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Phosphorylated pullulan as a local drug delivery matrix for cationic antibacterial chemicals to prevent oral biofilm

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Abstract

Background Preventing oral infections, such as oral caries and periodontal disease, helps reduce the risks of various systemic diseases. In this study, the polysaccharide pullulan produced by the black yeast *Aureobasidium pullulans* was modified in combination with the cationic surfactant cetylpyridinium chloride (CPC) to create a local drug delivery system, and its antibacterial potential on oral bacteria was examined in vitro.

Methods Pullulan was phosphorylated at the CH₂OH residue of α6 in the maltotriose structure and mixed with CPC. Bacterial attachment of cariogenic *Streptococcus mutans* on hydroxyapatite plates (HAPs) treated with the phosphorylated pullulan (PP) and CPC compound (0.01% PP and 0.001–0.03% CPC, and vice versa) was assessed by observing bacteria using a field emission scanning electron microscope (FE-SEM) and quantified through 16 S rRNA amplification via real-time polymerase chain reaction (PCR). Additionally, the quartz crystal microbalance (QCM) method was employed to evaluate the sustained release of CPC.

Results PP-CPC compound maintained significant bactericidal activity even at 0.01%, which is one-fifth of the conventional applicable concentration of CPC. Additionally, a residual mixture was detected by the hydroxyapatite sensor of the crystal oscillator microbalance detector, suggesting an unknown molecular interaction that enables the sustained release of CPC after attachment to hydroxyapatite.

Conclusions The combination of PP and CPC may contribute to the low concentration and effective prevention of oral infections, such as dental caries.

Keywords Phosphorylated Pullulan, Local drug delivery system, Cationic antimicrobial agents, Cetylpyridinium chloride, Oral biofilm

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Background

Biofilms composed of diverse bacterial flora within the oral cavity adhere to tooth surfaces and the gingival sulcus [1, 2]. These oral bacteria, such as *Streptococcus mutans* and *Porphyromonas gingivalis*, not only contribute to infections such as dental caries and periodontal disease, but they are also implicated in systemic conditions, including atherosclerosis [3, 4] and aspiration pneumonia, which are particularly prevalent among the elderly [5, 6]. Thus, the prevention of oral infectious diseases plays a crucial role in maintaining both oral and systemic health. Reducing attachment and growth of early colonizers of the oral microbiome may be an advantage for healthcare providers in intervening in these patients less frequently.

Antibacterial agents effectively inhibit biofilm formation and prevent oral infections [7, 8]. Various dental materials and oral care products that incorporate antibacterial agents have been investigated and developed. Among these, chlorhexidine gluconate (CHX), cetylpyridinium chloride (CPC), and isopropylmethylphenol (IPMP) have been widely applied to dental materials. CHX and CPC are cationic compounds, while IPMP is a neutral compound. CHX-containing products are considered the gold standard for chemical plaque control in Europe and the United States [9–13]; however, high concentrations applied to oral mucosa have been associated with anaphylactic reactions [14, 15]. Although IPMP exhibits inferior antibacterial activity compared to CHX and CPC, it demonstrates superior biofilm permeability [16, 17]. CPC possesses potent bactericidal activity even at low concentrations and is commonly used not only in dentifrices and mouthwashes but also in throat lozenges. However, its limited adhesion to tooth surfaces restricts its efficacy [18, 19]. When used in mouthwashes, the bactericidal effect of CPC is transient, raising concerns regarding its sustainability. Additionally, in Japan, the Pharmaceutical Affairs Law mandates that the concentration of CPC in oral care products must not exceed 0.05%. Although this concentration is lower than that associated with anaphylactic reactions, achieving sufficient antibacterial activity at even low concentrations would be preferable for long-term use. Therefore, the development of an antibacterial local drug delivery system (LDDS) that ensures sustained efficacy at low concentrations is essential for preventing oral infections.

To address this issue, we focused on pullulan as a delivery material. Pullulan is a polysaccharide with the general formula $\{[C_6H_{10}O_5]_n\}_m$, composed of maltotriose units (degree of polymerization: 100–5,000), linked via α -1,6 glycosidic bonds. It is synthesized by *Aureobasidium pullulans* (*A. pullulans*) using starch as a substrate. In Japan, pullulan has been widely utilized as a food additive for over 20 years, serving as a thickener, stabilizer,

glue, and adhesive. Additionally, it is classified as generally recognized as safe (GRAS) in the United States [20]. The functionality of pullulan can be further enhanced by introducing charged or reactive groups, enabling its application in various fields such as food, cosmetics [21, 22], and multifunctional drug delivery systems [23, 24]. Among these modifications, negatively charged phosphorylated pullulan (PP), particularly its sodium salt (sodium PP) [25–27], has been reported to adhere to hydroxyapatite, the primary component of teeth (Fig. 1), and undergo gradual autolysis, thereby releasing the encapsulated compounds [28, 29]. Based on these properties, we hypothesized that pullulan could serve as a carrier for antibacterial agents in LDDS. This study aimed to evaluate the potential of the PP-CPC complex as LDDS by analyzing the sustained release of CPC and its inhibitory effect on the growth of *Streptococcus mutans* (*S. mutans*), a key cariogenic bacterium.

Materials and methods

Preparation of phosphorylated pullulan (PP) and starches

The synthesis of phosphorylated polysaccharides was performed according to the report by Yonehiro et al. [25]. 8.5 g of pullulan 21 $\{[C_6H_{10}O_5]_n\}_m$, extracted from *A. pullulans* (Hayashihara Co., Ltd., Okayama, Japan), was dissolved in 38 mL of distilled water. Two hundred mL of a 1 mol/L aqueous solution of phosphoric acid was then added, and the reaction was carried out at 170 °C for 5 h. The phosphorylated compound was recovered by ethanol precipitation [25, 30]. As previously reported [25], chemical phosphorylation occurred at the CH₂OH residues at the α -6 position of the maltotriose units within the pullulan structure, yielding sodium PP (Fig. 1). For comparison, additional phosphorylated polysaccharides were examined as control materials, including phosphorylated polysaccharide Sunus 514 (derived from the tapioca starch family) and Trecomex AET4 (derived from the potato starch family), both obtained from Nihon Denpun Kogyo KK (Kagoshima, Japan). Additionally, glucose 6-phosphate (Merck, Darmstadt, Germany), a metabolite involved in glycolysis in vivo, was evaluated. All phosphorylated polysaccharides were stored in sealed containers under cool and dry conditions and were dissolved in double-distilled water prior to use.

Inorganic materials

The cationic cetylpyridinium chloride (CPC: SIGMA) (Fig. 1) was employed as a bactericidal agent and dissolved in double-distilled water for use. Synthetic sintered hydroxyapatite plates (HAPs: 10 × 10 × 2 mm) polished to a mirror finish (Apatite: APP-101, Pentax, Tokyo, Japan) were used as an artificial model for tooth enamel surfaces.

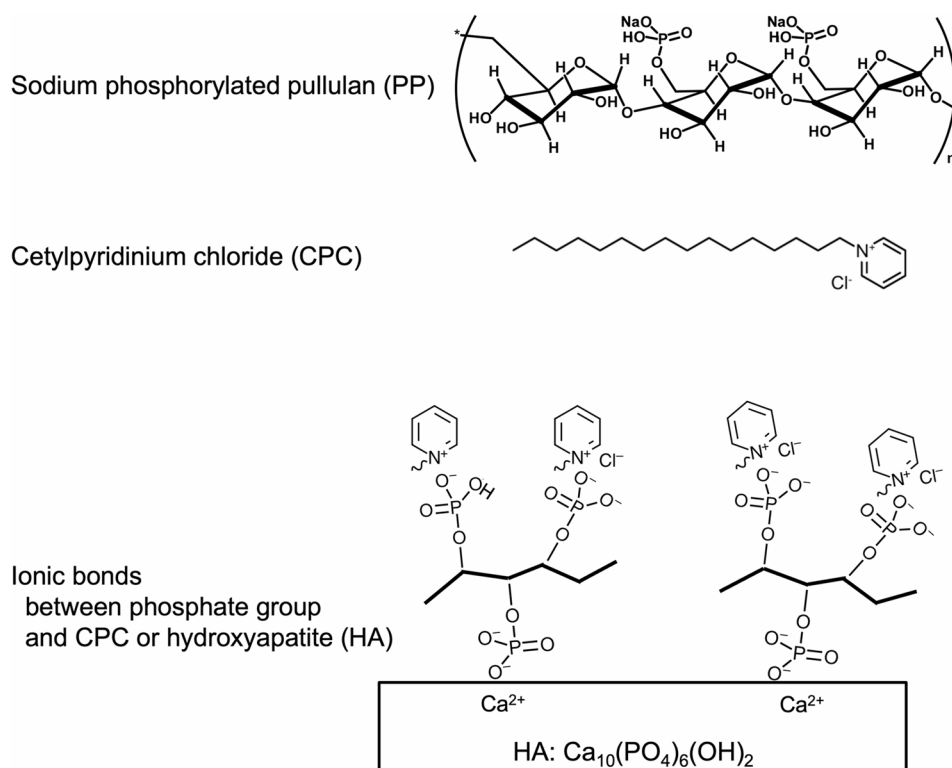


Fig. 1 Schematic representation of the proposed ionic interaction between PP-CPC and hydroxyapatite. CPC bound to the sulfate groups of PP is hypothesized to attach to the hydroxyapatite surface through ionic interactions between the sulfate groups of PP and the calcium ions present in hydroxyapatite

Microbial cultures

The procedures were conducted following the method described in our previous study [31]. *S. mutans* 854 S, cariogenic gram-positive anaerobic bacteria, was cultured in tryptic soy broth medium (TSBY: Becton, Dickinson and Company, Sparks, MD, USA) with 0.5% yeast extract (Becton, Dickinson and Company) and 10 µg/mL erythromycin (SIGMA, St. Louis, MO, USA) [32]. TSBY with 5% sucrose was used as the culture medium for the biofilm formation test. The bacterial suspension was cultured until it reached the logarithmic growth phase and then suspended in the medium to achieve 1.0×10^5 CFU/mL by measuring the absorbance at a wavelength of 570 nm (SPECTRONIC 20 A: Shimadzu Corporation, Kyoto, Japan).

Human saliva

Fifteen mL aliquots of human saliva were collected from three healthy volunteers without brushing or dietary changes over a period of two hours (Ethics Committee Approval #1089). The saliva supernatant was centrifuged at $1,710 \times g$ for 20 min at 4 °C to reduce saliva-derived microorganisms. The saliva samples from individuals were pooled after centrifugation to reduce individual differences, and the mixture was used in this study.

Procedure for bactericidal test

The procedures were conducted following the method described in our previous study [31]. HAPs were immersed in 4 mL of PP-CPC solution and incubated at 37 °C for up to 12 h in the wells of 12-well culture plates (Costar, Corning Inc., Corning, NY, USA). The HAPs were rinsed twice in distilled water and dried using gentle air blows. Subsequently, the HAP samples were exposed to *S. mutans* by adding 4 mL of microbial suspension (1.0×10^5 CFU/mL) in new 12-well culture plates. The culture plates were incubated at 37 °C for up to 12 h to allow bacterial growth. Following incubation, the HAPs were removed from the wells and subjected to further analysis as described in the next sections.

The bacterial growth on PP-CPC-coated HAPs in the presence of a salivary pellicle was also evaluated. After coating the HAPs with PP-CPC, the samples were immersed in 4 mL of human saliva (collected as described above) and incubated overnight at 4 °C to allow pellicle formation in new 12-well culture plates. Subsequently, the HAPs were gently rinsed by pipetting with phosphate-buffered saline (PBS: pH 7.2) in the same plates by changing solution in wells, then immersed in an *S. mutans* suspension (1.0×10^5 CFU/mL) and incubated at 37 °C for 12 h in new 12-well culture plates.

Observation using field emission scanning electron microscopy

The procedures were conducted following the method described in our previous study [31]. Bacterial growth and morphology on the tested HAPs were observed using a field emission scanning electron microscope (FE-SEM: Topcon DS-720, Tokyo, Japan). The HAPs were washed twice with a 0.15 mol/L NaCl solution containing 0.01 mol/L cacodylate buffer (pH 7.0) and then fixed with 1% glutaraldehyde. The sample HAPs were subsequently washed twice with the same buffer solution and dehydrated using an ascending ethanol series (50, 70, 90, 95%, and absolute ethanol). The HAPs were immersed in 3-methylbutyl acetate for 1 h to facilitate substitution, followed by critical point drying (JCPD-5, JEOL, Tokyo, Japan). Finally, a platinum-palladium coating was applied to the surfaces of the HAPs (Eiko IB-3 Ion Coater, Eiko Engineering, Ibaraki, Japan), and the HAP samples were observed under FE-SEM at an acceleration voltage of 15 kV.

Quantification of microbial 16 S rRNA using real-time PCR

Viable bacterial amounts on the HAP surfaces were evaluated by quantifying 16 S ribosomal ribonucleic acid (rRNA) [33]. The quantification of the bacterial 16 S rRNA was performed as previously described [34] using the quantitative reverse transcription polymerase chain reaction (RT-PCR) protocol (SYBER® Green; PE Applied Biosystems, Foster City, CA, USA). Prior to RNA extraction, the HAPs were washed twice with PBS. Total RNA was then extracted using TRIZOL LS Reagent® (Invitrogen, Carlsbad, CA, USA). To remove genomic deoxyribonucleic acid (DNA) contamination, the extracted RNA was treated with 0.2 unit/μL of DNase I (Takara Bio, Shiga, Japan) and 0.4 unit/μL of RNase Inhibitor (Invitrogen) in the presence of the associated enzyme reaction buffer (total volume: 50 μL) and incubated at 37 °C for 30 min. Complementary DNA (cDNA) synthesis was performed via reverse transcription at 42 °C for 50 min using 50 ng of random primers, 1 unit/μL reverse transcriptase, and 2 nmol/L dithiothreitol in the presence of 0.2 mmol/L dNTP mix (all reagents from Invitrogen), in a total reaction volume of 20 μL. The quantification of the 16 S rRNA gene was conducted via real-time PCR using GeneAmp® 5700 Sequence Detection System (PE Applied Biosystems). A 25 μL mixture was prepared, containing 20 pmol of each universal primer (forward: 5'-GTGSTGCAYGGYTGTCTGCA-3'; reverse: 5'-ACGT-CRTCCMCACCTTCCTC-3') and 2 × SYBR® Green. PCR amplification was carried out for 40 cycles, consisting of denaturation at 95 °C for 15 s, followed by annealing and extension at 60 °C for 60 s. Fluorescence emission from the PCR product was measured using GeneAmp® 5700 SDS software (PE Applied Biosystems).

Evaluation of sustained CPC release via quartz crystal microbalance

The delivery of CPC to HAP surfaces by PP and its sustained release from the surface were evaluated using the quartz crystal microbalance (QCM) method. A QCM apparatus (QCM-D300, Q-Sense, Sweden) equipped with an HAP-coated sensor was employed for these measurements. The pellicle layer on the HAP surface was formed using synthetic saliva (50 mmol/L HEPES buffer, 1.09 mmol/L CaCl₂, 0.68 mmol/L KH₂PO₄, 30 mmol/L KCl, and 2.6 μmol/L F) prepared according to the method reported by Sieck et al. [35]. The measurement protocol involved the sequential flow of different solutions through the sample chamber: distilled water for 5 min (0–5 min), the test solution for 60 min (5–65 min), followed by another wash with distilled water for 70 min (65–135 min). Real-time analysis of CPC absorption and dissociation, as well as its interactions with the carrier and composite, was performed based on the frequency shifts of the crystal oscillator.

Statistical analysis

The statistical analysis of the results from the real-time PCR method was conducted using one-way ANOVA and Scheffe's F-test. The software used for analysis was Stat-View (Version J-4.5, Abacus Concepts, Berkeley, CA), and the statistical significance level set was $p < 0.05$.

Results

PP-CPC decreased bacterial growth on HAPs

The effects of a mixed solution of PP and CPC at various concentration ratios on *S. mutans* were observed using FE-SEM (Fig. 2A and B). The CPC concentration was maintained below 0.05%, in accordance with the upper limit stipulated by the Japanese Pharmaceutical Affairs Law, to ensure suitability for long-term use, considering cytotoxicity concerns. FE-SEM analysis revealed an inhibitory effect on *S. mutans* biofilm formation when treated with a combination of 0.01% PP and 0.01% CPC or 0.02% PP and 0.01% CPC (Fig. 2A and B). Based on these findings, subsequent experiments were conducted using a CPC concentration of 0.01% or lower.

Polysaccharides from other foods did not inhibit bacterial growth

As comparative polysaccharides, tapioca starch and potato starch were phosphorylated using the same method as for pullulan. Additionally, glucose-6-phosphate was employed as a monomeric control, and native pullulan was used as a negative control. Each of these polysaccharides and glucose-6-phosphate was mixed with 0.01% CPC, and their effects on *S. mutans* growth were evaluated using FE-SEM as previously described (Fig. 2C). Among the tested combinations, only the PP

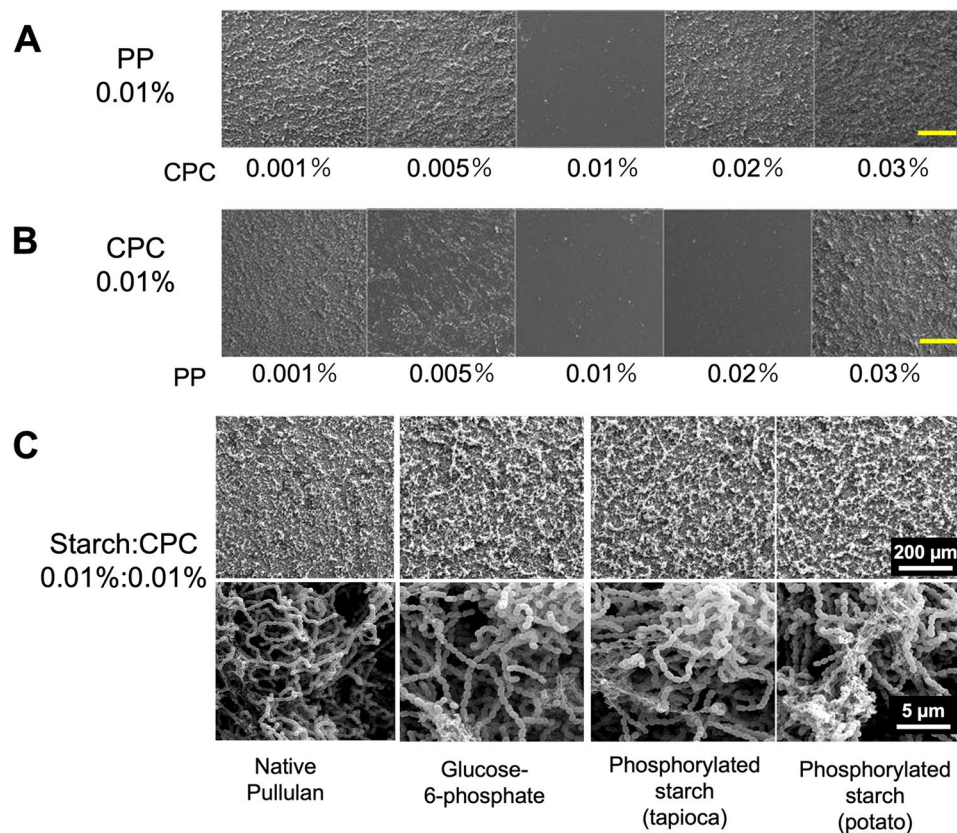


Fig. 2 Bacterial growth on HAPs treated with PP-CPC and other phosphorylated starches. **A** HAP samples were treated with a mixture of PP and CPC for 2 h, washed twice with distilled water, slightly dried, and then incubated in a bacterial solution at 37 °C for 12 h. The FE-SEM image shows *S. mutans* colonization on HAP treated with 0.01% PP and 0.001–0.03% CPC. Scale bar: 100 μm. **B** HAP samples were treated as described in (A), but with varying concentrations of PP (0.001–0.03%) and a fixed concentration of CPC (0.01%). The FE-SEM image illustrates the adherence of *S. mutans* under these conditions. Scale bar: 100 μm. **C** Two types of starch, derived from tapioca and potato, were phosphorylated using the same procedure applied to pullulan. The antibacterial activities of these phosphorylated starches, combined with CPC at 0.01% vs. 0.01%, were assessed against *S. mutans*. Similar to native pullulan and glucose-6-phosphate, the phosphorylated starches showed no antibacterial effect (Starch: CPC = 0.01%:0.01%). Scale bars: upper panel, 200 μm; lower panel, 5 μm. These experiments were conducted in duplicate, involving at least three independent trials. Typical results are presented

and CPC mixture demonstrated an inhibitory effect on biofilm formation.

PP-CPC decreased bacterial 16 S rRNA on HAPs

Bactericidal activity was assessed by quantifying bacterial 16 S rRNA levels (Fig. 3A). The results showed a significant reduction in 16 S rRNA levels (approximately $1/10^4$) following treatment with 0.01% PP and 0.01% CPC, compared to the untreated control plate (negative control) (Fig. 3A). Furthermore, FE-SEM imaging revealed a minimal presence of *S. mutans* on HAP surfaces treated with 0.01% PP and 0.01% CPC, whereas extensive bacterial colonization was observed on HAP treated with 0.01% PP alone, which was comparable to that on untreated HAP (Fig. 3B). Although treatment with 0.01% CPC alone resulted in a slight reduction in *S. mutans* growth compared to untreated HAP, the combined treatment with 0.01% PP and 0.01% CPC exhibited markedly enhanced antibacterial efficacy effects.

PP-CPC reduced bacterial growth on HAPs with pellicle

Salivary deposits were observed on HAP surfaces following saliva treatment (saliva-treated HAP in Fig. 4), and *S. mutans* colonization was evident on these surfaces in the absence of antimicrobial agents (Control in Fig. 4). However, treating saliva-coated HAP with a combination of 0.01% PP and 0.01% CPC (PP + CPC in Fig. 4) significantly reduced bacterial growth. Bacteria were still detectable on the salivary deposits, but at considerably lower levels.

PP-CPC coating enabled the sustained release of CPC from the HAPs

To investigate the bonding and releasing mechanism of CPC on HAPs, QCM analysis was performed to monitor frequency changes associated with the adhesion of test materials to the HAP sensor surface. Injecting 0.01% CPC only onto untreated HAP resulted in minimal frequency changes before and after washing (Fig. 5; green line). In contrast, injecting a mixed solution containing

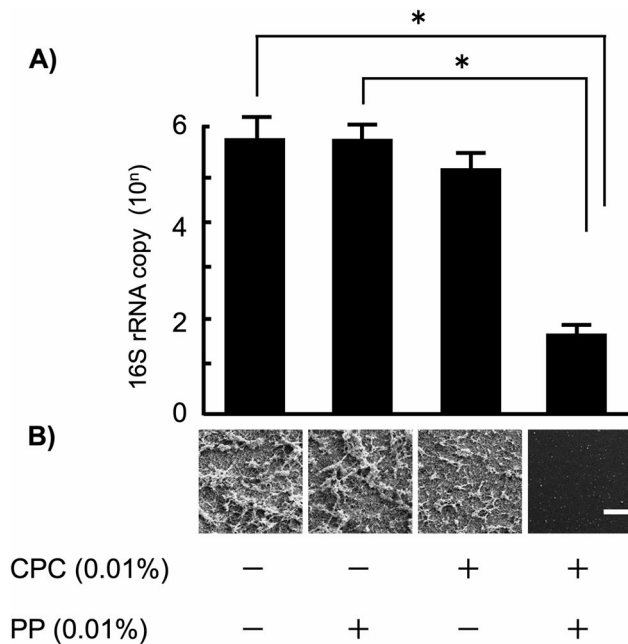


Fig. 3 Antibacterial activity of PP-CPC evaluated by quantifying *S. mutans* 16 S rRNA copies. HAP samples were treated with a mixture of PP and CPC for 2 h, washed twice with distilled water, slightly dried, and then incubated in a bacterial suspension at 37 °C for 12 h. These experiments were conducted in duplicate, involving at least three independent trials. **A** Total bacterial RNA was extracted from the HAP surface, and quantitative reverse transcription PCR (qRT-PCR) was conducted to measure the levels of *S. mutans* 16 S rRNA. The vertical axis indicates the quantity of 16 S rRNA, while the horizontal axis represents each treatment group. $n=6$, *: $P<0.05$. **B** Representative FE-SEM image of the HAP surface after treatment. Typical results are presented. Scale bar: 100 μ m

0.01% PP and 0.01% CPC into untreated HAP led to a decrease in frequency, indicating the adsorption of the PP-CPC mixture onto the HAP sensor surface (Fig. 5; blue line, before washing). Following washing, the frequency partially recovered, suggesting that while some of the adsorbed mixture was desorbed, a significant portion remained attached to the surface (Fig. 5; blue line, after washing). Similar trends were observed when the same mixed solution was injected onto HAP pretreated with synthetic saliva to form a pellicle; however, a greater frequency shift was recorded (Fig. 5; red line).

Discussion

FE-SEM observations revealed that the PP-CPC complex effectively inhibited the development of *S. mutans* on HAP surfaces at a CPC concentration of 0.01%. Quantitative analysis using bacterial 16 S rRNA measurements, alongside FE-SEM observations, further confirmed that the combination of 0.01% PP and 0.01% CPC most effectively suppressed bacterial growth (Figs. 2AB, 3). Moreover, although PP-treated and CPC-treated hydroxyapatite plates (HAPs) did not show bactericidal activity (Fig. 3), the mixture of 0.01% PP and 0.01% CPC could inhibit the growth of *S. mutans* even in the presence of saliva-derived pellicles on the HAP surface (Fig. 4). These observations suggest that CPC alone is easily washed off the HAP surface, and that PP alone, without bactericidal reagents, does not inhibit bacterial attachment. However, PP may persist on the HAP surface. The required concentration of CPC for antibacterial

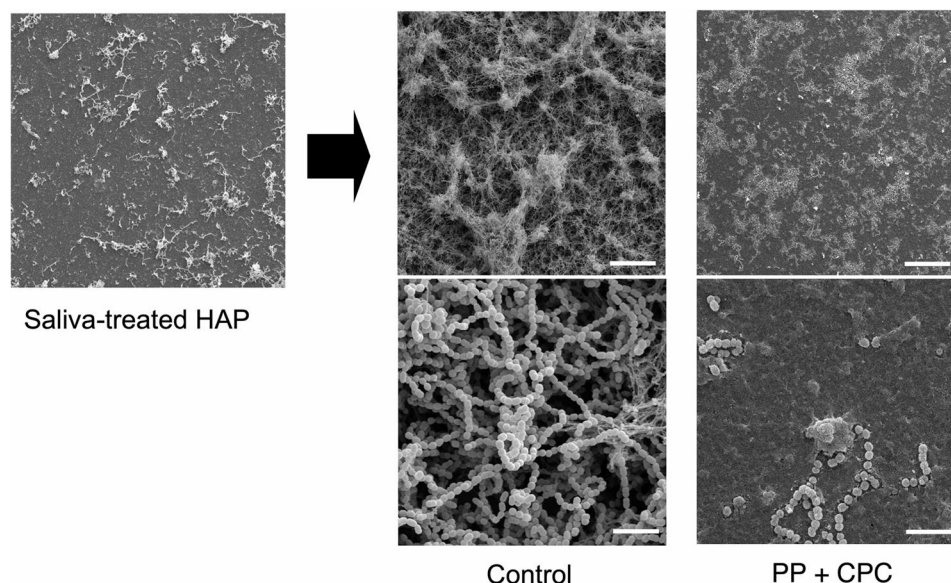


Fig. 4 Bacterial growth on HAP coated with saliva and treated with PP-CPC. HAP samples were pre-treated with human saliva for 1 h at room temperature to simulate oral conditions. Subsequently, the samples were treated with a mixture of PP and CPC for 2 h, rinsed twice with distilled water, slightly dried, and incubated in a bacterial suspension at 37 °C for 12 h. The FE-SEM images revealed extensive colonization of *S. mutans* on saliva-coated HAP; however, significantly fewer bacteria were observed in areas corresponding to saliva deposits on HAP samples treated with PP-CPC. These experiments were conducted in duplicate, involving at least three independent trials. Typical results are presented. Bar in the upper panel, 20 μ m; bar in the lower panel, 2 μ m

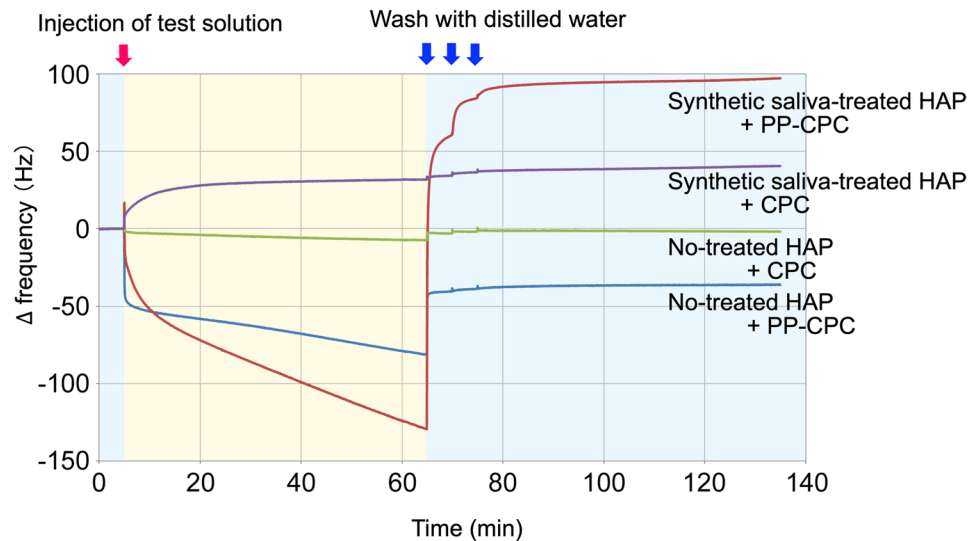


Fig. 5 Adsorption of PP-CPC on HAP surfaces. The adsorption and sustained release behavior of PP-CPC on HAP surfaces was evaluated using a QCM method. Upon injecting CPC or PP-CPC solutions onto the HAP surface, a notable decrease in frequency was observed with PP-CPC, while the decrease was less pronounced with CPC alone. After three rinses with distilled water, the frequency partially recovered but remained below the baseline, indicating that both CPC and PP-CPC stayed adsorbed on the HAP surface. In experiments using saliva-coated HAP, the frequency response differed significantly between CPC and PP-CPC treatments. The injection of CPC resulted in an increase in frequency above the baseline, suggesting a surfactant-like effect. In contrast, the injection of PP-CPC caused a substantial decrease in frequency, even greater than that observed with non-saliva-treated HAP, indicating stronger adsorption. Following rinsing, the frequency for both treatments rose noticeably above the baseline, which may reflect the removal of saliva-derived deposits from the HAP surface due to the surfactant effect of CPC. The y-axis represents frequency (Hz), and the x-axis represents time (minutes). These experiments were conducted in duplicate, involving at least three independent trials. Typical results are presented

efficacy was reduced to one-fifth when combined with PP. These findings suggest that the PP-CPC mixture may be suitable for long-term use without substantially disrupting the oral microbiota, as it demonstrates antimicrobial efficacy at concentrations lower than those conventionally employed.

As comparative polysaccharides, phosphorylated tapioca starch and potato starch were evaluated for their antimicrobial properties; however, they exhibited no inhibitory effect on bacterial growth (Fig. 2C). Given that glucose-6-phosphate contains a single phosphate group, it can interact only with either CPC or HAP surfaces, which likely explains its lack of antibacterial activity in this assay involving phosphorylated tapioca starch and potato starch. Tapioca starch comprises amylose, a linear α -1,4-linked glucose polymer, and amylopectin, a highly branched α -1,6-linked glucose polymer [36]. Potato starch contains linear amylose with approximately 1,000 glycosidic linkages and amylopectin with around 10,000 glycosidic linkages, exhibiting a more complex, luster-like branched structure [37]. In this study, basic characterization of the phosphorylated starches, such as the degree of phosphorylation, was not performed. Therefore, it is currently difficult to elucidate the reason for their lack of antibacterial activity. Further investigation will be necessary to address this issue.

Although several studies have reported on the preservation of dental pulp [25, 38, 39] and the promotion of

bone formation [30, 40–43], the detailed mechanism by which PP adheres to HAP and facilitates the release of CPC remains unclear. The results of QCM analysis in this study suggest that CPC adheres to the hydroxyapatite surface via ionic interactions mediated by PP and is subsequently released in a sustained manner to exert its antibacterial effects (Fig. 1, “Ionic bonds”). Moreover, the findings of this analysis indicate that the presence of a pellicle on the HAP surface enhances the adsorption of the PP-CPC complex, followed by partial desorption upon washing. Since untreated HAP exhibits stronger chemical binding of the PP-CPC complex compared to saliva-treated HAP, it is necessary to consider strategies such as applying the PP-CPC mixture after thoroughly cleaning and drying the tooth surface from a clinical perspective.

Hydrophobic polysaccharides modified with long-chain alkyl or cholesterol groups are known to self-assemble into nanoparticles with diameters of several tens of nanometers in dilute aqueous solutions [44–47]. A comparison of PP-CPC solutions at concentrations of 0.1%, 0.02%, and 0.01% showed that turbidity increased at both 0.1% and 0.01%, despite the solutions appearing transparent prior to mixing (Supplementary Fig. 1). It is known that such nanoparticles can be disassembled by dextrin, a feature that has drawn interest in drug delivery systems (DDS) for nanogel control [48–50]. These reports suggest that PP could form nanoparticles through

interactions with CPC. PP-CPCs exhibit potential as drug delivery carriers and represent promising materials for clinical applications. In this study, the antimicrobial efficacy was evaluated exclusively against one of the early colonizers, *S. mutans*. Future investigations should assess the antimicrobial activity against a broader range of early colonizers. Additionally, *in vivo* safety assessments using animal models and clinical trials are warranted to evaluate the feasibility of clinical application.

Conclusions

This study demonstrated that the effective concentration of CPC can be significantly reduced when PP is employed as a DDS carrier, compared to when CPC is used alone. QCM analysis further indicated that the PP-CPC mixture was adsorbed onto the surface of the apatite plate via interactions mediated by PP. These findings suggest that PP serves as an effective DDS carrier for the controlled delivery of antibacterial agents in the oral cavity. Moreover, since PP is expected to adhere to surfaces with a positive charge, its application as a DDS carrier may extend beyond dentistry to a broad range of biomedical fields.

Abbreviations

<i>A. pullulans</i>	Aureobasidium pullulans
cDNA	complementary DNA
CHX	Chlorhexidine gluconate
CPC	Cetylpyridinium chloride
DDS	Drug delivery system
DNA	Deoxyribonucleic acid
FE-SEM	Field emission scanning electron microscope
GRAS	Generally recognized as safe
HAP	Hydroxyapatite plate
IPMP	Isopropylmethylphenol
LDDS	Local drug delivery system
PBS	Phosphate-buffer saline
PP	Phosphorylated pullulan
QCM	Quartz crystal microbalance
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
<i>S. mutans</i>	<i>Streptococcus mutans</i>
TSBY	Tryptic soy broth medium

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12903-025-06666-z>.

Supplementary Material 1: Supplementary fig. 1. Turbidity changes due to concentration combinations of PP-CPC solutions. Photographs demonstrate the turbidity of PP-CPC solutions prepared at concentrations of 0.1%, 0.02%, and 0.01% for both PP and CPC. Note the differences in turbidity observed under top illumination. These experiments were conducted in duplicate, involving at least three independent trials. Typical results are presented

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Authors' contributions

Naoko Namba-Koide: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Funding acquisition. Yasuhiro Yoshida: Conceptualization, Methodology, Validation, Investigation, Resources, Visualization, Funding acquisition, Supervision. Yusuke Kawata: Investigation, Writing - Original Draft. Masahiro Ito: Formal analysis, Data Curation, Writing - Original Draft, Writing. Noriyuki Nagaoka, Takumi Okihara, Takashi Ito: Conceptualization, Methodology, Validation, Investigation, Resources, Visualization, Supervision. Yuki Shinoda-Ito, Kazu Takeuchi-Hatanaka, Kazuhiro Omori, Tadashi Yamamoto: Supervision, Writing - Review & Editing. Shogo Takashiba: Conceptualization, Validation, Writing - Review & Editing, Project administration, Funding acquisition.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study adhered to the Declaration of Helsinki, and approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (#1089). Written informed consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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