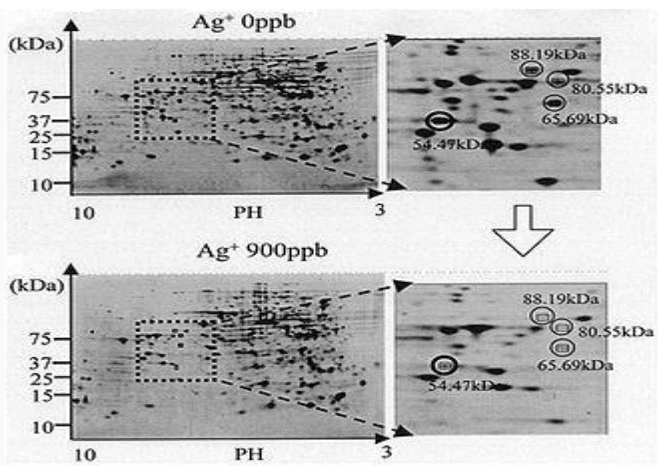
Introduction

The antimicrobial properties of silver have been known to cultures all around the world for many centuries. The Phonecians stored water and other liquids in silver coated bottles to discourage contamination by microbes (Wikipedia: Silver). Silver dollars used to be put into milk bottles to keep milk fresh, and water tanks of ships and airplanes that are "silvered" are able to render water potable for months (Saltlakemetals.com). In 1884 it became a common practice to administer drops of aqueous silver nitrate to newborn's eyes to prevent the transmission of *Neisseria gonorrhoeae* from infected mothers to children during childbirth (Silvestry-Rodriguez *et al.*, 2007).

In 1893, the antibacterial effectiveness of various metals were noted and this property was named the oligodynamic effect. It was later found that out of all the metals with antimicrobial properties, silver has the most effective antibacterial action and the least toxicity to animal cells (Guggenbichler *et al.*, 1999). Silver became commonly used in medical treatments, such as those of wounded soldiers in World War I, to deter microbial growth (Saltlakemetals.com).

Once antibiotics were discovered, the use of silver as a bactericidal agent decreased. However, with the discovery of antibiotics came the emergence of antibiotic-resistant strains such as CA-MRSA and HA-MRSA, the flesh-eating bacteria. Due to increasing antibiotic resistance, there has recently been a renewed interest in using silver as an antibacterial agent. The availability of new laboratory technologies such as radioactive isotopes and electron microscopy has greatly enabled us to investigate the antibacterial mechanism of silver in recent years (Fox and Modak, 1974; Feng *et al.*, 2000).

Mechanism of action



Description	M_r	Accession no. and database	Probability[a]
30S ribosomal subunit protein S2	54.47	26246115; NCBI	69
Succinyl-CoA synthetase	65.69	6980733; NCBI	64
Maltose transporter	88.19	37927753; NCBI	89
Hypothetical protein	80.55	26248233; NCBI	47
Fructose-bisphosphate adolase	36.33	P71295; SwissProt	121

[a] Probabilities were based on the Mowse scoring algorithm.

Figure 2. Protein expression is downregulated when bacteria are exposed to Ag^+. Shown are 2-D polyacrylamide gel electrophoresis protein patterns obtained from suspensions of *E. coli* after reactions with 0 ppb and 900 ppb Ag^+ solutions for 3 h. Some spots indicate decreased amounts of protein expression after reaction with a 900 ppb solution, compared to results after reaction with a 0 ppb solution. Four spots with a decrease to less than one-third of the original amount are marked with open circles. The circled spots were cut out from the gel and analyzed with MALDI-TOF mass spectrometry. The probable identity of the proteins represented by the circled spots were determined by the NCBI and Swiss-

Protdatabases. Fructose-bisphosphateadolase was identified as another possible downregulated protein through this same process (not shown on this gel). The identity of the protein marked "Hypothetical protein" could not be determined through protein databases (Yamanaka *et al.*, 2005).

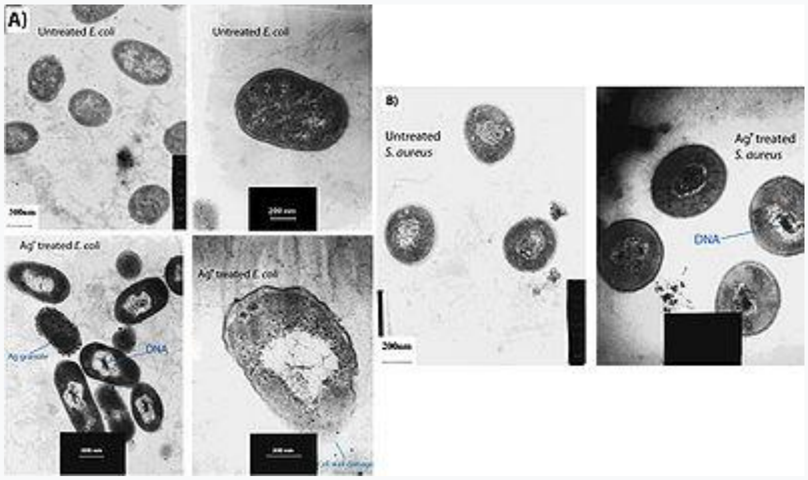


Figure 3. Treatment of cells with Ag^+ results in DNA condensation, cell wall damage, and silver granule formation. (A) *E. coli* and (B) *S. aureus* cells with and without Ag^+ treatment were observed with transmission electron microscopy (Feng *et al.*, 2000).

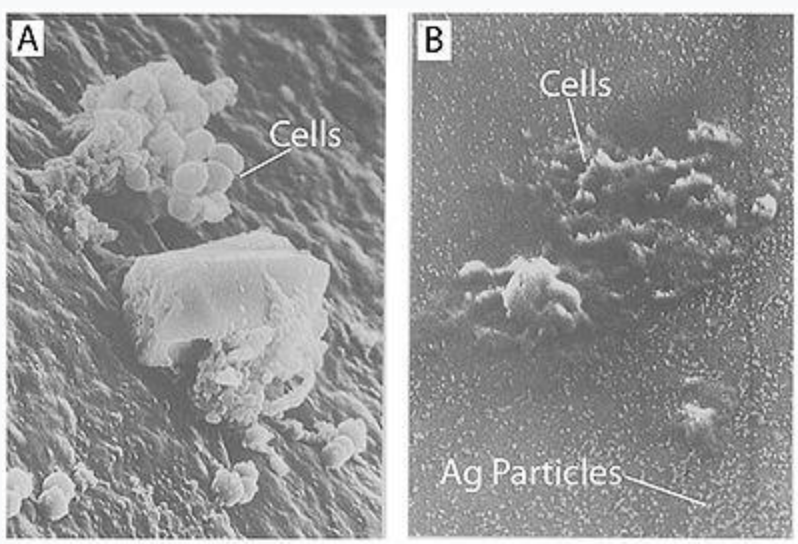


Figure 4. Treatment with silver leads to dehydration of microbial cells. A) *Staphylococcus aureus* without silver treatment as found on a catheter. B) *Staphylococcus aureus* on a silver-containing material with microdispersed silver particles throughout the matrix. Both images were captured using scanning electron microscopy. Notice the shrunken appearance of the cells (Guggenbichler *et al.*, 1999).

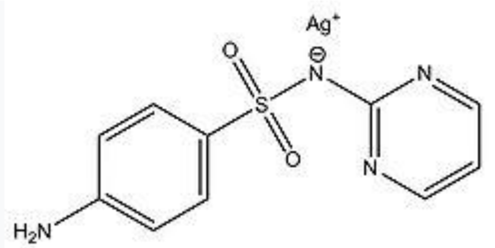


Figure 5.Structure of silver sulfadiazine.

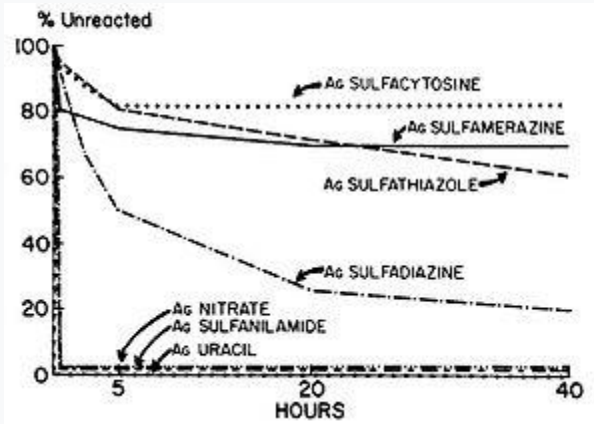


Figure 6.Percent completion of reactions of various silver compounds with human serum. Human serum was incubated with 10 μmol of each silver salt. At the intervals designated, portions were removed and centrifuged, and the clear supernatants were analyzed to determine the amount of unreacted silver compound. The amount of compound used was taken on 100% (Fox and Modak, 1974).

Protein inactivation

Although the antimicrobial properties of silver have been known for centuries, we have only recently begun to understand the mechanisms by which silver inhibits bacterial growth. It is thought that silver atoms bind to thiol groups (-SH) in enzymes and subsequently cause the deactivation of enzymes. Silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that are involved in transmembrane energy generation and ion transport (Klueh *et al.*, 2000). It is also believed that silver can take part in catalytic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R). Silver does this by catalyzing the reaction between oxygen molecules in the cell and hydrogen atoms of thiol groups: water is released as a product and two thiol groups become covalently bonded to one another through a disulfide bond (Davies and Etris, 1997). The silver-catalyzed formation of disulfide bonds could possibly change the shape of cellular enzymes and subsequently affect their function.

The silver-catalyzed formation of disulfide bonds can lead to changes in protein structure and the inactivation of key enzymes, such as those needed for cellular respiration (Davies and Etris, 1997). 30S ribosomal subunit protein, succinyl coenzyme A synthetase, maltose transporter (MalK), and fructose bisphosphateadolase were identified with high probability as proteins with decreased expression once cells are treated with a 900 ppb Ag^+ solution (Yamanaka *et al.*, 2005; Figure 2). It is hypothesized that silver ions bind to the 30S ribosomal subunit, deactivating the ribosome complex and preventing translation of proteins (Yamanaka *et al.*, 2005). The proteins that were found to be downregulated upon treatment with Ag^+ serve important functions to the cell: succinyl-coenzyme A synthetase, an enzyme involved in the TCA cycle, catalyzes the conversion of succinyl-CoA to succinate while phosphorylating ADP to produce ATP (Slonczewski and Foster, 2009); fructose bisphosphateadolase is an enzyme involved in glycolysis that catalyzes the breakdown of fructose-1,6-bisphosphate into glyceraldehydes 3-phosphate and dihydroxyacetone phosphate (Slonczewski and Foster, 2009); MalK is a cytoplasmic membrane-associated protein involved in the transport of maltose (Bavoil *et al.*, 1980). In one way or another, all of these proteins play a role in energy and ATP production for the cell, so the decreased expression of any one of these proteins could lead to cell death (Yamanaka *et al.*, 2005).

Ionized silver

In order for silver to have any antimicrobial properties, it must be in its ionized form (Lok *et al.*, 2007; Rai *et al.*, 2009). Silver in its non-ionized form is inert (Guggenbichler *et al.*, 1999), but contact with moisture leads to the release of silver ions (Radheshkumar and Munstedt, 2005). Thus, all forms of silver or silver containing compounds with observed antimicrobial properties are in one way or another sources of silver ions (Ag^+); these silver ions may be incorporated into the substance and released slowly with time as with silver sulfadiazine, or the silver ions can come from ionizing the surface of a solid piece of silver as with silver nanoparticles.

Observed effects of silver exposure

Feng *et al.* (2000) conducted a study to observe the effects of silver ions on gram-positive and gram-negative bacteria, namely *Staphylococcus aureus* and *Escherichia coli*. They treated cells with $AgNO_3$, which is a source of Ag^+ in aqueous environments, and looked at the structural and morphological effects of these silver ions on the cells. The cells were exposed to $AgNO_3$ for 4-12 hours before being prepared for microscopy. The cell were then fixed and sliced with an ultramicrotome to produce ultrathin sections for transmission electron microscopy (TEM). They observed that cells exposed to the Ag^+ ions seemed to have activated a stress response that led to the condensation of DNA in the center of the cell. They also observed cell membrane detachment from the cell wall, cell wall damage, and electron dense granules outside and, in some instances, inside the cell (Figure 3). It was proposed that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell (Feng *et al.*, 2000), however condensation of DNA could also prevent cell replication by preventing the DNA from being accessed by transcriptional enzymes such as DNA polymerase. The electron dense granules that formed inside and outside the cell were extracted and subjected to X-ray microanalysis to determine their composition. It was discovered that the granules were in part composed of silver and sulfur. This finding supports the idea that silver inactivates proteins by binding to sulfur-containing compounds (Klueh *et al.*, 2000). It was also observed that when treated with Ag^+, *E. coli*, a gram-negative bacterium, sustained more structural damages than the gram-positive *S. aureus* (Feng *et al.*, 2000).

It has also been shown that treating cells with silver leads to cell shrinkage and dehydration (Figure 4) (Guggenbichler *et al.*, 1999). The TEM images from Feng *et al.* (2000) (Figure 4) show that cells that sustained extensive damage eventually ended up with cell wall and cell membrane damage. Damage to the cell membrane could lead to the leaking of cytoplasm from the cell, which would result in dehydrated and shrunken cells as shown by the SEM images from Guggenbichler *et al.* (1999).

Attack on Gram-positive vs. Gram-negative

There are two explanations as to why gram-positive bacteria are less susceptible to Ag^+ than gram-negative bacteria. The first involves the charge of peptidoglycan molecules in the bacterial cell wall. Gram-positive bacteria have more peptidoglycan than gram-negative bacteria because of their thicker cell walls, and because peptidoglycan is negatively charged and silver ions are positively charged, more silver may get trapped by peptidoglycan in gram-positive bacteria than in gram-negative bacteria (Kawahara *et al.*, 2000). The decreased susceptibility of gram-positive bacteria can also simply be explained by the fact that the cell wall of gram-positive bacteria is thicker than that of gram-negative bacteria.

Silver nanoparticles

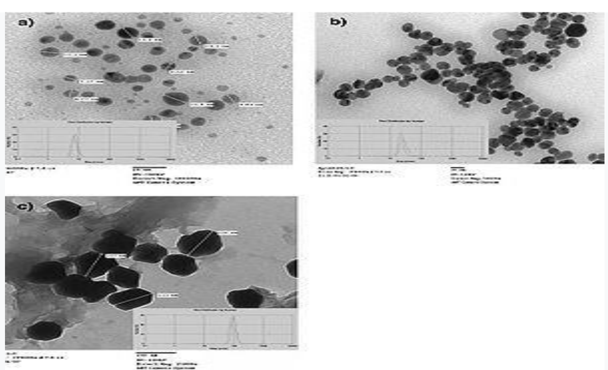


Figure 9. Different sizes of silver nanoparticles. 7-nm (a), 29-nm (b), and 89-nm (c) diameter nanoparticles were able to be synthesized using silver nitrate and gallic acid and by varying pH or UV light exposure. Images were captured using transmission electron microscopy (Martinez-Castanon *et al.*, 2008).

Silver nanoparticles have been heavily studied as antimicrobial materials. Their simple synthesis (Figure 8) and highly effective observed antibacterial activity make them a very attractive form of silver administration.

Nanoparticle size

Martinez-Castanon *et al.* (2008) studied the effect of nanoparticle size on antibacterial effectiveness. To begin, nanoparticles with 7-nm, 29-nm, and 89-nm diameters were synthesized. The syntheses of the three different sized nanoparticles all used silver nitrate as a supply of silver ions and gallic acid as a reducing and stabilizing agent. The different sizes were produced by simply either altering the pH or irradiating the solutions with UV light. Transmission electron microscopy was used in the characterization of the nanoparticles to determine their average sizes (Figure 9).

Once the sizes of the nanoparticles were confirmed, MIC assays were performed on *E. coli* and *S. aureus* using the synthesized nanoparticles. The results of the MIC assays showed that smaller nanoparticles have more of an inhibitory effect than larger nanoparticles and that *S. aureus* is more resistant to silver nanoparticles than *E. coli*. The result that *S. aureus* is more resistant than *E. coli* to silver agrees with findings obtained by Kawahara *et al.* (2000).

The increased antimicrobial activity of the smaller nanoparticles could be due to the fact that smaller particles have an easier time getting through the cell membrane and cell wall and that relative to larger nanoparticles, smaller particles have a greater surface area to volume ratio (Martinez-Castanon *et al.*, 2008). The greater surface area to volume ratio of smaller nanoparticles means that per unit mass of silver, the smaller nanoparticles have more silver atoms in contact with the solution than do larger nanoparticles. For smaller nanoparticles, this means that more of the silver atoms contained in the nanoparticle are able to take part in cell destruction processes. If only the outer layer of silver atoms of a silver nanoparticle are able to be ionized to silver ions, then a few large nanoparticles should produce less silver ions than a lot of small nanoparticles. Because silver ions are what impart antibacterial properties to a given silver-containing material, it makes sense that smaller silver nanoparticles have more antimicrobial effectiveness than larger silver nanoparticles.

Nanoparticle shape

Sample	Minimum inhibition concentration (μg/mL)	
	Bacteria	
	E. coli	*S. aureus*
7-nm silver nanoparticles	6.25	7.5
29-nm silver nanoparticles	13.02	16.67
89-nm silver nanoparticles	11.79	33.71
Gallic acid	–[a]	–[a]

[a] No antibacterial activity was found with the concentrations used in this work

Figure 10.Minimum inhibitory concentrations of Ag nanoparticles. Smaller nanoparticles have greater antibacterial activity. The concentration difference between 29-nm and 89-nm nanoparticles for *E. coli* was not significant (Martinez-Castanon *et al.*, 2008).

In addition to size, nanoparticle shape also plays a role in antibacterial activity. Pal *et al.* (2007) synthesized spherical, rod-shaped, and triangular silver nanoparticles and tested each of them for antimicrobial activity using *E. coli*. *E. coli* was streaked onto agar plates, and 1 μg, 12.5 μg, 50 μg, or 100 μg of each of the three types of synthesized silver nanoparticles as well as $AgNO_3$ were added to the streaked plates. The number of colonies that formed for each of the conditions was counted and graphs relating the number of formed colonies and silver concentration for each of the four types of silver tested were constructed (Figure 11). It was determined that the order of most antibacterial to least antibacterial of the four silver-containing compounds was triangular, spherical, rod-shaped, and $AgNO_3$. This order of antibacterial activity is explained by the different types of facets on the nanoparticles. The triangular nanoparticles had more active facets (electron dense facets) than did the spherical nanoparticles. The spherical nanoparticles, which weren't perfectly spherical, had more active facets than the rod-shaped nanoparticles (Pal *et al.*, 2007). Thus, shape does have an effect on antibacterial activity of silver nanoparticles and nanoparticles with more active facets have more antibacterial activity.

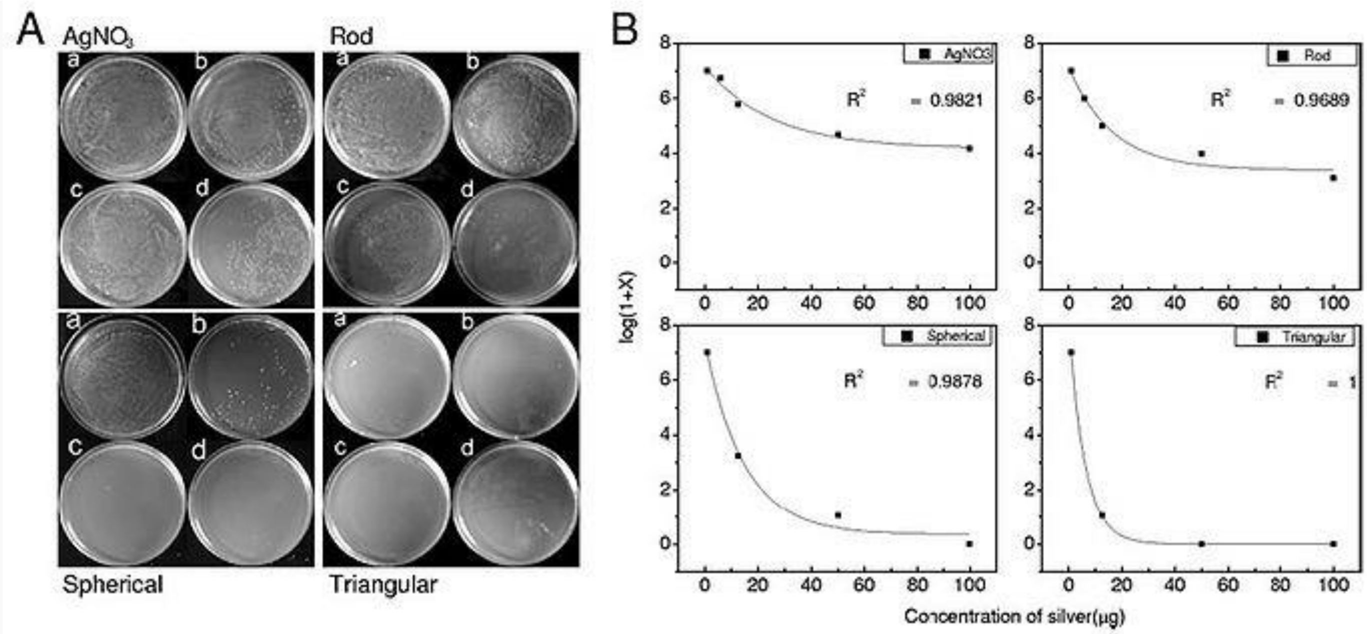


Figure 11. Antibacterial ability of silver nanoparticles is related to nanoparticle shape. A) Agar plates were streaked with 107 colony forming units/ml of *E. coli* and incubated with silver in different forms (either Ag^+ ($AgNO_3$), silver rod nanoparticles, spherical nanoparticles, or triangular nanoparticles). Each type of silver was administered in four quantities, 1 μg (a), 12.5 μg (b), 50 μg (c), and 100 μg (d). B) log(1 + # of colonies formed) was plotted against concentration of silver (μg) for each of the types of silver administered. R2 values indicate the fit of the plot to a first-order exponential decay curve (Pal *et al.*, 2007).

Figure 12. Colloidal silver, commonly sold as a health supplement, can cause argyria, an irreversible condition where the skin discolors to a bluish-gray tone (www.drugstore.com).

Current uses

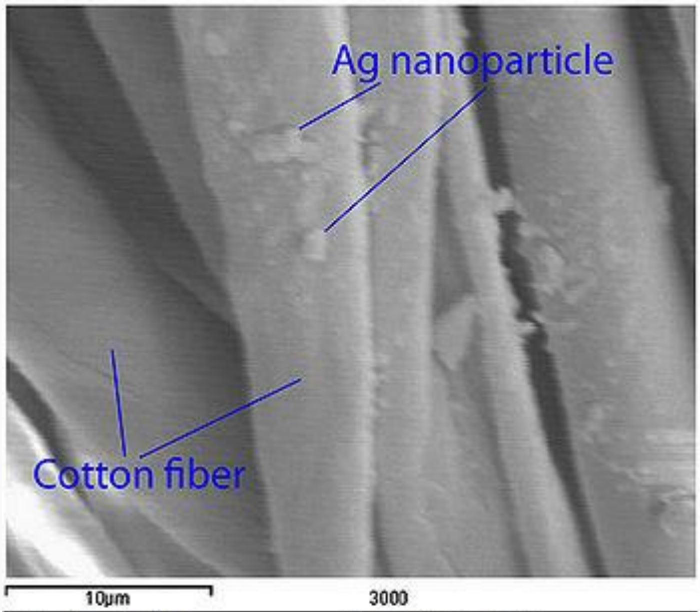
Wound dressings

Novel wound dressings have been developed that use silver to help prevent wound infections. Silver nanoparticles are incorporated into the wound dressing, and the silver-enhanced wound dressings were found in vitro to consistently kill *Pseudomonas aeruginosa* cultures entirely and kill *Staphylococcus aureus* cultures with >99.99% efficiency (Ong *et al.*, 2008). In mice, the silver-enhanced wound dressings were also found to reduce mortality from *Pseudomonas aeruginosa* wound infections from 90% to 14.3% (Ong *et al.*, 2008).

Endotracheal tubes

Among hospital patients that require ventilator-assisted breating, ventilator-associated pneumonia is the most common illness (Olson *et al.*, 2002). Endotracheal tubes are used by patients needing ventilator-assisted breathing. Silver coatings on the inside of endotracheal tubes have been shown to delay the appearance of bacteria on the insides of these tubes, and subjects that used the silver-coated tubes also showed decreased lung colonization by *Pseudomonas aeruginosa* (Olson *et al.*, 2002). Kollef *et al.* (2008) showed that silver-coated endotracheal tubes actually do reduce the incidence or increase the onset time of ventilator-associated pneumonia in patients using a ventilator.

Surgical masks



Number of washing	Bacterial reduction (%)	
	S. aureus	*E. coli*
Before washing	97	91
After 5 cycles	96.8	88.4
After 10 cycles	96	87.4
After 20 cycles	94	85

Figure 13.Scanning electron micrograph showing silver nanoparticles on cotton fibers and table showing antimicrobial efficacy of silver-implemented cotton after repeated laundering cycles. 54 ppm silver nanoparticle solutions were applied to cotton fibers and a binder was then applied to the cotton to help retain silver nanoparticles on the material (El-Rafie *et al.*, 2010).

Studies have examined the antibacterial properties of surgical masks coated with silver nanoparticles (Li *et al.*, 2006). Nanoparticle coated masks were capable of a 100% reduction in viable *E. coli* and *S. aureus* cells after incubation. Additionally, the study reported no signs of skin irritation in any of the persons wearing the masks (Li *et al.*, 2006).

Cotton fibers

Silver nanoparticles have been used to impart antimicrobial activity to cotton fibers. Cotton samples were immersed in silver nanoparticle solutions and then subjected to a curing process to allow the nanoparticles to adhere to the cotton (El-Rafie *et al.*, 2010). A chemical binder was then applied to the fabric to help maintain nanoparticle-cotton binding. Cotton samples prepared in this manner were able to reduce *S. aureus* and *E. coli* cell counts by 97% and 91% respectively. Even after subjecting the fabric to 20 laundry cycles, the cotton samples were still able to reduce *S. aureus* and *E. coli* cell counts by 94% and 85% respectively (Figure 13). Cotton prepared in this manner could be used by individuals working in the medical field or those who often work with microbes to prevent the spread of infectious bacteria (El-Rafie *et al.*, 2010).

Glass

AGC Flat Glass Europe has developed a glass with antimicrobial properties (AGC: Antibacterial glass). Silver ions incorporated into the glass are responsible for the antimicrobial activity. The company reports that 99.9%

of bacteria that come in contact with the surface of the glass are killed. The glass was produced to help prevent the spread of pathogens in a hospital setting. It could also be used to maintain the integrity of sterile workspaces.

Food packaging

Various types of food packaging have been supplemented with silver-containing compounds to deter microbial growth and extend product shelf life. Some of these packaging types include bulk food storage containers, paperboard cartons, plastic or paper food wraps, and milk containers (Appendini and Hotchkiss, 2002). Silver zeolite is the silver-containing compound used in food packaging (Appendini and Hotchkiss, 2002). Although few silver-containing compounds are approved by the FDA for direct food contact, silver-incorporated food packaging is quite widespread in Japan (Appendini and Hotchkiss, 2002).

Conclusion

Recent studies have revealed that the antimicrobial properties of silver are due to its ionized form, Ag^{+}, and its ability to cause damage to cells by interacting with thiol-containing proteins and DNA. These effects have been visualized (Feng *et al.*, 2000) as well as quantified through many experiments. Silver nanoparticles are a form of silver of particular interest because of their easy production, high antimicrobial activity, and ability to be incorporated into a diverse range of products. With the ever increasing number of antibiotic-resistant strains of bacteria and silver's low toxicity to humans, the use of silver as an antimicrobial agent is an exciting topic with a great deal of relevance to many fields of study and industry.

References

"AGC Flat Glass Europe launches world’s first antibacterial glass." AGC Glass Europe, 9 April 2007. http://www.agc-flatglass.eu/AGC+Flat+Glass+Europe/English/Homepage/News/Press+room/Press-Detail-Page/page.aspx/979?pressitemid=1031.

Appendini, P. and Hotchkiss, J.H. "Review of antimicrobial food packaging." *Innovative Food Science & Emerging Technologies*. 2002. Volume 3, issue 2. p. 113-126.

"Argyria." *Wikipedia*, 19 March 2010. http://en.wikipedia.org/wiki/Argyria.

Bavoil, P., Hofnung, M., Nikaido, H. "Identification of a Cytoplasmic Membrane-associated Component of the Maltose Transport System of Escherichia coli." *The Journal of Biological Chemistry*. 1980. Volume 255, No. 18. p. 8366-8369.

Breck D.W. Ion exchange reactions in zeolites, zeolite molecular sieves, structure, chemistry and use, New York: Wiley, 1974. p. 529.

Davies, R.L. and Etris S.F. "The Development and Functions of Silver in Water Purification and Disease Control." *Catalysis Today*. 1997. Volume 36. p. 107–114.

El-Rafie, M.H., Mohamed, A.A., Shaheen, T.I., Hebeish, A. "Antimicrobial effect of silver nanoparticles produced by fungal process on cotton fabrics." *Carbohydrate Polymers*. 2010. Volume 80, issue 3. p. 779-782.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O. "A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*." *Journal of Biomedical Materials Research Part A*. 2000. Volume 52, issue 4. p. 662-668.

Fox, C.L. and Modak, S.M. "Mechanism of Silver Sulfadiazine Action on Burn Wound Infections." *Antimicrobial Agents and Chemotherapy*. 1974. Volume 5, No. 6. p. 582-588.

Guggenbichler, J.P., Boswald, M., Lugauer, S., Krall, T. "A New Technology of Microdispersed Silver in Polyurethane Induces Antimicrobial Activity in Central Venous Catheters." *Infection*. 1999. Volume 27. p. 16-23.

Kawahara, K., Tsuruda, K., Morishita, M., Uchida, M. "Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions." *Dental Materials*. 2000. Volume 16, issue 6. p. 452-455.

Klueh, U., Wagner, V., Kelly, S., Johnson, A., Bryers, J.D. " Efficacy of Silver-Coated Fabric to Prevent Bacterial Colonization and Subsequent Device-Based Biofilm Formation." *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2000. Volume 53. p. 621-631.

Kollef. M.H, Afessa, B., Anzueto, A., Veremakis, C., Kerr, K.M., Margolis, B.D., Craven, D.E., Roberts, P.R., Arroliga, A.C., Hubmayr, R.D., Restrepo, M.I., Auger, W.R., Schinner, R. "Silver-Coated Endotracheal Tubes and Incidence of Ventilator-Associated Pneumonia." *The Journal of the American Medical Association*. 2008. Volume 300, No. 7. p. 805-813.

Li, Y., Leung, P., Yao, L., Song, Q.W., Newton, E. "Antimicrobial effect of surgical masks coated with nanoparticles." *Journal of Hospital Infection*. 2006. Volume 62, issue 1. p. 58-63.

Liau, S.Y., Read, D.C., Pugh, W.J., Furr, J.R., Russell, A.D. "Interaction of silver nitrate with readily identifiable groups : relationship to the antibacterial action of silver ions." *Letters in Applied Microbiology*. 1997. Volume 25, issue 4. p. 279-283.

Lok, C., Ho, C., Chen, R., He, Q., Yu, W., Sun, H., Tam, P.K., Chiu, J., Che, C. "Silver nanoparticles: partial oxidation and antibacterial activities." *Journal of Biological Inorganic Chemistry*. 2007. Volume 12, No. 4. p. 527-534.

Martinez-Castanon, G.A., Nino-Martinez, N., Martines-Gutierrez, F., Martinez-Mendoza, J.R., Ruiz, F. "Synthesis and antibacterial activity of silver nanoparticles with different sizes." *Synthesis and antibacterial activity of silver nanoparticles with different sizes*. 2008. Volume 10, No. 8. p. 1343-1348.

Monteiro, D.R., Gorup, L.F., Takamiya, A.S., Ruvollo-Filho, A.C., de Camargo, E.R., Barbosa, D.B. "The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver." *International Journal of Antimicrobial Agents*. 2009. Volume 34, issue 2. p. 103-110.

Moudgal, C.J., Lipscomb, J.C., Bruce, R.M. "Potential health effects of drinking water disinfection by-products using quantitative structure toxicity relationship." *Toxicology*. 2000. Volume 147, issue 2. p. 109-131.

Olson, M.E., Harmon, B.G., Kollef, M.H. "Silver-Coated Endotracheal Tubes Associated With Reduced Bacterial Burden in the Lungs of Mechanically Ventilated Dogs." *Chest*. 2002. Volume 121. p. 863-870.

Ong, S., Wu, J., Moochhala, S.M., Tan, M., Lu, J. "Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties." *Biomaterials*. 2008. Volume 29, issue 32. p. 4323-4332.

Pal, S., Tak, Y.K., Song, J.M. "Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*." *Applied and Environmental Microbiology*. 2007. Volume 73, No. 6. p. 1712-1720.

Radheshkumar, C. and Munstedt, H. "Antimicrobial polymers from polypropylene/silver composites—Ag^+ release measured by anode stripping voltammetry." *Reactive & Functional Polymers*. 2006. Volume 66. p. 780-788.

Rai, M., Yadav, A., Gade, A. "Silver nanoparticles as a new generation of antimicrobials." *Biotechnology Advances*. 2009. Volume 27. p. 76-83.

"Seconday Drinking Water Regulations: Guidance for Nuisance Chemicals." *Ground Water & Drinking Water*. United States Environmental Protection Agency, November 2006. http://www.epa.gov/safewater/consumer/2ndstandards.html.

Silvestry-Rodriguez, N., Sicairos-Ruelas, E.E., Gerba, C.P., Bright, K.R. "Silver as a Disinfectant." *Rev Environ ContamToxicol*. 2007. Volume 191. p. 23–45.

"Silver." *Wikipedia*, 16 April 2010. http://en.wikipedia.org/wiki/Silver.

"The antibacterial effects of silver and its compounds." Salt Lake Metals, November 2008. http://www.saltlakemetals.com/Silver_Antibacterial.htm.

"ToxFAQs for Silver." Agency for Toxic Substances and Disease Registry, July 1999. http://www.atsdr.cdc.gov/tfacts146.html.

World Health Organization.Guidelines for Drinking-Water Quality, 2nd Ed. 1996 WHO, Geneva, Switzerland.

Yamanaka M., Hara, K., Kudo, J. "Bactericidal Actions of a Silver Ion Solution on Escherichia coli, Studied by Energy-Filtering Transmission Electron Microscopy and Proteomic Analysis." *Applied and Environmental Microbiology*. 2005. Volume 71, No. 11. p. 7589-7593.