

Effects of silver nitrate and a silver nanoparticle biomaterial additive on *E. coli* growth, determined by isothermal micro-nano calorimetry (IMNC)

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INTRODUCTION: Silver has long been used as an antimicrobial; e.g. in disinfectant solutions and ointments for burns. Problems with antibiotic resistance have spurred renewed interest in silver, and in new forms, such as nanoparticles, to be added to or coated on medical materials. Using our isothermal micro-nano calorimetry (IMNC) method, we evaluated the effect of both silver nitrate solutions and silver nanoparticles (in an amorphous silica agglomerate carrier) on growth of *E. coli*.

METHODS: Heat is atomic-scale kinetic energy, transferred within liquids by mechanical interactions. Cultured cell metabolism and growth processes produce heat which is transferred to the medium. IMNC measures transfer rate changes as low as 22 nano J/sec--equivalent to an increase of only $\sim 10^4$ in the number of bacteria in a culture.

E. coli W3110 was cultured in LB broth at 37°C. An overnight culture was adjusted to an OD₆₀₀ of 0.01, and 1% of this solution was used as inoculum. Minimum inhibitory concentrations (MICs) were determined according to CLSI standards¹ with one change: Instead of using a single twofold serial dilution row, a second was overlaid (Fig. 1). Both silver nitrate solutions and micron-range amorphous silica agglomerates with 5, 9 and 20 % (w/w) of 5-20 nm silver particles were studied.

Growth over 24 h at 37 °C was monitored in sterile sealed 4 ml microcalorimetry ampoules. Before sealing, ampoules were filled with 2.97 ml LB broth supplemented with dilutions of silver nitrate or silica/nanosilver plus the 30 µl *E. coli* inoculum. Before heat transfer measurement, ampoules were equilibrated for 45 minutes in the microcalorimeter chambers to eliminate transient heat phenomena.

To confirm calorimetry results, parallel ampoules were incubated at 37 °C and evaluated for growth both visually and by OD₆₀₀ determinations.

RESULTS: For silver nitrate, a MIC (no growth at 24 hours) of 8 mg l⁻¹ was determined using IMNC. This result was confirmed in parallel ampoules using traditional visual/optical (turbidity) methods. Calorimetry (Fig.1) also showed something new. Subinhibitory concentrations of silver nitrate did not alter the time history of heat production (corresponding to bacterial growth). Instead, the effect of silver nitrate was only to delay the start of

growth. For example 6 mg l⁻¹ AgNO₃ delayed growth for 600 min compared to the control (culture medium and *E. coli* alone).

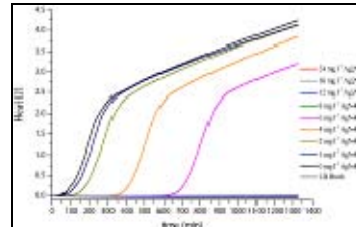


Fig. 1: IMNC change in total heat as $f(t)$ & AgNO₃ concentration, *E.coli* growth in LB broth @37°C.

The MIC for the silica/nanosilver determined by IMNC was 1 mg ml⁻¹ for 5 % silver, 0.5 mg ml⁻¹ for 9 % silver and 0.25 mg ml⁻¹ for 20 % silver. The MIC for silica/nanosilver was thus inversely proportional to the percent silver. The effect of subinhibitory concentrations on *E. coli* growth was the same as for silver nitrate (data not shown).

DISCUSSION & CONCLUSIONS: Heat transfer in liquids is a nanomechanical process, and IMNC can measure rates as low as 22 nano J/sec. Here, it precisely determined the MIC of two forms of silver for *E. coli*. IMNC also measured something missed by traditional methods--the real time effects of subinhibitory concentrations of silver on bacterial growth. Subinhibitory concentrations of silver delay growth, but once it starts, initial concentration has no effect on the rate. Further IMNC studies will measure the effect of silver additives on energetics of bacterial adhesion. IMNC can also determine MICs of antibiotics² and measure rate processes in other types of cultured cells, biomaterials and their interactions.

REFERENCES: ¹ Clinical & Laboratory Standards Institute (2006) M07-A7, CLSI, USA. ² von Ah, Wirz, Daniels, J Clinical Microbiology (accepted 04/2008).

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