“Microbiological characterization of the biofilm on the carious surface in preschoolers aging 36-60 months with early childhood caries”

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Abstract
The aim of this study was to identify species of the microbiota present in biofilm at different stages of early childhood caries. Biofilm samples of 75 preschool children aging 36-60 months from Piracicaba-SP, were collected, identified among the different groups (no caries surface, white spot lesion and cavitated caries lesion) and stored under cooling. It was carried out DNA purification, quantification of samples by spectrophotometry (Nanodrop® 2000) and quantification of total bacteria using 16S gene by qPCR.

Key words: Early childhood caries, polymerase chain reaction, biofilm.

Introduction
Early childhood caries (ECC) is defined as the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger¹ and due to its high prevalence in Brazil, is considered a public health problem. Several studies have assessed the microbiological etiology of ECC and showed that S. mutans is particularly related to the development of caries in children², but there are no studies showing the microbial profile of biofilm involved in the different stages of the disease. The aim of this study was to quantify and identify the microbiota on the tooth surface, at different stages of the disease, in order to increase the knowledge related to the progression of disease, allowing the implementation of strategies for the prevention and control. Thus, 75 preschool children aging 36-60 months were divided among the following groups: 1. Caries free children (CF) (n=20); 2. White spot lesion children (WSL) (n=9); 3. Cavitated caries lesion (CCL) (n=46).

Results and Discussion
In the biofilm the proportion of Actinomyces naeslundii, Bifidobacterium spp., Lactobacillus acidophilus, Streptococcus mutans, Veillonella dispar, Streptococcus mitis and Streptococcus gordonii were measured by qPCR (Quantitative-Polymerase Chain Reaction.) All children were subjected to clinical examination for the presence of clinically visible biofilm in the upper incisors and the diagnosis of caries (Nyvad criteria modified for deciduous teeth). The results of qPCR analysis showed the highest amounts of Streptococcus mutans, Lactobacillus acidophilus e Bifidobacterium spp. In addition, Actinomyces spp and Veillonella spp showed similar values among the different groups.

Conclusions
Through this work it was possible to quantify the bacteria at different stages of the disease, concluding that the species Streptococcus mutans , Lactobacillus acidophilus and Bifidobacterium spp were significantly higher in children with caries already cavitated.

Acknowledgement
We would like to thank the brazilian National Council for Scientific and Technological Development - CNPq for the scholarship.