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In situ effect of Tooth Mousse containing CPP-ACP on human enamel subjected to in vivo acid attacks

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ABSTRACT

Objective: This *in situ* study aimed to evaluate the protective effect of Tooth Mousse (GC) containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on human enamel erosion and to compare the difference in erosion between the anteriorly and posteriorly positioned human enamel.

Methods: This study used a 2-treatment (7 days each) crossover design with 12 healthy volunteers wearing intraoral appliances. Each appliance contained 4 human enamel specimens positioned on the buccal surfaces of the volunteers' maxillary central incisors and first molars. The specimens were intraorally treated withTooth Mousse (CPP-ACP group) or deionized water (control group) for 3 min and then exposed to *in vivo* acid attacks by rinsing with 150 ml of a cola drink (4 × 5 min/day). The surface microhardness (SMH) of the specimens was measured and used to calculate the percentage of SMH loss (%SMH_l). Erosion effect on enamel was also investigated by scanning electron microscopy (n = 4) at the end of study. The data were statistically analysed using two-way analysis of variance (ANOVA) and Tukey's test at a level of $P < 0.05$.

Results: A significant decrease in %SMH_l was observed for the specimens of CPP-ACP group compared to that for the controls ($P = 0.007$). The specimens positioned posteriorly exhibited a significantly lower %SMH_l than those positioned anteriorly ($P = 0.033$). Samples of CPP-ACP group showed fewer etching patterns than those of the control group.

Conclusions: In this *in situ* model, application of Tooth Mousse containing CPP-ACP before erosion reduced the %SMH_l of human enamel. Enamel located in different positions showed different patterns of erosion.

Clinical significance: Application of Tooth Mousse containing CPP-ACP could be considered as a suitable preventive strategy against enamel erosion.

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1. Introduction

Dental erosion is defined as the loss of tooth substance caused by acids without the involvement of microorganisms [1–3]. The increasing prevalence of dental erosion worldwide has led to increased attention from both clinicians and researchers [4,5]. Dental erosion is considered a multifactorial disease, and its increased prevalence observed in recent years has been related to the increased consumption of soft drinks [6,7].

Currently, strategies for preventing dental erosion include fluoride application, and modification of acidic beverage, and laser irridation, *etc.* [8–12]. Given that most fluoridated products show only a slight preventive effect against erosion [10], extensive attempts have been made to seek alternative preventive methods. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) which has shown anti-caries potential [13,14], has been recently considered as a potential candidate for erosion prevention [15]. However, the current literature offers contradictory findings regarding the erosion-inhibiting effects of CPP-ACP on dental enamel. Previous *in situ* studies showed a greater increase in surface microhardness (SMH) of previously eroded enamel after use of chewing gum containing 1% CPP-ACP compared to a conventional chewing gum [16,17]. Manton et al. [18] reported that the erosivity of soft drinks after the addition of 0.2% CPP-ACP was similar to that of distilled water. Ranjitkar et al. [19] reported that the application of 10% CPP-ACP paste reduced enamel and dentin wear due to erosion and abrasion. Similar findings were also reported by an *in vitro* study using atomic force microscopy and scanning electron microscopy

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[20]. Despite these positive results, some *in situ* and *in vitro* investigations found no positive effect of CPP-ACP on dental erosion [16,21–24]. The differences in the study designs (*in vitro*, *in situ / ex vivo*, *in situ / in vivo*) could account, at least in part, for the discrepancy in the above-mentioned reports.

The effects of test products against erosion should ideally be investigated using *in vivo* models. However, measurement of the erosive outcomes *in vivo* with acceptable accuracy is difficult. Therefore, *in vitro* and *in situ* studies with standardized conditions have been advocated for testing the effectiveness of preventive strategies against dental erosion [25]. Although most of the published studies were conducted *in vitro*, *in vitro* models cannot replicate the oral environment and can overestimate erosive changes by approximately 10-fold [26,27]. *In situ* models allow erosion changes to be measured over time in a natural environment of saliva and pellicle. In previous *in situ* studies, specimens were eroded either *in vivo* (intraorally) or *ex vivo* (extraorally). Although most of the published *in situ* studies were performed with *ex vivo* erosion [16,17,21,25], the best available method to simulate the clinical environment is an *in situ* study with *in vivo* erosion since the natural oral environment offers extra resistance to erosion compared to only the presence of the applied pellicle. A recently published study reported that surface roughness and SMH of specimens eroded *in vivo* were significantly different from those eroded *ex vivo* for 45 min [26]. Moreover, dental erosion is proven to have distinctive characteristics in location based on clinical evaluation. As for intrinsic erosion, erosion is generally present on the palatal surface of the maxillary teeth and occlusal surface of the mandibular posterior teeth. With regard to extrinsic erosion, the affected areas are often observed on the labial surface of anterior teeth and occlusal surface of the mandibular posterior teeth [1]. However, no *in situ / in vivo* model has been designed to evaluate the effects of the specimens' location on the erosion susceptibility of human enamel and the effects of CPP-ACP on dental erosion.

Thus, the aim of this *in situ* study was to determine the effects of Tooth Mousse containing CPP-ACP on human enamel subjected to *in vivo* erosion and to compare the difference in erosion between the anteriorly and posteriorly positioned human enamel. The following null hypotheses were tested: 1) the application of Tooth Mousse containing CPP-ACP would not affect the erosion susceptibility of human enamel; 2) the location of samples would not affect the erosion susceptibility of human enamel.

2. Methods and materials

This study was a single-blinded, controlled, randomized, two-treatment crossover *in situ* study, in accordance with the consolidated standards of reporting trials (CONSORT) guidelines. The overall observation period was 2 × 7 days with a washout period of 7 days. The protocol was approved by the Ethics Committee of the local university (ref 16-FMUSS-L81) and was registered under NCT03426150. The present study was performed following the Declaration of Helsinki and the Guidelines of Good Clinical Practice. The study was conducted at the Hospital of Stomatology of the local university from March 2017 to October 2017.

2.1. Inclusion and exclusion criteria

Healthy volunteers were examined and recruited based on the following inclusion criteria: (a) at least 18 years old and in good general health, (b) absence of caries, periodontal disease, and erosion, and (c) stimulated and unstimulated physio-logical saliva flow rates > 1 mL/min and > 0.25 mL/min, respectively. The following exclusion criteria were applied: (a) systemic diseases or oral mucosal disorders, (b) fixed or removable orthodontic appliances or removable prostheses, (c) pregnancy, (d) known allergies to the experimental drink, (e) history of gastric regurgitation or any current use of medication causing gastric reflux or xerostomia, and (f) any condition that precluded consumption of 600 ml of cola drinks per day for 7 consecutive days.

At the screening visit, the selected volunteers gave their written informed consent to participate in the study.

2.2. Specimen preparation

Enamel blocks were prepared from extracted non-carious human third molars from 18- to 35-year-old subjects of either gender [28]. After extraction, the teeth were stored in sealed containers with 0.1% thymol solution. Buccal enamel blocks (3 mm × 3 mm) were cut with a low-speed saw (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. The enamel blocks were further embedded with acrylic resin (Paladur, Heraeus Kulzer, Germany) using a custom-made silicone mould. The enamel surfaces were ground flat and polished using a series of carborundum discs (600#, 1200#, 2400#, and 4000#; Buehler) under water cooling. The specimens were cleaned in an ultrasound bath for 10 min after polishing. The final dimensions of the specimens were as follows: top surface 3 mm × 3 mm, bottom surface 4 mm × 4 mm, and thickness 2.2 mm. The absence of white spots and cracks on the enamel surface was confirmed with a stereomicroscope (MM400, Nikon, Tokyo, Japan). The SMH of the samples was measured using a Vickers microhardness tester (HXD-1000 TMC, Shanghai Taiming Optics Ltd., Shanghai, China) with a 50-g load and 15-s dwell time. Only samples with SMH values ranged from 310 to 360 VHN were included in the study. Prior to the *in situ* experiment, the specimens were stored in aqueous thymol solution for 2 weeks and 70% ethanol for 30 min for disinfection [29,30].

2.3. Intraoral appliance

Maxillary and mandibular impressions of each participant were taken using alginate materials (Jeltrate Alginate, Dentsply Detrey GmbH, Konstanz, Germany). The impressions were poured with dental hard stone (Heraeus, Hanau, Germany). The lower mouthguard was fabricated with a 0.035-in-thick soft-tray sheet (Ultradent Products Inc., South Jordan, UT, USA) and a heat/vacuum tray-forming machine (Ultraform, Ultradent Products Inc). The upper intraoral appliance was prepared in a similar manner but with 4 niches (slots) on the buccal surfaces of the central incisors and first molars (2 on the left and 2 on the right). An opening of 3 mm × 3 mm was made on each niche with a sharp scalpel (Fig. 1). The specimens were then fixed in the 4 niches with sticky wax approximately 0.5 mm beneath the appliance surface to avoid any abrasion from the buccal mucosa and tongue. The intraoral appliance was designed to protect the volunteers' natural dentition and expose the enamel surfaces of the specimens to *in vivo* acid challenges.

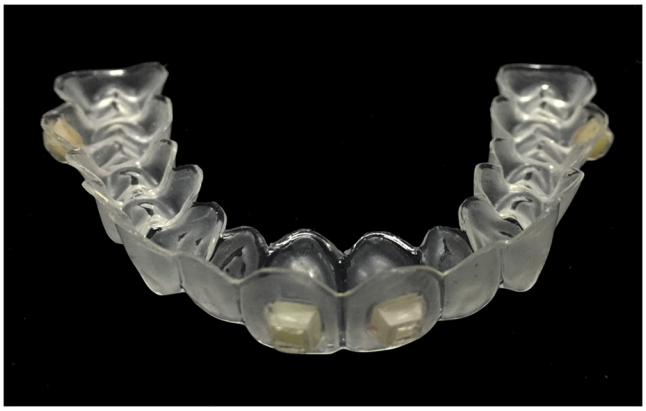

Fig. 1. Intraoral appliances containing 4 specimens. Each niche has 1 opening for acid exposure, and the specimen was fixed 0.5 mm beneath the appliance surface to prevent abrasion from the tongue and oral mucosa.