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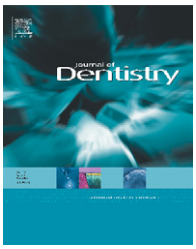

Characterization of oral films formed in the presence of a CPP–ACP agent: An *in situ* study

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ABSTRACT

Objectives: The aim of this study was to compare the morphological appearance and the molecular composition of intraoral integuments formed *in situ* on germanium (Ge) crystals in the presence or absence of the commercially available casein-phosphopeptide–amorphous calcium phosphate (CPP–ACP) cream agent.

Methods: Six volunteers participated in the study. Impression of maxillary arch was taken for each patient, and a removable orthodontic appliance with a custom-made retainer was fabricated. Clean Ge crystals mounted in the retainers were placed intraorally for 30 min, 8, 24 h and 1-week period. The free sampling surface of another series of Ge crystals was treated with the commercial CPP–ACP agent (Tooth Mousse), mounted in the retainers and placed intraorally for the same period as above. The free exposed surfaces in oral cavity of the specimens in all subjects were examined as follows: (a) reflected light microscopy, (b) micro-MIR-FTIR spectroscopy and (c) scanning electron microscopy (SEM) plus energy-dispersive X-ray microanalysis analysis (XEDS).

Results: The light microscopic observations revealed that there was a delay in biofilm formation on Ge surfaces treated with agent in comparison to those ones without treatment. The micro-MIR-FTIR spectra from the surfaces with Tooth Mousse showed an increase in intensity and a left shift of PO_4^{3-} peak (1064 cm^{-1}). Finally, the PO_4^{3-} peak at lower bands (564 cm^{-1}) and the low-wave bands at 525–530 cm^{-1} increased at 1-week interval. The SEM revealed the dendritic development of microbes. The XEDS analysis showed a significant increase in Cl/O, K/O, K/Cl and a decrease in Ca/O and P/O ratios on the crystal Ge without surface treatment. On contrary, on the crystal Ge with surface treatment an increase in Ca/O, Ca/P and a decrease in K/Cl ratios were found.

Conclusion: The results show that the presence of CPP–ACP agent delays the biofilm formation and favored the nucleation and crystallization of calcium phosphates, possibly in apatitic form, in matured biofilms.

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1. Introduction

Dental caries is the most common oral disease in children and adolescences. Although great efforts have been undertaken to reduce the incidence of caries in general population, the DMFS index still remains high.[1] The conventional treatment approach for all caries affected teeth includes removal of carious tissues and placement of a restorative material. However, a contemporary approach has been adopted late, by the non-invasive intervention of carious lesions. Non-cavitated as well as carious lesions extending up to dentin–enamel junction can be arrested if the cariogenic challenge of the lesion micro-environment is sufficiently controlled or therapeutic agents are applied for tissue healing.[2] Professional fluoride-delivery methods, such as gels and varnishes, are routinely applied to remineralize high-risk tooth areas (i.e. white-spots) or even early cavitated carious lesions.

During the last decade, bioactive agents based on milk products have been developed that, under cariogenic conditions, can release elements enhancing enamel and dentin remineralization. As a result, they intervene and arrest the progression of carious lesions and allow healing of the affected tissue, when used at the initial stages of the disease.[3] Recently, such an agent has become commercially available in a topical cream form (Tooth Mousse, GC International, Itabashi-ku, Tokyo, Japan) based on a nanocomplex of the milk protein casein-phosphopeptide (CPP) with amorphous calcium phosphate (ACP).

A multifactorial anticariogenic mechanism has been proposed for CPP–ACP. It has been claimed that it promotes remineralization of the carious lesions by maintaining a supersaturated state of enamel minerals, whilst it hinders colonization of dental surfaces by cariogenic bacteria.[4] Furthermore, it has been shown to act as a buffering agent, which may prevent pH reduction in the oral micro-environment.[5] Although CPP–ACP has been already shown to prevent enamel demineralization and promote remineralization of subsurface enamel lesions in animal and *in situ* human caries models, there is still no published report on the efficiency of the commercially available topical cream formula.[6–8]

Previous studies have shown that germanium (Ge) crystals provide a relevant *in situ* model system for studying the primary intraoral adhesive contacts between biofilms and solid surfaces; a model system that further allows for the consecutive application of a variety of surface sensitive analytical techniques.[9–11]

The high smoothness, which precludes surface roughness interferences, the surface energy properties that match that of tooth enamel and the lack of toxicity and solubility are some of the reasons that have long established Ge crystals as a suitable substrate for *in situ* biofilm studies.[9,12] Moreover, Ge crystals provide highly reflective surfaces for optical microscopic investigation of unstained biofilms, can be used as internal reflection elements in infrared spectroscopy for the molecular characterization of the adsorbed biofilms and can be combined with other analytical techniques (destructive or non-destructive).

The aim of this study was to compare the morphological appearance and the molecular composition of intraoral integuments formed *in situ* on Ge crystals in the presence or absence of the commercially available CPP–ACP cream agent. The research hypothesis was: (a) the agent adheres on Ge surfaces, delaying biofilm formation; (b) the agent promotes calcium–phosphate crystal growth on Ge surfaces.

2. Materials and methods

Volunteers were recruited after ethical approval was obtained from the Ethics Committee of the University of Athens School of Dentistry (Ref 47/2002) and informed consent forms were signed. Each volunteer completed a medical history, and examined to assess caries experience. Volunteers were admitted to the panel if they showed evidence of non-active caries and possessed at least 22 natural teeth. Exclusion criteria included age (<20 or >45 years), resting saliva pH (<6.5 or >7.5), saliva flow rate (<0.2 ml/min unstimulated or <0.8 ml/min stimulated), evidence of poor oral health including periodontal disease, recent professional fluoride therapy (≤ 2 weeks), uptake of fluoridated water or any medication that could affect oral flora, and finally pregnancy. Six volunteers participated in the study, four males and two females of 33 years mean age. None was smoking cigarettes. All of them strictly adhered to the study protocol.

Impression of maxillary arch was taken from each volunteer, and a removable orthodontic appliance with a custom-made retainer was fabricated. The retainer was placed in front of the right buccal surfaces of the first molar for mounting Ge internal reflection crystals (Spectra Systems, Irvington, NY, USA) (Fig. 1). The crystal dimensions were 10 mm × 5 mm × 0.5 mm with 45° edge angles.

Clean sterilized Ge crystals mounted in the retainers were placed intraorally in each volunteer for 30 min, 8 h, 24 h and 1-week period, with 1-week interval between each testing period. The free sampling surface of another series of Ge crystals was treated with the commercial CPP–ACP agent (Tooth Mousse, Lot No. 04008181, Table 1). The agent's film was left undisturbed for 5 min and the crystals were then mounted in the retainers and placed in the same volunteers for the same periods as above.

After intraoral exposure, the crystals were demounted, rinsed with 20 ml of distilled water to remove any loosely bound integuments, dried in a dessicator and examined as follows:

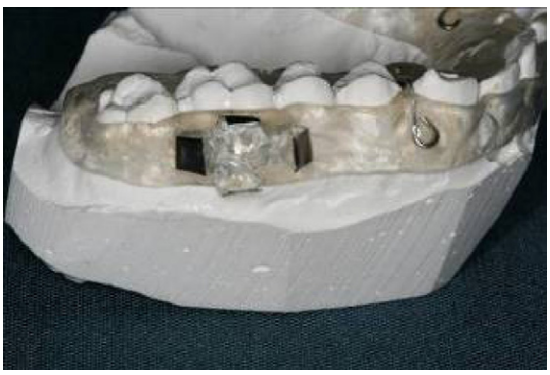

Fig. 1 – The orthodontic appliance with a custom made retainer for the Ge crystals.