Antimicrobial Agents

Background on Antimicrobial Agents

Each year, over 17 million people die from microbial diseases around the world. For example, MRSA is a microbial disease that killed an estimated 19,832 people in 2017. Because microbial diseases can be deadly, microbiologists are researching antimicrobial agents. Antimicrobial agents are substances that can kill harmful microbes. An example of an antimicrobial agent is an antibiotic. An antibiotic is a drug that can kill bacteria, which are microbes. You might have taken antibiotics if you have had strep throat!

How Antibiotics Work

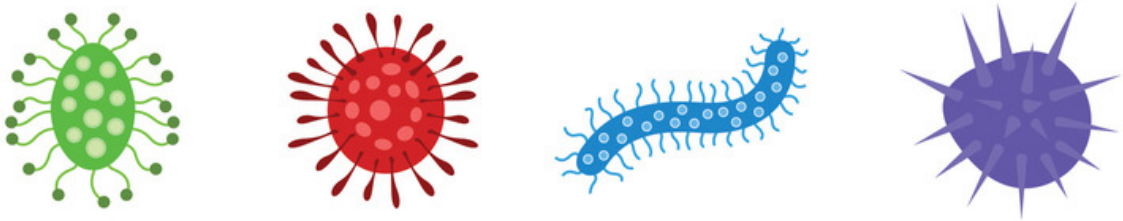
Antibiotics work on a cellular level to kill bacteria. Some antibiotics disrupt protein synthesis. Protein synthesis occurs when cells create proteins to help the bacterial cell grow and reproduce. When protein synthesis is disrupted, bacteria cannot properly develop or reproduce. The antibiotic can also disrupt bacteria's DNA. When protein synthesis or the DNA of the cell is disrupted, the bacterial cell will die because it cannot develop or reproduce.

Antibiotic Resistance

Even though antibiotics are effective at preventing bacteria growth, some bacteria can actually develop resistance to antibiotics. This means that an antibiotic that was once able to kill off bacteria no longer can. Instead of the antibiotic making the person healthy again, the bacteria continue to replicate and infect people.

Once the antibiotic resistant bacteria multiply, the resistance is passed on, and the new bacteria are also resistant to that particular antibiotic. In a short amount of time, millions of individual bacterial cells will have grown with resistance—meaning that the entire population of bacteria will be resistant to the antibiotic!

Antibiotic resistance is a serious problem. Bacteria that have gained extensive resistance to different antibiotics are called superbugs. Once a person or animal is infected with a superbug, there is little doctors can do to treat the infection using antibiotics.



Antibacterial Properties of Silver

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Alternative to Antibiotics

Scientists are looking for new ways to stop the spread of bacteria growth. Microbiologists have identified that silver nanoparticles can be used as an antimicrobial agent. Silver nanoparticles (AgNPs) are tiny particles of silver. AgNPs are so small that they can enter bacterial cells to target and kill the cells.

Silver nanoparticles, or AgNPs, kill bacterial cells in three main ways:

- AgNPs can stick to the cell membrane of the bacterial cell. The cell membrane's function is to exchange nutrients and waste with the outside of the cell. When the AgNPs attach themselves to the cell membrane, the bacterial cell has much more difficulty exchanging nutrients. The cell cannot uptake nutrients from the outside of the cell and cannot get rid of wastes from inside the cell. With all of the waste stuck inside the cell, the cell can become too full, and rupture, killing the cell.

- When AgNPs attach themselves to the cell membrane, they create small holes. The AgNPs are small enough that they can squeeze through these holes and get inside the cell. Then, the AgNPs hunt for proteins inside the cell. DNA is made of proteins, so the AgNPs attack it and cause it to destabilize. This causes harm to the bacterial genome. Once the genome is harmed, the bacteria can not make copies of it to reproduce. This is called genotoxicity. AgNPs stop the reproduction of bacterial cells.

- AgNPs cause oxidation, or the release of electrons. The AgNPs oxidize enzymes inside of the bacterial cell. The bacteria use the enzymes to retrieve oxygen from the environment. The AgNPs destroy these enzymes, ripping them apart, which releases a free electron in the cell. The electron acts like a ricocheting bullet and tears apart anything in its path. The oxidation of the enzyme prevents the bacteria from acquiring oxygen, so the cell suffocates and dies before it can generate more enzymes.

Silver nanoparticles are a promising alternative to antibiotics. However, different concentrations of nanoparticles have varying effects on different strains of bacteria. As a result, it is important to determine which concentration is most potent for the bacteria of interest.

Silver Nanoparticle Research

Microbiologists have studied using silver nanoparticles as an antimicrobial agent because of its ability to destroy microorganisms. Silver nanoparticles can be used in many different applications. Here are some examples:

Bandages and Dressings for Wounds:
Silver nanoparticles infused into bandages and wound dressings significantly reduce rates of bacterial infections.

Surgical Masks:
Surgical masks worn by surgeons, nurses and other medical professionals infused with silver nanoparticles eliminate bacteria originating from the mouth. Additionally, the masks did not exhibit any side effects or irritation to the users.

Endotracheal Tubes:
Endotracheal tubes assist people unable to breathe on their own. Silver infused tubes reduce bacterial infections that can occur in these tubes, such as pneumonia.

Food Packages:
Silver lined packaging prevent bacteria from growing, keeping food fresh for longer periods of time.

Cotton Fibers:
Silver nanoparticles integrated into cotton fabrics significantly reduce the presence and spread of bacteria in clothing.

When microbiologists conduct research on silver nanoparticles, they use different concentrations of the AGNPs to determine how effective they are at preventing the growth of bacteria. Microbiologists must also consider that different concentrations of AgNPs are more effective at killing certain bacteria. For example, one concentration of AgNPs might be extremely effective against E. coli, but minimally effective against a strain of Streptococcus bacteria.

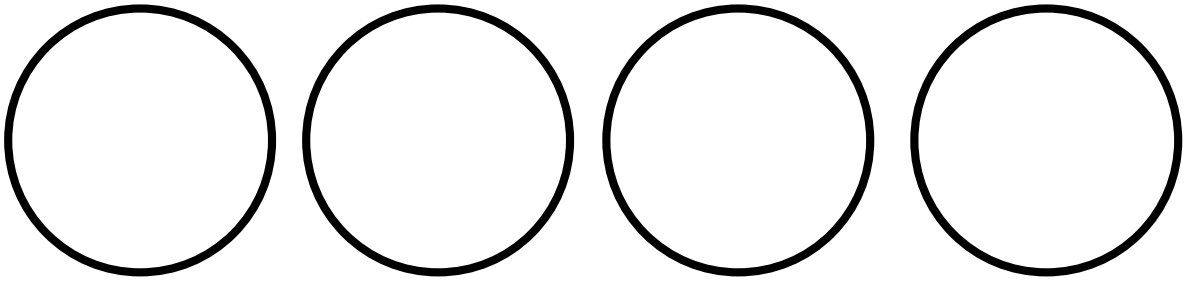
Here are some examples of different applications that require different concentrations of AgNPs:

- Diagnostic Applications: AgNPs can be used as a biological tag. This means that the nanoparticles are given to a patient, typically through injection. The AgNPs can then be observed traveling through the bloodstream with the use of a radiology device, such as an x-ray or MRI machine. The nanoparticles can make it easier to see damage to a person's internal organs, veins, ligaments, or tendons. The concentration of AgNPs varies in this application. A higher concentration means that the biological tag will last longer in the person's body, which enables doctors or scientists to make observations for a longer period of time.
- Optical Applications: AgNPs can be used in sensors that detect light. This is because AgNPs are very efficient at absorbing and scattering light. When white light is shone onto the silver nanoparticles, they emit a bright blue color. This application doesn't require a high concentration of AgNPs, because the AgNPs are very efficient.
- Conductive Application: AgNPs can be used as a conductor to help enhance thermal and electric conductivity. This application requires a high concentration of AgNPs. This is because wires are created using AgNPs, or a coating for the wire is created. This helps the wire conduct heat and electricity more efficiently.
- Antibacterial Applications: AgNPs can be used anywhere that bacteria exist! The AgNPs can be used in apparel, shoes and socks, bandages, makeup, face wash, paint, cleaners, and even appliances, such as meat grinders and ovens. The antibacterial properties of the AgNPs are then transferred to the object. This helps kill bacteria that are on the item, which keeps us safe from bacteria on our clothing, bandages, cosmetics, and homes. The concentration of AgNPs in the"
-]se situation varies, as it depends on the object that is being created to use the AgNPs. For instance, a different concentration is used on an oven versus a pair of socks!

Overview of Experiment

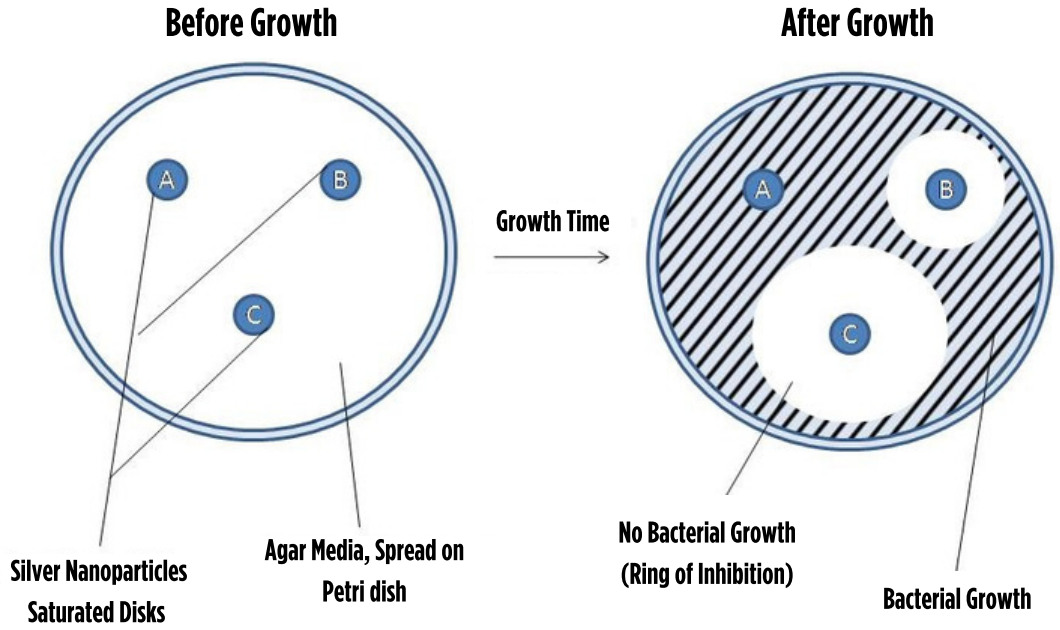
You will design an experiment to test the effects of different silver nanoparticle concentrations on the growth of bacteria.

The experiment will contain 4 agar plates with bacteria. One of these plates is a control, which has no silver nanoparticles added to it. The other three plates will contain different concentrations of silver nanoparticles. You will add the concentrations to each plate and then wait to see how much bacteria growth occurs.



Control **Concentration 1** **Concentration 2** **Concentration 3**

After the bacteria has had time to grow, you will use a ruler to measure the "ring of inhibition" around the silver nanoparticles. A ring of inhibition is an area where bacteria growth is inhibited by the silver nanoparticles. The larger the ring, the more effective the nanoparticles were at inhibiting bacterial growth.



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Designing an Experiment

Step 1: Brainstorm Ideas and Identify Variables

In this step, you will design an experiment to test the effects of different silver nanoparticle concentrations on the growth of bacteria.

- What is the independent variable in this experiment?
  - The independent variable is also called the manipulated variable.
  - This is the variable you purposely change or manipulate.
  - This variable will be the cause of the changes you measure in the experiment.
    - The independent variable in this experiment is:

- What is the dependent variable in this experiment?
  - The dependent variable is also called the responding variable.
  - This is the variable that responds in the experiment.
  - This is the variable you will measure after the experiment is set up, and will be the effect of the action you took.
    - The dependent variable in this experiment is:

- What variables do you need to control for?
  - These variables are factors that we want to remain the same throughout the experiment.
  - They are controlled to remain constant, so as to not affect the dependent variable.
  - Controlling these allows us to see how the independent variable affects the dependent variable.
    - The variables we need to control for are:

- Write a testable hypothesis for this experiment. Use information from the handouts as a basis for your hypothesis.
  - A hypothesis describes what you think is going to happen in the experiment.
  - Here is the format for a hypothesis:
    - If (the independent variable) is (increased, decreased, changed), then (the dependent variable) will (increase, decrease, change.), because (logical reasoning for what I think will happen and why)
    - Example: If the sunlight is increased, then the height of the plants will increase, because plants need sunlight to survive.
      - My hypothesis for this experiment is:

Step 2: Calculating the Silver Nanoparticle Solutions

In this step, you will use math to calculate the volumes of silver nanoparticles and water needed to create three different concentrations.

- Choose three concentrations of silver nanoparticles to use for the experiment. The concentration levels you choose must be between 50,000 - 500,000 micrograms/liter.
  - Concentration 1: _____
  - Concentration 2: _____
  - Concentration 3: _____
- You will be mixing the silver nanoparticles with water to dilute them. The amount of water that you add changes the concentration of the silver nanoparticles. Use the formula below to figure out the amount of water and silver nanoparticles needed to create each concentration.

Formula: $C_1 V_1 = C_2 V_2$

- C_1 = Concentration of the original solution.
  - For this experiment, the concentration of the original solution is 500,000 micrograms/liter
- V_1 = Volume of silver nanoparticles.
  - This is the number that we are solving for.
- C_2 = Concentration of the new solution.
  - This is the concentration that you chose in the first step.
- V_2 = Total volume of solution (silver nanoparticles + water)
  - All three of your solutions should have a final volume of 25 mL

First, solve for V_1. This will give you the volume of silver nanoparticles that are needed for the concentration.

Then, calculate the difference between V_1 and V_2 to determine the amount of water needed.

- Here is an example:
  - Concentration Desired: 40,000 micrograms/liter
    - C_1 = 500,000 micrograms/liter
    - V_1 = ?
    - C_2 = 40,000 micrograms/liter
    - V_2 = 25 ml

$$500{,}000\ (V_1) = 40{,}000\ (25)$$

$$500{,}000\ (V_1) = 1{,}000{,}000$$

$$V_1 = \frac{1{,}000{,}000}{500{,}000}$$

$$V_1 = 2\text{ml}$$

You would need 2ml of silver nanoparticles to make a solution with a concentration of 40,000 micrograms/liter.

Antibacterial Properties of Silver

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Use the space below to perform the calculations for each concentration you want to test:

Concentration 1:

Volume of silver nanoparticles needed: _____

Concentration 2:

Volume of silver nanoparticles needed: _____

Concentration 3:

Volume of silver nanoparticles needed: _____

Step 3: Mixing the Silver Nanoparticle Solutions

Follow the steps below to mix the silver nanoparticle solutions.

1. Wash your hands.
2. Wear safety gloves and goggles.
3. Sterilize work area with the bleach solution provided by your teacher.
4. Label three glass jars with the concentrations of silver in micrograms/liter you have chosen to test.
    a. Example: 50,000 mg/L
5. Label a fourth glass jar as "water."
6. Based on the measurements that you calculated in Step 2, use a graduated cylinder or syringe to add the correct amount of water to each jar.
    a. Be sure there are no air bubbles in the liquids when using the syringe. If there are, release the liquid, and try again.
7. Based on the measurements that you calculated in Step 2, use a syringe to add the correct amount of silver nanoparticles to the jar.
    a. Be sure there are no air bubbles in the liquids when using the syringe. If there are, release the liquid, and try again.
    b. Be sure to get a new syringe for each concentration to avoid cross contamination and changing the concentrations in the jar.
8. Use the syringe tip to swirl around the liquids. You can also use the syringe to pick up and release the liquid back and forth into the jar.
9. Dip the tweezers into isopropyl alcohol to ensure that they are completely clean. Wave in the air to dry.
    a. Do NOT blow on the tweezers to dry them.
10. Use the tweezers to add 3-5 sterile disks into each jar and make sure they are submerged.
    a. Clean the tweezers in the isopropyl alcohol and wave them dry before moving to the next jar to avoid cross contamination.

Step 4: Inoculate Agar Plates with Bacteria

Follow the steps below to add bacteria and the silver nanoparticle solutions to the agar plates.

1. Put on safety goggles and gloves.
2. Use a permanent marker to label each agar plate with the concentration of silver you will be placing on it. Then, label the agar plate that is the control. Add your initials to each plate.
3. Remove the lids to the agar plates. Do not touch the agar with your fingers or other objects.
4. Have one of your group members hold the lid to the agar plate. Don't set the lid down on any surface because this could contaminate the lid.
5. Dip a sterile cotton swab into the bacteria.
6. Wipe the swab onto the agar starting at one end and create a straight line down the middle of the plate.
7. Go back to the top and weave the swab back and forth between each side until you get to the bottom of the plate.
8. Turn the plate 90 degrees and repeat the last two steps with a second dose of bacteria to be sure the entire plate is covered.
9. Use this technique to cover the other three agar plates with bacteria.

Step 5: Add Silver Nanoparticles to the Agar Plates

Follow the steps below to add silver nanoparticles to the agar plates.

1. Dip the tweezers in isopropyl alcohol to make sure that they are clean from Step 3.
    a. Make sure that you clean the tweezers between each transfer of the disks.
2. Match the glass jar labeled “Control” to the agar plate labeled “Control”.
3. Using the tweezers, remove one disk from the jar and place it on the corresponding agar plate.
4. Repeat this process for the other two disks in the same jar.
    a. The disks should be placed an equal distance apart on the agar plate.
5. Repeat steps 1-4 for the other three jars.
    a. Be careful that you are matching the labels on the agar plate and the corresponding glass jar.
6. Once you are finished transferring the disks, cover all of the plates and TAPE THE DISH CLOSED.
7. Wait a few minutes for the disks to adhere to the agar.
8. Carefully carry the agar plates to a fume hood or other safe, well ventilated spot in the classroom to store them. The storage time will allow the bacteria time to grow.

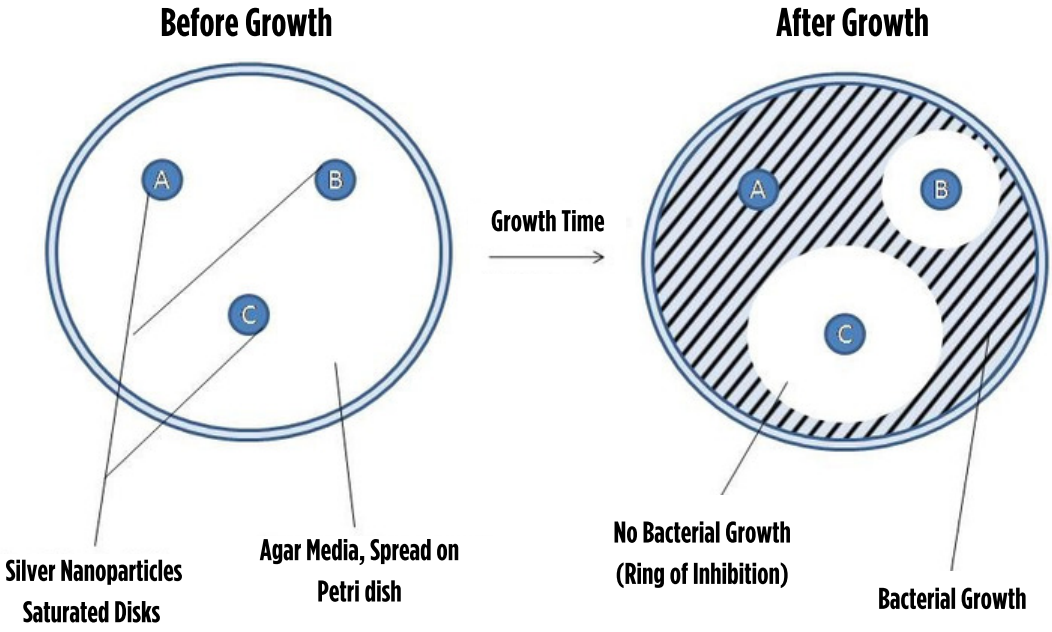
Experiment Observations

Follow the steps below to make observations of your experiment.

Step One: Group Data

- Look at the bacterial growth of the control group versus the others. Draw or write down any differences observed in the space below.

- Determine whether there are rings of inhibition on any of the plates. If there are rings present, you will see a ring of agar around the edge of the disk. On the outside of the ring, the bacteria will be growing. If there is a ring present, there will not be any bacteria growing inside the ring around the disk.



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Antibacterial Properties of Silver

- For each agar plate, measure the zone of inhibition using the ruler. Measure from the edge of the disk to the outer rim of the ring. Then, calculate an average size for each plate.
- Important note: place the ruler on the lid of the plate. Do NOT OPEN THE LID. If there is no zone of inhibition, write "0" in the data table.

Concentration of Silver Nanoparticle Solution	Average Size of Ring of Inhibition (mm)

- Write a conclusion that describes your data. Think about how the results vary based on the concentrations that you used in your experiment.
- Did the results of your experiment confirm or deny/falsify your hypothesis?

Step 2: Class Data

- Look at the results of the entire class experiment. Analyze the data for patterns and trends.
- Discuss the following questions with your group:
  - Are there any outliers in the class data (any rings that are extremely large or extremely small)?
  - What do you think these outliers mean?
  - Did the results of the class confirm or falsify your hypothesis?
  - If silver is effective on preventing bacterial growth, why do you think doctors don't use it for every surgery?
- Based on the new data, write a new conclusion. Think about how the results varied based on the different concentrations used in the entire class.

Class Data Sheet

Name of Student(s)	#1 Silver nanoparticle concentration (micrograms/liter)	#1 Average size of ring of inhibition (mm)	#2 Silver nanoparticle concentration (micrograms/liter)	#2 Average size of ring of inhibition (mm)	#3 Silver nanoparticle concentration (micrograms/liter)	#3 Average size of ring of inhibition (mm)	Average size of ring of inhibition control group (mm)