Size Dependent Biocidal Action of Nitric Oxide-Releasing Silica Nanoparticles

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Statement of Purpose: The broad-spectrum antimicrobial properties of nitric oxide (NO) suggest that designing new antimicrobial agents based on NO may decrease the prevalence of resistant microbes.[1] The gaseous nature of NO necessitates carriers capable of NO storage and release. Previously, we have demonstrated the efficacy of NO-releasing silica as antibacterial agents against both planktonic bacteria and bacterial biofilms.[2, 3] *N*-diazeniumdiolate NO donors were formed on secondary amines incorporated within a silica scaffold by exposing them to high pressures of NO in the presence of a base. In the presence of a proton source, NONOates decompose into the parent amine and two equivalents of NO. Herein, we describe how particle size influences particle-bacteria interactions and ultimately bactericidal efficacy of these NO-releasing materials. Using the reverse microemulsion technique, nanoparticles of distinct sizes (50, 100 and 200 nm) were synthesized with excellent stability and monodispersity. The particles were designed with constant NO release such that the influence of particle size on the particle-bacteria interactions could be studied.

Methods: Hybrid amine-containing silica nanoparticles were formed in a reverse microemulsion via the sol-gel reaction of N-(6-aminohexyl)aminopropyltrimethoxy-silane (AHAP) with tetraethoxysilane (TEOS). The reverse microemulsion was composed of an organic phase (pentane or heptane), a surfactant (Triton X100), a cosurfactant (1-hexanol), and an aqueous phase (H_2O and NH_4OH). Transmission electron microscopy (TEM) and dynamic light scattering (DLS) were used to characterize nanoparticle size, distribution, and solution stability. Cross polarization/magic angle spinning (CP/MS) solid state ^{29}Si NMR and CHN elemental analysis were used to confirm covalent incorporation of the aminosilane in the silica matrix. Nitric oxide release was measured via chemiluminescence with a Sievers nitric oxide analyzer (NOA, Boulder CO). Minimum bactericidal concentrations (MBC) of the NO-releasing particles against *Pseudomonas aeruginosa* were determined under static conditions (i.e., in PBS). Particle uptake by the bacteria was investigated with fluorescently-modified particles (RITC). Cytotoxicity of the particles against L929 mouse fibroblast cells was evaluated using the MTS assay.

Results: Monodisperse amine-containing silica nanoparticles were successfully synthesized by adopting a core-shell design, where an AHAP/TEOS shell was grown around a TEOS "seed" particles. Nanoparticle size was controlled by changing the type of organic solvent, surfactant to organic solvent ratio, and/or the reaction time. NO-releasing particles of three distinct sizes were synthesized: 52.68 ± 3.32 nm, 98.38 ± 6.73 nm, and 190.98 ± 1.84 nm. Of note, the particles were characterized by excellent monodispersity as indicated by their low PDI values (<0.150). By tuning the concentration of base used during the formation of the NO donor, the NO released from small, medium and large particles was held constant (i.e., 1.26±0.37, 1.35±0.10, and 1.13±0.18 μmol NO/mg particle, respectively) over the course of the bacteria assay (i.e., 1 h).

A direct relationship was found between particle size and the MBC against *P. aeruginosa*. Decreasing particle size was found to improve the bactericidal efficacy of the NO-releasing particles (Fig 1). The MBC for the smallest diameter particle was 50 μg mL^{-1}, while the MBC values increased to 100 μg mL^{-1} and 200 μg mL^{-1} for the medium and large particles, respectively. Confocal microscopy studies indicated that this greater efficacy for smaller particles was due to the ability of a higher concentration of these particles to enter the bacteria cells under shorter exposure times compared to larger particles. As a result, higher NO doses were delivered to the bacteria. Slower uptake of the larger particles results in lower doses of NO delivered to the bacteria as some of the NO was released prior to the particles entering the cell, thus MBC values were increased. No significant cytotoxicity against mammalian cells was observed with either NO-releasing or control particles regardless of size after 24 h exposure. Indeed, even at twice the highest bactericidal concentration cell viability remained above 80% (normalized to control).

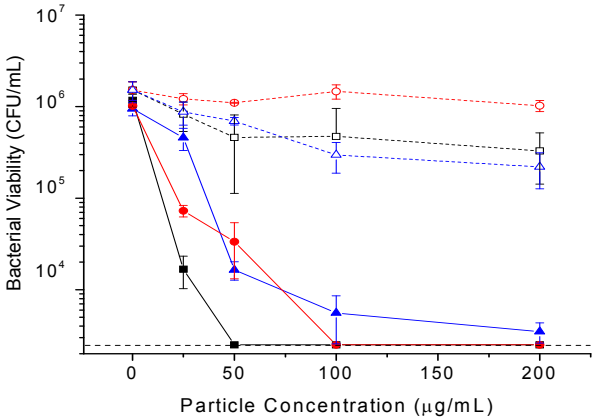

Figure 1. Bactericidal efficacy against *P. aeruginosa* as a function of dose (μg/mL) and nanoparticle size (black, 50 nm; red = 100 nm; and blue = 200 nm) for both control (open) and NO-releasing (solid) nanoparticles.

Conclusions: Bactericidal efficacy of NO-releasing nanoparticles against *P. aeruginosa* was found to improve by decreasing the particle size. Due to the short half-life of NO, the ability to deliver NO directly to bacteria cells is essential for NO-based therapies. Future studies will include evaluating the effect of size on particle uptake by other microbes and probing the influence of particle surface charge as well as NO release kinetics on biocidal activity.

References: 1. Eliopoulos GM Clin. Microbiol. Rev. 1988;1:139-156. 2. Hetrick EM ACS Nano 2008;2:235-246. 3. Hetrick EM Biomaterials 2009; 30;2782-2789.