

Lecture #1-2**Oral Ecology, Bacterial Plaque Biofilm Development, and Caries****LECTURE OBJECTIVES:****Be Able To:**

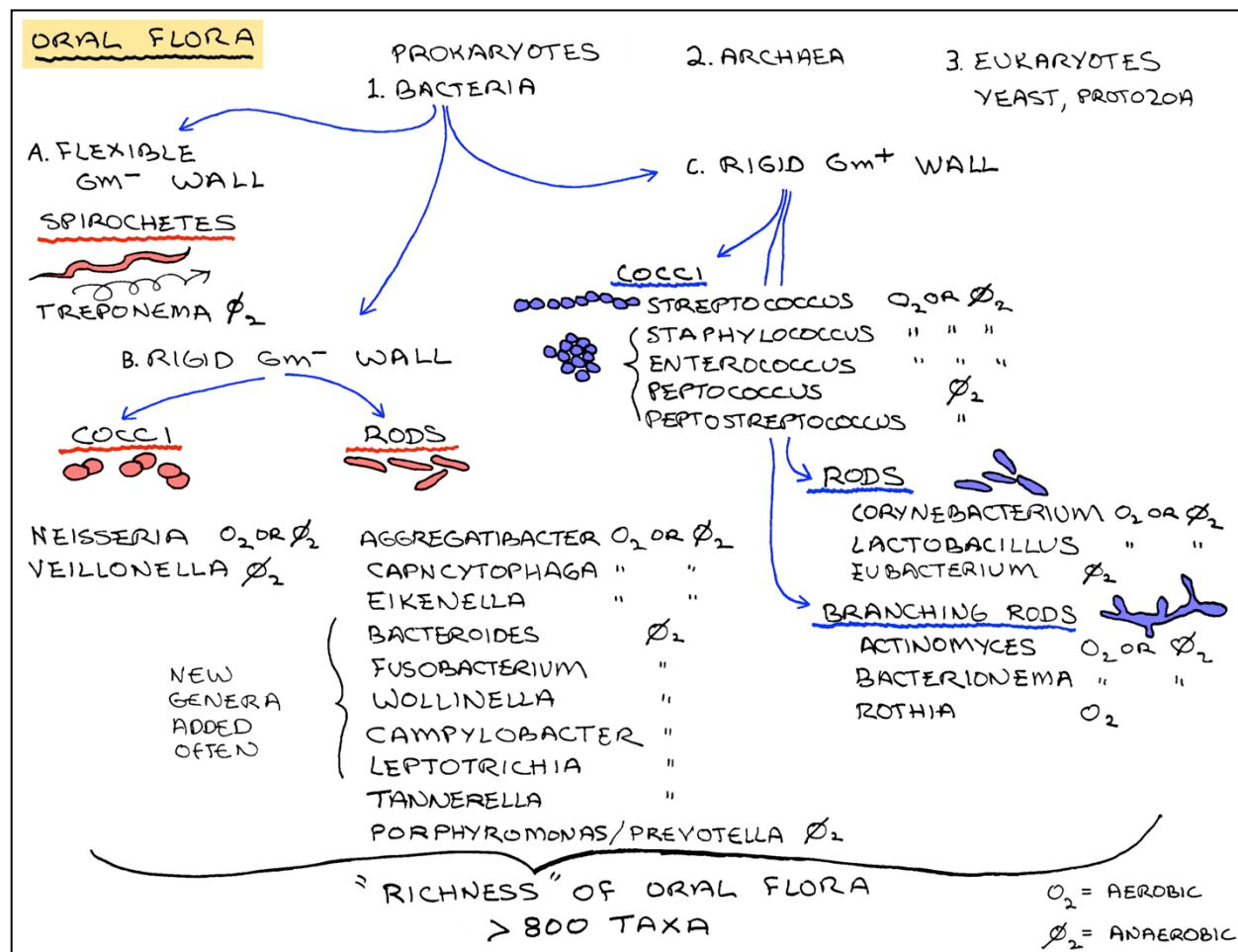
1. Discuss the complex nature of the oral flora and plaque.
2. Outline and give examples of the symbiotic and antagonistic relationship some bacteria have within the oral microbiota.
3. Discuss the relationship of certain gram-positive oral bacteria with hard-tissue disease (e.g., caries).
4. Discuss the relationship of certain gram-negative oral bacteria with soft-tissue inflammation (e.g., gingivitis, periodontitis, etc.).
5. Explain how a bacteria's ability to deal with oxygen will greatly influence where it colonizes in the mouth.
6. Define and give oral examples of:
 - Aerobic bacteria
 - Anaerobic bacteria
 - Facultative bacteria
 - Microaerophilic bacteria
 - Capnophilic bacteria
 - Saccharolytic bacteria
 - Asaccharolytic bacteria
 - Proteolytic bacteria
7. Explain how a bacteria's ability to deal with acid will influence where it colonizes in the mouth. Define acidogenic, aciduricity, and acidophilic.
8. Explain the value of normal flora bacteria. Also, describe what supplemental and transient floras are. Compare to pathogens.
9. Define and describe biofilm, with particular reference to oral biofilms. Define planktonic, and discuss its relationship to biofilms.
10. List the major advantages to the microbe to being part of a biofilm.
11. Describe how bacteria typically colonize the oral cavity, and the characteristic sequence of this colonization.
12. Describe the major niches in the mouth, with emphasis on plaque biofilms, and what types of bacteria colonize each site and why. How does oxygen, exposure to saliva, and food source impact colonization patterns?
13. Describe the major differences between supragingival versus subgingival dental plaque biofilms.
14. Explain why *Strep.* species are such key colonizers in the mouth.
15. Explain the ecological changes that occur as plaque biofilm grows thicker (i.e., ‘matures’) and begins to exclude saliva and oxygen.

16. Discuss how knowledge of biofilms might someday be applied to treatment of plaque biofilm-related diseases of all sorts.

Theme. Oral plaque ecology: the oral cavity is the 'home' of hundreds of different bacterial species. It has a wide variety of niches available. It's warm, moist, and there are multiple daily feedings! (Obviously it's a great place to raise a family!) The 'normal' oral flora (i.e., microbiota) is a complex 'mix' of 100's of different species, almost all bacteria. Both the total and relative number of these bacterial species at any given time varies dramatically with oral health (density of plaque, etc.), systemic health (especially as it impacts immunity), and diet (affecting plaque extra-cellular matrix, and acid production). They are mostly arranged into complex **biofilms**, which are by definition matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces. There are multiple complex environments that can be colonized in the mouth. Each of these niches presents distinct advantages and disadvantages that certain species prefer. As long as these normal flora, bacterial biofilms are fed properly and maintained well they generally cause little problem. If, however, they are fed improperly (e.g., too many sweets), or not maintained (e.g., brush & floss) then disease can, and usually does follow.

Keep the lawn mowed and properly fed, and you won't have trouble with weeds!

Taxonomy of oral flora. Wow, what a large number and diverse group microbes call the oral cavity home! The figure and legend below give a brief overview.



The number and types of microorganisms that normally inhabit the mouth are immense. They include Eukaryotic microorganisms such as protozoa (e.g., *Entamoeba gingivalis*) and fungi (e.g., *Candida albicans*). There are also 100's of different species of Bacteria that call the mouth home. [Note: Archaea have also been shown to inhabit at least some perio-pockets and infected pulp chambers.] The mouth contains many, many niches, with the majority of microorganisms living in plaque and tongue crypts. How a bacterium deals with oxygen is an important determinant of where it colonizes. There are few true obligate aerobes in the mouth. The vast majority of bacteria are facultative, microaerophilic, or anaerobic. Supragingival plaque biofilm is primarily made of facultative and microaerophilic bacteria; with various *Strep. species* making up a large percentage of the bacteria at some sites. Facultative and anaerobic bacteria make up most of subgingival plaque biofilm. Deep perio-pockets yield very high concentrations of strict anaerobes (e.g., *Porphyromonas*, *Treponema*, etc.).

Supragingival plaque biofilm bacteria – including, gram-positive *Streptococcus sp.*, *Lactobacillus sp.*, and *Actinomyces sp.* – cause dental caries. Subgingival plaque biofilm bacteria – mainly various gram-negative, facultative & anaerobic rods – are the major cause of periodontal disease and endodontic infections. Gingivitis is generally caused by a 'simple' overgrowth of plaque biofilm, and thus both gram-positive and gram-negative bacteria are associated with it.

Biofilms. A relatively recent shift in philosophy has occurred when thinking of microbial colonization of living hosts. This new thinking revolves around the realization that microbes do not typically live as separate individual colonies, but rather within complex communities call **biofilms**. A biofilm can be defined as a **microbially community characterized by 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.**

Basic Biofilm Properties:

- Cooperating community of different microorganisms
 - Microorganisms are arranged in microcolonies
 - Microcolonies are surrounded and attached together by protective matrix
 - Within, and between, the microcolonies are differing environments
 - Microorganisms have primitive communication system
 - Microorganisms in biofilms are more resistant to antibiotics, antimicrobials, and host responses
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In the mouth, the most conspicuous biofilm is dental plaque. **Plaque can be like a well-cared-for 'lawn' or an uncontrolled 'weed patch.'** As the plaque grows thicker it becomes less permeable to oxygen and saliva. Also, thicker plaque builds up higher concentrations of toxic products, acids, and inflammatory bacterial components. These changes with increased plaque thickness and density **result in a greatly altered environment that encourages the growth of different species**, changing the character of the plaque biofilm. With time the supragingival plaque become less aerobic, with more rods and branching forms. Some increase in gram-negative bugs also occurs, but the dominant bugs are still gram-positive bacteria, such as *Strep.* and *Actinomyces*. The increased inflammatory materials (e.g., FMLP, cell wall products, and probably most importantly LPS) around the gingival crevice causes, at first, gingivitis and eventually a pseudo-pocket and then real pockets with the increased growth of gram-negative organisms, best suited for the anaerobic, CO₂-rich, asaccharolytic environment of the deeper pockets. These **bacterial communities, which result from the 'uncared for lawn,' cause gingivitis, dental caries, intermittently produce the loss of attachment we call periodontal disease, and are available to infect the tooth pulp if the hydroxyapatite 'wall' is breached.**

TAG: Remember that all cells, including bacteria, can be infected by viruses. The viruses that infect bacteria have only one really big decision to make: Stay in the host cell or kill it. That is, either remain under the radar inside its host, or activate the kill sequence that involves creating thousands of viral offspring that burst out, killing the current host and launching themselves out towards new host cells.

There's an inherent risk in choosing the kill option: If there are no other hosts nearby, then the virus and all its progeny just died... Turns out that at least one virus has found a way to take the risk out of the decision. It listens, by eavesdropping on the bacteria's quorum-sensing communication, for the bacteria to announce that they are in a crowd, upping the chances that when the virus kills, the released viruses immediately encounter new hosts. What brilliant strategy!

This very new discovery (see article below) from the same lab that first discovered bacteria quorum-sensing, again shows how we continually underestimate 'our little friends,' and why I predict that one day Dr. Bonnie Bassler will be awarded a Nobel Prize.

<https://doi.org/10.1016/j.cell.2018.10.059>

Normal flora. Normal flora biofilm is of value, and usually is 'well behaved.' Normal flora provides many benefits to the host (e.g., inhibit colonization of pathogenic microorganisms, produce vitamins that are required by the host, etc.). Supplemental flora are found in a minority of the population, where they generally act like normal flora. Transient flora consists of microorganisms that are brought to the area from somewhere else. They almost always have a very difficult time colonizing and competing in the area, and are thus generally cleared within a few hours or days. Of course, true pathogens also will occasionally end up in an area. If they become established, these can cause overt disease, but usually will be cleared by the subsequent immune response directed against them.

MICROBIAL FLORA

① NORMAL FLORA ≈ INDIGENOUS FLORA
 ↳ DYNAMIC EQUILIBRIUM

MEMBERS - SITE SPECIFIC

- DIFFICULT TO CLEAR

- TEND TO RECOLONIZE

* - NOT USUALLY PATH AT THAT SITE

SPACE
NUTRITION
PH
ANTI-MICROBIAL FACTORS

ROLES OF NORMAL FLORA :

1. COMPETITION
2. VITAMIN K SYNTHESIS
3. IMMUNE STIMULI: LPS → GUT ↑ DTH, etc.
4. SOURCE OF OPPORTONISTS
5. ETIOLOGIC AGENTS OF CARIERS & CHRONIC INFLAMM., PERIODONTAL DISEASE

② SUPPLEMENTAL FLORA

- BEHAVES AS ABOVE IN A MINORITY OF POPULATION

e.g. VAGINAL S. aureus
 GUT Pseudomonas
 ORAL Coliforms

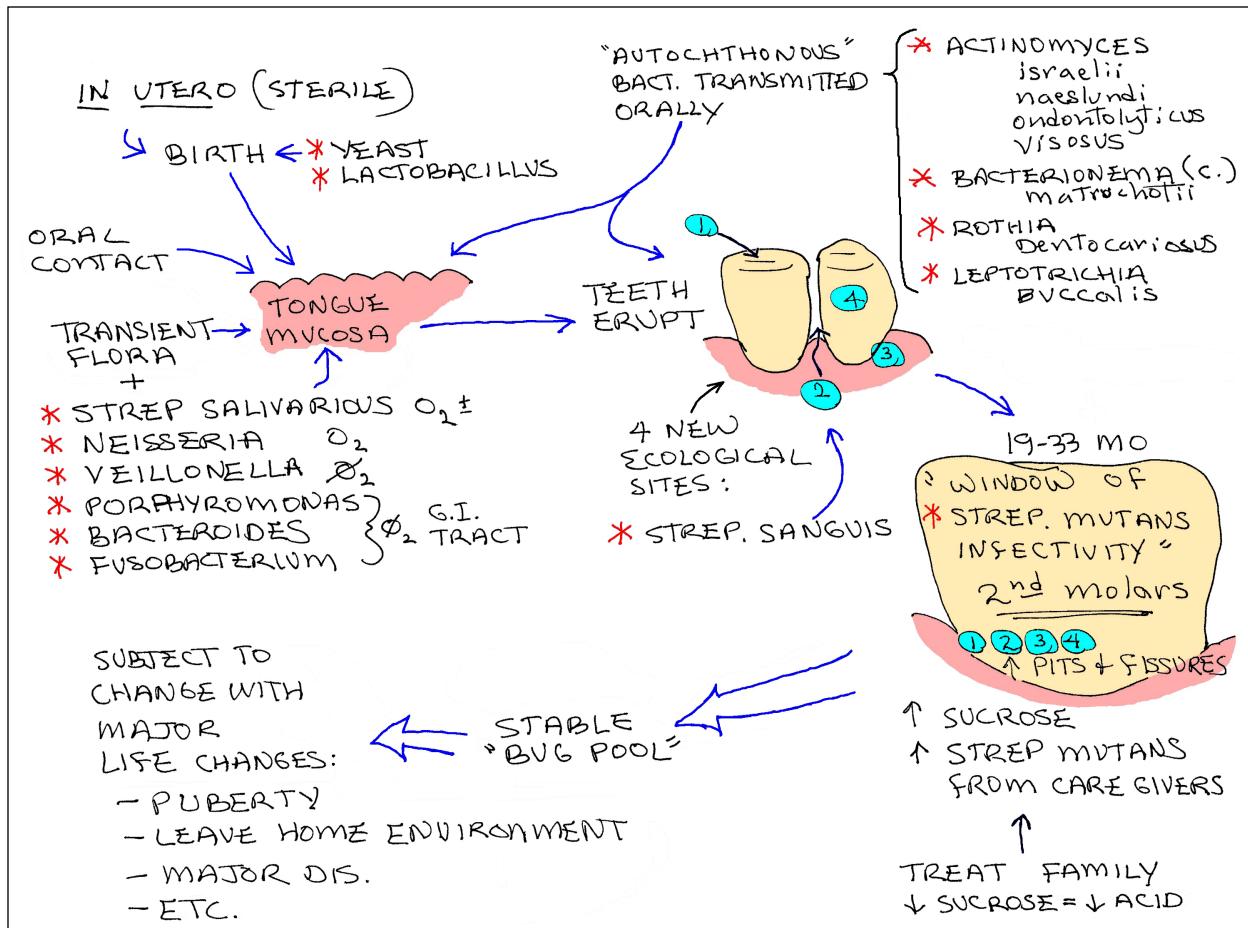
③ TRANSIENT FLORA

- CARRIED TO SITE BY CONTAMINATION
- CLEARED NATURALLY HOURS - DAYS
- WASHES AWAY (ATTACH. POOR)

Microbial flora falls into three major groups: 1) **Normal flora** - microbes that exist in 'equilibrium' with the host, and generally do not cause disease. Normal flora are usually our 'friends,' who among other things inhibit the colonization of more pathogenic microorganisms. 2) **Supplemental flora** - microbes that exist within the normal flora in some individuals. For example, some women carry *Staph. aureus* in their vaginal flora, where it doesn't usually cause disease. 3) **Transient flora** - microbes that are not normally found in that location. These are usually carried to the site by contamination. These microbes don't usually compete well in these environments, and are cleared naturally in a few hours or days. These microbes may cause disease at these new locations. (**Pathogens** are usually not considered part of this scheme since they almost always cause disease, which induces an immune response that generally is successful in clearing them from that site.)

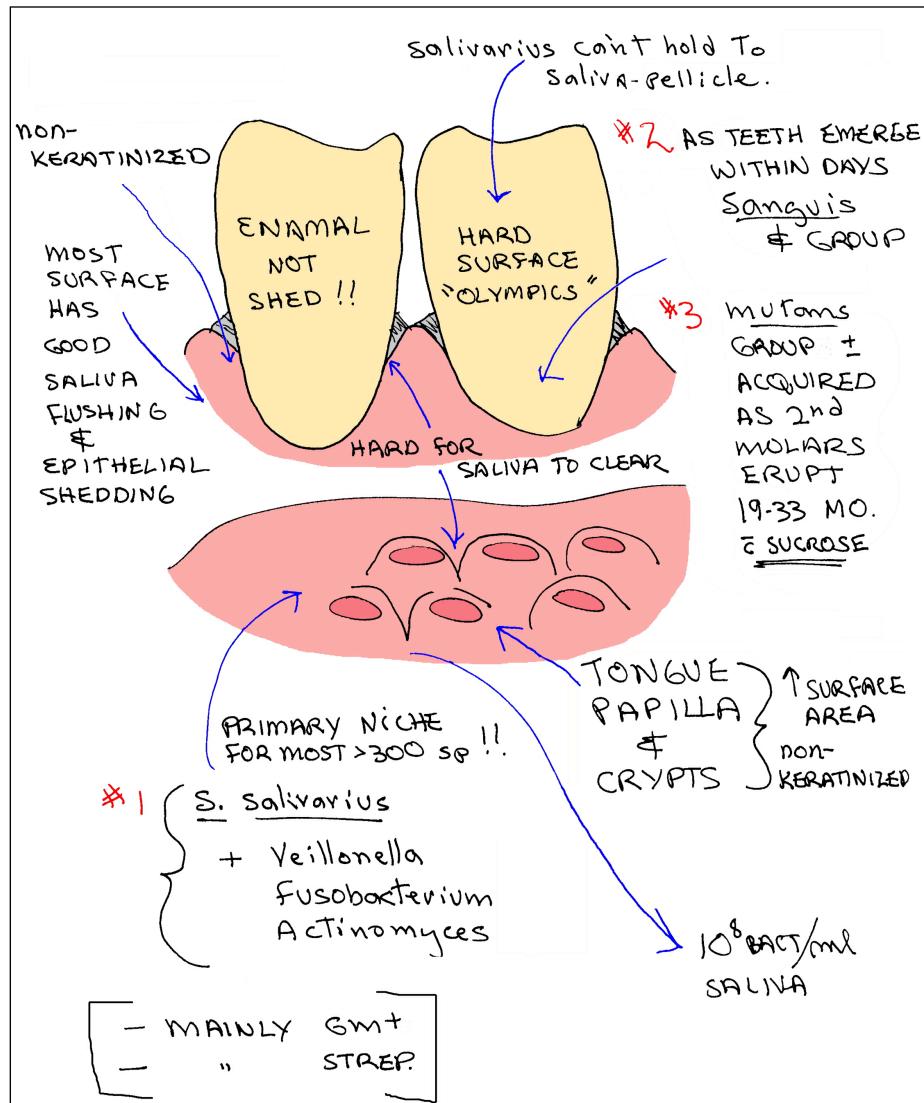
Q? We 'allow' normal flora to live exactly on our body?

Oral bacterial colonization. Generally human fetuses develop within a relatively sterile environment. This all changes at birth when the infant is exposed to microbes from the mother and environment. Almost immediately normal flora microorganisms begin to colonize, and change with time as different niches appear and competition stiffens for dwindling available sites.



Oral ecology - colonization of the oral cavity and formation of plaque biofilm. During birth, a child's oral cavity is first successfully colonized by vaginal microorganisms that can survive in the mouth (e.g., *Candida*, *Lactobacillus*, etc.). In the first days and weeks after birth, the soft tissues of the mouth are colonized usually via all the kisses bestowed upon them! When the teeth erupt into the mouth, four new niches develop (fissures, approximal surfaces, smooth surfaces, and gingival-tooth interfaces). This allows dental plaque biofilm to first form. Initial plaque colonizers must be able to adhere very well to tooth structure (i.e., they must not be washed away by saliva, or food & drink). Some bacteria such as *Strep. mutans* finally find a place where they can colonize and compete well with the appearance of the deep pits & fissures of the second molars. With the eruption of the second molars at about 2-years of age, the dental plaque ecology becomes relatively 'stable.' This 'stable' plaque biofilm does however 'evolve' with major life changes (e.g., puberty, major diseases, oral care, etc.).

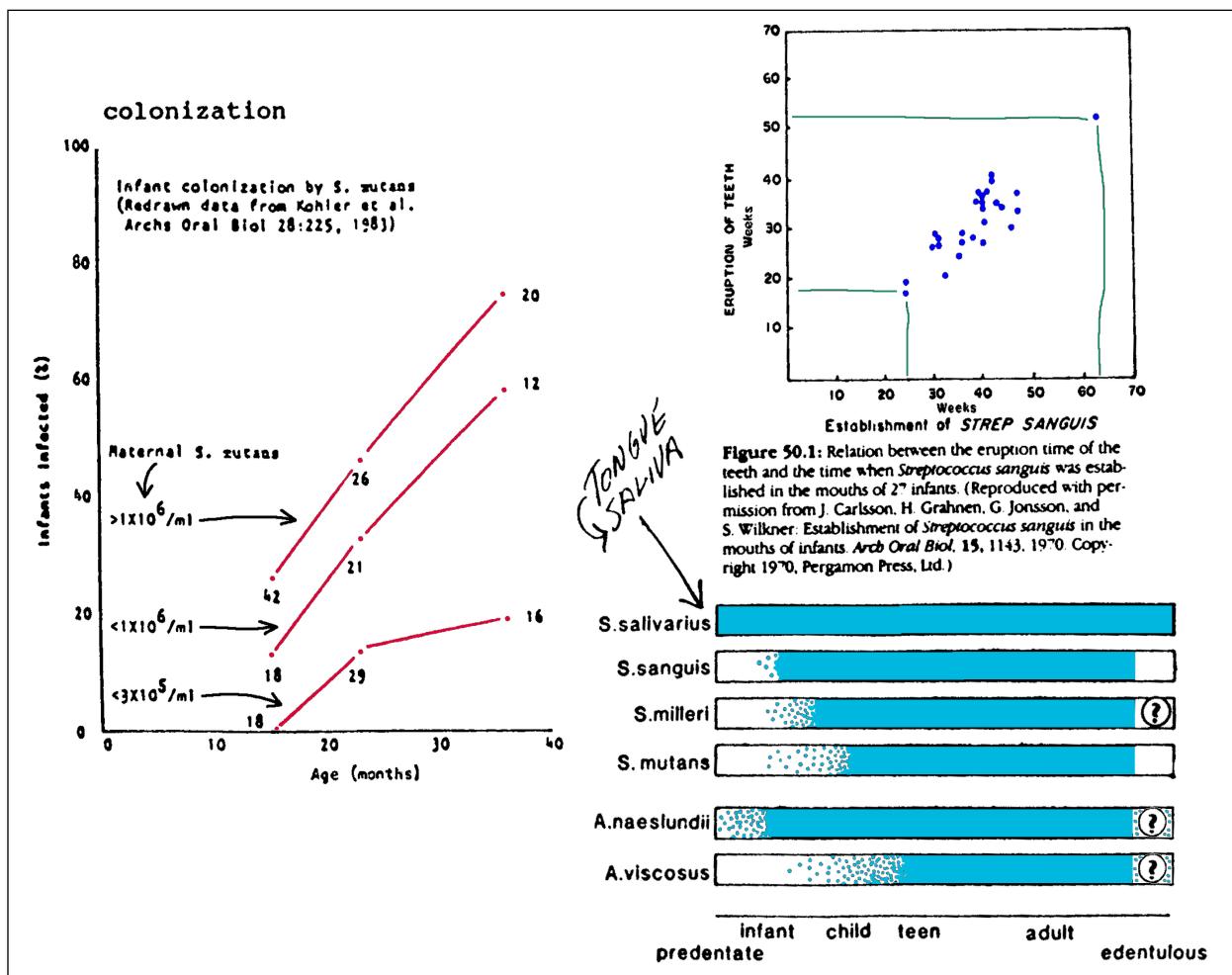
Q? How do you think C-section births might impact the above?



Dental Olympics – Let the competitions begin! Competition for a spot to call home in the mouth is fierce! Most surfaces of the oral cavity have good saliva-flushing and epithelial shedding. This makes it next to impossible for most microorganisms to colonize, and even those who can don't form large colonies. Most bacteria, of those that can colonize the mouth, call the tongue papilla and crypts their home. Tongue crypts present a very large surface area that is protected from the flushing of saliva. This allows many bacteria to colonize, and bacteria numbers get very high. After the teeth erupt, any bacteria that can adhere strongly to the pellicle (also called 'dental pellicle' or 'acquired pellicle') find a place to live (e.g., *S. sanguinis*, *S. mitis*, etc.). These pioneer species provide further binding sites for secondary colonizers in the growing plaque biofilm. As the plaque thickens the washing effect of saliva lessens. This allows more and more bacterial species to colonize plaque biofilm, which becomes increasingly complex.

Random Thought: Since we acquire much of our normal flora from our family, it follows that the microbes of the British royal family are probably descendants of the microbes of their ancestors during Victorian times – it's a whole parallel royal family, but of microbes!

Most of the early oral colonization of babies likely comes from their mother (typically their primary care-giver). (Another reason to choose your parents well!) This is illustrated with *Strep. mutans* where there is usually a direct link between mom's *S. mutans* and baby's.



Many bacteria require the hard surface of teeth to effectively colonize and remain in the mouth. *Strep. sanguinis* is an example of a 'pioneer' species in tooth plaque development. They do not appear in significant numbers in the mouth until after the teeth erupt (top right figure). They are one of the first bacteria that can adhere to pellicle proteins, and thus start dental plaque development. *Strep. mutans*, which also require teeth hard surfaces to effectively colonize, typically first appear in large numbers when the second molars emerge, with their large pits and fissures, at about 2-years. This is because *Strep mutans* don't usually compete as well on smooth tooth surfaces. Why? Note that the relative number of *S. mutans* a child harbors often relates directly to the number the mother has (left figure). (Remember to treat the family!) Also, note that some bacteria (e.g., *A. viscosus*) do not appear in appreciable numbers until later in a life (lower left figure).

Oral ecology. Oral bacterial species are not evenly distributed, but rather find their own ecological niche.

Intraoral Site Distribution of Various Indigenous and Supplemental Members of the Oral Flora				
SPECIES	SALIVA	TONGUE	PLAQUE	
			SUPRAGINGIVAL	SUBGINGIVAL
<i>Streptococcus salivarius</i>	+++	+++		
<i>S. sanguis</i>	++	++	+++	+
<i>S. mitior</i>	++	++	++	++
<i>S. milleri</i>	±	±	+ to +++	0
<i>S. mutans</i>	± to +	±	+ to +++	0
<i>Lactobacillus</i> sp.	± to +	+	+	±
<i>Actinomyces</i> sp.	+	+	++	± to ++
<i>Fusobacterium</i> sp.	0	0	±	± to ++
<i>Capnocytophaga</i>	0	0	±	± to +
<i>Treponema</i> sp.	0	0	±	± to + ++
<i>Prevotella melaninogenicus</i>	0	0	±	± to +
<i>Porphyromonas gingivalis</i>	0	0	0	0 to +
<i>Actinobacillus actinomycetemcomitans</i>	0	0	±	0 to +
<i>Veillonella</i>	+	+	++	++

0 = not usually detected; ± = rarely present; + = usually present in low proportions; ++ = usually present in moderate proportions; +++ = usually present in high proportions.

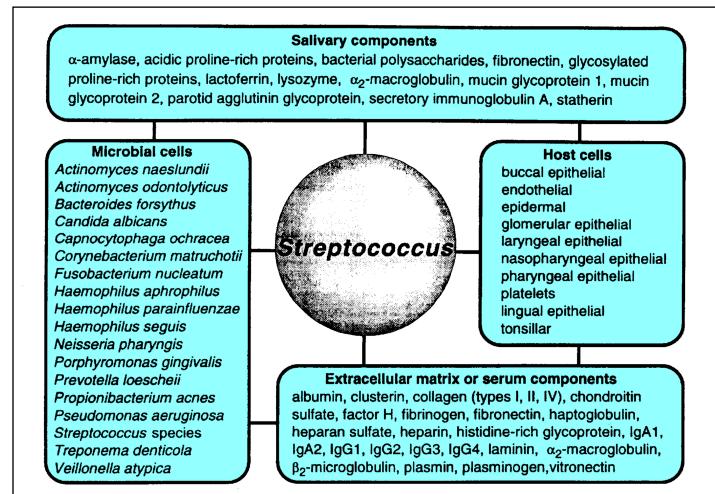
The various bacteria species in dental plaque are not distributed evenly throughout the plaque biofilm. Supragingival plaque contains mostly gram-positive bacteria such as *Streptococcus* and *Actinomyces*, with few gram-negative bacteria. Subgingival plaque contains significant numbers of gram-negative bacteria such as *Treponema* and *Fusobacterium*, with few gram-positive bacteria.

As might be expected, the species that do well supragingivally (because they can adhere to the surface and not be rinsed off, etc.), are different from the subgingival flora where other factors are more important (like being able to live on protein substrate, and in the absence of oxygen).

Characteristic	Supragingival	Subgingival
Gram Reaction	Mostly Gram ⁺	Mostly Gram-
Morphology	Coccii, Branched Rods	Mostly Rods, including Spirochetes
Oxygen ‘Relationship’	Facultative & Some Anaerobes	Mostly Anaerobes & Facultative
Energy Sources	Carbohydrates (Fermented)	Proteins
Motility	Firmly Adherent to Plaque	Adherence Less Pronounced, With Many Motile Forms
Diseases	Caries and Gingivitis	Gingivitis and Periodontitis

Some important differences between mature supragingival and subgingival plaque.

Some *Strep.* (e.g., *mutans*) appear to be dependent on the enamel surface for colonization, other *Strep.* (e.g., *salivarius*) are not. *Strep.* have many adherence factors that aid them in being one of the primary colonizers of the oral cavity. In the Dental Olympics, we'd be hearing their national anthem played often!



Multiple surface adhesion molecules expressed by *Streptococci* species allow them to adhere to a wide variety of substrates. Not all *Streptococcal* species adhere to all the substrates shown, and *Streptococci* species may utilize different mechanisms in binding. All of the adhesion molecules allow *Streptococci* species to be one of the major bacteria in the oral cavity, since they can colonize most niches. (The ‘cockroaches’ of the oral environment. Continuing with this absurd analogy, which bacteria do you think would be the Panda Bear?)

Even similar sites on the same tooth differ considerably in plaque bacterial composition:

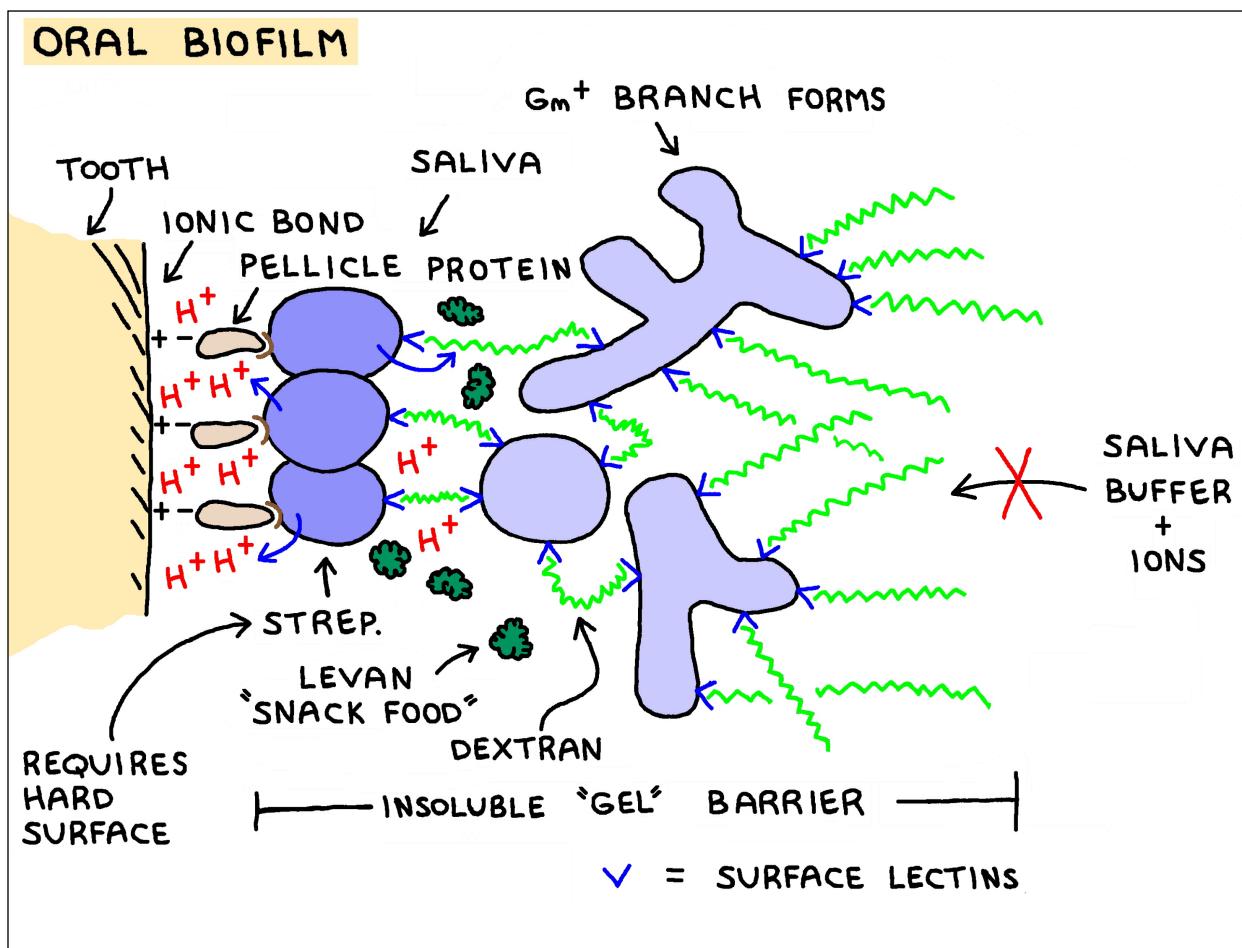
The cultivable microflora from three sites on the approximal surface of an extracted tooth from a schoolchild			
Bacterium	Viable count (colony forming units)		
	site 1	site 2	site 3
Total count	1.7×10^4	6.8×10^3	7.9×10^3
<i>Streptococcus</i>	0	0	6.1×10^5
<i>Actinomyces</i>	5.8×10^2	0	1.8×10^5
<i>Neisseria</i>	25	25	1.5×10^4
<i>Veillonella</i>	0	0	6.5×10^4
<i>Capnocytophaga</i>	0	0	1.3×10^2
<i>Haemophilus</i>	1.7×10^4	6.0×10^3	0
Individual species			
<i>mutans streptococci</i>	0	0	3.7×10^4
<i>S. sanguis</i>	0	0	1.1×10^5
<i>S. oralis</i>	0	0	1.0×10^4
<i>S. salivarius</i>	0	0	1.8×10^4
<i>A. viscosus</i>	5.8×10^2	0	1.8×10^5
<i>A. naeslundii</i>	0	0	6.5×10^3
Gram-negative ‘spreading’ filament	0	+	0

marsh et al

Even within very small areas of dental plaque, bacterial species are not evenly spread. This can lead to pathologies (e.g., caries, peritonitis, etc.) at one site on an otherwise healthy tooth.

Bacteria in mature plaque biofilms tend to grow more slowly and generally grow perpendicular to the tooth surface in micro-colonize.

The bacteria often form multicellular, multispecies complexes held together by linking polymers = **biofilms**.



Dental plaque is a biofilm. Plaque biofilm is a complex grouping of various bacteria species that adhere to one another, bacteria products, and salivary proteins. As the plaque matures it begins to exclude saliva, oxygen, and some food sources. This causes bacterial growth to slow, and pathologies to increase.

Q? What are the major component(s) of the extracellular matrix (ECM) of supragingival plaque biofilm?

Even with careful plaque removal, plaque regenerates rapidly in a coordinated manner reforming the biofilm. (“Leo, brush your teeth!” “But mom, I already did it this week!”)