PERIODONTAL MICROBIOLOGY

Dr. Parag Hadge

INTRODUCTION

- More than 500 microbial species in oral cavity
- Some beneficial, some harmful
- Inside uterus – fetus – sterile
- At birth – acquires vaginal and fetal microorganisms
- By 2 weeks – mature microbiota formed

- After 2 years – complex microbiota formed – approximately 10^{14} microbial cells present – from this point our body has 10 times more bacteria than human cells
- For healthy being – bacterial population comprises 2 kg of total body weight – brain 1.4 kg

- 2nd day onwards – anaerobic bacteria detected - increases gradually
- *Streptococcus salivarius* and *Streptococcus mitis* – first and most dominant microbes to colonize oral cavity of infants

ORAL CAVITY AND MICROORGANISMS

- *Streptococcus salivarius*
- *Streptococcus mitis*
- *Streptococcus Sanguinis*
- *Streptococcus pneumoniae*
- ***Fusobacterium nucleatum***
- ***Porphyromonas gingivalis***
- ***Prevotella intermedia***
- ***Tanerella forsythia***
- ***Aggregatibacter actinomycetemcomitans***
- ***Treponema pallidum***

- Oral cavity communicates – oral pharynx – **Open Growth System**
- Equilibrium among – bacterial adhesion Vs host removal forces e.g. GCF, ciliary motion etc.
- ***Ability of microorganism to adhere to host – crucial*** – for induction of disease/inflammation

Six major ecosystems – niches

1. Intraoral supragingival hard surfaces – teeth, implants, restorations, prosthesis
2. Subgingival regions – periodontal / periimplant pocket
3. Buccal, palatal epithelium and epithelium of floor of mouth
4. Dorsum of tongue
5. Tonsils
6. Saliva

BIOFILM

- Biofilms are composed of microbial cells encased within a matrix of extracellular polymeric substances (EPS) such as polysaccharides, proteins, and nucleic acids.

Bacteria in biofilm are ***1000 times*** more resistant to antimicrobial agents than their planktonic counterparts

1. One organism removes – waste products of other – utilizes them to make energy
2. They compete with each other in – secreting antimicrobial products e.g. bacteriocins, H_2O_2
3. Cell to cell signaling – ***quorum sensing***
4. DNA exchange

FEATURES OF BIOFILMS

Biofilms are heterogenous?

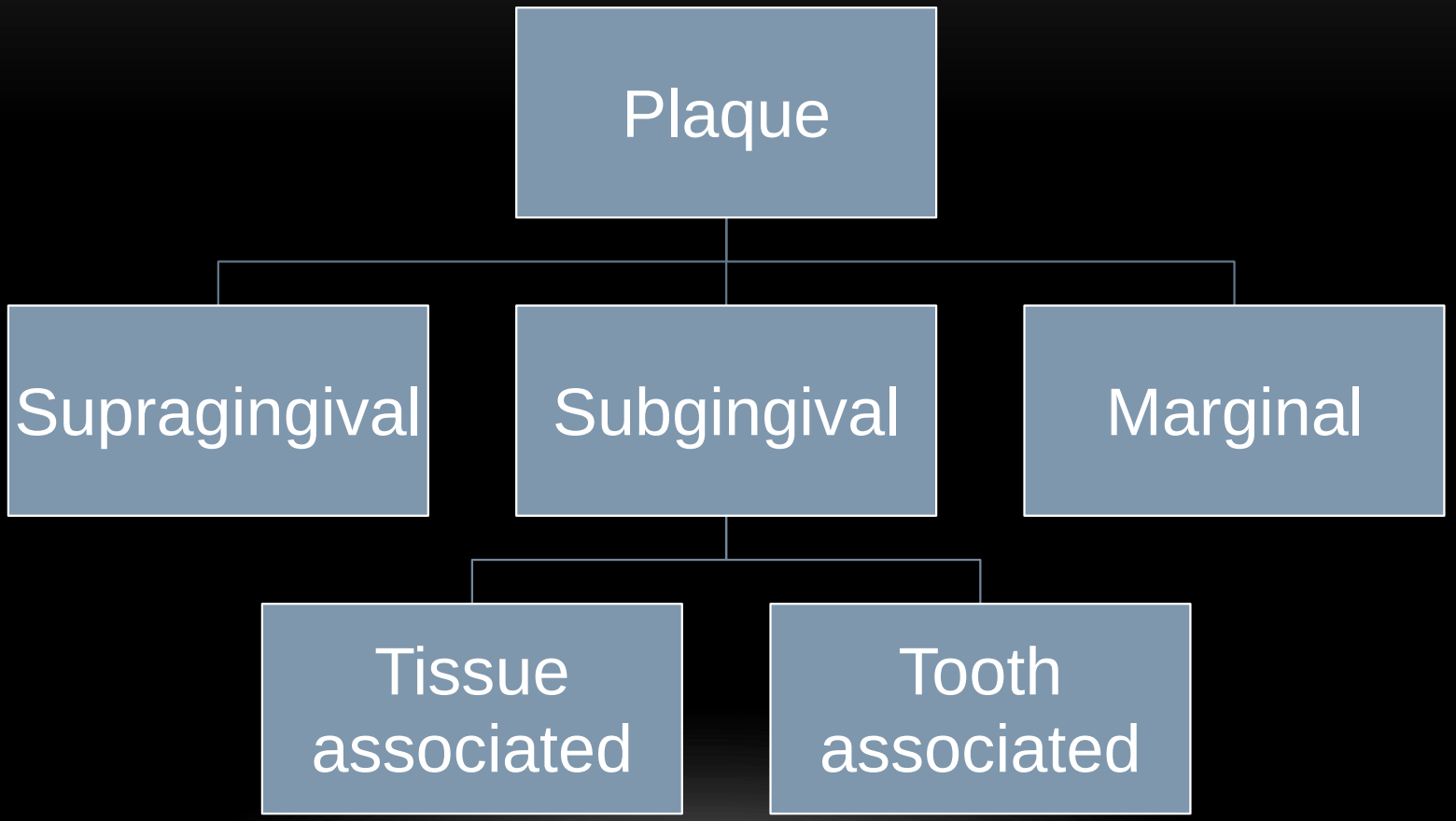
Variations within and different types of biofilms

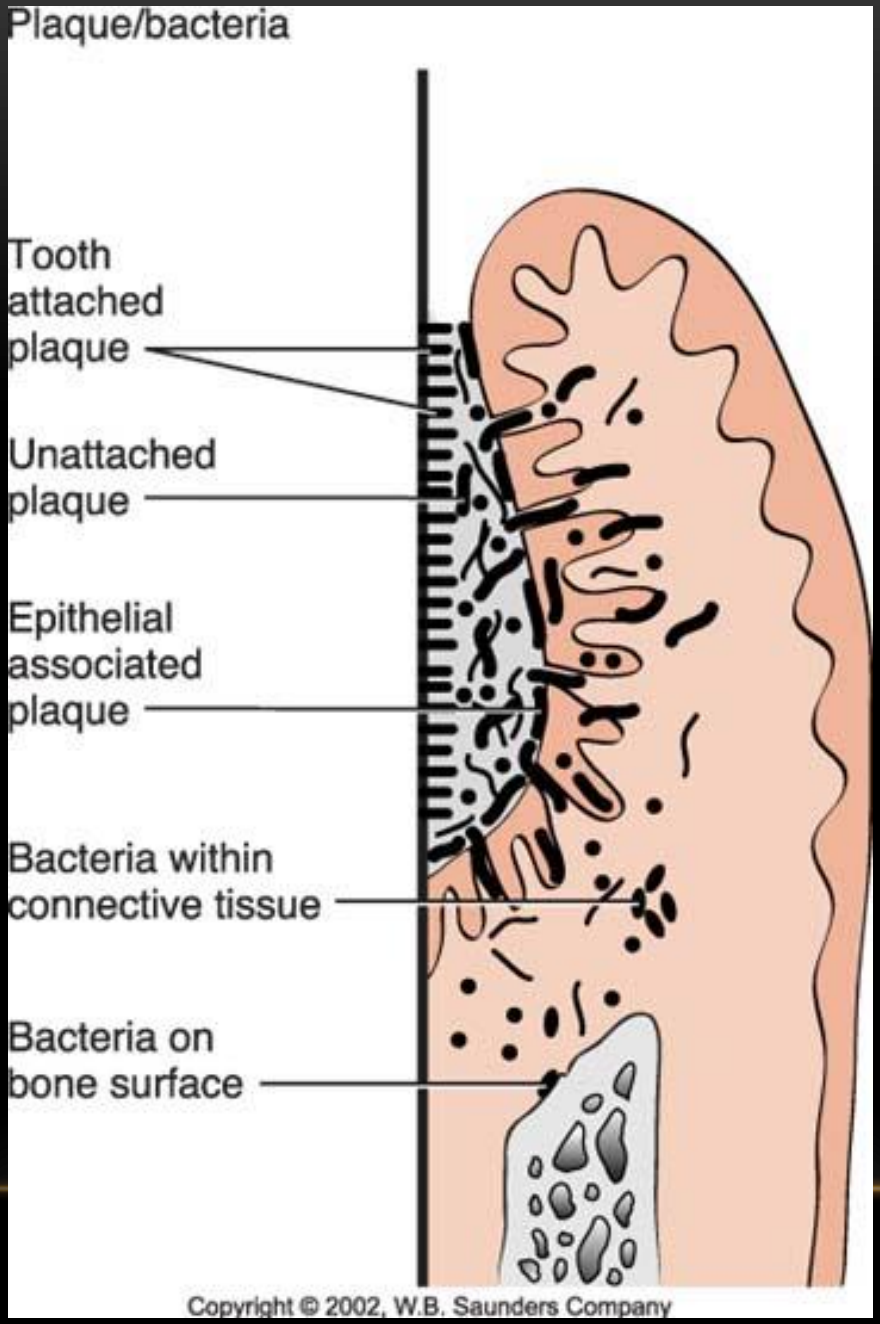
1. Micro-colonies
2. Water channels – act as primitive circulatory system – nutrition and waste removal
3. Surface structures – fronds – dissipate surface energy
4. Steep chemical gradient exists – pH or Oxygen

DENTAL PLAQUE – HOST ASSOCIATED BIOFILM

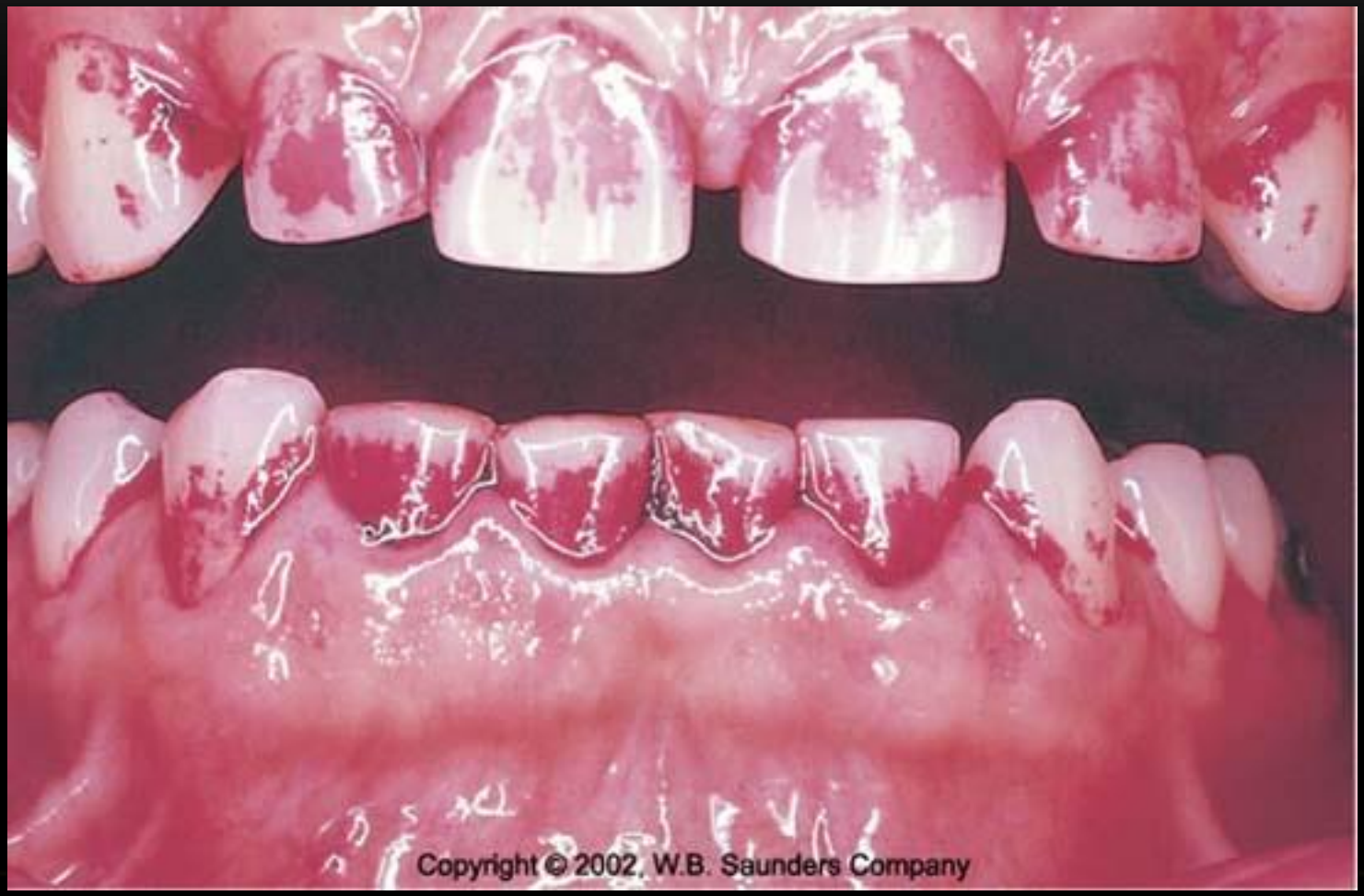
Definition (WHO 1978)

- ***Dental Plaque is defined as a specific but highly variable structural entity resulting from sequential colonization and growth of micro organisms on the surfaces of teeth and restoration*** consisting of micro organisms of various strains and species which are embedded in the extra cellular matrix, composed of bacterial metabolic products and substance from serum, saliva and blood.


Plaque
Supragingival
Subgingival
Marginal
Tissue associated
Tooth associated


Plaque/bacteria
Tooth attached plaque
Unattached plaque
Epithelial associated plaque
Bacteria within connective tissue
Bacteria on bone surface
Copyright © 2002, W.B. Saunders Company

PLAQUE SEEN WITH DISCLOSING AGENT



COMPOSITION

- 1 gm of plaque contains 2 $\times 10^{11}$ bacteria (70%)
- More than 500 different microbial species are found in plaque
- Non bacterial microorganisms in plaque - mycoplasma, yeast, protozoa, virus
- Intercellular matrix (20-30 %)

  1 organic

  2 inorganic

Sources

1. Saliva
2. GCF
3. Host & bacterial products

Organic components	Source
1.Glycoproteins - role in pellicle formation	Saliva
2 Polysaccharides - maintains integrity of plaque matrix	Bacteria
3 Proteins (albumin)	G C F
4 Lipids	Membranes of disrupted bacteria, host cells & food debris

Inorganic

Calcium & Phosphorus - major

trace amount of sodium, potassium, fluoride

Source

Saliva – supragingival plaque

GCF- subgingival plaque

- As the mineral component increases plaque mass becomes calcified to become calculus
- Usually near source of minerals from saliva

1. Lingual surfaces of mandibular anteriors
2. Buccal surfaces of maxillary first molars

Materia Alba	Dental Plaque	Calculus
Soft accumulations of bacteria, salivary proteins, food matter and tissue cells	Primarily composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides	Hard deposit – formed by mineralization of plaque
Lacks organized Structure - Simple	Highly organized - Complex	Usually covered by unmineralized plaque
Easily displaced by water spray	Impossible to remove by rinsing or by spray	
White cheeselike accumulation	Resilient clear to yellowish-grayish	

Significance

1. Marginal plaque - gingivitis
2. Supragingival and tooth associated subgingival plaque - calculus formation & root caries
3. Tissue associated plaque - soft tissue destruction

PLAQUE FORMATION

1. Formation of pellicle on tooth surface
2. Initial adhesion/attachment of bacteria
3. Colonization/plaque maturation

1. Formation of the pellicle

- All surfaces in oral cavity – hard & soft tissues – coated with a layer of organic material – ***acquired pellicle***
- Pellicle forms by ***selective adsorption*** of environmental macromolecules

- More than 180 peptides, proteins, glycoproteins, keratins, mucins – which act ***as adhesion sites (receptors)*** for bacteria
- By 2 hours after cleaning – pellicle is in equilibrium between adsorption and detachment
- **Pellicle – 2 layers**

a) ***Thin basal layer*** – difficult to remove with mechanical and chemical means

b) ***Thicker globular layer*** **-** 1µm or more – easier to detach

- Thus bacteria attaching to tooth surface – do not adhere directly – but interact through acquired enamel pellicle
- No biofilm could be grown on a pellicle coming from a different individual? ***(Walker et al 2007)***

2. Initial adhesion/attachment of Bacteria

- Toothbrushing removes most but not all bacteria
- Recolonization starts as fast as within 3 minutes
- Specific interaction between microbial cell surface ***'adhesin'*** molecules and receptors in salivary pellicle – which decides their adhesion

- First 4-8 hours – 60-80% are of genus *Streptococcus* – other are obligate aerobes *(Haemophilus and Neisseria)* & facultative anaerobes *(Actinomyces and Veillonella)*
- These are considered as Primary Colonizers

Primary Colonizers:

1. Provide new binding sites for adhesion to other bacteria
2. Modifies local microenvironment – makes it favorable to further microbes e.g. removing oxygen which helps survival and growth of Obligatory Anaerobes

Steps in Initial Adhesion

1. Transport to the surface
2. Initial adhesion
3. Strong attachment

Transport to the Surface

- Initial transport of bacteria to tooth surface
- Random contacts occur through
  - ❖ Brownian movement (40µm/hour)
  - ❖ Sedimentation
  - ❖ Liquid flow
  - ❖ Active bacterial movement (chemotactic activity)
- Note: few bacteria are motile and thus forces such as saliva flow and mechanical contact between oral soft and hard tissues – more important than mere swimming of bacteria for bringing to tooth

Initial adhesion

- Initial, reversible adhesion of the bacteria
- This occurs when bacteria come in close proximity to tooth surface (approx 50 nm)
- Long and short range forces – ***van der Waal's attractive forces*** and ***electrostatic repulsive forces***, ***Hydrophobic forces*** play important role

- ***Derjaguin-Landau-Verwey-Overbeek (DLVO)*** theory of colloidal stability – bacteria behave according to this theory

Total Interaction (total Gibb's) energy G_{TOT} =

Attractive forces G_A + Electrostatic Repulsion G_R

$G_{TOT} = G_A + G_R$

- At a distance of 10nm bacteria are reversibly bound
- Certain factors helps for irreversible binding

Strong Attachment

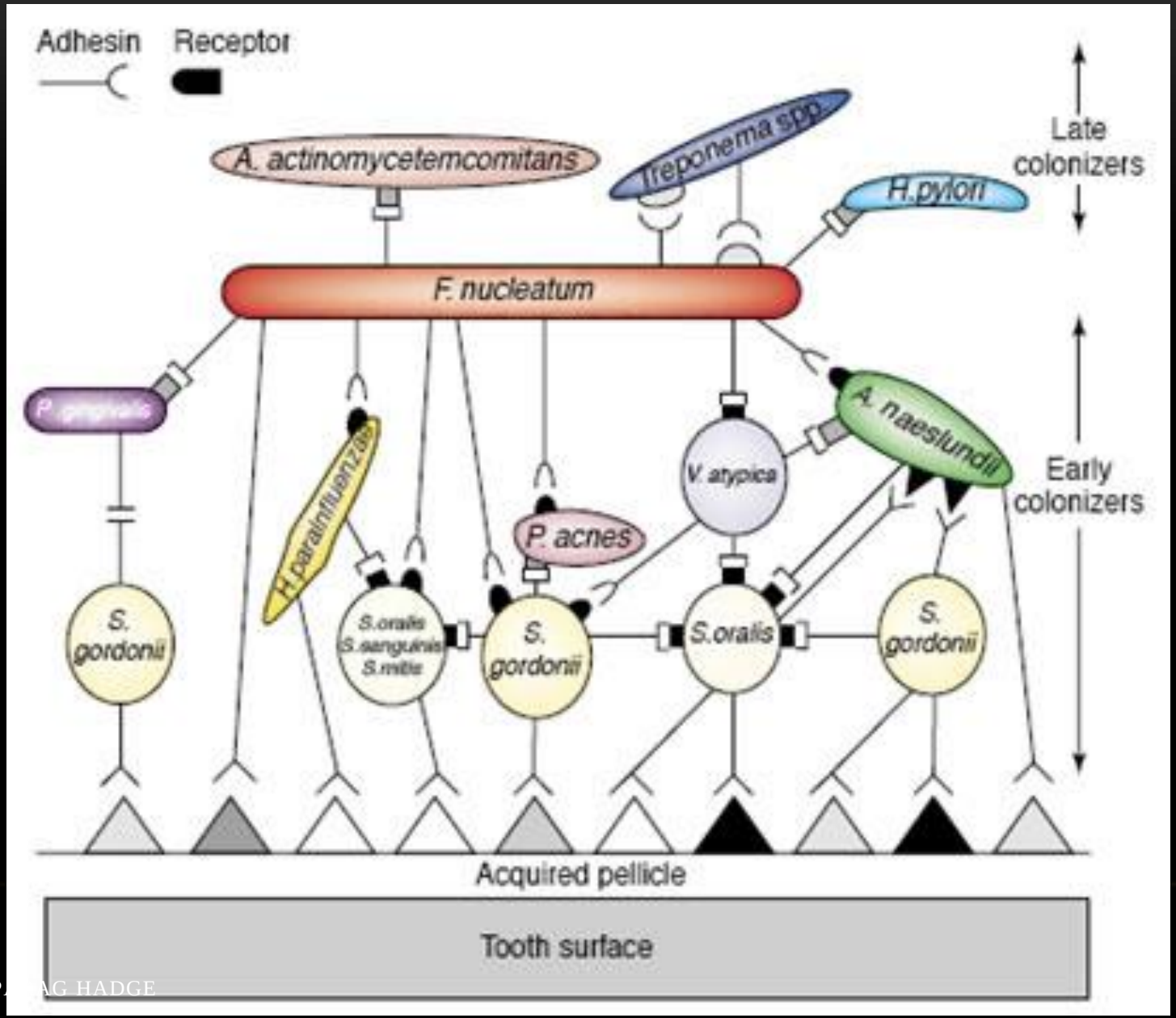
- Specific ***Adhesins on bacterial surface*** – bind with ***receptors*** (acid proline rich proteins, statherin, salivary agglutinin glycoprotein gp340) in acquired pellicle – ***strong attachment***
- E.g Antigen I/II family adhesins of oral Streptococci and gp340
- Certain conformational changes – opens more receptors
- Also on rough surface bacteria are more protected against shear forces – so irreversible binding occurs more easily.

Colonization and Plaque maturation

- Primary colonizers – provide – new receptors for attachment by other bacteria – process known as ‘coadhesion’
- Adherent microorganisms grow – along with coadhesion – microcolonies – mature biofilm (plaque)

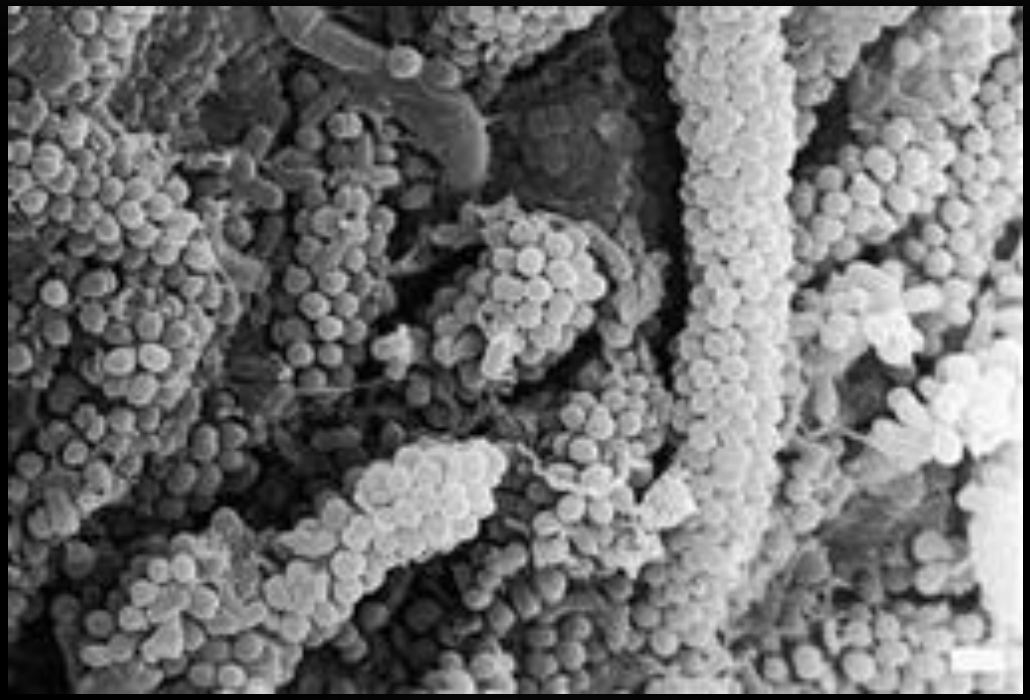
- ***Coaggregation*** – cell to cell adhesion between genetically distinct cells/bacteria
- Initial contact of bacteria – through active or passive transport – weak binding through ***van der Waal's, electrostatic***, and ***Hydrophobic forces***
- Later strong cell to cell specific binding – presence of protein or carbohydrate adhesins on one partner and complementary counterpart receptor on the other
- ***Fusobacteria*** – coaggregate with all human oral bacteria
- ***Veillonella, Capnocytophaga, Prevotella*** – with ***streptococci*** ***and/or*** ***actinomyces***

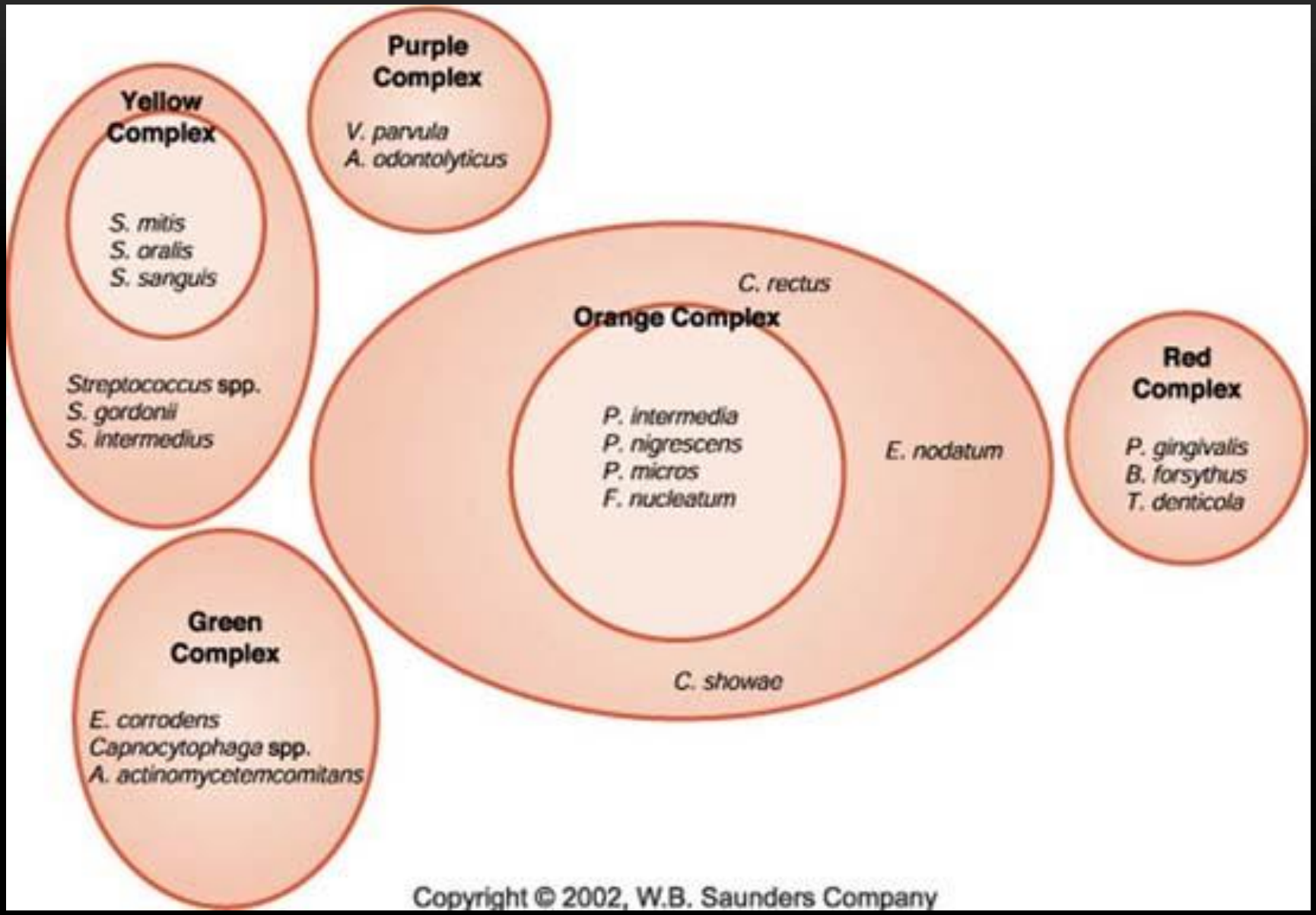
- Each newly added cell provide new surface and acts as coaggragation bridge
- Thus secondary colonizers which were unable to bind clean surface can bind to primary colonizers
- ***Early supragingival dental plaque to mature subgingival dental plaque – shows shift from primarily gram-positive bacteria to high numbers of gram-negative bacteria***


Adhesin
Receptor
A. actinomycetemcomitans
Treponema spp.
H.pylori
Late colonizers
F. nucleatum
P. gingivalis
H.parainfluenzae
P. acnes
V. atypica
A. naeslundii
Early colonizers
S. gordonii
S.oralis
S.sanguinis
S.mitis
S. gordonii
S.oralis
S. gordonii
Acquired pellicle
Tooth surface

Corn-cob formation

- Special example of coaggragation
- There is *streptococci* adhere to filaments of *Cornybacterium matruchotii* or *Actinomyces*
- Central filament and over cocci
- And the test tube brush is composed of filamentous bacteria to which gram negative rods adhere




Purple Complex
V. parvula
A. odontolyticus
Yellow Complex
S. mitis
S. oralis
S. sanguis
Streptococcus spp.
S. gordonii
S. intermedius
C. rectus
Orange Complex
P. intermedia
P. nigrescens
P. micros
F. nucleatum
E. nodatum
C. showae
Red Complex
P. gingivalis
B. forsythus
T. denticola
Green Complex
E. corrodens
Capnocytophage spp.
A. actinomycetemcomitans
Copyright © 2002, W.B. Saunders Company

FACTORS AFFECTING SUPRAGINGIVAL PLAQUE FORMATION

- Surface microroughness – threshold level for surface roughness 0.2 µm – above which bacterial adhesion will be faciliated
- Individual variation – heavy (fast) & light (slow) plaque formers
- Variation with dentition – lower jaw, buccal surfaces of molars
- Gingival inflammation & saliva
- Age – no effect

PLAQUE HYPOTHESES

- Non-specific plaque hypothesis
- Specific plaque hypothesis
- Ecologic plaque hypothesis

Non-specific plaque hypothesis

- It states that periodontal disease result from noxious products of entire plaque flora
- Less plaque – host neutralizes noxious stimuli
- More plaque – overcomes host defense - disease

Arguments

- Certain people with more plaque – no progression to periodontitis
- Certain people – less plaque – more disease severity – tissue destruction
- Site specificity?
- Further studies – concluded that all plaque is not harmful

- Though outdated – still our clinical treatment (surgical as well as non-surgical), oral hygiene methods follows this hypothesis – i.e. removal of all plaque

Specific plaque hypothesis

- Only certain plaque is harmful – pathogenicity depends on presence and/or increase in specific microorganisms
- A.a. in localized aggressive periodontitis
- Advanced methods to isolate and detect microorganisms responsible for periodontal disease

Ecologic plaque hypothesis

- *1990s Marsh et al* developed this hypothesis
- It states ‘both total amount of dental plaque and specific microbial composition – contribute to transition from health to disease
- There is ‘microbial homeostasis’ – host controls with immune response and GCF flow

Disease can occur when

- Excessive accumulation (non-specific) of plaque or by plaque independent host factors e.g. immune disturbance, hormonal imbalance/changes, environmental factors (smoking, diet), GCF flow
- These factors can disturb the harmony – leading to tissue destruction and periodontal disease

- It stresses on elimination of disease inducing stimulus – microbial / host / environmental – so that microbial homeostasis will be restored

- Thank You

- Microbes

CRITERIA FOR IDENTIFICATION OF PATHOGEN

- KOCH"S POSTULATES (ROBERT KOCH 1870)

The causative agent must

1. Be routinely isolated from diseased individuals
2. Be grown in pure culture in lab
3. Produce a similar disease when inoculated into susceptible lab. animals
4. Be recovered from lesions in diseased (experimental) animals

This criteria were difficult to apply in case of periodontitis because of:

- Inability to culture all the organisms that have been associated with disease.
- Difficulty in defining & culturing sites of active diseases
- Lack of good animal models for study of periodontitis

SIGMUND SOCRANSKY'S CRITERIA

ASSOCIATION

a potential pathogen must be associated with disease as evident by increase in number of organisms at diseased site

ELIMINATION

be eliminated /decreased in sites which shows disease resolution

HOST RESPONSE

Alteration in host cellular & humoral immune response

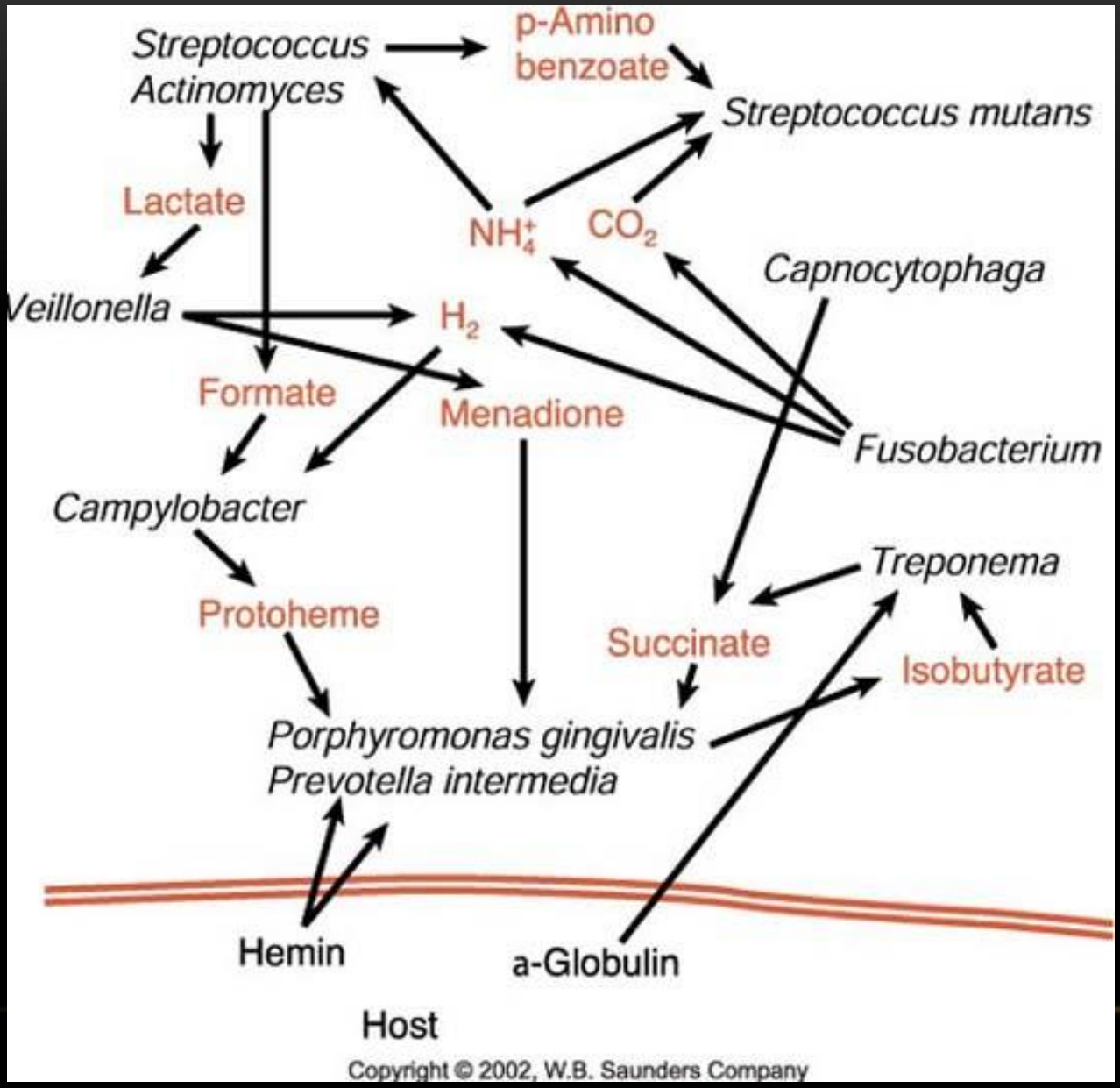
VIRULENCE

demonstrate virulence factors capable of causing destruction of tissue.

ANIMAL STUDIES

be capable of causing disease in experimental animal models

- As we discussed earlier
- ***Early colonizers make plaque/biofilm environment favorable for late colonizers***
- Early colonizers – use sugars
- Late colonizers - asaccharolytic and use amino acids & peptides as energy sources


Streptococcus
Actinomyces
p-Amino
benzoate
Streptococcus mutans
Lactate
NH_4^+
CO_2
Capnocytophaga
Veillonella
H_2
Formate
Menadione
Fusobacterium
Campylobacter
Treponema
Protoheme
Succinate
Isobutyrate
Porphyromonas gingivalis
Prevotella intermedia
Hemin
a-Globulin
Host
Copyright © 2002, W.B. Saunders Company

- Increase in steroid hormones – increase in ***Prevotella intermedia*** – during puberty
- While their growth is inhibited by ***S. mitis, S. salivarius, S. sanguinis***
- ***Quorum sensing?***

Biofilm & Antimicrobial resistance

- Bacterial 1000 to 1500 times – more resistant to antimicrobials - than in their planktonic or unattached state
- Iron exchange resin in plaque matrix – removes highly reactive agents – so they fail to reach
- ß-lactamases, dehydrogenases – inactivate antimicrobials
- Exchange of genetic material

Bacterial Transmission & Translocation

- Intraoral, Vertical or Horizontal
- Controversial

NON-BACTERIAL INHABITANTS

- Viruses
- Fungi
- Archaea
- Protozoa

VIRUSES

- Recent research – association in periodontal diseases
- Ulcerogenic & Tumerogenic
- In contrast to bacteria – they replicate only when present in eukaryotic (animals, plants, protists and fungi) or prokaryotic (bacteria, archaea) cells

Four major viral families

1. Herpes viruses - 8 members – enveloped double stranded DNA viruses
2. Human papillomaviruses – 5 genere - nonenveloped double stranded DNA viruses
3. Picornaviruses - Nonenveloped single stranded RNA viruses
4. Retroviruses - enveloped double stranded RNA viruses

Herpesviruses

- HSV-1, HSV-2, Vericalla- zoster virus, Ebstein Barr Virus, Human Cytomegalo virus, HSV-6, HSV-7, HSV-8

Papillomaviruses

- Warts, focal epithelial hyperplasia, oral squamous cell carcinoma, verrucous carcinoma

- **Picornaviruses**
- Nine genera e.g. Hepatitis A virus, poliovirus, rhinovirus, hand foot and mouth disease virus
- Enteroviruses: Coxsackievirus, Echovirus, Enterovirus

Retroviruses

- HIV-1, HIV-2
- T-lymphotropic virus

YEASTS/ FUNGI

- Major ***Candia*** especially ***Candida albicans***
- Opportunistic infections
- Other fungi ***Aspergillus, Cryptococcus*** etc.

PROTOZOA

- Mouth – entry to many parasites
- Only few parasites affect oral cavity
- Poor oral hygiene – low standards of living – decreased immunity
- ***Entamoeba gingivalis, Trichomonas tenax***
- Painful oral ulcers – cases in association with HIV-1 only

ARCHAEA

- Single celled organisms
- Recent research started to explore their role in oral diseases
- Methanogenic archeae- produce methane from H_2 and CO_2
- Not clear – they cause or take advantage for growth in periodontal diseases

VIRULENCE FACTORS

1. ***Factors that promote colonization (adhesins)***
2. ***Toxins and enzymes that degrade host tissues***
3. ***Mechanisms that protect pathogenic bacteria from host***

ADHESIVE SURFACE PROTEINS & FIBRILS

- Fimbriae – pilli – are small polymeric fibrils

P. gingivalis

- Major and minor fimbriae

Major sheath protein of ***T. denticola***

FACTORS PROMOTING TISSUE DESTRUCTION

Aggregatibacter actinomycetemcomitans

- It has ability to invade the epithelial and endothelial cells.
- It produces ***Leukotoxin & cytolethal distending toxins (CDT)***

Leukotoxin

- Affects PMNs, monocytes, lymphocytes
- Does not affect human platelets, fibroblast, endothelial cells, epithelial cells
- Mechanism of action:

  necrosis – at higher conc.

  apoptosis - at lower conc.

- Higher conc. of Leukotoxin - pores in cell membrane, water influx & osmotic lysis

Porphyromonas gingivalis

- Forms black pigmented colonies

Produce various proteases:

1. ***gingipains***
2. periodontain
3. prolyl tripeptidase
4. prolyl dipeptidase
5. ***collagenase***

Gingipains:

2 TYPES

ARGININE GINGIPAINS – cleave peptide bond following arginine residue

LYSINE GINGIPAINS - cleave peptide bond following lysine residue

Effect of Gingipains on

1. PMNs

- cleavage of PMN receptor for C5a so *decreased phagocytosis*
- degradation of C3 & Ig G so *decreased opsonization & phagocytosis*

2 INNATE IMMUNE RESPONSE:

Gingipains *degrade CD14* ,which is receptor for LPS so inability of host cells to recognized & response to bacteria

3 CYTOKINE NETWORK:

Degradation of IL -1β, IL-8, IL-6, TNF -α so *disrupted host response to infection*

4 KININ SYSTEM:

Activation with release of bradykinin, *increased vascular permeability* & supply of nutrients for bacteria

5 COAGULATION / FIBRINOLYSIS SYSTEM:

Paradoxical effect in activation of clot formation & degradation ***increased BOP***

Mechanisms that protect pathogenic bacteria from host

1. *Production of an extracellular capsule*
2. *Proteolytic degradation of host innate/aquired immune system components*
3. *Modulation of host response by binding serum components*
4. *Invasion of gingival epithelial cells*

MMPs – Matrix Metalloproteinases

- Family of proteolytic enzymes (MMP-1 to MMP-20)– degrade extracellular matrix molecules
- MMP-1 – collagenase
- Secreted by neutrophils, macrophages, fibroblasts, epithelial cells, osteoblasts, osteoclasts and sometimes ***A. a.*** & ***P. g.***
- **TIMPs – tissue inhibitors of MMPs**

- Endotoxins – Lipopolysaccharides (LPS) – these are recognized through human receptors – ***Toll like receptors***
- Prostaglandins

INFLAMMATORY MEDIATORS

- ***Chemokines***
- ***Interleukins*** (previously known as lymphokines) – more than 33
- ***TNF-α – tumor necrosis factor-α***

ANTI-INFLAMMOTORY MEDIATORS

- ***Lipoxins*** (LXA_4, LXB_4) & ***Resolvins*** (derived from omega-3 fatty acids eicosapentaenoic acid ***RvE*** and docosahexanoic acid ***RvD***)
- Appear during resolution of inflammation
- They have potent anti-inflammatory & immunoregulatory effects

- Thank You