11 Systems approaches to oral microbiology

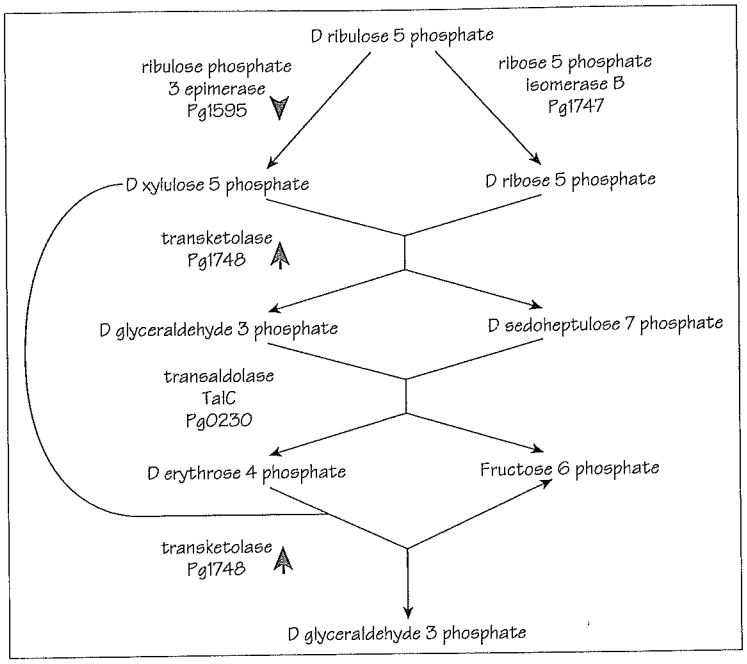


Figure 11.1 Example of ontology for *P. gingivalis* showing the pentose phosphate (non-oxidative) pathway populated from a differential expression proteomics experiment and showing protein abundance changes. Proteins catalyzing each step in the pathway are shown by their *P. gingivalis* gene designation number and protein name. Green downward arrows indicate decreased abundance. Red upward arrows indicate increased abundance. Yellow squares indicate no abundance change.

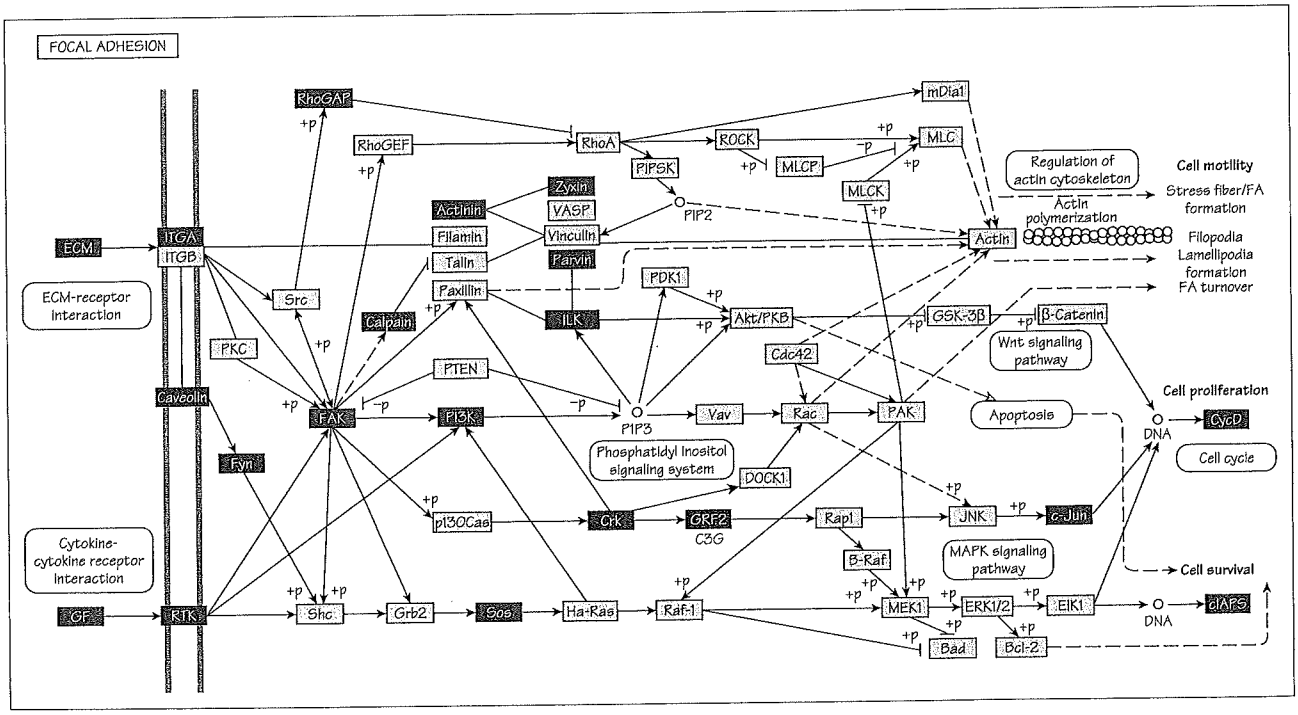


Figure 11.2 Example of ontology for host cell biological pathways involved in focal adhesion. Pathway populated from a differential expression transcriptomics experiment using a microarray to measure mRNA levels in human epithelial cells following challenge with *P. gingivalis*. Red boxes indicate elevated expression and blue boxes indicate reduced expression. Green boxes indicate no expression change.

As the complete genome sequences of oral bacteria are becoming available for an increasing number of organisms, it is now possible to explore bacterial processes such as pathogenicity on a global scale. Genomic sequence can be mined for genes or proteins that are differentially expressed under conditions relevant to disease, such as during infection in humans or in experimental animals, or in *in vitro* models. Genes expressed, up-regulated or otherwise involved in growth or survival under these conditions are candidates for virulence determinants; targets for vaccines or novel therapeutic agents; or have potential for use as diagnostic tools. A number of genome-wide approaches are available, the so called 'omics' disciplines.

Transcriptomics

Transcriptomics is the measurement of the levels of all mRNAs expressed under a particular condition. The transcriptome thus reflects all genes being expressed at a given time. Currently, transcriptomics is usually accomplished using high-throughput techniques based on DNA microarray technology. Microarrays contain spots of DNA target sequence from all genes of the organism. Several unique regions of each gene are amplified or synthesized and attached to a solid support such as a glass slide. RNA is isolated from the organism of interest. Usually the experimental design is to culture the organism under two different conditions. Fluorescently labeled cDNA is prepared from each RNA population, with different fluorescent dyes (usually Cy3 and Cy5) applied in order to distinguish the RNA populations. The labeled cDNA populations are mixed and hybridized to the slide which is then scanned for fluorescent signal intensity attached to the target spots. The strength of fluorescent signal then reflects the level of gene expression.

Proteomics

There is not always a good correlation between the levels of mRNA and the amount of the corresponding protein. As proteins are the major effector molecules of the bacterial cell, measurement of proteins can provide a more accurate view of physiological and metabolic status. Early techniques separated proteins by electrophoresis (either 1D or 2D), and then proteins were identified by sequencing. However, these techniques lacked sensitivity. Currently, the most sensitive technique is to separate whole bacterial lysates into fractions by high pressure liquid chromatography and then feed these fractions directly into a mass spectrometer for protein sequencing. Two bacterial populations can be compared by the differential labeling of one set of proteins with ^{13}C, ^{15}N or ^{18}O which causes a mass offset easily detectable by mass spectrometry. Mass spectrometry also allows the identification of post-translational modifications of proteins, which often dictate protein activity.

Gene ontology

The predictive power of regulation of individual genes/proteins is limited due to the extensive interconnectivity among bacterial physiological and regulatory networks, and functional redundancy (more than one protein being able to accomplish the same task). Thus, the assembly of regulated genes/proteins into biologically relevant pathways has greater biological resolution. The process of population of pathways with regulated components is known as gene ontology. Successful ontology requires information regarding metabolic and regulatory pathways of the organism under investigation. Such databases are now becoming available for oral bacteria (Figure 11.1). As these pathways become better defined it is possible to predict the system-wide effect of up or down-regulation of any bacterial protein, which then can be confirmed experimentally. For an opportunistic pathogen such as *P. gingivalis*, these approaches hold much promise for further elucidation of pathophysiology and mechanisms of virulence. Human pathway databases can be used to examine host responses to bacteria on a global scale (Figure 11.2).

Post-translational networks

Metabolomics is the comparative, non-targeted analysis of the complete set of metabolites (small organic molecules) in a cell. The power of the metabolome is that it directly reflects the physiological status of a cell. The metabolome is diverse and is continually changing, making it difficult to measure. However, high throughput chromatography and mass spectrometry can provide comprehensive datasets. The interactome is the complete set of molecular interactions in a cell. Examples include protein-protein interactions (the protein interaction network, PIN) or the protein-DNA interactome (important in gene regulation). Other 'omes' being established to provide global information about bacterial cells include the lipidome (total lipids) and the glycome (total carbohydrates).

Tiled arrays

Investigation of regulatory networks is facilitated by the use of tiled arrays. Rather than limiting targets to ORFs (as in conventional microarrays), tiled arrays contain overlapping sequences from the complete genome sequence, which allows identification of elements that bind to regulatory (non-coding) sequences.

12 Oral streptococci

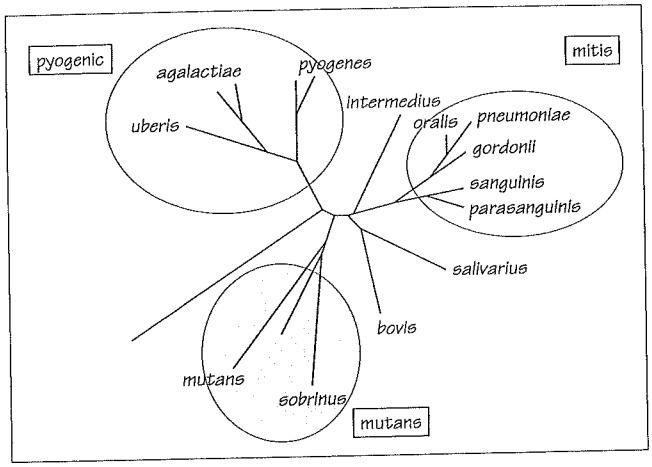
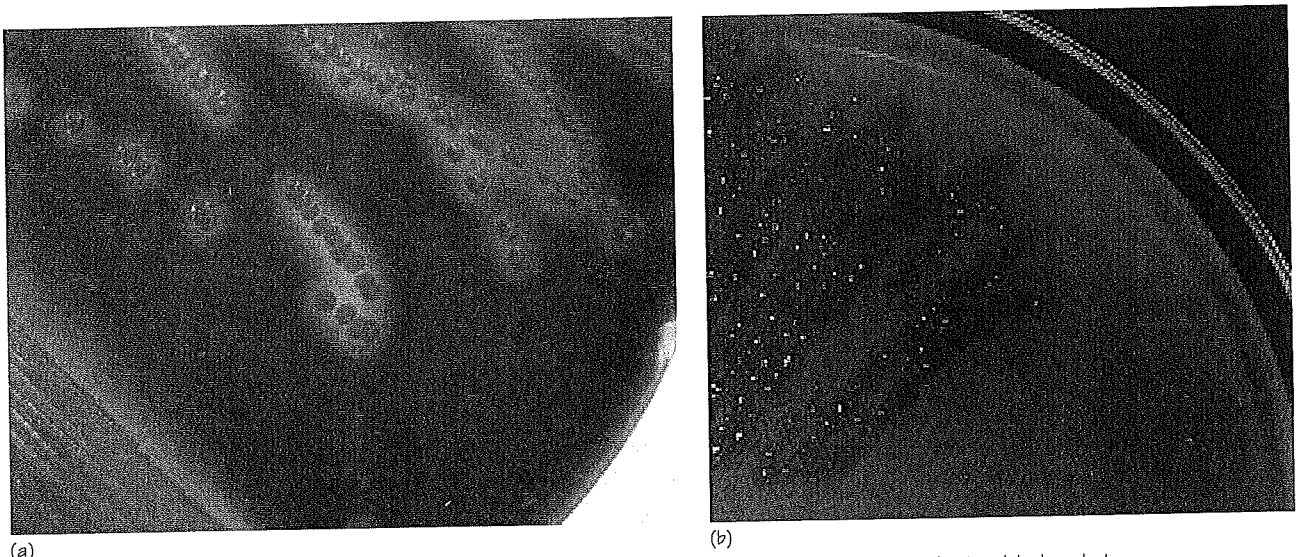


Figure 12.1 Relatedness within three of the major groupings of streptococci. Adapted with permission from Whiley RA, Beighton D Current classification of the oral streptococci. *Oral Microbiology and Immunology* (1998): 13, 195–216.



(a) (b)

Figure 12.2 (a) Group A streptococci on blood agar showing beta hemolysis. (b) Viridans streptococci on blood agar showing alpha hemolysis.

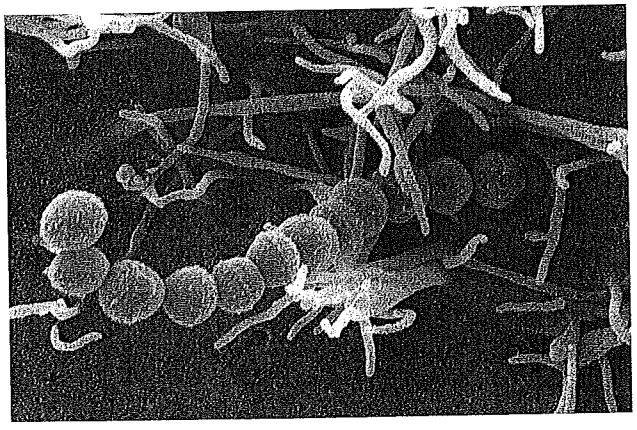


Figure 12.3 *Streptococcus pyogenes* interacting with the surface of epithelial cells. The cell membrane forms projections that entwine the bacteria, while uptake of the streptococcal cells occurs through caveolae formation (specific endocytic vesicles lined with the protein caveolin).

The first oral microorganisms to be cultivated were *Streptococcus mutans* and *Lactobacillus*. These organisms are amongst the family collectively designated as lactic acid bacteria. The group includes the mutans group streptococci, some other oral streptococci (although not all streptococci), and the genus *Leuconostoc*. Lactic acid bacteria characteristically ferment sugars through the glycolytic pathway to form pyruvate, which is then converted to lactate (lactic acid). The amounts of lactic acid produced by the individual bacteria depend upon the environmental conditions, e.g. pH, oxygen. They also depend upon the complement of enzymes present within the bacteria that produce alternative fermentation end products, like acetate (acetic acid), butyrate, propionate and ethanol. The genus *Lactobacillus* contains organisms that are highly acidogenic. This means that they produce large amounts of acid and, as a result, they are also aciduric (meaning able to survive, and sometimes grow, at very low pH). The lactic acid bacteria are characteristically associated with the fermentation of milk, and the generation of fermented milk products, e.g. cheese, yogurt, etc. There is a theory, therefore, that these organisms evolved alongside the emergence of mammals.

The genus *Streptococcus*

Over one hundred species of *Streptococcus* are recognized. Of these, about 50% may be found at some time within the human mouth. Almost all streptococci species are associated with animal hosts, including primates, ruminants, rodents and fish. Interestingly, dogs do not normally carry oral streptococci. Although there are many clear species designations of *Streptococcus*, the individual strains within a species are often very divergent in genotype and phenotype. This is because there is a high frequency of gene transfer between or across the different streptococci. The preponderance for horizontal gene transfer results in a genetic spectrum across the genus *Streptococcus*. Therefore, as more molecular information is obtained from genetic studies, it becomes harder to define individual species within the genus. There is so much variability within the species *S. mitis* and *S. oralis*, which are early colonizers of the teeth and oral mucosa, that individual strains might represent new species in classic terminology. In general though, the human-colonizing *Streptococcus* may be classified into three families: pyogenic (pathogenic), mitis (commensals and pathogens) and mutans streptococci (see Figure 12.1).

Viridans streptococci

The classical name for the oral streptococci is the viridans group. Species of viridans streptococci include *Streptococcus anginosus*, *gordonii*, *intermedius*, *sanguinis*, *parasanguinis* and the mutans group streptococci. The terminology comes from the observations that many of the oral streptococcal colonies produce a green to brown halo when grown on blood agar. The greening is termed alpha-hemolysis and is a result of the bacteria excreting hydrogen peroxide (H_2O_2) as a by-product of metabolism (Figure 12.2). Oxygen, and other oxidative products, e.g. superoxide ions, which are harmful to the bacteria, are converted to H_2O_2, which then oxidizes the heme present within hemoglobin to form green to brown pigments. In contrast, pathogenic streptococci, e.g. *Streptococcus pyogenes*, produce beta-hemolysis on blood agar. This is exhibited as complete clearing around colonies, caused by lysis of the red blood cells by cytotoxins (Figure 12.2). In *S. pyogenes* these toxins are streptolysin S and streptolysin O.

Lancefield grouping

Many species of streptococci can be assigned to the Lancefield grouping system, pioneered by Rebecca Lancefield at the Rockefeller Institute, New York, from 1929. The Lancefield grouping system depends on a panel of antibodies that react specifically with different carbohydrate antigens present within the cell wall of streptococci. The groups comprise A, B, C, D, E, F, G, H, K, L and R/S. Some of the viridans group streptococci can be Lancefield typed, e.g. *S. mutans* (F), *S. sanguinis* (H), *S. salivarius* (K), but some strains within these species, and *S. pneumoniae*, do not type and are referred to as non-Lancefield type organisms. *S. pyogenes* is a group A *Streptococcus* (GAS). *S. agalactiae* is a group B *Streptococcus* (GBS). GAS strains are further serotyped according to their surface fibrillar M protein. This is anti-phagocytic (protects against phagocytosis) and there are over 150 M types now recognized.

Relatedness and pathogenicity of streptococci

Although it is probably most convenient to use the pyogenic, mitis and mutans groupings, this simplifies a very complex genus. The pyogenic organisms are intracellularly invasive (Figure 12.3), cause invasive diseases and are responsible for pharyngitis, tonsillitis, rheumatic heart disease, acute glomerulonephritis, necrotizing fasciitis (GAS) and neonatal (newborn) infections (GBS). *S. pneumoniae* (also termed pneumococcus) causes serious lung infections (pneumonia) and meningitis, and is responsible for most childhood middle ear infections (otitis media). The mutans group organisms cause dental caries, but when they get into the bloodstream they may also cause infective endocarditis (Chapter 31). The mitis group organisms (but not *S. pneumoniae*) are generally very efficient colonizers of the oral cavity. They orchestrate the development of plaque and so contribute to subsequent development of caries, gingivitis and periodontal diseases.

13 Microbial adherence

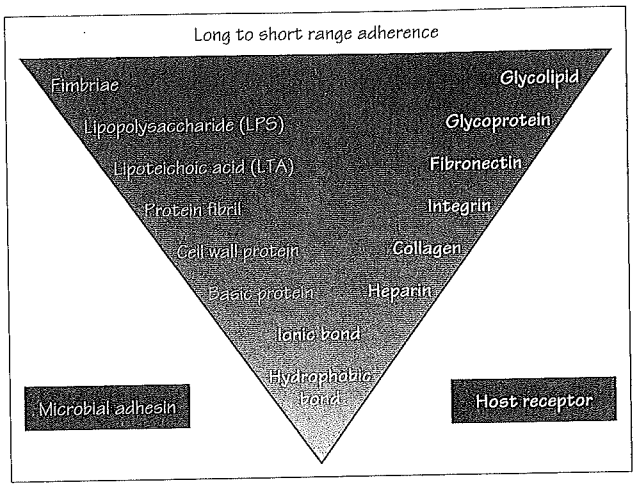


Figure 13.1 Examples of microbial adhesins recognizing host receptors at different ranges.

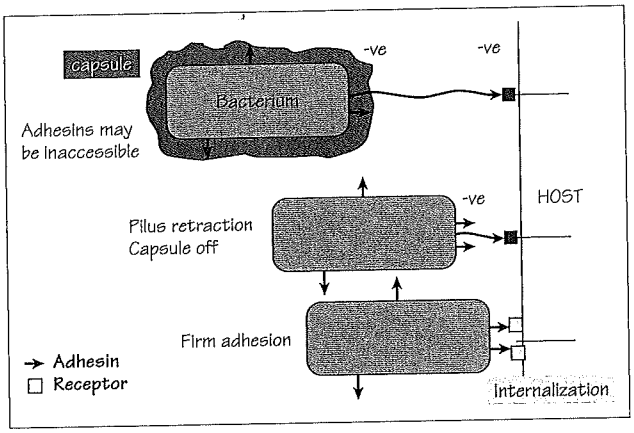


Figure 13.2 Bacterial appendages that mediate long-range adherence and functional consequences of their action.

Table 13.1 Long through short-range adherence.

Host receptor	Adhesin	Microorganism
Glycolipid	Fimbrial (type 1)	*Escherichia coli*
Glycoprotein	Lipopolysaccharide (LPS)	*Pseud. aeruginosa*
β_1 Integrin	Hemagglutinin (Fha)	*Bordetella pertussis*
Fibronectin	Lipoteichoic acid (LTA)	*Strep. pyogenes*
CEACAM (CD66)	Opa	*N. meningitidis*
Fibronectin	PrtF(Sfb)	*S. pyogenes*
Fibrinogen	ClfA	*Staph. aureus*
Heparin	Basic protein (HlpA)	*Streptococcus spp.*
Bound Ca^{2+}	Electrostatic (-ve)	*Strep. sanguinis*
Hydrophobic	Hydrophobic	*Candida albicans*

Most natural environments in which bacteria exist are open flow systems. This means that to grow and survive under such conditions bacteria must adhere to surfaces and form microbial communities (biofilms). Adherence specificity, together with metabolic processes, determines the ability of a microorganism to colonize an animal host. In the oral cavity, successful colonizers must resist the flushing action of salivary flow together with the mechanical shearing forces of the tongue and lips. Adherence is a potential target for novel therapeutics designed to inhibit bacterial colonization. Infections of catheters, hip replacement joints, contact lenses, dental implants and dentures are major problems that are inadequately controlled by antibiotics and could benefit from such an approach.

Adherence

Bacteria colonize different sites in the human body because they express specific adhesins (usually proteins) (Figure 13.1). These recognize complementary or cognate receptors, often sugars or oligosaccharides, present at different sites. Thus, the buccal mucosal surface comprised of keratinized epithelial cells expresses different receptors from, for example, those present in salivary pellicle. The requirement for a specific complementary receptor to be engaged by a microbial adhesin imparts specificity of bacterial adherence and colonization. Following the initial adherence processes, bacteria will only grow and survive if the immediate chemical environment, e.g. pH, oxygen levels and redox potential, is conducive. It is important to note that adherence is a dynamic process. It is advantageous for bacteria to detach from a surface if the growth conditions are, or become, unfavorable. Consequently, microorganisms have evolved methods for detachment as well as attachment.

Long-range adherence

Bacteria first localize at sites that are thermodynamically favorable. This involves overcoming the repulsive forces that occur between negatively charged surfaces (bacterial and host). One means to accomplish this is through surface appendages such as fimbriae (or pili), which are made up of multiple protein sub-units (polymeric). These extended structures allow for long-range adhesion (across 1 μm or more) to occur. Non-covalent forces such as van der Waals forces, along with electrostatic (if there are positively charged amino acids exposed in the fibrillar proteins) and hydrophobic attraction mediate this adhesion. As the bacterial and host surfaces become closer, hydrogen bonding and divalent cation bridges can also stabilize the interaction (Table 13.1). The combined strength of these bonds can be sufficient for bacteria to remain at a surface long enough to form interbacterial linkages and to allow penetration of the mucous or slime layers present on the surfaces of tissues.

Specific adhesion

Long-range adhesive forces are sufficient for initial attachment but lack specificity, and bacteria can be easily dislodged from the surface. Higher affinity adhesion is provided by complementary adhesins and receptors that fit together much as in an antibody-antigen interaction. Adhesins may be proteins or carbohydrates linked directly to the cell surface, or components of surface structures, e.g. fimbriae that are projected away from the confines of the cell wall (Table 13.1) (Figure 13.2). The protein sub-units of fimbriae (pili) may themselves mediate adherence, or carry the adhesins along their lengths and at their tips. Specificity of microbial adhesion is often associated with protein-carbohydrate (lectin-like) reactions involving specific oligosaccharide receptors. Many microbial adhesins recognize galactose or sialic acid carbohydrate chains or oligosaccharides. However, protein receptors are also common.

Oral bacterial adhesins and receptors

Oral streptococci often possess multiple adhesins which will both increase the affinity of binding and the range of substrates available. Examples, many of which are conserved across species, include (adhesin-receptor): AgI/II family-gp340 and collagen; glucan binding proteins (GBPs)-glucan; AbpA/B-amylase; Hsa-NeuNAc; FbpA-fibronectin. Fibrils on *S. gordonii* (CshA/B-fibronctein) and *S. parasanguinis* (Fap1 protein) also mediate adhesion. *Actinomyces naeslundii* type 1 fimbriae adhere to proline rich proteins (PRPs) present in salivary pellicle, while type II fimbriae attach to sugar residues on epithelial cells. *Porphyromonas gingivalis* long fimbriae (FimA) adhere to PRPs and to salivary statherin in pellicle, as well as directly to host epithelial cell integrins. The *tad* (tight adherence) locus of *Aggregatibacter actinomycetemcomitans* includes genes for the biogenesis of Flp pili, which are necessary for bacterial adhesion to surfaces. Another *A. actinomycetemcomitans* adhesion is Ema, a non-fimbrial protein that binds to collagen. Surface protein adhesin-receptors of other periodontally relevant bacteria include: *Fusobacterium nucleatum* FadA-epithelial cells; *Treponema denticola* Msp-matrix proteins; *Tannerella forsythia* BspA-host cells and matrix proteins. Bacterial surface polymers containing saccharides can also mediate or promote adhesion. Lipoteichoic acids are thought to assist adherence of these bacteria to surfaces. They may operate by ensuring correct presentation of protein adhesins towards their receptors. Polysaccharides that contain sialic acid may promote adhesion of bacteria to human cells, and the LPS of *A. actinomycetemcomitans* is involved in adherence. Adherence processes lead to several possible outcomes: colonization, superficial infection of tissues, intracellular invasion or systemic spread of bacteria throughout the body. Moreover, specific co-adhesion among bacterial species drives the development of the dental plaque community (see Chapter 14).

14 Complex communities

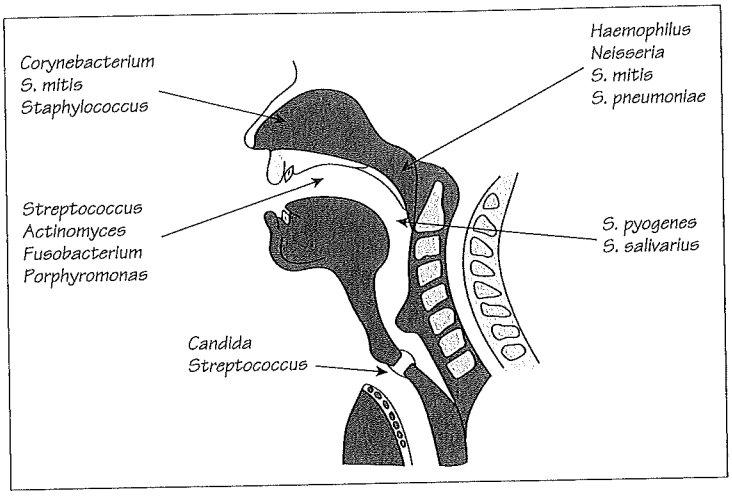


Figure 14.1 Site specificity of microbial adherence and colonization.

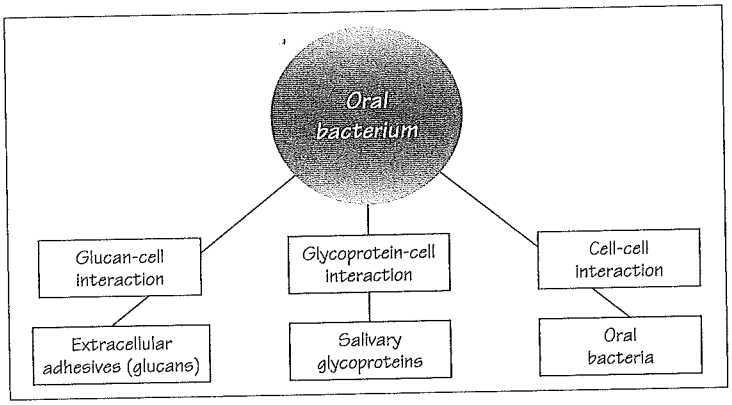


Figure 14.2 Major adherence mechanisms of oral bacteria.

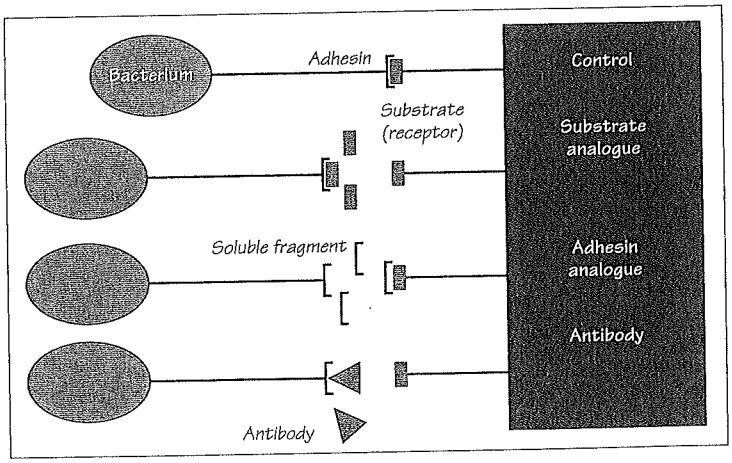


Figure 14.3 Strategies to inhibit bacterial adhesion.

Specificity of adhesion (Chapter 13), along with environmental constraints arising from host and other bacterial influences, imparts specificity to microbial colonization patterns (Figure 14.1). The first stages of dental plaque formation, already discussed, involve the attachment of bacteria to salivary proteins and glycoproteins that are deposited as pellicle on the surfaces of teeth and other hard surfaces (like dentures). The pioneering bacteria are *Streptococcus*, *Gemella*, *Granulicatella* and *Actinomyces*. As they build up on surfaces so they form the base layers for the development of complex dental plaque biofilms. Complex plaque begins to develop on a clean tooth surface after 4–6 hours. Plaque develops more quickly at the gingival margins where salivary flow forces are lower, and the micro-environment is more protected.

Inter-microbial reactions

The development of plaque is an ordered, temporal sequence of events, rather than random coming together of different bacteria. The primary colonizing bacteria adhere directly to receptors in the acquired salivary pellicle (Chapter 4). These bacteria compete for binding sites, so a tenaciously adhering bacterial species might effectively exclude other less-adherent species. Therefore, inter-microbial adherence (co-adhesion or co-aggregation) is important because it allows those bacteria that are less competitive in binding to salivary pellicle to nevertheless become incorporated into the biofilm (Figure 14.2). Streptococci and *Actinomyces* are the exemplars of this, frequently binding to each other as well as to salivary pellicle. Co-adherence is mediated by the type 2 fimbriae of *Actinomyces* binding to receptor polysaccharide on the streptococci. As the complex microbial community develops, so components from saliva continue to get incorporated into the matrix. One of the reasons that primary colonizers are often found at different levels throughout plaque is because they bind to salivary components that get deposited onto bacterial cells during the accumulation processes. Bacterial polysaccharides are also important in maintaining the cohesion of plaque.

Co-adhesion

Co-adhesion or co-aggregation is displayed by almost all successful colonizers of plaque that have been tested. Secondary colonizers such as *Fusobacterium* and *P. gingivalis* are especially effective in attaching to earlier plaque colonizers. *Fusobacterium* is very commonly isolated from oral microbial communities. *Fusobacterium* cells express a suite of protein adhesins on their surfaces, such as the arginine-specific RadD, and galactose and N-acetylneuraminic acid-specific lectins which are responsible for intergeneric binding. Multiple direct contacts between bacterial cells lead to a comprehensive network of physical associations being built up. *P. gingivalis* also co-adheres with earlier colonizers such as streptococci and actinomyces, as well as with later colonizers such as *Tannerella forsythia* and *Treponema denticola*. Specific adhesion to *S. gordonii* is mediated by two sets of adhesin receptor pairs. The long fimbriae (FimA) bind to glyceraldehyde-3-phosphate dehydrogenase present on the streptococcal surface, and the short fimbriae (Mfa) engage the streptococcal SspA and SspB (Antigen I/II) adhesins. Finding new compounds to disrupt co-aggregation might enable control of the development of microbial communities (Figure 14.3).

Metabolic associations

Oral bacteria tend to accumulate into communities that are metabolically compatible. This can be manifest as simple protection whereby facultative anaerobes such as streptococci remove oxygen that is toxic for the anaerobic secondary colonizing periodontal organisms. Nutritional interrelationships among oral bacteria are common. One of the best defined is between *Veillonella* and *Streptococcus*. Lactate produced by streptococci is utilized directly by *Veillonella* for growth. *Veillonella* lack a fully functional glycolytic pathway; therefore hydroxyl acids e.g. lactate, or carboxylic acids e.g. malate, provide sources of carbon and energy. Thus, levels of *Veillonella* in plaque usually increase with numbers of streptococci. Moreover, as lactate is removed from the immediate environment by *Veillonella*, so the flux of glucose to lactate increases, thus enhancing growth of streptococci. Such co-dependent nutritional associations may account, at least in part, for the difficulty in cultivating a large percentage of the oral microbes.

Antagonism

Not all interbacterial interactions are conducive to mutual colonization. Competition for nutrients and for attachment sites are examples of interbacterial antagonistic relationships. Many oral streptococci produce hydrogen peroxide that can damage and kill other bacteria through the generation of oxidizing free radicals. Streptococci and a variety of oral bacteria including *A. actinomycetemcomitans* produce bacteriocins that are lethal to other bacteria. Actinobacillin, produced by *A. actinomycetemcomitans* is toxic to streptococci and actinomyces and may contribute to the inverse relationship between levels of *A. actinomycetemcomitans* and streptococci/actinomyces in the plaque of patients with localized aggressive periodontitis. Lantibiotics are a class of bacteriocins that contain the post-translationally modified amino acids lanthionine and/or methyllanthionine, and are produced by various members of the oral streptococci. For example, Salivaricin A is produced by *S. salivarius* and is toxic to most strains of *S. pyogenes*. Contact-dependent signaling is also involved in antagonistic interactions. Initial contact between *P. gingivalis* and arginine deiminase on the surface of *Streptococcus cristatus* results in activation of a regulatory cascade that decreases transcription of the gene for FimA. As a result, biofilm accretion does not occur with *P. gingivalis* and *S. cristatus*.

15 Biofilms

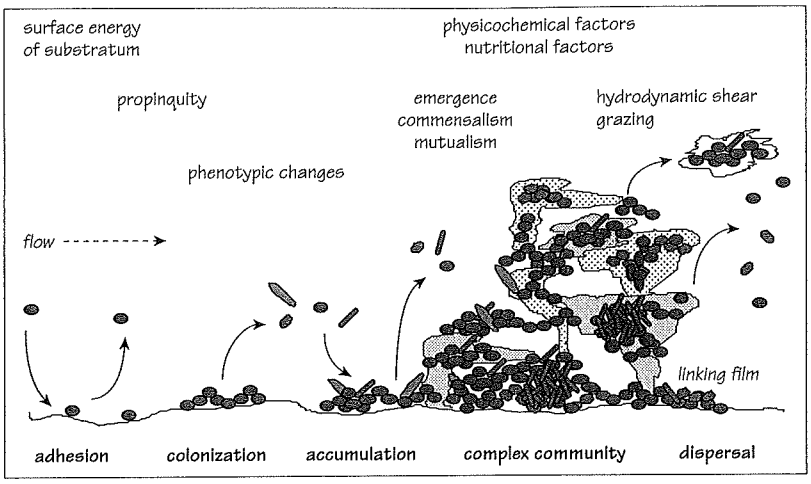


Figure 15.1 Process of biofilm formation. Reproduced with permission from Jenkinson HF, Lappin-Scott HK Biofilms adhere to stay. *Trends in Microbiology* (2001): 9, 9–10.

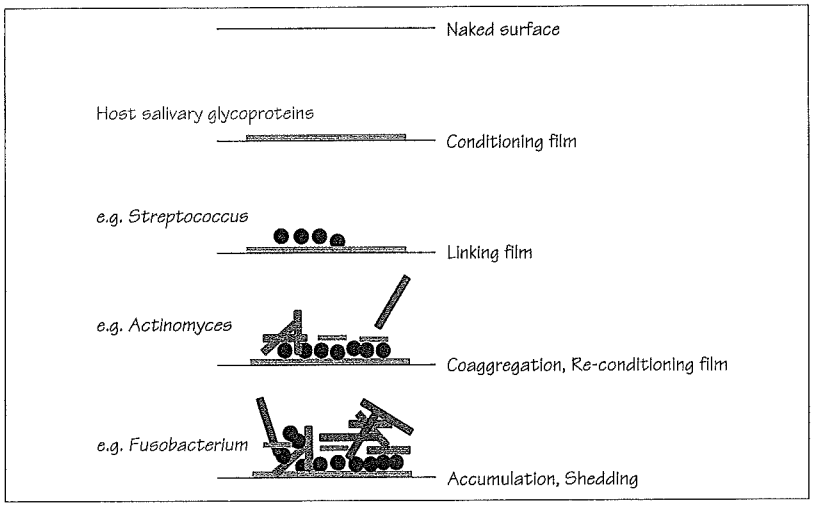


Figure 15.2 Stages in the formation of an oral biofilm community.

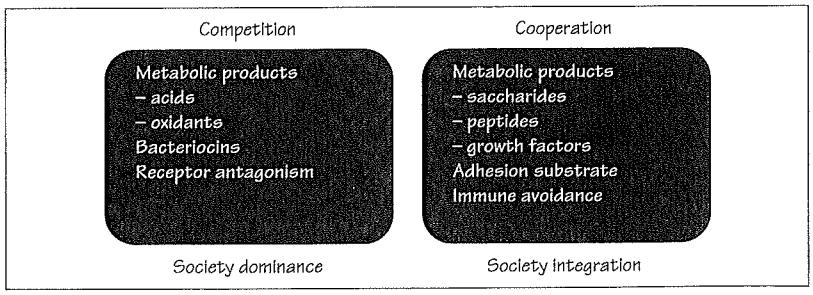


Figure 15.3 Competition and cooperation mechanisms in a multi-society community.

Bacteria in their natural environments do not generally exist as isolated cells, but grow and survive in organized communities. The microbial communities that develop at phase interfaces, such as solid/liquid or air/liquid, are termed biofilms. One feature of biofilms is that microbial cells with very different metabolic requirements can exist successfully in communities. The collective strength of a microbial biofilm community in terms of metabolic efficiency and survival is much greater than the sum of the components. In this respect, the biofilm could be considered as an evolutionary unit.

Biofilm development

The life of an oral biofilm may be depicted as a developmental cycle (Figure 15.1). The various stages of the cycle are determined by physical, biological and environmental factors. Initial adhesion of microbial cells to a biologically conditioned surface, e.g. saliva coated tooth enamel, is a random event influenced by surface free energy and propinquity (nearness) of bacterial cells. If microbial cells adhere, then phenotypic changes occur as the cells divide and form accumulations, generating a linking film. Onto this linking film, new microbial cells may attach and further accumulations of cells occur. During the development of polymicrobial populations, such as dental plaque, every new organism that binds to the linking film presents a new surface (Figure 15.2). This therefore forms the basis for accretion of defined organism groupings. The linking film also provides a means for stabilizing microbial communities that are continuously subject to physical shear forces, e.g. fluid flow, tongue movement. The biofilm community is a dynamic entity: cells continually enter or leave the community, promoting diversiftcation or dispersal, while protozoal grazing and shear forces in flow systems, such as in the oral cavity, lead to biofilm structure remodeling. Within biofilms, constituent species can be in competition or can co-operate with one another (Figure 15.3). Constraints on biofilm accumulation may be beneficial by maintaining an optimal surface area/volume ratio to facilitate diffusion of nutrients and removal of metabolic end-products.

Microbial recognition of surfaces and interbacterial communication

The metabolic processes occurring within microbes undergoing growth on a surface are vastly different from those occurring within microbes in liquid suspension (planktonic). Surface growth is associated with global changes in gene expression. Between 30–50% of genes transcribed in bacteria or fungi can be affected following attachment to substrata. Changes in gene expression result from sensing and recognition of abiotic surfaces and other bacteria, and through interbacterial communication systems. In biofilms, microbial cells in close proximity send signals to each other, such as acyl homoserine lactones (AHLs) in Gram-negative bacteria (although generally not oral Gram-negatives), oligopeptides in Gram-positive bacteria (e.g. CSP, see Chapter 18), or AI-2 (produced by the action of the LuxS enzyme) in both Gram-positives and Gram-negatives. These signaling molecules can act as cell-density-dependent regulators of gene expression and metabolism. They play roles in extracellular polysaccharide (EPS) matrix deposition, maintenance of optimal biofilm architecture and dispersal of bacteria from biofilms. For example, AI-2 dependent polysaccharide synthesis by *S. gordonii* is necessary for optimal heterotypic biofilm formation with *P. gingivalis*. In addition, contact between the Mfa fimbriae of *P. gingivalis* and the AgI/II family protein (SspA/B) of *S. gordonii* initiates a phosphorylation dependent signal transduction cascade in *P. gingivalis* that regulates EPS production and AI-2 levels. *Veillonella atypica* communicates with *S. gordonii* by means of a short-range diffusible signal that increases amylase production in *S. gordonii*, thus providing additional fermentation substrates for *V. atypica*.

The biofilm matrix, resilience and resistance

Many biofilms contain extracellular polymeric materials that contribute to structural integrity. A major component of the *Aggregatibacter actinomycetemcomitans* biofilm matrix is poly-β-1, 6-*N*-acetyl-D-glucosamine (PGA), a hexosamine-containing polysaccharide that mediates intercellular adhesion. Extracellular DNA also contributes to the matrix in *A. actinomycetemcomitans* biofilms. The role of glucans in *S. mutans* biofilms is discussed in Chapter 16.

The increased resistance properties of biofilm cells to external influences such as antibiotics, host defenses, antiseptics and shear forces, are of major concern in dentistry, medicine and industry. Extracellular polymeric substances, while providing mechanical strength, do not necessarily provide a barrier to inhibitory compounds. Bacteria within biofilms can be inherently more resistant to antimicrobial compounds. For example, small colony variants of *Staphylococcus aureus*, generated within biofilm communities on medical devices, have diminished metabolic rates that make them less susceptible to antibiotics. It is probable that these so-called persister cells with reduced antibiotic susceptibility are commonly produced within biofilms. In mixed biofilms, one β-lactamase producing bacterial species may protect cells of another species from penicillin, while streptococci or staphylococci growing in mixed biofilms with *Candida albicans*, reduce the susceptibility of *C. albicans* to antifungal agents. One of the many challenges is to devise effective means to prevent biofilm infections of indwelling medical devices. Impregnation of biomaterials with slow-release antimicrobials is one approach.

16 Bacterial polysaccharides

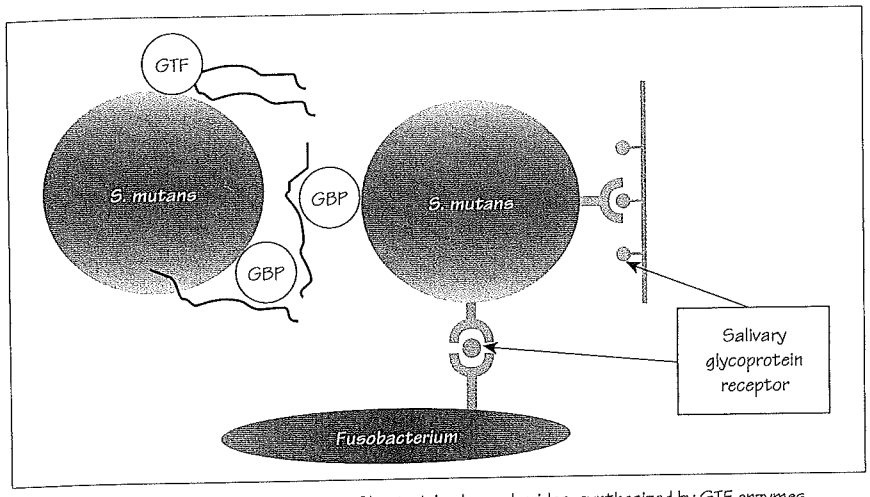


Figure 16.1 Diagrammatic representation of bacterial polysaccharides, synthesized by GTF enzymes, facilitating build up of communities through recognition by glucan binding proteins (GBP). Salivary glycoprotein receptor provides a substrate for adherence to tooth surfaces and for co-adhesion with other bacteria.

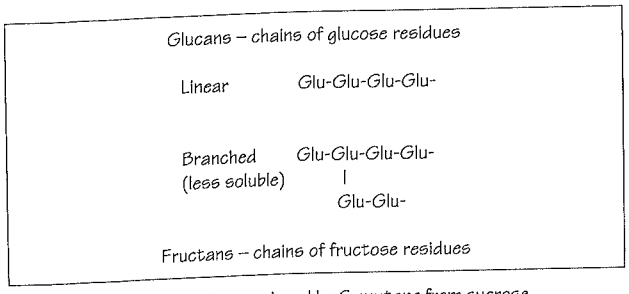


Figure 16.2 Polysaccharides produced by *S. mutans* from sucrose.

Table 16.1 Properties of glucans produced by *S. mutans*.

Streptococcus mutans produces from sucrose
(1) Water-soluble glucans
(a) Readily degraded for energy source
(b) Formation of lactic acid
(2) Water-insoluble glucans
(a) Sticky and hard, act as cement
(b) Promote accumulation of plaque

Many of the oral bacteria produce polysaccharides. Streptococcus strains produce extracellular polysaccharides (EPS) which are usually glucose or fructose polymers. They also accumulate intracellular polysaccharides that are glucose polymers, rather similar in structure to liver glycogen. Actinomyces strains produce mixed sugar polysaccharides. Bacterial polysaccharides provide a matrix that facilitates the accumulation of oral biofilms (Figure 16.1).

Extracellular polysaccharide production

Although EPS production is less important for development of subgingival plaque, it is significant in enhancing colonization of *S. mutans* and other streptococci associated with dental caries (enamel caries and root caries). Bacterial polysaccharides are synthesized by enzymes termed glycosyltransferases. *S. mutans* and several other viridans group streptococci, e.g. *S. salivarius* and *S. gordonii*, can express a number of different enzymes that are active in the synthesis of glucans (chains of glucose residues) and fructans (fructose polymers). The glucan chains may be either linear, or contain branches of chains (Figure 16.2). The branched chains are usually less soluble than the linear chains, and they need more complex sets of enzymes for degradation (Table 16.1). The linear chains are broken down more easily than branched chains by dextranases (DexA in *S. mutans*). The released sugars are taken up by the cells for growth. The glucose sub-units in linear glucan are generally linked by α-1,6 O-glycosidic bonds. α-1,3 bonds cause branched linkages and increased insolubility. α-1,4 linkages are less common as these are a target for digestion by salivary amylase. Fructans can be degraded by fructanase and are a good source of energy for bacteria, as fructose metabolism is quicker and requires one less phosphorylation step than glucose in the glycolytic pathway.

Glycosyltransferases and fructosyltransferases

Glucans and fructans are produced from sucrose (disaccharide of glucose and fructose). Water soluble glucans are produced both inside and outside the cells by glucosyltransferase (GTF) enzymes. These provide a mechanism for the bacteria to conserve sucrose, when it is in good supply (e.g. after meals or sugary drinks), by converting excess sucrose into glucans. Glucans can be broken down later into glucose residues a few hours after dietary intake has ceased. Water insoluble glucans are produced outside the cells. These are more long-term storage compounds, more difficult to break down, but therefore longer lasting in the oral environment. These glucans are sticky and so they can cement plaque together and promote development of microbial communities within a matrix of polysaccharides. GTF enzymes are produced by streptococci and are secreted onto the bacterial cell surface as well as into the environment and can remain active on tooth surfaces. The mechanism of action is first cleavage of sucrose into glucose and fructose. Then, in a two-step reaction, the glucose is added onto the end of a growing polysaccharide chain using the energy released from cleavage of the disaccharide bond. Some enzymes specifically synthesize linear chains (e.g. GtfC and GtfD of *S. mutans*), others catalyze production of a mixture of linear and branched chains (e.g. GtfB of *S. mutans*). The enzymes all have an amino acid repeat block structure running through the carboxy terminal half of the protein. These repeats, designated YG repeats (Y, tyrosine; G, glycine), form the glucan binding domain while enzyme activity is in the amino terminus region. Fructosyltransferases (FTF) specifically make fructan chains and these are often linear (β-2,1 and β-2,6 linkages).

Glucan binding proteins

GTF enzymes on their own are useful for making polysaccharide, but for the polysaccharide to be effective as a storage source of energy it has to be held onto by the cells. To do this the bacteria express glucan-binding proteins (GBPs). These are essentially lectins that bind glucans. GBPs are found on the cell surface of *S. mutans*, and some other streptococci. Some GBPs contain sequences that are similar to the YG repeats in GTFs. In addition to holding onto the polysaccharide for the bacteria to digest, they act as additional cementing agents in the build up of plaque. The GBPs are necessary for optimal *S. mutans* biofilm formation and antibodies raised to GBPs can be protective against development of caries by *S. mutans* in animal studies.

EPS produced by oral Gram-negatives

EPS can be produced by Gram-negative anaerobes such as *P. gingivalis*. At least six polysaccharide capsular serotypes are produced by *P. gingivalis*. The EPS capsule protects against phagocytosis and tends to be lost on laboratory subculture. Antibodies to *P. gingivalis* EPS are protective in animal models. EPS is also involved in *P. gingivalis-S. gordonii* biofilm development. *P. gingivalis* EPS can mask LPS and reduce its activity.

17 Microbiology of caries

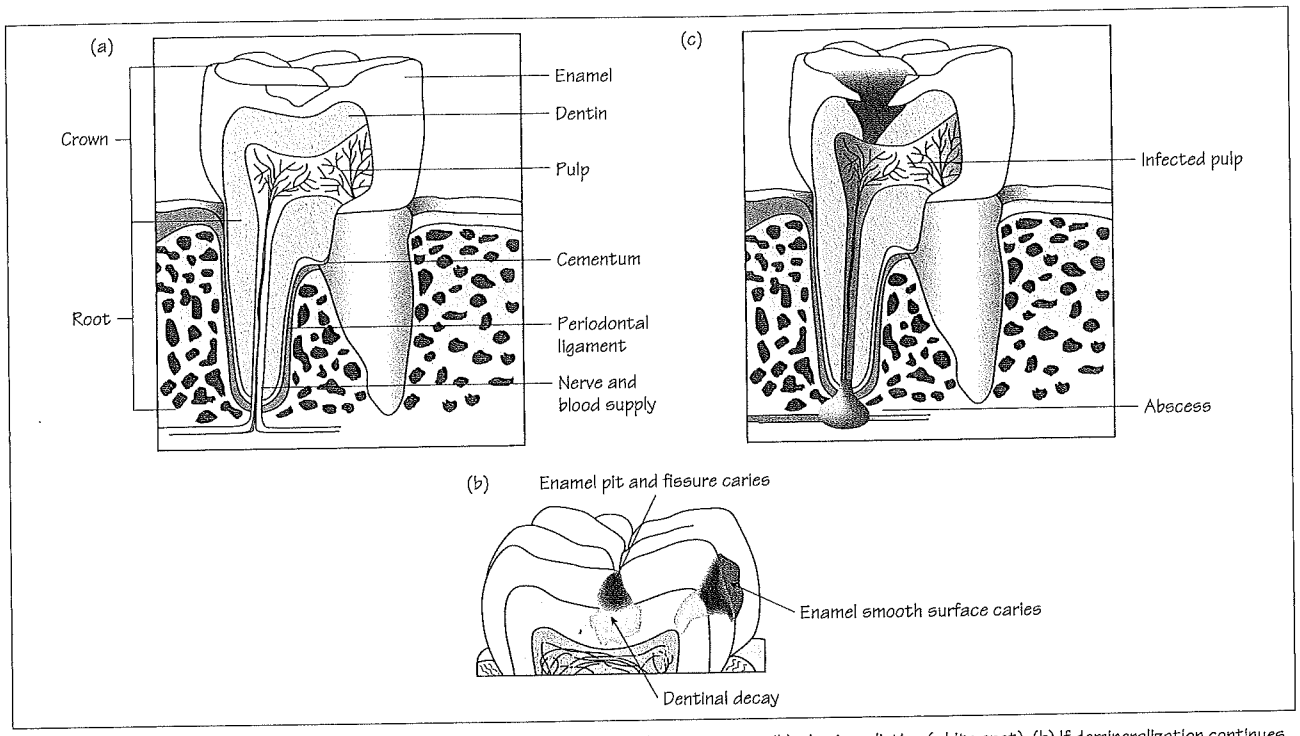


Figure 17.1 (a) Vertical sections showing major components of the tooth. Caries begins as reversible demineralization (white spot). (b) If demineralization continues, the enamel decays and destruction eventually spreads through the dentin to the pulp chamber, which can lead to a periapical abscess (c).

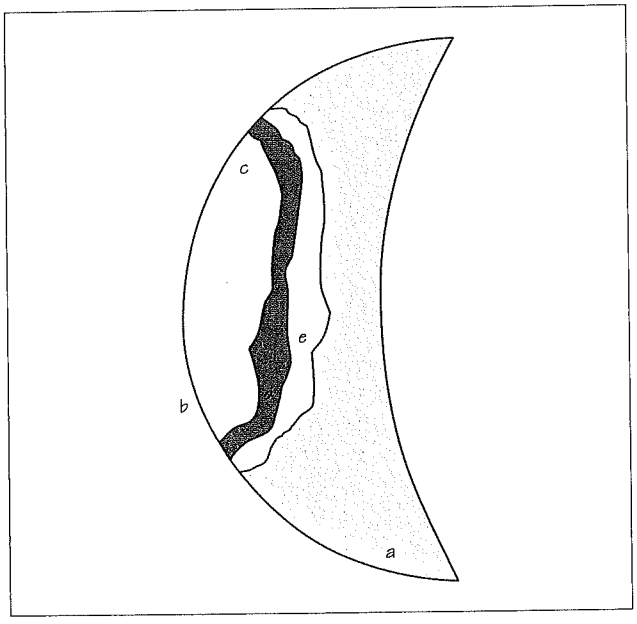


Figure 17.2 Schematic of a section through an early caries lesion on a smooth enamel surface. (a) Sound enamel. (b) Surface enamel slightly demineralized, but appears intact. (c) Body of the lesion with loss of about 25% mineral. (d) Dark zone with loss of about 5% of mineral. (e) Translucent zone with about 1% enamel loss.

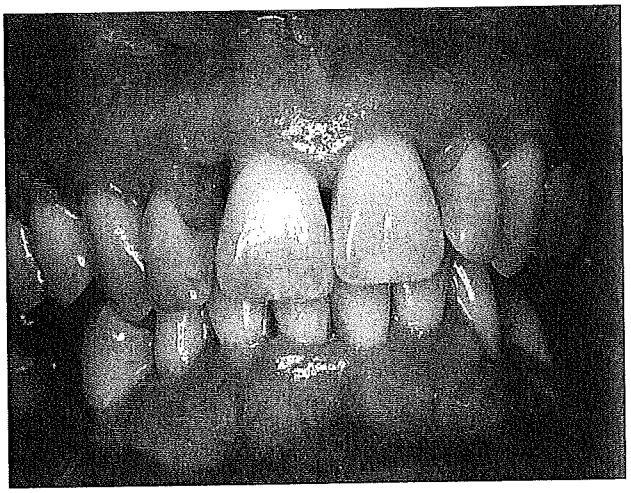


Figure 17.3 Early stages of root caries (discoloration) presenting on multiple teeth.

Dental caries is under control in many human populations. However, there are still major problems with dental decay in children regularly fed high sugar diets or drinks. There are also small populations throughout the world where good dental hygiene is not practiced. A change in diet towards higher sugar intake in these instances is very conducive to caries onset.

Structure of teeth (Figure 17.1)

The surface layer of the coronal part (crown) of the tooth is composed of enamel, a substituted hydroxyapatite (crystalline calcium phosphate). Enamel is highly mineralized and is the hardest substance in the body. Supporting the enamel is dentin, a less mineralized organic matrix of collagenous proteins. Beneath the dentin is the pulp chamber that contains blood vessels and nerves and extends down into the gum as root canals. The roots are composed of dentin and covered with cementum which is a hydroxyapatite and collagen matrix, and is about half as mineralized as enamel. Dentin has microscopic channels, called dentinal tubules, which radiate outward from the pulp chamber to the exterior cementum or enamel border.

Dental caries

Dental caries is the localized destruction of the tissues of the tooth by bacterial action (Figure 17.1). Enamel or cementum is demineralized by microbial acids (predominantly lactic acid) produced by fermentation of dietary sugars. The initial caries lesion is sub-surface, due to acid diffusion (Figure 17.2). The primary lesion that is detectable clinically is known as a white spot and can be reversed by remineralization and regrowth of hydroxyapatite crystals, a process enhanced by fluoride. Advanced caries results in cavitation, and can progress to the dentin and into the pulp chamber ultimately causing necrosis and periapical abscesses.

Types of dental caries

The differing topology and degree of mineralization of the different tissues of the tooth provide unique challenges to bacteria with cariogenic potential.

(1) Enamel smooth surface caries These surfaces are easy to keep clean and are continuously exposed to saliva, and thus difficult for bacteria to colonize.

(2) Pit and fissure and interproximal caries Bacteria can become physically entrapped in these areas without specialized adherence mechanisms.

(3) Root caries Cementum (or dentin when cementum is lost) is more easily demineralized than enamel (Figure 17.3). Roots become exposed to oral bacteria as the gingiva recedes with age or after periodontal surgery. Root caries is thus becoming more prevalent.

(4) Recurrent caries This occurs around existing restorations.

(5) Rampant caries This involves widespread and severe lesions, usually in people with reduced salivary flow (xerostomia).

(6) Early childhood caries (nursing or baby bottle caries) This is rampant caries of the primary dentition of infants and toddlers. The exact causal mechanisms are uncertain but unrestricted access to night-time bottles with fruit juice or sweetened formula is a major contributor.

Important bacteria in caries

Streptococcus mutans

Gram-positive cocci in chains. More accurately, a collection of closely related species known as mutans streptococci and comprising seven species and eight serotypes, a–h. *S. mutans* serotypes c, e and f and *S. sobrinus* serotypes d and g are most closely associated with human disease. *S. cricetus*, *S. ferus*, *S. rattus*, *S. macacae* and *S. downei* are more usually found in animals. Mutans streptococci possess adhesins for salivary receptors allowing attachment to saliva-coated smooth surfaces. In addition, these organisms produce extracellular polysaccharides from sucrose that facilitate retention on surfaces (these virulence factors are discussed in more detail in Chapters 16 and 18). Mutans streptococci are associated with all forms of caries.

Lactobacilli

Gram-positive rods. lactobacilli are efficient producers of lactic acid and are tolerant to low pH values (two important caries associated traits, Chapter 18). However, lactobacilli are poor colonizers of smooth surfaces and probably do not initiate caries at these sites. Most likely lactobacilli are secondary colonizers of established caries lesions, where their aciduric properties allow them to out compete other organisms. Acid production will then exacerbate the lesion and facilitate extension into the dentin. If lactobacilli become embedded in pits and fissures they may be able to initiate caries at these sites. Different species and strains of lactobacilli exhibit differing cariogenic potential.

Actinomyces species

Gram-positive rods. Actinomyces, especially *A. naeslundii*, are frequently isolated from root caries lesions and can cause root caries in experimental animals. However, the organisms are also commonly found on healthy root surfaces so the role of actinomyces in the disease process has been unclear. More recent molecular detection techniques (see below) are re-establishing the importance of *Actinomyces* species in both root and coronal caries.

Emerging and polymicrobial pathogens

Culture independent molecular based techniques such as 16S rRNA sequencing are altering our view of caries etiology. Bacteria that are emerging as important in caries progression include species of the genera *Veillonella*, *Bifidobacterium*, and *Propionibacterium* and *Atopobium*, along with low-pH non-mutans streptococci. Putative etiological agents of root caries are now thought to include species of *Atopobium*, *Olsenella*, *Pseudoramibacter*, *Propionibacterium* and *Selenomonas*. Dentinal caries is associated with *Rothia dentocariosa* and *Propionibacterium* spp. The collective outcome of these studies is shifting our understanding of caries toward a complex community disease.

18 Virulence factors of *S. mutans*

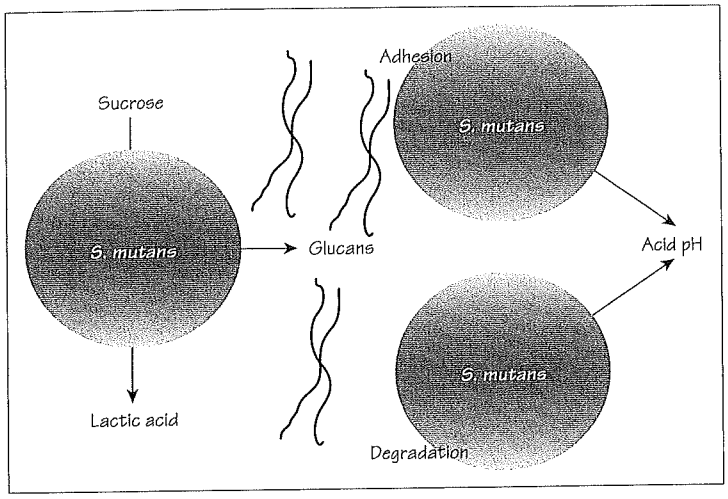


Figure 18.1 The combined effects of sucrose utilization, glucan production, adhesion and production of lactic acid in the generation of dental caries.

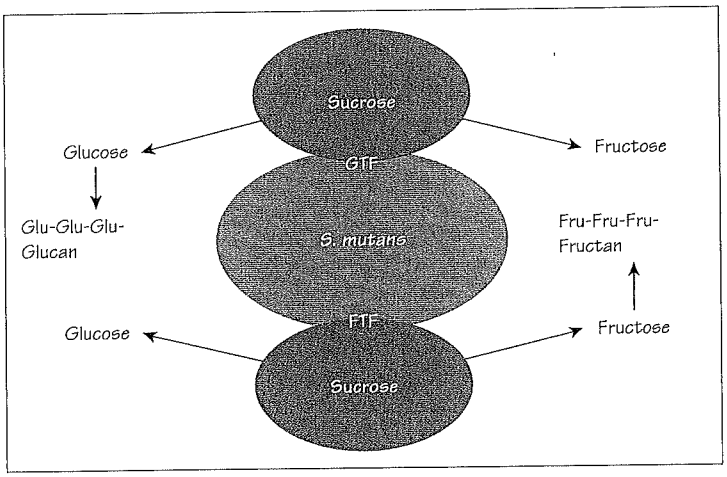


Figure 18.2 Glucosyltransferase (GTF) and fructosyltransferase (FTF) on the surface of *S. mutans* synthesize glucans and fructans from sucrose with the release of fructose (GTF) or glucose (FTF).

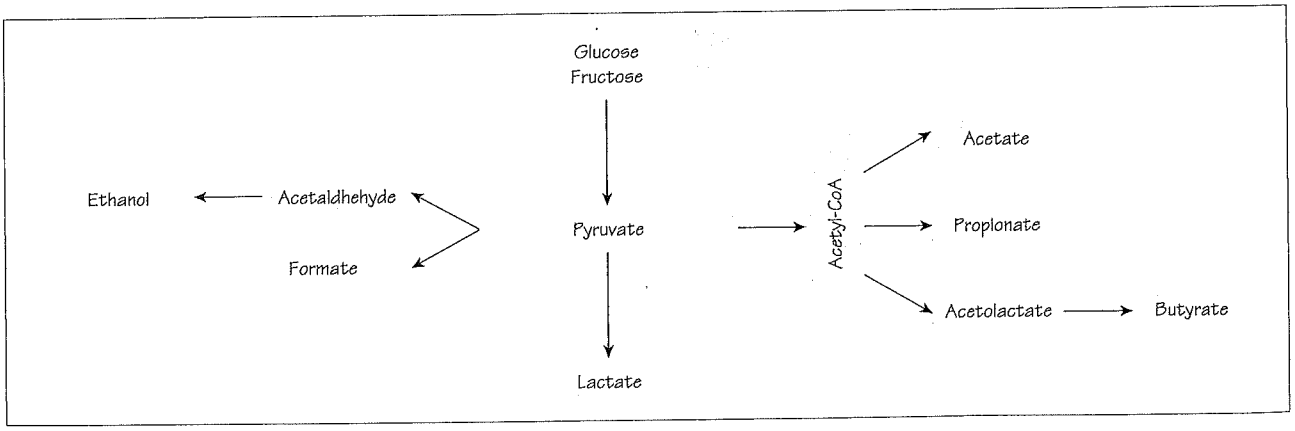


Figure 18.3 Simplified pathway showing major end products derived from hexose sugars via pyruvate in glycolytic fermentation. Streptococci such as *S. mutans* are usually homofermentative in producing lactate from glucose or fructose. Fructose fermentation is more efficient than that of glucose.

S. mutans possess several attributes that contribute to its success as a cariogenic organism: (1) ability to adhere to the tooth surface and develop plaque communities; (2) production of glucans and other polysaccharides from excess carbohydrate (often sucrose) in the diet, leading to plaque accumulation; (3) production of acids (principally lactic acid), that generate a low pH environment and enrich for aciduric organisms (Figure 18.1). Organisms such as *Lactobacilli* that produce and tolerate large amounts of lactic acid are not thought to be the initiators of smooth surface caries as they lack specialized colonization mechanisms. Actinomyces and non-mutans streptococci produce less acid and so may be more important in root caries as cementum (and dentin) is less mineralized and thus more easily dissolved, as compared to enamel.

Initial attachment to tooth surfaces

A major surface protein produced by *S. mutans* is the AgI/II family protein called SpaP (or P1). This protein contributes to the fibrillar layer that is on the outside of *S. mutans* cells, made up of proteins, polysaccharides and teichoic acids. The AgI/II polypeptide of *S. mutans* mediates attachment to salivary pellicle, principally through binding to gp340. AgI/II contains approximately 1500 amino acid residues, and several different regions that bind to salivary glycoproteins, collagen and fibronectin. *S. mutans* can also adhere to earlier colonizing streptococci, a process that can be enhanced by bridging gp340 molecules.

Polysaccharide production

As discussed in Chapter 16, *S. mutans* produces polymers of glucan and fructan from dietary sucrose through glucosyl- and fructosyl-transferases (Figure 18.2). *S. mutans* produces three glucosyltransferases designated GtfB, GtfC and GtfD, and one fructosyltransferase (Ftf). Glucan and fructan can serve as reserves of fermentable carbohydrate, allowing *S. mutans* to continue to metabolize and produce acid when dietary carbohydrates are no longer available. *S. mutans* possesses four glucan binding proteins (Chapter 16), GbpA, B, C and D, and so insoluble glucan also contributes to the cohesiveness and retention of *S. mutans*-rich plaque. Within the cytoplasm, glucose can be polymerized into intracellular polysaccharide (IPS), a glycogen-like polymer that can be mobilized for glycolysis and extend the duration of acidification.

Acid production

S. mutans can metabolize a variety of sugars, resulting in the production of a number of weak acids, including lactic, formic and acetic acids (Figure 18.3). Lactic acid is the strongest of these acids, with an ionization constant (pK_a) of 3.5. When the plaque pH drops below about 5.5 the balance between enamel demineralization and remineralization shifts toward solubility and the caries process is initiated. Sucrose is the most cariogenic sugar because it can be processed into glucan and fructan, and because it is efficiently fermented into lactic acid. Sucrose and other sugars are transported into *S. mutans* cells by the high affinity and high capacity phosphenolpyruvate (PEP) sugar: phosphotransferase (PTS) uptake system. Sucrose is accumulated as sucrose-6-phosphate which is then hydrolyzed to glucose-6-phosphate and fructose which are metabolized via the glycolytic pathway. *S. mutans* also has other sugar uptake systems including proton motive force (PMF) driven transport. Glycolysis of one C6 sugar yields two molecules of pyruvate (C3). The enzyme lactate dehydrogenase then converts pyruvate into lactic acid using NADH as an electron donor. Organisms such as *S. mutans* are considered homofermenters in that as much as 90% of pyruvic acid is converted to lactic acid.

Acid tolerance

S. mutans is highly aciduric, and resistance to the adverse effects of low pH is accomplished by several mechanisms: (1) extrusion of H^+ ions from the cell through a proton translocating F_1-F_0 ATPase (this maintains the cytoplasm at a pH closer to physiological levels); (2) increase in the proportion of mono-unsaturated membrane fatty acids, which will decrease proton permeability; (3) conversion of the arginine derivative agmatine to putrescine, ammonia and CO_2 by the agmatine deiminase system (AgDS); (4) malolactic fermentation, whereby the dicarboxylic L-malate, a major acid in fruits, is converted to the monocarboxylic lactic acid and CO_2; (5) up-regulation of molecular chaperones, proteases and DNA repair enzymes.

Biofilm adaptation

S. mutans is adapted to the biofilm lifestyle and there is coordinated production of bacteriocins along with an increase in competence in high density situations. *S. mutans* may thus acquire DNA from other organisms in close proximity either for nutrition or increasing genetic diversity or both. These processes are controlled by competence stimulating peptide (CSP), a 21-amino-acid peptide pheromone. CSP is a quorum sensing (QS) signal that is secreted into the milieu and initiates transcriptional activity in the bacterial cells after exceeding a threshold level. Biofilm formation and metabolic activity are controlled on multiple levels. Many environmental signals funnel through the nutritional alarmone (p)ppGpp. There are also multiple two-component signal transduction systems (TCS) in the organism, such as the ComDE TCS that responds to CSP.

19 Host and environmental factors in caries

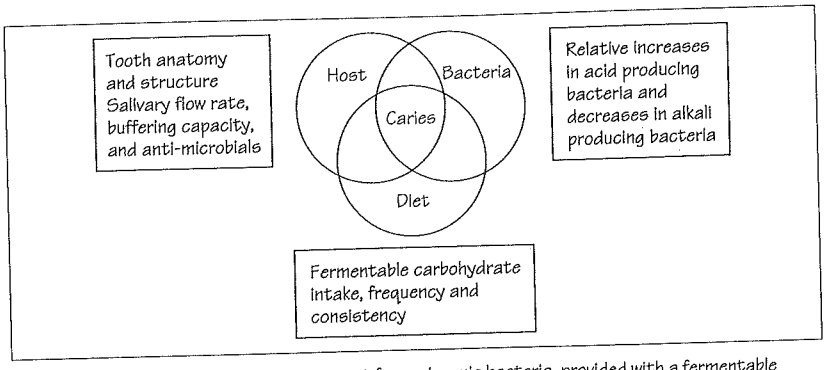


Figure 19.1 Interdependence and requirement for cariogenic bacteria, provided with a fermentable substrate in a susceptible host in order for caries to occur.

Table 19.1 Measurable parameters that are associated with risk of caries development.

Low caries risk	High caries risk
Alkali producing bacteria such as *S. sanguinis*	Acid producing bacteria such as mutans streptococci and lactobacilli
Unstimulated salivary flow rate > 1 ml/min	Unstimulated salivary flow rate < 0.7 ml/min
Infrequent sucrose consumption	Frequent consumption of high levels of sucrose and other fermentable carbohydrates particularly in retentive forms
Fluoride intake to levels allowing production of fluorapatite	Little or no fluoride intake

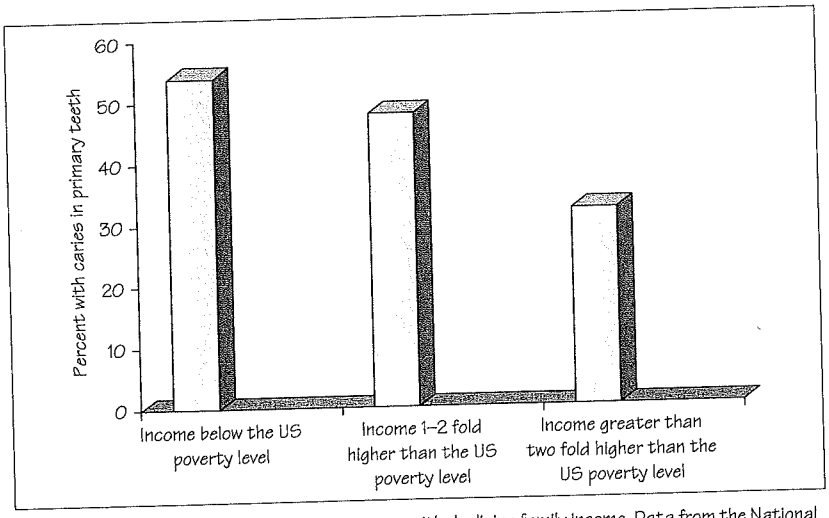


Figure 19.2 Caries incidence in children increases with declining family income. Data from the National Health and Nutrition Examination Survey (NHANES, 1999–2004).

The initiation and progression of caries requires that host, diet and bacterial factors are all conducive to disease (Figure 19.1). In this chapter, host and dietary factors will be considered.

Host factors

(1) **Teeth** Teeth become less susceptible to caries over time. Such post-eruptive resistance is due in part to an increase in the concentration of fluoride in the surface layer of enamel. Fluoride ions substitute for hydroxyl ions in hydroxyapatite, forming fluorapatite which is less soluble in acid than hydroxyapatite (Chapter 20). Tooth morphology also contributes to caries susceptibility on the basis of ease of bacteria colonization and accessibility to saliva as discussed in Chapter 17.

(2) **Saliva** There are several important aspects of saliva that contribute to caries resistance:

(a) Flow rate The flow of saliva physically washes away weakly attached bacteria and acids, and delivers salivary buffers. Xerostomia (low salivary flow, < 0.1 ml/min) leads to rampant caries. Xerostomia can be the result of Sjögren's syndrome, or occur after radiation for head and neck cancer when the salivary glands are damaged. Methamphetamine use also damages salivary glands and methamphetamine users often experience severe oral health problems. Certain medications can reduce salivary flow, particularly psychoactive drugs.

(b) Buffering capacity Saliva has two major buffering systems: bicarbonate-carbonic acid and phosphate. Bicarbonate is the most important as it buffers rapidly and is effective at pH values found in plaque. Buffering by saliva helps prevent bacterial acids, from reducing the pH to levels that dissolve apatite. The levels of ammonia and urea in saliva may also contribute to resistance to pH decline.

(c) Supersaturation At physiological pH saliva is supersaturated with respect to calcium and phosphate. This helps prevent loss of calcium and phosphate from enamel mineral. Anionic proline-rich proteins and a basic proline-rich glycoprotein are responsible for most of the calcium binding. Statherin is an active inhibitor of calcium phosphate precipitation.

(d) Antimicrobial factors Lysozyme, salivary peroxidase, mucins, agglutinins and immunoglobulins (IgA from saliva, IgG and IgM from serum via GCF) all possess antimicrobial properties as discussed in Chapters 6 and 7.

Dietary factors

In order to produce acid, cariogenic bacteria require a fermentable carbohydrate substrate, in particular sucrose. Studies have shown that in addition to total consumption, the frequency of intake and physical form of the sucrose containing food are important. The classical Vipeholm study found that in an institutionalized population the retentiveness of the food and frequency of intake were more important than total sucrose consumption.

The potential cariogenicity of food can be assessed by measuring the pH changes in plaque over time following ingestion. In general, there is a rapid pH drop followed by a slow rise back to resting pH, a pattern known as the Stephan curve after the first person to perform these measurements. Sucrose, glucose and fructose produce a more sustained and lower pH drop than some other sugars, such as lactose and starch. The bacterial metabolic pathways that lead to acid production from sugars are discussed in Chapter 18. Sucrose has the additional cariogenic property of providing a substrate for bacterial glucosyltransferases and fructosyltransferases. As discussed in Chapter 16, these enzymes produce polymers of glucan and fructan respectively from sucrose, both of which provide long-term energy storage. In addition, glucans can be insoluble and act as a cohesive matrix for the development and retention of cariogenic plaque.

Caries risk assessment

As the etiology of caries has become better understood, attempts have been made to measure known risk factors in order to assess caries risk. This would allow more proactive preventive measures when risk is high, for example in low income populations (Figure 19.2). Also, identification of children at high risk for caries would allow tailored pediatric preventive dentistry. Caries risk assessment should include at a minimum: bacterial factors (*S. mutans* and lactobacilli levels), salivary parameters (flow rate and buffering capacity), and a diet analysis (amount of fermentable carbohydrate). Fluoride levels in saliva and levels of salivary components such as gp340 could also be useful (Table 19.1). Unfortunately there are several drawbacks to these kinds of tests. It is not easy to distinguish the more cariogenic *S. mutans* or lactobacilli strains from less cariogenic strains, although nucleic acid or monoclonal antibody approaches may resolve this issue in the future. Salivary parameters vary considerably according to time of day or emotional state of the patient. An accurate chair-side test is therefore not yet available.

20 Fluoride

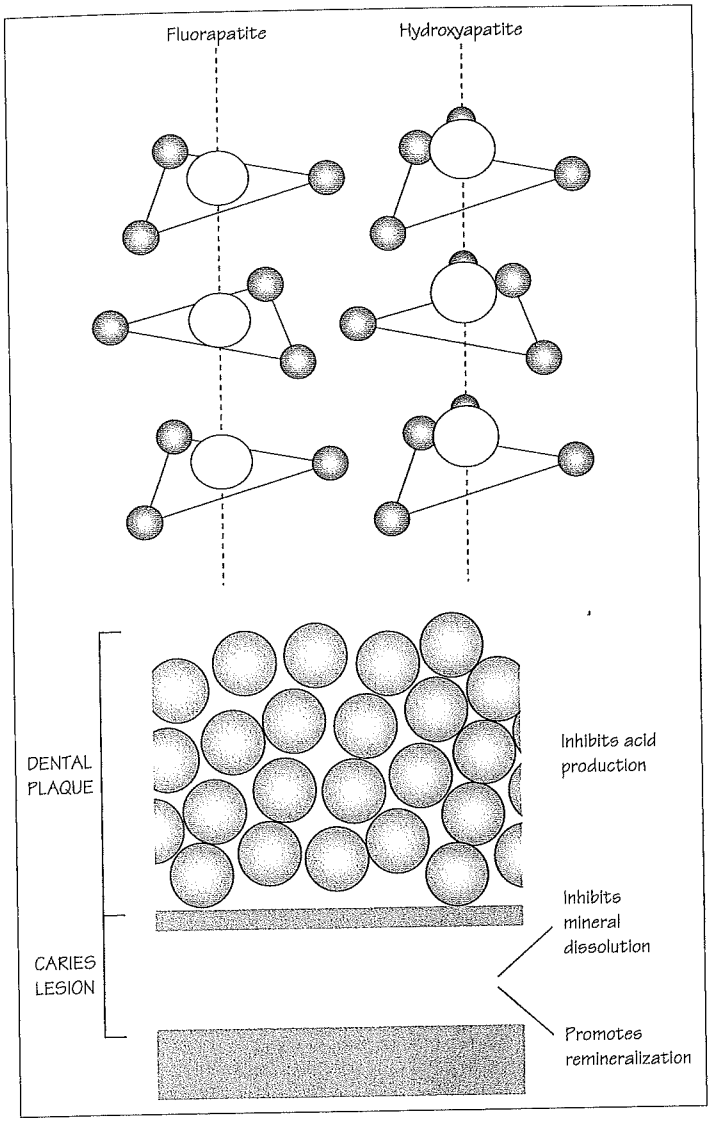


Figure 20.1 Upper panel: Crystal structure showing fluorapatite is a more compact structure than hydroxyapatite. Lower panel: Mechanisms of action of fluoride. Fluoride inhibits acid production and mineral dissolution, and promotes remineralization.

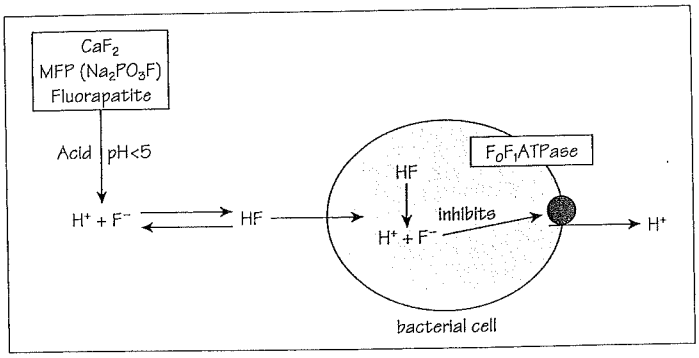


Figure 20.2 Fluoride from diet, toothpaste (MFP, sodium monofluorophosphate) or as fluorapatite can be released under low pH (acid). Fluoride ions interact with hydrogen ions to form hydrogen fluoride which enters bacterial cells. Dissociation again into hydrogen and fluoride ions leads to direct inhibition of the major energy and pH regulating transporter protein complex designated F_0F_1ATPase. Inhibition of this leads to the build up of hydrogen ions within the cell and therefore lessens the ability of the cells to tolerate acid (hydrogen ions) in the environment. One mechanism by which fluoride inhibits streptococci is therefore through reducing the ability of the bacterial cells to withstand acidic pH.

All hard tissues (bone, dentin, cementum and enamel) are made up of crystals of mineral (calcium phosphate) together with organic matrix and water. Mineralization of hard tissues is a biologically controled precipitation process. In precipitation, dissolved mineral ions leave a supersaturated solution and aggregate to form solid mineral. The mineral of bones and teeth are impure (partial carbonate substitution for phosphate) forms of hydroxyapatite. This is the least soluble simple calcium phosphate at neutral pH. The structure consists of phosphate ($PO_{4\ 3-}$) ions held together by Ca^{2+} ions. The Ca^{2+} ions form triangles and a spiral enclosing a channel. The OH- ions are located inside this channel (see Figure 20.1). In hydroxyapatite, the OH- ions lie slightly outside of the triangles because they are too large to fit exactly. Fluorapatite is formed when F- ions substitute for OH- and is less soluble than hydroxyapatite. In fluorapatite the smaller F- ions fit within the triangles. This results in a more compact and stable structure. Enamel has a much higher mineral content than dentin, resulting in greater density, stiffness and hardness. Because hydroxyapatite and fluorapatite are salts of phosphoric acid, solubility of all these solids increases as pH decreases.

Modes of action of fluoride

The presence of topical fluoride has three major effects (Figure 20.1): (1) Inhibition of acid production by dental plaque (see below). (2) Inhibition of mineral dissolution at the site of carious lesion. Fluoride present in the plaque fluid at the time that the bacteria generate acid will travel with the acid into the subsurface of the tooth and adsorb to the crystal surface protecting against being dissolved. (3) Enhancement of remineralization resulting in a remineralized layer that is less soluble and resistant to acid attack. Fluoride enhances remineralization by adsorbing to the crystal surface and attracting calcium and phosphate ions, leading to new mineral depositing on existing crystal nucleators. This newly formed layer excludes carbonate and is less soluble than carbonated hydroxyapatite.

Strategies for fluoride delivery

Consumption of fluoridated water during tooth development raises the fluoride content of tooth mineral. However, continual local presence of fluoride in the aqueous phase is essential to maintain beneficial effects. Fluoride tablets or clinically-applied topical fluoride boosts the fluoride concentrations of tooth surfaces. Fluoride toothpastes provide reservoirs of fluoride on the tooth surfaces (as calcium fluoride), on mucosa and in plaque (bound to surfaces of bacteria). At dose levels represented by normal dental use, there is no compelling evidence for toxic effects. Dental fluorosis, caused by impaired mineralization only occurs with very high fluoride concentrations that occur naturally in some water supplies. Mild cases show as white patches of enamel (mottling). In severe cases there is enamel hypomineralization and discoloration due to staining of porous enamel.

Anti-microbial effects of fluoride

Fluoride affects the physiology of microbial cells. There are two main mechanisms: inhibition of enzymes in intact cells, either directly or in the form of metal complexes at sub-millimolar levels; or increasing proton permeability of cell membranes by HF acting as a transmembrane proton carrier, which discharges the ΔpH across the cell membrane (Figure 20.2). This latter effect is the major activity leading to inhibition of acid production by bacterial cells in biofilms at low pH. Lowering of ΔpH by fluoride affects the energy status of the cell. The re-entry of protons across the membrane increases the ATP demand for pH regulation, so the result is intracellular stress.

In addition, fluoride in combination with aluminum inhibits the activity of proton translocating F-ATPase. Cytoplasmic acidification caused by fluoride disrupts glycolytic acid production, and the formation of intracellular storage polysaccharides. Fluoride at low concentrations can also affect the formation of *S. mutans* biofilms. Reduced extracellular glucan polysaccharide production in these biofilms may be related to partial inhibition of GTF by fluoride. At sub-millimolar levels, fluoride inhibits a variety of enzymes including enolase, urease, P-ATPase, phosphatases and heme peroxidase, by direct binding of HF or F-. Binding of metal-F complexes inhibits F-ATPase and RecA (needed for DNA repair). Dissipation of the proton gradient (as described above) inhibits glycolysis, sugar uptake through the phosphotransferase systems (PTS), and polysaccharide production.

Enhancing anti-microbial effects

The effectiveness of fluoride may be enhanced when combined with other cariostatic agents. Most of the agents used to date to enhance fluoride biological activity are based on non-specific antimicrobial agents such as chlorhexidine, triclosan and metal ions/cations. Two naturally occurring compounds, apigenin and tt-farnesol, found in propolis (a resinous beehive product) and fruits, show inhibitory activities against *S. mutans*. Apigenin inhibits GTF while tt-farnesol disrupts proton permeability of the cell membrane. These compounds enhance the biological effects of fluoride, reducing the amount of glucans in *S. mutans* biofilms, the biomass and the acidogenicity.