

# Nitric Oxide Nanoparticle Technology

## A Novel Antimicrobial Agent in the Context of Current Treatment of Skin and Soft Tissue Infection

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### ABSTRACT

*Staphylococcus aureus* infections account for the majority of skin and soft tissue infections in the United States. *Staphylococcus aureus* is rapidly evolving resistance to contemporary topical as well as systemic antibiotics. Alternatives to current treatment options for skin and soft tissue infections are needed for more effective treatment now and in the future. Nitric oxide's proven roles in both wound repair and as an antimicrobial agent make it an excellent candidate for the treatment of skin infections. Recent attempts at novel nitric oxide therapies, in the form of nitric oxide donors, have shown limited potential in treating cutaneous infection. However, more recent developments in nitric oxide delivery, using nitric oxide nanoparticle technology, demonstrate substantial promise in the promotion of wound repair and eradication of skin and soft tissue infections. (*J Clin Aesthetic Dermatol.* 2010;3(6):45–50.)

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**S***taphylococcus aureus* is a gram-positive coccus, currently responsible for the majority of skin and soft tissue infections (SSTIs) in the United States.<sup>1</sup> This bacterium is commonly found asymptotically in healthy individuals, colonizing the anterior nares and other sites of the body, such as the skin and gastrointestinal tract. However, *S. aureus* can be extraordinarily pathogenic, causing a broad range of morbid states from serious skin infections, such as cellulitis and abscesses, to endocarditis and sepsis. As the most commonly isolated bacteria from SSTIs worldwide, it poses a significant public health problem of epidemic proportions.

The current threat of *S. aureus* is more than the sheer number of infections it causes worldwide. Resistance of this bacterium to antibiotics is on the rise. Since its discovery in the 1880s, *S. aureus* has evolved resistance to antibiotics, beginning with penicillin. In 2002, *S. aureus* was reported to have resistant strains to vancomycin, a drug to which it had previously been uniformly sensitive.<sup>2</sup> Vancomycin-resistant strains are still rare, but methicillin-resistant strains (methicillin-resistant *S. aureus*, MRSA) are amplifying rapidly. The percentage of MRSA isolates in United States intensive care units

increased from two percent in 1974 to 64 percent in 2004, one of many startling statistical trends highlighting the rapid expansion of MRSA infections.<sup>3</sup> This escalation in methicillin-resistant isolates highlights the need for newer and different means of treating cutaneous infection.

### TOPICAL ANTIMICROBIALS FOR TREATMENT OF SKIN INFECTION

Despite the lack of standardized testing and formal evaluation, topical antimicrobials are still considered an essential component of wound care. Topical antimicrobials fall into two main categories: antiseptics and antibiotics.

**Antiseptics.** Antiseptics are disinfectants that have a broad antimicrobial spectrum, but are often toxic to host tissues.<sup>4</sup> There is much debate about the use of antiseptics on open wounds and their beneficial or detrimental outcomes on tissue healing. One major advantage of antiseptics is that they rarely select for resistant microbial strains,<sup>5</sup> making them preferable to antibiotics with regard to the development of bacterial resistance. Some antiseptics have been found to be cytotoxic *in vitro* to both micro-organisms and the host's cells.<sup>5</sup> The potential for cell toxicity is a leading argument against antiseptic use. When choosing a treatment for skin infection, one

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should analyze the risk of local toxicity versus the benefit of antibacterial action.<sup>5</sup> Newer antiseptic dressings, particularly those with slow and sustained release of the drug, are generally thought of as safe.<sup>5</sup>

Commonly used antiseptics include hydrogen peroxide, chlorhexidine, iodines, and silver compounds, each with varying degrees of antimicrobial and debriding efficacies.

*Hydrogen peroxide.* Hydrogen peroxide is a widely used topical antiseptic that damages cellular components of many bacteria on account of its highly reactive hydroxyl radical, but it must be used in very high concentrations because of the catalase activity of many pathogenic bacteria.<sup>4</sup>

*Chlorhexidine.* Chlorhexidine has been used for more than 50 years and has lower irritancy to the skin relative to other antiseptics.<sup>4</sup> It is a biguanide that exerts its antimicrobial effect by disrupting cytoplasmic membranes and is more effective against gram-positive than gram-negative bacteria with little activity on fungi.<sup>4</sup> Chlorhexidine may be a useful topical therapeutic agent for SSTIs, although it has not been thoroughly investigated.<sup>5</sup> It has been traditionally used for prophylactic means, such as presurgically or before laser skin resurfacing.<sup>6</sup> Of note, chlorhexidine is ototoxic and therefore is contraindicated for use in the ear.

*Iodines.* Iodines have been used for more than 150 years without bacteria developing resistance<sup>7</sup> and have been shown to be effective against MRSA *in vitro* and in clinical studies.<sup>5</sup> Modern formulations of iodophors, such as povidone iodine (PVP-I) and cadexomer iodine, provide sustained release of low levels of free iodine optimizing activity and reducing toxicity.<sup>5</sup> Despite United States Food and Drug Administration (FDA) approval of the PVP-I, iodine's clinical application for wound treatment is controversial.<sup>4</sup> Disadvantages of iodophors include skin irritation, allergy, and toxicity in susceptible patients. Iodophors are capable of percutaneous and mucous membrane absorption, and as a result should not be used in pregnant women, newborns, or patients with thyroid disorders.<sup>6</sup>

*Silver.* Silver compounds have proven efficacy against MRSA and vancomycin-resistant enterococci (VRE) as well as extended spectrum beta-lactamase producers. Resistance to silver compounds is rare and they may be active against biofilm.<sup>7</sup> Silver's therapeutic properties were recently highlighted by Liu et al,<sup>8</sup> who demonstrated accelerated wound closure through the proliferation and migration of keratinocytes as well as differentiation of fibroblasts in wounds treated with silver nanoparticles. Silver sulfadiazine continues to be frequently used on account of its low toxicity, low hypersensitivity, and low incidence of resistance.

*Honey.* Honey has been used for thousands of years as a topical treatment for wound infections and has demonstrated efficacy against more than 50 species of bacteria, including MRSA and VRE, with no reported microbial resistance.<sup>7</sup>

**Antibiotics.** Antibiotics are chemicals, produced

synthetically or naturally, that act on specific targets to kill micro-organisms resulting in a narrower spectrum of activity than antiseptics. Antibiotics are often less cytotoxic than antiseptics; however, they are more likely to lose their efficacy to bacterial resistance.<sup>7</sup> Another known disadvantage of topical antibiotics is the occurrence of contact allergy.<sup>5</sup> The contact allergy is sometimes secondary to the antibiotic, but more often a reaction to preservatives in the delivery vehicle. The ideal preservative, both effective and devoid of irritant or sensitizing potential, has yet to be discovered. Therefore, the gold standard for SSTIs remains systemic antibiotics, with or without topical antiseptics.<sup>5</sup>

*Neomycin.* Neomycin is an aminoglycoside antibiotic that inhibits protein synthesis by binding ribosomal ribonucleic acid (RNA). It is bactericidal against most gram-negative bacteria and some gram-positive bacteria. Neomycin is commonly indicated for treatment of superficial infections, infection prophylaxis in minor and postoperative wounds, and burns. Allergic contact dermatitis is an adverse effect noted in 1 to 6 percent of the population, and those with damaged skin are even more susceptible with an estimated incidence of contact dermatitis as high as 30 percent.<sup>6</sup> Additionally, the potential for delayed hypersensitivity, IgE-mediated reactions, and anaphylactic reaction exists.

*Bacitracin.* Bacitracin is one of the most popular topical antibiotics, bactericidal against a variety of gram-positive and gram-negative organisms. It is like neomycin, however, it is not indicated in the treatment of chronic ulcers where damaged skin poses an increased risk of sensitization. Occurrences of hypersensitivity reaction also exist in the literature. Allergic contact dermatitis is a well-documented reaction to bacitracin, making it a less favorable treatment for cutaneous infection.<sup>9</sup>

*Polymyxin.* Polymyxin, like bacitracin, is isolated from the bacteria *Bacillus* and, therefore, shares allergic cross reactivity with bacitracin. Its spectrum of activity is limited to some gram-negative bacteria, but it is active against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcescens*, *Escherichia coli*, *Enterobacter*, and *Klebsiella*. Therefore, polymyxin is more frequently used in combinations with bacitracin or neomycin (Neosporin, Johnson & Johnson Consumer Products Company; Division of Johnson & Johnson Consumer Companies, Inc., Skillman, New Jersey and Polysporin, Johnson & Johnson Consumer Products Company) to increase its spectrum of activity.<sup>6</sup>

*Mupirocin.* Mupirocin is a naturally occurring antibiotic that inhibits protein synthesis by binding to isoleucyl transfer-RNA synthetase, thereby preventing the incorporation of isoleucine into the protein. It is highly active against gram-positive cocci. Resistance and cross reactivity with other antimicrobials is low due to its unique mechanism of action. Indications for mupirocin include operative wounds, burns, skin infections, superinfection of chronic dermatoses, and eradication of nasal carriage of *S. aureus*. Mupirocin has also proven useful in the

management of secondary pyodermas.<sup>6</sup> While mupirocin is a highly effective antibiotic found to be similar in efficacy to oral antibiotics it is important to note that it is only proven to be beneficial in the reduction of methicillin-sensitive *S. aureus* and only marginally effective in eradicating MRSA.<sup>6</sup>

There is little evidence for the clinical efficacy of topical antimicrobials in the care of infected wounds, and, as a result, the mainstay of treatment remains systemic antibiotics. However, there are cases in which the use of topical antimicrobials is appropriate as a replacement for or in addition to systemic therapy.

Topical antiseptics with sustained release may be indicated if healing is delayed or if there are overt signs of infection.<sup>5</sup> Antiseptics work locally at the site of action to eradicate bacteria making their use appropriate for burns and ischemic ulcers in which blood vessels to the skin are destroyed or bacterial burden and biofilms inhibit healing. There is still a major need for innovative antimicrobial agents. The emergence of antibiotic resistance, the avascular nature of nonhealing wounds, and the presence of a complex network of bacterial biofilms, hinder the effect of systemically administered conventional antibiotics.<sup>10</sup> Topical antimicrobials must be used discriminatively. There is concern that resistance selection may be even worse for topical antibiotics than for systemic antibiotics.

## **EMPIRIC TREATMENT OF CUTANEOUS *S. AUREUS* INFECTIONS WITH SYSTEMIC ANTIBIOTICS: EMERGING RESISTANCE**

Systemic antibiotics are becoming first-line treatment for infections, such as impetigo and abscesses, because topical treatments are often ineffective. The resultant widespread use of systemic as well as topical antibiotics has led to the emergence of resistant bacterial strains.<sup>5</sup> Between the 1940s and 1960s, *S. aureus* made the transformation from being over 90 percent susceptible to penicillin to approximately 90 percent resistant. At that time, it was still uniformly susceptible to anti-staphylococcal penicillins (cloxacillin, dicloxacillin, flucloxacillin, oxacillin, methicillin, and nafcillin).<sup>11,12</sup> During the 1970s, MRSA strains emerged, resulting in diminished efficacy of anti-staphylococcal penicillins and early generation cephalosporins, thereby greatly reducing the antimicrobial options available for treatment.

Consequently, vancomycin or piperacillin/tazobactam are now considered first-line empiric therapy for nosocomial skin infections.<sup>12</sup> Community-acquired SSTIs, less likely caused by resistant pathogens, are still treated empirically with anti-staphylococcal penicillins, such as nafcillin/oxacillin or cefazolin.<sup>11</sup> Regrettably, the increasing and widespread use of vancomycin and other glycopeptide antibiotics has ushered the emergence of glycopeptide-resistant organisms.<sup>11</sup>

Many of the newer antimicrobial agents, such as the carbapenems, oxazolidinones, and streptogramins, are effective treatments for complicated SSTIs. Their proven activity against highly resistant organisms, including

MRSA and vancomycin-resistant enterococci, warrants their selective use in life-threatening situations when resistant pathogens are suspected in order to reduce the emergence of resistant bacterial strains.<sup>12</sup>

While the majority of serious SSTIs caused by *S. aureus* or beta-hemolytic streptococci are still methicillin/oxacillin susceptible, the obvious trend in the development of drug-resistant strains is of particular concern.<sup>11</sup> It has become crucial in the fight against chronic wound infection and management of *S. aureus* infections to focus on new directions for research and development of antimicrobial drug delivery systems.<sup>10</sup>

## **NITRIC OXIDE: A FAMILIAR ANTIMICROBIAL WITH A NEW ROLE IN TREATMENT OF SSTIs**

Once referred to as endothelium-derived relaxing factor (EDRF), because of its involvement in vasodilatation, nitric oxide (NO) is now recognized as a crucial molecule responsible for a tremendous array of physiological actions. Among those diverse functions, NO is a regulator of wound healing<sup>13</sup> and serves as an antimicrobial agent against a wide range of organisms.<sup>14</sup> NO is a diatomic, lipophilic, naturally occurring free radical. As a free radical with a short half-life and high reactivity, its site of action is likely only to extend around 100µm from its origin. In fact, NO's sphere of influence is roughly the length of a few cell diameters.<sup>15</sup> NO readily crosses most physiological barriers<sup>16</sup> and, as such, relies on its ability to be produced or released at the required site at a rate high enough to generate a concentration gradient.<sup>17</sup>

NO is synthesized by any one of three forms of the enzyme, nitric oxide synthases (NOS), that use arginine as their substrate, but can also be synthesized non-enzymatically by the reduction of nitrite and nitrosothiols. Constitutively expressed forms of NOS are represented by neuronal (nNOS) and endothelial cells (eNOS) and provide for homeostatic functions, such as the regulation of blood flow and melanogenesis.<sup>17,18</sup> A third isoform, initially found in macrophages, but expressed by most cells, produces NO using an inducible form of NOS (iNOS). Unlike the constitutively expressed NOS isoforms that carry out homeostatic functions, iNOS has been implicated in pathological states of the skin and is stimulated by tissue injury, cytokines, and/or bacterial products.<sup>13</sup>

## **NITRIC OXIDE AND WOUND HEALING: A CLOSER LOOK**

Numerous studies have pointed out the contribution of NO in skin homeostasis and wound repair. The role of NO in normal wound healing was emphasized in murine models by demonstrating impaired (excisional) wound closure in iNOS-/- mice, corrected following adenoviral replacement of the gene.<sup>13</sup> Another study found that L-arginine, the substrate for NOS, enhanced collagen deposition and wound strength, implicating NO as a factor in the promotion of wound healing.<sup>13</sup> In addition to wound

repair, NO appears to play a role in pathological states of the skin, such as psoriasis and lupus erythematosus, which were shown to have deregulated expression of iNOS.<sup>18</sup>

On a molecular level, NO modulates cytokines, which in turn control the various phases of wound healing.<sup>13</sup> One study identified NO as a potent mediator of keratinocyte proliferation by demonstrating that treatment of mice with iNOS inhibitor during wound healing resulted in delayed re-epithelialization of wounds secondary to a reduced number of proliferating keratinocytes.<sup>18</sup> NO's known roles in angiogenesis, collagen deposition, and keratinocyte proliferation indicate that its generation is an integral component of wound healing.<sup>17</sup>

## NITRIC OXIDE AS AN ANTIMICROBIAL

In addition to NO's involvement in wound repair, there is ample evidence for its role as an antimicrobial agent. On a molecular level, when microbial pattern recognition receptors in phagocytic cells are stimulated, iNOS transcription is upregulated and high-output NO production ensues. Studies have shown that NO interferes directly with DNA replication<sup>19</sup> and cell respiration<sup>20</sup> by inactivating zinc metalloproteins, as well as by interacting with reactive oxygen species to create reactive nitrogen intermediates. The above reactions allow for finely regulated production of antimicrobial effector molecules that act within the microbial cell.<sup>17</sup>

The efficacy of NO as a broad-spectrum, multifaceted antimicrobial has stimulated an intense race to translate our vast understanding to the bedside. There are numerous NO-releasing coatings on biomaterials currently under investigation, many of which have demonstrated decreased incidence of biomaterial-associated infections. NO-releasing carbon-based coatings added to monofilament polypropylene meshes, as a means of reducing infectious complications after abdominal wall surgeries, had a significant bactericidal effect on *in-vitro* biofilms of *S. aureus* and other pathogens.<sup>21</sup> Similarly, coating medical grade silicone elastomer implants with a sol-gel-derived film capable of storing and releasing NO in a murine model, resulted in an 82-percent reduction in the number of infected subcutaneous implants inoculated with *S. aureus* prior to wound closure.<sup>22</sup>

NO's ability to diminish bacterial burden and accelerate wound healing inspired the widespread development of NO in a range of vehicle formulations. Despite the plethora of NO donor drugs, discovery of an ideal delivery mechanism for NO has proven elusive. Recent investigation of NO-releasing nanoparticles holds tremendous potential as a suitable drug for topical treatment in cutaneous and subcutaneous wounds.

## BRIEF HISTORY OF NITRIC OXIDE DONORS

An NO donor is a molecular carrier of NO capable of stabilizing the radical until its intended release and delivery to its target. The ideal donor would store NO at room temperature for an extended period of time and release it consistently, when required, at the pharmacologically

specified dose and for a duration long enough to exert its biological action. The ideal donor agent would be nontoxic and noninflammatory.

There are several NO donors currently under investigation that have been proven to accelerate healing in experimental wound models, but they each have characteristics that limit their widespread use and efficacy in treating skin infections.

## LIMITATIONS OF NITRIC OXIDE DONORS

**Exogenous NO gas (gNO).** gNO is an NO donor that exhibits potent antimicrobial effects *in vivo* and has little toxicity to human skin cells *in vitro*.<sup>10</sup> Ghaffari et al<sup>10</sup> postulated that gNO applied at high doses could serve as an antimicrobial agent for the treatment of chronic nonhealing ulcers or burns without compromising the viability and function of skin cells. Nevertheless, gNO has inescapable limitations as a topically applied agent.<sup>10</sup> It requires the use of gas cylinders and cannot be exposed to oxygen, making gNO difficult to handle and impractical for topical application.

**Diazeniumdiolates (NONOates).** Diazeniumdiolates were first synthesized in the 1960s. They are composed of a nucleophile adduct that binds NO, associated with a primary, secondary amine, or polyamine. The advantage of this class of NO donor is that it can release NO within seconds, minutes, hours, or even days depending on the formulation.<sup>23</sup> Diazeniumdiolates have proven efficacy in illnesses, such as cancer and cardiovascular diseases; however, their clinical utility remains dubious because of potential toxicity, formation of carcinogenic secondary nitrosamines, and possible counteracting biological effects of their metabolic byproducts.<sup>15,24</sup>

**S-nitrosothiols (S-Ni-troso-N-acetyl-D,L-penicillamine).** S-nitrosothiols occur endogenously in blood plasma and are another group of NO donor drugs that consist of a thiol group and an NO moiety. S-nitrosothiols can be designed to release NO upon decomposition at varying rates and are not only tissue selective, but possess less stringent metabolic requirements for release.<sup>15</sup> Despite the above advantages, S-nitrosothiols have yet to be thoroughly studied in the context of skin disease and lack the stability needed for localized and topical delivery because light, heat, enzymes, and other compounds capable of releasing NO from the S-NO bond severely limit their suitability as a topical agent.

**NO hybrid drugs.** NO hybrid drugs are a range of drugs that have been structurally modified to incorporate NO. A recent study in 2008 suggested the efficacy of NO-ketoconazole hybrids as an antifungal agent, but more studies are needed to understand the potential strengths and limitations of this class of NO donor.<sup>25</sup>

**An acidic mixture of ascorbic acid and nitrite.** An acidic mixture of ascorbic acid and nitrite is a chemical system that utilizes sodium nitrite and ascorbate. This ascorbic system has been successful in treating cutaneous fungal, leishmanial, and mycobacterial infections<sup>26</sup> and hastens wound healing in diabetic mice.<sup>27</sup> However, it has limited sustained release and requires a barrier to prevent

skin and wound irritation because of its inflammatory effects.<sup>24</sup>

**Zeolites.** Zeolites are a new class of NO donors that are composed of a framework of metal ions that bind NO. The advantage of this class of donors is that they are stable and able to store NO and the rate of release is modifiable. Although NO-releasing zeolites have proven antibacterial properties against clinically relevant strains of bacteria, namely gram-negative *Pseudomonas aeruginosa* and gram-positive methicillin-sensitive and methicillin-resistant *S. aureus*, studies are needed to assess their applicability for SSTIs.<sup>28</sup>

## **NITRIC OXIDE NANOTECHNOLOGY: NANOPARTICLES, AN IDEAL VEHICLE FOR NITRIC OXIDE DELIVERY?**

Discovering a means of storing a small gaseous free radical, such as NO, has been a technical challenge.<sup>17</sup> Nanotechnology offers a new and exciting platform for generating powder formulations that can be used topically to enhance wound healing and fight infection. NO-releasing nanoparticle (NO-np) technology, described by Freidman et al,<sup>14</sup> possesses many of the characteristics needed for an ideal vehicle.

Using silane hydrogel-based nanotechnology,<sup>14</sup> NO remains trapped and stable within a dry matrix until the matrix is exposed to moisture. The dry matrix allows for NO nanoparticles to be easily stored and applied. Once exposed to moisture, the drug is released from the nanoparticle over an extended period of time at a relatively fixed concentration. This sustained release distinguishes nanoparticles from other vehicles, such as injections, that release a large concentration of the drug with a rapid return to baseline. The release rate and total concentration of NO delivered from the NO-nps to the affected area can be modulated by altering the production method of the nanoparticles. To illustrate, changing the molecular weight of polyethylene glycol, an ingredient used in the formulation of nanoparticles, or the concentration of nitrite encapsulated alters the release rate and concentration of drug delivered to the site of action respectively. The ease of storage, application, and the ability to alter release rate and concentration with minimal risk of toxicity make this powder formulation ideal for cutaneous delivery. Of note, the stability of the compound makes NO-nps an excellent bridging medication for natural disasters or warfare where topical treatment can be applied to wounds in the field before receiving hospital care.

Multiple studies have already demonstrated the efficacy and safety of NO-nps in treating cutaneous infection. As an already naturally occurring molecule, topically applied NO toxicity is extremely low and NO-nps did not show any *in-vitro* toxicity.<sup>14</sup> One study tested the biological effect of NO-nps on *S. aureus* in murine model by applying NO-nps, in powder formulation, on wounds infected with MRSA. As hypothesized, reduced bacterial load and acceleration of wound closure was demonstrated both clinically and histologically in the NO-np-treated group as compared to controls. Results from the study also

indicated that the healing effect of NO-nps might be attributable to the promotion of collagen deposition.<sup>29</sup>

The efficacy of NO-nps in treating infection, specifically MRSA, was further corroborated by a study that tested NO-nps on MRSA infected abscesses in mice.<sup>30</sup> The study concluded that the NO-np-treated wounds showed a marked reduction in bacterial load and the size of the infected area. It has been demonstrated, therefore, that the NO-nps have antimicrobial and wound healing effects in both cutaneous and subcutaneous wounds. While *S. aureus* was the bacteria under investigation in the above studies, NO-nps will likely show efficacy against a broad spectrum of bacteria. In fact, a recent study tested NO-nps on mouse wounds infected with *Acinetobacter baumannii* and concluded that treatment significantly reduced healing time.<sup>31</sup>

## **CONCLUSION**

Multidrug-resistant SSTIs remain a significant therapeutic problem. As bacteria, such as *S. aureus*, develop resistance to antibiotics, conventional treatments will continue to lose efficacy. Current topical antiseptics and antimicrobials as well as systemic antibiotics are limited in their ability to treat cutaneous and subcutaneous infections. In light of the limitations of existing topical and systemic therapies, a novel therapeutic to which bacteria are susceptible is crucial for the future management of SSTIs.

NO is a well-known molecule that has wound healing and antimicrobial properties. Designing a vehicle for NO delivery that is both practical and therapeutic has proven challenging. Research efforts studying various NO donor classes have presented significant obstacles for ideal delivery of NO. Nanoparticle technology may offer a more practical means of storing and delivering NO.

NO-releasing nanoparticles have the potential to serve as a novel class of topically applied antimicrobials for the treatment of cutaneous infections and wounds.<sup>29</sup> As a nanoparticle powder, this class of drugs is cheap; easy to use, store, and apply; and has exhibited a steady, slow release allowing for constant penetration into the infected wound as opposed to other formulations that have an initial peak without extended release of the drug. As a topical therapy, this class of drugs offers the advantage of working at the site of action and avoiding systemic toxicity and can be used as a monotherapy, bridge, or in addition to systemic treatment depending on the clinical indications. Further research studying the topical application of NO-nps on drug-resistant wounds and wounds that have poor circulation, such as burns and decubiti, is advised. NO-np technology offers a new, needed, and effective formulation in the treatment of cutaneous infections.

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