PCR reaction and dot-blot hybridization to monitor the distribution of oral pathogens within plaque samples of periodontally healthy individuals

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Running title: PCR and dot-blot hybridization to monitor bacteria in plaque

Abstract

The purpose of this study was to determine the distribution of the putative periodontal pathogens *Prevotella intermedia, Prevotella nigrescens,* the three oral *Capnocytophaga* species (*C. ochracea, C. sputigena, C. gingivalis*) as well as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in plaque samples of periodontally healthy individuals. We chose a newly developed 16S rDNA directed polymerase chain reaction (PCR) and a previously described dot-blot hybridization assay to detect, differentiate and quantify these bacteria directly in clinical samples. The subjects of these investigations were 66 sulcus fluid samples from 17 children (age 3-5) attending a kindergarten, 48 sulcus fluid samples from 12 children (age 9-10) from a primary school and 25 subgingival plaque samples isolated from 6 different periodontally healthy dental students (age 24-27). We were able to demonstrate the presence of *P. nigrescens* in 54

(kindergarten: 5/ primary school: 33/ students: 16) samples by PCR and quantified it by dot-blot hybridization. In addition, we found *C. ochracea* in 12 (kindergarten: 2/ primary school: 10) samples by PCR reaction only. The other tested bacterial species were absent by the methods used. Furthermore we confirmed the specificity of our *P. nigrescens*-PCR in selected samples by enzyme electrophoresis.

Key Words: Periodontitis/microbiology; *Prevotella intermedia*, *Prevotella nigrescens*, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Capnocytophaga* spp./etiological importance; Polymerase Chain Reaction; dot-blot hybridization.