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Efficacy of enzymatic toothpastes for immobilisation of protective enzymes in the *in situ* pellicle

C. Hannig [a,b,*], B. Spitzmüller [b], H.C. Lux [b], M. Altenburger [b], A. Al-Ahmad [b], M. Hannig [c]

[a] Clinic of Conservative Dentistry, Faculty of Medicine 'Carl Gustav Carus', Technical University of Dresden, 01307 Dresden, Germany
[b] Department of Operative Dentistry and Periodontology, University of Freiburg, Hugstetter Str. 55, D-79106 Freiburg, Germany
[c] Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, University Hospital, Saarland University, Building 73, D-66421 Homburg/Saar, Germany

ARTICLE INFO

Article history:
Accepted 24 March 2010

Keywords:
Lysozyme
Peroxidase
Glucoseoxidase
Enzyme
Pellicle
Immobilisation
Tooth paste

ABSTRACT

Aim: Different enzyme-containing toothpastes are available on the market. The aim of the present *in situ* study was to investigate their efficacy for immobilisation of protective enzymes in the pellicle layer.

Methods: Pellicle formation took place *in situ* on bovine enamel slabs fixed to individual upper jaw splints carried by 6 subjects. After pellicle formation for 1 min, brushing was performed for 3 min with the commercially available toothpastes Enzycal, biotène and BioXtra, respectively. Before as well as 0, 20 and 40 min after brushing, samples were removed from the splints and tested for lysozyme, peroxidase and glucoseoxidase activity. The assays for the respective enzyme activities were based on fluorogenic substrates. Separate experiments were conducted for the different enzymes and toothpastes.

Results: Brushing with the toothpastes caused an extensive increase of glucoseoxidase activity in the pellicle, but it was of low tenacity whereas peroxidase activity was enhanced considerably. However, targeted accumulation of lysozyme in the pellicle was not very pronounced. Brushing without toothpaste had no effect on enzyme activities in the acquired pellicle.

Conclusion: Targeted immobilisation of enzymes in the *in situ* pellicle can be achieved with toothpastes.

1. Introduction

Bioinspired and biomimetic strategies are of considerable interest for oral health care to avoid disturbance of the ecological equilibrium in the oral cavity. For years, enzymes have been used for this purpose as components of mouth rinses and toothpastes.[1–5] With respect to the substantivity and efficacy of these compounds the accumulation and immobilisation of the contained enzymes in the acquired pellicle is required due to the high clearance of the oral fluids.[1]

The proteinaceous pellicle layer formed almost immediately on all oral hard and soft tissues is the mediator between the tooth surface and the oral fluids.[1,6,7] Enzymes are key components of the pellicle with high relevance for its protective properties.[1] This applies especially for the antibacterial lysozyme and for peroxidases preventing oxidative stress in the oral cavity by hydrolysis of peroxides and radicals.[8,9] Both are detectable in the pellicle in an active conformation within 1 min.[9,10] However, the antioxidative capacity of salivary peroxidase is limited due to inactivation by

* *Corresponding author at*: Clinic of Conservative Dentistry, University Hospital, University of Dresden, Fetscherstr. 74, 01307 Dresden, Germany. Tel.: +49 351 458 2713; fax: +49 351 458 5318.
E-mail address: christian.hannig@uniklinikum-dresden.de (C. Hannig).
0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2010.03.020

the substrate.[10] Lactoperoxidase from cow milk and lysozyme from hen egg white are cheap and rather similar as compared with their counterparts in human saliva.[3] Thus, they are typical ingredients of enzymatic oral health care compounds.[1,3] Glucoseoxidase is no physiological component of the saliva. It occurs sporadically in the oral cavity due to fungi and due to the fact that this enzyme is admixed to several foodstuffs.[11] Nonetheless, it is sometimes added to enzymatic toothpastes and mouth rinses.[1] It decomposes glucose and therewith detracts this substrate from cariogenic microorganisms. Furthermore, the byproduct of this hydrolysis is hydrogen peroxide which serves as a substrate for peroxidase during formation of antibacterial hypothiocyanite from thiocyanate.[11,12]

Enzymatic mouth rinses may be helpful for patients suffering from xerostomia as a substitute for lacking saliva. However, targeted accumulation of protective enzymes cannot be achieved with these compounds.[2] Beside these rinses also enzymatic toothpastes are available on the market. It is to be expected that tooth brushing allows direct interaction of the enzymatic compounds of the toothpastes with the pellicle layer for facilitated immobilisation of enzymes.[2,7]

The aim of the present study was to investigate the efficacy of enzymatic toothpastes for targeted immobilisation of protective enzymes in the acquired pellicle as a sequel to a previous study on enzymatic mouth rinses.[2]

2. Materials and methods

2.1. Toothpastes

The following enzymatic toothpastes were evaluated in the experiments: biotène (Laclede International, Brussels, Belgium, PZN 3819858), BioXtra (*John O. Butler*, Kriftel, Germany, PZN 1553824) and Enzycal (Curaprox, Curadent AG, Kriens, Switzerland, Lot No. # 828702) (Table 1).

2.2. Subjects and samples

Six healthy volunteers, members of the laboratory staff, participated in the study. A visual oral examination was carried out by an experienced dentist, the subjects showed no signs of gingivitis or caries. The subjects had a physiological salivary flow rate. Informed written consent was given by the subjects about participation in the study. The study design was reviewed and approved by the Medical Ethic Committee of the Medical Association of Saarland, Germany (52/05). Cylindrical enamel slabs (diameter 5 mm, 19.63 mm^2 surface area, height 1.5 mm) were prepared from labial surfaces of bovine incisors. The surfaces were polished by wet grinding with abrasive paper (400–4000 grit). The smear layer on the slabs was removed by ultrasonication with NaOCl for 3 min.[13,14] Afterwards, the samples were disinfected in ethanol (70%) for another 3 min, washed in distilled water and stored in distilled water for 24 h before adoption in the *in situ* experiments.[15]

2.3. *In situ* experiments[2]

For *in situ* pellicle formation, individual upper jaw splints were vacuum-formed from 1.5 mm thick methacrylate foils. A number of 8 cavities were prepared on the left and right buccal aspects of the splints at the sites of the premolars and the 1st molar. The slabs were fixed on the splints with polyvinyl siloxane impression material (Aquasil, Dentsply, Konstanz). The splints were carried intraorally for 1 min for pellicle formation. Thereafter, the subjects brushed the samples' surfaces *in situ* with 1.5 g tooth paste for 3 min, the remnants of the paste were spitted out afterwards. Details on the enzymatic composition of the adopted toothpastes are given in Table 1. Control experiments were carried out without tooth paste.

Before brushing as well as 0, 20 and 40 min afterwards two enamel slabs were removed from the splints and rinsed thoroughly with running distilled water for 5 s in order to remove non-adsorbed salivary remnants. In the following, the pellicle samples were tested immediately for the immobilised enzyme activities.

2.4. Enzymatic assays

All enzymatic assays were performed with a Tecan Infinite 200 plate-reader at a gain of 100 (Tecan, Crailsheim, Germany). The enzyme activities were calculated per cm^2 enamel surface, considering the diameter of the slabs (5 mm).

2.5. Peroxidase assay[10,16]

Peroxidase activity was determined as described previously.[10] In the presence of peroxidase and hydrogen peroxide, the fluorogenic 2′,7′-dichlorofluorescin (LDCF) is oxidised to the fluorescing dichlorofluorescin (DCF). Stock solutions of the stable reagent 2′,7′-dichlorofluorescin diacetate (LDADCF) were stored at −80 °C (5×10^{-5} M in absolute ethanol). Every day, the fluorogenic substrate LDCF was prepared hydrolytically from 2′,7′-dichlorofluorescindiacetate (LDADCF). One vol. of LDADCF solution was admixed to 9 vol. of 0.01 M sodium hydroxide, and incubated for 30 min. The reaction was

Table 1 – Enzyme activities and pH of the toothpastes tested.

	Enzyme activities of the toothpastes (U/g)			pH
	Lysozyme	Lactoperoxidase	Glucoseoxidase	
BioXtra	2824	17.27	5.17	6.4
Biotène	11,171	4.1	3.1	5.6
Enzycal	Not contained	2.13	16.8	5.2