Antimicrobial Effects of Silver Nanoparticles

www.nano-cemms.uiuc.edu
Description:
Students make silver nanoparticles using a quick, simple and safe procedure. They then design experiments to test the effectiveness of the nanoparticles as an antimicrobial agent.

Prerequisites:
Students should have an introductory knowledge of nanotechnology that can be provided by the Introduction to Nanotechnology kit. The Gold and Silver Nanoparticles lab is a good companion activity.

Instruction Time:
Approximately three 50-minute class periods. The first period is for the PowerPoint presentation, the second is for the lab, the third is for collecting data.

Audience:
Middle or high school General Science, Biology, or Chemistry students.

Lesson Objectives:
Students will make silver nanoparticles and design an experiment to test the effectiveness of silver nanoparticles as an antimicrobial agent.

National Science Education Standards:
Content Standard C: The Cell
Content Standard E: Understandings about Science and Technology.

Illinois State Learning Standards:
11.A.4b Conduct controlled experiments or simulations to test hypotheses.
12.B.4b Simulate and analyze factors that influence the size and stability of populations.
12.C.5b Analyze the properties of materials in relation to their physical and/or chemical structures.
13.B.5b Analyze and describe the processes and effects of scientific and technological breakthroughs.
Instructional Method:
The instructor gives a PowerPoint presentation about the anti-bacterial properties of silver nanoparticles. Students then do a lab where they make and test the effectiveness of nanoparticles.

Background Information:
Nanotechnology is an emerging industry which is bringing us exciting new products and promises to change the way we live and work in the future. Several new products are using silver nanoparticles to generate antimicrobial surfaces. Silver nanoparticles are integrated into fabrics to prevent clothes from developing foul odors, doorknobs have silver nanoparticles embedded in their surfaces—even silver nanoparticle-treated pacifiers are on the market. For a list of hundreds of nanotechnology products using silver nanoparticles see http://nanotechproject.org/44.

Scientists are very interested in nanoparticles as it pertains to nanotechnology. Nanoparticles are often defined as having dispersed particles in the size range 1-100 nm. Gold nanoparticles are finding applications in cancer treatment, and silver nanoparticles are found to have antimicrobial properties.

Though there is a serious lack of information to describe the mechanism in which the silver nanoparticles actually prevent bacterial growth, most research points to interactions with the bacterial cell wall, and regulation of materials across the membrane. This could also be why there are different results in gram positive and gram negative bacterial strains. E. coli is a common gram negative bacteria used in microbiology, and is safely cultured and maintained. For quick results E. coli should grow overnight when incubated at 37°C.

Overview:
In this lab students will make a silver colloidal mixture. Silver solution will be reduced and stabilized with sodium citrate in a boiling water bath. The colloidal dispersions formed will be poured into petri dishes, soaked up into filters, and placed on top of a bacterial agar plate. The silver colloid should be yellow in color. Silver colloidal particles are 20-50 nm in size. If the silver nanoparticles prevent bacterial growth, there should be a ring of inhibition around the location where the filters soaked with nanoparticles are placed on the bacterial agar plate.
Materials:

- 1 mM silver nitrate
- *E. coli* bacterial culture
- 1% sodium citrate
- Coffee filter/filter paper
- Small test tube
- Q-tip swab
- 250-mL beaker
- Disposable transfer pipettes
- Incubator
- Agar plate
- Hot plate
- Scissors
- Tongs
- Tweezers
- Small containers for soaking filter paper
- Test tube rack

Safety:

Goggles, gloves and aprons should be worn as in all chemistry laboratory activities. The hot water baths should be handled with care to avoid burns. Any liquids spilled on skin can be washed off with water.

This lab uses *Escherichia coli*, *E. coli*, a gram-negative rod-shaped bacterium that is part of the normal intestinal fauna in mammals. Some strains, particularly O157:H7, are pathogenic to humans; most strains, however, are benign. *E. coli* it has become the “workhorse” of microbiology because it can be easily cultured. *E. coli* strains available for use in laboratories and classrooms grow very well on petri dishes but very poorly in intestines. The *E. coli* used in this lab is nonpathogenic; it would not likely live in a human intestine even if ingested in large amounts.

Preparation:

Procure sterile bacterial media. Culture bacteria 1-2 days before activity. Get the PowerPoint presentation or overheads ready. Make a hot water bath using 100 mL beakers half filled with boiling distilled water. Set out the solutions and equipment.

You will need to prepare the following:

- Sterile media (2-3 days before lab)
- Liquid bacteria culture (1-2 days before lab)
- Hot water baths consisting of 100 mL beakers half-filled with boiling distilled water on a hot plate

You will need to prepare stock solutions of:

- 1 mM AgNO₃ (.34 g of AgNO₃ in 2000 mL of distilled water)
- 1% Na₃C₆H₅O₇ (0.5 g of the solid in 50 mL of distilled water)
**Procedure:** Make Silver Nanoparticles

1. Add 2 mL of 1 mM silver nitrate to a small test tube.

2. Place this test tube in a 250-mL beaker of boiling water. While waiting start on *Plate Bacteria and Add Variables* section below.

3. Leave the test tube in the boiling water bath for 10 minutes.

4. Add 7 drops of 1% sodium citrate to the test tube containing the hot silver nitrate.

5. Continue to heat until the silver nitrate solution changes color (yellowish). ~15 minutes for silver.

6. Remove the test tube and set it in a test tube rack to cool.
Soak Filter in Nanoparticles

7. While waiting for the silver nanoparticles to form, cut the filter paper (or coffee filters) into small squares about 2 cm across.

8. Place the filter paper squares in a small container and pour the test tube of silver nanoparticles over them. Let the filter paper squares soak for about 10 minutes. While waiting, start on the next step.

Plate Bacteria and Add Variables

9. Mark the bottom of an agar plate with your initials, divide the bottom into sections and label each of the sections. Remember to set up your plate with a control for comparison.

10. Put 1 to 2 drops of bacterial culture on the agar plate using a 1-mL disposable transfer pipette.
Plate Bacteria and Add Variables

11. Spread the drops of bacteria culture on the agar plate using a Q-tip swab.

12. Place your nanoparticle-soaked filter paper squares and your control(s) in the designated areas.

Incubate and Check Results

13. Incubate your agar plate for 24 hours at 37°C.

14. Examine the petri plate. Record results.
ESEM Images:

These images were taken of bacterial cells using an ESEM microscope. It is impossible to see our nanoparticles using a light microscope since they are smaller than the wavelength of light. Instead of focusing light, a SEM uses a beam of electrons which allows us to see objects smaller than the wavelength of light.

E. coli Images:

Healthy *E. coli* cells 1000x (no nanoparticles)

*E. coli* is known as the “workhorse” of microbiology because it is easy, safe, and easy to manipulate.

Healthy *E. coli* cells 20000x (no nanoparticles)

Notice the biofilm that *E. coli* makes; the weblike structure shown is a mucus barrier that covers the surface of *E. coli*. It looks very similar to a web if dry.

Healthy *E. coli* cells 10000x (no nanoparticles)

Healthy *E. coli* cells 50000x (no nanoparticles)
**E. coli Images:**

Unhealthy *E. coli* cells 8000x (with nanoparticles)

There are not as many cells present since you can see the honeycomb lattice of the filter.

Unhealthy *E. coli* cells 20000x (with nanoparticles)

There are nanoparticles interacting with the surface of bacteria. Bacteria look deflated and elongated.

Unhealthy *E. coli* cells 50000x (with nanoparticles)

The bacteria surfaces do not look the same as the healthy cells.

Unhealthy *E. coli* cells 20000x (with nanoparticles)

More cells are deflated and elongated. The elongation is a sign of cell distress; the cell cannot divide or doesn’t recognize when it should divide.
**B. subtilis Images:**

Healthy *B. Subtilis* 65000x (no nanoparticles)

Another rod-shaped bacteria. This one is gram-positive. *B. subtilis* is an endospore-forming soil bacteria.

Unhealthy *B. Subtilis* 65000x (with nanoparticles)

These cells do not look shriveled or deflated, but you can notice nanoparticles on the surface of the cells.

**S. lutea Images:**

Healthy *S. Lutea* 35000x (no nanoparticles)

A gram-positive micrococcus bacteria. They grow in clusters of 4 and are often found on the skin of mammals.

Unhealthy *S. Lutea* 35000x (with nanoparticles)

Noticeable surface differences between these cells and healthy cells.
Sock Images

Fibers of "silver nanoparticle treated" socks 350x
Denser elements (silver) are highlighted.

Fibers of a regular white sock 160x

Fibers of "silver nanoparticle treated" socks 88x
Denser elements (silver) are highlighted.

Fibers of a regular white sock 80x
Presentation Details:

Slide 1 (Antibacterial Properties of Silver Nanoparticles): Our focus today and tomorrow will be on the antimicrobial properties of silver nanoparticles.

Slide 2 (Objective): Nanotechnology is a field that involves controlling matter on an atomic or molecular scale to make functional devices. During this presentation you will learn about some nanotechnology products. Even though the products come in standard sizes, they have all been altered using nanometer-sized particles of silver which have special properties.

Slide 3-4 (Motivation): It is often difficult to define size, especially without a point of comparison, but the nanoscale is usually defined as being in the range of 1 to 100 nanometers. The size scale and accompanying graphics on this slide allow you to compare the size of items you can see, such as an ant and a strand of human hair (macroscale), with things you can only see with the help of an optical microscope (microscale), such as blood cells. You can also compare these items with things that you can only detect with a scanning electron microscope (nanoscale), such as strands of DNA, molecules, and individual atoms. This last category of items that can only be detected with a very powerful microscope can be classified as nanoscale.

Slide 5-6 (Motivation): A lot of nanotechnology products are being developed both internationally and domestically. In the slides that follow, you will learn about only a few of these products. There are thousands of companies and research laboratories who are working in the area of nanotechnology development. However, as you learn about products, keep in mind that in many cases products have not yet been regulated or approved by the government. Nanotechnology is a very exciting field, but with changes happening so quickly there may be some societal, health and financial implications.

Slide 7 (Food Containers): Generally both new and leftover foods will stay fresh for only a couple of days, even when they are kept in the refrigerator. This is unfortunate since certain foods such as fresh produce can be quite expensive. With “Fresh Box” food containers, you can keep foods fresh, healthy and tasty for much longer. This prevents food from having to be thrown away and saves money in the process. These special airtight containers utilize a silver nanoparticle technology that can decrease bacteria levels by as much as 99.9%.

Slide 8 (Baby Bottles): Most parents are especially concerned with hygiene when it comes to their child’s health. A Korean company has developed a special milk bottle and mug for babies. The company describes the hygienic, medical, scientific and ergonomic properties of the product on their website. This product claims to be enhanced using a silver nano poly technology, a system that prevents 99.9% of germs through an anti-bacterial deodorizing function.

Slide 9 (Toothpaste): This toothpaste contains a silver powder with properties that sterilize and disinfect bacteria and prevent various oral problems. Gum disease, including gingivitis and periodontitis, is a serious bacterial infection that can lead to tooth loss. This product can be used by people suffering from
these conditions to promote healing and decrease inflammation. It is also possible that some people may use this product as a preventative measure.

**Slide 10 (Toothbrush):** This toothbrush, developed by Songsing Nanotechnology Company, suppresses the growth and spread of bacteria. It can be used for a comparable amount of time as regular toothbrushes and maintains its antiseptic properties.

**Slide 11 (Cutting Board):** A Korean company by the name of Nano Silver Clean has created a product somewhat similar to the food storage containers. This cutting board is embedded with silver nanoparticles such that this product has a 99.9% antibacterial effect. The company website explains that all surfaces of their products are made using pure silver. Unlike some of the other companies, Nano Silver Clean’s website contains the result of research that compared the bacteria growth rate of standard products to nano products.

**Slide 12 (Computer Mouse):** IOGEAR’s Personal Security Mouse is coated with a titanium Dioxide (TiO2) and a silver (Ag) nano-particle compound. The coating uses two mechanisms to deactivate enzymes and proteins to prevent a wide spectrum of bacteria, fungi and algae from surviving on the surface of the mouse.

**Slide 13 (Antibacterial Athletic Socks):** These socks made by Sharper Image are fairly typical sports socks (cushioned, fitted, quarter-length). However, the standard fabric has been interwoven with a cotton material containing millions of invisible (to the naked eye) silver nanoparticles. The socks are advertised as being non toxic and non allergenic.

http://www.avid4men.co.uk/nano_tech_sock.htm

**Slide 14 (Cotton Sheets):** If you are looking for health benefits or a more restful night of sleep, these sheets may be the solution. The sheets are 100% cotton and have been treated with SilverSure to help in the fight against cross infection of superbugs such as MRSA.

**Slide 15 (Washing Machine):** Globally recognized for its high-tech and futuristic appliances, Samsung’s Silver Nano C1235A 5.2 kg Drum Type washing machine incorporates a technology called Silver Wash, an advanced washing technology that kills bacteria faster and helps to sterilize your clothes. Samsung’s website explains that by using a cleaning solution containing dissolved silver ions, this product is capable of affecting “your clothes at an almost molecular level.” The sterilization is listed as 99.9% and the antibacterial effect will last up to one month.

http://ww2.samsung.co.za/silvernano/silvernano/washingmachine.html

**Slide 16 (Today’s Activities):** Students will first make nanoparticles to test the effectiveness of silver nanoparticles and an antimicrobial agent. They will soak the nanoparticles in filters. The filters will allow the nanoparticles to slowly diffuse away from the filters and stop the bacteria from growing next to the filter –that is, if the nanoparticles work as an antimicrobial agent. Students will inoculate the plates, place
the soaked filters on them, and check the results after growing the bacteria overnight at 37C (human body
temperature).

**Slide 17 (How to Make Silver Nanoparticles):** We will start with individual atoms of silver and stick
them together to make our silver nanoparticles. To get these atoms of silver we will start with a
compound called silver nitrate. It has a silver atom bonded to a nitrate group.

(It is not critical that you measure exactly 5 ml, in fact, you can just eye-ball how full to make your test
tubes. The reaction will work if you are off even by quite a lot.)

**Slide 18 (How to Make Silver Nanoparticles):** We are going to heat this compound up so it can react
quickly with another substance. (Give appropriate warnings about hot materials.)

Heat the test tubes containing their solutions in a boiling water bath. It is a good idea to use distilled water
in the water bath because salts will form larger pieces of gold that are purple or blue instead of the ruby
red color that should form. Results will not be affected even if some boiling distilled water bubbles into
the test tube.

(Alternately, the students can microwave their solutions for a few seconds in a small flask. Do not boil the
solution—just heat it to right before the boiling point.)

**Slide 19 (How to Make Silver Nanoparticles):** Sodium citrate will free the silver atoms from the silver
nitrate. Use a disposable pipette to add only ½ a milliliter of the sodium citrate. It is not important to get
exactly ½ a milliliter—it can be slightly less or slightly more.

**Slide 20 (How to Make Silver Nanoparticles):** After the solution heats for about 20 minutes, the silver
will start to form colloids and change to a yellow color. Be sure to leave it in the boiling water bath for a
couple extra minutes.

(Provide appropriate warnings about removing the hot test tubes from the boiling water bath.)

**Slide 21 (How to Make Silver Nanoparticles):** Here is the procedure on one slide.

**Slide 22 (Growth of Bacteria):** A colony is a large number of bacteria growing from a single cell—each
of the dots” on the top plate are a colony. As the bacteria divide, they grow outward and increase in
number. The bacterial cells can be seen as a dot, just like sand on a beach can be seen from an airplane—
individual bacterial cells are not seen, but the large number growing next to each other are seen as a
colony.

A lawn consists of many bacteria cells growing together on the plate. Individual colonies are not seen,
instead a smooth lawn of bacteria can be viewed.
Slide 23 (Bacterial Antibiotic Sensitivity): On this lawn of bacteria you can see regions where no growth occurs. These regions can be seen around disks soaked with antibiotics. The antibiotic molecules diffuse out from the disk and inhibit the growth of bacterial cells. This is seen as a cleared area of no growth around the disk.

As the distance from the disk increases, the diffusion of antibiotic molecules decreases. At some point, there are so few antibiotic molecules that bacteria can grow. This is seen as the growth of a lawn of bacteria.

Slide 24 (Procedure): We need to prepare our disks to test the antimicrobial properties of the silver nanoparticles. Cut a filter paper into small pieces and soak them in the silver nanoparticles for about 10 minutes.

Slide 25 (Procedure): (It is helpful to pass around a fresh agar plate for students to touch. (It will become contaminated and will later be discarded.) Students can gain a sense of how hard they can push on the plate without ripping the agar, which will be useful for them to know when they inoculate their own plate.

When students get their experimental plate, they will label and divide the plate into sections by marking it with a sharpie pen on the bottom of the plate.)

Plates are inoculated by putting about two drops of a liquid culture of bacteria on the plate and then spreading the drop over the plate with a sterile Q-tip or a bacteria spreader sterilized by flaming.

Slide 26 (Procedure): After the liquid bacteria media sits on the plate for a few minutes to allow the media to be absorbed into the agar, the prepared filter papers are placed on the plates. At least one of the filter squares should have been soaked in silver nanoparticles. The others can be treated several ways as controls (for example, they could be dry or soaked in sterile water). The plates should be incubated at 37°C overnight.

Slide 29 (Results): Both of the left filter squares are controls that were soaked in water. The right filters have both been soaked in silver nanoparticles and show a halo where no bacteria grew around the filter squares.

Slide 30 (Results): A close-up of a filter square soaked in silver nanoparticles displays the halo where no bacteria could grow.

Slide 31 (Results): The left filter square is a control – bacteria grew right up to the filter square. The right filter square shows a halo zone where no bacteria could grow because of the diffusing silver nanoparticles.

Slide 32 (Results): Sample 1 is a dry unsoaked filter. Sample 2 is a filter soaked in sterile water. Sample 3 was soaked in silver nanoparticles diluted 1:1 with sterile water. Sample 4 was soaked in full strength silver nanoparticles.
Slide 33 (Results): Close-up of previous slide

Slide 34 (Results): Previous slide opened and viewed from the top.

Slide 35 (Results): Close-up of previous slide
Purpose:

Nanotechnology is an emerging industry that is bringing us exciting new products and promises to change the way we live and work in the future. Several new products use silver nanoparticles to generate antimicrobial surfaces. Silver nanoparticles are integrated into fabrics to prevent clothes from developing foul odors. Doorknobs have silver nanoparticles embedded in their surfaces. Even silver nanoparticle-treated pacifiers are on the market.

Scientists are very interested in nanoparticles as they pertain to nanotechnology. Nanoparticles are often defined as having dispersed particles in the size range 1-100 nm. Gold nanoparticles are finding applications in cancer treatment, and silver nanoparticles are found to have antimicrobial properties.

Though there is a serious lack of information to describe the mechanism in which the silver nanoparticles actually prevent bacterial growth, most research points to interactions with the bacterial cell wall and regulation of materials across the membrane. This could also be why there are different results in gram positive and gram negative bacterial strains. *E. coli* is a common gram negative bacteria used in microbiology and is safely cultured and maintained.

Safety:

Goggles, gloves and aprons should be worn as in all chemistry laboratory activities. The hot water baths should be handled with care to avoid burns. Any liquids spilled on skin can be washed off with water.

Materials:

- 1 mM silver nitrate
- 38.8 mM (1%) sodium citrate
- Hot plate
- Small test tubes
- 250 mL beaker
- Disposable transfer pipettes
- Agar plates
- Scissors
- Tweezers
- *E. coli* bacteria culture
- Filter paper
- Q-tip swabs
- Incubator
- Small plastic petri dishes
Procedure:

1. Make colloidal silver using the following method:
   a. Add 2 mL of silver nitrate to a small test tube.
   b. Place this test tube in a 250 mL beaker of hot water.
   c. Leave in the beaker of hot water for about 10 minutes.
   d. Add 7 drops of sodium citrate to the test tube containing hot silver nitrate.
   e. Continue to heat until the silver nitrate solution turns color (yellowish).
   f. Remove test tubes and set in a test tube rack to cool.
2. Cut filter paper into small squares.
3. Place filter paper squares in a small petri dish and pour the test tube of colloidal silver over the squares.
4. Let the filter paper squares soak for about 10 minutes (can be even longer).
5. While waiting for the filter paper to soak, mark the bottom of a petri plate with initials, divide the bottom into sections and label each of the sections. Remember to set up control for comparison.
6. Use a 1 mL disposable transfer pipette and spread 1 drop of the bacteria culture on the agar plate using a Q-tip swab.
7. After 10 minutes of soaking, place filter paper squares in the designated areas.
8. Incubate the petri plates overnight at 37 degrees Celsius.
9. On the second day of this activity, the petri plates should be removed from the incubator and examined.

Analyze Data:

1. Draw your petri plate; be sure to include your labels.

2. Describe the bacterial growth in each labeled section.

3. How do you explain any differences that you observe?
Questions:

1. Are products containing silver nanoparticles justified in claiming that these products have antimicrobial effects?

2. What conclusions can you draw about the effect of silver nanoparticles to bacterial cell growth based on these images?

SEM images of Healthy *E. coli* (left) and *E. coli* cultured in the presence of silver nanoparticles (right)
Antimicrobial Effects of Silver Nanoparticles – Student Lab Key

**Purpose:**

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Scientists are very interested in nanoparticles as they pertain to nanotechnology. Nanoparticles are often defined as having dispersed particles in the size range 1-100 nm. Gold nanoparticles are finding applications in cancer treatment, and silver nanoparticles are found to have antimicrobial properties.

Though there is a serious lack of information to describe the mechanism in which the silver nanoparticles actually prevent bacterial growth, most research points to interactions with the bacterial cell wall and regulation of materials across the membrane. This could also be why there are different results in gram positive and gram negative bacterial strains. *E. coli* is a common gram negative bacteria used in microbiology and is safely cultured and maintained.

**Safety:**

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9. On the second day of this activity, the petri plates should be removed from the incubator and examined.

**Analyze Data:**

1. Draw your petri plate; be sure to include your labels.
   
   *Answers may vary.*

2. Describe the bacterial growth in each labeled section.
   
   *Answers should describe each quadrant labeled in question 1.*

3. How do you explain any differences that you observe?
   
   *Answers may vary, should include information from teacher presentations and background information.*
Questions:

1. Are products containing silver nanoparticles justified in claiming that these products have antimicrobial effects?
   
   Answers may vary, but students should support their conclusions with data from the lab.

2. What conclusions can you draw about the effect of silver nanoparticles to bacterial cell growth based on these images?
   
   Research leads us to believe that the nanoparticles are interacting with the cell wall of the bacterium and preventing a bacterium from regulating transport across that cell membrane. The density of cells and the shapes of the cells are good indicators of cell health.

SEM images of Healthy E. coli (left) and E. coli cultured in the presence of silver nanoparticles (right)
Established in 2003, the Center for Nanoscale Chemical-Electrical-Mechanical Manufacturing Systems (Nano-CEMMS) is funded by the National Science Foundation. Partnering Institutions include the University of Illinois, North Carolina Agriculture and Technical State University, Stanford University, University of Notre Dame, University of California – Irvine, and Northwestern University. Researchers are developing a nanomanufacturing system that will build ultrahigh-density, complex nanostructures. The Center’s research will ultimately result in a new way of working and has the potential to create millions of jobs for American workers. Our nation’s school children must be prepared to assume the new roles that will be the inevitable outcome of these emerging technologies.

This learning module is one of a series that is designed to interest middle and high school students in pursuing this new field. The Center also offers ongoing professional development for teachers through a continuous series of workshops and institutes. To sign up for a workshop or to order more learning modules, visit our website at http://www.nano-cemms.illinois.edu.

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