Chairside Diagnosis for Plaque-Associated Oral Infections

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Abstract

The aim is to acquaint students with some of the infections associated with plaque, specifically dental caries (tooth decay) and periodontal (gum) diseases, and to enable them to carry out some simple diagnostic tests which are potentially available to the dentist. An indication of plaque quantity is obtained using disclosing tablets; calculation of decayed, missing and filled teeth (DMFT) could provide information on past dental procedures and caries experience. The exercise is designed to be completed within one laboratory session, so that incubation and culture of microorganisms is not required.

Students carry out Gram staining and darkground microscopy of plaque; darkground microscopy enables the visualization of motile, anaerobic spirochetes which, if present in sufficient numbers, may be associated with gingivitis and gum disease. Incubation of saliva with sucrose and monitoring of the pH enables comparison of acidogenicity, which may be an indicator of plaque cariogenicity (caries-inducing ability).

The lab report enables more in-depth exploration of additional chairside procedures available to dentists, including visual inspection of teeth and gums, X-rays, and some of the more recently developed diagnostic methods. The policy of "minimum intervention" in dentistry continues the preventative rather than curative strategy which is the normal procedure in today's profession.

A more sophisticated exercise is described elsewhere within ASM curriculum resources: "A laboratory class exploring oral biofilms and the contamination of toothbrushes." Information therein may be useful for supplementation.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Objectives.
At completion of this activity students will be able to:

- Appreciate the complexity of dental plaque.
- Describe the etiology of tooth decay and gum disease.
- Understand the impact of different factors on oral health.
- Recognize the microscopic appearance of plaque and properties of key species (cocci, rods, spirochete motility, large gram-negative rods, microcolonies, coaggregation, and host epithelial cells).
- Collate and present data pertaining to acid production in saliva.
- Review diagnostic procedures for oral infection.
- Understand the importance of good oral hygiene.

Background.
This exercise is extremely flexible in terms of target audiences. Nonmajors, science education majors, and allied health majors should have some idea of oral hygiene, the difference between tooth decay and gum disease, and the roles of microorganisms in these infections. If they are not familiar with Gram staining, then demonstrations can be prepared and presented; likewise the darkground microscopy can be carried out by a demonstrator. Data presentation and interpretation skills are necessary.

In addition to the knowledge noted above, microbiology and biology majors should be able to carry out Gram stains, understand the principles of darkground microscopy, and have some knowledge of the formation and composition of dental plaque. Properties of biofilms are implicit in this understanding; plaque is an extremely good example of a biofilm.

PROCEDURE

Materials.

- Plaque disclosing tablets (commercially available from a pharmacist or ask a local dentist or dental health

organization)
- Digital camera
- Darkground microscope with attached camera
- Microscope slides and cover slips
- Sterile toothpick
- Physiological saline
- Gram reagents
- Test tubes (of a size compatible with pH meter probe)
- Sucrose solution (5% in water, need not be sterile)
- pH meter (one per six students preferable)
- Toothbrush and toothpaste (one per student)
- Mirror

Student Version.

Chairside diagnoses for plaque-associated oral conditions

The oral microflora have a significant role in oral disease: dental caries, periodontal diseases, denture-associated stomatitis, and oral malodour have all been associated with the normal oral flora.

In the surgery, the clinician examines the gums and teeth (as well as other tissues in the cavity) for evidence of various disease conditions. In terms of those diseases associated with microorganisms, policy has switched significantly over the past decades from one of treatment to one of prevention. One strategy for reinforcing a prevention message is to utilize methods which enable indicators of oral disease to be demonstrated to patients at the chairside.

This class will explore some of those methods.

*1. Darkground microscopy and the gingival flora***.**

Periodontal (gum) diseases result from the loss of attachment of tooth and gum. A gingival crevice develops and is filled with periodontopathogenic microorganisms (particularly gram-negative anaerobes) which produce enzymes and toxins which further aggravate the condition. Eventually the pocket depth is so significant that even the underlying bone is damaged, and the tooth is loosened and lost.

Good oral hygiene (i.e., plaque removal) provides an excellent strategy for helping prevent periodontal disease. Gingivitis, inflammation of the gums, is generally deemed to be a possible initial stage of gum disease. In all cases, in any study, gingivitis is an inevitable consequence of the absence of oral hygiene, but unlike periodontal disease, it is reversible.

In order to demonstrate a more pathogenic gingival flora, plaque sampled from the gingival margin can be examined using darkground microscopy. The anaerobic, motile spirochetes are very difficult to culture, but their typical corkscrew motility is easily seen in such a specimen, and their presence is indicative of a more mature, potentially pathogenic gingival flora.

Activity.

Place a drop of physiological saline on a microscope slide. Using a sterile toothpick, remove some plaque from the tooth-gum interface (use back teeth and take care not to injure gums), and mix gently with the drop of saline on the slide. Place a coverslip over the sample, and IMMEDIATELY examine using darkground microscopy. Look for spirochetes, clusters of microorganisms of different shapes, and epithelial cells and take a representative image.

Now perform a Gram stain of a plaque sample. Examine using brightfield microscopy under oil immersion. Note different Gram reactions and shapes of cells. Look for gram-positive cocci (probably streptococci), gram-positive or variable staining rods (e.g., *Actinomyces*), filamentous, thin, long gram-negative rods (e.g., putative *Fusobacterium*, long rods with pointed ends; *Prevotella* or *Porphyromonas*, large rods) and possibly corncobs (cocci attached to rods).

2. Acidogenic properties of the oral flora

Dental caries (tooth decay) is the dissolution of tooth enamel caused by organic acid produced during carbohydrate fermentation by acidogenic members of the plaque flora. *Streptococci* are particularly important, with the species *Streptococcus mutans* having a significant association with dental caries in the literature. *Lactobacilli* are also acidogenic and aciduric but are generally deemed to be secondary colonizers of the lesion. Thus, plaque containing a higher proportion of these acidogenic microorganisms may be more cariogenic. It is not easy to sample plaque to demonstrate this acidogenicity, but saliva as a transport medium may be used to indicate the prevalence of these species in the oral cavity.

Activity.

Collect 2 ml of saliva into each of two sterile test tubes and measure the pH. This provides an indication of the resting pH. Put 2 ml of sucrose solution (5% weight per volume) into a third test tube and measure the pH. Add 2 ml of the sucrose solution provided to one of the saliva test tubes and 2 ml of saline to the other two tubes and immediately note the pH. Place the test tubes in a water bath (37°C) and read the pH of all three test tubes (saliva, saliva plus sucrose, sucrose) after 2 hours. Record initial and end pH for yourself and the rest of the group. Is there any difference in acidogenicity for the different samples?

Plaque quantity

The presence of plaque on teeth is inevitable, but good oral hygiene can help keep it under control. In order to demonstrate the presence of plaque to children, disclosing tablets are used. These contain a vegetable dye which stains plaque (and tongue) and enables the extent of coverage to be seen. It can also be used after cleaning teeth to determine how effective the brushing has been.

Activity.

Chew a disclosing tablet and examine your plaque. Take a picture of your disclosed plaque. Now clean your teeth!! NEW TOOTHBRUSHES AND TOOTHPASTE WILL BE PROVIDED.

Decayed, missing, and filled teeth

The decayed missing and filled teeth (DMFT) index provides an indication of the health status of the mouth (the higher the DMFT, the worse the health status) and is particulary useful for young children (less so for you as adults). The decayed, missing and filled surfaces (DMFS) accounts for teeth which may have more than one filling.

Activity.

Using the chart provided, count the number of decayed (shouldn't be any!) surfaces, missing teeth (possibly several), and filled (several) surfaces. Add these numbers together to obtain your DMFS value.

Record class results.

Practical report (marks out of 100)

(20) Write a general introduction to the oral flora and dental plaque: what it is, its composition, etc. (approximately one side).

For each of the oral conditions explored in the class, write around half a page outlining the condition and its microbiological origin. Include information on oral malodour and denture stomatitis.

Thinking about the aims of the class, remind yourself about what a dentist does when you visit.

(20) For **dental caries**, describe the different methods available to the dentist for diagnosing the condition.

Describe and discuss the work that you have done (include graphs). Is there any relationship between the DMFS for an individual and their plaque acidogenicity? Would Stephan curves (pH versus time) help? (correlation?). Do you think the methods would work well in a dental surgery? How valid is DMFT-DMFS for your age group for assessing caries susceptibility?

Review the methods available for prevention and treatment.

(20) For **periodontal disease**, describe the different methods available to the dentist for diagnosing the condition.

Describe and discuss the work that you have done (include photograph). Do you think the method would work well in a dental surgery?

Review the methods available for prevention and treatment.

(20) **Concluding remarks**

Consider the following:

The success of chairside demonstrations to inform the patient regarding oral hygiene?
Other chairside methods for diagnosing oral health?
New strategies, for example, quantitative light-induced fluorescence.
Other methods for informing the public about dental hygiene (leaflets, etc.)?
Other strategies to improve the oral health of the general population? (fluoride?)
Any information about minimum intervention strategy.

(10) **References**

Use of some introductory material from text books is acceptable, but mainly please use up-to-date references from the literature.

Oral Microbiology by Marsh and Martin provides an excellent introduction (not much on malodour or denture stomatitis).

You can use websites and leaflets, etc., for general public health information.

(10) **Presentation**

This report should be informative and could be very attractive!! You have images of your own mouth, and there are several additional images on the internet, etc.

Faculty Version.
STUDENTS MUST BE TOLD NOT TO CLEAN THEIR TEETH PRIOR TO THE CLASS: this is to enable the development of at least a 12-hour plaque, which may then present more indicators of maturity (more anaerobes, more diversity, more quantity).

The exercise is straightforward and the sheets provided to students give an outline of the background of the work and the etiology of the different infections, but the sequence of activities is important.

Key points to reinforce:

- The treatment of dental caries and gum disease is extremely costly due to the high levels of incidence of these preventable infections.
- Personal oral hygiene is easy to maintain.
- Regular visits to the dentist are important.
- Prevention strategies are essential in oral care; the dentist and hygienist can help reinforce appropriate behaviour.

Chairside demonstrations can help in this context.

Microscopy

Although logically the extent of plaque coverage on teeth should be the first step (disclosing agent), this activity may damage the viability of anaerobic microorganisms visualized using darkground microscopy. Thus the plaque Gram stain and darkground microscopy must be done first. Wet specimens must be examined immediately for darkground microscopy because the delicate anaerobes may die, and the liquid may dry out. The Gram smear may be left to dry and be fixed, stained, and examined later in the class.

I usually assemble the class around the darkground microscope, where the image is displayed on a screen, so that interesting and key features (microcolonies of rods and cocci, often microcolonies of different morphologies physically attached as they would be in plaque, "corncobs"—where cocci are attached to a rod, epithelial cells, isolated cells moving via Brownian motion, and spirochetes demonstrating corkscrew motility) may be pointed out. Photographs are taken of representative fields so that each student has a copy. Students prepare their specimen as required so that "fresh" specimens are examined, one by one. Using the Gram stain, the diversity of cells can also be noted but without motility. This can be done by students at their own bench; photomicrographs of their stains may be taken.

If the class is too large to gather around the microscope, a television monitor enables the whole class to observe microscopic fields.

Monitoring acid production by salivary flora

This part of the exercise takes longest (incubation period), so could be started next. There should be a pH fall over time, so if the class ends before 2 hours are up, take readings as late as possible in the class. The pH should fall when saliva is incubated with sucrose. At a pH of 5.5 or below enamel may demineralize. (Thus repeated "acid attack" caused by snacking during the day will generate more frequent pH falls and predispose to caries). There should be a difference between starting and ending pH. There may be a fall in the saliva control; this is due to metabolism of intracellular polysaccharide by the "starved" microorganisms and should be smaller than in the saliva-sucrose test mixture. There should not be a fall in pH of the sucrose solution. (Note: sometimes the pH of this solution is much lower than would be expected, sometimes due to the quality of the lab water supply. It is a good idea to check beforehand using different water supplies to ensure that this pH is as near neutral as possible. No buffers though!)

Disclosing plaque

Plaque may then be disclosed, images taken, and students issued with toothbrushes and toothpaste so that they can clean their teeth. It is important to provide this facility, both for oral hygiene purposes and since the disclosing agent stains oral tissues an unattractive bright red color (easily removed by brushing). Brushes and paste can often be obtained free from local oral health organizations or commercial producers. There is no harm in asking!

This method is used to demonstrate to children the efficiency of their brushing (disclose after brushing instead of before). It is also used in many research projects to estimate the efficiency of oral hygiene products. A measurement of coverage of surface by plaque is made, often using computer-based image analysis.

Calculating decayed, missing, and filled surfaces (DMFS)

When breath is fresh and teeth are clean, this is a good time to calculate DMFS. Students use a guide which indicates where teeth should be and can note where teeth are absent or filled. There should not be any decay. Students can assess their own DMFS using a mirror or ask a (good) friend to help! Decayed, missing, and filled teeth (DMFT) measures only total teeth affected; DMFS takes into account teeth which have more than one affected site (i.e., more than one filling).

This method is used to determine the oral health of children, where the main cause of tooth loss is tooth decay. The higher the DMFT, the worse the oral health status. In older individuals, the reasons for tooth loss are more varied, thus the DMFT cannot be used in the same way. However, the number of fillings should provide an indicator of past caries experience, which is interesting, but which is not necessarily related to current cariogenicity or acidogenicity of plaque (as indicated by monitoring pH changes). Often older (mature) students have had a higher caries incidence, reflecting the different approach to dental treatment in the past and present (treatment as opposed to prevention). The impact of fluoride in toothpaste on caries incidence has been significant. The DMFS index enables the incorporation of this information, where for example there may be several fillings on one tooth, which will give a higher DMFS than DMFT.

Class data are pooled at the end of the exercise, and general trends noted. The initial pH values may differ between students. In order to compare pH fall, the difference between initial and final pH may be calculated and used to plot data. There should be a range of properties demonstrated across the class. Any relationship to DMFT could be discussed (i.e., a DMFT of zero and a negligible pH fall would be logical, as would the opposite, a high DMFT and large pH fall. However, since DMFT is recording past dental history, this relationship may not be obvious, especially if teeth have been removed for reasons other than for tooth decay).

Safety Issues.

The main safety issue in this exercise is the sampling of plaque. Some states will not allow this procedure. Faculty should check this.

Ethical considerations should be made; ethical approval may be required.

Students should sample only their own plaque.

If students are permitted to sample plaque in their own home (rather than in the laboratory) for carriage to the laboratory, then a sterile reduced transport fluid is required to extend the potential viability of the delicate anaerobes; a small volume (0.5 ml) in a sterile, small, screw-topped container should be provided to the students, along with a sterile toothpick. Plaque should be sampled in the morning. Teeth should not be cleaned that day until the end of the appropriate laboratory

exercises. If sampling at home, students should carry out the procedure in the bathroom, using a mirror to locate the tooth-gum margin of the back teeth. By gently scraping the toothpick across the margin, plaque should be collected on the point. The toothpick plus plaque is then placed in the transport fluid. The stick should be bent or broken so that the top can be screwed on tightly.

Since there is no cultivation of microorganisms, the need for aseptic technique to reduce contamination post sampling is obviated. Reagents used in the class need not be sterile.

It is not necessary for all students in a class to examine their own plaque; general principles are conveyed after, usually, three or four samples are visualized. It is preferable that plaque is fresh and not brought from home, since many of the anaerobes, particularly the spirochetes, will not survive. Perhaps teaching assistants or faculty could use their own plaque samples, if permitted by state regulations.

ML Safety Statement regarding Environmental Isolates

The Curriculum Resources Committee recognizes that isolated organisms can be a powerful learning tool as well as a potential biological hazard. We strongly recommend that:

- Environmental enrichment laboratories should only be performed in classes in which students have been trained to work at a BSL2.
- Direct environmental samples (eg. soil, water) which are known to contain infectious organisms should be handled according to the biosafety level of that infectious agent.
- Cultures of enriched microorganisms, derived from environmental samples, should be handled using Biosafety Level 2 precautions.
- Mixed, enriched or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a biosafety cabinet if available.
- Where possible, media used for the enrichment of environmental isolates should contain an appropriate anti-fungal agent.
- Instructors should be aware if they are teaching in regions with endemic fungi capable of causing systemic infections, and should avoid environmental isolations.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
Students are provided with information indicating requirements on their procedure sheet. A report of the exercise, accompanied by additional background information, provided the assessment of the exercise. Images taken in the laboratory, as well as images sourced from the internet were to be included, as well as any appropriate data presentation and analysis.

Field Testing.
This class has been carried out once (2004) as an emergency measure due to laboratory remodelling. The class of 16 had to perform the exercise and obtain results in one session. Learning was significantly reinforced and evidenced by the laboratory reports produced for assessment.

The exercise was submitted to the American Society for Microbiology for discussion at the 2004 Undergraduate Microbiology Education Conference, prior to submission for Curriculum Resources, since the author felt that the content might be of value particularly for faculty involved with allied health or nonmajor students. During discussion at the conference, this premise was reinforced by contributions from the appropriate sectors. Other points raised have been addressed in the supplementary materials section below.

SUPPLEMENTARY MATERIALS

Possible Modifications.
This exercise lends itself to considerable modification and development.

Simplification:
Extend the exercise across two sessions: one addressing plaque quantity, staining, and examination and the other examining acid production. This would allow more time to discuss principles and examine findings.

Acid production—instead of measuring pH, a universal pH indicator (Fisher) could be included in the test tube mixes, with the color change being compared with standards already provided.

Sophistication:
1. Plaque quantity. The amount of plaque on teeth could be quantified by some sort of area coverage estimate (Lennox) and comparisons made between individuals and/or teeth in the same mouth. Some individuals accumulate plaque more easily or rapidly than others. Plaque accumulates particularly at the tooth-gum interface, within defects on the enamel, and on teeth which are less exposed to "cleaning" by lips, cheeks, and tongue movement.

2. pH changes. Monitoring change in pH over time during incubation of saliva with sucrose will enable the construction of Stephan curves (pH versus time). The largest changes occur within the first few minutes of incubation. Pure cultures of microorganisms could be compared with plaque suspensions. *Streptococcus mutans* for example, is the most acidogenic species, and generally acknowledged to be cariogenic. For such comparisons however, there needs to be some standardization of inoculum in the incubation mix. High concentrations of cells are required to enable rapid pH changes to be monitored in a fairly short time.

A different approach could be to use different food substrates to monitor pH change, such as sucrose, glucose, lactose, sorbitol, xylitol, and other sucrose substitutes (e.g., saccharin and aspartame).

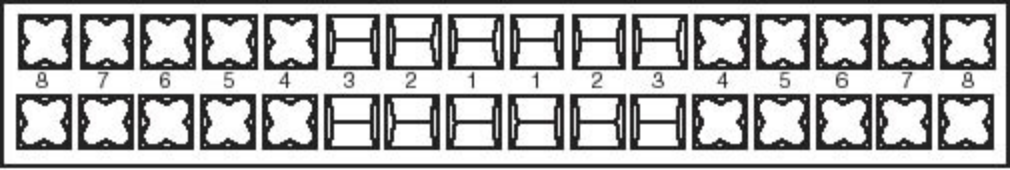
Students could be set a challenge to devise their own experiment using the selected substrate and microorganism.

3. Microbiology of plaque. Culture of plaque samples using selective media (see *A Laboratory Class Exploring Oral Biofilms and the Contamination of Toothbrushes* exercise), investigation of obligate anaerobic species, preliminary identification of predominant isolates, use of molecular identification techniques, etc.

References.

Verran, J. 2004. A laboratory class exploring oral biofilms and the contamination of toothbrushes. ASM MicrobeLibrary. [Online.] http://archive.microbelibrary.org//asmonly/details.asp?id=1437.

Information on Stephan curves methods:
Drucker, D. B., and J. Verran. 1980. Comparative effects of the substance-sweeteners glucose, sorbitol, sucrose, xylitol and trichlorosucrose on lowering of pH by two oral *Streptococcus mutans* strains in vitro. Arch. Oral Biol. **24:**965–970.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