

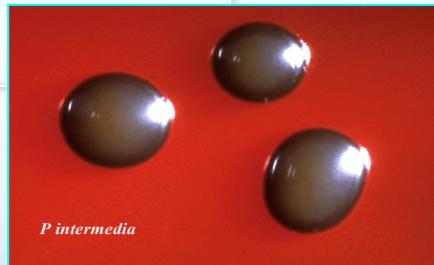


Fusobacterium nucleatum

Microbiology of Health, Gingivitis and Periodontitis



Culture of subgingival sample



Prevotella intermedia

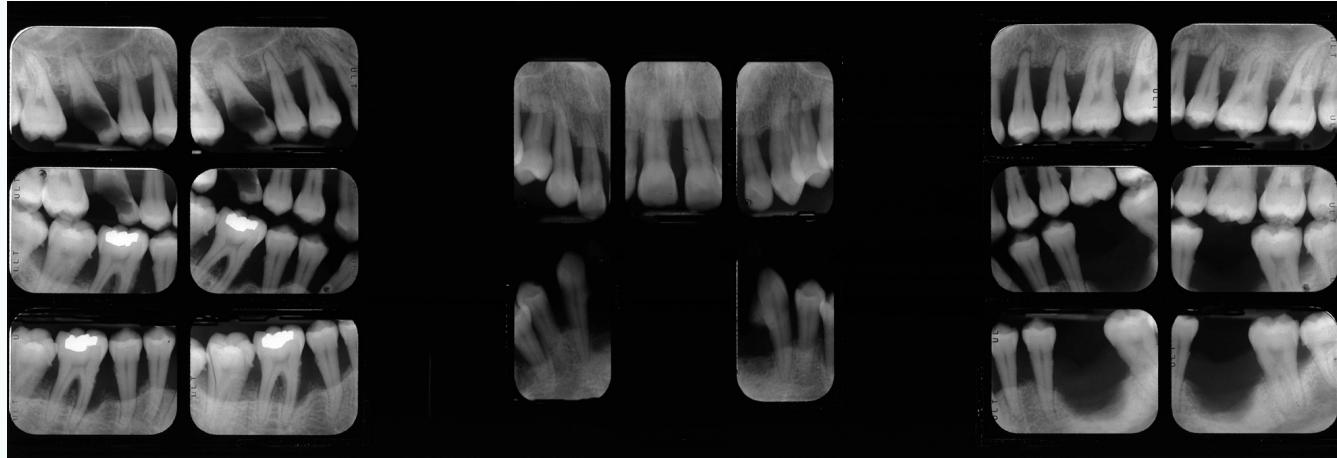
Objectives

- You should be able to name and briefly describe the bacteria associated with:
 - Periodontal Health
 - Periodontal Disease
- You should be able to discuss the current concept of tissue destruction in periodontal diseases
- You should be able to describe the main features of subgingival biofilms



Wow!! How/Why did this happen???

What can we do to stop/prevent it??



Most microbial infections are treated with antibiotics. Caries and Periodontal diseases are microbial infections but are not (usually) treated with antibiotics.

Why?

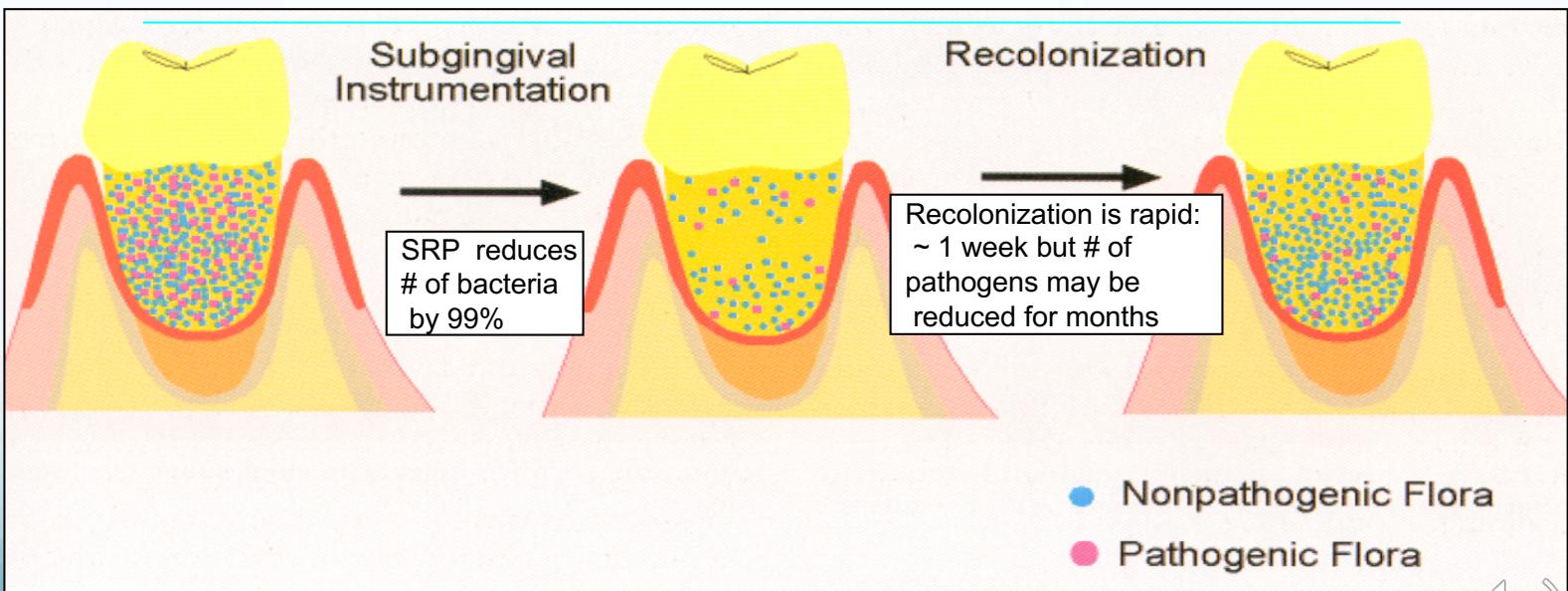


Why aren't antibiotics routinely used?

- Microorganisms are accessible: i.e., they can be removed from the tooth surface by oral hygiene measures or professional cleaning
- Bacteria in biofilms are resistant to antibiotics but are not resistant to physical removal
- After physical removal or after taking antibiotics, bacteria repopulate. You can't keep giving antibiotics but you can repeat physical removal



Effective tooth cleaning disrupts the biofilm.
Re-colonization may occur with predominantly
non-pathogenic bacteria in biofilms. It takes at least 2 months
for the biofilm to re-establish itself.



In all subjects, plaque biofilms associated on teeth elicit gingival inflammation but it is not associated with significant tissue destruction in most people.



In some people, the balance (homeostasis) between plaque biofilm and the host is disturbed and tissue destruction results. It is currently thought that the principal mechanisms involved in tissue destruction are host-derived.

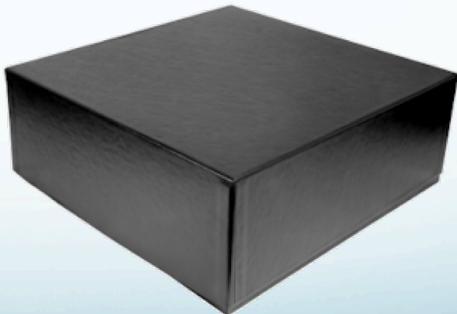


The microbial biofilm is therefore necessary but not sufficient to cause periodontitis, you need a susceptible host



The oral microbiota

- The oral microbiota is very complex
- There are many hundreds and possibly thousands of different species that live in the mouth, many of which cannot be cultivated



Periodontal Diseases are associated with increased numbers of subgingival microorganisms

- Healthy sites: $10^2 - 10^3$ isolates – mostly Gram **positive** Streptococci (facultative anaerobes) and Actinomyces sp. with about 15% gram negative rods including *Fusobacterium nucleatum* mostly motile
- Periodontally diseased sites: $10^5 - 10^8$ isolates with increased gram **negative**, obligate anaerobes, and motile species (15-50%)

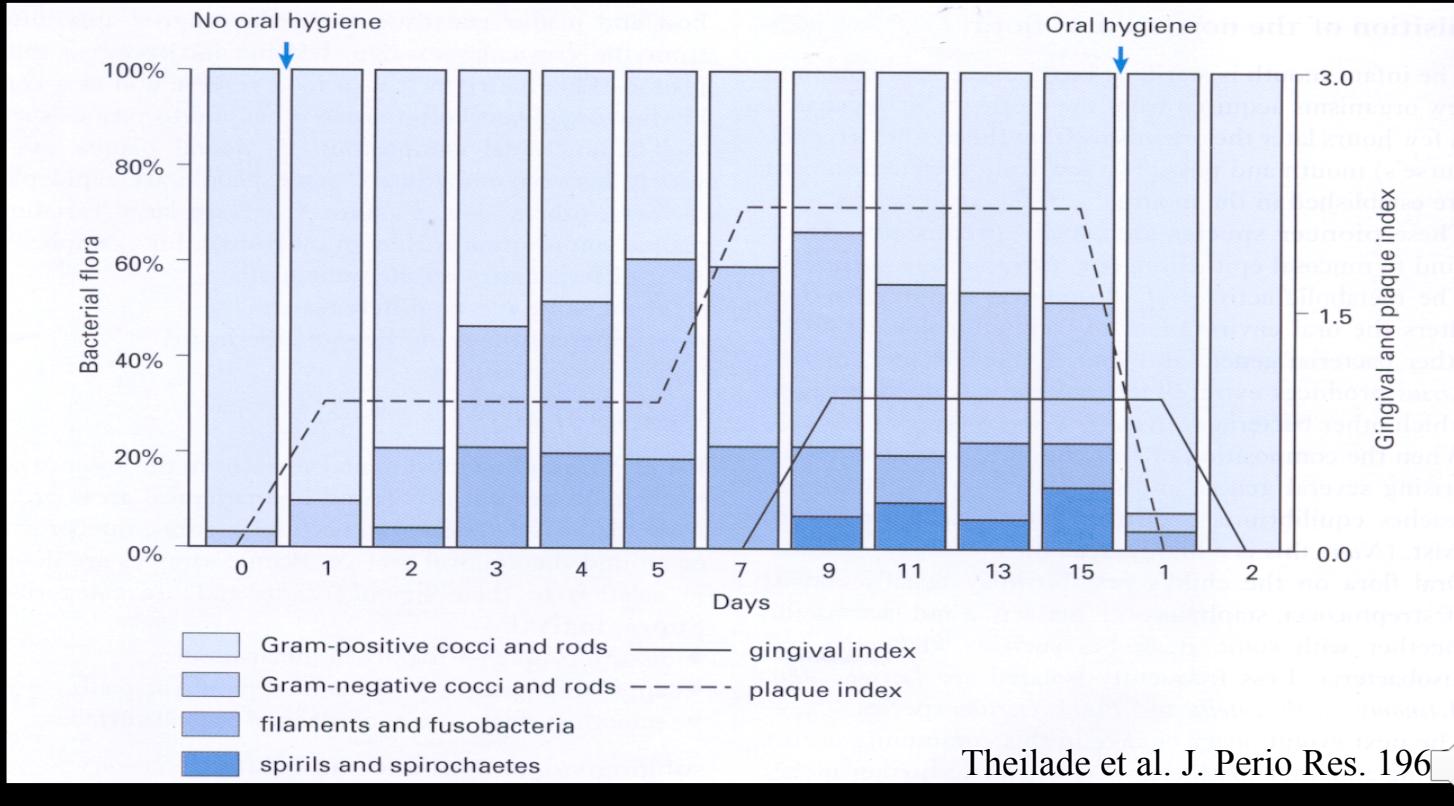


Why So Much Plaque?

- On a single tooth there may be 300 million bacteria.
- Why are teeth so attractive to bacteria?
 - Because a tooth is a non-shedding surface



It has been known for nearly 50 years that there are different bacteria associated with health and disease



So it is very clear that microbial biofilms associated with healthy periodontal tissues are different than biofilms associated with periodontal diseases.

However it is **still not clear** what specific bacteria species are causally related to periodontal diseases.



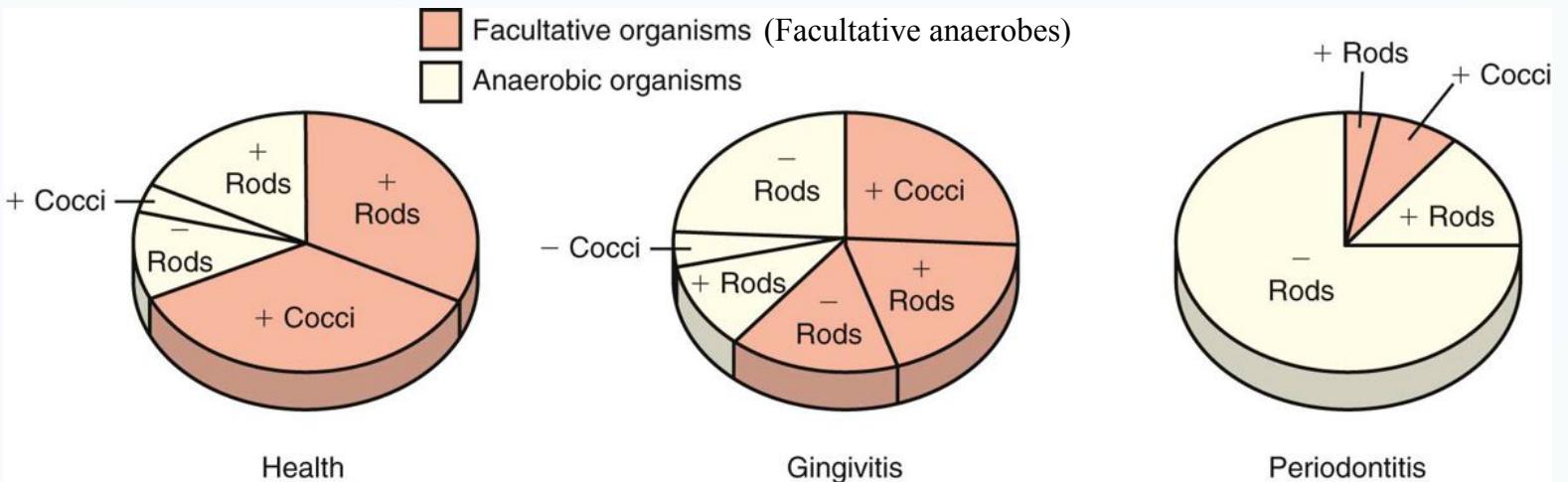
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© Mike Baldwin / Cornered



"We also find the defendant's lawyer
guilty by association."



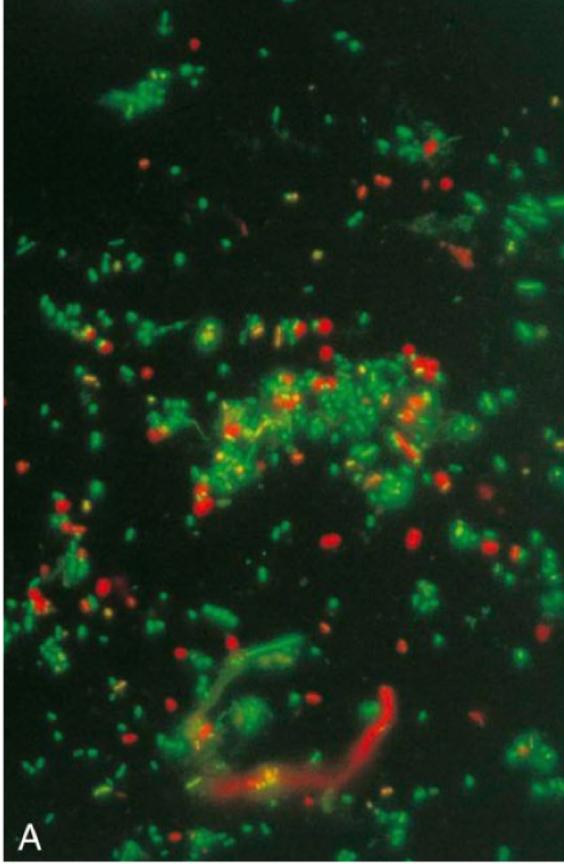


Carranza's Clinical Periodontology, 11th ed.

- Compared with Health, Gingivitis is associated with increased numbers of gram negative species
- Compared with Gingivitis, Periodontitis is associated with significant increase in Anaerobic species
- Periodontitis is associated with large numbers of Gram negative rods

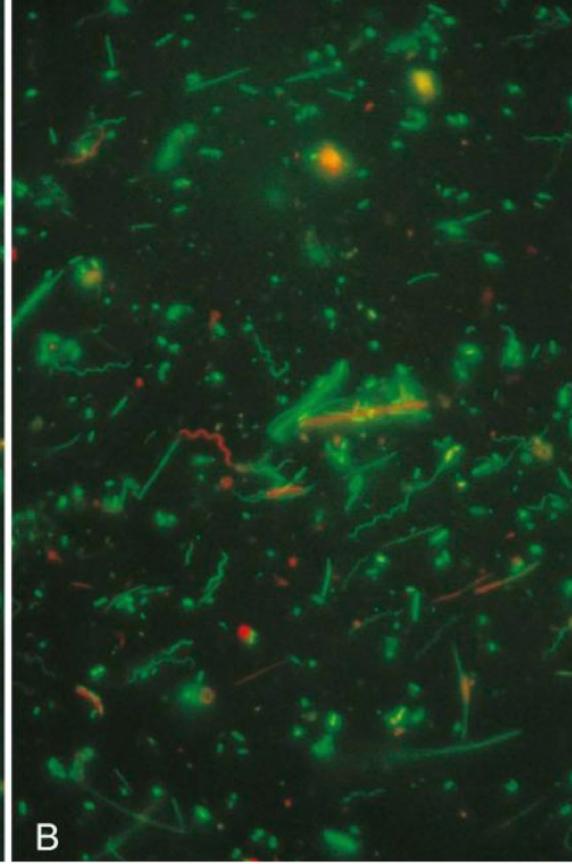


mostly cocci



A

Healthy

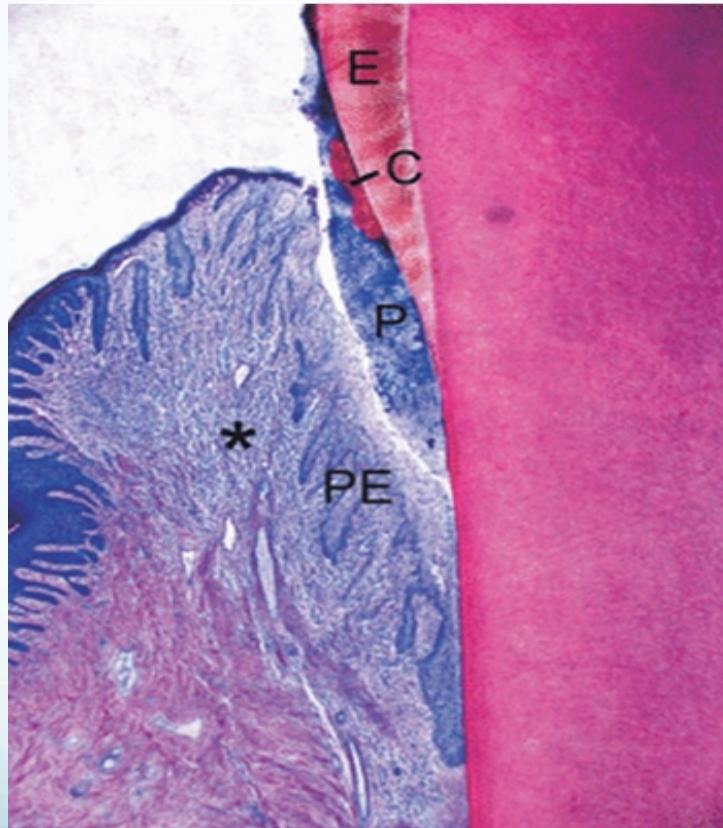


B

Periodontitis

more
rods
filaments
spirals
motile bac





E enamel

C calculus

P plaque

PE pocket epithelium

→ past C E J down to root surface

↳ attachment loss

- lots of dark dots:

- plasma cells

- macrophages

- white spc = vasodilation

Jepsen et al. Periodontology 2000 55; 167 -188: 2011



Gram positive cocci associated with periodontal health

- *Gram +, cocci, facultative anaerobes*
 - *Streptococcus sanguinis*
 - *Streptococcus gordonii*



Gram positive rods associated with gingivitis

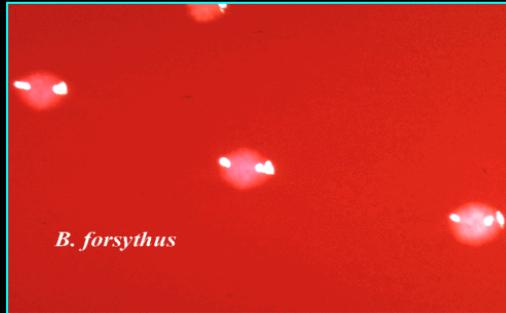
- *Actinomyces israelii*
- *Actinomyces naeslundii*



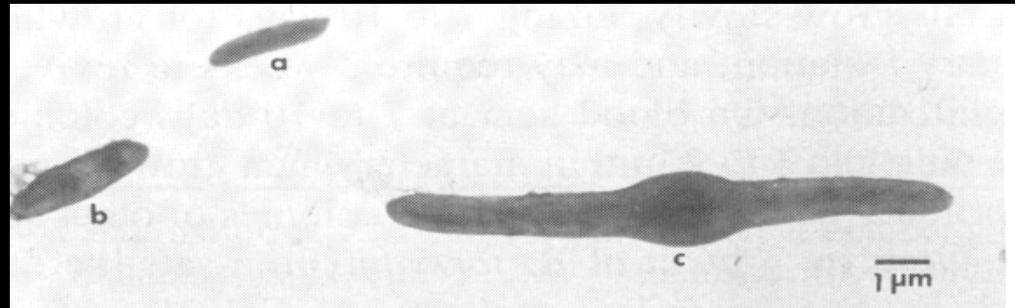
Gram negative bacteria associated with tissue destruction in Periodontitis:

- *Gram negative obligate anaerobes*
 - *Porphyromonas gingivalis*
 - *Tannerella forsythia*
 - *Treponema denticola*
 - This is the so called “**Red Complex**”
 - ↳ for boards
 - in general, there's a shift in bac type

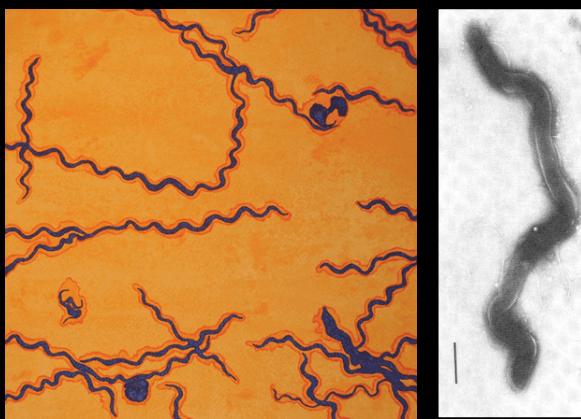




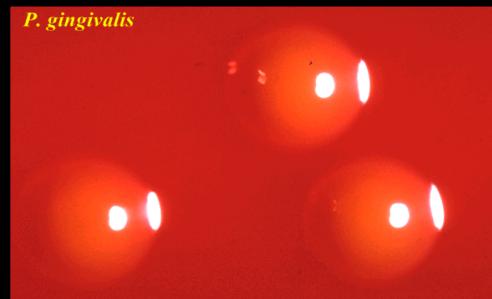
B. forsythus



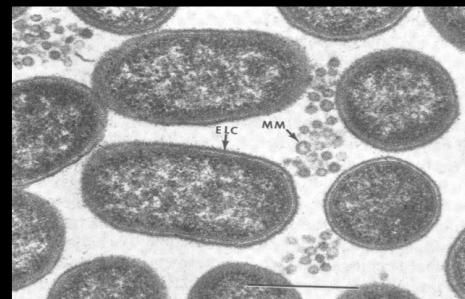
Tannerella forsythia



Treponema denticola



P. gingivalis



Porphyromonas gingivalis



Spirochetes (Treponema)

- *Treponema denticola*

(A recent study identified 10 known Treponema species and 47 unknown species in subgingival plaque)



Porphyromonas gingivalis

- Gram negative, anaerobic rod
- Periodontally healthy young children and adolescents harbor very few or no *Porphyromonas gingivalis*
- Most patients have a single clone of *Porphyromonas gingivalis*
- Strongly associated with Periodontitis
- Multiple virulence factors- which include proteases like collagenase



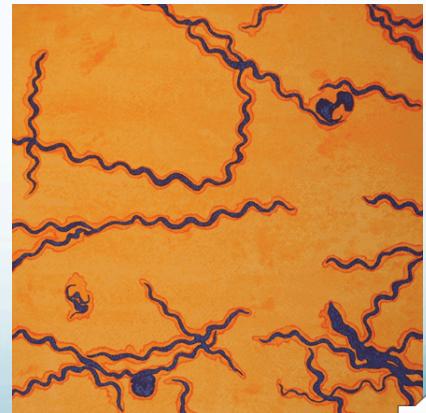
Tannerella forsythia

- Gram negative anaerobic rod
- Strongly associated with Periodontitis



Treponema denticola

- Gram negative
- Strict anaerobe – found in large numbers at the base of pockets
- Strongly associated with Periodontitis



Aggregatibacter actinomycetemcomitans



coccus



Aggregatibacter actinomycetemcomitans

- Gram negative coccobacillus
- Strongly associated with **some cases** of periodontitis with a circumpubertal onset- formally called “Localized Aggressive Periodontitis (LAP)” in the old classification system
- Localized aggressive periodontitis is now the ‘molar-incisor pattern of periodontitis, generalized aggressive periodontitis no longer recognized as a unique periodontitis diagnosis
- In one study, >95% of LAP patients are colonized by *Aggregatibacter actinomycetemcomitans*, < 25% of healthy adults are*
- *Aggregatibacter actinomycetemcomitans* can make up 90% of bacteria recovered from LAP lesions
- Some strains produce a toxin that kills neutrophils

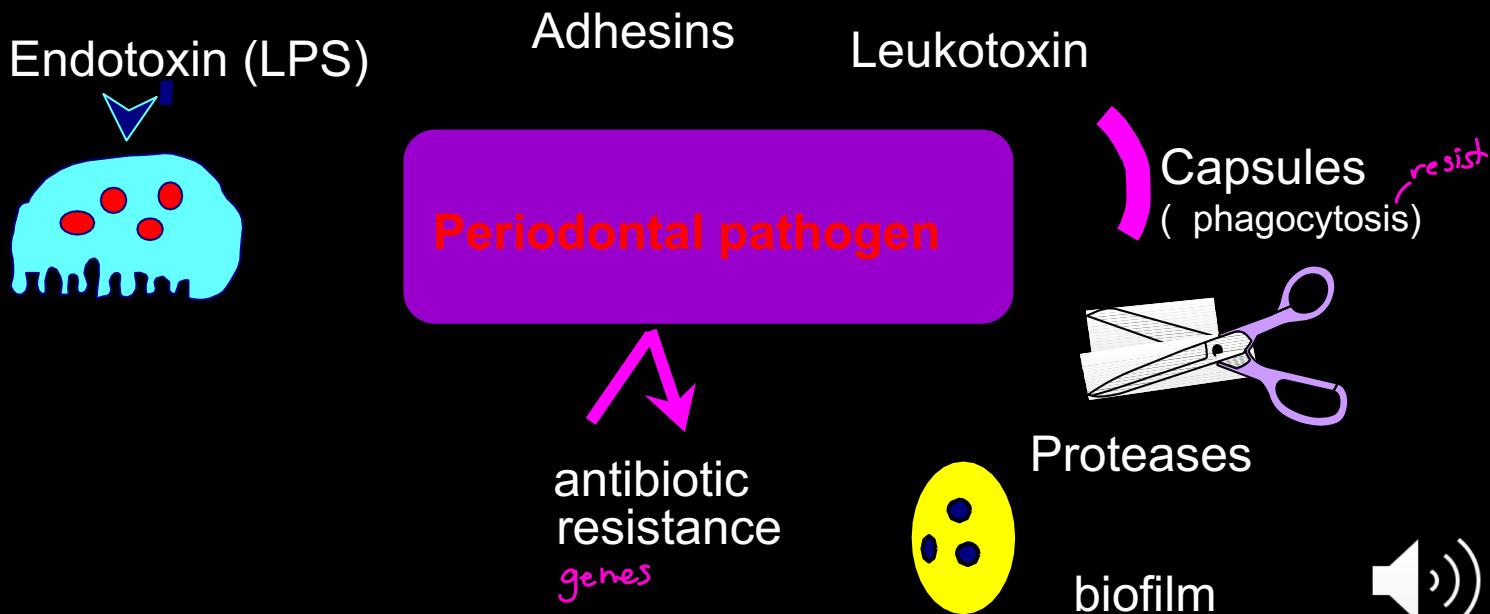
*Zambon et al. J. Periodontol. 54: 707; 1983



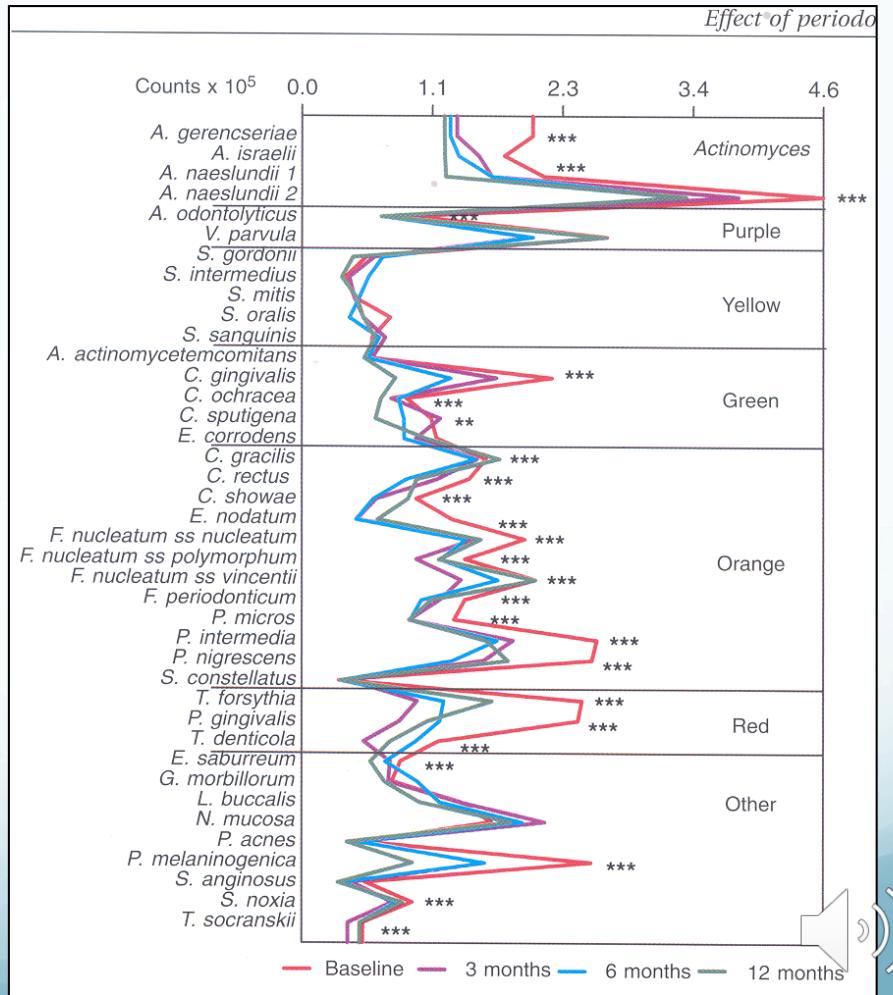
Periodontal pathogens express multiple virulence factors and their presence is necessary for periodontal disease to occur.



A Summary of Selected Virulence factors



Effect of treatment on subgingival microorganisms



Prevalence of Microbial Species with Various Clinical Forms of Periodontitis

Species	FORMS OF PERIODONTITIS		
	Chronic Periodontitis	Localized Aggressive Periodontitis	Aggressive Periodontitis
<i>Aggregatibacter actinomycetemcomitans</i>	28.2%	81.8%	40.9%
<i>Porphyromonas gingivalis</i>	53.8%	13.3%	79.6%
<i>Prevotella intermedia/ nigrescens</i>	50.2%	53.4%	71.4%
<i>Tannerella forsythia</i>	50.6%	O	50.8%
<i>Campylobacter rectus</i>	40.3%	12.5%	47.8%

Adapted from Haffajee AD, Socransky SS: *Periodontol 2000* 5:78, 1994; and Mombelli A, Casagni F, Madianos PN: *J Clin Periodontol* 29 Suppl 3:10, 2002.

O, Occasionally isolated.



Prevalence of Key Pathogens in Healthy Subjects and Patients with Periodontitis

Species	PREVALENCE		SIGNIFICANCE OF DIFFERENCE	
	Health	Periodontitis	P-Value	Odds Ratio*
<i>Aggregatibacter actinomycetemcomitans</i>	12.8%	31.0%	0.002	3.1
<i>Porphyromonas gingivalis</i>	10.6%	59.5%	<0.001	12.3
<i>Prevotella intermedia/nigrescens</i>	69.1%	87.9%	0.001	3.3
<i>Tannerella forsythia</i>	47.9%	90.5%	<0.001	10.4
<i>Fusobacterium nucleatum</i>	85.1%	95.7%	0.014	3.9
<i>Parvimonas micra</i>	67%	94%	<0.001	7.7
<i>Campylobacter rectus</i>	13.8%	20.7%	NS	1.6

Adapted from Van Winkelhoff, Loos BG, van der Reijden WA, et al: *J Clin Periodontol* 29:1023, 2002.

NS, Not significant.



Summary of Species Significantly Associated with Different Clinical Conditions

Health	Gingivitis	Chronic Periodontitis	Localized Aggressive Periodontitis	Generalized Aggressive Periodontitis
<i>A. naeslundii</i>	<i>A. naeslundii</i>	<i>Eubacterium DO6</i>	<i>E. nucleatum</i>	<i>A. naeslundii</i>
<i>S. sanguis</i>	<i>E. nucleatum</i>	<i>A. naeslundii</i>	<i>E. nodatum</i>	<i>E. nucleatum</i>
<i>K. parvula</i>	<i>K. parvula</i>	<i>E. nucleatum</i>	<i>E. timidum</i>	<i>S. sanguis</i>
<i>E. nucleatum</i>	<i>S. sanguis</i>	<i>E. nodatum</i>	<i>A. naeslundii</i>	<i>P. micros</i>
<i>S. oralis</i>	<i>C. ochracea</i>	<i>C. rectus</i>	<i>L. uli</i>	<i>B. gracilis</i>
<i>S. intermedius</i>	<i>A. israelii</i>	<i>V. parvula</i>	<i>P. micros</i>	<i>C. concisus</i>
<i>Actinomyces</i>	<i>C. gingivalis</i>	<i>L. uli</i>	<i>Eubacterium DO6</i>	<i>E. timidum</i>
serotype 963	<i>C. sputigena</i>	<i>S. oralis</i>	<i>P. intermedia</i>	<i>S. oralis</i>
<i>P. micros</i>	<i>S. noxia</i>	<i>E. timidum</i>	<i>S. intermedius</i>	<i>L. uli</i>
<i>Streptococcus</i>	<i>C. rectus</i>	<i>P. micros</i>	<i>Streptococcus D39</i>	<i>S. sputigena</i>
<i>D06</i>	<i>B. gracilis</i>	<i>P. oris</i>	<i>P. denticola</i>	<i>S. intermedius</i>
<i>G. morbillorum</i>	<i>P. nigrescens</i>	<i>E. alocis</i>	<i>L. rimae</i>	<i>A. odontolyticus</i>
<i>C. ochracea</i>	<i>S. oralis</i>	<i>L. rimae</i>	<i>E. brachy</i>	<i>L. rimae</i>
<i>H. segnis</i>	<i>E. brachy</i>	<i>Actinomyces</i>	<i>E. alocis</i>	<i>E. nodatum</i>
<i>H. parainfluenzae</i>	serotype 963	<i>S. mitis</i>	<i>P. oris</i>	<i>V. parvula</i>
<i>S. epidermidis</i>	<i>E. timidum</i>	<i>P. denticola</i>	<i>V. parvula</i>	<i>P. nigrescens</i>
<i>C. gingivalis</i>	<i>G. morbillorum</i>	<i>P. anaerobius ID</i>	<i>S. noxia</i>	<i>E. alocis</i>
<i>C. rectus</i>		<i>E. saphenum</i>	<i>P. acnes</i>	<i>C. ochracea</i>
<i>B. gracilis</i>		<i>A. odontolyticus</i>	<i>E. saphenum</i>	<i>C. rectus</i>
<i>A. odontolyticus</i>	<i>P. catoniae</i>	<i>A. actinomycetemcomitans</i>	<i>E. saphenum</i>	<i>P. gingivalis</i>
<i>P. acnes</i>	<i>H. segnis</i>	<i>A. meyeri</i>	<i>A. israelii</i>	<i>V. atypica</i>
	<i>C. concisus</i>	<i>Prevotella M1</i>	<i>C. ochracea</i>	<i>A. israelii</i>
<i>P. denticola</i>	<i>A. odontolyticus</i>	<i>P. intermedia</i>	<i>S. sp. tigena</i>	<i>P. anaerobius ID</i>
<i>E. timidum</i>		<i>S. sputigena</i>	<i>E. alactolyticum</i>	<i>A. gerencseriae</i>
Facultative		<i>S. mitis</i>	<i>P. anaerobius ID</i>	<i>E. saphenum</i>
gram-negative		<i>E. brachy</i>	<i>P. buccae</i>	<i>P. anaerobius II</i>
rod D24	<i>Actinomyces</i>	<i>B. gracilis</i>	<i>A. meyeri</i>	<i>G. morbillorum</i>
<i>S. noxia</i>	<i>A. meyeri</i>	<i>P. oralis</i>	<i>A. meyeri</i>	<i>S. infelix</i>
<i>S. faecium</i>	<i>E. yurii</i>	<i>C. ochracea</i>	<i>P. anaerobius II</i>	<i>E. alactolyticum</i>
Facultative	<i>S. infantis</i>	<i>P. gingivalis</i>	<i>P. anaerobius ID</i>	<i>P. denticola</i>
gram-positive		<i>S. noxia</i>	<i>P. buccae</i>	
coccus	<i>E. fluegei</i>	<i>P. nigrescens</i>	<i>A. meyeri</i>	
D40	<i>E. alocis</i>	<i>D. pneumosintes</i>	<i>C. curvus</i>	
<i>A. meyeri</i>	<i>E. saphenum</i>	<i>P. anaerobius II</i>	<i>S. mutans</i>	
<i>S. warneri</i>	<i>C. curvus</i>	<i>P. oralis</i>	<i>E. brachy</i>	
<i>E. saburreum</i>	<i>P. oris</i>	<i>A. israelii</i>	<i>C. sputigena</i>	
Leptotrichia		<i>P. micros</i>	<i>Campylobacter X</i>	
D16		<i>P. melaninogenica</i>	<i>S. mitis</i>	
Facultative		<i>S. intermedius</i>	<i>S. parasanguis</i>	
serotype 963		<i>G. morbillorum</i>	<i>D. pneumosintes</i>	
gram-positive		<i>Campylobacter X</i>	<i>A. gerencseriae</i>	
coccus		<i>S. sputigena</i>	<i>P. tannerae</i>	
D40		<i>E. alactolyticum</i>	<i>S. oralis</i>	
<i>E. nodatum</i>		<i>B. capillosum</i>	<i>P. gingivalis</i>	
Campylobacter X		<i>Streptococcus SM</i>		
<i>K. atypica</i>				
<i>S. capitis</i>				
<i>N. mucosa</i>				
<i>Bacillus D06</i>				
<i>P. propionicum</i>				
<i>B. forsythus</i>				
<i>P. denticola</i>				
Prevotella				
DIC20				



The bottom line

The problems:

- There are enormous numbers of subgingival species
- We have little knowledge about many of these bacteria.
- There are complex interactions within the biofilm

However:

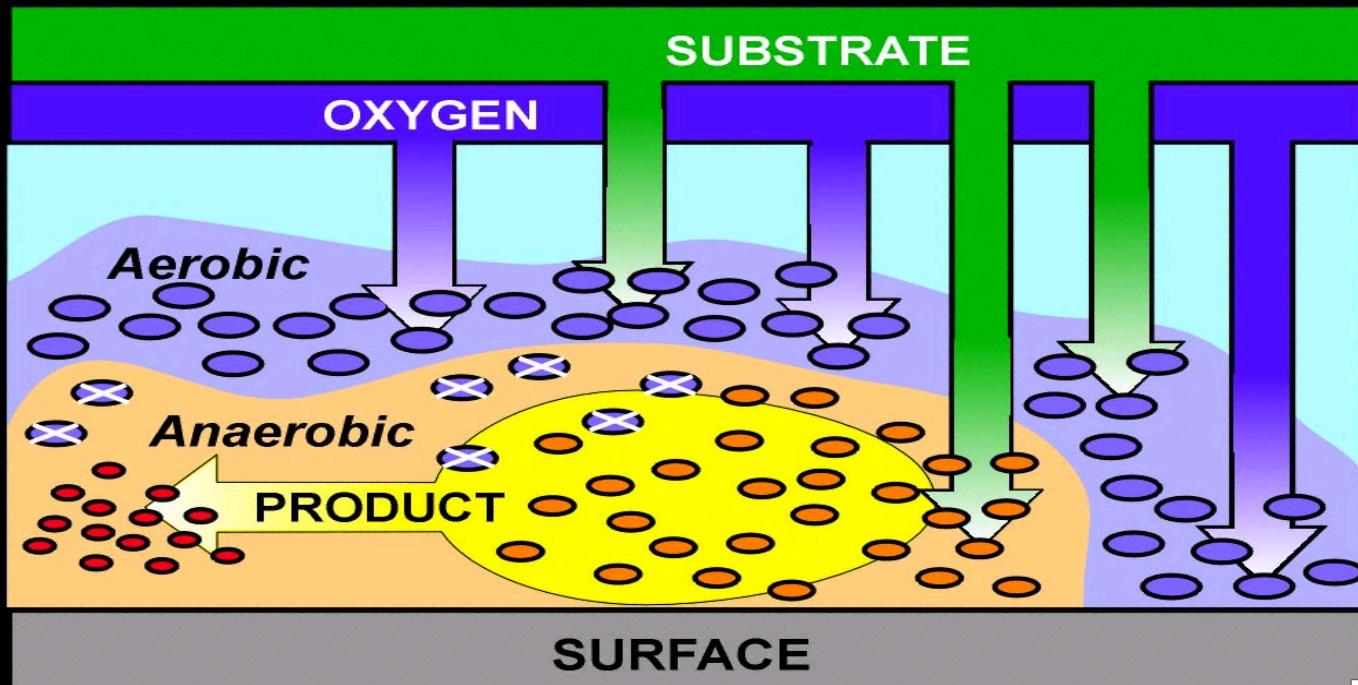
- Microbial species associated with health are very different from those associated with Gingivitis and Periodontitis
- *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* are very likely to be pathogens associated in some way with Periodontitis and *Aggregatibacter actinomycetemcomitans* is very likely to be a pathogen associated with some cases of periodontitis with a circumpubertal onset



Biofilms

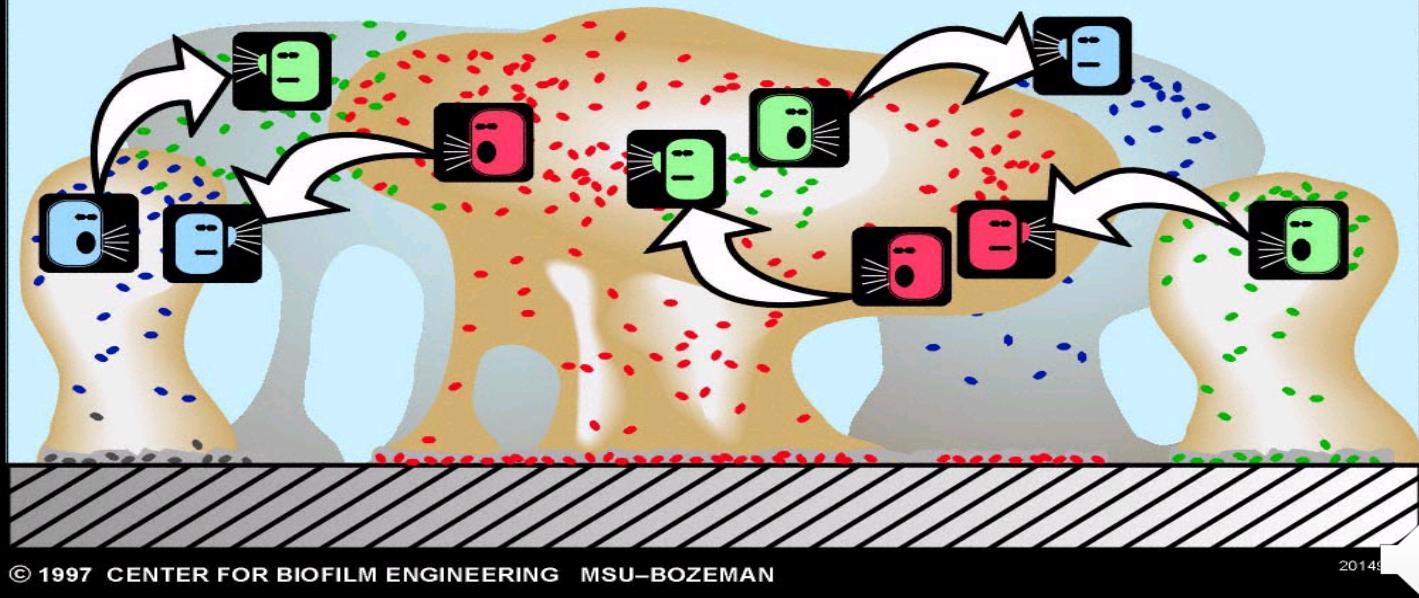
- Biofilm formation occurs in 3 stages:
 1. attachment of pioneer bacteria to pellicle
 2. colonization of other microorganisms by attachment to pioneer bacteria
 3. microbial growth and matrix production
- Bacterial associations are not random
- Bacterial genes are switched on soon after attachment to surface
- Genes may be switched on as a result of “quorum sensing” –the build up of signaling molecules when bacteria are present in aggregates
- Molecules involved in quorum sensing may switch on multiple virulence genes

Biofilms



Biofilms

Cell-Cell Communication

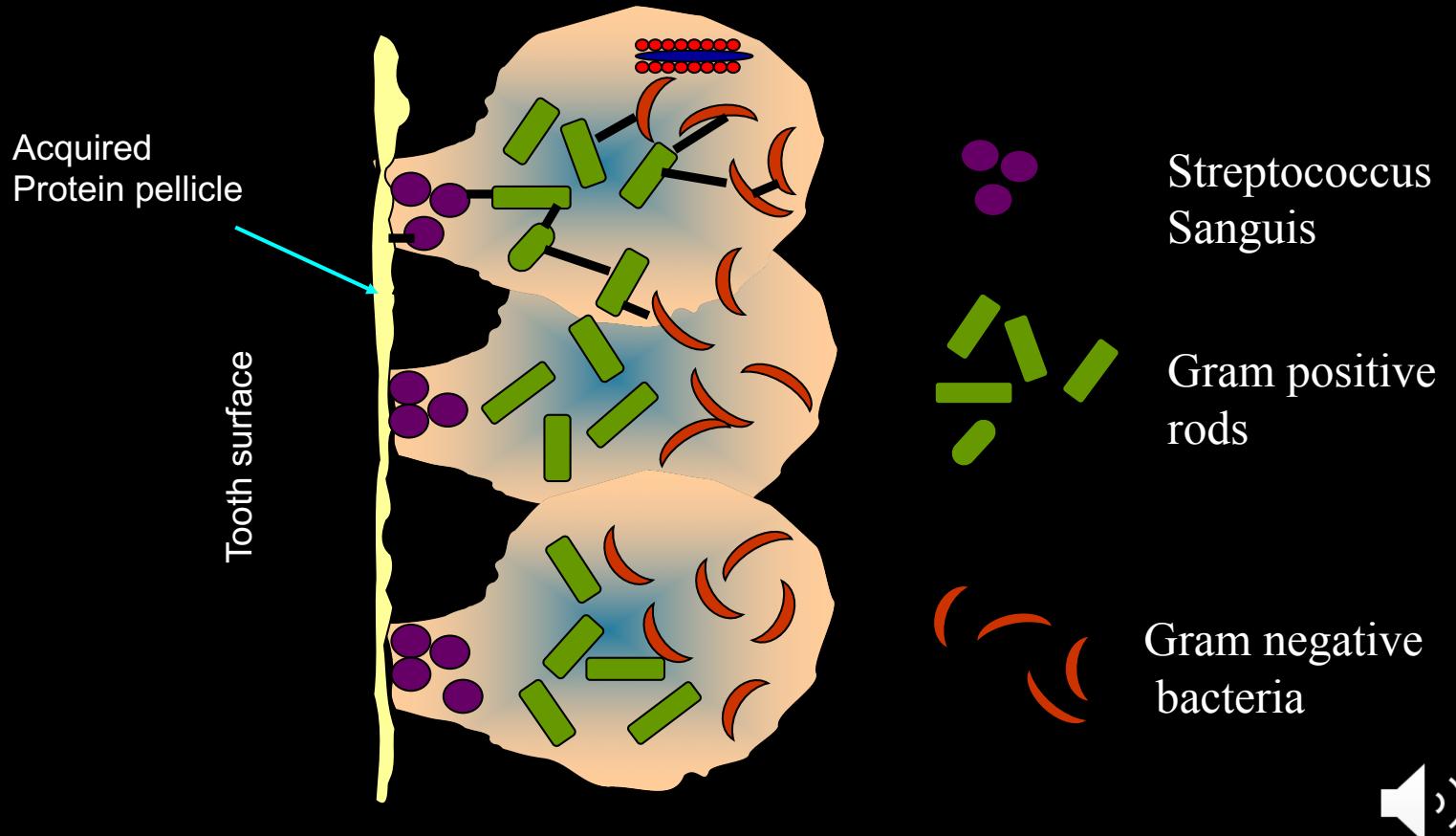


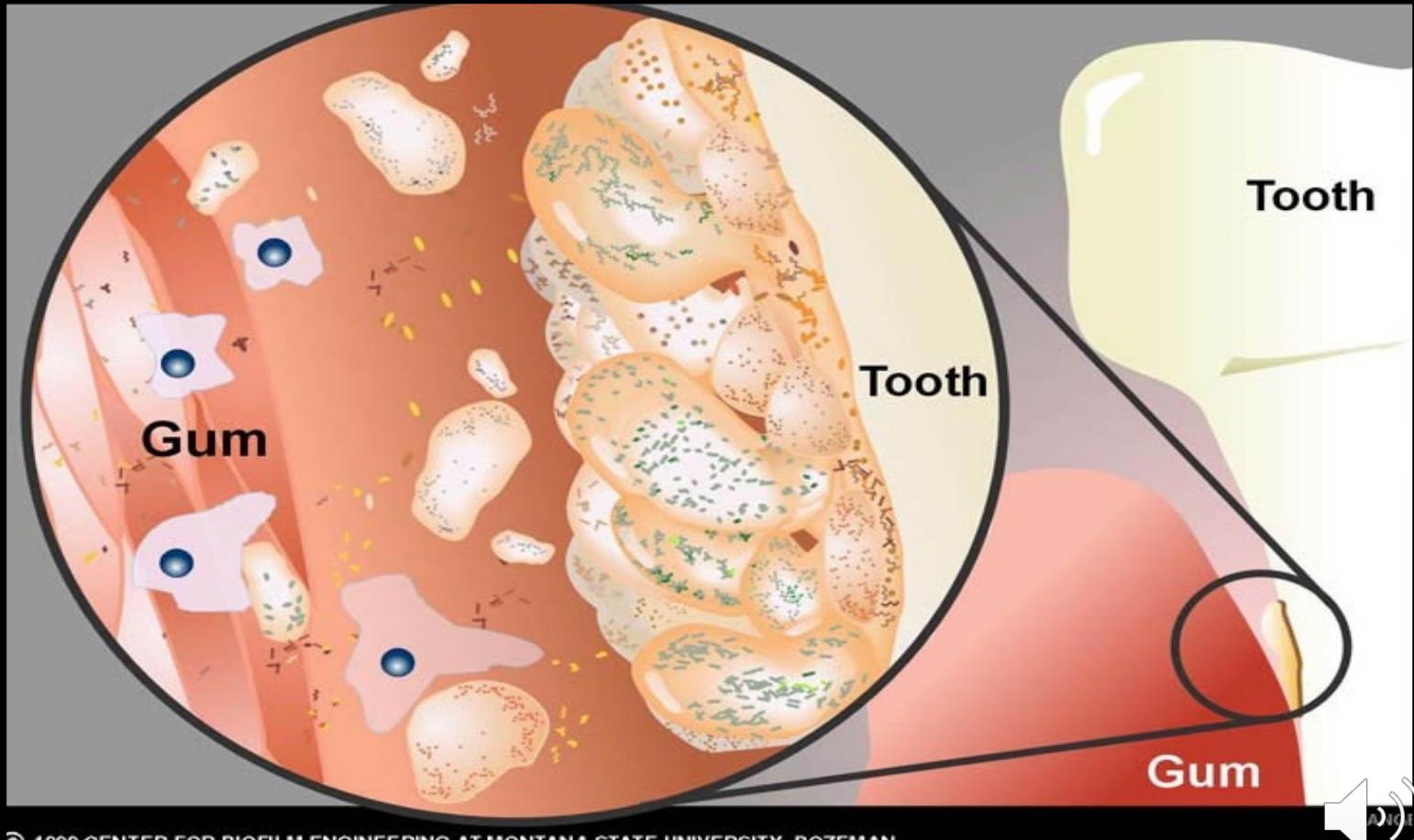
Diseases associated with biofilms

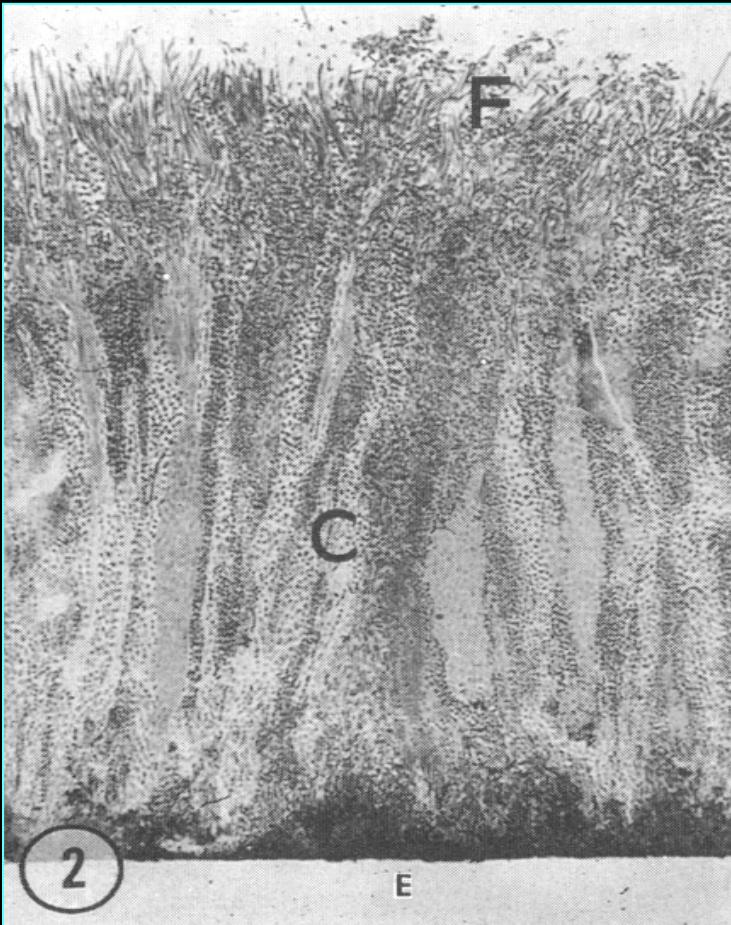
- Periodontal diseases
- Prostate infections
- Kidney stones
- Tuberculosis
- Legionnaire's disease
- Some middle ear infections



The formation of plaque biofilms

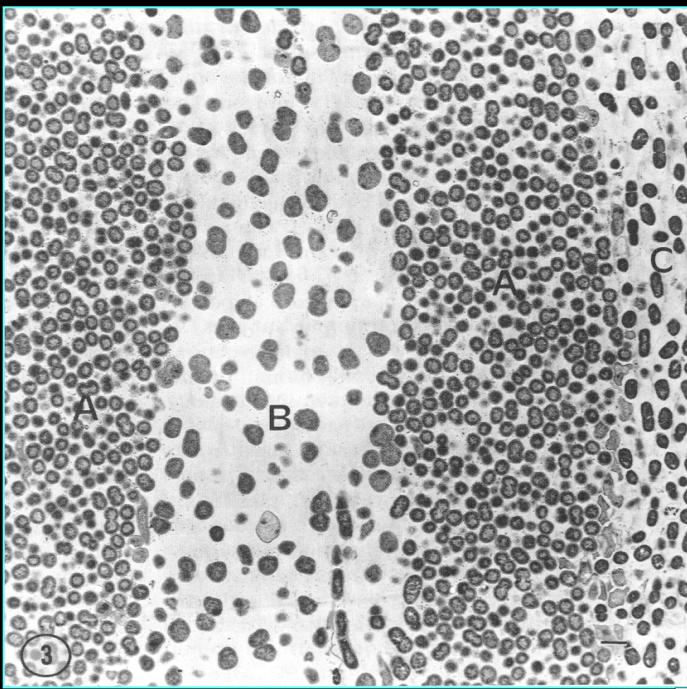




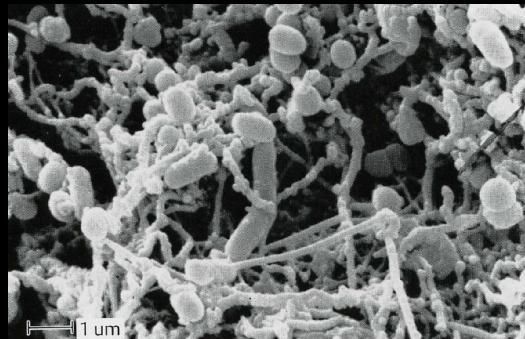
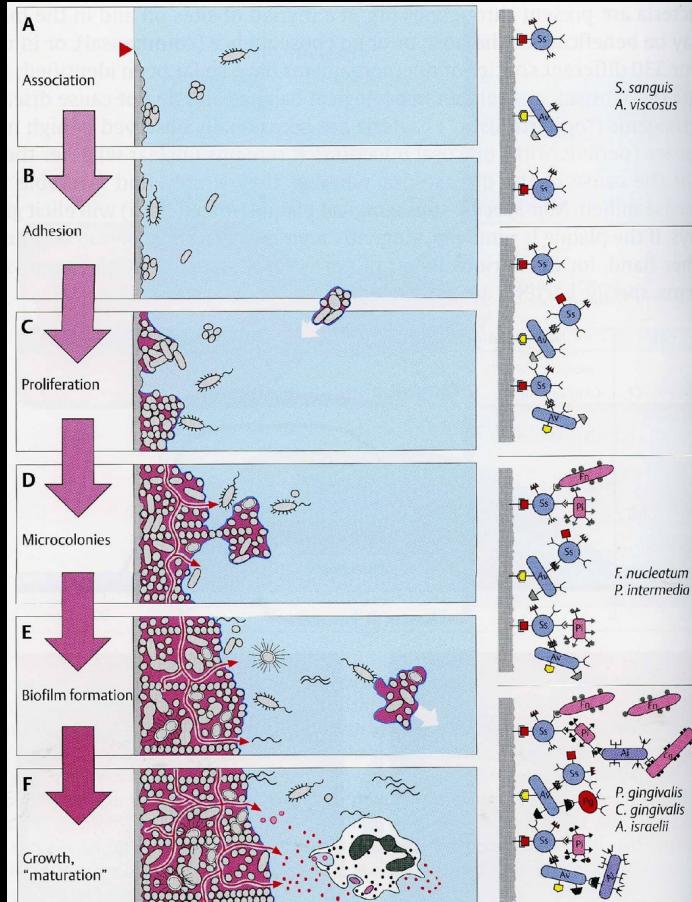


Low power

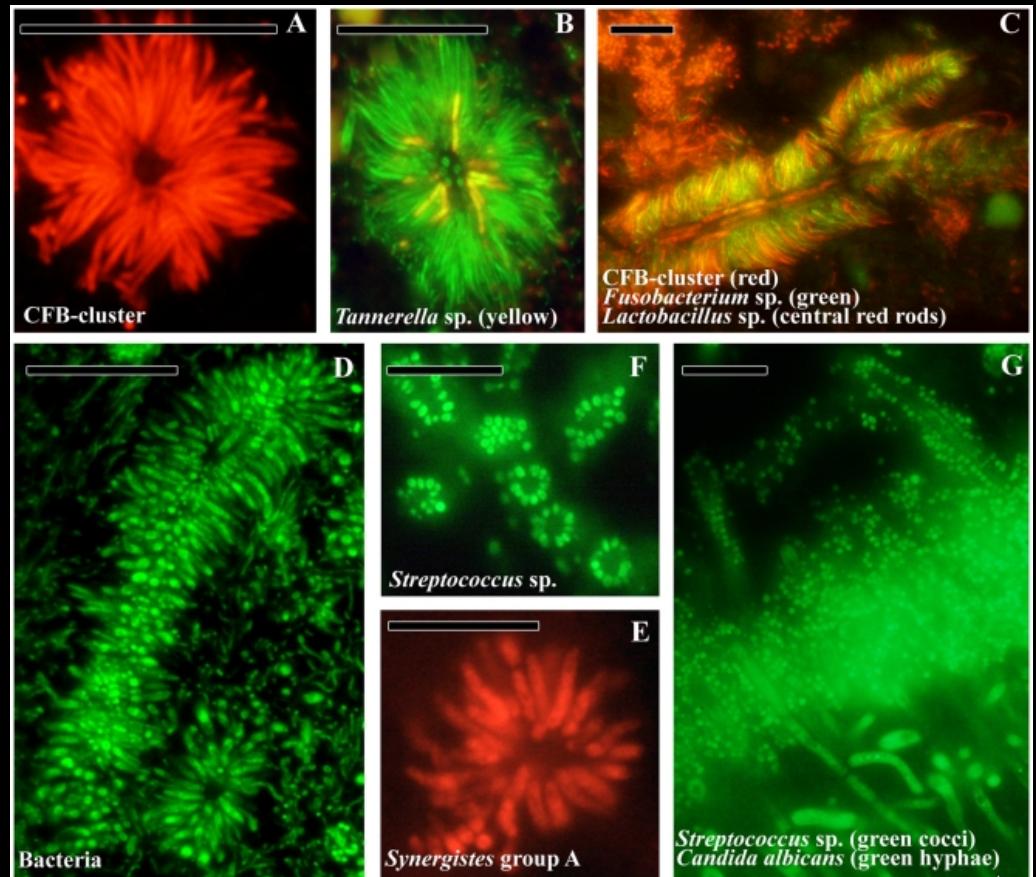
Microbial plaque grown on epoxy resin crown showing features of a biofilm.



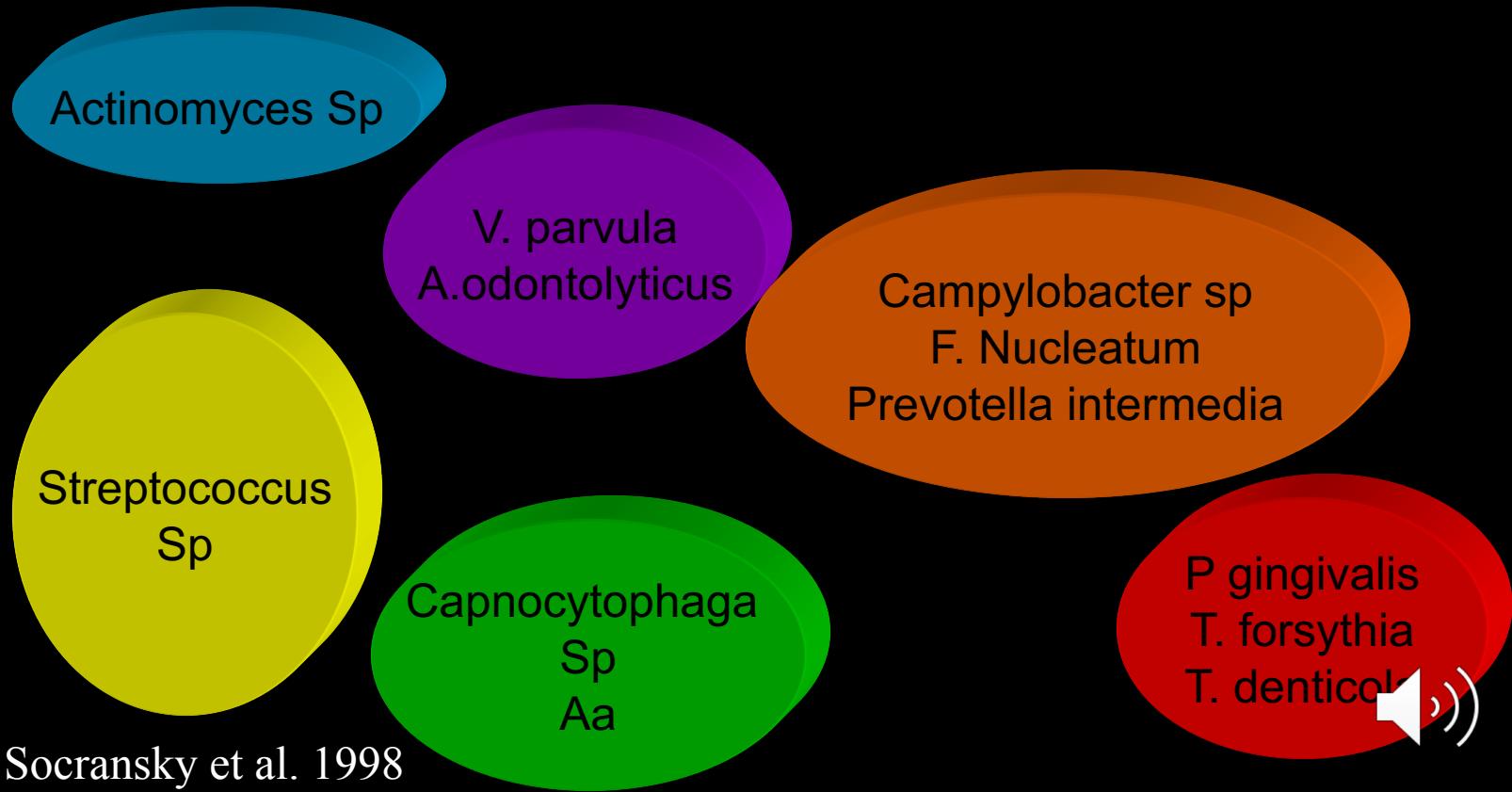
High power



Microbial aggregates
from superficial zones
of subgingival biofilms



Association between different microbial species in the subgingival biofilm is not random



Why is the concept of the Biofilm important?

- Bacteria behave differently in a biofilm compared with free living forms:
 - May be protected from host response
 - May be more resistant to antibiotics



Future Strategies to combat biofilms ?

- Anti-adhesins
- Replacement therapy
- Block quorum-sensing molecules
- Enzymes that degrade matrix molecules



Questions? Discussion

