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Role of the oral micro flora in human population: A brief review

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Abstract

The mouth contains both distinct mucosal (lips, cheek, tongue, palate) and, uniquely, non-shedding surfaces (teeth) for microbial colonisation. Each surface harbours a diverse but characteristic micro flora, the composition and metabolism of which is dictated by the biological properties of each site. The resident oral micro flora develops in an orderly manner via waves of microbial succession (both autogenic and allogenic). Pioneer species (many of which are sIgA protease-producing streptococci) colonise saliva-coated surfaces through specific stereo-chemical, adhesin-receptor interactions. The metabolism of these organisms modifies local environmental conditions, facilitating subsequent attachment and growth by later, and more fastidious, colonisers. Eventually, a stable bio film community develops, that plays an active role in (a) the normal development of the physiology of the habitat, and (b) the innate host defences (colonisation resistance). Thus, when considering treatment options, clinicians should be aware of the need to maintain the beneficial properties of the resident oral micro flora.

Key-Words: Resident Oral Micro Flora, Microbial Succession, Dental Plaque, Bio Film, Colonisation Resistance

Introduction

Far from having a passive relationship with the host, recent research has confirmed earlier (and largely forgotten) studies that demonstrated that the resident micro flora of animals and humans plays a positive role in the normal development of the host. This resident micro flora also plays an active role in the maintenance of the healthy state by contributing to the host defences and preventing colonisation by exogenous microorganisms. Implicit in this statement is that disease can be a consequence of disruption of this resident micro flora. A deeper understanding of the relation between the host and its resident micro flora may lead to new strategies for the prevention of disease via the active maintenance of a health-associated micro flora. It has been estimated that the human body is made up of 10^{14} cells of which only 10% are mammalian (1). The remainder are the microorganisms that make up the resident micro flora of the host. The composition of this micro flora varies at distinct habitats, but is relatively consistent over time at each individual site among individuals.

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The mouth is similar to other environmentally-exposed sites in the body in having a characteristic (autochthonous) and diverse micro flora in health. Curiously, this micro flora consists of organisms with apparently contradictory requirements; for example, facultative, microaerophilic, capnophilic and obligatory anaerobic species (with either saccharolytic or asaccharolytic metabolic lifestyles) are able to co-exist. Bacteria that are presently uncultivable can also be detected by molecular techniques in samples from the mouth (2, 3).

The organisms making up the oral micro flora are regularly transferred to neighbouring habitats via saliva. Of significance, however, only 29 out of >500 microbial taxa recovered from the mouth are cultivated from faecal samples, despite the continuous passage of these bacteria into the gut (4), while oral bacteria do not establish on the skin surface. Thus, the resident micro flora is directly influenced by the environmental conditions prevailing in a particular habitat. Following on from this, the composition of the resident oral micro flora shows local variations in composition on distinct surfaces (e.g. tongue, cheek, teeth) due to differences in key environmental conditions. The environmental factors that help define the mouth as a microbial habitat will now be reviewed.

THE MOUTH AS A MICROBIAL HABITAT

As stated above, the mouth is not a homogeneous environment for microbial colonisation. Distinct habitats exist, for example, the mucosal surfaces (such as the lips, cheek, palate, and tongue) and teeth which, because of their biological features, support the growth of a distinctive microbial community.

The mouth is continuously bathed with saliva, and this has a profound influence on the ecology of the mouth (5). The mean pH of saliva is between pH 6.75–7.25, which favours the growth of many microorganisms, and the ionic composition of saliva promotes its buffering properties and its ability to remineralise enamel. The major organic constituents of saliva are proteins and glycoprotein, such as amylase, mucin, immunoglobulin (mainly sIgA), lysozyme, lactoferrin and sialoperoxidase. These influence the oral micro flora by: (a) adsorbing to oral surfaces, especially teeth, to form a conditioning film (the acquired pellicle) to which microorganisms can attach. Adherence involves specific intermolecular interactions between adhesins on the surface of the microorganism and receptors in the acquired pellicle. Oral organisms are not distributed randomly but usually display tissue tropisms, i.e. they selectively attach and grow on certain surfaces (6, 7), (b) acting as primary sources of nutrients (carbohydrates and proteins) which foster the growth of the resident micro flora without inducing a damaging pH fall (8, 9), (c) binding to the surface of bacteria to mask bacterial antigens, thereby making the organism appear more host like, (d) aggregating microorganisms and thereby facilitating their clearance from the mouth by swallowing; the flow of saliva will also wash away weakly-adherent cells, and (e) inhibiting the attachment and growth of some exogenous microorganisms, via their role as components of the host defences.

The properties of some of the major habitats in the mouth will change during the life of an individual. For example, during the first few months of life the mouth consists only of mucosal surfaces for microbial colonisation. Hard non-shedding surfaces appear with the development of the primary dentition, providing a unique surface in the body for microbial colonisation. The eruption of teeth also generates another habitat, the gingival crevice (where the tooth emerges from the gums), and an additional major nutrient source for that site (gingival crevicular fluid, GCF). Teeth allow the accumulation of large masses of microorganisms (predominantly bacteria) and their extracellular products (collectively, this is termed dental plaque), especially at stagnant or retentive sites. In contrast, elsewhere in the body, desquamation ensures that the microbial load is relatively light on most mucosal surfaces. In addition, ecological conditions within the mouth will also be affected by the eruption and loss of teeth, the insertion of prostheses such as dentures, and any dental treatment including scaling, polishing and restorations. Transient fluctuations in the stability of the oral ecosystem may be induced by the frequency and type of food ingested, periods of antibiotic therapy, and variations in the composition and rate of flow of saliva. For example, a side-effect of medication can be a reduction in saliva flow, and this can predispose a site to caries. In old age, the activity of the host defences can wane, and this might explain the increased isolation of staphylococci and entero bacteria from the oral cavity of the elderly (10, 11). In general, however, the composition of the micro flora at a site remains relatively constant over time (microbial homeostasis) despite regular minor perturbations (12).

The health of the mouth is dependent on the integrity of the mucosa (and enamel) which acts as a physical barrier preventing penetration by microorganisms or antigens. The host has a number of additional defence factors present in oral secretions (saliva and GCF) which play an important role in maintaining the integrity of these oral surfaces. As stated earlier, saliva contains several anti-bacterial factors (5), including sIgA which can reduce or prevent microbial colonisation of oral surfaces. Antimicrobial peptides are present, including histidine-rich polypeptides (histatins), and cystatins, which may control the levels of yeasts, and a range of active proteins and glycoprotein (lysozyme, lactoferrin, sialoperoxidase).

Serum components can reach the mouth via GCF (13). The flow of GCF is relatively slow at healthy sites, but increases during the inflammatory responses associated with periodontal diseases. IgG is the predominant immunoglobulin in GCF; IgM and IgA are also present, as is complement. GCF contains large numbers of viable neutrophils, as well as a minor number of lymphocytes and monocytes. GCF can influence the ecology of the site by: (a) removing weakly-adherent microbial cells, (b) introducing additional components of the host defences, and (c) acting as a novel source of nutrients for the resident microorganisms. The growth of several fastidious obligate anaerobes is dependent on haemin, which can be derived from proteolysis of haemoglobin, haemopexin, and transferrin, etc.

Although temperature remains relatively constant, it can increase in the sub-gingival area during an inflammatory response if plaque accumulates beyond levels compatible with health. The local pH in dental plaque can fall rapidly to ca. pH 4.0–5.0 following the intake of fermentable dietary carbohydrates, before

returning to resting values (6.75–7.25). Gradients in oxygen concentration and redox potential develop over relatively short distances in plaque, which enables the growth of large numbers of obligate anaerobes recovered from the mouth (14).

DEVELOPMENT OF THE RESIDENT ORAL MICROFLORA

The foetus in the womb is normally sterile. Acquisition of the resident micro flora of any surface depends on the successive transmission of microorganisms to the site of potential colonization. In the mouth, this is by passive transfer from the mother, from organisms present in milk, water (and eventually food), and the general environment, although saliva is probably the main vehicle for transmission (15–18). Microorganisms such as lactobacilli and

Candida may also be acquired transiently from the birth canal. The mouth is highly selective for microorganisms even during the first few days of life. Only a few of the species common to the oral cavity of adults, and even less of the large number of bacteria found in the environment, are able to colonize the mouth of the newborn.

The first microorganisms to colonize are termed pioneer species, and collectively they make up the pioneer microbial community. In the mouth, the predominant pioneer organisms are streptococci and in particular, *S. sali6arius*, *S. mitis* and *S. oralis* (19, 20). Some pioneer streptococci possess IgA1 protease activity (21), which may enable producer and neighbouring organisms to evade the effects of this key host defence factor that coats most oral surfaces. With time, the metabolic activity of the pioneer community modifies the environment thereby providing conditions suitable for colonization by a succession of other populations. This may be by: (a) changing the local Eh or pH, (b) modifying or exposing new receptors on surfaces for attachment ('cryptitopes'; (22)), or (c) generating novel nutrients, for example, as end products of metabolism (lactate, succinate, etc.) or as break-down products (peptides, haemin, etc.) which can be used as primary nutrients by other organisms as part of a food chain.

Microbial succession eventually leads to a stable situation with increased species diversity (climax community). The component species interact, and there is the opportunity for a genuine division of labour; this may involve chemical signalling among members of the community to co-ordinate gene expression (23, 24). This results in participating organisms having a broader habitat range (e.g. obligate anaerobes grow in an overtly aerobic habitat), a more efficient metabolism (i.e. consortia are able to more fully catabolise complex host macromolecules such as mucin, against which the individual organisms have only limited metabolic activity), and are more tolerant of environmental stress and to antimicrobial action. A climax community reflects a highly dynamic state; a change in the environment can lead to a reaction from the micro flora resulting in an altered composition and metabolic activity, and can also predispose a site to disease.

The oral cavity of the newborn contains only epithelial surfaces for colonization. The pioneer populations consist of mainly aerobic and facultative anaerobic species. In a study of 40 full-term babies, a range of streptococcal species were recovered during the first three days of life, and *Streptococcus oralis*, *S. mitis* biovar 1 and *S. sali6arius* were the numerically dominant species (20). The diversity of the pioneer oral community increases during the first few months of life, and several species of Gram-negative anaerobes appear. In a study of edentulous infants with a mean age of 3 months (range: 1–7 months), *Pre6otella melaninogenica* was the most frequently isolated anaerobe, being recovered from 76% of infants (25). Other commonly isolated bacteria were *Fusobacterium nucleatum*, *Veillonella* spp., and non-pigmented *Pre6otella* spp.. In contrast, *Capnocytophaga* spp., *P. loescheii* and *P. Intermedia* were recovered from 4–23% of infants, while *E. Corrodens* and *Wolinella succinogenes* were only found in a single mouth. The number of different anaerobes in the same mouth varied from 0–7 species (25).

The same infants were followed longitudinally during the eruption of the primary dentition (26). Gram-negative anaerobic bacteria were isolated more commonly, and a greater diversity of species were recovered from around the gingival margin of the newly erupted teeth (mean age of the infants -32 months) (26). Also, *Streptococcus mutans* and *S. sanguis* appear in the mouth following tooth eruption (27, 28). These findings confirm that a change in the environment, such as the eruption of teeth, has a significant ecological impact on the resident micro flora.

Bacteriocin-typing and genotyping of strains have established the vertical transmission of many oral bacteria (e.g. *S. sali6arius*, *Streptococcus mutans*, *Porphyromonas gingi- 6alis* and *Actinobacillus actinomycetemcomitans*) from mother to child (15–18). Generally, similar clonal types of species are found within family groups, while different patterns are usually observed between such groups. The genotypes of *Streptococcus mutans* found in children appeared identical to those of their mothers in 71% of 34 infant-mother pairs examined (29). No evidence of father

infant (or father-mother) transmission of *Streptococcus mutans* was observed, although horizontal transmission of some periodontal pathogens, such as *P. gingi6alis*, may occur between spouses (17, 18).

During the first year of life, members of the genera *Neisseria*, *Veillonella*, *Actinomyces*, *Lactobacillus*, and *Rothia* are commonly isolated, particularly after tooth eruption (30). Some of the genera (*Porphyromonas* and *Actinobacillus*) associated with the aetiology of periodontal disease have been cultivated from the plaque of infants aged around 12 months, albeit infrequently and in low numbers (31). This suggests that these diseases result from a change to the balance of the components of the resident micro flora, presumably due to an alteration to the ecology of the affected site.

The acquisition of some bacteria may occur optimally only at certain ages. Studies of the transmission of *Streptococcus mutans* to children have identified a specific 'window of infectivity' at 19–31 months (median age: 26 months) (32). This opens up the possibility of targeting preventive strategies over this critical period to reduce the likelihood of colonisation in the infant. Indeed, reducing the carriage of *Streptococcus mutans* in mothers can reduce transmission of these potentially cariogenic bacteria to their offspring, and delay the onset of caries.

The development of a climax community at an oral site can involve examples of both allogenic and autogenic microbial succession. In allogenic succession, factors of non-microbial origin are responsible for an altered pattern of community development. For example, *Streptococcus mutans* and *S. sanguis* only appear in the mouth following tooth eruption (19, 27, 28) or the insertion of artificial devices such as acrylic obturators in children with cleft palate. Community development is also influenced by microbial factors (autogenic succession). Examples include (a) the lowering of the redox potential and consumption of oxygen in plaque by pioneer species, thereby facilitating colonisation by obligate anaerobes, (b) the development of food chains and food webs, whereby the metabolic end product of one organism becomes a primary nutrient source for a second, and (c) the exposure of new receptors on host macromolecules for bacterial adhesion by the metabolism of the resident micro flora ('cryptitopes'; (22)).

EVASION OF THE HOST DEFENCES

One of the least understood phenomena is the ability of the resident micro flora to persist in the presence of a broad range of specific and innate host defence factors. Some of the proposed mechanisms that might explain this apparent paradox in the context of the oral cavity are listed in Table IV. Oral bacteria use salivary molecules as receptors when they attach to a surface; the binding of such molecules will also mask microbial antigens and make the cell appear 'host-like'. Some clones of species such as *S. mitis* appear to persist for long periods at a site whereas others appear to be transient, and undergo replacement by distinct clones (33, 34). Wide variations in the expression of carbohydrate and protein antigens were found among different genotypes of *S. mitis* biovar 1 within a family, suggesting that this 'clonal turnover' might play a role as an 'immune-evasion' mechanism for this species.

The resident oral micro flora comprises a broad range of gram-negative species that contain potentially inflammatory molecules such as LPS in their cell wall. Although local inflammation is a hallmark of periodontal diseases (see Liljemark, this volume), such a reaction by the host is relatively uncommon. Recently, it has been proposed that the resident micro flora and mucosal tissues exist in a balanced state due to active signalling between the bacteria and the epithelial cells. This 'cross-talk' would suppress inflammation and enable the micro flora to grow and enter a symbiotic relationship with the host ('commensal communism', (35)), for example, the flora derives nutrients from the host while also contributing to the host defences (see later).

FORMATION OF DENTAL PLAQUE

Most studies have focussed on the development and properties of dental plaque, because of its role in caries and periodontal diseases. The formation of dental plaque involves an ordered colonization (microbial succession) by a range of bacteria (36). Host and bacterial molecules are adsorbed to clean tooth surfaces. The early colonisers interact with, and adhere to, this conditioning film initially via long range, non-specific physico-chemical interactions followed by irreversible, short range stereo-chemical adhesin-receptor interactions (6, 7, 37). Later colonisers bind to already attached species by co-adhesion (38).

A range of habitats are associated with distinct tooth surfaces, each of which is optimal for colonization and growth by different microorganisms. Again, this is due to the physical nature of the particular surface and the resulting biological properties of the area. The areas between adjacent teeth (approximal) and in the gingival crevice afford protection from the normal removal forces, such as mastication, salivary flow and oral hygiene practices. Both sites have a low redox potential (Eh) and, in addition, the gingival crevice region is bathed in the nutritionally- rich GCF, particularly during inflammation, and so these areas are able to support a more diverse microbial community including

higher proportions of obligatory anaerobic bacteria. Pits and fissures of the biting (occlusal) surfaces of the teeth also offer protection from some of these environmental factors and, in addition, are also susceptible to food impaction. Such protected areas are associated with the largest microbial communities and, in general, the most disease (see other chapters, this volume). In contrast, smooth surfaces are more exposed to the prevailing environmental conditions and, consequently, are colonized by only limited numbers of organisms.

Plaque is an example of a bio film (39, 40), and, while it is found naturally in health, in disease there is a shift in the composition of the plaque micro flora away from the species that predominate in health (see the other Chapters in this volume; (41)). Bacteria growing on a surface as a bio film display an altered phenotype, and are also more resistant to antimicrobial agents (42–44).

FUNCTIONS OF THE RESIDENT ORAL MICROFLORA IN HEALTH

The presence of a resident micro flora is not essential for life, but is an important component if the host is to have a normal existence. Information on the beneficial role of the resident microbial flora has come from early studies comparing the physiology of germ-free and conventional laboratory animals, and from humans in whom the normal flora has been disrupted by long-term administration of antibiotics. Most studies have focused on the gut micro flora but, in general, such findings are also relevant to the mouth. The role played by the resident micro flora is one of the most poorly understood topics in microbiology, and has been neglected for many years; further research applying modern approaches to this area would be valuable.

The gut of germ-free animals is poorly developed, and these animals may suffer from an enlarged caecum, poor nutrient adsorption and from vitamin deficiencies; in addition, the development of the host defences is impaired (45, 46). Also, components of the micro flora modify the differentiation programmes of intestinal epithelial cell lineages at critical points during their morphogenesis. However, many of these anatomical and physiological deficiencies can be reversed when these animals are colonised by members of the resident micro flora.

The presence of a resident micro flora prevents disease by reducing the chance of colonisation by exogenous species. Thus, germ-free animals are highly susceptible to disease, so that if they are introduced to a conventional environment they suffer from diarrhoea and have a high death rate (47). It has been determined experimentally that the use of antibiotics can suppress the normal flora of the digestive tract resulting in a reduction in the infective dose of pathogens such as *Salmonella enteritidis* from ca. 10^6 to as low as 10 cfu (48). In humans, the use of antibiotics is associated with overgrowth in the gut by *Clostridium difficile* leading to severe diarrhoea. In the mouth, antibiotic usage perturbs the resident micro flora resulting in overgrowth by drug-resistant, but previously minor, components of the oral micro flora (49), or colonisation by exogenous and potentially pathogenic organisms, including yeasts (1, 50).

These observations suggest that the resident micro flora of all sites in the body can influence the physiology of the host and contribute to the innate host defences, by being a barrier to exogenous species. This barrier effect is termed 'colonisation resistance' (51). The mechanisms involved in colonisation resistance include competition for (a) nutrients and (b) attachment sites. Natural selection has probably ensured that the most competitive and efficient strains in terms of metabolism and colonisation are already members of the oral resident micro flora. In addition, colonisation resistance will be maintained by (c) the production of inhibitory metabolites, and (d) the creation of unfavourable environmental conditions for exogenous organisms (48). Some strains of *S. sali6arius* strains produce a bacteriocin (termed enocin or salivaricin) with activity against Lancefield Group A streptococci (52). Bacteriocin production by such strains in the pharynx may reduce or prevent colonisation of the mouth by this pathogen. Similarly, many oral bacteria produce other inhibitors such as volatile fatty acids or hydrogen peroxide, or they change local environmental conditions (e.g. pH or redox potential), which may exclude exogenous species and suppress opportunistic pathogens. For example, the production of hydrogen peroxide by members of the *S. mitis*-group can suppress the growth in plaque of potential periodontal pathogens, such as *A. actinomycetemcomitans*.

Conclusion

The arguments outlined above clearly demonstrate that the resident oral micro flora plays an active role in the normal development of the mouth and in the maintenance of health at a site. Clinicians need to be aware of the beneficial properties of the resident micro flora, and their treatment strategies should be focussed on the control rather than the elimination of these organisms, especially in dental plaque. In the future, it may be feasible to target treatment more specifically at particular 'pathogens' (e.g. immunotherapy (53)), or more imaginative approaches could be used to prevent disease. For example, it may be possible to remove the environmental pressures that favour the selection of the organisms associated with disease (54), and

'prebiotics' (agents that encourage the growth of the normal micro flora) and 'probiotics' (the deliberate use of organisms to restore colonisation resistance) may become available. Such approaches are currently finding increasing favour in those attempting to enhance the natural properties of the gut micro flora (55). In this context, the outcome of current clinical trials with bacteriocin-producing, nonacidigenic but highly competitive strains of *S. mutans* (replacement therapy) will be of great relevance (56).

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