Oral Biofilms Lab Activity – Teacher Preparation and Hints

Materials needed per student:

1 dental disclosing tablet
1 toothbrush (consider having students bring their own from home)
Toothpaste
1 travel sized toothpaste
OR have students bring their own tube from home
OR dispense a small amount to each student on a piece of waxed paper/foil/etc to avoid contamination of the tube from individual toothbrushes
1 bathroom cup for rinsing after brushing
2 glass or plastic microscope slides
2 glass or plastic coverslips
2 toothpicks
1 copy of Oral Biofilms Lab Activity Sheet
1 copy of Oral Biofilms Data and Question Sheet

Shared materials:

1 bottle of mouthwash (dispensed in bathroom cups to students who are using the mouthwash only protocol of cleaning)
Microscopes (ideally no more than two students per microscope)

For best results, have students skip brushing their teeth the night before and morning of this lab activity, particularly if students will perform this activity in the morning.

Dental disclosing tablets may stain surfaces and clothing. Caution students to wipe up spills and to rinse sinks thoroughly.

Decide which students will perform each protocol for cleaning teeth. After they they perform their second observation of dental plaque, they will be able to brush appropriately (2 minutes) with toothpaste to remove the stains. Any stain left on their tongues or lips will wear away shortly.

If students are familiar with the use of oil immersion microscopy, encourage them to do this when observing the biofilm slide, as the bacteria are quite small.

When students are comparing their results, encourage them to focus on the **change** in the amount of plaque after they complete their protocol rather than the **amount** of plaque left on their teeth, as some students may be very sensitive about how much plaque is present on their teeth.

Proper care should be taken to disinfect work spaces after this laboratory exercise. Use of a biohazard bag for used toothpicks, slides, and coverslips, followed by sterilizing in an autoclave or by soaking in a bleach solution is recommended to prevent the spread of harmful microbes.

Oral Biofilms Data and Question Sheet – Teacher Key

Method used to clean teeth _____as assigned by teacher_____

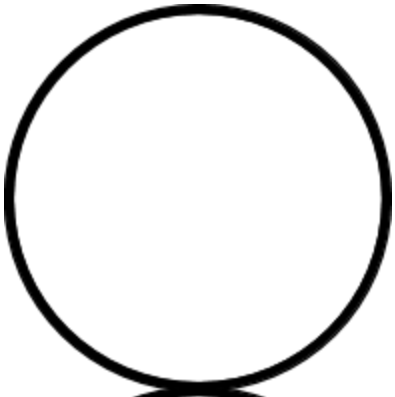
Tooth	Plaque index for buccal (cheek) side of tooth		Plaque index for lingual (tongue) side of tooth	
	Before	After	Before	After
Upper incisor	For each of these, except just using mouthwash, students should observe a decrease in amount of plaque after they clean their teeth. The greatest impact should be with toothbrush and toothpaste.			
Lower incisor				
Upper molar				
Lower molar				

Sketch of cheek cells

Magnification __likely 400_____X

Total magnification will be objective lens X ocular

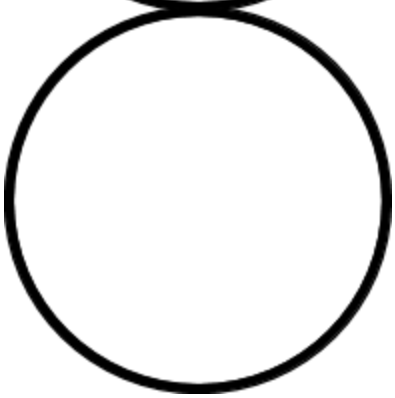
Label the cell membrane, nucleus, and cytoplasm of one cheek cell. Use this drawing to help you identify cheek cells that may be in your biofilm slide



Sketch of oral biofilm

Magnification _likely 400_____X

Total magnification will be objective lens X ocular



How many different shapes do you observe?

Answers will vary, but students will likely be able to spot at least one shape – round balls, possibly in chains, as the dominant species of bacteria in new plaque is *Streptococcus mutans*. Strepto refers to chains and coccus refers to round balls. As the biofilm matures, other bacteria may join the biofilm and students may see rods of lactobacilli or filaments of *Actinomyces israelii*. Other bacteria may also be present. It is important to remind students to not confuse cheek cells for the bacteria. The bacteria may be in clumps that are similar size to clumps of cheek cells.

Questions

1. What must be done, according to the class data, to effectively remove dental plaque?

   Student data should show that brushing is necessary to effectively remove dental plaque. Mechanical agitation of the biofilm is required to remove it.

2. What difference did the use of toothpaste make?

   Data should show that bushing with toothpaste was most effective at removing dental plaque. Toothpastes have ingredients to help disrupt the biofilm.

3. Did mouthwash use have any significant impact on the dental plaque? Explain.

   It is expected that the use of mouthwash alone does little to decrease dental plaque. Mouthwash has ingredients designed to kill bacteria, but not to remove them. Manual agitation is necessary to remove oral biofilms.

4. Do you think these results are meaningful and reliable? Explain.

   Student answers will vary, but students may raise issues of not brushing quite as normally as they would at home, use of different brands of toothpaste/toothbrushes/mouthwash (if students bring their own), small sample size, etc.

5. What modifications or additional questions would you like to see to this lab?

   Student answers will vary.

6. Investigate ways that might *prevent* the formation of tartar (calculus) which forms when plaque hardens? (Consider toothpaste ingredient labels, online sources, or other sources of information to help you answer this.)

   Prevention of the formation of tartar begins with proper oral hygiene, including brushing twice a day and flossing once a day to remove food and plaque stuck between teeth. Removal of plaque on a regular basis decreases the growth of the oral biofilm. Many toothpastes contain ingredients designed to remove plaque and prevent tartar buildup. Some of these ingredients are abrasive to help remove the plaque, such as silicates and calcium carbonate. Other active include: enzymes, amine alcohols, herbal or natural products, triclosan, bisbiguanides (chlorhexidine), quaternary ammonium compounds (cetylpyridinium chloride) and different metal salts (zinc salts, stannous fluoride, stannous fluoride with amine fluoride). (Monogr Oral Sci. 2013;23:27-44. doi: 10.1159/000350465. Epub 2013 Jun 28) Tricolsan is an antibiotic which can kill some of the bacteria.
   Additionally, avoiding sugary foods and drinks helps prevent biofilm formation because it limits the amount of food source for the biofilm.

7. Dental caries (cavities) are one health issue associated with oral biofilms. Investigate at least one other health issue that results from the microorganisms found in an oral biofilm. In other words, why is good oral health so vital to overall health?

   Poor oral hygiene is linked to a variety of issues, including diabetes, heart disease

   https://www.mayoclinic.org/healthy-lifestyle/adult-health/in-depth/dental/art-20047475