Student 1:

Draw and label (hint: draw large & bold) a tooth surface and the adjacent oral gingival and junctional epithelium.

With the help of your fellow students indicate on the diagram what bacteria will initially colonize the surface of a new tooth. What causes them to be the 'pioneers' or 'primary colonizers'? When are they typically first acquired & from where? Which types of bacteria colonize next? What is the role of salivary proteins, and carbohydrate polymers in all of this?

Streptococcus mitis group species (S. sanguinis, S. mitis, etc.) are the initial pioneers/primary colonizers of a tooth's surface. They are pioneers because they can adhere to the pellicle proteins on the tooth to start plaque formation thanks to their many surface adhesion molecules. They are typically first acquired at birth from the mother's oral, GI, and/or vaginal mucosa. The next type of bacteria to colonize are the secondary colonizers (P. intermedia, Fusobacterium nucleatum, P. gingivalis, Lactobacillus species, etc.) that can bind to binding sites that the pioneer colonizers created. The salivary proteins are what bind to the charged hydroxyapatite on the tooth surface, creating a binding site for Strep. mitis group bacteria to bind in turn. Carbohydrate polymers can be created and metabolized by the bacteria in biofilms to create dextrans, which form the extracellular matrix of the plaque, and also store levans as food sources even after initial food sources in the mouth are gone.

What will the ecology of early (i.e., first hours after thorough brushing & cleaning) supra-gingival plaque biofilm look like in terms of the following:

Sensitivity to oxygen

Facultative bacteria only

Substrate (i.e., What do they eat?')

Fermented carbohydrates from anything we've eaten after brushing.

Acid production

Slightly acidic

Inflammatory potential

Minimal

Motility

Non-motile to ensure adherence

Attachment requirements

Need to be able to bind the pellicle (pioneer species) and stay tightly bound or be washed away by saliva

Broadly speaking, what is the approximate percent of gram+ vs. gram- bacteria in the supragingival plaque biofilm at this early time?

Mostly Gram Positive

Will the supragingival plaque biofilm become more acidic with time? Why?

Yes, as the biofilm builds up, acid-producing species can better build up as well within the thicker biofilm and create an acidic environment that doesn't get washed away or diluted, only becoming more acidic over time.

How might some Neisseria and Veillonella species affect the level of acid?

Neisseria and Veillonella species metabolize lactate/lactic acid produced by other bacteria into less acidic products like CO_2 and acetate, which can help to raise the pH

Student 2:

What is a biofilm?

"Matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces"
"Microbial cells that are firmly attached to a substrate or to each other, are embedded in an extracellular matrix that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription"

Where do biofilms exist? Where do biofilms exist in a dental office?

Biofilms are found everywhere (showers, water bottles, your dexter).
In the dental office, they're found mostly in patient's mouths both supragingivally and subgingivally.

What advantages do biofilms have for microorganisms?

Microorganisms in biofilms are more resistant to antibiotics, antimicrobials, and host responses. Biofilms can create environments that allow certain microorganisms to flourish and also provide sources of food and allow for communication (quorum sensing) that benefits the entire microbiome.

What problems do biofilms present for dentists and their patients?

Biofilms are much more difficult to remove than free bacteria.

What holds biofilms together?

An extracellular matrix that the bacteria themselves produce, commonly made of carbohydrate polymers like dextrans.

What are the major components of dental plaque biofilm extracellular matrix (ECM)?

Carbohydrate polymers such as glucans and dextrans, fructans and levans, and other polysaccharides that form the main component of the matrix.
Also contains protein, nucleic acids, and more.

Are there oral hygiene products/procedures designed to break apart plaque biofilm ECM?

…brushing, flossing

How is normal oral flora beneficial to us?

Normal flora help defend us from pathogenic organisms that want to colonize, produce essential vitamins and nutrients, and help us break down our food.

Is S. mutans part of normal oral flora? When is it usually acquired?

It is normal flora that can commonly become an opportunistic cariogenic bacteria. It is usually acquired around 2 years of age, when the second molars emerge. This is because they can only really colonize hard surfaces with pits and fissures.

If we could permanently eliminate Strep. mutans from a mouth what might be the impact? Would dental caries be eliminated?

If Strep. mutans was eliminated, caries would be significantly reduced, but not completely eliminated. While Strep. mutans alone is capable of causing caries, it is not the only cariogenic bacteria, and therefore other cariogenic bacteria (Other Strep, Actinomyces, or Lactobacillus species) could still cause caries.

What is the "Specific Plaque Hypothesis"? What is the "Non-Specific Plaque Hypothesis"? What is the "Dysbiosis Hypothesis"? What are "Keystone Microbes"? Might Strep. mutans be a Keystone Microbe?

The 'Specific Plaque Hypothesis' claims that only CERTAIN plaque is pathogenic. Certain bacteria within the plaque produce more substances or actions that lead to the destruction of periodontal tissues.

The 'Non-Specific Plaque Hypothesis' claims that ALL plaque is bad. Small amounts of plaque are 'neutralized' by the host, but large amounts of plaque are able to produce disease. Plaque control is the treatment and much of clinical treatment is based on this theory.

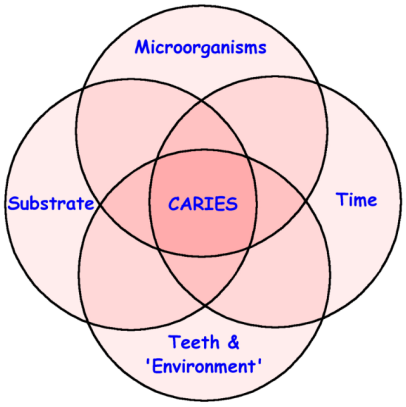
The 'Dysbiosis Hypothesis' claims that ecological imbalance of the plaque biofilm results in disease. This may involve Keystone Pathogens.

Keystone microbes are microbes that have a disproportionately large effect compared to their numbers.

Yes.

Student 3:

Draw (large) a Venn-type diagram of the key elements of tooth caries development.



Label and explain the key elements that are required for caries to develop.
Describe both the qualitative and quantitative aspects of each:

Bacteria

You need cariogenic bacteria (qualitative) in sufficient number (quantitative)

Substrate

You need fermentable substrates like simple sugars with sticky consistencies (qualitative) in sufficient amounts (quantitative)

Time

More constant and frequent presence of sugars and bacteria (qualitative) with greater time (quantitative)

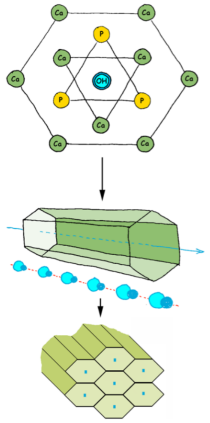
Teeth

The anatomy, mineralization, and location of teeth (qualitative) and the number of teeth (quantitative)

What is the exact chemical formula of hydroxyapatite(HA)? Why are the () crucial? Spatially how are the major constituents of HA arranged?

$Ca_{10}(PO_4)_6(OH)_2$.

Each one is a constituent ion that can be substituted for something else by a substitution ion. They are arranged in layers of pentagon with calcium and phosphate ions in rings and the hydroxyl group in the center.



What are 'substitute ions' of HA, why are they important, and what are the major ones in tooth HA?

Substitution ions are ions that can take the place of Ca, PO_4, or OH within hydroxyapatite. They are important because they can increase or decrease the susceptibility of hydroxyapatite to insults. The major substitution ions for calcium are: lead, magnesium, strontium, and radium. The major substitution ion for phosphate is carbonate. The major substitution ions for hydroxide are fluorine and chlorine.

How does the HA mineral structure of teeth make them susceptible to mild acids?

Since it is salt, it is held together by ionic bonds and can be dissolved in aqueous solutions with acid, breaking apart the ionic bonds and demineralizing the tooth.

What types of bonds hold tooth HA together?

Ionic

How do these bonds encourage remineralization?

Ionic bonds can reform just as easily as they are broken

What does Critical pH of enamel mean? Do you think the Critical pH of root cementum is different? How about the Critical pH of dentin?

The critical pH is the pH at which demineralization is greater than remineralization. The critical pH of root cementum would be higher (more vulnerable to demineralization) because it has the lowest percentage of hydroxyapatite. The critical pH of dentin would be between enamel and cementum since its hydroxyapatite content is between the two as well.

Student 4:

List and explain the key contributions of saliva to overall oral health, including:

- **Tooth Pellicle formation, including:**
  - **Among the major proteins that make up the Pellicle are Statherin, Mucins, and Proline-Rich Proteins (PRPs). What is the source of these proteins?**

These proteins are produced by the salivary glands and released into the oral cavity in saliva.

  - **What is the major function(s) of the Pellicle proteins?**

Pellicle proteins function to provide binding sites for primary colonizers, but their intended function by the body is to help with remineralization and also prevent excessive mineral precipitation on teeth. They also cover our mucous membranes to create a chemical and thermal barrier to the things we put in our mouths, helping to protect the epithelium.

- **Innate defenses, including:**
  - **Washing**

Washing of leftover carbohydrates and bacteria.

  - **Diluting toxins & damaging end-products**

That is what it do

  - **Contains many 'generic' antimicrobials**

Lysozyme and Abs

- **Adaptive defense:**
  - **Antibody**
    - **Class(isotype)? How many identical Ag-binding sites does it have?**

Mostly sIgA which has 4 identical binding sites.

    - **How does this Ab actually protect orally?**

It blocks colonization and neutralizes toxins (does NOT opsonize or activate complements)

- **Protection against caries development:**
  - **Washes away fermentable CHO substrates**

Simple sugars go away

  - **Contains anti-bacterials (both generic & specific)**

Lysozymes, lactoperoxidase (generic)
IgA (specific)

  - **Contains buffers**

Keeps mouth pH near neutral to prevent dimineralization.

- **Contains $Ca^{2+}$and PO_4^{3-} required for tooth remineralization**

More calcium and phosphate means that the equilibrium favors remineralization and makes critical pH higher

- **Contains 'recycled' topical fluoride (Where does this come from?)**

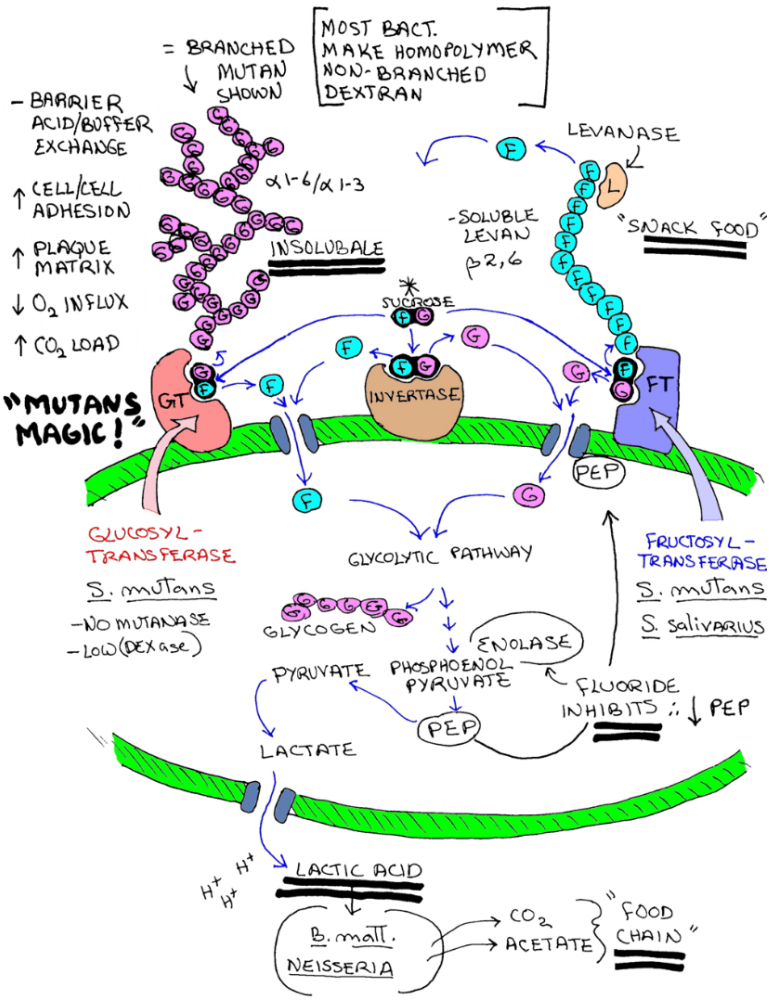
Fluoride from the food and water that we consume gets absorbed into our GI, where the fluoride ions can enter the bloodstream and get back into our mouths within saliva

- **Explain how both a quantitative lack of saliva (Xerostomia) or a qualitative lack of key saliva components can impact the protective value of saliva.**

If you don't have enough saliva, you lose all of the above benefits.
If your saliva is very viscous or is missing important salivary proteins, it inhibits the ability to perform the above functions as well.

Student 5:

Draw a Streptococcus mutans bacterium and explain why it is considered highly cariogenic.



- **Explain and discuss the key cariogenic properties of S. mutans, including:**
    - **Adherence to teeth biofilm**

While Strep. mutans are not a pioneer species, they can still attach to plaque biofilm which many bacteria simply cannot.

    - **Sugar transport at low pH**

Strep. mutans are able to effectively transport sugar at low pH, which most oral bacteria have difficulty doing

    - **Homolactic fermentation**

Homolactic fermentation means that Strep. mutans produces two lactic acids from monosaccharide rings, rather than just one (as is the case with bacteria that are heterolactic). Lactic acid is the leading cariogenic acids.

    - **Acidogenicity**

S. mutans makes a lot of acid.

    - **Aciduricity**

S. mutans is able to tolerate acidic environments.

    - **Acidophilic**

S. mutans actually prefers and grows best in acidic environments.

    - **Production of "mutan," an insoluble, extracellular glucose-polymers (glucan)**

The glucosyl-transferase enzyme on S. mutans surface creates the insoluble 'mutan/dextran', which is a branched glucose polymer (glucan). This is the key component of plaque biofilms that creates a resistant and sticky extracellular matrix that is difficult to wash away (because it's insoluble). This also decreases the ability for buffer in the saliva to buffer the acid within the biofilm, which increases acid concentration.

    - **Production of "levan," an extracellular fructose-polymer 'snack food' (fructan)**

The fructosyl-transferase enzyme on S. mutans surface creates 'levan', which is a fructose polymer, allowing it to hoard a store of energy for long-term use even if additional sugars aren't eaten for a while.

    - **Production and secretion of bacteriocins (define)**

Bacteriocins are small molecular weight protein antimicrobials that are produced by bacteria themselves to beat up other bacteria. This lets them kill off all their competitors and dominate.

- **Discuss the roles, including substrate(s) and products, of invertase, glucosyl transferase (GTF), fructosyl transferase (FTF), and fructanase in the cariogenicity of S. mutans.**

Invertase, glucosyl-transferase, and fructosyl transferase use sucrose as their substrate. Fructanase (levanase) is used to break down levans that have been made and access the energy that was stored.
Invertase breaks down sucrose into its constituents, glucose and fructose, which can then be transported into the cell and through the glycolytic pathway to produce lactate/lactic acid.
Glucosyl-transferase splits sucrose as well, letting the cell take up the fructose to be made into lactate, while the glucose is kept to form mutan/dextran.
Fructosyl-transferase splits sucrose as well, letting the cell take up the glucose to be made into lactate, while the fructose is kept to form levan.
Every one of these serve to make S. mutans hardier and more acidogenic and therefore cariogenic.

- **Do all the major cariogenic bacteria show aciduricity? What are some of the other known, highly cariogenic bacteria?**

Actinomyces and Lactobacillus species are the other major cariogenic bacteria, and they do demonstrate aciduricity, which makes sense because they should be able to resist acid when they make so much of it.

Group questions:

1. List 5 bacteria (Genus species) characteristically found supra-gingivally and explain why they exist there. Where do these bacteria come from?
Corynebacterium
Actinomyces
Neisseria
Streptococcus
Lactobacillus

They are normal flora. They come from yo momma.

2. List 5 bacteria (Genus species) characteristically found subgingivally and explain why they exist there. Where do these bacteria come from?
Fusobacterium
Filifactor alocis
Aggregatibacter
Porphyromonas
Treponema

Same as above

3. If a Streptococcus mutans bacteria is given one molecule of sucrose (e.g., ordinary 'table sugar'), how many molecules of lactic acid can it produce? (Hint: Slow down and think about your answer.)
Depending on which enzyme it touches first.
If it's processed by the invertase, it generates 4 molecules of lactic acid, 2 from each monosaccharide.
If it's processed by either of the transferases, it will only generate 2 molecules.

4. If supragingival plaque biofilm is fed a simple sugar such as glucose or sucrose, what acids are characteristically produced? After the sugar has been completely metabolized what happens to this acid profile? Why?
Lactic acid
Popionic acid
Formic acid
Succinic acid
Acetic acid

After all the sugar has been metabolized, the mouth will slowly raise the pH back to normal thanks to buffering and dilution by saliva.

5. What is bacterial progression/succession? How can dental treatment impact and even reverse such progression/succession?
Bacterial progression/succession is the natural process that occurs as dental plaque matures, with inner areas of the biofilm becoming increasingly anaerobic and acidic, encouraging the proliferation and organization of aciduric bacteria towards the tooth surface. Dental treatment can undo the biofilm and set it back to step 1.

6. What types of bacteria generally cause root caries?
S. mutans
A. viscosus
A. odontolyticus
Lactobacilli species

Gram positive, acidogenic, facultative bacteria

7. What is the average pH supra-and subgingival? What sustains this pH?
Supragingival slightly acidic (<7) due to sugar fermentation and digestion (lactic acid)

Subgingival slightly basic (>7) due to protein digestion (ammonia)

8. Besides the biofilm blocking diffusion/penetration of antibiotics into the biofilm, describe a plausible reason for antibiotics working so poorly against bacteria within biofilms.
Some of the bacteria in biofilms can create enzymes that can destroy the antibiotics, giving that benefit to all the bacteria in the biofilm.

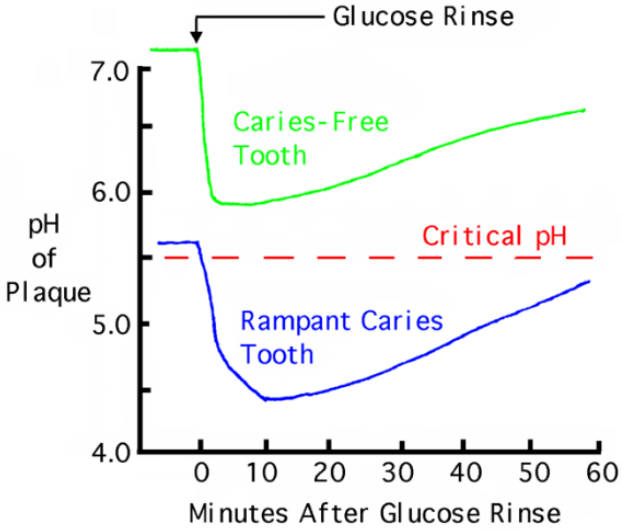
9. Is dental caries an infectious disease? An inevitable result of bacterial succession? An ecological disaster? What evidence supports this?
No, it is not infectious, but there is a correlation with specific species and variants of those species that increases one's risk and those bacteria can also be passed down among families. Research into mothers that have higher amounts of S. mutans found more and earlier caries in their children.

10. What is the typical saliva flow rate of an individual? (Yes, I know this would 'not be a good question' on an exam, but answer it the best you can.) Clinically, what is hyposalivation exactly?
1 liter a day. Hyposalivation is where your mouth isn't dry (i.e. xerostomia), but you are still producing less than normal, which would likely impair the benefits and defenses you would normally get.

11. Amylase is found in human saliva. What is its role there? Does it promote or protect against dental caries?
Amylase breaks down polysaccharides, aiding in digestion. This would promote dental caries since it can make complex carbohydrates into simpler ones that could be used by cariogenic bacteria.

12. What does supersaturated mean with regard to Ca^{2+} and PO_4^{3-} in saliva? What is/are the consequence/s of this? What would the consequence of lower Ca^{2+} levels in saliva mean for an individual? For 'their' critical pH? For calculus (tartar) formation?
Supersaturated means you have big big number of calcium and phosphate ions in saliva, which would promote remineralization and inhibit demineralization. The consequence of lower calcium ion levels in saliva would mean the opposite.
More calcium and phosphate ions in saliva than normal would lower the critical pH, making it more resistant to demineralization as pH would have to go much lower than before demineralization can occur.
Conversely, this would make calculus formation easier.

13. Draw & explain the typical supra-gingival plaque biofilm pH responses to simple carbohydrates (i.e., a simple 'Stephan's Curve'). What is the difference between a pH of 7.0 (a nice cool drink of water) and a pH of 3.0 (a nicer cool drink of diet Dr Pepper)?



Sugar go in, pH go down. pH of 3.0 is 10,000x more acidic than pH of 7.0. Teeth go away quick.

14. Historically what was the caries experience of our ancient ancestors? Why? Our ancestors became 'agriculturists' starting about 10,000 years ago. This resulted in what specific changes in S. mutans? Why? Did the same thing happen to 'perio bacteria' such as Tannerella Forsythia? Why?

Ancestors no have caries because they not eat sugar because they too poor. S. mutans get lot of sugar and grow fast. Gum germs eat protein so change not same

15. Do you think patients with chronic renal failure would be at increased risk of dental caries? How about patients with diabetes? Why? Just for fun: Does this give you any ideas about possible products/treatments to reduce the risk of caries?

Yes. Yes. ?? No.