Studies on Pellicle and Early Dental Plaque in Relation to Periodontal Conditions

AKADEMISK AVHANDLING

som för avläggande av odontologie doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i föreläsningssal 3, institutionen for odontologi, Medicinaregatan 12E, Göteborg onsdagen den 5 december 2012, kl. 9.00

av

Stefan Rüdiger leg. tandläkare, odont. lic.

Fakultetsopponent Professor Anders Gustafsson, institutionen för odontologi, Karolinska institutet, Huddinge

Avhandlingen baseras på följande delarbeten:

- I. Carlén A, Rüdiger SG, Loggner I, Olsson J. Bacteria-binding plasma proteins in pellicles formed on hydroxyapatite *in vitro* and on teeth *in vivo*. *Oral Microbiology and Immunology* 2003;18:203-207.
- II. Rüdiger SG, Carlén A, Meurman JH, Kari K, Olsson J. Dental biofilms at healthy and inflamed gingival margins. *Journal of Clinical Periodontology* 2002;29:524-530.
- III. Rüdiger SG, Dahlén G, Carlén A. Pellicle and early dental plaque in periodontitis patients before and after surgical pocket elimination. *Acta Odontologica Scandinavica* 2012;70:615-621.
- IV. Rüdiger SG, Dahlén G, Carlén A. Proteins and bacteria on root surfaces exposed after periodontal surgery. Submitted.



UNIVERSITY OF GOTHENBURG

Abstract

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Background and Hypothesis: Bacterial receptors in dental pellicles may influence colonisation and subsequent plaque formation. Studies on such receptors in the dental pellicles and bacterial adherence have mostly been performed *in vitro* and focused on proteins of salivary origin. We have only a limited knowledge of the receptor functions of plasma proteins and even less on the *in vivo* situation where they could reach the pellicle via the gingival crevicular fluid. Our hypothesis was that plasma proteins in the gingival crevicular fluid affect pellicle formation and the establishment of the early dental microflora on the tooth surface

Material and Method: In the present series of studies, plasma proteins in pellicles formed on hydroxyapatite *in vitro* and on teeth *in vivo* and the adherence of bacteria to these pellicles were examined. *In vivo* studies were performed at different periodontal conditions in periodontally healthy and diseased subjects. Samples were taken at healthy and experimentally inflamed gingival margins as well as before and after surgical pocket elimination. The samples were taken from the gingival and incisal parts of teeth and, in one study group, even from surgically exposed root surfaces. Pellicle proteins were analysed using sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunoblotting and image analysis. Bacterial adherence *in vitro* was examined using radiolabelled bacteria and liquid scintillation. *In vivo* plaque samples were analysed by culturing and the PCR technique.

Results: Components from plasma were readily incorporated into the experimental pellicles and into natural pellicles on tooth surfaces in vivo. These components mediated the adherence of Porphyromonas gingivalis, Fusobacterium nucleatum and Actinomyces spp. in vitro and were found to a higher extent in pellicles formed at the gingival part of the tooth surface than at the incisal part. The amount of pellicle proteins and the numbers of bacteria were higher in the presence of periodontal inflammation. In experimentally inflamed gingival margins of periodontally healthy individuals, this observation was pronouncedly seen on the incisal parts of the tooth surfaces. In the presence of periodontal pockets, higher amount of pellicle proteins and numbers of bacteria was seen on the gingival tooth surfaces when compared with the situation after surgical pocket elimination. In periodontally healthy individuals, the bacterial findings indicated a pattern of less streptococci and Actinomyces spp. and more bacteria associated with periodontitis in the 4-hour dental plaque formed during experimentally inflamed conditions, compared with healthy conditions. Periodontitis-associated bacteria were also more frequently found in the 4-hour plaque in the presence of periodontal pockets compared with the status after pocket elimination surgery. Pellicle and early dental plaque on surgically exposed root surfaces contained significantly more plasma proteins and total numbers of bacteria compared with the adjacent gingival enamel surfaces. Actinomyces spp. were found in comparably high numbers on the exposed root surfaces.

Conclusions: Plasma proteins with the ability to mediate the *in vitro* adherence of periodontitis-associated bacteria are important components of the *in vivo* pellicle, particularly in the presence of periodontal inflammation. As the gingival crevicular fluid flow increases so does the relative amounts of plasma proteins in the pellicle, thereby modifying bacterial attachment and early dental plaque composition. Surgically exposed root surfaces were found to bind significantly higher amounts of plasma proteins and total number of bacteria than the adjacent enamel surfaces. Further on, the extent of the root surface exposure significantly reduced the amount of plasma proteins binding to the adjacent enamel surface. On the basis of our observations, we suggest that the bacterial composition of early dental plaque may be governed by the presence of plasma proteins in the pellicle and the presence of exposed root surfaces.

Key words: bacterial adherence, *in vitro* pellicle, *in vivo* pellicle, periodontitis-associated bacteria, gingival crevicular fluid, plasma proteins, dental biofilm, experimental gingivitis, chronic periodontitis, periodontal pocket, exposed root surfaces

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