Testing a degradable topical varnish of cetylpyridinium chloride in an experimental dental biofilm model

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Dental biofilms are highly associated with the development of dental caries. Novel drug delivery systems are being developed in order to eliminate cariogenic bacteria from the dental biofilms. We formulated two degradable sustained release varnishes, based on acrylic resin, with cetylpyridinium chloride (CPC) as the active agent. These formulations were tested in a dental biofilm model. The retention of CPC in the biofilm was dependent upon the pharmaceutical additives of the varnish. Both varnishes decreased bacterial adhesion, while also demonstrating marked antibacterial properties against the bacteria in the biofilm.

Introduction
Dental biofilms are among the most virulent factors associated with dental disease. Biofilms are biological deposits on tooth surfaces containing salivary constituents, bacterial cell-free enzymes, such as fructosyltransferase (FTF) and glucosyltransferase (GTF), polysaccharides and bacteria. Elimination of cariogenic bacteria, which are immobilized in the dental biofilm, is an important step in the prevention and treatment of dental caries. The biofilm ecosystem is generally characterized by a lower susceptibility to antibacterial agents compared with planktonic bacteria. Given these differences, it is posited that testing a drug or drug delivery system in a biofilm model reflects more accurately the oral environment conditions than a planktonic environment.

Several topical varnishes intended for application directly on to the tooth surface have been developed to treat dental caries. These preparations differ in the polymeric matrix, pharmaceutical additives and the therapeutic agent. Most of these dental varnishes have incorporated active agents such as fluoride or chlorhexidine. Cetylpyridinium chloride (CPC) has high antimicrobial activity against oral bacteria, yet it has been used in only a few types of dental varnishes. It has been shown that increasing the retention of CPC in the biofilm results in enhanced antibacterial effect. This may be attributed to the ability of CPC to adsorb on to coated pellicle. Therefore, a locally applied degradable sustained release varnish (DSRV) of CPC, which exhibits increased contact time between the active agent and the dental biofilm, may enhance the microbial and clinical effects of CPC.

In this study, we tested two pharmaceutical formulations of locally applied degradable varnishes supplemented with CPC. Drug retention in the biofilm, antibacterial efficacy and anti-adherent properties of these varnishes were examined in a dental biofilm model.

Materials and methods
Preparation of the DSRV
Two different pharmaceutical DSRVs of acrylic resin polymer (Eudragit; Rohm Pharma, Darmstadt, Germany) were formulated based on preparations of Kozlovsky et al. as follows. (i) CPC-1: Eudragit, CPC, glycerin, sorbitol, menthol, sodium saccharin and arginine in ethanol–water solution; and (ii) CPC-2: Eudragit, CPC, glycerin, sorbitol, menthol, sodium saccharin, CaCl2 and NaOH in ethanol–water solution. As a control, placebo formulations were prepared as above, without CPC.

Construction of the experimental dental plaque biofilm
Glass rods of 5 × 0.3 cm were immersed in 10% NaOH. The rods were then washed thoroughly in distilled water. After autoclaving, the sterile rods were equilibrated with sterilized buffered KCl (50.0 mmol/L KCl, 0.65 mmol/L KH2PO4, 0.35 mmol/L K2HPO4, 1.0 mmol/L CaCl2, 0.1 mmol/L MgCl2)
Retention of CPC in the biofilm

The degradable varnish (total amount 1 mg CPC) was applied to the biofilm-coated rods by means of a soft brush. The varnish was dried completely and the coated rods were immersed in KCl buffer. The rods were transferred every 15 min to a new leaching buffer solution (pH 6.5). The leaching media were stored and analysed for CPC as follows. The CPC was examined by HPLC (Waters Instruments, MA, USA) (Ciano column of 10 μm particle size, 4.6 × 250 mm) connected to an automatic sampler. The mobile phase was prepared by mixing a buffer of 0.02 M tetramethylammonium hydroxide, adjusted to pH 3.5 by acetic acid, with acetonitrile, at a ratio of 70:30. The flow rate of the mobile phase was adjusted to 1 mL/min. The injection volume was 20 μL and retention time 5.2 min. The eluted CPC was detected by UV spectrophotometry at 258 nm.

The release profiles of CPC are presented as a percentage of the total amount of drug loaded.

Viability of bacteria in biofilm

The biofilms were covered by varnishes for a period of 2 h, as described above. The immobilized bacteria were detached from the biofilm by means of sonication (Simgen, Elma, Germany). Samples from the supernatant were serially diluted in saline. Selective agar media, mitis salivarius agar supplemented with bacitracin (MSB), was used for enumeration of S. sobrinus.® Fifty microlitres of the diluted samples were plated on to MSB plates using a manual turntable. The plates were incubated for 48 h at 37°C in air supplemented with 5% CO₂. Following incubation, the number of bacterial colonies grown on each plate was counted using a colony counter (New Brunswick Scientific, New Brunswick, NJ, USA).

Bacterial adhesion

The effect of the degradable varnish coatings on bacterial adhesion was examined as follows: ³H-radiolabelled S. sobrinus were prepared by supplementing the bacterial growth medium with 10 μCi/mL [³H]thymidine (ARC, St Louis, MO, USA) for 18 h. After incubation, the bacteria were washed three times with saline. Three millilitres of bacteria at an OD (540 nm) of 1.2 were introduced to rods coated with the varnish preparations. Following 2 h incubation, the rods were washed three times and transferred into scintillation vials containing 10 mL scintillation fluid (Eco-scint A; National Diagnostics, Manville, NJ, USA). The amount of adsorbed bacteria was measured in a scintillation counter (BETAmatic; Kontron, Basel, Switzerland), and was determined as a percentage of the radioactivity counts of the control group, which contained no active agent.

Statistical analysis

The results are presented as the mean of five different samples. Statistical significance was determined using the two-way ANOVA test. The level of significance was P < 0.05.

Results and discussion

Spurred by the debate regarding which drug or drug delivery system is optimal, the search continues for new antibacterial preparations to treat dental diseases. In this study, we have tested several properties of a new degradable varnish containing CPC, using a closed culture biofilm model comprised of host, cell-free enzymes, polysaccharides and oral bacteria. CPC was incorporated into degradable acrylic resins supplemented with pharmaceutical additives such as CaCl₂, NaOH or arginine in order to enhance the therapeutic efficacy of CPC. Our results indicate that several properties of the varnishes could be altered by changing the additives in the pharmaceutical formulation.

This type of manipulation affects the retention rate of CPC in the matrix. The CPC-1 formulation exhibited a slow, gradual retention profile of CPC in the biofilm (Figure). About 70% of the loaded drug was maintained on the surface for the first 15 min, after which a gradual decline in drug retention was recorded. After 90 min, about 25% of the loaded drug still remained on the surface, while the rest of the loaded drug was released within 24 h. When substituting the arginine component in the CPC-1 formulation with CaCl₂ and NaOH, there was a marked effect on the retention profile of CPC in the biofilm. About 90% of the drug remained in the biofilm during the initial 15 min of incubation. However, a sharp decline in retention was observed compared with the CPC-1 formulation. Only about 10% of CPC remained after 90 min; the rest of the loaded drug was released within 24 h.
Degradable varnishes tested in dental biofilm

The present study indicates that CPC can be incorporated into an acrylic resin varnish while maintaining its antibacterial and anti-adhesion properties. These effects were significant compared with the control. Although others have reported on SRVs with an extended drug release period, it seems that in order to overcome the low susceptibility of bacteria in the biofilm, a DSRV applied directly on to the biofilm is beneficial.

Several biofilm models are reported in the literature. Since a biofilm model represents more accurately the tooth surface environment, the use of a biofilm experimental model can offer preliminary indications of the basic properties of a formulation before commitment to further laboratory and human clinical trials.

One of the main pharmaceutical goals in preventing dental caries is to decrease the viable biofilm mass. One possible avenue for achieving this goal is by minimizing bacterial adhesion to the tooth surface, and consequently reducing the amount of bacteria in the biofilm. Any coating—biological or chemical—on the tooth surface may influence bacterial adhesion. The tested formulations had an inhibitory effect on the adhesion of S. sobrinus. The anti-adhesion effect was found with the CPC-2 formulation (P < 0.05). A similar anti-adhesion effect was also found with the CPC-1-coated biofilm (P < 0.05).

Another approach to influencing bacteria in the biofilm is by using antibacterial agents against bacteria harboured in the biofilm. Reducing bacterial counts in the biofilm requires usually higher doses of antibacterial agents than those required for planktonic bacteria. The local application of the varnish, coupled with a prolonged retention time of the drug, may lead to better antibacterial results against the immobilized bacteria in the biofilm. Our results show that both varnishes tested reduced bacterial viability in the biofilm significantly compared with the placebo.

Table. The anti-adhesion and antibacterial effects of degradable varnish containing CPC on accumulation of bacteria in biofilm

<table>
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<tr>
<th>Adhesion (%)</th>
<th>CPC-1</th>
<th>CPC-2</th>
<th>Control</th>
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<tr>
<td>32 ± 12a</td>
<td>55 ± 35a</td>
<td>100 ± 14</td>
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The anti-adhesion results are expressed as percentage of control, and the antibacterial effect on bacteria adsorbed on to the biofilm (cfu). *P < 0.05.

References


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