Iron Oxide Nanoparticles Induce Pseudomonas Aeruginosa Growth, Biofilm Formation, and

Inhibit Antimicrobial Peptide Function[†]

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Iron Oxide Particles. The smallest iron oxide nanoparticles investigated, 2 ± 1 nm in size, were prepared using a synthesis method similar to that used previously for other metal oxide nanoparticles reported earlier by Wu *et al*¹. In the synthesis, 2.35 g (8.7 mmol) of iron chloride hexahydrate precursor was added to 40 ml methanol. The solution was the refluxed for several minutes to dissolve the precursor and 3 ml of water was added to the resulting solution. In the next step, 30 ml of methanol solution containing 1.04 g (26.1 mmol) of NaOH was added dropwise and refluxed. After 48 hours of reaction at reflux temperature, a brown-red precipitate was collected by centrifugation at 10,000 rpm for 30 minutes. The isolated precipitates were washed three times using a 1:1 ratio of ethanol/acetone and dried under vacuum for one day. The weight of the iron oxide product from a 3 ml water addition reaction was 0.82 g corresponding to a 60 % yield based on the iron precursor reactant. Other iron oxide nanoparticle samples were purchased from commercial sources including Nanostructured & Amorphous Materials (43 nm), Alfa Aesar (85 nm) and Sigma Aldrich (540 nm).

Antimicrobial Peptide (AMP) Activity. A schematic representation of this assay is shown in Figure S2.



Figure S2. Schematic representation of AMP experiment. AMPs activity following exposure to iron oxide particles were tested using a radial diffusion assay. First, in separate experiments, FeCl₃ (control), $2 \text{ mm} \alpha$ -Fe₂O₃ and 540α -Fe₂O₃ were incubated with AMPs for one hour at 37°C. The samples were centrifuged to remove particles. The supernatant was added to the underlay for three hours before the overlay was added and the zone of inhibition, as determined by the diameter of killing bacteria, was measured after overnight incubation.

References

1. Wu, C.-M.; Baltrusaitis, J.; Gillan, E. G.; Grassian, V. H., Sulfur dioxide adsorption on ZnO nanoparticles and nanorods. *J. Phys. Chem. C* 2011, *115* (20), 10164-10172.