# 公益財団法人 セコム科学技術振興財団 研究成果報告書

#### 研究課題名

ヒト・デンタルバイオフィルムの次世代シーケンス網羅的解析に基づく制御法の開発

## Development of control methods of human dental biofilms based on comprehensive analyses using next-generation sequencing

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#### Abstract

We investigated the effect of sleep on the microbiome of oral biofilm (buccal mucosa, palate, tongue, gingival mucosa, tooth surface, saliva). *Prevotella* had higher relative abundance on awaking than before sleep in all location of the oral cavity, whereas *Streptococcus* tended to be higher before sleep than awaking. Furthermore, we investigated the effect of sleep on the dental biofilm using an *in situ* biofilm model. No significant changes in the numbers of biofilm –forming bacteria were caused by sleep. However, the relative abundance of *Fusobacterium* and *Prevotella* were significantly higher after awaking. Confocal laser scanning microscopy showed that the volume of exopolysaccharide was significantly larger before sleep than awaking. We clarified that although no quantitative changes due to sleep were observed, qualitative changes such as the bacterial flora and three-dimensional structure of the biofilm occurred.

The microbiota related to root caries was investigated. The relative abundance of *Actinomyces* and *Streptococcus* were higher in the caries-affected dentin compared to the healthy root surfaces. In addition, these bacteria have a higher relative abundance in initial caries than in advanced caries and deeper site in the cavities. We showed that the biofilm microbiota was different between the healthy and caries-affected sites, and that the microbiota was different depending on the progress of root surface caries.

We have been investigated the healthy human core microbiome. For this purpose, we tried to elucidate healthy core microbiome of Japanese older adults at the age of 90 and compared the core microbiome of the children living isolated area in Myanmar. We also tired selective tooth surface disinfection by antimicrobials chlorhexidine for the patients with fixed orthodontic appliance.

The core microbiome of the healthy Japanese older adults at the age of 90 was abundant in Firmicutes. Many species that can use for probiotics were abundant. However, some species that are the agents of pneumonia were abundant. This tendency was similar to the result of our previous report that investigated the core microbiome of the four Japanese centenarians. In contrast, Proteobacteria was abundant in the core microbiome of the children living in isolated area in Myanmar. These results indicated that core microbiome was affected by the living area or lifestyles.

In the study of selective tooth surface disinfection, we analyzed the microbiome of the patients with orthodontic treatment by fixed orthodontic appliance. The study design was open labeled randomized controlled trial. The patients were randomly allocated to conventional preventive treatment with fluoride application groups and additional tooth surface disinfection groups. The oral microbiome was analyzed twice at the baseline and after 6 months of preventive treatment. In addition, tooth white spot lesions were also investigated. Salivary levels of mutans streptococci were significantly reduced in tooth surface disinfection group. In addition, some species that have been suggested to be the pathogens for pneumonia were reduced in tooth surface disinfection group. However, at the phylum levels, drastic changes of the microbiome was not observed. Some species had significantly strong correlation with area and volume of white spot lesions. These results indicated that even by using antimicrobials, drastic changes of microbiome compositions were not induced. Therefore, microbiome may be stable in the short span of human life.

In summary, there may be healthy core microbiome that consisted by beneficial species for human body. However, human oral core microbiome included some pathogenic species. And composition of oral microbiome was affected by the living area and lifestyles. It is stable during short span of human life.