Dental plaque - A microbial perspective and recent concepts as a host and guest relation

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Abstract
Dental plaque is defined clinically as a structured, resilient yellow grayish substance that adheres tenaciously to the intra-oral hard surfaces i.e teeth including removable and fixed restorations. It is primarily composed of micro-organisms. One gram of plaque contains approximately 10^{11} bacteria. Any individual may harbor 150 or more different species. Non bacterial microorganisms that are found in plaque include archaea, yeasts, protozoa and viruses. Nowadays application of novel imaging and molecular techniques has created great understanding about dental plaque. This article is an insight of host and guest relation of dental plaque in terms of micro-organisms as guest and humans as host.

Keywords: Dental plaque, Biofilm, Oral Microflora.

Introduction
The existence of bacteria in nature is mostly in the form of biofilm. Biofilm is comprised of consortium of interacting species. Studies on microbial ecology[1] and the knowledge of the interaction between the resident human microflora and the host has provided sound base for our understanding regarding microbial behaviour. The gene expression in microflora is highly affected by changes in host environment which also has great impact on the metabolic activity, competitivity and composition of that microbial community. In turn these resident microorganisms also have impact on host physiology as well as pathology. Nowadays application of novel imaging and molecular techniques has created great understanding about dental plaque.

Out of 1014 cells in human body only 10% are mammalian.[2,3] The environmentally exposed surfaces of the body consists of majority of microorganisms of the resident flora whose combined metabolic activity is reported as equivalent to that of the human liver. Despite of potential movements of the microorganisms between different sites of body sites, the distinct biological and physical properties of skin, mouth, digestive and reproductive tracts etc results in distinctive micro flora of each site.[3]

These findings conclude about habitat's selective and dictating properties which decides the colonization, membership and growth of respective microbes. Similarly the oral cavity also has a characteristic microbial community benefiting the host. Warmth and moisture of mouth support the growth of certain specific group of microorganisms (viruses, mycoplasma, bacteria, Archaea, fungi and protozoa),[4] out of which bacteria are the most numerous group.[5,6] Less than 50% of the resident oral micro flora can currently be cultivated in pure culture in the laboratory.[7,8] Newer culture-independent molecular approaches have greatly improved our concepts about the richness and diversity of the resident oral micro flora. Amplification, cloning and sequencing techniques of the 16S rRNA gene have identified approximately 700 species in the mouth while the number of species per individual mouth ranged from 34 to 72.[9] However, the use of a more discriminatory pyro-sequencing rendered above as an under-estimation as this advanced approach detected several thousands of phylotypes in samples of saliva and supra-gingival plaque from healthy adults.[10] In this, a next-generation high-throughput sequencing technique is used which resulted in an increased number of clones that can be sequenced causing increased the chances of detection of the less abundant taxa.

Resident micro flora obtains their primary nutrients mainly from endogenous sources such as amino acids, proteins and glycoproteins in saliva and gingival crevicular fluid whereas the host diet has a minor role to plays. Combined co-operated metabolic capabilities of the microbial consortia helps in catabolism of the more complex host molecules.[11-16] Neutral pH of saliva also promotes the growth of many microorganisms. Early aerobic bacterial colonizers (e.g. Neisseria spp.) or facultatively anaerobic (e.g. Streptococcus and Actinomyces spp.) and other gases (CO2, H2) and reduced lower the redox potential of dental plaque which create environment for the growth of obligate anaerobes.[4] In this way a precise spatial organization of interacting bacteria is created in the plaque (e.g- Streptococci and Fusobacterium nucleatum) whose composition also varies within distinct sites in oral cavity.

Formation of dental plaque
This involve certain steps. At first there is formation of enamel pellicle by adsorbtion of molecules

from saliva and gingival crevicular fluid to the tooth surface. Then reversible attachment of microoraganisms to it occurs by weak, long-range, physico-chemical interactions between the charge on the microbial cell surface and that produced by the conditioning film i.e pellicle.[17,18] Transport of microorganisms is usually passive to the surface by the flow of saliva or gingival crevicular fluid whereas a few species (e.g.Wolinella, Selenomonas and Campylobacter spp.) are found subgingivally and have flagella and are motile. Then microorganisms get attached irreversibly by strong and short distance interactions between adhesins on microbes and receptors present in the acquired pellicle which opens the gateway for novel interventions aimed at blocking colonization.[19] Co-attachment i.e co-adhesion takes place where secondary and late colonizers adhere via cell-surface adhesins to receptors on already attached bacteria.[20,21] The multiplication of the adherent cells by increase in biomass and synthesis of matrix of the biofilm occurs by synergistic interaction between neighbouring species. Bacteria adapts to fluctuating environmental changes and contradictory growth requirements by altering their patterns of gene expression.[4,22-24]

In deteriorated circumstances some species (e.g. Prevotella loescheii and Aggregatibacter actinomycetem comitans) upregulate enzymes that cleave their adhesions and enable them to detach and colonize elsewhere.[25,26] The changes in community composition and activity may predispose a site to disease. Different forms of microbial arrangements (e.g. corn cob, test tube, brush or rosette) exist within subgingival dental plaque.

Properties of dental plaque as biofilm

Dental plaque as biofilm holds many important properties.[4] They have an open architecture with presence of channels and voids. They provide microbial protection, host protection, colonization and gene transfer. Neutralization of inhibitors also occurs within it by beta-lactamase production by neighbouring cells to protect sensitive organisms. Novel gene expression occurs via coordinated gene responses by producing bacterial cell-to-cell signalling molecules (e.g. CSP, AI-2). They also communicate with host for e.g down regulation of pro-inflammatory responses by resident oral bacteria. Co-adhesion is also a prime feature. Biofilm has a broader habitat range (obligate anaerobes in an overtly aerobic environment). Efficient metabolism like complete catabolism of complex host macromolecules also takes place. It also has enhanced virulence.

Interactions within biofilm

Metabolic product of one organism serves as the primary nutrient for another microorganism. Bacterial collaboration also aids in catabolism of complex host molecules (proteins, glycoproteins).[11,16] Similarly obligately anaerobic bacteria such as P. gingivalis can survive in aerobic environments on their collaboration with oxygen-consuming species such as Neisseria.[27,28] So some bacteria appear in plaque biofilms as discrete clusters of cells. Bacterial gene expression occurs in a coordinated manner in dental plaque via quorum sensing.[29-31] Transformation frequency of biofilm-grown S. mutans is increased 10-600 folds via quorum sensing mediated by CSP i.e competence stimulating peptide.[32] It also increases cells property of tolerance to acids.[33] Similarly Lux-S dependent signaling enhances efficiency of A. actinomycetemcomitans and P.gingivalis[34] (11). These molecules play evident role in intra and inter-species communication and coordination as AI-2 produced by A. actinomycetemcomitans complemented a lux-S mutation in P. gingivalis and AI-2 secretion by P. gingivalis could stimulate biofilm formation by F. nucleatum.[31,35] Another mechanism of cellular interaction within biofilms is horizontal gene transfer. For e.g transfer of conjugative transposons encoding tetracycline resistance amongst streptococci.[36] The presence of pathogenicity islands in periodontal pathogens such as P. gingivalis may explain the evolution of more virulent strains due to horizontal gene transfer.[37]

Microbial gene expression in biofilm

Microorganisms also alter their gene expression in order to survive[38-40] in the changing habitat environment as in case of disease. Increased sugar consumption frequency results in rapid formation of acid from fermentable carbohydrates causing caries. This cause the decrease in local pH and increase in acidogenic and acidophilic bacterial growth.[38,41,42] Similarly in periodontal disease, apart from providing components of the host defences, increased gingival crevicular flow also introduces a range of host proteins and glycoproteins which are used as substrates for growth by many of the obligate anaerobic and proteolytic species present in subgingival biofilms.[16,43] The inflammation also alters gene expression for example, P. gingivalis become more proteolytic with higher gingival pain activity in response to an increase in haemin availability[44,45] due to differential expression of 70S proteins[41] whereas a high temperature resulted in down-regulation of protease activity in P. gingivalis.[46] So the changes in subgingival environment results in a shift in both the competitiveness and aggressiveness of previously minor components of the microflora. This may result in increased risk of disease due to disruption of the natural balance of organisms and modification of the composition of the microflora.[41] Genes associated with glucan and fructan synthesis in S.mutans i.e matrix formation were found to be differentially regulated in biofilms.[47] In early biofilm formation (<48 h) surface growth didn't influenced gene expression much while in older biofilms(7-day), glucan expression was

markedly upregulated and fructan activity was repressed.[47,48] When P. gingivalis was grown as a biofilm, approximately 18% of the genome was differentially expressed.[48]

Impact of dental plaque as a biofilm on host

There is a major role of resident microflora in aiding host defence by "colonization resistance". It also enhances physiological and nutritional development of host as the gut of germ free animals was poorly developed. When these animals are colonized with members of resident microflora, the deficiencies were reversed.[49-54] Resident microflora also determine normal expression of immune mediators.[55] The disruption of host-microbe balance leads to disease. [52,53,56,57] Gene expression in both bacterial and host cells is affected by signaling amongst them via receptors for e.g Toll-like receptors and NOD-like recetors.[51,52,56,58] By inhibiting the nuclear factor kappa B pathway, Streptococcus salivarius K12 down-regulates epithelial cell inflammatory responses as well as stimulates type I and II interferon responses and also exerts significant effects on the cytoskeleton and adhesive properties of the host cell.[59] Formation of integrin-associated focal adhesions which cause remodelling of the actin and tubulin cytoskeleton in primary gingival epithelial cells is induced by Fimbriated P. gingivalis cells.[60] In healthy states, putative periodontal pathogens are noncompetitive with beneficial micro flora and remain at low levels. Host inflammatory responses are mounted if plaque accumulates beyond health compatible levels. This leads to increased gingival crevicular flow which further provides nutrient source to proteolytic gram-nagative anaerobes predisposing disease sites. Resultant proteolysis increase local pH causing upregulation of virulence factors of these putative pathogens (e.g. gingival pain activity by P. gingivalis) promoting their growth at the expense of health related microbes.[16,43,61] This dynamic relationship, resulting in disease process is explained by 'ecological plaque hypothesis'.[41,62] Replacement therapy with 'beneficial' bacteria and manipulation by use of pre- or probiotics is also under evaluation.[63,64]

Dental plaque: resistance against antimicrobial agents

Being in a community, the biofilm provides a broader habitat range to microorganisms.[18,28,65] It promotes more efficient metabolism of complex host derived products[15,66] . It reinforces microbes to tackle inhibitory agents and host defenses more efficiently[67-69] as well as enhance their virulence.[70-74]

300 times greater concentrations of chlorhexidine and 75 times greater concentrations of amine fluoride than the minimum bactericidal concentration against planktonic cells are required to kill Streptococcus sobrinus growing as an established biofilm.[75] The more the age of biofilm, the more will be the tolerance.[76,77] Biofilms with more diverse environment have proved to be more tolerant to amoxycillin, doxycycline, minocycline and metronidazole than planktonic cells.[77-80] Either the quenching of the agent at the biofilm surface or a lack of penetration causes chlorhexidine to only affect the outer layers of cells in 24 and 48 hrs plaque biofilms as shown in conofocal microscopy of in-situ established natural biofilms.[81] Similarlly fluoride also shows uneven distribution within biofilm.[82] Bacteria that grow in the depths of biofilms generally divide slowly and thus are always less sensitive to antimicrobial agents.

Conclusion

There occurs a harmonious relationship between host and resident flora. Change in local environmental factor causes disruption of host-microbe balance predisposing and potentiating disease process. Rather than just focusing upon mere presence or absence of putative pathogenic species, it is needed to expand the dimensions of knowledge regarding their proportions and combinations. Additionally role of reduction in beneficial bacteria in disease process must be considered. The aim of oral health care programme should concern regarding controlling the levels and activity of the oral microflora, rather than focusing on their elimination.

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