Mission Folder: View Mission for 'Silver Bullet'

State	Texas
Grade	7th
Mission Challenge	Food, Health and Fitness
Method	Scientific Inquiry using Scientific Practices
Students	anthrobones dinosaur24 silvershade15 skittles02

Team Collaboration

(1) Describe the plan your team used to complete your Mission Folder. Be sure to explain the role of each team member and how you shared and assigned responsibilities. Describe your team's process to ensure that assignments were completed on time and deadlines were met.

The United States is one of the wealthiest nations on Earth, yet serious health issues still affect many Americans. A crisis is mounting as the number of people diagnosed with diabetes skyrockets. More than 29 million people are infected with this disease, and developing a chronic wound that refuses to heal is an expected complication. Diabetics are particularly in fear of wounds because if they wait too long, it can get to the point of no return and eventual amputation. This is just one of the very scary and common problems for diabetics across the country. As middle school students, we studied the facts and as if that was not reason enough to work on the problem, each of us knows someone personally who suffers from diabetes and who has experienced issues with wounds. For these reasons, the team selected this challenging national issue.

Silver Bullet is a team of seventh graders that began work in April of 2015. We began studying a substance known as bacterial biofilms that are the root of the problem in chronic wounds. This Ecybermission project seeks to build awareness of biofilms, infections, and contribute to advancing a way to fight the problem.

Our team is involved in many different school activities such as football, basketball, science/math competitions, music, and theatre. We had to find time in our schedules when everyone could meet. Our school has set aside time for extra help on Wednesday afternoons and we would meet then to share research, conduct experiments, and work with community partners on the project. We also met on Sunday afternoons in the school science lab for experimentation and after school as needed so that deadlines were always met. Several field trips were taken during school because the principal is supportive of STEM. Everybody was very determined to find an answer to the problem of chronic wounds.

The plan to complete the project was to meet regularly, assign tasks and hold ourselves to deadlines, search for experts who would allow us to collaborate with them and use their labs, and agree to fight procrastination. The team completed the Mission Folder answers by dividing the questions into groups, and assigning the questions as evenly as possible to each person, while keeping in mind the strengths and weaknesses of the team members. When there were conflicts about what to do next or how to read the data, the team talked through all possible answers and then came to a unanimous decision. Since team members have completed projects before, working together and maximizing time was not difficult.

Each team member worked hard on each aspect of the project. We followed our action plan, kept a binder in the classroom that kept everyone on track, and each person helped the others stay focused. Other Ecybermission teams at our school did not finish, but it was great to be part of this team!

To encourage each other this year, we kept a positive attitude during team meetings and never allowed put-downs of anybody or any idea. We also complimented each other on ideas, even if we personally thought there was a better way to do something. Together, we celebrated successes along the way, texted each other, and talked about the project all year. In all these ways, team members encouraged one another to finish the project.

During team meetings, a deadline was assigned for all questions to be completed and turned in to our Team Advisor so it could be read by an adult. Suggestions were made for editing and then the Team Advisor submitted the final copy of the Mission Folder with the uploaded files as evidence of the work we accomplished this year. The experience of being part of Ecybermission and working towards a common goal was exciting and allowed many opportunities to work in university laboratories and meet with doctors that became role models.

Elizabeth fulfilled the role of leader, organizer, writer, and artist. She scheduled meetings and kept the team informed of upcoming events and deadlines.

Kaden's roles included data analyzer, creative thinker, troubleshooting, research online, and presentations.

Blade was the dreamer, public speaker, team motivator, website builder, and took charge during in vitro and in vivo testing.

Kaylah was in charge of interviews, public speaking, engineering hydrogels with silver, and research.

A key factor in the ability to complete the mission was teamwork.

Please open the table showing the specific roles and responsibilities of each member, the timeline for the project, and an overall view of the project in the uploaded pdf files titled “Team Responsibilities,” “Action Plan,” and “Timeline.”

Uploaded Files:

- [View] **Team Responsibilities** (By: dinosaur24, 02/24/2016, .pdf)

  A chart was created to show the roles and responsibilities each of us fulfilled during this project.
- [View] **Action Plan** (By: anthrobones, 02/27/2016, .pdf)

  A graphic organizer and flow chart was created to show how our team went from a community problem, to a series of hypotheses, to a set of experiments, and finally to community solutions to the problem of chronic wound care.
- [View] **Timeline** (By: anthrobones, 02/27/2016, .pdf)

  A timeline of the project is included to demonstrate progress from April 2015 - May 2016. Even though the competition deadline is February 29, there are exciting events scheduled throughout the spring.

Scientific Inquiry

Problem Statement

(1) What problem in your community did your team try to solve? Why is this problem important to your community?

Oil field workers, welders, farmers, and ranchers – these are the working people of our community. According to Covenant Hospital, they are also the people who suffer from multiple injuries and oftentimes have wound issues. While most wounds are relatively harmless and heal quickly, an unattended wound on an individual with compromised health can become a serious infection.

Chronic wounds are rarely seen in individuals who are healthy. In fact, most people suffering from chronic wound complications are affected with other diseases such as obesity or diabetes. The United States National Institutes of Health states that chronic wounds affect around 6.5 billion people. For diabetics, the leading health concern in this state, the unfortunate solution is oftentimes amputation or death.

According to Centers for Disease Control and Prevention, over 29 million Americans suffer from diabetic wounds over the years. This shocking statistic was the motivation for selecting this community problem. In a county with a high rate of poverty, a large elderly population, and diabetes, finding a solution for healing wounds would have a lasting change on this community and many others. The National Institutes of Health states that $25 billion is spent to treat life-threatening wounds. It goes without saying that even though healing wounds is expensive, having a chronic wound healed is worth every penny.

Problem Statement: Can an affordable and effective material and bandage be developed to aid in the control and treatment of diabetic chronic wounds?

(2) List at least 10 resources you used to complete your research (e.g., websites, professional journals, periodicals, subject matter experts).

Chronic wounds are an issue in this community and throughout the nation. The team began research by identifying subject matter experts, community leaders, and organizations near our town that would provide a strong foundation for this project. The information came from webinars, TED talks, newspapers, online podcasts, interviews, professional journals, emails, websites, and newspapers. Government agencies and independent studies were trusted research sites. The hypotheses and experiments are based on what was learned from these sources in hopes for a solution that will benefit many individuals. Please see three uploaded files “Bibliography,” “Collaboration with Community Experts,” and “Contact List” for evidence of thorough and valid research.

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(3) Describe what you learned in your research.

Biodegradable Bandages --- Biodegradable bandages are being created that act as a new skin forming over the wound. First the doctors attach the patient to a scaffold before the dressing is applied over the wound. Then the doctors take a biopsy of the patient’s skin cells. The skin cells multiply and grow over the scaffold so when the bandage eventually dissolves, all you have left is the patient’s own skin cells. What if this technology could be combined with the hydrogel technology and wounds could be covered with medicated and moist bandages that would never need to be removed? If that happened, biofilms might not be able to get ahead.

History of Wounds --- Wound healing is an important process that scientists have been trying to make better for hundreds of years. Natural and artificial ways of wound healing exist as doctors fight biofilms and infection. For replacing tissue in chronic wound patients, scientists are turning to biocompatible and biodegradable polymers. These are alternatives to painful skin grafting, in addition to tissue engineering to speed up wound healing.

Herbal Uses --- As an accelerant of wound healing (to speed it up), polymer solutions were spread onto cotton gauze and Bletilla striata, an herb, was added to the gauze to increase wound healing. The cotton gauze was tested and showed to have rapid success when closing up the chronic wound.

Bandages --- Bandages for wounds come in many shapes and sizes but there is a new kind of bandage called a “hydrogel” that may speed up the healing process. It has several advantages that others do not such as being very flexible so it will stay in place no matter where the wound occurs. It is made of a material that is mostly water but it is also stretchy. Since the human body is wet and soft, this bandage is more similar to the needs of the wound. What if hydrogels could be injected with medications that would fight biofilms? If so, the hydrogel could be placed on the wound, it would stay in place and keep the wound moist, and the material would fight biofilms and infections they cause.

Biofilms --- Biofilms are like communities of bacteria but they are very well-organized. They stick to each other and they are microorganisms sometimes called “slime” in the medical field. Usually they are on the surface of tissues and also on surfaces in hospitals or other settings. Biofilms cause more than 65% of infections with their polysaccharide covers. The problem with biofilms comes when it allows the bacterial colony to communicate with each other and fight any kind of treatment that a doctor may be applying. Some antibiotics produce glycogen and that gives the biofilms plenty of food. Sometimes, what a person thinks is an antibiotic resistant infection that will not clear up, may really be the result of biofilms working overtime.

Resistance to Antibiotics --- Bacteria can be grouped as “gram negative” or “gram positive” and it depends on what kind of cell walls they have. Some can be stopped by certain kinds of antibiotics but according to an immunology professor at the University of British Columbia Bob Hancock, “Our entire arsenal of antibiotics is gradually losing effectiveness.” This is where the problem of chronic wounds comes in – antibiotics cannot always get rid of the infection in that wound because the biofilms are so good at keeping them out. Since many antibiotics are becoming ineffective in the fight against infection, what about trying different types of materials that the biofilms and bacteria may not have seen before?

Biomaterial-based Dressing --- Closing wounds and burns completely can be done through a method called autografting that moves skin into a new position on the body from which it was removed. However inadequate donor areas for large led to a new search for a new tissue source. Biological dressings are natural dressings with collagen-type structures.

Water Based Dressing --- Scientist Xuanhe Zhao has designed a new Water-based bandaid that will help promote healing faster. First he makes sure that the bandage will stay bonded to really flexible areas of the body such as a knee or elbow. Then Zhao gets the hydrogel which is a rubbery material mostly composed of water. Zhao then connects the conductive wires to the bandage with the semiconductor chips, and temperature sensors. Electronics are usually hard and dry but in this case the human body is soft and wet. These two systems have drastically different properties. So when you make the electronics devices you want to make them soft and stretchy to fit the environment of the human body. His team came up with a way to use hydrogels by mixing water with biopolymers to create stretchy materials with also stiffness so they will hold. Zhao also added LED lights that could attach to different sections of the body.

Hypothesis

(4) State your hypothesis. Describe how your hypothesis could help solve your problem.

From research to interviews, several substances were chosen to be tested in the development of a wound ointment and enhanced bandage. The materials were selected based on their properties and potential to fight biofilms surrounding bacteria in wounds. The following would be used in forming the hypotheses:

Colloidal silver is an antibacterial material chosen because of its astonishing ability to control antibiotic resistant superbugs in other tests. In addition, silver is antimicrobial and anti-inflammatory, therefore it would possibly be a key in healing stubborn wounds.

Xylitol was recommended by Dr. Wolcott because it is easily accessible for use in the lab. Bacteria in wounds often feed on glucose and since xylitol is a sugar alcohol, it blocks the energy-producing pathway of the bacteria - basically robbing it of food. According to the research, xylitol may eliminate harmful bacterial colonies by 27% to 75% in the mouth, therefore it would be tested in vitro for possible success in topical wounds.

Probiotics were selected because it is a well-known fact that probiotics add good bacteria to the gut and can fight off bad bacteria internally. However, after visiting an Ecybermission recommended site called LiveScience, the team learned some evidence is linked to its benefit as a topical treatment as well. A common probiotic, fructooligosaccaride, was selected for in vitro experimentation.

The following hypotheses were written to specifically address the problem statement. They are able to be measured quantitatively and qualitatively, repeated by anyone for reliable results, and are research-based to be valid.

Hypothesis 1 - In Vitro Experiment: If a composite material of xylitol plus colloidal silver is created and is effective in preventing the spread of bacteria during in vitro experimentation, (as quantified by measuring zones of inhibition), then the substances could be useful as a topical ointment for chronic wounds.

Hypothesis 2 -In Vivo Experiment: If a material can successfully lead to cell regrowth, as evidenced by in vivo experiments by decreasing the length of incisions on invertebrates, then the substance could be used to promote healing of chronic wounds.

Hypothesis 3 – Bandage Enhancement: If colloidal silver, xylitol, silver/xylitol composite, or probiotics could be successfully applied within a bandage, then the enhanced bandage could be more effective in sealing wounds from airborne bacteria and in fighting biofilms and infection with its embedded ointment.

(5) Identify the independent variables and the dependent variables in your hypothesis.

For hypothesis 1 - In Vitro Experiment - The independent variable is the material applied to the Petri dish agar to fight bacteria. This includes colloidal silver, probiotics, xylitol, and the xylitol/silver composite. The dependent variable is the size of the zone of inhibition measured in millimeters surrounding each of the materials within the Petri dishes.

For hypothesis 2 -In Vivo Experiment – The material most effective in preventing bacterial growth during the in vitro experiment will become the independent variable in this experiment – either silver, xylitol, probiotics, or the silver/xylitol composite. The dependent variable is the size of the invertebrate's wound as measured in millimeters over a period of time.

For hypothesis 3 – Bandage Enhancement: The independent variable is the kind of ointment applied to the bandage, and the dependent variable is the amount of ointment remaining in the bandage over a period of time.

(6) How did you measure the validity of your hypothesis? (In other words, how did you determine that your hypothesis measures what it is SUPPOSED to measure?)

In vitro hypothesis validity - If the zone of inhibition surrounding the applied materials (silver, xylitol, xylitol + silver, or probiotic) is at least 10 mm in diameter, the hypothesis will be proven valid. This would indicate a clear area free of bacterial growth as a result of the material application. Measurements will be made using millimeters from the center of a material-soaked disk outward to the nearest bacterial colony present. The disks are used to control the size of application of the material within the Petri dish for accurate and reliable measurements. According to Dr. Rumbaugh in the Texas Tech wound care laboratory, a zone at least double the size of disk application would indicate a successful treatment.

In vivo hypothesis validity – The validity of the hypothesis will be proven if the length of the incision on the earthworm decreases over time as a result of the applied material. Testing will only be conducted on earthworms and NOT on vertebrate organisms. Care will be taken to conduct the experiment in a professional and careful manner, knowing that even invertebrates are animals to be respected.

Bandage enhancement validity – The validity of the hypothesis will be supported if at least 80% of the healing material is able to be embedded in the bandage for application. If the bandage is able to keep the material as part of its structure, then the ointment would be available on the wound surface and could promote healing.

Experimental Design

(7) List the materials you used in your experiment. Include technologies you used (e.g., scientific equipment, internet resources, computer programs, multimedia, etc.).

In Vitro Experiment ---

Pipette
Gloves
Goggles
Petri dishes
Saucepan and wood spoon
Hotplate
Triple beam balance
Beakers
Thermometer
Scoop
Incubator
Distilled Water
Graduated Cylinders
Metric measuring spoons
Coffee Filters
Hole Punch
Alcohol for sterilizing equipment
Nutrient agar
Colloidal Silver – 500 ppm
Xylitol Crystals
Fructooligosaccaride – Probiotic

In Vivo Experiment ---

Colloidal Silver – 500 ppm
Xylitol
Alcohol for sterilizing equipment
Metric measuring spoons
Graduated Cylinders
Distilled Water
Thermometer
Beakers
Triple beam balance

Earthworms, Lumbricus terrestris
Scalpel
Forceps
Metric ruler
Pyranine
Yellow Highlighter Markers
Black Light
Syringe and needle
Sterile bowls
Soil
Goggles
Gloves
Pipette

Bandage Enhancement ---

Hydrogel
Pads
Syringe and needles
Distilled water
Beaker
Metric measuring spoons
Silver
Pyranine fluorescent chemical

Technology Used ---

Compound Light Microscope
Apple Computer
YouTube instructional videos
TED X Talks – microbiology
Black light
Mac Book
Cell phone
Incubator
Digital Camera
Microsoft ExCel
Microsoft PowerPoint
Microsoft Word
Google SketchUp
Weebly Website Builder
Google Docs
Google Drive

(8) Identify the control group and the constants in your experiment.

In vitro Experiment

Control – There are often two controls used when conducting tests on bacterial growth and this experiment utilized both controls. The first control is a Petri dish containing only nutrient agar and no bacteria. The second control is a Petri dish containing nutrient agar and bacteria, but lacking a substance to control its growth. Both control dishes will be placed alongside the test samples for all three trials/tests being conducted.

Constants – Constants are the quantities in the experiment that are not allowed to be changed, either because they cannot be or because the scientist does not choose to change them. Constants can be physical (such as pi, average body temperature, boiling point of water) or can be control constants that are kept the same for the purpose of having an experiment that can be reproduced by someone else (such as holding the temperature steady, or the length of time the experiment is run, or volume of liquids tested).

The physical constant in this experiment is bringing water to a boil at 100oC for preparing the agar properly. The control constants include following the written procedures exactly for all three trials; maintaining the incubator at a temperature of 37oC to mimic body temperature for bacterial growth; using the same size disks for each material; collecting the data 16 hours after incubation for all tests; using the same amount of water and agar for preparing the Petri dishes; adding the same amount of agar to each dish; soaking the filter paper in the same amount of each material for the same amount of time; using standard-sized Petri dishes divided into equal quadrants for multiple trials; measuring the zone of inhibition from the center of each paper disk outward to the first colony of bacteria present.

In vivo Experiment

Controls – Earthworms with incisions the same length as that of the three test subjects will be used to compare the effectiveness of the colloidal silver. There will be no ointment applied to the control earthworm.

Constants – The control constants include using isopropyl alcohol to clean scalpels for exactly 45 seconds each time before making any incisions; making a 1.0 centimeter incision in each earthworm tested; measuring the length of the incision using a flexible dissecting ruler each time; measuring the control and the test subjects within the same hour; applying the same amount of silver ointment 0.5 ml to each incision.

Bandage Enhancement Experiment

Controls – Hydrogel pads without materials or ointment added to the pad will be used as the control.

Constants – The amount of colloidal silver injected into the hydrogel will be kept at 1.0 ml; the temperature of the conditions will be 25oC; the size of the hydrogel pads will be kept at 7 cm x 5 cm.

(9) What was your experimental process? Include each of the steps in your experiment.

** Please open the PDF file titled “Experiments” to see photos of the team working on all three experiments, plus explanations, procedures, and data analysis for every experiment.

In testing bacterial growth, the team was resourceful in identifying affordable ingredients that were antimicrobial. Colloidal silver, probiotics, and xylitol are common substances that can be purchased over-the-counter by any consumer. Each of these materials, plus the unique and novel “colloidal silver + xylitol” composite that will be created by the team, will be tested in vitro experimentation using bacteria-inoculated agar in Petri dishes. Circular disks of filter paper will be saturated with each solution and then placed in Petri dishes and incubated at 37oC for 16 hours. The team will measure the diameter of the disk outward to the closest bacterial colony. This area free from bacteria surrounding the disk is called the “zone of inhibition.” The greater the zone, the more effective the material was in fighting biofilms, and the closer the team would be to identifying an alternative solution for chronic wounds.

** This process will be repeated three times for reliability. Specific procedures are listed below to allow anyone to replicate this experiment and duplicate the results of these tests for verification.

In Vitro Experiment -

To prepare Petri dishes for inoculation, use this procedure:

1) Weighed out the following into a 1 L flask:
6 grams agar
250 ml of distilled water
2) Heat to boiling for one minute in a pan placed on a hotplate
3) Stir to mix and dissolve the agar.
4) Pour agar until it just covers the bottom of Petri dishes.
5) Place lid on Petri dishes, invert, and allow to cool at room temperature.

To test the antibacterial disks against bacteria, use this procedure:

1) Cut circular disks out of filter paper soaked with the solution used – silver, xylitol, probiotic, or silver + xylitol – a composite material.
2) Measure to ensure each disk is 5 mm in diameter.
3) Label each Petri dish with the solution being tested – silver, xylitol, probiotic, or silver + xylitol – a composite material.
4) As directed by Dr. Rumbaugh of the Texas Tech Medical School, gently touch the agar in the dish to inoculate with bacteria common to skin. Give one short cough on the agar to inoculate with airborne bacteria.
5) Divide each Petri dish into quadrants and place four disks, each soaked with a solution, into the separate sections of the plate.
6) Place Petri dishes into an incubator, inverted to prevent condensation from forming on the agar.
7) Set incubator to body temperature, 37o Celsius.
8) Measure the zones of inhibition (mm) surrounding each disk after 16 hours.
9) Analyze the most effective material by looking at the data for the disk with the greatest average zone of inhibition.
10) Repeat this procedure on two additional sets of experiments.
11) Use the material identified as most effective in the following in vivo experiment.

In Vivo Experimentation

** To test whether or not colloidal silver would be successful when applied to an incision on an earthworm, the team used the novel idea of combining pyranine, a chemical commonly found in highlighter markers and which is non-toxic, with the colloidal silver so that the incisions on the earthworms could be seen more easily. Without the use of pyranine under the black light, it was difficult to locate and measure the incisions.

1) Obtain 20 earthworms, species Lumbricus terrestris.
2) Prepare four containers with soil for housing earthworms, labeled A, B, C, D.
3) Place three uncut worms into container A, as a control. Label.
4) Cut an incision 1.0 cm in length below the clitellum band on each of nine earthworms.
5) In a 40 ml beaker, add the felt of a yellow highlighter marker to extract the chemical pyranine. (non- toxic, glows under black light)
6) Add 0.5 ml of colloidal silver to an equal amount of pyranine.
7) With the exception of our control experiment, each worm’s incision was treated with colloidal silver using a sterile swab.
8) Once the solution is applied, each worm is put into its own container and set aside overnight.
9) Under black lights, remove worms from soil and locate the incision as it glows in the dark.
10) Each day the worms’ incisions will be measured in millimeters.
11) Repeat the in vivo experiment two times for reliability.
12) Data will be analyzed to determine if the colloidal silver increased the rate in which the incisions healed.

Bandage Enhancement

When testing the effect of colloidal silver when applied to a hydrogel bandage, use this procedure.

Hydrogel Test:
1) A syringe was filled with 0.5 ml of colloidal silver
2) The hydrogel was peeled back and the silver was injected into the side of the dressing. Repeat on two other pads.
3) Measure the amount of material that enters the hydrogel in milliliters.
4) Observe hydrogel pads daily for leakage.
5) Collect qualitative data including moisture level and amount of silver seen inside the transparent hydrogel.
6) Collect quantitative data – amount of colloidal silver leaking out of hydrogel.
7) Repeat experiment two additional times for reliable results.
8) Analyze qualitative and quantitative data.

Data Collection and Analysis

(10) Describe the data you collected and observed in your testing (use of data tables, charts, and/or graph is encouraged).

** Please see the three uploaded ExCel spreadsheets titled “In Vitro Experiment Data,” “In Vivo Experiment Data,” and “Bandage Enhancement Data,” that contain data tables, charts, and bar graphs (including explanations) of the results of the experiments.

** Please also open the PDF file titled “Experiments” to see photos of the team working on all three experiments, plus explanations, procedures, and data analysis for every experiment.

In Vitro Experiment – Colloidal silver consistently produced the largest zones of inhibition surrounding the treated disks when compared with the other experimental groups. In all three tests and in every trial within each test, the disk treated with silver alone was effective in fighting biofilms and bacteria.

In Vivo Experiment – Since silver was the standout during in vitro testing for all three experiments, it was used during in vivo testing on earthworms. Again, it was very successful in promoting cell regrowth and closing the incision wound of the worm in a short amount of time.

Bandage Enhancement – With the success of silver fighting bacteria off in vitro and promoting cell regrowth in vivo experimentation, it was used as an added material to hydrogel pads. The injections were effective in keeping the medication as part of the bandage which would continually release the colloidal silver onto wounds.

(11) Analyze the data you collected and observed in your testing. Does your data support or refute your hypothesis? Do not answer with a yes or no. Explain your answer using one of the following prompts: 'Our data supports/refutes the hypothesis because...'

In Vitro Testing:

The data refutes the hypothesis that stated, the composite material of silver plus xylitol we created would show greater ability to fight bacterial colonies. In the experiment, colloidal silver by itself was most effective in preventing bacterial colonies from overtaking the test materials. Silver had the largest zone of inhibition surrounding the test disks in the Petri dishes, which means the biofilms detected silver and could not grow in its presence.

In Vivo Testing -

The data from this experiment supports the hypothesis stating that silver would be one of the best candidates for encouraging cell regrowth and closing wounds. The colloidal silver prevented the bacteria from making a biofilm over the incisions on the earthworms and healing occurred under the experimental conditions.

Bandage Enhancement -

The data collected during the bioengineering of a bandage supported the hypothesis. Hydrogel pads were able to be embedded with colloidal silver and the material remained in the bandage throughout testing. With a bandage that maintains the ointment inside the pad, gradual release will allow bacteria to be reduced throughout the process, and less changes of bandages is needed with moist hydrogels.

(12) Explain any sources of error and how these could have affected your results.

** Please see the uploaded data table titled, "Experimental Errors"

Every experiment has sources of error and although good scientists try to keep these to a minimum, they do affect results.

During the bacterial growth experiments, the saturated filter made punching out disks a disadvantage. Cutting out the disks from the filter paper prior to its saturation proved to be much easier. The size and shape of the disks were more precise and constant during experimentation. Before applying the saturated filters to the agar, allow them to dry out. Changing the procedure resulted in more accurate measurements in millimeters during the zone of inhibition data collection.

A systemic error we experienced was setting the Petri dishes in the incubator face up during the trial run before actual experimentation began. Because the Petri dish was face up, condensation was collecting at the top of the dish, not allowing us to observe the inside clearly. After consulting with medical students at Texas Tech we learned for a more accurate outcome, to put the Petri dishes in the incubator face down. Once we began the actual experiment, it was easier to evaluate zones of inhibition through a clear lid.

A systemic error made was using an incubator that is not new and did not always keep the environment at 37oC – it fluctuated from 36o to 38o Celsius. After redoing the experimentation, the results proved to be repeatable and valid.

Drawing Conclusions

(13) Interpret and evaluate your results and write a conclusion statement that includes the following: Describe what you would do if you wanted to retest or further test your hypothesis. Evaluate the usefulness of the data your team collected. What changes would you make to your hypothesis and/or experimental design in the future, if any?

The result of team Silver Bullet is exciting. The colloidal silver used during experimentation was by far the most successful, common, affordable ingredient in fighting bacteria and was a key component in destroying biofilms. Biofilms plague the planet - causing slow healing, blood clots, infections leading to amputations, and heart disease. Through this project, the true colors of biofilms were seen and defeated in preliminary testing.

It is important to continue searching for treatments to conquer chronic wounds because of the incredible number of people who suffer.

To further test the hypothesis, colloidal silver should be combined with other materials and composites tested. At the nano level, silver could be used internally and the fight could result from the inside out. Gold nanoparticles have shown antimicrobial promise, so if combined with silver, in vitro and in vivo testing could result in an even more effective treatment.

A new hypothesis would be tested as well. Certain essential oils (frankincense, cedar) are also showing evidence of killing bacteria resistant to over-prescribed antibiotics. Using these in testing would allow for collaborations with many organizations today that are popularizing the use of oils and diffusers.

Working with Dr. Kendra Rumbaugh, a biofilms expert at Texas Tech and globally recognized for her work with biofilms and mice, the tests performed on invertebrate earthworms could be tested under controlled conditions in her laboratory using vertebrates. This next step of testing would be important in bringing a solution to reality. In fact, the data collected by our team is useful only because of our connections with doctors working in clinics and medical schools whose focus is on chronic wounds. Our experiment was not performed on an island – the information we learned was requested by these leaders in the field of wound care because they recognize that alternative treatments are essential in a world steeped in antibiotic-resistance.

The experiments conducted over and over led to better techniques in testing and more reliable results for society. Changes in our procedures for the future include experimenting with different concentrations of colloidal silver to find the most ideal percentage while balancing affordability.

Uploaded Files:

- [View] **Contact List** (By: anthrobones, 02/25/2016, .pdf)
  Major supporters of the team this year are included in this list. They helped by allowing us to visit their laboratories and they gave us advice on how to best test for the growth of bacteria in vitro.
- [View] **Collaboration with Community Experts** (By: anthrobones, 02/26/2016, .pdf)
  Work with many regional experts in chronic wounds and biofilms is shown in this pdf file. Included are notes from interviews, brainstorming pages from the team, photos of interviews and lab training, and exciting collaborations with experts in the community and beyond.
- [View] **Bibliography** (By: anthrobones, 02/27/2016, .pdf)
  Bibliography includes websites, professional journals, personal interviewees, newspaper & magazine articles, TED talks, webinars, and relevant organizations and university resources.
- [View] **In Vitro Experiments Data** (By: anthrobones, 02/29/2016, .xlsx)
  Data Tables, charts, graphs, and explanations of the analysis are found in this ExCel spreadsheet. They are also included in the PDF upload titled "Experiments."
- [View] **Data Table - Experimental Errors** (By: anthrobones, 02/29/2016, .pdf)
  This data table accompanies the answers to the question about errors that may have affected the experiments.
- [View] **In Vivo Experiments Data** (By: anthrobones, 02/29/2016, .xlsx)
  In Vivo experiments are those conducted on living organisms. INVERTEBRATES ONLY WERE USED DURING THIS PROJECT, THEREFORE NO VERTEBRATE FORM IS REQUIRED. This ExCel spreadsheet shows the data collected and analyzed, has an explanation included, and contains data tables, charts and graphs for all three trials of all three experiments conducted using earthworms and silver.
- [View] **Bandage Experiment Data** (By: anthrobones, 02/29/2016, .xlsx)
  The ability to load colloidal silver into hydrogel using a sterile needle and syringe was an important test to see if there could be a solution in the form of an enhanced bandage. This ExCel spreadsheet shows a table and explanation of the 5 trials conducted. It can also be found in the Experiments PDF Upload containing photos and data from every experiment.
- [View] **Experiments** (By: anthrobones, 02/29/2016, .pdf)
  A MUST-SEE Please open this PDF file converted from PowerPoint that includes many photos of the team working on all three experiments. Steps are included, data tables, graphs, charts, explanations of data, and the story of this project can be seen in this document. A MUST-SEE
- [View] **Bacteria Experiment Lab Report** (By: anthrobones, 02/29/2016, .pptx)
  Lab report with details about the bacteria experiment.

Community Benefit

(1) How could your experiments and data help solve your problem and benefit your community? Describe next steps for further research/experimentation and how you have or how you could implement your solution in the future.

The data and experiments could solve the problem of chronic wounds because a material was identified that is already FDA approved for topical use and is an affordable solution that kills bacteria. An ointment and hydrogel bandage was bioengineered for treating chronic wounds in not just this community, but in the nation. In our community, the oil field and agriculture are responsible for the predominant economy, yet they are the two most dangerous fields of work in the West. Numerous injuries are reported every year due to these occupations. Combined with diabetes which is a leading cause of death in our county, chronic wounds are far too common. Our colloidal silver hydrogel-bandage will prevent dangerous bacterial infections in chronic wounds if further experimentation leads this data to a university laboratory and medical school. Implementing the solution in the future will only result if the project continues past the competition deadline. Silver Bullet is more than a short term project; we are interested in this solution for the long run. Our community has benefited from our data and experiments because of the public awareness campaigns of which we are a part. People with diabetes have been targeted as those with great interest in this solution. After consulting their doctor, the ointment we recommend from data analysis would be affordable in this county and is available locally.

The following is a list of ways our team has implemented the solution and is making a difference in the community and changing the results of chronic wounds:

• IRB approval to test colloidal silver on vertebrates through Texas Tech
• Preliminary results already reported to Dr. Rumbaugh in the medical school
• Presentation to the Cochran County Senior Citizens Center
• Presentation to the Community Health Fair
• Develop a commercial name for the ointment of colloidal silver
• Manufacture colloidal silver-embedded bandage of hydrogel
• Reports to all doctors who advised this team
• April poster presentation given alongside graduate students at Texas Tech
• Website is continuing to expand the information as pages are added
• Media from the regional newspapers are reporting our story of success
• Certain geometric shapes from nature (biomimicry) may prevent bacteria from spreading deeper into wounds, therefore bandage shape is a new development

Our team knows we chose a topic this year that is a great challenge. There is not one answer. There is no easy answer. There are many searching for the solution. That doesn't mean people like us shouldn't join the fight.

To summarize this project:

Chronic wounds have become a significant health issue and a $25 billion business in the United States. With an aging population and a steep rise in diabetes and obesity, heartbreaking wound complications can result in blood clots, heart disease, and amputation. The goal of cost effective ways to heal wounds in a world with antibiotic-resistant bacteria was the team challenge. Research and interviews led to experiments using xylitol, probiotics, and colloidal silver as possible ways to attack biofilms in chronic wounds.

The team tested the effectiveness of these materials individually and created composite materials. In vitro tests used Petri dishes of nutrient agar inoculated with airborne bacteria and colonies from direct contact. The results identified colloidal silver as the material with the largest zone of inhibition and greatest ability to fight bacteria. On treated discs, bacteria could not form at all, and in tests with established bacterial colonies, silver fought back. It was the most cost effective healing substance at approximately one dollar per application, as opposed to much higher costs for gold nanoparticle ointments or oxygen chamber treatments.
During in vivo invertebrate testing, silver again showed great promise by speeding cell growth and closing wounds on earthworms. When silver was injected into hydrogel, a water-based pad, its potential was fully realized as a bandage with benefits of moisture, comfort, healing properties of colloidal silver, and affordability. Working with university research scientists, this STEM solution is designed to benefit the 6.5 billion people suffering from chronic wounds.

The battle with chronic wounds continues, but with the information learned this year, we are one step closer to conquering the ugliness of wounds that refuse to heal.
“We don’t grow when things are easy. We grow when we face challenges.” – Joyce Meyer

Uploaded Files:

- [**View**] **Website URL and QR Code** (By: anthrobones, 02/28/2016, .pdf)
  The Silver Bullet website spreads awareness of chronic wounds and alternative treatments becoming available. The website's URL and QR code are included in this document.
- [**View**] **Community Health Fair** (By: anthrobones, 02/28/2016, .pdf)
  A flyer was distributed to raise awareness of the health fair and information the team would present on "Middle School STEM Involvement and Chronic Wounds." There are alternative treatments for chronic wounds of which many people are unaware. Silver Bullet aims to change that.
- [**View**] **Senior Citizens Presentation** (By: anthrobones, 02/28/2016, .pdf)
  A flyer advertising the presentation from the Silver Bullet team to the senior citizens meeting of Cochran County is included in the uploads. Some of the most vulnerable people for chronic wounds live in our county and education here is important for them.

Mission Verification

(1) Does your Mission Folder project involve vertebrate testing, defined as animals with backbones and spinal columns (which include humans)? If yes, team must complete and attach an IRB approval form.

No

(2) Did your team use a survey for any part of your project? If yes, team must complete and attach a survey approval form.

No

(3) You will need to include an abstract of 250 words or less. As part of the abstract you will need to describe your project and explain how you used STEM (Science, Technology, Engineering and Mathematics) to improve your community

Chronic wounds have become a significant health issue and a $25 billion business in the United States. With an aging population and a steep rise in diabetes and obesity, heartbreaking wound complications can result in blood clots, heart disease, and amputation. The goal of cost effective ways to heal wounds in a world with antibiotic-resistant bacteria was the team challenge. Research and interviews led to experiments using xylitol, probiotics, and colloidal silver as possible ways to attack biofilms in chronic wounds.
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Silver Bullet

Join the fight against biofilms and prevent infection in chronic wounds

Blade	Kaden	Kaylah	Elizabeth
Enthusiasm For The Project Coordinated the Brainstorming Sessions	Records Notes during Interviews with Community Experts	Communication Specialist Strong Leadership Skills	Works Together well with Anyone Good Listener
Interviewing Specialist Writes Thank-you notes	Problem Solver/Trouble Shooter Asks tough questions	Enjoys Experiments Good Lab Manager	Graphs, Charts, Data Generator
Data Collection & Measurements	Thorough Researcher	Strong Lab Scientist	Time Management Specialist
Public Speaker during Presentations	Idea Generator Studies Paleontology	Strong Writer Coordinated Outreach with Dr. Lehman	Strong Writing Skills Organizer for Meetings
Sees the Big Picture Easily Grand Ideas and dreams	Innovator and Creative Advanced Mathematician	Interacts well with Medical Scientists Highly Verbal Strong Academics	Prepared Support Materials Artist
Thorough during Research & Summaries	Driven and Determined Poster Presentations	Organized On Time	High Vocabulary in Verbal and Written Communication
Takes notes during interviews	Detail-oriented Advance Calculator Skills	Analyzes Data Well Draws Conclusions	Independent Worker Highly Dependable
Encourager to the Team and an Outgoing Team Member	Physically Outgoing Flexible & Compromises	Data Collection Created Tables & Qualitative Data Sheets	Academically Advanced Memory for Details
Good Listener Appreciative of Others Ideas	Prepared Power Points and Presentations Thought of our Team Name	Socially Outgoing Works well with Other People of all Ages	Logical & a Voice of Reason for the Team Keeps others on Track

Community Problem:
Chronic wounds affect 5.7 million patients due to bacterial infections that are difficult to treat. This problem is particularly hard to treat in diabetics.

Hypothesis:
If probiotics can provide a zone of inhibition double the size of application in vitro, then it could serve as an effective material in fighting bacterial biofilms found in chronic wounds.

Experiment :
What effect do probiotics have on bacterial colonies when tested in vitro?

Solution-
Probiotics were ineffective in fighting bacterial colonies during in vitro experimentation. Our hypothesis was refuted and another material should be tested for antimicrobial properties.

Hypothesis:
If silver and xylitol prevent the growth of bacterial colonies in vitro, then it will provide greater effectiveness in the fight with biofilms when combined as a composite material.

Experiment :
What effect does colloidal silver and xylitol have on bacterial colonies when tested individually and in a composite form during in vitro testing?

Solution-
Silver alone provided the greatest amount of protection against bacterial colonies during in vitro testing. Xylitol was in effective and also seemed to compromise the effects of silver when used as a composite. For an effective application against bacterial infection, colloidal silver would be an excellent choice as a material.

Hypothesis:
If colloidal silver maintains a zone of inhibition around itself during in vitro testing, then what effect does it have on bacterial colonies that are already established in vitro: Will it protect itself when in direct contact with bacteria?

Experiment:
What effect does colloidal silver have on bacterial colonies that are already established in vitro: can it protect itself when in direct contact with bacterial colonies and inhibit future growth?

Solution-
When an ointment is needed in a wound that is already showing signs of bacterial infection, colloidal silver would be an effective material to use because of its ability to repel the colonies of bacteria and disrupt their biofilms.

Hypothesis:
If colloidal silver can show visible signs of promoting cell growth during in vivo experimentation on invertebrates, then it could be an effective treatment in chronic wounds .

Experiment:
Does colloidal silver have the ability to promote cell regrowth during in vivo experimentation ?

Solution:
During in vivo experimentation on invertebrates, colloidal silver inhibited bacterial growth and promoted cell regrowth over a week's time, showing its potential as an ingrendient in ointments for chronic wounds.

Silver Bullet

Timeline for eCYBERMISSION Project

April 2015

Research & topic chosen: Ways to effectively break down bacteria that would be cost effective.

May 2015

Team and Action Plan Development

August 2015

Ecybermission Registration:

Team was completed with members Elizabeth, Kaylah, Kaden, and Blade.

Sept 2015

Team Meetings – Action Plan Developed

Chose a schedule for completion of Mission Folder & Experiments

Oct 2015

Began Experimentation:

Chose cost effective substances with healing properties.

Nov 2015

Consulted with Josie Musico

She interviewed us to spread the news of wound healing.

April 2015 ~ May 2016

Silver Bullet Timeline Continued ...

Jan 2016

Set up interviews with professional doctors.

Continued doing research for the project Silver Bullet.

Feb 2016

Contacted Other Experts

Conducted tests with medical researchers, Dr. Kendra Rumbaugh, Randall Wolcott, in the Biopolymer Institute, Medical Biofilm Research Institute and Texas Tech Health and Sciences Center.

Tested the chosen substances to break down the bacteria

March 2016

Future Plans for the Project is to develop a hydrogel bandage that will deliver the colloidal silver directly and effectively to the wound

April 2016

Poster Presentation

Prepare poster for Texas Tech University Graduate School.

May 2016

Start a public awareness campaign about wound care.

Contact List for Silver Bullet

Major supporters, subject matter experts, and collaborations during this project are listed below with contact information for judges' convenience. Only those people with whom the team had continuous contact are included on this list.

Gregory Rogers, MD
Covenant Hospital Levelland
Family and Emergency Medicine
1900 College Avenue
Levelland, Texas 79336
806-894-4963

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Department of Surgery, MS 8312
Texas Tech University Health Sciences Center
3601 4th Street
Lubbock, Texas 79430
kendra.rumbaugh@ttuhsc.edu

Randall Wolcott, MD
Southwest Regional Wound Center
2002 Oxford Avenue
Lubbock, TX 79410
(806) 793-8869

Michael Lehman, MD
Lehman Dermatology
3715 21st Street
Lubbock, Texas 79410
806-795-0617

Many thanks to the individuals who allowed us to visit with them about the issue of chronic wounds. Their identity is being kept hidden for privacy.

CHRONIC WOUNDS/BIOFILMS COMMUNITY EXPERTS

A collection of interviews and collaborations between the team and regional experts

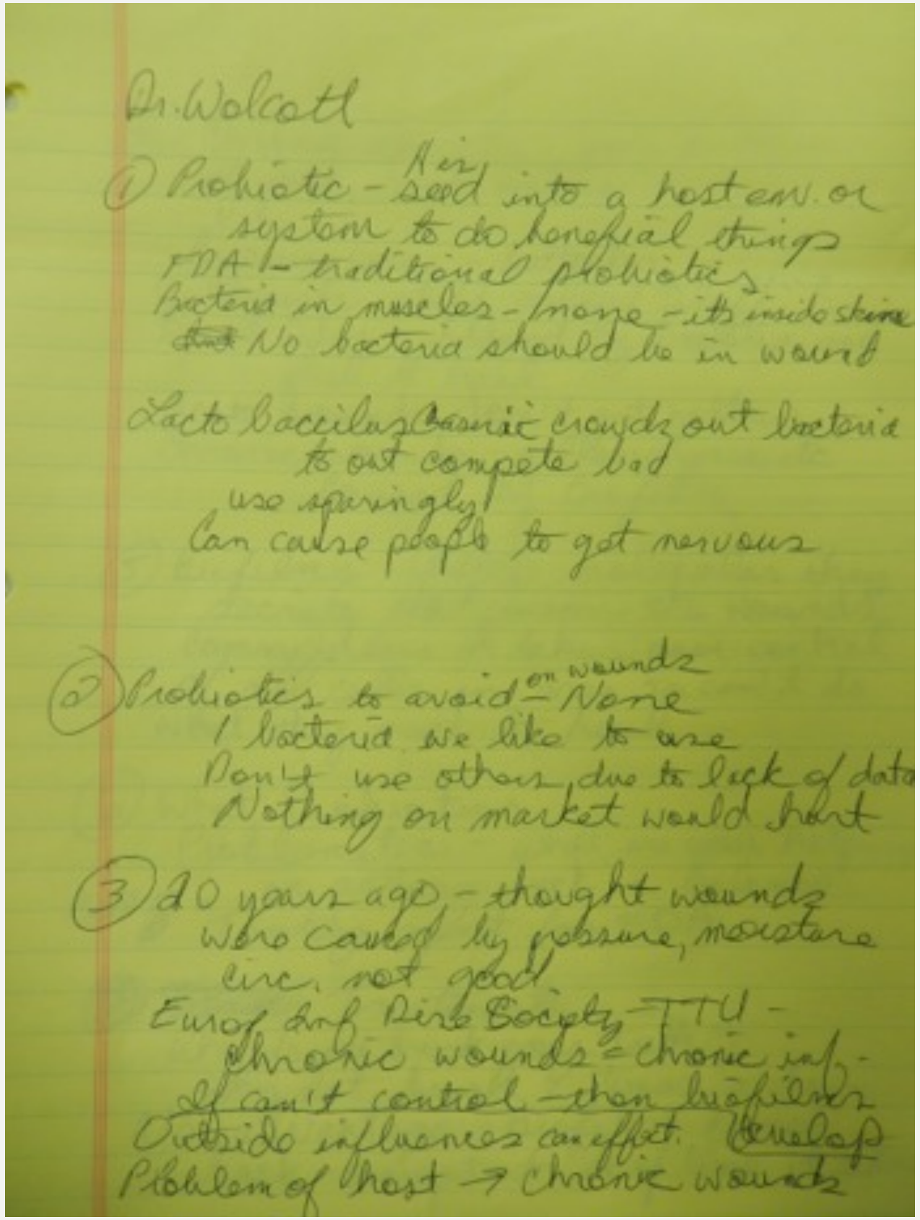
Questions Answers Teamwork Photos Lab Experiences Advice

SILVER BULLET
ECYBERMISSION 2016

INTERVIEWS & COLLABORATIONS

- Dr. Michael Lehman, MD Lehman Dermatology, Lubbock, Texas – Wound & Skin Disease Specialist
- Dr. Angel Cueva, Texas Tech Medical School, Lubbock, Texas – Laboratory research, advanced technology
- Dr. Hannah Zhao, Texas Tech University Health Sciences Center, Department of Surgery, Lubbock, Texas, Microbiologist
- Dr. Greg Rogers, MD – Lab and medical techniques, General Physician, Covenant Hospital, Levelland, Texas
- Michael Briggs, Neuropathy patient with chronic wounds, Lubbock, Texas
- Dr. Randy Wolcott, MD – Southwest Regional Wound Care Center, chronic wound care specialist, Lubbock, Texas
- Dr. Kendra Rumbaugh, PhD, Texas Tech University Health Sciences Center, Microbiologist, Department of Surgery, Texas Tech Medical School, Chronic Wounds Laboratory, Lubbock, Texas

INTERVIEW NOTES TAKEN BY THE TEAM



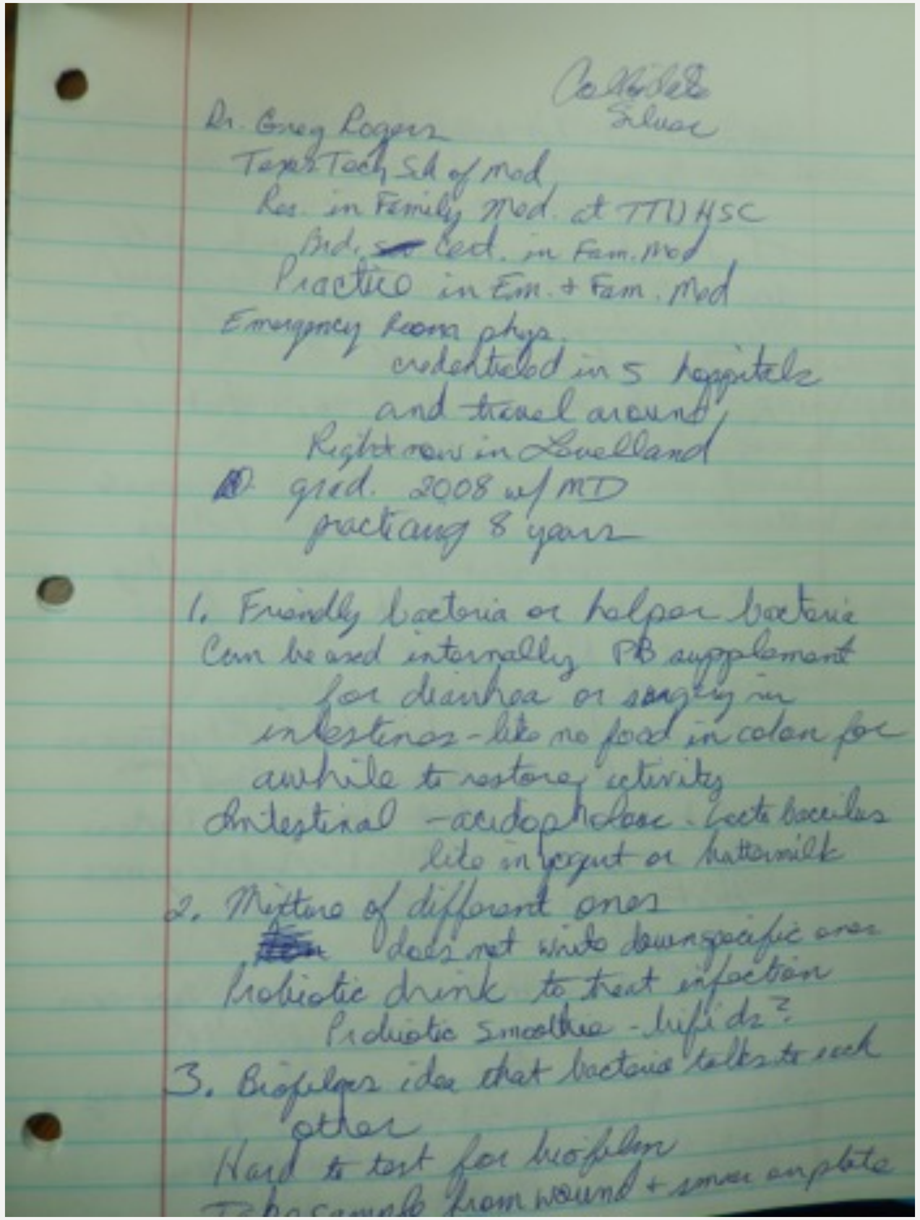
Dr. Wolcott

(1) Probiotic – seed into a host env. or system to do beneficial things
FDA – traditional probiotics
Bacteria in muscles – none – its inside skin
No bacteria should be in wound

Lacto baccilus casei crowds out bacteria to out compete bad
use sparingly!
Can cause people to get nervous

(2) Probiotics to avoid on wounds – None
1 bacteria we like to use
Don't use others due to lack of data
Nothing on market would hurt

(3) 20 years ago – thought wounds were caused by pressure, moisture circ. not good
Euro. Inf. Bio Society – TTU –
Chronic wounds = chronic inf.
If can't control – then biofilm
Outside influences can effect. Develop
Problem of host → chronic wounds



Colloidal Silver

Dr. Greg Rogers
Texas Tech Sch of Med
Res. in Family Med. at TTUHSC
Brd. cert. in Fam. Med
Practice in Em. + Fam. Med
Emergency Room phys.
credentialed in 5 hospitals and travel around
Right now in Levelland
grad. 2008 w/ MD
practicing 8 years

1. Friendly bacteria or helper bacteria
Can be used internally PB supplement for diarrhea or surgery in intestines – like no food in colon for awhile to restore activity
Intestinal – acidophilus – lacto baccilus like in yogurt or buttermilk
2. Mixture of different ones
does not write down specific ones
Probiotic drink to treat infection
Probiotic smoothie – bifids?
3. Biofilms idea that bacteria talks to each other
Hard to test for biofilm
Take sample from wound + smear on plate

QUESTIONS AND BRAINSTORMING BY THE TEAM

Elizabeth Casarez

Natural ways – Herbal methods:
Clove, Probiotics, False black pepper, Apple cider vinegar
Vitamins:
A, C, E
Mineral:
Zinc
Best species of clove is Syzygium aromaticu
Maybe this mixed with a vitamin that pro
wound healing?

~MIX ALL?~

Blue cheese mold? – Probiotic
Kefir – Probiotic
Sour Pickle – Probiotic

Interview Notes from Dr. Randy Wolcott

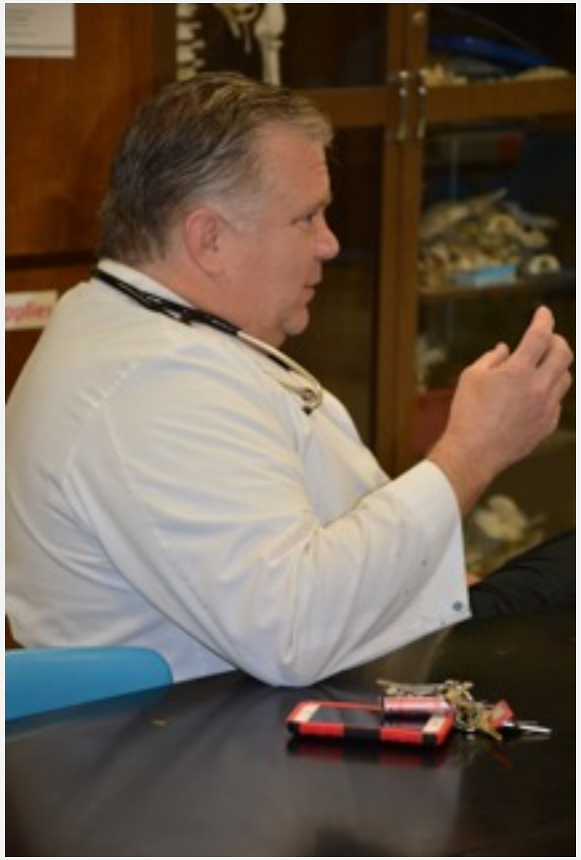
We had a great visit with Dr. Wolcott! He gave our team lots of information on biofilms and even helped us come up with another approach to the experiment. He gave us his email address and told us to pass it on to our team advisor in case we need help later on. He has some articles to send about different things we talked about.

The only probiotic they use is lactobacillus gaserii and it is used sparingly. He suggested testing Hydrogel with xylitol, colloidal silver, and arithrotal (which are are things you can order online. He suggested trying the hydrogel once with the zylotal and then without, etc. You could test it 3 different ways to see which mixture is the least irritating on the skin.

We took lots of pictures, and he also gave us a tour of the lab. He was a very nice man!

1) What natural probiotics, if any, have you used in the past?
2) Out of the ones you've worked with which one do you think worked the best and why?
3) What kind of medicine or probiotic works best when breaking down biofilms without applying damage to the skin?
4) What are some probiotics that do too much damage to the skin that we should avoid using?
5) How has wound healing changed in the last twenty years ago to today?
6) What herbal treatments or supplements aid wound healing?
7) What kind of medicine ointment or treatments do you use to normally heal wounds?
8) How often do you see wounds that have biofilms in/on wounds?
9) Have you ever used medicine containing probiotics to help heal a wound what were the medicines called?
10) How has biofilms effected any of your patients healing treatment?

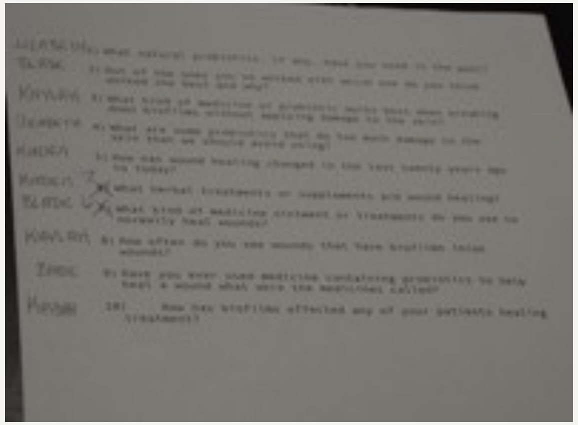
COLLABORATION PHOTOS DR. ROGERS



Dr. Rogers had expertise in dealing with chronic wounds because he has many patients in rural clinics with diabetic complications.

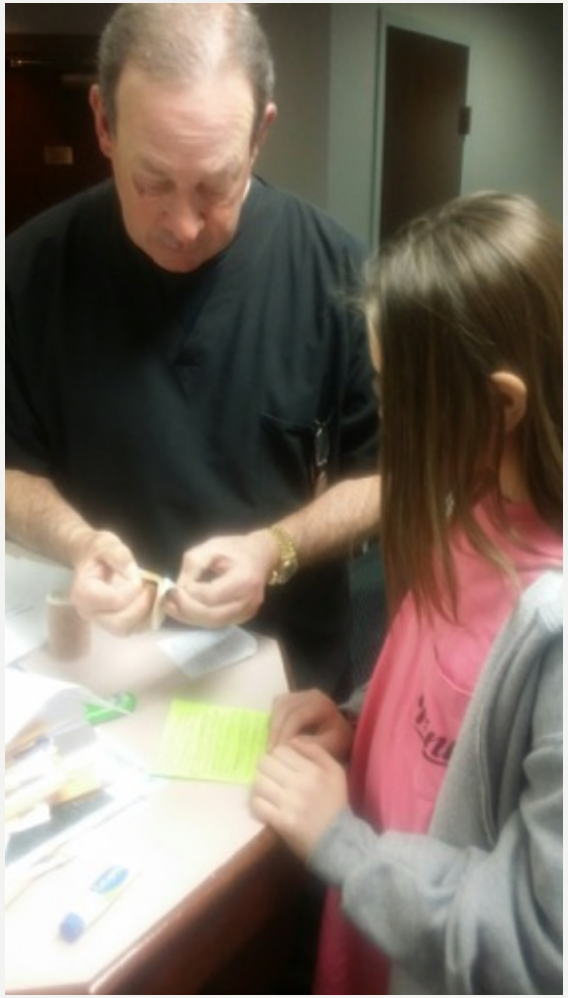
Silver Bullet team invited Dr. Rogers to the science lab to see the progress of the in vitro experiments and ask advice on in vivo experimentation.

Dr. Rogers helped us understand the difference between a wound, a serious wound, and a chronic wound that can be fatal. He answered all of the questions we prepared in advance.



COLLABORATION PHOTOS DR. LEHMAN

Dr. Lehman demonstrated the proper way to apply a moist bandage to prevent infection. We wanted to be certain we followed proper protocols



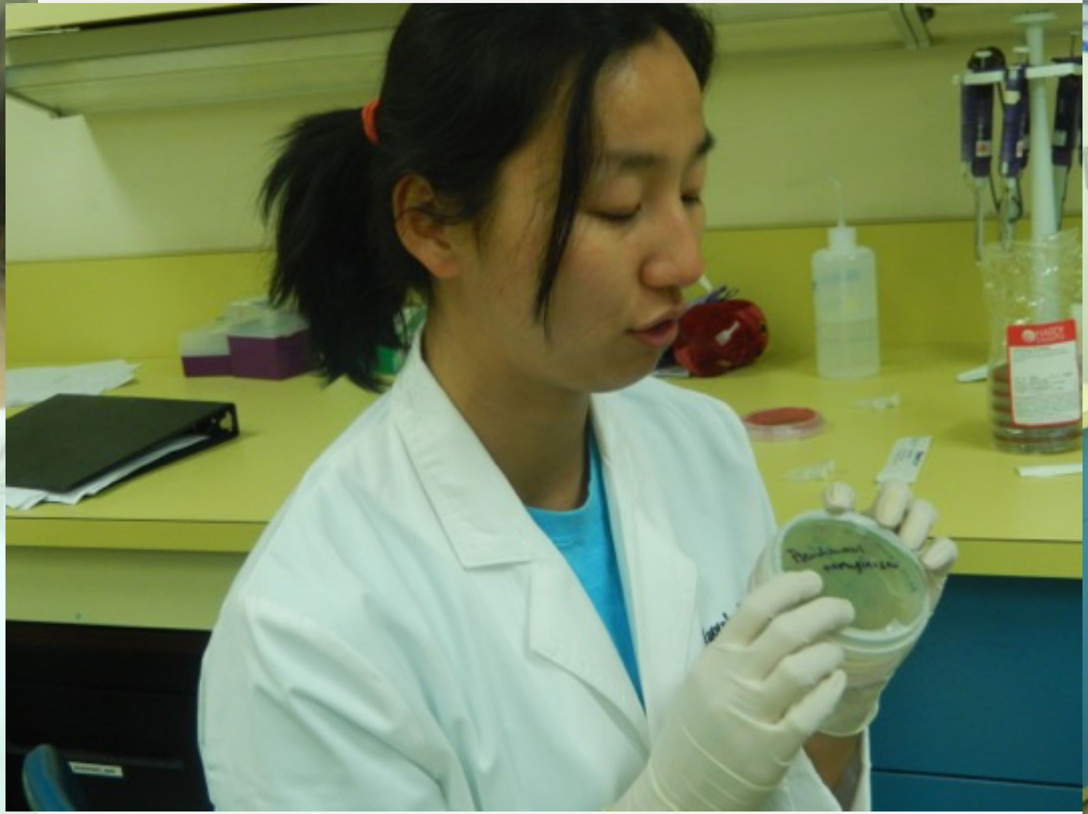
Our team asked Dr. Lehman for advice about our methods of engineering a bandage to fight biofilms in chronic wounds.

Kaylah and Dr. Lehman in the dermatology clinic after discussing wound care.

COLLABORATION PHOTOS DR. ZHAO

Dr. Zhao described types of beneficial and dangerous bacteria – and how the LOCATION of that bacteria makes the difference.

Notetaking – a real world skill

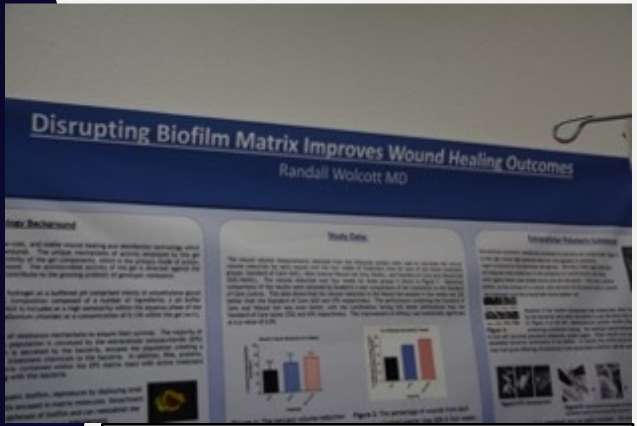


COLLABORATION PHOTOS DR. WOLCOTT

Learning how to disrupt a matrix of biofilms that allow bacteria to communicate. If silver and xylitol can stop the communication, wounds could heal.

The Silver Bullet team with Dr. Wolcott in the clinic learning about cutting edge research in the field of chronic wounds.

COLLABORATION PHOTOS DR. WOLCOTT



Dr. Wolcott's poster on Disrupting Biofilm Matrices – the very issue we were studying.

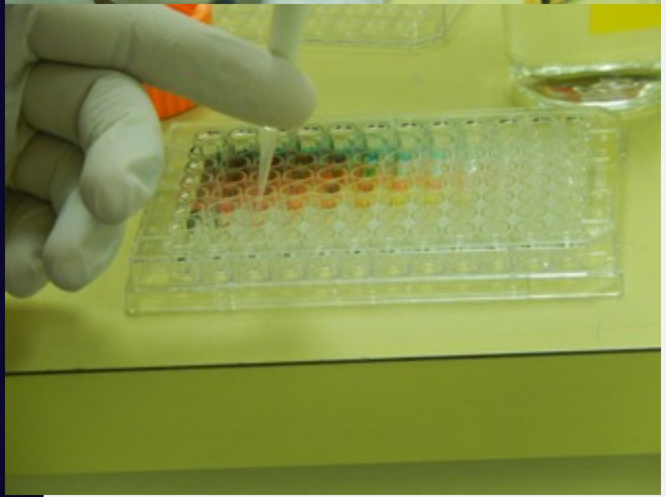
Technology such as chambers of oxygen and ozone are also being used to treat chronic wounds that do not respond to medications.

Demonstrating the protocols for testing materials and their abilities to fight infection in vitro experiments.

Dr. Cueva taught us to load and deliver liquids through a pipette

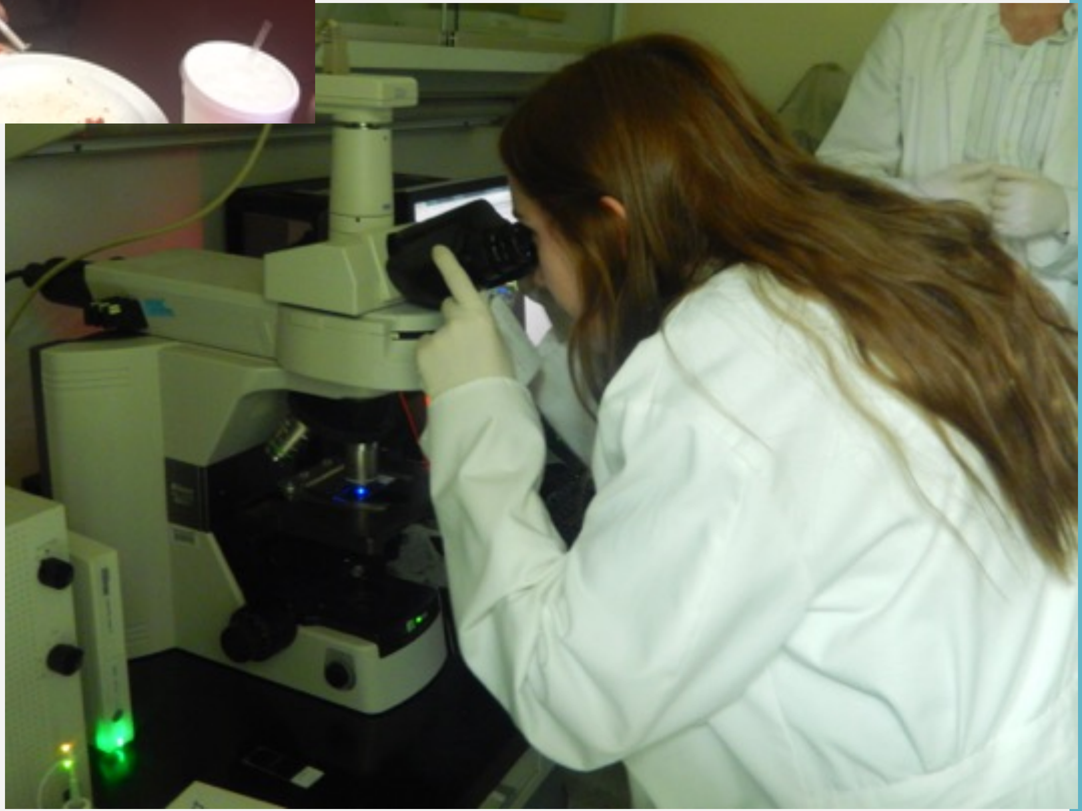
COLLABORATION PHOTOS DR. CUEVA

Practicing the use of a pipette to make concentration gradients



Technology such as chambers of oxygen and ozone are also being used to treat chronic wounds that do not respond to medications.

COLLABORATION PHOTOS DR. CUEVA DR. RICHARDS

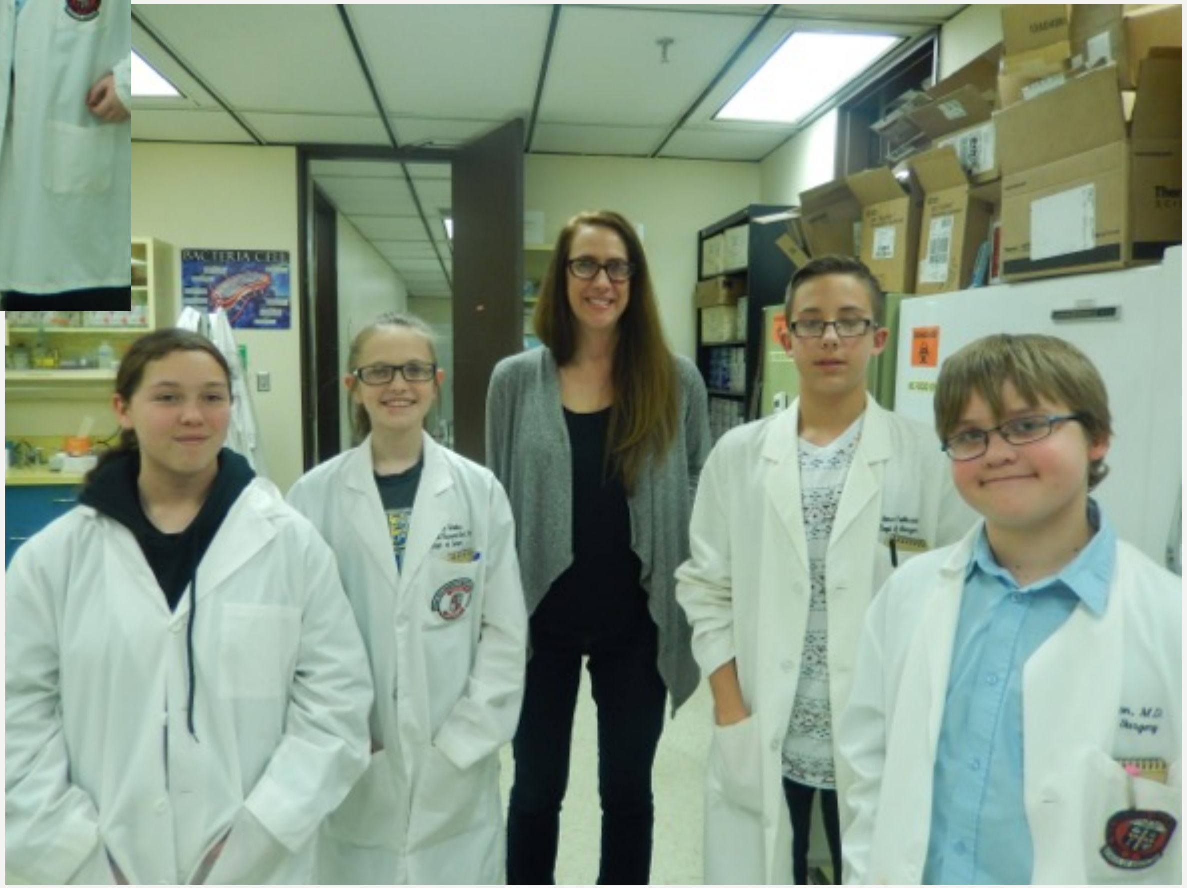


Always ask a university if you need the use of better and more advanced equipment than what your school can provide! They say YES!

Practice makes perfect

COLLABORATION PHOTOS DR. RUMBAUGH'S LAB

Ecybermission led us to a fantastic role model



We were so excited to be able to work with Dr. Kendra Rumbaugh who is a global leader in the field of biofilms and chronic wounds!

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Testing the Effectiveness of Materials in **Preventing Bacterial Growt**

Trial 1			
Material	**Sample 1**	**Sample 2**	**Sample 3**
	Size of Zone of Inhibition Surrounding the Disk		
Probiotics	9	8	6
Xylitol	4	12	5
Silver	**15**	**15**	**5**
Composite - Silver + Xylitol	6	12	4
Control	5	3	2

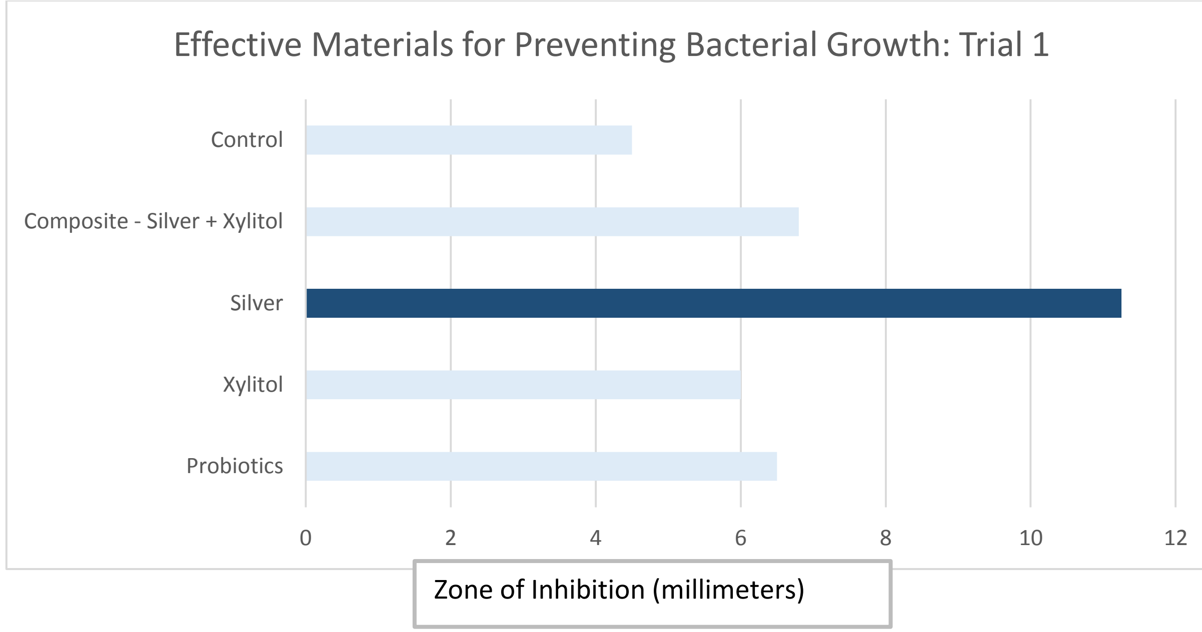
Silver was the most effective ingredient tested in vitro for stopping bacterial growth. In
3 consistent results were obtained in sample after sample tested. Our hypothesis was re
expected the composite of silver + xylitol to "outperform" silver treatments alone. Base
repeated testing, silver will now be tested in vivo to learn if similar success in fighting ba
cell regrowth/healing will occur on a living organism. Testing will NOT involve vertebrat
will be used for experimentation.

Trial 2			
Material	**Sample 1**	**Sample 2**	**Sample 3**
	Size of Zone of Inhibition Surrounding the Disk		
Probiotics	6	8	5
Xylitol	4	11	6
Silver	**20**	**25**	**15**
Composite - Silver + Xylitol	3	12	7
Control	5	3	2

Trial 3			
Material	**Sample 1**	**Sample 2**	**Sample 3**
	Size of Zone of Inhibition Surrounding the Disk		
Probiotics	7	7	10
Xylitol	2	7	9
Silver	**24**	**23**	**19**
Composite - Silver + Xylitol	1	9	6
Control	2	3	8

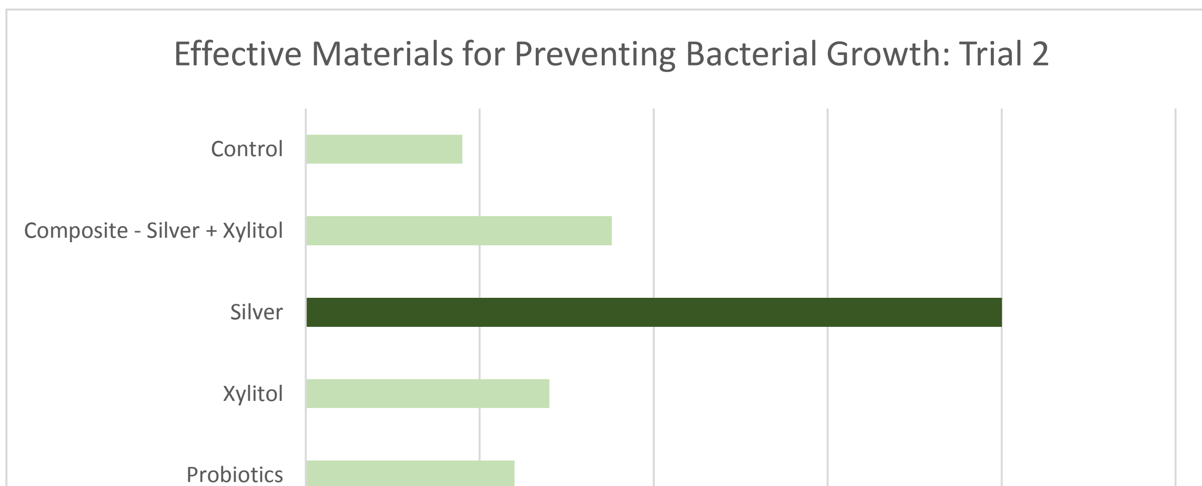
th In Vitro

Sample 4	Mean
((mm)	
3	6.5
3	6
10	**11.25**
5	6.8
8	4.5

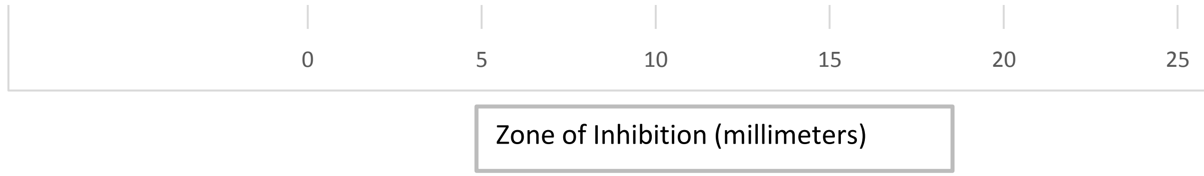


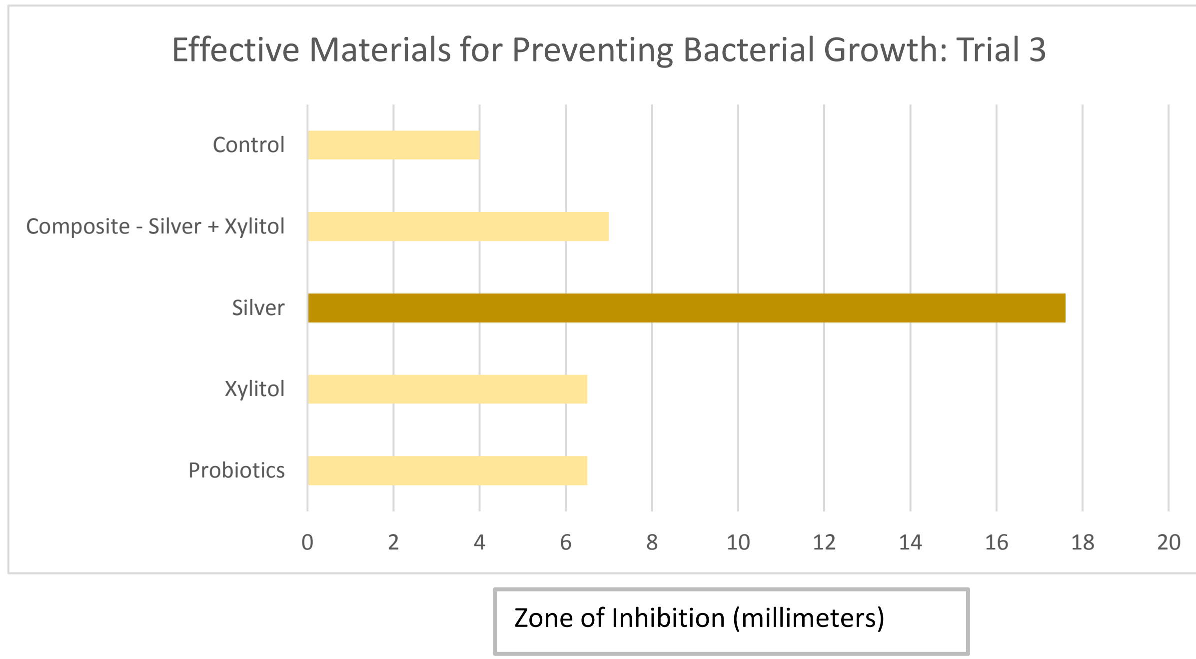
Trial 1, Trial 2, and Trial
efuted because we
d on the results of
acteria and promoting
es - invertebrates only

Sample 4	**Mean**
((mm)	
5	6
7	7
20	**20**
13	8.8
8	4.5



Sample 4	**Mean**
(mm)	
2	6.5
8	6.5
22	**17.6**
12	7
3	4

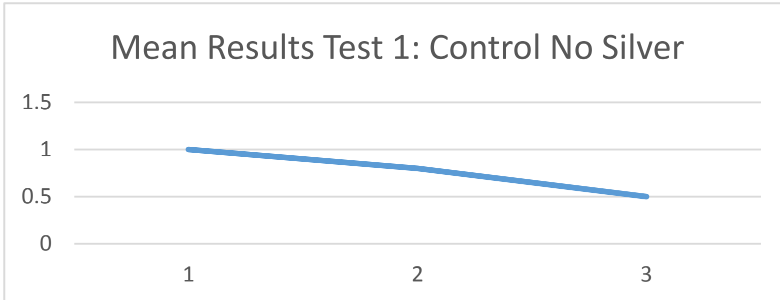




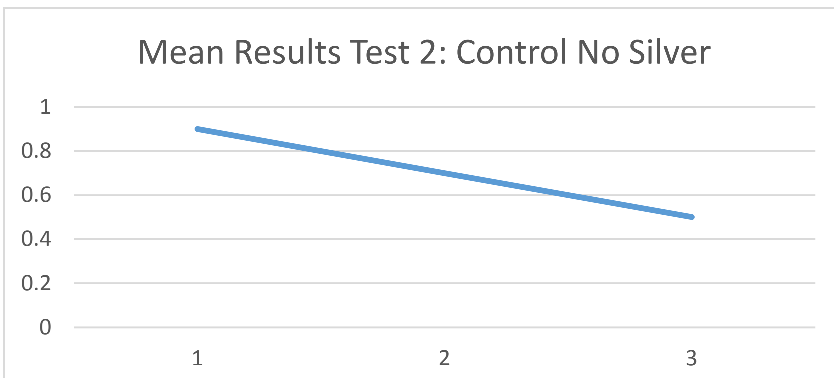
Sources of Experimental Errors

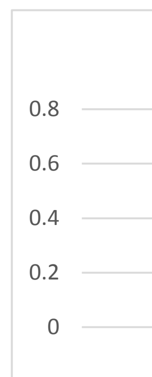
Source of Error	Experiment Affected	Possible Error Recorded	Changes that Could Improve Results in the Future
Control of incisions to the earthworms	In Vivo Testing	Incisions made on the earthworms were approximately the same length but not exact	Use of a dissecting microscope for better viewing; remeasuring incision multiple times as the earthworm stretched and contracted
Ointment applied to earthworms with incisions	In Vivo Testing	No ointment was applied to the control earthworm	Use an ointment without an active ingredient such as Vaseline next time to compare with the ointment with tested ingredients
Incubator temperature	In Vitro testing	Temperature was set for 37°C or body temperature but fluctuated between 36° and 38° C	Conduct in vitro experiments in a university lab with newer incubators

In Vivo Experimentation - Test 1 - Measurement of I					
Condition Applied	Day 3			Day 4	
	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2
Control No Silver	1	1	0.8	0.7	0.8
Colloidal Silver	0.7	0.7	0.6	0.3	0.4

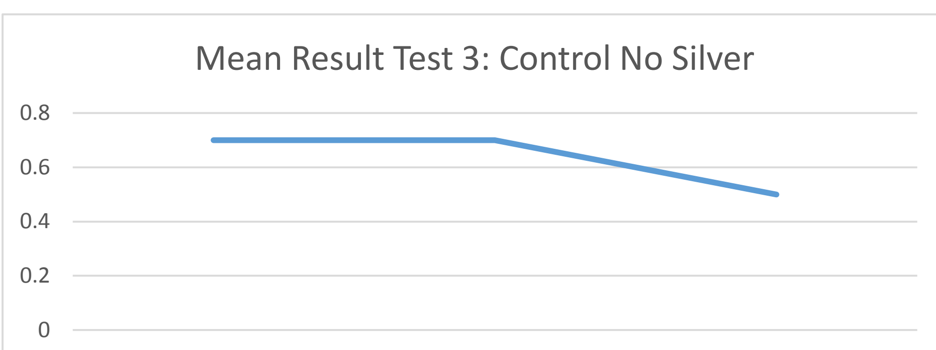


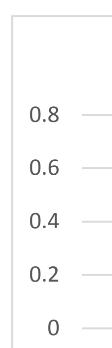
In Vivo Experimentation - Test 2 - Measurement of I					
Condition Applied	Day 3			Day 4	
	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2
Control No Silver	0.9	0.8	1	0.7	0.5
Colloidal Silver	0.8	0.7	0.6	0.4	0.3





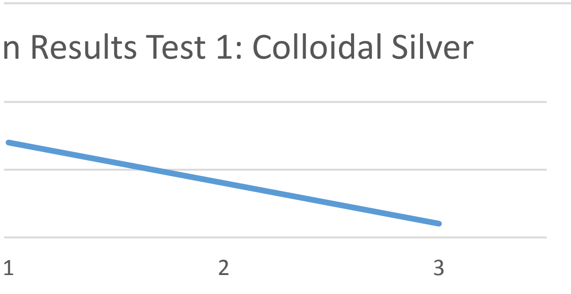
In Vivo Experimentation - Test 3 - Measurement of					
Condition Applied	Day 3			Day 4	
	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2
Control No Silver	0.9	1	0.7	0.8	0.8
Colloidal Silver	0.6	0.7	0.5	0.3	0.4



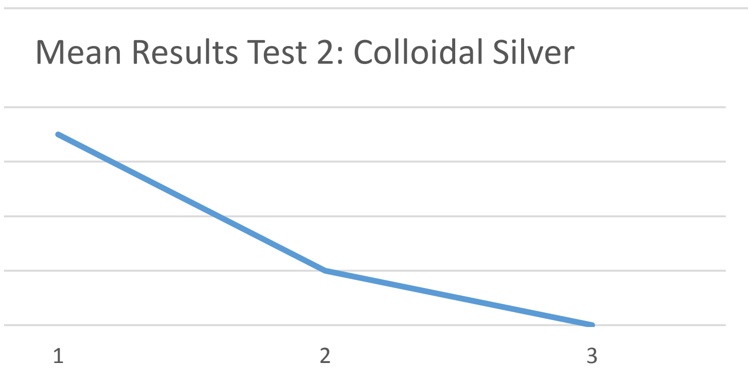


1	2	3

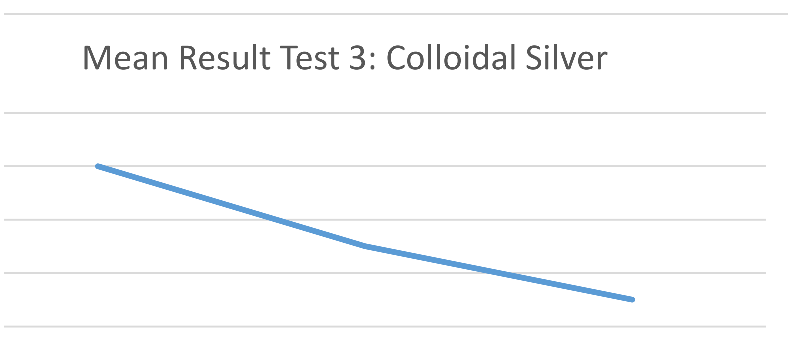
ncision (Initial = 1.0 cm)			
	Day 5		
Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3
0.8	0.5	0.5	0.4
0.5	0	0.1	0.2



ncision (Initial = 1.0 cm)			
	Day 5		
Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3
0.7	0.5	0.4	0.5
0.2	0	0	0



Incision Initial = 1.0 cm			
	Day 5		
Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3
0.7	0.6	0.4	0.5
0.2	0.1	0.2	0



During tests conducted in vivo using the earthworm Lubricus terrestris for testing, a 1 cm incision was made into the skin of the worm just below the clitellum band. Silver was mixed with pyranine, a non-toxic chemical that glows brilliantly under UV black light. Under a black light, it was easy to see the site of the incision and make measurements, whereas it was extremely difficult before this treatment.

Then, the silver was applied to the wound. Earthworms were returned to their containers and measurements were made in cm on Day 3, 4, and 5.

The worms treated with silver had greater cell growth and wound healing faster than those with no silver applied. These results were consistent during testing and show that colloidal silver does speed cell regrowth and healing on living organisms.

1	2	3

Trial	Amount of Silver in Syringe (ml)
1	0.5
2	0.5
3	0.5
4	0.5
5	0.5

In every test except Test 3, 100% of the silver that wa
transferred into the hydrogel pad. The silver remaine
testing. This hydrogel enhanced bandage with colloic
airborne bacteria, and in contact with colloidal silver

Percent of Silver Successfully Transferred to Hydrogel (ml)
100%
100%
85%
100%
100%

as contained in the syringe was able to be successfully
ed in the hydrogel as part of the bandage throughout
dal silver would keep a wound moist, protected from
during the time the bandage remains on the wound.

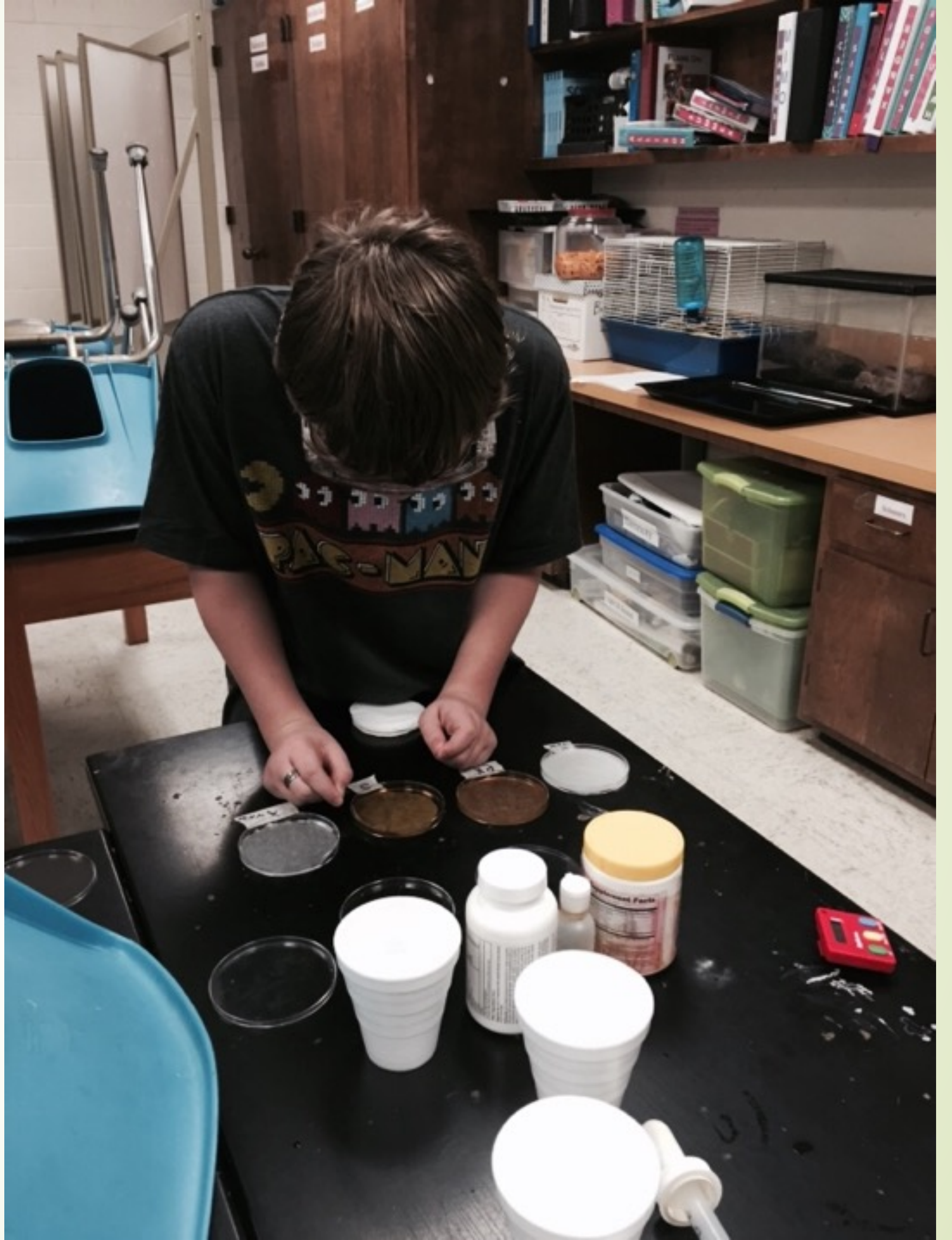
In Vitro Experiments

Photos * Data * Tables * Graphs

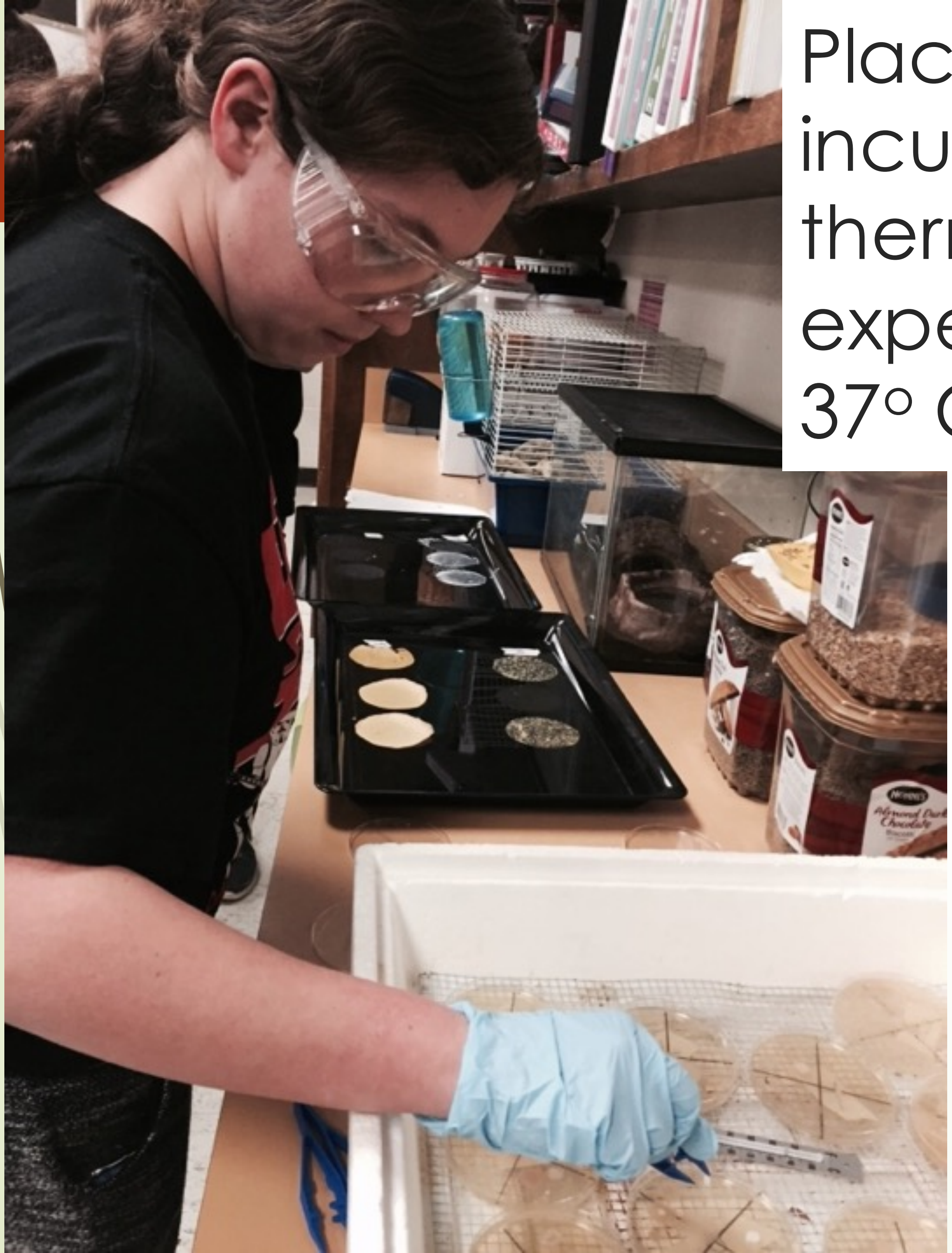
Silver Bullet Ecybermission Team 2016

Preparing Petri dishes with agar and placing disks to fight bacteria

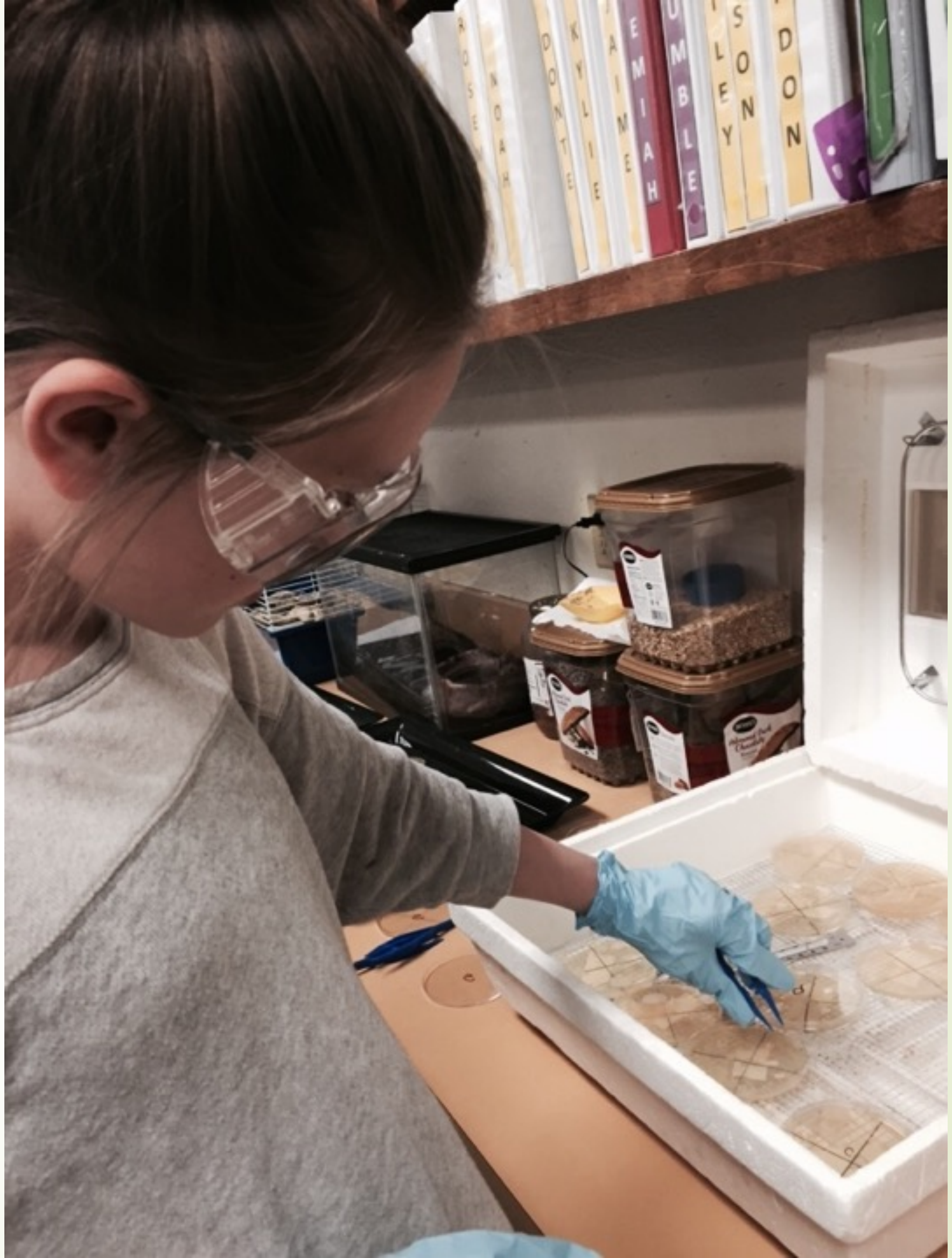
Labelling Petri dishes and inoculating with probiotics, colloidal silver, xylitol, and silver/xylitol composite.



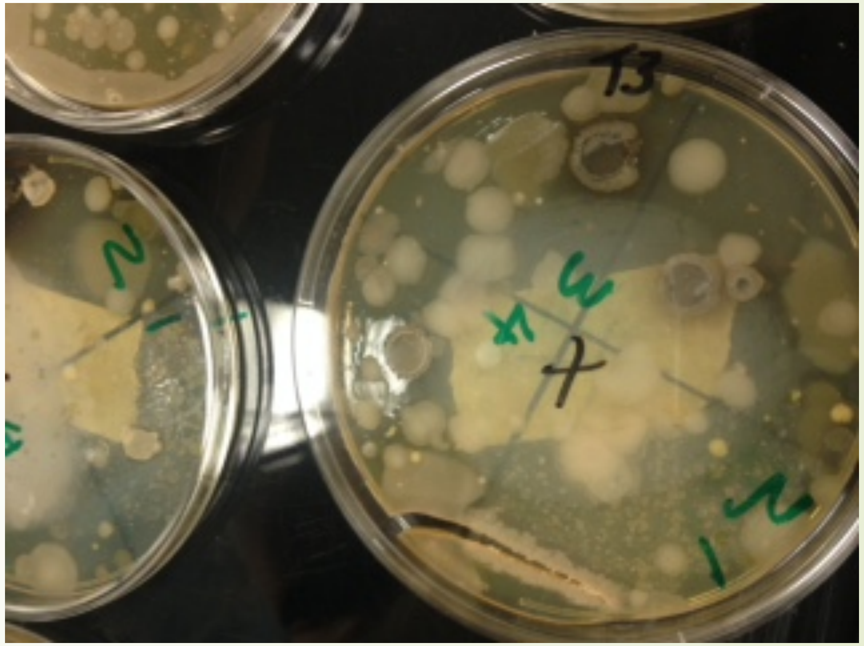
Placing Petri dishes into the incubator and setting the thermometer to control the experiment at body temperature or 37° C



Ready to measure the zones of inhibition using millimeters.



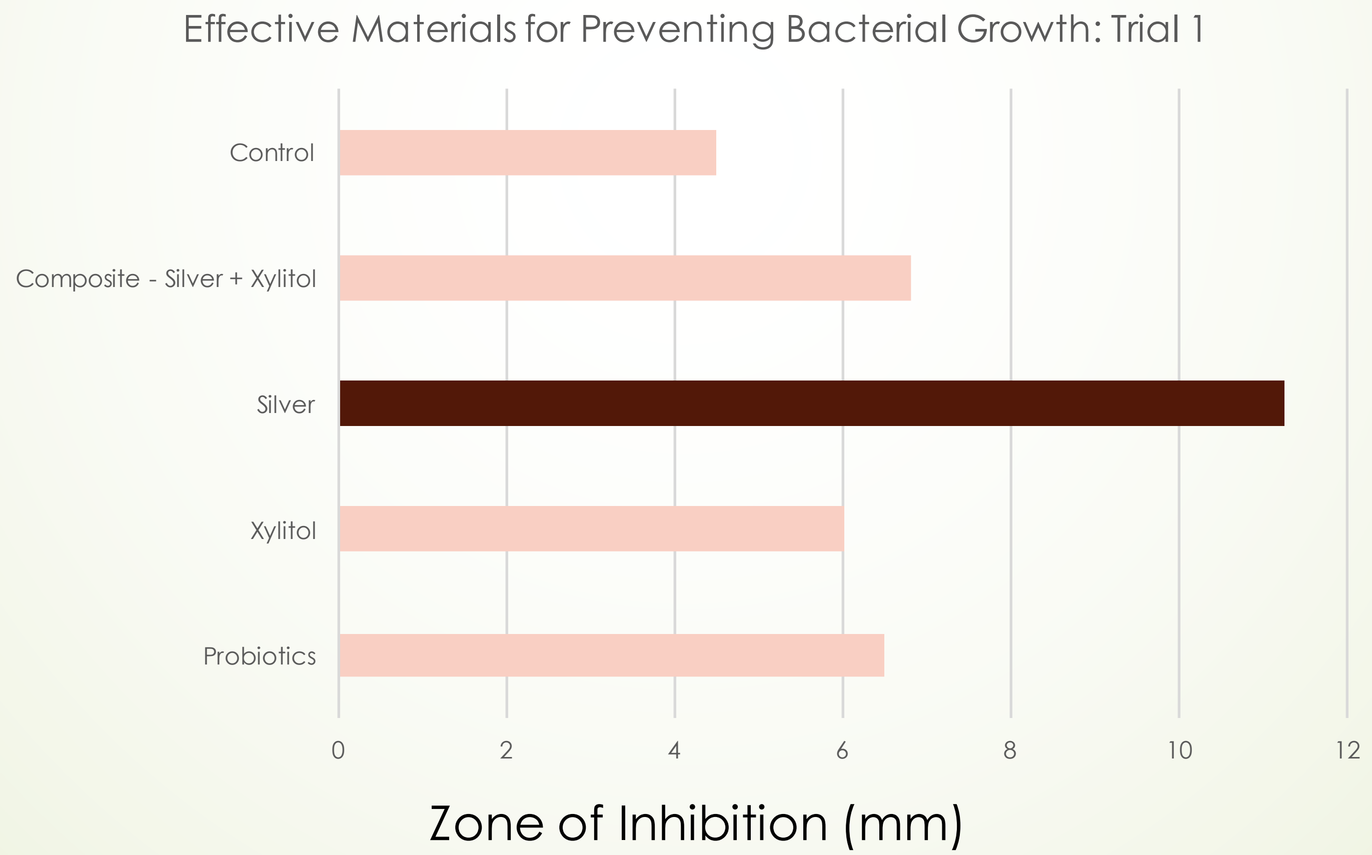
Zones of Inhibition were measured (mm) from the center of the paper disks outward to the nearest bacterial colony.



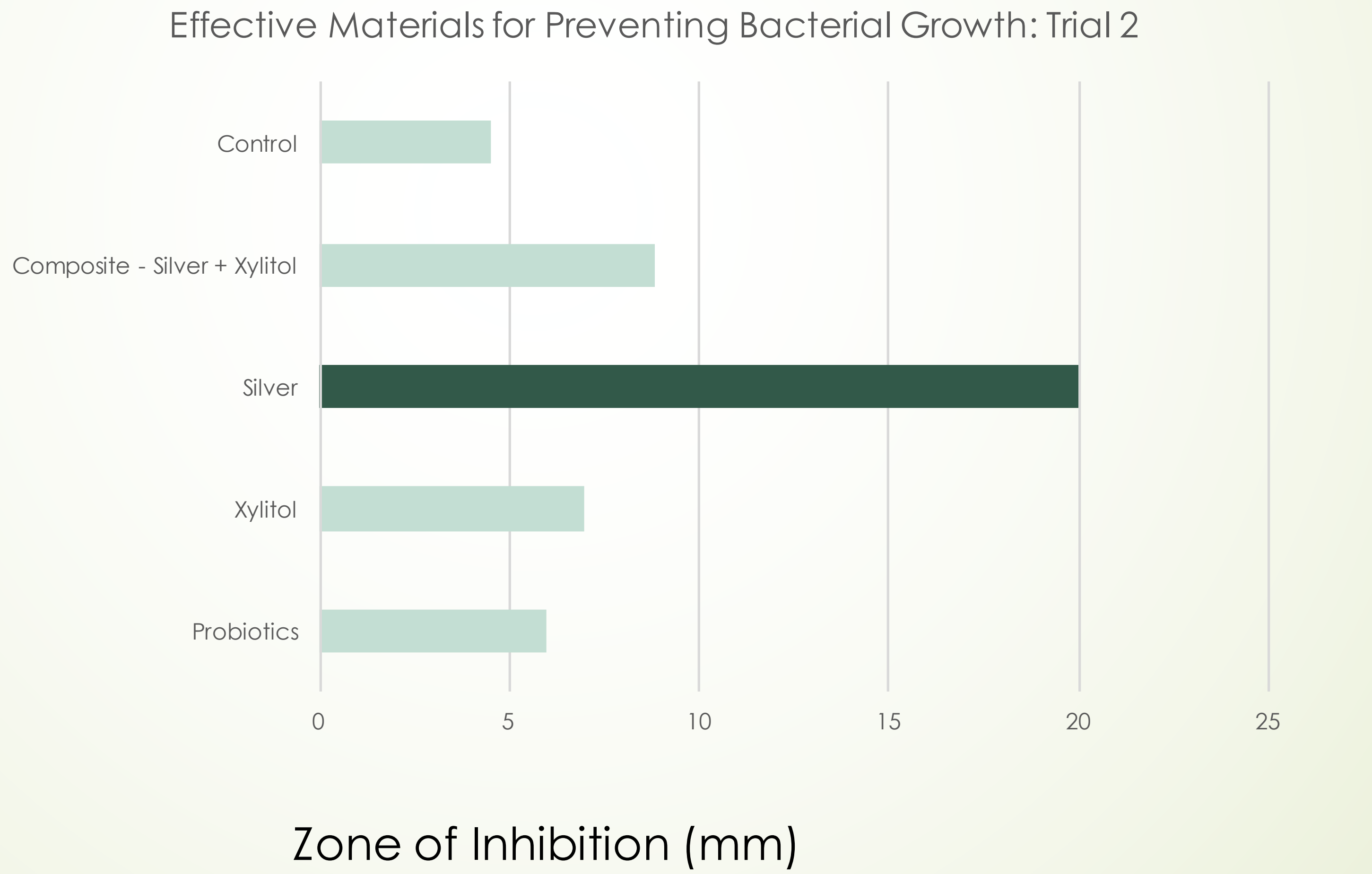
Testing the Effectiveness of Materials in **Preventing Bacterial Growth In Vitro**

Trial 1

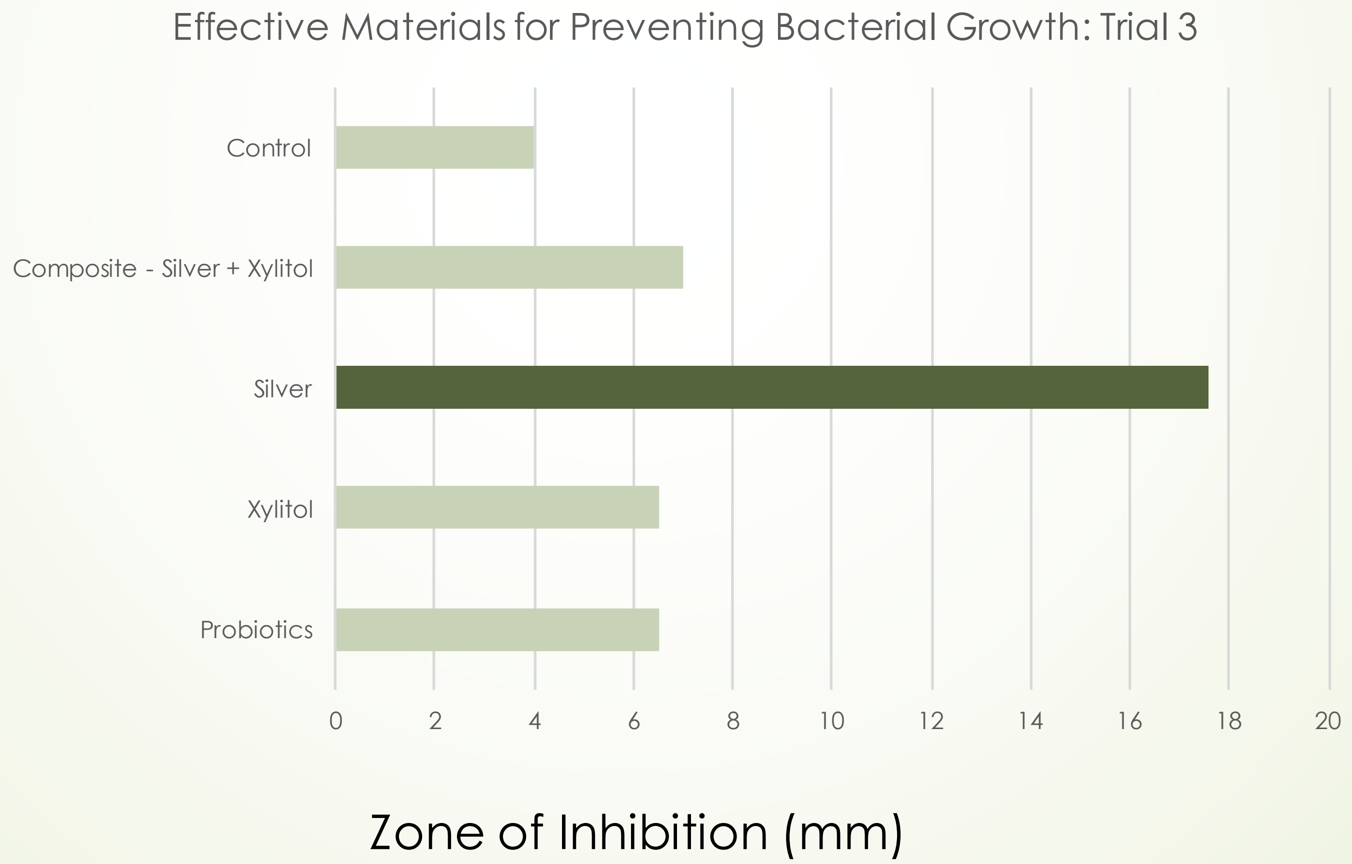
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	9	8	6	3	6.5
Xylitol	4	12	5	3	6
Silver	**15**	**15**	**5**	**10**	**11.25**
Composite - Silver + Xylitol	6	12	4	5	6.8
Control	5	3	2	8	4.5

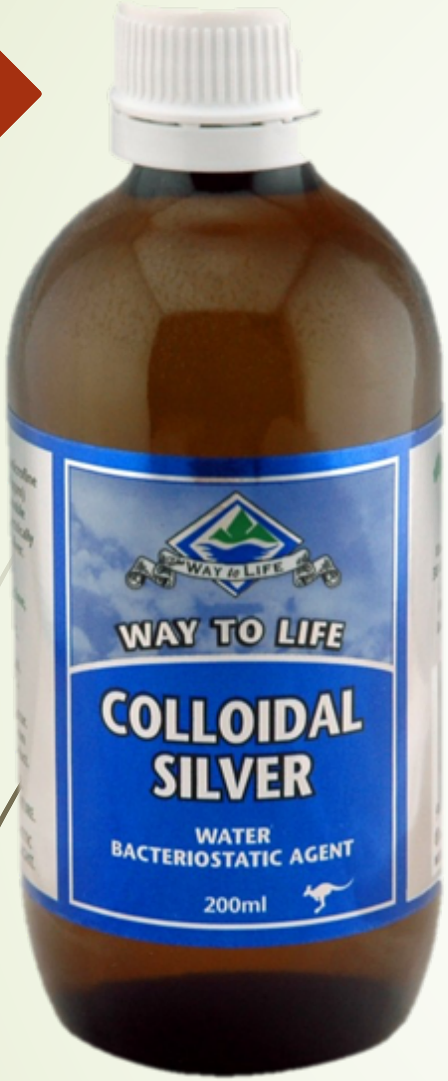


Material	Trial 2				Mean
	Sample 1	Sample 2	Sample 3	Sample 4	
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	6	8	5	5	6
Xylitol	4	11	6	7	7
Silver	**20**	**25**	**15**	**20**	**20**
Composite - Silver + Xylitol	3	12	7	13	8.8
Control	5	3	2	8	4.5



Trial 3					
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	7	7	10	2	6.5
Xylitol	2	7	9	8	6.5
Silver	**24**	**23**	**19**	**22**	**17.6**
Composite - Silver + Xylitol	1	9	6	12	7
Control	2	3	8	3	4





Colloidal silver was the most effective in stopping bacterial growth. Since it was successful during in vitro testing, it would be used during in vivo testing.

Silver was the most effective ingredient tested in vitro for stopping bacterial growth. In Trial 1, Trial 2, and Trial 3 consistent results were obtained in sample after sample tested. Our hypothesis was refuted because we expected the composite of silver + xylitol to "outperform" silver treatments alone. Based on the results of repeated testing, silver will now be tested in vivo to learn if similar success in fighting bacteria and promoting cell regrowth/healing will occur on a living organism. Testing will NOT involve vertebrates - invertebrates only will be used for experimentation.

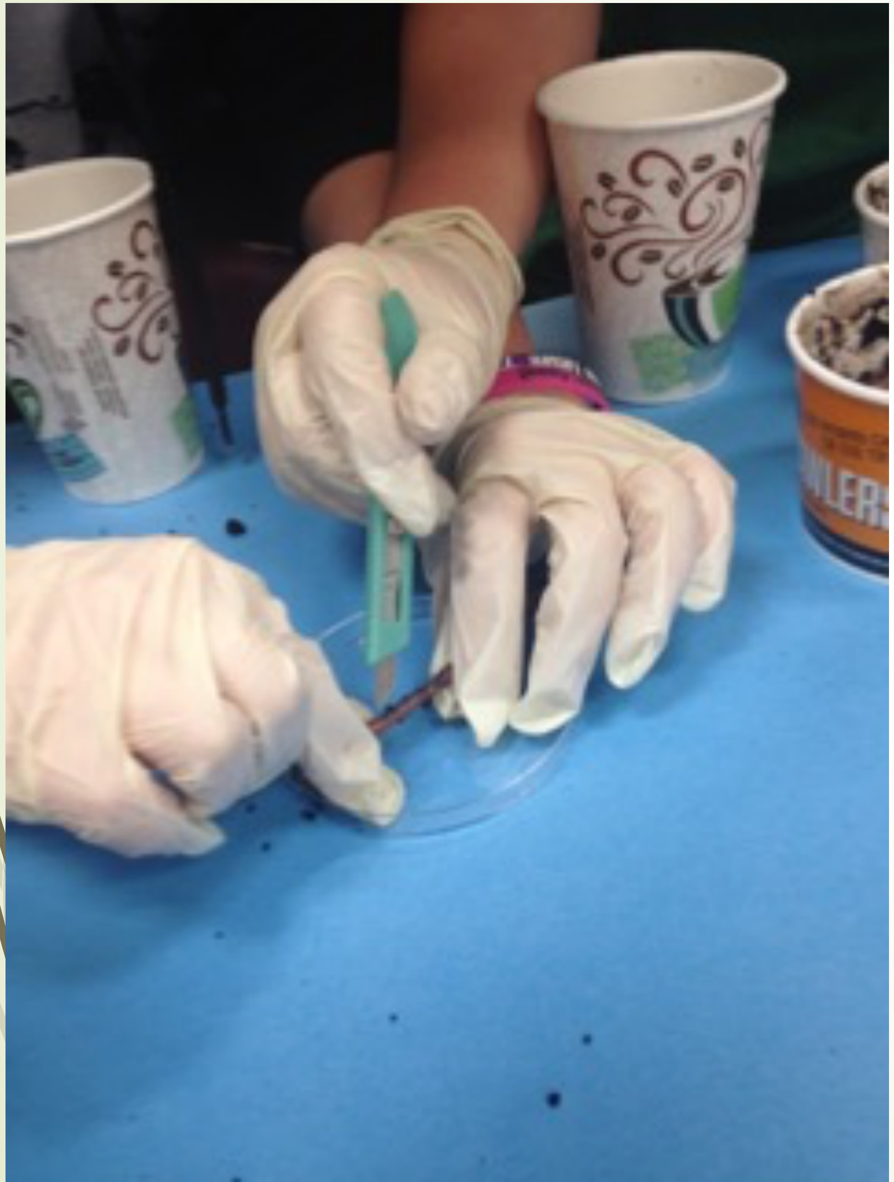
Image from www.rochway.com.au

In Vivo Experiments

Photos * Data * Tables * Graphs

Silver Bullet Ecybermission Team 2016

Working together to make a 1 cm incision in the earthworm

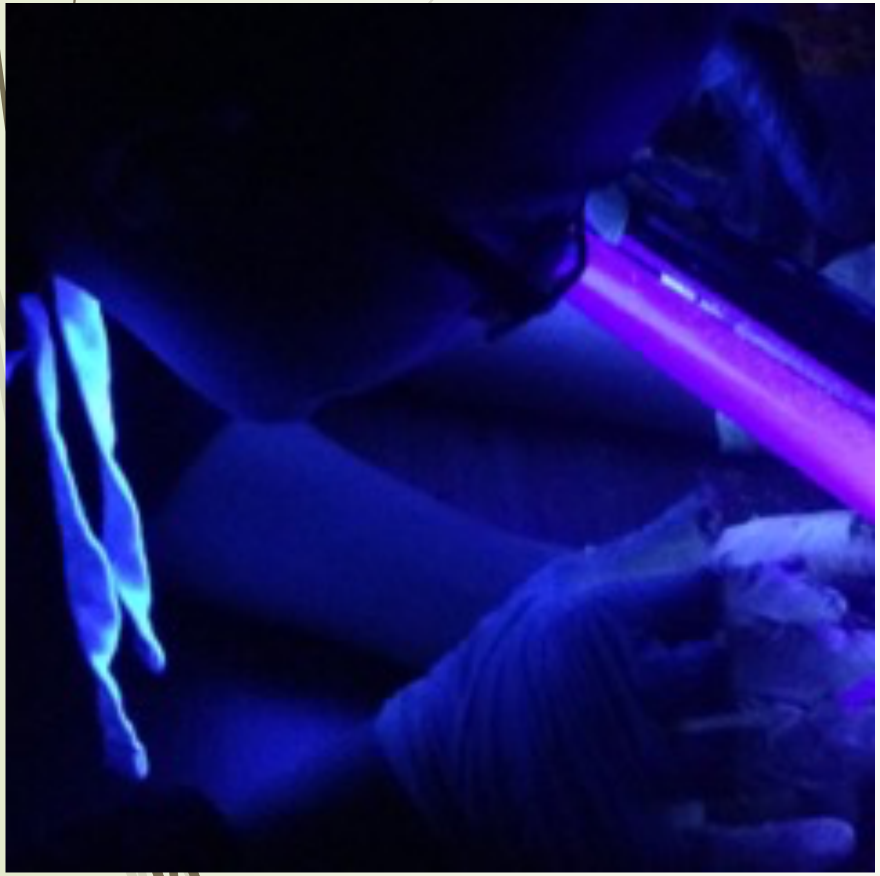


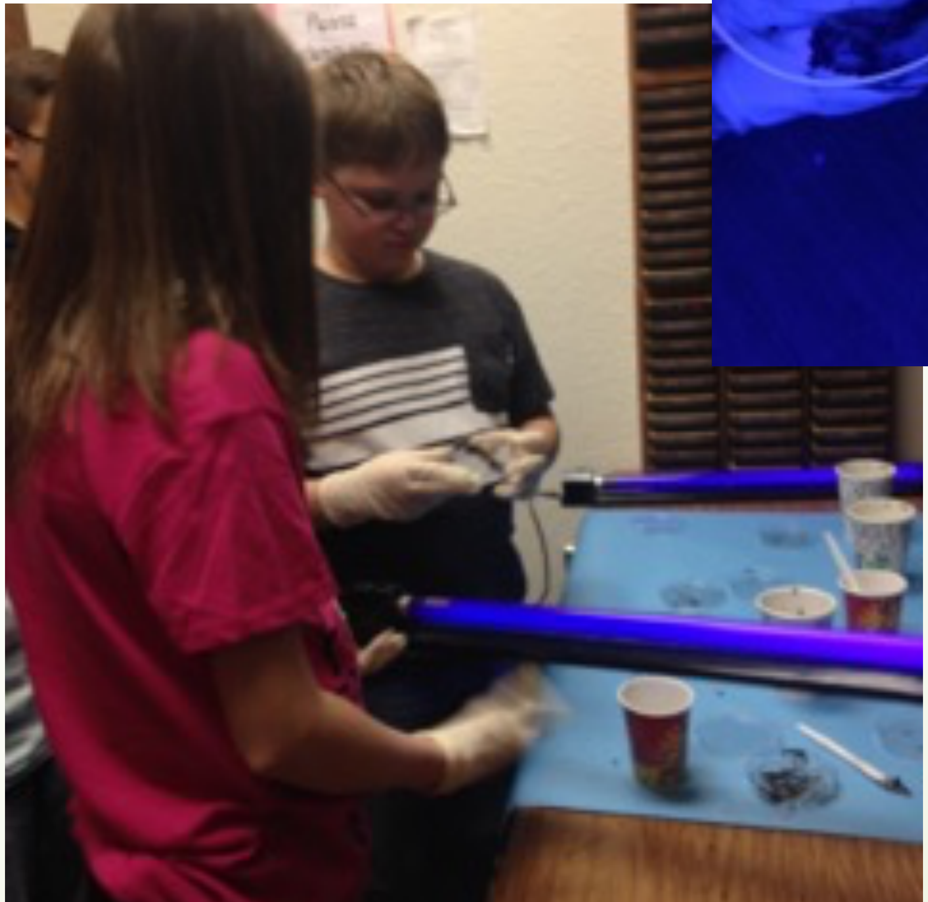
Sunday afternoon in the school library separating earthworms, preparing habitats, and treating incisions with silver.

Applying colloidal silver to the wound on the earthworm ….

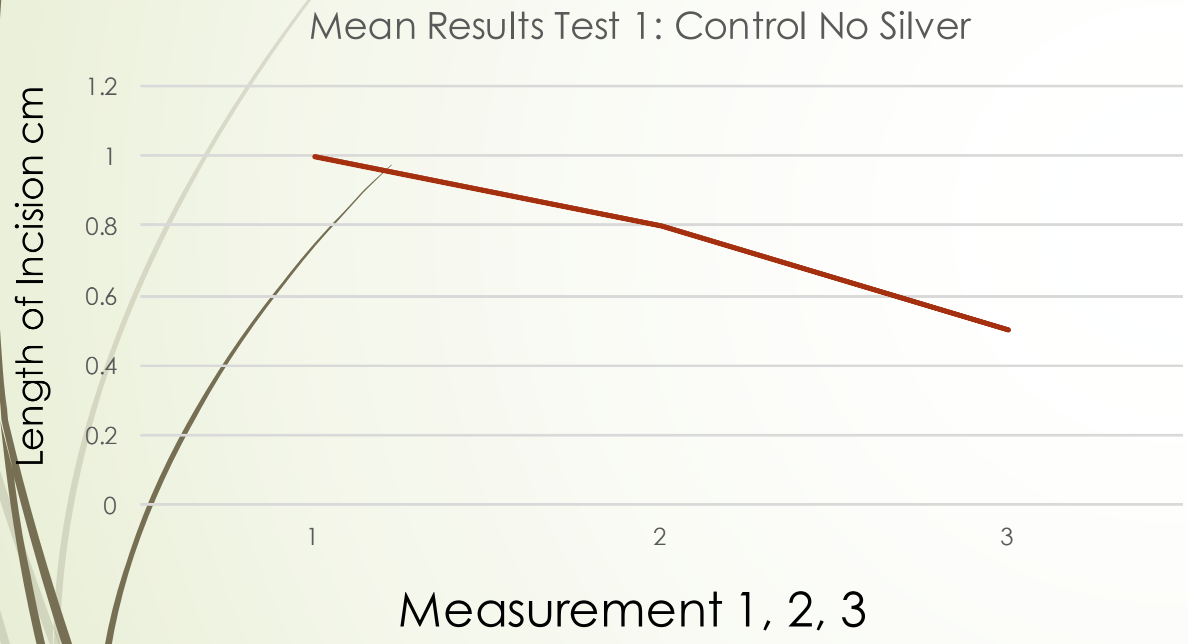
Measuring regrowth from day to day

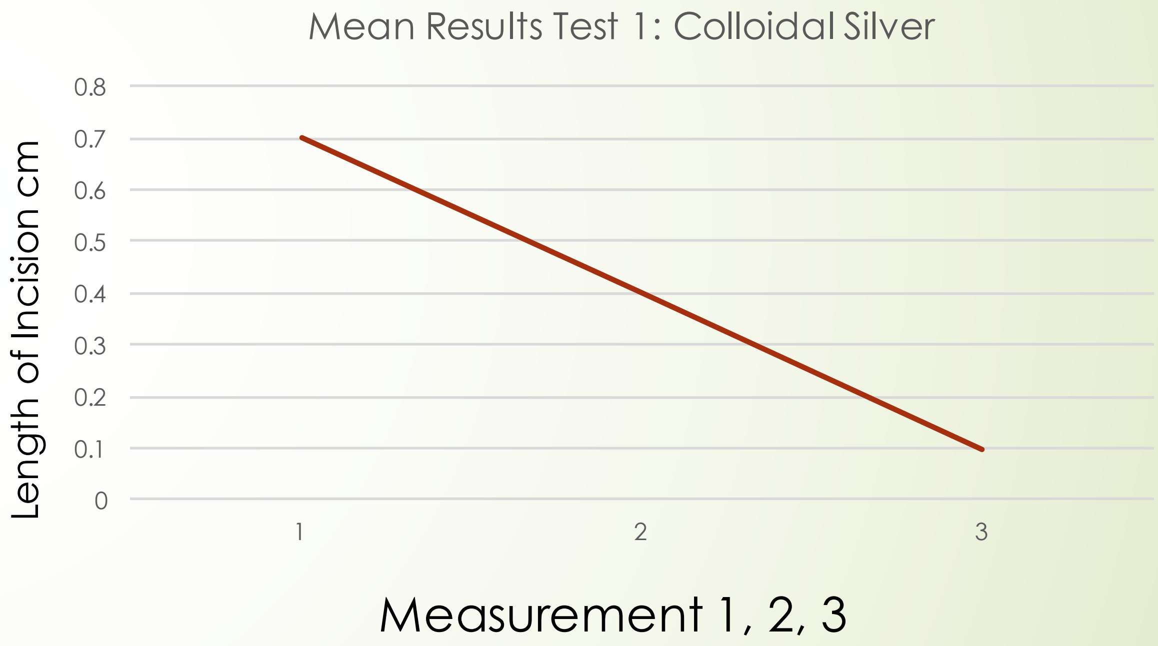
We had to get creative in finding the wounds and measuring them. Under a black light, the pyranine made the silver easier to see and measure.



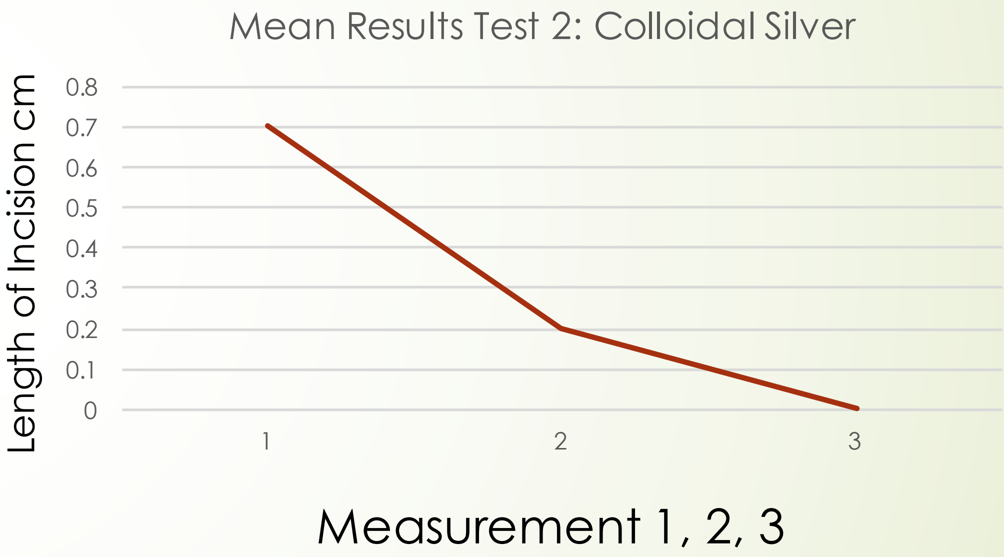


In Vivo Experimentation - Test 1 - Measurement of Incision (Initial = 1.0 cm)									
Condition Applied	Day 3			Day 4			Day 5		
	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3
Control No Silver	1	1	0.8	0.7	0.8	0.8	0.5	0.5	0.4
Colloidal Silver	0.7	0.7	0.6	0.3	0.4	0.5	0	0.1	0.2

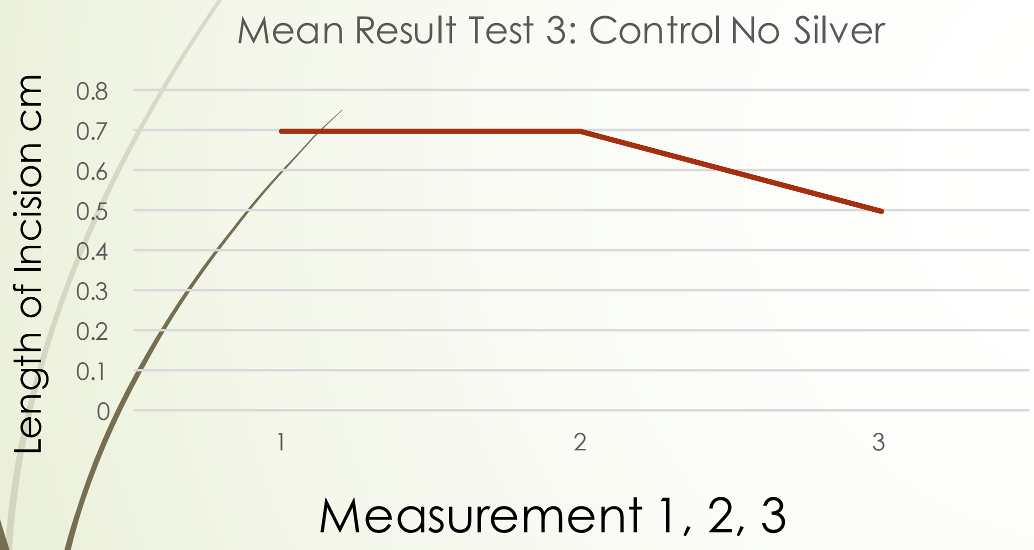


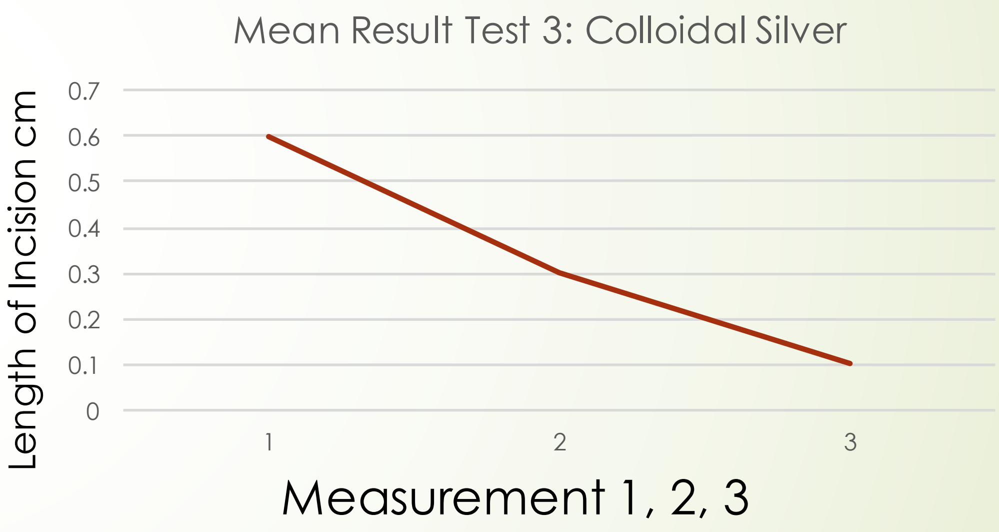


In Vivo Experimentation - Test 2 - Measurement of Incision (Initial = 1.0 cm)									
Condition Applied	Day 3			Day 4			Day 5		
	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3
Control No Silver	0.9	0.8	1	0.7	0.5	0.7	0.5	0.4	0.5
Colloidal Silver	0.8	0.7	0.6	0.4	0.3	0.2	0	0	0



In Vivo Experimentation - Test 3 - Measurement of Incision Initial = 1.0 cm									
Condition Applied	Day 3			Day 4			Day 5		
	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3	Earthworm 1	Earthworm 2	Earthworm 3
Control No Silver	0.9	1	0.7	0.8	0.8	0.7	0.6	0.4	0.5
Colloidal Silver	0.6	0.7	0.5	0.3	0.4	0.2	0.1	0.2	0





During tests conducted in vivo using the earthworm Lubricus terrestris for testing, a 1 cm incision was made into the skin of the worm just below the clitellum band. Silver was mixed with pyranine, a non-toxic chemical that glows brilliantly under UV black light. Under a black light, it was easy to see the site of the incision and make measurements, whereas it was extremely difficult before this treatment.

Then, the silver was applied to the wound. Earthworms were returned to their containers and measurements were made in cm on Day 3, 4, and 5.

The worms treated with silver had greater cell growth and wound healing faster than those with no silver applied. These results were consistent during testing and show that colloidal silver does speed cell regrowth and healing on living organisms.

Bandage Enhancement

- Photos * Data * Tables * Graphs

- Silver Bullet Ecybermission Team 2016

Embedding Hydrogel with Silver

Trial	Amount of Silver in Syringe (ml)	Percent of Silver Successfully Transferred to Hydrogel (ml)
1	0.5	100%
2	0.5	100%
3	0.5	85%
4	0.5	100%
5	0.5	100%

In every test except Test 3, 100% of the silver that was contained in the syringe was able to be successfully transferred into the hydrogel pad. The silver remained in the hydrogel as part of the bandage throughout testing. This hydrogel enhanced bandage with colloidal silver would keep a wound moist, protected from airborne bacteria, and in contact with colloidal silver during the time the bandage remains on the wound.

Adding silver from a syringe to a hydrogel pad to enhance the bandage's ability to fight bacteria.

Effective Materials in Preventing Bacterial Growth and Halting Biofilms

IN VITRO EXPERIMENTATION

SILVER BULLET
eCYBERMISSION
7th Grade 2016

Purpose

- To identify a material that will be highly effective in preventing bacterial growth for the purpose of engineering an ointment for chronic wounds

Hypothesis

- Following extensive research, it is believed that if a composite material of silver + xylitol is applied in vitro, then bacterial growth will be stopped more effectively than with the application of silver, xylitol, or probiotics alone.

Validity of the Hypothesis

- During in vitro tests, if the zone of inhibition surrounding the applied materials (silver, xylitol, xylitol + silver, or fructooligosaccaride probiotic) is at least 10.0 mm in diameter, the hypothesis will be proved valid. This would indicate a clear area free of bacterial growth as a result of the material application.
- Measurements will be made using a metric ruler from the center of a material-soaked disk outward to the nearest bacterial colony.
- The disks are used to control the size of application of the material within the Petri dish for accurate and reliable measurements. According to Dr. Rumbaugh in the wound care laboratory, a zone at least double the size of application would indicate a successful treatment.

Controls and Constants

- Controls – A Petri dish containing agar and bacteria will be compared to the experimental Petri dishes
- Constants – To each dish in every trial, the temperature will be maintained at 37°C for incubation, the same amount of material will be applied to the filter paper, and the zone of inhibition around each sample will be read at the same time.

Manipulated Independent Variable

- The independent variable in this experiment is the type of material used in the Petri dish to stop the bacteria.
- Probiotics Colloidal Silver Xylitol Silver + Xylitol

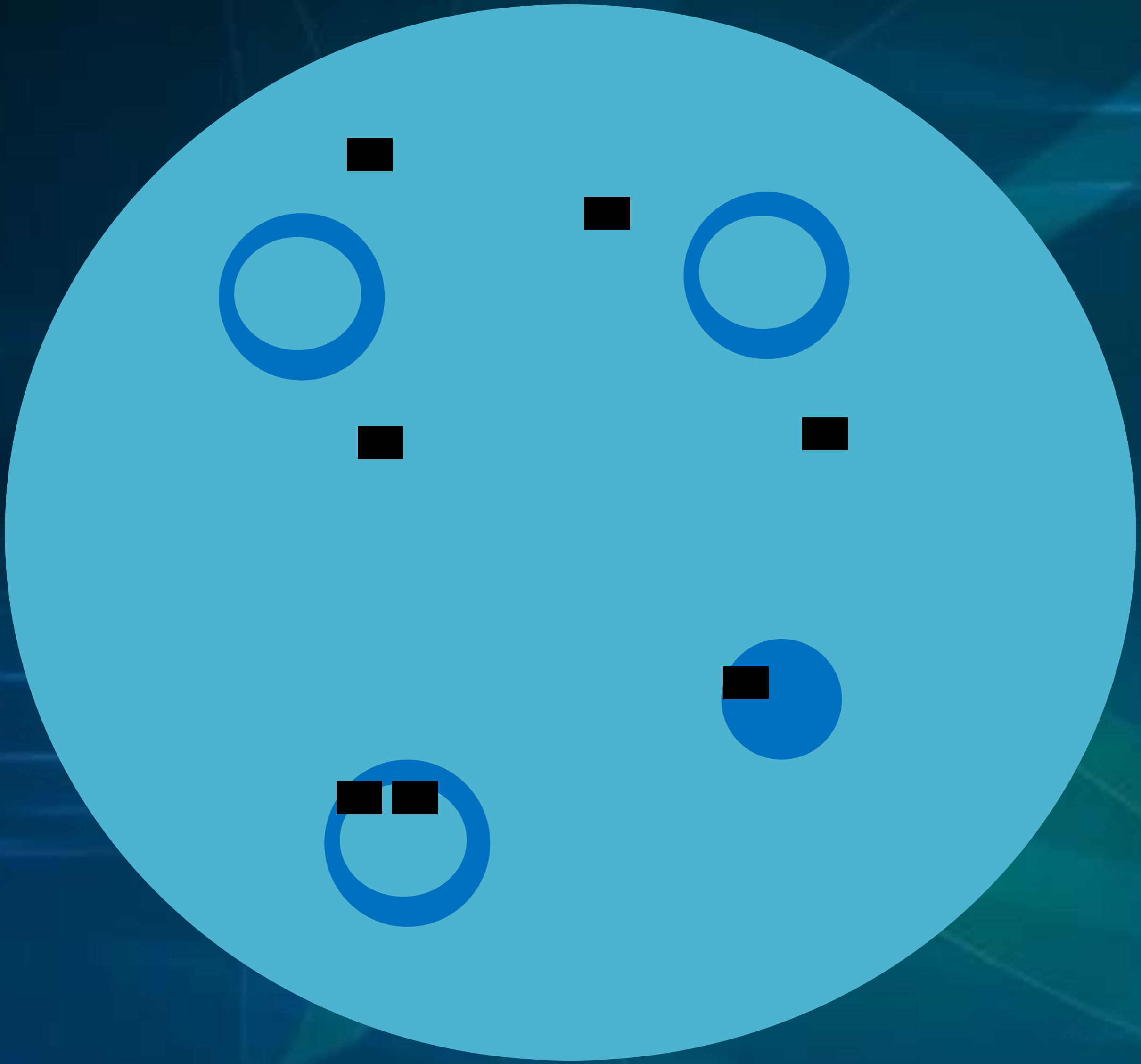
Responding Dependent Variable

- The dependent variable is the measurement taken called the Zone of Inhibition. The area surrounding the material disk and which is free from bacteria will be measured in millimeters. This is a measure of how effective the material is in stopping the growth of bacteria

Procedure for In Vitro Experimentation

1. Measure 23 grams of nutrient agar using a triple beam balance.
2. Measure one liter of distilled water into a saucepan and add the 23 grams of agar to the pan.
3. Heat and stir until the nutrient agar dissolves and then boil for one minute.
4. Carefully pour the solution into sterile Petri dishes, being sure to completely cover the bottom of the Petri dish.
5. Place lids on the Petri dishes, turn them upside down to prevent condensation from collecting on the agar, and allow to cool to 50°C and until the gel is set.
6. Follow Dr. Rumbaugh's lab directions and touch the agar gently to inoculate it with bacteria that would commonly be found near chronic wounds. Cough on the agar dish one time as well to simulate the bacteria found in the air that could cause infection in wounds.
7. Place paper disks saturated in xylitol, colloidal silver, probiotics, and silver + xylitol into four divided sections of the Petri dishes.
8. Incubate at 37°C or body temperature for 16 hours.
9. Remove Petri dishes from incubator and measure (mm) the Zone of Inhibition from the edge of the disk to the area clear of bacteria surrounding the disk.

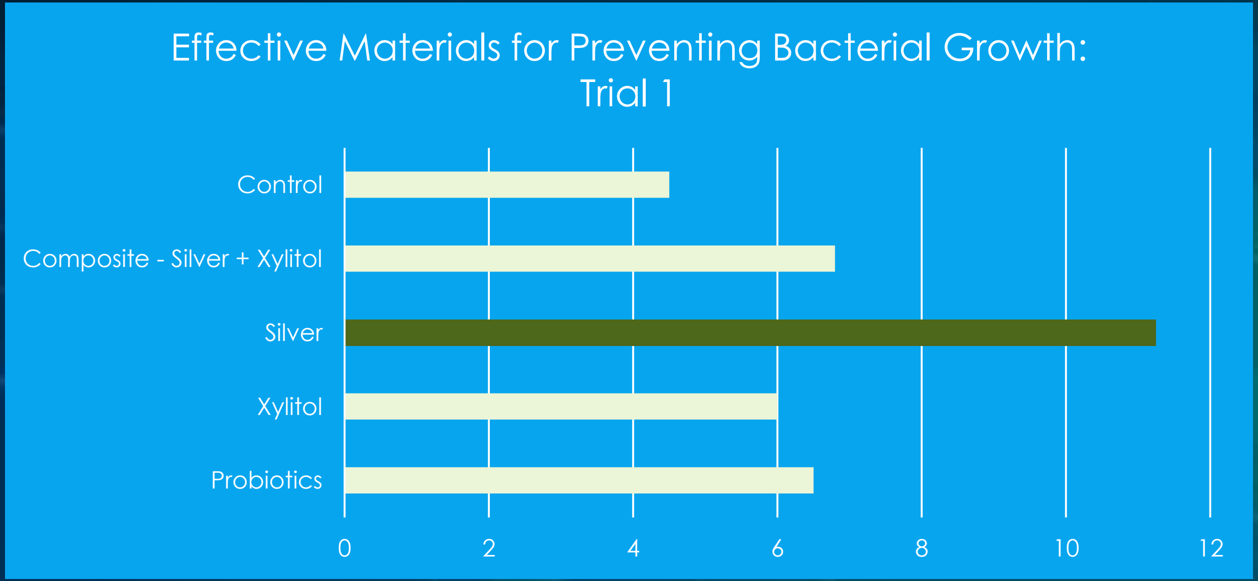
Zone of Inhibition Measurements



The black represents the bacteria and the blue spots represent the saturated paper disk of colloidal silver, xylitol, probiotics, or silver + xylitol. The ring around the material disk is clear of bacteria on some samples and bacteria has overtaken the disk in others. The most effective materials are those that prevent bacteria from getting near.

In Vitro Experimentation: Trial 1

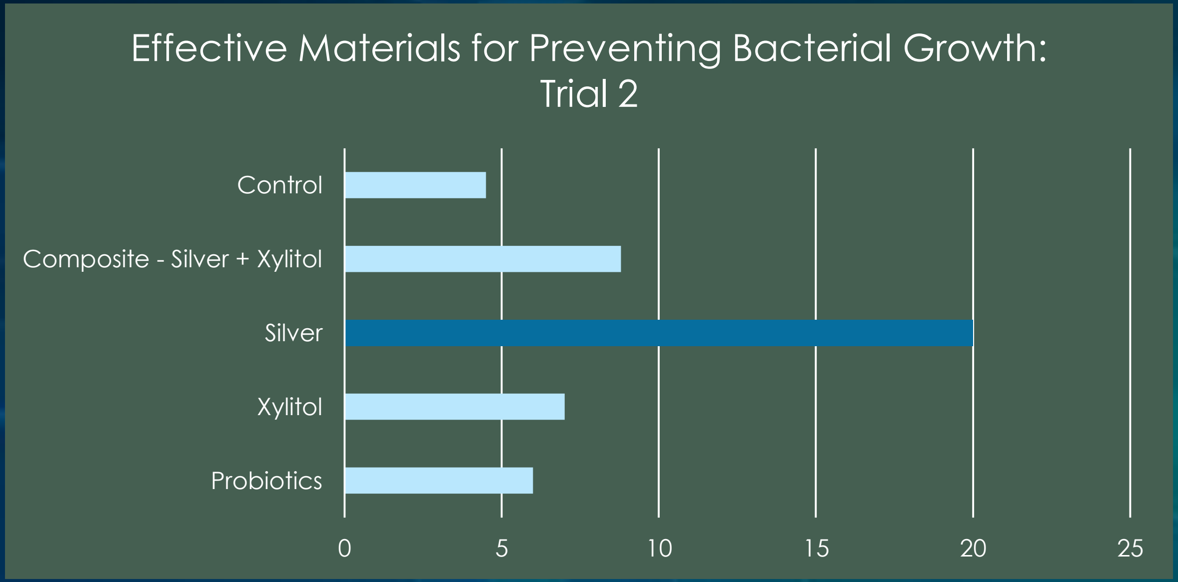
Testing the Effectiveness of Materials in **Preventing Bacterial Growth In Vitro**					
Trial 1					
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	9	8	6	3	6.5
Xylitol	4	12	5	3	6
Silver	**15**	**15**	**5**	**10**	**11.25**
Composite - Silver + Xylitol	6	12	4	5	6.8
Control	5	3	2	8	4.5



Zone of Inhibition (millimeters)

In Vitro Experimentation: Trial 2

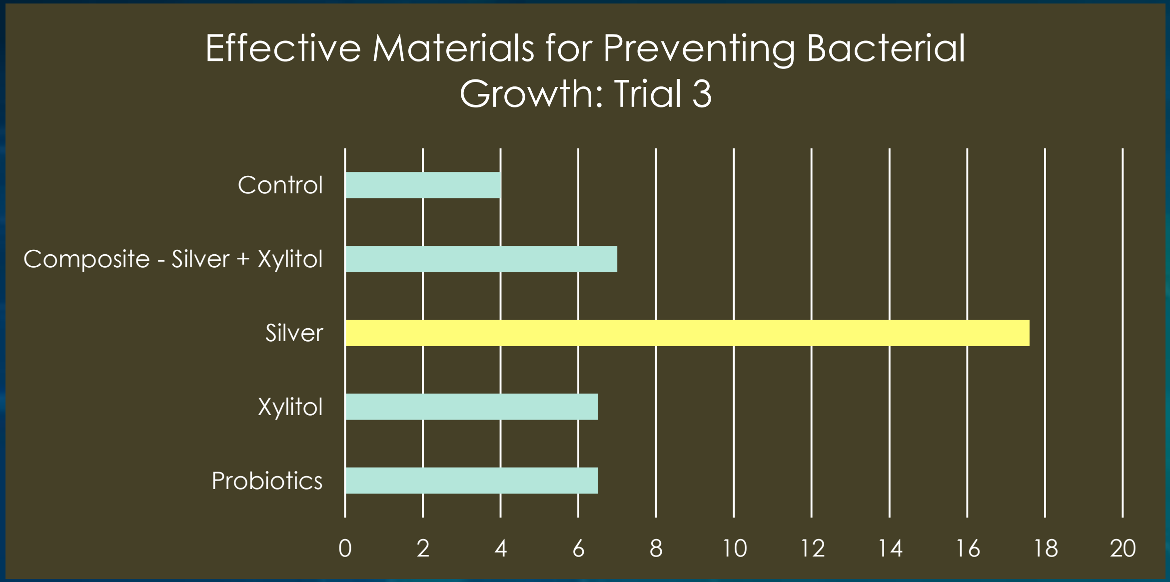
Trial 2					
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	6	8	5	5	6
Xylitol	4	11	6	7	7
Silver	**20**	**25**	**15**	**20**	**20**
Composite - Silver + Xylitol	3	12	7	13	8.8
Control	5	3	2	8	4.5



Zone of Inhibition (millimeters)

In Vitro Experimentation: Trial 3

Material	Trial 3				
	Sample 1	Sample 2	Sample 3	Sample 4	Mean
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	7	7	10	2	6.5
Xylitol	2	7	9	8	6.5
Silver	**24**	**23**	**19**	**22**	**17.6**
Composite - Silver + Xylitol	1	9	6	12	7
Control	2	3	8	3	4



Zone of Inhibition (millimeters)

Qualitative Data/Observations/Results

Silver was the most effective ingredient tested in vitro for stopping bacterial growth. In Trial 1, Trial 2, and Trial 3 consistent results were obtained in sample after sample tested. Our hypothesis was refuted because we expected the composite of silver + xylitol to "outperform" silver treatments alone. Based on the results of repeated testing, silver will now be tested in vivo to learn if similar success in fighting bacteria and promoting cell regrowth/healing will occur on a living organism. Testing will NOT involve vertebrates - invertebrates only will be used for experimentation.

Conclusion

- The most effective material tested in fighting biofilms and bacteria that cause infection was colloidal silver. Our hypothesis was refuted – the composite of silver + xylitol was ineffective, while silver alone consistently showed great potential in fighting bacteria.
- With the knowledge of silver and its effectiveness in stopping bacteria, the next stage of testing will involve the invertebrate earthworms and in vivo experimentation.

Progression from In Vitro Testing to the Development of a Salve for Chronic Wounds

- In vitro testing
- Identification of silver as antimicrobial

Step 1
In Vitro

Step 2
In Vivo

- In vivo testing
- Silver used on earthworms with cuts

- Engineer an ointment
- Silver used in hydrogels

Step 3
Ointment

Effective Materials in Preventing Bacterial Growth and Halting Biofilms

IN VITRO EXPERIMENTATION

SILVER BULLET
eCYBERMISSION
7th Grade 2016

Purpose

- To identify a material that will be highly effective in preventing bacterial growth for the purpose of engineering an ointment for chronic wounds

Hypothesis

- Following extensive research, it is believed that if a composite material of silver + xylitol is applied in vitro, then bacterial growth will be stopped more effectively than with the application of silver, xylitol, or probiotics alone.

Validity of the Hypothesis

- During in vitro tests, if the zone of inhibition surrounding the applied materials (silver, xylitol, xylitol + silver, or fructooligosaccaride probiotic) is at least 10.0 mm in diameter, the hypothesis will be proved valid. This would indicate a clear area free of bacterial growth as a result of the material application.
- Measurements will be made using a metric ruler from the center of a material-soaked disk outward to the nearest bacterial colony.
- The disks are used to control the size of application of the material within the Petri dish for accurate and reliable measurements. According to Dr. Rumbaugh in the wound care laboratory, a zone at least double the size of application would indicate a successful treatment.

Controls and Constants

- Controls – A Petri dish containing agar and bacteria will be compared to the experimental Petri dishes
- Constants – To each dish in every trial, the temperature will be maintained at 37ºC for incubation, the same amount of material will be applied to the filter paper, and the zone of inhibition around each sample will be read at the same time.

Manipulated Independent Variable

- The independent variable in this experiment is the type of material used in the Petri dish to stop the bacteria.
- Probiotics Colloidal Silver Xylitol Silver + Xylitol

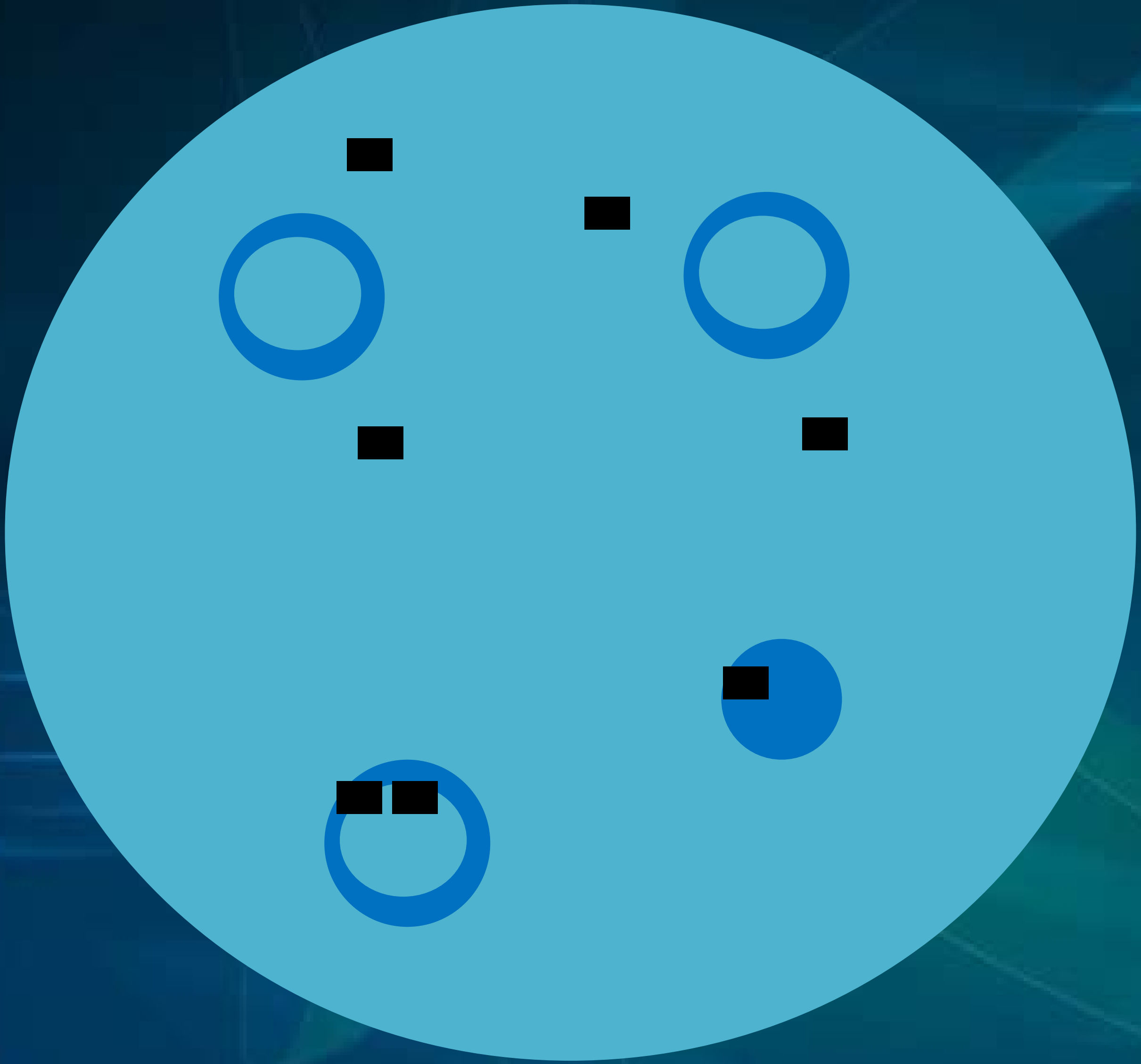
Responding Dependent Variable

- The dependent variable is the measurement taken called the Zone of Inhibition. The area surrounding the material disk and which is free from bacteria will be measured in millimeters. This is a measure of how effective the material is in stopping the growth of bacteria

Procedure for In Vitro Experimentation

1. Measure 23 grams of nutrient agar using a triple beam balance.
2. Measure one liter of distilled water into a saucepan and add the 23 grams of agar to the pan.
3. Heat and stir until the nutrient agar dissolves and then boil for one minute.
4. Carefully pour the solution into sterile Petri dishes, being sure to completely cover the bottom of the Petri dish.
5. Place lids on the Petri dishes, turn them upside down to prevent condensation from collecting on the agar, and allow to cool to 50^{o}C and until the gel is set.
6. Follow Dr. Rumbaugh's lab directions and touch the agar gently to inoculate it with bacteria that would commonly be found near chronic wounds. Cough on the agar dish one time as well to simulate the bacteria found in the air that could cause infection in wounds.
7. Place paper disks saturated in xylitol, colloidal silver, probiotics, and silver + xylitol into four divided sections of the Petri dishes.
8. Incubate at 37^{o}C or body temperature for 16 hours.
9. Remove Petri dishes from incubator and measure (mm) the Zone of Inhibition from the edge of the disk to the area clear of bacteria surrounding the disk.

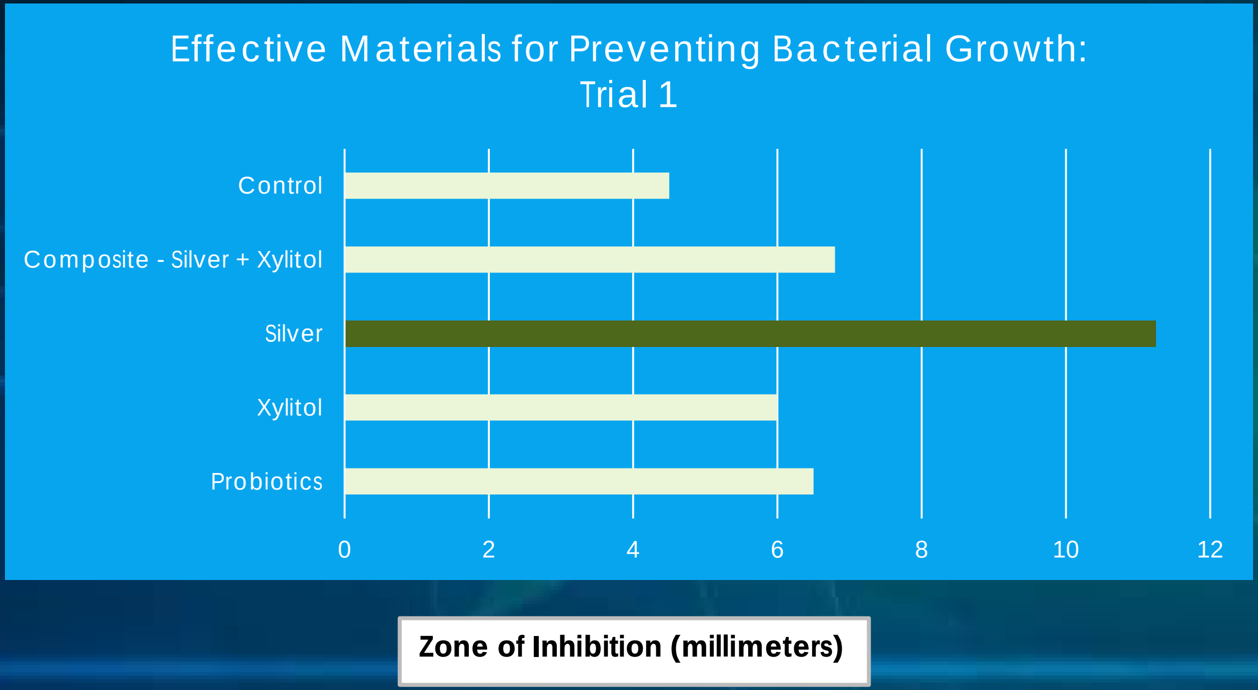
Zone of Inhibition Measurements

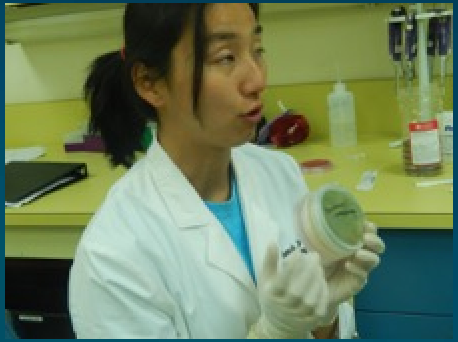


The black represents the bacteria and the blue spots represent the saturated paper disk of colloidal silver, xylitol, probiotics, or silver + xylitol. The ring around the material disk is clear of bacteria on some samples and bacteria has overtaken the disk in others. The most effective materials are those that prevent bacteria from getting near.

In Vitro Experimentation: Trial 1

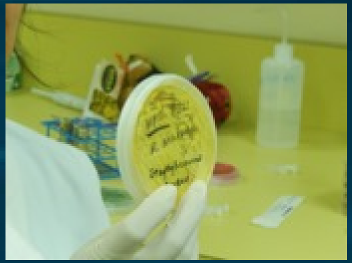
Testing the Effectiveness of Materials in **Preventing Bacterial Growth In Vitro**					
Trial 1					
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	9	8	6	3	6.5
Xylitol	4	12	5	3	6
Silver	**15**	**15**	**5**	**10**	**11.25**
Composite - Silver + Xylitol	6	12	4	5	6.8
Control	5	3	2	8	4.5

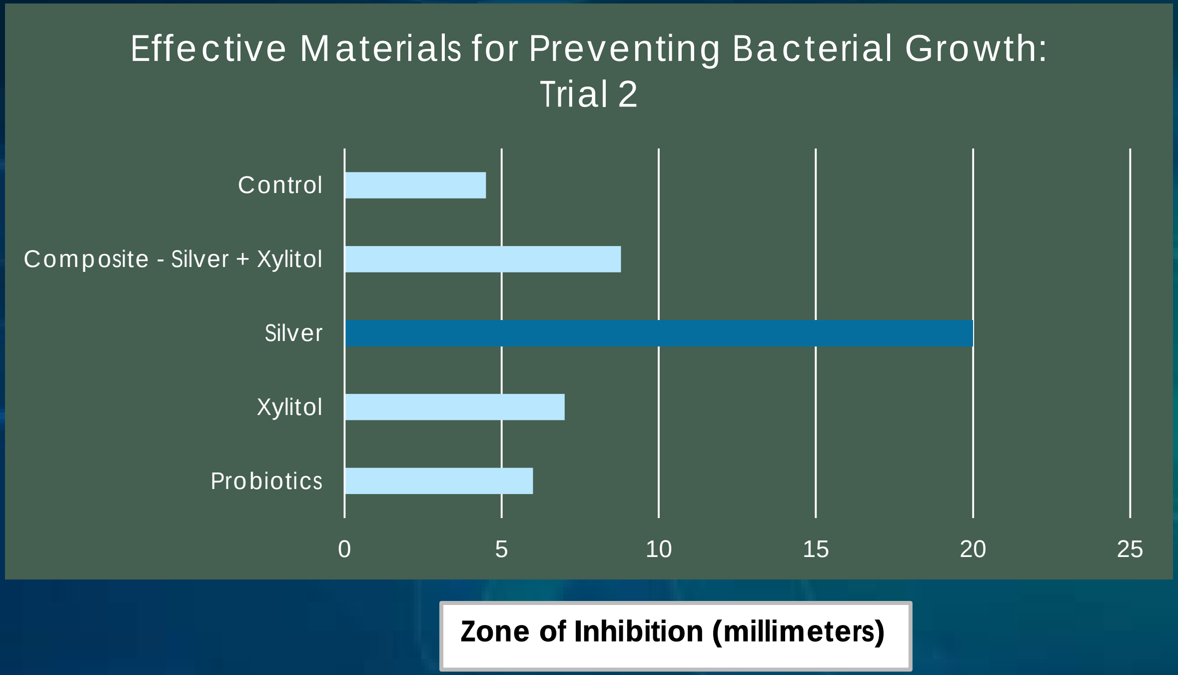




In Vitro Experimentation: Trial 2

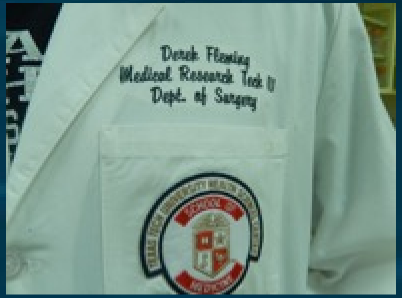
	Trial 2				
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	6	8	5	5	6
Xylitol	4	11	6	7	7
Silver	**20**	**25**	**15**	**20**	**20**
Composite - Silver + Xylitol	3	12	7	13	8.8
Control	5	3	2	8	4.5

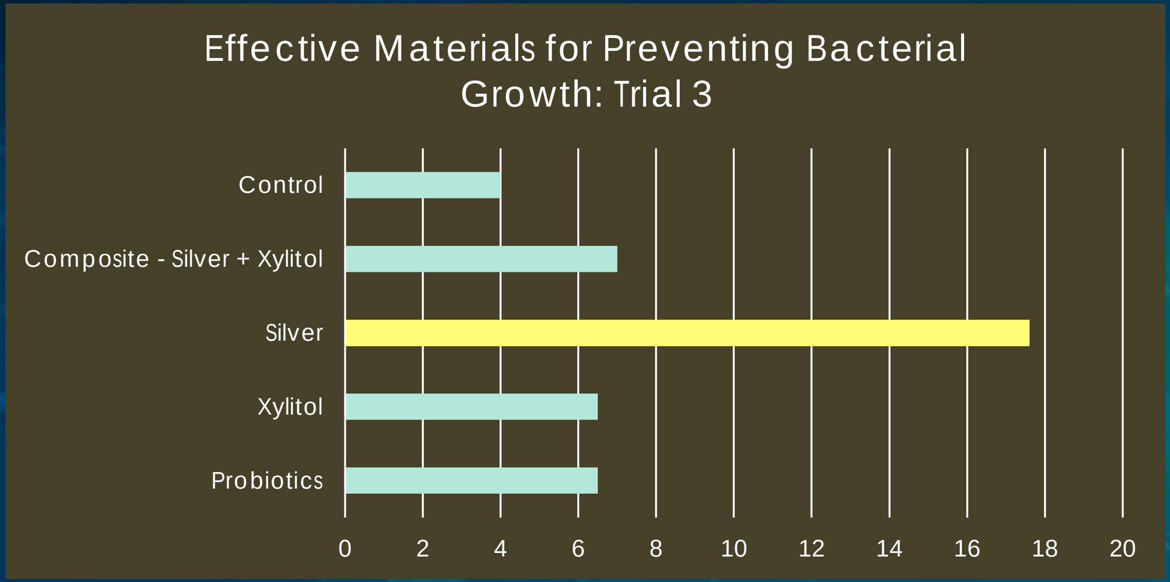




In Vitro Experimentation: Trial 3

Trial 3					
Material	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
	Size of Zone of Inhibition Surrounding the Disk (mm)				
Probiotics	7	7	10	2	6.5
Xylitol	2	7	9	8	6.5
Silver	**24**	**23**	**19**	**22**	**17.6**
Composite - Silver + Xylitol	1	9	6	12	7
Control	2	3	8	3	4





Zone of Inhibition (millimeters)

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Silver Bullet Website

The team is developing a website to spread awareness of chronic wounds and the alternative methods of treatment that may be available to stop infection. This is the first website I've built and I'm learning a lot about how to add important features that will meet the needs of people with chronic wounds. Continue checking back to see the progress being made.

http://silverbulletecm.weebly.com

QR Code

To make it easy for people to visit our website, the team created a QR code to the website on posters for the places we would be presenting information. This is also available on our business cards and in brochures.

Silver Bullet Wound Care

Come visit us at the Whiteface Community Center for an introduction to a new wound healing bandage. We will be explaining the benefits of this treatment, what we learned from the Texas Tech laboratories, and how alternative medicine may play a role in healing chronic wounds. We would enjoy having you. See you there!

Silver Bullet 7th Grade Ecybermission Team*: Elizabeth Casarez, Blade Henry, Kaden Moses, and Kaylah Nunn*

For more information email us at
sciencerocksu@yahoo.com

Silver Bullet Wound Care

Cochran County Senior Citizens

You are invited to a presentation about our project – CHRONIC WOUNDS. We will share:

- Alternative materials for wound care
- Results of our lab experiments
- Photos of visits to Tech laboratories

Silver Bullet 7th Grade Ecybermission Team: *Elizabeth Casarez, Blade Henry, Kaden Moses, and Kaylah Nunn*

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