SO MANY BIOFILMS, SO LITTLE TIME!

A STUDY OF BIOFILMS IN MEDICINE

BY DR. CLAUDIA SINGKORN RAT
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Introduction

ABOUT THIS LESSON

I chose this to work on biofilms in this unit because I learned about their importance in disease and health during a 2-week lab visit to Dr. Gregory Schultz’s wound research lab at University of Florida. There I found out how important biofilms are in medicine and I was exposed to great research involving biofilm pathology and treatment research. Since these biofilms are such an important part of human anatomy dysfunction, I decided to design a whole unit about this topic.

The lessons were written as one unit, which would work very well at the end of the school year, as by this time students have been exposed to all or the majority of the body systems. This unit involves the integumentary, digestive, immune and cardiovascular systems.

The flow of the unit is as follows:

1. It starts off with a medical mystery in which students discover that the patient has endocarditis, which was caused by a bacterial infection during a root canal.
2. Then, lesson two first exposes dental plaque as a biofilm. Experiments allow a visual of a biofilm, as well as an exploration on the difficulty of eradicating these biofilms.
3. Lesson three expands on the problems that biofilms cause to the human body and on how researchers are trying to address these. It focuses on wound research, and the development of biofilm preventive techniques. A lab allows for the verification that certain dressings can help in addressing the problem.

The sequencing of the lesson plan is based on the following causal path:

As an alternative, the unit can be broken up into 3 discrete lessons. Lesson 1 can be presented during the cardiovascular system, lesson 2 during the digestive system, and lesson 3 during the integumentary system.
The unit was written as an explorative lesson and there is very little lecture time (30 minutes in 7 periods) and conventional testing involved. This was done for several reasons:

1. I wanted to take this topic and present it in a way that was relevant to the students with real-life applications without them worrying about “what will be on the test”.
2. I wanted to expose the students to the medical and research fields in a reality oriented way.
3. It allows students to apply their critical thinking skills to form conclusions in medical cases.
4. It shows students that they can read medical records and interpret the findings for a better understanding of a condition.
5. It gives them an understanding of the factors, trials and satisfaction involved in creating and executing new experiments.
6. It empowers students to take intellectual risks, formulate their own ideas, and evaluate information and ethical questions.

AUDIENCE:

<table>
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<tr>
<th>SCIENCE SUBJECT</th>
<th>Anatomy and Physiology</th>
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<tr>
<td>GRADE LEVEL</td>
<td>11th and 12th Grade</td>
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<td>ABILITY LEVEL</td>
<td>Honors</td>
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Tips About this Curriculum

Each lesson has the same structure as the following components:

**KEY QUESTION(S):** Identifies key questions the lesson will explore.

**OVERALL TIME ESTIMATE:** Indicates total amount of time needed for the lesson, including advanced preparation.

**LEARNING STYLES:** Visual, auditory, and/or kinesthetic.

**VOCABULARY:** Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

**LESSON SUMMARY:** Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

**STUDENT LEARNING OBJECTIVES:** Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

**STANDARDS:** Specific state benchmarks addressed in the lesson. Also collected in one list.

**MATERIALS:** Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

**BACKGROUND INFORMATION:** Provides accurate, up-to-date information from reliable sources about the lesson topic.

**ADVANCE PREPARATION:** This section explains what needs to be done to get ready for the lesson.

**PROCEDURE WITH TIME ESTIMATES:** The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

**ASSESSMENT SUGGESTIONS:** Formative assessment suggestions have been given. Teachers should feel free to create additional formative and summative assessment pieces.

**EXTENSIONS: (ACTIVITIES/LITERATURE)** There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

**RESOURCES/REFERENCES:** References used in the lesson are listed. A section of resources follows each lesson.
<table>
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<tr>
<th>DAY</th>
<th>LESSON</th>
<th>LESSON COMPONENTS</th>
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| 1   | LESSON 1: What Happened to Ginny Cardoza? A Medical Mystery | ▪ Medical Mystery  
▪ Video of a heart valve repair |
| 2   | LESSON 2: Do I Really Need to Floss? | ▪ Virtual root canal procedure  
▪ Relationship of dental procedures and endocarditis Worksheet  
▪ Evaluating dental prophylaxis guidelines  
▪ Inquiry Lab: Is mouthwash effective against dental plaque? |
| 3-4 | LESSON 3: Watch Those Biofilms! | ▪ Video on biofilms  
▪ Designing a Flyer/brochure/ Prezi of biofilm characteristics  
▪ Lecture on biofilms in chronic wounds  
▪ Experiment: Can Wound Dressing Inhibit Bacterial Growth  
▪ Ted Talk on Bacterial Quorum Sensing |
| 8-10| New lessons. Allow 10-15 minutes during each day to work on the inquiry lab of lesson 2 |
Unit Vocabulary

**Acute:** having a sudden onset, sharp rise, and short course

**Antimicrobial:** destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms

**Appendectomy:** removal of the appendix

**Arthralgia:** joint pains

**Biofilm:** A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

**Biopsy:** is a sample of tissue taken from the body in order to examine it more closely. A doctor should recommend a biopsy when an initial test suggests an area of tissue in the body isn't normal.

**Blood cultures:** is a microbiological culture of blood. It is employed to detect infections that are spreading through the bloodstream.

**C-reactive protein:** protein found in the blood that indicates inflammation and disease

**Cationic polymer:** a natural or synthetic compound of usually high molecular weight consisting of up to millions of repeated positively charged linked units, each a relatively light and simple molecule.

**Dental caries:** tooth decay or a cavity

**Dental plaque:** biofilm that develops naturally on the teeth.

**Dentin:** yellowish tissue that makes up the bulk of all teeth. It is harder than bone but softer than enamel and consists mainly of apatite crystals of calcium

**Dressing:** A dressing is an adjunct used by a person for application to a wound to promote healing and/or prevent further harm. A dressing is designed to be in direct contact with the wound, which makes it different from a bandage, which is primarily used to hold a dressing in place.

**Echocardiography:** also known as echocardiogram; procedure which uses ultrasound technology which can examine the heart or blood vessels.

**Enamel:** The hard, calcareous substance covering the exposed portion of a tooth.

**Endocarditis:** is an inflammation of the inner layer of the heart, the endocardium. It usually involves the heart valves.

**Genetic expression:** is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as rRNA genes or tRNA genes, the product is a functional RNA. The process of gene expression is used by all known life.

**Gingiva:** Also known as gum. It consists of the mucosal tissue that lies over the mandible and maxilla inside the mouth.

**Gingivitis:** inflammation of the gum/gingiva.
**Gutta percha:** A tough plastic substance from the latex of several Malaysian trees used to plug the root cavity in a tooth.

**Hemostasis:** is a process, which causes bleeding to stop.

**Infection:** is the invasion of a host organism's bodily tissues by disease-causing organisms, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce.

**Inflammation:** is the body's attempt at self-protection; the aim being to remove harmful stimuli, including damaged cells, irritants, or pathogens - and begin the healing process.

**Malaise:** a feeling of general discomfort or uneasiness, of being "out of sorts", often the first indication of an infection or other disease.

**Musculoskeletal** pertaining to the muscular and skeletal systems

**Myalgia:** muscle pains

**Neutrophils:** specific kind of white blood cells indicative of an infection

**Oral cavity:** the inside of the mouth.

**Planktonic:** state in which bacteria are free-living and not associated with a biofilm.

**Polysaccharides:** Large carbohydrates. Usually made out of hundreds of subunits, Examples are starches and cellulose.

**Proliferation:** To grow or multiply by rapidly producing new tissue, parts, cells, or offspring

**Prophylactic:** preventive measure; acting to defend against or prevent something, especially disease; protective.

**Pulp cavity:** The central cavity of a tooth containing the pulp (including the root canal).

**Quorum sensing:** system of stimulus and response correlated to population density. Many species of bacteria use quorum sensing to coordinate gene expression according to the density of their local population.

**Root canal:** Procedure in which the tooth’s root canal is cleaned out and sealed

**Sub acute:** An abnormal condition present in a person who appears to be clinically well; Between acute and chronic

**Superbugs:** Term refers to antibiotic resistant organisms.

**T cells:** specific immune system cells. Severely decreased in AIDS

**Tartar:** Calculus or tartar is a form of hardened dental plaque. It is caused by the continual accumulation of minerals from saliva on plaque on the teeth.

**Undiagnosed:** not having been identified

**Urological:** pertaining to the urinary system (kidneys and bladder)

**Valve dysfunctions:** The heart has four valves that open and close as the heart pumps. When any of the four valves fails to function properly, it is known as cardiac valvular dysfunction.

**Vegetation:** an abnormal growth of microorganisms upon a body part

**White blood cells:** are cells of the immune system involved in defending the body against both infectious disease and foreign materials.
<table>
<thead>
<tr>
<th>Standard Code</th>
<th>Standard Description</th>
<th>LESSON 1</th>
<th>LESSON 2</th>
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<tbody>
<tr>
<td>SC.912.L.16.7</td>
<td>Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.</td>
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<tr>
<td>SC.912.L.14.2</td>
<td>Relate structure to function for the components of plant and animal cells. Explain the role of cell membranes as a highly selective barrier (passive and active transport).</td>
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<tr>
<td>SC.912.L.14.6</td>
<td>Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.</td>
<td>X</td>
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<tr>
<td>SC.912.L.14.36</td>
<td>Describe the factors affecting blood flow through the cardiovascular system.</td>
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<tr>
<td>SC.912.L.14.46</td>
<td>Describe the physiology of the digestive system, including mechanical digestion, chemical digestion, absorption and the neural and hormonal mechanisms of control.</td>
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<td>X</td>
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<tr>
<td>SC.912.L.14.52</td>
<td>Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.</td>
<td>X</td>
<td>X</td>
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<tr>
<td>SC.912.N.1.4</td>
<td>Identify sources of information and assess their reliability according to the strict standards of scientific investigation.</td>
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<tr>
<td>SC.912.N.1.6</td>
<td>Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.</td>
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<td>X</td>
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<tr>
<td>SC.912.N.1.7</td>
<td>Recognize the role of creativity in constructing scientific questions, methods and explanations.</td>
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<tr>
<td>SC.912.N.2.1</td>
<td>Identify what is science, what clearly is not science, and what superficially resembles science (but fails to meet the criteria for science).</td>
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<tr>
<td>SC.912.N.4.1</td>
<td>Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.</td>
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<tr>
<td>SC.912.N.2.4</td>
<td>Explain that scientific knowledge is both durable and robust and open to change. Scientific knowledge can change because it is often examined and re-examined by new investigations and scientific argumentation. Because of these frequent examinations, scientific knowledge becomes stronger, leading to its durability.</td>
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<tr>
<td>Common Core Code</td>
<td>Standard Description</td>
<td>Lesson Number</td>
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<tr>
<td>RST.11-12.1.</td>
<td>Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.</td>
<td>X</td>
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<tr>
<td>RST.11-12.2.</td>
<td>Determine the central ideas or conclusions of a text; summarize complex concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.</td>
<td>X</td>
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<tr>
<td>RST.11-12.3.</td>
<td>Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks; analyze the specific results based on explanations in the text.</td>
<td>X X X</td>
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<tr>
<td>RST.11-12.4.</td>
<td>Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11–12 texts and topics.</td>
<td>X X X</td>
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<tr>
<td>RST.11-12.7.</td>
<td>Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.</td>
<td>X</td>
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<tr>
<td>RST.11-12.9.</td>
<td>Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.</td>
<td>X X</td>
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<tr>
<td>RST.11-12.10.</td>
<td>By the end of grade 12, read and comprehend science/technical texts in the grades 11–12 text complexity band independently and proficiently.</td>
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Background Information

Much of the material in this section on biofilms was abstracted from Costerton & Stewart - Scientific American July 2001 and the American Society for Microbiology

Biofilms are composed of populations or communities of microorganisms adhering to environmental surfaces. These microorganisms are usually encased in an extracellular polysaccharide that they themselves synthesize. Biofilms may be found on essentially any environmental surface in which sufficient moisture is present. Their development is most rapid in flowing systems where adequate nutrients are available.

Biofilms may form:

- on solid substrates in contact with moisture.
- on soft tissue surfaces in living organisms.
- at liquid air interfaces.

Typical locations for biofilm production include rock and other substrate surfaces in marine or freshwater environments.

Biofilms are also commonly associated with living organisms, both plant and animal. Tissue surfaces such as teeth and intestinal mucosa which are constantly bathed in a rich aqueous medium rapidly develop a complex aggregation of microorganisms enveloped in an extracellular polysaccharide they themselves produce.

Here, human dental plaque has been exposed to 5 % sucrose for 5 minutes, after which Gram's iodine (0.33% Iodine in 0.66% KI) was applied. The sucrose solution was applied to the left central incisor (which appears on the right) while the right central incisor served as a control.
Biofilms can also be beneficial to us. Scientists have made considerable use of microbial biofilms, primarily in the area of habitat remediation. Water treatment plants, waste water treatment plants and septic systems associated with private homes remove pathogens and reduce the amount of organic matter in the water or waste water through interaction with biofilms. Oil spills and biodegradation of resistant chemicals are other areas we use biofilms.

**How do biofilms form?**

Typically, within minutes, an organic monolayer adsorbs to the surface of the slide substrate. This changes the chemical and physical properties of the glass slide or other substrate. These organic compounds are found to be polysaccharides or glycoproteins. These adsorbed materials condition the surface of the slide and appear to increase the probability of the attachment of planktonic bacteria.

Free floating or planktonic bacteria encounter the conditioned surface and form a reversible, sometimes transient attachment often within minutes.

This attachment called **adsorption** is influenced by electrical charges carried on the bacteria, by Van der Waals forces and by electrostatic attraction although the precise nature of the interaction is still a matter of intense debate. In some instances, as for example, in the association between a pathogen and the receptor sites of cells of its host there may be a stereospecificity which though still reversible is stronger than that achieved strictly by ionic or electrostatic forces.

If the association between the bacterium and its substrate persists long enough, other types of chemical and physical structures may form which transform the reversible adsorption to a permanent and essentially
irreversible attachment.

The final stage in the irreversible adhesion of a cell to an environmental surface is associated with the production of extracellular polymer substances or EPS. Most of the EPS of biofilms are polymers containing sugars such as glucose, galactose, mannose, fructose, rhamnose, N-acetylglucosamine and others.

This layer of EPS and bacteria can now entrap particulate materials such as clay, organic materials, dead cells and precipitated minerals adding to the bulk and diversity of the biofilm habitat. This growing biofilm can now serve as the focus for the attachment and growth of other organisms increasing the biological diversity of the community.

Biofilms can show surprising variation in environmental conditions within very short internal distances.

Large oxygen variations occur within a few hundredths of a millimeter and significant diffusion gradients of nutrients can also be established if they are used by the bacteria in the biofilm. Another effect is that of protection of the bacteria deeper in the biofilm against toxic chemicals.

Groups of bacteria (consortia) grown on surfaces (biofilms) have been shown to be shock-resistant relative to cultures of a single type of bacteria. Growth on a surface is advantageous when compared to that in liquid because it increases the local density of the organisms, may facilitate the concentration of nutrients (especially important in low nutrient environments such as contaminated subsurface waters) and reduce exposure to shear stresses. In addition, consortia have diverse metabolic capabilities simply as a result of the genetic diversity present within the biofilm conferring to them a selective advantage over individual organisms within the environment.

Biochemistry and Interactions in Biofilms:

Some recent observations:

Bacteria such as *Pseudomonas aeruginosa* have genes that are turned on in about 15 mins after they attach to a surface - one gene is algC and is needed to make alginate - one of the components of the polysaccharide matrix material.

Many biofilm bacterial cells typically make dozens or hundreds of proteins not found in "free-floating" cells.

The cells signal to each other as the approach the "quorum" or number required to initiate biofilm formation. It seems as if a certain number of cells are needed to produce enough of the signal molecules to "switch over" the cells to matrix production - this is the "quorum".

In *Pseudomonas aeruginosa* and similar cells, the signal molecule is known - they are acylated homoserine lactones. If the genes for these compounds are missing - no biofilms are formed.

Some red algae produce compounds called substituted furanones - they have almost no biofilm on their fronds in seawater. It seems that they block the signal transmissions due to the acylated homoserine lactones since they bind to the receptor sites normally used for signaling.
LESSON 1 – What’s Wrong with Ginny? A Medical Mystery

KEY QUESTION(S): How are dental plaque and endocarditis related? What clues in a medical history can lead to a diagnosis of a disease?

SCIENCE CONCEPTS: Spreading of a disease, immune system response to disease, relationships among body systems,

OVERALL TIME ESTIMATE: 1 Class period of 50 minutes

LEARNING STYLES: Visual, and auditory.

VOCABULARY:

Acute: having a sudden onset, sharp rise, and short course

Antimicrobial: destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms

Appendectomy: removal of the appendix

Arthralgia; joint pains

Biofilm: A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

Blood cultures: is a microbiological culture of blood. It is employed to detect infections that are spreading through the bloodstream

C-reactive protein: protein found in the blood that indicates inflammation and disease

Echocardiography: also known as echocardiogram; procedure which uses ultrasound technology which can examine the heart or blood vessels.

Endocarditis: is an inflammation of the inner layer of the heart, the endocardium. It usually involves the heart valves.

Infection: is the invasion of a host organism's bodily tissues by disease-causing organisms, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce.
**Malaise:** a feeling of general discomfort or uneasiness, of being "out of sorts", often the first indication of an infection or other disease.

**Musculoskeletal** pertaining to the muscular and skeletal systems

**Myalgia:** muscle pains

**Neutrophils:** specific kind of white blood cells indicative of an infection

**Prophylactic:** preventive measure; acting to defend against or prevent something, especially disease; protective.

**Sub acute:** An abnormal condition present in a person who appears to be clinically well; between acute and chronic

**T cells:** specific immune system cells. Severely decreased in AIDS

**Undiagnosed:** not having been identified

**Urological:** pertaining to the urinary system (kidneys and bladder)

**Vegetation:** an abnormal growth of microorganisms upon a body part

**White blood cells:** are cells of the immune system involved in defending the body against both infectious disease and foreign materials.

**LESSON SUMMARY:**

The lesson summary will explore the causal relationship between the heart condition Endocarditis and dental plaque. Students will obtain a patient’s history and will apply research and their knowledge to diagnose the patient’s disease. Then they will see a short animation of the disease progression.

**STUDENT LEARNING OBJECTIVES**

The student will be able to:

1. Research and use new anatomical vocabulary
2. Relate symptoms to specific diseases
3. Infer where the disease originated from

**STANDARDS:**

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<th>COMMON CORE</th>
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<tbody>
<tr>
<td>SC.912.L.14.6</td>
<td>RST.11-12.4</td>
</tr>
<tr>
<td>SC.912.L.14.36</td>
<td>RST.11-12.4</td>
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<tr>
<td>SC.912.L.14.46</td>
<td>RST.11-12.7</td>
</tr>
<tr>
<td>SC.912.L.14.52</td>
<td>RST.11-12.9</td>
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</tbody>
</table>

**MATERIALS:**
**Student Materials:**
- Student handout: Medical Mystery: What’s Wrong with Ginny? (one copy per student)
- Computers, tablets, or smart phones with internet connectivity: (ideally one per student, but technology can be shared as needed)

**Teacher Materials:**
- Teacher handout: Medical Mystery: What’s Wrong with Ginny?
- Animation: Robotic Mitral Valve Repair works. The website is: http://www.baymedical.org/Services/Heart-Institute/Heart-Surgery.aspx

**BACKGROUND INFORMATION:**

**BACTERIAL ENDOCARDITIS**

1. Also known as Sub acute Bacterial Endocarditis (SBE) or Infective Bacterial Endocarditis (IBE).

2. It is a condition in which the interior of the heart is infected and it usually is the result of a blood infection. Bacteria can enter the bloodstream after a medical procedure such as dental work and it can settle on damaged heart valves (2). The microorganisms embed themselves in a meshwork of fibrin and other materials that are similar to a blood clot. Because it resembles a blood clot, it can hide from the immune system and it is uncommon to find white blood cells in the area of infection, In addition, these organisms can also form a biofilm, which makes the bacteria even more difficult to eradicate.

If left untreated, it will lead to death. Common treatment is intense antimicrobial therapy (intravenous antibiotics) for 2-6 weeks (3). Some patients also need surgery to remove the infected tissue and to repair damaged heart valves. (1)

Symptoms that may indicate endocarditis vary from mild to severe and they are quite non-specific. They are: low grade fever, headache, muscle aches, joint pains, weakness, tiredness, low appetite, and unexplained weight loss and heart murmur (4, 6).

There are two types of endocarditis, one with a rapid onset in which the patient is unwell in a few days and the other type in which the infection can take one to several weeks to settle in the heart. Since symptoms are vague, it may go undiagnosed for several months. Needless to say, the later it is diagnosed, the more difficult it is to treat it.

Not all bacteria can attach to the heart and the organism that is responsible for 50% of all the infections is Streptococcus viridans (2). This is a bacterium commonly found in the mouth, where it is harmless.

The best tests to diagnose are threefold: first a good history may reveal hints, such as dental work within the last two months; second blood cultures often can identify S. viridans, though sometimes they will not be seen, as these germs do
not grow well in laboratory settings (2). The third identifier is an **echocardiography**, which can show the **vegetation** in the affected part of the heart (3)

Below is a diagram of endocarditis on the heart valves:

![Diagram of Endocarditis on Heart Valves](image)

3. As a preventive measure, many doctors would prescribe **prophylactic** antibiotics in patients with heart problems, such as valve deformities. But studies question antibiotic prophylaxis since the benefit has not been evidenced, and there is considerable danger to developing antibiotic-resistant bacteria (1, 5).

Experts agree that good oral hygiene, which includes brushing and flossing teeth, is the best prevention known at this time.

**ADVANCE PREPARATION:**

1. Copy student handouts
2. Procure technology (computers)
3. Preview 3-minute Animation: **Robotic Mitral Valve Repair** works. The website is: [http://www.baymedical.org/Services/Heart-Institute/Heart-Surgery.aspx](http://www.baymedical.org/Services/Heart-Institute/Heart-Surgery.aspx)
4. Read the medical mystery. Two web sites that are great in explaining bacterial endocarditis are:
   a. [http://circ.ahajournals.org/content/107/20/e185.full#sec-2](http://circ.ahajournals.org/content/107/20/e185.full#sec-2)

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

1. Start class by posting the following question on the board or PowerPoint slide:
   How many of you know of a case where somebody went to the hospital and they were misdiagnosed?
2. Discussion (5 minutes):
   a. Allow 2-3 students to relate their experiences
b. Ask: How can that be? Very often students will blame it on doctor and staff incompetence. If they do not, ask them if this can be the case. Once they are incensed about this incompetence introduce the Activity

3. **Activity: Medical Mystery (35 minutes)**
   a. Start by stating: Let’s see how difficult or easy it is to diagnose a patient. I will give you a scenario and you will be able to use the Internet and your knowledge to come up with the answers.
   b. Distribute the handouts and give these instructions:
      i. Obtain a computer
      ii. At first you will work alone and answer questions 1-3 of the worksheet
      iii. You will have 25 minutes. I will tell you when time is up.
   c. Write the time that they are supposed to be finished on the board. Walk around to make sure that the students are on task and to answer questions. Make sure you do not help them with the diagnosis – many students will be uncomfortable at first with the struggle but they will get into the project once they start working on it. (25 minutes)
   d. Once time is up tell students that they have 5 minutes to find 1-2 persons to discuss their findings and to firm up their diagnosis.
   e. Discuss the diagnosis as a class and reveal what happens to Ginny (5 minutes).
   f. Show 3 minute video clip of the robotic mitral repair and answer questions (5 minutes)

4. **Wrap-up of lesson (10 minutes)**:
   a. Finish up by re-visiting the question about misdiagnoses by asking students: Can you see how difficult is sometimes is to diagnose a disease? (Mention that this happens to be a fairly straightforward case). Allow for students to discuss their reflections (5 minutes and write a short statement about it on the back of their worksheet (5 minutes).
   b. Collect the worksheet as they walk out.

**ASSESSMENT SUGGESTIONS:**
- Student worksheet addresses objectives 1, 2 and 3
- The Reflection on the case addresses objective 3

**EXTENSIONS**
1. Another good visual is a 4 minute video from Cleveland Clinic which shows an actual robotically-assisted repair of the mitral valve in a patient with endocarditis: [http://my.clevelandclinic.org/heart/disorders/valve/valve_videos.aspx](http://my.clevelandclinic.org/heart/disorders/valve/valve_videos.aspx) (view video beforehand- it is graphic! ).

2. There are 2 medical cases of endocarditis that are interesting. They can be found the following websites:
   - [http://www.biomedcentral.com/1471-2334/6/179](http://www.biomedcentral.com/1471-2334/6/179): Case of a male with ta tooth abscess that developed osteomyelitis and endocarditis
   - [http://www.casesjournal.com/content/2/1/6857](http://www.casesjournal.com/content/2/1/6857): Case history of endocarditis caused by dental problems

**RESOURCES:**
There are 2 resources for this lesson: the student handout for the medical mystery and the teacher handout. They are attached at the end of the lesson.

REFERENCES:


MEDICAL MYSTERY: WHAT’S WRONG WITH GINNY?

Read the following medical case. Use the computer to research any information that is not clear to you. Then use the handout to find the diagnosis of the patient.

ADMISSIONS INFORMATION:
On July 14th, 2013, Ginny Cardoza, a 22-year-old College student, came into the emergency room complaining of fever, malaise, headaches, and myalgia.

HISTORY:
A. PRESENTING COMPLAINTS:
Ms. Cardoza states that she has been feeling unwell for about two months. She complains of malaise and anorexia (loss of appetite) and she has lost 25 lbs. in the two month. When asked, she affirms that she has been having intermittent episodes of low-grade fever in the afternoons, but they would be gone by the next morning. She decided to come in today, because in addition of her fever and malaise, she started to develop joint pains in multiple areas and she is worried that the condition is getting worse rather than better. The patient has been taking Tylenol for the fever, headache and myalgia; patient negates use of any other medications, except for occasional use of Midol for menstrual cramps. Patient denies shortness of breath, abdominal pains, and digestive difficulties except for loss of appetite. She states that she has not tried to lose weight and has not changed her dietary habits. She has not traveled or had contact with animals over the past three months. The patient affirms that she is sexually active, having had the same sexual partner over the last 6 months.

B. PAST MEDICAL HISTORY:
Ms. Cardoza was a premature birth and had to stay incubated for three weeks; she developed pneumonia and a mitral valve prolapse. At age 7, she had a fracture of her right greater tuberosity of the humerus, and at age 12 she had an appendectomy. Her dentist performed a root canal 12 weeks ago.

C. FAMILY HISTORY
Unremarkable

D. MEDICAL EXAM:
Height: 5’5”; Weight: 110 lbs., Temperature 101.3°F, Blood Pressure 120/80 (normal), Pulse 90 beats per minute (elevated), heart murmur over mitral valve. Abdominal, respiratory, neurological, musculoskeletal, urological examinations are unremarkable.

E. LAB REPORT:
Elevated neutrophils, C-reactive protein elevated, normal T cell count. Lab culture shows presence of Streptococcus viridans. Echocardiography visualizes vegetation on the mitral valve.
NAME: __________________________________________

**DIAGNOSE GINNY’S PROBLEM USING THE FOLLOWING STEPS:**

1. For each section, look up terms that you do not understand. Then write explain each section in your own words.

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<thead>
<tr>
<th>ADMISSIONS INFORMATION:</th>
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<table>
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<tr>
<th>PRESENTING COMPLAINTS:</th>
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<th>PAST MEDICAL HISTORY:</th>
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<tr>
<th>FAMILY HISTORY</th>
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<table>
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<tr>
<th>MEDICAL EXAM</th>
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</table>
2. In the 2 columns, list the pertinent information for this case and the information that has no bearing on the case:

<table>
<thead>
<tr>
<th>IMPORTANT INFORMATION:</th>
<th>EXTRANEOUS INFORMATION:</th>
</tr>
</thead>
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</table>

3. Now state 1 – 3 diseases this could be. Justify each of your choices by referencing the medical information. Discuss your option with 2 colleagues

A. ________________________________

B. ________________________________

C. ________________________________

4. What do you think is the definite diagnosis of the condition. Explain what the telltale indicators are and deduce how the patient contracted it.
5. Research the treatment that the patient is likely to obtain.

Once you figure it all out, you will find out to what happened to Ms. Ginny Cardoza.

**REFLECTION:**
ANSWER KEY

MEDICAL MYSTERY:

Read the following medical case. Use the computer to research any information that is not clear to you. Then use the handout to find the diagnosis of the patient.

ADMISSIONS INFORMATION:

On July 14th, 2013, Ginny Cardoza, a 22-year-old College student, came into the emergency room complaining of fever, malaise, headaches, and myalgia.

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A. PRESENTING COMPLAINTS:

Ms. Cardoza states that she has been feeling unwell for about two months. She complains of malaise and anorexia (loss of appetite) and she has lost 25 lbs. in the two month. When asked, she affirms that she has been having intermittent episodes of low-grade fever in the afternoons, but they would be gone by the next morning. She decided to come in today, because in addition of her fever and malaise, she started to develop joint pains in multiple areas and she is worried that the condition is getting worse rather than better. The patient has been taking Tylenol for the fever, headache and myalgia; patient negates use of any other medications, except for occasional use of Midol for menstrual cramps.

Patient denies shortness of breath, abdominal pains, and digestive difficulties except for loss of appetite. She states that she has not tried to lose weight and has not changed her dietary habits. She has not traveled or had contact with animals over the past three months. The patient affirms that she is sexually active, having had the same sexual partner over the last 6 months.

B. PAST MEDICAL HISTORY:

Ms. Cardoza was a premature birth and had to stay incubated for three weeks; she developed pneumonia and a mitral valve prolapse. At age 7, she had a fracture of her right greater tuberosity of the humerus, and at age 12 she had an appendectomy. Her dentist performed a root canal 12 weeks ago.

C. FAMILY HISTORY

Unremarkable

D. MEDICAL EXAM:
Height: 5’5”; Weight: 110 lbs., Temperature 101.3°F, Blood Pressure 120/80 (normal), Pulse 90 beats per minute (elevated), heart murmur over mitral valve. Abdominal, respiratory, neurological, musculoskeletal, urological examinations are unremarkable.

E. LAB REPORT:

Elevated neutrophils, C-reactive protein elevated, normal T cell count.

Lab culture shows presence of Streptococcus Viridans

Echocardiography visualizes vegetation on the mitral valve.

IF TEACHER DOES NOT WANT TO CLASSIFY PATIENT AS SEXUALLY ACTIVE:

DELETE SENTENCE FROM HISTORY

MODIFY PARAGRAPH:

B. PAST MEDICAL HISTORY:

Ms. Cardoza was a premature birth and had to stay incubated for three weeks; she obtained two blood transfusions, developed pneumonia and a mitral valve prolapse. At age 7, she had a fracture of her right greater tuberosity of the humerus, and at age 12 she had an appendectomy. Her dentist performed a root canal 12 weeks ago.

(since one of the detractors should be HIV, this scenario will also work)

DIAGNOSE GINNY’S PROBLEM USING THE FOLLOWING STEPS:

6. For each section, look up terms that you do not understand. Then write explain each section in your own words.

ADMISSIONS INFORMATION:

Patient comes in with fever, tiredness and achiness (Malaise), headaches, and muscle pains (myalgia)

PRESENTING COMPLAINTS:

2 months of feeling poorly: tired and achy (malaise), loss of appetite, significant weight loss, mild fever on and off in the afternoons.

New Symptom: developed joint pains on top of the other symptoms

She is worried that she is getting worse rather than better.
Medications:

1. Only taking Tylenol for fever, muscle pains and headache
2. Occasional Midol for menstrual cramps (irrelevant for this case)

Patient does not have breathing problems, or digestive problems other than no appetite.

She has not picked up a disease from traveling or from animals.

She is sexually active, having one partner for 6 months. (Significant: patient could have HIV infection – many symptoms fit)

PAST MEDICAL HISTORY:

Premature baby: pneumonia (which is long healed) and a heart valve defect (that is there now)

Broken bone (detractor; irrelevant to the case)

Had her appendix taken out long ago (detractor; irrelevant to the case)

Dental procedure: root canal (significant, but the students will not be able to know that until they look at the lab tests; it can be used for sections 3 and 4)

FAMILY HISTORY

Nothing that helps with the case
MEDICAL EXAM

Normal blood pressure

Fever and elevated pulse (indicates that the body is struggling)

Heart murmur (need to figure out if it is from birth defect or from the disease – helps once the student knows about the condition to verify it)

The rest of the exam is normal

LAB REPORT

C-reactive protein indicates that there is inflammation in the body

Normal T-cell count rules out HIV/AIDS

S. Viridans: a bacterium that most often causes bacterial endocarditis

Vegetation on the heart valve damaged at infancy also points to bacterial endocarditis

7. Now make two columns listing all the pertinent information in one column and all the normal findings/non-pertinent in the other.

<table>
<thead>
<tr>
<th>SIGNIFICANT INFORMATION FOR THIS CASE</th>
<th>EXTRANEOUS INFORMATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Recent dental procedure</td>
<td>Appendix removal</td>
</tr>
<tr>
<td>Sexually active</td>
<td>Fracture</td>
</tr>
<tr>
<td>Heart murmur</td>
<td></td>
</tr>
<tr>
<td>Normal T cells</td>
<td></td>
</tr>
<tr>
<td>Presence of S. viridans vegetation on heart valve</td>
<td></td>
</tr>
</tbody>
</table>
8. Now state 1 – 3 diseases this could be. Justify each of your choices by referencing the medical information. Discuss your option with 2 colleagues.

This will vary.

Many students will include AIDS/HIV. Explain that the T cell count does not support this diagnosis.

If research was done in depth, one diagnosis should be Endocarditis (or subacute infectious endocarditis or infective endocarditis). If it is not a choice, have the student go back and research S. viridans, which will link it to the disease and to dental problems.

Any other diagnosis given can be countered by: follow-up lab procedures are negative for this disease.

9. What do you think is the definite diagnosis of the condition. Explain what the telltale indicators are and deduce how the patient contracted it.

Endocarditis:

Recent dental appointment

fever

Defective heart valve

Murmur

Bacterial growth on the heart valve

How it occurred:

At the root canal, S viridans was introduced into the bloodstream and attached to defective valve. There it reproduced causing the symptoms and malfunction. (Without treatment the patient will die)

10. Research the treatment that the patient is likely to obtain.

2-6 weeks of intravenous antibiotics. Sometimes surgery repair the structure is required
Once you figure it all out, you will find out to what happened to Ms. Ginny Cardoza.

This is up to the teacher: in my case, Ginny has to stay in the hospital for 6 weeks on an antibiotic drip and a mitral valve repair surgery.

Show one of the two following short videos on heart repair:

http://www.baymedical.org/Services/Heart-Institute/Heart-Surgery.aspx ; is an excellent 3 minute animation of the procedure of robotic valve repair.

There is a 4 minute video from Cleveland video that show robotically assisted repair of the mitral valve in a patient with endocarditis: http://my.clevelandclinic.org/heart/disorders/valve/valve_videos.aspx

(view video beforehand it is graphic!)

(SIDE NOTE: the patient’s name is based on the two related diseases of this case: Ginny from gingivitis and Cardoza from cardio)

LESSON 2 – Do I Really Need to Floss?
KEY QUESTIONS:
1. How can an infection enter the body?
2. Can infections from medical procedures be prevented?
3. Why are medical guidelines not always universal and precise?
4. Are antimicrobials such as mouthwash effective in the prevention of infections?

SCIENCE CONCEPTS: Spreading of a disease, immune system response to disease, designing and executing an experiment, natural and artificial causes of disease.

OVERALL TIME ESTIMATE:
The lesson and part A of the experiment will take 3 full lessons. Part B of the experiment is optional but highly recommended; it takes 10-15 minutes a period over approximately 7 days.

LEARNING STYLES: visual, auditory and kinesthetic

VOCABULARY:
Biofilm: A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).
Dental caries: tooth decay or a cavity
Dental plaque: biofilm that develops naturally on the teeth.
Dentin: yellowish tissue that makes up the bulk of all teeth. It is harder than bone but softer than enamel and consists mainly of apatite crystals of calcium
Enamel: The hard, calcareous substance covering the exposed portion of a tooth.
Endocarditis: Endocarditis is an infection of the inner lining of your heart (endocardium); it usually also affects the heart valves.
Gingiva: Also known as gum. It consists of the mucosal tissue that lies over the mandible and maxilla inside the mouth.
Gingivitis: inflammation of the gum/gingiva.
Gutta percha: A tough plastic substance from the latex of several Malaysian trees used to plug the root cavity in a tooth.
Prophylactic: Preventive measure; acting to defend against or prevent something, especially disease; protective.
Pulp cavity: The central cavity of a tooth containing the pulp (including the root canal).
Root canal: Procedure in which the tooth’s root canal is cleaned out and sealed
Superbugs: Term refers to antibiotic resistant organisms.
**Tartar:** Calculus or tartar is a form of hardened dental plaque. It is caused by the continual accumulation of minerals from saliva on plaque on the teeth.

**Valve dysfunctions:** The heart has four valves that open and close as the heart pumps. When any of the four valves fails to function properly, it is known as cardiac valvular dysfunction.

**LESSON SUMMARY:**
The lesson will explore how a root canal is performed and how organisms can be introduced into the body to set up an infection. The students then will learn about the composition and importance of dental plaque and they will perform an experiment that demonstrates the growth of a biofilm that mimics plaque. Afterwards, Students will design and execute an experiment to test the efficacy of mouthwash and other antiseptics on eliminating bacteria. Students will also evaluate the guidelines and concerns of prophylactic antibiotic treatment during dental work.

**METHODS OF DELIVERY:** A short PowerPoint acts as an introduction and goes over tooth anatomy needed to understand the ensuing activity. A virtual lab will demonstrate a root canal procedure, this is followed by a worksheet that applies the information learned during the virtual lab and it allows students to discuss ethical and practical issues in health. A lab consisting of two parts wraps up this lesson. Part A demonstrates the formation of a biofilm similar to dental plaque and part B in an inquiry lab allows the student to test efficacy of antiseptics.

**STUDENT LEARNING OBJECTIVES**
The student will be able to:
1. Describe the procedure of a root canal
2. Predict how pathogens can invade the body during a root canal
3. Evaluate the benefits and dangers of prophylactic antibiotic treatment
4. Discuss about the role of the doctor in this particular disease scenario
5. Design an experiment and predict the outcome

**STANDARDS:**

**NGSSS:**
- SC.912.L.14.6
- SC.912.L.14.52
- SC.912.N.1.6
- SC.912.N.1.7
- SC.912.N.2.1
- SC.912.N.4.1

**COMMON CORE:**
- RST.11-12.1
- RST.11-12.3
- RST.11-12.4
- RST.11-12.7
- RST.11-12.9
- RST.11-12.10

**MATERIALS:**
**Student Materials:**
- Worksheet for the virtual root canal (1 copy per student)
- Lab handout (1 copy per student)
- Lab equipment as described in the Teacher copy of the Lab
Optional: computers for the whole class.
- Mimio clickers or other polling devices

**Teacher Materials:**
- PowerPoint on Tooth Anatomy
- Website for the virtual root canal
- Teacher copy of the worksheet
- Teacher copy of the Lab procedures
- Lab supplies as outlined in the Teacher lab procedures

**BACKGROUND INFORMATION:**

**LESSON 2: DO I REALLY HAVE TO FLOSS?**

**BACKGROUND INFORMATION**

**Dental plaque** is generally known as a sticky, colorless film of bacteria that forms on the teeth. These bacteria

metabolize carbohydrates producing acid as a result. If the frequency of carbohydrate intake increases, then bacteria in the plaque will produce acid, which dissolves the **enamel**, leading to tooth decay, known as **dental caries**. (5) The plaque can also form at and under the **gingiva** (gum), which then can lead to tooth root decay and breakdown of the bone supporting the tooth (2). Furthermore, mineral deposits can harden plaque, causing **tartar**, which can only be removed through professional dental cleaning. (6) Not only will tartar contribute to tooth and gum decay, but it makes it difficult to clean in between teeth (leading to more decay) and it often leads to **gingivitis** (gum tissue inflammation), which can lead to even more oral disease. (5).

Current guidelines to maintain dental health include brushing teeth, flossing and mouthwash. While brushing teeth is effective in removing plaque, it does not reach all the surfaces and, therefore, flossing is important to eradicate the plaque in these places (7). Mouthwashes are recommended since there is strong evidence that they are good anti-plaque, anti-gingivitis agents.

Other guidelines given by the ADA to minimize plaque build-up are avoidance of drinks with sugar or sugar substitutes in-between meals (as these will be used by bacteria to produce acid) and to chew sugar-free gum in order to stimulate salivary flow (saliva restores pH levels).

When a cavity forms, the acids can further destroy the underlying **dentin** and reach the **pulp cavity** and the **root canal**. This could allow the introduction of **pathogenic** bacteria into the bloodstream causing **septicemia** and other infections such as **endocarditis**. (7)
If the cavity poses this threat, dentist will perform a root canal. In the procedure they drill into the cavity, clean and disinfect it, and plug it. The sealer is a paste and a rubber compound called gutta percha. The exterior access hole created at the beginning of treatment, is sealed with a filling.

During this procedure there is a danger of infection that can lead to septicemia and endocarditis. Introducing contamination, improper sealing of the canal or the cavity, or a weakened immune system are causes for infection.

During dental procedures such as the root canal, in which there is a chance of infection, dentist use to prescribe prophylactic medications patients with heart diseases (such as valve dysfunctions) or other risk factors in order to protect them from complications. New guidelines indicate that prophylaxis has not been proven to be effective and that there is a danger to creating superbugs. Presently the routine prescription of antibiotics for at-risk patients is not recommended anymore and the physician has to evaluate each case separately. (8)

ADVANCE PREPARATIONS:

1. Look at the Teacher’s copy of the lab and plan accordingly. Preparation starts 3 days prior to the lab
2. Copy Activity handout and the lab handout for students.
3. Review the virtual surgery

PROCEDURE AND DISCUSSIONS WITH TIME ESTIMATES:

DAY 1:

Starting the lab (30 Minutes):

- At the beginning of class, hand out the lab procedures and go over the procedures. Tell them that they will start the lab today and finish it over the next two days. Direct students to form groups of 4. Have students assign roles (one that gives the instructions, one or two that perform the lab, one that is responsible for the materials and the cleanup). Tell students that they will perform only steps 1-4 of the lab today. Go over safety tips prior to starting the activity.

2. Virtual Root Canal Activity (20 Minutes):
• Distribute the handout Do I Need to Floss? and instruct students that they will use it during the upcoming activity. If computers are available ask students to pair up and to go through the activity as directed in the directions. Tell students that they need to fill Item 1 out while performing the activity. (Alternative way of instruction: the teacher can present the virtual surgery). (10 minutes)
• Direct students to put the computers away and to complete all the items in sections A and B. Students working in pairs should be discussing the issues and form their own opinion. Tell the students that they have 10 minutes. (10 minutes)

DAY 2:
1. Virtual Root Canal Activity Continued: (35 minutes)
   • Start the day with a discussion of the worksheet questions. The questions: “Was it the dentist’s fault? Should he pay for Ginny’s hospital bill?”, should bring about interesting ethics questions. (15 minutes)
   • Have students work alone for part C. Direct them to read the article and to answer the questions. Tell the students that they have 15 minutes. Allow 10 minutes to share opinions with their evidence. Take a poll on how many students think that prophylaxis should be given either always, sometime, or not at all. (20 minutes).
2. Continuing the lab - part A: (15 minutes)
   • have students re-form their groups and continue with the experiment, steps 5-9.

DAY 3:
Finishing the lab - part A (10 minutes)
• Finish the lab by performing steps 10 and 11 (10 minutes on Day 3) Remind students that they need to keep their results as a reference for part B.
Lab – part B (40 minutes)
• Students will brainstorm in their groups on how they plan to set up and run their experiment. Each group needs to turn in their worksheet to be checked prior to being allowed to start the lab. Once the procedure has been cleared they run the experiment. (40 minutes).

DAYS 4-10: (10-15 minutes each day)
• Student groups will run their individual labs. Allot 15 minutes of class time for the next 7 days.

ASSESSMENT SUGGESTIONS:
❖ Discussions provide for assessment of objectives: 3 and 4
❖ The handout: Do I Need to Floss can be sued for assessing objectives 1-4
❖ The lab write-up assesses objective 5.

EXTENSIONS:
1. Run a lab for bacterial transformation and discuss antibiotic resistance in medicine
2. Research the different guidelines for prophylactic treatments and compare and contrast them
3. Research nosocomial infections.
4. Watch Contagion and discuss the likelihood of it occurring.
RESOURCES:

There are 5 resources for this lesson

- The introductory PowerPoint (in PDF format)
- The student handout and the teacher handout for the activity Virtual Surgery Activity
- The student handout and the teacher handout for the Lab

They are attached at the end of the lesson.

REFERENCES:


NAME: ____________________________________________________________

STUDENT WORKSHEETS: DO I HAVE TO FLOSS?
A. ROOT CANAL
   - Go do www.Surgerysquad.com
     - Click on the tab: Oral/Dental
— Select root canal

• While doing the activity answer the following questions:
  — A-1 Summarize the procedures of a root canal

A-2: Deduce in which steps bacteria could have gotten into the bloodstream.

B. WHO IS RESPONSIBLE FOR GINNY CARDOZA'S DISEASE?

B-1: Based on this information, explain how Ginny contracted endocarditis:
C. PROPHYLACTIC TOOTH CARE

C1. Do you recommend for dentists to give preventive antibiotic treatment to patients at risk for developing endocarditis? Why?

C2. Review the article given to you. What is your opinion now on prophylactic antibiotic treatment before a dental procedure? Prove your point by quoting specific parts of the article next to your comments. Use at least 2 quotes.
EMPIRIC APPROACH TO INFECTIVE ENDOCARDITIS PROPHYLAXIS: CAUTION IS THE WATCHWORD!

Prevention of infective endocarditis, potentially a life-threatening condition, has been given serious consideration by the entire medical community including physicians, surgeons, and dentists. Guidelines for preventing infective endocarditis related to medical procedures have been developed by professional societies like the American Heart Association (AHA) since the 1950s. The guidelines were empiric, based more on theory and less on clinical evidence. Since both endothelial injury (mucus membranes in the mouth) and a valve defect are requisite for development of infective endocarditis on heart valves, patients with risk factors undergoing medical procedures which can cause temporary bacteremia (presence of bacteria in the blood) were considered to be at high risk for developing infective endocarditis (inflammation of the inside of the heart, often the valves). Since certain groups of bacteria (e.g., Streptococci, Staphylococci, Enterococci) are commonly known to adhere to defective heart tissues causing infective endocarditis, procedures that caused bacteremia from these organisms were considered as high-risk procedures. Prevention of bacteremia with appropriate antibiotics could in theory prevent or reduce bacteremia and minimize the risk of infective endocarditis. There is more than one guideline since different organizations define high-risk patients slightly differently. (Dentists need to choose which guidelines to adhere to)

EVIDENCE-BASED APPROACH TO INFECTIVE ENDOCARDITIS PROPHYLAXIS: DO NO HARM!

While in theory, use of appropriate antibiotic prophylaxis (preventive treatment) for bacteremia causing procedures in patients with cardiac risk factors should lead to decreased incidence of infective endocarditis, this has not been shown out in studies. Several factors contribute to this. It is common knowledge, and studies have shown, that procedures like dental extraction cause bacteremia, but so do everyday activities like brushing teeth. While the degree of bacteremia caused by routine activities at a particular point in time may or may not be on the same magnitude as dental procedures, the overall burden of bacteremia over extended periods of time from daily activities would definitely be stronger than the temporary bacteremia seen after medical procedures. The cumulative exposure to bacteremia from routine daily activities in 1 year may be as high as 5.6 x 10^6 times greater as that resulting from a single tooth extraction. Studies have shown only a small fraction, if any, of the infective endocarditis cases were probably caused by dental procedures. Another weak link is
the efficacy of antibiotic prophylaxis in preventing or reducing bacteremia, as this is the main argument for empiric approach. The evidence for bacteremia reduction or prevention by antibiotic prophylaxis is conflicting.\textsuperscript{8–11} Even studies which show reduction in bacteremia do not show reduction in endocarditis.\textsuperscript{8,9} Studies in dental procedures have failed to show a clear benefit for antibiotic prophylaxis.

Evidence on the cost effectiveness of antibiotic prophylaxis for at-risk patients undergoing these procedures is contradictory as well.\textsuperscript{12–16} Without any clear evidence of benefit, the traditional approach for antibiotic prophylaxis has been questioned and fell out of favor. Emergence of antibiotic resistance as a significant public health concern combined with the risk of antibiotic-related adverse effects, albeit infrequent, such as anaphylaxis or \textit{Clostridium difficile} colitis, has prompted a steady move towards an evidence-based approach to infective endocarditis prophylaxis. This is reflected in the recommendations of different professional societies in the last decade.

NOTE: This is an excerpt of the full article. The explanations in parenthesis in bold letters were added by the teacher and are not part of the original article.

**ANSWER KEY**

**WORKSHEETS FOR: DO I HAVE TO FLOSS?**

A. ROOT CANAL
   - Go do [www.Surgeryquad.com](http://www.Surgeryquad.com)
     - Click on the tab: Oral/Dental
— Select root canal

• While doing the activity answer the following questions:

— A1. Summarize the procedures of a root canal

In the procedure The dentist will
1. drill into the cavity,
2. clean and disinfect it, and
3. plug it. The sealer is a paste and a rubber compound called gutta percha.
4. The exterior access hole created at the beginning of treatment, is sealed with a filling.

— A2. Deduce in which steps bacteria could have gotten into the bloodstream.

Each one of these steps can cause bacteremia.
1. During the drilling blood there is an opening in which bacteria can find their way into the bloodstream,
2. If the cleaning and disinfecting is done improperly
3. If the plug is infected or it does not plug the opening fully.
4. If the filling is improper.

B. WHO IS RESPONSIBLE FOR GINNY CARDOZA’S DISEASE?

• B1. Based on this information, explain how Ginny contracted endocarditis:

During her root canal, the bacteria S. viridans found its way into the bloodstream, settled on the defective mitral heart valve and began to multiply
B2. Was it the dentist’s fault? Should he pay for Ginny’s hospital bill? Please explain and elaborate

Cannot be determined.
An examination of the plug and the plugged cavity may indicate a problem, but it could have simply occurred during the procedure even if the dentist did everything 100% correctly.

C. PROPHYLACTIC TOOTH CARE:

- Do you recommend for dentists to give preventive antibiotic treatment to patients at risk for developing endocarditis? Why?

Answers vary

- Review the article given to you. What is your opinion now on prophylactic antibiotic treatment before a dental procedure? Prove your point by quoting specific parts of the article next to your comments. Use at least 2 quotes.

Answers vary, but students should be reflecting on some of the following points:
1. New guidelines indicate that prophylaxis has not been proven to be effective
2. There is a danger to creating superbugs. Presently the routine prescription of antibiotics for at-risk patients is not recommended anymore and the physician has to evaluate each case separately
3. It is not cost-effective

**Infective Endocarditis: Rationale for Revised Guidelines for Antibiotic Prophylaxis**

Prabhakaran P. Gopalakrishnan, MD, Sanjay K. Shukla, PhD, and Tahir Tak, MD, Ph.D, FACC

This article has been cited by other articles in PMC.
EMPIRIC APPROACH TO INFECTIVE ENDOCARDITIS PROPHYLAXIS: CAUTION IS THE WATCHWORD!

Prevention of infective endocarditis, potentially a life-threatening condition, has been given serious consideration by the entire medical community including physicians, surgeons, and dentists. Guidelines for preventing infective endocarditis related to medical procedures have been developed by professional societies like the American Heart Association (AHA) since the 1950s. The guidelines were empiric, based more on theory and less on clinical evidence. Since both endothelial injury (mucus membranes in the mouth) and a valve defect are requisite for development of infective endocarditis on heart valves, patients with risk factors undergoing medical procedures which can cause temporary bacteremia (presence of bacteria in the blood) were considered to be at high risk for developing infective endocarditis (inflammation of the inside of the heart, often the valves). Since certain groups of bacteria (e.g., Streptococci, Staphylococci, Enterococci) are commonly known to adhere to defective heart tissues causing infective endocarditis, procedures that caused bacteremia from these organisms were considered as high-risk procedures. Prevention of bacteremia with appropriate antibiotics could in theory prevent or reduce bacteremia and minimize the risk of infective endocarditis. There is more than one guideline since different organizations define high-risk patients slightly differently. (Dentists need to choose which guidelines to adhere to)

EVIDENCE-BASED APPROACH TO INFECTIVE ENDOCARDITIS PROPHYLAXIS: DO NO HARM!

While in theory, use of appropriate antibiotic prophylaxis (preventive treatment) for bacteremia causing procedures in patients with cardiac risk factors should lead to decreased incidence of infective endocarditis, this has not been shown out in studies. Several factors contribute to this. It is common knowledge, and studies have shown, that procedures like dental extraction cause bacteremia, but so do everyday activities like brushing teeth. While the degree of bacteremia caused by routine activities at a particular point in time may or may not be on the same magnitude as dental procedures, the overall burden of bacteremia over extended periods of time from daily activities would definitely be stronger than the temporary bacteremia seen after medical procedures. The cumulative exposure to bacteremia from routine daily activities in 1 year may be as high as 5.6 x 10^6 times greater as that resulting from a single tooth extraction. Studies have shown only a small fraction, if any, of the infective endocarditis cases were probably caused by dental procedures. Another weak link is the efficacy of antibiotic prophylaxis in preventing or reducing bacteremia, as this is the...
main argument for empiric approach. The evidence for bacteremia reduction or prevention by antibiotic prophylaxis is conflicting.\textsuperscript{8–11} Even studies which show reduction in bacteremia do not show reduction in endocarditis.\textsuperscript{8,9} Studies in dental procedures have failed to show a clear benefit for antibiotic prophylaxis.

Evidence on the cost effectiveness of antibiotic prophylaxis for at-risk patients undergoing these procedures is contradictory as well.\textsuperscript{12–16} Without any clear evidence of benefit, the traditional approach for antibiotic prophylaxis has been questioned and fell out of favor. Emergence of antibiotic resistance as a significant public health concern combined with the risk of antibiotic-related adverse effects, albeit infrequent, such as anaphylaxis or \textit{Clostridium difficile} colitis, has prompted a steady move towards an evidence-based approach to infective endocarditis prophylaxis. This is reflected in the recommendations of different professional societies in the last decade.

NOTE: This is an excerpt of the full article. The explanations in parenthesis in bold letters were added by the teacher and are not part of the original article.

LAB: DOES MOUTHWASH AFFECT BIOFILM?

\textbf{BACKGROUND INFORMATION:}

\textbf{Dental plaque} is generally known as a sticky, colorless film of bacteria that forms on the teeth. These bacteria \textbf{metabolize} carbohydrates producing acid as a result. If the frequency of carbohydrate intake increases, then bacteria in the plaque will produce acid; this dissolves the \textbf{enamel}, leading to tooth decay, known as \textbf{dental caries}. The plaque can also form at and under the \textbf{gingiva} (gum), which then can lead to tooth root decay and breakdown of the bone supporting the tooth. Furthermore, mineral deposits can harden plaque, causing \textbf{tartar}, which can only be removed through professional dental cleaning. Not only will tartar contribute to tooth and gum decay, but it makes it difficult to
clean in between teeth (leading to more decay) and it often leads to **gingivitis** (gum tissue inflammation), which can lead to even more oral disease. Current guidelines to maintain dental health include brushing teeth, flossing and mouthwash. While brushing teeth is effective in removing plaque, it does not reach all the surfaces and, therefore, flossing is important to eradicate the plaque in these places. Mouthwashes are recommended since there is strong evidence that they are good anti-plaque, anti-gingivitis agents.

A biofilm is composed of bacteria and other microorganisms that are embedded in a self-produced matrix of **extracellular polymeric substance (EPS)**. This gives biofilm a slimy feeling and it is made out of extracellular DNA, proteins and **polysaccharides**. Due to the structure and the different **genetic expression**, bacteria growing in a biofilm are highly resistant to **antimicrobial agents**, up to 1,000 times more resistant than the same bacteria not growing in a biofilm.

**PART ONE: GROWING A BIOFILM**

In this part we will create a biofilm by growing a biofilm on plastic teeth.

**Materials:**

1. sterile 50mL plastic conical centrifuge tube
2. 1 calibrated cylinder (10mL)
3. 10 ml of Luria broth
4. 30 micro-liters of E. coli suspension
5. plastic teeth
6. micropipette
7. tube rack
8. Incubator
9. 2 agar plates
10. glass beads
11. 3 inoculating loops

**Procedure:**

1. Prepare one tube by pouring in 10 ml of sterile Luria broth into the tube. Cover tube
2. Pipette 30 microliters of the E. coli suspension into your tube with Luria broth. Gently mix
3. With sterilized tweezers, gently place the plastic teeth into the tube so that they are partially immersed
4. Cover tube, place into a rack and incubate it at 37 degrees Celsius for 72 hours.
5. Bring tube out and observe the slimy film on the plastic teeth. This is the biofilm.
6. Label 2 agar plates: one planktonic (for the free living bacteria in the broth) and one biofilm
7. Using a micropipette, transfer 25 microliters of the fluid in the tube onto the agar plate labeled planktonic and use glass beads to spread the bacteria. Cover plate.
8. Using an inoculation loop, scrape off a small amount of biofilm and spread it onto the plate labeled biofilm.
9. Incubate the plates upside down for 24 hours.
10. Bring the plates out count how many colonies formed on each.
11. On a sheet of paper record your results and keep them as a reference for part 2.
PART 2: ARE ANTIMICROBIAL AGENTS EFFECTIVE AGAINST BIOFILM?

Using the procedures above, design and execute an experiment that tests the efficacy of an antimicrobial agent of your choice (bleach, mouthwash, Ampicillin, hand sanitizer, etc.).

First decide whether you want to test if the agent prevents biofilm formation or if the agent destroys the biofilm. Please clear your choice of antimicrobial with the teacher before you design the lab.

Prior to the lab write up:

1. Your hypothesis
2. The materials and procedures you plan to use in as much detail as possible (see our initial lab)
3. Your independent variable, dependent variable, and your control
4. Make sure you only have one independent variable!!
5. Explain how you will quantify your data

Turn in your information. Once I clear it, you will run your experiment.

Make sure you document any changes in your materials and procedures.

Record and analyze your results.

Finally, discuss:

1. how your findings related to the hypothesis,
2. any significant errors,
3. a reflection on what this experiment means for health issues and
4. what good follow-up step in testing biofilms would be.

TEACHER’S NOTES

LAB: DOES MOUTHWASH AFFECT BIOFILM?

BACKGROUND INFORMATION:

Dental plaque is generally known as a sticky, colorless film of bacteria that forms on the teeth. These bacteria metabolize carbohydrates producing acid as a result. If the frequency of carbohydrate intake increases, then bacteria in the plaque will produce acid, which dissolves the enamel, leading to tooth decay, known as dental caries. The plaque
can also form at and under the **gingiva** (gum), which then can lead to tooth root decay and breakdown of the bone supporting the tooth. Furthermore, mineral deposits can harden plaque, causing **tartar**, which can only be removed through professional dental cleaning. Not only will tartar contribute to tooth and gum decay, but it makes it difficult to clean in between teeth (leading to more decay) and it often leads to **gingivitis** (gum tissue inflammation), which can lead to even more oral disease.

Current guidelines to maintain dental health include brushing teeth, flossing and mouthwash. While brushing teeth is effective in removing plaque, it does not reach all the surfaces and, therefore, flossing is important to eradicate the plaque in these places. Mouthwashes are recommended since there is strong evidence that they are good anti-plaque, anti-gingivitis agents.

A biofilm is composed of bacteria and other microorganisms that are embedded in a self-produced matrix of **extracellular polymeric substance (EPS)**. This gives biofilm a slimy feeling and it is made out of extracellular DNA, proteins and **polysaccharides**.

Due to the structure and the different **genetic expression**, bacteria growing in a biofilm are highly resistant to **antimicrobial agents**, up to 1,000 times more resistant than the same bacteria not growing in a biofilm.

**PART ONE: GROWING A BIOFILM**

In this part we will create a biofilm by growing E. coli on plastic teeth. **(I recommend buying the fake vampire teeth at toy stores or Halloween stores and then cutting them into sections)**

**PRE-LAB PREPARATIONS:**

You will need the following materials per student for part A. I recommend that you have 3 times of the perishable items at hand so that students can run at least two trials of their own experiments.

**Materials:**

1. 1 sterile 50mL plastic conical centrifuge tube
2. calibrated cylinder (10mL)
3. 10 ml of Luria broth
4. 30 micro-liters of E. coli suspension
5. plastic teeth
6. micropipette
7. tube rack
8. Incubator
9. 2 agar plates
10. glass beads
11. 3 inoculating loops

**Pour agar plates three days before the lab. Leave them at room temperature for two days and then store in the refrigerator.**

**Order lyophilized E.coli HB101. Rehydrate the E. coli 24 hours before the start of the experiment:**

1. add 250 microliters of sterile Luria broth to the vial of lyophilized E coli
2. Incubate at 37 Degrees Celsius

**Procedure:**

12. Prepare one tube by pouring in 10 ml of sterile Luria broth into the tube. Cover tube
13. Pipette 30 microliters of the E. coli suspension into your tube with Luria broth. Gently mix
14. With sterilized tweezers, gently place the plastic teeth into the tube so that they are partially immersed
15. Cover tube, place into a rack and incubate it at 37 degrees Celsius for 72 hours.
16. Bring tube out and observe the slimy film on the plastic teeth. This is the biofilm.
17. Label 2 agar plates: one planktonic (for the free living bacteria in the broth) and one biofilm
18. Using a micropipette, transfer 25 microliters of the fluid in the tube onto the agar plate labeled planktonic and use glass beads to spread the bacteria. Cover plate.

19. Using an inoculation loop, scrape off a small amount of biofilm and spread it onto the plate labeled biofilm.

20. Incubate the plates upside down for 24 hours.

21. Bring the plates out count how many colonies formed on each.

22. Repeat steps 9 & 10 twice, for a total of 3 observations

(Extensions: look at biofilm under microscope, gram stain bacteria)

**PART 2: Are Antimicrobial Agents Effective Against Biofilm?**

Using the procedures above, design and execute an experiment that tests the efficacy of an antimicrobial agent of your choice (bleach, mouthwash, Ampicillin, hand sanitizer, etc.).

First decide whether you want to test if the agent prevents biofilm formation or if the agent destroys the biofilm.

Science is leaning more toward prevention because it is very difficult to kill bacteria in a biofilm. The bacteria in the biofilm will take longer to grow, but most of the well-established biofilms can withstand biocidal agents and will reform.

Please clear your choice of antimicrobial with me before you design the lab.

Prior to the lab write up

6. Your hypothesis

7. The materials and procedures you plan to use in as much detail as possible (see our initial lab)

8. Your independent variable, dependent variable, and your control

9. Make sure you only have one independent variable!!

10. Explain how you will quantify your data (I suggest counting colonies on agar plates)

Turn in your information. Once I clear it, you will run your experiment.

Make sure you document any changes in your material and procedures.

Record and analyze your results.

Finally, discuss:

5. how your findings related to the hypothesis,

6. any significant errors,

7. a reflection on what this experiment means for health issues and

8. what good follow-up step in testing biofilms would be.

**LESSON 3 - Watch Those Biofilms!**
KEY QUESTION(S): How do scientists deal with the challenges of biofilms in the disease process?

SCIENCE CONCEPTS: Formation and behavior of biofilms, immune system response, research in medicine, performing labs to verify information.

OVERALL TIME ESTIMATE: 3 periods of 50 minutes

LEARNING STYLES: Visual, auditory, and kinesthetic.

VOCABULARY:

Acute: having a sudden onset, sharp rise, and short course

Antimicrobial: antimicrobial is an agent that kills microorganisms or inhibits their growth.

Biofilm: A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

Biopsy: is a sample of tissue taken from the body in order to examine it more closely. A doctor should recommend a biopsy when an initial test suggests an area of tissue in the body isn't normal.

Cationic polymer: a natural or synthetic compound of usually high molecular weight consisting of up to millions of repeated positively charged linked units, each a relatively light and simple molecule.

Dental plaque: biofilm that develops naturally on the teeth

Dressing: A dressing is an adjunct used by a person for application to a wound to promote healing and/or prevent further harm. A dressing is designed to be in direct contact with the wound, which makes it different from a bandage, which is primarily used to hold a dressing in place.

Endocarditis: is an inflammation of the inner layer of the heart, the endocardium. It usually involves the heart valves.

Genetic expression: is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as rRNA genes or tRNA genes, the product is a functional RNA. The process of gene expression is used by all known life

Hemostasis: is a process, which causes bleeding to stop.

Inflammation: is the body's attempt at self-protection; the aim being to remove harmful stimuli, including damaged cells, irritants, or pathogens - and begin the healing process.

Oral cavity: the inside of the mouth.

Planktonic: state in which bacteria are free-living and not associated with a biofilm.

Polysaccharides: Large carbohydrates. Usually made out of hundreds of subunits, Examples are starches and cellulose.

Proliferation: To grow or multiply by rapidly producing new tissue, parts, cells, or offspring

Quorum sensing: system of stimulus and response correlated to population density. Many species of bacteria use quorum sensing to coordinate gene expression according to the density of their local population.
**Sub-optimal conditions:** less than optimal; falling short of a standard

**LESSON SUMMARY:**

The lesson focuses on how biofilms relate to Anatomy. It shows how biofilms cause dysfunction of normal body metabolism. It then explains what researchers are doing to address these issues, using an example of research on chronic wound healing. Finally it tests the claim made by scientists that certain wound dressings arrest bacterial growth and, therefore, aid in the healing of wounds.

Students will investigate the structure and physiology of a biofilm by reading 2 scientific articles, answering questions and designing a flyer, brochure or Prezi. The wound healing research will be a PowerPoint presentation with the information provided by Dr. Schultz, the researcher working on wound care. The testing of the claim about dressings will be a lab.

**OBJECTIVES:**

The student will be able to:

1. Explain the relationship between biofilms and disease
2. Conclude if biofilm works in the laboratory
3. Discuss the structure and physiology of a biofilm
4. Discover how research contributes to better medical care

**STANDARDS**

**NGSS:**

- SC.912.L.16.7
- SC.912.L.14.2
- SC.912.N.1.4
- SC.912.N.2.1
- SC.912.N.2.4

**COMMON CORE:**

- RST.11-12.1.
- RST.11-12.3.
- RST.11-12.4.
- RST.11-12.7.
- RST.11-12.9.
- RST.11-12.10.

**MATERIALS:**

**Student Materials**

- Flyer Handout (one per student)
- Lab Handout one per student
- Quiz (One per student)
- 2 articles (1 set per student)
- Lab materials as described in the teacher’s lab handout
- Unit Assessment

**Teacher Materials**

- PowerPoint Presentation
- Teacher copy of the flyer handout
Teacher copy of the lab handout
Quiz Key
Ted talks: Bonnie Bassler: How Bacteria Talk
http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate.html
Downloaded YouTube video: What Are Bacterial Biofilms? A Six Minute Montage
https://www.youtube.com/watch?v=lpI4WCM_9pM
Materials for the lab as described in the teacher’s lab handout
Unit assessment Key

BACKGROUND INFORMATION:
Tie in to the previous unit:

Dental plaque is classified as a biofilm. According to Marsh, Dental plaque can be defined as the diverse community of microorganisms found on the tooth surface as a biofilm, embedded in an extracellular matrix of polymers of host and microbial origin (1).

Microorganisms from the oral cavity may attach and develop biofilms on components of mechanical heart valves and damaged heart valve and surrounding tissues of the heart, leading to a condition known as endocarditis.

Biofilm Structure and Function
A biofilm is any group of microorganisms in which cells stick to each other on a surface.

Bacteria occur in two states in nature, as planktonic individuals and as a group of organisms in a biofilm
A biofilm is composed of bacteria and other microorganisms that are embedded in a self-produced matrix of extracellular polymeric substance (EPS). This gives biofilm a slimy feeling and it is made out of extracellular DNA, proteins and polysaccharides. (2) The biofilm is formed when microorganisms recognize attachment sites, when there are nutritional or other molecular cues. Studies have shown that large groups of genes are differently regulated in the organisms when they are in a biofilm as compared to being planktonic (3). Interestingly enough, the bacteria can communicate with each other through quorum sensing and will be able to allow the biofilm to function as a unit, increasing the chance of survival even in sub-optimal conditions. As a matter of fact biofilms often form in response to environmental crises. (4)
Biofilms may be found on living and non-living substances and they are prevalent in natural, industrial and hospital settings. Some examples are showers, toilets, water and sewage pipes, within the body, in rivers, on boat hulls, etc.

Biofilms can have a positive impact on its surroundings: biofilms are used for sewage treatment and to remove petroleum from contaminated waters, help us process foods in our digestive tract. But there are also many negative impacts and they can be very challenging in treating diseases. Due to the structure and the different genetic expression, bacteria growing in a biofilm are highly resistant to antibiotics, up to 1,000 times more resistant than the same bacteria not growing in a biofilm. Standard antibiotic therapy is often useless. (5) Even increased amounts of antibiotics will not affect the biofilm because they cannot penetrate it. Some of the surface bacteria may die, but the ones on the inside survive and continue to create havoc.

Current objectives on biofilm research focus on prevention, avoiding re-growth, better imaging and lab cultures for identification and determining characteristics, and genetic expression of the microbes.

Research of Biofilm on Chronic Wounds by Dr. Schultz at University of Florida:

The wound healing process has four stages: hemostasis (blood clot forms), inflammation (blood vessels then dilate to allow essential cells; antibodies, white blood cells, growth factors, enzymes and nutrients to reach the wounded area), proliferation (the wound is ‘rebuilt’ with new granulation tissue) and maturation (remodeling with collagen).

When a wound gets stuck in the inflammatory phase, it is called a chronic wound. A chronic wound is the ideal environment for biofilm, which establishes itself quickly and delays or inhibits healing of the wound. 60% of biopsies of chronic wounds have biofilm, whereas only 6% of acute (normal) wound have it. (6)

The biofilm is difficult to treat, because antibiotics cannot penetrate it to eradicate it. One of the main pathogens is Pseudomonas aeruginosa. This microbe is ubiquitous and harmless on our intact skin, but it forms a pathogenic biofilm.
in wounds. Planktonic P. aeruginosa is easily killed by antibiotics such as Tobramycin, but the antibiotics cannot kill the biofilm version. (6)

Wound biofilms are currently treated by removing the biofilm first (either by scraping it off, or suctioning it off) (7) and then trying to prevent the re-formation of the film with antibiotics, effective dressings, antimicrobials and/or antiseptics. It has been shown that biofilm takes 3 days to establish itself either initially or after removal. (6) During this window treatment needs to get started and then it needs to be continued daily until wound healing occurs. A big help in preventing bacteria from attaching to the wound and forming the biofilm has been special gauze called Biofilm, which was developed by Dr. Schultz. Since Biofilm does not secrete any substances, it prevents bacteria from growing on the dressing and shedding onto the wound surface, while protecting surrounding tissues. The mechanism it does so is by a bound cationic polymer that disrupts the bacterial membrane. The biofilm has been successfully used in even the most difficult infections, including MRSA, Staph. aureus and Pseudomonas infections.

Dr. Schultz is currently working on the suction technique, in which he applies suction with different antimicrobial agents to pigskin that has been infected with Pseudomonas, to see if delivering antimicrobials along with the suction will decrease the biofilm, speed up healing time and decrease pain of the treatment.

ADVANCE PREPARATIONS:
1. Copy handouts
2. Prepare lab materials as outlined in the teacher lab handout. These need to be started at least 3 days before the lab.
3. Download the YouTube video and preview the videos.
PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

DAY 1

A. Introduction (10 minutes):
   - Introduce the lesson by showing the video: What Are Bacterial Biofilms? A Six Minute Montage
     https://www.youtube.com/watch?v=lpI4WCM_9pM (6 Minutes)
   - Answer any questions students may have. Check their understanding by asking: (4 Minutes)
     1. Do biofilms cause chronic or acute infections (A: chronic)
     2. What does planktonic mean? (A: free living; not in a biofilm)
     3. Where do you find biofilm infections in the body (A: on heart valves, bones=osteomyelitis, urinary infections)
     4. What are the two main concerns of biofilms in medicine? (A: the immune system cannot eradicate them and they are antibiotic resistant)

B. Design a Flyer, Brochure or Prezi (30 minutes)
   - Hand out Instructions and 2 articles for the activity: WATCH THOSE BIOFILMS!" -DESIGN A FLYER, BROCHURE Or PREZI.
   - Instruct the students to use the articles to find the information needed for their flyer. Let them know that they have 30 minutes to complete the brochure.

C. Quiz (10 minutes)
   - Hand out the quiz
   - Tell students they have 10 minutes to complete it and turn it in.

DAY 2:

A. Lecture on Biofilms in wound care (20 minutes)
B. Start Experiment (30 minutes)
   - Hand out procedures
   - Have students form groups of 4
   - Have them read the lab and perform steps 1-7

DAY 3:

A. Have the student journal on: Based on yesterday’s lecture can you express how research on biofilms contributes to better medical care?
B. Finish the lab (30 minutes)
   - Students perform steps 8-10
   - Students fill out the worksheet and turn it in
C. Show Video from Ted Talks Bonnie Bassler: How Bacteria Talk

ASSESSMENT SUGGESTIONS:
- The Flyer assesses objective 1 and 3
- The quiz assesses objective 3
- The lab assessment assesses objective 2
- The journal entry assesses objective 4
- The Unit assessment addresses all three lessons

EXTENSIONS:
- Research other medical conditions due to biofilms and what is being done for those diseases
Read the book Oxygen by Carol Cassella to obtain a view of what physicians go through when patients die under their care.

RESOURCES:
There are 11 Resources attached at the end of the unit. They are:
- PowerPoint on Wounds and Biofilm
- Teacher and student copy of the flyer handout
- Teacher and student copy of the lab handout
- Teacher and student copy of the Quiz
- Teacher and student copy of the Assessment Questions for the Unit
- 2 Articles for the flyer activity

REFERENCES:
WATCH THOSE BIOFILMS!

DESIGN A FLYER, BROCHURE OR PREZI

The purpose of this flyer/brochure/Prezi is to educate the public about biofilms. Utilize the documents given to you as resources to answer the following questions:

- What is a biofilm?
- Explain the composition (include a description of the EPS)
- How does biofilm form?
- How quickly do biofilms form?
- Where are biofilms found?
- List some positive and some negative effects of biofilms
- How do biofilms delay wound healing?

Use relevant pictures to make it more appealing. You can draw your own images or import them from the Internet.

Make sure you reference the sources in the text and list them at end of your brochure.
LESSON 3 ACTIVITY FOR “WATCH THOSE BIOFILMS!”
DESIGN A FLYER, A BROCHURE or PREZI

The purpose of this flyer/brochure/Prezi is to educate the public about biofilms. Utilize the documents given to you as resources to answer the following questions:

What is a biofilm
A biofilm is any group of microorganisms in which cells stick to each other on a surface.

Explain the composition (include a description of the EPS)
A biofilm is composed of bacteria and other microorganisms that are embedded in a self-produced matrix of extracellular polymeric substance (EPS). The EPS is made out of extracellular DNA, proteins and polysaccharides.

How does biofilm form
The biofilm is formed when microorganisms recognize attachment sites, when there are nutritional or other molecular cues. There are 3 stages:

Stage one: Reversible surface attachment
Microbes attach to the surface

Stage two: Permanent surface attachment
Microbes multiply and become more firmly attached. They differentiate by changing gene expression patterns, which helps them, survive.

Stage three: Slimy protective matrix/biofilm
Bacteria now secrete the protective EPS and the biofilm is established

How quickly do biofilms form?
Bacteria attach within minutes and colonies can become firmly attached in 2-4 hours. The EPS is developed within 6-12 hours. Biofilms will become strong and resistant to antibiotics within 2-4 days. This is also the phase they can start spreading either planktonic bacteria or pieces of the biofilm.

Where are biofilms found?
Everywhere in nature where there is moisture including rivers, rocks, soft tissues surfaces in organisms, toilet bowl, oceans, etc.

List some positive and some negative effects of biofilms
Vary:
Positive: protect against invading microbes (example biofilms in the human intestinal tract), help clean up oil spills, used to degrade plastics, etc.
Negative: invade chronic wounds and medical equipment increasing disease, clog pipes, degrade ship hulls, etc.

How do biofilms delay wound healing?
They stimulate chronic inflammation in the wound and will not react to the immune system. The increased inflammation causes the tissues to secrete more exudate, which then can be used as nutrition source by the biofilm.

Use relevant pictures to make it more appealing. You can draw your own images or import them from the Internet.

Make sure you reference the sources in the text and list them at end of your brochure.
Check Your Understanding on Biofilms

1. Free living bacterial cells are said to be ____________.
   a. Planktonic
   b. Ascribed
   c. Liberated
   d. Sessile
   f. Biofilmed

2. What role do biofilms **NOT** play?
   *Please choose the **incorrect** statement:*
   a. Biofilm can play a role in chronic conditions
   b. Biofilms protects bacteria from chemical disinfectants
   c. Biofilms can make many antibiotics less effective
   d. A biofilm helps prevent bacteria from attaching to any one specific surface

3. Dental plaque is one of the best-known examples of a biofilm. What organism is known to be involved with the formation of dental plaques?
   a. B. cereus
   b. S mutans
   c. S. viridans
   d. E. coli
   e. N. subflava

4. In which of the following conditions would you not expect to find a biofilm
   a. On a wet rock
   b. In the lungs
   c. On a dried out log
   d. On the fiberglass surface of a boat

5. The function of the EPS is to
   a. Control environmental policy
   b. Protect body cells from biofilm
   c. Destroy bacterial cell membrane
   d. Attach, protect and nourish the biofilm
Check Your Understanding on Biofilms

1. Free living bacterial cells are said to be _______________.
   
   a. Planktonic  
   b. Ascribed  
   c. Liberated  
   d. Sessile  
   f. Biofilmed

2. What role do biofilms **NOT** play?  
   
   Please choose the **incorrect** statement:
   
   a. Biofilm can play a role in chronic conditions  
   b. Biofilms protects bacteria from chemical disinfectants  
   c. Biofilms can make many antibiotics less effective  
   d. A biofilm helps prevent bacteria from attaching to any one specific surface (One of the main characteristics of biofilm is attachment to many different kinds of surfaces)

3. Dental plaque is one of the best-known examples of a biofilm. What organism is known to be involved with the formation of dental plaques?
   
   a. B. cereus  
   b. S. mutans  
   c. S. viridans (discussed in detail in Lesson 1)  
   d. E. coli  
   e. N. subflava

4. In which of the following conditions would you not expect to find a biofilm
   
   a. On a wet rock  
   b. In the lungs  
   c. On a dried out log (biofilm form on moist surfaces and in water; they do not exist under dry conditions)  
   d. On the fiberglass surface of a boat

5. The function of the EPS is to
   
   a. Control environmental policy  
   b. Protect body cells from biofilm  
   c. Destroy bacterial cell membrane  
   d. Attach, protect and nourish the biofilm
LAB: CAN WOUND DRESSINGS INHIBIT BACTERIAL GROWTH?

INSTRUCTIONS:
1. Read the background information
2. You will be given different types of wound dressings. Examine them, then formulate a hypothesis on whether one or both can help healing or hinder healing chronic wounds. Discuss your thoughts with your lab partners.
3. Perform the experiment
4. Record the results
5. Write a discussion on how the results relate to your hypothesis.
6. Write a reflection on what these results may mean to the medical world.

BACKGROUND INFORMATION:
When a wound gets stuck in the inflammatory phase, it is called a chronic wound. A chronic wound is the ideal environment for biofilm, which establishes itself quickly and delays or inhibits healing of the wound. 60% of biopsies of chronic wounds have biofilm, whereas only 6% of acute (normal) wound have it. The biofilm is difficult to treat, because antibiotics cannot penetrate it to eradicate it. One of the main pathogens is Pseudomonas aeruginosa. This microbe is ubiquitous and harmless on our intact skin, but it forms a pathogenic biofilm in wounds. Planktonic P. aeruginosa is easily killed by antibiotics such as Tobramycin, but the antibiotics cannot kill the biofilm version.
Wound biofilms are currently treated by removing the biofilm first (either by scraping it off, or suctioning it off) and then trying to prevent the re-formation of the film with antibiotics, effective dressings, antimicrobials and/or antiseptics.
It has been shown that biofilm takes 3 days to establish itself either initially or after removal. During this window, treatment needs to get started and then it needs to be continued daily until wound healing occurs. The question that arises is whether the wound dressing can increase or delay healing.

MATERIALS
1. 200 microliters of E. coli suspension
2. 1 strip of each gauze (a and B)
3. 4 agar plates
4. Luria broth
5. Micropipette
6. 2 Inoculating loops
7. 4 sterilized tweezers

PROCEDURES
1. Label one agar plate “Gauze A” and one “Gauze B”
2. With a micropipette transfer 100 microliters of bacterial suspension onto the center of the plate labeled gauze A. Use an inoculating loop to spread the bacteria evenly over the plate.
3. Repeat procedure for Plate B
4. With sterilized Tweezers, dip gauze A strip into the Luria broth until it is completely moistened. Let the excess drip off in the broth container.
5. When it stopped dripping, carefully spread the gauze with the tweezers on Plate A. Cover the plate.
6. Repeat steps 4 and 5 for gauze B strip, placing it into plate B.
7. Incubate plate for 24 hours.
8. Open each plate and carefully lift one part of the gauze with a sterilized tweezers. Write down your observations of the gauze strip and the plate. Discard gauze as directed by your instructor. Count colonies under the gauze and record the number. Then count the colonies around the gauze and record that number.
9. Count how many colonies formed on each plate
10. Discard everything as per teacher instructions
NAME________________________________________________

WORKSHEET FOR: LAB- CAN WOUND DRESSINGS INHIBIT BACTERIAL GROWTH?

HYPOTHESIS

RESULTS:

Observation of gauze A and B:

Table:

<table>
<thead>
<tr>
<th></th>
<th>Initial Plate</th>
<th>Second Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Colonies under the Gauze</td>
<td># of Colonies around the Gauze</td>
<td># of colonies</td>
</tr>
<tr>
<td>Gauze A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gauze B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION (How do the findings relate to your hypothesis?)

REFLECTION (What do these results mean to the medical world)

If there is not enough space, please continue the discussion and reflection on the back of the sheet.
TEACHER’S COPY
LAB: CAN WOUND DRESSINGS INHIBIT BACTERIAL GROWTH?

INSTRUCTIONS:
1. Read the background information
2. You will be given different types of wound dressings. Examine them, then formulate a hypothesis on whether one or both can help healing or hinder healing chronic wounds. Discuss your thoughts with your lab partners
3. Perform the experiment and
4. Record the results and
5. Write a discussion on how the results relate to your hypothesis.
6. Write a reflection on what these results may mean to the medical world.

BACKGROUND INFORMATION:
When a wound gets stuck in the inflammatory phase, it is called a chronic wound. A chronic wound is the ideal environment for biofilm, which establishes itself quickly and delays or inhibits healing of the wound. 60% of biopsies of chronic wounds have biofilm, whereas only 6% of acute (normal) wound have it. The biofilm is difficult to treat, because antibiotics cannot penetrate it to eradicate it. One of the main pathogens is Pseudomonas aeruginosa. This microbe is ubiquitous and harmless on our intact skin, but it forms a pathogenic biofilm in wounds. Planktonic P. aeruginosa is easily killed by antibiotics such as Tobramycin, but the antibiotics cannot kill the biofilm version.
Wound biofilms are currently treated by removing the biofilm first (either by scraping it off, or suctioning it off) and then trying to prevent the re-formation of the film with antibiotics, effective dressings, antimicrobials and/or antiseptics.
It has been shown that biofilm takes 3 days to establish itself either initially or after removal. During this window, treatment needs to get started and then it needs to be continued daily until wound healing occurs. The question that arises is whether the wound dressing can increase or delay healing.

PREPARATION PRIOR TO THE LAB:
1. Obtain gauze and cut to a size of approximately 0.75” x 0.75”. One type of gauze can be the normal roll that is sold in drugstores and the other one has to be Biofilm.
2. Pour 4 Luria agar plates per group 3 days – 1 week prior to the lab. The plates have to stay in room temperature for 2 days and then they need to be placed into the refrigerator.
3. Hydrolyze the E. coli in Luria broth 24 hours before the experiment and incubate it.
4. Prepare Luria broth prior to the lab. Each group needs about 100 ml to dunk the gauze in.
5. On the day of the lab, pull the plates out of the refrigerator early enough so that they are at room temperature when used.

MATERIALS
8. 200 microliters of E. coli suspension
9. 1 strip of each gauze (a and B)
10. 4 agar plates
11. Luria broth
12. Micropipette
13. 2 Inoculating loops
14. 4 sterilized tweezers

PROCEDURES
11. Label one agar plate “Gauze A” and one “Gauze B”
12. With a micropipette transfer 100 microliters of bacterial suspension onto the center of the plate labeled gauze A. Use an inoculating loop to spread the bacteria evenly over the plate.
13. Repeat procedure for Plate B
14. With sterilized Tweezers, dip gauze A strip into the Luria broth until it is completely moistened. Let the excess drip off in the broth container.
15. When it stopped dripping carefully spread the gauze with the tweezers on Plate A. Cover the plate.
16. Repeat steps 4 and 5 for gauze B strip, placing it into plate B.
17. Incubate plate for 24 hours.
18. Open each plate and carefully lift one part of the gauze with a sterilized tweezers. Write down your observations of the gauze strip and the plate. Discard gauze as directed by your instructor. Count colonies under the gauze and record the number. Then count the colonies around the gauze and record that number.
19. Scrape the surface of gauze A that was in contact with the agar. Use the loop to streak a new plate labeled “Gauze A -2”
20. Repeat steps 8 and 9 for Gauze B.
21. Incubate plates for 24 hours
22. Count how many colonies formed on each plate
23. Discard everything as per teacher instructions

(Worksheet answers on next sheet)
STUDENT'S WORKSHEET:

NAME__________________________________________________________

WORKSHEET FOR: LAB- CAN WOUND DRESSINGS INHIBIT BACTERIAL GROWTH?

HYPOTHESIS

Varies; has to be a testable statement

RESULTS:

Observation of gauze A and B:
Students should be able to determine if there is colony growth on the plates and if the gauze has a slimy look to them. If it is slimy then biofilm, grew.

Table:

<table>
<thead>
<tr>
<th></th>
<th>Initial Plate</th>
<th>Second Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Colonies under the Gauze</td>
<td># of Colonies around the Gauze</td>
<td># of colonies</td>
</tr>
<tr>
<td>Gauze A</td>
<td>&gt; 0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>Gauze B (Bioguard)</td>
<td>0</td>
<td>&gt;0</td>
</tr>
</tbody>
</table>

There should be growth under and around the normal gauze, and there should be no growth under the Bioguard, but there should be growth around it. On the second plate only the plate that had the streaking from the normal gauze should have growth

DISCUSSION (How do the findings relate to your hypothesis?)

Varies

REFLECTION (What do these results mean to the medical world)

Varies:
Students should be able to convey that Bioguard will help chronic wounds heal better and faster because it inhibits bacterial growth.

If there is not enough space, please continue the discussion and reflection on the back of the sheet
ASSESSMENT QUESTIONS:

1. If you had a choice, would you rather have an infection of planktonic bacteria or bacteria in a biofilm. Why?

2. Why do bacteria act differently in a biofilm than when they are planktonic? Explain at least two reasons.

3. Can you treat chronic wounds with just antibiotics? Elaborate

4. How does Biofilm differ from normal gauze so that it inhibits bacterial growth?

5. You are treating a chronic wound of a 72-year-old diabetic male. It has been open for three weeks and has not healed. You just found out about Bioguard and want to give it a try. How would you “prep’ the wound prior to applying the gauze?

6. Explain in terms of biofilm, why it is not a good idea to go to bed without brushing your teeth.

7. Pseudomonas are a formidable challenge as biofilms in chronic wounds. But they live harmlessly on our skin. Can you explain that?
ANSWER Key

Name: _________________________________________

ASSESSMENT QUESTIONS:

1. If you had a choice, would you rather have an infection of planktonic bacteria or bacteria in a biofilm. Why?

Planktonic bacteria are preferable because they are easier to treat

2. Why do bacteria act differently in a biofilm than when they are planktonic? Explain at least two reasons.

Varies; some of the reasons are:

- They are protected from the biocidals due to the EPS
- Other bacteria may provide and transfer genetic material that conveys antibiotic resistance
- Bacterial genetic expression changes to withstand treatments
- Antibiotics cannot penetrate biofilm
- Biocidals may not be able to detach biofilm

3. Can you treat chronic wounds with just antibiotics? Elaborate

No, because of the above given reasons. The wound has to be cleared of the biofilm first and then treated within the first 24-36 hours in order to avoid for the biofilm to become established. The wound needs continuous treatment until it heals.

4. How does Biofilm differ from normal gauze so that it inhibits bacterial growth?

It contains bound microcide that does not leach out onto the wound. The microcide disrupts the bacterial membranes, which kills the bacteria.

5. You are treating a chronic wound of a 72-year-old diabetic male. It has been open for three weeks and has not healed. You just found out about Bioguard and want to give it a try. How would you “prep’ the wound prior to applying the gauze?

First the wound needs to be cleaned by debridement. The biofilm can be either scratched off or suctioned off. Then, within 24 hours a microcide needs to be applied and then the Bioguard dressing. Every 24-36 hours the microcide needs to be re-applied and the dressing needs to be changed.

6. Explain in terms of biofilm, why it is not a good idea to go to bed without brushing your teeth.
Leaving carbohydrates on the teeth provides bacteria with nutrition, which they will metabolize, producing acid, which can turn into cavities. The nourished bacteria in biofilms can produce plaque, tartar and gingivitis.

7. *Pseudomonas* are a formidable challenge as biofilms in chronic wounds. But they live harmlessly on our skin. Can you explain that?

The skin is a dry environment, which does not allow biofilm to grow on or procure nutrients for maintenance.
Teeth, Biofilm and Endocarditis

“I also do Bluetooth if you’re interested.”
Teeth

- The role is to masticate (chew) food
- Humans have two sets of teeth
  - Deciduous
  - Permanent
Deciduous Teeth

- Primary Teeth
- Post-natal development spans 2-1/2 years
- Usually 20 in number
  - 4 incisors
  - 2 canines
  - 4 molars per arch
Regions of a Tooth

- **Crown** – exposed part
  - Outer enamel
  - Dentin
  - Pulp cavity
- **Neck**
  - Region in contact with the gum
  - Connects crown to root
- **Root**
  - Root canal carrying blood vessels and nerves

Figure 14.10
Importance of Enamel

• Enamel:
  – hardest and most highly mineralized substance of the body
  – It helps protect your teeth from daily use such as chewing, biting, crunching, and grinding; it also insulates the teeth
  – It can chip or break
  – It dissolves in acid
    • Sodas, fruit juices, acid reflux
    • Plaque has bacteria that produce acid when metabolizing carbohydrates
Dental Plaque

- sticky, colorless film of bacteria that forms on the teeth
- bacteria metabolize carbohydrates producing acid as a result.
Cavities

Notice where both cavities are located!
What areas of teeth are most susceptible to cavities? Why?
Consequences of compromised enamel

- acids can further destroy the underlying dentin, reach the pulp cavity and the root canal. This could allow the introduction of pathogenic bacteria into the bloodstream.
Gum Disease can also lead to a systemic infection

- **Gingivitis** = inflammation of the gums
- When gums are infective compromised bacteria can also invade the body
- It can lead to tooth loss, bone destruction and septicemia
• Progression in Gingivitis:
gums recede exposing the root of the tooth

Notice the sticky film on the teeth – this is plaque
Tartar

- When plaque hardens with minerals it is called tartar
- Tartar contributes to tooth decay and it makes it harder to clean between the teeth
Check Your Understanding

- What does the acid do to teeth?
- Plaque can also form at and under the gingiva. What can this lead to?
- Using this information, can you explain when you would need a root canal?
WHAT FILLING WOULD YOU LIKE?

CHOCOLATE. PLEASE.
Activity

• Go do www.Surgerysquad.com
  – Click on the tab: Oral/Dental
  – Select root canal

• While doing the activity answer the following questions:
  – Summarize the procedures of a root canal
  – Deduce in which steps bacteria could have gotten into the bloodstream.
Now back to Ginny Cardoza
Please form groups of 2-4 persons to work on the following questions:

• Based on this information, explain how Ginny contracted endocarditis

• Was it the dentist’s fault? Should he pay for Ginny’s hospital bill? Please explain and elaborate

• Do you recommend for dentists to give preventive antibiotic treatment to patients at risk for developing endocarditis? Why?
Review the article given to you. What is your opinion on prophylactic antibiotic treatment before a dental procedure?

Prove your point by quoting specific parts of the article next to your comments. Use at least 2 quotes.
Biofilms in Medicine
Biofilms present many problems in medicine

- Chronic conditions such as skin wounds, urinary tract infections, osteomyelitis heart infections are often difficult to treat due to biofilm.

- They are a big concern for implanted medical devices such as eye contacts, prosthetic joints, and pacemakers.

- They also affect catheters (tubes inserted in the body) which then can lead to infections.
Current objectives on biofilm research focus on

- prevention,
- stopping re-growth,
- better imaging and lab cultures for identification and
- determining characteristics, and genetic expression of the microbes.
Innovations in Chronic Wound Care

Slides by Gregory Schultz, Ph.D.
Florida Research Foundation Professor
Institute for Wound Research
Department of Obstetrics and Gynecology
University of Florida
CONFLICT OF INTEREST


• Dr. Schultz has significant financial interests in QuickMed Technologies, Inc., Differential Diagnostics, LLP, Biosara, LLP, and Excaliard Pharmaceuticals, LLP

• Dr. Schultz has research grant funding from Hollister Wound Care, Inc., Healthpoint Ltd., Differential Diagnostics, LLC, and Kinetic Concepts Inc.,

• Dr. Schultz is a frequent speaker for 3M, Smith & Nephew Ltd, and Derma Sciences, Inc.

• Dr. Schultz is an inventor of BIOGUARD® dressing, EXC001 antiscarring drug, and SPR-DDx rapid point-of-care diagnostic
Sequence of Molecular and Cellular Events in Skin Wound Healing

Four Phases of Healing
1. Hemostasis
2. Inflammation
3. Repair
4. Remodeling

1. Clotting
2. Vascular Response
3. Inflammation
4. Scar Formation
5. Epithelial Healing
6. Contraction
7. Scar Remodeling
Think of Wound Healing as a Spectrum of Clinical Outcomes

Inadequate Healing (Chronic)
Normal Healing (Repair)
Excessive Healing (Fibrosis)

Venous Leg Ulcer
Good Skin Scar
Hypertrophic Scar
Is There a Common Molecular Pathology Of Chronic Wounds?

Diabetic foot ulcer

Pressure ulcer

Acute wound dehiscence

Venous ulcer
Chronic Wounds Get ‘Stuck’ in the Inflammatory Phase Of Wound Healing

Sequential phases of healing interrupted.

Sequential phases of healing

Chronic inflammation

BIOFILMS

Chronic wound

Healed wound
Confocal laser scanning microscopy (top view) of stained four-day *Pseudomonas aeruginosa* biofilm

(A) Area containing planktonic bacteria formed on pig skin and
(B) bacterial clusters forming a biofilm. Red bacteria are dead and green/yellow bacteria are alive.
(C) Schematic representation of polymicrobial bacterial biofilm formation (side view).
Biofilms Identified in **60%** of Biopsies of Chronic Wounds but in Only **6%** of Acute Wounds

Garth James et al, Wound Repair Regen, 2008
Qingping Yang et al, submitted
Biocides verses Biofilms
Bacteria are Hard to Kill in Biofilms

After 60 minutes of exposure to dilute bleach (Dakin’s solution), many bacteria in this biofilm were dying (green cells), but many cells in the interior of the biofilm were still alive (orange cells)

Costerton, Sci Am, 2001

Tobramycin rapidly kills planktonic Pseudomonas aeruginosa (●) very effectively, but is not effective against biofilm Pseudomonas (○).
Question: If bacteria in biofilms are difficult to kill with topical or systemic antibiotics, antimicrobials, or antiseptics, how can we treat biofilms?

Answer: LOCATE and REMOVE biofilms by effective debridement techniques then PREVENT THE REFORMATION OF BIOFILMS by applying effective dressings, antibiotics, antimicrobials, or antiseptics.
Wound Slough Harbors Bacterial Biofilms
Biofilm Maturity Studies Indicate Sharp Debridement Opens a Time-Dependent Therapeutic Window

Biopsies from three patients with large (>10 cm²) venous ulcer were split into two tubes containing saline (control) or saline with 200 ug/ml gentamicin (treatment), and after 24 hours of incubation, samples were disperse biofilm into microcolonies and CFU/5 gm. were measured. Total levels of bacteria at 0, 1, 2, and 3 days after initial debridement remained consistently high. However, in two of the three wounds, all bacterial were “planktonic” at 1 and 2 days after debridement (full kill by exposure to gentamicin), but by 3 days post-debridement, all three wounds had re-established substantial levels of biofilm bacteria (10³ – 10⁵ CFU/5 gm.).

Question: What are ways to prevent biofilms from reforming on a wound after debridement?

Answer:

- Use a wound dressing that is an effective barrier for bacterial penetration onto the wound surface
- BIOGUARD® microbicidal dressing that has a bound microbicide that is not released into wound
- Prevent bacteria from growing in the dressing then shedding back onto the wound surface
- Dressings that release microbicides silver, PHMB, chlorhexidine, are effective but toxic to wound cells
Normal bacterial membranes (Panel A) are stabilized by Ca$^{+2}$ ions binding negatively-charged phospholipids. Cationic quat-polymer (NIMBUS) rapidly displaces Ca$^{+2}$ (Panel B) leading to loss of fluidity (Panel C) and eventual phase separation of different lipids. Domains in the membrane then undergo a transition to small micelles which leads to membrane disruption and bacterial cell death. Development of microbial resistance to cationic polymer is essentially impossible since bacteria cannot simultaneously mutate their basic membrane structure.
Normal gauze dressing (left) or microbicidal gauze dressing (right) were inoculated with 2 ml of PBS containing $1 \times 10^4$ cfu of E. coli then incubated for 15 hours at 37°C tryptic soy agar (Difco) containing 0.01% TTC. Red color indicates areas of bacterial growth.
A Closer Look at Cell Destruction

Bacterial Cells Collapse After Contact with BIOGUARD™

Staphylococcus aureus

Escherichia coli

Before | After | Before | After
---|---|---|---
# Broad-Spectrum Highly Effective Rates of Kill

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>ATCC#</th>
<th>PERCENT REDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>12600</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>15597</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13833</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>51447</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>13115</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>13880</td>
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</tr>
<tr>
<td>Enterococcus faecalis</td>
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<td>&gt;99.9999%</td>
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<tr>
<td>Enterobacter aerogenes</td>
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<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
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<td>&gt;99.9999%</td>
</tr>
<tr>
<td>MRSA</td>
<td>BAA-44</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>VRE</td>
<td>700221</td>
<td>&gt;99.9994%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriophage</td>
<td>MS-2 (RNA virus)</td>
<td>&gt;99.994%</td>
</tr>
<tr>
<td>Bacteriophage</td>
<td>PRD1 (DNA virus)</td>
<td>&gt;99.87%</td>
</tr>
</tbody>
</table>

*Tested in 10% bovine serum (except viruses) after 18 hours of exposure*
Activity starts immediately, even in high challenge environments*...

Percentage Reduction Within Indicated Time

<table>
<thead>
<tr>
<th>Time</th>
<th>Staph. aureus</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>99.98780 %</td>
<td>96.99842%</td>
<td>99.98205%</td>
</tr>
<tr>
<td>10 min</td>
<td>99.99415%</td>
<td>99.99763%</td>
<td>99.98564%</td>
</tr>
<tr>
<td>20 min</td>
<td>99.99268%</td>
<td>99.99938%</td>
<td>99.99397%</td>
</tr>
<tr>
<td>30 min</td>
<td>99.99878%</td>
<td>99.99972%</td>
<td>99.99746%</td>
</tr>
<tr>
<td>60 min</td>
<td>99.9999%</td>
<td>99.99946%</td>
<td>99.99936%</td>
</tr>
<tr>
<td>4 hrs</td>
<td>99.9999%</td>
<td>99.99981%</td>
<td>99.99996%</td>
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<tr>
<td>8 hrs</td>
<td>99.9999%</td>
<td>99.99997%</td>
<td>99.99996%</td>
</tr>
<tr>
<td>12 hrs</td>
<td>99.9999%</td>
<td>99.99997%</td>
<td>99.99996%</td>
</tr>
</tbody>
</table>

... and is extremely long lasting due to its permanent bond

* Tested in 10% bovine serum
Microbicidal gauze dressing (right panels) or silver releasing dressing (left panels) were placed on agar plate with complete lawn of E. coli (top panels) or placed on cultures of skin fibroblasts (lower panels) then incubated overnight. Silver ions released from dressing produced a zone of killing for bacteria (top panel) but also killed wound fibroblasts (bottom panel). Non-leaching microbicidal gauze dressing did not leach microbicide from the dressing (no zone of bacterial kill) and did not kill skin fibroblasts in culture.
Day 7 -- Left thigh donor site wound. Dressings: TheraBond, Brunswick, Kerlix followed by Ace wraps. Note the characteristic bright-green color of exudates indicative of bacterial colonization.

Day 7 -- Right Knee graft site. Dressings: BIOGUARD® followed by ace wraps- Shows exudate present but no bright green drainage. No odor reported by nursing staff.
Research in Dr. Schultz’s Lab

COUNTERTHINK

They appear to be intelligent, but display an irresistible attraction to sugar.

CONCEPT-MIKE ADAMS  ART-DAN BERGER  WWW.NEWSTARGET.COM
Using Ultrasound to destroy Biofilm

- First they grow biofilm on pigskin.
- Then scrape it off while using ultrasound.
- Study if the biofilm gets broken apart and broken.
Using vacuum pumps with antibiotic delivery to destroy the biofilm

- Cycles of suction followed by delivery of antibiotics are administered for three days.
- Then quantify the number of live cells