The Great Bacterial Reef: Communication and development in human oral bacterial biofilms

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Consider that The Great Barrier Reef is home to thousands of species of plants and animals with spatiotemporally predictable fish communities on coral reefs, and compare this with the fact that human oral bacteria develop spatiotemporally predictable dental plaque communities on enamel after each oral hygiene procedure. This reassembling of oral bacterial communities over a time interval of only a few hours offers an opportunity to investigate the role of communication in community architecture and composition. Coaggregation, the physical cell-to-cell recognition system among genetically distinct cell types, has been shown to be an integral component of the development of oral communities as demonstrated by a retrievable enamel chip *in vivo* model system[1] as well as in *in vitro* model systems[2,3]. Juxtaposition of signal-producing and signal-receiving species in a saliva-based flowing environment is critical for communication[4]. Small molecule signals have an important role in developing the architecture of single[5] and dual species biofilms[6]. The understanding of the interactions occurring among initial colonisers will be greatly augmented by the use of *in vitro* communities reconstituted from *in vivo* biofilms. We are currently developing novel micromanipulation techniques to isolate intact *in vivo* communities that can then be analysed and reassembled *in vitro*.

We can identify those bacteria that adhere to enamel in the first few hours after an oral hygiene procedure such as brushing and flossing. Bacteria removed from a retrievable enamel chip carried in the oral cavity for four and eight hours have been characterised by culture methods[7] and by DNA sequence-based, non-culture techniques that rely on amplifying by PCR the 16S rRNA genes from initial colonising bacteria followed by cloning and sequencing of the amplicons *(Diaz et al., manuscript in preparation)*. The results of both methods unequivocally show streptococci as the dominant group in the first 8 hours with low numbers of other bacterial genera such as *Actinomyces* and *Veillonella.* The diversity increases at later times to include fusobacteria, which we have proposed to hold an important role in bacterial succession as a coaggregation bridge, because fusobacteria coaggregate with both early and late colonising species[8].

The adherence characteristics of the early colonising streptococci are highly significant towards understanding the role of these dominant early colonisers. The differential binding specificities of oral streptococcal antigen I/II family of adhesins mediating coaggregation and binding to salivary agglutinin glycoprotein (SAG or gp340), a component of the acquired pellicle on enamel, have been reported[2]. The antigen I/II adhesins, called SspA and SspB in *S. gordonii,* demonstrated distinct and specific adhesin-mediating properties; SspB mediated coaggregation with *Actinomyces naeslundii* T14V, whereas SspA was integral to coaggregations with *A. naeslundii* PK606. Both adhesins mediated high levels of binding to gp340. A 20-amino acid peptide within the C-terminal region of SspA and SspB inhibited binding of antigen I/II polypeptides to gp340 but did not inhibit antigen I/II-mediated coaggregation with actinomyces. These results indicate that antigen I/II-mediated binding to actinomyces and to host glycoproteins occurs by fundamentally distinct mechanisms. Adherence to host salivary receptors in the acquired pellicle on the enamel surface or to bacterial cells is the first step in the repetitive formation of initial communities *in vivo*.

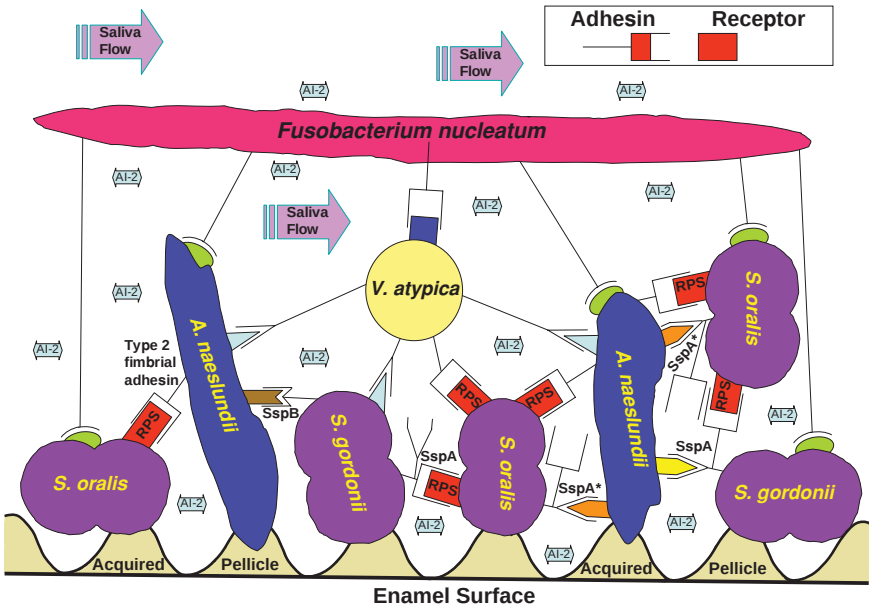
Using an *in vitro* model flowcell system with saliva as the sole nutritional source, we showed that signaling between two of these early colonisers, *S. gordonii* DL1 and *Veillonella atypica* PK1910, occurred only when the two cell types were in juxtaposition in the flowing environment[4]. These results suggest that building a reoccurring natural multispecies community *in vivo* in salivary flow might require coaggregation between any two complementary signal-producing and -receiving cells.

One signaling molecule called autoinducer-2 (AI-2) is produced by *S. gordonii* DL1 and several other oral bacterial species[8,9] and has been proposed

to be a universal inter-species signal [10]. Even in monocultures of *S. gordonii,* the absence of AI-2 has profound effects on the biofilm architecture of *S. gordonii* [5]. We have recently shown that different concentrations of this small signaling molecule are likely to regulate responses by the receiving bacteria and influence inter-generic commensalism within dual-species biofilms [6].

Culturing and identifying the initial bacteria repopulating a cleaned enamel surface is the first step in reconstructing the *in vivo* communities in an *in vitro* setting. It is possible to micromanipulate discrete communities of bacteria after 4 to 8 hours development on a retrievable enamel surface. The retrieved community can be investigated by reassembling the component species as a completely restored community *in vitro*. As an example, an early colonising community consisting of *Streptococcus oralis, S. gordonii, A. naeslundii,* and *V. atypica* could be investigated (Figure 1) in a saliva-based flowcell model system. Each pair can be introduced into the flowcell, and growth on saliva as the sole nutrient can be measured. Then, communities of three or all four species in the flowcell can be investigated for growth and for effects of small molecule signals such as AI-2. Reconstructing communities *in vitro* from species obtained from a single, isolated community formed *in vivo* will aid our understanding of how initial communities develop spatiotemporally *in vivo*.

Figure 1. Diagrammatic representation of a community of bacteria on an enamel chip *in vivo*. The enamel surface is at the bottom and is coated with an acquired pellicle that consists primarily of host-derived molecules such as statherin, sialylated mucins, proline-rich proteins, salivary agglutinin (gp340), and alpha-amylase. Each of these molecules is known to bind specific oral bacteria including oral streptococci and actinomyces. Interactions between bacteria are indicated as complementary sets of symbols of different shapes. One set is depicted in the box at the upper right. Rectangular symbols represent lactose-inhibitable coaggregation. Other symbols represent components that have no known inhibitor. Proposed adhesins (symbols with a stem) represent cell surface components that are heat inactivated (cell suspension heated to 85˚C for 30 min) and protease sensitive; their complementary receptors (symbols without a stem) are unaffected by heat or protease. Identical symbols represent components that are functionally similar but may not be structurally identical. An example is SspA and SspA* which are adhesins shown on *S. gordonii* and *S. oralis*, respectively. SspA is a multi-functional adhesin in that it mediates a lactose-inhibitable coaggregation between *S. gordonii* and *S. oralis*, and it mediates a different interaction between *S. gordonii* and *A. naeslundii* (lower right). The interaction between type 2 fimbrial adhesin and its cognate streptococcal receptor polysaccharide (RPS) is shown in the lower left. The small signaling molecule AI-2 is shown throughout the community. *Fusobacterium nucleatum* is shown as a coaggregation partner with all of the initial colonisers to indicate the next possible step in colonisation of enamel.



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