Study of Silver Nanoparticles Biocidal Impact on Escherichia coli Using Optical and Atomic Force Microscopy

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Abstract

Silver's biocidal properties have been known to affect cellular metabolism and inhibit cell growth; therefore it is expected that it will affect *Escherichia coli*, a bacteria known as one of the many species of bacteria living in the lower intestines of mammals. One of the primary adverse effects of the bacteria is the ability to cause food-borne illness. Atomic force microscopy (AFM) is a high-resolution imaging technique that can resolve features as small as an atomic lattice in real space. One of its many advantages is that it allows molecular scale resolution in liquid or without vacuum; therefore it has been immediately extended to biological systems. The focus of the present study was to investigate the structural and surface alterations induced in *E. coli* by the exposure of silver nanoparticles and image these changes using AFM. It is expected we will obtain easily achievable images by AFM with different orientations in space and accurate measurements of the morphology of normal versus affected *E. coli*. Optical microscopy was used for preliminary experiments to confirm that silver nanoparticles can affect the *E. coli* [1-3].

Objective

Investigate the effects induced on *Escherichia coli* when exposed to a biocidal compound such as silver nanoparticles and image them using optical and atomic force microscopy.

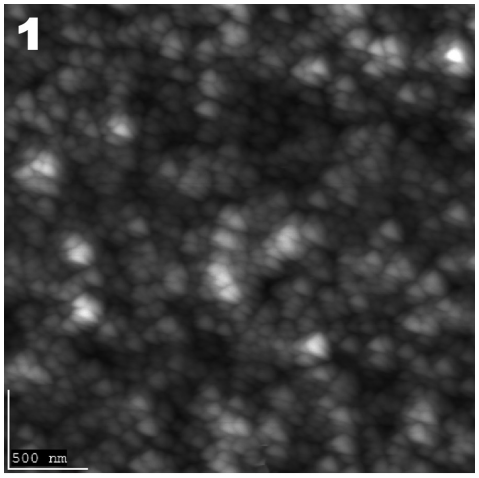
Materials and Methods

Surface Characterizations. Silver/silicon (AgSi) and Si wafer samples were studied using AFM. AgSi samples were prepared by DC magnetron sputtering of Ag and Si targets to deposit films onto a Si wafer. The Ag content for the samples was expected to be from 18-20%. Characterization was done by observing patterns on the surface topography and by studying data provided by the AFM, such as the average roughness of each sample. Contact and tapping mode was used, the latter being the ideal method because of its use in biological applications.

Preparation of Bacteria Samples. *E. coli* was grown in two different mediums: brain heart infusion (BHI) broth and nutrient agar. Flame inoculation was used to probe the *E. coli* into glass slants containing each medium. *E. coli* slants were left in an incubator at 37°C overnight to observe bacterial growth. After bacterial growth was evident, slants were labeled as stock solutions.

Serial Dilutions. Phosphate buffer was used to prepare solutions of decreasing bacterial concentration (10^{-1} to 10^{-6}) from *E. coli* BHI stock solution. These were labeled according to their bacterial concentration. The most diluted solutions (10^{-4} to 10^{-6}) were plated in a BHI agar and left to incubate at 37°C overnight to observe and quantify colonies.

Optical Microscopy. *E. coli* BHI stock solution and diluted solutions were observed on depression slides by optical microscopy using a (40x) objective. 500 μL of each *E. coli* solution were added to the slides with a sterile pipette. *E. coli* behavior was observed and recorded for 5 minutes prior to exposure to silver nanoparticles.



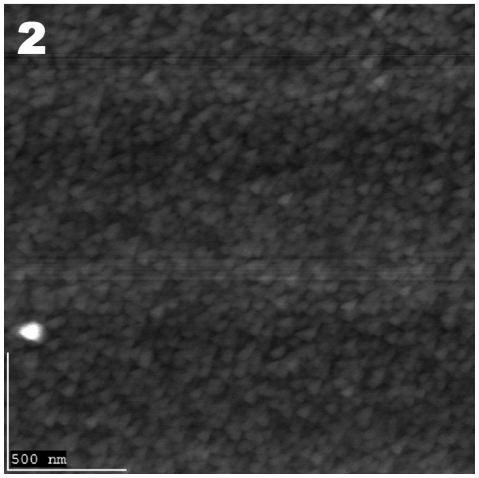


Figure 1, top: AgSi (3 µm scan) (Ra: 8.27 nm).

Figure 2, bottom: Si (3 µm scan) (Ra: 0.829 nm).

Silver Nanoparticles. Ag nanoparticle solution was prepared in a RPMI 1640 medium. This solution contained 10^{14} Ag nanoparticles per milliliter. Slides containing *E. coli* were exposed to 200 μL of Ag nanoparticles solution after 5 minutes of observation. *E. coli* behavior after exposure to Ag nanoparticles was observed for 35 minutes.

Results and Conclusions

Surface Characterization. Different patterns were observed when imaging surfaces by non-contact mode on the AFM. When the Si wafer samples were studied, surface topography images seemed smooth and very uniform contrary to AgSi samples that showed a rougher and more complex surface topography. AgSi samples appeared to have small scattered clusters along the surface. When average roughness (Ra) data was obtained, it showed that Si wafer surfaces had a (Ra = 0.829 nm) and that AgSi samples had a (Ra = 8.27 nm), confirming what was previously observed on surface topography images. Additionally, when the (Ra) for the AgSi sample and Si wafer sample were subtracted, a value of approximately 7 nm was obtained. This value confirmed that silver nanoparticles were embedded in the surface since it was the expected value for the size of the silver nanoparticles when prepared by DC magnetron sputtering. (See Figures 1 and 2.)

***E. coli* and Silver Nanoparticles.** Before silver nanoparticles exposure, *E. coli* seemed healthy and moving freely in solution. *E. coli* was observed for a period of 35 minutes after exposure to silver nanoparticles solutions. During the 35 minute period, the *E. coli* movement began to decrease and *E. coli* began to collide with each other forming clusters. After the 35 minute period, the *E. coli* movement stopped completely and a large number of clusters were observed. (See Figures 3 and 4.)

Future Work

Future work is expected to include: further study of surface characterization of AgSi and Si wafer surfaces by AFM, and *E. coli* growth on mica surfaces. In both studies, *E. coli* will be exposed to silver nanoparticles and the *E. coli* behavior will be observed *ex-situ* and *in-situ,* anticipating that it will die. Additionally it is expected that quantifying the *E. coli* in each sample will enable calculation of the rate at which the *E. coli* dies.

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References

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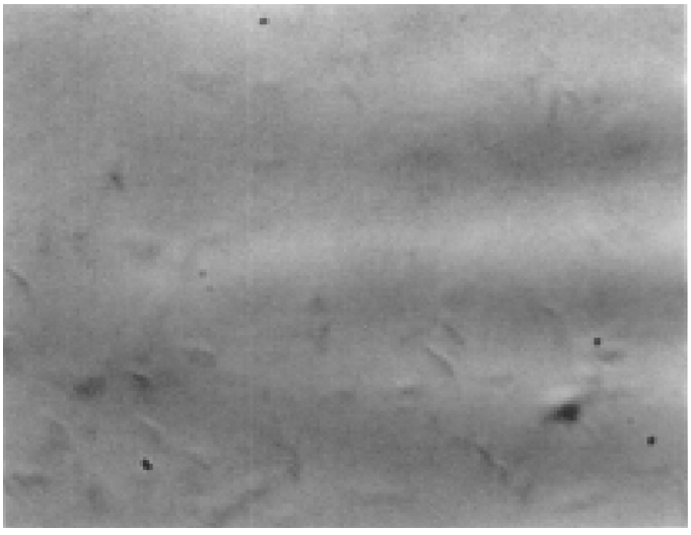

Figure 3: Before exposure to silver nanoparticles.

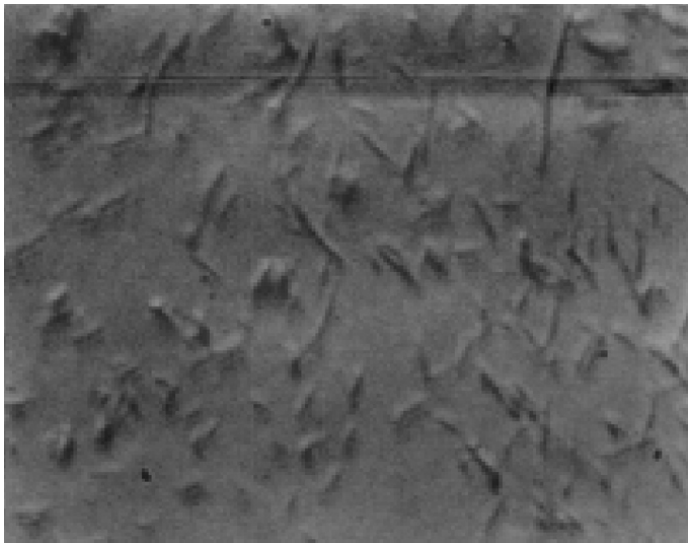

Figure 4: 40 minutes after exposure.