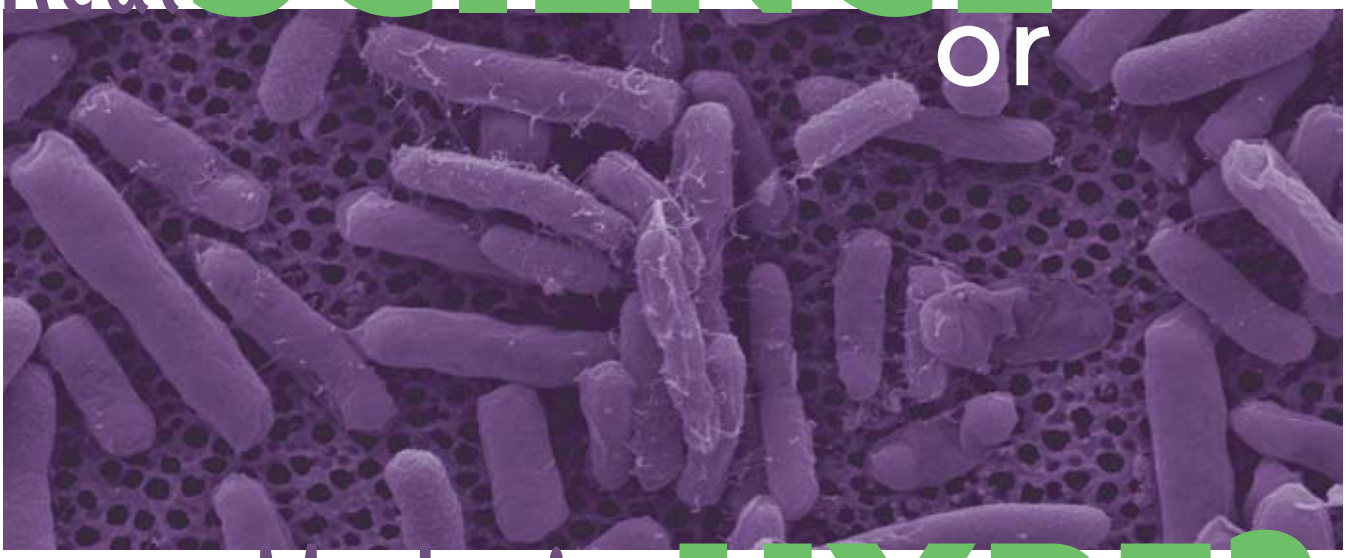
Real SCIENCE or Marketing HYPE?



Student-designed experiments test the antimicrobial effects of silver nanoparticles

Joseph Muskin, Janet Wattnem, Matthew Ragusa, and Barbara Hug

Teachers have been asked to engage their students in more and more cutting-edge science (Committee on Prospering in the Global Economy of the 21st Century 2007). One way of doing this is to bring nanotechnology into the classroom. The Center for Nanoscale Chemical–Electrical–Mechanical Manufacturing Systems (Nano-CEMMS) at the University of Illinois, in collaboration with local Champaign-area teachers, has developed classroom activities designed to introduce nanotechnology to secondary students. One such activity investigates the antimicrobial effect of silver nanoparticles, or very small pieces of silver. The diameters of these silver pieces range in size from about 5–50 nm across; for comparison, three or four atoms placed end-to-end are typically about 1 nm in length. These nanoparticles are so small that they remain suspended in water.

Silver nanoparticles have been used to generate antimicrobial surfaces in several new products, including fabrics (to prevent clothes from developing foul odors), doorknobs, and even pacifiers (see "On the web" at the end of this article). With hundreds of new products claiming antimicrobial properties, we at Nano-CEMMS thought it appropriate to put silver nanoparticles to the test.

This article describes a quick, simple, and safe classroom activity in which students make silver nanoparticles and design experiments to test their effectiveness. In less than 30 minutes, students make nanoparticles by mixing a few readily available solutions on a hotplate. Students also generate their own experiments to test the "antimicrobial properties" of the silver nanoparticles they have created by growing bacteria in the absence or presence of these nanoparticles. Based on the level of teacher involvement, the activity can be modified for students with a range of ability levels. Therefore, the activity is appropriate for advanced chemistry or biology classes, yet still suitable for introductory biology or general science classes.

Connections to student learning goals

This investigation is highly adaptive to the needs of individual teachers and can be incorporated into the established curriculum to address a number of key National Science Education Standards focusing on scientific inquiry or discipline-specific concepts (NRC 1996). Regardless of how this investigation is included, students engage in their own inquiry about the antimicrobial effect of silver nanoparticles. Depending on the amount of scaffolding, students can engage in an open inquiry investigation or a more closely scaffolded activity. The intended learning goals determine the type of inquiry undertaken.

In addition to the inquiry standards, different content standards can also be addressed depending on the context in which the activity is embedded. If a more chemical approach is taken, this investigation could address Content Standard B by examining how the structure and properties of matter change following a chemical reaction (NRC 1996, pp. 178, 179). While the context of testing the properties is a biological one, the content focus would be chemistry; this can be used to show the interdisciplinary nature of science.

For illustrative purposes, we have chosen to highlight experimental design in the description of this activity. Research has shown that students have difficulty designing controlled experiments (Chen and Klahr 1999; Toth, Klahr, and Chen 2000). In this article, we provide a description of how a chemistry and biology teacher supports her students as they engage in the activity, which focuses on the identification of experimental variables to control.

The activity

In the teacher's biology class, students were introduced to this activity through a PowerPoint presentation on the making of silver nanoparticles. Similarly, teachers can introduce students through a presentation that reviews student understanding of colloidal chemistry—the study of mixtures containing particles between 1 and 1,000 nm in size—and relates colloids to particle size (nm). A second PowerPoint presentation could introduce students to microbiology, common terms, and techniques and provide them with the background information needed to better comprehend the goal of this activity and evaluate their data. A slide of the halo effect, or area of inhibition that is often observed when testing bacteria for antibiotic sensitivity, could also be included.

On the first day of this activity, students make silver nanoparticles using the recipe found in Figure 1. While waiting the required 10 minutes for the silver nitrate to heat, students can cut their filter paper squares, which will be used to test for antimicrobial effects. Depending on the student's experimental design, some of these squares should be placed in a small container for later soaking with colloidal silver; the rest should be used as a control. (**Note:** In the design of their experiment, students should be allowed to determine the necessary controls, such as filter paper soaked with silver nanoparticles, filter paper soaked with plain water, unsoaked filter paper, or no filter paper).

After students have cut their squares, the instructor passes around an agar plate, which consists of two pieces: a bottom and a lid that fits over top of it. The agar is poured into the bottom of the plates and covered with the lid. Plates should remain agar side up to prevent contamination from airborne sources. Next, the instructor removes the lid from an agar plate that will not be used in the activity and allows students to feel the agar with their fingers—this allows students to get a better idea of how much pressure to apply to the agar when they spread their bacteria. Students should not dig into the agar when spreading bacteria over its surface; introducing tears into the agar makes it difficult to evaluate plate results.

After the sodium citrate—which is used to synthesize the silver nanoparticles—has been added to the test tubes and students are waiting for an observable color change, they can label their agar plates. The plates should be handed out agar side up, and students should be reminded not to open them. Students should label the agar side of their plates because the lid rotates and can change the position of the labels in relation to the sample. By this time, a change from a clear to a yellow or golden color should be noticeable in the heated test tubes, indicating silver nanoparticles have formed. If needed, this is a good overnight stopping point.

Once test tubes have cooled, students pour their silver nanoparticles over the selected filter paper squares in their small container. The squares should soak for about 10 minutes. During this time, students inoculate their plates with

Safety note.

Because of their small size, nanoparticles of otherwise benign materials may in fact be hazardous. For example, like other small molecules, some may be able to cross the cell membrane, whereas the bulk material is too large to do so. As with all chemical use in a laboratory, appropriate gloves and chemical-splash safety goggles should be worn and other standard safety precautions should be taken.

This lab also makes use of commercially obtained *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. Because *E. coli* is so easy to culture, it has become the "workhorse" of microbiology. For safety reasons, strains for use in laboratories and classrooms are derived from *E. coli* that grows very well on petri dishes but very poorly in the intestines. The *E. coli* used in this lab is nonpathogenic and therefore would not be likely to survive in a human intestine even if large amounts of the bacteria were ingested—it could not out-compete the naturally occurring bacterial fauna, including benign forms of *E. coli*, already present in the gut.

FIGURE 1

Lab activity: Bacterial sensitivity to silver nanoparticles.

Purpose

This lab was developed to test claims that silver nanoparticles have antimicrobial effects. Many products on the market now have nanoparticles of silver in them—including socks, clothing, and food containers. One advertising claim states that silver nanoparticles inhibit the growth of bacteria and, hence, reduce odors or spoilage.

In the activity below, which takes two to three days to complete, filter paper soaked in silver nanoparticles will be used to test antimicrobial effects. Be sure to include a control in your experimental design so that you can determine whether the silver nanoparticles are effective. At the end of the activity, you will be asked to analyze your data.

Materials

- 1 mM silver nitrate
- 1% sodium citrate
- small test tube
- 250 mL beaker
- 1 mL disposable transfer pipets
- agar plate
- *E. coli* bacterial culture
- filter paper
- cotton swab
- small containers for soaking filter paper
- incubator
- hot plate

Experimental procedure

1. Make silver nanoparticles using the following method:
   a. Add 2 mL of 1 mM silver nitrate to a small test tube.
   b. Place this test tube in a 250 mL beaker of boiling water. While waiting for it to boil, start on step 2.
   c. Leave test tube in boiling water bath for 10 minutes.
   d. Add 7 drops of 1% sodium citrate to the test tube containing hot silver nitrate.
   e. Continue to heat until the silver nitrate solution changes color (from clear to yellow or golden).
   f. Remove test tubes and set in a test tube rack to cool.
2. While waiting for silver nanoparticles to form, decide what controls need to be run and cut filter paper into small squares (about 2 cm across) to accommodate the silver and all needed controls.
3. Place filter paper squares in small containers and pour the test tube of silver nanoparticles over the squares in one container. Prepare the controls needed in the other containers.
4. Let the filter paper squares soak for about 10 minutes (or overnight). While waiting, start on step 5.
5. Mark the bottom of an agar plate with your initials, divide the bottom into sections to accommodate the silver-soaked filter and all controls, and label each section. Remember to set up your plate with a control for comparison.
6. Put one to two drops of bacterial culture on the agar plate using a 1 mL disposable transfer pipet.
7. Spread the drops of bacteria culture on the agar plate using a cotton swab.
8. Place your nanoparticle-soaked filter paper squares and your control(s) in the designated areas.
9. Incubate your agar plate for 24 hours at 37°C.
10. Examine your agar plate the next day. Record data and analyze.

Record data

1. Draw your agar plate; be sure to include your labels.
2. Describe the bacterial growth in each labeled portion.

Analyze data

1. How do you explain any differences that you observe?

Conclusion

1. Are advertisements for products containing silver nanoparticles justified in claiming that these products have antimicrobial effects? Include evidence from your experiment that supports your answer.

a commercially obtained *Escherichia coli (E. coli)* bacteria culture. First, students turn their plate over so the agar side is facing downwards. Next, students use a 1 mL disposable transfer pipet to place one to two drops of the culture onto the surface of the agar plate. It is important that students lift the lid of the plate only enough to place the drops of culture in the center of the agar surface, so as to prevent contamination of the agar with additional bacteria. While keeping the lid slanted over the surface, students spread the culture using a cotton swab in a back-and-forth motion and rotating the plate a quarter of a turn each time.

The plates should be set agar side down while the filter paper squares finish soaking. This allows the liquid from the culture to soak into the plate. After the squares have soaked for 10 minutes, students place them in the sections designated as silver nanoparticles in their setup. Analysis and evaluation of different setups can be done on the next day of this activity through classroom discussion in which students answer the question: "Do silver nanoparticles inhibit bacterial growth?"

After the squares are placed, the plates should set for another 10 minutes while the squares adhere to the

surface of the agar. Then, these inoculated plates should be placed agar side up in an incubator at 37°C for about 24 hours. The next day, plates are removed and students record and analyze the data. A halo of inhibition, or halo effect, should be observed only around the squares that contain silver nanoparticles.

Initially, students investigate the question of whether or not the silver nanoparticles are effective antimicrobial substances, but by the end of the activity, they have also learned about basic experimental design. Using what they learn from this activity, students can identify and manipulate additional variables (e.g., incubation temperature, concentration of nanoparticles, amount of bacteria, and type of bacteria), building on results from previous investigations.

Teacher preparation

Preparation of the stock solutions needed for this lab is straightforward. The solutions are inexpensive and easy to make, and the chemicals needed to make silver nanoparticles are stable and have a long shelf life. The 1 mM silver nitrate solution can be prepared by dissolving 0.17 g of solid silver nitrate in 1,000 mL of distilled water. This solution should be stored in an amber bottle because it is photoreactive and will break down under extended exposure to light. The 1% sodium citrate is prepared by dissolving 0.5 g of solid sodium citrate in 50 mL of water.

Stock *E. coli* can be obtained from several scientific supply houses on sealed agar plates or in culture tubes. The K12 strain or most others sold for biological research are safe options. Nutrient agar or Luria-Bertani (LB) agar plates are used to grow the *E. coli*. These agar plates can be purchased ready to use from a commercial supplier or can be made. To make plates, add 20 g of LB media and 15 g of agar to 1 L of distilled water in a large flask and autoclave for 20 minutes. When the mixture cools, pour the mixture into individual plates.

Students need a liquid broth culture of the *E. coli* to inoculate their agar plates with bacteria. The culture is prepared by pouring 3 mL of nutrient broth or LB into sterile tubes. Sterile culture tubes can be purchased at a commercial supplier or prepared by the teacher if an autoclave is available. A toothpick is used to transfer the *E. coli* into the broth by touching it to the stock *E. coli*, which could be on a commercially obtained plate or tube, and dropping the inoculated toothpick into the liquid broth. Test tubes should then be incubated at 37ºC overnight. (**Note:** If an incubator is not available, an inexpensive insect incubator could work instead; leaving the media out at room temperature may also work.) When the *E. coli* grows, the broth should become a uniform cloudy consistency. If it does not look like this, contamination may be to blame. Several tubes can be prepared in case one or two become contaminated.

Finally, to dispose of the bacteria after the lab, a 10% bleach solution must be added to the test tubes and agar plates and allowed to sit for one hour. The 10% bleach solution should be made daily, as the bleach will break down. Unused bleach solution can be used to wipe down the lab surfaces after the activity has been completed.

Outcomes

On the second day of this activity, students observe their plates and record, analyze, and share their data (Figure 1, p. 59). Students share their self-designed experiment with classmates by, for instance, drawing it on a whiteboard. Analysis of the different agar plate setups and evaluation of the conclusions reached by the different student groups are discussed and shared in a meaningful and considerate manner.

In the teacher's biology class, student plates consistently showed the halo effect around the squares that had been soaked in colloidal silver. As in antibiotic testing with bacteria, this halo was evidence of bacterial sensitivity. There were no halos around any of the other filter paper squares. Through dialogue, many aspects of experimental design and the process of science were identified and reviewed. Student conclusions of analyzed data demonstrated an understanding of content and a critical analysis of information obtained from different trials done in different ways. For example, one group of students put silver nanoparticles on all of their filter paper squares. Because of this, they could not eliminate other variables as the cause of inhibition (e.g., the filter paper itself or the liquid from the filter paper washing the bacteria away from the soaked filter paper), and therefore learned the importance of using controls in experimental design.

From this lab, students can identify a relationship between bacterial growth and the presence of silver nanoparticles. To investigate the relationship further, students can examine scanning electron microscope (SEM) pictures of bacteria with and without exposure to silver nanoparticles (Figure 2). Evidence of stress in the bacterial population exposed to silver nanoparticles can be seen in its shape. SEM images may be necessary to substantiate the relationship between bacterial growth and the presence of these nanoparticles—this is because the most powerful light microscopes cannot magnify an *E. coli* cell enough for one to see a structural indication of cell death or stress. With careful dehydration procedures, it is possible to take SEM pictures of the cell. Electronic copies of SEM pictures and other resources available for this lab can be found online (see "On the web").

Conclusion

As an extension in the teacher's biology class, a student athlete tested a commercially available athletic sock that incorporates silver nanoparticles in the fabric to inhibit bacterial growth. The foul odor associated with worn socks results from this bacterial growth. This student used good experimental design by wearing a nanoparticle-embedded sock on one foot and a standard sock on the other foot during a workout. He tested for odor after the workout and

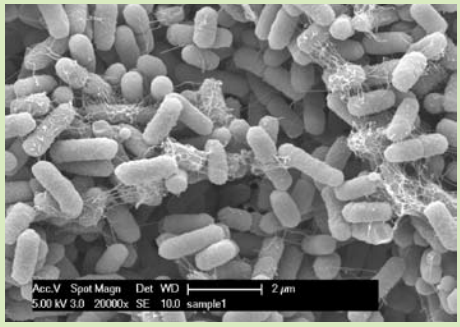
FIGURE 2

Images of *E-coli* cells taken under an SEM microscope.

These images of bacterial cells were taken using an SEM microscope. It is impossible to see nanoparticles using a light microscope because they are smaller than the wavelength of light. Instead of focusing light, an SEM microscope uses a beam of electrons, allowing one to see objects smaller than the wavelength of light.

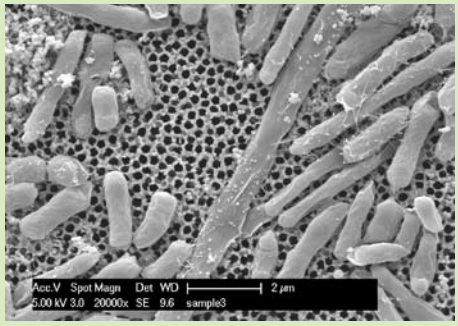
Healthy *E. coli* cells (without silver nanoparticles).

E. coli creates a biofilm, like the one pictured (top right). The weblike structure seen here is a mucus barrier that covers the surface of *E. coli.* It looks very similar to a web when it is dry.



Unhealthy *E. coli* cells (with silver nanoparticles).

In this *E. coli* sample (bottom right), which was cultured in the presence of silver nanoparticles, there are not as many bacteria cells present. Note the honeycomb lattice of the filter used to isolate the bacteria; the nanoparticles can be seen interacting with the surface of the bacteria. All of the bacteria look "deflated" and several are elongated. The elongation is likely the result of inhibited cell division.



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after three days. There was a very obvious difference between the odors detectable from the two socks. The original activity lab ignited interest in further research and laid the groundwork for conducting this research using good experimental design.

Based on the scientific practices that students use in this activity, the learning goals of scientific inquiry are met. We want students to understand the importance of designing controlled experiments to eliminate other variables that could be causing the observed phenomenon. We also want students to critically evaluate data obtained from different experimental designs. Through this activity, students begin to see faults in their own experiments and are able to point these faults out when discussing their results. At the activity's conclusion, students are also able to suggest ways to improve their design. For weeks after the activity, students in the biology class talked about how easy it was to make silver nanoparticles and the different products that incorporate them. ■

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On the web

Nanotechnology Consumer Products Inventory: *http://nanotechproject.org/44*

Nano-CEMMS: *www.nano-cemms.uiuc.edu/content/education/online_labs*

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