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 within hard-to-heal wounds, such as diabetic foot ulcers (DFUs).

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 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 bacteria.[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).**
- Several RNS have been shown to depolymerise polysaccharides causing them to fragment, reducing the structural integrity of the biofilm matrix.[6]
- eDNA promotes cell-cell adhesion and biofilm stability,[7] and may be targeted by the RNS peroxynitrite ($OONO^-$) resulting in cleavage of the sugar-phosphate backbone.[8] Breakdown of eDNA would therefore result in reduced microbial adhesion and dispersal of biofilm.[9]
- Structural proteins and free proteins within the matrix[10] may be targeted by RNS, breaking them apart, changing the structure, and ultimately inactivating them.[5,11]

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

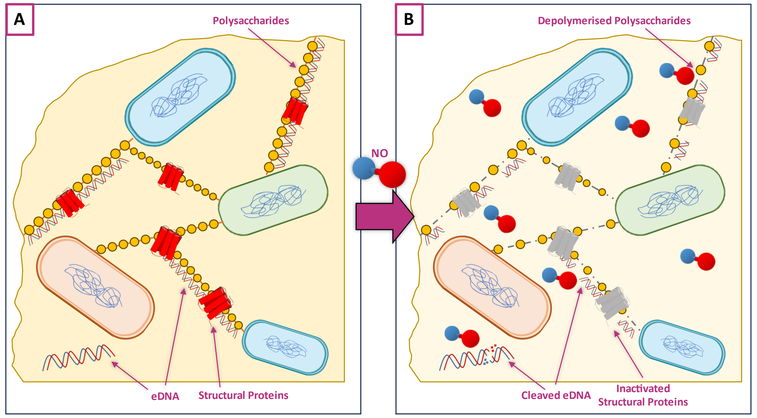

Figure 1. The composition of biofilm containing polysaccharides, structural proteins and eDNA **(A)** before the introduction of NO, and **(B)** the disrupted EPS components after the introduction of NO.

Induction of biofilm dispersal

- Cyclic-diguanylate-guanosine monophosphate (c-di-GMP) within bacteria regulates their aggregation phenotype. As c-di-GMP concentration increases, so does biofilm formation[11] **(Figure 2A).**
- NO reduces the concentration of intracellular c-di-GMP, thereby inducing biofilm dispersal[11] **(Figure 2B).**
- It is theorized that c-di-GMP binds to protein regulators of dispersal proteins, such as proteins for flagellum movement, inactivating them[11]:
  - As biofilm matures, NO is naturally synthesized by the 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.

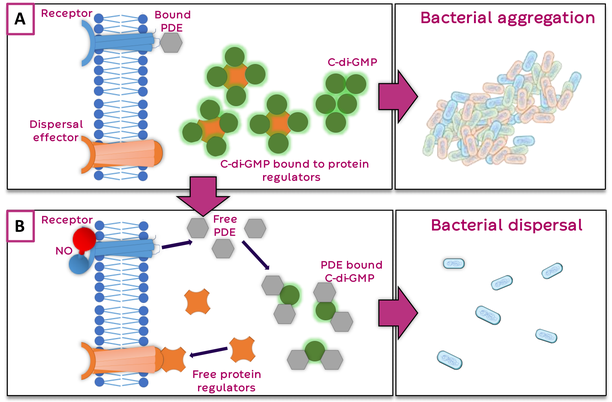

Figure 2. The c-di-GMP regulation of biofilm formation in bacteria. **(A)** C-di-GMP bound to dispersal effector regulators resulting in aggregation. **(B)** NO-induced release of PDE quenching c-di-GMP, resulting in biofilm dispersal.

Interference of quorum sensing systems

- 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 other bacteria when they bind to specific receptors, this can include decreasing antimicrobial susceptibility, signaling bacteria to disperse, and, vice versa, reducing motility so bacteria aggregate more.[12]
- NO can deactivate the AgrA quorum sensing system in *S. aureus* preventing the autoinducing peptide pathway from completing and reducing the virulence of the bacteria **(Figure 3A).**[13]
- In bacteria NO can be sensed 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).**[13]

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

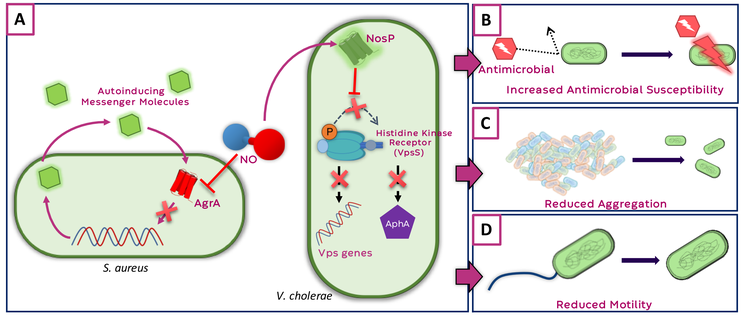

Figure 3. (A) NO inhibits the AgrA quorum sensing system of *S. aureus*, switching off the autoinducing peptide cascade. NO activates NosP of *V. cholerae* which inhibits autophosphorylation of histidine kinase receptor (VpsS), preventing the activation of Vps genes and AphA. Pathway interference results 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 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, such as DFUs, that are impeded by biofilm.

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