LETTER TO EDITOR

Extracellular Polysaccharides Matrix — An Often Forgotten
Virulence Factor in Oral Biofilm Research

Hyun Koo*, Jin Xiao, Marlise I. Klein
Eastman Department of Dentistry and Center for Oral Biology, University of Rochester Medical Center, USA

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Oral diseases related to dental biofilms continue to afflict the majority of the world’s population. Among them, dental caries continues to be the single most prevalent and costly oral infectious disease (Marsh, 2003; Dye et al., 2007). Dental caries results from the interaction of specific bacteria with constituents of the diet within a dental biofilm known as plaque (Bowen, 2002). Sucrose is considered to be the “arch criminal” from the dietary aspect because it serves as a substrate for synthesis of extracellular (EPS) and intracellular (IPS) polysaccharides in dental biofilm and is also fermentable (Bowen, 2002). However, it is important to emphasize that additional sugars and starch can certainly contribute to the pathogenesis (Bowen et al., 1980; Firestone et al., 1982; Thurnheer et al., 2008). Streptococcus mutans (S. mutans), a member of the oral microbial community, is generally regarded as the primary microbial culprit although additional microorganisms may be involved (Hamada and Slade, 1980; Loesche, 1986; Beighton, 2005). This bacterium (i) effectively utilizes dietary sucrose (and possibly starch) to synthesize large amounts of EPS through glucosyltransferases (Gtfs) and a fructosyltransferase (Ftfs), (ii) adheres tenaciously to glucan-coated surfaces, and (iii) is also acidogenic and acid-tolerant, which are critical virulence properties involved in the pathogenesis of dental caries.

Biofilms and dental caries — the role of exoenzymes and EPS synthesis

In nature, most of the biofilms develops from initial microbial attachment on a surface followed by formation of highly structured cell clusters (or microcolonies) and further development and stabilization of the microcolonies, which are occurring in a complex extracellular matrix (Branda et al., 2005). The majority of biofilms matrices are rich in polysaccharides, and dental biofilms are no exception; up to 40% of the dry-weight of dental biofilm is composed of polysaccharides (as reviewed in Paes Leme et al., 2006). All the available evidence shows clearly that the primary sources of EPS in dental biofilms are products from the interaction of Gtfs and Ftfs with sucrose and starch (as reviewed in Vaca-Smith et al., 1996; Kopec et al., 1997; Hayacicbara et al., 2004; van Hijum et al., 2006; Klein et al., 2009).

S. mutans is a key contributor to the formation of exopolysaccharide-matrix in dental biofilms. This bacterium produces three Gtfs, products of gtfB, gtfC and gtfD genes (Kuramitsu, 2003): GtfB, which synthesizes mostly insoluble glucans containing elevated amounts of α1,3-linked glucose; GtfC, which synthesizes a mixture of insoluble and soluble glucans [rich in α(1,6) linkages]; and GtfD which synthesizes predominantly soluble glucans. In addition, S. mutans produces a Ftfs, the
These observations show very clearly all these dental caries (Koo et al., 1974; Schilling and Bowen, 1992; Cross et al., 2007). The Gtfs secreted by S. mutans, particularly GtfC, bind avidly to the pellicle formed on the tooth surface in an active form; a layer of polysaccharides is formed rapidly on the surfaces in the presence of sucrose (Rolla et al., 1983; Scheie et al., 1987; Schilling and Bowen, 1988; Vacca-Smith and Bowen, 1998; Hannig et al., 2008). In addition, starches can be digested by salivary α-amylases to maltose, maltodextrins and other oligosaccharides, some of which can be acceptors during glucan synthesis by Gtfs increasing the overall exopolysaccharide production (Fukui and Moriyama, 1983; Fu and Robyt, 1991; Vacca-Smith et al., 1996). The polysaccharides on the surface provide binding site for colonization by S. mutans (Schilling and Bowen, 1992) through several surface-proteins capable of binding glucans, including the Gtfs and specific non-enzymatic glucan binding proteins (Banas and Vickerman, 2003). Moreover, it is apparent that glucan synthesized in situ by Gtf in pellicle provides enhanced binding for several oral microorganisms, including other oral streptococci, and Lactobacillus and Actinomyces species (Vacca-Smith et al., 1996; Bowen, 2002).

The Gtfs, especially GtfB, also adhere to bacterial surfaces, and furthermore adhere to surfaces of bacteria that do not make enzyme, thereby converting them into de facto glucan producers (McCabe and Donkersloot, 1977; Hamada et al., 1978; Vacca-Smith and Bowen, 1998). Thus, glucans, and at a lesser extent fructans, formed in situ promote the accumulation of microorganisms on the tooth surface and to each other, and may explain the electron micrographs of even early dental biofilm-plaque, which display microorganisms enmeshed in and attached to polysaccharide on the surface of saliva-coated hydroxyapatite (Vacca-Smith and Bowen, 2000). The attachment of bacterial cells on surfaces and formation of cell-cluster (or microcolonies) within an extracellular matrix are critical steps for the initial formation and further development of pathogenic biofilms.

The surface-adsorbed Gtfs and initial bacterial adherence on apatitic surfaces

S. mutans cells can attach initially to saliva coated surfaces through sucrose-independent mechanisms mediated primarily by lectin-like interactions between specific pellicle proteins and bacterial adhesins (Gibbons, 1996). However, this bacterium binds to the glucan-coated surfaces, especially those synthesized by GtfB and GtfC, in larger numbers and with higher adhesion strength than to uncoated or saliva-coated apatitic surfaces (Kuramitsu, 1974; Schilling and Bowen, 1992; Cross et al., 2007). The Gtfs secreted by S. mutans, particularly GtfC, bind avidly to the pellicle formed on the tooth surface in an active form; a layer of polysaccharides is formed rapidly on the surfaces in the presence of sucrose (Rolla et al., 1983; Scheie et al., 1987; Schilling and Bowen, 1988; Vacca-Smith and Bowen, 1998; Hannig et al., 2008). In addition, starches can be digested by salivary α-amylases to maltose, maltodextrins and other oligosaccharides, some of which can be acceptors during glucan synthesis by Gtfs increasing the overall exopolysaccharide production (Fukui and Moriyama, 1983; Fu and Robyt, 1991; Vacca-Smith et al., 1996). The polysaccharides on the surface provide binding site for colonization by S. mutans (Schilling and Bowen, 1992) through several surface-proteins capable of binding glucans, including the Gtfs and specific non-enzymatic glucan binding proteins (Banas and Vickerman, 2003). Moreover, it is apparent that glucan synthesized in situ by Gtf in pellicle provides enhanced binding for several oral microorganisms, including other oral streptococci, and Lactobacillus and Actinomyces species (Vacca-Smith et al., 1996; Bowen, 2002).

The role of EPS in the development of cariogenic biofilms

If the initial biofilm is allowed to remain on tooth surfaces with a frequent consumption of a high carbohydrate diet (especially sucrose and starch), S. mutans, a constituent of the biofilm community, will continue to synthesize exopolysaccharides and metabolize the sugars to organic acids. The elevated amounts of EPS, which may involve up-regulation of gtf genes in response to pH and carbohydrate availability (Li and Burne, 2001), increase the accumulation of bacterial cells on the surface and the bulk and stability of the biofilm, enhancing its virulence (Bowen, 2002). Recently, we examined the structural relationships between the bacterial cells and EPS using a novel fluorescence imaging technique (Klein et al., 2009; Xiao and Koo, 2009), which allows for visualization and quantification of exopolymers and bacterial cells simultaneously within intact biofilms (see Figure 1). The rendered images showed EPS closely associated with bacterial cells and microcolonies.
Figure 1  Fluorescence imaging showed the structural relationships between the bacterial cells and EPS

(A): Rendered 3-D images of the structural organization of the biofilms. Green: bacteria; Red: EPS. (B): Close-up view of the EPS: bacteria structural relationship.

throughout the biofilm development process (1) serving as a matrix holding bacterial cells on the surface allowing for the initial clusterization, and further development into microcolonies, and (2) providing supporting frame for continuous growth of the microcolonies (by enmeshing and filling the spaces between bacterial cells). The formation of specific EPS domains could be explained by the presence of enzymatically active Gtfs (particularly GtfB and GtfC) bound to saliva-coated apatitic and bacterial surfaces (Schilling and Bowen, 1988; Venkitaraman et al., 1995; Vaaca-Smith and Bowen, 1998; Hannig et al., 2008). The production of exopolysaccharides by Gtfs acting in concert at different sites is a highly efficient mechanism by which S. mutans colonize the surface and maintain the microcolony structure, allowing them to persist on the tooth surface for prolonged period. This EPS-dependent mechanism could explain why S. mutans, either alone or mixed with other species, can only build up microcolonies when sucrose is available (Kreth et al., 2008; Xiao and Koo, 2009); strongly suggesting that sucrose may increase the competitiveness of this bacterium in a mixed species environment. Considering that bacterial fitness in biofilms may be linked with micro-colonies formation, EPS may modulate the colonization and survival of S. mutans within complex biofilms in a context not previously considered.

The presence of these structured EPS micro-domains within and surrounding microcolonies across the biofilm depth may influence the diffusion properties and also protect the bacteria from inimical influences of antimicrobials and other environmental assaults (Dibdin and Shellis, 1988; Thurnheer et al., 2003; Reese and Guggenheim, 2007; Kreth et al., 2008; Xiao and Koo, 2009). In addition, the ability of S. mutans to utilize some exopolysaccharides (soluble glucans and fructans) as short term storage compounds offers an additional ecological benefit, and simultaneously, increases the amount of acid production and the extent of acidification. The persistence of this acidic condition leads to selection of highly acid tolerant (and acidogenic) flora (Quivey et al., 2001; Marsh, 2003; Marquis et al., 2003); the low pH microenvironment in the biofilm’s matrix results in dissolution of enamel of tooth surface, thus contributing to the pathogenesis of dental caries disease. Furthermore, glucans synthesized by other oral streptococci (e.g. S. oralis and S. sanguinis) may also contribute to the overall polysaccharide synthesis in the extracellular matrix.
Extracellular Polysaccharides Matrix  Koo et al.  http://www.ijos.org.cn

(Tamesada et al., 2004; Reese and Guggenheim, 2007). Clearly, EPS, particularly glucans synthesized by Gtfs, are essential for the formation and establishment of cariogenic biofilms.

A number of in vivo studies investigated the influence of Gtfs in sucrose-dependent colonization of teeth and in the pathogenesis of dental caries disease using animal models (Tanzer et al., 1985; Munro et al., 1991; Yamashita et al., 1993). Among them, Yamashita et al. (1993) conducted a comprehensive study using S. mutans mutants defective of each of the three gtf genes in a glucan-dependent rat model system to determine the precise role of these enzymes in the expression of virulence in vivo. Mutants defective in gtfB, gtfC and gtfBC exhibited marked reductions (up to 90%) in smooth-surface caries relative to that from parental organism. The gtfD mutant also induced significantly fewer smooth-surface lesions than did the parental organism albeit with less pronounced effect than gtfBC mutants; GtfD enzyme appears to be important for maximum development of smooth-surface carious lesions in the oral cavities of rats (Yamashita et al., 1993). Furthermore, several clinical studies have demonstrated that insoluble glucans produced by GtfB and GtfC in the extracellular matrix augment the cariogenicity of dental biofilms in humans (as reviewed in Paes Leme et al., 2006). Recently, it was shown that GtfB levels in saliva correlate strongly with presence of clinical caries and with number of carious lesions in young children (Vacca-Smith et al., 2007). Overall, the observations from in vitro, in vivo and clinical studies are highly supportive of the importance of the Gtfs and EPS in the pathogenesis of dental caries. Therefore, biofilm-control strategies based on disruption of EPS offer an attractive and alternative approach to the traditional chemotherapeutic approaches based on use of broad spectrum microbiocides, which require elevated concentration of agents due to reduced susceptibility of microorganisms in biofilms (Koo and Jeon, 2009).

Summary and future directions

The presence, composition and structure of the EPS-matrix influence the physical and biochemical properties of biofilm; the matrix may be as variable as the microbial constituents and is influenced by local environmental conditions and even changes with time. All the available evidences indicate that EPS, particularly glucans produced by S. mutans Gtfs, can contribute to the pathogenesis of dental caries in animals and humans by at least 6 distinct routes: (1) enhancing tight adherence and further accumulation of S. mutans and other oral species on the surface, (2) providing mechanical integrity/stability to the extracellular matrix allowing the microbial cells to establish microcolonies (and increasing the bulk of the biofilms), (3) protecting microorganisms from inimical influences of antimicrobials and other environmental assaults, (4) acting as reserve source of energy, (5) limiting diffusion of substances into and out of biofilm, and (6) helping to concentrate metal ions and other physiological nutrients within a microenvironment. Thus, elucidation of how the exopolymers synthesized by these enzymes affects the biophysical, structure, and diffusion properties of the matrix would advance our understanding of the exact mechanisms by which S. mutans influences the formation and pathogenicity of oral biofilms, and possibly identify novel therapeutic targets for effective anti-biofilm therapies.

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