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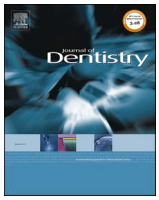

Erosion protective properties of the enamel pellicle in-situ

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ABSTRACT

Objectives: Previous studies on short- and long-term pellicles showed that the enamel pellicle provides partial protection against erosion. The aim of the present study was to investigate the protective properties of clinically relevant pellicles formed within 2 to 24 h. The hypothesis was that factors such as pellicle formation time, intraoral location, and acidic challenge severity would not influence the erosion-protective properties of the pellicle.

Methods: Six subjects participated in the study. Bovine enamel specimens were prepared and intraorally exposed at buccal or palatal sites for 2, 6, 12, and 24 h to allow pellicle formation, followed by erosion using 0.1 % or 1 % citric acid. Calcium release and surface microhardness were measured, and specimens were analysed using scanning and transmission electron microscopy. Quantitative data were statistically analysed with three-way ANOVA and Tuckey's multiple comparison test ($p = 0.05$).

Results: Pellicle formation time and intraoral location did not significantly influence the erosion-protective properties of the pellicle, while citric acid concentration significantly affected enamel erosion. The pellicle thickness increased with longer formation times and on buccal sites, but decreased or was entirely removed following treatment with 0.1 % or 1 % citric acid, respectively. The enamel surface exhibited a characteristic erosion pattern.

Conclusions: This study underscores the importance of investigating pellicle properties within the critical 2- to 24-h timeframe and highlights the significance of pellicle thickness in acid resistance.

Clinical significance: These findings provide valuable insights into the factors influencing the protective properties of enamel pellicles and could guide preventive measures in dental practice.

1. Introduction

Changes in consumption patterns and intensive marketing resulted in an increase in dental erosion [1]. Defined as the loss of dental hard tissue trough acids and/or chelation and without bacterial involvement [2], dental erosion poses a challenge in oral health management. Among various factors affecting erosive tooth wear, saliva plays an important protective role. Saliva not only neutralizes and removes acids from the oral cavity but also supplies minerals and proteins that adhere to the dental surface to form the protective pellicle [3].

Pellicle formation is initiated by the adsorption of precursor proteins to the dental surface through ionic interactions. Further salivary proteins and protein aggregates adhere to this basal layer, which appears as a thin electron-dense layer in transmission electron microscopy. They bind via hydrophobic interactions and Van der Waals interactions, forming what appears as a loose outer layer [4]. Pellicle formation is a selective and highly individual process that determines its protective potential against erosion [5]. The structure of the pellicle varies depending on its intraoral location and the duration of pellicle formation. For instance, while the pellicle on buccal sites undergoes a noticeable thickening over time, characterized by a globular-granular outer layer, the palatal pellicle maintains a thinner granular outer layer [4].

The pellicle is considered a semi-permeable membrane with erosion-protective properties. It acts as a diffusion barrier for both acids and released ions, and it binds released calcium via calcium-binding proteins [6]. The impact of location and formation time on the pellicle's protective properties is a topic of ongoing discussion, owing to variations in pellicle structure and maturation processes, including protein adsorption and desorption, enzymatic processing, and the prevalence of erosive tooth wear on certain dental surfaces [4,6,7]. While previous studies examined the short- and long-term pellicle with a pellicle formation time of up to 2 h and 7 d, respectively [8-16], investigation of the 2- to 24-h-pellicle is of clinical relevance for nutritional and oral hygiene

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practices.

The aim of the present study was to comprehensively investigate the erosion-protective properties of the pellicle under several conditions, using both quantitative and qualitative analyses, since previous studies predominantly focused on individual conditions and analyses [8,13]. The hypothesis is that pellicle formation time, intraoral location, and the severity of acidic challenge do not influence the erosion-protective properties of the pellicle.

2. Materials and methods

2.1. Subjects

The present study was performed with 6 subjects who gave their written informed consent. The subjects had no active carious lesions, periodontitis, oral mucosal or systemic diseases, did not smoke or take any medication. The study protocol was approved by the Medical Ethics Committee of the Medical Association of Saarland (238/03, 2016).

2.2. Specimens

Enamel specimens were made from the teeth of 2-year-old cattle from the slaughterhouse in Zweibrücken. The bovine teeth were processed into rectangular specimens (5 × 5 × 3 mm) using a circular saw and wet grinding machine (Buehler, Düsseldorf, Germany). For microhardness measurement, specimens were additionally embedded in epoxy resin and ground plane-parallel, while for calcium release measurement, all surfaces but the test surface were covered by nail polish. After specimens were polished up to 4000 grit using silicon carbide grinding paper (Buehler, Duesseldorf, Germany), they were disinfected for 30 min in 70 % isopropanol, and the smear layer was removed by ultrasonication for 10 min in 3 % sodium hypochlorite. Finally, specimens were rehydrated in sterile water and fixed to individual upper splints made of methacrylate (Scheu Dental, Iserlohn, Germany) using silicone impression material (Coltène/Whaledent, Langenau, Germany) (Fig. 1).

2.3. In-situ trial

The splints were inserted into the oral cavity for durations of 2, 6, 12, or 24 h to enable pellicle formation. They were inserted at 9am for durations of 2, 6, and 24 h, and at 7pm for a duration of 12 h, with each subject undergoing all time periods. For intraoral exposure times of 12 or 24 h, the splints were temporarily removed and stored in a moist chamber to allow for food intake. To prevent biofilm formation, specimens were cleaned with calibrated brushing movements using a standard toothbrush (Oral B 40, Gilette, Kronberg, Germany). Therefore, subjects were trained to clean the specimens with five unidirectional movements from apical to occlusal at a loading weight of 50 g. Following pellicle formation, the specimens were removed, salivary residues were rinsed off with sterile water, and then the specimens were subjected to erosion with 1 ml of 0.1 % (pH 2.63) or 1 % (pH 2.09) citric acid (Merck, Darmstadt, Germany) under agitation (600 min^{-1}). Specimens without erosion served as control (electron microscopy only). Finally, specimens or erosion solutions were prepared for subsequent analyses.

2.4. Calcium release

The surface area of specimens for calcium release measurement (n = 192, in total) was measured with analySIS (Soft Imaging Systems, Muenster, Germany). After erosion, the citric acid solution was mixed with lanthanum chloride to eliminate the interference of other ions. The solution was atomized at 2800 °C using a flame atomizer. The calcium concentration was measured at 422.7 nm using atomic absorption spectroscopy (Unicam Solar 969, Unicam, Offenbach, Germany) and normalized to the surface area of specimens, expressed in µg/mm^2.

2.5. Surface microhardness

Specimens for measurement of surface microhardness (n = 96, in total) were examined before intraoral exposure and after erosion using the universal hardness tester Fisherscope H100 (Helmut Fischer, Sindelfingen, Germany). The mean Vickers microhardness value was determined after four indentations at a loading weight of 100 g for 10 s, the hardness loss was calculated and given in ΔHV.

2.6. Statistics

Statistical analysis was performed with GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA). Data of calcium release and surface microhardness measurement were tested for normal distribution using Shapiro-Wilk test. Differences between the factors pellicle formation time, location of specimens, and concentration of citric acid, were tested

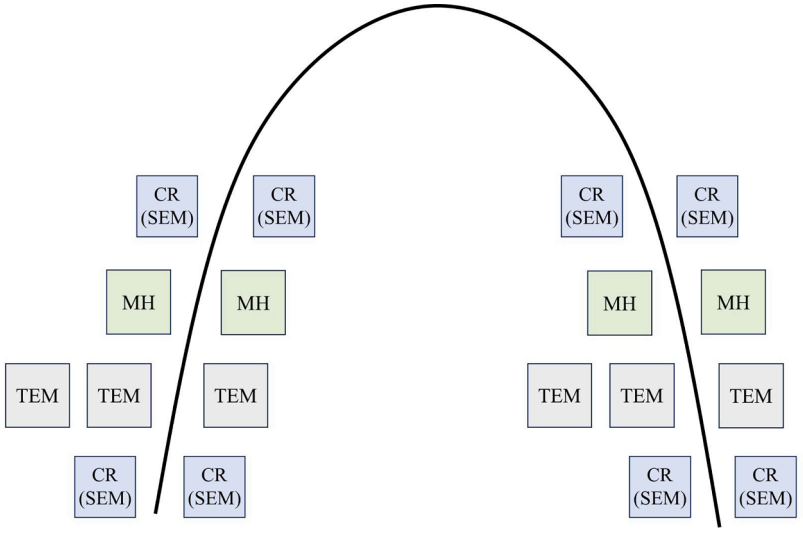

Fig. 1. Schematic illustration of specimens for calcium release measurement (CR), scanning electron microscopy (SEM), microhardness measurement (MH) and transmission electron microscopy (TEM) fixed on individual upper splints. The specimens were positioned on both the palatal (inside) and buccal (outside) sites of the dental arch.

with 3-way ANOVA and Tukey's multiple comparisons test ($p = 0.05$).

2.7. Scanning electron microscopy

Randomly selected specimens of the calcium release measurement were additionally investigated by scanning electron microscopy ($n = 48$, in total). The pellicle was removed by ultrasonication in 3 % sodium hypochlorite for 15 min. Afterwards, specimens were air-dried, coated with gold and examined for erosion patterns using the scanning electron microscope XL 30 ESEM FEG (FEI Company, Eindhoven, The Netherlands).

2.8. Transmission electron microscopy

Specimens for transmission electron microscopy ($n = 144$, in total) were fixed for 2 h in a fixing solution containing 1 % glutaraldehyde and 1 % paraformaldehyde in phosphate buffer and post-fixed in 2 % osmium tetroxide (Chempur, Karlsruhe, Germany). After drying in an ascending ethanol series and propylene oxide, specimens were embedded in araldite (Serva, Heidelberg, Germany), the enamel was dissolved with 0,1 N hydrochloric acid and the samples counter-embedded with araldite. Ultrathin sections were cut with an ultramicrotome (Reichert, Benzheim, Germany) and diamond knife (Microstar Technologies, Huntsville, AL, USA), and mounted on pioloform-coated cooper grids (Plano, Wetzlar, Germany). The sections were contrasted with uranyl acetate and lead citrate (Science Services, Munich, Germany). Finally, the ultrastructure of the pellicle was investigated using the transmission electron microscope TEM 109 (Carl Zeiss, Oberkochen, Germany).

3. Results

3.1. Quantitative results

Erosion of enamel specimens with citric acid resulted in calcium release and surface microhardness loss (Tables 1 and 2). While 1 % citric acid led to significantly more calcium release than 0.1 % citric acid, the location of specimens within the oral cavity and pellicle formation time had no significant impact. The microhardness loss was not significantly altered by any of the three factors, but there tended to be a greater loss of surface microhardness after 1 % citric acid than with 0.1 % citric acid.

The same uppercase letters in a row indicate significant differences at different concentrations of citric acid. The same lowercase letters in a column indicate significant differences for different groups of pellicle formation times ($p = 0.05$).

3.2. Qualitative results

The ultrastructure of the pellicle was characterized by a basal and outer layer (Fig. 2). The thin basal layer was electron dense and homogenous, while the heterogenous outer layer consisted of globular and granular parts. The thickness of the outer layer depended on the location of specimens in the oral cavity and pellicle formation time, with the thickest pellicle formed on buccal specimens after 24 h of intraoral exposure. When specimens were eroded with 0.1 % citric acid, the thickness of the outer layer was reduced, and the continuity of the basal layer was interrupted. After erosion with 1 % citric acid, a pellicle could no longer be detected. After removal of the pellicle for the SEM analysis, the micrographs show an enamel surface with a typical erosion pattern (Fig. 3). The central and peripheral parts of the enamel prisms were partially dissolved. The erosion patterns of buccal and palatal specimens were similar, but the prisms were less dissolved the longer specimens were exposed to the oral cavity.

Table 1
Means and standard deviations (SD) of calcium release (µg/mm^2).

Pellicle formation time	Location	0.1 % citric acid	1 % citric acid
2 h	buccal	0.453 (0.154) ^{A}	1.89 (0.444) ^{A}
	palatal	0.59 (0.126)	1.854 (0.66)
6 h	buccal	0.431 (0.198) ^{A}	2.168 (0.872) $^{A, a}$
	palatal	0.562 (0.19) ^{A}	2.544 (0.866) $^{A, b}$
12 h	buccal	0.193 (0.093) ^{A}	1.275 (0.442) ^{A}
	palatal	0.367 (0.116) ^{A}	1.464 (0.0469) $^{A, b}$
24 h	buccal	0.201 (0.196)	1.012 (0.606) ^{a}
	palatal	0.372 (0.199) ^{A}	1.75 (0.962) ^{A}

Table 2
Means and standard deviations (SD) of surface microhardness loss (ΔHV).

Pellicle formation time	Location	0.1 % citric acid	1 % citric acid
2 h	buccal	40.12 (38.6)	76.7 (28.05)
	palatal	30.82 (38.71)	62.43 (27.52)
6 h	buccal	51.72 (42.3)	111.11 (14.9)
	palatal	45.54 (31.17)	99.69 (26.21)
12 h	buccal	1.78 (40.74)	39.44 (27.37)
	palatal	18.73 (21.66)	46.8 (41.47)
24 h	buccal	−1.97 (25.49)	69.89 (58.36)
	palatal	35.61 (38.84)	58.17 (44.31)

There were no significant differences at different concentrations of citric acid (row) or groups of pellicle formation times (column) ($p = 0.05$).

4. Discussion

The findings of the present study demonstrate the impact of pellicle formation time, location within the oral cavity, and citric acid concentration on the enamel pellicle's potential to protect against erosion. The results emphasize that among all the factors investigated, only the concentration of citric acid significantly influenced the extent of enamel erosion.

Enamel is less susceptible to erosion when covered by a pellicle [8-11,15-17], which acts as a semi-permeable membrane, reducing acid diffusion and ion release. This occurs through its function as a diffusion barrier and via calcium-binding proteins present in the pellicle [6]. However, the impact of intraoral localization and pellicle formation time remains unclear. Depending on the locally available saliva, a thin granular pellicle layer forms at palatal oral sites and a thicker globular-granular pellicle layer forms on buccal surfaces [9,18], with the globular components attributed to protein aggregates from the parotid gland [19]. In agreement with other studies, no site-specific differences were found regarding the erosion protective properties of the enamel pellicle [13,15,17]. This finding supports the hypothesis that the protection is mainly due to the electron dense basal layer, which forms rapidly on enamel irrespective of the intraoral location [18]. In contrast, the study by Amaechi et al. (1999) found an inverse relationship between pellicle thickness and the degree of erosion [9]. This may be attributed to the fact that after an erosive challenge, thicker pellicle layers are only reduced, while thinner layers are completely lost and thus unable to provide protection against erosion. The present results suggest that pellicle thickness primarily affects acid resistance rather than overall protective properties.

Pellicle formation time, in line with most studies, did not significantly influence the erosion-protective properties of the pellicle in the present study [8,16,17]. However, a more recent in-situ study revealed that the pellicle provides significantly better protection compared to a pellicle-free control, but only after 2 h of pellicle formation [13]. The authors suggested that pellicle maturation plays a role when mechanical interferences are absent. In their study, bovine enamel specimens were shielded from the mechanical influences of oral soft tissues by an orthodontic wire [13], a scenario that rarely occurs in clinical practice. Although pellicle formation time appears to have no effect on the erosion-protective properties of the pellicle under clinical conditions, it does impact the acid resistance of the pellicle itself. Consistent with the study by Hannig et al. [8], the dissolution and detachment of the 2-h

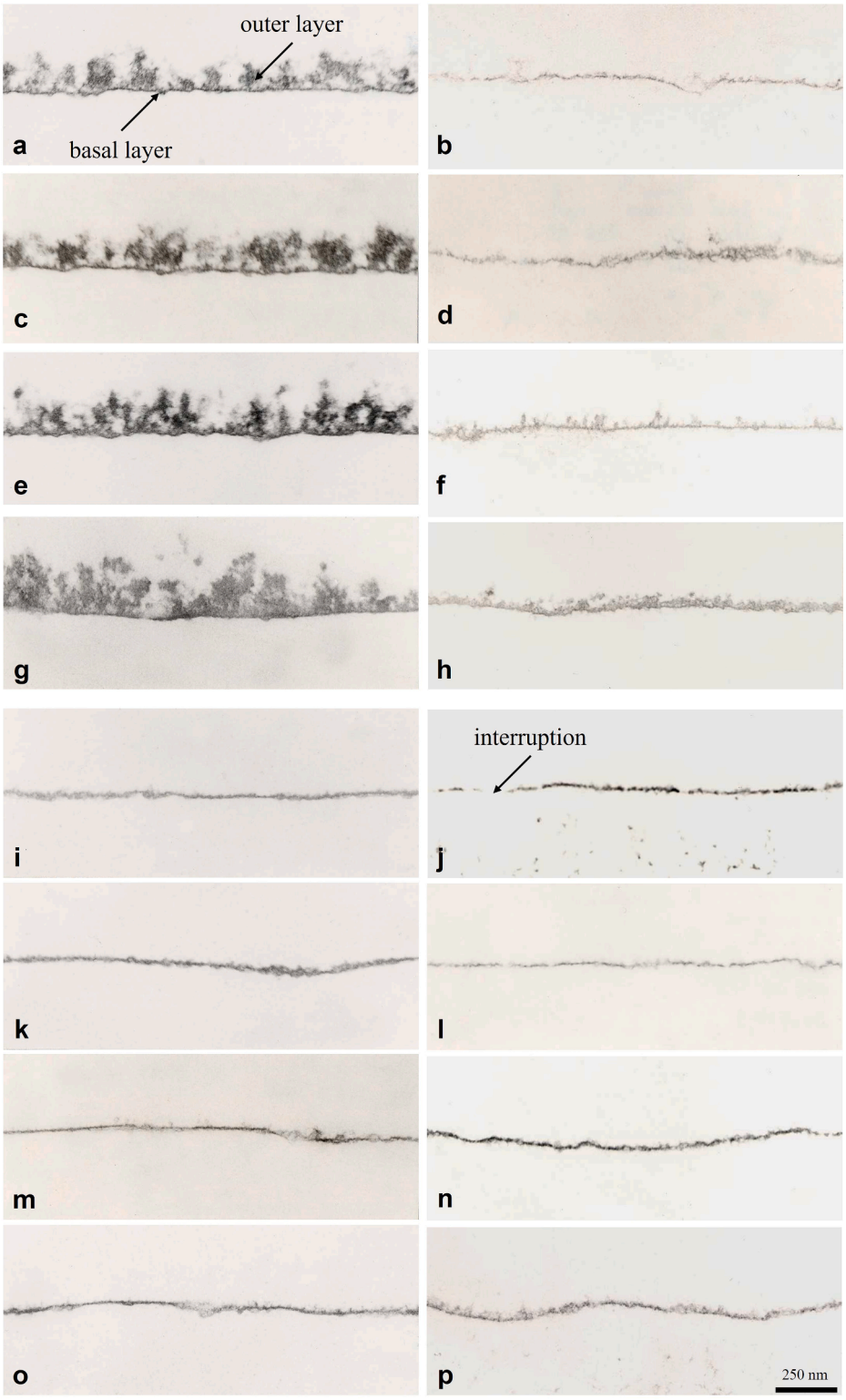

Fig. 2. Representative transmission electron micrographs of the enamel pellicle formed on buccal (a-h) and palatal (i-p) specimens for 2 h (a, b, i, j), 6 h (c, d, k, l), 12 h (e, f, m, n) and 24 h (g, h, o, p) in-situ. Pellicle-covered specimens were eroded with 0.1 % citric acid (b, d, f, h, j, l, n, p), 1 % citric acid (not shown), or stayed untreated and served as controls (a, c, e, g, I, k, m, o). The enamel was dissolved prior to sectioning (bottom site). Original magnification: 30,000-fold.

pellicle were observed more frequently compared to older pellicles. With prolonged or severe erosive challenge, the pellicle and thus its erosion-protective properties are lost. In the present study, the pellicle was no longer detectable after treatment with 1 % citric acid, and significantly higher calcium release and microhardness loss were measured. The exact relationship between the ultrastructure of the pellicle and its acid resistance requires further investigation.

The qualitative and quantitative methods used are established in identifying early erosion. In addition to SEM, which enables a qualitative evaluation of erosion through etching patterns and exposure of

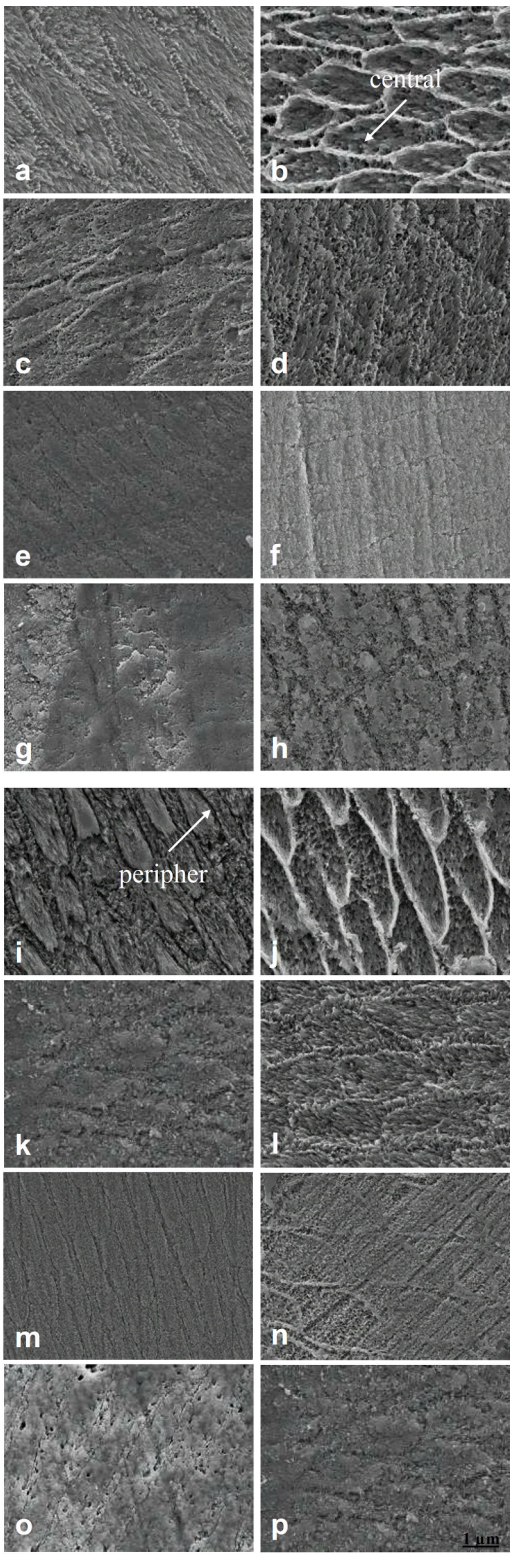

Fig. 3. Representative scanning electron micrographs of the enamel surface. Enamel specimens were exposed to the oral cavity for 2 h (a, b, i, j), 6 h (c, d, k, l), 12 h (e, f, m, n) and 24 h (g, h, o, p) at buccal (a-h) and palatal (i-p) sites. After erosion with 0.1 % (b, d, f, h, j, l, n, p) or 1 % citric acid, the pellicle was intentionally removed, revealing the typical erosion pattern with partially dissolved central and peripheral parts of the enamel prisms. Original magnification: 5000-fold.

enamel prisms, measuring calcium release and surface microhardness loss offers direct and indirect quantitative analysis, respectively. Calcium release measurement allows for highly selective detection of calcium released during erosion, while microhardness measurements provide insights into acid-induced softening of the enamel surface [20]. The results of the microhardness measurements should be interpreted with caution, as the pellicle was not removed before the analysis. The hardness of the pellicle itself is not yet known, which might affect the accuracy of the measurements. In contrast to in vitro conditions, pellicle formation in this study was conducted under oral conditions, which better represent the clinical scenario. Pellicle formation in the oral cavity is influenced by several factors, including selective protein adsorption, enzymatic protein degradation, protein-protein interactions, constant salivary flow, and contact with the oral mucosa [21, 22]. However, the pellicles analysed at 12 and 24 h should be considered toothbrushing-modified, as the specimens were cleaned with controlled brush strokes to inhibit biofilm formation and simulate routine oral hygiene. Another limitation is the performance of erosion ex-vivo, which means that the protective properties of saliva, such as dilution, buffering, clearance, and provision of minerals, were not considered [3]. By applying acids outside the oral cavity, more subjects could be motivated to participate. Nevertheless, it was difficult to recruit a sufficient number of subjects, and the sample size was based on comparable studies. Consequently, the statistical analysis is underpowered. In this study, bovine teeth were used as substitutes for human teeth. A recent study found that incubation in hydrochloric acid released significantly more calcium from bovine enamel than from human enamel, attributed to post-eruptive maturation of human teeth. However, this difference disappeared in the presence of a 30-min pellicle, which exhibited a similar structure on bovine and human enamel. Bovine enamel was chosen for practical reasons. They are large enough to produce several specimens, are available in sufficient quantity and quality, and exhibit high homogeneity due to constant nutrition and slaughter age [23].

5. Conclusions

In summary, the present study reveals how pellicle formation and enamel protection from erosion are interconnected. Although pellicle formation time and location within the oral cavity did not significantly affect the erosion-protective properties of the pellicle, the concentration of citric acid had a notable impact on enamel erosion. Thicker pellicle layers showed better resistance against acids, highlighting their role in frequent or severe erosive challenges. The present study demonstrates the importance of studying pellicle dynamics within the 2- to 24-h timeframe relevant to clinical settings and emphasizes the significance of pellicle thickness in resisting acids. These findings provide more insights into the understanding of dental erosion mechanisms and could guide preventive measures in dental practice.

CRediT authorship contribution statement

Anton Schestakow: Writing – original draft, Visualization, Formal analysis. **Björn Echterhoff:** Writing – review & editing, Investigation. **Matthias Hannig:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] D. Gambon, H. Brand, E. Veerman, Dental erosion in the 21st century: what is happening to nutritional habits and lifestyle in our society? Br. Dent. J. 213 (2) (2012) 55–57, https://doi.org/10.1038/sj.bdj.2012.613.

[2] T. Imfeld, Dental erosion. Definition, classification and links, Eur. J. Oral Sci. 104 (2) (1996) 151–155, https://doi.org/10.1111/j.1600-0722.1996.tb00063.x.

[3] M.A.R. Buzalaf, A.R. Hannas, M.T. Kato, Saliva and dental erosion, J. Appl. Oral Sci. 20 (2012) 493–502, https://doi.org/10.1590/S1678-77572012000500001.

[4] M. Hannig, A. Joiner, The structure, function and properties of the acquired pellicle, Monogr. Oral Sci. 19 (2006) 29, https://doi.org/10.1159/000090585.

[5] T. Martini, D. Rios, L.P.S. Cassiano, C.M.d.S. Silva, E.A. Taira, T.M.S. Ventura, H.A. B.S. Pereira, A.C. Magalhães, T.S. Carvalho, T. Baumann, A. Lussi, R.B. Oliveira, R. G. Palma-Dibb, M.A.R. Buzalaf, Proteomics of acquired pellicle in gastroesophageal reflux disease patients with or without erosive tooth wear, J. Dent. 81 (2019) 64–69, https://doi.org/10.1016/j.jdent.2018.12.007.

[6] M. Hannig, C. Hannig, The pellicle and erosion, in: A. Lussi, C. Ganss (Eds.), Erosive Tooth Wear, Karger, Monographs in Oral Science, 2014, pp. 206–214.

[7] T. Jaeggi, A. Lussi, Prevalence, incidence and distribution of erosion, in: G. M. Whitford (Ed.), Dental Erosion, Karger, Monographs in Oral Science, 2006, pp. 44–65.

[8] M. Hannig, N.J. Hess, W. Hoth-Hannig, M. De Vrese, Influence of salivary pellicle formation time on enamel demineralization–an in situ pilot study, Clin. Oral Investig. 7 (3) (2003) 158–161, https://doi.org/10.1007/s00784-003-0219-2.

[9] B.T. Amaechi, S.M. Higham, W.M. Edgar, A. Milosevic, Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion, J. Dent. Res. 78 (12) (1999) 1821–1828, https://doi.org/10.1177/00220345990780120901.

[10] R.V. Moazzez, R.S. Austin, M. Rojas-Serrano, G. Carpenter, E. Cotroneo, G. Proctor, L. Zaidel, D.W. Bartlett, Comparison of the possible protective effect of the salivary pellicle of individuals with and without erosion, Caries Res. 48 (1) (2014) 57–62, https://doi.org/10.1159/000352042.

[11] A. Wiegand, S. Bliggenstorfer, A.C. Magalhaes, B. Sener, T. Attin, Impact of the in situ formed salivary pellicle on enamel and dentine erosion induced by different acids, Acta Odontol. Scand. 66 (4) (2008) 225–230, https://doi.org/10.1080/00016350802183401.

[12] A.P. Boteon, N.M. Dos Santos, L. Lamana, I.M.B. Rosa, C.C.L. Di Leone, R. A. Caracho, T.S. Carvalho, H.M. Honório, D. Rios, Erosion-inhibiting and enamel rehardening effects of different types of saliva, Arch. Oral Biol. 154 (2023) 105755, https://doi.org/10.1016/j.archoralbio.2023.105755.

[13] F.L. Mendonça, M.C. Jordão, F.Q. Ionta, M.A.R. Buzalaf, H.M. Honório, L. Wang, D. Rios, In situ effect of enamel salivary exposure time and type of intraoral appliance before an erosive challenge, Clin. Oral Investig. 21 (8) (2017) 2465–2471, https://doi.org/10.1007/s00784-016-2043-5.

[14] Y. Nekrashevych, M. Hannig, L. Stosser, Assessment of enamel erosion and protective effect of salivary pellicle by surface roughness analysis and scanning electron microscopy, Oral Health Prev. Dent. 2 (1) (2004) 5–11.

[15] M. Hannig, M. Balz, Protective properties of salivary pellicles from two different intraoral sites on enamel erosion, Caries Res. 35 (2) (2001) 142–148, https://doi.org/10.1159/000047446.

[16] M. Hannig, M. Balz, Influence of in vivo formed salivary pellicle on enamel erosion, Caries Res. 33 (5) (1999) 372–379, https://doi.org/10.1159/000016536.

[17] M. Hannig, M. Fiebiger, M. Guntzer, A. Dobert, R. Zimehl, Y. Nekrashevych, Protective effect of the in situ formed short-term salivary pellicle, Arch. Oral Biol. 49 (11) (2004) 903–910, https://doi.org/10.1016/j.archoralbio.2004.05.008.

[18] M. Hannig, Ultrastructural investigation of pellicle morphogenesis at two different intraoral sites during a 24-h period, Clin. Oral. Investig. 3 (2) (1999) 88–95, https://doi.org/10.1007/s007840050084.

[19] M. Rykke, G. Smistad, G. Rölla, J. Karlsen, Micelle-like structures in human saliva, Colloids Surf. B. Biointerfaces 4 (1) (1995) 33–44, https://doi.org/10.1016/0927-7765(94)01152-U.

[20] T. Attin, F.J. Wegehaupt, Methods for Assessment of Dental Erosion, in: A. Lussi, C. Ganss (Eds.), Dental Erosion, Karger, Monographs in Oral Science, 2014, pp. 152–172.

[21] Y. Yao, J. Grogan, M. Zehnder, U. Lendenmann, B. Nam, Z. Wu, C.E. Costello, F. G. Oppenheim, Compositional analysis of human acquired enamel pellicle by mass spectrometry, Arch. Oral Biol. 46 (4) (2001) 293–303, https://doi.org/10.1016/s0003-9969(00)00134-5.

[22] V.T. Pelá, J.G.Q. Lunardelli, T.M.O. Ventura, G.D. Camiloti, T. Baumann, T. S. Carvalho, A. Lussi, M.A.R. Buzalaf, Proteomic profiles of the acquired enamel pellicle formed in vitro, in situ, or in vivo, Eur. J. Oral Sci. 128 (6) (2020) 487–494, https://doi.org/10.1111/eos.12744.

[23] S. Hertel, S. Basche, V. Schmidt, C. Staszyk, C. Hannig, T. Sterzenbach, M. Hannig, Erosion behaviour of human, bovine and equine dental hard tissues, Sci. Rep. 13 (1) (2023) 19617, https://doi.org/10.1038/s41598-023-46759-9.