Effect of Safflower Oil on the Protective Properties of the in situ Formed Salivary Pellicle

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Abstract

Aim: The prevalence of dental erosion is still increasing. A possible preventive approach might be rinsing with edible oils to improve the protective properties of the pellicle layer. This was tested in the present in situ study using safflower oil. *Methods:* Pellicle formation was carried out in situ on bovine enamel slabs fixed buccally to individual upper jaw splints (6 subjects). After 1 min of pellicle formation subjects rinsed with safflower oil for 10 min, subsequently the samples were exposed in the oral cavity for another 19 min. Enamel slabs without oral exposure and slabs exposed to the oral cavity for 30 min without any rinse served as controls. After pellicle formation in situ, slabs were incubated in HCl (pH 2; 2.3; 3) for 120 s, and kinetics of calcium and phosphate release were measured photometrically (arsenazo III, malachite green). Furthermore, the ultrastructure of the pellicles was evaluated by transmission electron microscopy (TEM). *Results:* Pellicle alone reduced erosive calcium and phosphate release significantly at all pH values. Pellicle modification by safflower oil resulted in an enhanced calcium loss at all pH values and caused an enhanced phosphate loss at pH 2.3. TEM indicated scattered accumulation of lipid micelles and irregular vesicle-like structures attached to the oil-treated pellicle layer. Acid etching affected the ultrastructure of the pellicle irrespective of oil rinsing. *Conclusion:* The protective properties of the pellicle layer against extensive erosive attacks are limited and mainly determined by pH. The protective effects are modified and reduced by rinses with safflower oil.

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The prevalence of dental erosion is still increasing as shown in many epidemiological studies [Larsen et al., 2005; Jaeggi and Lussi, 2006]. Stages from initial tooth surface alterations with microscopically detectable enamel loss to more than 50% of tooth surface loss can be found [Lussi et al., 2006]. Demineralization is induced by intrinsic or extrinsic acids. Main causes of erosion are different dietary substances containing acids, especially the extensive consumption of acidic beverages by vast parts of the population [Lussi et al., 2004]. The erosive effects of acidic foodstuffs or beverages suggest that dietary additives providing pH-buffering effects could be one ap-

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proach for the prevention or reduction of erosive mineral loss, e.g. calcium or milk derivatives such as casein phosphopeptides could be added to orange juice [Reynolds, 1998; Hannig and Hannig, 2010; Wegehaupt et al., 2011]. Moreover, fluoridated toothpaste has been shown to provide a protective effect against enamel erosion, if used regularly [Magalhaes et al., 2008]. Furthermore, in different culture groups mouth rinsing or oil pulling with edible oils is already part of body care. During oil pulling, a tablespoon of edible oil is taken into the mouth, sucked, sipped and pulled between the teeth over minutes, sometimes till the viscous oil turns thin and foamy. It is assumed that this procedure helps to remove bacteria from the oral cavity [Asokan, 2008; Asokan et al., 2011]. Typical examples for oil pulling agents are olive oil, sesame oil, safflower oil or linseed oil. Oil pulling could also mean an alternative approach to prevent erosive damage by adding more hydrophobic properties to the tooth surface due to accumulation of lipids in the acquired pellicle layer. The pellicle formed on all solid substrates exposed to the oral fluids is regarded as one key factor in the physiological prevention of dental erosion [Hannig and Joiner, 2006]; it has been shown that lipids account for 22–23% of the dry weight of the pellicle [Slomiany et al., 1986, 1990]. Though knowledge on lipids in the acquired pellicle is based on only these two publications [Slomiany et al., 1986, 1990], their possible impact on the protective properties has been discussed extensively [Hannig and Joiner, 2006]. Furthermore, several in vitro studies neglecting the in situ pellicle indicated that vegetable oil mouthrinses or olive oil-containing preparations might form a protective coating on dental hard tissues decreasing erosive mineral loss [Buchalla et al., 2003; Wiegand et al., 2007]. Irrespective of the lipid composition the pellicle is regarded as a semi-permeable barrier and buffer reducing erosive mineral loss [Hara et al., 2006; Hannig et al., 2009]. Due to its mesh-like ultrastructure, it provides a medium through which ions such as fluoride, calcium or phosphate are delivered during de- and re-mineralization [Schüpbach et al., 2001; Hannig, 2002; Hannig and Joiner, 2006]. Nevertheless, the protective properties are limited and the ultrastructure of the pellicle is degraded continuously at low pH levels [Hannig and Balz, 1999, 2001; Hannig et al., 2003, 2009].

Many edible oils have a pronounced particular flavor, whereas safflower oil is rather tasteless. There are two types of safflower oil which are regarded as beneficial in preventive medicine. One type is rather rich in monounsaturated oleic acid, the other in linoleic acid [Krist et al., 2008]. The high concentration of unsaturated fatty acids, especially linoleic acid, is considered to be valuable for the reduction of cholesterol levels [Czernichow et al., 2010; Higa et al., 2010; Suzuki et al., 2010]. Due to the dominance of essential ω-6 fatty acids, safflower oil was also used as a component of parenteral nutrition solutions. The aim of the present in situ/in vitro study was to evaluate the effect of safflower oil on the protective properties of the in situ formed pellicle. For this purpose, pellicles formed in situ on bovine enamel slabs and modified by rinses with safflower oil were incubated in hydrochloric acid in vitro. Thereby, the study focussed on short-term erosive effects simulating the situation in the oral cavity. The erosive effects were measured via determination of calcium and phosphate release. In addition, transmission electron microscopic investigations were performed to visualize the impact of rinsing with oil on pellicle ultrastructure in an acidic milieu.

Methods

Subjects

A number of 6 healthy volunteers, members of the laboratory staff (age 24–42), participated in the study. Visual oral examination was carried out by an experienced dentist. The subjects showed no signs of gingivitis or caries; they had a physiological salivary flow rate as determined qualitatively during oral examination. Informed written consent had been given by the subjects about participation in the study. The study design was reviewed and approved by the Ethics Committee of the University of Freiburg in Germany (Proposal 222/08).

Specimen Preparation

Enamel slabs with a diameter of 5 mm were prepared from bovine incisor teeth (2-year-old cattle). In total, 398 slabs were prepared. According to the size of the teeth, between 2 and 3 samples were gained from each bovine incisor. Specimens were etched at all sites except for the outer enamel surface for 15 s with 37% phosphoric acid gel (Etching gel, DMG, Hamburg, Germany) and treated for 30 s with Optibond Primer (Kerr, Karlsruhe, Germany). Optibond Adhesive was applied afterwards and light-cured for 30 s. Application of the adhesive was performed 3 times. Subsequently, the unsealed enamel surfaces were ground flat and polished (grit 4000) [Hannig et al., 2007a]. In a standardized grinding procedure, approximately 200 μm of the enamel was removed. Specimens with structural alterations of the enamel were excluded from the study. The smear layer on the slabs was removed with a steam jet and by ultrasound with NaOCl for 3 min [Hannig et al., 2007b]. Then the slabs were washed twice in distilled water for 5 min also by ultrasound. Afterwards, the samples were disinfected in ethanol (70%) for another 10 min (ultrasound), washed in distilled water and stored in distilled water for 24 h before exposure in the oral cavity.

Different experimental setups were tested to evaluate the effect of rinsing with safflower oil on the acquired pellicle and on erosive mineral loss (fig. 1).
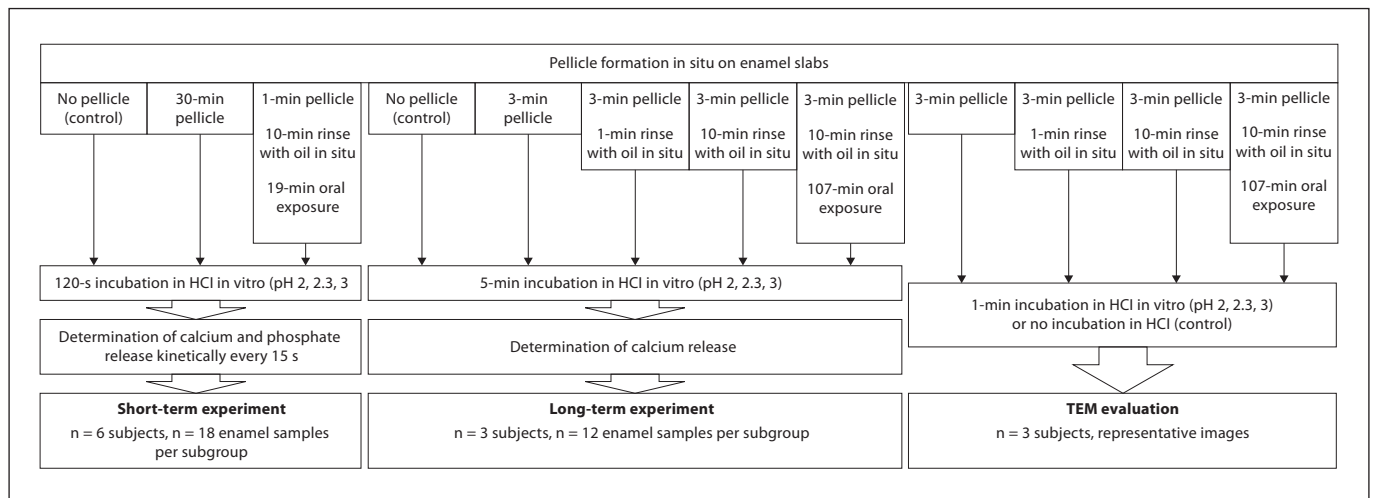
Fig. 1. Flow chart of all experiments.

In situ Pellicle Formation and in vitro Erosion

For in situ pellicle formation, individual upper jaw splints were vacuum-formed from 1.5-mm-thick methacrylate foil for each volunteer. Cavities were prepared in the buccal aspects of the splints (in the region of 16, 15, 14, and the region of 24, 25, 26, respectively). A number of 6 slabs were fixed on each splint with polyvinylsiloxane impression material (President Light Body, Coltène, Switzerland) in such a way, that only the specimen's surfaces were exposed to the oral environment. The splints were carried intraorally for 1 min to allow initial pellicle formation on the specimen surfaces. Afterwards, the subjects rinsed thoroughly with 8 ml safflower oil (Brölio Distelöl, Brökelmann & Co. Ölmühle GmbH & Co., Hamm, Germany) for 10 min. The splints remained in the oral cavity for further 19 min; this regime gave a total intraoral exposure time of 30 min. After intraoral exposure, the slabs were quickly removed from the splints and rinsed thoroughly with running water for 5 s. In vitro erosion was performed by incubating each sample in 1,000 μl hydrochloric acid (pH 2, 2.3, 3) to provide an excess of acid in order to maintain constant pH during the incubation period of 120 s (short-term experiment, fig. 1). Before exposure to HCl, the enamel slabs were embedded in silicone impression material at the bottom of a 2-ml Eppendorf cup. The acid was moved continuously by pumping with a 100-μl pipette (1 lift/s). Every 15 s, 100 μl of the acid was removed for photometric analysis and replaced by 100 μl of fresh acid. For control purposes, enamel specimens coated with a 30-min salivary pellicle (without oil rinsing) and specimens without pellicle were incubated in HCl.

Furthermore, calcium loss after 5-min incubation in HCl was measured in order to investigate the protective effect of the pellicle during longer incubation in an acidic agent (long-term experiment, fig. 1). In this additional experiment the following pretreatments of the enamel slabs under in situ conditions were tested: no pellicle; 3-min pellicle formation followed by 1-min rinse with oil; 3-min pellicle followed by 10-min rinse with oil and 107-min further oral exposure in order to have a total intraoral expo-

sure time of 120 min (120-min pellicle); 3-min pellicle; 3-min pellicle followed by 10-min rinse with oil. Three subjects took part in this additional experiment, each subject carried 4 samples per subgroup in the oral cavity (12 enamel samples).

Photometric Determination of Calcium and Phosphate Release

Mineral dissolution caused during incubation in HCl was determined by measuring photometrically calcium and phosphate release into the solution in double assays using the arsenazo III method (Fluitest®, Ca-A-II, Analyticon, Lichtenfels, Germany) and the malachite green assay, as described previously [Attin et al., 2005a, b; Hannig et al., 2005, 2007a].

Arsenazo III reacts with calcium in an acidic solution to form a blue purple complex. The resulting intensity is proportional to the calcium concentration. Absorption can be determined at $\lambda = 650$ nm according to standard curves. The reagent for determination of calcium was composed of 100 mM imidazole buffer (pH 6.5) and 0.12 mM arsenazo III. A volume of 10 μl from the sample was pipetted to 100 μl arsenazo reagent [Hannig et al., 2005, 2007a]. Two samples were measured for each specimen, and the average absorption was calculated.

Malachite green reacts with phosphate to form a colored complex which can be determined photometrically at $\lambda = 650$ nm. For the test reagent 0.045 mg of malachite green dissolved in 100 ml distilled water was mixed with 12.69 g of ammonium molybdate dissolved in 300 ml HCl (4 M). The reagent was stirred for 30 min afterwards and filtered (pore size 0.22 μm).

A volume of 10 μl from the sample was pipetted to 200 μl malachite reagent; the absorption was measured after 15 min [Attin et al., 2005b]. Two samples were measured for each specimen, and the average absorption was calculated. Calcium and phosphate release were calculated based on the mean photometric absorption values for the specimens and their surface areas (based on 5 mm diameter). The assays were also used for determination of calcium and phosphate concentrations of the saliva and of the oil.

Transmission Electron Microscopy (fig. 1)

In addition, in situ pellicle samples were prepared for electron microscopic analysis in order to visualize the impact of rinses with safflower oil on the ultrastructure of the pellicle and on its protective properties. Pellicle formation was performed for 3 min followed by a 10-min rinse with safflower oil. Then the samples were removed from the splints and transferred to the in vitro experiments, or carried in the oral cavity for another 107 min in order to have a total intraoral exposure time of 120 min. The samples were incubated in HCl (pH 2; 2.3, 3) in vitro for 1 min, or prepared for transmission electron microscopy (TEM) without incubation in HCl. Enamel samples with 3-min pellicles and 120-min pellicles, but without rinsing with safflower oil, served as controls. Immediately after the erosion experiments, the enamel slabs were fixed in glutaraldehyde for 2 h (2.5% glutaraldehyde, 1.5% formaldehyde in phosphate buffer, pH 7.4). Afterwards, the specimens were washed 5 times in phosphate buffer. Post-fixation for visualization of organic structures took place in 1% osmium tetroxide for 2 h. The specimens were dehydrated in increasing concentrations of alcohol and embedded in Araldite M (Serva, Darmstadt, Germany). The dentine was removed from the samples with a diamond bur, and the samples were decalcified in 1 M HCl. Re-embedding was performed with Araldite. Ultrathin sections of the pellicle samples were cut in series with an ultramicrotome (Ultracut E, Reichert, Bensheim, Germany), using a diamond knife. The ultrathin sections were mounted on mesh grids (Plano, Wetzlar, Germany) and contrasted with uranyl acetate and lead citrate. TEM investigation took place at 3,000- to 50,000-fold magnification in a TEM TECNAI 12 Biotwin (FEI, Eindhoven, The Netherlands).

Statistics

Data of the short-term experiments were evaluated by one-way ANOVA followed by the Scheffé procedure in order to detect significant influences of pellicle formation and safflower oil-rinsing on calcium and phosphate loss during incubation in HCl. Statistical analysis was performed considering the data from the 18 enamel slabs from all 6 subjects of each experimental subgroup for calculation of the means that were compared for significant differences. Normal distribution was checked using the Kolmogorov-Smirnov test; the software used was SPSS 17.0 (StatSoft, Hamburg, Germany). The level of significance was set at $p < 0.05$.

Results

Photometric Determination of Calcium and Phosphate Release

In general, the erosive mineral loss was predominantly determined by the pH of the hydrochloric acid, irrespective of whether the pellicle was modified or not by rinsing with safflower oil (fig. 2, 3, table 1). The kinetics of calcium and phosphate release over 120 s were almost linear irrespective of the pellicle layer or its modification by rinsing with oil. However, the 30-min non-rinsed pellicle reduced the erosive mineral loss at all pH values.

Due to the linearity of calcium and phosphate release, statistical evaluation was conducted for the cumulative 120-second data. During exposure in HCl for 120 s, calcium release was significantly reduced by the 30-min pellicle layer at pH 2.0 (–20%), pH 2.3 (–25%), and pH 3.0 (–64%) compared with the controls without pellicle (table 1). Rinsing with safflower oil led to a slightly higher loss of calcium from the pellicle-covered specimens than from slabs with 30-min non-rinsed pellicles at all pH values (pH 3.0: $p < 0.05$).

In part, divergent effects were observed for the kinetics of phosphate release recorded over 120 s. At all pH values the physiological 30-min pellicle (without oil rinsing) reduced the erosive phosphate loss significantly (pH 2: –25%, pH 2.3: –45%, pH 3: –41%). In contrast to the kinetics of calcium release, the results for phosphate indicate that rinsing with safflower oil did not significantly impair the protective effect of the 30-min non-rinsed pellicle at pH 2.0 and 3.0. However, at pH 2.3, a significantly higher phosphate dissolution from enamel samples covered by an in situ pellicle modified by a rinse with safflower oil was observed compared to the 30-min non-rinsed pellicle ($p < 0.05$).

In contrast to the results gained after 120-second HCl exposure, application of safflower oil in situ had no inhibiting influence on the cumulative erosive mineral loss after 5-min exposure to HCl (fig. 4). Thereby, different rinsing modes were tested without observing any significant effect.

The calcium and phosphate concentration of the safflower oil were very low: calcium concentration = 1.61×10^4 nM; phosphate = 3.95×10^4 nM. The calcium concentration in the saliva ranged between 1 and 1.5×10^6 nM, the phosphate concentration between 2.5 and 3×10^6 nM.

Transmission Electron Microscopy

The TEM images gave additional insight into the interaction of safflower oil with the pellicle layer; the effect of HCl on physiological and modified pellicles was visualized (fig. 5, 6). Rinses with oil for 10 min induced ultrastructural alterations of the 3-min pellicle layer (fig. 5). Small oil micelles (200–400 nm in diameter) were detected on the surface of the pellicle. However, the principal structure of the basal pellicle was not changed, large areas of the pellicle looked like typical physiological 3-min pellicles. If the samples were carried in the oral cavity for another 107 min (120-min pellicles), after rinsing with safflower oil, for further pellicle formation, a different ultrastructure as compared with the controls was observed. The pellicle was of lower density, including some irregularly shaped vesicle-like structures.
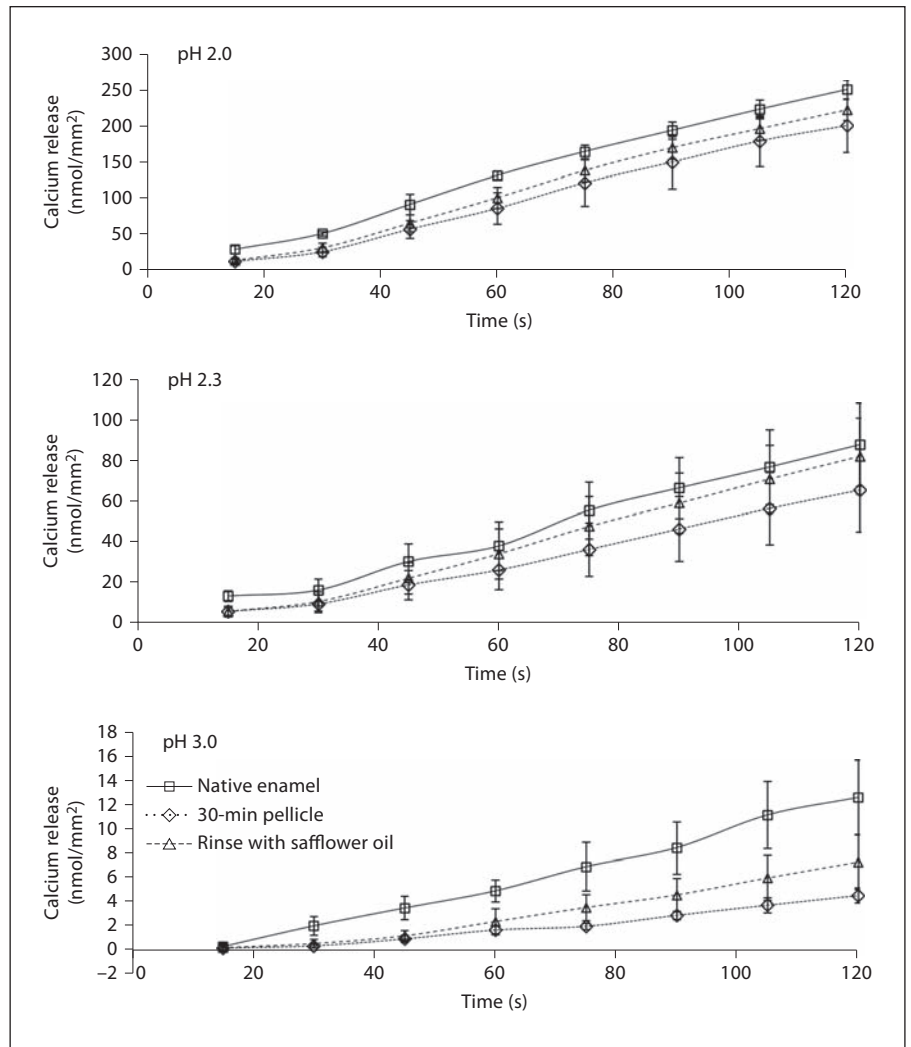
Fig. 2. Kinetics of calcium loss during incubation of enamel slabs for 120 s with and without safflower oil rinsing in situ. Physiological 30-min pellicles (without rinse) and specimens without pellicle served as controls. Note the different scale of the experiments performed at pH 3; n = 6 subjects, n = 18 enamel samples per subgroup, mean values ± standard deviation.

Table 1. Cumulative calcium and phosphate loss (nmol/mm^2) from control and test enamel slabs following incubation for 2 min in HCl at different pH values

| | Cumulative mineral loss over 120 s, nmol/mm^2 | | | | | |
| | calcium release | | | phosphate release | | |
	pH 2.0	pH 2.3	pH 3.0	pH 2.0	pH 2.3	pH 3.0
Enamel without pellicle	249.92 ± 13.41[a]	87.69 ± 20.35[a]	12.63 ± 3.09[a]	132.52 ± 3.97[a]	64.76 ± 8.59[a]	10.79 ± 1.27[a]
Enamel with 30-min pellicle	199.85 ± 37.11[b]	65.32 ± 20.39[b]	4.55 ± 0.62[b]	99.06 ± 7.92[b]	29.47 ± 2.16[c]	6.36 ± 0.84[b]
Enamel with safflower oil-modified 30-min pellicle	221.71 ± 14.53[b]	82.01 ± 18.67[a, b]	7.31 ± 2.26[c]	103.53 ± 11.26[b]	52.99 ± 11.30[b]	7.09 ± 1.25[b]
Corresponding ANOVA data, p value	<0.001	0.012	<0.001	<0.001	<0.001	<0.001

Physiological 30-min pellicles (without oil rinse) and specimens without pellicle served as controls; n = 6 subjects, n = 18 enamel samples per subgroup. Data significantly different within one column are followed by distinct superscripts (p < 0.05, Scheffé procedure).
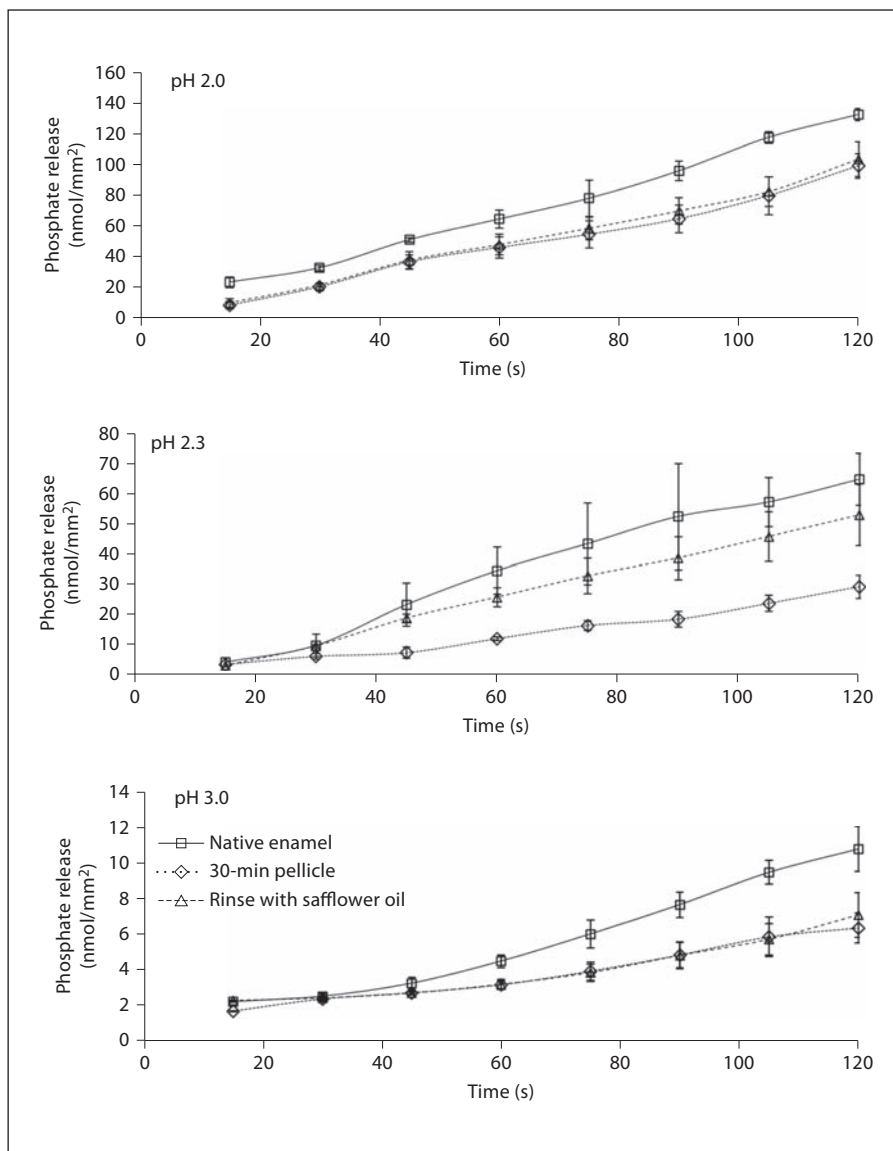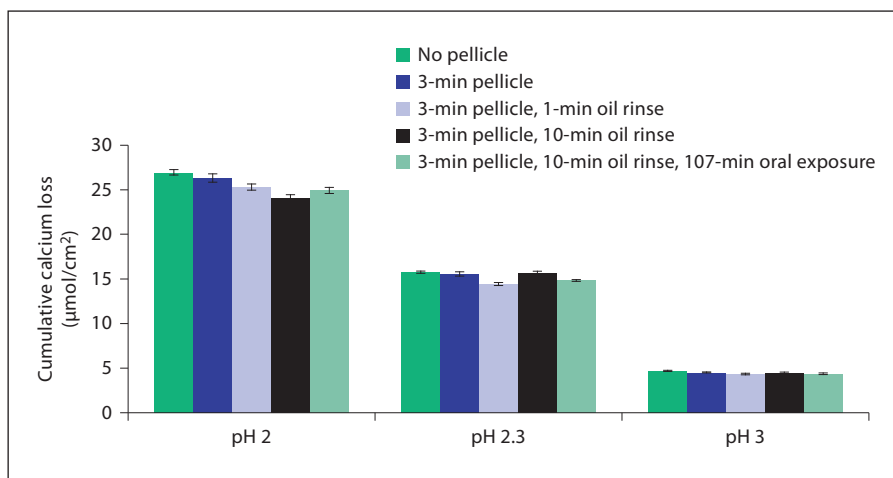
Fig. 3. Kinetics of phosphate loss during incubation of enamel slabs for 120 s with and without safflower oil rinsing in situ. Physiological 30-min pellicles (without rinse) and specimens without pellicle served as controls. Note the different scale of the experiments performed at pH 3; n = 6 subjects, n = 18 enamel samples per subgroup, mean values ± standard deviation.

Fig. 4. Incubation of enamel samples for 5 min in HCl after different modes of pellicle formation in situ with and without safflower oil rinsing; cumulated loss of calcium; n = 3 subjects, n = 12 enamel samples per subgroup, mean values ± standard deviation.

Fig. 5. TEM micrographs of 3-min pellicles (**a**, **d–g**) and 120-min pellicles (**b**, **c**, **h**, **i**) with and without 10-min oil rinsing. The 3-min pellicle without oil application showed a fine electron-dense structure (basal pellicle layer, **a**). Rinses with oil for 10 min mainly yielded a pellicle with a similar basal structure (**d**), in some cases, small irregular vesicle-like structures were visible (**e**, arrow). Lipid micelles (arrows) were observed attached randomly to the pellicle layer (**f**, **g**). The 120-min pellicle without oil application showed a fine granular structure on top of an electron-dense basal layer (**b**, **c**). Rinses with oil for 10 min after 3-min pellicle formation followed by a 107-min oral exposure (120-min pellicles) yielded a pellicle with a lower density (**h**, **i**), sometimes irregular vesicle-like structures are visible (**i**, triangle). Note that the enamel was removed during the preparation of the samples, the former enamel site is marked with asterisks.
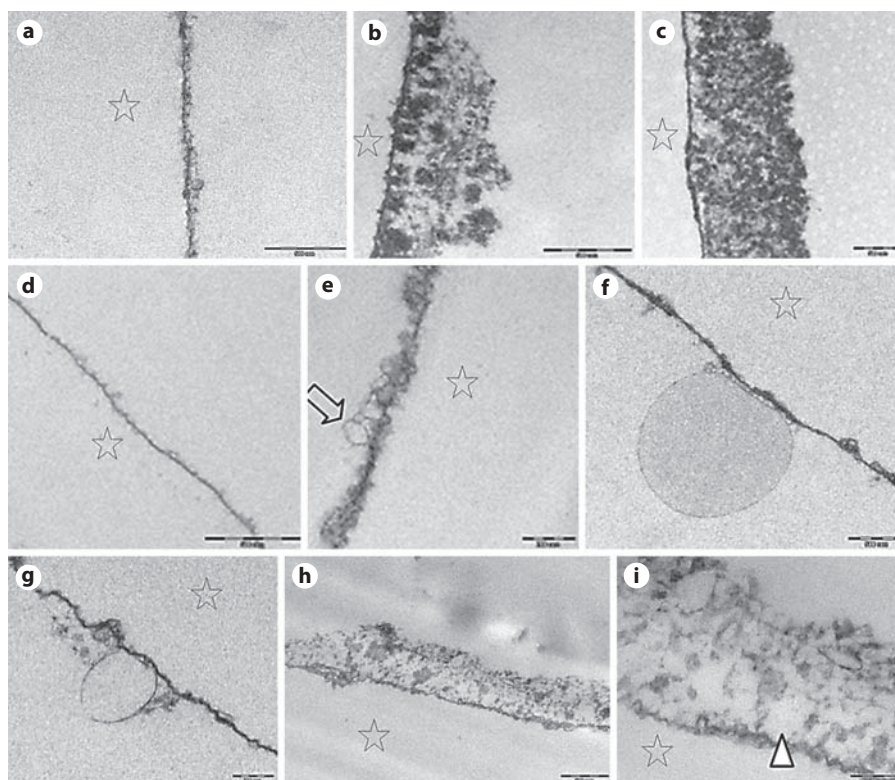
The ultrastructure of the pellicle was degraded during incubation in HCl in a pH-dependent manner (fig. 6). Thereby, the preliminary rinses with the edible oil had no protective effect on the degradation of the pellicle layer. Sometimes, the degradation process of the proteinaceous layer seemed to be more pronounced in the oil-rinsed specimens. This applied for the 3-min as well as for the 120-min samples and corresponded to the results of the calcium and phosphate assay.

Discussion

The present investigation based on in situ formed pellicles monitored short-term erosive effects occurring during the first 5 min of exposure to acidic agents [Attin, 2006]. Thereby, strong emphasis was put on short-term experiments, measuring the erosive mineral loss from 15 to 120 s in a kinetic manner. To the best knowledge of the authors, erosive mineral loss from enamel slabs coated with an in situ pellicle has not been investigated in such short intervals until now. The different results observed in the present study within 120 s and after 5 min of erosion in HCl underline the necessity of short-term experiments.

The photometric calcium and phosphate assays are characterized by very high sensitivity and precision [Attin, 2006; Hannig et al., 2008]. Their only disadvantage is that they cannot be used in situ or in vivo [Attin et al., 2005a, b; Hannig et al., 2008]. Accordingly, a combined in situ/in vitro approach was chosen in the present study to ensure formation of physiological (without rinse) and modified (oil-rinsed) in situ pellicles followed by incubation with HCl in vitro under standardized conditions simulating short-term exposure to acidic agents [Hannig et al., 2007a]; pH values were chosen according to a previous study on erosive calcium and phosphate release [Hannig et al., 2005].

Several edible oils have been discussed for application in preventive dentistry and preventive medicine [Wiegand et al., 2007; Asokan et al., 2009]. The present study focussed on safflower oil, which is rather tasteless. In contrast to the widely used olive oil, it does not contain many polyphenolic components that will modulate the taste and possibly result in denaturation of the pellicle. Furthermore, it is noteworthy that the mineral content of safflower oil itself is very low and will not affect the erosive process.
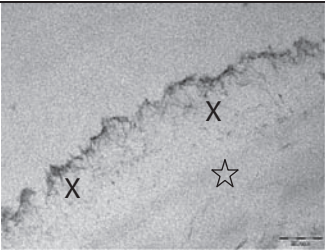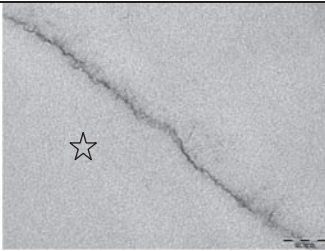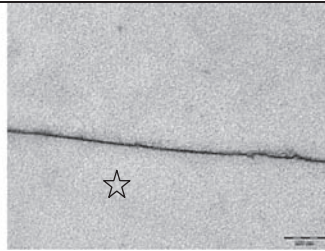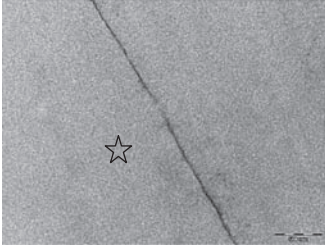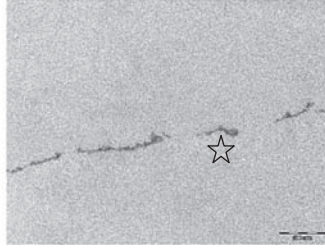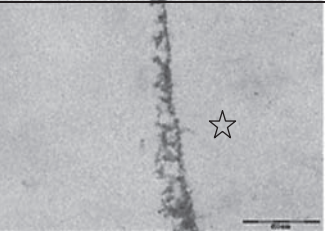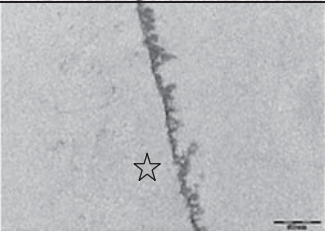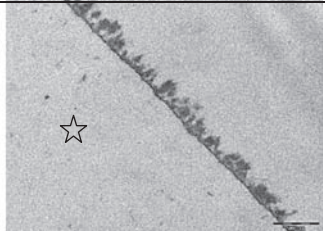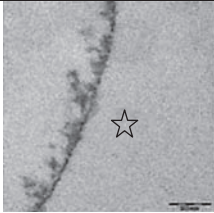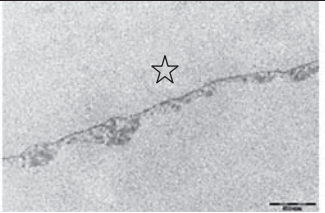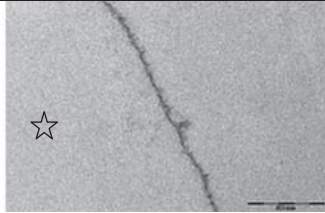
|	pH 2	pH 2.3	pH 3
3-min pellicle	X X ☆	☆	☆
3-min pellicle 10-min rinse with safflower oil	no pellicle detectable	☆	☆
120-min pellicle	☆	☆	☆
3-min pellicle, 10-min rinse with safflower oil followed by 107-min pellicle formation	☆	☆	☆

Fig. 6. Acid etching with HCl for 1 min destroyed the pellicle structure in a time- and pH-dependent manner. No protective effect of the previously performed oil rinsing was visible. Sometimes, the pellicle modified by safflower oil rinsing was degraded in a more pronounced manner than the physiological pellicle (without rinse). At pH 2, acid etching caused considerable destruction of the physiological 3-min pellicle (without rinse) and induced extensive erosion refilled with adsorbed proteins (×). Following oil rinsing, no residual pellicle structures were detectable after acid etching at pH 2 in many cases. Note that the enamel was removed during the preparation of the samples, the former enamel sites are marked with asterisks.

In general, the present study showed that rinses with safflower altered calcium and phosphate loss as compared with non-rinsed pellicle samples; in many cases an enhanced mineral loss was recorded. The electron microscopic analysis, though based on 3-min pellicles in contrast to the 1-min specimens adopted in the calcium and phosphate assay, offers an explanation for this phenomenon. In this context, it should be noted that previous investigations on the ultrastructure of the pellicle clearly indicated that the 1- and the 3-min pellicle are virtually identical [Hannig, 1999, 2002]. The TEM images clearly indicate that the application of safflower oil did not retard pellicle disintegration under acidic conditions, though accumulation of lipid vesicles at the pellicle sur-

Fig. 7. Model for the initial stage of pellicle formation. Pellicle precursor proteins interact with a layer of calcium and phosphate ions. In the presence of saliva, the calcium ions of the enamel structure have a stronger tendency to dissolve than the phosphate ions. This leads to a negative charge and results in a layered arrangement of the ions [Hay et al., 1982; Moreno et al., 1982; Hannig and Joiner, 2006]. This will have an impact on the kinetics of mineral loss during the initial phase of an erosive attack. Figure from Hannig and Joiner [2006], modified.
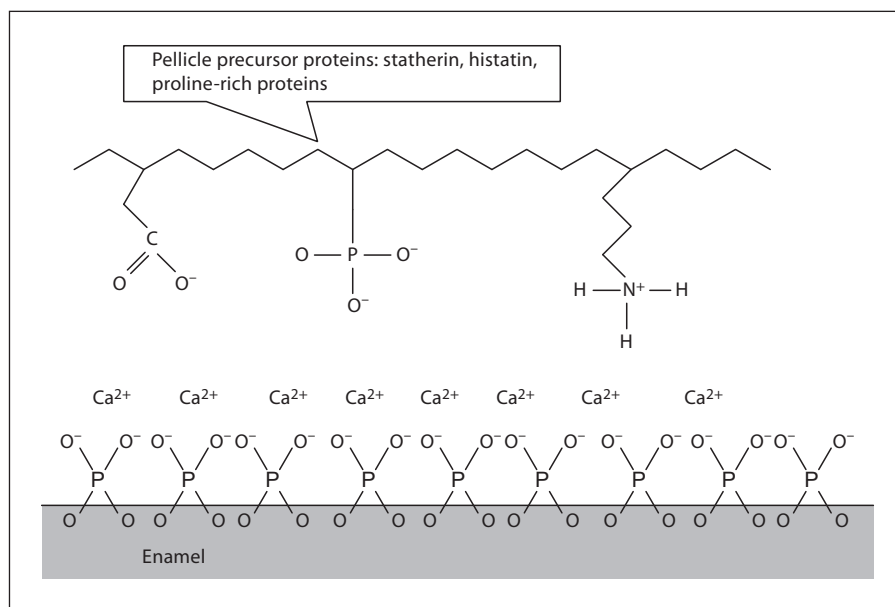
face and an altered ultrastructure were observed after rinsing with safflower oil. The adsorbed lipid micelles caused a scattered accumulation of lipids on the pellicle surface, but virtually no substantial integration into the pellicle. Apparently, the structure of the 120-min pellicle after initial rinsing with safflower oil was even of lower tenacity and loosened density in some cases. Several mechanisms may account for these observations. On the one hand, limited accumulation of the lipophilic molecules from the safflower oil in the pellicle layer is conceivable due to the high selectivity of pellicle formation, as indicated by the loosely adsorbed lipid micelles [Hannig and Joiner, 2006]. On the other hand, the abundant lipids of the safflower oil may even deplete relevant lipophilic molecules from the pellicle, impairing its protective properties [Hannig et al., 2012]. Taking together the present findings, the rinse with safflower oil certainly modulates the ultrastructure and the properties of the acquired pellicle, especially with respect to demineralization processes, but does not improve its protective properties. Interestingly, rinses with edible oils have also no impact on the initial bacterial colonization of enamel surfaces as observed in situ over 8 h [Hannig et al., 2012]. Thus, the relevance of edible oils in preventive dentistry seems to be limited with respect to dental erosion or caries, respectively.

Possibly other lipids with higher affinity to the pellicle structure are more appropriate than trigylcerides, mak-

ing up the most relevant fraction of the safflower oil. Amphiphilic lipids such as phospholipids might be more suitable for sustainable integration into the pellicle structure and effective protective modification of pellicle ultrastructure [Slomiany et al., 1990; Hannig and Joiner, 2006; Hannig et al., 2012]. The protective properties could also be optimized by tanning agents such as polyphenols rather than by lipids [Joiner et al., 2003, 2004].

In the present study, erosive calcium and phosphate loss from enamel samples with an in situ formed pellicle depended predominantly on the pH of the acid. This is in line with the results of a previous study on erosive mineral loss from specimens without pellicle. This previous study was also based on photometric measurement of calcium and phosphate release at pH 2, 2.3 and 3 [Hannig et al., 2005]. During short-term exposure to solutions of pH 3, the physiological in situ pellicle diminished erosive mineral loss clearly, but did not offer complete protection; at lower pH, the protective effect is less pronounced. This corresponds to the findings of an in situ study based on the determination of microhardness of enamel slabs after consumption of different erosive beverages [Hannig et al., 2009]. Fast drinking of orange juice had no erosive effect, whereas Coke light and Sprite light caused a significant reduction of microhardness, meaning effective and comprehensive protection only against short and moderate erosive attacks due to pellicle and saliva [Hannig et al., 2009].

Generally, in contrast to previous postulates, the in situ pellicle layer has limited potential to protect the underlying enamel surface against erosion by hydrochloric acid or other erosive agents. The in situ formed pellicle acts mainly as an ion-permeable network, rather than a diffusion barrier [Hannig and Joiner, 2006]. Differing effects of the pellicle and of the modified pellicle on calcium and phosphate release were observed in the present study. The model for pellicle formation and for arrangement of calcium and phosphate ions at the tooth surface offers a possible explanation for the different behavior of calcium and phosphate ions (fig. 7) [Hannig and Joiner, 2006]. In the presence of saliva, the calcium ions of enamel have a more pronounced tendency than the phosphate ions to dissolve; this results in a double layer of ions at the enamel surface [Hay et al., 1982; Moreno et al., 1982; Hannig and Joiner, 2006]. The layer of calcium ions that are directly interacting with the pellicle precursor proteins of the basal layer is more prone to loss and might affect the solubility of the respective ions. Furthermore, the ionic radius of calcium (Ca^{2+}) amounts to 0.106 nm, the negatively charged phosphate ions and hydrogen phosphate ions have a greater diameter. Besides the charge of the ions, their size might have an impact on the kinetics of demineralization and on the retention in the physiological as well as in the modified pellicle layer after application of safflower oil. This applies also for the interaction with the pellicle proteins and their functional groups at different pH values. A pH-dependent modification of ion-binding pellicle proteins offers also an explanation for the unexpected, inhomogeneous effect of the pH on calcium and phosphate release through pellicles with and without application of oil. The application of the oil might modulate the interaction of the ions with these functional groups. This could be the reason for the increased phosphate release at pH 2.3 following oil treatment.

Conclusions

(1) During short-term exposure to acidic agents, the physiological pellicle offers protection against erosive mineral loss in a pH-dependent manner.

(2) The pellicle has to be regarded as a semi-permeable barrier.

(3) Rinsing with safflower oil modulates the ultrastructure of the pellicle and the passage of calcium and phosphate ions through the pellicle layer, but impairs protection against HCl.

(4) Erosive processes at the tooth surface are rather complex and should be investigated with a combination of several methods at very short time intervals.

Disclosure Statement

The authors declare that they have no conflict of interest.

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