molecular weight chitosan (CS) conjugates, enabling simultaneous, controlled delivery of methicillin and NO (CS-PAMAM-MET/NONOate), leading to significant bacterial killing and improved wound healing against Gram-positive, Gram-negative, and MRSA infections.[130]

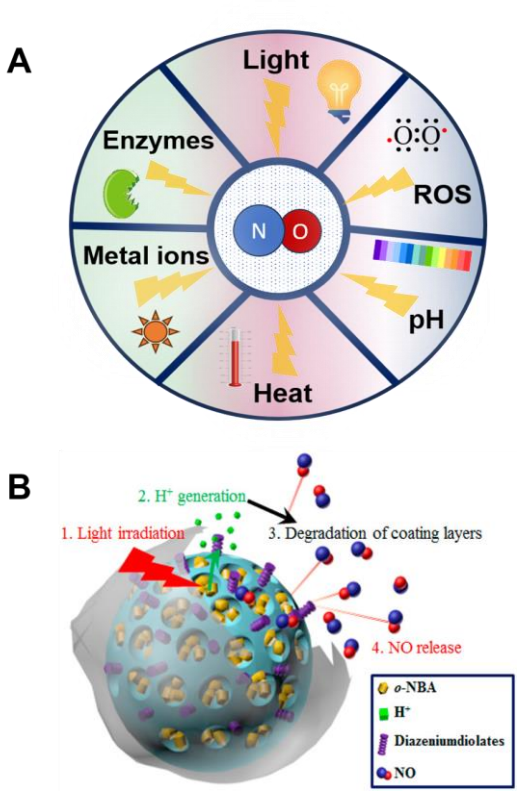


Figure 3. A. Various physiological and external stimuli utilized for NO release**;** B. Schematic of pH@MSN-CaP-NO mediated smart NO release. Reprinted with permission from ref.[103] Copyright 2016. American Chemical Society.

3.3.1.2. Inorganic Nanoparticles

Mesoporous silica nanoparticles (MSNs) are frequently used due to their large surface area and porous structure, allowing efficient loading and release of NO donors in a controlled manner.[63,131,132] Studies indicate that silica nanoparticles releasing NO exhibit superior antibacterial efficacy against *P. aeruginosa* in comparison to small-molecule NO donors, while concurrently minimizing cytotoxicity towards healthy cells.[131] These nanoparticles have additionally shown effective control and killing of *P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis*, and *C. albicans* biofilms.[22,63] Furthermore, metallic nanoparticles, such as gold and silver, have distinct optical and electronic characteristics that aid in NO delivery and can be combined with MSNs for multifunctional applications.[63] Gold Core@Shell MSNs combined photothermal therapy with NO release have been reported to significantly reduce *S. aureus* biofilm integrity in literature.[114,133]

3.3.1.3. Lipid-Based Systems

Liposomes, with their lipid bilayer structure, can encapsulate NO donors, prevent rapid decomposition and allowing for

prolonged NO release. They offer good biocompatibility and can be modified for targeted delivery.[63] Cholesterol moieties can enhance donor affinity and facilitate liposome transport. Liposomal encapsulation of NO precursors like ISMN has been shown to substantially increase their anti-biofilm effects against *S. aureus*, positioning them as a potential agent for topical clinical administration.[134] Solid lipid nanoparticles also serve as a more robust and regulated release platform for NO compared to conventional liposomes.[63,115]

3.3.2. Polymeric Materials and Coatings

Integrating NO donors directly into polymeric materials or developing NO-releasing coatings for surfaces is a key strategy for preventing infections, particularly on medical devices.[19,24,135–137] NO-releasing polymeric coatings are applied to catheters, stents, and other implants to prevent adhesion and consequent bacterial biofilm formation.[24,135,138] These materials can continuously release NO at the surface, creating an antimicrobial environment. SNAP has been extensively incorporated into biomedical-grade polymers, showing potential for long-term applications.[139,140] Elast-Eon E2A polymer catheters doped with SNAP showed a significant reduction in thrombosis and bacterial adhesion during implantation for 7 days in sheep veins.[141,142] Silicone Foley urinary catheters infused with NO-releasing materials have been developed for the prevention of catheter-associated urinary tract infections (CUTIs).[77,143] CarboSil 2080A, releasing NO with an SP60D60 top-coated polymer, reduced *S. aureus* cell count by 96% compared to control.[144,145] Advanced NO-releasing catheter models using diblock copolymer brushes made with uniform high-density precision have been reported to reduce >99.99% biofilm of various Gram-positive and Gram-negative bacteria, even outperforming commercial silver catheters.[146] Polymeric films and electrospun fibers incorporated with NO donors have served as active wound dressings and in other surface applications.[18,24,139,147–151] Electrospun polyurethane fibers doped with silica particles releasing NO have demonstrated sustained NO release for up to two weeks, allowing release for a longer duration compared to fibers doped directly with NO donors.[152]

3.3.3. Hydrogels and Topical Formulations

Hydrogels, as biocompatible, soft, and water-swollen polymer networks, are excellent candidates for localized and topical NO delivery.[26,153,154] Hydrogels can encapsulate NO donors and release NO in a controlled manner, making them suitable for treating skin infections, chronic wounds, and other localized conditions where direct application is feasible.[153,155] Their tunable structures allow for flexible control over NO release, as has been reported by altering the weight content of polyethylene glycol (PEG).[26] NO-releasing ointments have been shown to improve healing activity in skin-wounded animal models, promoting re-epithelialization, granulation formation, collagen deposition, and angiogenesis in the early phases of wound healing.[26,156] An L-Arg- and H_2O_2-encapsulated hydrogel has been reported to continuously generate NO, mediating chemotaxis of macrophages and fibroblasts to the site of wound and promoting synthesis of collagen, thereby accelerating wound closure and dermal regeneration.[157] Studies have also shown that NO released from NO-containing graphene oxide nanocarriers embedded in hydrogels can accelerate the scarless repair of burned skin by inhibiting microorganisms and promoting pro-vascularization activities.[118,158] NO-loaded metal-organic frameworks have also demonstrated potential for skin repair, increasing wound closure in vitro.[112,159,160] Cream formulations containing GSNO have also demonstrated significant killing against *S. epidermidis*, *S. aureus*, and *P. aeruginosa*.[45,66]

3.4. Gaseous NO Delivery

While most current research focuses on donor-based systems, direct administration of gaseous NO has specific clinical applications, primarily for pulmonary conditions [20,95,161,162]. Inhaled NO is an FDA-approved treatment for pulmonary hypertension.[161] It has also shown promise in limited clinical studies for its antimicrobial activity against non-tuberculous mycobacterial lung disease.[163] Studies have demonstrated that exogenous gaseous NO has a significant effect on the *P. aeruginosa* viability in rat lungs and eradicates MDR *S. aureus* and *E. coli* strains *in vitro* with intermittent exposure over 4 hours (160–200 ppm for 30 min).[164] Clinical findings indicate that high-dose inhaled nitric oxide is a promising therapeutic option, particularly in cases involving highly resistant bacterial strains.[100] However, direct delivery of gaseous NO to deep-seated or localized infection sites is therapeutically challenging due to its short half-life and potential toxicity at high concentrations if uncontrolled.[100] Thus, it is imperative to explore novel strategies for inhaled NO therapy to improve patient outcomes.

Table 1. Recent advanced NO delivery platforms for infection-associated biomedical applications

Donor class	NO Delivery System	Stimulus	Applications	Ref
N-Diazeniumdiolates (NONOates)	PLGA-PEI/NONOate nanoparticles	—	MRSA biofilm eradication in diabetic wound infection	122
	Cinnamaldehyde-derived T^2A^2 (Cin-T^2A^2) with diethylenetriamine (DETA)-NONOate	—	Bactericidal against MDR S. Staphylococci and its biofilm	165
	PVP and EC nanofibers loaded with Proline (PROLI), dipropylenetriamine (DPTA), and DETA NONOates	—	Growth inhibition of *S. aureus* and *P. aeruginosa*	166
	Diethylamine (DEA)	—	*E. coli* infection	167

	NONOate		treatment, proof of concept to pursue as hand sanitizers	
	Chitosan (CS)-Poly(amidoamine) (PAMAM)-MET/NONOate	—	MRSA infection treatment via biofilm penetration	130
	pH@MSN-CaP-NO, pH-jump reagent 2-nitrobenzaldehyde loaded into MSNs coated with NONOates and calcium phosphate	Light irradiation at 365 nm and pH 5.0	Targeted NO delivery for corneal wound healing	103
	NO-releasing polymer coatings of PET and SE immobilized with aminosilane precursors tethered with NONOates	—	Reduction of *P. aeruginosa* adhesion and biofilm formation	168
	Dendritic Fe_3O_4@Poly(dopamine) PDA@PAMAM@NONOate nanocomposite	808 nm NIR light	Synergistic Photothermal Therapy against *E. coli* and *S. aureus*	128
S-Nitrosothiols (RSNOs)	SNAP coupled to ampicillin	—	Antibiotic potentiation of ampicillin against biofilm formation	169
	SNAP-Se polymer composites	Selenium (Se)	Growth reduction of adhered *S. aureus* and *E. coli* cells	170
	Elast-eon E2As polymer coatings doped with SNAP	—	Reduction of bacterial adhesion and thrombusafter 7 days of intravascular implantation in sheep	141
	CuS/NO hydrogel	1065 nm Near-Infrared light, Copper ions	Photothermal therapy involving infection elimination and wound repair	171
	GSNO-doped nanofibrous scaffolds made of zein (ZN) and silk fibroin (SF)	—	Biodegradable fibers killing adhered *S. aureus* and *E. coli*	172
	Chitosan (CS)/GSNO film	—	Antibacterial activity against *S. aureus* and *P. aeruginosa* with accelerated healing and epithelialization in a rat wound model	173
	GSNO-doped Tryptophan-poly(ester urea) (PEUs)/PCL electrospun composite mat	—	*S. aureus* biofilm inhibition and wound healing	174
Furoxans	3-nitro-4-phenylfuroxan	Thiol-mediated activation	Pseudomonas biofilm inhibition activity	82
	FuNPs combined with Polymyxin B	Thiol-mediated activation	Activity against *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* strains	175
	FOTyr-AMP	Thiol-mediated activation	Dual antibiofilm efficacy against *E. coli* and *S. aureus*	89
	Nitrofuroxanoquinoline	Thiol-mediated activation	—	176
Metal Nitrosyl Complexes	Ti (Titanium)-PDA@SNP-OGP (osteogenic growth peptide)	808 nm NIR light	Photothermal therapy eradicating MRSA biofilms and enhancing osteointegration in Ti bone implants *in vivo*	177
	A tannic acid-thioctic acid (TATA) based supramolecular hydrogel (MPCST) with SNP and CeO_2 loaded on MoS_2 nanoflakes coated with PDA	808 nm	PTT resulting in antibacterial and healing activity in oral ulcers	178
	SNP@PCN@Gel hydrogels in carboxymethyl chitosan	660 nm light	PTT, PDT, chemodynamic, gas and ion therapy to promote healing in *S.*	179

	(CMCS) polymer matrix		*aureus* infected wounds.	
Inorganic Nitrite ($NaNO_2$)	NO-TS (thiolated starch) nanoparticles with gelatin	—	Antibacterial activity against *E. coli*	[180]
L-Arginine	CAT/bArg/GSNO hydrogel	Nitric Oxide Synthase activation	Anti-*S. aureus* and *E. coli* activity and promotes chronic wound healing	[181]
	L-Arg/GOx@CuBDC (MOFs) composite	Enzymatic activation by Glucose oxidase (GOx)	Biocompatible, potent activity against *E. coli* and *S. aureus*	[182]
	L-Arg- Gold nanoparticles loaded (AG)-DMSN dendritic mesoporous nanoparticles	Enzymatic activation by GOx	Non-toxic, selective elimination of *S. aureus* biofilms in *vivo*	[183]
	Platelet rich fibrin A-PRF/CS/PEG sponge layer and L-Arg/chitosan nanofiber	—	Antibacterial activity against *E. coli* and *S. aureus*, wound healing and skin tissue regeneration	[184]
	BH + POM@L-Arg bilayer hydrogel	Peroxide microenvironment	Antibacterial, anti-inflammatory and diabetic wound healing activity	[185]
	CMCS-HA hydrogel incorporating PDA, porphyrin and L-Arg	660 nm and 808 nm, ROS	PTT, PDT and gas therapy resulting in activity against *S. aureus* and MRSA, also promotes wound healing	[186]
	GOA@HG cryogel loaded with glucose oxidase and L-Arg	Glucose oxidase mediated activation	Infected diabetic wound dressing	[187]
N-Nitrosamines	Palladium (II) tetraphenyltetrabenzoporphyrin (PdTPTBP) incorporated coumarin-based delivery micelles (CuON(NO)-R) loaded with N-Nitrosamine and Ciprofloxacin	630 nm Red Light	Photoresponsive antibiotic combination treatment for *P. aeruginosa* biofilm dispersal and wound abscess.	[188]
Gaseous NO	Delivery via inhalation	Direct administration	Lung infections	[162]

Abbreviations: PLGA: Poly(lactic-co-glycolic acid), PEI: Polyethyleneimine; T^2A^2: Two tailed antimicrobial amphiphiles, PVP: Polyvinylpyrrolidone, EC: Ethyl Cellulose, MET: Methicillin, MSN: Mesoporous Silica Nanoparticles, CaP: Calcium phosphate, PET: Poly(ethylene terephthalate), SE: Silicone elastomer, SNAP: S-Nitroso-N-acetylpenicillamine, CuS: Copper Sulfide, FuNPs: Furoxan-based nanoparticles, PCL: Polycaprolactone, FOTyr-AMP: Antimicrobial peptide conjugated 4-(4-(l-alanine methyl ester-3-yl)-phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (FOTyr), PDA: Poly(dopamine), SNP: Sodium Nitroprusside, PAMAM: Polyamidoamine, MoS_2: Molybdenum sulfide, PCN: Polymeric Carbon Nitride, CAT: adhesive hydrogels prepared using adenine- and thymine-modified chitosan (CSA and CST), GSNO: S-nitrosoglutathione, CuBDC: Copper metal organic framework, PEG: Polyethylene glycol, BH + POM@L-Arg: Bilayer hydrogel containing Polyvinyl alcohol (PVA), hydroxypropyl methyl cellulose and chitosan loaded with L-Arginine modified polyoxometalate nanoclusters, CMCS: Carboxymethyl chitosan, HA: Hyaluronic acid, GOA@HG: Glucose oxidase (GO) and L Arginine (A) incorporated into HA aldehyde methacryloyl (H) and gelatin methacryloyl (G) cryogels.

3.5. Synergistic Therapies

3.5.1. Antibiotic Potentiation

NO can act synergistically with other therapeutic modalities, significantly enhancing their antibacterial efficacy and potentially reducing the dosage of conventional treatments required.[71,106,189] NO can increase bacterial susceptibility to antibiotics by disrupting protective biofilm structures, interfering with bacterial stress responses, and enhancing antibiotic penetration into bacterial cells (Figure 4A).[190] Studies have confirmed that combinations of NO with various antibiotics, like tobramycin, ciprofloxacin and colistin, are often synergistic or additive, with no antagonism observed against multiple MDR bacteria.[25] NO, combined with tobramycin and colistin enhanced the susceptibility of *S. aureus* and *P. aeruginosa*, while NO-donating fluoroquinolone/oxime hybrids were found to be more potent than their parent compounds.[42] The combined use of nitroxide carboxy-TEMPO (4-carboxy 2,2,6,6tetramethylpiperidine 1-oxy) with fluoroquinolone ciprofloxacin demonstrated synergistic effect toward Gram-negative bacterial biofilms, reducing them by over 93%.[191] Polymeric nanoparticles co-delivering NO and gentamicin have shown synergistic effects, with NO exhibiting biofilm dispersal into a planktonic state and gentamicin killing the dispersed bacteria more effectively under photoinduction.[106]

3.5.2. Combination with Physical Therapies

NO therapy, when combined with photothermal (PTT) and photodynamic (PDT) studies, presents a powerful strategy for treating diverse bacterial infections. Multiple studies have reported the synergistic approach effectively combats drug-resistant strains by enhancing antimicrobial effects and improving the efficacy of light-based therapies.[28,31,114,117,128,133,159,160,192–200] In PDT, NO can react with ROS generated by photosensitizers (e.g., singlet oxygen) to produce more reactive and oxidative peroxynitrite ($ONOO^-$), thereby enhancing the killing effect.[201] In PTT, NO can enhance the photothermal effect, allowing for effective bacterial killing at lower temperatures, which can protect healthy tissues.[87] Several studies highlight its effectiveness against specific pathogens. MDR Gram-negative bacteria and their biofilms have been targeted through platforms that integrate single near-infrared laser (NIR)-triggered PTT with NO release.[23,28,66,138] *P. aeruginosa* infections were eradicated using red-light responsive NO donor micelles, often in conjunction with PTT utilizing photosensitizers and photothermal agents.[188,202] MRSA biofilms have been shown to be susceptible to combined PDT destruction by NO and NIR-stimulated NO-releasing nanocages.[200,201,203] *S. aureus* biofilms were eradicated by nanoplatforms combining NO with PDT and low-temperature PTT. An integrated phototherapeutic nanoplatform, termed AI-MPDA, was developed by Zhang et al. for effective *S. aureus* biofilm eradication. This platform is composed of mesoporous polydopamine functionalized with L-arginine on its surface and subsequently loaded with indocyanine green (ICG) via π-π stacking. Upon near-infrared exposure, the AI-MPDA generates heat and ROS, initiating a cascade catalytic release of NO from the L-Arg (Figure 4B). This system leverages NO-enhanced PDT alongside low-temperature PTT (PTT, ≤45 °C). In an abscess model, this comprehensive phototherapy platform achieved nearly 100% biofilm removal, leading to rapid recovery of infected wounds and a significant reduction in bacterial colonization.[28] Hence, synergistic PDT/gas/PTT therapy using NO holds significant promise for the future treatment of bacterial infections.[87] This combined therapeutic strategy leverages NO's antimicrobial properties and the precision of light-activated treatments to overcome bacterial resistance, making it a crucial area of research. Future investigations should aim to fine-tune NO release kinetics and light irradiation parameters to enhance biofilm eradication while preserving surrounding tissue integrity.[204,205]

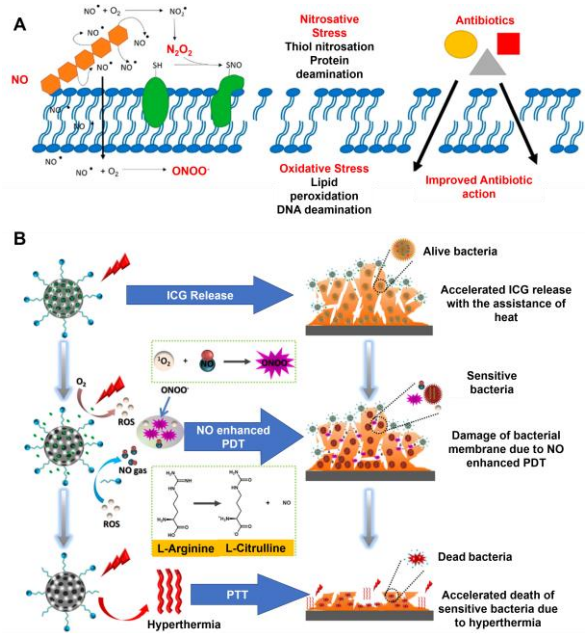


Figure 4. A. Schematic of mechanisms of antibiotic potentiation by NO. Adapted with permission from ref.[190] Copyright 2021. American Chemical Society; B. Schematic illustration of an integrated phototherapeutic nanoplatform, termed AI-MPDA conjugated with indocyanine green dye (ICG) for ROS production and L-Arginine for NO release for *S. aureus* biofilm eradication. Adapted with permission from ref.[28] Copyright 2020. American Chemical Society.

4. Challenges and Future Prospects

Despite significant advancements, the clinical translation and widespread adoption of NO-based antibacterial therapies still face several challenges that require ongoing research and innovation

4.1. Improving Stability and Shelf-Life of NO Delivery Systems

The inherent instability and highly reactive nature of NO, with a short half-life of only a few seconds to minutes, pose significant challenges for developing practical and storable NO delivery systems.[15,61] NO donors can degrade prematurely during storage, handling, or before reaching the target site, leading to reduced therapeutic efficacy.[43] This is particularly true for small-molecule NO donors, which are prone to spontaneous decay under physiological factors, often resulting in quick and uncontrolled release.[43] NONOate compounds are reported to have a half-life of just a few minutes at 25°C, making their incorporation into drug delivery systems complex.[25] The hydrophilic nature and lability of the S-NO bond in RSNOs also add to the complexity of incorporating them into formulations, limiting their use with certain delivery systems.[25] Future research must focus on designing more stable NO donor molecules and advanced encapsulation strategies to extend the half-life and improve the shelf-life of NO-releasing materials. Macromolecular NO donors, such as those conjugated to polymeric scaffolds, show promise in improving stability, biodistribution, and

circulation time.[73] Novel materials and functionalization techniques are needed to protect NO donors from degradation under various storage and physiological conditions.[206]

4.2. Strategies for Targeted Delivery to Infection Sites

Achieving precise and localized delivery of NO to specific infection sites is extremely important to maximize therapeutic efficacy while reducing potential off-target systemic toxicity.[25] NO's short half-life means its effective range from the production site is limited to about 100-200 µm, making highly targeted and selective delivery essential.[15] Non-specific NO release can lead to reduced efficacy and potential adverse effects on healthy host cells and tissues, as high concentrations of NO can be cytotoxic.[32] Traditional NO delivery often results in a rapid burst of NO followed by a progressive decay, which is not ideal for sustained antibacterial action. Research is moving towards developing "smart" delivery systems that can respond to specific biomarkers or environmental cues prevalent in infected tissues, such as changes in pH, redox potential, or the presence of bacterial enzymes.[207] Enzymatic prodrug systems where NO production is controlled by specific enzymes or substrates can finely tune delivery rates.[208,209]

4.3. Emerging Approaches: Computational tools and Machine Learning in NO-based Therapeutics and Delivery Systems

The integration of advanced computational tools and machine learning approaches offers powerful new avenues for accelerating the design, optimization, and understanding of NO delivery systems.[37]

4.3.1. Modeling Antimicrobial Mechanisms

Computational approaches can be used to understand the mechanisms underlying the antimicrobial action of NO.[34,35,38] To comprehensively assess these mechanisms, multiscale simulations should be employed, pairing molecular dynamics (MD) simulations to capture NO's molecular-level damage with cellular-scale models that reflect its systemic impact on microbes. This combined approach would help predict how NO and reactive derivatives like peroxynitrite induce nitrosative and oxidative stress on bacterial membranes, proteins, and DNA. Additionally, computational modeling of biofilms is essential: simulations could elucidate how NO compromises the extracellular polymeric substance (EPS) and disrupts quorum-sensing communication, thereby destabilizing biofilm structure and enhancing susceptibility to treatment. This includes predicting anti-biofilm activity of various molecules and training strain-specific models for targeted treatments.[39]

4.3.2. Designing and Optimizing NO-Releasing Systems

Computational studies can be used to engineer better NO-releasing systems. A major challenge is controlling the release of NO, which has a very short half-life. Quantitative models can predict the rate and duration of NO release from different delivery systems, such as nanoparticles or hydrogels, helping us adjust their design.[32] Using computational methods like molecular docking and SAR modeling allows scientists to forecast how effective and specific certain compounds will be against various microbes.[42] Also, machine learning (ML) can be a powerful tool.[37] ML approaches can derive enhanced broad-spectrum antimicrobial peptides by relating descriptors to activity.[39] By training ML algorithms on existing data, we can predict the properties of new NO donors and rapidly identify the most promising compounds for synthesis.[210] A recent *Cell* study demonstrated the power of AI-driven molecular generation in antibiotic discovery by combining fragment-guided and unconstrained generative strategies to explore vast chemical space. It filtered millions of candidates for synthesizability and activity and validated promising leads both *in vitro* and *in vivo*.[40] This framework provides a useful guide for applying the generative deep learning technique to compounds that release NO. Unconstrained generation allows for the discovery of new scaffolds, while established NO-donor motifs can be used as guiding components. The development of structurally distinct NO-based treatments with potentially novel mechanisms of action against resistant microbes could be accelerated by implementing predictive filters for NO-release kinetics, antimicrobial activity, and safety, as well as by using an iterative cycle of generation, prediction, synthesis, and experimental validation. Likewise, the deep learning-guided discovery of antibiotic structural classes was made possible by a recent study by Wong F. et al., which showed that ML models in drug development can be explained and offer insights into the chemical substructures underlying selective antibiotic activity.[41] These methodologies could be directly applied to research on NO-releasing moieties, where a comprehensive collection of compounds that produce or release NO, along with the associated cellular toxicity profiles and antimicrobial properties, could be used to train an AI model (Figure 5). Researchers could forecast and create novel compounds with improved antibacterial properties by employing explainable AI to help the model identify the precise chemical characteristics or structural configurations responsible for the most efficient and selective NO delivery.

Furthermore, computational fluid dynamics (CFD) and MD can simulate how NO-releasing nanoparticles interact with biological fluids and penetrate complex tissues, informing the design of systems for targeted delivery to infected sites.[38] To improve NO delivery systems, there is a need to enhance their effectiveness and reduce side effects. Computational modeling can aid in designing nanoparticles, hydrogels, and other drug delivery platforms that ensure controlled and targeted release of NO. For example, technologies such as NO-releasing porous silicon nanoparticles and polymer-based systems are promising areas for exploration to enhance antimicrobial efficacy.[18,62] A crucial aspect of developing NO-releasing antimicrobials is ensuring their safety and biocompatibility. Computational models can predict the cytotoxicity and pharmacokinetic profiles of NO

donors, assisting in the design of compounds with minimal adverse effects on mammalian cells.

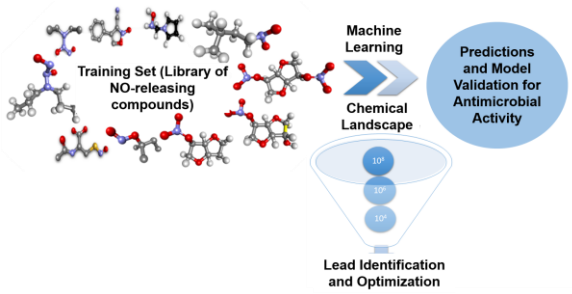


Figure 5. Schematic diagram of how Machine learning can effectively provide predictions and model validation for antimicrobial activity using a library of NO-releasing compounds as a training set to predict novel NO-releasing compounds**.**

5. Conclusion and Outlook

NO stands as a powerful and versatile tool in the ongoing battle against antibiotic resistance. Its broad-spectrum antibacterial activity, potent anti-biofilm capabilities, and multiple mechanisms of action offer a robust alternative to conventional antibiotics. Significant advancements in the development of sophisticated NO delivery systems, including various nanoparticles, polymeric materials, and hydrogels, have addressed the challenges of NO's inherent instability, enabling controlled, sustained, and targeted release. These innovations have opened diverse biomedical applications, particularly in combating persistent biofilm infections, promoting wound healing, and preventing infections on medical devices.

While challenges remain concerning the long-term stability, precise targeted delivery, and clinical translation of NO-based therapies, the outlook is highly promising. Emerging strategies, such as stimulus-responsive systems and the integration of computational and machine learning approaches, are poised to revolutionize the design and optimization of NO delivery. Continued innovation in material science, nanomedicine, and computational biology will be crucial for overcoming these hurdles. By facilitating the successful clinical translation of NO-based antibacterial therapies, we can significantly contribute to a new era of antimicrobial drugs, thereby safeguarding global public health against the ever-growing threat of drug-resistant pathogens.

6. Acknowledgements

GK thanks the Department of Science and Technology (DST), Government of India, for the DST INSPIRE Faculty Fellowship. SV thanks MOE-STARS (Ministry of Education, Government of India) and National Technical Textile Mission (Ministry of Textiles, Government of India) for financial support.

7. References

1. GBD 2021 Antimicrobial Resistance Collaborators. Global Burden of Bacterial Antimicrobial Resistance 1990–2021: A Systematic Analysis with Forecasts to 2050. *The Lancet* **2024**, *404* (10459), 1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1.
2. Ho, C. S.; Wong, C. T. H.; Aung, T. T.; Lakshminarayanan, R.; Mehta, J. S.; Rauz, S.; McNally, A.; Kintses, B.; Peacock, S. J.; de la Fuente-Nunez, C.; Hancock, R. E. W.; Ting, D. S. J. Antimicrobial Resistance: A Concise Update. *Lancet Microbe* **2024**. https://doi.org/10.1016/j.lanmic.2024.07.010.
3. Koo, H.; Allan, R. N.; Howlin, R. P.; Stoodley, P.; Hall-Stoodley, L. Targeting Microbial Biofilms: Current and Prospective Therapeutic Strategies. *Nat Rev Microbiol* **2017**, *15* (12), 740–755. https://doi.org/10.1038/NRMICRO.2017.99.
4. Guyer, R. L.; Koshland, D. E. The Molecule of the Year. *Science (1979)* **1989**, *246* (4937), 1543–1546. https://doi.org/10.1126/science.2688087.
5. Fang, F. C. Mechanisms of Nitric Oxide-Related Antimicrobial Activity. *Journal of Clinical Investigation* **1997**, *99* (12), 2818–2825. https://doi.org/10.1172/JCI119473.
6. Lancaster, J. R. Nitric Oxide: A Brief Overview of Chemical and Physical Properties Relevant to Therapeutic Applications. *Future Sci OA* **2015**, *1* (1). https://doi.org/10.4155/FSO.15.59.
7. Lundberg, J. O.; Weitzberg, E. Nitric Oxide Signaling in Health and Disease. *Cell* **2022**, *185* (16), 2853–2878. https://doi.org/10.1016/J.CELL.2022.06.010.
8. Adusumilli, N. C.; Zhang, D.; Friedman, J. M.; Friedman, A. J. Harnessing Nitric Oxide for Preventing, Limiting and Treating the Severe Pulmonary Consequences of COVID-19. *Nitric Oxide* **2020**, *103*, 4–8. https://doi.org/10.1016/J.NIOX.2020.07.003.
9. Carpenter, A. W.; Schoenfisch, M. H. Nitric Oxide Release: Part II. Therapeutic Applications. *Chem Soc Rev* **2012**, *41* (10), 3742–3752. https://doi.org/10.1039/C2CS15273H.
10. Privett, B. J.; Broadnax, A. D.; Bauman, S. J.; Riccio, D. A.; Schoenfisch, M. H. Examination of Bacterial Resistance to Exogenous Nitric Oxide. *Nitric Oxide* **2012**, *26* (3), 169–173. https://doi.org/10.1016/J.NIOX.2012.02.002.
11. Barraud, N.; Kelso, M.; Rice, S.; Kjelleberg, S. Nitric Oxide: A Key Mediator of Biofilm Dispersal with Applications in Infectious Diseases. *Curr Pharm Des* **2014**, *21* (1), 31–42. https://doi.org/10.2174/1381612820666140905112822.
12. Arora, D. P.; Hossain, S.; Xu, Y.; Boon, E. M. Nitric Oxide Regulation of Bacterial Biofilms. *Biochemistry* **2015**, *54* (24), 3717–3728. https://doi.org/10.1021/BI501476N.
13. Thompson, C. M.; Tischler, A. H.; Tarnowski, D. A.; Mandel, M. J.; Visick, K. L. Nitric Oxide Inhibits Biofilm Formation by Vibrio Fischeri via the Nitric Oxide Sensor HnoX. *Mol Microbiol* **2019**, *111* (1), 187–203. https://doi.org/10.1111/MMI.14147.
14. Plate, L.; Marletta, M. A. Nitric Oxide Modulates Bacterial Biofilm Formation through a Multicomponent Cyclic-Di-GMP Signaling Network. *Mol Cell* **2012**, *46* (4), 449–460. https://doi.org/10.1016/J.MOLCEL.2012.03.023.
15. Andrabi, S. M.; Sharma, N. S.; Karan, A.; Shahriar, S. M. S.; Cordon, B.; Ma, B.; Xie, J. Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications. *Advanced Science* **2023**, *10* (30). https://doi.org/10.1002/ADVS.202303259.

16. Hall, J. R.; Rouillard, K. R.; Suchyta, D. J.; Brown, M. D.; Ahonen, M. J. R.; Schoenfisch, M. H. Mode of Nitric Oxide Delivery Affects Antibacterial Action. *ACS Biomater Sci Eng* **2020**, *6* (1), 433–441. https://doi.org/10.1021/ACSBIOMATERIALS.9B01384.

17. Ali, R.; Sen, S.; Hameed, R.; Nazir, A.; Verma, S. Strategies for Gaseous Neuromodulator Release in Chemical Neuroscience: Experimental Approaches and Translational Validation. *J Controlled Release* **2024**, 132–160. https://doi.org/10.1016/j.jconrel.2023.11.014.

18. Sadrearhami, Z.; Nguyen, T. K.; Namivandi-Zangeneh, R.; Jung, K.; Wong, E. H. H.; Boyer, C. Recent Advances in Nitric Oxide Delivery for Antimicrobial Applications Using Polymer-Based Systems. *J Mater Chem B* **2018**, *6* (19), 2945–2959. https://doi.org/10.1039/C8TB00299A.

19. Chug, M. K.; Brisbois, E. J. Recent Developments in Multifunctional Antimicrobial Surfaces and Applications toward Advanced Nitric Oxide-Based Biomaterials. *ACS Materials Au* **2022**, *2* (5), 525–551. https://doi.org/10.1021/ACSMATERIALSAU.2C00040.

20. Yang, Y.; Qi, P. K.; Yang, Z. L.; Huang, N. Nitric Oxide Based Strategies for Applications of Biomedical Devices. *Biosurf Biotribol* **2015**, *1* (3), 177–201. https://doi.org/10.1016/J.BSBT.2015.08.003.

21. Yang, Y.; Huang, Z.; Li, L. L. Advanced Nitric Oxide Donors: Chemical Structure of NO Drugs, NO Nanomedicines and Biomedical Applications. *Nanoscale* **2021**, *13* (2), 444–459. https://doi.org/10.1039/D0NR07484E.

22. Yang, T.; Zelikin, A. N.; Chandrawati, R. Progress and Promise of Nitric Oxide-Releasing Platforms. *Advanced Science* **2018**, *5* (6). https://doi.org/10.1002/ADVS.201701043.

23. Wang, Z.; Jin, A.; Yang, Z.; Huang, W. Advanced Nitric Oxide Generating Nanomedicine for Therapeutic Applications. *ACS Nano*. American Chemical Society May 23, 2023, pp 8935–8965. https://doi.org/10.1021/acsnano.3c02303.

24. Rong, F.; Tang, Y.; Wang, T.; Feng, T.; Song, J.; Li, P.; Huang, W. Nitric Oxide-Releasing Polymeric Materials for Antimicrobial Applications: A Review. *Antioxidants (Basel)* **2019**, *8* (11), 556. https://doi.org/10.3390/antiox8110556.

25. Poh, W. H.; Rice, S. A. Recent Developments in Nitric Oxide Donors and Delivery for Antimicrobial and Anti-Biofilm Applications. *Molecules* **2022**, *27* (3), 674. https://doi.org/10.3390/MOLECULES27030674.

26. Kim, T.; Nah, Y.; Kim, J.; Lee, S.; Kim, W. J. Nitric-Oxide-Modulatory Materials for Biomedical Applications. *Acc Chem Res* **2022**, *55* (17), 2384–2396. https://doi.org/10.1021/ACS.ACCOUNTS.2C00159.

27. Zhao, Z.; Li, H.; Tao, X.; Xie, Y.; Yang, L.; Mao, Z. W.; Xia, W. Light-Triggered Nitric Oxide Release by a Photosensitizer to Combat Bacterial Biofilm Infections. *Chemistry - A European Journal* **2021**, *27* (17), 5453–5460. https://doi.org/10.1002/CHEM.202004698.

28. Yuan, Z.; Lin, C.; He, Y.; Tao, B.; Chen, M.; Zhang, J.; Liu, P.; Cai, K. Near-Infrared Light-Triggered Nitric-Oxide-Enhanced Photodynamic Therapy and Low-Temperature Photothermal Therapy for Biofilm Elimination. *ACS Nano* **2020**, *14* (3), 3546–3562. https://doi.org/10.1021/ACSNANO.9B09871.

29. Zhou, Y.; Jiang, Y.; Cai, J.; Wang, J.; Li, S.; Wang, M.; Zhou, X.; Wang, X.; Zhao, X.; Ren, L. A Core/Shell Nanogenerator Achieving PH-Responsive Nitric Oxide Release for Treatment of Infected Diabetic Wounds. *Nanoscale* **2022**, *14* (40), 14984–14996. https://doi.org/10.1039/D2NR03704A.

30. Hibbard, H. A. J.; Reynolds, M. M. Enzyme-Activated Nitric Oxide-Releasing Composite Material for Antibacterial Activity against Escherichia Coli. *ACS Appl Bio Mater* **2020**, *3* (8), 5367–5374. https://doi.org/10.1021/ACSABM.0C00670.

31. Yu, Y. T.; Shi, S. W.; Wang, Y.; Zhang, Q. L.; Gao, S. H.; Yang, S. P.; Liu, J. G. A Ruthenium Nitrosyl-Functionalized Magnetic Nanoplatform with Near-Infrared Light-Controlled Nitric Oxide Delivery and Photothermal Effect for Enhanced Antitumor and Antibacterial Therapy. *ACS Appl Mater Interfaces* **2020**, *12* (1), 312–321. https://doi.org/10.1021/ACSAMI.9B18865.

32. Sivaloganathan, D. M.; Brynildsen, M. P. Quantitative Modeling Extends the Antibacterial Activity of Nitric Oxide. *Front Physiol* **2020**, *11*. https://doi.org/10.3389/FPHYS.2020.00330.

33. Robinson, J. L.; Brynildsen, M. P. A Kinetic Platform to Determine the Fate of Nitric Oxide in Escherichia Coli. *PLoS Comput Biol* **2013**, *9* (5). https://doi.org/10.1371/JOURNAL.PCBI.1003049.

34. Bignon, E.; Rizza, S.; Filomeni, G.; Papaleo, E. Use of Computational Biochemistry for Elucidating Molecular Mechanisms of Nitric Oxide Synthase. *Comput Struct Biotechnol J* **2019**, *17*, 415–429. https://doi.org/10.1016/j.csbj.2019.03.011.

35. Rousseau, B. J. G.; Soudackov, A. V.; Tuttle, R. R.; Reynolds, M. M.; Finke, R. G.; Hammes-Schiffer, S. Computational Insights into the Mechanism of Nitric Oxide Generation from S-Nitrosoglutathione Catalyzed by a Copper Metal-Organic Framework. *J Am Chem Soc* **2023**, *145* (18), 10285–10294. https://doi.org/10.1021/jacs.3c01569.

36. Sharma, P.; Chowdhury, P. R.; Jain, A.; Patwari, G. N. Machine Learned Potential Enables Molecular Dynamics Simulation to Predict the Experimental Branching Ratios in the NO Release Channel of Nitroaromatic Compounds. *Journal of Physical Chemistry A* **2024**, *128* (47), 10137–10142. https://doi.org/10.1021/acs.jpca.4c04703.

37. Kleandrova, V. V.; Cordeiro, M. N. D. S.; Speck-Planche, A. Perturbation-Theory Machine Learning for Multi-Objective Antibacterial Discovery: Current Status and Future Perspectives. *Applied Sciences (Switzerland)* **2025**, *15* (3). https://doi.org/10.3390/app15031166.

38. Mikayilov, E.; Zeynalov, N.; Taghiyev, D.; Taghiyev, S. Role of Computational Modeling in the Design and Development of Nanotechnology-Based Drug Delivery Systems. *Chem Biochem Eng Q* **2024**, *38* (2), 97–110. https://doi.org/10.15255/CABEQ.2024.2290.

39. An, A. Y.; Choi, K. Y. G.; Baghela, A. S.; Hancock, R. E. W. An Overview of Biological and Computational Methods for Designing Mechanism-Informed Anti-Biofilm Agents. *Front Microbiol* **2021**, *12* (April), 1–24. https://doi.org/10.3389/fmicb.2021.640787.

40. Krishnan, A.; Anahtar, M. N.; Valeri, J. A.; Jin, W.; Donghia, N. M.; Sieben, L.; Luttens, A.; Zhang, Y.; Modaresi, S. M.; Hennes, A.; Fromer, J.; Bandyopadhyay, P.; Chen, J. C.; Rehman, D.; Desai, R.; Edwards, P.; Lach, R. S.; Aschtgen, M.-S.; Gaborieau, M.; Gaetani, M.; Palace, S. G.; Omori, S.; Khonde, L.; Moroz, Y. S.; Blough, B.; Jin, C.; Loh, E.; Grad, Y. H.; Saei, A. A.; Coley, C. W.; Wong, F.; Collins, J. J. A Generative Deep Learning Approach to de Novo Antibiotic Design. *Cell* **2025**. https://doi.org/10.1016/J.CELL.2025.07.033.

41. Wong, F.; Zheng, E. J.; Valeri, J. A.; Donghia, N. M.; Anahtar, M. N.; Omori, S.; Li, A.; Cubillos-Ruiz, A.; Krishnan, A.; Jin, W.; Manson, A. L.; Friedrichs, J.; Helbig, R.; Hajian, B.; Fiejtek, D. K.; Wagner, F. F.; Soutter, H. H.; Earl, A. M.; Stokes, J. M.; Renner, L. D.; Collins, J. J. Discovery of a Structural Class of Antibiotics with Explainable Deep Learning. *Nature* **2024**, *626* (7997), 177–185. https://doi.org/10.1038/S41586-023-06887-8.

42. Aziz, H. A.; Moustafa, G. A. I.; Abuo-Rahma, G. E. D. A.; Rabea, S. M.; Hauk, G.; Krishna, V. S.; Sriram, D.; Berger, J. M.; Abbas, S. H. Synthesis and Antimicrobial Evaluation of New Nitric Oxide-Donating Fluoroquinolone/Oxime Hybrids. *Arch Pharm (Weinheim)* **2021**, *354* (1). https://doi.org/10.1002/ARDP.202000180.

43. Chen, J.; Liu, L.; Wang, W.; Gao, H. Nitric Oxide, Nitric Oxide Formers and Their Physiological Impacts in Bacteria. *Int J Mol Sci* **2022**, *23* (18), 10778. https://doi.org/10.3390/IJMS231810778.

44. Terasaka, E.; Yamada, K.; Wang, P.-H.; Hosokawa, K.; Yamagiwa, R.; Matsumoto, K.; Ishii, S.; Mori, T.; Yagi, K.; Sawai, H.; Arai, H.; Sugimoto, H.; Sugita, Y.; Shiro, Y.; Tosha, T. Dynamics of Nitric Oxide Controlled by Protein Complex in Bacterial System. *Proceedings of the National Academy of Sciences* **2017**, *114* (37), 9888–9893. https://doi.org/10.1073/pnas.1621301114.

45. Mendhi, J.; Asgari, M.; Ratheesh, G.; Prasadam, I.; Yang, Y.; Xiao, Y. Dose Controlled Nitric Oxide-Based Strategies for Antibacterial Property in Biomedical Devices. *Applied Materials Today*. Elsevier Ltd June 1, 2020. https://doi.org/10.1016/j.apmt.2020.100562.

46. Yang, L.; Teles, F.; Gong, W.; Dua, S. A.; Martin, L.; Schoenfisch, M. H. Antibacterial Action of Nitric Oxide-Releasing Hyperbranched Polymers against Ex Vivo Dental Biofilms. *Dental Materials* **2020**, *36* (5), 635–644. https://doi.org/10.1016/J.DENTAL.2020.03.012.

47. Ischiropoulos, H.; Al-Mehdi, A. B. Peroxynitrite-Mediated Oxidative Protein Modifications. *FEBS Lett* **1995**, *364* (3), 279–282. https://doi.org/10.1016/0014-5793(95)00307-U.

48. Radi, R. Oxygen Radicals, Nitric Oxide, and Peroxynitrite: Redox Pathways in Molecular Medicine. *Proc Natl Acad Sci U S A* **2018**, *115* (23), 5839–5848. https://doi.org/10.1073/PNAS.1804932115.

49. Arkenberg, A.; Runkel, S.; Richardson, D. J.; Rowley, G. The Production and Detoxification of a Potent Cytotoxin, Nitric Oxide, by Pathogenic Enteric Bacteria. *Biochem Soc Trans* **2011**, *39* (6), 1876–1879. https://doi.org/10.1042/BST20110716.

50. Gusarov, I.; Shatalin, K.; Starodubtseva, M.; Nudler, E. Endogenous Nitric Oxide Protects Bacteria against a Wide Spectrum of Antibiotics. *Science (1979)* **2009**, *325* (5946), 1380–1384. https://doi.org/10.1126/SCIENCE.1175439.

51. Mogen, A. B.; Carroll, R. K.; James, K. L.; Lima, G.; Silva, D.; Culver, J. A.; Petucci, C.; Shaw, L. N.; Rice, K. C. Staphylococcus Aureus Nitric Oxide Synthase (SaNOS) Modulates Aerobic Respiratory Metabolism and Cell Physiology. *Mol Microbiol* **2017**, *105* (1), 139–157. https://doi.org/10.1111/MMI.13693.

52. Shu, X.; Shi, Y.; Huang, Y.; Yu, D.; Sun, B. Transcription Tuned by S-Nitrosylation Underlies a Mechanism for Staphylococcus Aureus to Circumvent Vancomycin Killing. *Nat Commun* **2023**, *14* (1). https://doi.org/10.1038/S41467-023-37949-0.

53. Zhao, A.; Sun, J.; Liu, Y. Understanding Bacterial Biofilms: From Definition to Treatment Strategies. *Front Cell Infect Microbiol* **2023**, *13*. https://doi.org/10.3389/FCIMB.2023.1137947.

54. Ha, D.-G.; O'Toole, G. A. C-Di-GMP and Its Effects on Biofilm Formation and Dispersion: A Pseudomonas Aeruginosa Review . *Microbiol Spectr* **2015**, *3* (2). https://doi.org/10.1128/MICROBIOLSPEC.MB-0003-2014.

55. Barraud, N.; Schleheck, D.; Klebensberger, J.; Webb, J. S.; Hassett, D. J.; Rice, S. A.; Kjelleberg, S. Nitric Oxide Signaling in Pseudomonas Aeruginosa Biofilms Mediates Phosphodiesterase Activity, Decreased Cyclic Di-GMP Levels, and Enhanced Dispersal. *J Bacteriol* **2009**, *191* (23), 7333–7342. https://doi.org/10.1128/JB.00975-09.

56. Jo, J.; Price-Whelan, A.; Dietrich, L. E. P. Gradients and Consequences of Heterogeneity in Biofilms. *Nat Rev Microbiol* **2022**, *20* (10), 593–607. https://doi.org/10.1038/S41579-022-00692-2.

57. Barnes, R. J.; Bandi, R. R.; Wong, W. S.; Barraud, N.; McDougald, D.; Fane, A.; Kjelleberg, S.; Rice, S. A. Optimal Dosing Regimen of Nitric Oxide Donor Compounds for the Reduction of Pseudomonas Aeruginosa Biofilm and Isolates from Wastewater Membranes. *Biofouling* **2013**, *29* (2), 203–212. https://doi.org/10.1080/08927014.2012.760069.

58. Barraud, N.; Storey, M. V.; Moore, Z. P.; Webb, J. S.; Rice, S. A.; Kjelleberg, S. Nitric Oxide-Mediated Dispersal in Single- and Multi-Species Biofilms of Clinically and Industrially Relevant Microorganisms. *Microb Biotechnol* **2009**, *2* (3), 370–378. https://doi.org/10.1111/J.1751-7915.2009.00098.X.

59. Mihu, M. R.; Cabral, V.; Pattabhi, R.; Tar, M. T.; Davies, K. P.; Friedman, A. J.; Martinez, L. R.; Nosanchuk, J. D. Sustained Nitric Oxide-Releasing Nanoparticles Interfere with Methicillinresistant Staphylococcus Aureus Adhesion and Biofilm Formation in a Rat Central Venous Catheter Model. *Antimicrob Agents Chemother* **2017**, *61* (1). https://doi.org/10.1128/AAC.02020-16.

60. Henares, B. M.; Xu, Y.; Boon, E. M. A Nitric Oxide-Responsive Quorum Sensing Circuit in Vibrio Harveyi Regulates Flagella Production and Biofilm Formation. *Int J Mol Sci* **2013**, *14* (8), 16473–16484. https://doi.org/10.3390/IJMS140816473.

61. Duong, H. T. T.; Kamarudin, Z. M.; Erlich, R. B.; Li, Y.; Jones, M. W.; Kavallaris, M.; Boyer, C.; Davis, T. P. Intracellular Nitric Oxide Delivery from Stable No-Polymeric Nanoparticle Carriers. *Chemical Communications* **2013**, *49* (39), 4190–4192. https://doi.org/10.1039/C2CC37181B.

62. Kafshgari, M. H.; Cavallaro, A.; Delalat, B.; Harding, F. J.; McInnes, S. J. P.; Mäkilä, E.; Salonen, J.; Vasilev, K.; Voelcker, N. H. Nitric Oxide-Releasing Porous Silicon Nanoparticles. *Nanoscale Res Lett* **2014**, *9* (1), 1–9. https://doi.org/10.1186/1556-276X-9-333.

63. Tortella Fuentes, G.; Fincheira, P.; Rubilar, O.; Leiva, S.; Fernandez, I.; Schoebitz, M.; Pelegrino, M. T.; Paganotti, A.; dos Reis, R. A.; Seabra, A. B. Nanoparticle-Based Nitric Oxide Donors: Exploring Their Antimicrobial and Anti-Biofilm Capabilities. *Antibiotics 2024, Vol. 13, Page 1047* **2024**, *13* (11), 1047. https://doi.org/10.3390/ANTIBIOTICS13111047.

64. Pieretti, J. C.; Pelegrino, M. T.; Silveira, N. M.; Rodrigues, M. G.; Seabra, A. B. State-of-the-Art and Perspectives for Nanomaterials Combined with Nitric Oxide Donors: From Biomedical to Agricultural Applications. *ACS Appl Nano Mater* **2024**, *7* (16), 18590–18609. https://doi.org/10.1021/ACSANM.3C03482.

65. Cai, Y. ming; Webb, J. S. Optimization of Nitric Oxide Donors for Investigating Biofilm Dispersal Response in Pseudomonas Aeruginosa Clinical Isolates. *Appl Microbiol*

Biotechnol **2020**, *104* (20), 8859–8869. https://doi.org/10.1007/S00253-020-10859-7.

66. Wang, T. Y.; Zhu, X. Y.; Wu, F. G. Antibacterial Gas Therapy: Strategies, Advances, and Prospects. *Bioact Mater* **2023**, *23*, 129–155. https://doi.org/10.1016/J.BIOACTMAT.2022.10.008.

67. Jones-Carson, J.; Laughlin, J. R.; Stewart, A. L.; Voskuil, M. I.; Vázquez-Torres, A. Nitric Oxide-Dependent Killing of Aerobic, Anaerobic and Persistent Burkholderia Pseudomallei. *Nitric Oxide* **2012**, *27* (1), 25–31. https://doi.org/10.1016/J.NIOX.2012.04.001.

68. Vumma, R.; Bang, C. S.; Kruse, R.; Johansson, K.; Persson, K. Antibacterial Effects of Nitric Oxide on Uropathogenic Escherichia Coli during Bladder Epithelial Cell Colonization-A Comparison with Nitrofurantoin. *Journal of Antibiotics* **2016**, *69* (3), 183–186. https://doi.org/10.1038/JA.2015.112.

69. Yang, L.; Wang, X.; Suchyta, D. J.; Schoenfisch, M. H. Antibacterial Activity of Nitric Oxide-Releasing Hyperbranched Polyamidoamines. *Bioconjug Chem* **2018**, *29* (1), 35–43. https://doi.org/10.1021/ACS.BIOCONJCHEM.7B00537.

70. Ahonen, M. J. R.; Suchyta, D. J.; Zhu, H.; Schoenfisch, M. H. Nitric Oxide-Releasing Alginates. *Biomacromolecules* **2018**, *19* (4), 1189–1197. https://doi.org/10.1021/ACS.BIOMAC.8B00063.

71. Nguyen, T. K.; Selvanayagam, R.; Ho, K. K. K.; Chen, R.; Kutty, S. K.; Rice, S. A.; Kumar, N.; Barraud, N.; Duong, H. T. T.; Boyer, C. Co-Delivery of Nitric Oxide and Antibiotic Using Polymeric Nanoparticles. *Chem Sci* **2016**, *7* (2), 1016–1027. https://doi.org/10.1039/C5SC02769A.

72. Jin, H.; Yang, L.; Ahonen, M. J. R.; Schoenfisch, M. H. Nitric Oxide-Releasing Cyclodextrins. *J Am Chem Soc* **2018**, *140* (43), 14178–14184. https://doi.org/10.1021/JACS.8B07661.

73. Yang, L.; Feura, E. S.; Ahonen, M. J. R.; Schoenfisch, M. H. Nitric Oxide–Releasing Macromolecular Scaffolds for Antibacterial Applications. *Adv Healthc Mater* **2018**, *7* (13). https://doi.org/10.1002/ADHM.201800155.

74. Park, D.; Kim, J.; Lee, Y. M.; Park, J.; Kim, W. J. Polydopamine Hollow Nanoparticle Functionalized with N-Diazeniumdiolates as a Nitric Oxide Delivery Carrier for Antibacterial Therapy. *Adv Healthc Mater* **2016**, *5* (16), 2019–2024. https://doi.org/10.1002/ADHM.201600150.

75. Shim, J. S.; Park, D. S.; Baek, D. H.; Jha, N.; Park, S. I.; Yun, H. J.; Kim, W. J.; Ryu, J. J. Antimicrobial Activity of NO-Releasing Compounds against Periodontal Pathogens. *PLoS One* **2018**, *13* (10), e0199998. https://doi.org/10.1371/JOURNAL.PONE.0199998.

76. Rouillard, K. R.; Markovetz, M. R.; Bacudio, L. G.; Hill, D. B.; Schoenfisch, M. H. Pseudomonas Aeruginosa Biofilm Eradication via Nitric Oxide-Releasing Cyclodextrins. *ACS Infect Dis* **2020**, *6* (7), 1940–1950. https://doi.org/10.1021/ACSINFECDIS.0C00246.

77. Colletta, A.; Wu, J.; Wo, Y.; Kappler, M.; Chen, H.; Xi, C.; Meyerhoff, M. E. S-Nitroso-N-Acetylpenicillamine (SNAP) Impregnated Silicone Foley Catheters: A Potential Biomaterial/Device to Prevent Catheter-Associated Urinary Tract Infections. *ACS Biomater Sci Eng* **2015**, *1* (6), 416–424. https://doi.org/10.1021/ACSBIOMATERIALS.5B00032.

78. Tuttle, R. R.; Finke, R. G.; Reynolds, M. M. CuIILewis Acid, Proton-Coupled Electron Transfer Mechanism for Cu-Metal-Organic Framework-Catalyzed NO Release from S-Nitrosoglutathione. *ACS Catal* **2022**, *12* (13), 8055–8068. https://doi.org/10.1021/ACSCATAL.2C00477.

79. Luo, Z.; Ng, G.; Zhou, Y.; Boyer, C.; Chandrawati, R. Polymeric Amines Induce Nitric Oxide Release from S-Nitrosothiols. *Small* **2023**, *19* (13). https://doi.org/10.1002/SMLL.202200502.

80. Sadrearhami, Z.; Namivandi-Zangeneh, R.; Price, E.; Krasowska, M.; Al-Bataineh, S. A.; Whittle, J.; Wong, E. H. H.; Blencowe, A.; Boyer, C. S-Nitrosothiol Plasma-Modified Surfaces for the Prevention of Bacterial Biofilm Formation. *ACS Biomater Sci Eng* **2019**, *5* (11), 5881–5887. https://doi.org/10.1021/ACSBIOMATERIALS.9B01063.

81. Poh, W. H.; Barraud, N.; Guglielmo, S.; Lazzarato, L.; Rolando, B.; Fruttero, R.; Rice, S. A. Furoxan Nitric Oxide Donors Disperse Pseudomonas Aeruginosa Biofilms, Accelerate Growth, and Repress Pyoverdine Production. *ACS Chem Biol* **2017**, *12* (8), 2097–2106. https://doi.org/10.1021/ACSCHEMBIO.7B00256.

82. Orlandi, V. T.; Bolognese, F.; Rolando, B.; Guglielmo, S.; Lazzarato, L.; Fruttero, R. Anti-Pseudomonas Activity of 3-Nitro-4-Phenylfuroxan. *Microbiology (United Kingdom)* **2018**, *164* (12), 1557–1566. https://doi.org/10.1099/MIC.0.000730.

83. Hasan, S.; Thomas, N.; Thierry, B.; Prestidge, C. A. Biodegradable Nitric Oxide Precursor-Loaded Micro- and Nanoparticles for the Treatment of Staphylococcus Aureus Biofilms. *J Mater Chem B* **2017**, *5* (5), 1005–1014. https://doi.org/10.1039/C6TB03290G.

84. Hasan, S.; Thomas, N.; Thierry, B.; Prestidge, C. A. Controlled and Localized Nitric Oxide Precursor Delivery From Chitosan Gels to Staphylococcus Aureus Biofilms. *J Pharm Sci* **2017**, *106* (12), 3556–3563. https://doi.org/10.1016/j.xphs.2017.08.006.

85. Tang, Q.; Ye, W.; Chandarajoti, K.; Ge, R.; Lv, S.; Zhang, K.; Han, X.; Wang, C.; Bai, H.; Wang, X.; Zhou, W. Discovery of Xanthone-Based Nitric Oxide Donors Targeting Biofilm Clearance. *Arch Pharm (Weinheim)* **2025**, *358* (1), e2400793. https://doi.org/10.1002/ARDP.202400793.

86. Hibbard, H. A. J.; Reynolds, M. M. Synthesis of Novel Nitroreductase Enzyme-Activated Nitric Oxide Prodrugs to Site-Specifically Kill Bacteria. *Bioorg Chem* **2019**, *93*, 103318. https://doi.org/10.1016/J.BIOORG.2019.103318.

87. Jin, X.; Wang, J.; Ruan, Y.; Li, J.; Kong, X.; Xia, J.; Yang, J.; Zhang, Q.; Liu, J.; Pi, J. Nitric Oxide Therapeutics: New Hopes for More Effective Tuberculosis Treatment Combine with Targeted and Controlled Nanotechnology. *International Journal of Nanomedicine*. Dove Medical Press Ltd 2025, pp 9195–9218. https://doi.org/10.2147/IJN.S531255.

88. Böger, R. H. L-Arginine Therapy in Cardiovascular Pathologies: Beneficial or Dangerous? *Curr Opin Clin Nutr Metab Care* **2008**, *11* (1), 55–61. https://doi.org/10.1097/MCO.0B013E3282F2B0C3.

89. Fei, Y.; Wu, J.; An, H. W.; Zhu, K.; Peng, B.; Cai, J.; Zhang, Y.; Li, L. L.; Wang, H.; Huang, Z. Identification of New Nitric Oxide-Donating Peptides with Dual Biofilm Eradication and Antibacterial Activities for Intervention of Device-Related Infections. *J Med Chem* **2020**, *63* (17), 9127–9135. https://doi.org/10.1021/ACS.JMEDCHEM.9B01832.

90. Chen, M.; Zhou, J.; Ran, P.; Lei, F.; Meng, J.; Wei, J.; Li, X. Photoactivated Release of Nitric Oxide and Antimicrobial Peptide Derivatives for Synergistic Therapy of Bacterial Skin Abscesses. *Adv Healthc Mater* **2022**, *11* (12). https://doi.org/10.1002/ADHM.202200199.

91. Mondal, A.; Paul, S.; De, P. Recent Advancements in Polymeric N-Nitrosamine-Based Nitric Oxide (NO) Donors and Their Therapeutic Applications. *Biomacromolecules*.

American Chemical Society September 9, 2024, pp 5592–5608. https://doi.org/10.1021/acs.biomac.4c00685.

92. White, C. J.; Lehnert, N.; Meyerhoff, M. E. Electrochemical Generation of Nitric Oxide for Medical Applications. *Electrochemical Science Advances* **2022**, *2* (5). https://doi.org/10.1002/ELSA.202100156.

93. Lee, W. H.; Ren, H.; Wu, J.; Novak, O.; Brown, R. B.; Xi, C.; Meyerhoff, M. E. Electrochemically Modulated Nitric Oxide Release from Flexible Silicone Rubber Patch: Antimicrobial Activity for Potential Wound Healing Applications. *ACS Biomater Sci Eng* **2016**, *2* (9), 1432–1435. https://doi.org/10.1021/ACSBIOMATERIALS.6B00360.

94. He, X.; Zou, C.; Zhang, L.; Wu, P.; Yao, Y.; Dong, K.; Ren, Y.; Hu, W.; Li, Y.; Luo, H.; Ying, B.; Luo, F.; Sun, X. Advances in Electrochemical Nitrite Reduction toward Nitric Oxide Synthesis for Biomedical Applications. *Adv Healthc Mater* **2025**, *14* (6), 2403468. https://doi.org/10.1002/ADHM.202403468.

95. Jin, J.; Mao, J.; Wu, W.; Jiang, Y.; Ma, W.; Yu, P.; Mao, L. Highly Efficient Electrosynthesis of Nitric Oxide for Biomedical Applications. *Angewandte Chemie* **2022**, *134* (41). https://doi.org/10.1002/ANGE.202210980.

96. Sen, S.; Kaul, G.; Verma, S.; Shantanu, S.; Grace, K.; Sandeep, V.; Sen, S.; Kaul, G.; Verma, S. Nucleobase and Peptide-Based Designed Molecules for Biomedical Applications. *Current Indian Science* **2024**, *2* (1), 1–27. https://doi.org/http://dx.doi.org/10.2174/012210299X308571240521052924.

97. Pal, H. A.; Mohapatra, S.; Gupta, V.; Ghosh, S.; Verma, S. Self-Assembling Soft Structures for Intracellular NO Release and Promotion of Neurite Outgrowth. *Chem Sci* **2017**, *8* (9), 6171–6175. https://doi.org/10.1039/c6sc05017d.

98. Pal, H. A.; Singh, A.; A.sheikh, P.; Panjla, A.; Kumar, A.; Verma, S. Peptide-Based Scaffold for Nitric Oxide Induced Differentiation of Neuroblastoma Cells. *ChemBioChem* **2018**, *19* (11), 1127–1131. https://doi.org/10.1002/CBIC.201800065.

99. Bormon, R.; Srivastava, E.; Ali, R.; Singh, P.; Kumar, A.; Verma, S. Anti-Proliferative, -Migratory and -Clonogenic Effects of Long-Lasting Nitric Oxide Release in HepG2 Cells. *Chemical Communications* **2024**. https://doi.org/10.1039/d4cc00232f.

100. Okda, M.; Spina, S.; Safaee Fakhr, B.; Carroll, R. W. The Antimicrobial Effects of Nitric Oxide: A Narrative Review. *Nitric Oxide* **2025**, *155*, 20–40. https://doi.org/10.1016/J.NIOX.2025.01.001.

101. Hu, Y.; Ding, M.; Lv, X.; Jiang, J.; Zhang, J.; Yang, D. Stimuli-Responsive NO Delivery Platforms for Bacterial Infection Treatment. *Adv Healthc Mater* **2024**, *13* (31). https://doi.org/10.1002/ADHM.202402240.

102. Liu, T.; Wei, J.; Fu, G.; Zhang, P.; Zhang, Z.; Guo, D. S.; Yang, X. Surface Charge Switchable Nanoparticles Capable of Controlled Nitric Oxide Release for the Treatment of Acidity-Associated Bacterial Infections. *Polym Chem* **2021**, *12* (7), 1023–1029. https://doi.org/10.1039/D0PY01460E.

103. Choi, H. W.; Kim, J.; Kim, J.; Kim, Y.; Song, H. B.; Kim, J. H.; Kim, K.; Kim, W. J. Light-Induced Acid Generation on a Gatekeeper for Smart Nitric Oxide Delivery. *ACS Nano* **2016**, *10* (4), 4199–4208. https://doi.org/10.1021/ACSNANO.5B07483.

104. He, H.; Liu, Y.; Zhou, Z.; Guo, C.; Wang, H. Y.; Wang, Z.; Wang, X.; Zhang, Z.; Wu, F. G.; Wang, H.; Chen, D.; Yang, D.; Liang, X.; Chen, J.; Zhou, S.; Liang, X.; Qian, X.; Yang, Y. A Photo-Triggered and Photo-Calibrated Nitric Oxide Donor: Rational Design, Spectral Characterizations, and Biological Applications. *Free Radic Biol Med* **2018**, *123*, 1–7. https://doi.org/10.1016/J.FREERADBIOMED.2018.04.563.

105. Li, L.; Lin, Z.; Lu, X.; Chen, C.; Xie, A.; Tang, Y.; Zhang, Z. Photo-Controlled and Photo-Calibrated Nanoparticle Enabled Nitric Oxide Release for Anti-Bacterial and Anti-Biofilm Applications. *RSC Adv* **2022**, *12* (51), 33358–33364. https://doi.org/10.1039/D2RA05352G.

106. Duan, Y.; Zhang, M.; Shen, Z.; Zhang, M.; Zheng, B.; Cheng, S.; Hu, J. Photoresponsive Vesicles Enabling Sequential Release of Nitric Oxide (NO) and Gentamicin for Efficient Biofilm Eradication. *Macromol Rapid Commun* **2021**, *42* (18). https://doi.org/10.1002/MARC.202000759.

107. Heilman, B. J.; Halpenny, G. M.; Mascharak, P. K. Synthesis, Characterization, and Light-Controlled Antibiotic Application of a Composite Material Derived from Polyurethane and Silica Xerogel with Embedded Photoactive Manganese Nitrosyl. *J Biomed Mater Res B Appl Biomater* **2011**, *99B* (2), 328–337. https://doi.org/10.1002/JBM.B.31904.

108. Sharma, K.; Sengupta, K.; Chakrapani, H. Nitroreductase-Activated Nitric Oxide (NO) Prodrugs. *Bioorg Med Chem Lett* **2013**, *23* (21), 5964–5967. https://doi.org/10.1016/j.bmcl.2013.08.066.

109. Yepuri, N. R.; Barraud, N.; Mohammadi, N. S.; Kardak, B. G.; Kjelleberg, S.; Rice, S. A.; Kelso, M. J. Synthesis of Cephalosporin-3'-Diazeniumdiolates: Biofilm Dispersing NO-Donor Prodrugs Activated by β-Lactamase. *Chemical Communications* **2013**, *49* (42), 4791–4793. https://doi.org/10.1039/C3CC40869H.

110. Barraud, N.; Kardak, B. G.; Yepuri, N. R.; Howlin, R. P.; Webb, J. S.; Faust, S. N.; Kjelleberg, S.; Rice, S. A.; Kelso, M. J. Cephalosporin-3'-Diazeniumdiolates: Targeted NO-Donor Prodrugs for Dispersing Bacterial Biofilms. *Angewandte Chemie - International Edition* **2012**, *51* (36), 9057–9060. https://doi.org/10.1002/ANIE.201202414.

111. Dharmaraja, A. T.; Ravikumar, G.; Chakrapani, H. Arylboronate Ester Based Diazeniumdiolates (BORO/NO), a Class of Hydrogen Peroxide Inducible Nitric Oxide (NO) Donors. *Org Lett* **2014**, *16* (10), 2610–2613. https://doi.org/10.1021/OL5010643.

112. Garren, M.; Maffe, P.; Melvin, A.; Griffin, L.; Wilson, S.; Douglass, M.; Reynolds, M.; Handa, H. Surface-Catalyzed Nitric Oxide Release via a Metal Organic Framework Enhances Antibacterial Surface Effects. *ACS Appl Mater Interfaces* **2021**, *13* (48), 56931–56943. https://doi.org/10.1021/ACSAMI.1C17248.

113. Liu, S.; Li, G.; Ma, D. Controllable Nitric Oxide-Delivering Platforms for Biomedical Applications. *Adv Ther (Weinh)* **2022**, *5* (3). https://doi.org/10.1002/ADTP.202100227.

114. Yuan, X.; Zhao, X.; Xia, R.; Xie, Z.; Lin, Y.; Su, Z. Photothermally Responsive Magnetic Nanoparticles for Nitric Oxide Release to Combat Staphylococcus Aureus Biofilms. *ACS Appl Nano Mater* **2022**, *5* (12), 18799–18810. https://doi.org/10.1021/ACSANM.2C04561.

115. Arabestani, M. R.; Bigham, A.; Kamarehei, F.; Dini, M.; Gorjikhah, F.; Shariati, A.; Hosseini, S. M. Solid Lipid Nanoparticles and Their Application in the Treatment of Bacterial Infectious Diseases. *Biomedicine & Pharmacotherapy* **2024**, *174*, 116433. https://doi.org/10.1016/J.BIOPHA.2024.116433.

116. Adnan, N. N. M.; Sadrearhami, Z.; Bagheri, A.; Nguyen, T. K.; Wong, E. H. H.; Ho, K. K. K.; Lim, M.; Kumar, N.; Boyer, C. Exploiting the Versatility of Polydopamine-Coated

Nanoparticles to Deliver Nitric Oxide and Combat Bacterial Biofilm. *Macromol Rapid Commun* **2018**, *39* (13). https://doi.org/10.1002/MARC.201800159.

117. Lv, K.; Li, G.; Pan, X.; Liu, L.; Chen, Z.; Zhang, Y.; Xu, H.; Ma, D. Bacteria-Targeted Combined with Photothermal/NO Nanoparticles for the Treatment and Diagnosis of MRSA Infection In Vivo. *Adv Healthc Mater* **2023**, *12* (20). https://doi.org/10.1002/ADHM.202300247.

118. Cao, J.; Hlaing, S. P.; Lee, J.; Kim, J.; Lee, E. H.; Kang, S. H.; Hong, S. W.; Yoon, I. S.; Yun, H.; Jung, Y.; Yoo, J. W. Bacteria-Adhesive Nitric Oxide-Releasing Graphene Oxide Nanoparticles for MRPA-Infected Wound Healing Therapy. *ACS Appl Mater Interfaces* **2022**, *14* (45), 50507–50519. https://doi.org/10.1021/ACSAMI.2C13317.

119. Dolanský, J.; Henke, P.; Malá, Z.; Žárská, L.; Kubát, P.; Mosinger, J. Antibacterial Nitric Oxide- and Singlet Oxygen-Releasing Polystyrene Nanoparticles Responsive to Light and Temperature Triggers. *Nanoscale* **2018**, *10* (5), 2639–2648. https://doi.org/10.1039/C7NR08822A.

120. Martinez, L. R.; Han, G.; Chacko, M.; Mihu, M. R.; Jacobson, M.; Gialanella, P.; Friedman, A. J.; Nosanchuk, J. D.; Friedman, J. M. Antimicrobial and Healing Efficacy of Sustained Release Nitric Oxide Nanoparticles against Staphylococcus Aureus Skin Infection. *Journal of Investigative Dermatology* **2009**, *129* (10), 2463–2469. https://doi.org/10.1038/JID.2009.95.

121. Gupta, A.; Landis, R. F.; Li, C. H.; Schnurr, M.; Das, R.; Lee, Y. W.; Yazdani, M.; Liu, Y.; Kozlova, A.; Rotello, V. M. Engineered Polymer Nanoparticles with Unprecedented Antimicrobial Efficacy and Therapeutic Indices against Multidrug-Resistant Bacteria and Biofilms. *J Am Chem Soc* **2018**, *140* (38), 12137–12143. https://doi.org/10.1021/JACS.8B06961.

122. Hasan, N.; Cao, J.; Lee, J.; Naeem, M.; Hlaing, S. P.; Kim, J.; Jung, Y.; Lee, B. L.; Yoo, J. W. PEI/NONOates-Doped PLGA Nanoparticles for Eradicating Methicillin-Resistant Staphylococcus Aureus Biofilm in Diabetic Wounds via Binding to the Biofilm Matrix. *Materials Science and Engineering C* **2019**, *103*. https://doi.org/10.1016/J.MSEC.2019.109741.

123. Tang, R.; Jiang, F.; Wen, J.; Deng, Y.; Sun, Y. Managing Bacterial Biofilms with Chitosan-Based Polymeric Nitric Oxides: Inactivation of Biofilm Bacteria and Synergistic Effects with Antibiotics. *J Bioact Compat Polym* **2016**, *31* (4), 393–410. https://doi.org/10.1177/0883911515623798.

124. Pelegrino, M. T.; Lima, B. de A.; do Nascimento, M. H. M.; Lombello, C. B.; Brocchi, M.; Seabra, A. B. Biocompatible and Antibacterial Nitric Oxide-Releasing Pluronic F-127/Chitosan Hydrogel for Topical Applications. *Polymers (Basel)* **2018**, *10* (4). https://doi.org/10.3390/POLYM10040452.

125. Liu, Y.; Chen, X.; Lai, X.; Dzuvor, C. K. O.; Lyu, L.; Chow, S. H.; He, L.; Yu, L.; Wang, Y.; Song, J.; Hsu, H. Y.; Lin, T. W.; Chan, P. W. H.; Shen, H. H. Coassembled Nitric Oxide-Releasing Nanoparticles with Potent Antimicrobial Efficacy against Methicillin-Resistant Staphylococcus Aureus (MRSA) Strains. *ACS Appl Mater Interfaces* **2022**, *14* (33), 37369–37379. https://doi.org/10.1021/ACSAMI.2C08833.

126. Reighard, K. P.; Hill, D. B.; Dixon, G. A.; Worley, B. V.; Schoenfisch, M. H. Disruption and Eradication of p. Aeruginosa Biofilms Using Nitric Oxide-Releasing Chitosan Oligosaccharides. *Biofouling* **2015**, *31* (9), 775–787. https://doi.org/10.1080/08927014.2015.1107548.

127. Lu, Y.; Slomberg, D. L.; Shah, A.; Schoenfisch, M. H. Nitric Oxide-Releasing Amphiphilic Poly(Amidoamine) (PAMAM) Dendrimers as Antibacterial Agents. *Biomacromolecules* **2013**, *14* (10), 3589–3598. https://doi.org/10.1021/BM400961R.

128. Yu, S.; Li, G.; Liu, R.; Ma, D.; Xue, W. Dendritic Fe3O4@Poly(Dopamine)@PAMAM Nanocomposite as Controllable NO-Releasing Material: A Synergistic Photothermal and NO Antibacterial Study. *Adv Funct Mater* **2018**, *28* (20). https://doi.org/10.1002/ADFM.201707440.

129. Garzón-Porras, A. M.; Bertuzzi, D. L.; Lucas, K.; Ornelas, C. Well-Defined Bifunctional Dendrimer Bearing 54 Nitric Oxide-Releasing Moieties and 54 Ursodeoxycholic Acid Groups Presenting High Anti-Inflammatory Activity. *ACS Biomater Sci Eng* **2022**, *8* (12), 5171–5187. https://doi.org/10.1021/ACSBIOMATERIALS.2C00713.

130. Liu, S.; Cai, X.; Xue, W.; Ma, D.; Zhang, W. Chitosan Derivatives Co-Delivering Nitric Oxide and Methicillin for the Effective Therapy to the Methicillin-Resistant S. Aureus Infection. *Carbohydr Polym* **2020**, *234*. https://doi.org/10.1016/j.carbpol.2020.115928.

131. Hetrick, E. M.; Shin, J. H.; Stasko, N. A.; Johnson, C. B.; Wespe, D. A.; Holmuhamedov, E.; Schoenfisch, M. H. Bactericidal Efficacy of Nitric Oxide-Releasing Silica Nanoparticles. *ACS Nano* **2008**, *2* (2), 235–246. https://doi.org/10.1021/NN700191F.

132. Hetrick, E. M.; Shin, J. H.; Paul, H. S.; Schoenfisch, M. H. Anti-Biofilm Efficacy of Nitric Oxide-Releasing Silica Nanoparticles. *Biomaterials* **2009**, *30* (14), 2782–2789. https://doi.org/10.1016/J.BIOMATERIALS.2009.01.052.

133. Ma, H.; Tang, Y.; Rong, F.; Wang, K.; Wang, T.; Li, P. Surface Charge Adaptive Nitric Oxide Nanogenerator for Enhanced Photothermal Eradication of Drug-Resistant Biofilm Infections. *Bioact Mater* **2023**, *27*, 154–167. https://doi.org/10.1016/J.BIOACTMAT.2023.03.022.

134. Jardeleza, C.; Rao, S.; Thierry, B.; Gajjar, P.; Vreugde, S.; Prestidge, C. A.; Wormald, P. J. Liposome-Encapsulated ISMN: A Novel Nitric Oxide-Based Therapeutic Agent against Staphylococcus Aureus Biofilms. *PLoS One* **2014**, *9* (3), e92117. https://doi.org/10.1371/JOURNAL.PONE.0092117.

135. Beurton, J.; Boudier, A.; Barozzi Seabra, A.; Vrana, N. E.; Clarot, I.; Lavalle, P. Nitric Oxide Delivering Surfaces: An Overview of Functionalization Strategies and Efficiency Progress. *Adv Healthc Mater* **2022**, *11* (13). https://doi.org/10.1002/ADHM.202102692.

136. Pant, J.; Gao, J.; Goudie, M. J.; Hopkins, S. P.; Locklin, J.; Handa, H. A Multi-Defense Strategy: Enhancing Bactericidal Activity of a Medical Grade Polymer with a Nitric Oxide Donor and Surface-Immobilized Quaternary Ammonium Compound. *Acta Biomater* **2017**, *58*, 421–431. https://doi.org/10.1016/j.actbio.2017.05.061.

137. Sadrearhami, Z.; Shafiee, F. N.; Ho, K. K. K.; Kumar, N.; Krasowska, M.; Blencowe, A.; Wong, E. H. H.; Boyer, C. Antibiofilm Nitric Oxide-Releasing Polydopamine Coatings. *ACS Appl Mater Interfaces* **2019**, *11* (7), 7320–7329. https://doi.org/10.1021/ACSAMI.8B16853.

138. Li, J.; Zhang, G.; Zyryanov, G. V; Shabunina, O. V; Guo, X.; Zhu, M.; Jin, Y.; Wang, Z.; Li, J.; Zhang, G.; Guo, X.; Zhu, M.; Jin, Y.; Wang, Z.; Zyryanov, G. V; Shabunina, O. V. Nitric-Oxide-Enhanced Positively Charged Semiconductor Conjugated Polymer Composite Nanomaterials for Antibiofilm In Vivo. *Adv Funct Mater* **2025**, *35* (6), 2415134. https://doi.org/10.1002/ADFM.202415134.

139. Workman, C. D.; Hopkins, S.; Pant, J.; Goudie, M.; Handa, H. Covalently Bound S-Nitroso- N-Acetylpenicillamine to Electrospun Polyacrylonitrile Nanofibers for Multifunctional Tissue Engineering Applications. *ACS Biomater Sci Eng*

2021, *7* (11), 5279–5287. https://doi.org/10.1021/ACSBIOMATERIALS.1C00907.

140. Estes Bright, L. M.; Mondal, A.; Pinon, V.; Kumar, A.; Thompson, S.; Brisbois, E. J.; Handa, H. Investigation of the Susceptibility of Clinical Infection Loads to Nitric Oxide Antibacterial Treatment. *Nitric Oxide* **2025**, *154*, 19–28. https://doi.org/10.1016/j.niox.2024.11.003.

141. Brisbois, E. J.; Davis, R. P.; Jones, A. M.; Major, T. C.; Bartlett, R. H.; Meyerhoff, M. E.; Handa, H. Reduction in Thrombosis and Bacterial Adhesion with 7 Day Implantation of S-Nitroso- N-Acetylpenicillamine (SNAP)-Doped Elast-Eon E2As Catheters in Sheep. *J Mater Chem B* **2015**, *3* (8), 1639–1645. https://doi.org/10.1039/C4TB01839G.

142. Brisbois, E. J.; Handa, H.; Major, T. C.; Bartlett, R. H.; Meyerhoff, M. E. Long-Term Nitric Oxide Release and Elevated Temperature Stability with S-Nitroso-N-Acetylpenicillamine (SNAP)-Doped Elast-Eon E2As Polymer. *Biomaterials* **2013**, *34* (28), 6957–6966. https://doi.org/10.1016/j.biomaterials.2013.05.063.

143. Homeyer, K. H.; Goudie, M. J.; Singha, P.; Handa, H. Liquid-Infused Nitric-Oxide-Releasing Silicone Foley Urinary Catheters for Prevention of Catheter-Associated Urinary Tract Infections. *ACS Biomater Sci Eng* **2019**, *5* (4), 2021–2029. https://doi.org/10.1021/ACSBIOMATERIALS.8B01320.

144. Wo, Y.; Li, Z.; Brisbois, E. J.; Colletta, A.; Wu, J.; Major, T. C.; Xi, C.; Bartlett, R. H.; Matzger, A. J.; Meyerhoff, M. E. Origin of Long-Term Storage Stability and Nitric Oxide Release Behavior of CarboSil Polymer Doped with S-Nitroso-N-Acetyl- d -Penicillamine. *ACS Appl Mater Interfaces* **2015**, *7* (40), 22218–22227. https://doi.org/10.1021/acsami.5b07501.

145. Singha, P.; Pant, J.; Goudie, M. J.; Workman, C. D.; Handa, H. Enhanced Antibacterial Efficacy of Nitric Oxide Releasing Thermoplastic Polyurethanes with Antifouling Hydrophilic Topcoats. *Biomater Sci* **2017**, *5* (7), 1246–1255. https://doi.org/10.1039/C6BM00948D.

146. Hou, Z.; Wu, Y.; Xu, C.; Reghu, S.; Shang, Z.; Chen, J.; Pranantyo, D.; Marimuth, K.; De, P. P.; Ng, O. T.; Pethe, K.; Kang, E. T.; Li, P.; Chan-Park, M. B. Precisely Structured Nitric-Oxide-Releasing Copolymer Brush Defeats Broad-Spectrum Catheter-Associated Biofilm Infections In Vivo. *ACS Cent Sci* **2020**, *6* (11), 2031. https://doi.org/10.1021/ACSCENTSCI.0C00755.

147. Craven, M.; Kasper, S. H.; Canfield, M. J.; Diaz-Morales, R. R.; Hrabie, J. A.; Cady, N. C.; Strickland, A. D. Nitric Oxide-Releasing Polyacrylonitrile Disperses Biofilms Formed by Wound-Relevant Pathogenic Bacteria. *J Appl Microbiol* **2016**, *120* (4), 1085–1099. https://doi.org/10.1111/JAM.13059.

148. Choi, M.; Hasan, N.; Cao, J.; Lee, J.; Hlaing, S. P.; Yoo, J. W. Chitosan-Based Nitric Oxide-Releasing Dressing for Anti-Biofilm and in Vivo Healing Activities in MRSA Biofilm-Infected Wounds. *Int J Biol Macromol* **2020**, *142*, 680–692. https://doi.org/10.1016/j.ijbiomac.2019.10.009.

149. Dolanský, J.; Demel, J.; Mosinger, J. Multifunctional Polystyrene Nanofiber Membrane with Bounded Polyethyleneimine and NO Photodonor: Dark- and Light-Induced Antibacterial Effect and Enhanced CO2 Adsorption. *J Mater Sci* **2019**, *54* (3), 2740–2753. https://doi.org/10.1007/S10853-018-2982-0.

150. Dolanský, J.; Henke, P.; Kubát, P.; Fraix, A.; Sortino, S.; Mosinger, J. Polystyrene Nanofiber Materials for Visible-Light-Driven Dual Antibacterial Action via Simultaneous Photogeneration of NO and O2(1δg). *ACS Appl Mater Interfaces* **2015**, *7* (41), 22980–22989. https://doi.org/10.1021/ACSAMI.5B06233.

151. Sundaram, J.; Pant, J.; Goudie, M. J.; Mani, S.; Handa, H. Antimicrobial and Physicochemical Characterization of Biodegradable, Nitric Oxide-Releasing Nanocellulose-Chitosan Packaging Membranes. *J Agric Food Chem* **2016**, *64* (25), 5260–5266. https://doi.org/10.1021/ACS.JAFC.6B01936.

152. Koh, A.; Carpenter, A. W.; Slomberg, D. L.; Schoenfisch, M. H. Nitric Oxide-Releasing Silica Nanoparticle-Doped Polyurethane Electrospun Fibers. *ACS Appl Mater Interfaces* **2013**, *5* (16), 10.1021/am402044s. https://doi.org/10.1021/AM402044S.

153. Jang, -H ; Jeong, H.-J. ; Yoon, G.-J. ; Hydrogel, J.-K.; Heo, T.-H.; Jang, H.-J.; Jeong, G.-J.; Yoon, J.-K. Hydrogel-Based Nitric Oxide Delivery Systems for Enhanced Wound Healing. *Gels 2025, Vol. 11, Page 621* **2025**, *11* (8), 621. https://doi.org/10.3390/GELS11080621.

154. Davis, S. C.; Gil, J.; Solis, M.; Strong, R.; Cassagnol, R. Efficacy of a Topical Nitric Oxide-Releasing Gel on Polymicrobial Wound Infections. *Mil Med* **2025**, *190* (5–6), 1037–1044. https://doi.org/10.1093/MILMED/USAE551.

155. Urzedo, A. L.; Gonçalves, M. C.; Nascimento, M. H. M.; Lombello, C. B.; Nakazato, G.; Seabra, A. B. Cytotoxicity and Antibacterial Activity of Alginate Hydrogel Containing Nitric Oxide Donor and Silver Nanoparticles for Topical Applications. *ACS Biomater Sci Eng* **2020**, *6* (4), 2117–2134. https://doi.org/10.1021/ACSBIOMATERIALS.9B01685.

156. Kang, Y.; Kim, J.; Lee, Y. M.; Im, S.; Park, H.; Kim, W. J. Nitric Oxide-Releasing Polymer Incorporated Ointment for Cutaneous Wound Healing. *Journal of Controlled Release* **2015**, *220*, 624–630. https://doi.org/10.1016/J.JCONREL.2015.08.057.

157. Yang, Y.; Yu, J.; Zhang, R.; Chen, B.; Liu, X.; Jia, Q.; Wang, X.; Yang, Z.; Ning, P.; Wang, Z.; Yang, Y.; Yu, J.; Zhang, R. L.; Chen, B.; Liu, X.; Jia, Q.; Wang, X.; Yang, Z.; Ning, P. B.; Wang, Z. L. Injectable Reactive Oxygen Species-responsive Hydrogel Dressing with Sustained Nitric Oxide Release for Bacterial Ablation and Wound Healing. *Wiley Online LibraryJ Yu, R Zhang, B Chen, X Liu, Q Jia, X Wang, Z Yang, P Ning, Z Wang, Y YangAdvanced Functional Materials, 2022•Wiley Online Library* **2022**, *32* (33). https://doi.org/10.1002/ADFM.202202857.

158. Garren, M.; Ashcraft, M.; Crowley, D.; Brisbois, E. J.; Handa, H. Derivatization of Graphene Oxide Nanosheets with Tunable Nitric Oxide Release for Antibacterial Biomaterials. *J Biomed Mater Res A* **2023**, *111* (4), 451–464. https://doi.org/10.1002/JBM.A.37493.

159. Zeng, Y.; Wang, C.; Lei, K.; Xiao, C.; Jiang, X.; Zhang, W.; Wu, L.; Huang, J.; Li, W. Multifunctional MOF-Based Microneedle Patch With Synergistic Chemo-Photodynamic Antibacterial Effect and Sustained Release of Growth Factor for Chronic Wound Healing. *Adv Healthc Mater* **2023**, *12* (19). https://doi.org/10.1002/ADHM.202300250.

160. Yao, S.; Wang, Y.; Chi, J.; Yu, Y.; Zhao, Y.; Luo, Y.; Wang, Y. Porous MOF Microneedle Array Patch with Photothermal Responsive Nitric Oxide Delivery for Wound Healing. *Advanced Science* **2022**, *9* (3). https://doi.org/10.1002/ADVS.202103449.

161. Creagh-Brown, B. C.; Griffiths, M. J. D.; Evans, T. W. Bench-to-Bedside Review: Inhaled Nitric Oxide Therapy in Adults. *Crit Care* **2009**, *13* (3), 221. https://doi.org/10.1186/CC7734.

162. Del Sorbo, L.; Michaelsen, V. S.; Cypel, M.; Ali, A.; Wang, A.; Ribeiro, R. V. P. High Doses of Inhaled Nitric Oxide as an Innovative Antimicrobial Strategy for Lung Infections. *Biomedicines* **2022**, *10* (7). https://doi.org/10.3390/BIOMEDICINES10071525.

163. Flume, P. A.; Garcia, B. A.; Wilson, D.; Steed, L.; Dorman, S. E.; Winthrop, K. Inhaled Nitric Oxide for Adults with Pulmonary Non-Tuberculous Mycobacterial Infection. *Respir Med* **2023**, *206*, 107069. https://doi.org/10.1016/J.RMED.2022.107069.

164. Miller, C.; McMullin, B.; Ghaffari, A.; Stenzler, A.; Pick, N.; Roscoe, D.; Ghahary, A.; Road, J.; Av-Gay, Y. Gaseous Nitric Oxide Bactericidal Activity Retained during Intermittent High-Dose Short Duration Exposure. *Nitric Oxide* **2009**, *20* (1), 16–23. https://doi.org/10.1016/J.NIOX.2008.08.002.

165. Hu, X.; Li, Y.; Piao, Y.; Karimi, M.; Wang, Y.; Wen, F.; Li, H.; Shi, L.; Liu, Y. Two-Tailed Dynamic Covalent Amphiphile Combats Bacterial Biofilms. *Advanced Materials* **2023**, *35* (33), 2301623. https://doi.org/10.1002/ADMA.202301623.

166. Wang, D. C.; Clark, J. R.; Lee, R.; Nelson, A. H.; Maresso, A. W.; Acharya, G.; Shin, C. S. Development of Antimicrobial Nitric Oxide-Releasing Fibers. *Pharmaceutics 2021, Vol. 13, Page 1445* **2021**, *13* (9), 1445. https://doi.org/10.3390/PHARMACEUTICS13091445.

167. Sysel, A. M.; Dunphy, M. J.; Bauer, J. A. Antimicrobial Properties of Diethylamine NONOate, a Nitric Oxide Donor, against Escherichia Coli: A Pilot Study. *Journal of Antibiotics* **2021**, *74* (4), 260–265. https://doi.org/10.1038/S41429-020-00397-9;TECHMETA.

168. Fleming, G.; Aveyard, J.; Fothergill, J. L.; McBride, F.; Raval, R.; D'Sa, R. A. Nitric Oxide Releasing Polymeric Coatings for the Prevention of Biofilm Formation. *Polymers (Basel)* **2017**, *9* (11). https://doi.org/10.3390/POLYM9110601.

169. Estes Bright, L. M.; Garren, M. R. S.; Douglass, M.; Handa, H. Synthesis and Characterization of Nitric Oxide-Releasing Ampicillin as a Potential Strategy for Combatting Bacterial Biofilm Formation. *ACS Appl Mater Interfaces* **2023**, *15* (12), 15185–15194. https://doi.org/10.1021/ACSAMI.3C00140.

170. Mondal, A.; Douglass, M.; Hopkins, S. P.; Singha, P.; Tran, M.; Handa, H.; Brisbois, E. J. Multifunctional S-Nitroso-N-Acetylpenicillamine-Incorporated Medical-Grade Polymer with Selenium Interface for Biomedical Applications. *ACS Appl Mater Interfaces* **2019**, *11* (38), 34652–34662. https://doi.org/10.1021/ACSAMI.9B10610.

171. Jia, P.; Zou, Y.; Jiang, J. S-Nitrosylated CuS Hybrid Hydrogel Patches with Robust Antibacterial and Repair-Promoting Activity for Infected Wound Healing. *Small* **2024**, *20* (21), 2307629. https://doi.org/10.1002/SMLL.202307629.

172. Ghalei, S.; Douglass, M.; Handa, H. Nitric Oxide-Releasing Nanofibrous Scaffolds Based on Silk Fibroin and Zein with Enhanced Biodegradability and Antibacterial Properties. *ACS Biomater Sci Eng* **2022**, *8* (7), 3066–3077. https://doi.org/10.1021/ACSBIOMATERIALS.2C00103.

173. Kim, J. O.; Noh, J. K.; Thapa, R. K.; Hasan, N.; Choi, M.; Kim, J. H.; Lee, J. H.; Ku, S. K.; Yoo, J. W. Nitric Oxide-Releasing Chitosan Film for Enhanced Antibacterial and in Vivo Wound-Healing Efficacy. *Int J Biol Macromol* **2015**, *79*, 217–225. https://doi.org/10.1016/J.IJBIOMAC.2015.04.073.

174. Li, M.; Qiu, W.; Wang, Q.; Li, N.; Liu, L.; Wang, X.; Yu, J.; Li, X.; Li, F.; Wu, D. Nitric Oxide-Releasing Tryptophan-Based Poly(Ester Urea)s Electrospun Composite Nanofiber Mats with Antibacterial and Antibiofilm Activities for Infected Wound Healing. *ACS Appl Mater Interfaces* **2022**, *14* (14), 15911–15926. https://doi.org/10.1021/ACSAMI.1C24131.

175. Lai, X.; Yu, L.; Huang, X.; Gardner, W.; Bamford, S. E.; Pigram, P. J.; Wang, S.; Brun, A. P. L.; Muir, B. W.; Song, J.; Wang, Y.; Hsu, H. Y.; Chan, P. W. H.; Shen, H. H. Enhanced Nitric Oxide Delivery Through Self-Assembling Nanoparticles for Eradicating Gram-Negative Bacteria. *Adv Healthc Mater* **2024**, *13* (32), 2403046. https://doi.org/10.1002/ADHM.202403046.

176. Fedik, N. S.; Kletskii, M. E.; Burov, O. N.; Lisovin, A. V.; Kurbatov, S. V.; Chistyakov, V. A.; Morozov, P. G. Comprehensive Study of Nitrofuroxanoquinolines. New Perspective Donors of NO Molecules. *Nitric Oxide* **2019**, *93*, 15–24. https://doi.org/10.1016/J.NIOX.2019.08.007.

177. Yu, Y. L.; Wu, J. J.; Lin, C. C.; Qin, X.; Tay, F. R.; Miao, L.; Tao, B. L.; Jiao, Y. Elimination of Methicillin-Resistant Staphylococcus Aureus Biofilms on Titanium Implants via Photothermally-Triggered Nitric Oxide and Immunotherapy for Enhanced Osseointegration. *Mil Med Res* **2023**, *10* (1), 1–23. https://doi.org/10.1186/S40779-023-00454-Y/FIGURES/6.

178. Cheng, H.; Tian, G.; Liu, H.; Bai, D.; Zhang, Y.; Wang, Q.; Zhao, M.; Cao, S.; Deng, D.; Wang, X. A Molybdenum Sulfide Based Nitric Oxide Controlled Release Oral Gel for Rapid Healing of Oral Mucosal Ulcers. *J Colloid Interface Sci* **2025**, *678*, 560–571. https://doi.org/10.1016/J.JCIS.2024.08.210.

179. Xin, J.; Yang, Z.; Zhang, S.; Sun, L.; Wang, X.; Tang, Y.; Xiao, Y.; Huang, H.; Li, W. Fast Fabrication of "All-in-One" Injectable Hydrogels as Antibiotic Alternatives for Enhanced Bacterial Inhibition and Accelerating Wound Healing. *J Nanobiotechnology* **2024**, *22* (1), 1–31. https://doi.org/10.1186/S12951-024-02657-4/FIGURES/10.

180. Davari, N.; Nourmohammadi, J.; Mohammadi, J. Nitric Oxide-Releasing Thiolated Starch Nanoparticles Embedded in Gelatin Sponges for Wound Dressing Applications. *Int J Biol Macromol* **2024**, *265*, 131062. https://doi.org/10.1016/J.IJBIOMAC.2024.131062.

181. Zheng, Y.; Yang, D.; Gao, B.; Huang, S.; Tang, Y.; Wa, Q.; Dong, Y.; Yu, S.; Huang, J.; Huang, S. A DNA-Inspired Injectable Adhesive Hydrogel with Dual Nitric Oxide Donors to Promote Angiogenesis for Enhanced Wound Healing. *Acta Biomater* **2024**, *176*, 128–143. https://doi.org/10.1016/J.ACTBIO.2024.01.026.

182. Cheng, X.; Zhang, S.; Liu, H.; Chen, H.; Zhou, J.; Chen, Z.; Zhou, X.; Xie, Z.; Kuang, Q.; Zheng, L. Biomimetic Metal–Organic Framework Composite-Mediated Cascade Catalysis for Synergistic Bacteria Killing. *ACS Appl Mater Interfaces* **2020**, *12* (33), 36996–37005. https://doi.org/10.1021/ACSAMI.0C12159.

183. Zheng, J.; Wang, W.; Gao, X.; Zhao, S.; Chen, W.; Li, J.; Liu, Y. N. Cascade Catalytically Released Nitric Oxide-Driven Nanomotor with Enhanced Penetration for Antibiofilm. *Small* **2022**, *18* (52), 2205252. https://doi.org/10.1002/SMLL.202205252.

184. Mirhaj, M.; Tavakoli, M.; Varshosaz, J.; Labbaf, S.; Salehi, S.; Talebi, A.; Kazemi, N.; Haghighi, V.; Alizadeh, M. Preparation of a Biomimetic Bi-Layer Chitosan Wound Dressing Composed of A-PRF/Sponge Layer and L-Arginine/Nanofiber. *Carbohydr Polym* **2022**, *292*, 119648. https://doi.org/10.1016/J.CARBPOL.2022.119648.

185. Zhao, B.; Ren, Y.; Zhang, K.; Dong, Y.; Wang, K.; Zhang, N.; Li, J.; Yuan, M.; Wang, J.; Tu, Q. Hydroxypropyl Methylcellulose Reinforced Bilayer Hydrogel Dressings Containing L-Arginine-Modified Polyoxometalate Nanoclusters to Promote Healing of Chronic Diabetic Wounds. *Carbohydr Polym* **2024**, *342*, 122396. https://doi.org/10.1016/J.CARBPOL.2024.122396.

186. Xu, C.; Chen, Y.; Lin, C.; Xiao, J. A.; Li, P.; Su, W. NIR Photo-Responsive Injectable Chitosan/Hyaluronic Acid Hydrogels with Controlled NO Release for the Treatment of MRSA Infections. *Int J Biol Macromol* **2025**, *300*, 140304. https://doi.org/10.1016/J.IJBIOMAC.2025.140304.

187. Hu, J.; Duan, K.; Zhao, Y.; Xv, H.; Ge, X.; Lin, M.; Zhu, H.; Chen, D.; Deng, H.; Lee, B. H. Hyperglycemia-Responsive Nitric Oxide-Releasing Biohybrid Cryogels with Cascade Enzyme Catalysis for Enhanced Healing of Infected Diabetic Wounds. *Journal of Controlled Release* **2025**, *378*, 912–931. https://doi.org/10.1016/J.JCONREL.2024.12.061.

188. Shen, Z.; Zheng, S.; Xiao, S.; Shen, R.; Liu, S.; Hu, J. Red-Light-Mediated Photoredox Catalysis Enables Self-Reporting Nitric Oxide Release for Efficient Antibacterial Treatment. *Angewandte Chemie - International Edition* **2021**, *60* (37), 20452–20460. https://doi.org/10.1002/ANIE.202107155.

189. Privett, B. J.; Deupree, S. M.; Backlund, C. J.; Rao, K. S.; Johnson, C. B.; Coneski, P. N.; Schoenfisch, M. H. Synergy of Nitric Oxide and Silver Sulfadiazine against Gram-Negative, Gram-Positive, and Antibiotic-Resistant Pathogens. *Mol Pharm* **2010**, *7* (6), 2289–2296. https://doi.org/10.1021/MP100248E.

190. Rouillard, K. R.; Novak, O. P.; Pistiolis, A. M.; Yang, L.; Ahonen, M. J. R.; McDonald, R. A.; Schoenfisch, M. H. Exogenous Nitric Oxide Improves Antibiotic Susceptibility in Resistant Bacteria. *ACS Infect Dis* **2021**, *7* (1), 23–33. https://doi.org/10.1021/ACSINFECDIS.0C00337.

191. Reffuveille, F.; de la Fuente-Núñez, C.; Fairfull-Smith, K. E.; Hancock, R. E. W. Potentiation of Ciprofloxacin Action against Gram-Negative Bacterial Biofilms by a Nitroxide. *Pathog Dis* **2015**, *73* (5), 16. https://doi.org/10.1093/FEMSPD/FTV016.

192. Liu, H.; Zhu, X.; Guo, H.; Huang, H.; Huang, S.; Huang, S.; Xue, W.; Zhu, P.; Guo, R. Nitric Oxide Released Injectable Hydrogel Combined with Synergistic Photothermal Therapy for Antibacterial and Accelerated Wound Healing. *Appl Mater Today* **2020**, *20*. https://doi.org/10.1016/J.APMT.2020.100781.

193. He, J.; Li, Z.; Wang, J.; Li, T.; Chen, J.; Duan, X.; Guo, B. Photothermal Antibacterial Antioxidant Conductive Self-Healing Hydrogel with Nitric Oxide Release Accelerates Diabetic Wound Healing. *Compos B Eng* **2023**, *266*. https://doi.org/10.1016/J.COMPOSITESB.2023.110985.

194. Liu, G.; Wang, L.; He, Y.; Wang, L.; Deng, Z.; Liu, J.; Peng, D.; Ding, T.; Lu, L.; Ding, Y.; Zhang, J.; Liu, P.; Cai, K. Polydopamine Nanosheets Doped Injectable Hydrogel with Nitric Oxide Release and Photothermal Effects for Bacterial Ablation and Wound Healing. *Adv Healthc Mater* **2021**, *10* (23). https://doi.org/10.1002/ADHM.202101476.

195. Huang, S.; Liu, H.; Liao, K.; Hu, Q.; Guo, R.; Deng, K. Functionalized GO Nanovehicles with Nitric Oxide Release and Photothermal Activity-Based Hydrogels for Bacteria-Infected Wound Healing. *ACS Appl Mater Interfaces* **2020**, *12* (26), 28952–28964. https://doi.org/10.1021/ACSAMI.0C04080.

196. Wang, X.; Zheng, H.; Wu, Y.; Sun, H.; Chang, A.; Zhou, Z.; Bao, W.; Li, C.; Zhang, D.; Wu, C. J.; Zhu, A.; Zhu, H. Cyclodextrin-Modified Amphiphilic Microgel with Bifunctional Domains for Infected Wound Healing via Photothermal Antibacterial Therapy and Nitric Oxide Release. *ACS Appl Mater Interfaces* **2023**, *15* (23), 27548–27559. https://doi.org/10.1021/ACSAMI.3C02115.

197. Lv, J.; Qi, Y.; Tian, Y.; Wang, G.; Shi, L.; Ning, G.; Ye, J. Functionalized Boron Nanosheets with Near-Infrared-Triggered Photothermal and Nitric Oxide Release Activities for Efficient Antibacterial Treatment and Wound Healing Promotion. *Biomater Sci* **2022**, *10* (14), 3747–3756. https://doi.org/10.1039/D2BM00519K.

198. Guo, W.; Hao, J.; Wang, M.; Huang, T.; Miao, C.; Yin, L.; Ji, H.; Gao, F.; Zhou, B. Polypyrrole Hydrogel with Near-Infrared Light-Driven Nitric Oxide Release and Photothermal Activities for Rapid Synergistic Sterilization and Infected Wound Therapy. *Colloids Surf A Physicochem Eng Asp* **2023**, *677*. https://doi.org/10.1016/J.COLSURFA.2023.132411.

199. Hu, D.; Deng, Y.; Jia, F.; Jin, Q.; Ji, J. Surface Charge Switchable Supramolecular Nanocarriers for Nitric Oxide Synergistic Photodynamic Eradication of Biofilms. *ACS Nano* **2020**, *14* (1), 347–359. https://doi.org/10.1021/ACSNANO.9B05493.

200. Lv, X.; Jiang, J.; Ren, J.; Li, H.; Yang, D.; Song, X.; Hu, Y.; Wang, W.; Dong, X. Nitric Oxide-Assisted Photodynamic Therapy for Enhanced Penetration and Hypoxic Bacterial Biofilm Elimination. *Adv Healthc Mater* **2023**, *12* (29). https://doi.org/10.1002/ADHM.202302031.

201. Lin, J.; Cao, M.; Wang, S.; Wu, X.; Pan, Y.; Dai, Z.; Xu, N.; Zuo, L.; Liu, J.; Wang, Y.; Zhong, Q.; Xu, Y.; Wu, J.; Gui, L.; Ji, X.; Liu, H.; Yuan, Z. Deep Red-Light-Mediated Nitric Oxide and Photodynamic Synergistic Antibacterial Therapy for the Treatment of Drug-Resistant Bacterial Infections. *Small* **2025**, *21* (6). https://doi.org/10.1002/smll.202408759.

202. Cheng, J.; Gan, G.; Zheng, S.; Zhang, G.; Zhu, C.; Liu, S.; Hu, J. Biofilm Heterogeneity-Adaptive Photoredox Catalysis Enables Red Light-Triggered Nitric Oxide Release for Combating Drug-Resistant Infections. *Nat Commun* **2023**, *14* (1), 1–12. https://doi.org/10.1038/S41467-023-43415-8;TECHMETA.

203. Tang, Y.; Wang, T.; Feng, J.; Rong, F.; Wang, K.; Li, P.; Huang, W. Photoactivatable Nitric Oxide-Releasing Gold Nanocages for Enhanced Hyperthermia Treatment of Biofilm-Associated Infections. *ACS Appl Mater Interfaces* **2021**, *13* (43), 50668–50681. https://doi.org/10.1021/ACSAMI.1C12483.

204. Wang, Y.; Shi, H. dong; Zhang, H. L.; Yu Chen, Y.; Ren, B.; Tang, Q.; Sun, Q.; Zhang, Q. L.; Liu, J. G. A Multifunctional Nanozyme with NADH Dehydrogenase-Like Activity and Nitric Oxide Release under Near-Infrared Light Irradiation as an Efficient Therapeutic for Antimicrobial Resistance Infection and Wound Healing. *Adv Healthc Mater* **2023**, *12* (25). https://doi.org/10.1002/ADHM.202300568.

205. Gao, L.; Cheng, J.; Shen, Z.; Zhang, G.; Liu, S.; Hu, J. Orchestrating Nitric Oxide and Carbon Monoxide Signaling Molecules for Synergistic Treatment of MRSA Infections. *Angewandte Chemie - International Edition* **2022**, *61* (3). https://doi.org/10.1002/ANIE.202112782.

206. Kumar, R.; Chug, M. K.; Brisbois, E. J. Long-Term Storage Stability and Nitric Oxide Release Behavior of (N-Acetyl- S-Nitrosopenicillaminyl)- S-Nitrosopenicillamine-Incorporated Silicone Rubber Coatings. *ACS Appl Mater Interfaces* **2022**, *14* (27), 30595–30606. https://doi.org/10.1021/acsami.2c06712.

207. Wang, J.; Wang, Y.; Su, Y.; Gong, H.; Han, X.; Ma, S.; Sun, D.; Liu, Y.; Chen, W.; Zhang, M.; Zhao, J. *Emerging Trends in Design of Nitric Oxide Stimuli-Responsive Release Materials for Antibacterial Applications*; Elsevier B.V., 2025; Vol. 182.

208. Hou, J.; Pan, Y.; Zhu, D.; Fan, Y.; Feng, G.; Wei, Y.; Wang, H.; Qin, K.; Zhao, T.; Yang, Q.; Zhu, Y.; Che, Y.; Liu, Y.; Cheng, J.; Kong, D.; Wang, P. G.; Shen, J.; Zhao, Q. Targeted Delivery of Nitric Oxide via a 'Bump-and-Hole'-

Based Enzyme–Prodrug Pair. *Nat Chem Biol* **2019**, *15* (2), 151–160. https://doi.org/10.1038/S41589-018-0190-5.

209. Hou, L.; Zhang, Y.; Yang, X.; Tian, C.; Yan, Y.; Zhang, H.; Shi, J.; Zhang, H.; Zhang, Z. Intracellular NO-Generator Based on Enzyme Trigger for Localized Tumor-Cytoplasm Rapid Drug Release and Synergetic Cancer Therapy. *ACS Appl Mater Interfaces* **2019**, *11* (1), 255–268. https://doi.org/10.1021/ACSAMI.8B17750.

210. Bhat, A. R.; Ahmed, S. Artificial Intelligence (AI) in Drug Design and Discovery: A Comprehensive Review. *In Silico Research in Biomedicine* **2025**, *1*, 100049. https://doi.org/10.1016/J.INSI.2025.100049.

8. About the author(s)

Prof Sandeep Verma has been working at Indian Institute of Technology Kanpur since 1997. He is also a Distinguished Visiting Professor, Shiv Nadar Institution of Eminence, NCR, Honorary Adjunct Professor at School of Science, Royal Melbourne Institute of Technology University, Melbourne, and School of Agriculture, Biomedicine and Environment, La Trobe University, Melbourne, Australia. His research interests include chemical neuroscience, new antibiotics, and hand-held microfluidic detection devices in healthcare. With over 250 publications and several patents to his credit, his work has been recognized by Shanti Swarup Bhatnagar Prize, Distinguished Alumnus Award of Banaras Hindu University, Society of Materials Chemistry Gold Medal (DAE), JC Bose Fellowship (DST), Abdul Kalam Technology Innovation National Fellowship (INAE), CRS Gold Medal, CRSI Silver Medal, and Swarna Jayanti Fellowship. He is an elected Fellow of Indian National Science Academy, Indian Academy of Sciences, National Academy of Sciences, India, Indian National Academy of Engineering, and Royal Society of Chemistry (UK). He also serves as an Associate Editor of *Chemical Communications* (RSC, UK) and is on the Editorial Advisory Board of *ChemBioChem* (Wiley, Germany).

Dr Grace Kaul is a DST INSPIRE Faculty Fellow in the Department of Chemistry at the Indian Institute of Technology Kanpur (IITK), India. She has formerly worked as an Institute Postdoctoral Fellow in Prof. Sandeep Verma's research group, in the Department of Chemistry, IITK. She received her doctorate in Biological Sciences from CSIR-Central Drug Research Institute (CDRI), Lucknow, India, where her work encompassed investigation of antibacterial resistance mechanisms, rational design and development of novel antibacterials, and implementation of drug-repurposing strategies. She previously earned her Master's and Bachelor's degrees in Biotechnology from Dr. R. M. L. Avadh University, Ayodhya, Uttar Pradesh, India. Her current research focuses on how bacterial pathogens adapt to therapeutic pressure and exploring peptide-based and small-molecule interventions to overcome resistance. Dedicated to translational impact, she pursues interdisciplinary approaches to address the global challenge of drug-resistant infections.

Dr Asmita Shrestha received her Ph.D. in Chemistry in 2024 from Tribhuvan University, Nepal. Her doctoral research focused on the identification of a drug candidate that can inhibit SARS-CoV-2. She received a UGC Fellowship "PhD-78/79-S&T-06" during her Ph.D. Currently, she is working as a Postdoctoral Research Fellow with Prof. Sandeep Varma and Prof. Nisanth N. Nair at Indian Institute of Technology Kanpur, India. She is working on designing new antibiotics against class C β-lactamase using computational methodologies.

Article Note: A collection of invited papers based on *Award Lecture* presentations in the 21st CRS® International Symposium *'Science Beyond Boundary: Invention, Discovery, Innovation and Society Rasayan 21'* held at Manipal Academy of Higher Education, India on August 1-2, 2025.