Can an ecological approach control or prevent dental disease?

P. D. Marsh

Health Protection Agency,
Centre for Emergency Preparedness & Response (CEPR),
Salisbury, & Leeds Dental Institute.
Can an ecological approach control or prevent dental disease?

- relationship between man & microbes
- contemporary view of plaque in health & disease
- dynamic relationship between the environment and dental plaque
- an “ecological” approach to dental disease
- implications for disease control
- summary
MICROBIAL COMMUNITIES OF HUMANS

Homo sapiens:
- $10^{14}$ cells
- 10% mammalian

Benefits to host:
- normal development of host physiology & host defences
- exclusion of exogenous microbes (colonisation resistance)
Functions of the resident oral microflora that contribute to colonization resistance

Function

• Competition for receptors for adhesion

• Competition for essential endogenous nutrients

• Creation of microenvironments that discourage the growth of exogenous species

• Production of inhibitory substances (bacteriocins, H₂O₂, bacteriophage, etc)
DISTRIBUTION OF THE RESIDENT HUMAN MICROFLORA

SKIN
Staphylococcus
Micrococcus
Propionibacterium
Corynebacterium

NASO-PHARYNX
Streptococcus
Neisseria
Moraxella
Haemophilus

MOUTH
Streptococcus
Actinomycetes
Prevotella
Fusobacterium

GUT
Peptostreptococcus
Eubacterium
Clostridium
Bifidobacterium
Bacteroides

URO-GENITAL TRACT
Streptococcus
Staphylococcus
Lactobacillus
Candida

HABITAT SELECTS
SITE VARIATION
MICROFLORA OF THE HUMAN MOUTH

TEETH:
- S. mutans
- S. oralis
- S. mitis
- A. naeslundii
  Gram-negative anaerobes

CHEEK:
- S. salivarius
- S. mitis

TONGUE:
- S. salivarius
- S. mitis
- Haemophilus
- Neisseria
- Veillonella
- Rothia

HABITAT SELECTS
Site Variations in Dental Plaque Microflora

Fissure Plaque:
- streptococci
- few Gram negative bacteria
- high redox potential
- neutral – low pH

Gingival Crevice Plaque:
- obligate anaerobes
- assaccharolytic bacteria
- low redox potential (anaerobic)
- neutral – alkaline pH

SALIVA FLOW

GCF:
- haeme-containing proteins

MICRO-HABITAT SELECTS
ECOLOGICAL INTERPRETATION

• habitat selective

• habitat is hostile

• direct relationship between environment & microflora
Can an ecological approach control or prevent dental disease?

- relationship between man & microbes
- contemporary view of plaque in health & disease
- dynamic relationship between the environment and dental plaque
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- summary
The community of micro-organisms found on the tooth surface as a biofilm embedded in a matrix of polymers of salivary and bacterial origin.

Plaque is natural, normal & has benefits for the host.
DENTAL PLAQUE BIOFILMS – NEW PERSPECTIVES

Imaging
[confocal microscopy]

Molecular detection
[16S rRNA; FISH]

Gene expression
[transcriptomics; proteomics]

Typing & virulence
[virulent clones]
STAGES IN DENTAL PLAQUE BIOFILM FORMATION

1. conditioning film (acquired pellicle)
2. transport of microbes to tooth surface
3. reversible phase: long range, physico-chemical
4. irreversible phase: adhesin - receptor
5. co-aggregation/co-adhesion
6. growth (biofilm formation)
7. detachment
PLAQUE FORMATION: STAGES 1 – 3

1. Pellicle
   - weak, long range, van der Waals

2. Transport - passive

3. Reversible attachment
PLAQUE FORMATION: STAGES 4 & 5

4. adhesin-receptor
irreversible, specific, short range

5. co-adhesion

ENAMEL
## STAGES IN PLAQUE FORMATION

Examples of adhesin - receptor interactions:

<table>
<thead>
<tr>
<th>BACTERIUM</th>
<th>ADHESIN</th>
<th>RECEPTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>antigen I/II</td>
<td>salivary agglutinin</td>
</tr>
<tr>
<td>mutans streptococci</td>
<td>glucan-binding protein</td>
<td>glucan</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em></td>
<td>type 1 fimbriae</td>
<td>proline-rich proteins (PRP)</td>
</tr>
</tbody>
</table>
5. CO-ADHESION

Acquired Pellicle

ENAMEL
PLAQUE FORMATION: STAGE 6

- metabolic interactions
- environment modification
- gradients
- matrix formation
- cell-cell signalling

6. Biofilm formation
INTERACTIONS IN PLAQUE BIOFILMS:

BENEFICIAL
food chain / food web
enzyme complementation
cell-cell signalling
inhibitor neutralisation
gene transfer
- cell signalling
- genetic competence
- horizontal gene transfer
- acid tolerance

- new opportunities for control?

Streptococci – peptides (competence stimulating peptide)
Gram negative anaerobes – autoinducer-2 (global?)
PLAQUE FORMATION: STAGE 6

- metabolic interactions
- environment modification
- gradients
- matrix

- antagonism
- bacteriocins
- \( \text{H}_2\text{O}_2 \)
- acids
- nutrient competition

6. Biofilm formation
PLAQUE FORMATION: STAGE 7

- metabolic interactions
- environment modification
- gradients
- matrix

6. Biofilm formation
7. Detachment

detachment (protease)
PLAQUE: GRADIENTS

- exogenous nutrients
- metabolic products
- pH (caries)
- pH (periodontal)
- $O_2 / E_h$
Biofilm gradients - pH heterogeneity

TPEM + FLIM

x - z

50μm

pH 5.0

pH 7.5

explain co-existence of incompatible species

Vroom et al., 1999
Bacterial composition of dental plaque

Culture:
- selective & non-selective agars
- range of atmospheric conditions
- prolonged incubation
PLAQUE COMPOSITION

**Gram positive**

- Streptocococcus
- Peptostreptococcus
- Actinomyces
- Bifidobacterium
- Corynebacterium
- Eubacterium
- Lactobacillus
- Propionibacterium
- Pseudoramibacter
- Rothia

**Gram negative**

- Neisseria
- Moraxella
- Veillonella
- Aggregatibacter (Actinobacillus)
- Campylobacter
- Capnocytophaga
- Eikenella
- Fusobacterium
- Haemophilus
- Leptotrichia
- Porphyromonas
- Prevotella
- Selenomonas
- Treponema
- Wolinella
DENTAL PLAQUE – changing perspectives

Bacterial composition of dental plaque

Molecular:
- DNA extraction
- 16S rRNA gene amplification with universal primers
- partial sequencing
- search for homology in databases
- probes for subsequent analysis (e.g. FISH)

- 50% unculturable
- 47 uncultivated *Treponema* spp.
- TM7 clade
<table>
<thead>
<tr>
<th>PLAQUE COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GRAM-POSITIVE</strong></td>
</tr>
<tr>
<td><strong>COCCI</strong></td>
</tr>
<tr>
<td>Abiotrophia</td>
</tr>
<tr>
<td>Enterococcus</td>
</tr>
<tr>
<td>Finegoldia</td>
</tr>
<tr>
<td>Gemella</td>
</tr>
<tr>
<td>Granulicatella</td>
</tr>
<tr>
<td>Micromonas</td>
</tr>
<tr>
<td>Peptostreptococcus</td>
</tr>
<tr>
<td>Streptococcus</td>
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</tbody>
</table>
DENTAL PLAQUE – consequences for treatment

Mechanisms of Biofilm Tolerance

- Antimicrobial Depletion
- Antimicrobial agent
- Gene transfer
- Slow growth rate
- Novel phenotype

Slow Penetration
Stress Response
Altered Microenvironment
Persisters
# DENTAL PLAQUE BIOFILM

Antimicrobial tolerance [laboratory models]:

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Antimicrobial agent</th>
<th>Biofilm effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strep. sanguinis</em></td>
<td>chlorhexidine</td>
<td>10 – 50x MIC*</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>metronidazole</td>
<td>2 – 8x MBC+</td>
</tr>
<tr>
<td></td>
<td>doxycycline</td>
<td>4 – 64x MBC</td>
</tr>
<tr>
<td></td>
<td>amoxycillin</td>
<td>2 – 4x MBC</td>
</tr>
</tbody>
</table>

* MIC = Minimum Inhibitory Concentration  
+ MBC = Minimum Bactericidal Concentration

- **BIC, BKC, BEC**  
  [“biofilm inhibitory concentration”]

<table>
<thead>
<tr>
<th>Agent</th>
<th>MBC (µM)</th>
<th>BKC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amine F</td>
<td>20</td>
<td>1,500</td>
</tr>
<tr>
<td>CHX</td>
<td>5</td>
<td>1,500</td>
</tr>
</tbody>
</table>

*Strep. sobrinus*
Plaque - biofilm & microbial community

Antibiotic resistance:

Penicillin Binding Proteins

S. oralis  ↔  S. pneumoniae

60% \( \beta \)-lactamase positive sites had enzyme levels sufficient to inactivate penicillins in GCF

CROSS-PROTECTION
DENTAL PLAQUE – MICROBIAL COMMUNITY

- broader habitat range
  - *anaerobes in an overtly aerobic habitat*

- increased metabolic diversity & efficiency
  - *metabolism of complex host glycoproteins*

- increased tolerance to antimicrobial agents, inhibitors, and host defences
  - *cross protection by β-lactamase*

- enhanced pathogenicity
  - *pathogenic synergism/polymicrobial disease*
Plaque - biofilm & microbial community

**BIOFILM**
- altered gene expression
- spatially organised
- matrix embedded *(slime)*
- cell-cell communication: 
  - gene transfer
  - signalling

**MICROBIAL COMMUNITY**
- diverse composition
- broader habitat range
- more efficient metabolism
- increased stress resistance
- enhanced virulence

- novel phenotype
- co-ordinated activities
- functionally-organised
- increased drug resistance
MICROBIAL HOMEOSTASIS

Ecological stress

- host defences / antimicrobials
- diet
- age
- exogenous species

community balance

negative feedback

Homeostatic mechanisms

antagonistic & synergistic interactions
CONSEQUENCES OF MICROBIAL HOMEOSTASIS

normal diet

diligent oral hygiene

de-/re-mineralisation in equilibrium

community balance

low inflammation/slow GCF
BREAKDOWN OF MICROBIAL HOMEOSTASIS

Selection of "pathogens"

- normal diet
- demineralisation
- caries
- diligent oral hygiene
- inflammation
- periodontal disease
MICROBIAL HOMEOSTASIS

Environmental overload

- acne?
- colitis?
- dental diseases ??

out-growth of minor components

re-arrangement of community structure

“Ecological catastrophe”
HEALTH

CARIES

PERIODONTAL DISEASE

**Why?**

**How?**

**Where?**

**HEALTH**

**dental plaque**

**Strep. mitis / oralis**

**Actinomyces spp.**

**Haemophilus spp**

**Neisseria spp**

**Fusobacterium**

**CARIES**

**Strep. mutans**

**Lactobacillus spp**

- acid production
- acid tolerance
- EPS & IPS

**PERIODONTAL DISEASE**

**Gram negative anaerobes**

- *Porphyromonas gingivalis*
- *Aggregatibacter actinomycetemcomitans*
- spirochaetes
- *Tannerella forsythia*
- unculturables
- proteases
- cytotoxins
- immune modulators
- inflammatory response
WHERE DO “PATHOGENS” COME FROM?

PREVALENCE OF ORAL PATHOGENS AT HEALTHY SITES

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Method</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutans streptococci</td>
<td>IF</td>
<td>60%</td>
</tr>
<tr>
<td>Mutans streptococci</td>
<td>culture</td>
<td>46%</td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td>PCR</td>
<td>55%</td>
</tr>
<tr>
<td>T. forsythia (children)</td>
<td>PCR</td>
<td>65%</td>
</tr>
<tr>
<td>P. gingivalis (children)</td>
<td>PCR</td>
<td>49%</td>
</tr>
<tr>
<td>P. gingivalis (teens)</td>
<td>PCR</td>
<td>13%</td>
</tr>
<tr>
<td>P. gingivalis (adults)</td>
<td>PCR</td>
<td>33%</td>
</tr>
<tr>
<td>P. gingivalis (infants)</td>
<td>CKB</td>
<td>69%</td>
</tr>
<tr>
<td>T. forsythia (infants)</td>
<td>CKB</td>
<td>29%</td>
</tr>
</tbody>
</table>
AETIOLOGY OF DENTAL DISEASES

Where do pathogens come from?

- re-arrangement of endogenous species
- acquisition of exogenous species
AETIOLOGY OF DENTAL DISEASES

Transmission

Major ecological pressure

Health

Health

Disease

diet
inflammation ·
host defences ·
pH
AETIOLOGY OF DENTAL DISEASES

Health
Health
Disease

Major ecological pressure

diet
inflammation • host defences • pH
Can an ecological approach control or prevent dental disease?

- relationship between man & microbes
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ECOLOGICAL INFLUENCES

NUTRIENTS
- Exogenous (sugar frequency)
- Endogenous (GCF - haeme)

pH
- Caries = acid stress
- Gum = rise in pH (alkaline)

ATMOSPHERE
- Aerobic / anaerobic

ANTIMICROBIALS / HOST DEFENCES
- Neutrophil defects
- Reduction in saliva flow
ENVIRONMENTAL INFLUENCES

Pure culture

pH, sugar

Phenotypic properties:
- acid production
- proteases
• environment changes during disease

• oral bacteria respond to environmental change

• disease-related environmental changes favour growth and survival of “pathogens”
  - mutans streptococci & lactobacilli thrive at low pH & high sugar (e.g. faster acid production, etc)
  - *P. gingivalis*: proteases up-regulated at alkaline pH & high haemin; growth optimum @ pH 7.5

• genes that are up-regulated relate to “virulence”
ENVIRONMENTAL INFLUENCES

Pure culture

- pH, sugar

Phenotypic properties:
- acid production
- proteases

Mixed culture

- pH, sugar

Consequences:
- competitiveness
- community structure
Modelling studies – Mixed cultures

• defined consortium
  - species relevant in health & disease
  - ease of identification
  - storage / replicate
  - add / replace

• habitat-simulating medium (mucin – based)

• chemostat
  - long-term studies
  - controlled conditions
  - cause-and-effect relationships
  - surfaces; 2-stage system

• Constant Depth Film Fermenter (CDFF)
Two-Stage Chemostat System

Stage 1
Chemostat
“Conventional”

Stage 1 Culture
50ml.h⁻¹, D=0.1.h⁻¹
75ml.h⁻¹, D=0.1.h⁻¹

HA DISKS

Stage 2 Chemostat
Aerated

HABITAT-SIMULATING MEDIUM (MUCIN-BASED)

CDFF
- biofilms of pre-determined depth
Microbial community - modelling studies

10 species mucin metabolism

<table>
<thead>
<tr>
<th>GRAM-POSITIVE</th>
<th>GRAM-NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. sanguis</strong> tr</td>
<td><strong>N. subflava</strong> tr</td>
</tr>
<tr>
<td><strong>S. oralis</strong> sialidase</td>
<td><strong>V. dispar</strong> tr</td>
</tr>
<tr>
<td><strong>S. mutans</strong> tr</td>
<td></td>
</tr>
<tr>
<td><strong>A. naeslundii</strong> sialidase</td>
<td><strong>P. nigrescens</strong> tr</td>
</tr>
<tr>
<td><strong>L. rhamnosus</strong> fucosidase</td>
<td><strong>P. gingivalis</strong> protease</td>
</tr>
<tr>
<td></td>
<td><strong>F. nucleatum</strong> tr</td>
</tr>
</tbody>
</table>
INCREASED METABOLIC DIVERSITY & EFFICIENCY

Endogenous nutrients:

- serine/threonine
- N-acetyl galactosamine
- galactose
- sialic acid
- fucose
- protein
- glycoprotein
Increasingly Complex Communities

No. of Bacteria in Chemostat Community

- Five
- Seven
- Eight
- Nine
- Ten

Sg, Sm, Fn, Vd, Ns
+ So, An

BASAL

FUCOSIDASE

SIALIDASE

PROTEASE

+ Pg

+ Lr

+ Pn

Newly Added
Increase in Existing
Existing

Total CFU/ml (millions)
Nutrient acquisition

Endogenous nutrients:
concerted & sequential action

glycoprotein

organism 1
Nutrient acquisition

Endogenous nutrients:

concerted & sequential action

glycoprotein

organism 2
Nutrient acquisition

Endogenous nutrients:
concerted & sequential action

glycoprotein

organism 3
Nutrient acquisition

Endogenous nutrients:
concerted & sequential action

glycoprotein
organism 4
Microbial community - modelling studies: oxygen

<table>
<thead>
<tr>
<th>GRAM-POSITIVE</th>
<th>GRAM-NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep. sanguis</td>
<td>Neisseria subflava</td>
</tr>
<tr>
<td>Strep. oralis</td>
<td>Veillonella alcalescens</td>
</tr>
<tr>
<td>Strep. mutans</td>
<td></td>
</tr>
<tr>
<td>Actinomyces naeslundii</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus rhamnosus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prevoitella nigrescens</td>
</tr>
<tr>
<td></td>
<td>Porphyrromomonas gingivalis</td>
</tr>
<tr>
<td></td>
<td>Fusobacterium nucleatum</td>
</tr>
</tbody>
</table>

two-stage chemostat: stage 2 - aerated + HA surfaces

Bradshaw et al., 1996
AERATION, WITHOUT NEISSERIA

PLANKTTONIC

- Anaerobes: 57%
- Facultatives: 43%

BIOFILM

- Anaerobes: 86.5%
- Facultatives: 13.5%

SECOND STAGE

- O₂ < 50%
- Eₜ +50 mV
### OBLIGATE ANAEROBES

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Half-Life in Planktonic Aerated Stage (mins.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Veillonella parvula</em></td>
<td>5.0</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em></td>
<td>4.4</td>
</tr>
<tr>
<td><em>Prevotella nigrescens</em></td>
<td>&lt; 2.8</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em></td>
<td>&lt; 3.7</td>
</tr>
</tbody>
</table>

SECOND STAGE

Bradshaw et al., 1997
<table>
<thead>
<tr>
<th>Anaerobe - Aerobe Pair</th>
<th>Pair-wise</th>
<th>Post <em>F. nucleatum</em> addition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. gingivalis</em> + <em>N. subflava</em></td>
<td>0 - 1</td>
<td>2 - 3</td>
</tr>
<tr>
<td><em>P. nigrescens</em> + <em>N. subflava</em></td>
<td>0 - 1</td>
<td>2 - 3</td>
</tr>
<tr>
<td><em>V. dispar</em> + <em>N. subflava</em></td>
<td>1 - 2</td>
<td>2</td>
</tr>
<tr>
<td><em>F. nucleatum</em> + <em>N. subflava</em></td>
<td>4</td>
<td>---</td>
</tr>
</tbody>
</table>

0 = no coaggregation

4 = immediate, complete coaggregation

Bradshaw et al., 1998
Co-aggregation

Neisseria subflava + Prevotella nigrescens

Neisseria subflava + Prevotella nigrescens + Fusobacterium nucleatum
Co-aggregation: role of *F. nucleatum* in biofilms

<table>
<thead>
<tr>
<th></th>
<th>Percentage viable count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with <em>F. nucleatum</em></td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>15%</td>
</tr>
<tr>
<td><em>P. nigrescens</em></td>
<td>6%</td>
</tr>
</tbody>
</table>

aerated second stage
ECOLOGICAL CHANGES IN DISEASE

CARIES

Sugar

pH

PERIODONTAL DISEASES

GCF

pH
CARBOHYDRATE & pH - *In Vitro*

10 Daily Glucose Pulses, at Constant pH 7

Effect of Glucose *Per Se*

Effect of Glucose, Plus Low pH

Chemostat Mixed Culture 10 Species Steady-State (incl. *S. mutans*)

10 Daily Glucose Pulses, No pH Control

[Bradshaw et al, 1989]
HOMEOSTASIS: EVIDENCE FOR BREAKDOWN

Chemostat microbial community
- low MS
- low lactobacilli

Glucose Pulse, Constant pH 7
- Flora Unchanged
- MS + Lactobacilli = <1%

Glucose Pulse, Low pH
- >50% MS + Lactobacilli
  “Healthy species” reduced
  - “dose dependent”
EFFECT OF pH ON MICROBIAL COMMUNITIES

Terminal pH

Final proportion

- 7
- 5.5
- 5
- 4.5
- no control

S. mutans
lactobacillus

Bradshaw & Marsh, 1998
EFFECT OF pH ON MICROBIAL COMMUNITIES

Bradshaw & Marsh, 1998
CARBOHYDRATE & pH: IMPLICATIONS

• low pH rather than carbohydrate availability _per se_ selects for cariogenic bacteria

• indirect relationship: lower pH = higher levels of cariogenic bacteria

• could inhibitors of acid production can prevent selection?
Carbohydrate, pH, & Fluoride - *In Vitro*

10 Daily Glucose Pulses, no pH Control

- Effect of Glucose, Plus Low pH
  - F: enamel effects
    - antimicrobial?

Chemostat Mixed Culture 10 Species Steady-State (incl. *S. mutans*)

10 Daily Glucose Pulses, with Fluoride [0.5 or 1 mM; 10 or 20 ppm]

- Effect of Glucose, Low pH Plus Fluoride

Bradshaw et al., 1990, 2001
### Fluoride

**Culture:**

<table>
<thead>
<tr>
<th>glucose pulsing</th>
<th>pH change</th>
<th>% composition glucose pulsing</th>
</tr>
</thead>
<tbody>
<tr>
<td>-F^-</td>
<td>4.41</td>
<td>pre -F^- S. mutans 4</td>
</tr>
<tr>
<td>+F^-</td>
<td>4.81</td>
<td>+F^- 23 &lt;3*</td>
</tr>
</tbody>
</table>

\[(p = 0.0001; paired t-test)\]

F^- 10 ppm
Fluoride

Final [H+ ion] vs Pulse

- Glucose
- G + F
Fluoride

Time to reach pH 5.0 (hours)

Pulse

- Glucose
- G + F
Fig 1. Final pH 6 h after pulses of glucose (G), xylitol/glucose (X) & sorbitol/glucose (S).

Fig 2. Time to reach pH 5.0 after pulses of glucose (G), xylitol/glucose (X) & sorbitol/glucose (S).

Proportions

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>G+S</th>
<th>G+X</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans</td>
<td>19%</td>
<td>41%</td>
<td>3%</td>
</tr>
</tbody>
</table>
PERIODONTAL DISEASES

Novel Nutrients In Gingival Crevicular Fluid (GCF):

- albumin
- haemoglobin
- complement
- haptoglobin
- immunoglobulins
- haptoglobin
- haemopexin
- transferrin

SERUM used as surrogate for GCF
### PERIODONTAL DISEASES

**Enrichment of *P. intermedia* on human serum**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Enrichment step no.:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>plaque</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>plaque</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>10</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>plaque</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>&lt;1</td>
<td>7</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

*ter Steeg et al, 1987*
PERIODONTAL DISEASES

Protease activity: low
*P. gingivalis*: low

Protease activity: high
*P. gingivalis*: high

pH 7.5

Eh - 330 mV

pH 6.95

Eh - 390 mV

Serum added

Chemostat Run (hrs)
EFFECT OF pH ON COMPETITION AMONG BLACK-PIGMENTED ANAEROBES

<table>
<thead>
<tr>
<th>pH</th>
<th>Growth Percentage Viable Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Prevotella</strong></td>
</tr>
<tr>
<td>6.70</td>
<td>98</td>
</tr>
<tr>
<td>7.00</td>
<td>57</td>
</tr>
<tr>
<td>7.25</td>
<td>28</td>
</tr>
<tr>
<td>7.50</td>
<td>&lt;1</td>
</tr>
<tr>
<td>8.00</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Health vs Disease
ECOLOGICAL INTERPRETATION

- habitat selective
- habitat is hostile
- direct relationship between environment & microflora
- relationship is active & dynamic
- relationship can be manipulated?
Can an ecological approach control or prevent dental disease?

- relationship between man & microbes
- contemporary view of plaque in health & disease
- dynamic relationship between the environment and dental plaque
- an “ecological” approach to dental disease
- implications for disease control
- summary
Aetiology of Plaque-Mediated Diseases

Specific plaque hypothesis (Loesche, 1976)

Non-specific plaque hypothesis (Theilade, 1986)

ECOLOGICAL PLAQUE HYPOTHESIS

• Aetiology need not be mono-specific:
  - many species can contribute
    (pathogenic synergism)

• Carriage of “pathogens”
  - clinically-insignificant levels

• Disease preventable/controllable by:
  - direct inhibition of causative organisms
  - maintenance of microbial homeostasis
  - interference with factors driving deleterious
    shifts in microflora
Shifts in Microflora Associated with Caries

- Excess Sugar
- Stress
- Acid Production
- Neutral pH
- Environmental Shift
- Low pH
- S. sanguinis, S. gordonii
- Ecological Shift
- mutans-streps lactobacilli
- Health
- Disease
- Caries
Reduced sugar/low pH challenge:

- fluoride
- dietary control
- sugar substitutes
- stimulation of saliva flow
- antimicrobial agents (sub-MIC)
Effect of Sugar Substitutes on Community Structure

- sucrose can be replaced by non-fermentable sweeteners (sugar substitute)
- intense (saccharin, aspartame, etc)
- bulk (sorbitol, xylitol, etc)
- prevent acid production; stimulate saliva
- xylitol - interferes with sugar transport in S. muta...
Shifts in Microflora Associated with Gingivitis

- Reduced Plaque
- Reduced Inflammation
- Low GCF flow
- G + ve Flora
- Stress
- Environmental change
- Ecological Shift
- Disease
- Increased Plaque
- Increased Inflammation
- High GCF flow, bleeding, raised pH & temp
- G - ve Flora Anaerobes

Gingival Health
Gingivitis

GCF: Gingival Crevice Fluid
PREVENTION STRATEGIES & THE ECOLOGICAL PLAQUE HYPOTHESIS

Altered sub-gingival environment:

- oxygenating or redox agents
- reduced inflammation/GCF flow
- antimicrobial agents (sub-MIC)
ORAL MICROBIAL COMMUNITIES - HARMONY & HAVOC

- Microbe – microbe interactions
- Host – microbe interactions

Diet
- Hormones
- Oral hygiene
- Host defences
- Antimicrobial agents

Dynamic & Active

MICROBIAL HOMEOSTASIS
ORAL MICROBIAL COMMUNITIES - HARMONY & HAVOC

HORMONES

DIET↑

MICROBE - microbe interactions

HOST - microbe interactions

ORAL HYGIENE

HOST DEFENCES↓

Antimicrobial agents

Dynamic & Active

MICROBIAL HOMEOSTASIS
ECOLOGICAL PLAQUE HYPOTHESIS: HOLISTIC APPROACH TO DISEASE CONTROL

- Identify risk factors for individual patients
- Tailor preventive strategies to patient’s needs
SUMMARY

• Dental plaque is a biofilm & microbial community

• Dental plaque is natural & beneficial to health

• Plaque-mediated diseases due to:
  - change in local environment
  - enrichment of minor bacterial populations
  - i.e. (micro)-ecological catastrophes

• Ecological concept offers novel therapeutic & educational / communication opportunities