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Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach

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Setting time

Summary *Objectives*: Since atraumatic restorative treatment (ART) involves removal of carious lesions with manual instruments, improvement of filling materials to guarantee greater success should be considered. This study aimed to evaluate antibacterial, physical, and bonding properties of glass-ionomer cements (GIC) containing chlorhexidine (CHX), and to determine optimal concentrations for incorporation of agents to obtain antibacterial GICs for use with the ART approach.
Methods: CHX diacetate combined with CHX dihydrochloride was added to control GIC powder to obtain concentration ratios of 1/0, 2/0, 3/0, 1/1, or 2/2% w/w. Antibacterial activity of each cement against *Streptococcus mutans*, *Lactobacillus casei* or *Actinomyces naeslundii* was examined using agar-diffusion methods, and release of CHX was analyzed by HPLC. Compressive strength, bond strength to dentin, and setting time were measured, and compared with those of control samples.
Results: All experimental GICs exhibited inhibition of three bacteria, but sizes of inhibition zones and concentrations of CHX released were not dependent upon CHX content. Incorporation of CHX diacetate at 2% or greater, significantly decreased compressive strength, and bond strength to dentin was adversely affected by addition of CHX diacetate at 2% or more ($p<0.05$, ANOVA, Fisher's PLSD test), although setting time was extended a little by addition of any concentrations of CHX.
Significance: The present results demonstrate that experimental GICs containing CHX are effective in inhibiting bacteria associated with caries, and incorporation of 1% CHX diacetate is optimal to give appropriate physical and bonding properties.
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Introduction

Atraumatic restorative treatment (ART) is one of minimal intervention approaches in which demineralized tooth tissues are removed using manual instruments, and the cavity including adjacent pits and fissures is restored using a filling material, usually a glass-ionomer cement (GIC) [1-3]. The annual failure rate of single-surface ART restorations in permanent dentitions is about 4–5% [4]. Since ART can be performed under circumstances where neither electricity nor local anesthesia is required, it is possible that insufficient carious tissues are removed in the process of cavity cleaning. The prevalence of secondary caries with ART restorations in permanent dentitions over 3-6 years is reported to be 1.5–2.4% [5-7], and improvement of filling materials to overcome the problems caused by incomplete removal of infected dentin will be beneficial for increasing success rate of ART further.

Several attempts in developing GICs with antibacterial effects by the addition of bactericides such as chlorhexidine (CHX) have been reported [8-11]. However, incorporation of antibacterial agents frequently results in changes in physical properties of restorative materials [11-14]. Since the ART approach using GICs is indicative for use in posterior teeth, it is critical that the type of restorative material shows strong enough physical properties to resist occlusal load. Therefore, antibacterial GICs for use in the ART approach require an optimum amount of antibacterial agents, which should not jeopardize the basic properties of the parent materials.

This study aimed to evaluate antibacterial effects, physical properties and bond strength of GICs containing CHX diacetate and/or CHX dihydrochloride, and to determine the optimal concentration of CHX incorporation for obtaining antibacterial GICs for use with the ART approach.

Materials and methods

Materials

A conventional powder/liquid type GIC (Fuji IX, GC, Tokyo, Japan) was used as control. Experimental GICs were prepared by incorporating CHX diacetate (Sigma Aldrich, Steinheim, Germany) and/or CHX dihydrochloride (Sigma Aldrich) into the powder of control GIC at various concentrations (Table 1).

Table 1 Control and experimental GICs containing CHX tested in this study.

Code	Additives (w/w %)
Control	-
1/0	CHX diacetate 1%
2/0	CHX diacetate 2%
3/0	CHX diacetate 3%
1/1	CHX diacetate 1%, CHX dihydrochloride 1%
2/2	CHX diacetate 2%, CHX dihydrochloride 2%

Agar-diffusion tests

Antibacterial activities of unset or set cements against *Streptococcus mutans* NCTC10449, *Lactobacillus casei* ATCC4646, and *Actinomyces naeslundii* (formerly *viscosus*) ATCC19246 were assessed using agar-diffusion tests. Each bacterial strain from stock cultures stored in 50% glycerol at −20 °C was cultivated in Brain Heart Infusion (BHI; Becton Dickinson, Sparks, MD, USA) broth at 37 °C, and a loopful inoculum was transferred to 10 mL of BHI broth. After incubation for 24 h, for *S. mutans;* or 48 h, for *L. casei* and *A. naeslundii,* 350 μL bacterial suspension was spread onto a BHI agar plate and left for 30 min at room temperature.

Powder and liquid of each material were mixed for 30 s (P/L ratio = 3.6). The paste was then put into a mold (10 mm in diameter, and 2 mm thickness), and allowed to set for 30 min at 25 °C after covering the surface with a glass slide. The set disc-shaped specimens were placed onto a BHI agar plate, inoculated with each bacterial strain. For unset specimens, a well of 10 mm diameter was punched in a bacterium-inoculated BHI agar plate and filled with the paste using a syringe.

Plates were incubated at 37 °C for 48 h, and diameters of zones of inhibition produced around specimens were measured using a digital caliper (Mitsutoyo, Tokyo, Japan) at three different points. Sizes of inhibition zones were calculated by subtracting the diameter of the specimen (10 mm) from the average of the three measurements of the halo. Three specimens were tested for each material.

Release of CHX

The disc-shaped specimen (10 mm in diameter, 2 mm thickness) of each cement was allowed to set for 30 min (P/L ratio = 3.6), and then immersed in 1 mL of distilled water. After being stored at 37 °C for 24 h, concentration of eluted CHX was

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