Teacher's Preparatory Guide

Assessment of Inhibition of Bacteria by Silver Colloid-Impregnated Bandages

Purpose: This lab is designed to have students develop and implement their own experiment to test the antimicrobial properties of silver nanoparticles. Students will be required to document and communicate the entire process via their scientific notebook. Students will also consider the implications and applications of nanotechnology by evaluating scientific literature.

Time required: Two 50 minute periods

Level: High school biology or chemistry

Big Ideas in Nanoscale Science: Size Dependent Properties; Models and Simulations

Teacher Background: The antimicrobial properties of silver have been documented throughout human history. Silver (Ag) is a natural antimicrobial agent however, it must directly be in contact with the wound to work effectively. Silver interferes with bacterial growth by disrupting the cell membrane, binding to the DNA of cells, and blocking the metabolism of the bacteria. The increased surface area to volume ratio of nanoparticles allows more of the surface to enter into the chemical reaction and in this case, be an effective antimicrobial agent. The use of silver as a "preservative" goes back to ancient times. You may want to read *A Brief History of the Health Support Uses of Silver* at: http://www.purestcolloids.com/history-silver.htm or Silver Nanoparticles – How They Are Providing Environmentally Friendly Antibacterial Properties in Consumer Goods at: http://www.azonano.com/details.asp?ArticleID=1695.

Manufacturers have recently been introducing products that contain silver colloids or silver nanoparticles, marketing its antimicrobial properties. In these products, the silver has been added with the intent of inhibiting microbial growth. One example is silver-impregnated bandages designed to reduce wound infection by inhibiting the growth of bacteria in the wounds. It is not clear that the addition of silver will significantly reduce bacterial growth in this context, so in this project, teachers will prepare their own silver nanoparticles and assess their ability to inhibit bacterial growth in bandages. Commercial silver bandages and bandages impregnated with various silver colloids will be added to various agars and broths to determine if the silver has a significant negative effect on the growth of *Micrococcus luteus*, an example of common skin bacteria. Another common skin bacteria that can be used is *Staphylococcus epidermis*. Comparisons of potential inhibition of microbial growth will be made with Neosporin-impregnated and metal-free control bandages.

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Materials per group (4 students):

- 4 Band-aids: 3 standard band-aids and 1 commercial silver band-aid CURAD© is a company that currently manufactures and sells silver bandages and a silver solution to apply to wounds. Check local drugstores for the product. Many online merchants also carry these products.
- triple antibiotic ointment
- Ag colloid [May use colloid synthesized from "Synthesis and Investigation of Silver Nanoparticles" lab on the NNIN education portal. The nanoparticle sizes from this lab are approx. 12 nm. in size or purchase Mesosilver® which can be obtained at http://www.colloidsforlife.com/ 250 ml. $24.97] or other online sources or your local health and nutrition stores.
- 4 petri dishes
- Agar
- Pure bacteria strain of *Micrococcus luteus or Staphylococcus epidermis.*
- cotton swabs
- goggles and lab gloves

The microbiology supplies were purchased from Flinn Scientific but may also be purchased from Carolina Biological. The list below is for Flinn Scientific:

Catalog No.	Description	Contents	Price
FB0526	Prepared nutrient agar plates	10 prepared plates	19.95
N0019	Nutrient agar	100 g (makes ~ 4 liters	28.95
AB1470	Disposable petri dishes	1 pkg of 20	5.00
LM1007	*Micrococcus. luteus* culture	1 culture tube (screw top)	10.95
AP1051	Inoculating loop	1 wire loop	2.45
AP8328	Forceps	Nickel plated fine point forcep	1.95

Advanced Preparation: Agar dishes can be purchased pre-poured. Bulk agar can be purchased and prepared to pour into sterile petri dishes, which may be more cost effective. Teachers may wish to cut the band-aids beforehand to ensure sterility. From stock culture inoculate broth and allow incubating overnight. Due to exponential growth there will an estimated 10^8 bacteria per ml. Follow the procedure outlined in the figure below for serial dilutions. Prior to the activity, demonstrate how students can sterilize the scissors and forceps. Dip both utensils into a beaker of isopropyl alcohol. Lift both utensils out of the beaker; gently shake off any excess alcohol being careful not to allow any of the

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alcohol to spill onto hands. Pass both utensils over a lighted burner until all the alcohol has been burned off. Urge caution when using an open flame and alcohol.

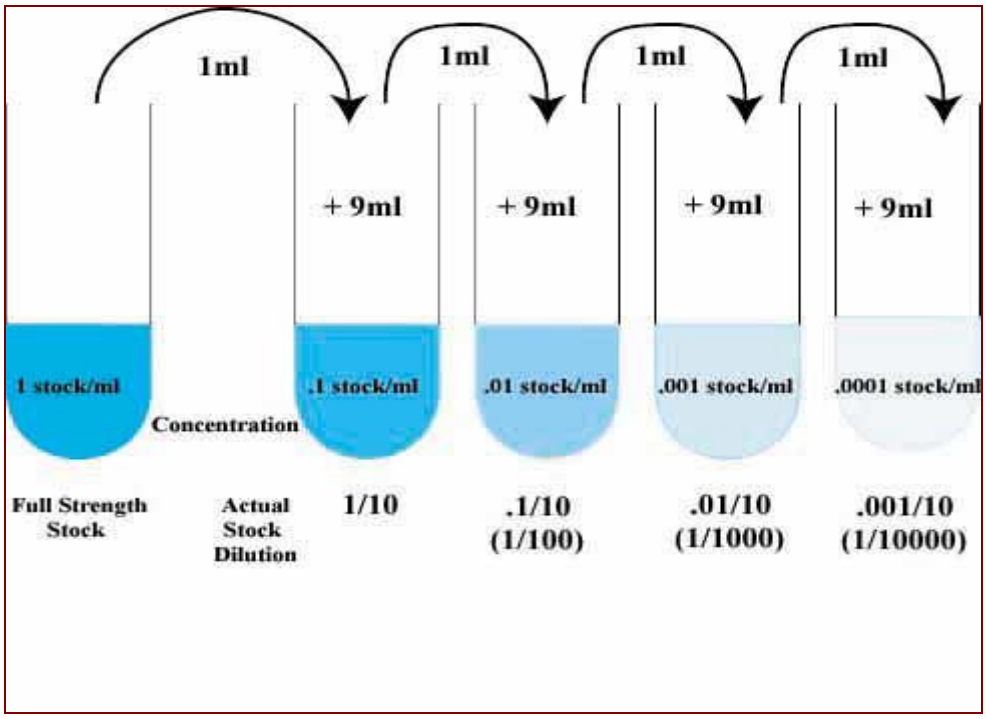

Image source from *Basic Microbiology* - http://xnet.rrc.mb.ca/davidb/applied_microbiology.htm

Safety Information:
Micrococcus luteus and Staphylococcus epidermis are found in soil, dust, water and air, and as part of the normal flora of the mammalian skin. It poses little threat in small concentrations. Students should wear goggles at all times. Latex/nitrile gloves are suggested to maintain a sterile envrionment.

Directions for Activity:
This activity should be approached as an inquiry based activity for students. Present students with the material and have students develop their own experiment to test the antimicrobial properties of silver. Students should have a copy of the attached science notebook form and rubric. Students should complete and submit first side of notebook form before beginning their experiment.

Procedure (from Student Guide):

- Each group of students should be given a test-tube with the bacteria in broth.
- Each group will obtain four petri dishes that have been pre-poured with agar.
- Using sterilized cotton swabs they will spread the bacteria from the test-tube over the entire surface of the agar using the procedure demonstrated by the teacher.
- Using sterilized scissors and forceps, they will cut out the pad of three untreated band-aids and one commercial silver band-aid

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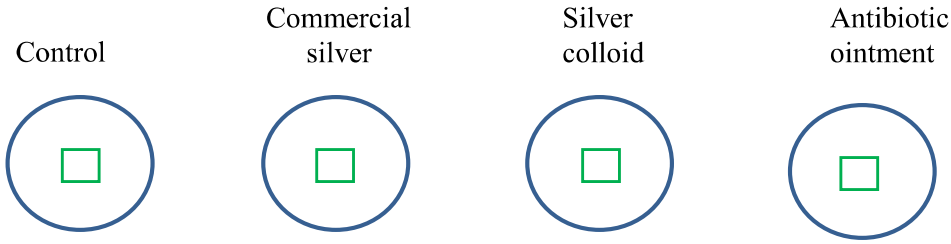
- On one of the untreated pads they will place 2.0 ml of silver colloid, on a second pad place 2.0 ml of triple antibiotic ointment. The third untreated band-aid is the control.
- Using sterilized forceps, students will place each pad face down into its own petri dish. Students will label each dish as shown in the figure below.
- Collect the petri dish and incubate for 24hrs in either a 37° oven or other warm place.



- After 24 hours, using metric rulers, students will measure zone of inhibition under and around the bandage. They will record their data.

Cleanup:
Ideally, petri dishes should be autoclaved before disposal but an effective alternative would be to treat the dishes with a bleach solution. Once treated, the dishes can be discarded.

Resources:
To learn more about nanotechnology, here are some web sites with educational resources:
Nanooze magazine for kids
http://www.nanooze.org/main/Nanooze/English.html
Science News for Kids
http://www.sciencenewsforkids.org
Nanoparticles.org
http://www.nanoparticles.org
Silver as an Antimicrobial Agent
http://microbewiki.kenyon.edu/index.php/Silver_as_an_Antimicrobial_Agent#Current_uses
General information:
http://www.nano.gov
http://www.education.nnin.org
Microbiology techniques and information:
http://xnet.rrc.mb.ca/davidb/applied_microbiology.htm

National Science Education Standards
- Content Standard A
  - Abilities necessary to do scientific inquiry
  - Understanding about scientific inquiry
- Content Standard B
  - Structure and properties of matter
  - Chemical reactions

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- Content Standard C
    - The cell
    - Behavior of organisms
- Content Standard E
    - Abilities of technological design
    - Understandings about science and technology
- Content Standard F
    - Natural and human-induced hazards

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