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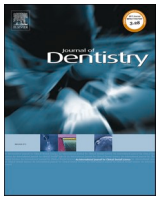

Review article

Impact of modifications on the characteristics of salivary pellicle on dental hard tissue: a scoping review

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ABSTRACT

Objectives: The salivary pellicle regulates interfacial events on dental surfaces and is a promising target for modification to improve dental health. This scoping review systematically examines substances that modify the pellicle, evaluating their potential and identifying knowledge gaps.
Data: This review followed the PRISMA-ScR guidelines. Studies investigating how substances applied during pellicle formation on enamel or dentin affect its structure, composition, or protective function were included.
Sources: A systematic search of Medline was conducted up to March 2024 using predefined terms. Only English-language research articles were included. No hand-searching was performed.
Study selection: A total of 864 records were identified. After title and abstract screening, 110 articles were assessed in full, resulting in 85 eligible studies for data charting. These studies investigated human saliva-derived pellicles on enamel or dentin, comparing their composition, structure, or protective properties with and without modification. Most studies, conducted equally in vitro and in situ, focused on fluorides and metals ($n = 28$), peptides and proteins ($n = 21$), and polyphenols ($n = 18$), while other substances were less frequently examined ($n = 17$). Modifications affected the pellicle's ultrastructure, altered its proteome, or enhanced its protective role against erosion, abrasion, and bacterial adherence.
Conclusions: Despite numerous studies and distinct substance classes, findings remain inconclusive and must be interpreted within the context of individual study designs.
Clinical significance: Modifying the pellicle can improve its protective function, presenting a promising approach for preventive dentistry. However, the long-term effects of these substances within the dynamic oral environment remain unclear. This review underscores the need for further research to close existing knowledge gaps and confirm clinical relevance.

1. Introduction

Recent advances in pellicle modification have introduced new strategies to enhance dental health by modifying the pellicle, a thin layer of salivary proteins and macromolecules that covers the tooth surface. Pellicle formation is a highly dynamic, selective, and individual process, eventually progressing to a biofilm as bacteria adhere [1,2]. While the pellicle serves as a substrate for bacterial colonization [3], it also protects teeth against attrition, abrasion, and erosion [4].

Initially, the pellicle forms as a thin, 10-nm basal layer composed of adsorbed precursor proteins, which grows as additional proteins bind through protein-protein interactions. Depending on the availability of local saliva, the pellicle can reach a thickness of approximately 1000 nm. Compared to the electron-dense basal layer, the outer layer of the pellicle is looser in structure and exhibits a globular-granular morphology on buccal surfaces due to protein aggregates from parotid saliva [1,5]. While the basal layer plays a major role in the pellicle's erosion-protective properties [6], the overall thickness may affect its durability during frequent chemical or mechanical challenges, as a thicker pellicle persists longer [7–9]. Specific proteins are also associated with the pellicle's protective properties. The pellicle is composed of several hundred salivary proteins [2,10], including haemoglobin, albumin, and cystatin, which contribute to erosion protection [11,12]. It also serves as a physical barrier that separates sliding surfaces and, together with lubricating proteins such as mucins and statherins, is associated with abrasion resistance [13,14]. In addition to its protective

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functions, the pellicle mediates early biofilm formation by presenting receptors for bacterial adhesins. Species-specific interactions with components such as proline-rich proteins, glucans or mucins influence the initial microbial colonization and shape the composition of the dental biofilm [4]. The structure and composition of the pellicle can be altered by various substances, optimizing its protective properties, for instance, by forming a denser, thicker pellicle and enriching it with protective proteins.

The pellicle offers multiple targets for modification and despite growing interest in pellicle modification, a comprehensive evaluation of the effects of various agents remains lacking. Therefore, this scoping review systematically examines the literature to explore how and by which agents the pellicle and its protective properties can be modified. By identifying research gaps, it highlights potential areas for future investigation and provide insights into how targeted modifications can improve the pellicle's properties, contributing to better oral health.

2. Methods

2.1. Eligibility criteria

The present scoping review was conducted in accordance with the PRISMA-ScR guidelines [15]. The protocol was published post-analysis on the Open Science Framework on 17th January 2025 (https://doi.org/10.17605/OSF.IO/MDPYH). Eligibility criteria were determined based on the research question: First, only pellicles formed on enamel or dentin in the human oral cavity or from human saliva were considered, while other substrates and edentulous subjects, or subjects with salivary gland diseases or on specific medications, were excluded. Second, substances had to be applied during pellicle formation and compared to an appropriate control, rather than being applied to an established biofilm. Third, studies were included only if they investigated the ultrastructure, protective properties (erosion, abrasion, attrition, bacterial adherence), or composition of the pellicle, excluding those that focused solely on fungal adherence. Fourth, only research articles were included; case studies and review articles were excluded.

2.2. Search strategy

The freely accessible database Medline was searched up to March 2024. The search strategy was initially drafted by A.S. and revised through discussions with co-authors. Medline was searched via PubMed using the search terms "(pellicle) AND (structure OR erosion OR abrasion OR attrition OR tooth wear OR bacterial adherence OR composition) AND (research article OR experimental study OR observational study OR in-vitro OR in-situ OR in-vivo) NOT (review article)". Only studies published in English were considered. Hand searching was not conducted. Two reviewers (J.V.F.S. and A.S.) independently screened the titles, abstracts, and full texts. Disagreements between reviewers were resolved through consensus.

2.3. Data charting

All authors designed the data-charting table in Excel. Two reviewers (J.V.F.C. and A.S.) independently extracted the following data from eligible studies: study design (in vitro, in situ, in vivo), pellicle formation (enamel, dentin, initial pellicle formation, saliva), intervention (substance, exposure time, frequence), control, methods (profilometry, calcium release, electron microscopy, others), and conclusions. The included studies were grouped according to the substances investigated. Within each group, the effects of these substances on the pellicle were discussed, with additional literature to contextualize and deepen the interpretation of findings. Both reviewers actively contributed to the synthesis and discussion across all substance groups. In addition, the analytical methods in eligible studies were critically evaluated using a customized bias assessment tool, adapted for this scoping review from the pilot risk of bias tool by Fox et al. (2024) and the RoBDEMAT by Delgado et al. (2022) [16,17].

3. Results

3.1. Study selection

Initially, 864 studies were identified and screened. From these, 110 studies met the eligibility criteria based on the titles and abstracts (Fig. 1). After screening the full texts, 85 eligible studies were included for data charting.

3.2. Summary of the results

The detailed study characteristics are given in Table S1. Half of the studies were conducted in vitro, the other half in situ, with only a few conducted in vivo. Bovine teeth and enamel were used as specimens more often than human teeth and dentin. Pellicles were formed for varying durations and interrupted by treatments, with the timing of pellicle formation before the first treatment summarized in Table S1. The first treatment was applied either before pellicle formation or up to 24 h afterward, most frequently within the first 2 h. Stimulated saliva was predominantly used in vitro studies. Substances were applied for durations ranging from 30 s to 2 h, most commonly for 1–2 min. Pellicles and their protective properties were analyzed based on outcomes such as tissue loss, hardness, morphology of dental hard tissue and pellicles, and proteome analyses. Three major substance classes emerged, fluorides and metals, peptides and proteins, and polyphenols, while other substances were discussed separately due to limited representation.

Fluorides and metals were the most extensively studied substance, included in a total of 28 studies. Of these, 20 focused on its role in dental erosion, 2 examined its effects on biofilm formation, and 1 study investigated both biofilm and erosion. Additionally, 1 study explored fluoride's impact on the pellicle proteome, while 4 studies assessed other aspects, such as stannous fluoride deposition in the pellicle layer, changes in amino acid composition, alterations in pellicle thickness and chemical properties, and the amount of calcium fluoride-like precipitate formed. Most studies were conducted in vitro (17 studies), followed by in situ studies (10), and 1 study that combined in situ and in vivo methods. No studies were performed exclusively in vivo. Regarding analytical methods, profilometry was most frequently used (11 studies), followed by calcium loss measurements (7), transmission electron microscopy (TEM) (4), and scanning electron microscopy (SEM) (3).

Polyphenols were investigated in 19 studies. Of these, 12 focused on erosion, 4 on biofilm, and 2 on both erosion and biofilm. One study explored the effect of polyphenols on the pellicle proteome. In terms of study design, 7 were conducted in vitro, 8 in situ, and 4 combined both methods. No in vivo studies were identified. TEM was the most commonly used analytical method (9 studies), followed by calcium loss (7), profilometry (3), and SEM (1).

Peptides were examined in 21 studies. Among these, 15 focused on erosion, 2 on biofilm, and 3 on proteomic changes. One study by Kensche (2019) specifically investigated whether bovine milk or milk protein isolates could alter the ultrastructure of the in-situ pellicle and potentially influence oral health. Most peptide studies were in vitro (14 studies), followed by in situ (3), in vivo (3), and 1 study combining in vitro and in situ methods. Profilometry was used in 8 studies, calcium loss in 6, TEM in 1, and SEM in 1.

Other substances were investigated in 18 studies. Of these, 9 focused on erosion, 4 on biofilm, 2 on proteomic changes, 1 on both erosion and biofilm, and 2 on saliva-related properties such as adsorption and lubrication. Study designs included 7 in vitro, 7 in situ, 2 in vivo, 1 combining in vitro and in situ methods, and 1 combining in vitro and in vivo. TEM and SEM were the most commonly used methods (6 studies), followed by calcium loss (3) and profilometry (1).

While fluorides, metals and polyphenols generally protected against

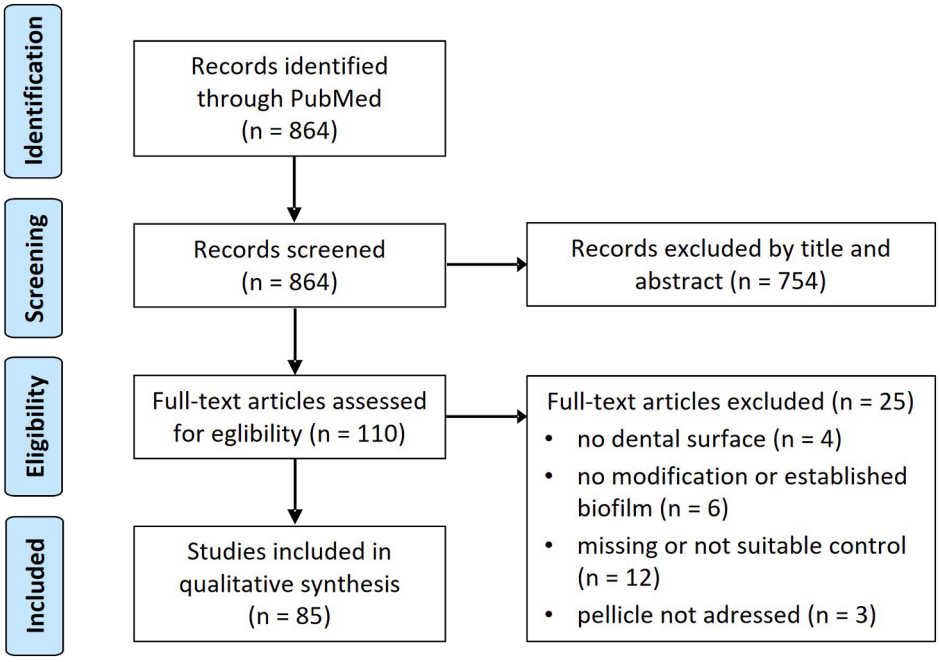

Fig. 1. Flow chart of study selection process.

erosion and reduced bacterial adherence, the effects of peptides and proteins were less consistent, and limited studies on other substances prevented a conclusive overview (Table 1).

3.3. Quality assessment

The detailed results of the quality assessment are provided in Table S2. Approximately half of the studies randomly allocated samples or subjects to treatment groups (39 studies). The relevance of randomization in pellicle research is unclear, as samples are often taken from a pool of teeth with unknown properties prior to analysis. For hardness tests, initial hardness values are typically collected before allocation, ensuring they are evenly distributed with similar mean values across groups. Only a few studies calculated an appropriate sample size (17 studies), raising concerns that the remaining studies may be underpowered. Most studies used bovine specimens (70 studies), which are more homogeneous than human specimens due to standardized feeding regimens and slaughter ages [18,19]. However, in several studies, test and control groups differed in variables beyond the active substance, such as concentration (9 studies), solvents (3), or pH values (9), which could significantly affect outcomes. This issue is pronounced with commercial products, which often vary in numerous other components. While most studies reported inclusion and exclusion criteria for saliva donors or subjects (58 studies), a few did not specify the number of donors (8), increasing the risk of bias, as saliva composition, and therefore pellicle formation, varies between individuals [2]. Using pooled saliva from multiple donors reduces this variability and produces more representative results. in-situ studies, a cross-over design was commonly used. However, if the washout period was not reported (12 studies), there is a higher risk of bias due to potential carry-over effects. Studies examining bacterial adherence or viability often used fluorescence microscopy, a semi-quantitative method prone to bias if ocular grids were not randomized or if analysis was performed by a single, unblinded investigator. As most studies did not report these details, it is difficult to reliably assess the associated risk. Blinding of investigators, particularly in-vitro and in-situ studies, is straightforward to implement and reduces bias, yet it was employed in only a small number of studies (13 studies). Statistical methods were reported in most studies, but many failed to describe how data distribution was analyzed. Decisions regarding parametric or non-parametric tests depend on a prior analysis of normal distribution, which was often not reported (27 studies). Overall, the included studies, based on the modified bias assessment tool, presented some concerns (36 studies) or a high risk of bias (39).

4. Discussion

4.1. Fluorides and metals

Fluorides and other metal salts are not traditionally considered as agents used in pellicle modification; however, they were included in this study due to the ongoing debate regarding their mode of action. Specifically, it remains unclear whether fluorides and other metal salts interact directly with the dental surface to form a calcium fluoride layer, for example, or if they are possibly stored within the pellicle, thereby altering its properties. The interaction of fluorides and tin with dental hard tissue is complex and multifactorial (see [20]).

With regard to pellicle modification, there appears to be a synergistic relationship. Fluorides and their cations, particularly tin, form precipitates on the dental surface that protect it from acids. On the one hand, these precipitates seem to promote protein adsorption [21], thereby altering the ultrastructure of the pellicle. For instance, tin-containing preparations increase the electron density of the basal pellicle layer, while amine fluorides lead to a thicker outer pellicle layer [22,23]. Additionally, it has been suggested that fluorides and tin can cross-link pellicle proteins [24], which, in turn, may enhance the resilience of the pellicle [25]. On the other hand, the pellicle facilitates the precipitation of calcium fluoride [26]. Bioinformatic studies suggest that human proteins, some of which are present in the pellicle [2], may have the ability to bind fluoride [27]. The pellicle, as a biomembrane, could modulate the adsorption, diffusion, and desorption of fluorides, thereby enriching the pellicle with protective precipitates [22,23].

The modification of the pellicle by fluorides leads to changes in its composition, including an enrichment with erosion-protective proteins, such as carbonic anhydrase [24,28,29]. Furthermore, tin-containing precipitates in the basal pellicle layer provide both protection against erosion and shielding the pellicle from acid-induced degradation [22,23]. Some fluoride compounds, such as sodium fluoride, monofluorphosphate, amine fluoride or stannous fluoride, also inhibit

Table 1
Findings as stated in the included studies.

substance classes	agents	modifications	protective properties
fluorides and metals	- sodium fluoride - titanium tetrafluoride - zirconium tetrafluoride - amine fluoride - sodium monofluorophosphate - hafnium tetrafluoride - stannous fluoride and stannous chloride	- alter pellicle ultrastructure - increase outer layer thickness (e.g., with amine fluoride) - increase electron density of basal layer (e.g., with tin) - promote protein adsorption - cross-link pellicle proteins - enrich pellicle with erosion-protective proteins - induce precipitations - modify surface hydrophobicity	- reduce demineralization - promote remineralization - reduce bacterial adherence
peptides and proteins	- caneCPI-5 - statherin-derived peptide - hemoglobin - casein - mucin	- modify protein adsorption - enrich pellicle with erosion-protective proteins	- reduce erosive tooth wear
poly-phenols	- polyphenol rich extracts (grapeseed, cranberry, grapefruit, acai, blueberry, red wine, hop, blackcurrant leaves, *Inula viscosa, Cistus incanus*, gallnut, black and green tea) - epigallocatechin gallate - ellagic acid - tannic acid - theaflavin - resveratrol	- protein aggregation - enrich pellicle with erosion-protective proteins - increase thickness and density - increase chemical resistance - reduce receptor sites for bacteria	- reduce demineralization - reduce bacterial adherence
others	- calcium-based materials (calcium, hydroxyapatite nanoparticles, casein phosphopeptide-amorphous calcium phosphate)	- incorporation of a calcium phosphate reservoir - reduce receptor sites for bacteria	- reduce demineralization - promote remineralization - reduce bacterial adherence
	- lipids (edible oils, vitamin A, vitamin E, silicone oil)	- alter amino acid composition - enrich the pellicle with lipids - loosen pellicle structure	- transient or negligible effects
	- milk	- incorporation of lipid droplets and micelles	- transient or negligible effects
	- chitosan	- protein aggregation - increase thickness - forms acid-resistant and lubricating layer	- reduce demineralization - increase lubrication
	- chlorhexidine	- changes in pellicle proteome - alteration of pellicle structure - reduction of receptor sites for bacteria	- reduce bacterial adherence

bacterial adherence [22,30], and it has been suggested that amine fluorides reduce bacterial adhesion by modifying the hydrophobicity of the pellicle [31].

Overall, fluorides influence both the composition and ultrastructure of the pellicle, which, in addition to their inherent protective properties, may explain their synergistic effects in the presence of the pellicle. However, the molecular-level interactions between fluorides and pellicle proteins remain unclear and warrant further investigation.

4.2. Peptides and proteins

Various peptides, including cystatin-derived and statherin-derived peptides, as well as proteins such as hemoglobin, casein, and mucin, were identified and are discussed in the following paragraph regarding their roles in pellicle modification.

Cystatin, a phosphoprotein found in saliva and the pellicle, was identified as acid-resistant and may contribute to the pellicle's erosion-protective properties [32]. While it has potential for optimizing the pellicle, the high cost of commercially available human-recombinant cystatin limits its clinical use. To address this issue, natural homologs have been searched, leading to the identification of five sugarcane cystatins, including CaneCPI-5. CaneCPI-5 has demonstrated strong adherence to enamel [33]. Studies investigating CaneCPI-5 consistently report that its application to the pellicle reduces erosive tooth wear in enamel and dentin [33–42]. The observed effects have been attributed to several mechanisms, primarily associated with changes in the pellicle's proteome. CaneCPI-5 adheres strongly to enamel, likely due to interactions between its N-terminal amino acids and hydroxyapatite [32], resulting in reduced surface free energy and an increased number of acid-resistant proteins in the pellicle [34,43]. Additionally, as a cystatin-derived peptide, CaneCPI-5 could inhibit collagenases, which degrade dentin collagen exposed during acidic challenges [38]. However, a recent study found that pellicle modification using CaneCPI-5 did not protect enamel from hydrochloric acid erosion, representing intrinsic erosion caused by gastric acids, possibly due to the single application of CaneCPI-5 before pellicle formation and a one-time acid exposure [35]. This finding underscores the need for further research to clarify the relationship between the pellicle proteome and its erosion-protective properties.

Statherin, a phosphorylated salivary protein with calcium-binding capacity, is associated with the erosion-protective properties of the pellicle. Epidemiological data indicate that patients with dental erosion exhibit reduced concentrations of statherin in the pellicle. Similarly, pellicle collected in vivo from eroded regions of the same patient showed lower statherin levels compared to non-eroded regions [44]. The negatively charged phosphate residues in statherin's helical N-terminal region facilitate interactions with calcium and hydroxyapatite [45,46]. Furthermore, in vitro studies demonstrate that peptides derived from statherin can reduce enamel demineralization, though at least 15 N-terminal residues are required for this effect [47]. These protective mechanisms may involve the formation of a semi-permeable membrane, modulation of calcium concentration and solution saturation, and stabilization of the hydroxyapatite surface [48]. Studies also show that statherin-derived peptide treatment alters the pellicle proteome, promoting the accumulation of acid-resistant proteins [34]. Another study found that pre-treating polished enamel with statherin-derived peptide

also influenced pellicle proteome, suggesting that statherin forms the basal pellicle layer, which may alter the binding of other precursor proteins and pellicle growth through protein-protein interactions [49]. Despite these promising findings, experimental results on the erosion-protective properties of modified pellicles remain inconsistent. Variations in experimental conditions or the presence of native statherin in saliva may explain why statherin-derived peptides often provide no additional benefit [50]. Moreover, the protective effect of statherin-derived peptides appears highly concentration-dependent, with minor deviations diminishing their efficacy against initial erosion. This lack of a clear dose-response relationship raises concerns about the feasibility of consistently achieving optimal concentrations in clinical settings, limiting their practical application in enamel-protective treatments outside controlled environments [47,51].

Hemoglobin was recently identified in the acquired enamel pellicle, specifically in samples from posterior teeth, as earlier studies focused only on anterior teeth [44]. Elevated hemoglobin levels observed in patients with gastroesophageal reflux disease but without erosion suggest a potential protective role against intrinsic erosion [11]. The included studies of the present review show that pellicle modification with hemoglobin increases erosion-resistant proteins and enhances the pellicle's protective properties [34,35,37]. However, proteomic analyses lack details about the hemoglobin used, and hemoglobin itself was not detected in the modified pellicle [34,35], leaving the mechanism by which hemoglobin alters the pellicle unclear.

Casein and mucin are commonly tested in combination for pellicle modification. Casein, a milk-derived phosphoprotein, is negatively charged at neutral pH and binds to hydroxyapatite [52]. Mucin, a mucus-forming protein found in saliva, is considered an acid-resistant protein of the pellicle [32,53]. Both proteins can be incorporated into the pellicle [52,54], with casein potentially promoting mucin accumulation through protein-protein interactions [55]. Studies modifying pellicles with casein, mucin, or their combination investigated their protective effects against erosion, tooth wear, and bacterial adherence. While one study reported improved erosion protection [55], others found no sufficient improvements [56–60]. This discrepancy may arise because saliva and pellicle already contain mucins, diminishing any additional benefit from their application [53,61].

These findings on pellicle modification with peptides and proteins underscore the need for further research. Although peptides and proteins can form an artificial pellicle and offer some protection against erosion, the tooth surface is naturally covered by a pellicle. As these substances do not consistently improve the pellicle's protective properties, their clinical benefits remain unclear.

4.3. Polyphenols

Polyphenols are secondary plant metabolites that are abundant in nature, comprising a large and heterogeneous group of over several thousand substances. Due to their health-promoting properties, which include anti-inflammatory, antioxidant, and antibacterial effects, polyphenols are gaining increasing popularity in research. Their basic chemical structure includes more than one phenolic ring without any nitrogen-based functional groups in their basic structure, derived from the shikimate or polyketide pathway [62]. They can interact with proteins and are responsible for the astringent sensation in the oral cavity when consuming polyphenolic substances like green tea [63]. While polyphenols can exhibit specific interactions with proteins at an individual level, polyphenol-protein interactions are generally driven by non-specific interactions, where the phenolic functional groups of polyphenols primarily interact with the carbonyl groups of proteins through hydrophobic interactions and hydrogen bonding [64].

Polyphenols enhance the pellicle's ability to protect against erosion and reduce bacterial adherence, both of which depend on the pellicle's structure and composition. Polyphenols modify the pellicle by interacting with proteins both within the pellicle and from saliva. This interaction leads to the formation of protein aggregates in saliva [63,65,66], which contribute to pellicle formation. Similar to the deposition of amylase agglomerates from the parotid gland, which are known to be responsible for the thick, globular structure of the buccal pellicle [1,5], the deposition of these aggregates results in a thicker and denser pellicle. This effect is further enhanced by interactions with proteins already present in the pellicle [67–74]. Given the particular affinity of polyphenols for proline-rich proteins and statherins [64,75], these proteins are also increasingly incorporated into the pellicle [76], with the calcium-binding protein statherin being often credited with contributing to the pellicle's erosion-protective properties [6,11]. Polyphenols themselves are also incorporated into the pellicle [70]. Given that the hydroxyphenyl groups of polyphenols can chelate calcium, they may enhance the pellicle's protective function in a manner similar to statherin [77]. Finally, polyphenols also enhance the resistance of the pellicle to elution by solutions such as phosphate buffer, sodium dodecyl sulfate, and citric acid [70,78,79]. This property becomes increasingly important as the severity of acid attacks intensifies, since the pellicle, along with its protective functions, can be lost during a strong acid exposure [9]. In most erosion studies included in the present systematic review, polyphenols enhanced the protective properties of the pellicle. It remains unclear which specific structural or compositional changes resulting from pellicle modification had the greatest impact, but the improvement is likely due to a combination of multiple factors.

In addition to the antibacterial properties of polyphenols and their effects on established biofilms, which are not considered part of pellicle modification and are therefore not discussed further, treating the pellicle with polyphenols reduces bacterial adherence. Various mechanisms have been proposed, all of which are attributed to protein-polyphenol interactions [67,68,73,80–82]. Bacterial adherence typically occurs through interactions between bacterial adhesins and pellicle proteins or polysaccharides [4]. Polyphenols may, on one hand, mask these receptors or binding sites. However, molecular-level analyses are lacking, as they are difficult to perform due to the large variety of involved compounds. On the other hand, polyphenols are known to inhibit glycosyltransferases in the pellicle, thereby reducing glucan synthesis, which also serves as a receptor for bacterial adherence [83]. Clinical studies are still needed to confirm whether polyphenols effectively inhibit biofilm formation, making an evidence-based conclusion currently unavailable [84].

Overall, polyphenols are promising substances for pellicle modification, and their health-promoting properties, combined with their biocompatibility and renewability as natural compounds, could contribute to both general and dental health.

4.4. Others

4.4.1. Calcium-based materials

Calcium is a component of saliva and dental hard tissues, playing a key role in the formation of the electrostatic double layer on the tooth surface, which is essential for pellicle formation [4]. While saliva is naturally saturated with calcium relative to hydroxyapatite, increasing calcium levels might enhance the pellicle's protective properties. In a study by Zeng et al. (2019), human unstimulated saliva was supplemented with calcium, and pellicles were formed on human enamel for over 20 min. Despite interfering with initial protein adsorption that has limited clinical relevance, calcium contributed to increased pellicle thickness, along with the formation of surface projections. Additionally, the improved mechanical properties suggest better protection against abrasion. The authors suggest that calcium acts as a mediator between negatively charged or chelating functional groups of proteins, promoting their interaction [85]. The extent to which these structural and mechanical changes improve the pellicle's protective properties under oral conditions remains unclear, especially since physicochemical factors, such as salt concentration, can influence protein conformation and interactions. Nonetheless, this study highlights the physiological role

that calcium may play in supporting protein-protein interactions during pellicle formation.

Calcium, in the form of hydroxyapatite, has been also widely researched as a biocompatible material for various dental applications [86]. Since saliva is already saturated with calcium and phosphate in relation to hydroxyapatite, it is typically applied intraorally in the form of insoluble nanoparticles. The small size of calcium-based nanoparticles allows them to penetrate microdefects more effectively than larger particles, enabling deeper mineral deposition. Their similarity to dental hard tissue supports interaction with salivary proteins, while their high surface area facilitates contact with bacterial cells and biofilms, potentially disrupting adhesion. Unlike calcium phosphate solutions, these nanoparticles may also be retained in the pellicle or enamel surface, prolonging their protective effects [86]. Research has shown that hydroxyapatite particles, often forming aggregates, can be deposited in the outer layer of the pellicle without altering its basic morphology. However, over time, these particles gradually desorb [87,88]. In the study by Kensche et al. (2016), no clear improvement in the erosion-protective properties of the pellicle was observed, despite the incorporation of hydroxyapatite particles. However, the authors suggest that these particles may act as a calcium phosphate reservoir, potentially contributing to subsequent remineralization [87]. Nevertheless, it remains uncertain to what extent these reservoirs can promote remineralization in an already saturated environment, particularly in the absence of amorphous calcium phosphate stabilizers. The incorporation of hydroxyapatite nanoparticles may block receptors in the pellicle that facilitate bacterial adhesion, thereby inhibiting bacterial adherence, as demonstrated in the studies by Kensche et al. (2017) and partially in Hannig et al. (2013). However, since the hydroxyapatite nanoparticles were applied intraorally, the observed effect could also result from interactions with bacterial adhesins from planktonic bacteria [88,89]. Therefore, the specific role of pellicle modification in this context has not yet been fully clarified.

Calcium phosphate can be stabilized in a metastable solution by casein, a milk protein discussed in the peptides and proteins section, to form casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [90]. CPP-ACP can act as a reservoir for calcium and phosphate, preventing demineralization and promoting remineralization [91–93]. Recently, CPP-ACP was shown to interact with the pellicle, depositing micelle- and mineral-like structures [94]. In the included studies, CPP-ACP was investigated for its ability to enhance the pellicle's erosion-protective properties and reduce bacterial adherence. While CPP-ACP treatment deposited fine mineral precipitates in the pellicle and improved erosion protection, it was less effective than the positive control fluoride [87]. Furthermore, Grychtol et al. (2014) found that CPP-ACP had no effect on bacterial adherence, likely due to the masking effect of the pellicle, which compensate the ability of CPP-ACP to alter the pellicle's charge and long-range bacterial interactions [95].

4.4.2. Lipids

Similar to polyphenols, lipids represent a broad group of substances that share lipophilic or amphiphilic characteristics. Lipids can be categorized into various, sometimes overlapping, subgroups, including mono-, di-, and triglycerides of fatty acids (fats and oils), waxes, phospholipids, sterols, and fat-soluble vitamins. Given that the pellicle contains a substantial number of phospholipids and triacylglycerols [96,97], which can interact with other lipids [98], several studies have investigated optimizing pellicle properties through lipid incorporation.

Since lipids are lipophilic, they tend to form lipid droplets in water or aqueous environments, such as saliva in the oral cavity. These lipid droplets can accumulate on dental surfaces, with the pellicle serving as a prerequisite for this interaction [98]. However, this interaction is not stable; lipid droplets initially observed in the pellicle shortly after the application of edible oils are no longer detectable after several hours, suggesting desorption [98–100]. Edible oils can alter the composition of the pellicle by enriching it with lipids, which affects pellicle formation, resulting in a more heterogeneous and less dense structure. The lipophilic properties of the incorporated lipids may loosen the pellicle's structure [99,101].

The extent to which lipids can enhance the protective properties of the pellicle remains inconclusive. On the one hand, edible oils did not reduce bacterial adherence to the modified pellicle in the study by Hannig et al. (2013) [99], and most lipids and vitamin A failed to reduce erosion in the studies by Ionta et al. (2016) and Rios et al. (2021). On the other hand, both palm oil and vitamin E were shown to improve erosion-protective properties by interacting with the lipids and albumin in the pellicle, respectively. The authors suggested that edible oils likely reduce the degradation of the basal pellicle layer by erosive substances, thereby better preserving its protective function [102,103]. In contrast, the study by Hannig et al. (2012) demonstrated that the basal layer of a pellicle modified with safflower oil was less resistant to acids, as indicated by interruptions in its continuity following acid exposure. This, combined with structural loosening, led to reduced erosion-protective properties compared to the native pellicle [101].

The variability in the effects of edible oils may be attributed to differences in their composition, including the types of lipids, such as triacylglycerols, diacylglycerols, and free fatty acids, as well as minor lipid components like phospholipids and sterols. Additionally, non-lipid substances such as polyphenols and carotenoids may contribute to their biological activity [104]. However, it is important to note that the palm oil used in both studies was not further classified, despite its composition being dependent on production and processing methods [105].

In addition to edible oils, the effect of silicone oil, a synthetically produced silicone-based oil, on pellicle formation was also investigated in the last century. Despite technical limitations, it was shown that silicone oils are incorporated into the pellicle and alter its amino acid composition, resulting in impaired pellicle formation, though this effect diminished over time. The authors hypothesized that the low surface tension of silicone oils allows their adsorption onto the tooth surface, generating a water-repellent layer [106]. While the extent to which pellicle modification with silicone oil can optimize protective properties has yet to be investigated, it is known that a hydrophobic surface reduces contact with aqueous acid solutions and thus erosion [107], and can also modulate bacterial adherence [108].

While lipids show potential for pellicle modification, the results remain inconsistent and depend on the type of lipid, its composition, and its interaction with the pellicle, warranting further investigation into their precise mechanisms and long-term effects.

4.4.3. Milk

Frequent milk consumption is associated with a lower susceptibility to dental erosion [109,110], although its relationship with the pellicle remains unclear. Beyond its minerals and trace substances, lipids and proteins are the primary components discussed in relation to erosion protection. In this section, the focus is on different types of milk rather than individual constituents, which have already been covered in the sections above.

Milk types vary by fat content and processing methods, such as homogenization, where fat globules are broken into smaller lipid droplets, and ultra-heat treatment, which preserves milk but also alters its proteins and carbohydrates [111]. A study by Nekrashevych et al. (2021) found no effect of low- or high-fat milk on pellicle structure [112]. However, Kensche et al. (2019) observed deposits of lipid droplets and possibly casein micelles, noting that the influence on pellicle formation increased with higher fat content and homogenization. These changes were only evident 60 min after application, and by 120 min, the treated pellicles no longer differed from the native pellicle, likely due to the desorption of these components [113]. In a proteomic study by Cassiano et al. (2018), which examined only human proteins and not milk proteins, whole and fat-free milk were found to influence the pellicle proteome 60 min post-application. Proteins such as statherins were

enriched, while others, like proline-rich proteins, were reduced [114]. It remains unclear whether these changes, like those observed by Kensche et al. (2019), are temporary. In the only study that investigated the erosion-protective properties of a modified pellicle, milk did not result in any significant improvement [112].

Based on the limited literature, milk appears to have minimal relevance for pellicle modification, though it may reduce dental hard tissue loss through other mechanisms, such as acting as an antacid [115].

4.4.4. Chitosan

Chitosan, the deacetylated and water-soluble form of chitin, is a widely studied natural polycationic polymer known for its ability to interact with salivary proteins and the acquired enamel pellicle. These interactions induce protein aggregation, increase pellicle thickness, and impart a positive surface charge [66,116–119]. Depending on its degree of deacetylation and molecular weight, chitosan can be formulated as a solution or gel, often serving as a vehicle for active substances. Despite requiring an acidic pH to dissolve, which may risk demineralization of dental hard tissues [72], its erosion-protective effects vary across experimental models, sometimes offering protection [38], and at other times showing limited efficacy [42].

Mechanistically, chitosan binds electrostatically to negatively charged pellicle components, particularly mucins and glycoproteins, resulting in stable integration and overcharging of the pellicle surface [118]. This binding alters the ultrastructure of the pellicle, increasing its electron density and surface roughness, which has been confirmed through TEM and atomic force microscopy imaging [72,118]. These structural changes may contribute to the sensation of oral astringency but also enhance the pellicle's resilience against erosive and abrasive challenges.

Furthermore, when combined with protective agents such as CaneCPI-5, chitosan gels demonstrate synergistic effects. These formulations not only facilitate the delivery of bioactive proteins but also modulate pellicle composition and improve its protective function against enamel and dentin wear under in situ conditions [38,42]. Chitosan's mucoadhesive properties and its ability to stabilize and modify the pellicle underscore its potential in erosion-preventive strategies, particularly within the concept of acquired pellicle engineering.

4.4.5. Chlorhexidine

Chlorhexidine is often used as a positive control in antibacterial studies and is considered the gold standard for chemical biofilm control. While it is primarily known for its antibacterial effects [120], some of its properties stem from its interaction with the pellicle. Chlorhexidine interacts with salivary and pellicle proteins [121–123], leading to an altered pellicle structure characterized by more globular components and the incorporation of chlorhexidine into the pellicle [124,125].

Treatment of the pellicle with chlorhexidine induces changes in the pellicle proteome, leading to the detection of unique calcium-binding and acid-resistant proteins within the pellicle [76]. However, the extent to which these modifications provide protection against erosion has not yet been investigated. All included studies demonstrated an inhibitory effect of chlorhexidine on bacterial adherence [68,73,88,89,95,99,126]. However, in these studies, participants rinsed with chlorhexidine, making it difficult to differentiate between its effects on planktonic bacteria and those resulting from pellicle modification. It has been suggested that chlorhexidine may block receptors in the pellicle that facilitate bacterial adherence. Nevertheless, an older study found no inhibitory effect of pre-treating the pellicle with chlorhexidine on subsequent bacterial adherence [127]. Thus, the role of pellicle modification with chlorhexidine remains unclear.

4.4.6. Magnesium hydroxide

Some substances, such as magnesium hydroxide and alcohol, were only evaluated in single studies in this review, making their role in pellicle modification speculative. In the study by Passos et al. (2018), a pellicle was formed from human saliva on enamel daily for five days, followed by three daily demineralization cycles using citric acid. The enamel was treated with either non-fluoridated, sodium fluoride or magnesium hydroxide dentifrice. The magnesium-containing dentifrice was as ineffective as the other dentifrices in providing protection against erosion. However, the authors attributed protective properties to magnesium hydroxide likely due to the neutralization of citric acid through the formation of magnesium citrate and other neutralizing intermediates [128]. However, the effect of magnesium hydroxide on pellicle structure or its retention in the pellicle has not yet been studied. Therefore, its role in pellicle modification remains unclear and requires further investigation at both the elemental and ultrastructural levels.

4.4.7. Alcohol

In a study by Zeng et al. (2017), alcohol's influence on pellicle formation was examined. Human saliva was collected after rinsing with water or increasing concentrations of alcohol, and pellicles were formed in vitro for 1 min. Alcohol-stimulated saliva increased pellicle adhesion to enamel but reduced its lubricating properties. Scanning probe micrographs showed a smoother pellicle surface compared to the control. The authors suggested that alcohol alters the tertiary structure of proteins, unfolding them and exposing more functional groups for adhesion to the dental surface, which explains the stronger attachment and structural changes [129]. However, these laboratory conditions do not reflect clinical scenarios, where the dental surface is always covered by a pre-existing pellicle, and opportunities to modify initial protein adsorption are minimal. Still, the ability of alcohol to influence protein structure suggests that future studies should consider the solvent used for lipophilic substances when interpreting results.

4.5. Study designs

Each study design for pellicle research, in vitro, in situ, and in vivo, presents benefits and limitations that should be considered when conducting and interpreting experiments. in vitro designs, the pellicle is formed using collected human saliva applied to specimens. Saliva can be used from one individual for consistency or pooled from multiple donors for representative data [130]. Sterile filtration is commonly used to remove microorganisms, which also eliminates protein aggregates like amylase complexes from the parotid gland, preventing the formation of a typical globular outer layer [5]. Unlike natural conditions, where fresh saliva and proteolytically active proteins are constantly renewed, in vitro experiments often compensate by regularly exchanging saliva or adding protease inhibitors [131]. Despite these limitations, in vitro studies are useful for identifying potential substances for pellicle modification before testing under more complex conditions (supplement).

In situ designs involve pellicle formation on specimens attached intraorally to teeth or appliances, which can limit oral hygiene, nutrition, and comfort. These studies require strict inclusion criteria, such as uniform saliva flow, oral health status, and other factors influencing saliva composition, to minimize variability. Furthermore, specimens of human or animal origin must be sterilized, which may influence results depending on the specimen type and assessment method [132].

In vivo studies involve natural pellicle formation on teeth without the use of specimens, allowing for more realistic conditions. However, analyses are limited primarily to compositional studies, as pellicles must be removed from the teeth for examination. This removal process is challenging, particularly for the basal layer, which plays a critical role in the pellicle's erosion-protective properties [6,133]. Many proteomic studies on pellicles and erosion-protective proteins likely fail to completely remove the basal layer, limiting the interpretation of their results. Additionally, a recent study confirmed that the composition of pellicles differs depending on the study design [134].

4.6. Limitations

This scoping review applied strict eligibility criteria due to the large number of studies available, which represents its greatest limitation but still provides an overview of potential substances for pellicle modification. Using a single database and the absence of handsearching may have further limited the comprehensiveness of the search, potentially excluding studies from other sources. Medline was selected due to its broad and well-established coverage of biomedical and clinical literature, which aligned closely with the focus of this review. The search yielded 864 records, from which 85 studies were included, suggesting adequate topic saturation and a high yield of relevant studies. While the use of a single database is a limitation, it was addressed by thorough screening and is consistent with the exploratory nature of scoping reviews. The review was also restricted to enamel and dentin, even though pellicle structure and composition are not strongly substrate-dependent [135,136], leading to the exclusion of some studies. Moreover, as most studies were conducted in vitro or in vivo with short experimental durations, the effects of regular application and the long-term biocompatibility of the substances were not assessed.

4.7. Outlook

Substances that alter the ultrastructure and composition of the salivary pellicle can affect its protective properties. To improve pellicle research, it may be beneficial to standardize certain aspects of study design. For example, using consistent solvents and concentrations within test groups could enhance comparability. Expressing organic substance concentrations in molar terms, rather than weight or volume percentages, may also help address differences in molecular weight and number of functional groups. Furthermore, long-term studies with extended follow-up periods are lacking. Pellicle formation is a dynamic process involving continuous protein adsorption and desorption, as well as exposure to bacteria, chemical and mechanical challenges, and oral hygiene measures. The turnover rate of pellicle components and their modifications has been identified for only a few molecules and remains unknown in vivo [4,137–139]. Understanding these dynamics is essential for determining the optimal application duration and frequency, as well as the clinical feasibility of interventions. As highlighted in this review, lipids exhibit rapid desorption, limiting their clinical effectiveness. Regarding substantivity, chlorhexidine is considered the gold standard, remaining detectable in the oral cavity for up to 24 h after a single application [125,140]. In clinical practice, it is essential to determine whether pellicle modifications induced by substances such as fluorides and metals, peptides and proteins, and polyphenols persist long-term or are lost during pellicle remodelling. If frequent reapplication is required, this may increase the risk of adverse effects, such as tooth discoloration. While pellicle modification is still in its preliminary stages, primarily based on in vitro and in situ studies, it shows promising potential for future clinical research.

5. Conclusions

Various compounds demonstrated differing potentials to modify the pellicle's structure, composition, and protective functions, such as increasing its thickness and density, incorporating active substances, modulating protein interactions, blocking bacterial receptors, and enriching the pellicle with protective proteins. These modifications were partly associated with enhanced resistance to tooth wear and biofilm formation. Most studies employed in vitro and in situ designs, with limited in vivo evidence. While substances such as fluorides, metals, and polyphenols showed promising effects, others, including peptides, proteins, calcium-based agents, and lipids, yielded inconsistent or controversial results. The clinical relevance and long-term efficacy of these interventions remain uncertain. Future research should prioritize in vivo studies to assess the durability and effectiveness of pellicle modification under dynamic oral conditions, including the natural turnover of the pellicle. Key areas for investigation include the optimal concentration, formulation, frequency, and duration of application, as well as potential adverse effects. Clarifying these factors is essential to determine the most effective and safe strategies for enhancing pellicle function in the prevention and management of dental diseases.

CRediT authorship contribution statement

João Victor Frazão Câmara: Writing – original draft, Investigation. **Anton Schestakow:** Writing – original draft, Visualization, Methodology, Investigation. **Matthias Hannig:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Matthias Hannig reports financial support was provided by German Research Foundation. If there are other authors, they 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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Supplementary materials

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References

[1] 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.
[2] S. Trautmann, N. Künzel, C. Fecher-Trost, A. Barghash, P. Schalkowsky, J. Dudek, J. Delius, V. Helms, M. Hannig, Deep proteomic insights into the individual short-term pellicle formation on enamel—An In situ pilot study, PROTEOMICS – Clinical Applications 14 (3) (2020) 1900090, https://doi.org/10.1002/prca.201900090.
[3] D. Heller, E.J. Helmerhorst, A.C. Gower, W.L. Siqueira, B.J. Paster, F. G. Oppenheim, Microbial diversity in the early In vivo-formed dental biofilm, Appl. Environ. Microbiol. 82 (6) (2016) 1881–1888, https://doi.org/10.1128/aem.03984-15.
[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] 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.
[6] M. Hannig, C. Hannig, The pellicle and erosion, in: A. Lussi, C. Ganss (Eds.), Erosive Tooth WEar, Monographs in Oral Science, Karger, 2014, pp. 206–214.
[7] A. Joiner, A. Schwarz, C.J. Philpotts, T.F. Cox, K. Huber, M. Hannig, The protective nature of pellicle towards toothpaste abrasion on enamel and dentine, J. Dent. 36 (5) (2008) 360–368, https://doi.org/10.1016/j.jdent.2008.01.010.
[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] A. Schestakow, B. Echterhoff, M. Hannig, Erosion protective properties of the enamel pellicle in-situ, J. Dent. 147 (2024) 105103, https://doi.org/10.1016/j.jdent.2024.105103.
[10] H. Hu, M.F. Burrow, W.K. Leung, Proteomic profile of in situ acquired pellicle on tooth and restorative material surfaces, J. Dent. 129 (2023) 104389, https://doi.org/10.1016/j.jdent.2022.104389.
[11] 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.
[12] R. Alharthi, M. Mutahar, D. Bartlett, J. Jafari, R. Moazzez, Salivary proteins in Human acquired enamel pellicle (AEP) on eroded and uneroded teeth in patients with gastro-oesophageal reflux disease (GORD), Dentistry Journal 12 (8) (2024) 235, https://doi.org/10.3390/dj12080235.

[13] W.H. Douglas, E.S. Reeh, N. Ramasubbu, P.A. Raj, K.K. Bhandary, M.J. Levine, Statherin: a major boundary lubricant of human saliva, Biochem. Biophys. Res. Commun. 180 (1) (1991) 91–97, https://doi.org/10.1016/S0006-291X(05)81259-8.
[14] L.A. Tabak, In defense of the oral cavity: structure, biosynthesis, and function of salivary mucins, Annu. Rev. Physiol. 57 (1995) 547–564, https://doi.org/10.1146/annurev.ph.57.030195.002555. Volume 57.
[15] A.C. Tricco, E. Lillie, W. Zarin, K.K. O'Brien, H. Colquhoun, D. Levac, D. Moher, M.D. Peters, T. Horsley, L. Weeks, PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation, Ann. Intern. Med. 169 (7) (2018) 467–473, https://doi.org/10.7326/M18-0850.
[16] T. Fox, B.J. Hunt, R.A.S. Ariens, G.J. Towers, R. Lever, P. Garner, R. Kuehn, Systematic reviews of clinical laboratory studies: pilot risk of bias tool developed by consensus, Cochrane Evidence Synthesis and Methods 2 (8) (2024) e12098, https://doi.org/10.1002/cesm.12098.
[17] A.H.S. Delgado, S. Sauro, A.F. Lima, A.D. Loguercio, A. Della Bona, A. Mazzoni, F. M. Collares, F. Staxrud, J. Ferracane, J. Tsoi, J. Amato, K.W. Neuhaus, L. Ceballos, L. Breschi, M. Hannig, M.A. Melo, M. Özcan, N. Scotti, N. Opdam, S. Yamaguchi, S. Paris, L.S. Turkun, S. Doméjean, V. Rosa, W. Palin, F. Schwendicke, RoBDEMAT: a risk of bias tool and guideline to support reporting of pre-clinical dental materials research and assessment of systematic reviews, J. Dent. 127 (2022) 104350, https://doi.org/10.1016/j.jdent.2022.104350.
[18] F. Wegehaupt, D. Gries, A. Wiegand, T. Attin, Is bovine dentine an appropriate substitute for human dentine in erosion/abrasion tests? J. Oral Rehabil. 35 (5) (2008) 390–394, https://doi.org/10.1111/j.1365-2842.2007.01843.x.
[19] G.H. Yassen, J.A. Platt, A.T. Hara, Bovine teeth as substitute for human teeth in dental research: a review of literature, J. Oral Sci. 53 (3) (2011) 273–282, https://doi.org/10.2334/josnusd.53.273.
[20] L. Fiorillo, G. Cervino, A.S. Herford, L. Laino, M. Cicciù, Stannous fluoride effects on enamel: a systematic review, Biomimetics 5 (3) (2020) 41, https://doi.org/10.3390/biomimetics5030041.
[21] M. Rykke, J.E. Ellingsen, T. Sönju, Chemical analysis and scanning electron microscopy of acquired pellicle formed in vivo on stannous fluoride treated enamel, Scand. J. Dent. Res. 99 (3) (1991) 205–211, https://doi.org/10.1111/j.1600-0722.1991.tb01886.x.
[22] J. Kirsch, M. Hannig, P. Winkel, S. Basche, B. Leis, N. Pütz, A. Kensche, C. Hannig, Influence of pure fluorides and stannous ions on the initial bacterial colonization in situ, Sci. Rep. 9 (1) (2019) 18499, https://doi.org/10.1038/s41598-019-55083-0.
[23] A. Kensche, E. Buschbeck, B. König, M. Koch, J. Kirsch, C. Hannig, M. Hannig, Effect of fluoride mouthrinses and stannous ions on the erosion protective properties of the in situ pellicle, Sci. Rep. 9 (1) (2019) 5336, https://doi.org/10.1038/s41598-019-41736-7.
[24] A.A. Algarni, M.C. Mussi, E.B. Moffa, F. Lippert, D.T. Zero, W.L. Siqueira, A. T. Hara, The impact of stannous, fluoride ions and its combination on enamel pellicle proteome and dental erosion prevention, PLoS ONE 10 (6) (2015) e0128196, https://doi.org/10.1371/journal.pone.0128196.
[25] A. Kensche, J. Kirsch, S. Mintert, F. Enders, S. Pötschke, S. Basche, B. König, C. Hannig, M. Hannig, Impact of customary fluoride rinsing solutions on the pellicle's protective properties and bioadhesion in situ, Sci. Rep. 7 (1) (2017) 16584, https://doi.org/10.1038/s41598-017-16677-8.
[26] C. Ganss, N. Schlueter, J. Klimek, Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions—A comparison of in vitro and in situ results, Arch. Oral Biol. 52 (1) (2007) 9–14, https://doi.org/10.1016/j.archoralbio.2006.07.004.
[27] M.Q. Hasan, J. Yuen, G. Slaughter, Carbon nanotube-cellulose pellicle for glucose biofuel cell, Annu Int Conf IEEE Eng Med Biol Soc 2018 (2018) 1–4, https://doi.org/10.1109/embc.2018.8513229.
[28] M. Rykke, T. Sønju, K. Skjørland, G. Rølla, Protein adsorption to hydroxyapatite and to calcium fluoride in vitro and amino acid analyses of pellicle formed on normal enamel and on calcium-fluoride-covered enamel in vivo, Acta Odontol. Scand. 47 (4) (1989) 245–251, https://doi.org/10.3109/00016358909007709.
[29] N. Masson, R.R. Domingues, J.A. Cury, A.F. Paes Leme, Acidulated phosphate fluoride application changes the protein composition of human acquired enamel pellicle, Caries Res 47 (3) (2013) 251–258, https://doi.org/10.1159/000346280.
[30] N. Tinanoff, S.H.Y. Wei, F.M. Parkins, Effect of the acquired pellicle on fluoride uptake in tooth enamel in vitro, Caries Res 9 (3) (1975) 224–230, https://doi.org/10.1159/000260159.
[31] H.C. van der Mei, E. Engels, J. de Vries, H.J. Busscher, Effects of amine fluoride on biofilm growth and salivary pellicles, Caries Res 42 (1) (2007) 19–27, https://doi.org/10.1159/000111746.
[32] T.R. Delecrode, W.L. Siqueira, F.C. Zaidan, M.R. Bellini, E.B. Moffa, M.C. Mussi, Y. Xiao, M.A. Buzalaf, Identification of acid-resistant proteins in acquired enamel pellicle, J. Dent. 43 (12) (2015) 1470–1475, https://doi.org/10.1016/j.jdent.2015.10.009.
[33] A.C. Santiago, Z.N. Khan, M.C. Miguel, C.C. Gironda, A. Soares-Costa, V.T. Pela, A.L. Leite, J.M. Edwardson, M.A.R. Buzalaf, F. Henrique-Silva, A new sugarcane cystatin strongly binds to dental enamel and reduces erosion, J. Dent. Res. 96 (9) (2017) 1051–1057, https://doi.org/10.1177/0022034517712981.
[34] T.S. Carvalho, T.T. Araújo, T.M.O. Ventura, A. Dionizio, J.V.F. Câmara, S. M. Moraes, V.T. Pelá, T. Martini, J.C. Leme, A.L.B. Derbotolli, L.T. Grizzo, E. Crusca, P.Y.T. Shibao, R. Marchetto, F. Henrique-Silva, J.P. Pessan, M.A. R. Buzalaf, Acquired pellicle protein-based engineering protects against erosive demineralization, J. Dent. 102 (2020) 103478, https://doi.org/10.1016/j.jdent.2020.103478.
[35] T.S. Carvalho, T.T. Araújo, T.M.O. Ventura, A. Dionizio, J.V.F. Câmara, S. M. Moraes, J.C. Leme, L.T. Grizzo, E. Crusca, P.Y.T. Shibao, R. Marchetto, F. Henrique-Silva, J.P. Pessan, M.A.R. Buzalaf, Hemoglobin protects enamel against intrinsic enamel erosive demineralization, Caries Res. 58 (2) (2024) 90–107, https://doi.org/10.1159/000536200.
[36] A.A. de Oliveira, A.L. MarquesXavier, T.T. da Silva, A.L.B. Debortolli, A.C. A. Ferdin, A.P. Boteon, D.D.S. Martins, V.T. Pelá, M.A.R. Buzalaf, F. Henrique-Silva, H.M. Honório, D. Rios, Acquired pellicle engineering with the association of cystatin and vitamin E against enamelerosion, J. Dent. (2023) 104680, https://doi.org/10.1016/j.jdent.2023.104680.
[37] V.T. Pelá, M.A.R. Buzalaf, S.H. Niemeyer, T. Baumann, F. Henrique-Silva, D. Toyama, E. Crusca, R. Marchetto, A. Lussi, T.S. Carvalho, Acquired pellicle engineering with proteins/peptides: mechanism of action on native human enamel surface, J. Dent. 107 (2021) 103612, https://doi.org/10.1016/j.jdent.2021.103612.
[38] V.T. Pelá, C.C. Gironda, E.A. Taira, L. Brito, J.C. Pieretti, A.B. Seabra, C.A. B. Cardoso, M.C. Rodrigues, F. Henrique-Silva, M.A.R. Buzalaf, Different vehicles containing CaneCPI-5 reduce erosive dentin wear in situ, Clin. Oral Investig. (2023), https://doi.org/10.1007/s00784-023-05175-z.
[39] V.T. Pelá, T.M.O. Ventura, E.A. Taira, L.T.G. Thomassian, L. Brito, Y.E. Matuhara, F. Henrique-Silva, S. Groisman, T.S. Carvalho, A. Lussi, M.A.R. Buzalaf, Use of reflectometer Optipen to assess the preventive effect of a sugarcane cystatin on initial dental erosion in vivo, J Mech Behav Biomed Mater 141 (2023) 105782, https://doi.org/10.1016/j.jmbbm.2023.105782.
[40] V.T. Pelá, S.H. Niemeyer, T. Baumann, F.M. Levy, F. Henrique-Silva, A. Lussi, T. S. Carvalho, M.A.R. Buzalaf, Acquired pellicle engineering using a combination of organic (Sugarcane Cystatin) and inorganic (Sodium Fluoride) components against dental erosion, Caries Res 56 (2) (2022) 138–145, https://doi.org/10.1159/000522490.
[41] L.A. Santos, T. Martini, J.V.F. Câmara, F.N. Reis, A.C. Ortiz, G.D. Camiloti, F. M. Levy, P.Y.T. Shibao, H.M. Honorio, F. Henrique-Silva, J.C. Pieretti, A. B. Seabra, C.A.B. Cardoso, M.A.R. Buzalaf, Solutions and gels containing a sugarcane-derived cystatin (CaneCPI-5) reduce enamel and dentin erosion in vitro, Caries Res 55 (6) (2021) 594–602, https://doi.org/10.1159/000520261.
[42] V.T. Pelá, L. Brito, E.A. Taira, F. Henrique-Silva, J.C. Pieretti, A.B. Seabra, C.D. B. Cardoso, E.P. de Souza, S. Groisman, M.C. Rodrigues, A. Lussi, T.S. Carvalho, M.A.R. Buzalaf, Preventive effect of chitosan gel containing CaneCPI-5 against enamel erosive wear in situ, Clin. Oral Investig. 26 (11) (2022) 6511–6519, https://doi.org/10.1007/s00784-022-04600-z.
[43] C.C. Gironda, V.T. Pelá, F. Henrique-Silva, A.C.B. Delbem, J.P. Pessan, M.A. R. Buzalaf, New insights into the anti-erosive property of a sugarcane-derived cystatin: different vehicle of application and potential mechanism of action, J. Appl. Oral Sci. 30 (2022) e20210698, https://doi.org/10.1590/1678-7757-2021-0698.
[44] T. Ventura, L.P.S. Cassiano, E.S.C.M. Souza, E.A. Taira, A.L. Leite, D. Rios, M.A. R. Buzalaf, The proteomic profile of the acquired enamel pellicle according to its location in the dental arches, Arch. Oral Biol. 79 (2017) 20–29, https://doi.org/10.1016/j.archoralbio.2017.03.001.
[45] J. Li, E.J. Helmerhorst, Y. Yao, M.E. Nunn, R.F. Troxler, F.G. Oppenheim, Statherin is an in vivo pellicle constituent: identification and immunoquantification, Arch. Oral Biol. 49 (5) (2004) 379–385, https://doi.org/10.1016/j.archoralbio.2004.01.002.
[46] P.A. Raj, M. Johnsson, M.J. Levine, G.H. Nancollas, Salivary statherin. Dependence on sequence, charge, hydrogen bonding potency, and helical conformation for adsorption to hydroxyapatite and inhibition of mineralization, J. Biol. Chem. 267 (9) (1992) 5968–5976, https://doi.org/10.1016/S0021-9258(18)42650-6.
[47] E.A. Taira, G. Carvalho, C.R. Ferrari, T. Martini, V.T. Pelá, T.M.O. Ventura, A. S. Dionizio, E. Crusca, R. Marchetto, M.A.R. Buzalaf, Statherin-derived peptide protects against intrinsic erosion, Arch. Oral Biol. 119 (2020) 104890, https://doi.org/10.1016/j.archoralbio.2020.104890.
[48] J. Kosoric, R.A.D. Williams, M.P. Hector, P. Anderson, A synthetic peptide based on a natural salivary protein reduces demineralisation in model systems for dental caries and erosion, Int. J. Pept. Res. Ther. 13 (2007) 497–503, https://doi.org/10.1007/s10989-007-9085-0.
[49] E.A. Taira, C.R. Ferrari, G. Carvalho, T.M.O. Ventura, T. Martini, A.S. Dionizio, T. T. Araújo, E. Crusca, R. Marchetto, M.A.R. Buzalaf, Rinsing with statherin-derived peptide alters the proteome of the acquired enamel pellicle, Caries Res 55 (4) (2021) 333–340, https://doi.org/10.1159/000517959.
[50] F.N. Reis, M.M. Francese, N. Silva, V.T. Pelá, J.V.F. Câmara, J.S. Trevizol, J. C. Pieretti, A.B. Seabra, J.P. Pessan, H.M. Honório, E. Crusca, R. Marchetto, M.A. R. Buzalaf, Gels containing statherin-derived peptide protect against enamel and dentin erosive tooth wear in vitro, J. Mech. Behav. Biomed. Mater. 137 (2023) 105549, https://doi.org/10.1016/j.jmbbm.2022.105549.
[51] F.N. Reis, M.M. Francese, N.D.G. da Silva, V.T. Pelá, J.V.F. Câmara, J.S. Trevizol, H.M. Honório, E. Crusca, R. Marchetto, M.A.R. Buzalaf, Solutions containing a statherin-derived peptide reduce enamel erosion in vitro, Caries Res 57 (1) (2023) 52–58, https://doi.org/10.1159/000529016.
[52] P. Schupbach, J.R. Neeser, M. Golliard, M. Rouvet, B. Guggenheim, Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci, J. Dent. Res. 75 (10) (1996) 1779–1788, https://doi.org/10.1177/00220345960750101101.
[53] S.P. Humphrey, R.T. Williamson, A review of saliva: normal composition, flow, and function, J Prosthet Dent 85 (2) (2001) 162–169, https://doi.org/10.1067/mpr.2001.113778.

[54] I.E. Svendsen, L. Lindh, U. Elofsson, T. Arnebrant, Studies on the exchange of early pellicle proteins by mucin and whole saliva, J. Colloid Interface Sci. 321 (1) (2008) 52–59, https://doi.org/10.1016/j.jcis.2008.01.041.
[55] Z. Cheaib, A. Lussi, Impact of acquired enamel pellicle modification on initial dental erosion, Caries Res 45 (2) (2011) 107–112, https://doi.org/10.1159/000324803.
[56] Z. Cheaib, E. Rakmathulina, A. Lussi, S. Eick, Impact of acquired pellicle modification on adhesion of early colonizers, Caries Res 49 (6) (2015) 626–632, https://doi.org/10.1159/000442169.
[57] K.R. Sieber, C. Schmidt, T. Baumann, A. Lussi, T.S. Carvalho, Acquired enamel pellicle modification with casein and Mucin in different concentrations and its impact on initial dental erosion, Caries Res 53 (4) (2019) 457–466, https://doi.org/10.1159/000499579.
[58] T.S. Carvalho, J.E. Halter, D. Mucolli, A. Lussi, S. Eick, T. Baumann, Pellicle modification with casein and Mucin does not promote In vitro bacterial biofilm formation, Oral Health Prev. Dent. 18 (1) (2020) 475–483, https://doi.org/10.3290/j.ohpd.a43351.
[59] 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.
[60] T. Baumann, C. Schmidt, T.S. Carvalho, Pellicle modification with Casein and Mucin does not affect surface loss from erosion and abrasion, Caries Res 54 (5–6) (2020) 509–516, https://doi.org/10.1159/000510699.
[61] S. Trautmann, A. Barghash, C. Fecher-Trost, P. Schalkowsky, C. Hannig, J. Kirsch, S. Rupf, A. Keller, V. Helms, M. Hannig, Proteomic analysis of the initial oral pellicle in caries-active and caries-free individuals, PROTEOMICS – Clinical Applications 13 (4) (2019) 1800143, https://doi.org/10.1002/prca.201800143.
[62] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouységu, Plant polyphenols: chemical properties, biological activities, and synthesis, Angewandte Chemie International Edition 50 (3) (2011) 586–621, https://doi.org/10.1002/anie.201000044.
[63] J. Delius, S. Trautmann, G. Médard, B. Kuster, M. Hannig, T. Hofmann, Label-free quantitative proteome analysis of the surface-bound salivary pellicle, Colloids Surf. B. Biointerfaces 152 (2017) 68–76, https://doi.org/10.1016/j.colsurfb.2017.01.005.
[64] E. Haslam, Natural polyphenols (Vegetable Tannins) as drugs: possible modes of action, J. Nat. Prod. 59 (2) (1996) 205–215, https://doi.org/10.1021/np960040+.
[65] K. Hara, M. Ohara, I. Hayashi, T. Hino, R. Nishimura, Y. Iwasaki, T. Ogawa, Y. Ohyama, M. Sugiyama, H. Amano, The green tea polyphenol (-)-epigallocatechin gallate precipitates salivary proteins including alpha-amylase: biochemical implications for oral health, Eur. J. Oral Sci. 120 (2) (2012) 132–139, https://doi.org/10.1111/j.1600-0722.2012.00947.x.
[66] A. Schestakow, M.S. Guth, T.A. Eisenmenger, M. Hannig, Evaluation of anti-biofilm activity of mouthrinses containing tannic acid or chitosan on Dentin In situ, Molecules 26 (5) (2021) 1351, https://doi.org/10.3390/molecules26051351.
[67] Q. Xi, W. Hoth-Hannig, S. Deng, X. Jin, B. Fu, M. Hannig, The effect of polyphenol-containing solutions on in situ biofilm formation on enamel and dentin, J. Dent. 102 (2020) 103482, https://doi.org/10.1016/j.jdent.2020.103482.
[68] G. Wittpahl, I. Kölling-Speer, S. Basche, E. Herrmann, M. Hannig, K. Speer, C. Hannig, The polyphenolic composition of Cistus incanus herbal tea and its antibacterial and anti-adherent activity against Streptococcus mutans, Planta Med 81 (18) (2015) 1727–1735, https://doi.org/10.1055/s-0035-1557822.
[69] M.-T. Weber, M. Hannig, S. Pötschke, F. Höhne, C. Hannig, Application of plant extracts for the prevention of dental erosion: an in situ/in vitro study, Caries Res 49 (5) (2015) 477–487, https://doi.org/10.1159/000431294.
[70] A. Schestakow, W. Rasputnis, M. Hannig, Effect of polyphenols on the ultrastructure of the dentin pellicle and subsequent erosion, Caries Res (2024) 1–9, https://doi.org/10.1159/000536199.
[71] A. Schestakow, Y. Nekrashevych, W. Hoth-Hannig, M. Hannig, Influence of periodic polyphenol treatment on the anti-erosive potential of the acquired enamel pellicle—A qualitative exploratory study, J. Dent. 124 (2022) 104236, https://doi.org/10.1016/j.jdent.2022.104236.
[72] M. Rehage, J. Delius, T. Hofmann, M. Hannig, Oral astringent stimuli alter the enamel pellicle's ultrastructure as revealed by electron microscopy, J. Dent. 63 (2017) 21–29, https://doi.org/10.1016/j.jdent.2017.05.011.
[73] S. Hertel, L. Graffy, S. Pötschke, S. Basche, A. Al-Ahmad, W. Hoth-Hannig, M. Hannig, C. Hannig, Effect of Inula viscosa on the pellicle's protective properties and initial bioadhesion in-situ, Arch. Oral Biol. 71 (2016) 87–96, https://doi.org/10.1016/j.archoralbio.2016.07.006.
[74] J. Flemming, C.T. Meyer-Probst, K. Hille, S. Basche, K. Speer, I. Kölling-Speer, C. Hannig, M. Hannig, Olive oil as a transport medium for bioactive molecules of plants?-an In situ study, Molecules 28 (9) (2023), https://doi.org/10.3390/molecules28093803.
[75] S. Soares, R. Vitorino, H. Osório, A. Fernandes, A. Venâncio, N. Mateus, F. Amado, V. de Freitas, Reactivity of Human salivary proteins families toward food polyphenols, J. Agric. Food Chem. 59 (10) (2011) 5535–5547, https://doi.org/10.1021/jf104975d.
[76] C.M. de Souza-e-Silva, T.M. da Silva Ventura, L. de Pau, C. la Silva, A. de Lima Leite, M.A.R. Buzalaf, Effect of gels containing chlorhexidine or epigallocatechin-3-gallate on the protein composition of the acquired enamel pellicle, Arch. Oral Biol. 82 (2017) 92–98, https://doi.org/10.1016/j.archoralbio.2017.05.024.
[77] L. Tang, L. Zhu, Y. Liu, Y. Zhang, B. Li, M. Wang, Crosslinking improve demineralized dentin performance and synergistically promote biomimetic mineralization by CaP_PILP, ACS Omega 8 (16) (2023) 14410–14419, https://doi.org/10.1021/acsomega.2c07825.
[78] A. Joiner, D. Muller, U.M. Elofsson, T. Arnebrant, Ellipsometry analysis of the in vitro adsorption of tea polyphenols onto salivary pellicles, Eur. J. Oral Sci. 112 (6) (2004) 510–515, https://doi.org/10.1111/j.1600-0722.2004.00166.x.
[79] A. Joiner, D. Muller, U.M. Elofsson, M. Malmsten, T. Arnebrant, Adsorption from black tea and red wine onto in vitro salivary pellicles studied by ellipsometry, Eur. J. Oral Sci. 111 (5) (2003) 417–422, https://doi.org/10.1034/j.1600-0722.2003.00073.x.
[80] J. Flemming, C.T. Meyer-Probst, K. Speer, I. Kölling-Speer, C. Hannig, M. Hannig, Preventive applications of polyphenols in dentistry—A review, Int. J. Mol. Sci. 22 (9) (2021) 4892, https://doi.org/10.3390/ijms22094892.
[81] C. Hannig, J. Sorg, B. Spitzmüller, M. Hannig, A. Al-Ahmad, Polyphenolic beverages reduce initial bacterial adherence to enamel in situ, J. Dent. 37 (7) (2009) 560–566, https://doi.org/10.1016/j.jdent.2009.03.017.
[82] S. Hertel, S. Pötschke, S. Basche, J. Delius, W. Hoth-Hannig, M. Hannig, C. Hannig, Effect of tannic acid on the protective properties of the in situ formed pellicle, Caries Res 51 (1) (2017) 34–45, https://doi.org/10.1159/000451036.
[83] A. Yanagida, T. Kanda, M. Tanabe, F. Matsudaira, J.G. Oliveira Cordeiro, Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of mutans streptococci, J. Agric. Food Chem. 48 (11) (2000) 5666–5671, https://doi.org/10.1021/jf000363i.
[84] A. Schestakow, C.T. Meyer-Probst, C. Hannig, M. Hannig, Prevention of dental biofilm formation with polyphenols: a systematic review, Planta Med 89 (11) (2023) 1026–1033, https://doi.org/10.1055/a-1939-7615.
[85] Q. Zeng, J. Zheng, D. Yang, Y. Tang, Z. Zhou, Effect of calcium ions on the adsorption and lubrication behavior of salivary proteins on human tooth enamel surface, J Mech Behav Biomed Mater 98 (2019) 172–178, https://doi.org/10.1016/j.jmbbm.2019.06.019.
[86] M. Hannig, C. Hannig, Nanomaterials in preventive dentistry, Nat Nanotechnol 5 (8) (2010) 565–569, https://doi.org/10.1038/nnano.2010.83.
[87] A. Kensche, S. Potschke, C. Hannig, G. Richter, W. Hoth-Hannig, M. Hannig, Influence of calcium phosphate and apatite containing products on enamel erosion, Sci. World. J. 2016 (2016) 7959273, https://doi.org/10.1155/2016/7959273.
[88] A. Kensche, C. Holder, S. Basche, N. Tahan, C. Hannig, M. Hannig, Efficacy of a mouthrinse based on hydroxyapatite to reduce initial bacterial colonisation in situ, Arch. Oral Biol. 80 (2017) 18–26, https://doi.org/10.1016/j.archoralbio.2017.03.013.
[89] C. Hannig, S. Basche, T. Burghardt, A. Al-Ahmad, M. Hannig, Influence of a mouthwash containing hydroxyapatite microclusters on bacterial adherence in situ, Clin. Oral Investig. 17 (3) (2013) 805–814, https://doi.org/10.1007/s00784-012-0781-6.
[90] K. Cross, N. Huq, E. Reynolds, Casein phosphopeptides in oral health-chemistry and clinical applications, Curr. Pharm. Des. 13 (8) (2007) 793–800, https://doi.org/10.2174/138161207780363086.
[91] F. Cai, D.J. Manton, P. Shen, G.D. Walker, K.J. Cross, Y. Yuan, C. Reynolds, E. C. Reynolds, Effect of addition of citric acid and casein phosphopeptide-amorphous calcium phosphate to a sugar-free chewing gum on enamel remineralization in situ, Caries Res 41 (5) (2007) 377–383, https://doi.org/10.1159/000104796.
[92] F. Cai, P. Shen, G.D. Walker, C. Reynolds, Y. Yuan, E.C. Reynolds, Remineralization of enamel subsurface lesions by chewing gum with added calcium, J. Dent. 37 (10) (2009) 763–768, https://doi.org/10.1016/j.jdent.2009.06.003.
[93] D. Manton, F. Cai, Y. Yuan, G. Walker, N. Cochrane, C. Reynolds, L. Brearley-Messer, E. Reynolds, Effect of casein phosphopeptide-amorphous calcium phosphate added to acidic beverages on enamel erosion in vitro, Aust. Dent. J. 55 (3) (2010) 275–279, https://doi.org/10.1111/j.1834-7819.2010.01234.x.
[94] A. Schestakow, G.J. Lefering, M. Hannig, An ultrastructural, In-situ study on the impact of desensitizing agents on Dentin, Int. Dent. J. (2024), https://doi.org/10.1016/j.identj.2024.09.027.
[95] S. Grychtol, S. Basche, M. Hannig, C. Hannig, Effect of CPP/ACP on initial bioadhesion to enamel and dentin in situ, ScientificWorldJournal 2014 (2014) 512682, https://doi.org/10.1155/2014/512682.
[96] B.L. Slomiany, V.L. Murty, E. Zdebska, A. Slomiany, K. Gwozdzinski, I.D. Mandel, Tooth surface-pellicle lipids and their role in the protection of dental enamel against lactic-acid diffusion in man, Arch. Oral Biol. 31 (3) (1986) 187–191, https://doi.org/10.1016/0003-9969(86)90126-3.
[97] M. Reich, C. Hannig, M. Hannig, K. Kümmerer, A. Kensche, The lipid composition of the in situ pellicle, Arch. Oral Biol. 142 (2022) 105493, https://doi.org/10.1016/j.archoralbio.2022.105493.
[98] D.B. Peckys, D.E.J.N.M. Hannig, Oil droplet formation on pellicle covered tooth surfaces studied with environmental scanning electron microscopy, J Microsc 274 (3) (2019) 158–167, https://doi.org/10.1111/jmi.12794.
[99] C. Hannig, J. Kirsch, A. Al-Ahmad, A. Kensche, M. Hannig, K. Kümmerer, Do edible oils reduce bacterial colonization of enamel in situ? Clin. Oral Investig. 17 (2) (2013) 649–658, https://doi.org/10.1007/s00784-012-0734-0.
[100] A. Kensche, M. Reich, C. Hannig, K. Kümmerer, M. Hannig, Modification of the lipid profile of the initial oral biofilm In situ using linseed oil as mouthwash, Nutrients 13 (3) (2021), https://doi.org/10.3390/nu13030989.
[101] C. Hannig, C. Wagenschwanz, S. Pötschke, K. Kümmerer, A. Kensche, W. Hoth-Hannig, M. Hannig, Effect of safflower oil on the protective properties of the in

situ formed salivary pellicle, Caries Res 46 (5) (2012) 496–506, https://doi.org/10.1159/000339924.
[102] F.Q. Ionta, C.R.B. Alencar, P.P. Val, A.P. Boteon, M.C. Jordão, H.M. Honório, M.A. R. Buzalaf, D. Rios, Effect of vegetable oils applied over acquired enamel pellicle on initial erosion, J. Appl. Oral. Sci. 25 (4) (2017) 420–426, https://doi.org/10.1590/1678-7757-2016-0436.
[103] D. Rios, A.P. Boteon, C.C.L. Di Leone, T.T. Castelluccio, F.L. Mendonça, F.Q. Ionta, M.A.R. Buzalaf, T.S. Carvalho, Vitamin E: a potential preventive approach against dental erosion-an in vitro short-term erosive study, J. Dent. 113 (2021) 103781, https://doi.org/10.1016/j.jdent.2021.103781.
[104] F.D. Gunstone, Composition and properties of edible oils, Edible Oil Processing (2013) 1–39.
[105] F. Abdullah, N.A.S. Ramli, Identification of hydrophilic phenolic compounds derived from palm oil products, J. Oil Palm Res. 32 (2) (2020) 258–270, https://doi.org/10.21894/jopr.2020.0020.
[106] M. Rykke, G. Rölla, Effect of silicone oil on protein adsorption to hydroxyapatite in vitro and on pellicle formation in vivo, Scand. J. Dent. Res. 98 (5) (1990) 401–411, https://doi.org/10.1111/j.1600-0722.1990.tb00991.x.
[107] R. Patiño-Herrera, G. González-Alatorre, A. Estrada-Baltazar, S.E. Escoto-Chavéz, E. Pérez, Hydrophobic coatings for prevention of dental enamel erosion, Surf. Coat. Technol. 275 (2015) 148–154, https://doi.org/10.1016/j.surfcoat.2015.05.026.
[108] F. Song, H. Koo, D. Ren, Effects of material properties on bacterial adhesion and biofilm formation, J. Dent. Res. 94 (8) (2015) 1027–1034, https://doi.org/10.1177/0022034515587690.
[109] M. Salas, G. Nascimento, F. Vargas-Ferreira, S. Tarquinio, M. Huysmans, F. Demarco, Diet influenced tooth erosion prevalence in children and adolescents: results of a meta-analysis and meta-regression, J. Dent. 43 (8) (2015) 865–875, https://doi.org/10.1016/j.jdent.2015.05.012.
[110] H. Li, Y. Zou, G. Ding, Dietary factors associated with dental erosion: a meta-analysis, (2012). https://doi.org/10.1371/journal.pone.0042626.
[111] A. Haug, A.T. Høstmark, O.M. Harstad, Bovine milk in human nutrition–a review, Lipids Health Dis 6 (2007) 1–16, https://doi.org/10.1186/1476-511X-6-25.
[112] Y. Nekrashevych, A. Schestakow, W. Hoth-Hannig, M. Hannig, Influence of periodic milk or cream treatment on the anti-erosive potential of the acquired enamel pellicle, J. Dent. 115 (2021) 103858, https://doi.org/10.1016/j.jdent.2021.103858.
[113] A. Kensche, A. Durasch, B. Konig, T. Henle, C. Hannig, M. Hannig, Characterization of the in situ pellicle ultrastructure formed under the influence of bovine milk and milk protein isolates, Arch. Oral Biol. 104 (2019) 133–140, https://doi.org/10.1016/j.archoralbio.2019.05.021.
[114] L.P.S. Cassiano, T.M.S. Ventura, C.M.S. Silva, A.L. Leite, A.C. Magalhaes, J. P. Pessan, M.A.R. Buzalaf, Protein profile of the acquired enamel pellicle after rinsing with whole milk, fat-free milk, and water: an in vivo study, Caries Res 52 (4) (2018) 288–296, https://doi.org/10.1159/000485390.
[115] B. Lindquist, P. Lingström, L. Fändriks, D. Birkhed, Influence of five neutralizing products on intra-oral pH after rinsing with simulated gastric acid, Eur. J. Oral Sci. 119 (4) (2011) 301–304, https://doi.org/10.1111/j.1600-0722.2011.00841.x.
[116] M.N.V. Ravi Kumar, A review of chitin and chitosan applications, React. Funct. Polym. 46 (1) (2000) 1–27, https://doi.org/10.1016/S1381-5148(00)00038-9.
[117] M.H. Periayah, A.S. Halim, A.Z.M. Saad, Chitosan: a promising marine polysaccharide for biomedical research, Pharmacogn Rev 10 (19) (2016) 39, https://doi.org/10.4103/0973-7847.176545.
[118] R. Zimmermann, J. Delius, J. Friedrichs, S. Stehl, T. Hoffnann, C. Hannig, M. Rehage, C. Werner, M. Hannig, Impact of oral astringent stimuli on surface charge and morphology of the protein-rich pellicle at the tooth-saliva interphase, Colloids and Surfaces B-Biointerfaces 174 (2019) 451–458, https://doi.org/10.1016/j.colsurfb.2018.11.028.
[119] H.C. van der Mei, E. Engels, J. de Vries, R.J. Dijkstra, H.J. Busscher, Chitosan adsorption to salivary pellicles, Eur. J. Oral Sci. 115 (4) (2007) 303–307, https://doi.org/10.1111/j.1600-0722.2007.00454.x.
[120] C.G. Jones, Chlorhexidine: is it still the gold standard? Periodontol. 15 (1997) 55–62, https://doi.org/10.1111/j.1600-0757.1997.tb00105.x, 2000.
[121] L.B. Freitas, N. Vassilakos, T. Arnebrant, Interactions of chlorhexidine with salivary films adsorbed at solid/liquid and air/liquid interfaces, J. Periodontal Res. 28 (2) (1993) 92–97, https://doi.org/10.1111/j.1600-0765.1993.tb01055.x.
[122] L.G. Hjeljord, G. Rølla, P. Bonesvoll, Chlorhexidine–protein interactions, J. Periodontal Res. 8 (s12) (1973) 11–16, https://doi.org/10.1111/j.1600-0765.1973.tb02158.x.
[123] G. Rölla, H. Löe, C.Rindom Schiøtt, The affinity of chlorhexidine for hydroxyapatite and salivary mucins, J. Periodontal Res. 5 (2) (1970) 90–95, https://doi.org/10.1111/j.1600-0765.1970.tb00698.x.
[124] A. Schestakow, M. Hannig, Effects of experimental agents containing tannic acid or chitosan on the bacterial biofilm formation in Situ, Biomolecules 10 (9) (2020) 1315, https://doi.org/10.3390/biom10091315.
[125] B. Reda, K. Hollemeyer, S. Trautmann, D.A. Volmer, M. Hannig, First insights into chlorhexidine retention in the oral cavity after application of different regimens, Clin. Oral Investig. (2021) 1–10, https://doi.org/10.1007/s00784-021-03910-y.
[126] C. Hannig, A. Gaeding, S. Basche, G. Richter, R. Helbig, M. Hannig, Effect of conventional mouthrinses on initial bioadhesion to enamel and dentin in situ, Caries Res 47 (2) (2013) 150–161, https://doi.org/10.1159/000345083.
[127] D.G. Tustian, R.P. Ellen, Some effects of chlorhexidine acetate on the adherence of oral bacteria to dental enamel in vitro, J. Periodontal Res. 12 (5) (1977) 323–330, https://doi.org/10.1111/j.1600-0765.1977.tb01523.x.
[128] V.F. Passos, L.K.A. Rodrigues, S.L. Santiago, The effect of magnesium hydroxide-containing dentifrice using an extrinsic and intrinsic erosion cycling model, Arch. Oral Biol. 86 (2018) 46–50, https://doi.org/10.1016/j.archoralbio.2017.11.006.
[129] Q. Zeng, L. Zheng, J. Zhou, H. Xiao, J. Zheng, Z. Zhou, Effect of alcohol stimulation on salivary pellicle formation on human tooth enamel surface and its lubricating performance, J Mech Behav Biomed Mater 75 (2017) 567–573, https://doi.org/10.1016/j.jmbbm.2017.05.029.
[130] S. Wetton, J. Hughes, R.G. Newcombe, M. Addy, The effect of saliva derived from different individuals on the erosion of enamel and dentine: a study in vitro, Caries Res 41 (5) (2007) 423–426, https://doi.org/10.1159/000104802.
[131] T. Baumann, S.H. Niemeyer, M.A.R. Buzalaf, T.S. Carvalho, Protease-inhibitors added to saliva in vitro influence the erosion protective effect of enamel pellicles, Sci. Rep. 13 (1) (2023) 8618, https://doi.org/10.1038/s41598-023-35334-x.
[132] R.P. Shellis, C. Ganss, Y. Ren, D.T. Zero, A. Lussi, Methodology and models in erosion research: discussion and conclusions, Caries Res 45 (Suppl. 1) (2011) 69–77, https://doi.org/10.1159/000325971.
[133] M. Hannig, A.K. Khanafer, W. Hoth-Hannig, F. Al-Marrawi, Y. Acil, Transmission electron microscopy comparison of methods for collecting in situ formed enamel pellicle, Clin. Oral Investig. 9 (1) (2005) 30–37, https://doi.org/10.1007/s00784-004-0284-1.
[134] 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.
[135] M. Hannig, Transmission electron microscopic study of in vivo pellicle formation on dental restorative materials, Eur. J. Oral Sci. 105 (5 Pt 1) (1997) 422–433, https://doi.org/10.1111/j.1600-0722.1997.tb02139.x.
[136] S. Trautmann, N. Künzel, C. Fecher-Trost, A. Barghash, J. Dudek, V. Flockerzi, V. Helms, M. Hannig, Is the proteomic composition of the salivary pellicle dependent on the substrate material? Proteomics Clin. Appl. 16 (3) (2022) e2100109 https://doi.org/10.1002/prca.202100109.
[137] C. Hannig, B. Spitzmüller, H.C. Lux, M. Altenburger, A. Al-Ahmad, M. Hannig, Efficacy of enzymatic toothpastes for immobilisation of protective enzymes in the in situ pellicle, Arch. Oral Biol. 55 (7) (2010) 463–469, https://doi.org/10.1016/j.archoralbio.2010.03.020.
[138] C. Hannig, A. Ruggeri, B. Al-Khayer, P. Schmitz, B. Spitzmüller, D. Deimling, K. Huber, W. Hoth-Hannig, W.H. Bowen, M. Hannig, Electron microscopic detection and activity of glucosyltransferase B, C, and D in the in situ formed pellicle, Arch. Oral Biol. 53 (11) (2008) 1003–1010, https://doi.org/10.1016/j.archoralbio.2008.04.005.
[139] C. Hannig, B. Spitzmüller, W. Hoth-Hannig, M. Hannig, Targeted immobilisation of lysozyme in the enamel pellicle from different solutions, Clin. Oral Investig. 15 (1) (2011) 65–73, https://doi.org/10.1007/s00784-009-0357-2.
[140] B. Reda, K. Hollemeyer, S. Trautmann, M. Hannig, D.A. Volmer, Determination of chlorhexidine retention in different oral sites using matrix-assisted laser desorption/ionization-time of flight mass spectrometry, Arch. Oral Biol. 110 (2020) 104623, https://doi.org/10.1016/j.archoralbio.2019.104623.