Assessing the Antimicrobial Activity of Silver Nanoparticles Leached from Medical Devices

B.J. Casey* and B.J. Dair**

*United States Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Chemistry and Materials Science, 10903 New Hampshire Avenue, Silver Spring, MD, USA, brendan.casey@fda.hhs.gov
**United States Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Chemistry and Materials Science, 10903 New Hampshire Avenue, Silver Spring, MD, USA, benita.dair@fda.hhs.gov

ABSTRACT

Numerous analytical techniques including dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), ultraviolet-visible light spectroscopy (UV-Vis), atomic force microscopy (AFM), and transmission electron microscopy (TEM) were utilized to characterize the physical structure of particles leached from a silver nanoparticulate containing wound dressing. Concurrent research was conducted to investigate the effect that nanoparticle size has on antimicrobial activity. Antimicrobial activity was quantified by determining the reduction of viable colony forming units relative to a control. Results indicate that the size of the silver nanoparticle drastically influences antimicrobial activity providing further support for the proper characterization of leachates evolving from nanoparticulate containing medical devices.

Keywords: Silver nanoparticle, medical device, leachate, Escherichia coli, antimicrobial

1 INTRODUCTION

The antimicrobial properties of silver have been known and exploited for centuries. Silver has many advantages over traditional antibiotics including its low cost, robustness, e.g. ability to maintain effectiveness in a variety of environments, along with its ability to interfere with multiple bacterial cell components and functions [1]. Recently, there has been a growing trend to incorporate silver nanoparticles into various medical devices to inhibit or prevent bacterial growth. Although the antimicrobial effectiveness of silver ions is well accepted there is discrepancy in the field as to the efficacy of silver nanoparticles incorporated within medical devices, e.g. medical catheters [2-5].

The objective of this research is to properly characterize the structure of the silver that is leached from silver nanoparticulate containing medical devices utilizing a variety of analytical techniques. Experiments were also conducted to determine how particle size influences antimicrobial activity.

2 METHODS

2.1 Characterization of Leachates

100 mg of silver nanoparticulate embedded wound dressing was placed inside a 25 mL Erlenmeyer flask. 25 mL of deionized water (DI water) was then added to the flask which was then incubated in a shaker incubator (150 rpm, 37ºC) for 24 hours. Three separate trials were completed and analyzed (n=3). After 24 hours the water (leachate solution) was extracted from the flask and analyzed using DLS (Malvern Zetasizer Nano ZS), NTA (NanoSight NS500), UV-Vis (Molecular Devices Spectra MAX 190), AFM (Asylum Research MFP-3D) and TEM (JEOL JEM-1011). The leachate solution was analyzed directly for all modalities except for NTA where it was necessary to dilute the sample at a ratio of 3:2 (DI water:leachate solution).

2.2 Antimicrobial Experiments

Citrate capped silver nanoparticles of nominal diameters 10, 20, 50, and 100 nm were purchased from NanoComposix, Inc. The experimental protocol to determine antimicrobial activity was based on ASTM standard E2149-10. Briefly, silver nanoparticles were added to a solution of Escherichia coli (E. coli) bacteria and incubated (37ºC) under perturbation (shaker incubator) for 1 hour. After the 1 hour incubation aliquots of the solution were cultured on agar plates and incubated (37ºC) under static conditions for 24 hours. Concentrations of 1, 10, and 100 ng/mL were tested. Silver nitrate was also tested as a ionic control. Antimicrobial activity was quantified by determining the reduction of viable colony forming units relative to a control.

NSTI-Nanotech 2012, www.nsti.org, ISBN 978-1-4665-6274-5 Vol. 1, 2012

3 RESULTS

3.1 Characterization of Leachates

After 24 hours of agitated (150 rpm) incubation at 37ºC the water in all test flasks had turned light brown indicating the presence of silver within the solution. Characterization of the leachate solution using DLS and NTA revealed that the average hydrodynamic radius of the particulates was approximately 60 nm. Although the average hydrodynamic radius of the particles was determined to be approximately 60 nm it was apparent that the leachate included a broad distribution of particles indicated in DLS analysis by a high polydispersity index (PDI), between 0.2-0.4, and a broadened intensity peak. This broad distribution was further supported by the presence of multiple peaks observed in NTA.

The surface plasmon resonance (SPR) peak of the leachate solution was determined to be approximately 410 nm (UV-Vis). This SPR peak was correlated with an average particle size of approximately 20-30 nm based on empirical experimental data obtained from previous studies of silver nanoparticles of known diameter.

Subsequent TEM analysis revealed the presence of particles ranging from approximately 1 to over 100 nm (Figure 1). Particle analysis on the images indicated an average diameter of approximately 20 nm. AFM analysis was also completed on the samples but was not included due to the difficulty in accurately interpreting the results (inability to clearly differentiate individual particles).

The results from the light scattering techniques must be placed in context since DLS is incapable of resolving polydisperse samples and NTA is not able to accurately measure particles below 15 nm. Furthermore, since light scattering intensity is proportional to the radius (r^6) of the particle such anaytical techniques tend to disporportionately weight larger particles skewing the average particle size. As predicted, TEM analysis indicated that the leachate solution did in fact contain a broad distribution of nanoparticles. Furthermore the average particle size determined by TEM closely matched that obtained from UV-Vis data.

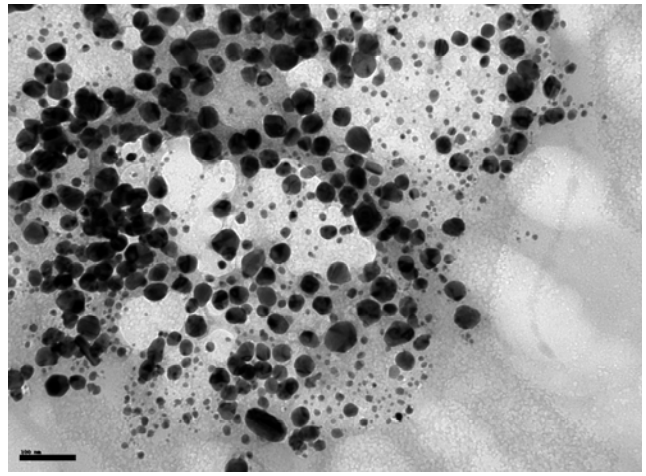
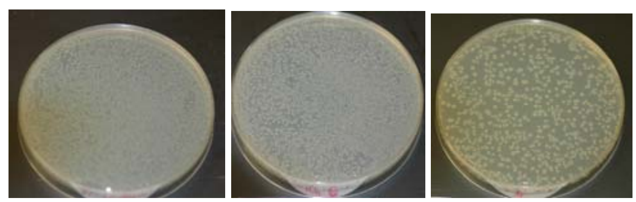


Figure 1: TEM image of the leachate solution obtained after a 24 hour extraction of the silver nanoparticulate containing wound dressing. 100 mg sections of the wound dressing were incubated (37ºC) in 25 mL of DI water under agitation (150 rpm) for 24 hours. 10 µL of the leachate solution was added directly to a TEM grid and imaged accordingly. Scale bar: 100 nm.

3.2 Antimicrobial Experiments

Results of the antimicrobial studies revealed that the size of the silver nanoparticle drastically affects antimicrobial effectivity. Figure 2 below shows agar plates of E. coli grown after introduction of 10 nm and 100 nm silver nanoparticles both at a concentration of 1 ng/mL.



(**A**) (**B**) (**C**)

Figure 2: Images of bacteria grown on nutrient agar after introduction to various sizes of silver nanoparticles (1 ng/mL). (A) Control (no silver nanoparticles). (B) 100 nm silver nanoparticles. (C) 10 nm silver naoparticles.

As Figure 2 shows at 1 ng/mL 100 nm silver nanoparticles showed little reduction in bacterial colonies compared to the control. Conversely 10 nm particles were able to induce a drastic reduction in the number of bacteria suggesting that that the antimicrobial activity of these particles was much higher than the 100 nm particles. This trend was also evident with the 20 and 50 nm particles and at different concentrations of silver (10 and 100 ng/mL).

4 CONCLUSION

The experimental results indicate that silver nanoparticles, differing in both structure and size, are leached from silver nanoparticulate containing wound dressings in simulated use. In any event, the research demonstrates that it is indeed feasible to accurately characterize the size, shape, and morphology of nanoparticulates leached from a medical device if the proper analytical techniques and protocols are employed. Corroborating research utizing E. coli bacteria revealed that the antimicrobial activity of a silver nanoparticle is directly influenced by its size.

The accurate characterization of leachates from nanoparticulate containing medical devices is vital since size and structure can directly impact toxicological profile, biodistribution, and in some cases, as with silver nanoparticles, antimicrobial efficacy [6-8]. Therefore, in order to better assess the safety and efficacy of a medical device containing nanomaterials and thus better protect the public health, it is imperative to develop the proper techniques and protocols necessary to ensure reproducible and accurate characterization of such nanoparticulate leachates.

Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

REFERENCES

[1] V. Kostenko, J. Lyczak, K. Turner and R.J. Martinuzzi, "Impact of Silver-Containing Wound Dressings on Bacterial Biofilm Viability and Susceptibility to Antibiotics during Prolonged Treatement," Antimicrobial Agents and Chemotherapy, 54, 5120-5131, 2010.

[2] M. Rai, A. Yadav and A. Gade, "Silver nanoparticles as a new generation of antimicrobials," Biotechnology Advances, 27, 76-83, 2009.

[3] D. Roe, B. Karandikar, N. Bonn-Savage, B. Gibbins and J.B. Roullet, "Antimicrobial surface functionalization of plastic catheters by silver nanoparticles," Journal of Antimicrobial Chemotherapy, 61, 869-876, 2008.

[4] J.M. Schierholz, L.J. Lucas, A. Rump and G. Pulverer, "Efficacy of silver-coated medical devices," Journal of Hospital Infection, 40, 257-262, 1998.

[5] U. Samuel and J.P. Guggenbichler, "Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter," International Journal of Antimicrobial Agents, 23S1, S75-S78, 2004.

[6] W.H. De Jong, W.I. Hagens, P. Krystek, M.C. Burger, A.J.A.M. Sips and R.E. Geertsma, "Particle size-dependent organ distribution of gold nanoparticles after intravenous administration," Biomaterials, 29, 1912-1919, 2008.

[7] K. Cha, H.W. Hong, Y.G. Choi, M.J. Lee, J.H. Park and H.K. Chae, "Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles," Biotechnology Letters, 30, 1893-1899, 2008.

[8] C. Carlson, S.M. Hussain, A.M. Schrand, L.K. Braydich-Stolle, K.L. Hess, R.L. Jones and J.J. Schlager, "Unique Celular Interaction of Silver Nanoparticles: Size-Dependent Generation of Reactive Oxygen Species," Journal of Physical Chemistry B, 112, 13608-13619, 2008.