

DENTAL PLAQUE AS A BIOFILM

THE POSSIBILITIES OF CHEMICAL PLAQUE CONTROL



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DENTAL PLAQUE: BIOLOGICAL SIGNIFICANCE OF A BIOFILM AND COMMUNITY LIFE-STYLE

- Most microorganisms in nature attach to surfaces and form biofilms.
- Biofilms are highly structured and often composed of consortia of interacting microorganisms,
- The properties of microbial communities, are more than the sum of the component species



PLAQUE FORMATION

The organism enharbour many billions of bacteria on its surfaces. Nevertheless the desquamation of the epithelial cells anticipates the long lasting bacterial coexistence in the body.

In the oral cavity the non shedding surfaces, like enamel, root cementum, restorations can promote permanent, long lasting bacterial adhesion and survival on the surfaces.



PLAQUE FORMATION

Löe - experimental gingivitis model (1965), proved that plaque accumulation can lead to gingivitis, and its removal can reverse the disease.

Similarly experimentally was proven that plaque accumulation can cause peri-implantitis (Pontoriero 1994).

Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR Land NP. Experimentally induced periimplant mucositis. A clinical study in humans. Clinical Oral Implants Research 5 254-259. 1994.



Plaque is natural and may exist in harmony with the host in health.

Maintenance of health depends on the balance between the bacterial challenge and the host .



Disease is the consequence of this balanced relationship breaking down, provoked by

- **either changes of the microbial challenge**
- **or the changes of the host response** (Socransky et al. 1998).



Most bacterial species implicated in periodontitis can be found in periodontally healthy subjects in low numbers.

In some geographical regions, some species are infrequently detected in health (Van Winkelhoff et al. 2002).



CHRONIC PERIODONTITIS AND HEALTHY CONTROLS THE OCCURENCE OF PERIODONTOPATHOGENIC STRAINS

THE NUMBER OF SUBJETS AND THE PROPORTION (%)

BACTERIUM	CHRONIC PERIO (n=29)	HEALTHY (n=20)
Treponema sp.	29 (100)	8 (40)
A.A.	26 (89.7)	1 (5)
P. gingivalis	29 (100)	6 (30)
Fusobacterium sp.	29 (100)	17 (85)
T. forsythia	28 (96.9)	11 (55)
P. intermedia	26 (89.7)	1 (5)
P. micros	28 (96.6)	6 (30)

Choi BK et al. Detection of major putative periodontopathogens in Korean advanced adult periodontitis patients using a nucleic acid-based approach J. Periodontol 2000;71:1387-1394

Most bacterial species currently implicated in periodontitis can be found in periodontally healthy subjects in low numbers.

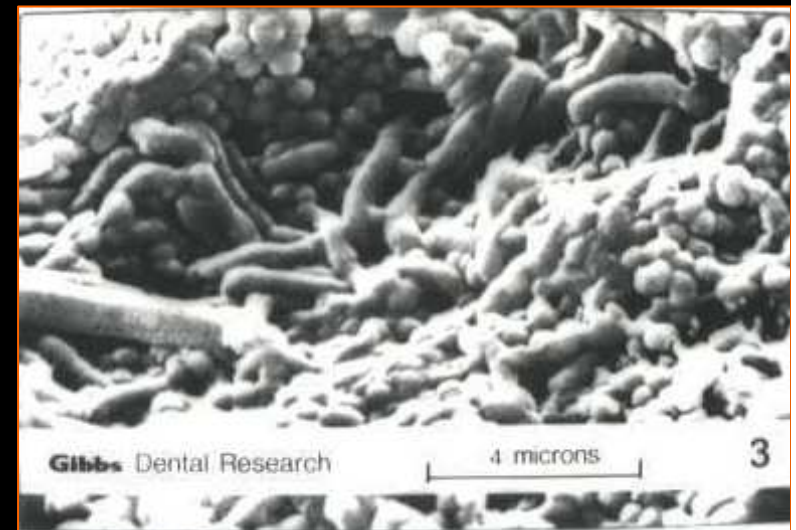
BACTERIAL BIOFILM

IS MADE UP OF

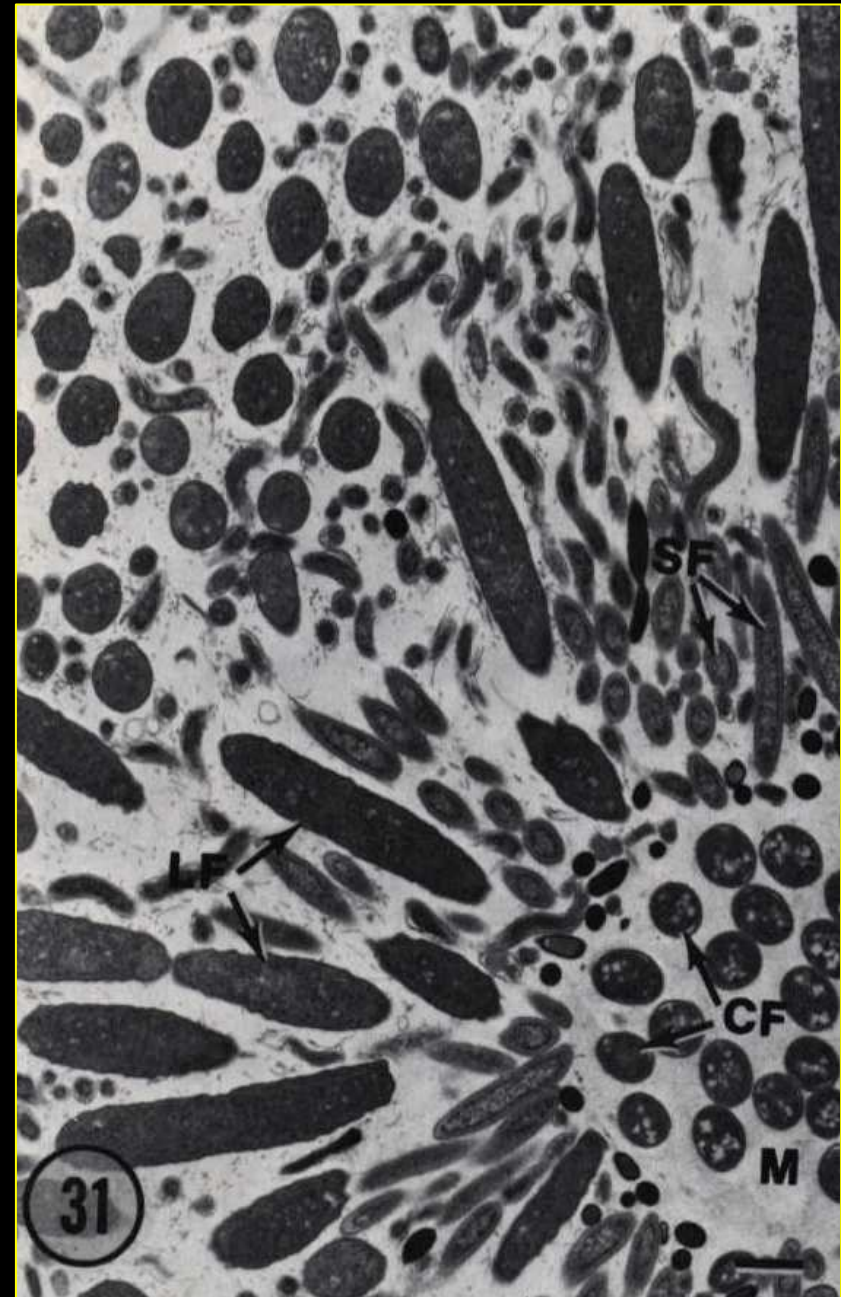
„FRIENDLY COMMENSAL BACTERIA

AND HOSTILE PERIODONTOPATHOGENIC STRAINS

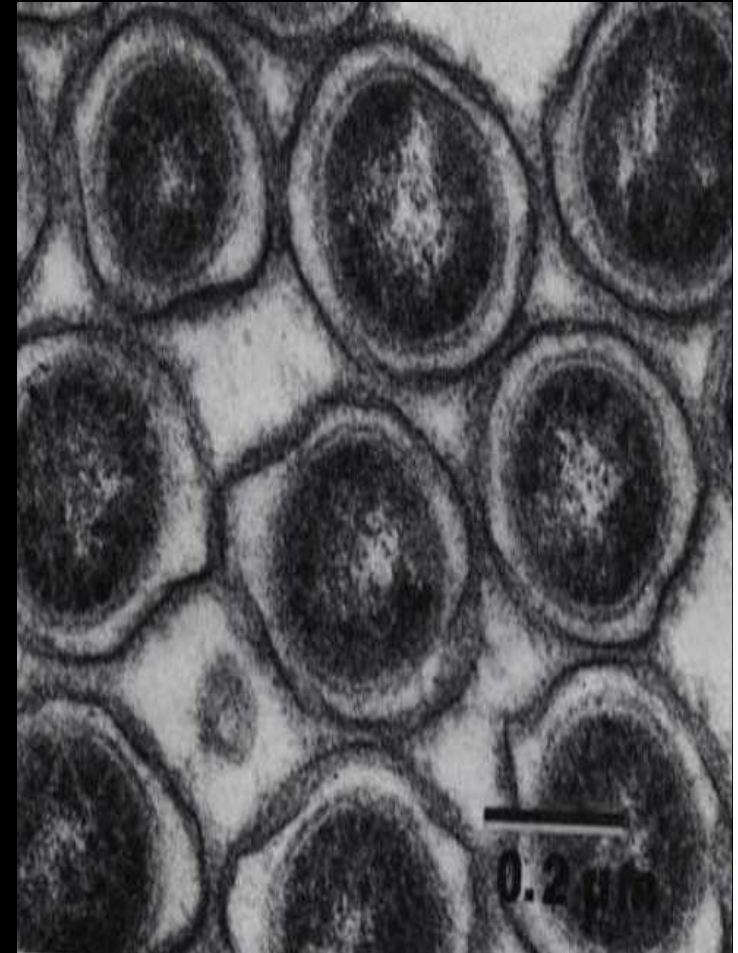
**THE MANIFESTATION OF
PERIODONTAL BREAKDOWN IS
DEPENDENT ON THE HOST'S
SUSCEPTIBILITY AND THE
VIRULANCE OF THE BIOFILM**



- Biofilms is a matrix-embedded microbial population, adherent to the surfaces or to each other (Costerton et al. 1995).
- Biofilms are usually highly structured with channels leading to the depth of the biofilm, creating a primitive circulation (Costerton et al. 1995).
- The component species are not randomly distributed
- spatially and functionally organized,
- natural biofilm has a highly diverse microflora.



- **Most natural biofilms contain multiple species and are termed microbial communities.**
- **The component organisms are not passive bystanders**
 - **they are involved in physical, metabolic and molecular interactions.**
 - **These interactions are essential for the attachment, growth and survival**
 - **to persist in a hostile environment.**



- Environmental heterogeneity in biofilms can accelerate phenotypic and genotypic diversity in bacterial populations
- Cells are better prepared to cope with adverse conditions

"biological insurance,,

(Boles et al. 2004).



THERE CAN BE PEACE BETWEEN
BIOFILM AND HOST – NO OVERT
INFLAMMATORY REACTION

OR

CLINICALLY MANIFEST INFLAMMATION



PERIODONTOGENIC BACTERIA IN ORAL CAVITY

PERIODONTAL DISEASE IS NOT CAUSED BY ONE SINGLE STRAIN

KOCH'S POSTULATE IS MODIFIED BY SOKRANSKY'S POSTULATE

(Sokransky 1992)

- THE ORGANISM SHOULD BE FOUND IN HIGH NUMBERS IN THE PROXIMITY TO THE PERIODONTAL TISSUES
- THE ORGANISM SHOULD BE ABSENT OR IN SIGNIFICANTLY SMALLER NUMBER IN HEALTHY PERIODONTAL TISSUES
- THE ORGANISM SHOULD HAVE HIGH TITER OF SERUM ANTIBODY
- THE ORGANISM SHOULD POSSESS MANY VIRULENCE FACTORS
- THE ORGANISM SHOULD PRODUCE PERIODONTAL INFLAMMATION IN ANIMAL MODELS
- FOLLOWING ERADICATION OF THE ORGANISM CLINICAL IMPROVEMENT SHOULD OCCUR
- THE ORGANISM MUST BE OF A VIRULENT CLONAL TYPE
- THE HOST SHOULD BE SUSCEPTIBLE TO THE ORGANISMS

**Most bacterial species implicated in periodontitis can be -
- in low numbers - found in periodontal health.-**



- it has been shown that there is often only one detectable clonal type of *A. actinomycetemcomitans* and *P. gingivalis* per subject,

- although many clonal types of these species have been identified in the whole population (Haubek et al. 2002).

-

- These observations have facilitated studies, which demonstrate that *A. actinomycetemcomitans* and *P. gingivalis* are transmittable, especially within families (Okada et al. 2004).

A PARODONTITISBEN SZENVEDŐK ÉS EGÉSZSÉGES KONTROLOK PERIOPATOGÉN TÖRZSEKRE POZITÍV PARODONTÁLIS TASAKJAINAK ARÁNYA

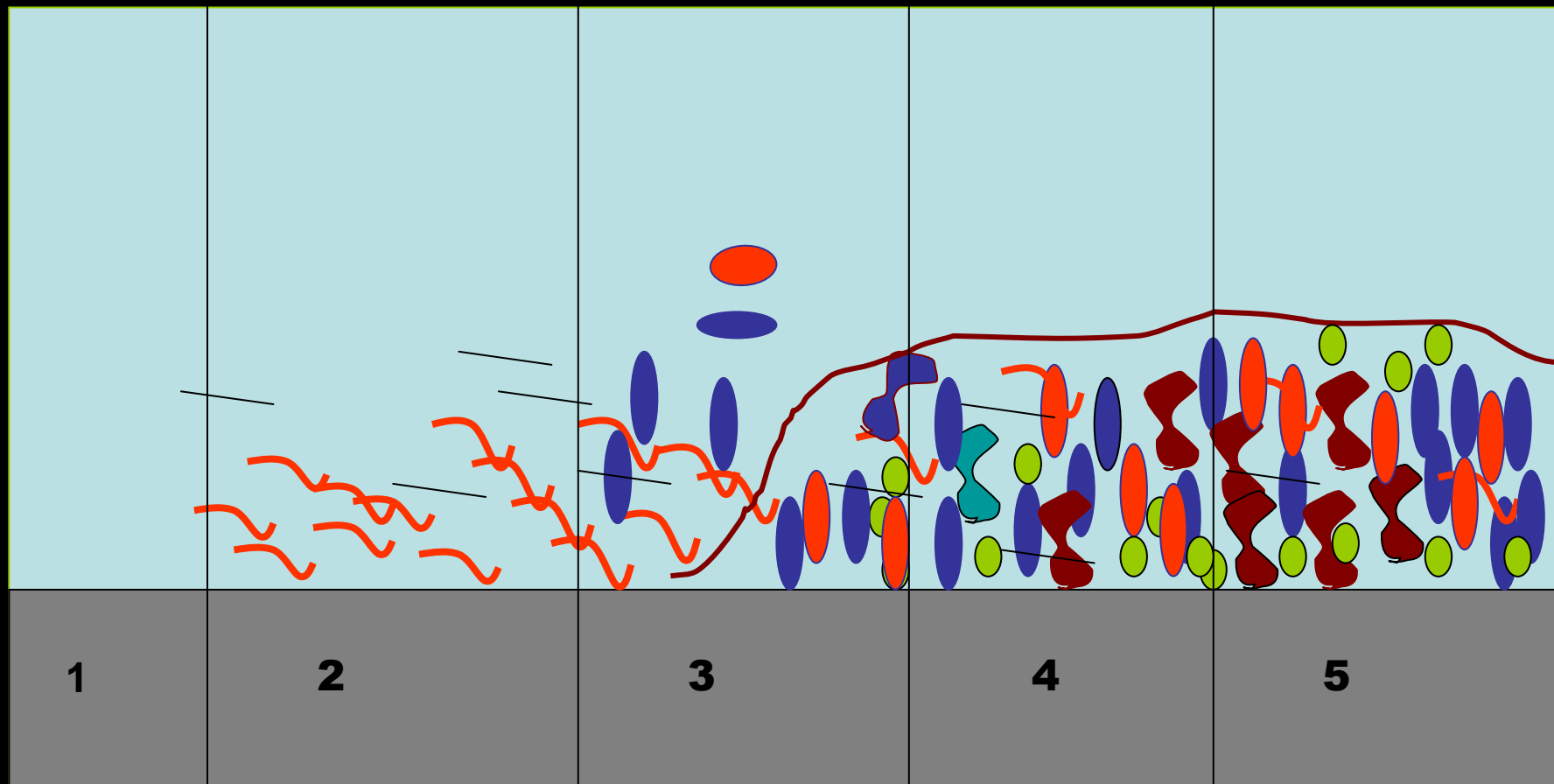
A POZITÍV TASAKOK SZÁMA ÉS ARÁNYA (%)

BAKTÉRIUM	KRÓNIKUS PARODONTITIS aktív tasak (n=116)	KRÓNIKUS PARODONTITIS inaktív tasak(n=28)	EGÉSZSÉGES KONTROL (n=100)
Treponema sp.	114 (98.3)	13 (46.4)	22 (22)
A.A.	86 (74.1)	8 (28.6)	1 (1)
P. gingivalis	113 (97.4)	14 (50)	18 (18)
Fusobacterium sp.	116 (100)	20 (71.4)	58 (58)
T. forsythia	112 (96.6)	9 (32.1)	18 (18)
P. intermedia	82 (70.7)	5 (17.9)	2 (2)
P. micros	95 (81.9)	10 (35.7)	8 (8)

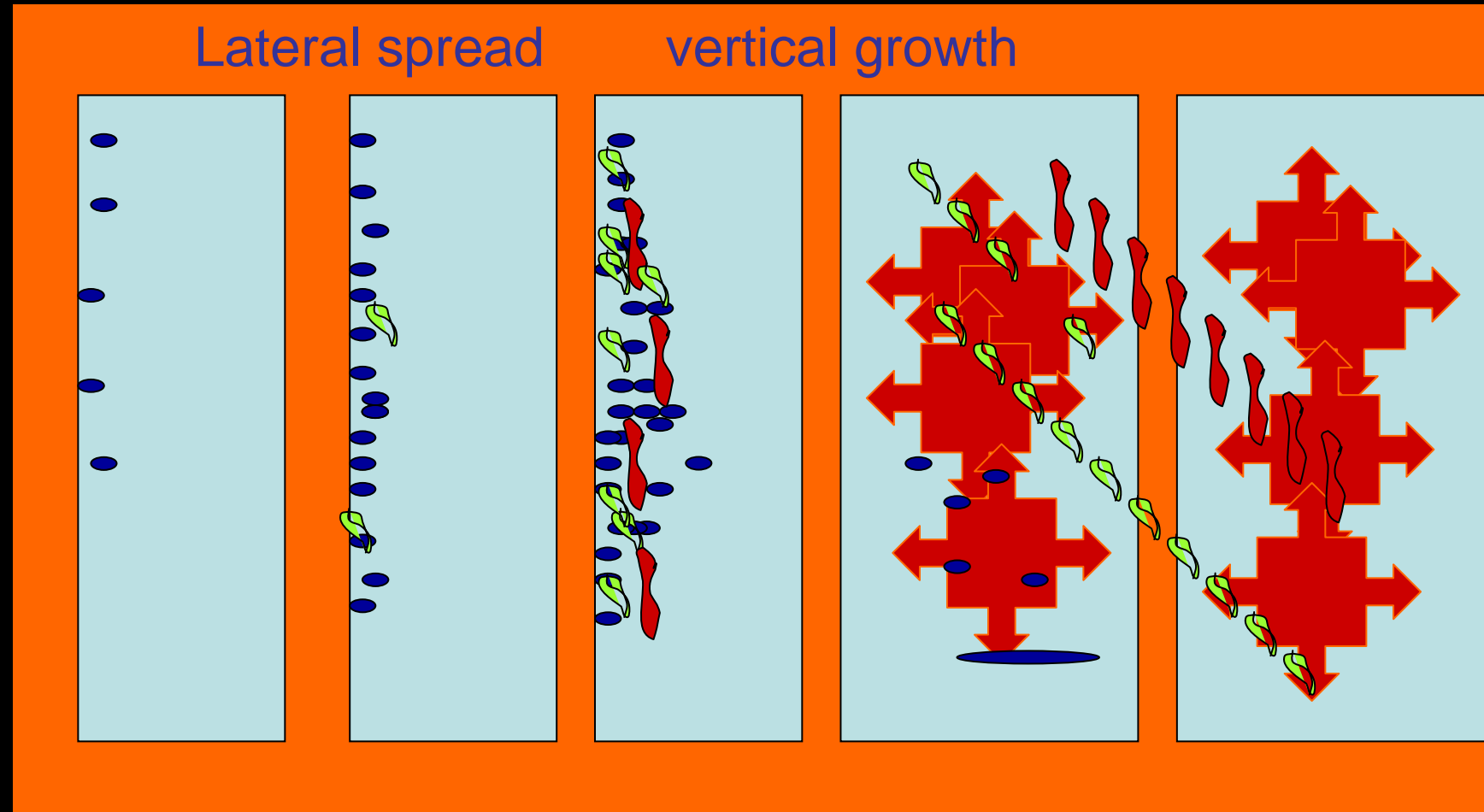
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Most bacterial species currently implicated in periodontitis can be found in periodontally healthy subjects in low numbers.

biofilm formation



THE DEVELOPMENT OF DENTAL BIOFILM

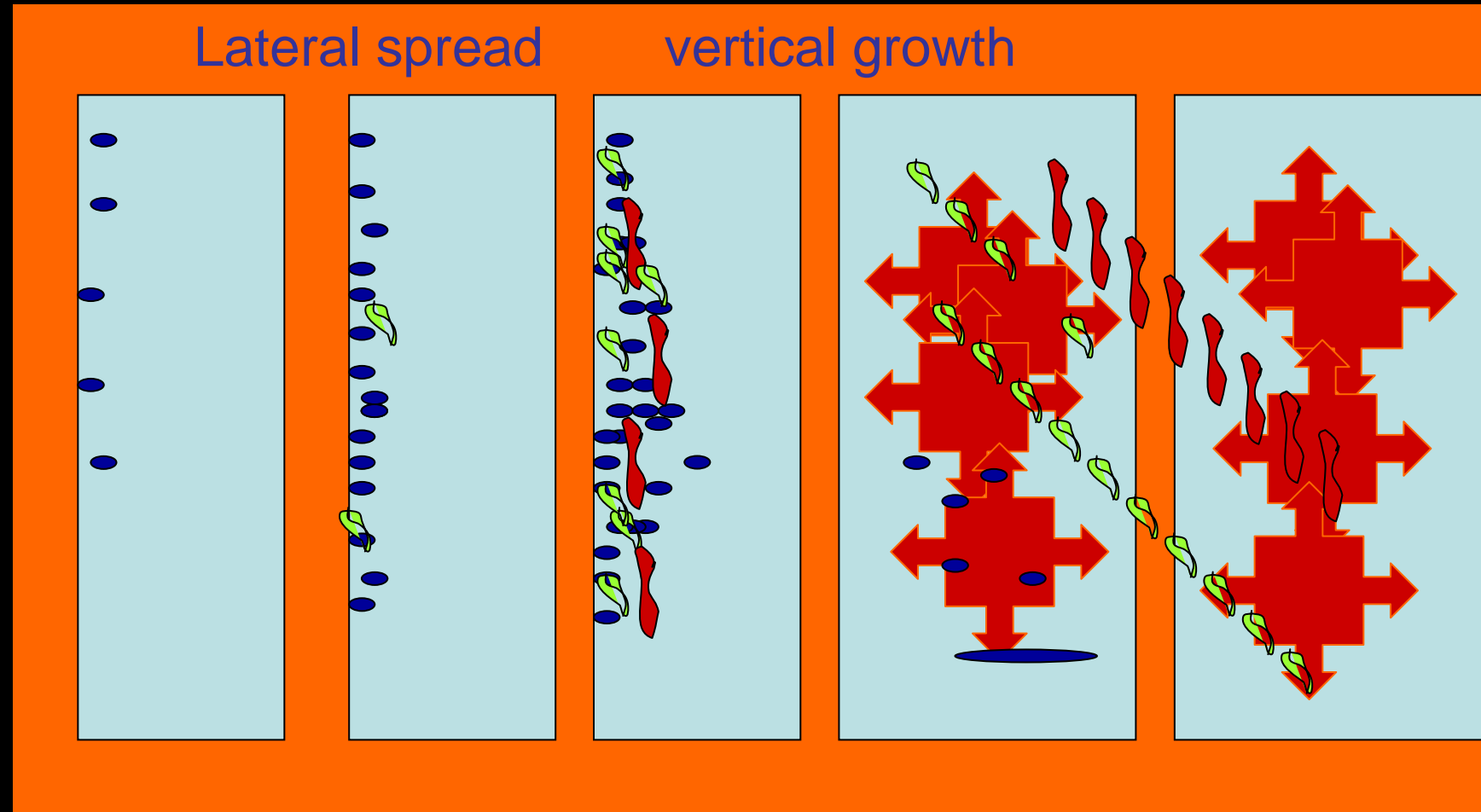


ATTACHMENT

COAGGREGATION

SPREAD

THE DEVELOPMENT OF DENTAL BIOFILM



ATTACHMENT



ADHERENCE OF PERIODONTOPATHOGENS TO PERIODONTAL TISSUES

A. actinomycetemcomitans

- AA, fimbriae also mediate binding of the bacteria to epithelial cells
- Clonal types with fimbriae bind three times more rapidly than bacteria without
- Carboxy terminal of the fimbriin is responsible for the binding to host proteins – adhesins, integrins, fibronectin
- By binding to integrins could damage the normal turnover of the connective tissue
- P. gingivalis* can bind to different bacterial surface proteins
- P. gingivalis* fimbriins is important for epithelial tissue invasion

ADHERENCE OF PERIODONTOPATHOGENS TO PERIODONTAL TISSUES

T. denticola

- *T. denticola* can bind to human epithelial cells
- can bind to host proteins – adhesins, integrins, fibronectin
- By binding to integrins could damage the normal turnover of the connective tissue
- *P. gingivalis* can bind to different bacterial surface proteins
- *P. gingivalis* fimbrellins is important for epithelial tissue invasion

ADHERENCE OF PERIODONTOPATHOGENS TO PERIODONTAL TISSUES

F. Nucleatum

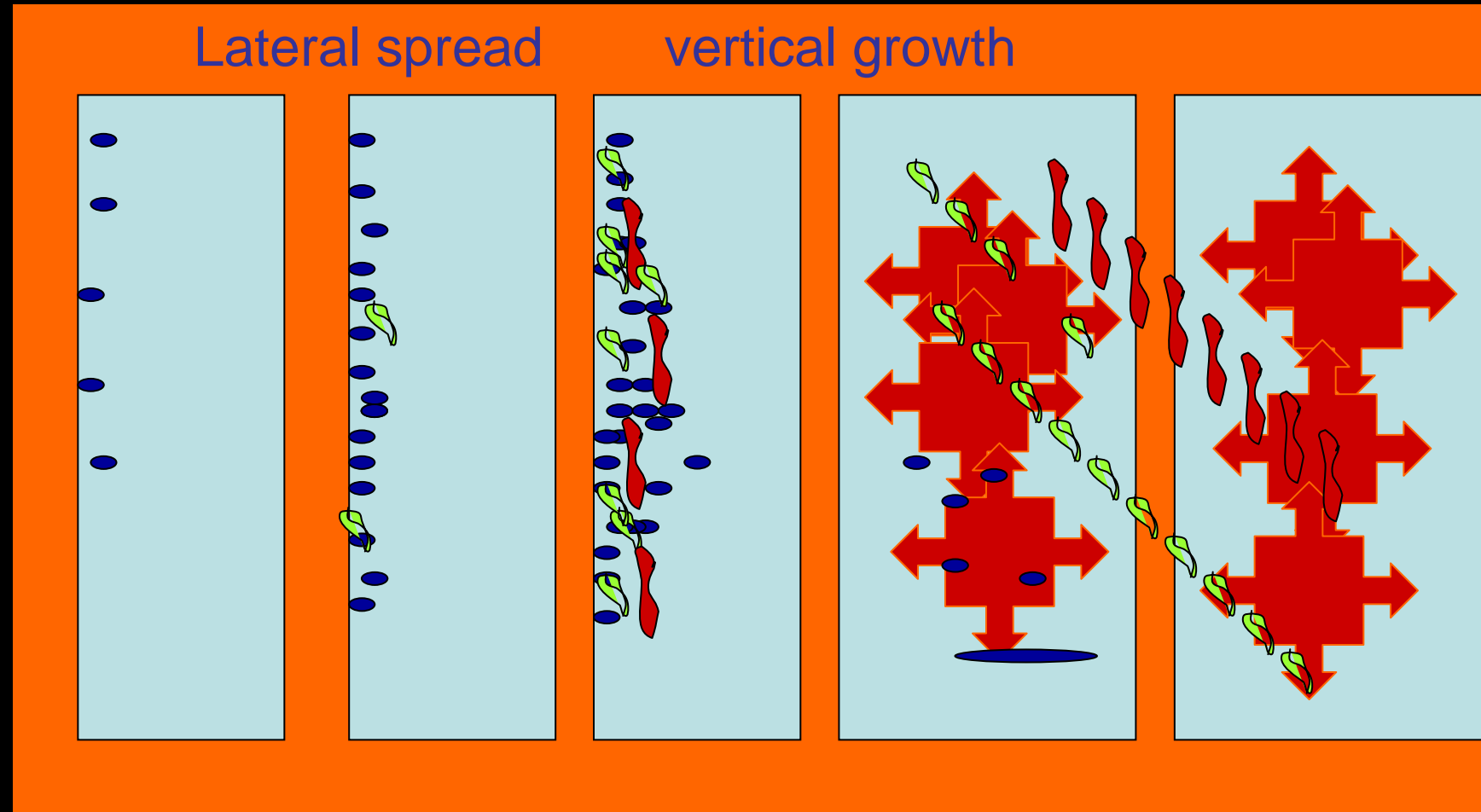
Is not member of the red complex

- Can bind to epithelial and endothelial cells, elicit IL-8 production

INVASION OF PERIODONTAL TISSUES BY PERIODONTOPATHOGENS

- Invasion is important component of virulence
- *AA* and *P. gingivalis* can be detected within mucosal epithelial cells
- *P. gingivalis* can rapidly invade epithelial cells
- *P. gingivalis* fimbriae play an important role
- In a study in which adhesion was reduced invasion decreased by 8 fold
- It involves an active interaction with epithelial integrins
- *P. gingivalis* inhibit IL-8 production and avoid PMN chemotaxis
- *P. ginigvlais* is a stealth bacterium

THE DEVELOPMENT OF DENTAL BIOFILM



SPREAD



INVASION OF PERIODONTAL TISSUES BY PERIODONTOPATHOGENS

- Invasion is important component of virulence
- *AA* and *P. gingivalis* can be detected within mucosal epithelial cells
- *AA.* can rapidly invade epithelial cells
- Fibrillated *AA* can be more aggressive in invasion than the smooth phenotype
- *AA* can enter host cells
- *AA* can invade endothelial cells
-

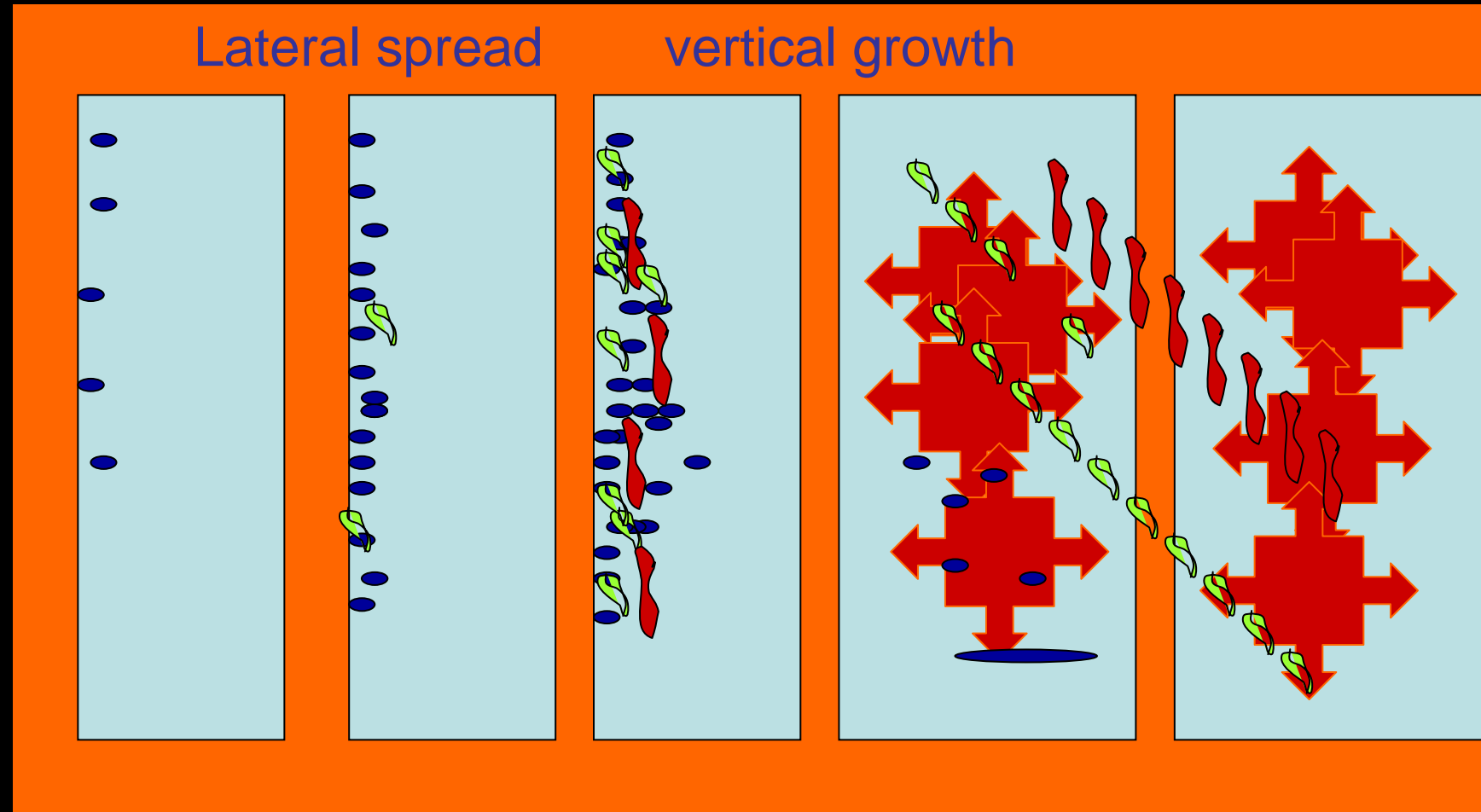
INVASION OF PERIODONTAL TISSUES BY PERIODONTOPATHOGENS

- Invaded epithelial cells demonstrate reduced adhesion to extracellular matrix, and lost its motility
- *T. forsythia* can also invade epithelial cells
- *E. corrodens* can also invade epithelial cells
- *F. nucleatum* can also invade epithelial cells
- *T. denticola* can also invade epithelial cells

A microbial consortium invades periodontal tissues
AA can invade endothelial cells

•

THE DEVELOPMENT OF DENTAL BIOFILM



SURVIVE



IMMUNEMODULATION BY PERIODONTOPATHOGENIC BACTERIA

- *P. gingivalis* inhibits PMN chemotaxis
- also inhibits transepithelial PMN migration
- by blocking IL-8 production and ICAM-1 in epithelial cells
- Down regulates Th-1 response
- Suppresses IL-1 beta production by macrophages

-

IMMUNEMODULATION BY PERIODONTOGENIC BACTERIA

- *Direct toxin production that kills immunocytes in the host*
- *AA leukotoxin*
- *low concentration creates PMN degranulation and release of MMP –8*
- *Higher concentration leads to cell lysis by pore formation in the cell membrane*

-

CYTOKINE MODULATION BY PERIODONTOPATHOGENIC BACTERIA

- *P. gingivalis* blocks IL-8 production in epithelial cells also enhanced by other bacteria
- Upregulates IL-1, IL-6, TNF production
- Suppresses IL-1 beta production by macrophages
- Later *P. gingivalis* can enhance chemokine production in the connective tissue
- *P. gingivalis* LPS can act on CD14 receptors and toll like receptors

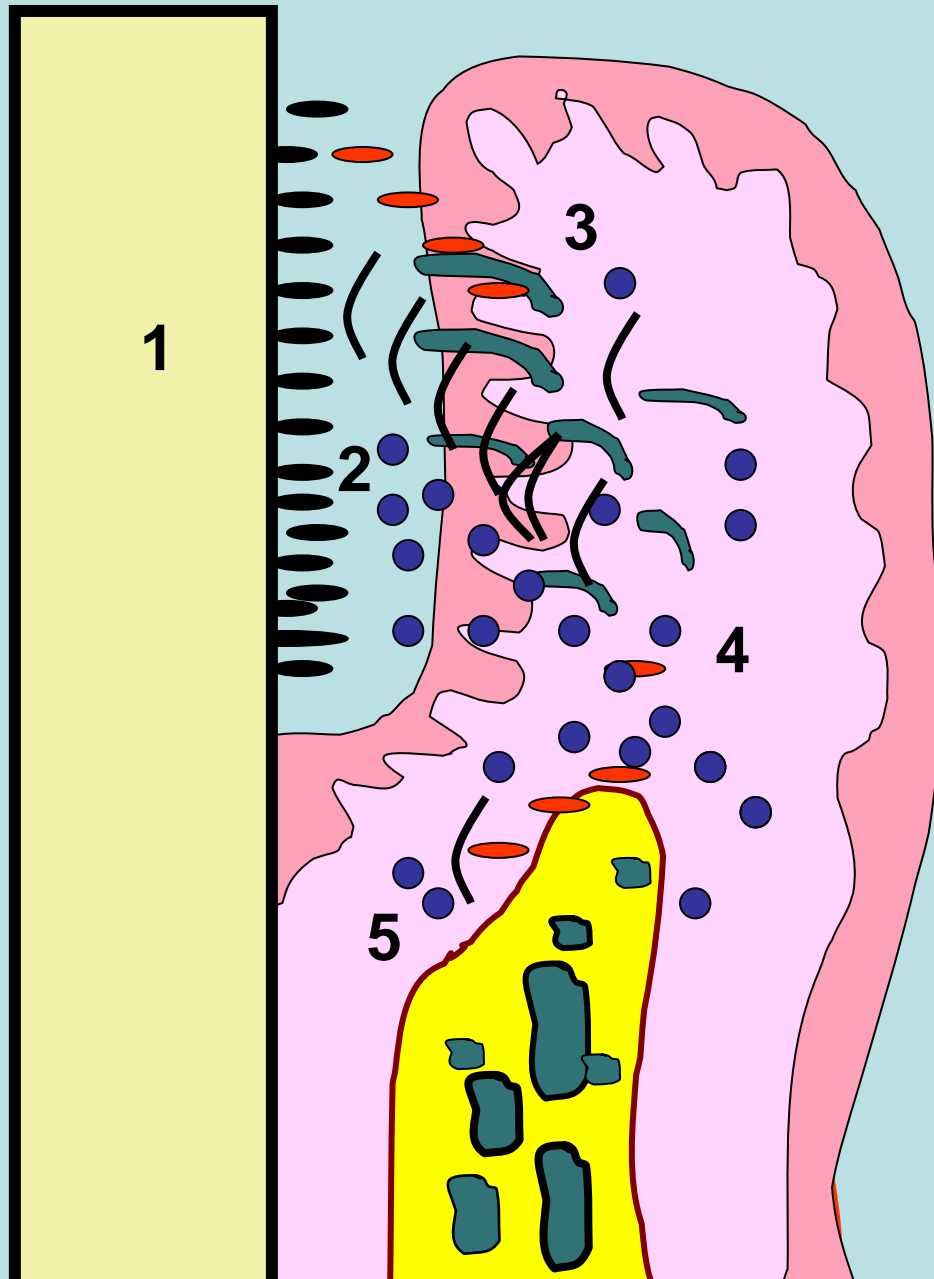
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CYTOKINE MODULATION BY PERIODONTOPATHOGENIC BACTERIA

- *P. gingivalis* endotoxin is less immunogenic
- AA can also modulate host immune response
- Kills PMN leukocytes by interacting on CD11a/CD18 receptors
- AA induces high level of IL-6 production by fibroblasts and IL-8
- Initial AA infection provokes a Th1 response and only after 30 days a more protecting Th2 response with Ig production and antiinflammatory cytokines

PROTEAZES PRODUCED BY PERIODONTOPATHOGENIC BACTERIA AND PERIODONTAL BREAKEDOWN

- PERIODONTOPATHOGENS PRODUCE *A LOT OF PROTEASES*
- *P. GINGIVALIS GINGIPAIN IS THE MOST AGRESSIVE ENZYME*
- *THE CONNECTIVE TISSUE NORMAL TURNOVER IS MAINTAINED BY NATIVE MMP AND ITS INHIBITORS SECRETED BY FIBROBLASTS, MONOCYTES*
- *PMN - MMP8, MMP9*
- *BACTERIAL PROTEASES RATHER ACTIVATE ENDOGENOUS PROTEASES THAN DIRECTLY DIGEST MATRIX*
- *P. GINGIVALIS CAN ACTIVATE MMP-1 FROM GINGIVAL FIBROBLASTS AND MMP8 FROM PMN LEUKOCYTES*
- *P GINGIVALIS CAN ALSO ACTIVATE MMP 2,*
- *BACTERIAL PROTEASES CAN ALSO INHIBIT NORMAL ENDOGENOUS PROTEASE INHIBITORS (A2 MACROGLOBULIN) AND ALSO CAN DIGEST RECEPTORS, IMMUNOGLOBULINS AND COMPLEMENT FACTORS*



1. Hard tissue associated

2. loose

3. Epithelial associated

4. Invasive

5. Bone associated

The most viable bacteria present in the central part of plaque, and lining the voids and channels (Auschill et al. 2001).

This more open architecture should enable molecules to readily move in and out of plaque

- **Histological sections of sub-gingival plaque viewed by conventional light microscopy show a complex organization of attached microorganisms**
- **tooth-associated**
- **epithelial cell-associated biofilms,**
- **a less dense zone of organisms between the two (Socransky & Haffajee 2002).**
- **may be more periodontal pathogens in the epithelial biofilm**

In deep periodontal pockets :

**the deepest zones were colonized mainly by
spirochaetes and Gram-negative bacteria**

**whereas shallow regions comprised predominantly
Gram-positive cocci (Wecke et al. 2000)**

SUBGINGIVAL BACTERIAL COMPLEXES

Actinomyces

V. pervula
A. odontolyticus

S. mitis
S. oralis
S. sanguis

Streptococcus

P. gingivalis
T. forshytia
T. denticola

C. rectus
P. intermedia
P. nigrescens
P. micros
F. nucleatum

E. nodatum

E. Corrodens
Campilobacter
A. actinomycetemcomitans

Sockransky 1998

THE PRINCIPLES OF MICROBIAL ECOLOGY

- BACTERIAL ECOSYSTEM
- HABITAT
- NICHES
- MICROBIAL SUCCESSION
- FACTORS LIMITING COLONIALIZATION
- DISSEMINATION OF THE ORGANISMS
- SUCCESSFUL COLONIALIZATION
- CLIMAX COMMUNITY

THE PRINCIPLES OF MICROBIAL ECOSYSTEM

- the receptors on the tooth surface are salivary proteins making up the acquired pellicle
- these proteins are differ from individual to individual
- the epithelial soft tissue is also differ from subject to subject
- Low crevicular fluid limit the growth of subgingival plaque while increased crevicular fluid will provide nutrients and foster the growth of *P. gingivalis* or *T. denticola*

DETERMINANTS OF SUBGINGIVAL BIOFILM

- **THE MICROBIAL COMPOSITION OF
SUBGINGIVAL BIOFILM**
- **THE INFLUENCE OF SURFACE ON THE
COMPOSITION**
 - **TOOTH, EPITHELIA, CREVICULAR FLUIDS**
- **THE BULK FLUID**
 - **SALIVA OR CREVICULAR FLUID**

THE PRINCIPLES OF MICROBIAL ECOLOGY

- BACTERIAL ECOSYSTEM
- HABITAT
- is the site at which a population of bacteria grows

THE PRINCIPLES OF MICROBIAL ECOLOGY

- **NICHES**
- the function of the bacteria in a habitat is its niche
- A bacterium can have one niche in one habitat and another niche in another habitat

THE PRINCIPLES OF MICROBIAL ECOLOGY

- MICROBIAL SUCCESSION
- Factors contributing to microbial succession:
 - Provision of nutrients
 - Altering the concentration of inorganic nutrients – like metals
 - Modifying the host tissues – like edema
 - Autointoxication
 - Elimination of organisms by physical means
 - Establishment of barriers

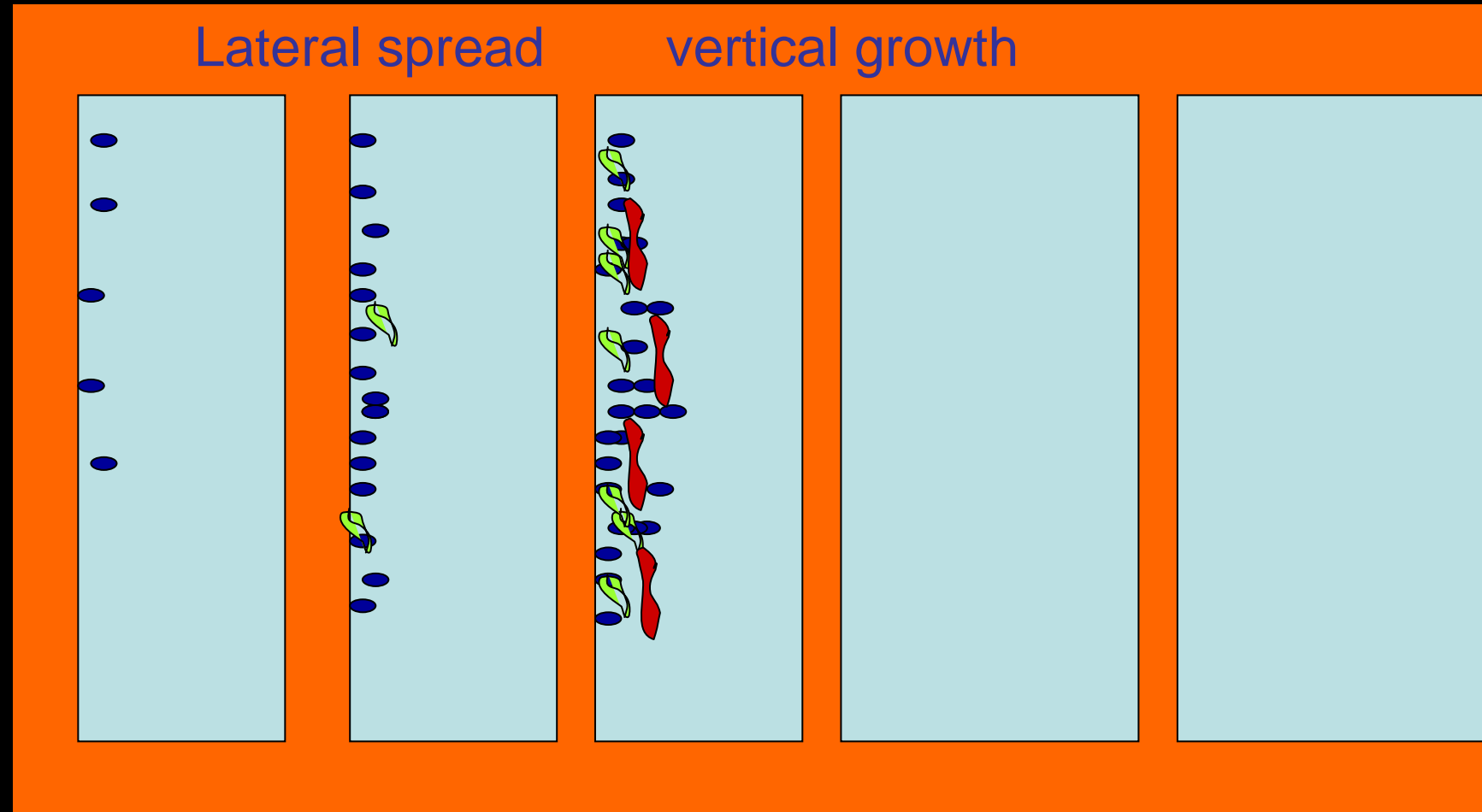
THE PRINCIPLES OF MICROBIAL ECOLOGY

- BACTERIAL ECOSYSTEM
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- **MICROBIAL SUCCESSION**
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- DISSEMINATION OF THE ORGANISMS
- SUCCESSFUL COLONIALIZATION
- CLIMAX COMMUNITY

THE PRINCIPLES OF MICROBIAL ECOLOGY

- **MICROBIAL SUCCESSION**
- **Autogenic succession** – the bacterial community changes the environment to favor the growth of other species
- **Allogenic succession** – the environment changes because of non microbial factors like restorations, pocket depth etc.

THE DEVELOPMENT OF DENTAL BIOFILM



ATTACHMENT

COAGGREGATION

SPREAD

THE PRINCIPLES OF MICROBIAL ECOSYSTEM

- the receptors on the tooth surface are salivary proteins making up the acquired pellicle
- these proteins are differ from individual to individual
- the epithelial soft tissue is also differ from subject to subject
- Low crevicular fluid limit the growth of subgingival plaque while increased crevicular fluid will provide nutrients and foster the growth of *P. gingivalis* or *T. denticola*

THE PRINCIPLES OF MICROBIAL ECOSYSTEM

- Early studies using electron microscopy, showed a compacted mass of microorganisms,
- Confocal laser scanning microscopy showed that supragingival plaque has a structured architecture
- Channels have been observed that link the plaque/oral environment interface to the tooth surface (Wood et al. 2000, Auschill et al. 2001, Zaura-Arite et al. 2001).

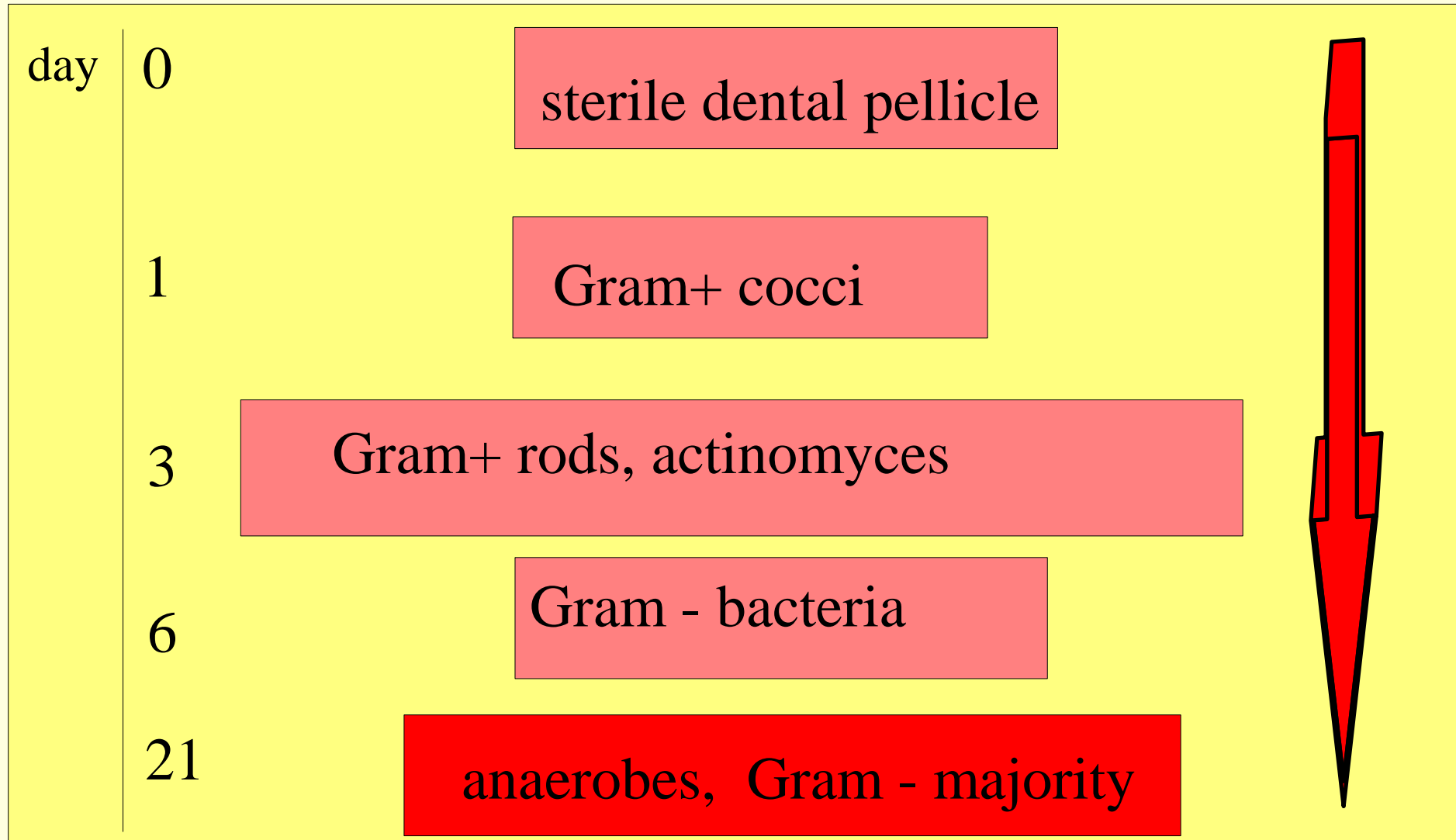
Biofilm regulation of gene expression

During the initial stages of biofilm formation by *S. mutans* (first 2 h following attachment), 33 proteins were differentially expressed (25 proteins were up-regulated; eight proteins down-regulated) (Welin et al. 2004).

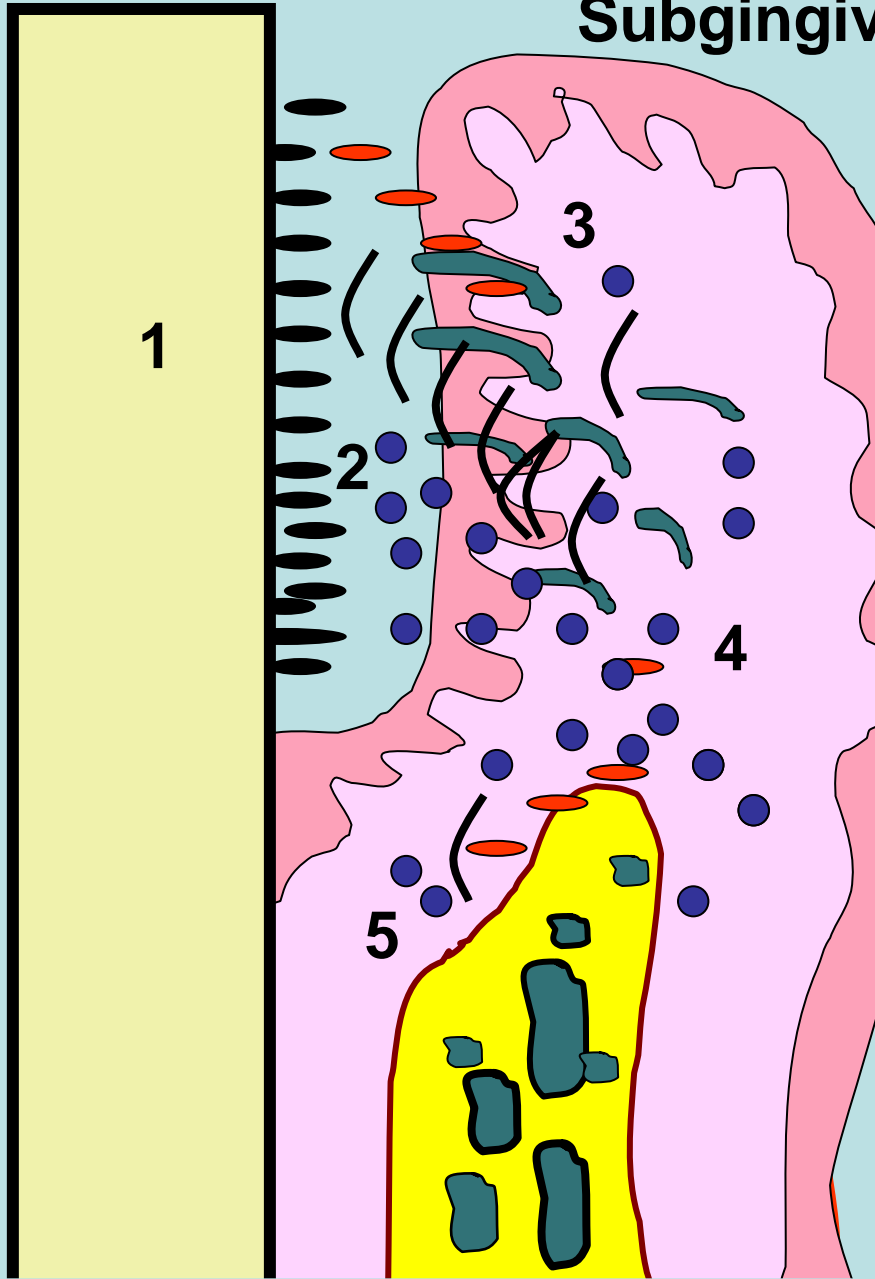
- There was an increase in the relative synthesis of enzymes involved in carbohydrate catabolism
- This is needed for energy production and act as adhesins on the cell surface.
- some glycolytic enzymes involved in acid production were down-regulated in older (3 day) biofilms, (Svensater et al. 2001).

- **In plaque, bacteria bind to many host proteins and co-aggregate with other organisms, can have potential impact on gene expression .**
- **Organisms from plaque have also been shown to communicate with one another in a cell density-dependent manner via small diffusible molecules, using strategies similar to those described for other biofilms**
- **Lysed cells in biofilms could act as donors of chromosomal DNA, thereby increasing the opportunity for horizontal gene transfer in dental plaque.**

THE MECHANISM OF PLAQUE ACCUMULATION



Subgingiv



Plaque/bacteria

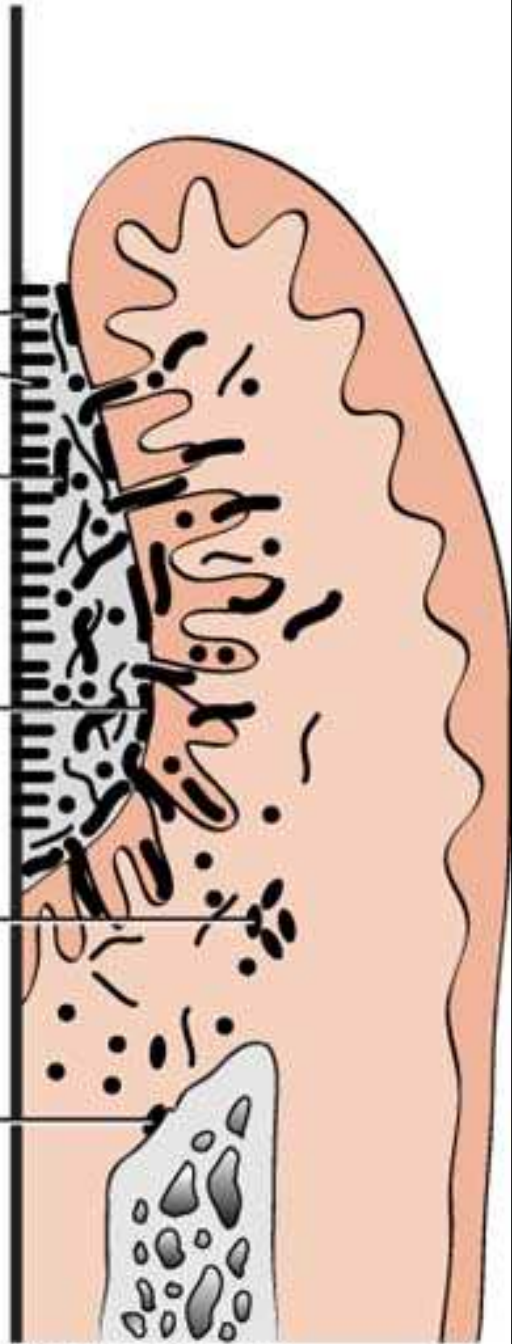
Tooth attached plaque

Unattached plaque

Epithelial associated plaque

Bacteria within connective tissue

Bacteria on bone surface



**PERIODONTAL DISEASE IS A POLYMICROBIAL
INFECTIOUS DISEASE**

**BENEFICIAL COMMENSAL AND PATHOGENIC
BACTERIA PLAY AN IMPORTANT ROLE IN THE
PATHOGENESIS OF PERIODONTAL DISEASE**



„COMPLEXES" OF BACTERIA ARE ASSOCIATED WITH EITHER HEALTH OR DISEASE

(Socransky et al. 1998, Socransky & Haffajee 2002).

Certain groups of bacteria are early colonizers of the tooth surface,

Others, such as members of the "red complex" (*Porphyromonas gingivalis*, *T. denticola*, *Tannerella forsythensis*), are associated more commonly with periodontal diseases, and are rarely detected in the absence of members of other "complexes" (e.g. the "orange complex"),

(Socransky et al. 1998, Socransky & Haffajee 2002).

**SUBGINGIVÁLIS
BAKTÉRIUM KOMPLEXEK**

Actinomyces

V. pervula
A. odontolyticus

S. mitis
S. oralis
S. sanguis
Streptococcus

P. gingivalis
T. forshytia
T. denticola

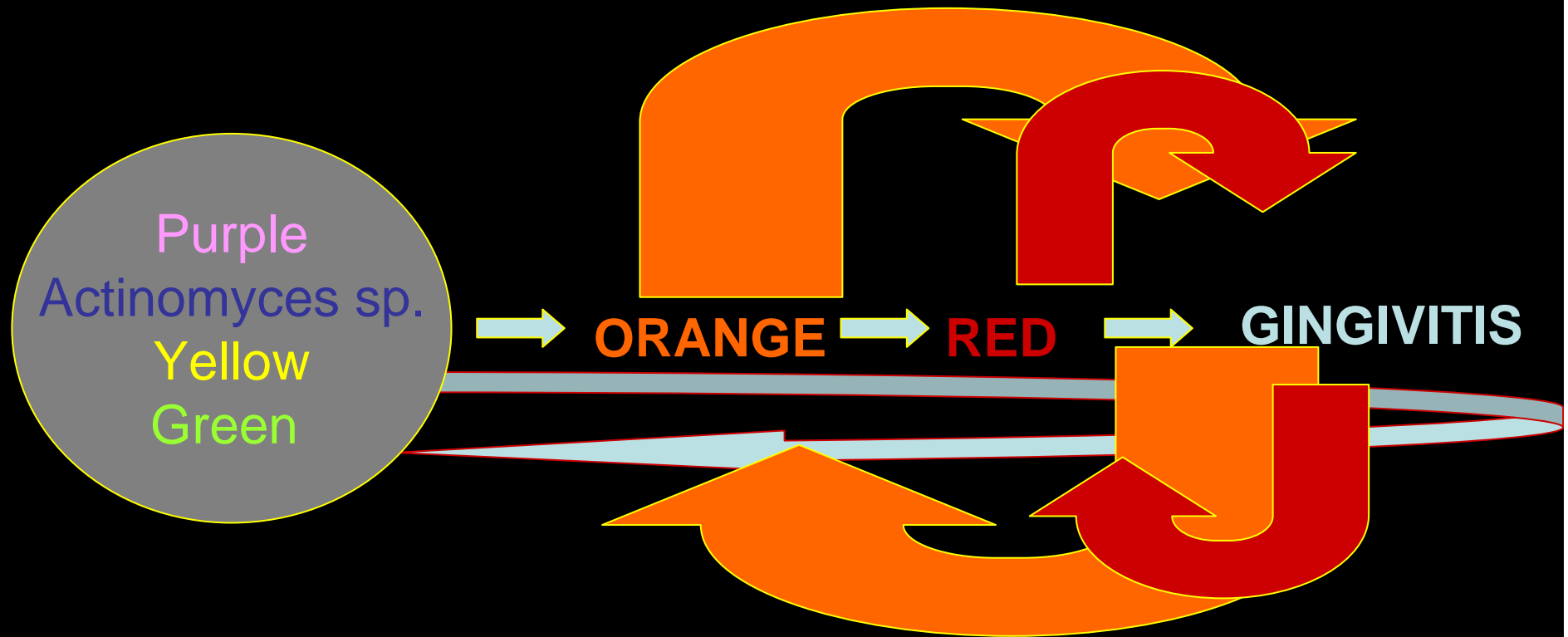
C. rectus
P. intermedia
P. nigrescens
P. micros
F. nucleatum
E. nodatum

E. Corrodens
Campilobacter
A. actinomycetemcomitans

Sockransky 1998

THE PRINCIPLES OF MICROBIAL ECOLOGY

- MICROBIAL SUCCESSION



COMMENSAL BACTERIAL FLORA

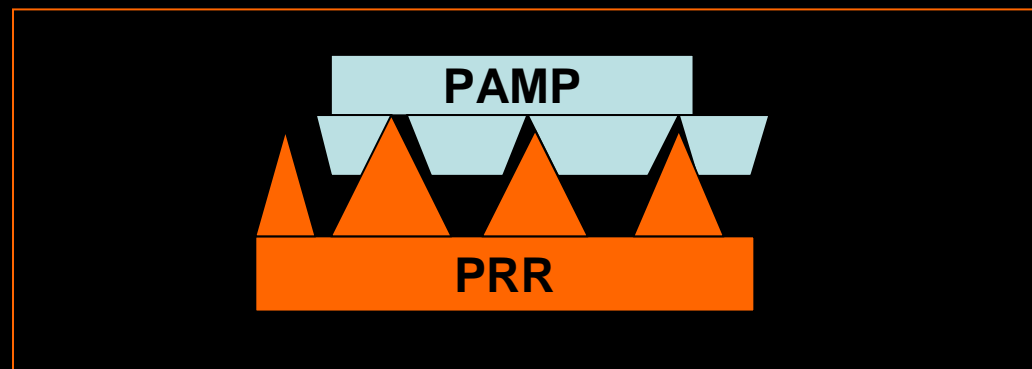
COMMENSAL MEANS – RELATIONSHIP BETWEEN ORGANISMS OF TWO DIFFERENT SPECIES IN WHICH ONE DERIVES NUTRIENTS FROM THE OTHERS WHILE THE OTHER REMAINS UNHARMED AND UNAFFECTED

COMMENSAL BACTERIA CAN BE PATHOGENIC IN OTHER LOCATIONS, LIKE ORAL OR GUT BACTERIA IN OTHER TISSUES

TOLL LIKE RECEPTORS AND THE COMMENSAL FLORA

Toll like receptors are to recognize **microbial molecular patterns**

by this host can discriminate between commensal and hostile bacteria



THE HUMAN HOST IS HEALTHY
DESPITE THAT THE 90% OF THE
CELLS IN THE HUMAN BODY
ARE BACTERIA (Henderson 1998)



COMMENSAL BACTERIA AND CYTOKINE PRODUCTION

- ALWAYS THE ACTUAL LEVEL OF PRO-AND ANTIINFLAMMATORY CYTOKINES AND OTHER FACTORS (PGE, NO etc.) CAN DETERMINE THE IMMUNE RESPONSE
- IL-2 PLAYS A CRUCIAL ROLE IN THE INITIATION OF INNATE REACTIONS
- COMMENSAL BACTERIA IN MUCOSAL CELLS INITIATE IL-2 SECRETION THAT IS IMPORTANT IN MAINTAINING HOMEOSTASIS BETWEEN COMMENSAL BACTERIA AND THE HOST

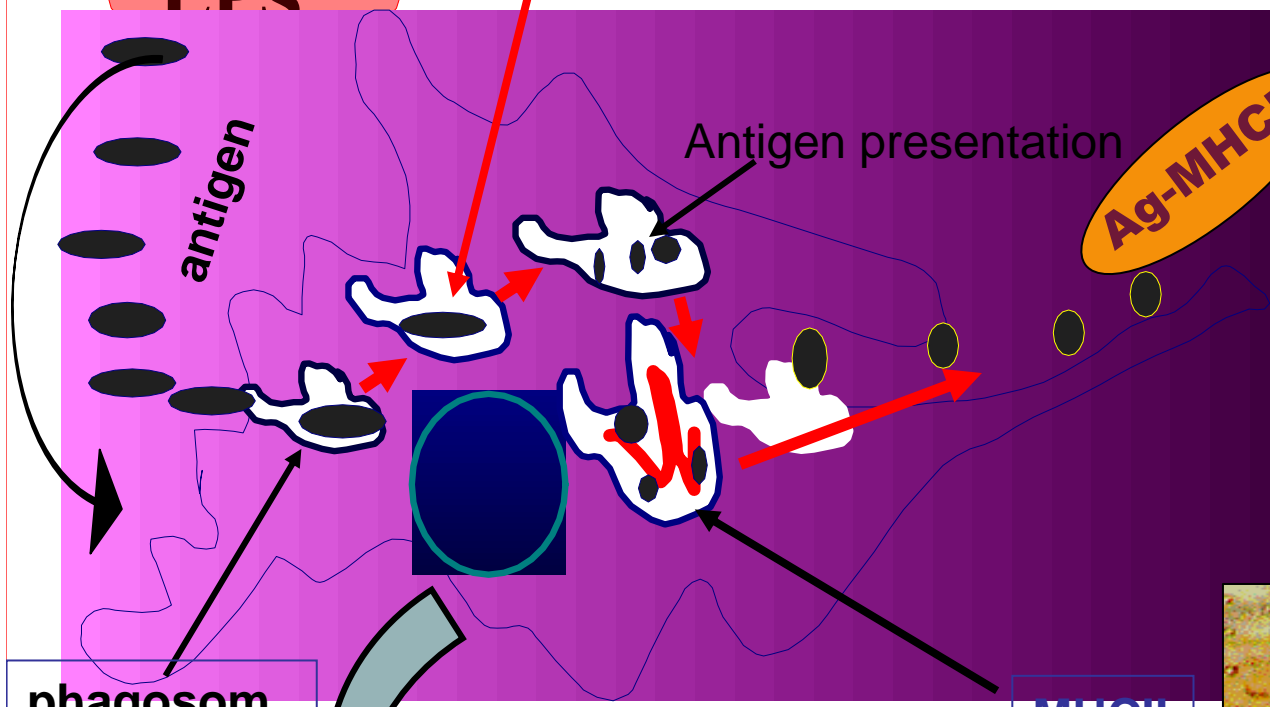
HOW COMMENSAL FLORA CAN MAINTAIN PERIODONTAL HEALTH?

- IN THE GUT THE NORMAL SALMONELLA FLORA CAN SUPPRESS LOCAL INFLAMMATORY CYTOKINE PRODUCTION
- IN THE ORAL CAVITY THE COMMENSAL FLORA CAN PREVENT THE HOST TO ACTIVATE THE IMMUNE SYSTEM
- BACTERIA CAN STIMULATE SUPPRESSIVE T LYMPHOCYTES TO PRODUCE INHIBITORY CYTOKINES (TGF, IL-10)
- **THE MAJOR DIFFERENCE BETWEEN COMMENSAL AND PATHOGENIC BACTERIA IS THAT THE ONE ELICIT IMMUNE TOLERANCE THE OTHER PROVOKE ADAPTIVE IMMUNE REACTION**
- COMMENSAL BACTERIAL ANTIGEN PRESENTATION TO DENDRITIC CELLS HAPPENS WITHOUT COSTIMULATION

T LYMPHOCYTA

Fago-lisosoma

LPS



antigen

Antigen presentation

Ag-MHCII

TCR

CD28

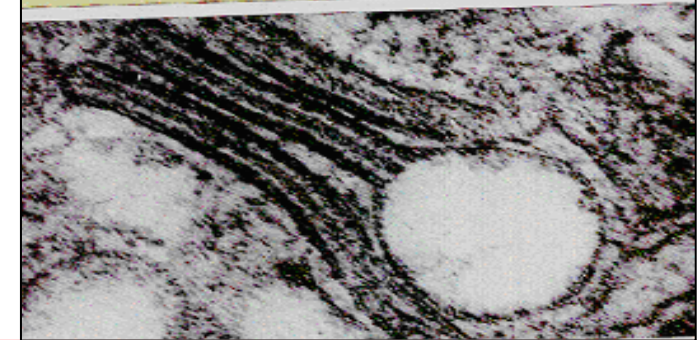
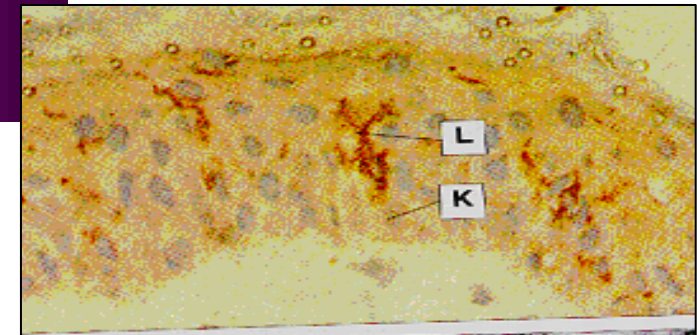
COSTIMULATION

phagosom

MHCII

MACROPHAGE

IL-1, IL-6, IL-8, IL-12, THF α TFG β , INF γ , FGF



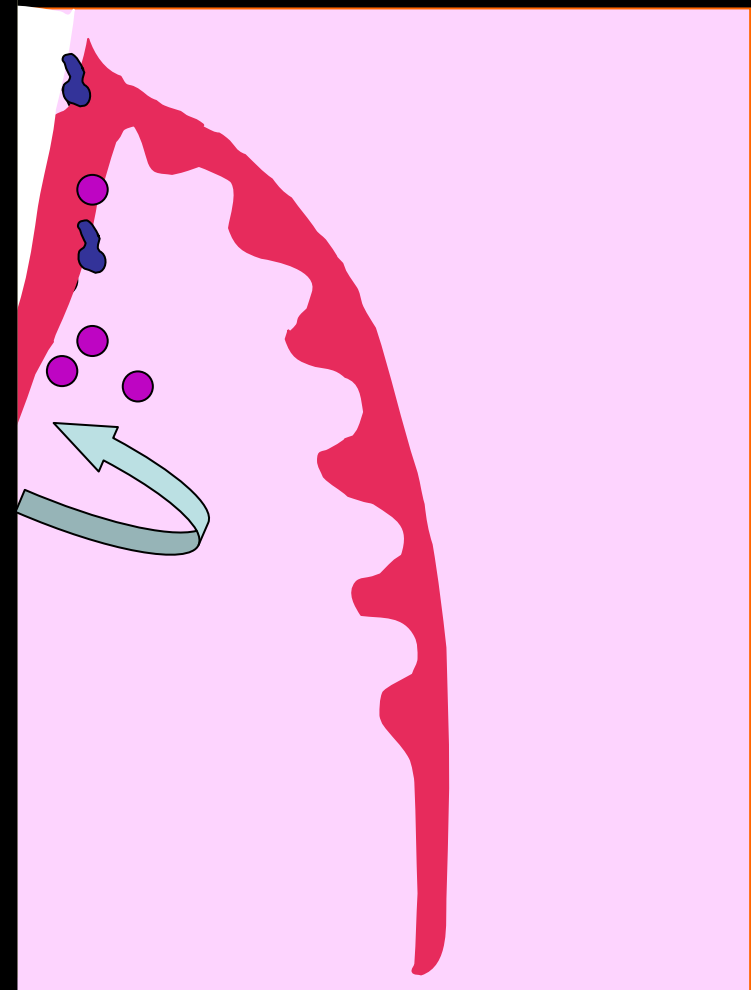
In the presence of commensal bacteria the mucosal antigen presenting cells suppress inflammation by maintaining low level of costimulatory molecules and favors Th2 cytokin production

FUSOBACTERIUM NUCLEATUM CAN ENHANCE MHC II EXPRESSION BUT DECREASES THE PRODUCTION OF COSTIMULATORY MOLECULES

P. GINGIVALIS WILL ENHANCE THE PRODUCTION OF COSTIMULATORY MOLECULES

COMMENSAL BACTERIA AND CYTOKINE PRODUCTION

- **COMMENSAL BACTERIA CAN PRODUCE**
- **CONSTANT IL 8 PRODUCTION** → **MILD PMN LEUKOCYTE EMIGRAION**
- **ADHESION MOLECULES** - → **ICAM-1, LCAM-1, PLATELET ADHESION MOLECULE 1, THAT ASSIST RECRUITING PMN LEUKOCYTES**
- **EPITHELIAL DERIVED ANTIBACTERIAL PEPTIDES** – → **DEFENSINS** –
- **THOSE BACTERIA ARE IMMUNE TO THOSE AGENTS !!!!!**



COMMENSAL BACTERIA AND EPITHELIAL ANTI-MICROBIAL PEPTIDES

- The harmless early colonizers of the dental pellicle (*streptococci*) keep unwanted pathogens off the plaque.
- Commensal bacteria promote the production of antimicrobial peptides that kill pathogens but has no effect on commensal bacteria
- *F. nucleatum* can protect the epithelial cells from *P. gingivalis* invasion by enhancing defensin-2 production
- Human *F. nucleatum* itself is resistant to defensins

COMMENSAL BACTERIA AND EPITHELIAL ANTI-MICROBIAL PEPTIDES

- Human *F. nucleatum* itself is resistant to defensins
- In other tissues human *defensin-2* can only be expressed in inflammation but in the gingiva it is expressed in clinical health
- It can be due to the phylogenetic coexistences of the human oral cavity and *F. nucleatum*
- *F. nucleatum* can invade amniotic fluid and infect fetus and also can cause widespread infection in other tissues

**SUBGINGIVÁLIS
BAKTÉRIUM KOMPLEXEK**

Actinomyces

V. pervula
A. odontolyticus

S. mitis
S. oralis
S. sanguis

Streptococcus

P. gingivalis
T. forshytia
T. denticola

C. rectus
P. intermedia
P. nigrescens
P. micros
F. nucleatum

E. nodatum

E. Corrodens
Campilobacter

A. actinomycetemcomitans

Sockransky 1998

PERIOPATHOGENIC BACTERIA AND PERIODONTAL TISSUE

The composition of the dental plaque dictates the degree of which the periodontium breaks down

Red complex bacteria

T. denticola

T. forsythia

P. gingivalis

The ability to- colonize subgingivally

The invasive capacity

Proteases and exotoxin production

To induce destructive immune reactions

Gene expression can alter markedly when cells form a biofilm,

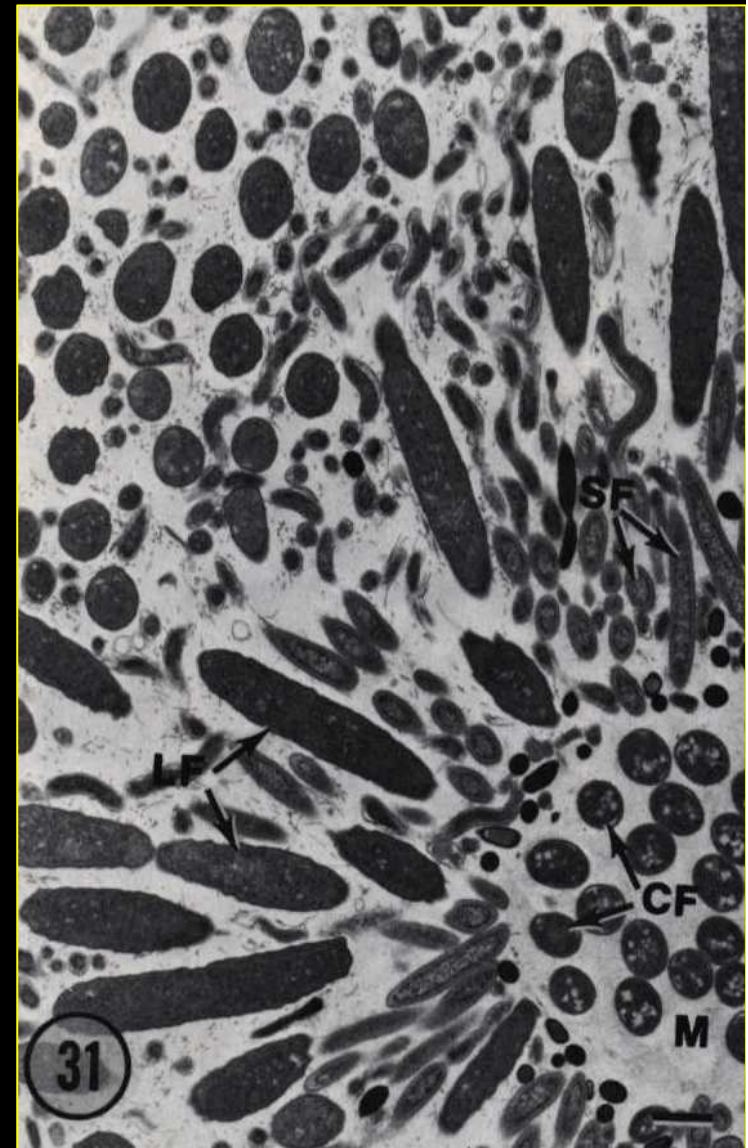
resulting in many organisms having a radically different phenotype following attachment to a surface

when compared with conventional liquid grown (planktonic) cells.

(Whiteley et al. 2001)



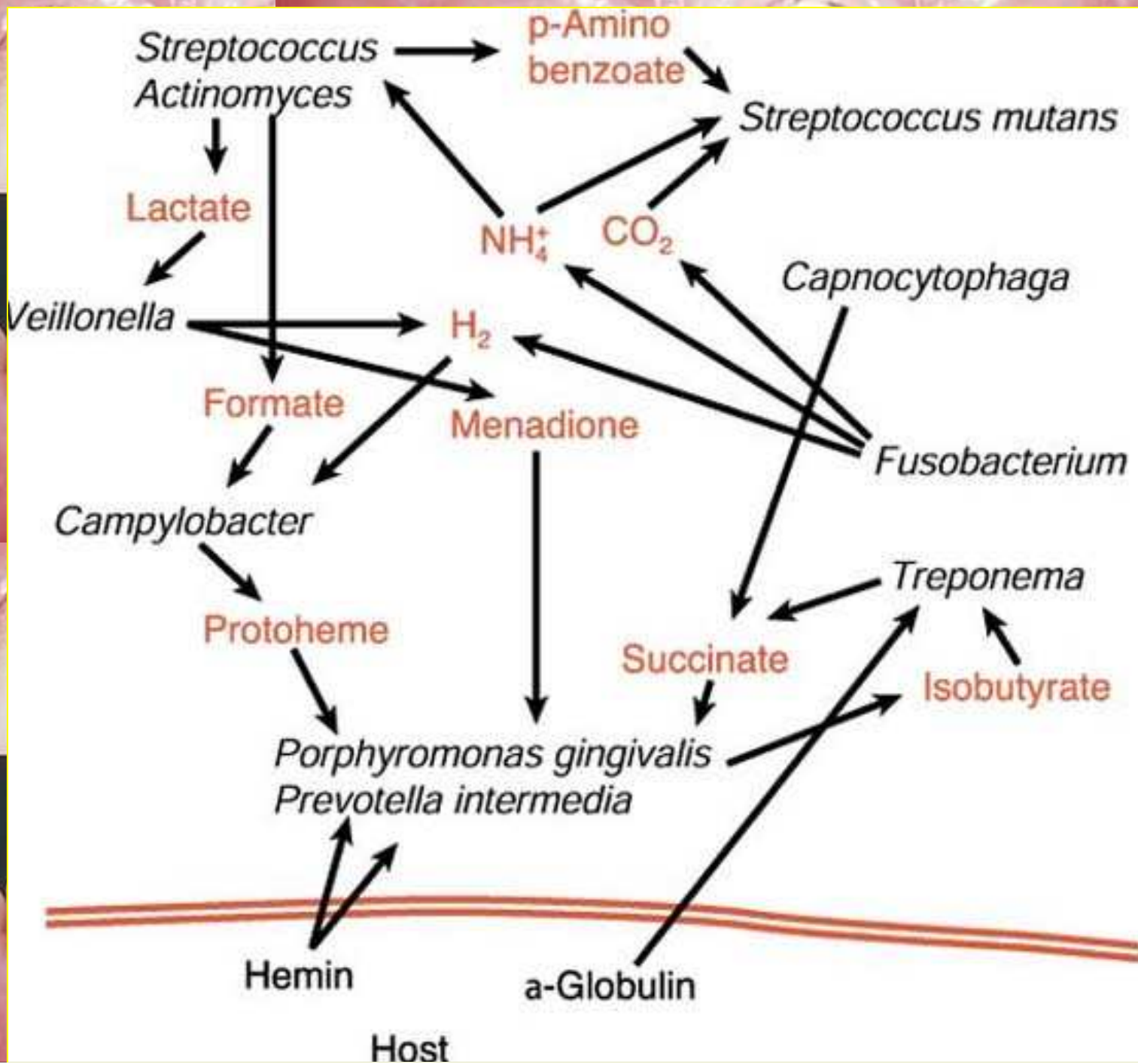
- The binding of bacteria to specific host receptors can also trigger significant changes in host cell patterns of gene expression (Abraham et al. 1998).
- As the biofilm matures, there is continued synthesis of exopolymers to form an **extracellular matrix**.
- The **matrix** is not only important physically as part of the scaffolding that determines the structure of biofilms, but it is also biologically active and can retain nutrients, water (thereby preventing desiccation) and key enzymes within the biofilm
- (Allison 2003, Branda et al. 2005).



This community life-style provides enormous potential benefits to the participating organisms

(Caldwell et al. 1997, Shapiro 1998, Marsh & Bowden 2000).

- (a) The metabolism of early colonizers alters the local environment, making conditions suitable for attachment and growth of later (and sometimes more fastidious) species. Thus, the diversity of the microflora increases over time because of microbial succession.
- (b) An increased metabolic diversity and efficiency; molecules that are normally recalcitrant to catabolism by individual organisms can often be broken down by microbial consortia
- (c) An enhanced resistance to environmental stress, antimicrobial agents and the host defences.



- **Within biofilms, sophisticated systems of**

- **CELL-TO- CELL COMMUNICATION**

are used by some bacteria to co-ordinate gene expression.

- **Gram-positive bacteria generally communicate via small diffusible peptides (Sturme et al. 2002),**

- **Gram-negative bacteria secrete acyl homoserine lactones (AHLs) (Whitehead et al. 2001),**

- **AHLs are involved in quorum sensing whereby cells are able to modulate gene expression in response to increases in cell density.**

An important clinical consequence of both the structural organization of biofilms and the subsequent altered pattern of gene expression therein is the **reduced susceptibility of cells to antimicrobial agents**

(Gilbert et al. 1997, 2002, Ceri et al. 1999, Stewart & Costerton 2001).



Recent studies suggest that the environmental heterogeneity generated within biofilms promotes accelerated genotypic and phenotypic diversity (even in mono-species biofilms of *P. aeruginosa*)

that provides a form of "biological insurance" that can safeguard the "microbial community" in the face of adverse conditions



This diversity can affect several key properties of cells,

- motility,
- nutritional requirements,
- secretion of products,
- detachment,
- biofilm formation;

this diversity better equips an organism or community to survive an environmental stress.

•Conventionally, the sensitivity of bacteria to antimicrobial agents is determined on cells grown in liquid culture by the measurement of the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC).

•Numerous studies have shown that the MIC of an organism growing on a surface can range from 2- to 1000-fold greater than the same cells grown planktonically

•(Stewart & Costerton 2001, Johnson et al. 2002).

ANTIMICROBIAL RESISTANCE

Bacteria growing in dental plaque also display an increased tolerance to antimicrobial agents, including those used in **dentifrices and mouthrinses**

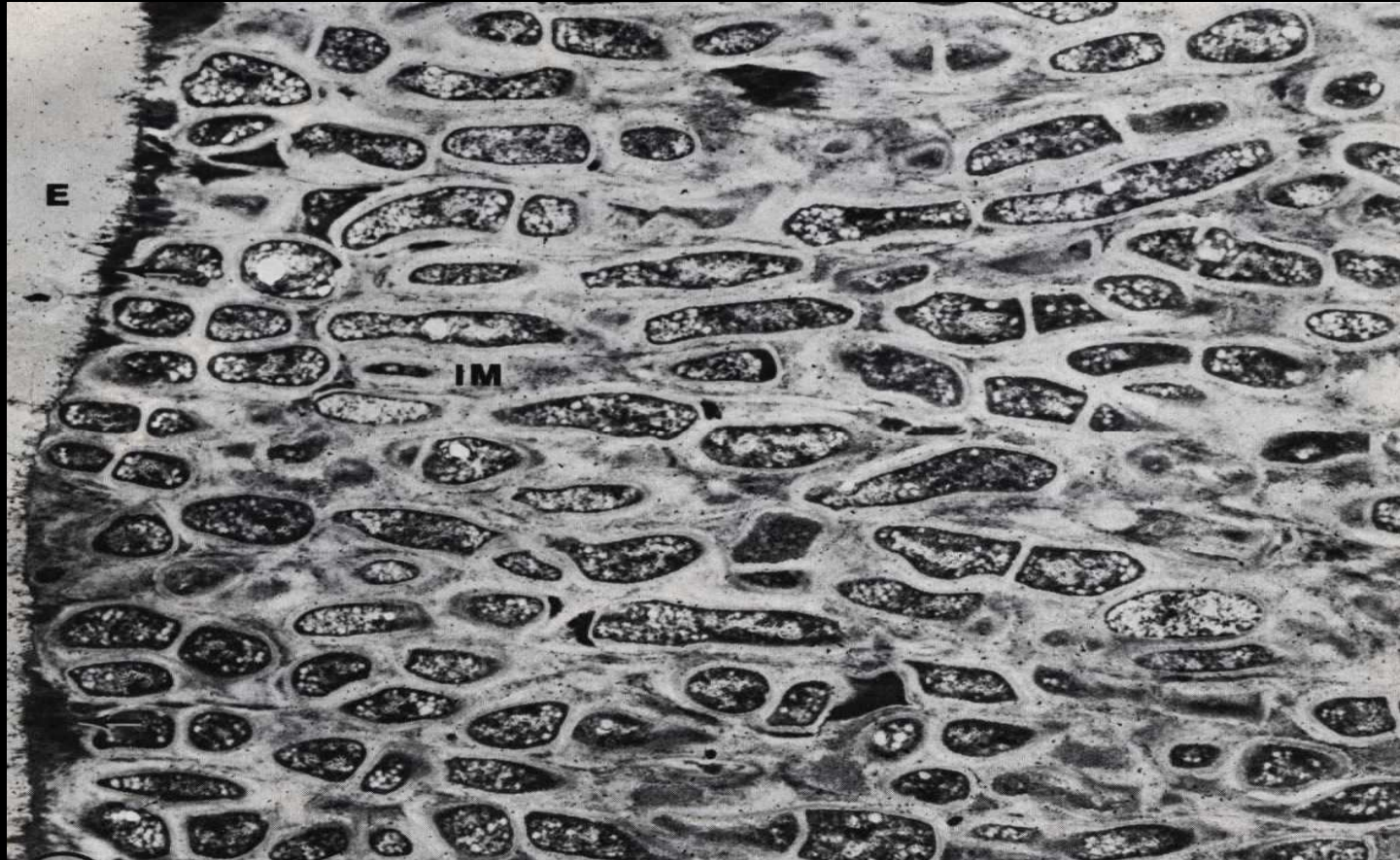
(Marsh & Bradshaw 1993, Kinniment et al. 1996, Wilson 1996, Pratten & Wilson 1999).

The BIC for chlorhexidine and amine fluoride was 300 times and 75 times greater, respectively, when *S. sobrinus* was grown as a biofilm compared with the MBC of planktonic cells

(Shani et al. 2000).

The age of the biofilm can also be a significant factor; older biofilms (72 h) of *S. sanguinis* were more resistant to chlorhexidine than younger (24 h) biofilms

(Millward & Wilson 1989).



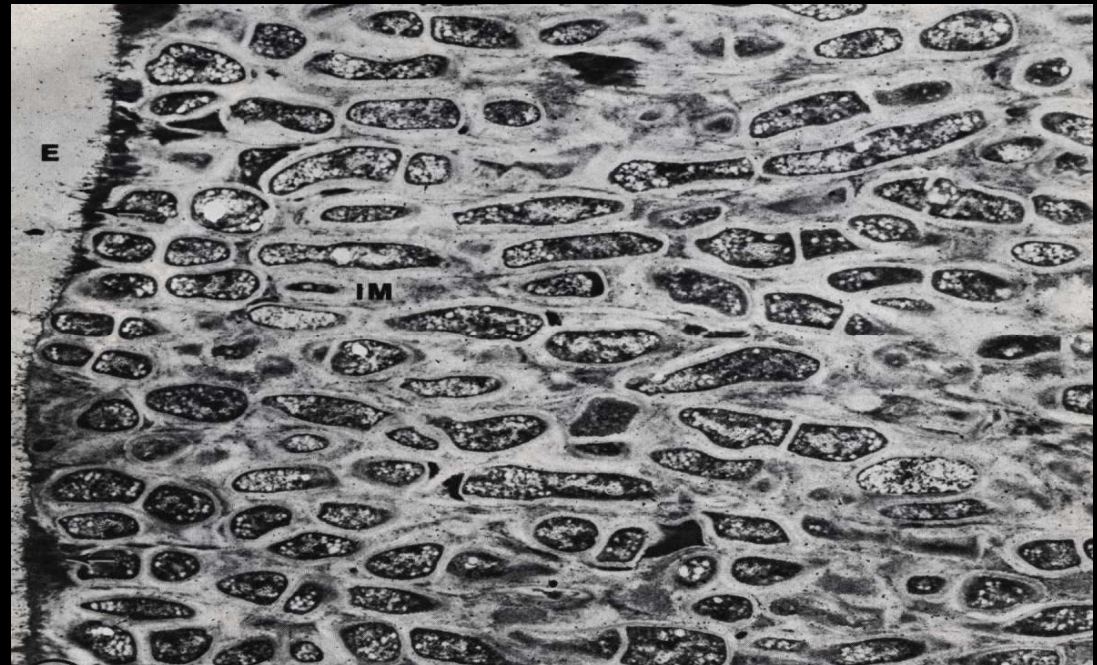
Biofilms of oral bacteria are also more tolerant of antibiotics than planktonic cells

(e.g. amoxicillin, doxycycline, minocycline, metronidazole)

(Larsen 2002, Socransky & Haffajee 2002, Noiri et al. 2003),

Biofilms of *P. gingivalis* tolerated 160 times the MIC of metronidazole that had been determined for planktonic cells

(Wright et al. 1997),



it would be more appropriate to determine the "biofilm inhibitory concentration" (BIC) of an agent (also described as the "biofilm eradicating concentration" or biofilm killing concentration)

(Anwar & Costerton 1990, Nichols 1994, Johnson et al. 2002).

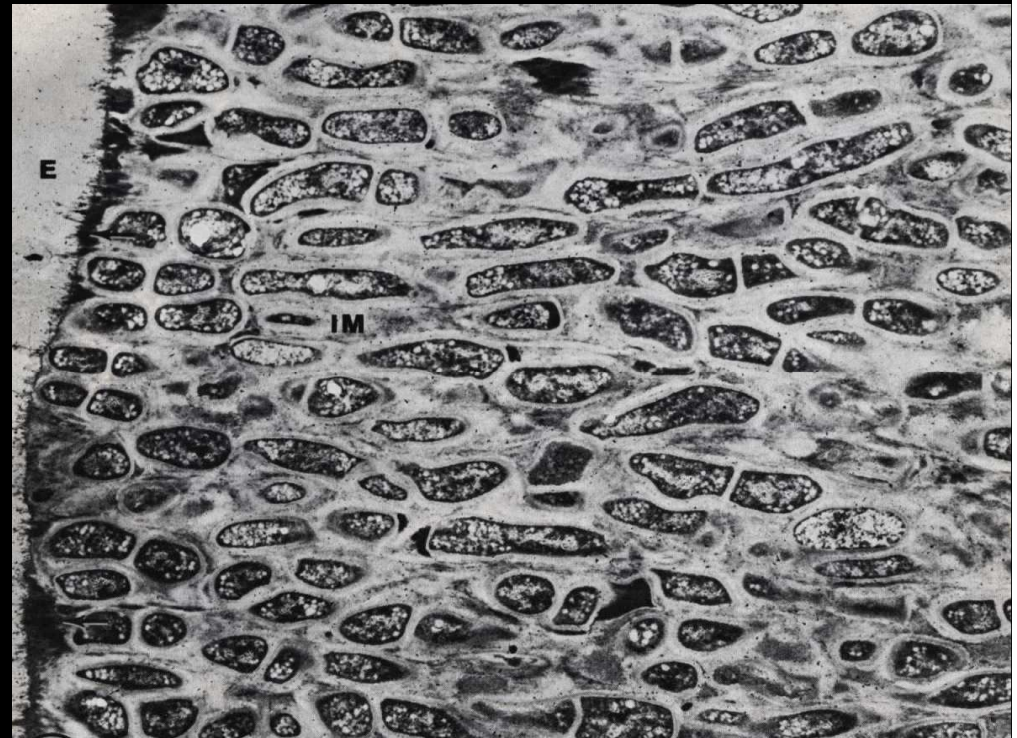


why,???

- The structure of a biofilm may restrict the penetration of the antimicrobial agent;
- charged inhibitors can bind to oppositely charged polymers that make up the biofilm matrix (diffusionreaction theory).
- The agent may also adsorb to and inhibit the organisms at the surface of the biofilm, leaving cells in the depths of the biofilm relatively unaffected.
- The matrix in biofilms can also bind and retain neutralizing enzymes (e.g. -lactamase) at concentrations that could inactivate an antibiotic or inhibitor (Allison 2003).

Bacteria grow only slowly under nutrient-depleted conditions in an established biofilm, and, as a consequence, are much less susceptible than faster dividing cells.

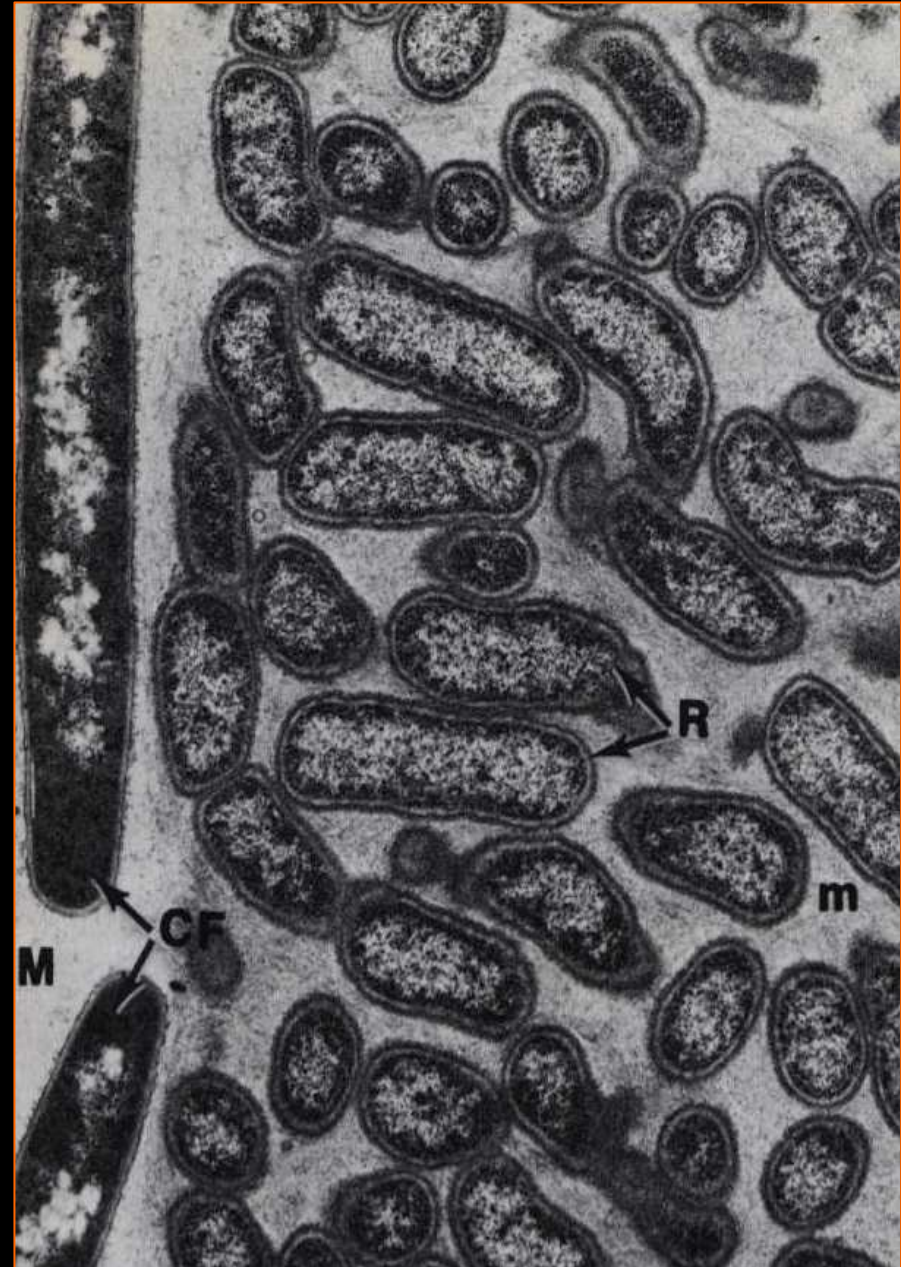
The environment in the depths of a biofilm may be unfavourable for the optimal action of some drugs (Gilbert et al. 2002).



Neighbouring cells of a different species can produce neutralizing enzymes (-beta lactamase, IgA protease, catalase, etc.) that protect inherently susceptible organisms from inhibitors (Brook 1989).

A penicillin-sensitive pathogen (*Streptococcus pyogenes*) can be protected during antibiotic treatment by -beta lactamase produced by other commensal strain (*Moraxella catarrhalis*)

(Hol et al. 1994).



During the inflammatory response to plaque accumulation, there is an increase in the flow of gingival crevicular fluid; this not only delivers components of the host defences but also provides an array of novel nutrients (proteins and glycoproteins) that favour the growth of organisms with an asaccharolytic metabolism

A further consequence of this metabolism is a rise in local pH and a reduction in redox potential.

Collectively, these changes in environment will selectively enrich for the proteolytic organisms associated with inflamed sites

CHEMICAL PLAQUE CONTROL

- ***chemoprophylaxis*** →



- ***chemotherapy*** →



CHEMOPROPHYLAXIS

to sustain the normal ecological balance of the oral cavity and control bacterial colonization .

CHEMOTHERAPY

kill subgingival bacteria
control bacterial invasion into the deeper periodontal tissues
assist periodontal healing

Periodontal chemoprophylaxis

Non-selective with total bacterial eradication

Non-selective with marked oral bacterial reduction

.

Selective chemoprophylaxis .

Ideal anti-plaque chemical or biological agent

- Can permanently inhibit bacterial adhesion
- The agent can penetrate and reach plaque bacteria
- Substantive
- Do not alter normal oral bacterial ecology
- Do not have cumulative or chronic irritative effects

aauthor	Time month	No of cases	agent	Plaque reduction	Gingivitis reduction
Grossman 1989	6	481	CHX 0,12%	49 %	31 %
Grossman 1986	6	380	CHX 0,12%	61 %	39 %
Löe és mts 1976	24	120	CHX 0,2%	45 %	27 %
Lang és mts1982	6	158	CHX 0,1% CHX 0,2%	16 % 19 %	67 % 80%
Gordon és mts 1985	9	85	Listerine EO	19,5%	23,9%
Lamster és mts 1983	6	145	Listerine EO	22,2%	28,2%
Overholser és mts 1990	6	124	Listerine EO	36,1%	35,9%
Charles és mts 2001	6	316	Listerine EO	56,1%	22,9%
DePaola és mts 1989	6	107	Listerine EO	34%	34%

ADHERENCE OF PERIODONTOPATHOGENS TO PERIODONTAL TISSUES

P. Gingivalis

- *P. Gingivalis* fimbriae mediate binding of the bacteria to epithelial cells
- Carboxy terminal of the fimbriin is responsible for the binding to host proteins – adhesins, integrins, fibronectin
- By binding to integrins could damage the normal turnover of the connective tissue
- *P. gingivalis* can bind to different bacterial surface proteins
- *P. gingivalis* fimbrellins is important for epithelial tissue invasion

In *S. mutans*, quorum sensing is mediated by a competence stimulating peptide (CSP) (Li et al. 2001).

This peptide also induces genetic competence in *S. mutans* so that the transformation frequency of biofilm-grown *S. mutans* was 10-600-fold greater than for planktonic cells (Li et al. 2002b).

- **CSP is also directly involved in biofilm formation;**
- **mutants in some of the genes involved in the CSP signalling system (*comC*, *comD*, *comE* and *comX*) produce defective biofilms.**
- **The quorum sensing system also functions to regulate acid tolerance in *S. mutans* biofilms (Li et al. 2002a).**
- ***S. mutans*, upon exposure to low pH, could release CSP, and initiate a co-ordinated "protective" response among neighbouring cells to such a potentially lethal stress.**

Mutants of the *luxS* gene that encodes for the AI-2 synthase in *S. mutans* and *S. gordonii* had an impaired ability to produce monospecies biofilms in vitro (Blehert et al. 2003, Merritt et al. 2003)

Several periodontal bacteria (*Fusobacterium nucleatum*, *Prevotella intermedia*, *P. gingivalis*, *Actinobacillus actinomycetemcomitans*) secrete a signal related to AI-2 (Fong et al. 2001, Frias et al. 2001).

In *A. actinomycetemcomitans*, *luxS*-dependent signalling induced expression of leukotoxin and a transport protein involved in iron acquisition.

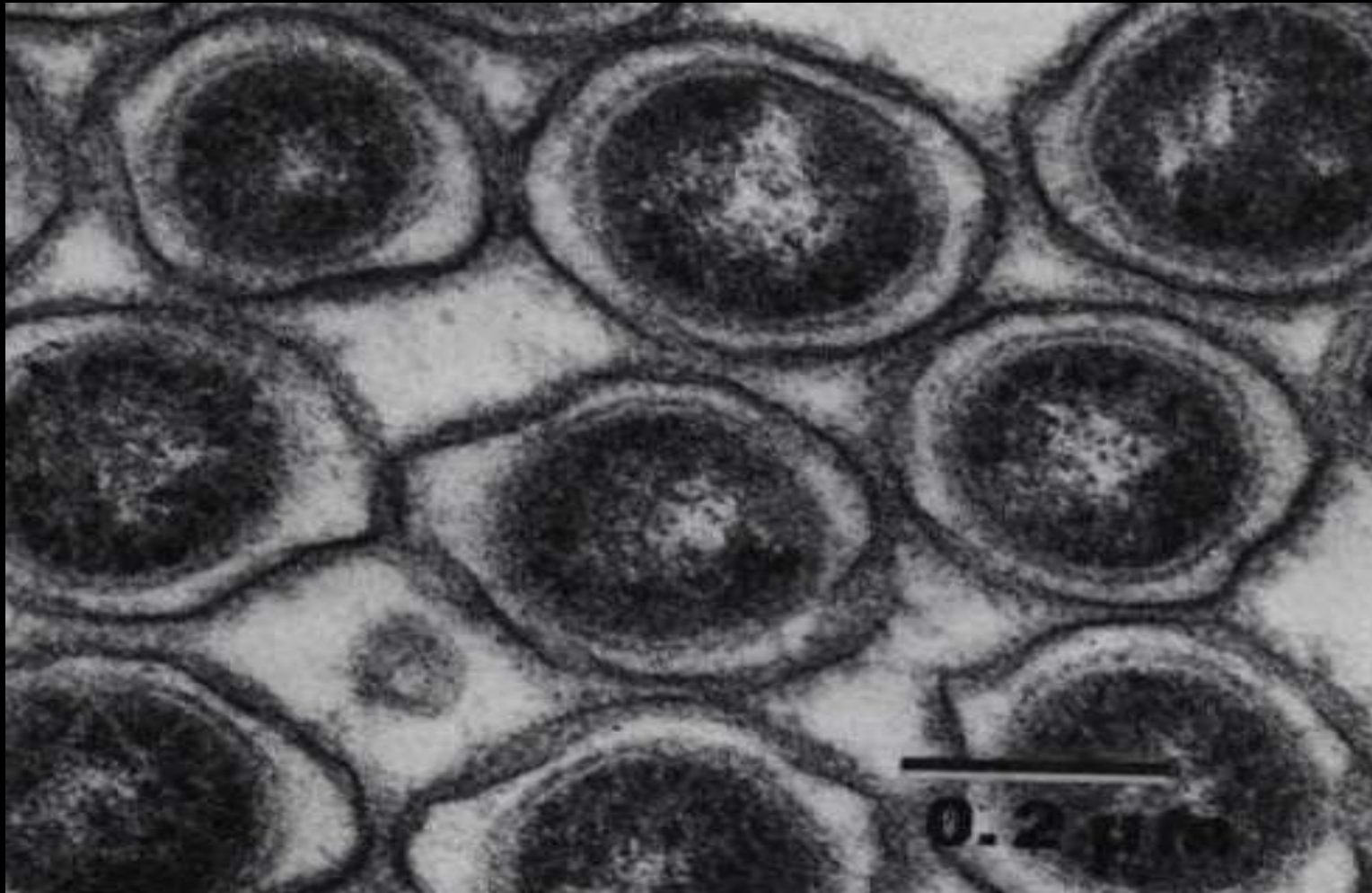
Cells also "communicate" and interact with one another in biofilms via horizontal gene transfer.

Signalling molecules such as CSP markedly increase the ability of recipient cells in biofilms to take up DNA (Li et al. 2002b).

The transfer of conjugative transposons encoding tetracycline resistance between streptococci has been demonstrated in model biofilms (Roberts et al. 2001).

The recovery of resident (*S. mitis*, *S. oralis*) and pathogenic (*S. pneumoniae*) bacteria from the nasopharynx with penicillin resistance genes showing a common mosaic structure confirms that gene transfer can occur in vivo (Dowson et al. 1990, Hakenbeck et al. 1998

These findings suggest that plaque can function as a "genotypic reservoir" by harbouring transferable mobile elements and genes.



Communication is not just between bacterial cells.

Surface components of sub-gingival bacteria are involved in adhesion to epithelial cells at the start of colonization and biofilm formation, and there is also evidence that they are involved in bacterium-host cell cross-talk.

Fimbriated *P. gingivalis* cells can induce formation of integrin-associated focal adhesions with subsequent remodelling of the actin and tubulin cytoskeleton in primary gingival epithelial cells (Yilmaz et al. 2003)

Plaque as a community

Oral bacteria do not exist as independent entities but rather function as a co-ordinated, spatially organized and metabolically integrated microbial community (Marsh & Bradshaw 1999, Marsh & Bowden 2000)

Benefits of a community life-style to plaque

(a) a broader habitat range for growth, e.g. oxygen-consuming species such as *Neisseria* spp. create environmental conditions suitable for colonization in plaque by obligate anaerobes (Bradshaw et al. 1996).

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(b) A more efficient metabolism, e.g. many complex host macromolecules, especially glycoproteins such as mucins, can only be degraded efficiently by consortia of oral bacteria (Bradshaw et al. 1994).

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Benefits of a community life-style to plaque

c) Increased resistance to stress and antimicrobial agents.

a sensitive organism can be rendered as being apparently "resistant" to an antibiotic if neighbouring, non-pathogenic cells produce a neutralizing or drug-degrading enzyme ("indirect pathogenicity")

In the mouth, GCF can contain sufficient β -lactamase to inactivate the concentrations of antibiotic delivered to the site (Walker et al. 1987, Herrera et al. 2000).

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Benefits of a community life-style to plaque

d) communities with varying bacterial composition have been found at sites with similar disease, and would be consistent with the concept of "complexes" associated with health and disease (Socransky & Haffajee 2002).

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Benefits of a community life-style to plaque

The shift towards communities containing increased proportions and numbers of anaerobic and proteolytic bacteria, as seen in periodontal disease, could be explained by the response of sub-gingival biofilms to changes in local environmental conditions and host responses. ")