# The antibiofilm effects of nitric oxide, a component of our innate immune system

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## Introduction

Nitric oxide (NO) is an innate molecule of the human immune response produced in the body by nitrogen synthases (NOS) from L-arginine through a series of oxidation reactions.[1] The immune system uses NO to kill bacteria encapsulated within phagosomes through protein and DNA disruption.[2] The ability of NO to disrupt proteins, DNA, and act as a signal molecule, means NO can also be effective as an antibiofilm agent. Generation of NO in a wound dressing would be an effective method of disrupting biofilm in hard-to-heal wounds

**Aim: To evaluate the potential of nitric oxide (NO) as an antibiofilm agent for the treatment of hard-to-heal wounds**

## Disruption of biofilm extracellular polymeric substances

- As bacteria aggregate, they produce and are held together by a matrix of extracellular polymeric substances (EPS). Hydrated EPS contains complex polysaccharides, proteins, extracellular DNA (eDNA) (and host components from the environment, i.e., wounds)[3] (Figure 1A)
- EPS supports adherence to the wound bed, protection from environmental and antimicrobial stresses, and movement of nutrients and signal molecules between microorganisms[4]
- As NO and other reactive nitrogen species (RNS) enter the biofilm they interact with biofilm components, targeting structural linkages that hold together the EPS[5] (Figure 1B)
- NO has been shown to depolymerize polysaccharides causing them to fragment, reducing the structural integrity of the biofilm matrix[6]
- NO regulates the expression of polysaccharide production genes, reducing biofilm aggregation[7]
- eDNA promotes cell-cell adhesion and biofilm stability,[8] and may be targeted by NO through cleavage of the backbone.[9] Breakdown of eDNA therefore results in reduced microbial adhesion and dispersal of biofilm[10]
- Structural proteins and free proteins within the matrix[11] are targeted by NO, breaking them apart, changing the structure, and ultimately inactivating them[12]

→ By targeting key EPS components, the microorganisms in wounds may become more exposed, increasing the effectiveness of antimicrobial action, whilst reducing the integrity of the biofilm

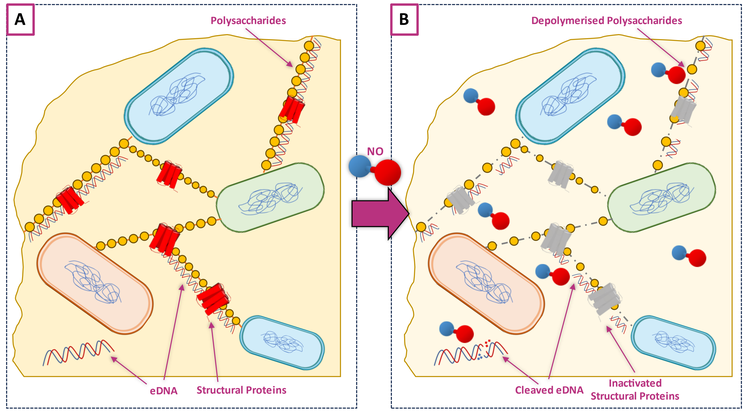


Figure 1. The composition of biofilm containing polysaccharides, structural proteins and eDNA (A) before the introduction of NO, and (B) the disrupted EPS components after the introduction of NO

## Induction of biofilm dispersal

- Cyclic-diguanylate-guanosine monophosphate (c-di-GMP) regulates the bacterial aggregation phenotype. As c-di-GMP concentration increases, so does biofilm formation[12] (Figure 2A)
- NO reduces the concentration of intracellular c-di-GMP, thereby inducing biofilm dispersal[12] (Figure 2B)
- It is theorized that c-di-GMP binds to protein regulators of dispersal proteins, such as proteins for flagellum movement, reducing motility[12]:
  - As biofilm matures, NO is naturally synthesized by bacteria to promote biofilm dispersal
  - NO binds to cell receptors which release phosphodiesterase (PDE) into the cell
  - PDE binds to c-di-GMP releasing the protein regulators, activating the dispersal proteins, reducing bacterial aggregation

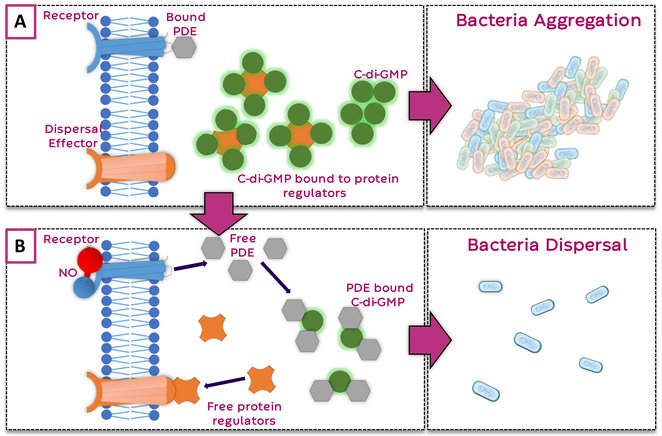


Figure 2. The C-di-GMP regulation of biofilm formation. (A) C-di-GMP bound to dispersal effector regulators resulting in aggregation. (B) NO-induced release of PDE, quenching c-di-GMP resulting in biofilm dispersal.

## Interfering with quorum sensing

- The biofilm EPS matrix allows communication between bacterial cells using small messenger molecules via quorum sensing
- Messenger molecules are produced by bacteria to induce a change in behavior of others when they bind to specific receptors. This can include decreasing antimicrobial susceptibility, signaling bacteria to disperse, and, vice versa, reducing motility so that bacteria aggregate more[13]
- NO can deactivate the AgrA quorum sensing system in *S. aureus*, preventing the autoinducing peptide pathway from completing and reducing bacterial virulence (Figure 3A)[14]
- Bacteria can detect NO by NO sensing protein (NosP), which inhibits phosphorylation reactions and therefore the movement and activation of messenger proteins in the cell. This leads to a reduction in the expression of biofilm promoting genes, and in *V. cholerae,* stops AphA enzyme activation to reduce virulence (Figure 3A)[14]

→ NO therefore interferes with normal cell-cell communication, increasing antimicrobial susceptibility, reducing aggregation and motility (Figure 3B-D), making biofilm weaker and less virulent

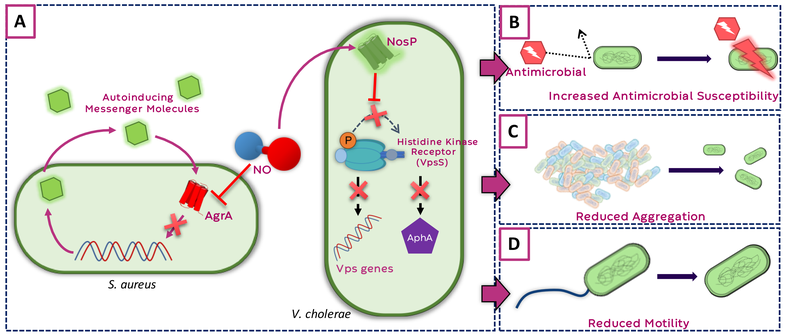


Figure 3. (A) NO inhibits AgrA of *S. aureus*, switching off the autoinducing peptide cascade. NO activates NosP of *V. cholerae* which inhibits autophosphorylation of histidine kinase receptor (VpsS), preventing the activation of Vps genes and AphA. Pathway interference results in (B) increased susceptibility to antimicrobials, (C) reduced aggregation, and (D) reduced motility; overall, reducing virulence, and biofilm formation.

## Discussion

The multiple effects that NO can exert on biofilm means there is a greater likelihood of EPS matrix disruption, enabling easier removal, increased susceptibility to antimicrobial agents, and reduction in biofilm virulence and spread. A NO-generating wound dressing may facilitate healing of hard-to-heal wounds that are impeded by biofilm.

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