

Ultrasmall gold nanoparticles as bactericidal agents against planktonic and biofilm enveloped Staphylococci

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Statement of Purpose:

The emergence of multidrug resistant bacterial strains necessitates the development of novel antimicrobial agents effective at doses below host toxic concentrations. The gram positive staphylococci are among the most pathogenic bacteria and have been implicated in implant failure due to prosthetic infection [1]. The treatment of such infections with metal nanoparticles (NPs) like Ag, Cu and metal oxide NPs like ZnO, NiO and CuO have met with considerable success along with the added risk of cytotoxicity to the host tissues. In the present study, the synthesis, characterization and antimicrobial efficacy of ultrasmall gold nanoparticles (AuNPs) is presented. Further, the antistaphylococcal potency of the AuNPs is evaluated against both planktonic and biofilm cultures.

Methods:

For the study, AuNPs were synthesized in different sizes ranging from ultrasmall (0.8 and 1.4 nm) to small nanoclusters (5.1, 8.7 and 10.4 nm). The AuNPs were decorated with monosulfonated phosphine ligands ensuring identical surface chemistry. The particles of different sizes were designated as Au0.8MS, Au1.4MS and so on. The AuNPs were characterized by UV/Vis spectroscopy, ³¹P NMR, Atomic force microscopy (AFM), Scanning transmission electron microscopy (STEM) and Dynamic light scattering (DLS) methods. The synthesized AuNPs were purified using centrifuge microfiltration devices. The concentrations stated in the results section refer to [Au], determined by atomic absorption spectroscopy (AAS). The AuNPs were tested against two gram positive pathogens – *S.aureus* and *S.epidermidis* as well as two gram negative – *E.coli* and *P.aeruginosa* strains. The protocols used for the tests were in compliance with the standard guidelines recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

Results:

The phosphine capped AuNPs were ineffective against the gram negative bacterial strains. However, the ultrasmall AuNPs (Au0.8MS and Au1.4MS) were highly efficacious against the planktonic cultures of gram positive staphylococci with minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of 25 μ M [Au]. This was also confirmed by the diameters of the inhibition zones formed in the disc agar diffusion (DAD) test for these strains. Similar sized Aurovist (1.9 nm), a thiol stabilized AuNP exhibited no inhibitory effect on bacterial growth. A 4-5 fold reduction in viable bacterial numbers was recorded

within 5h of treatment at MIC dosage with the ultrasmall AuNP. Scanning electron micrographs revealed membrane blebbing and lysis at sub-MIC of 6.25 μ M [Au]. Transmission electron microscopy (TEM) analysis suggested a significant decrease in the percentage of dividing cells along with a simultaneous reduction in the average cell wall thickness of the staphylococcal species treated at MIC for 2h. The induction of oxidative stress was also implicated to contribute significantly to the bactericidal activity elicited by the AuNPs.

With regard to mature staphylococcal biofilms grown for 48h, biochemical assays recorded ~ 80-90% reduction in bacterial viability (w.r.t. control), when treated with Au0.8MS and Au1.4MS at MIC and 2xMIC for 24h. Live/dead imaging of the AuNP treated biofilms at 2xMIC divulged a near complete bactericidal effect and destruction of the staphylococcal biofilms. Fig. 1 is the summary of the study depicted as a schematic.

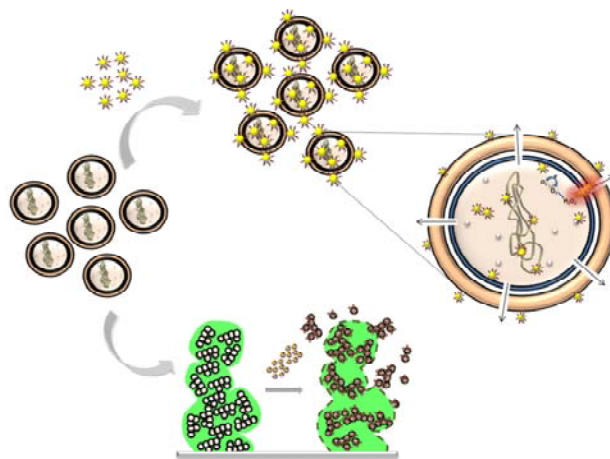


Fig. 1: Schematic (not to scale) showing the antimicrobial action of AuNPs against planktonic and biofilm enveloped staphylococci

Conclusions:

The size and surface chemistry are important parameters that can be manipulated to illicit antibacterial and anti-biofilm properties to NPs. The MIC and MBC (25 μ M [Au]) doses of the ultrasmall AuNPs are lower than the IC₅₀ values (50–250 μ M) determined against representative mammalian cell lines [2]. This presents the ultrasmall AuNP as potent antimicrobial agents.

References:

- [1] L. G. Harris, S. J. Foster and R. G. Richards, *Eur Cell Mater.*, 2002, **4**, 39-60.
- [2] Y. Pan et al., *Small*, 2007, **3**, 1941-1949