Determining binding effectiveness of silver nanoparticles to *Bacillus atrophaeus* spores

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Introduction

In 2001, anthrax-laced letters killed five people and infected seventeen others, yet the spores went undetected until after exposure. Soldiers and first responders therefore require sensors capable of detecting *Bacillus anthracis* spores at trace levels before dangerous concentrations are reached. *Bacillus atrophaeus* is used as a safe laboratory surrogate for *Bacillus anthracis* due to its near-identical spore structure and nonpathogenic nature.

Surface-Enhanced Raman Spectroscopy (SERS) is one of the most promising techniques for this purpose. Raman scattering is inherently weak, occurring in approximately 1 in 10^6–10^8 photons; however, noble metal nanoparticles amplify the electromagnetic field at their surface, dramatically increasing spectral signal intensity (Mosier-Boss, 2017). SERS effectiveness depends on nanoparticle size, surface charge, capping chemistry, and aggregation on the bacterial cell wall (Zhou et al., 2014), and inconsistent nanoparticle binding across trials limits its reliability for real-world biodefense detection.

This project sought to optimize the binding efficiency of silver nano-colloidal preparations on *Bacillus atrophaeus* spores by evaluating the effects of concentration and exposure time on percent surface coverage.

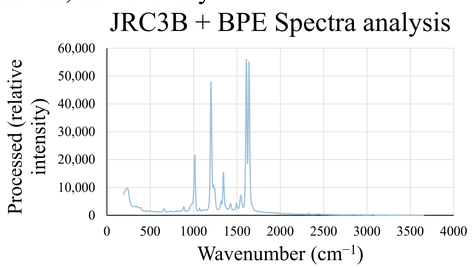
Methods and Materials

Four silver nano-colloidal suspensions were prepared in collaboration with Dr. Jason Guicheteau. Colloids were each named JRC2–JRC5. Each formulation varied in nanoparticle size, surface charge, and capping chemistry, and each was fractionated at three concentrations: 1× (stock), 5×, and 10× relative nanoparticle count per unit volume.

Nanoparticle binding was initially confirmed using Raman spectroscopy to verify spectral enhancement prior to imaging (Graph 1).

Bacillus atrophaeus spores (5×10^8 CFU/mL, ≥ 98% purity) were obtained from the DEVCOM laboratory and handled under approved biosafety protocols. Equal volumes of nano-colloidal suspension and spore solution were combined and incubated for three distinct exposure durations: 2–3 minutes, 2–3 hours, and 2–3 days.

Graph 1 (right): Preliminary colloid methodology testing of Raman spectra. Combining the colloid with BPE (trans-1,2-bis(4-pyridyl)ethylene) and optionally NaCl should result in Raman peaks at 1200, 1607, and 1639 cm^{-1} due to aggregation of nanoparticles.



Methods and Materials (continued)

Following incubation, 3 µL of each mixture were deposited onto aluminum-coated microscope slides (Figure 1). High-resolution scanning electron microscopy (SEM) was then performed on consistent fields of view to visualize nanoparticle distribution and aggregation on the bacterial surface.

A custom Python program was created that applies Gaussian blur for noise reduction, Otsu's threshold segmentation, and pixel-based analysis to calculate the percentage of the bacterial cell wall covered by nanoparticles, corroborating observed values and increasing reproducibility compared to manual estimation.

Figure 1 (left): Aluminum-coated microscope slide mounted on a circular SEM holder. Each column is a concentration (10×, 5×, and 1×) and each row is an exposure time. The holder ring has a diameter of one inch.

Results

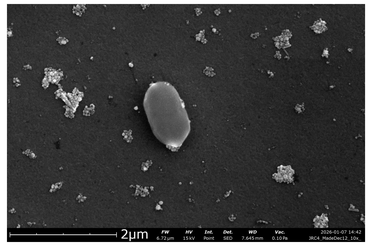


Figure 2 (above): Bacterial image from JRC4 with low nanoparticle coverage.

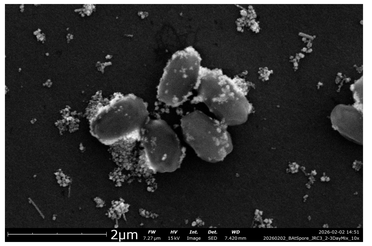


Figure 3 (above): Bacterial image from JRC3 with high nanoparticle coverage.

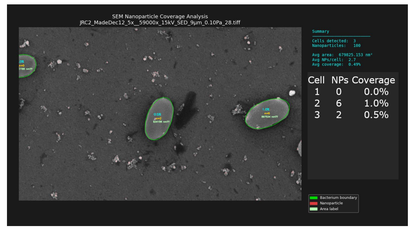
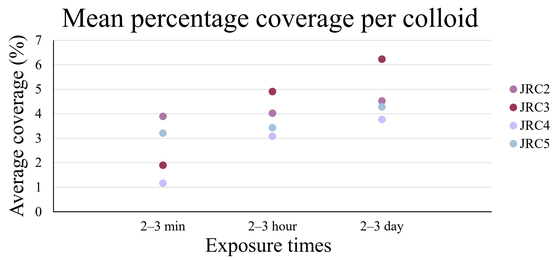
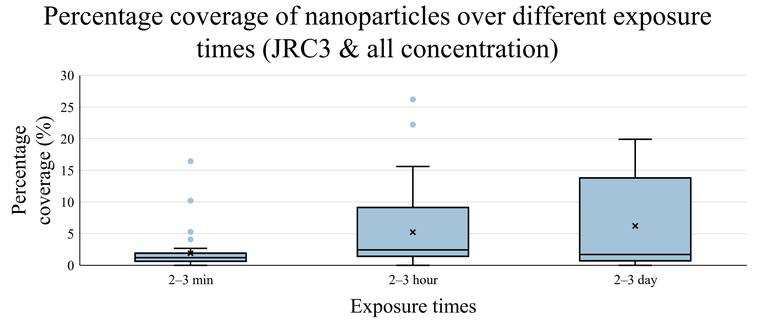


Figure 4 (left): Analyzed image of a JRC2 image inputted into Python script. Number of spores detected and average coverage of bacteria in image located at top right corner. Nanoparticle sizes ranged from 20 to 60 nm in diameter across the different colloids.



Graph 2 (left): Graph of each colloid over the 3 different exposure times. Data encapsulates all concentrations. JRC3 has the overall highest coverage with JRC4 having overall worst.

Results (continued)



Graph 3 (above): A box-and-whisker plot showing nanoparticle surface coverage across three exposure times for the colloid JRC3 ($n = 45$ per group). Tested hypotheses were H_0: $\mu_{2–3\ min} = \mu_{2–3\ hour} = \mu_{2–3\ day}$ and H_A: not all µ are equal. A one-way ANOVA found a significant effect of exposure time, $F(2, 132) = 6.20$, $p = .003$. Tukey's post hoc test showed the 2–3 min group ($M = 2.09\%$, $SD = 3.91\%$) differed significantly from both the 2–3 hour ($M = 5.23\%$, $SD = 5.85\%$) and 2–3 day groups ($M = 6.23\%$, $SD = 7.20\%$), while the latter two did not differ significantly. Results suggest increased coverage with longer exposure, though variability indicates additional influencing factors.

Conclusion

The purpose of this study was to optimize silver nanoparticle binding on *Bacillus atrophaeus* spores to improve the reliability of Surface-Enhanced Raman Spectroscopy detection. Results indicated that increasing nanoparticle concentration significantly increased percent surface coverage across all colloids tested, while the effect of exposure time was found to be dependent on colloid type. The JRC3 colloid exhibited the highest mean coverage at 2–3 hours of exposure, whereas JRC4 consistently produced the lowest coverage across all tested conditions. These findings are consistent with Zhou et al. (2014) and Mosier-Boss (2017), who demonstrated that nanoparticle concentration and surface properties are key determinants of SERS binding efficiency and signal reproducibility.

Future studies should investigate additional nanoparticle surface chemistries and extend testing to a broader range of relevant analytes, such as explosives and airborne pathogens, to further evaluate nanoparticle-based detection strategies.

References

Mosier-Boss, P. A. (2017). Review on SERS of bacteria. *Biosensors*, *7*(4), 51–77. https://doi.org/10.3390/bios7040051

Zhou, H., Yang, D., Ivleva, N. P., Mircescu, N. E., Niessner, R., & Haisch, C. (2014). SERS detection of bacteria in water by in situ coating with Ag nanoparticles. *Analytical Chemistry*, *86*(3), 1525–1533. https://doi.org/10.1021/ac402935p