Biocidal Activity of Nanocrystalline Silver Powders and Particles

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While the activity of the SMAD powders is lower than that of pure silver nitrate, it has the ability to kill bacteria very effectively and over long periods of time.

1. Introduction

Silver has been known for centuries to have antimicrobial properties. Some of the first recorded uses of silver date back to ancient Greece where silver coins were used as a disinfectant of water and to prevent spoilage of milk.[1] The role of silver for medicinal purposes expanded with time, and today silver is still used in the heath care system in a variety of ways. Silver metal coatings on instruments like catheters have cut down on the occurrence of infections. In other applications, delivery of silver ions is routinely accomplished through the use of either silver sulfadiazine cream or dilute silver nitrate solutions. Silver sulfadiazine cream has been found to be beneficial to treat ocular keratomycosis,[2] as well as being a common topical treatment on burn wounds.[3] Silver nitrate has been used to treat gonococcal and chlamydial ophthalmia neonatorum by using drops in the eyes of newborn children.[4]

As the nanotechnology field is blossoming, the design of multifunctional materials is growing as well. New delivery methods of silver have been devised to maximize the slow release of the antimicrobial metal into its environment. One notable example is polyelectrolyte multilayer films.[5] These films are membrane-like supports layered with alternating positive and negative ions incorporated between polymer sheets. The supports are biocompatible and can provide a sustained release of silver ions from the polymer membrane.[5] Sharma et al. described a procedure for impregnating the surface of alumina with silver metal as a method to produce a disinfecting filter material.[6] On a small scale this Ag/Al_2O_3 material was found to be an excellent disinfectant of drinking water, which they hope can be expanded into use in community water systems. Bioactive glasses are new materials that have the innate ability to bind to both hard and soft biological tissues. The incorporation of silver through silver oxide has added an antimicrobial property as silver is slowly released from the material.[7] These bioactive glasses are now much more resistant to infection and have increased the success rate of implantation in living organisms.

One of the first nanoparticle commercial products put forth by a private company was from Acticoat.[8] This firm incorporated silver nanoparticles into wound dressings. These bandages were found to be magnificent in preventing infection and were also shown to increase the rate of wound healing compared to standard treatments. Despite the large potential for nanoparticulate silver to be a more effective antimicrobial delivery system, few laboratories are doing research involving nanopreparations of silver. One method devised by Balogh and co-workers employed silver ions that were reduced to 1−3 nm particles inside dendrimer macromolecules.[9] The highly branched nature of the dendrimers stabilized the particles and the use of poly(amidoamine) (PAMAM) polymer chains as the branching units make them very compatible with living cells.

Silver nanoparticles with PVP as a capping agent have shown antibacterial properties.[10] Likewise, hydrogel−silver nanocomposites have been investigated, as well as silver-nanocoated fabrics.[11,12] Thus, there have been numerous studies directed toward improving the biocidal properties of silver and for producing more usable products.[13]

Recent efforts in our laboratories have been directed toward preparing the highest surface area nanomaterials in large scale. Such materials possess many reactive surface sites and, thereby, enhanced reactivities. To this end, we devised several preparations of silver nanoparticles each with unique characteristics. These include high-surface-area dry silver powders and nanoparticles with water-soluble capping agents. Other preparations were designed to emphasize exposure of the reactive metal powders to oxidizing agents in an attempt to change the surface chemistry of the particles and improve their activity, as some studies have shown that silver oxide may have an even greater toxic effect on microbes than silver metal.[8] The samples were tested against *Escherichia coli* and *Staphylococcus aureus* to judge the activity against both gram-

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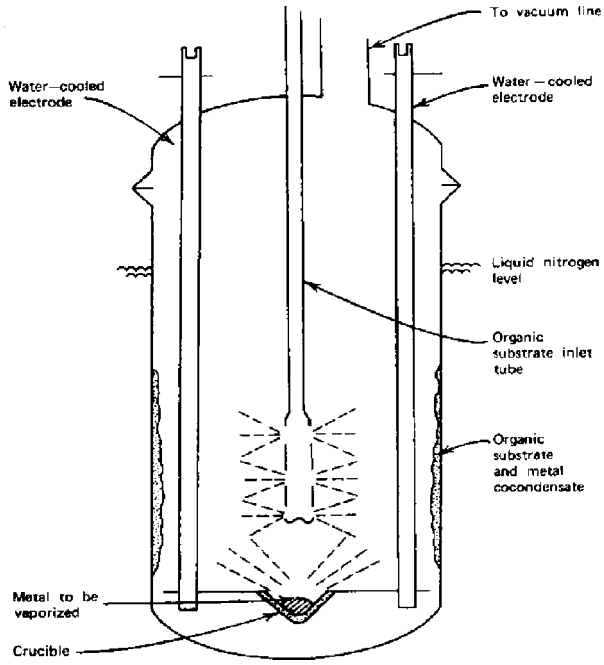

Figure 1. Schematic of SMAD reactor depicting silver metal being heated to evaporation by a tungsten crucible. The metal atoms are then embedded in a solvent matrix on the reactor walls cooled by a liquid nitrogen Dewar surrounding the vessel.

negative and gram-positive bacteria.

2. Experimental Methods

Several silver samples were made to take advantage of different chemical interfaces. All syntheses were prepared by the solvated metal atom dispersion (SMAD) method, which is described in detail in the literature.[14] This is a procedure involving covaporization of large quantities of metal along with a solvent onto a liquid nitrogen cooled surface. When the liquid nitrogen is removed the metal atoms and solvent warms causing aggregation of the metal atoms which is slowed and eventually terminated by interaction with the solvent molecules.

In a typical reaction silver shot (0.3 g) was placed into a tungsten crucible set up in a glass vessel attached to a vacuum line. A schematic of the assembly is shown in Figure 1. Two copper electrodes enter the chamber to provide current to the crucible. A glass tube was placed 4 cm over the crucible with several holes perforated near the end. The glass tube was connected to a Schlenk tube containing a degassed solvent. After the system was sealed and evacuated to vacuum, a large Dewar was placed around the reaction chamber and filled with liquid nitrogen. The liquid nitrogen was used to freeze solvent molecules on the walls of the reactor. Initially, 30 mL of solvent was evaporated and condensed in a uniform film on the glass wall. At this time the temperature of the crucible was brought up to the evaporation point of the silver metal. During reaction, the metal atoms evaporated from the crucible and became embedded into the solvent matrix on the glass walls. Excess solvent as a vapor was brought into the chamber and co-condensed throughout the reaction to trap the reactive metal atoms and physically separate them from one another. When the metal had completely vaporized, a final layer of solvent (30 mL) was applied over the walls of the reactor. Afterward, the Dewar was removed from the system and the reactor was allowed to warm to room temperature. During this time the metal atoms frozen in the solvent matrix interact and agglomerate, forming nanoparticles of various sizes stabilized only by the surrounding solvent.

Millipore water was used in all reactions unless otherwise stated. Silver metal was purchased from Strem chemicals. Organic reagents and solvents and the silver powders used as standards in microbiological testing were purchased from Aldrich. All preparations were viewed in a Phillips 101 transmission electron microscope (TEM) to analyze the particle size. The dry powders were further analyzed in a Bruker powder X-ray spectrophotometer using Cu Kα radiation. Surface area measurements were obtained on a Nova BET nitrogen absorber, and a Hitachi scanning electron microscope (SEM) was used to analyze powder morphology. All commercially purchased powders were subject to the same sampling conditions as our products.

2.1. SMAD Silver Powder. To maximize the surface availability of silver atoms to the environment, the initial nanoparticle products were produced without the presence of capping ligands included in the SMAD reactor. The particles were isolated by evaporation of the solvent, leaving a high surface area metal powder as the final form of the product. The size of the final particles was completely dependent on the ability of the solvent to passivate the metal atoms both during evaporation and after the warm-up process where the metal atoms interact with one another. The solvents used were pentane, toluene, THF, isopropanol, acetone, butanone, and water.

2.2. Ligand-Capped Silver Particles. In an effort to further decrease the size of the silver particles and prevent the sintering and agglomeration of the previously described powders, water-soluble ligands were introduced into the reaction chamber before metal vaporization. This was accomplished by dissolving the ligand in 40 mL of water and freezing the solution at the bottom of the reactor. The synthesis was very similar to that used in the literature with organic ligands and solvents.[14] A large molar excess of ligand to metal atoms (30:1) was used in the reactor. Acetone was used as the codeposition solvent. Acetone was removed from the colloid by evaporation under vacuum, leaving a product of silver particles ligated with a water-soluble ligand and excess ligand in aqueous solution.

Three different ligands were used for the experiments: 3-mercapto-1,2-propanediol, sodium 3-mercapto-1-propanesulfonate, and sodium 5-mercapto-1-tetrazoleacetate. These ligands were selected from a group of ligands previously used with gold aqueous colloids by Stoeva et al.[15] They are listed as having very low toxicity in their respective MSDSs and proved to be the most stable systems with the similar gold metal. Some attempts at digestive ripening were conducted on each silver colloid; however, the particles were not stable to this treatment. This is likely the result of lower binding affinity of the ligands to the silver metal or increased reaction with water at elevated temperatures.

2.3. Air−SMAD. We attempted to modify the surface of the particles using three procedures. In the first method, once the metal atoms are evaporated from the bulk phase in the SMAD reactor, they exist as highly reactive atoms/clusters frozen in a bed of solvent (acetone) cooled by liquid nitrogen. In an effort to oxidize the silver atoms the reactor was opened to the atmosphere at this low-temperature stage. This had a two-fold effect on the reaction. Primarily this allowed the atoms to interact with oxygen in the air. It also has the detrimental effect of warming the product much faster than in a standard experiment. This caused a more rapid agglomeration of the metal atoms and resulted in larger particles.

2.4. KOH−SMAD. In a second effort to modify the silver particles as they first form, potassium hydroxide was introduced in the reactor system. The KOH was dissolved in 60 mL of isopropanol and placed on the bottom of the reactor. When the end of a standard evaporation was over, the solvated metal atoms and clusters melted from the frozen solvent matrix and came into contact with the isopropanol/KOH solution.

Two preparations were attempted from this procedure. Isopropanol was used as the co-evaporation solvent in one reaction. This was done to improve the contact of the metal atoms with the basic solution in the reactor. Unfortunately, isopropanol proved to be a very poor solvent for the stabilization of the metal atoms and quickly led to

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agglomeration of the silver particles before reaching the hydroxide. Acetone was used in a second reaction to circumvent this problem and had sufficient miscibility with the basic solution.

2.5. SMAD−Heat Treatment. A third method of modifying the surface of the particles was to heat the isolated powder in the presence of air. This was done in a muffle furnace at temperatures of 60, 100, 150, 200, and 250 °C. Each sample was heated for 3 h.

2.6.1. Microbiology Testing. Bacteria were grown by standard methods by implanting a class strain of either *E. coli* or *S. aureus* into a flask containing Luria broth overnight in a shaking water bath set to 37 °C.[16] The bacterial solution was diluted to 10^5 CFU (colony forming units)/mL to meet with EPA standards for bacterial reduction in drinking water.[17] Typically 1−2 mL of bacterial solution was pipetted into a 10 mL centrifuge tube with 1 mL of a silver dispersion and 2 mL of Millipore H_2O. After the desired minutes of contact time the tubes were centrifuged at 2500 rpm for 1 min. This was found to be a treatment that could precipitate the silver powder but leave the bacteria suspended in solution. A 100 μL aliquot was removed from the supernatant of the sample, diluted, and plated in triplicate. The bacteria plates were of Tryptic Soy Auger and incubated overnight at 37 °C. Surviving colonies were counted and recorded.

2.6.2. Powder Activity vs Time. To test the activity of the silver powders as a function of time, 2 mL of 10^7 bacteria were introduced into an Erlenmeyer flask that contained 97 mL of autoclaved Millipore water and stirred constantly. To this mixture 1 mL of silver sample (nanoparticle silver−water slurry, soluble silver−ligand solution, or silver nitrate solution) was injected. Aliquots (1 mL) of the dispersion were removed before interaction and after 10 and 30 min of contact time. Once the sample was removed from solution, it was placed in a 1 mL Eppendorf vial and centrifuged at 2500 rpm for 10 s. This was sufficient to bring the silver nanoparticles to the bottom of the flask while leaving the bacteria suspended in solution. From this tube 100 μL of the supernatant was removed, then diluted to suspected concentrations of 3, 30, and 300 CFUs and plated in triplicate.

2.6.3. Presence of Ag Ions. To test for Ag^+ activity the following experiment was performed in five centrifuge tubes initially filled with 2 mL of water. The first sample was left unaltered and acted as a control. The second and third samples were spiked with the supernatant liquid from 150 mg of SMAD powder centrifuged at 2500 rpm for 3 min. The third tube had 1.0 g of NaCl added. The fourth and fifth samples were injected with 1 mL of water containing 15 mg of $AgNO_3$. The fifth sample also had 1.0 g of NaCl added. These samples were stirred vigorously in a vortex shaker. Then, 1 mL of 10^5 CFU/mL of bacteria was added to each. The samples were shaken again and allowed to be in contact for 10 min. The tubes were then centrifuged, and the supernatant was diluted and plated in triplicate.

2.6.4. TEM Preparation for Bacteria Samples. To prepare the specimens *E.* coli cells were first captured in the exponential growth phase. This was found to be after 13 h of incubation time under the laboratory conditions used. These bacteria were then allowed to interact with an excess of silver material needed to kill the pathogens from previous results. These dead bacteria were separated as much as possible from the silver sample by centrifugation. The bacteria were then fixed according to standard procedures described in the literature.[18,19] Briefly, the bacteria were fixed with a 2% solution of glutaraldehyde for 16 h and washed three times by shaking for 5 min with a sodium cacodylate buffer. After every wash the sample was centrifuged at 12 000 rpm for 1 min to separate the bacteria from solution. The sample was then stained with 1% osmium tetroxide for 1.5 h. The sample was washed another three times and then dehydrated by washing with 50%, 60%, 70%, 80%, 90%, 95%, and 100% water/acetone mixtures for 5 min apiece. The sample was then washed with a 1:1 acetone/resin mixture and finally set overnight in 100% resin. The sample tubes were heated in an oven to harden the resin and cut with a microtome diamond knife and viewed in the TEM.

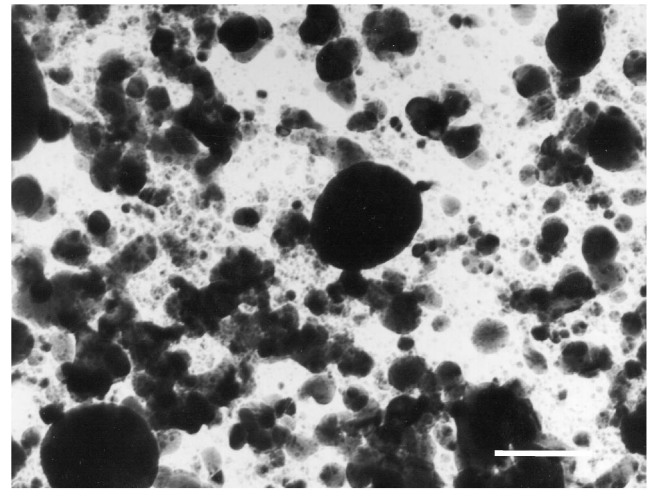

Figure 2. TEM image of as-prepared silver nanoparticles from an acetone slurry prepared by the SMAD method. Scale bar is 100 nm.

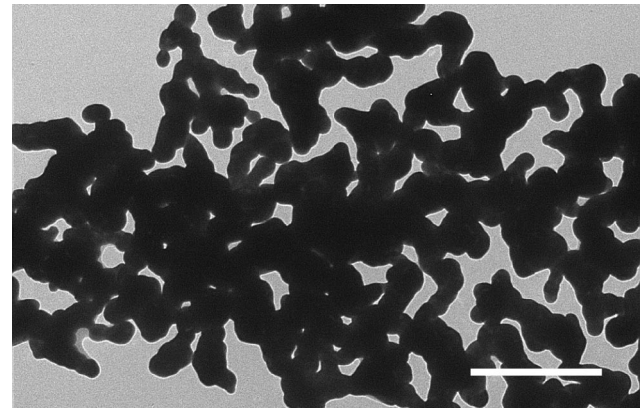

Figure 3. TEM image of silver powder after evaporation of acetone solvent exhibiting severe agglomeration. The scale bar is 500 nm.

3. Results

3.1.1. Particle Size and Morphology. The silver products were first observed by examining TEM pictures of the products. When the SMAD-prepared silver nanoparticles were first synthesized, they began as metal atoms embedded in a frozen organic solvent matrix. As this matrix was warmed from liquid nitrogen to room temperature the organic solvent melted and allowed collisions and interactions between the metal atoms. Once the slurry reached room temperature the product was relatively stable; however, it had agglomerated significantly to produce 3−100 nm particles of silver metal, as can be seen from Figure 2. In order to isolate dry metal nanoparticles, the solvent must be removed from the sample. This further induces aggregation as the solvent that was partially protecting the reactive metal particles from one another is removed. The resulting powder is somewhat porous but has distinct surface features in the nanosized regime. As can be seen from the images in Figure 3, the resulting powder is composed of micrometer-sized chunks of silver. The use of different organic solvents leads to a slight change in particle size in the order of butanone (smallest particles) < acetone < toluene < THF < pentane < isopropanol. Upon sonication when dispersed in water, the SMAD powders broke

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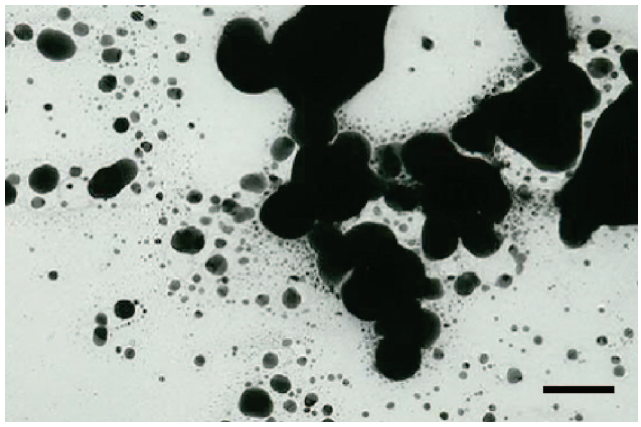

Figure 4. TEM image of SMAD-prepared silver powder after sonication in water. The scale bar is 200 nm.

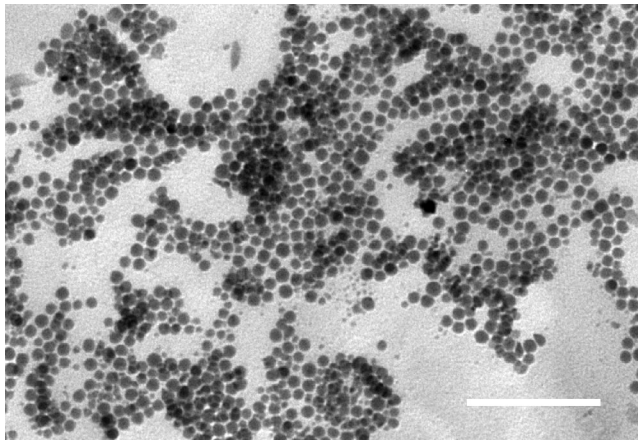

Figure 5. Silver nanoparticles capped with 3-mercapto-1,2-propanediol. The scale bar represents 100 nm.

apart significantly and regained much of their lost surface area shown in Figure 4.

3.1.2. Ligand−SMAD Colloids. In an attempt to prevent aggregation water-soluble ligands were placed in the reactor before metal evaporation. This was done so that when melting of the organic solvent took place they would come into contact with molecules that would bind to the surface of the silver and provide a buffer from further particle−particle collisions. Three separate ligands were used, which had varying success at trapping the forming silver nanoparticles and arresting their further growth. The ability of the ligand to protect the nanoparticles was in the order of 3-mercapto-1,2-propanediol > sodium propane sulfonate > sodium 5-mercapto-tetraazoleacetate. The results are displayed in Figures 5, 6, and 7. It is clear that the smallest, most mondisperse particles were obtained using the 3-mercapto ligand. With the other ligands, larger polydisperse particles were obtained. We believe these results are due to stronger binding of the 3-mercapto ligand, probably due to its polydentate nature. This stronger binding then protected the small particles from aggregating and further growth and amalgamation with nearby particles.

3.1.3. Air−SMAD Powder. The metal atoms in this reaction were exposed to air at the stage when they were just beginning to come into contact with one another during the melting of the organic solvent. Surprisingly, exposure to oxygen did not increase the size of the final particles greatly. However, there was some deviation from the mainly spherical particles. The air−SMAD

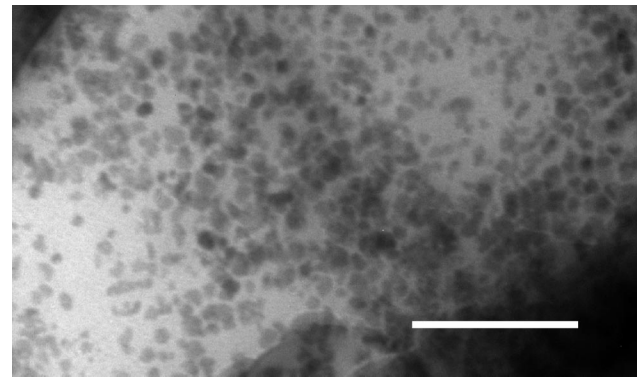

Figure 6. TEM image of silver nanoparticles coated with sodium propane sulfonate. The scale bar represents 100 nm.

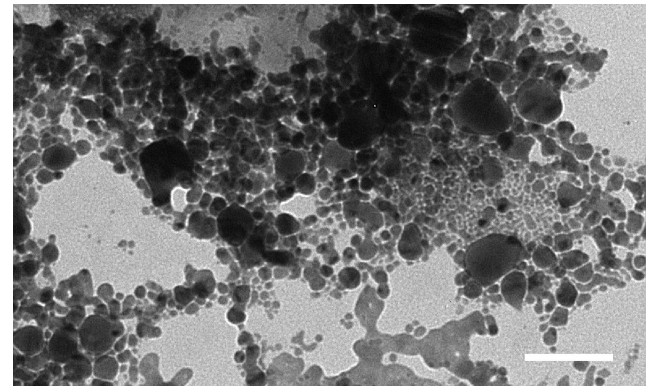

Figure 7. TEM image of silver nanoparticles coated with sodium 5-mercapto-tetraazoleacetate. The scale bar represents 100 nm.

sample displayed a propensity toward fibrous crystallites in addition to spherical agglomerates. TEM and SEM images of this sample are provided as Supporting Information.

3.1.4. KOH−SMAD Powder. The particle size from this preparation was increased markedly from other preparations even when acetone was used as the initial stabilizing agent in the synthesis. Isopropanol proved to be a very poor stabilizing agent, and while it was needed to dissolve the KOH, it had a detrimental effect on the size of the particles. TEM and SEM images of this sample are also provided as Supporting Information.

3.1.5. Commercial Silver Powders. Two silver powders were purchased from Aldrich to be used as standards of reactivity and compared to our samples. TEM and SEM images of the commercial "nanosized" powder do not show features that are smaller than 100 nm. The particle size is roughly 1 μm. The other standard used was a 60 mesh silver powder that appears very large in comparison, seen in Figure 8. The TEM pictures for the commercial samples are very complementary to the SEM pictures. They clearly show particle sizes of over 5 μm and the lack of any interesting surface morphology, increasing the likelihood of low chemical activity. The images for these samples are provided in the Supporting Information.

3.2. Surface Area BET Analysis. As one measure of the activity of the silver powders, the available surface area was measured via nitrogen absorption−desorption isotherms. Before testing all samples were heated under vacuum at 60 °C to remove water vapor. Higher temperatures led to a loss of surface area. A BET calculation gave the following values for the pore size and surface area of the samples (Table 1). BET data were taken on different preparative batches, and the values below represent average results of at least two separately prepared samples.

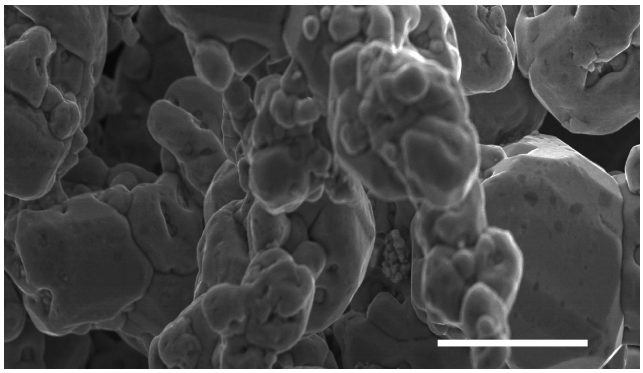

Figure 8. Commercial silver powder. The scale bar is 10 μm.

Table 1. Surface Area of SMAD-Prepared Powders and Commercial Silver Powder

solvent	surface area (m^2/g)
pentane	5 ± 1
toluene	8 ± 2
THF	6 ± 1
Aldrich nanosized	$\ll 1$

Commercially available "nanosized" samples did not have enough surface area to register on the instrument. The SMAD-prepared powders had the highest surface area as expected in lieu of the TEM data. The loss of surface area on going from the dispersed nanoparticles in the original organic solvent to a sintered dry powder is significant. However, in order to remove all traces of organic residues and make them more biocompatible, solvent removal was necessary.

3.3. X-ray Diffraction. To confirm creation of silver nanoparticles and test the purity of the sample, powder XRD spectra were obtained for all the powder samples. The major peaks for silver metal are accepted to be 38.1, 44.2, 64.4, and 77.5 2θ from the literature.[20] The acetone/SMAD powder was used as a representative spectrum here with nearly identical results from powders synthesized from other solvents. As can be seen in Figure 9, all samples display strong peaks at each of the standard values, and line broadening suggests crystallite sizes of 5−10 nm. However, these crystallites must be tightly agglomerated together since TEM and surface areas indicate that *particle* sizes are considerably larger than *crystallite* sizes. No other peaks can be discerned from the background of the diffractogram.

An XRD of the air−SMAD powder displays peaks solely for silver metal. Any silver oxide from the samples is either amorphous or present in very small quantities on the Ag metal surface. A comparison of the XRD spectra of this sample and commercially purchased Ag_2O and AgO are shown in Figure 10.

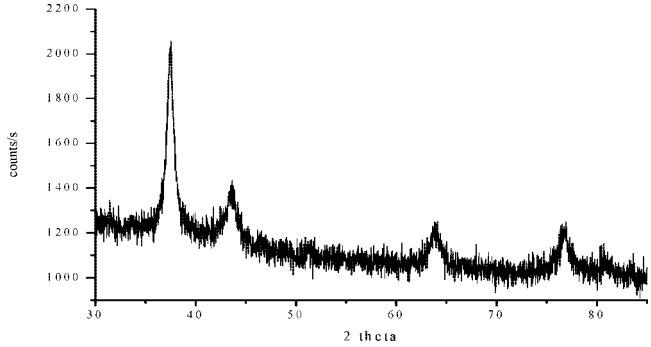

Figure 9. XRD spectrum for SMAD-prepared silver powder synthesized with acetone as the stabilizing solvent.

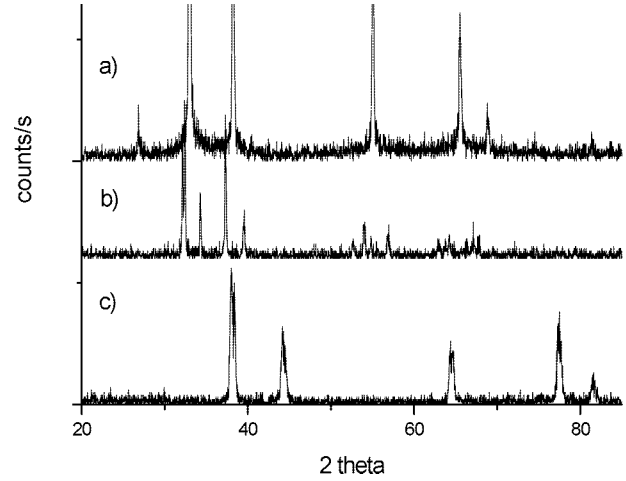

Figure 10. XRD spectra of (a) silver(I) oxide, (b) silver(II) oxide, and (c) air−SMAD powdered sample.

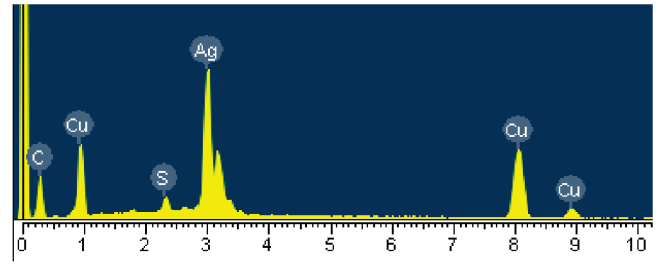

Figure 11. EDX spectra of SMAD-prepared silver powder. The carbon and copper peaks are due to the carbon-coated copper TEM grid used as a support for the powder.

The KOH−SMAD powder had a very similar spectrum to the air−SMAD sample displaying solely Ag metal peaks.

3.4. EDX. In a further test for the presence of oxygen incorporated into the silver nanocrystals EDX spectroscopy was conducted on the samples during SEM imaging. The SMAD-prepared silver sample (from acetone solvent, Figure 3) displayed no elemental oxygen peak; however, a small percentage of sulfur was observed in the sample, as seen in Figure 11. We believe this is due to atmospheric sulfur containing molecules being scavenged by the highly active surface of the powder. The lack of this sulfur peak in commercial samples indicates that the SMAD samples are considerably more reactive and sensitive. Despite the efforts to oxidize the surface of the powders in the air−SMAD and KOH−SMAD no traces of oxygen were found for these samples by EDX spectroscopy. This suggests oxygen concentrations of less than 1%. Apparently, a thin oxide coating was formed, which did provide a more potent biocidal nature by facilitating more ready access to surface silver ions. However, under the short times involved, the oxide coating protected against deeper oxidation.

4. Microbiology Testing

The rise of resistant strains of bacteria has introduced the need for a more general disinfectant that can be active against all types of single-celled pathogens. A class strain of *E. coli* represented gram-negative bacteria, while *S. aureus* was used as a gram-positive bacterium. Both bacterial strains are fairly robust and resist disinfection. The fundamental difference between gram-negative and gram-positive bacteria is the nature of the cell wall.

(20) JCPDS International Centre for Diffraction Data. 1998 v. *201*.

Table 2. Surviving Bacteria Colonies vs mg of Silver Product, Based on 10 min of Contact between Silver and Bacteria in Water[a]

sample	amount of silver sample (mg)	*E. coli* CFUs	*S. aureus* CFUs
no silver (control)	–	100 000	100 000
Powders			
Aldrich 60 mesh	15	79	53
Aldrich 60 mesh	1.5	0	72
Aldrich nano-sized powder	15	0	31
Aldrich nano-sized powder	1.5	0	84
$AgNO_3$	1.5	0	0
$AgNO_3$	0.15	0	0
$AgNO_3$	0.015	0	0
SMAD	15	0	0
SMAD	1.5	–	1
SMAD	0.15	–	53
air–SMAD	0.75	1	2
air–SMAD	0.15	1	0
KOH–SMAD–isopropanol	15	–	0
KOH–SMAD–isopropanol	1.5	7	153
Ligand-Capped Particles			
3-mercapto-1,2,propanediol	15	–	5000
3-mercapto-propanesulfonate	15	175	11
5-mercapto-tetrazolacetate	15	84	5000
pure ligand (no silver)	0.1 mL	100 000	100 000
ligand plus SMAD powder	15	10 000	1000
ligand plus $AgNO_3$	1.5	500	100

[a] All values are an average of CFUs taken in triplicate and were reproducible within roughly 5%. All samples were dispersed in water, except $AgNO_3$, which was dissolved in water.

Gram-positive bacteria have an additional thick peptidoglycan layer that appears purple when treated with crystal violet during the Gram stain. Gram-negative bacteria have an additional outer membrane but a much thinner peptidoglycan layer between its inner and outer membranes. The drastic difference in the nature of the cell boundary in contact with the environment often dictates the basic response of the cell to environmental forces such as antimicrobial agents.

4.1. Dry Silver Powder Testing. Dry powders were found to be initially difficult to disperse in an aqueous environment. This is likely due to pockets of air in the pores of the powders and an inability to break the surface tension of the water. Low-power sonication of the sample for 5 min along with vigorous shaking of the sample sufficiently disperses the sample in water for testing. Typically, 150 mg of silver powder was dispersed in 10 mL of Millipore water. Aliquots of this mixture were either tested directly or diluted to appropriate concentrations. The silver powders were removed from the bacterial solution using careful centrifugation of 2500 rpm for 3 min.

To remove Ag^+ from $AgNO_3$ samples, an excess of NaCl was added to the sample and resulted in a white precipitate. The salt same concentration was added to bacteria alone and was not found to have a detrimental effect on the cells. The same procedure was also used to precipitate the ligand–SMAD samples. The increase in ionic concentration of the solution caused the colloid to destabilize and flocculate which was then easily separated by centrifugation.

The results of these tests are displayed in Table 2. For each sample the concentration of silver in mg needed to completely kill the bacterial cells is typically an order of 10 greater than that displayed if there are surviving colonies. All samples were tested against 10^5 CFUs with 10 min of contact time, and shown are the number of survivors.

It was possible that the bacteria were merely sticking to the heavy silver powder and being precipitated in the centrifugation showing as a false positive. To test this, silver precipitate was taken from a sample after interaction with bacteria and smeared wholly on an agar plate and incubated. Still no growth of any bacteria was seen showing that the cells were in fact no longer able to reproduce if not destroyed (Table 3).

4.2. Active Silver Species. At this time the active agent interacting with the bacteria was unknown. The possible candidates are contact with the silver powder (presumably silver(0) atoms on the surface of the particle), Ag^+ dissolved into solution from the silver powder, or Ag(0) atoms eroded into solution from the silver powders.

If Ag^+ is the only active agent in the SMAD powdered sample, little to no activity should be found when NaCl is present. This was found to be the case, and a white precipitate was found in the SMAD supernatant sample, as well as with the $AgNO_3$ experiment. When either sample was introduced to a sample tube without NaCl, the bacteria were killed 100% (Table 4).

4.3. Nanoparticle and Bacteria Interaction–TEM Images. In a further attempt to understand the interaction of the silver nanopowders with bacteria, selected bacteria samples were stained and fixed after interaction with the silver products.

Our results for bacteria in contact with silver nitrate are very similar to the literature.[17,18] It appears the silver ions make it into the cell where they are reduced as the cell attempts to remove the toxin from the cell interior, as seen in Figure 12b. This most likely occurs through a reductase mechanism for cell self-defense.[21] The SMAD powder has a very similar effect on the bacteria. It appears in this case that there are silver nanoparticle deposits within the cell but are much smaller than the starting nanoparticles, as shown in Figure 12c.

The interaction of the air–SMAD sample with bacteria was even more effective; Figure 12d,e. Large portions of the cellular contents seemed to have been completely eaten away. The cell appears to have little defense against this type of attack. It was hoped that images from either silver(I) or silver(II) oxide would be very similar, but this was not found. Silver(II) oxide appears to be able to infiltrate the cell much more effectively than silver(I) oxide, as deduced from the images. Despite the visual differences, per gram each form of oxide displayed very similar antimicrobial activity. The damage to the cell from the two silver oxide samples is reminiscent of samples exposed to the air–SMAD powder, although some differences can be seen. TEM images are provided in the Supporting Information.

The interaction of 3-mercapto-1,2-propanediol-capped silver particles with bacteria was also observed in the TEM in an attempt to understand their low reactivity. The ligand–SMAD sample was seen to be incorporated into some of the bacterial cells but their small size is retained, suggesting that the silver core is still encapsulated in the initial ligand.

5. Discussion

5.1. SMAD Powders. The SMAD powders outperformed their commercial counterparts in bacterial mortality by more than an order of magnitude. This new material has a higher surface area and overall much smaller surface features than commercial samples. This translates into more active sites for the material. These features provide a quicker and more thorough means for the silver powder to erode silver ions into solution that interact and destroy the bacterial cells. The concentration of silver ions eroded into solution was measured by conductivity and were found to emit as much as 50 times as much silver as a solid silver pellet. The silver ions are then available to infiltrate the inside

(21) Rugh, C. L.; Wilde, H. D.; Stack, N. M.; Thompson, D. M.; Summers, A. O.; Meagher, R. B. *Proc. Natl. Acad. Sci.* **1996**, *93*, 3182–3187.

Table 3. Effect of Silver Samples on Bacteria with Time

sample	amount of silver sample (mg)	initial bacteria	surviving bacteria 5 min	surviving bacteria 30 min
SMAD	1.5	50 000 000	0	0
	0.15	5000	1840	0
	0.015	5000	3600	670
Aldrich nano-sized powder	1.5	12 400 000	1 000 000	32 000
Aldrich 60 mesh powder	1.5	11 000 000	3 000 000	28 000
silver pellet	1.5	303 000	279 000	94 000
air−SMAD	0.15	450 000	0	0
	0.015	5 000 000	4000	0
	0.0015	5000	40	0
KOH−SMAD	0.15	270 000	200 000	14 000
Ag_2O (commercial)	0.15	46 000	0	0
Ag_2O (commercial)	0.0015	10 000	0	0
AgO (commercial)	0.15	46 000	0	0
AgO (commercial)	0.0015	19 000	200	200
3-mercapto-propane sulfonate	1.5	256 000	233 000	133 000
5-mercapto-1-tetraazolacetate	1.5	256 000	105 000	0
3-mercapto-1,2,-propanediol	1.5	620 000	145 000	28 000
$AgNO_3$	0.0015	22 000	15 000	500

Table 4. Surviving Bacteria in the Presence of Silver Samples, with and without NaCl

test tube contents with bacteria	surviving bacteria
water (control)	1000
SMAD supernatant	0
SMAD supernatant + NaCl	500
$AgNO_3$	0
$AgNO_3$ + NaCl	500

of the *E. coli* cells. There some of the silver is reduced by the cell, and this chemistry denatures the proteins preventing cellular function, or reproduction in the case of DNA.

5.2. Ligand−SMAD Colloids. The ligand-passivated nanoparticle synthesis was very successful. Silver nanoparticles of 6 nm were prepared and were highly dispersible in aqueous solution. The increase in surface area of the particles and improved solubility were anticipated to improve the toxic effect of the nanoparticle on microbes. However, this was not the case. Very poor biocidal activity was observed. Apparently, the ligand coating prevented the erosion of silver ions into solution (which provides biocidal action). However, the ligand-coated nanoparticles did invade the cell. In fact, the presence of the ligand also had a detrimental effect on SMAD particles and on $AgNO_3$ biocidal activities, as shown in Table 2. Indeed, all three of the ligands exhibited these detrimental effects, in the order of 5-mercapto-1-tetraazolacetate > 3-mercapto-1,2-propanediol> 3-mercapto-propane sulfonate.

5.3. Air−SMAD Powder. The air−SMAD powders were created in an attempt to chemically modify the surface of the silver metal to an oxide. Upon doing this, the particle size was sacrificed only very minimally but the effect on the bacteria, which is evidenced in Figure 12, was dramatic. The huge holes in the *E. coli* cells after interaction and the decrease in concentration of silver needed to destroy the bacteria cells were striking. We believe this is due to high surface area and the presence of a thin oxide coating of the silver, and this thin oxide coating allowed even more silver ion to be eroded into solution.

5.4. Heat-Treated Powders. This powder was prepared with the intention of at least partially oxidizing the surface of the silver powders. High-surface-area SMAD-prepared silver powder was heated in the presence of oxygen. It was hoped the nanosized surface morphology would be maintained yet change the chemical activity. SMAD powder was heated at temperatures of 60, 100, 150, 200, and 250 °C for 3 h. Surprisingly, these powders acquired only a thin oxide coating and displayed only a slight increase in antimicrobial activity over the SMAD-prepared powder and showed negligible differences in physical properties.

5.5. KOH−SMAD Powder. This preparation was an attempt to modify the silver powders with liquid-phase chemistry in the reactor. Adding the basic agent to the reactor allows interaction with silver when the metal atoms are still very reactive immediately after melting. It was hoped that a much larger portion of the silver powder could be surface-modified in this manner. To encourage immediate reaction, isopropanol was used as the

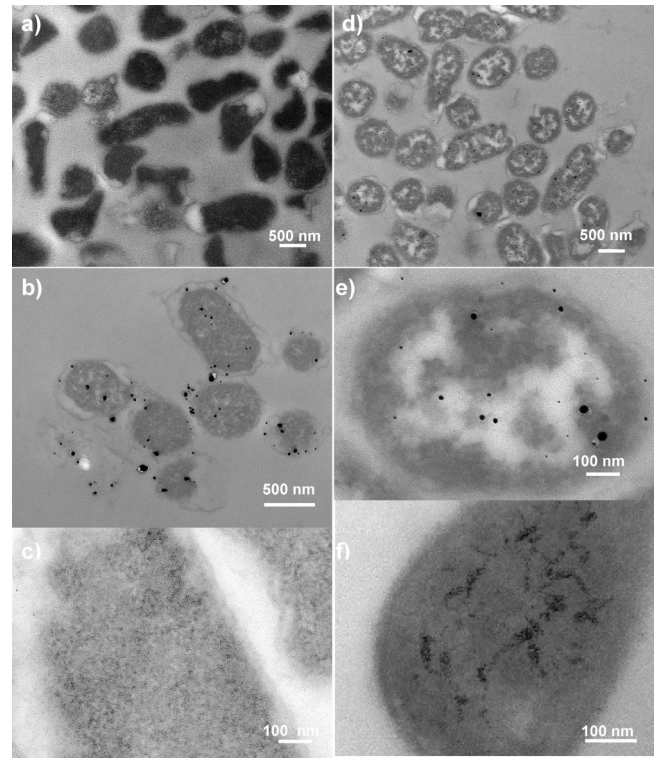

Figure 12. Interactions between silver nanoparticles and bacteria. (a) Healthy bacteria. (b) $AgNO_3$ interaction with bacteria; large deposits of reduced Ag ion (silver nanoparticles) can be seen and the cells were overwhelmed and destroyed. (c) SMAD powder interaction with bacteria; numerous small deposits of reduced Ag ion (silver nanoparticles) dispersed throughout the cell; the cell is destroyed. (d) Low-magnification air−SMAD powder interaction with bacteria. (e) Air−SMAD powder interaction with bacteria; severe damage to cell contents. (f) Ligand−SMAD colloid interaction with bacteria; ligated silver nanoparticles have infiltrated the cell, yet the cell lives.

initial solvent in the SMAD synthesis because of its ability to dissolve KOH so well. An initial amount of KOH was dissolved with the isopropanol on the bottom of the reaction to interact right after melt. Isopropanol proved to be a much poorer stabilizing agent for the initial silver atoms and led to rapid agglomeration and a much poorer particle size. Even when this reaction was attempted with acetone as the stabilizing solvent, the particle size was not much improved and this product did not perform better than standard SMAD-prepared samples.

6. Conclusions

While the activity of the SMAD powders is lower than that of pure silver nitrate, it has the ability to kill bacteria very effectively and over long periods of time. The powder can be dispersed in water as a slurry. Our SMAD-prepared powder was able to outperform the commercially available powders by a large margin. Further studies showed that an air-treated SMAD sample possessed remarkable killing power against bacteria and that it is a promising antibacterial product. We are currently investigating the surface chemistry of this sample, which exhibits this improved biocidal effect.

Overall, the following new and interesting observations have been made. (1) Very small, irregular surfaces are necessary for high biocidal activity. (2) Silver ions are the actual biocidal species, and these are provided by active surfaces of silver nanoparticles and silver oxide surfaces on these nanoparticles. (3) The silver ions enter the bacterial cells, are reduced, and agglomerate to form (re-form) silver metal nanoparticles inside the cell. (4) Water-soluble ligands that cap the silver nanoparticles greatly diminish biocidal activity. Indeed, these ligand-capped nanomaterials are of the highest quality with regard to size and monodispersity. Although these ligated particles readily invade the bacterial cell, the cell is not killed. (5) The most active samples for destroying gram-positive and gram-negative bacteria are nanocrystalline (5−10 nm crystallites), but these crystals are agglomerated into micrometer-sized particles (Figure 3). Moreover, this high biocidal activity can be further enhanced by partial surface oxidation.

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Supporting Information Available: Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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