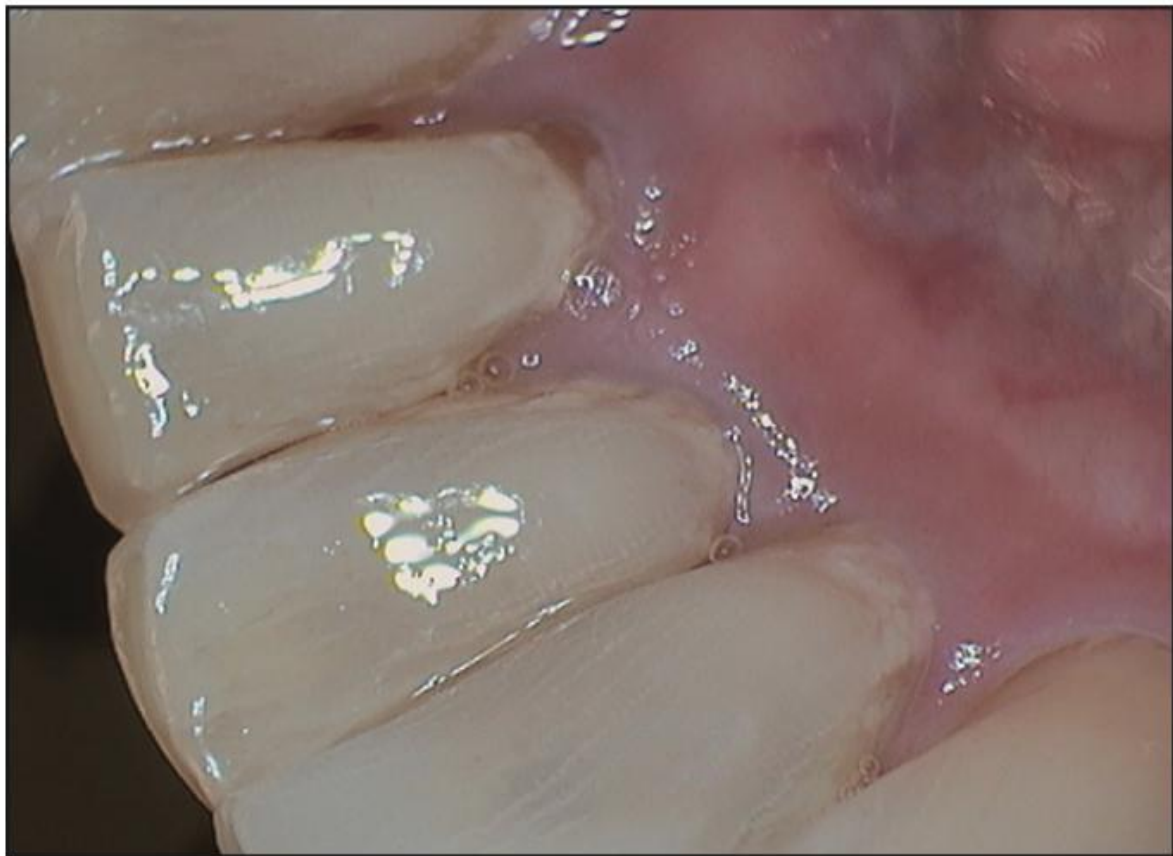


Oral Biofilms

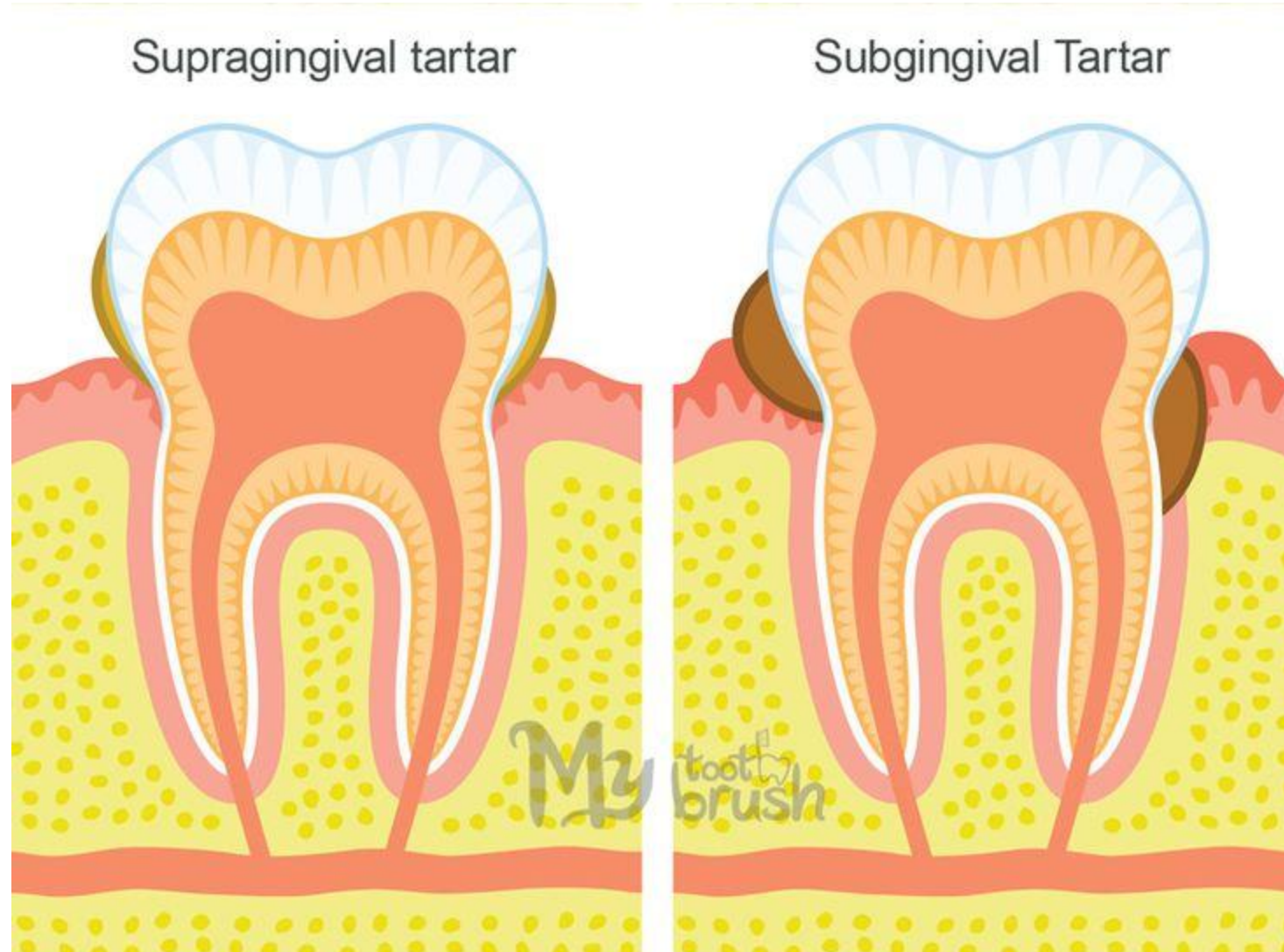
Matthew M. Ramsey, PhD

Department of Oral Immunology and
Infectious Disease, ULSD





Supra vs subgingival



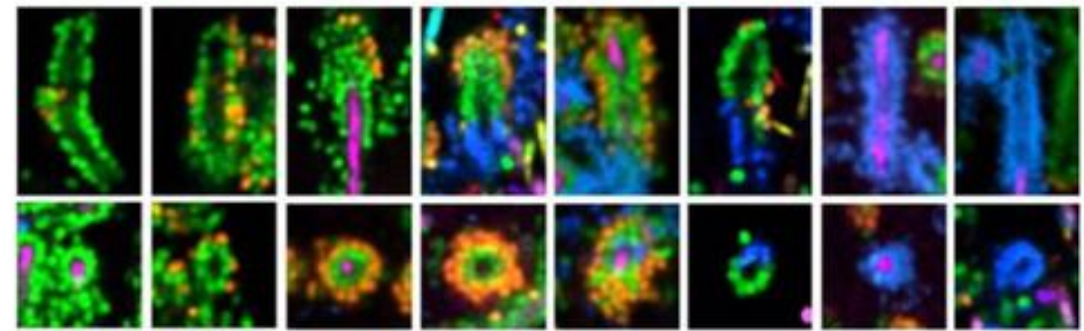
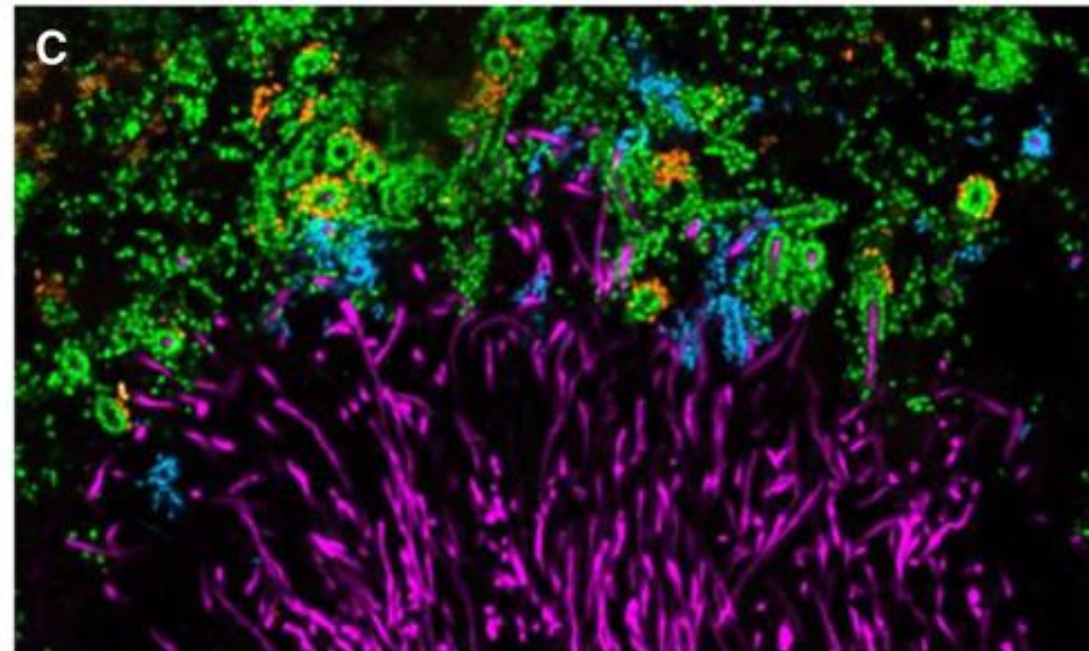
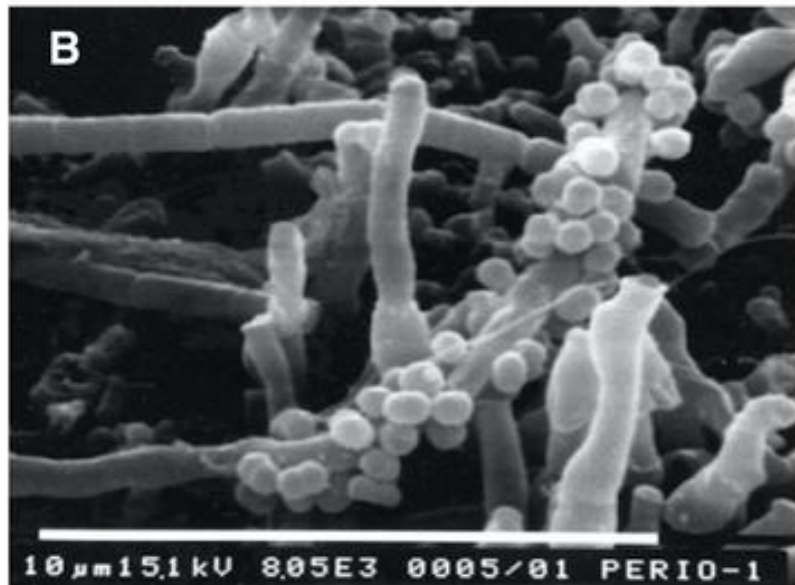
Supra vs subgingival

Subgingival tartar

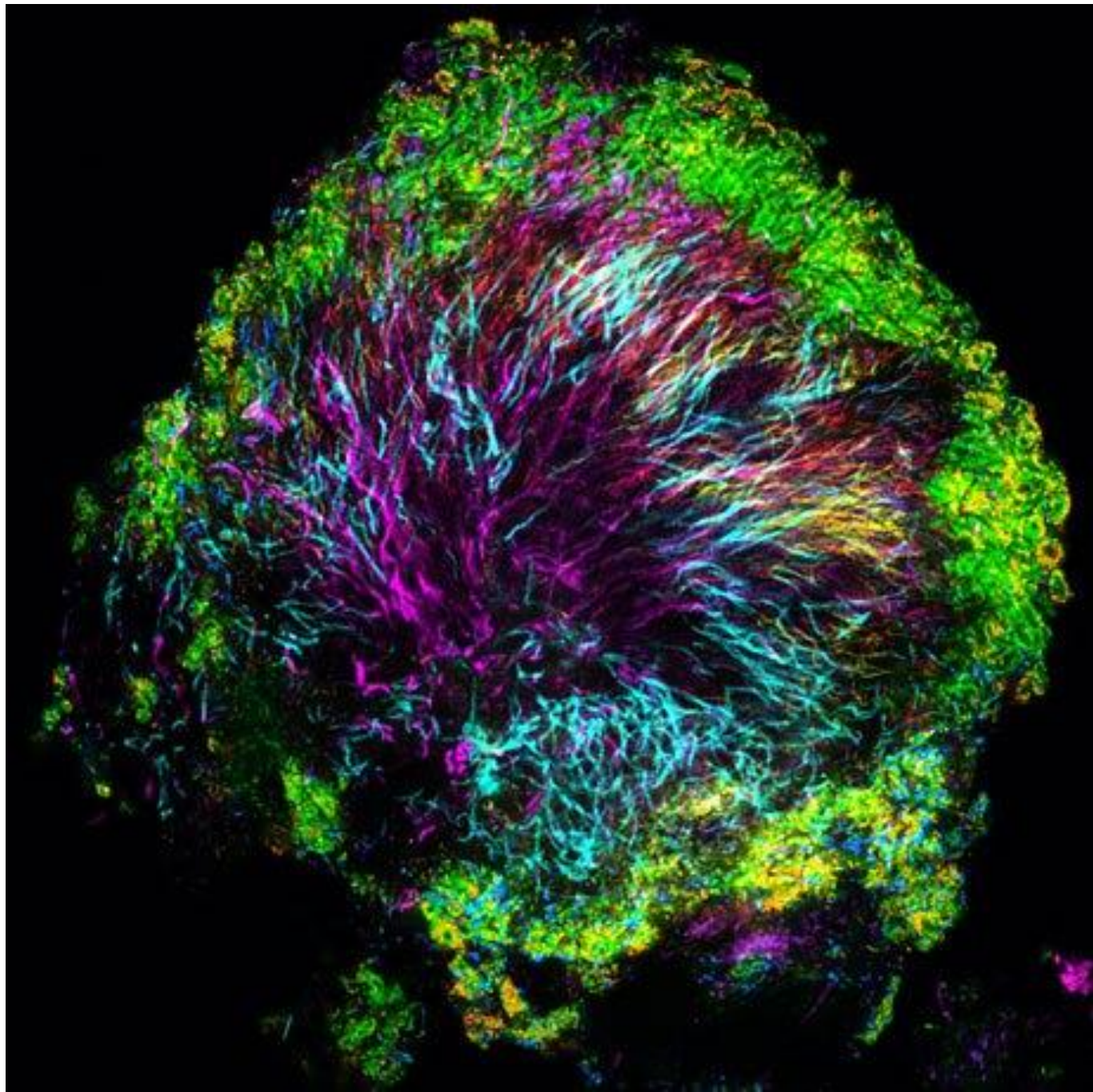


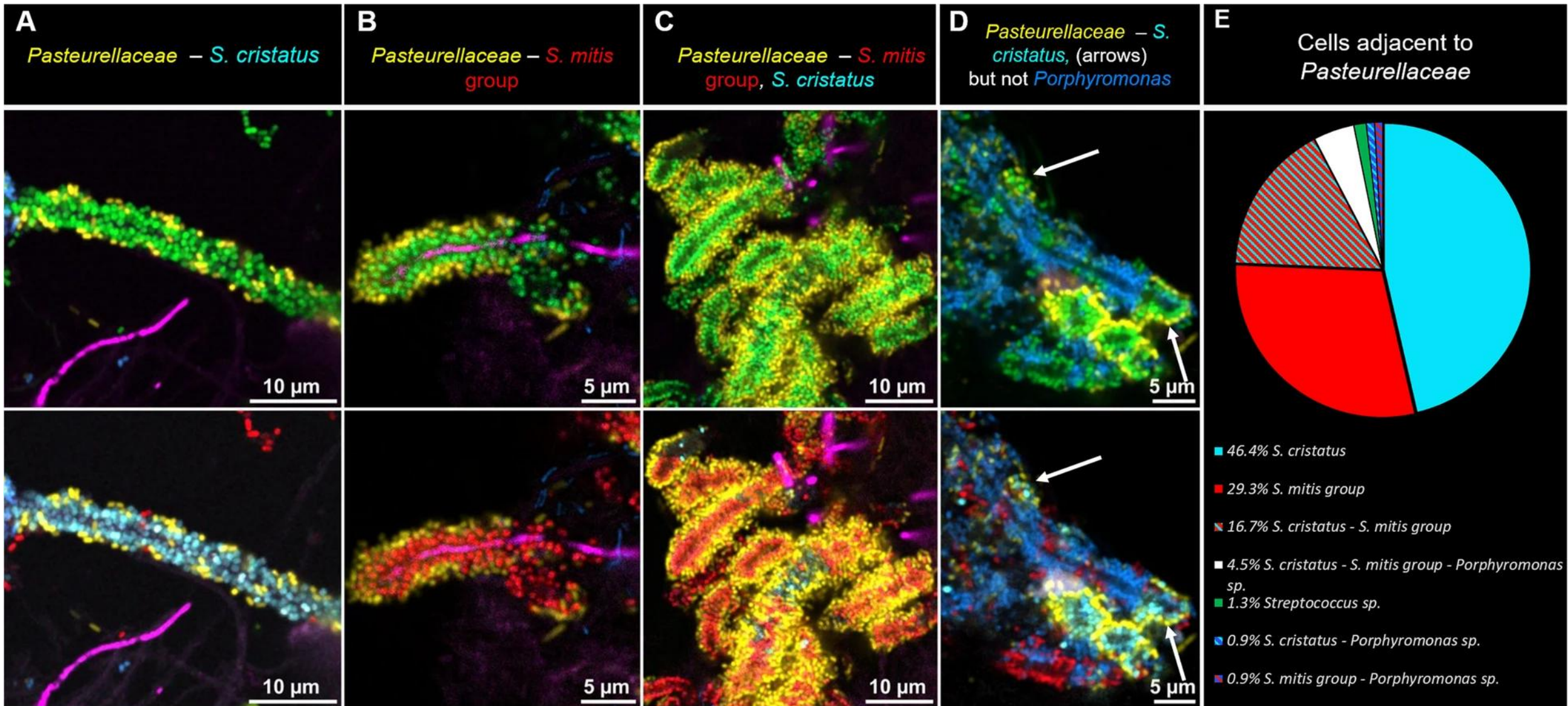
Supragingival tartar





■ <i>Corynebacterium</i>	■ <i>Fusobacterium</i>
■ <i>Streptococcus</i>	■ <i>Leptotrichia</i>
■ <i>Porphyromonas</i>	■ <i>Capnocytophaga</i>
■ <i>Haemophilus/Aggregatibacter</i>	■ <i>Neisseriaceae</i>





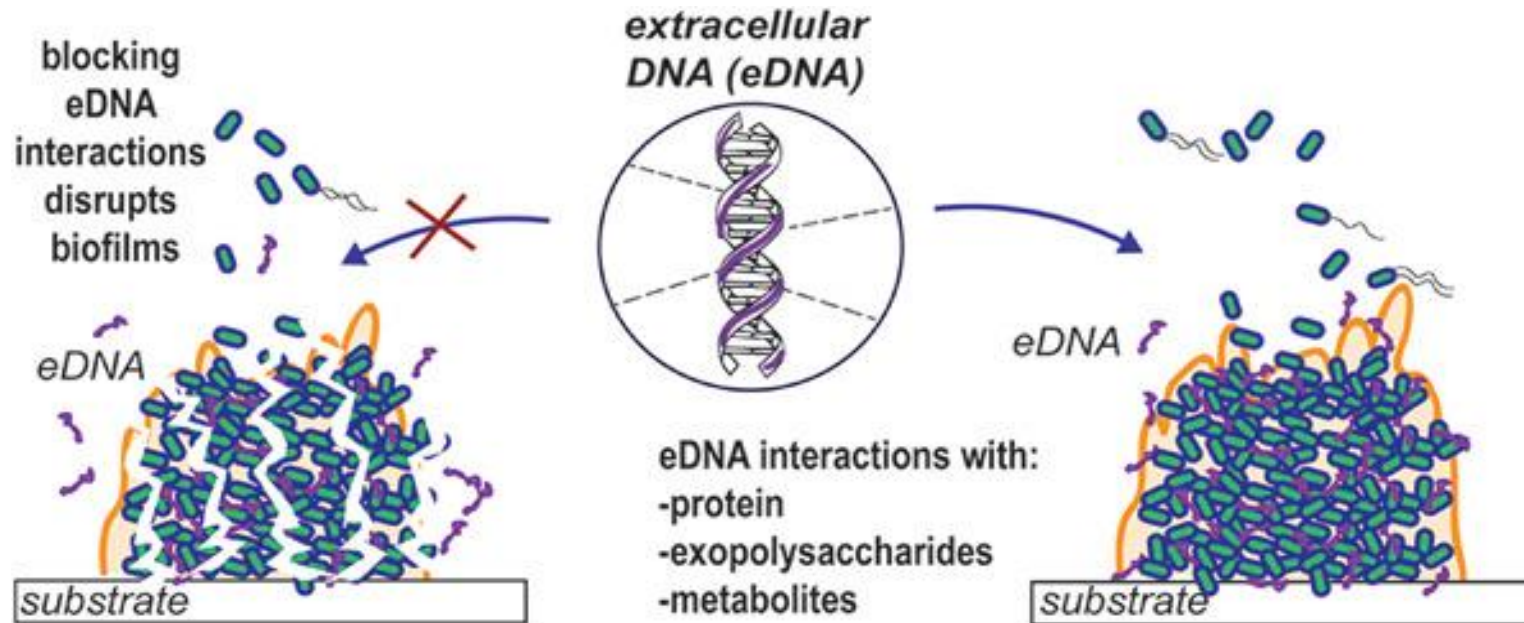
Definition of a biofilm

- Oral biofilms are functionally and structurally organized polymicrobial communities that are embedded in a **self-produced** extracellular matrix of exopolymers on mucosal and dental surface
- In general, there are 5 main components
 - Polysaccharide
 - Proteins
 - DNA
 - Metabolites
 - Lipids

Biofilm Characteristics

- Specific attachment via bacterial adhesins and receptors on surfaces.
- Co-aggregation—adhesins and bacterial receptors
- Cells embedded in a polysaccharide matrix or glycocalyx produced by the colonizing species
- Shared metabolic activities
- Transport of nutrients and waste products via water channels
- Communication - quorum sensing
- Protection
- Spread by detachment & subsequent reattachment

Extracellular DNA enhances biofilm development



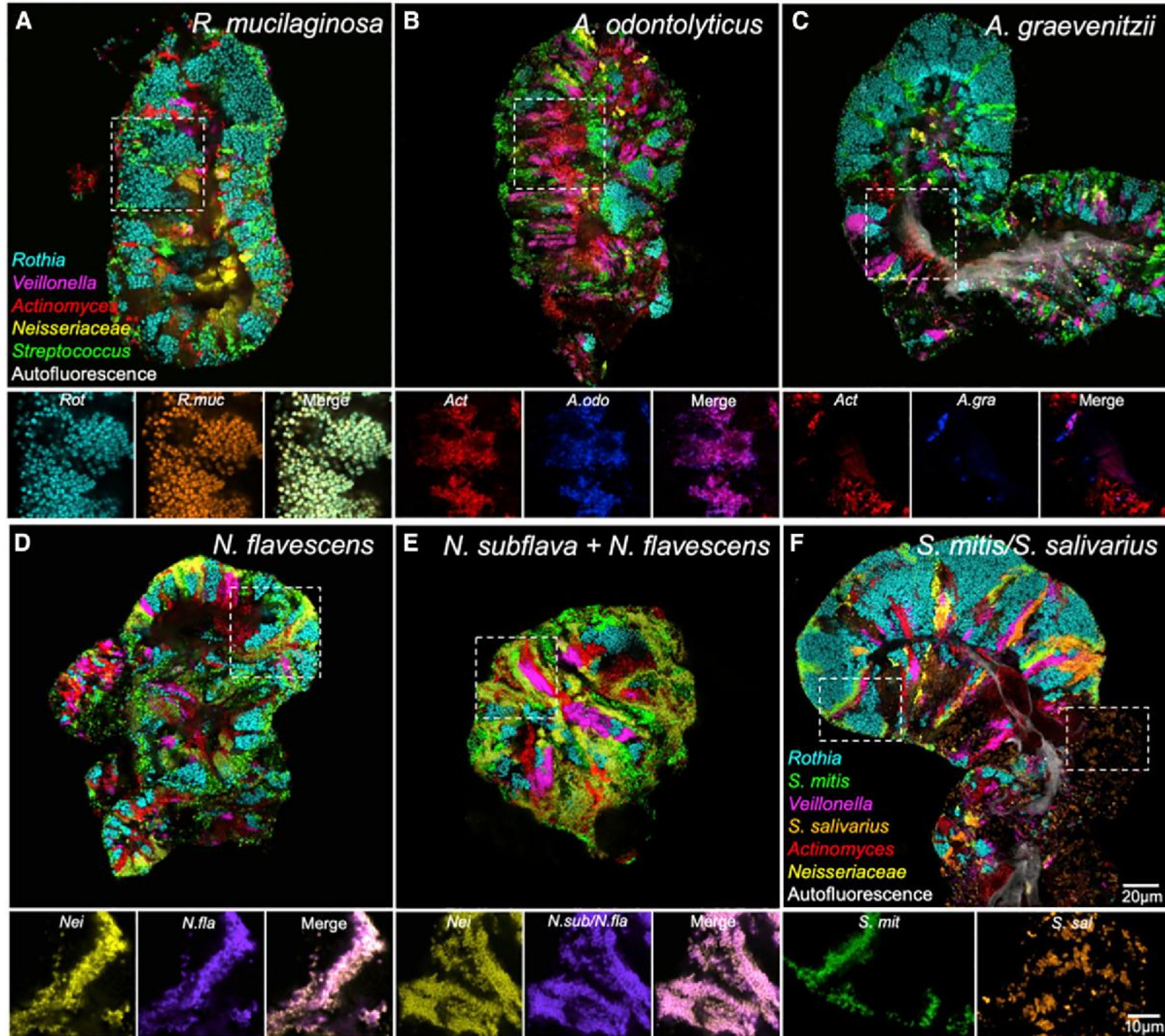
DNase may be a potential treatment

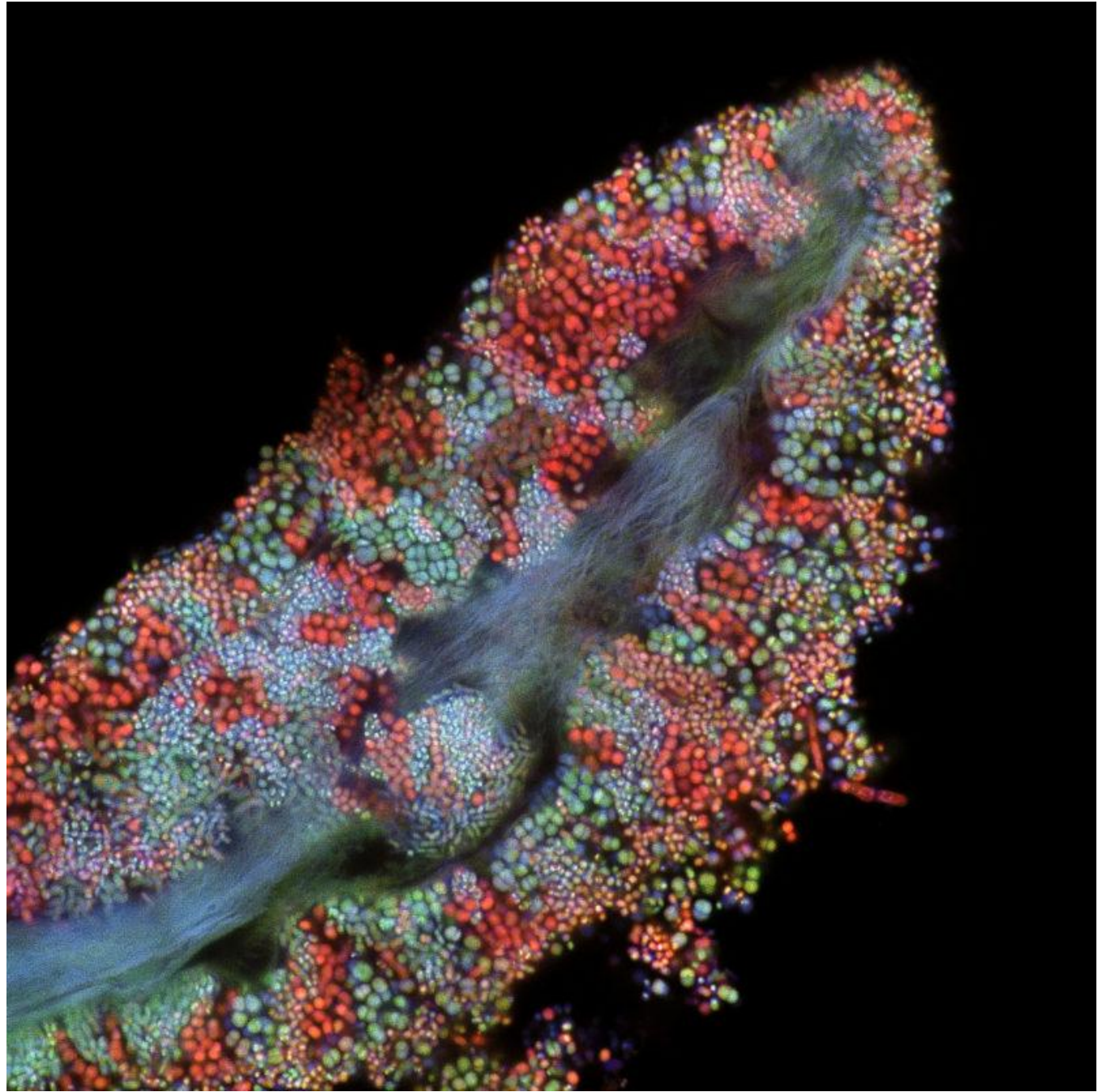
Natural cleansing

- Gingival crevicular fluid and salivary flow.
- Mastication and tongue movement
- Rapid turnover of epithelial cells, especially buccal
- Host defense mechanisms, e.g., Langerhans cells for immune response

Biofilms grow just about anywhere

- Tongue Dorsum
- Epithelial cells
- Inside spaceships
- OUTSIDE spaceships
- Rocks in streams
- Medical devices ☹️





<https://www.nikonsmallworld.com/galleries/2022-photomicrography-competition/bacterial-biofilm-on-a-human-tongue-cell>

Biofilms in dental unit waterlines



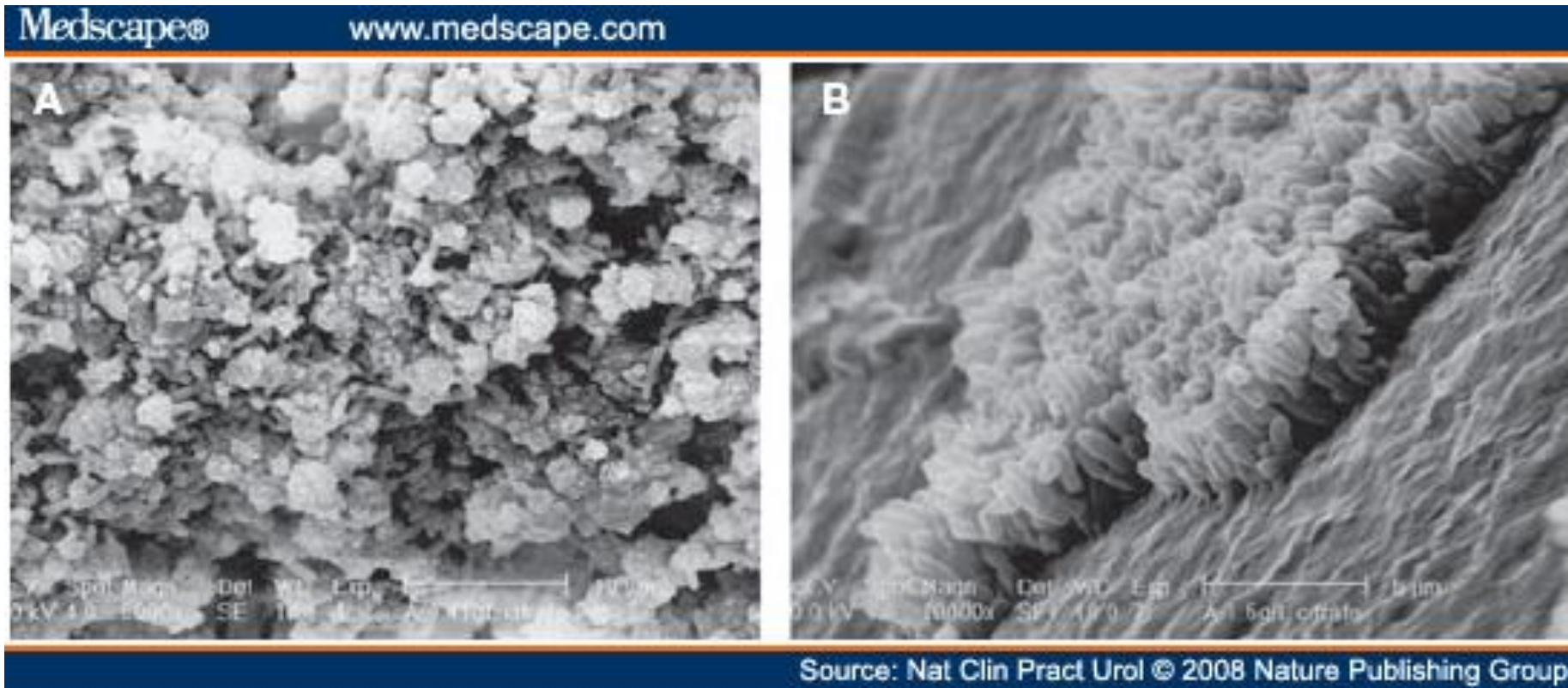
Clinical significance of biofilm formation

- CDC estimates that most bacterial infections are biofilm-based
- Typically, biofilm-based infections are chronic
- Resistance to antibiotic treatment
- Phenotypic changes in the bacteria
- Extracellular polymers inactivate antibiotic
- Antibiotic sequestration
- Nutrient limitation

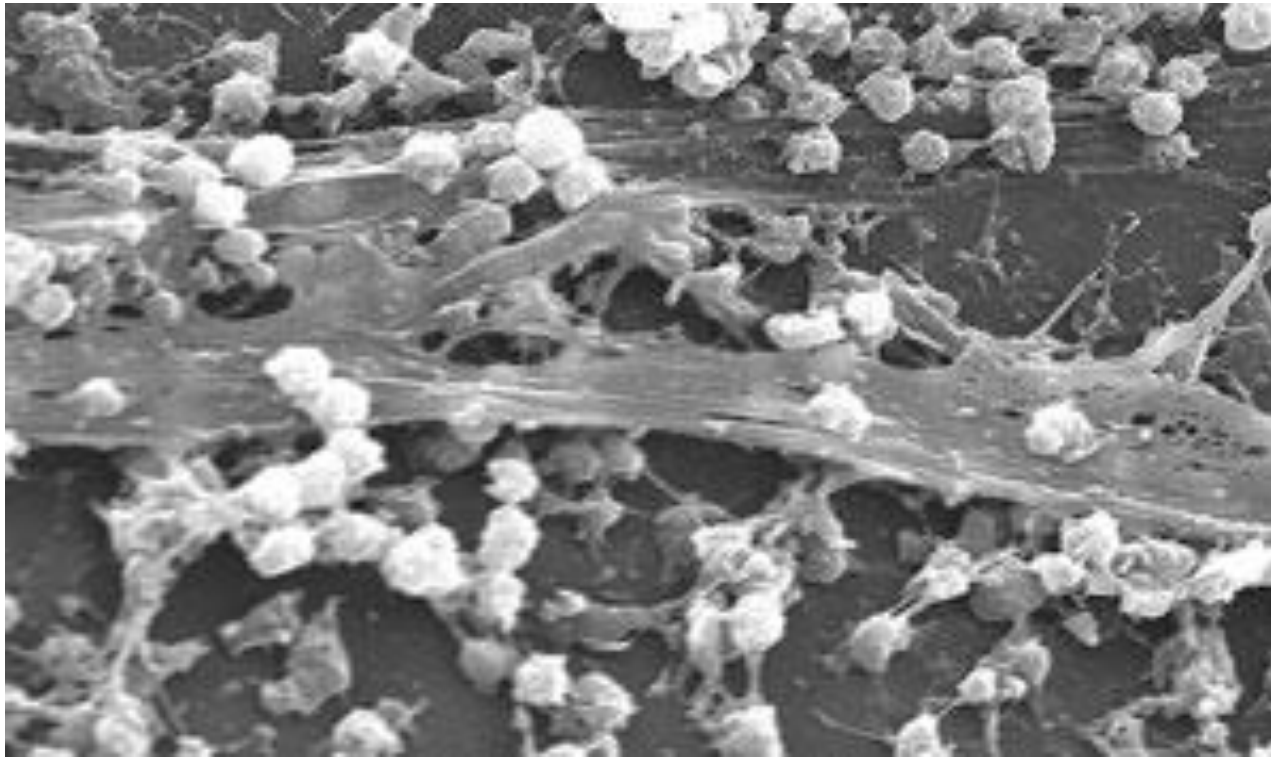
Examples of Biofilm-Based Infections

- Biofilms form on dead and living tissue
 - dental tissues
 - bladder infections
 - eye infections
 - ear - otitis media
 - sinusitis
 - tonsilitis
 - lung infections
 - wounds
- Biofilms form on medical implants / catheter / ventilator tubes

Biofilm in urinary catheter



Biofilm on contact lenses



Don't sleep with contact lenses

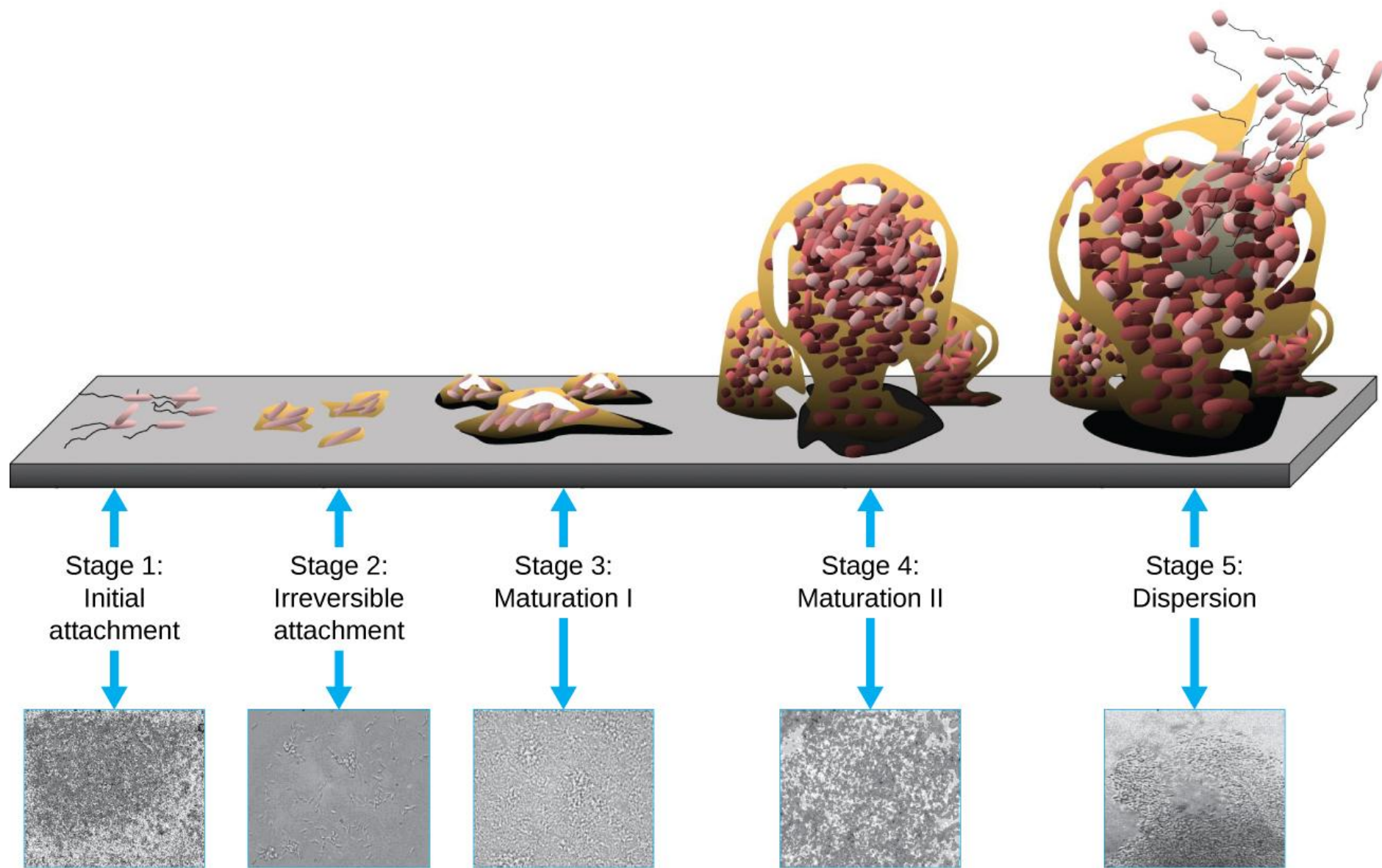
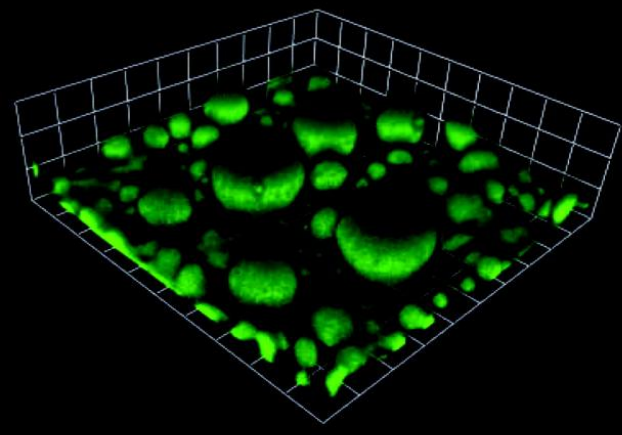
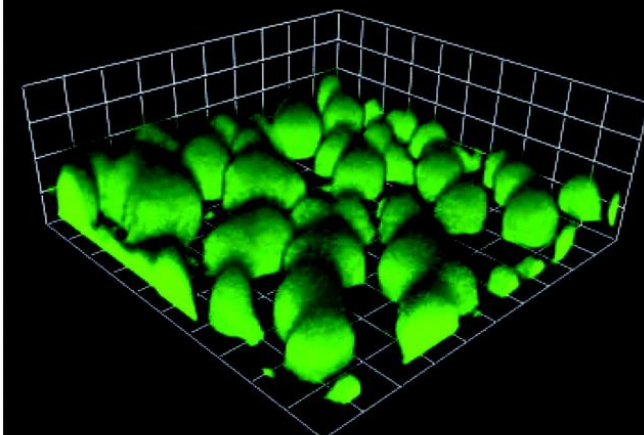
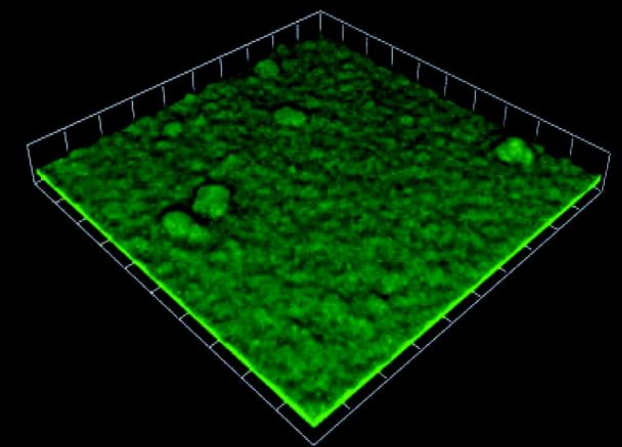
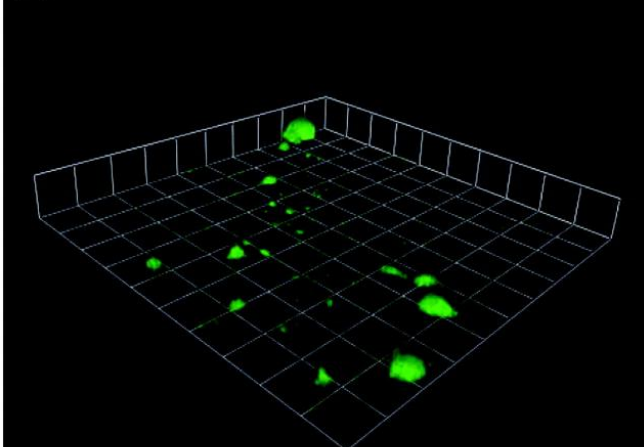
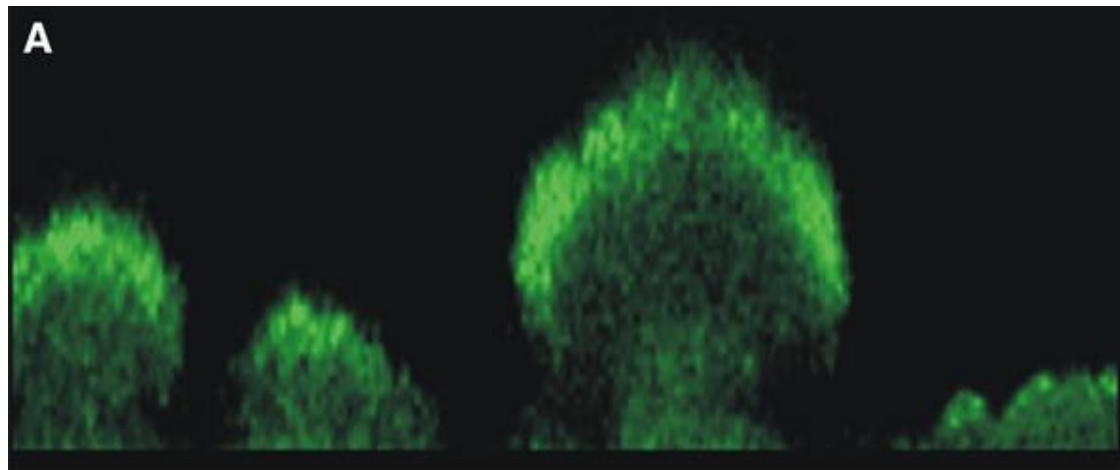
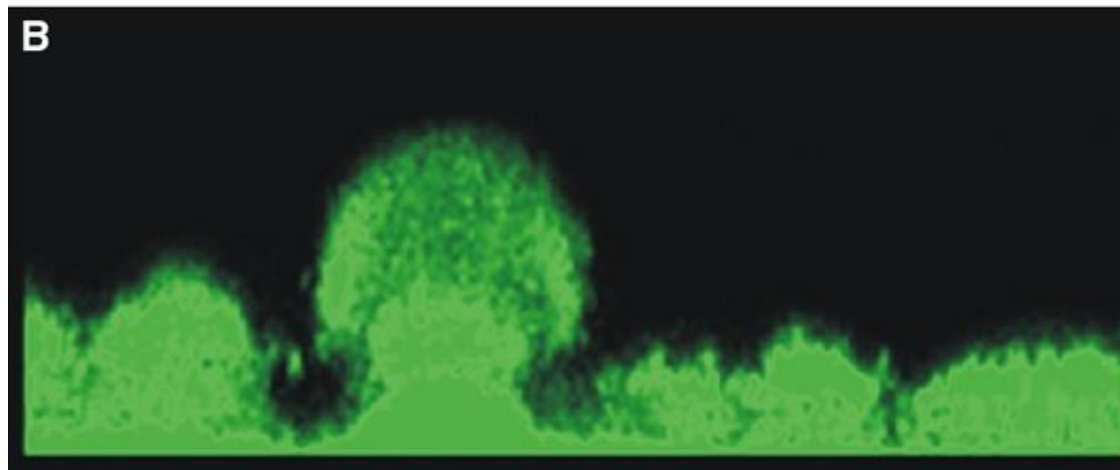


Diagram showing five stages of biofilm development of *Pseudomonas aeruginosa*. All photomicrographs are shown to same scale.

A**B****C****D****A****B**

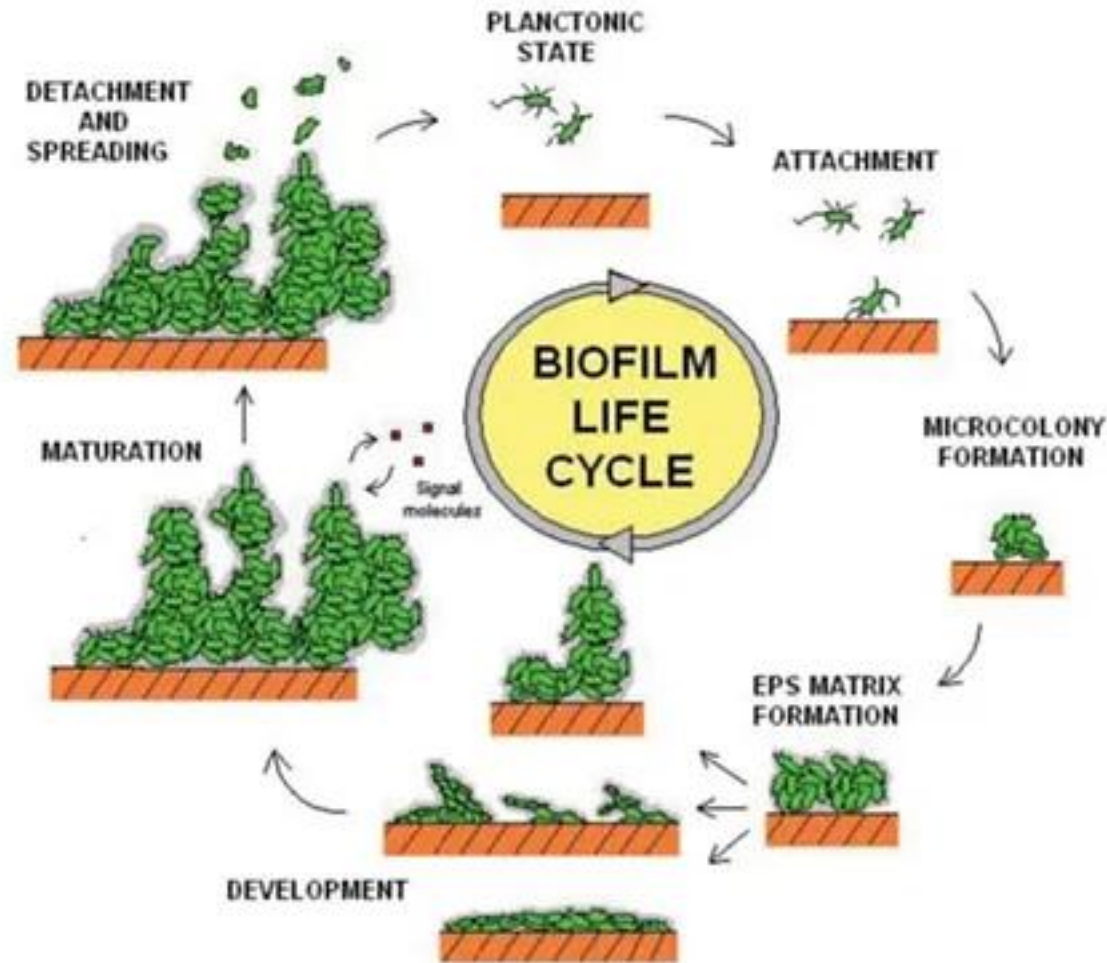
Confocal Microscopy

<https://doi.org/10.1073/pnas.0504266102>
<https://doi.org/10.1111/apm.12335>

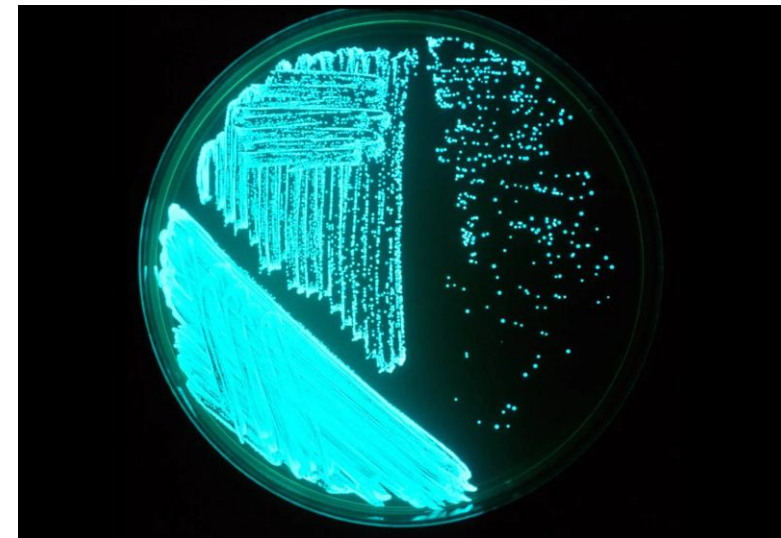
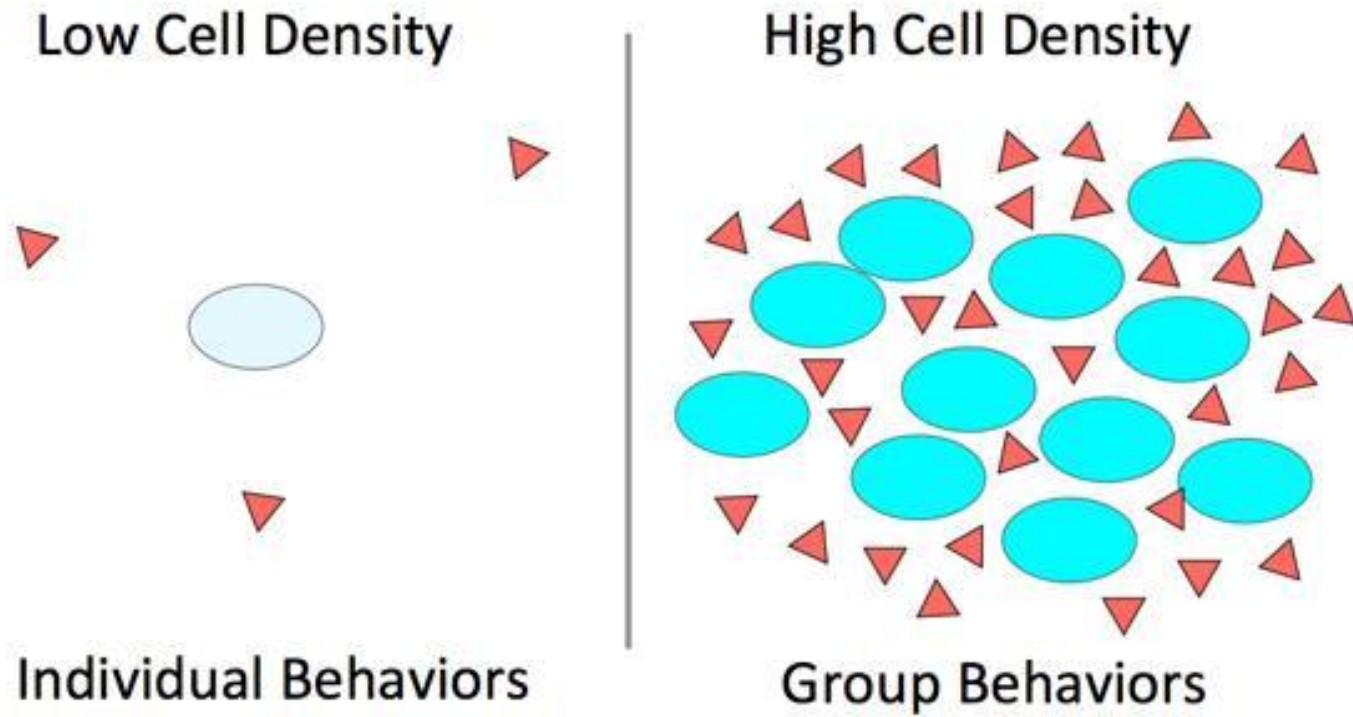
Why do we care about biofilms?

- When they produce exopolysaccharides or change other behaviors they can become extremely drug and disinfectant resistant.
- Bacteria can form biofilms on nearly any type of surface (species dependent)
- This causes problems with water quality, implantable medical devices etc.

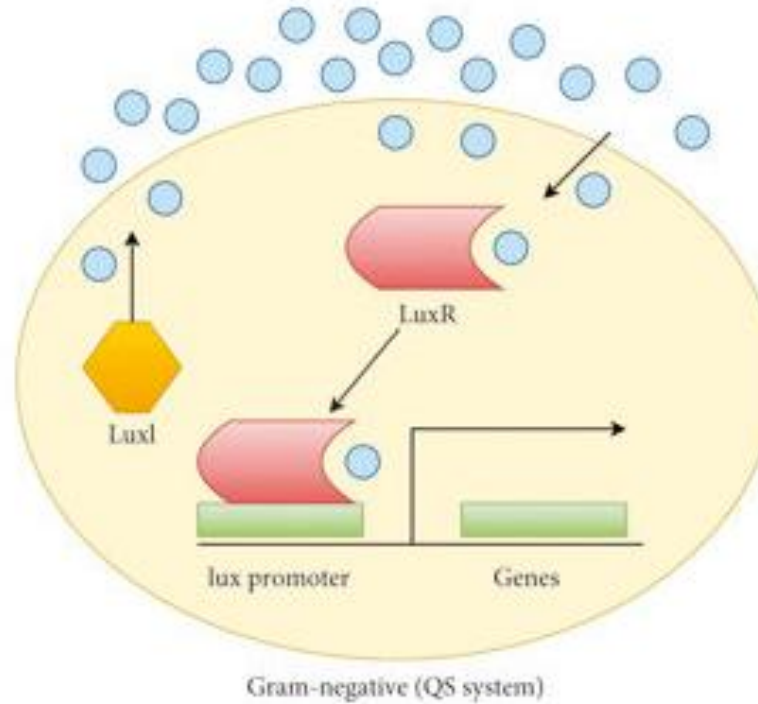
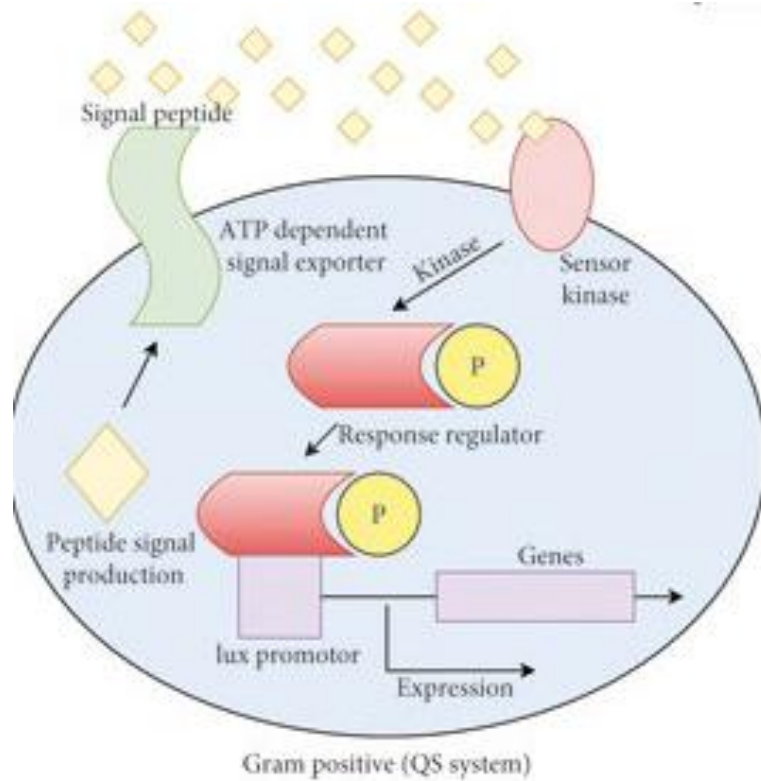
How do the cells know when to leave?



Bacterial Quorum Sensing



Quorum Sensing—Gram + vs Gram -

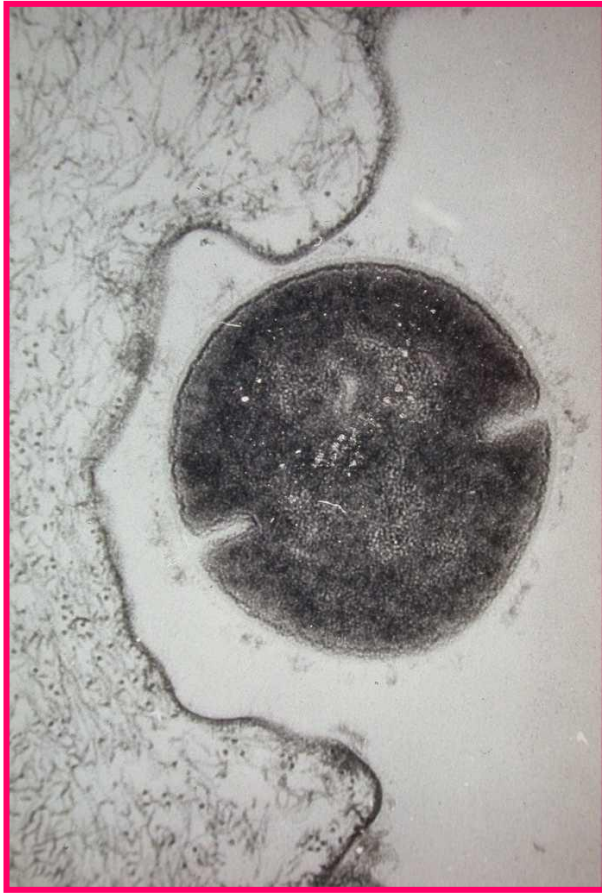


Gram positive bacteria are different from Gram negative bacteria

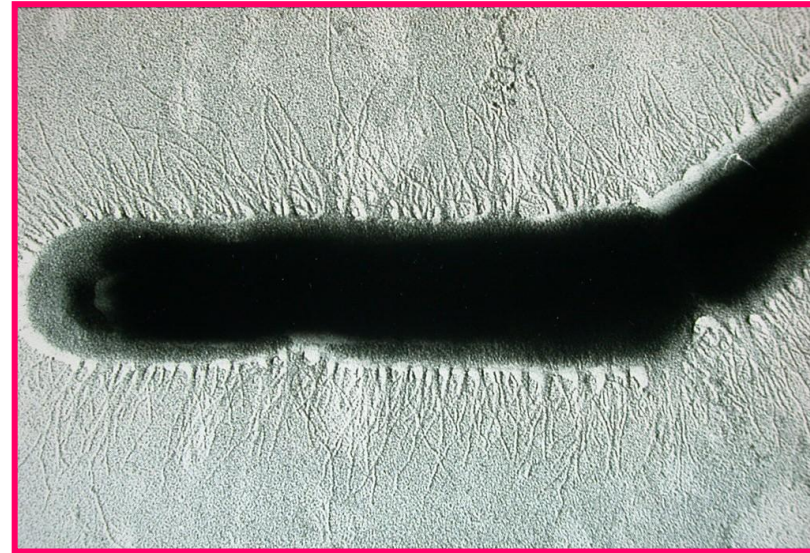
The impact of quorum sensing is large

- Involved in biofilm formation
- Ensures bacterial cells only do things when there are enough of them to matter
- Works in nature, and works in disease
- Can you make a drug that blocks quorum signaling?

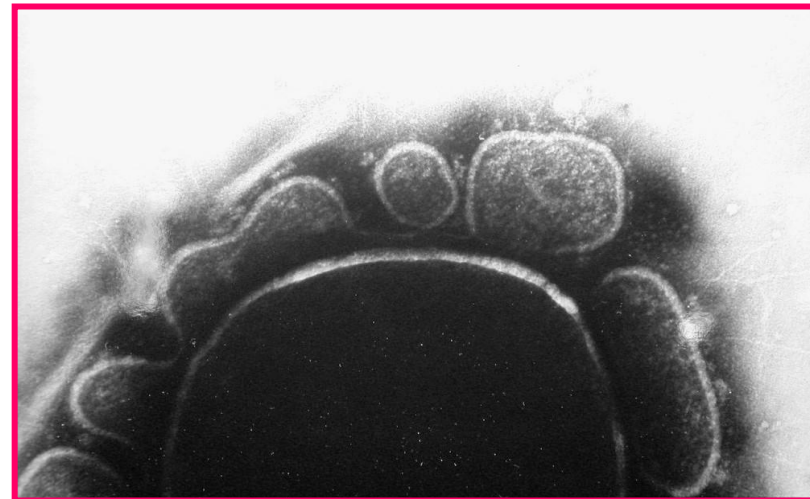
Bacteria have evolved complex surface structures



Short fibrils of streptococci

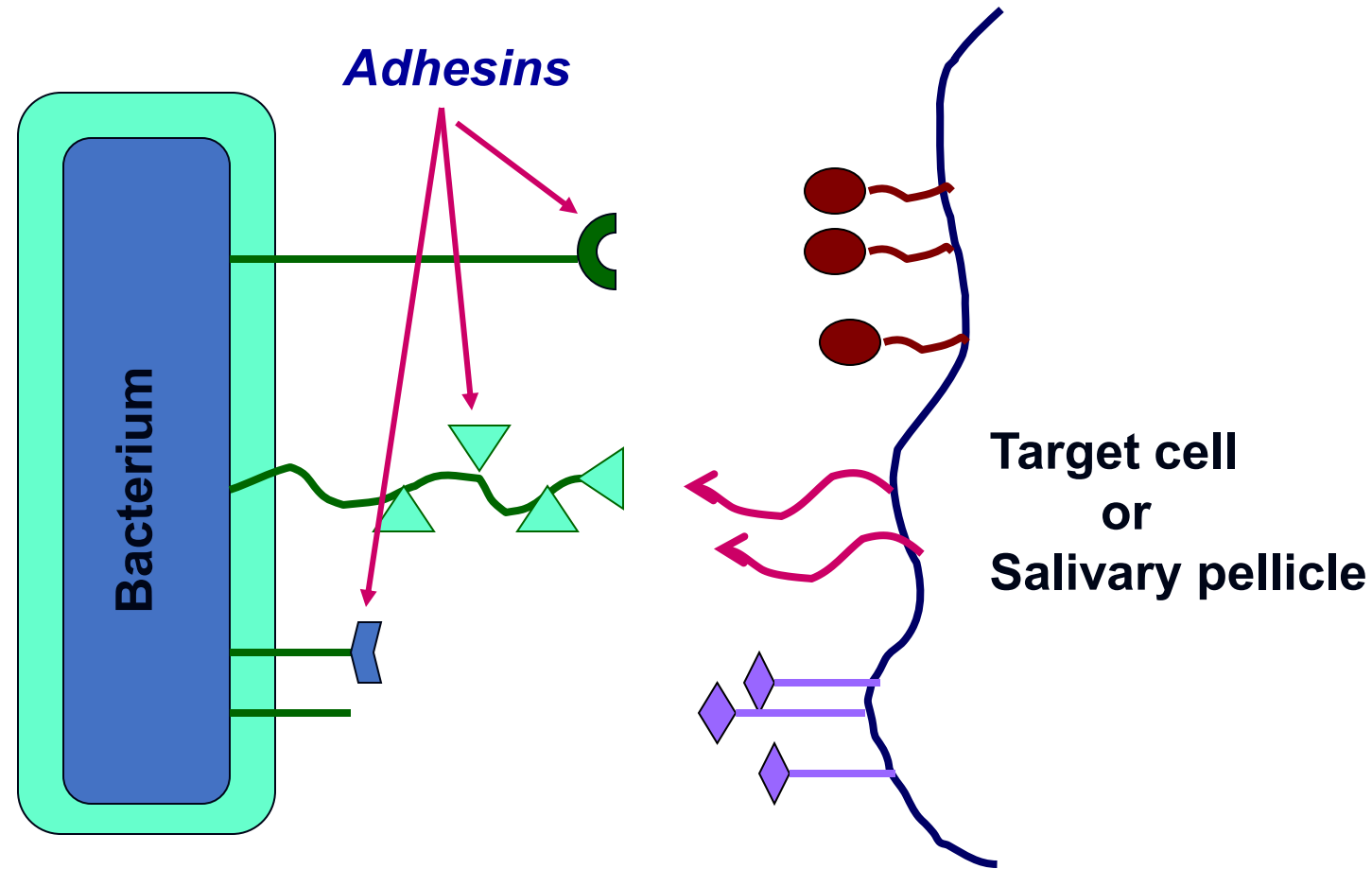


Long fimbriae of *Actinomyces* spp.



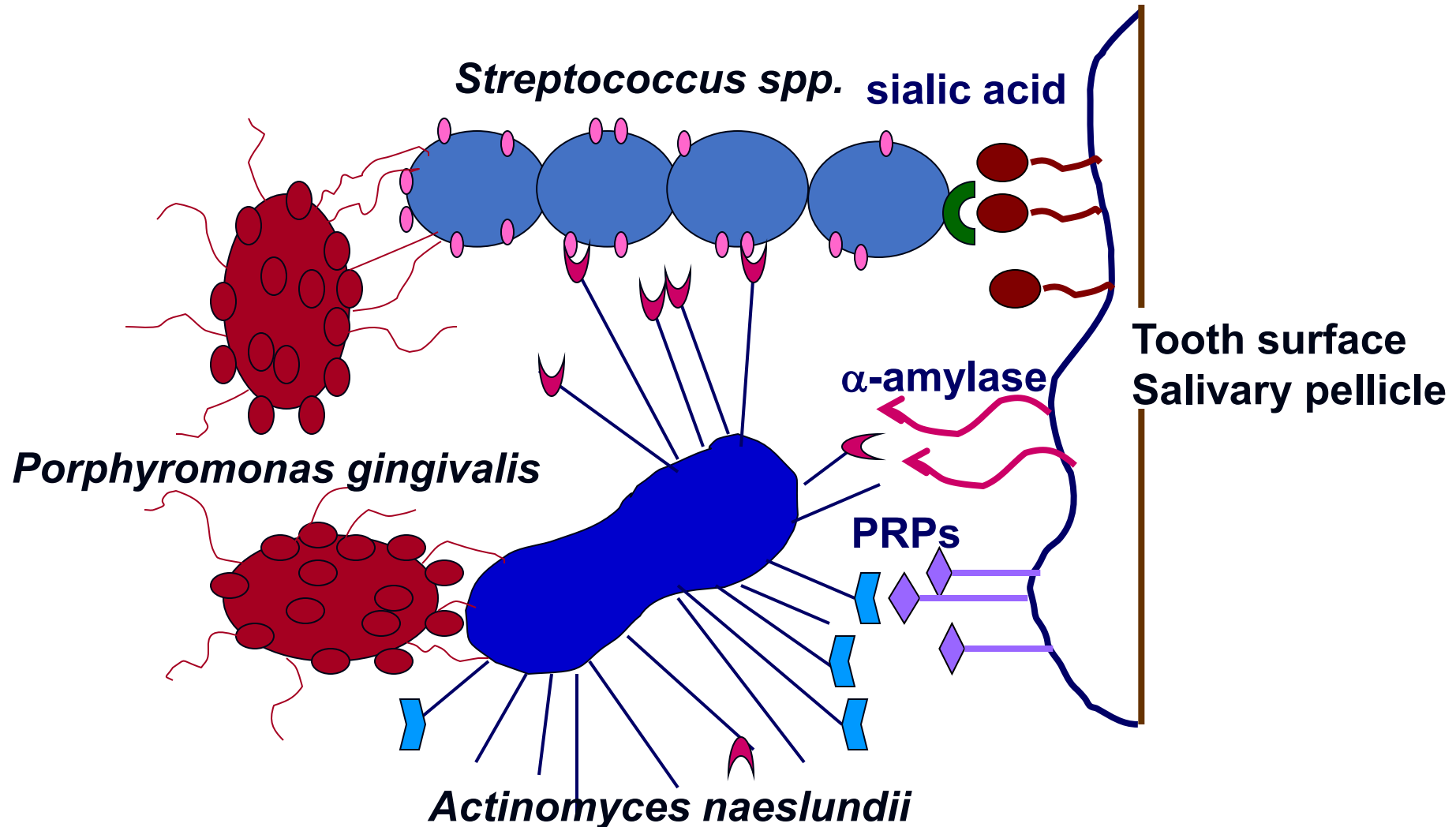
Fimbriae and vesicles of *P. gingivalis*

Bacteria express adhesins, molecules that bind host ligands and receptors stereochemically

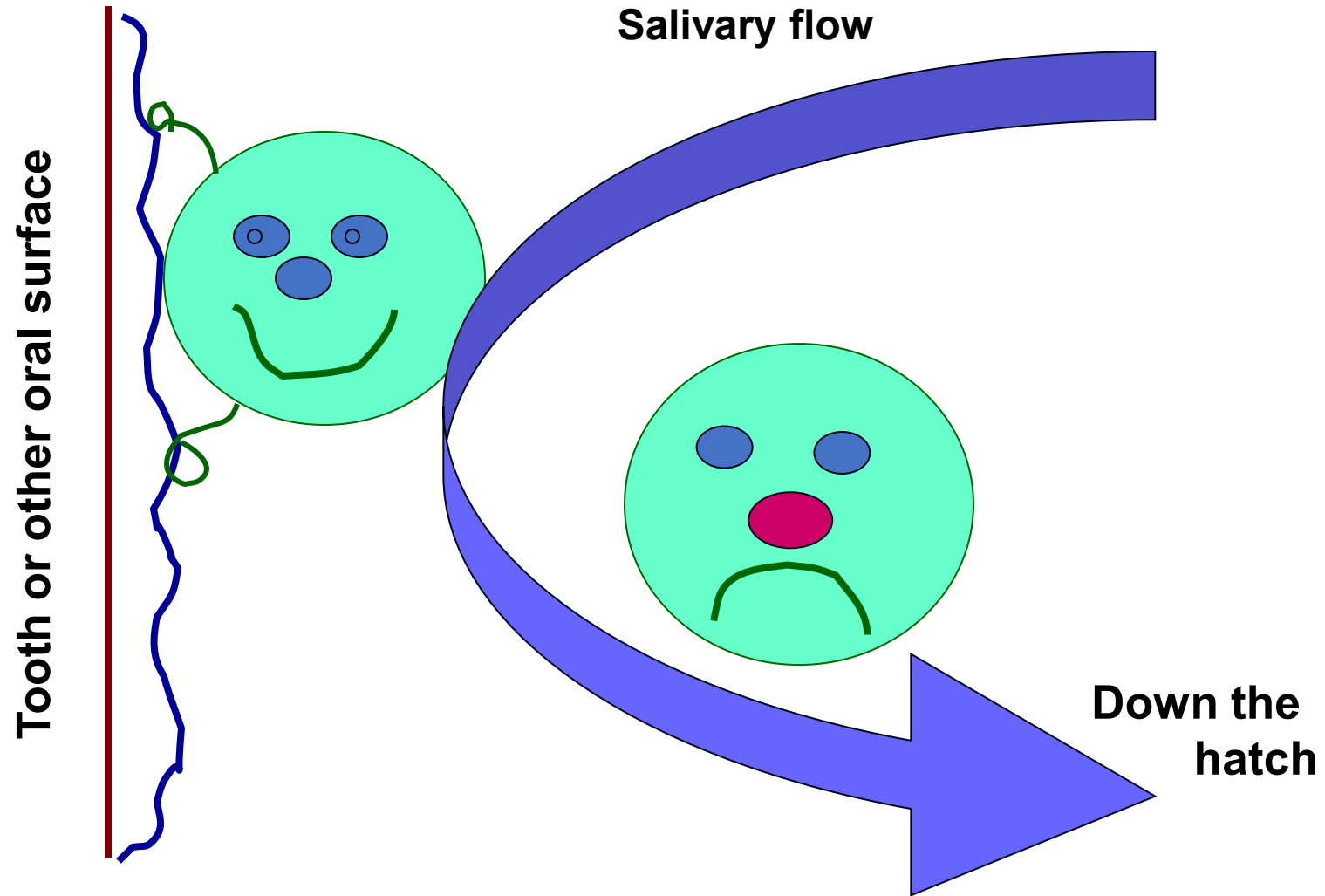


... or bind receptors on other bacteria

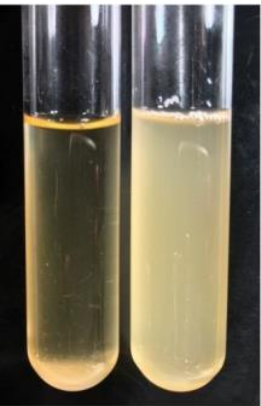
Stereochemical interactions promote coadhesion among bacteria in plaque



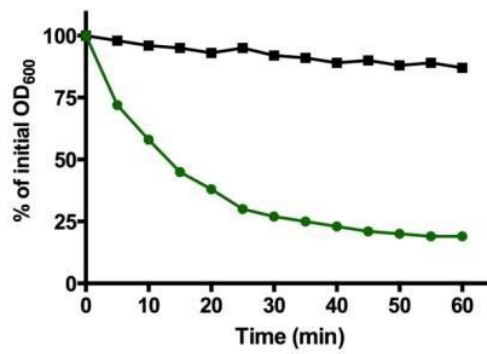
Bacteria that adhere to surfaces avoid removal by bathing secretions



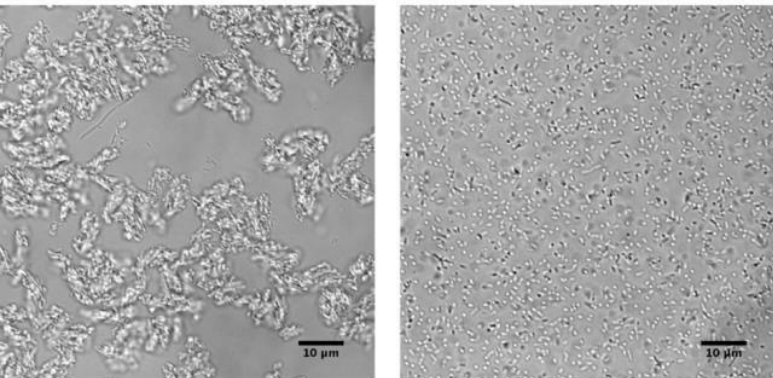
A



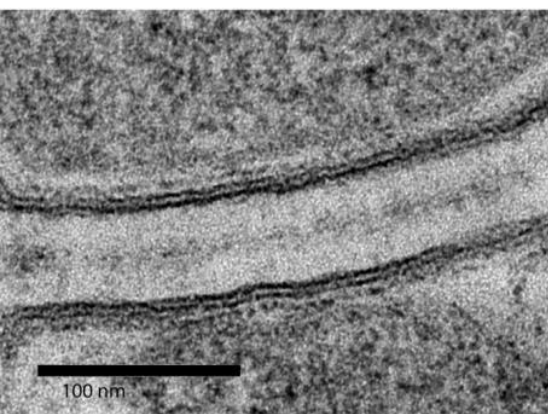
B



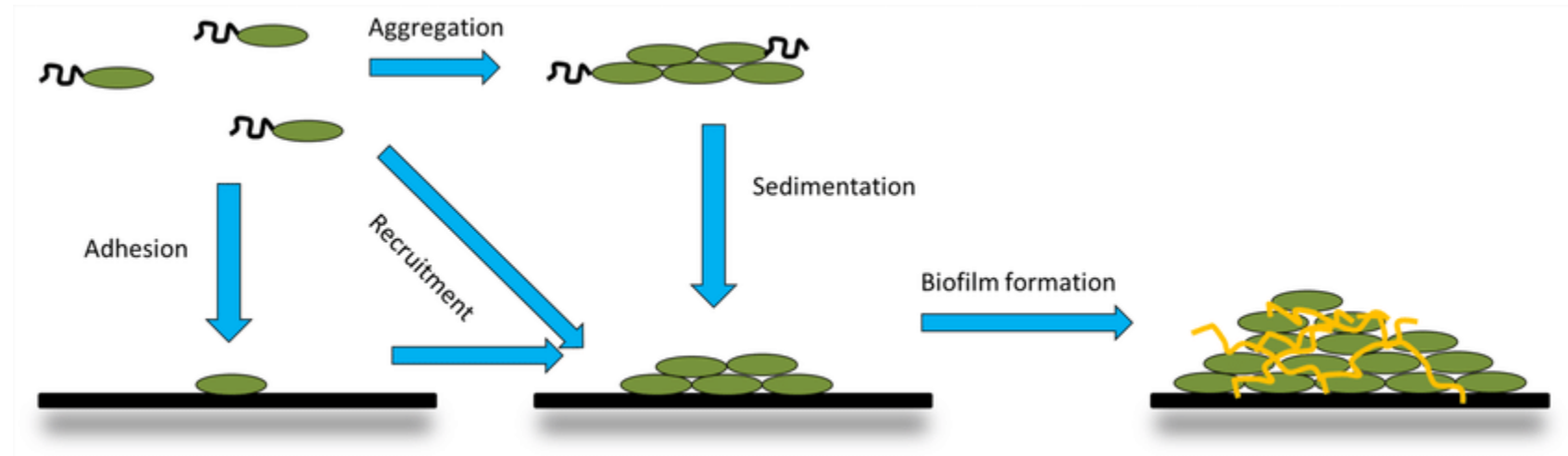
C



D

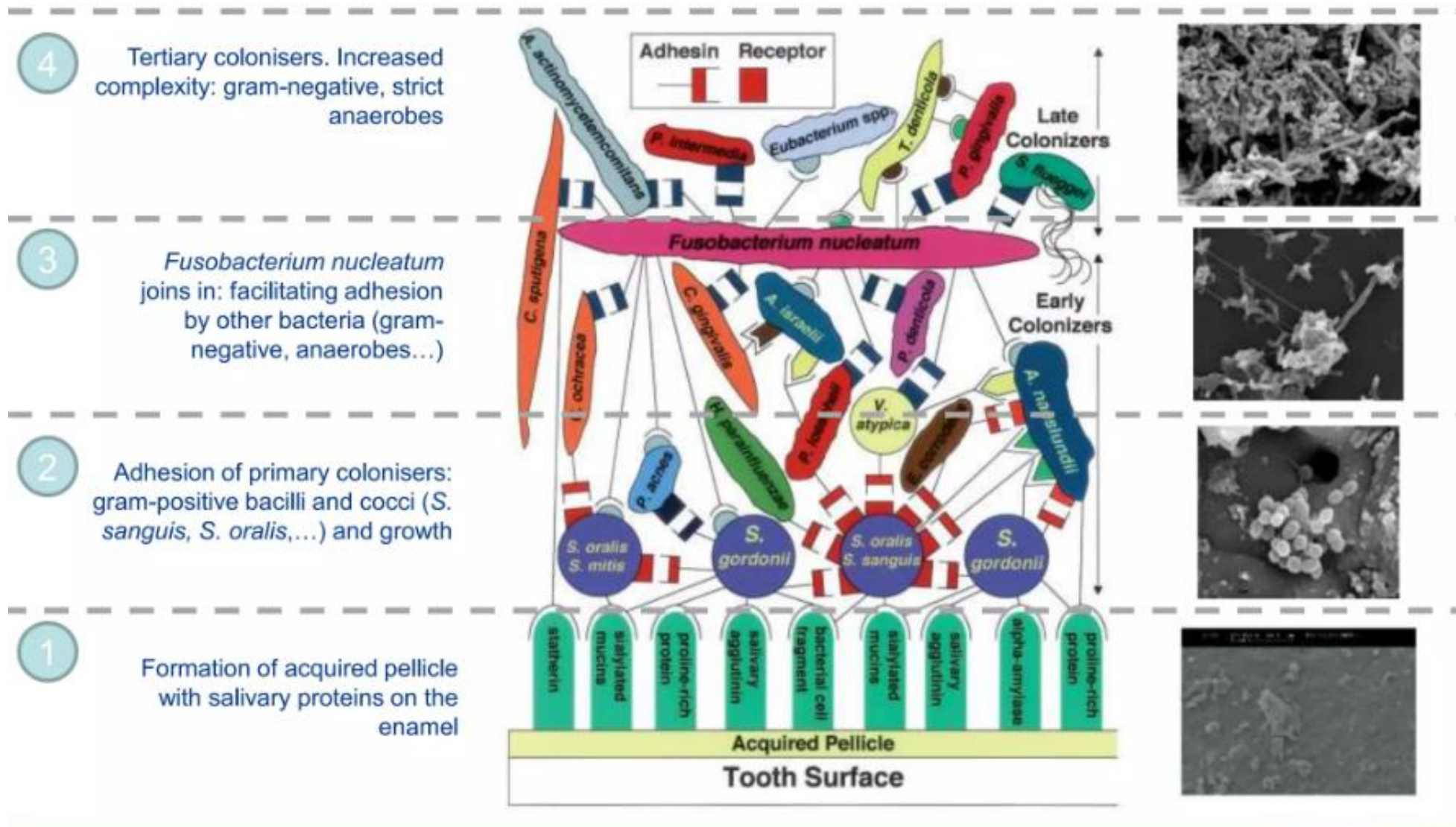


Co-Aggregation

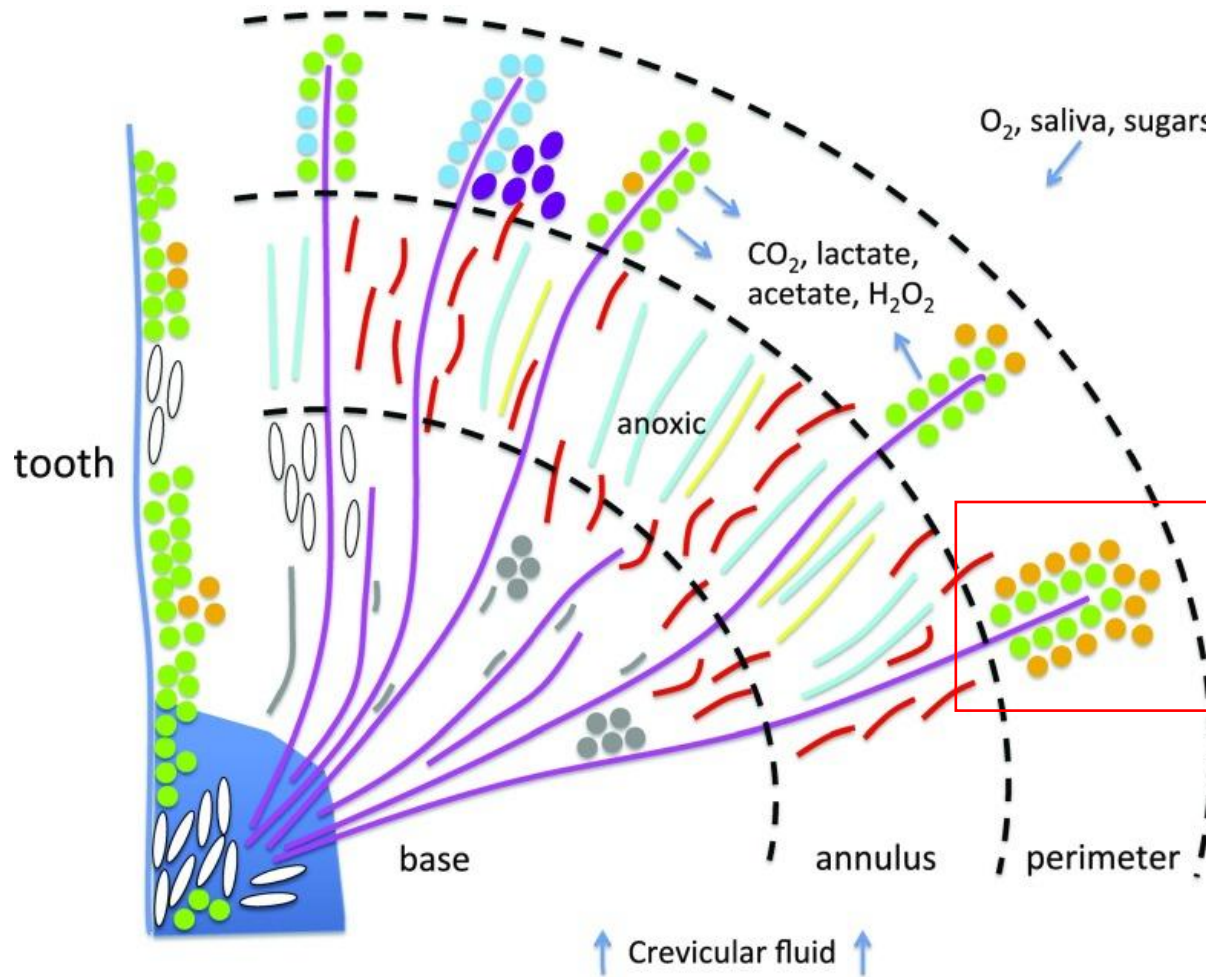


[10.3934/microbiol.2018.1.140](https://doi.org/10.3934/microbiol.2018.1.140)

Biofilm development



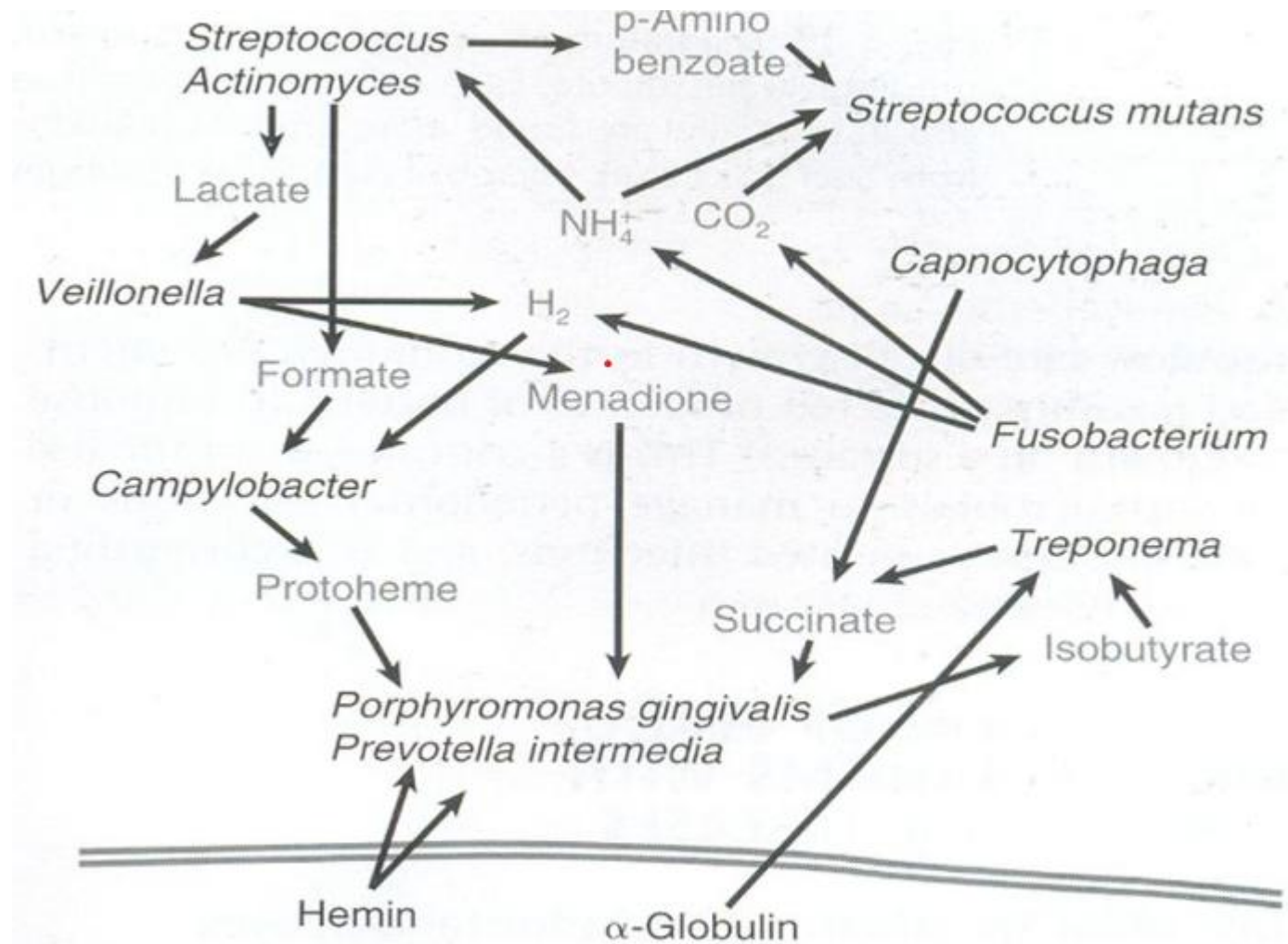
Model of supragingival plaque



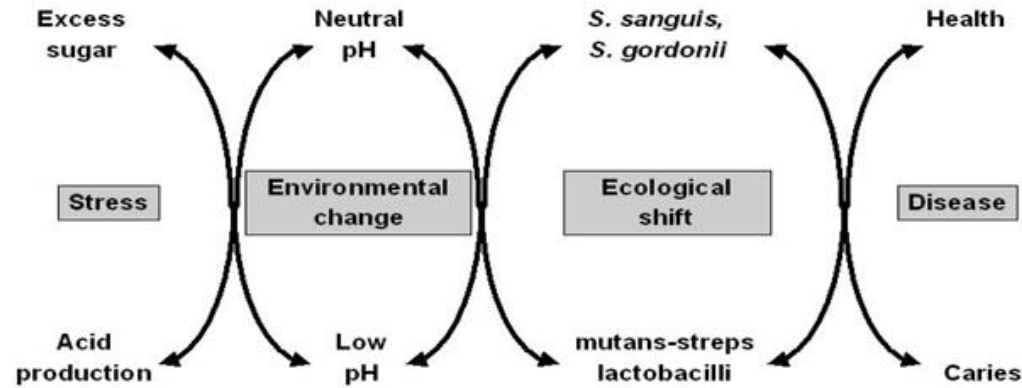
- | | | | |
|----------------------------------------------------------------|------------------------------------------------------------|--------------------------------------------------------------------|-------------------------------------------|
| ■ <i>Corynebacterium</i> | ■ <i>Porphyromonas</i> | ■ <i>Fusobacterium</i> | ■ other |
| ■ <i>Streptococcus</i> | ■ <i>Neisseriaceae</i> | ■ <i>Leptotrichia</i> | |
| ■ <i>Haemophilus/Aggr.</i> | ■ <i>Capnocytophaga</i> | □ <i>Actinomyces</i> | |

- [Proc Natl Acad Sci U S A. 2016 Feb 9; 113\(6\): E791–E800.](#)

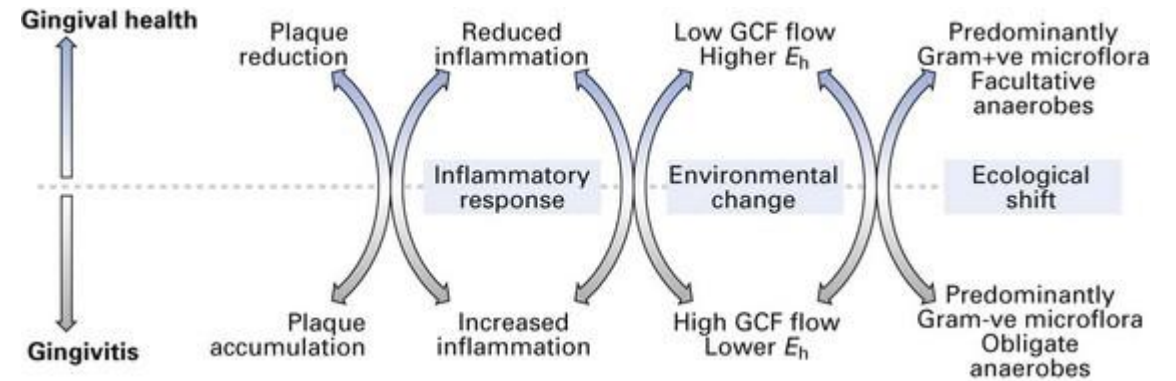
Metabolic interactions of an oral biofilm



Ecological plaque hypotheses



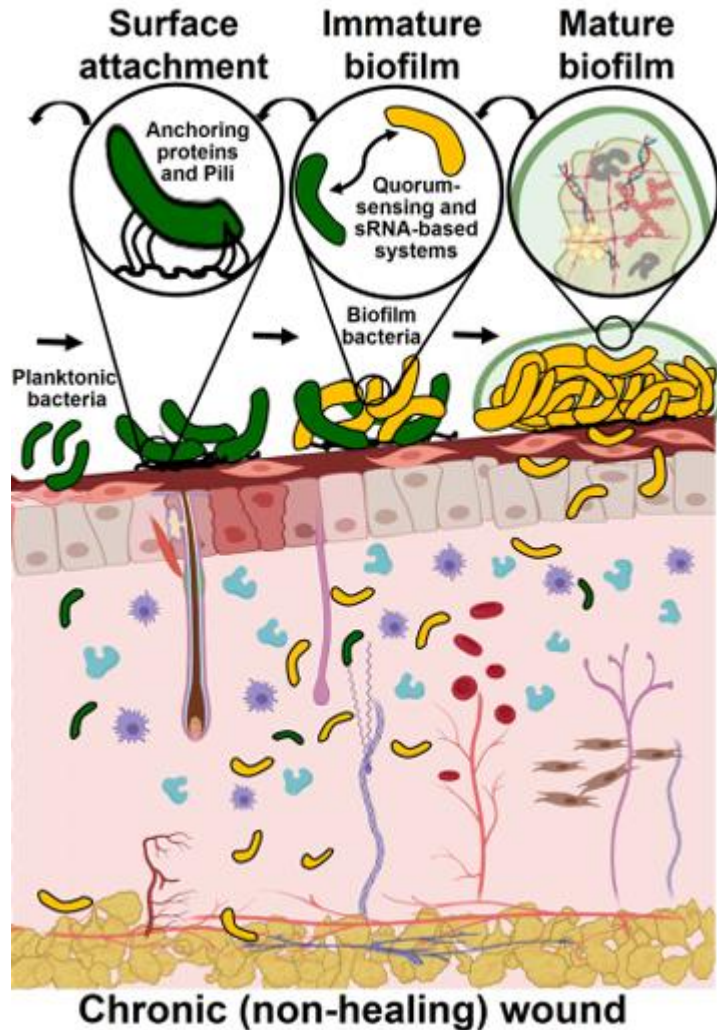
Caries



Periodontal disease

Ecological Plaque Hypothesis: certain species cause either disease, but are not always present in enough abundance to do so

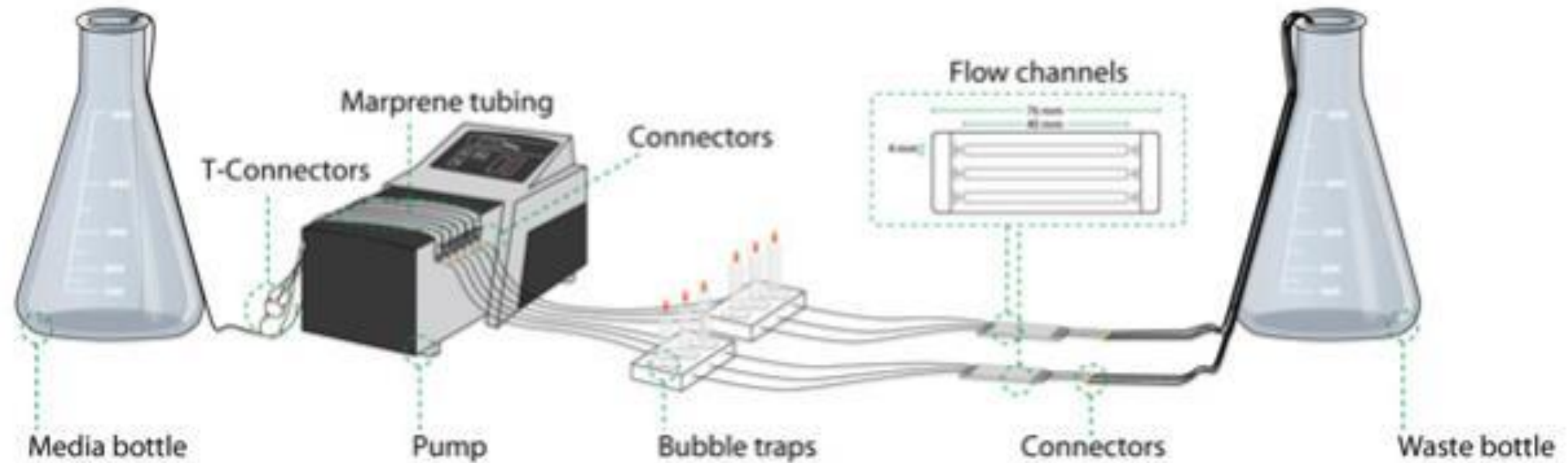
Why chronic wounds will not heal



Need the correct antibiotic, sooner than later. Otherwise, virulence genes factors will keep on expressing.

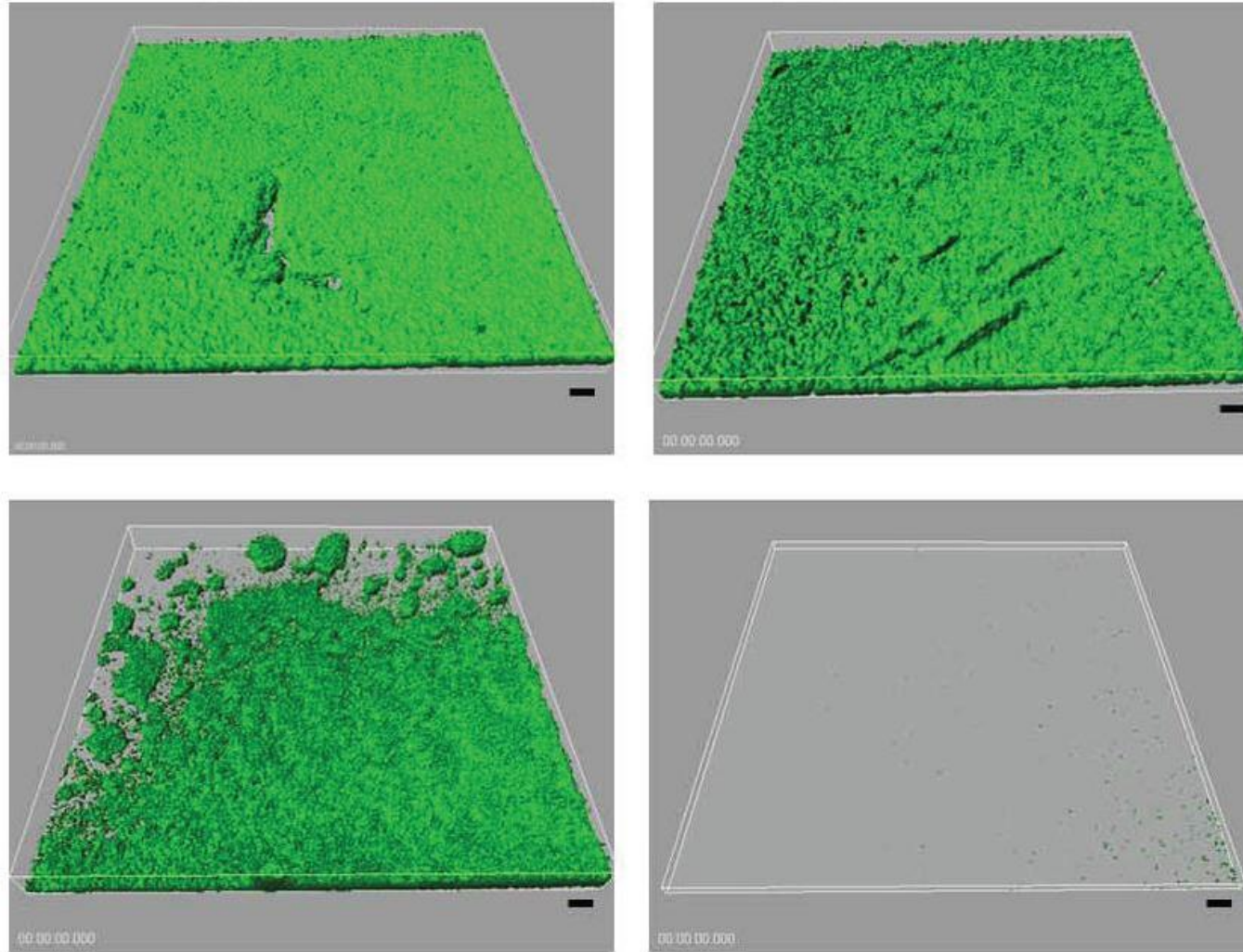
Remember, biofilms are often resistant to antibiotic treatment

Flow-cell system

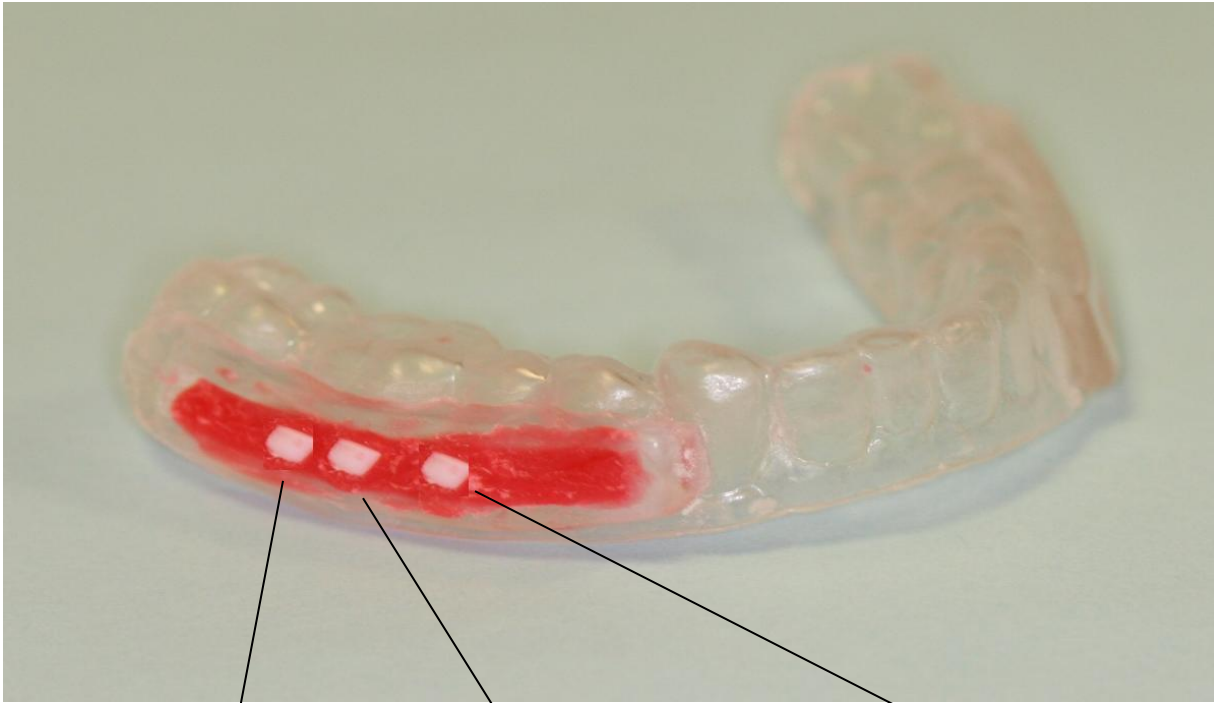


https://www.researchgate.net/figure/The-components-and-set-up-of-the-flow-cell-system-27_fig1_294575975

Flow-cell system



https://www.researchgate.net/figure/The-components-and-set-up-of-the-flow-cell-system-27_fig1_294575975



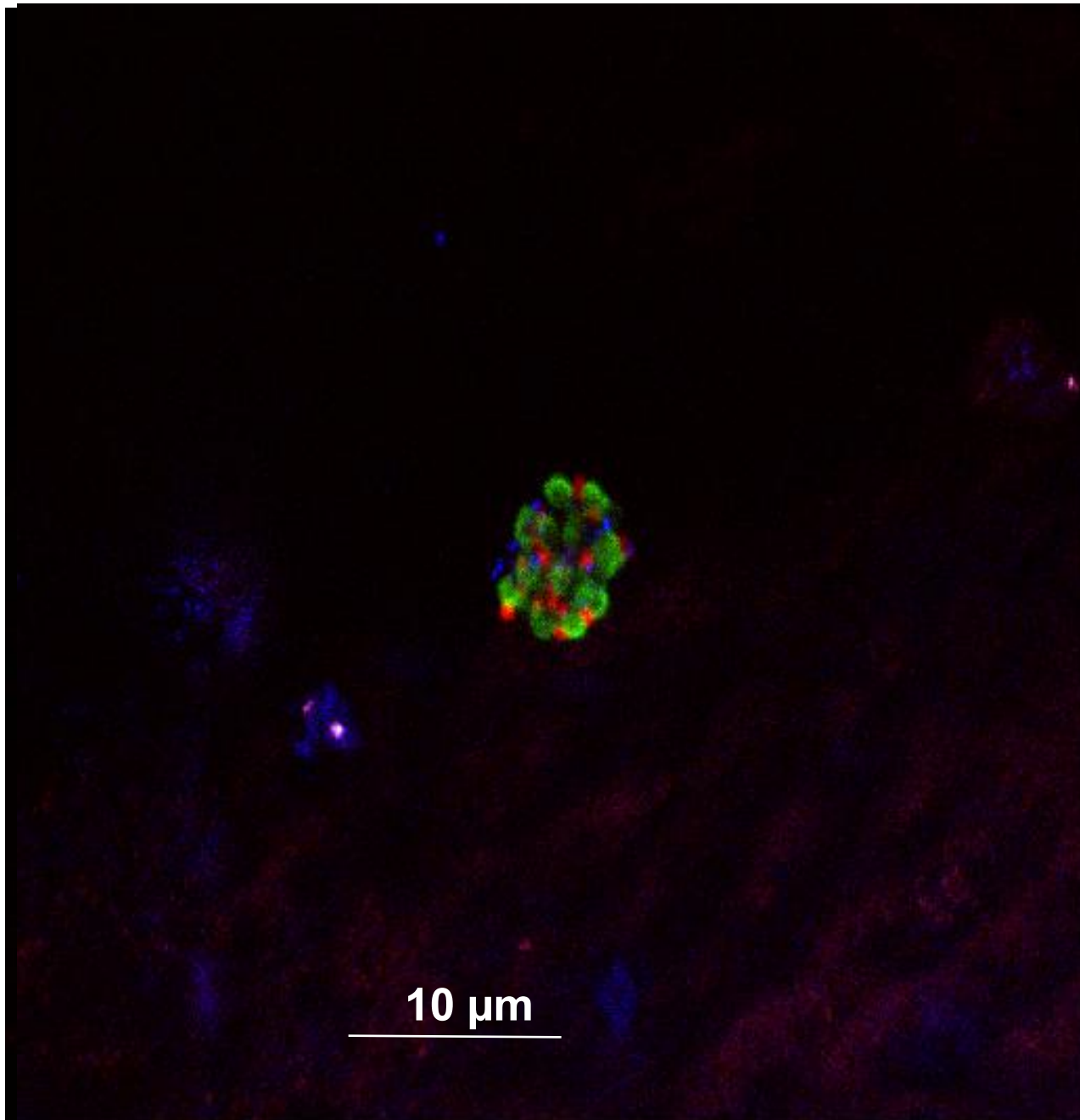
Palmer et al

4 hr and 8 hr timepoints

Molecular analysis

Isolation of bacterial pure cultures
phenotypic analysis

confocal microscopy
spatial organization within biofilm

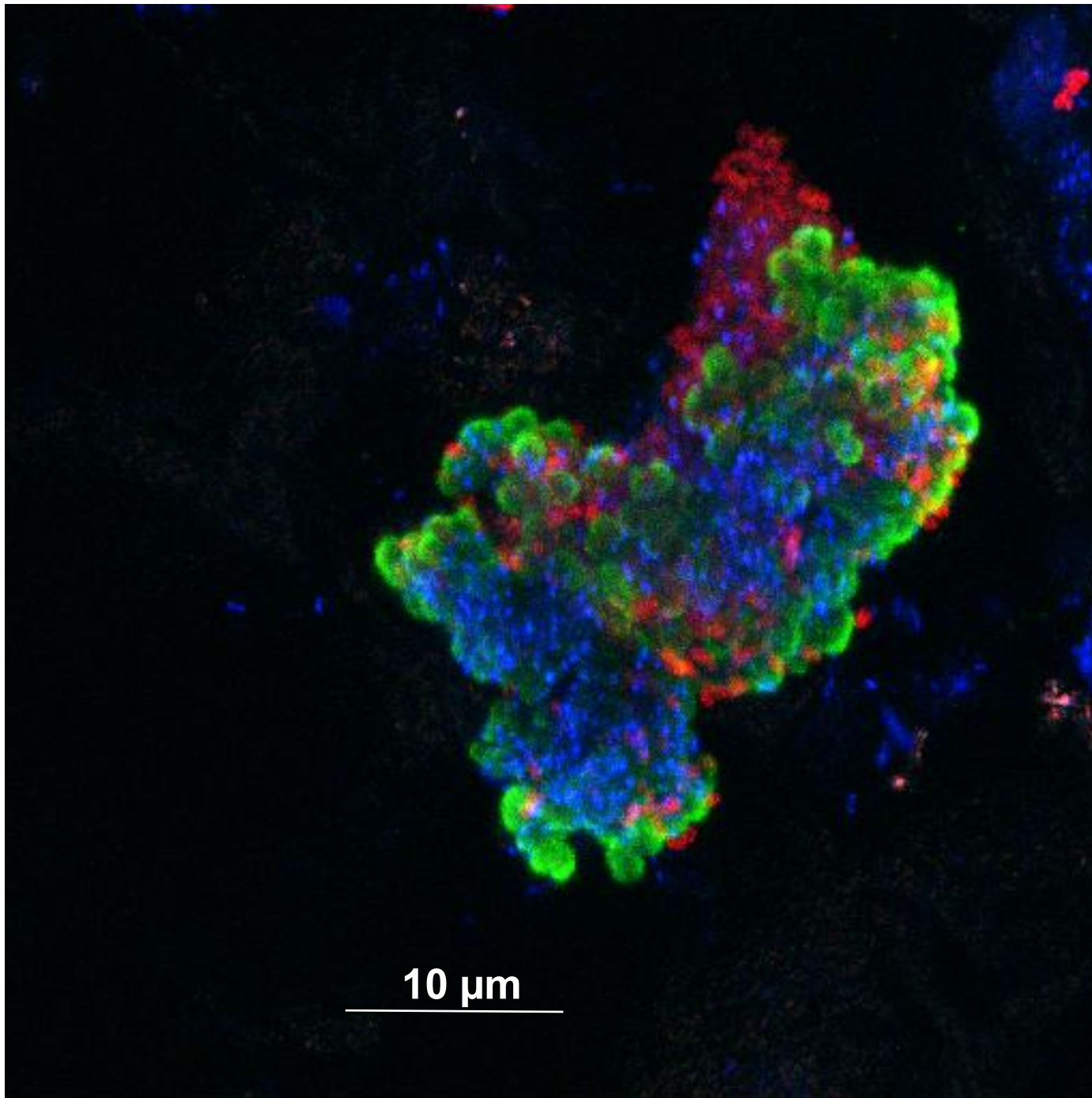


4 hrs

DAPI

Rothia

S. salivarius



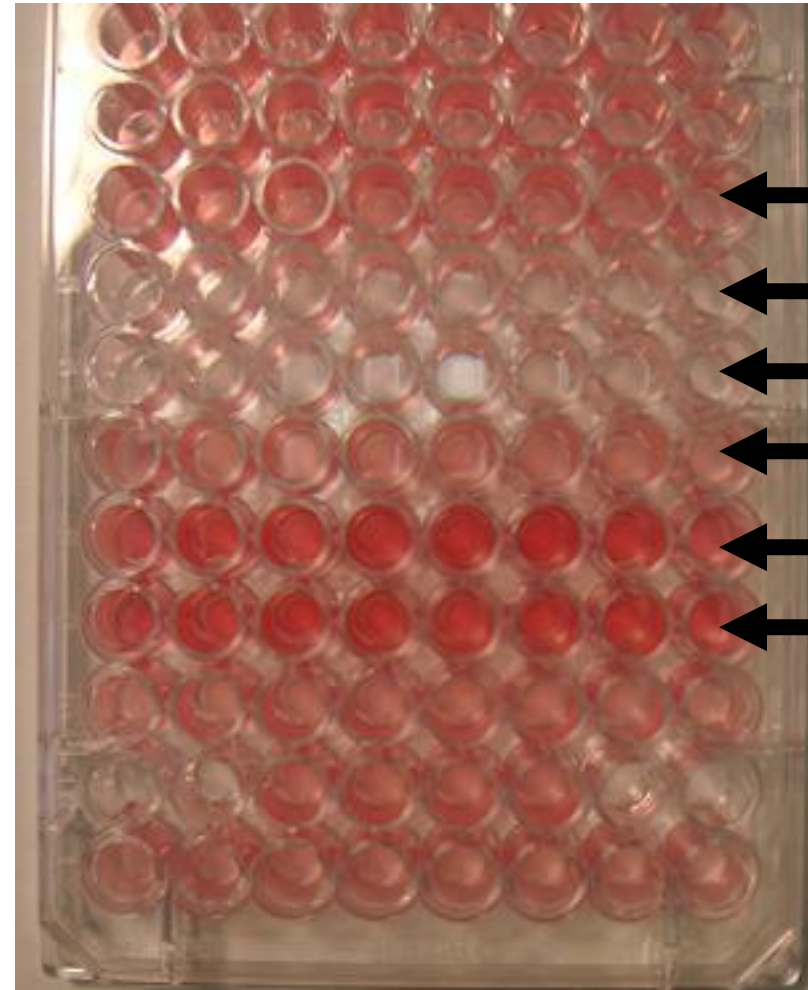
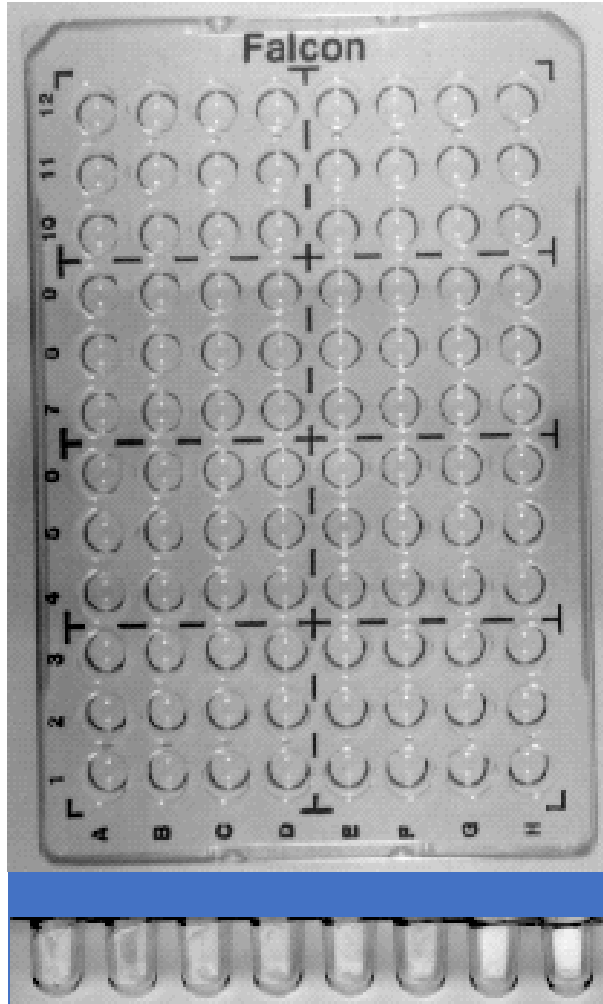
8 hrs

DAPI

Rothia

S. salivarius

Static systems



← Wild-type

← Deficient mutant

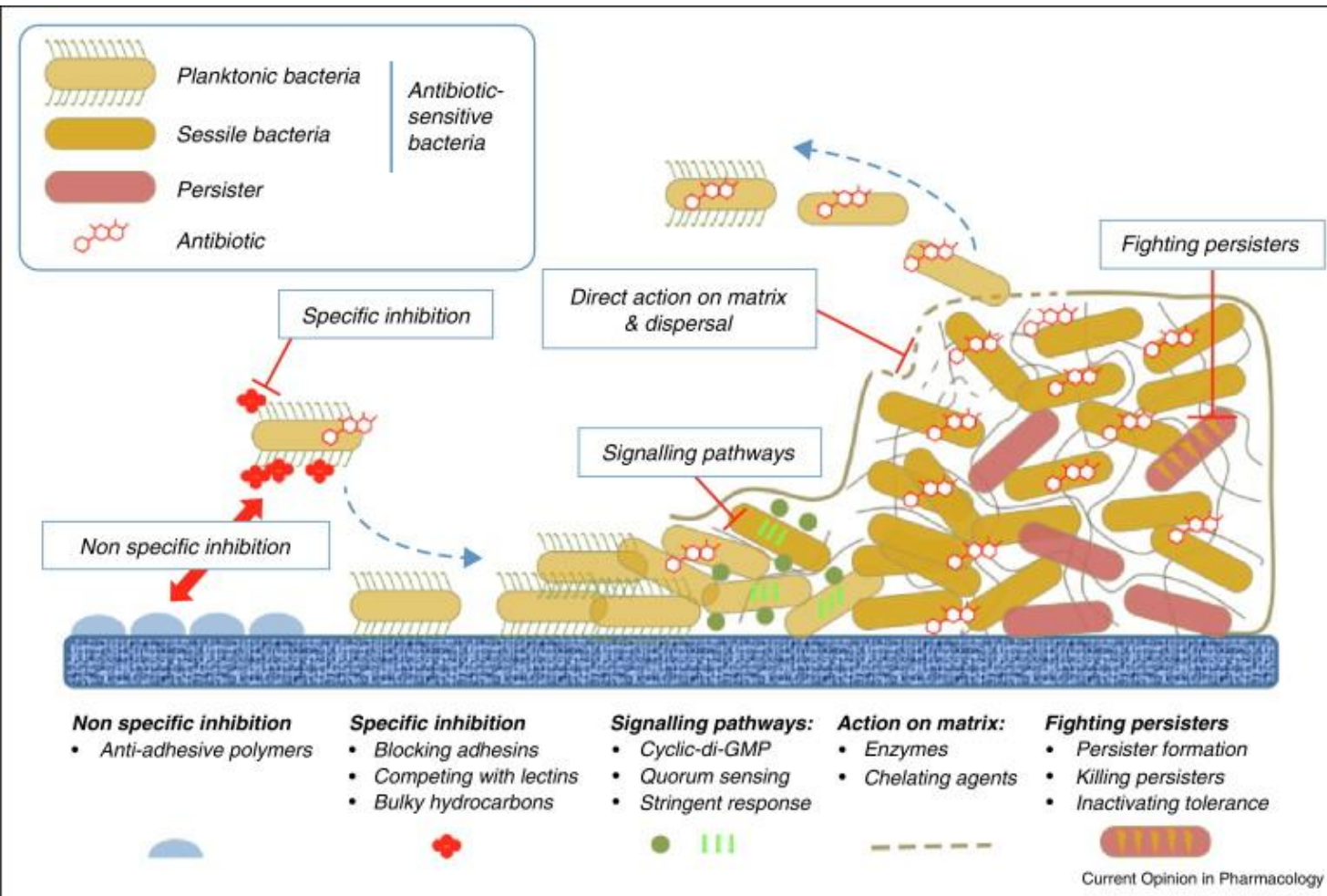
← Deficient mutant

← No effect

← Hyper mutant

← Hyper mutant

Combating biofilms



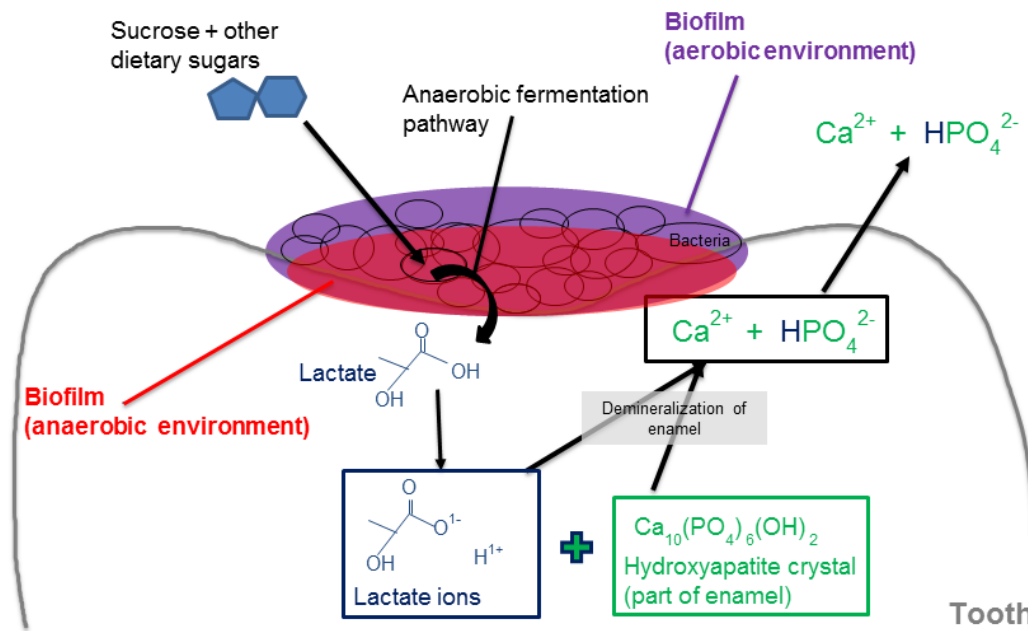
Significant focus has been to develop means to remove/combat biofilms

Five major approaches to combat biofilms are represented with their impact on biofilm formation or integrity and their possible combination with antibiotics.

Caries is a microbially-mediated biofilm disease

The primary pathogen associated with caries is *Streptococcus mutans* although the community context is important, and some other organisms may also cause caries

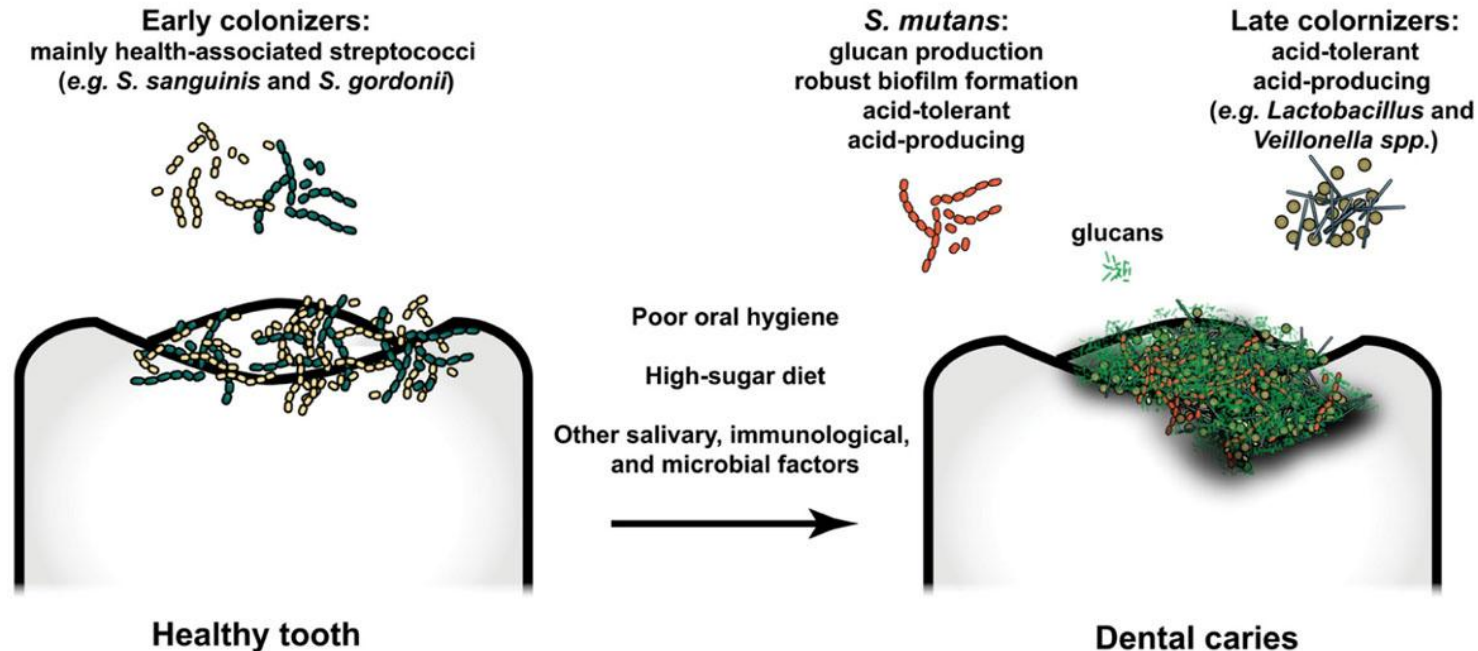
***S. mutans* efficiently produces organic acids as a product of its metabolism of carbohydrates**



High levels of organic acids (particularly lactic acid) lower the pH of the oral cavity

The solubility of hydroxyapatite (enamel and dentin) is greater under acidic conditions

***S. mutans* is an acidophile; it thrives in acidic environments that are lethal to many other oral organisms**



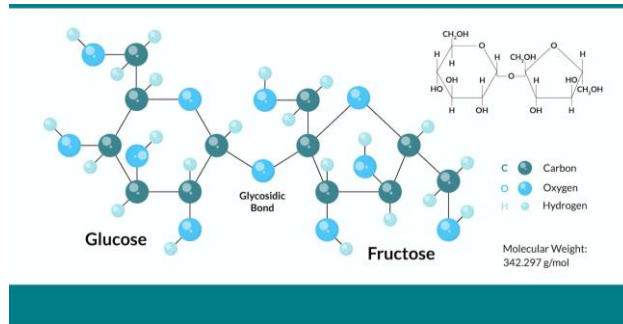
Acid-sensitive bacteria

S. mitis,
S. sanguinis,
S. gordonii

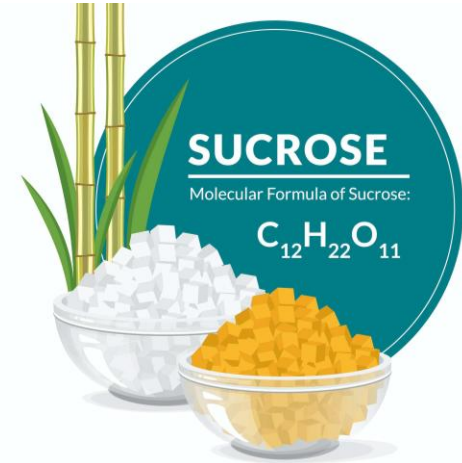
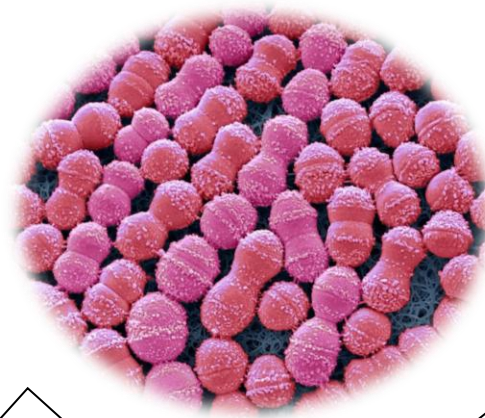
Highly acidogenic & aciduric (acid-tolerant)

S. mutans

Ecological Plaque Hypothesis: certain species cause caries,
but are not always present in enough abundance to do so



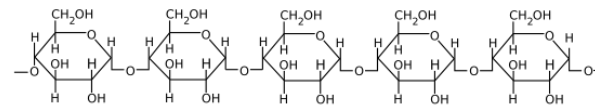
S. mutans



Lactic acid

Reduce the pH of the local environment
Kills acid-intolerant organisms

Glucans & Fructans
 (Glucansucrase)



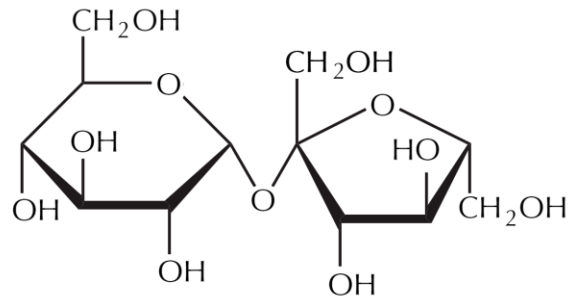
Attract more
***S. mutans* cells**

Bacteriocins
"mutacin"

Kills other bacteria
Reduces competition for niche

Sucrose and polymers

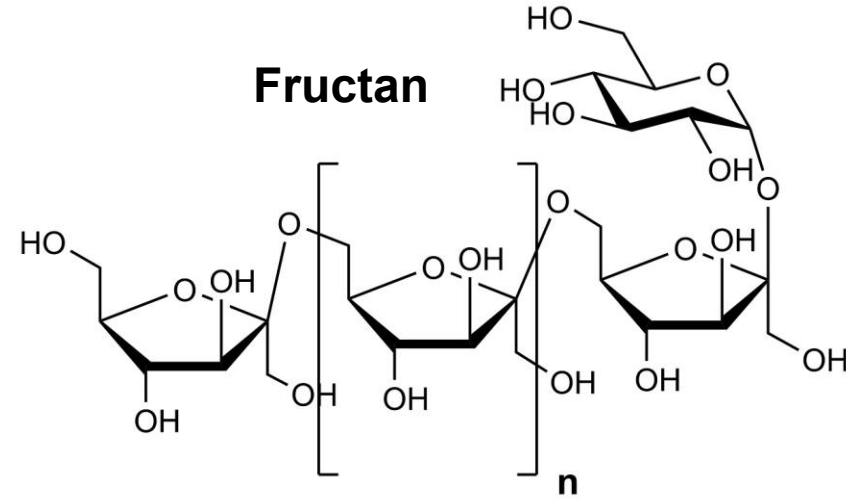
Sucrose



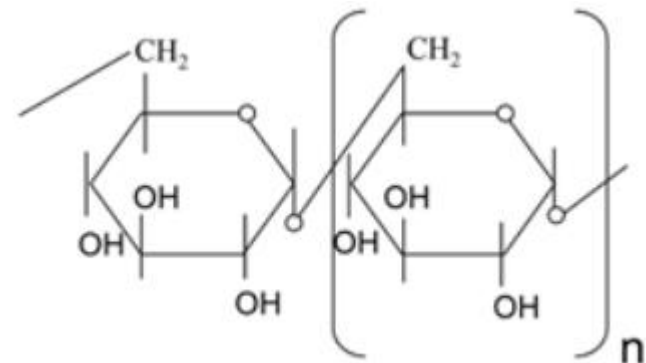
6C-glucose + 5C-fructose

These can be made extracellularly

Fructan

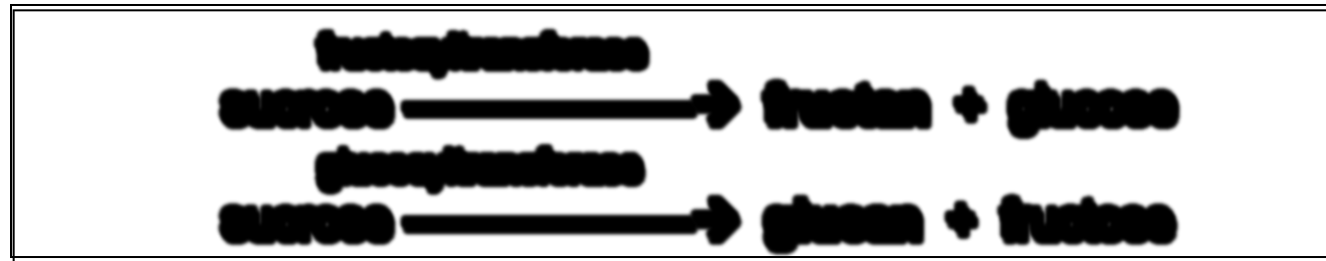


Glucan



Matrix Carbohydrates Contribute to Caries

1) *S. mutans* secretes enzymes that utilize sucrose to produce extracellular polymeric glucans and fructans.

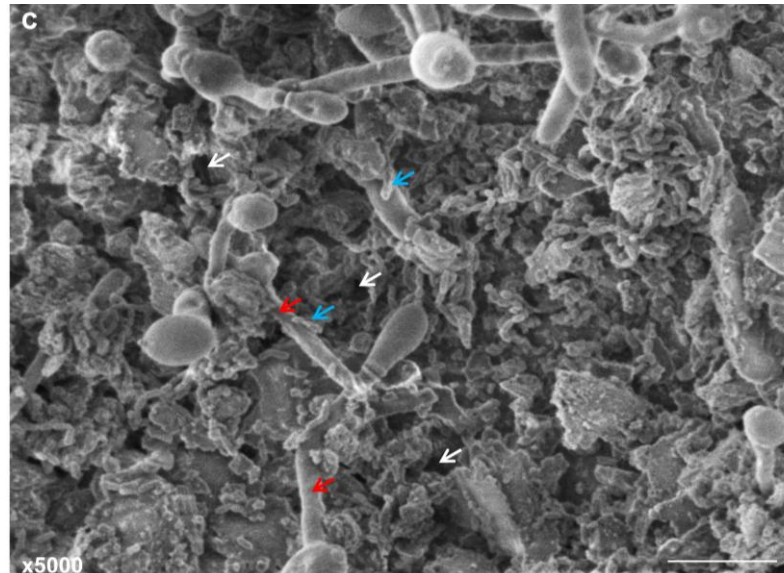
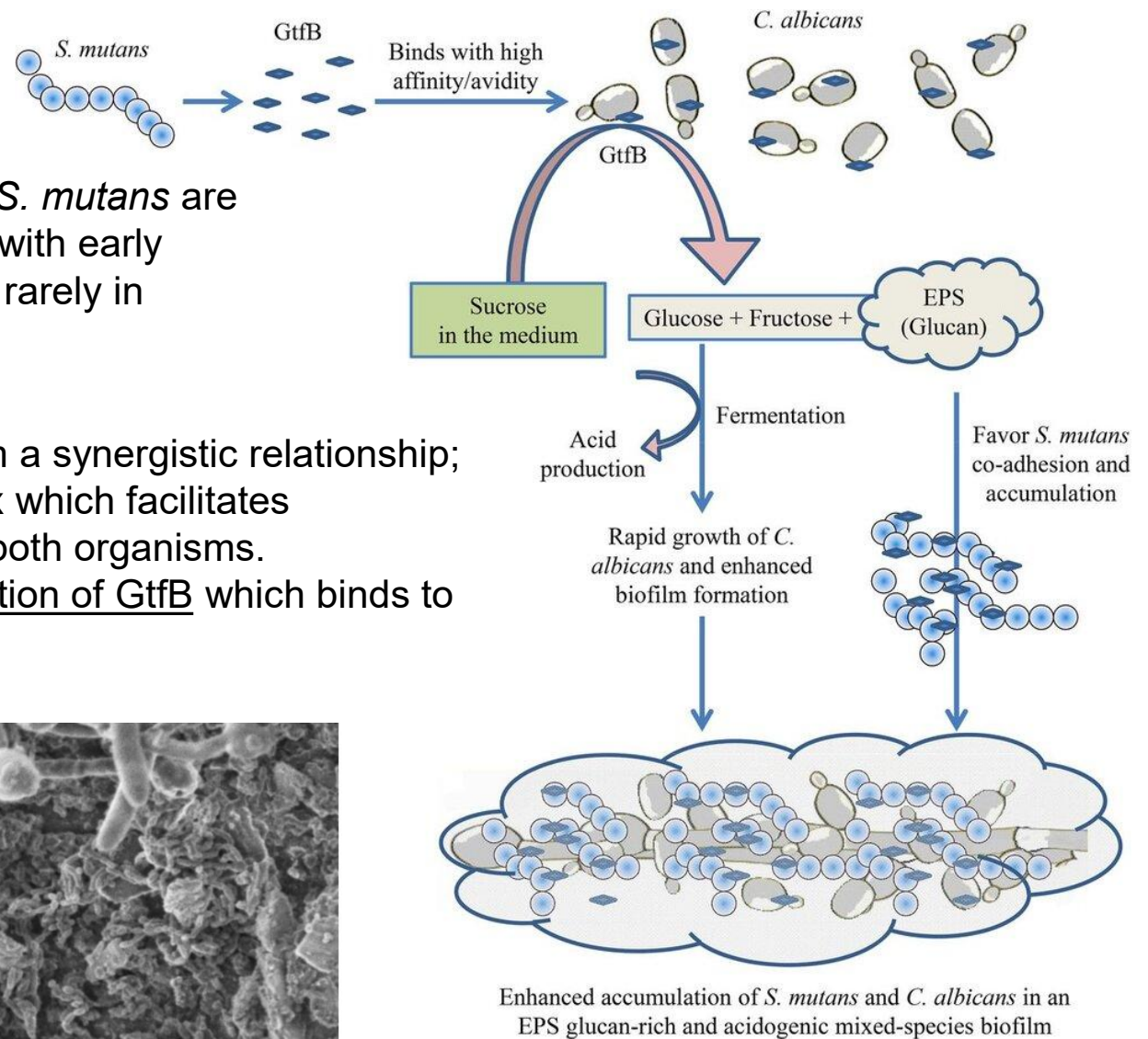


2) Glucans and fructans provide:

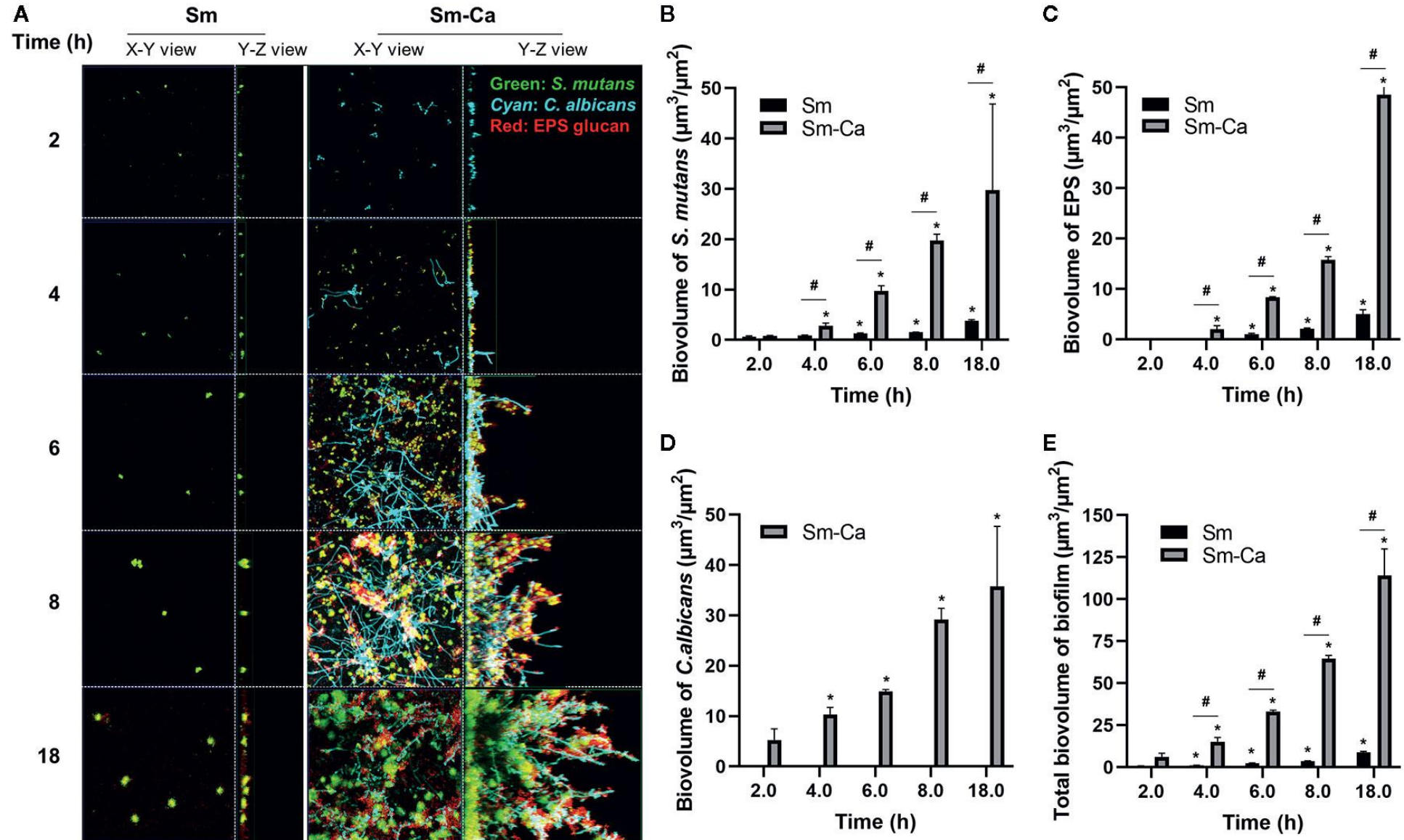
- Extracellular stores of carbohydrates for *S. mutans* (e.g., glucans or fructans cleaved by dextranase or fructanase to yield glucose or fructose, respectively)
- Promote attachment of additional *S. mutans* cells to plaque
- Protect attached *S. mutans* from antimicrobial effects of saliva

A role for fungi?

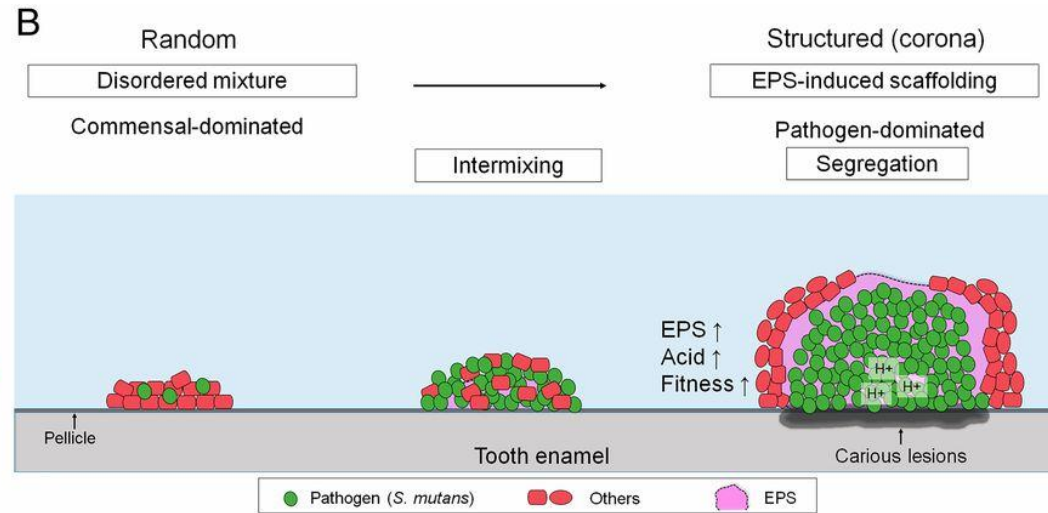
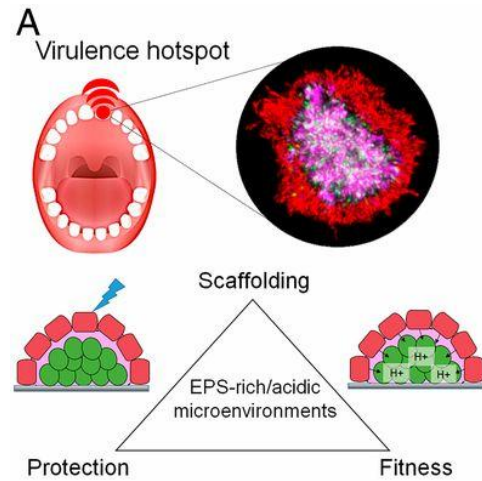
- High levels of *Candida* and *S. mutans* are found in biofilms in children with early childhood caries (ECC), but rarely in healthy children.
- *Candida* and *S. mutans* form a synergistic relationship;
- GtfB produces glucan matrix which facilitates colonization of the tooth by both organisms.
- *Candida* induces the production of GtfB which binds to fungal surface.



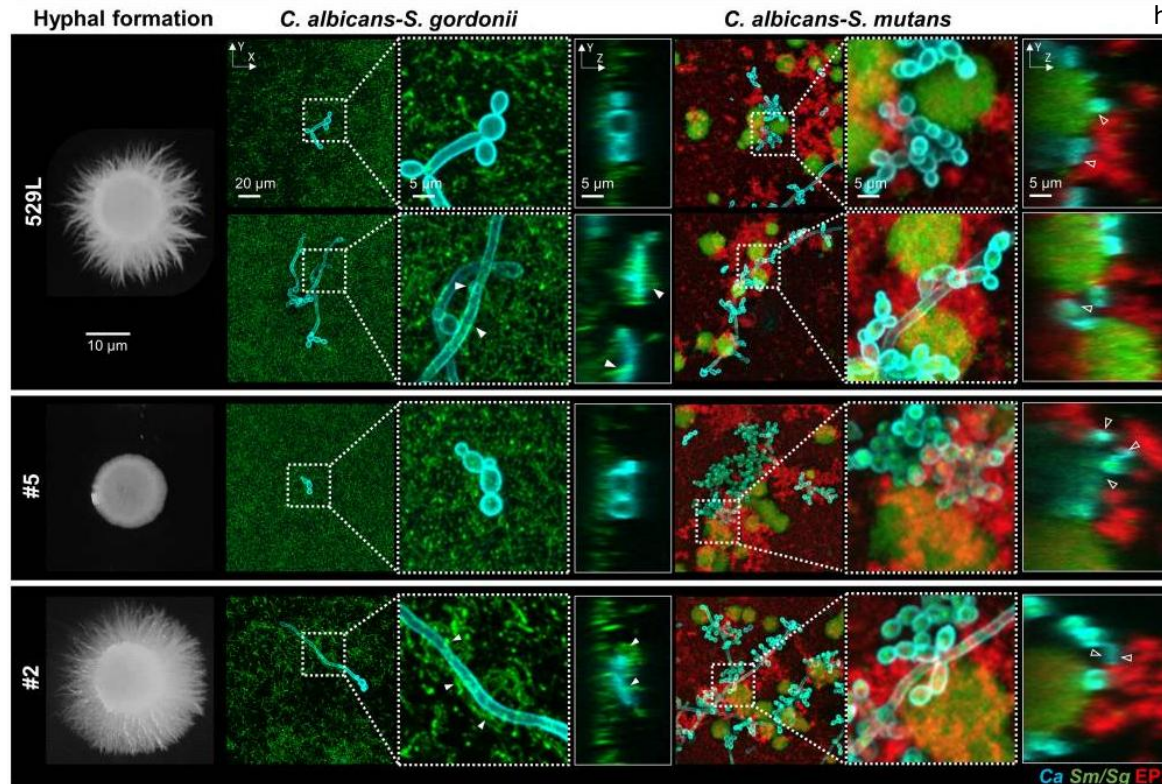
Synergism of *Streptococcus mutans* and *Candida albicans* Reinforces Biofilm Maturation and Acidogenicity in Saliva



Further microbe - *S. mutans* interaction



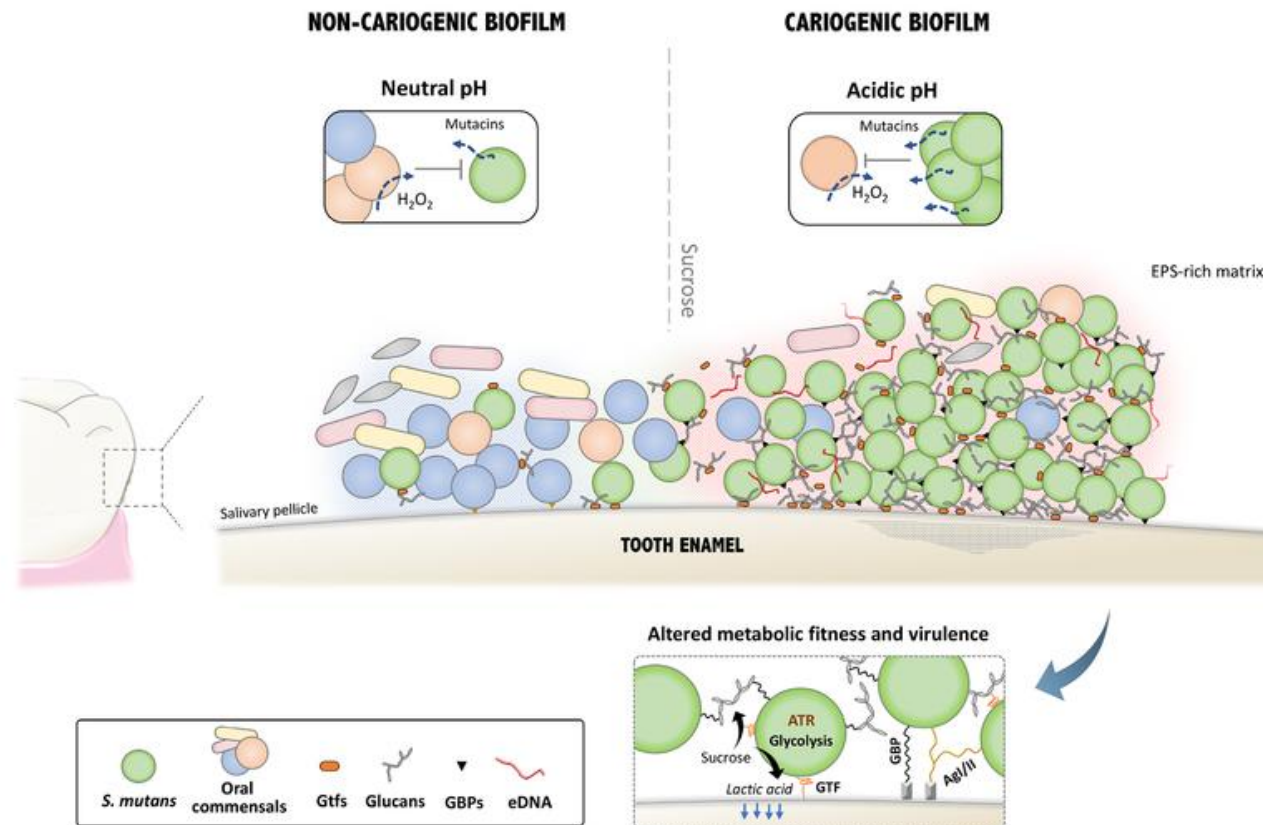
<https://www.pnas.org/doi/full/10.1073/pnas.1919099117>



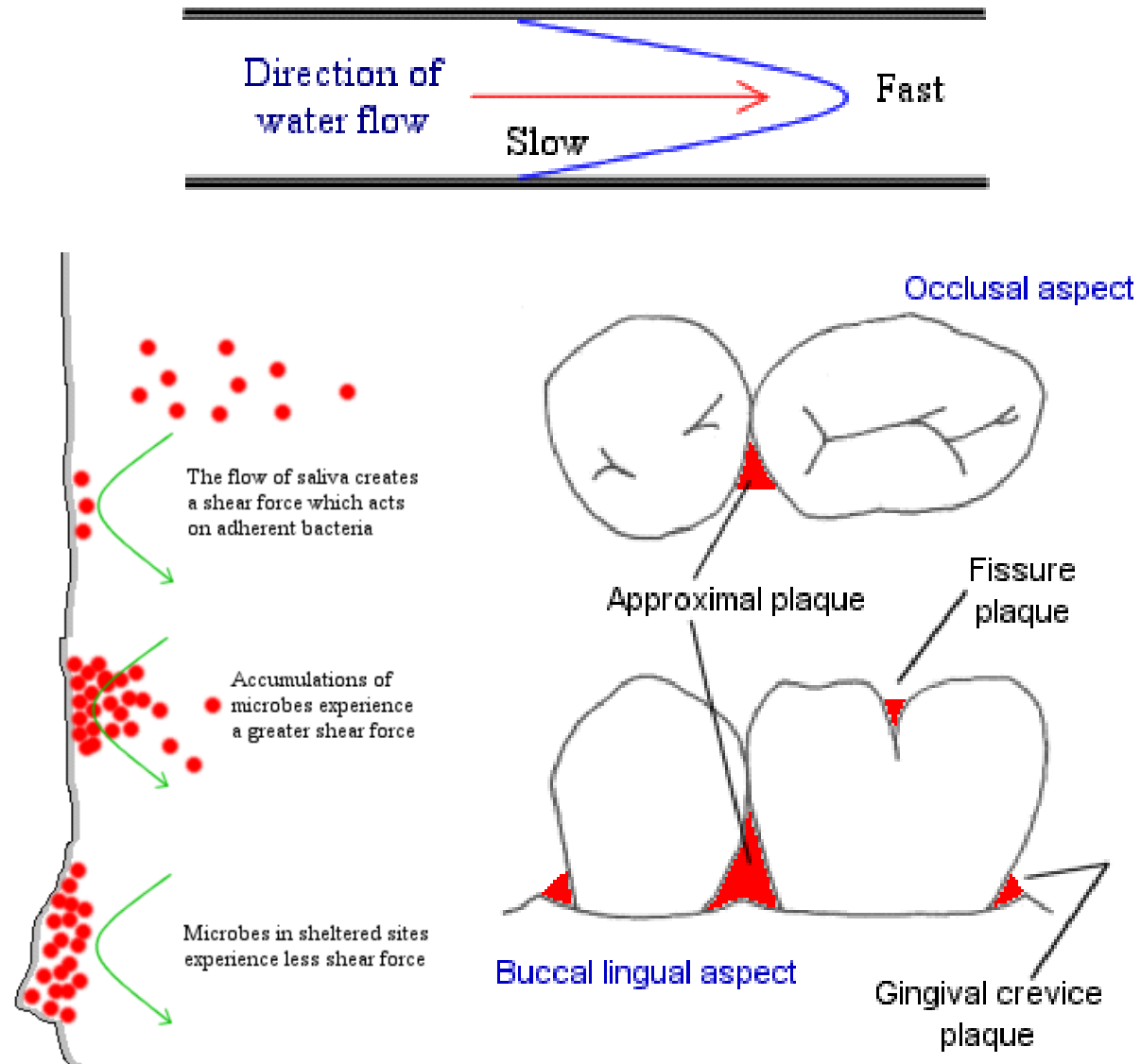
<https://pmc.ncbi.nlm.nih.gov/articles/PMC9973264/>

The Matrix Hypothesis

The main role of *S. mutans* may be to facilitate the development of the cariogenic biofilm through the production of extracellular matrix, which generates an environment that promotes the colonization and growth of other acidophiles, possibly at the eventual expense of *S. mutans* itself.



Salivary Flow Modulates Growth of Dental Plaque



Salivary Mucin influence on plaque

- Healthy salivary mucin structure limits quorum signaling in *S. mutans*
- Also promotes mixing of *S. mutans* with commensal species that limit its growth

