

Review article

Pellicle engineering with CaneCPI-5: a scoping review

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ABSTRACT

Objectives: The salivary pellicle regulates interfacial processes on dental surfaces, offering protection against erosion and influencing bacterial adhesion. CaneCPI-5, a sugarcane-derived peptide inspired by cystatins, has been proposed as a cost-effective agent for pellicle modification. This scoping review evaluates the potential of CaneCPI-5 in pellicle engineering, particularly regarding erosion prevention and bacterial adhesion.

Data: Studies investigating the effects of CaneCPI-5 on pellicles formed by human saliva on enamel or dentin were included.

Sources: A literature search was conducted in Medline, Scopus, and Web of Science up to May 2025. Only English-language research articles were considered. Hand-searching, including checking reference lists, was not conducted.

Study selection: A total of 131 records were identified. After removing duplicates, the titles, abstracts and full-text were screened, resulting in 20 included studies. Most studies were conducted on enamel pellicles ($n = 17$), with some on dentin ($n = 2$) or both ($n = 1$). CaneCPI-5 demonstrated enamel-binding ability and modified the pellicle proteome by increasing acid-resistant proteins, enhancing resistance to erosion. Its effects on bacterial adhesion and caries prevention were inconsistent. Combinations with other agents, particularly vitamin E, showed synergistic effects, though overall findings were mixed.

Conclusions: CaneCPI-5 shows promise as a pellicle-modifying agent for erosion protection. However, current evidence is limited, and findings remain inconclusive, merit further research under clinically relevant conditions.

Clinical significance: Intentional modification of the pellicle with peptides like CaneCPI-5 may improve its protective properties and support preventive measures. Nonetheless, the long-term effects and clinical applicability of such modifications remain unclear. Further studies are needed to confirm their efficacy in the dynamic oral environment.

1. Introduction

Saliva protects against tooth demineralization through multiple functions, including the formation of the pellicle [1,2]. This thin, protein-based layer forms in vivo via selective adsorption of proteins onto the dental surface. Most of these proteins originate primarily from saliva, with contributions from bacteria, crevicular fluid, and epithelial cells [3,4]. Pellicle formation is a dynamic and individual process characterized by continuous adsorption and desorption. It begins with the rapid adsorption of precursor proteins, such as proline-rich proteins, statherin, cystatin, and histatin, which form a basal layer on the dental surface within seconds, reaching a thickness of approximately 10–20 nm. Subsequently, protein aggregates bind to the basal layer, forming

the outer globular layer and contributing to the continued growth of the pellicle [3].

The amount and presence of specific proteins in the pellicle influences its protective capacity against demineralization. Patients with erosive tooth wear exhibit only half as many proteins in the 1-hour pellicle compared to healthy individuals [5]. Proteomic analyses have also revealed an accumulation of acid-resistant proteins in the pellicle. Notably, the relative expression of cystatin B increases 20-fold and 13-fold after exposure to citric and lactic acid, respectively, conditions that simulate erosive and cariogenic challenges [6]. Incorporating such proteins into dental products holds promise for enhancing protection against demineralization. However, recombinant human cystatin B remains expensive, highlighting the need for more cost-effective

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alternatives.

Inspired by natural cystatins, the first sugarcane-derived cystatin, CaneCPI-1, was produced using a heterologous expression system [7]. Building on its inhibitory activity against peptidases, several CaneCPI variants were developed [8]. The most recent variant, CaneCPI-5, demonstrated binding affinity to hydroxyapatite, as confirmed by atomic force microscopy. CaneCPI-5 also showed potential for pellicle engineering, as it improves the protective properties of the pellicle against erosion [9]. Furthermore, since dental biofilm formation is initiated by interactions between bacterial adhesins and specific receptors in pellicle proteins, enriching the pellicle with peptides such as CaneCPI-5 may also influence early bacterial adhesion to the tooth surface, offering potential strategies for caries management [10].

This scoping review aims to provide a concise summary and critical overview of recent findings on pellicle engineering with CaneCPI-5. The focus lies on its role in enhancing the erosion-protective properties of the pellicle, regulating bacterial adhesion, modifying pellicle composition, and exploring its potential synergy with other similar substances.

2. Methods

This scoping review was conducted in accordance with the PRISMA-ScR guideline [11]. Studies were considered eligible if they investigated pellicle engineering on enamel or dentin substrates using human participants or human saliva donors, under *in vitro*, *in situ*, or *in vivo* conditions. Interventions had to include CaneCPI-5, with water, phosphate-buffered saline (PBS), artificial saliva or placebo serving as negative controls. Eligible studies were required to assess at least one of the following outcomes: bacterial adherence, protective effects, pellicle composition, or mechanisms. Studies were excluded if they involved animal models, edentulous subjects, or did not investigate CaneCPI-5 specifically for pellicle engineering. Additional exclusions applied to studies using CaneCPI-5 in combination with other agents, unless a direct comparison to CaneCPI-5 alone was provided. Only English-language articles were included, based on feasibility of accurate interpretation and resource constraints. No restrictions were placed on publication date.

The literature search was performed in the Medline (via PubMed), Scopus, and Web of Science databases, covering all publications up to May 8, 2025. The search strategy was developed by B.M.A.R. and refined in collaboration with the co-authors. For PubMed, the search used the following terms: “(CaneCPI OR cystatin-derived peptides OR sugarcane-derived cystatin) AND dentistry.” No filters were applied, and the strategy was adapted as needed for the other databases. Search results were exported to Microsoft Word, and duplicates were removed manually. Hand-searching, including checking reference lists, was not conducted.

Two reviewers (B.M.A.R. and C.J.V.F.) independently screened the titles and abstracts of retrieved records and assessed the full texts of potentially eligible studies. Discrepancies were resolved by consensus. Data were charted using a predesigned table in Microsoft Word, developed and completed by C.J.V.F. and S.A. The charting form was not piloted or calibrated. Extracted information included: authors, study design, substrate surface, treatment (including concentration and duration), control, investigation, and measurement method. The results were synthesized narratively, with studies grouped into thematic categories based on their primary focus: erosion, caries, or combinations with other agents.

3. Results

A total of 131 records were identified through database searches (Fig. 1). After removing duplicates and screening titles and abstracts, 20 studies met the eligibility criteria and were included in this review (Table 1). Among the included studies, 12 were conducted *in vitro*, 4 *in situ*, and 4 *in vivo*. Most studies focused on enamel ($n = 17$), while two included dentin and one addressed both. Interventions involved CaneCPI-5 applied alone or in combination with other substances, with water, PBS, artificial saliva or placebo serving as negative controls. The majority of studies ($n = 14$) evaluated erosion or erosion/abrasion protection, consistently reporting improved outcomes compared to negative controls. Four studies investigated proteomic changes and found that CaneCPI-5 promoted the accumulation of acid-resistant proteins in the pellicle. Five studies addressed bacterial adhesion or

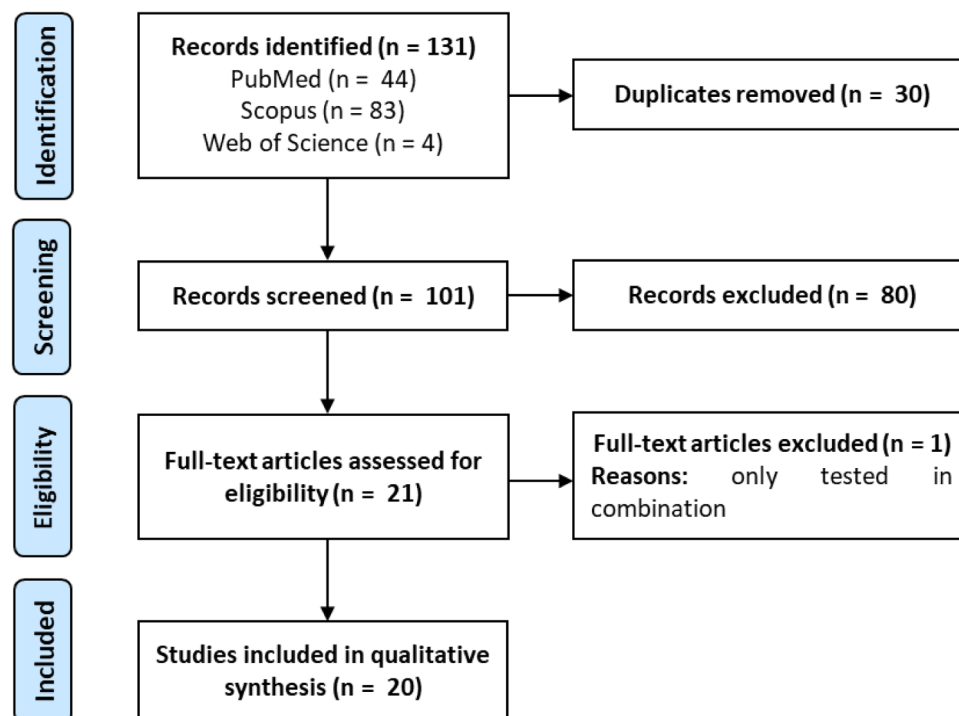


Fig. 1. Flow chart of study selection process.

Table 1

Overview of included studies aiming to investigate different aspects of modified pellicles engineered with CaneCPI-5.

Study	Aim	Design	Surface	Results
Araujo et al. (2021)	caries	in vitro	enamel	partially antibacterial effects; partial reduction lesion depth
Araujo et al. (2022)	proteome	in vivo	enamel	alteration of proteome
Araujo et al. (2024)	proteome; microbiome	in vivo	enamel	alteration of proteome and microbiome
Camara et al. (2021)	caries	in vitro	dentin	no effect
Carvalho et al. (2020)	proteome; erosion	in vivo	enamel	alteration of proteome; improved erosion protection
Carvalho et al. (2024)	proteome; erosion	in vivo	enamel	alteration of proteome; no effect on erosion protection
Da Silva et al. (2022)	caries	in vitro	enamel	no effect
De Oliveira et al. (2023)	erosion	in vitro	enamel	improved erosion protection
Ferrari et al. (2024)	erosion	in vitro	enamel	improved erosion protection
Gironda et al. (2022)	erosion; surface energy	in vitro	enamel	improved erosion protection at certain concentrations; reduced surface energy
Pela, Braga et al. (2021)	caries	in vitro	enamel	antibacterial effects; reduction of lesion depth
Pela, Lunardelli et al. (2021)	erosion; erosion/abrasion; cytotoxicity	in situ	enamel	improved erosion and erosion/abrasion protection; cytocompatibility
Pela, Buzalaf et al. (2021)	erosion	in vitro	enamel	improved erosion protection
Pela, Niemeyer et al. (2022)	erosion	in vitro	enamel	improved erosion protection
Pela, Brito et al. (2022)	erosion; erosion/abrasion	in situ	enamel	improved erosion and erosion/abrasion protection
Pela, Ventura et al. (2023)	erosion	in vivo	enamel	improved erosion protection
Pela, Gironda et al. (2023)	erosion; erosion/abrasion	in situ	dentin	improved erosion and erosion/abrasion protection
Pela et al. (2024)	erosion	in vivo	enamel	improved erosion protection
Santiago et al. (2017)	erosion; binding force	in vitro	enamel	improved erosion protection; relative high binding force
Santos et al. (2021)	erosion	in vitro	enamel; dentin	improved erosion protection

caries progression, with mixed results: some showed antibacterial effects or reduced lesion depth, while others reported no significant impact. One in vivo study found a synergistic effect when CaneCPI-5 was combined with vitamin E, whereas other combinations showed inconsistent or no added benefits. The authors frequently suggested that CaneCPI-5 enhances the protective properties of the pellicle against erosion likely through enamel binding and modulation of pellicle composition.

4. Discussion

4.1. Protection against erosion

Different analytical methods were used to evaluate protection against erosion, including measurement of calcium release using the Arsenazo III method or atomic absorption spectrometry, surface roughness analysis and substance loss through profilometry or surface reflection intensity, and morphological assessment by scanning electron microscopy. Calcium release and surface reflection intensity should be interpreted with caution, particularly in vivo studies where the pellicle is not removed, as both outcome measures may be affected not only by demineralization but also by the presence and modification of the pellicle [12].

Due to its reported binding affinity to enamel and presumed acid resistance, CaneCPI-5 was investigated for its effects on erosion protection in most included studies. However, this binding affinity was only assessed in comparison to mucin and casein [9]. Given that casein is not a typical oral protein and that other proteins commonly present in the pellicle were not included in the comparison [13–15], the classification of CaneCPI-5's binding affinity remains limited.

In order to control initial protein adsorption and guide pellicle formation, several in situ and in vitro studies applied CaneCPI-5 to pellicle-free surfaces [16–20]. However, this approach does not reflect the clinical situation, as pellicle-free surfaces do not occur in vivo due to the immediate adsorption of salivary proteins. One study that specifically investigated the sequence of application found that applying CaneCPI-5 prior to pellicle formation resulted in greater protective effects compared to post-pellicle application [16]. Nevertheless, protective effects were also observed when CaneCPI-5 was applied after pellicle formation, with repeated application, or under in vivo conditions.

Regarding application protocols, durations ranged from clinically relevant exposures of 1 min to experimental periods of up to 2 h, both of which improved pellicle protective properties. A concentration of 0.1 mg/mL was established as the standard, as saturation effects were observed at this level in the initial study [9]. In contrast, reduced efficacy at concentrations above 2 mg/mL was attributed to potential dimerization of CaneCPI-5, which may limit the availability of free peptides for enamel binding [18].

All studies assessed the effects of CaneCPI-5 against a negative control, typically using water for comparison. In some cases, positive controls consisting of the commonly used AmF/NaF/SnCl₂ were included. Notably, CaneCPI-5 consistently improved protective properties compared to water and, when positive controls were included, showed comparable effects [12,16,17,21–23]. However, it is important to note that the protective effects of the modified pellicle are limited and may be lost under severe erosive challenges, such as exposure to 0.01 M hydrochloric acid [24]. Beyond chemical erosion, the modified pellicle also showed improved protection against mechanical wear of previously eroded surfaces. Due to the absence of specific controls, however, the contribution of CaneCPI-5 to abrasion resistance remains unclear [21, 23,25].

Several mechanisms have been proposed for the effect of CaneCPI-5. It showed affinity for the enamel surface and may act as an acid-resistant pellicle component, thereby directly contributing to erosion protection [19]. In this context, CaneCPI-5 has been proposed to alter the selective adsorption of salivary proteins by binding to the dental surface and limiting available binding sites [19,22]. Alternatively, it may reduce surface free energy, thereby influencing protein selectivity [18]. From a pellicle engineering perspective, proteomic analyses showed that CaneCPI-5 alters pellicle composition, promoting the accumulation of acid-resistant and erosion-protective proteins [24,26–28]. In particular, the pellicle proteome has been shown to contain increased levels of cystatins and keratins within minutes after CaneCPI-5 rinsing [26]. These proteins are commonly associated with enhanced acid resistance [6]. In addition to its role in enamel protection, CaneCPI-5 also inhibits

cathepsins, which are involved in organic matrix degradation during the biphasic erosion process of dentin [9]. Although studies on dentin are limited, available data also suggest a protective effect of CaneCPI-5 [22, 25].

CaneCPI-5 shows potential as a pellicle-modifying agent with anti-erosive properties. Further studies are needed to elucidate its mechanism in the presence of a mature pellicle and its efficacy under clinical conditions, especially on dentin.

4.2. Prevention of caries

Despite established preventive measures in terms of nutrition, oral hygiene, and fluoridation, the global prevalence of caries remains high (Bernabe et al., 2025). Consequently, research into novel prevention strategies is ongoing.

In contrast to the effects of CaneCPI-5 on the erosion-protective properties of the pellicle, the results regarding biofilm and caries inhibition are less consistent. The methods used in these studies included metabolic assays, colony-forming unit counts, live/dead staining, and the assessment of mineral content and lesion depth in artificial carious lesions using transverse microradiography. CaneCPI-5 concentrations ranging from 0.05 mg/mL to 1.0 mg/mL were applied and chlorhexidine was most frequently used as a positive control. However, unlike fluorides, chlorhexidine lacks clear evidence of caries prevention [29], making it difficult to classify the anti-caries properties of CaneCPI-5. One included study demonstrated that daily treatment with CaneCPI-5 disrupted bacterial metabolism and inhibited biofilm formation, although the underlying mechanisms remain unclear. The authors suggested that changes in the pellicle, and consequently in bacterial adherence, may be responsible [10]. An in-situ study further showed that CaneCPI-5 can alter the microbiome, although the observed changes were minor. No significant differences in alpha or beta diversity were detected, likely because the 3-h biofilm was still in its initial state and limited to individual adherent bacteria [28]. However, the remaining studies did not show any significant reduction in bacterial metabolism or biofilm formation on enamel or dentin, nor was there a decrease in caries progression compared to the negative control. In contrast, the positive control consistently demonstrated significant effects [30–32]. Interestingly, unlike the erosion-protective properties, some studies observed a concentration-dependent effect on biofilm and caries-related outcomes [10,32]. This raises the question of whether dimerization of CaneCPI-5 plays a role in its antibacterial activity. A limiting factor in the in vitro studies was the preparation of saliva used for pellicle formation. The saliva was not only stored diluted with glycerol (7:3) but was also further diluted 1:50 with a protein-containing artificial saliva before use. As a result, the concentrations of native salivary proteins were distinctly reduced and no longer representative of natural saliva.

In summary, while CaneCPI-5 shows promising potential for pellicle engineering in erosion prevention, it demonstrates little to no effect on bacterial biofilm formation or caries lesion progression under the tested conditions.

4.3. Combination with other substances

Various combinations with CaneCPI-5 have been investigated. Incorporation into chitosan gel is not discussed here, as no chitosan-free CaneCPI-5 controls or consistent experimental conditions between groups were provided [22,23,25]. Among the included studies, the synergistic effects of CaneCPI-5 with hemoglobin and statherin-derived peptides were most frequently examined, as these salivary proteins had previously been identified as acid-resistant pellicle components [6]. The results, however, were inconsistent. In some cases, the combination was less effective in enhancing the erosion-protective properties of the pellicle than the individual application of each component [27]. In other studies, only one component improved protective properties, but not the

combination [24]. Contradictory findings within the same study were also reported, for instance, surface reflection improved significantly, while calcium release measurements showed no effect [19]. In other cases, neither the individual substances nor their combinations produced any measurable effect [31]. Overall, there appears to be a trend indicating that acid-resistant peptides or proteins with a high affinity for hydroxyapatite do not act synergistically under the chosen test conditions. The authors speculated that competitive selective binding may occur at the dental surface, which could hinder simultaneous adsorption [19,24,27,31].

Additionally, a combination with the lipophilic substance vitamin E was also studied. This was based on the rationale that the pellicle naturally contains lipids and that vitamin E possesses anti-erosive properties [33]. In the included study, CaneCPI-5 and vitamin E were applied sequentially and demonstrated an enhanced erosion-protective effect compared to each substance used alone. Beyond this additive effect, the authors also proposed a synergistic mechanism, suggesting that the interaction of CaneCPI-5 with the pellicle may be improved by the lipophilic properties of vitamin E [16].

In another study, CaneCPI-5 was combined with sodium trimetaphosphate, a substance previously shown to possess anti-erosive effects by reducing the free surface energy of enamel and increasing the number of electron donor sites, thereby promoting the adsorption of calcium and phosphate ions. However, the results were contradictory, with discrepancies between microhardness measurements and calcium release data, making it difficult to determine whether a synergistic effect occurred [17].

Overall, studies investigating combinations of CaneCPI-5 with other substances are heterogeneous. Due to the lack of research on the mechanisms of interaction, no definitive assessment can be made, and further investigations are necessary.

4.4. Limitations and outlook

The main limitation of this review is that all available studies on the substance were conducted by the authors' own research group. As a result, there is a high degree of self-citation and an increased risk of bias, further amplified by the narrative nature of the review. To enhance objectivity, the literature search was conducted systematically, and researchers from another working group were included for critical evaluation. Furthermore, a publication bias may be present concerning the erosion-protective properties, as studies with positive outcomes appear to be disproportionately published.

Moreover, there are methodological limitations in the included studies. The majority were conducted in vitro, which does not adequately represent the dynamic and individual conditions of the oral cavity. However, such studies do offer valuable initial insights into the potential effects of the substances. Regarding the caries-preventive effects, the available research is even more limited. No meaningful extrapolation to real-life conditions is possible, even from in vivo studies, due to the slow progression of caries over time.

Given the high production costs of CaneCPI-5 and the lack of studies on long-term effects, its use over more established substances, such as tin-containing fluoride compounds, which are known to have astringent properties and cause discoloration with frequent use [34], cannot currently be justified. However, so far, no side effects have been reported for CaneCPI-5 in short-term applications [21,27].

Further studies are required to clarify the mechanism of action of CaneCPI-5 in pellicle engineering. Previous studies have focused on the outcomes or changes in the pellicle proteome, but since CaneCPI-5 was primarily applied during initial pellicle formation, no definitive conclusions can be drawn about the exact mechanisms. Future research should include theoretical or experimental interaction studies with pellicle proteins, as well as investigations into the interaction with a mature pellicle, where access to the dental surface, and thus the often-cited binding to enamel, would be limited.

5. Conclusion

CaneCPI-5 shows promising potential for pellicle engineering, particularly in enhancing erosion protection through enamel binding and modification of pellicle composition. Its effects on bacterial adhesion and caries prevention remain limited and inconsistent. While combinations with other agents have shown mixed results, synergy with vitamin E appears encouraging. Despite these findings, further independent and clinically relevant studies are needed to clarify mechanisms, long-term efficacy, and practical application.

CRedit authorship contribution statement

João Victor Frazão Câmara: Writing – original draft, Investigation. **Anton Schestakow:** Writing – original draft, Investigation. **Carolina Ruis Ferrari:** Writing – review & editing, Investigation. **Matthias Hannig:** Writing – review & editing. **Marília Afonso Rabelo Buzalaf:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

I have nothing to declare.

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Supplementary materials

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