# THE IMPACT OF SMOKING ON ORAL MICROORGANISMS

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**Introduction.** Smoking and exposure to secondhand smoke causes various human diseases. The primary reasons for global tobacco control are based on evidence of causality between exposure to tobacco smoke and disease. Categories for judgment of causal inference were applied to oral diseases in national reports.

**Aim of the study.** In a works investigating the potential mechanisms linking smoking and oral and upper respiratory tract bacterial pathogens, findings regarding the impact of exposure to smoking and smoking cessation on the flora of otitis media and the nasopharynx, respectively, were strengthened in relation to the association between reduction in the numbers of species of the normal oral mucosa flora and a greater frequency of respiratory infections.

**Materials and methods.** Resolution of dysbiosis following treatment for periodontal disease and tobacco dependence has been reported in longitudinal intervention studies. In the present report, we evaluated the biological findings regarding the effect of smoking on the periodontal microbiome. A standardized electronic search was conducted using MEDLINE. Studies that addressed the relationship between tobacco and periodontal pathogens were included.

Results and discussion. Microorganisms in saliva include bacteria from the surfaces of different oral tissues. Microarray analysis of stimulated saliva from 292 individuals with low levels of dental caries and periodontal disease showed a higher abundance of two bacterial taxa, *Streptococcus sobrinus (S. gordonii)* and *Eubacterium brachy (E. brachy)*, in smokers as compared with nonsmokers. Current smoking was associated with the relative abundance profile of 13 bacterial genera including *Prevotella* and *Veillonella* among the core bacterial species as characterized by a higher ratio of the operational taxonomic units. 16S rRNA sequencing of oral wash samples from 1205 US adults showed differences in the overall microbiome composition between current and non-current smokers, and functional analysis from inferred metagenomes revealed that bacterial genera related to carbohydrate, energy, and xenobiotic metabolism were depleted by smoking. These findings suggest the potential role of smoking in the shift in functions of the oral microbiome in smoking-related oral diseases.

*Porphyromonas gingivalis (P. gingivalis)* adapts to CSE, an environmental stress, by altering the expression of its major fimbrial antigen in addition to its capsule. CSE upregulates the *P. gingivalis* major fimbrial antigen while suppressing polysaccharide production in the capsule that promotes *P. gingivalis* colonization and infection. *S. gordonii*, an early colonizer in oral cavities, interacts with *P. gingivalis*. CSE exposure promoted the formation of a *P. gingivalis* - *S. gordonii* biofilm in a dose-dependent manner, with a stronger *P. gingivalis* major fimbriae binding capacity than the control, but did not induce *P. gingivalis* auto-aggregation. These effects on the *P. gingivalis* phenotype could explain the alterations that promote colonization and infection by key periodontal pathogens, and the reversibility of the fimbrial alteration

observed *in vitro* suggests that there are benefits to smoking cessation. Treatment with nicotine at a range of concentrations in saliva of smokers, from 0 to over 2 mg/ml stimulated *S. gordonii* planktonic cell growth, increased biofilm formation and bacterial cell mass, and upregulated *S. gordonii* binding-related genes in planktonic cells.

Treatment of *S. gordonii* with nicotine may contribute to *P. gingivalis* - *S. gordonii* biofilm formation. However, the role of nicotine on *P. gingivalis* growth is not straightforward. Growth of *P. gingivalis* was inhibited by nicotine treatment in a dose-dependent manner by a single exposure method, but its growth rate increased with each subsequent exposure to nicotine in a multiple-treatment model. Exposure to 0.1 mg/ml of cotinine, a metabolite of nicotine, increased *P. gingivalis* association and invasion of epithelial cells, but this was not affected by exposure to nicotine. Inoculation of primary epithelial cell monolayers with nicotine and cotinine at a concentration of 1 mg/ml increased colonization of *Aggregatibacter actinomycetemcomitans* in a time-dependent manner, but decreased colonization of *P. gingivalis*. Nicotine and its metabolite cotinine may promote the formation of pathogenic biofilms by enhancing the *P. gingivalis* - *S. gordonii* interaction.

Smoking has been reported to be among the factors which may influence the subgingival microbiome. Subgingival plaque samples from 12 patients with chronic periodontitis showed a high relative abundance of *Parvimonans, Desulfubulbus, Paludibacter, Haemophilus*, and *Sphaerochaeta* compared with those from 8 healthy controls. Among the periodontitis patients, a major microbial community alteration was evident in 6 smokers when compared with 6 nonsmokers. Subgingival plaque samples taken from deep sites were different in abundance of 51 genera and 200 species compared with samples taken from shallow pockets in 88 patients with chronic periodontitis. Differences in the microbiome between deep and shallow sites were influenced independently by smoking among patient-level factors, indicating that smoking independently influences the difference in microbiome by pocket depth in periodontitis patients. The synergistic effect of smoking and hyperglycemia on disease-associated subgingival microbiomes was greater than sum of each individual effect.

**Conclusions.** Analyses of microbiome in saliva and oral wash samples implicate that the periodontal microbiome is the potential origin of alterations in the oral microbiome resulting from smoking. Nicotine treatment and exposure to cigarette smoke extract alter the function of key periodontal pathogens such as *P. gingivalis* and promote biofilm formation, colonization, and infection. New 16S rRNA sequencing technology has shed new light on the subgingival microbiome of smokers and quitters to oral health professionals. Dysbiosis of the periodontal microbiome was observed in smokers regardless of their periodontal condition (healthy, gingivitis, or periodontitis). Furthermore, dysbiosis in smokers remained significant even after the resolution of experimentally-induced gingivitis and following recovery of clinical signs of periodontitis by non-surgical periodontal treatment and over 3 months post-therapy. Smoking cessation in periodontitis patients may be beneficial in terms of compositional changes of the subgingival microbiome toward a healthy subgingival microbial community. Further studies are required to elucidate the impact of tobacco intervention on the prevention of recurrence of periodontal destruction for the full achievement of tobacco interventions in dental settings.