Inhibiting Infectivity of the Spindle Shaped Virus Using Silver Nanoparticles

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Abstract
Drug-resistant viruses have only expanded their frequency, with vaccines offering protection to only a small portion of the known viruses. Exploring new ways to combat these small, infectious agents is a last hope. Using silver nanoparticles (AgNP), viruses were shown to reduce their infectious ability previous research has explored the inhibition effects of AgNP on HIV-1, Monkeypox virus, Tacaribe virus, and Hepatitis B (2, 3, 4, 5). In my research, I have discovered that the Spindle Shaped virus (SSV) is also inhibited by AgNP; this was done through examining and analyzing plaque assays. The method of inhibition is still unknown, but the nanoparticles have a significant effect nonetheless.
Introduction
Viruses have shown to be a threat to human lives since humanity began. Even now, the majority of known viruses that are a threat to humans are drug resistant. Finding new ways to combat these viruses involves bringing in new approaches, such as using silver nanoparticles (AgNP). In past research, AgNP has shown to inhibit viruses such as HIV-1, Monkeypox virus, Tacaribe virus, and Hepatitis B in vitro (2, 3, 4, 5). With HIV-1, the AgNP seem to be binding onto the glycoprotein knobs on the surface of the virus, and therefore not allowing the virus to bind to their host (Figure 1). The antibacterial effects of silver are also well known, but when it comes to antivirals, the area is understudied. Showing that AgNP can inhibit the infectivity of most viruses is a significant step towards fighting the infectious agents. The virus used in this study, the Spindle Shaped Virus (SSV), thrives in acidic (pH below 4) and hot (75°C) environments, such as geothermally heated hot springs, showing that the virus has unique properties that allow it to survive in such extreme environments. Finding whether SSV can be inhibited by AgNP will show that silver may be able to inhibit the infectious ability of a wide range of viruses.

![Figure 1: a) AgNP-exposed HIV-1 virus (attached to glycoprotein knobs) b) HIV-1 virus not exposed to AgNP](Source: Journal of Nanobiotechnology 2005)

Methods
Silver Nanoparticle Synthesis
The AgNP were made in Dr. Mackiewicz’s lab. Specifically, the shape of the AgNP was a sphere and the size was about 15 nm. They were made by reducing Ag⁺ into Ag⁰ using NaBH₄, and then stabilizing the product using ligand so that it could be soluble in water. The resulting concentration of the spherical AgNP was 2.47×10¹⁴ np/L.
Testing AgNP with SSV

Plaque assays were used to test the SSV with AgNP. The general method is shown in figure 2. With my process of doing plaque assays, I take 30 μl of concentrated virus stock, and I mix it with 270 μl of yeast-soybean based broth. From that mixture I took 30 μl and mixed it with another 270 μl of YS broth. I continued to do this until a $10^{-5}$ dilution was obtained. In order to incorporate the AgNP, I mixed 100 μl of AgNP with 1000 μl of concentrated virus, and I let them sit together for 0, 24, 72, and 96 hours. I diluted normally with that mixture. Afterwards, 100 μl of each dilution was mixed with 0.5 mL of concentrated Sulfolobus cells. The mixture was filled with 5 mL of a YS/gelrite mixture and immediately plated on a petri dish. The dilutions were then put into a 75°C incubator for 72 hours. Each trial was run with SSV on its own, as well as SSV with AgNP.

![Figure 2: The general method for doing plaque assays.](image)

Results

After 0 hours of incubation, the plaque count for SSV at $10^3$ on its own was too many to count (TMTC). For SSV + AgNP, the plaque count was 1,680,000 pfu/mL (figure 3). After 24 hours of incubation of the SSV and AgNP, the plaque count for SSV at $10^{-4}$ dilution on its own was $2.5\times10^7$ pfu/mL, while for SSV and AgNP, the plaque count was $2\times10^6$ pfu/mL at the same dilution. After 72 hours, the plaque count for SSV was TMTC at $10^{-4}$ dilution, while the plaque count for SSV + AgNP was $2.05\times10^7$ pfu/mL. After 96 hours, the plaque count for SSV was $1.3\times10^7$ pfu/mL at $10^4$ dilution, while the plaque count for SSV + AgNP was $3.6\times10^6$ pfu/mL at the same dilution (figure 4).
Figure 3: (Left) Plaque assay with mixture of Wild Type and NPs at $10^3$ (1,680,000 pfu/ml). (right) Plaque assay with Wild Type at $10^3$ (TMTC)

Figure 4: (left) SSV with AgNP at $10^4$ (3.6*10^6 pfu/mL) (right) SSV with no AgNP at $10^4$ (1.3*10^7 pfu/mL)

**Conclusion**
Knowing that there is some kind of mode of inhibition for the AgNP on SSV, we can conclude that AgNP can act as an antiviral on viruses that infect *Sulfolobus* in an acidic and hot environment. This repeated pattern of inhibition also shows that the AgNP is interacting with the virus or the cell in such a way that the virus is less infectious. Further studies could possibly explore the exact mode of inhibition, and specifically how the AgNP is interacting with SSV. Further studies can also be done with other viruses to confirm that the AgNP act as a general
antiviral, rather than being specific to a single virus. Using different nanoparticles, ones that are different in size and shape could also be studied, as well as using differing concentrations. In conclusion, this field of study has yet to be fully explored and has a significant amount of potential for finding new ways of combating life-threatening viruses.

Sources
3. Rogers, James V. Et al. A Preliminary Assessment of Silver Nanoparticle Inhibition of Monkeypox Virus Plaque Formation.