Original Article

**ADSORPTION OF ORAL BACTERIA TO POROUS TYPE CALCIUM CARBONATE**

AYUMI YAMANAKA, YOJI SAEKI*, TETSUYA SEKI**, TETSUO KATO and KATSUJI OKUDA

*Department of Microbiology, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan

* Food Material Section, LOTTE Co., LTD. Central Laboratory, 3-1-1 Numakage, Urawa, Saitama 336-0027, Japan

** Chewing Gum Section, LOTTE Co., LTD. Central Laboratory, 3-1-1 Numakage, Urawa, Saitama 336-0027, Japan

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**Abstract**

The purpose of this study was to investigate the adsorption of \[^3\]H\]-thymidine labeled oral microorganisms to porous type calcium carbonate (PCC) beads in a buffer containing human parotid saliva and to PCC combined chewing gum sheets. Adsorption rates of *Streptococcus sobrinus* B13 and 6715, *Streptococcus mutans* MT8148R and *Actinomyces naeslundii* T14V with PCC were significantly higher than those with calcium carbonate (CC) beads \((p<0.01)\). Adsorption rates of *S. sobrinus*, *S. mutans* and *A. naeslundii* with PCC combined chewing gum were significantly higher than those with CC combined chewing gum \((p<0.01)\). The present results suggested that the chewing gum containing PCC may be able to exclude oral bacteria, including cariogenic and periodontopathic bacteria, for prevention of dental caries and periodontal disease.

Key words: Porous type calcium carbonate—Bacterial adsorption—Mutans streptococci—Actinomyces naeslundii

**INTRODUCTION**

There are multi-aetiological factors in dental caries, but infection by specific bacteria is a key factor in the disease. Mutans streptococci such as *Streptococcus mutans* and *Streptococcus sobrinus* have been strongly implicated as causative organisms of dental caries\(^5\). The adsorption of mutans streptococci to the tooth surface is an essential step in the development of dental caries.

*Actinomyces* species in plaque are associated with developing gingivitis and root surface caries\(^5\). It has been suggested that *Actinomyces naeslundii* T14V (formerly *Actinomyces viscosus* T14V) possesses two types of fimbriae that mediate its adsorption to surfaces of the mouth\(^6\). The colonization of this organism is considered to be an important step in the etiology and pathogenesis of dental caries\(^2\).

It has been noted that the exclusion or reduction of pathogenic bacteria is beneficial in controlling oral infections such as dental caries and periodontitis\(^6\). Porous type cal-

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Calcium carbonate (PCC) is known to adsorb glucans of extracellular polysaccharides and odor molecules. The authors propose the hypothesis that PCC is able to reduce specific bacteria in human dental plaque when used in a chewing gum base. The purpose of the present study was to investigate the adsorption of oral microorganisms to PCC beads and CC combined chewing gum sheets.

**MATERIALS AND METHODS**

1. **Strains and culture conditions**

The organisms used in this study were *S. sobrinus* B13 and 6715, *S. mutans* MT8148R, and *A. naeslundii* T14V. Strains of streptococci were stored in Todd Hewitt broth containing 50% glycerol at −20°C until use, then subcultured anaerobically in 5 ml of Todd Hewitt broth (BBL Microbiology Systems, Cockeysville, MD) in a chamber filled with an atmosphere of 80% N₂, 10% H₂, and 10% CO₂ at 37°C. *A. naeslundii* T14V was stored in 10% skim milk at −20°C until use, then subcultured onto Trypticase soy agar (BBL Microbiology Systems) plates supplemented with 10% defibrinated horse blood, hemin (5.0 µg/ml), and menadione (0.5 µg/ml).

To radiolabel bacteria, the organisms were anaerobically grown at 37°C to the early stationary phase in the following way. Mutans streptococci were grown in Todd Hewitt broth (BBL Microbiology Systems) which contained [³H]-thymidine (10 µCi/ml). *A. naeslundii* T14V was grown in broth supplemented with [³H]-thymidine (10 µCi/ml) and 1% yeast extract (Difco Laboratories, Detroit, MI). The harvested cells were washed three times in buffered KCl to produce a suspension containing 1.0 × 10⁸–2.0 × 10⁹ cells per ml.

2. **Preparation of PCC or CC beads and combined chewing gum**

A human parotid saliva sample was collected from a healthy adult donor with collecting devices. The salivary flow was stimulated by an acid candy. The saliva was stored at −20°C and was used for the following experiments.

Bacterial attachment to calcium carbonate was studied using saliva mixed porous type calcium carbonate (PCC) beads (Shiraishi Calcium Co. Ltd.) or calcium carbonate (CC) beads. PCC has an area of 26.1 m²/g and an average diameter of 3.3 µm (Table 1).

Bacterial attachment to calcium carbonate combined gum was studied using PCC or CC combined chewing gum flattened on OHP film (1 × 1 cm). These gum sheets contained 10.5 mg of CC and PCC, respectively, and their average weight was 91.5 mg per sheet. The composition of the tested chewing gum was the following: gumbase 27%, palatinose 28%, maltitol 24%, isomalt 6%, xylitol 6%, maltitol syrup 7%, peppermint flavor 1%, and softener 1%.

3. **Adsorption assay to PCC or CC beads and to combined chewing gum**

A mixture of a suspension of 60 µl bacterial cells (2.0 × 10⁷ cells per ml) and 60 µl of saliva was incubated with 5 mg of PCC or CC beads in a rotator (RT 50, Taitec Co., Tokyo) at 5 rpm for 1 hr. After washing three times with buffered KCl, the number of bacterial cells which had attached to PCC or CC beads was determined by direct scintillation counting.

To measure adsorption to chewing gum, a mixture of a suspension of 500 µl bacterial cells (2.0 × 10⁶ cells per ml) and 500 µl of

<table>
<thead>
<tr>
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<th>area surface (m²/g)</th>
<th>diameter (µm)</th>
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<tr>
<td>PCC</td>
<td>26.1</td>
<td>3.3</td>
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<tr>
<td>CC</td>
<td>12.6</td>
<td>5.5</td>
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Table 1 Characterization of PCC and CC used in the study
saliva was incubated with each sheet of PCC or CC gum in a rotator at 5 rpm for 1 hr. After washing three times with buffered KCl, the number of bacterial cells which had attached to the PCC or CC chewing gum was determined by direct scintillation counting, and each percentage adsorption rate was calculated. The experiments were done six times and expressed as mean values. Statistical differences were calculated by a one-way classification ANOVA.

RESULTS AND DISCUSSION

The adsorption rate of four kinds of bacterial cells to PCC or CC beads was examined. As shown in Fig. 1, the adsorption rates of all bacterial strains tested to PCC were significantly higher than those to CC beads ($p<0.01$). Adsorption rates of S. sobrinus B13, S. sobrinus 6715, S. mutans and A. naeslundii strains to PCC were 1.8, 3.2, 2.0, and 2.0 times higher than those to CC, respectively (Fig. 1). Because the surface area of PCC was twice as
great as that of CC, the differences between these values may reflect the differences in surface area between the two types of calcium carbonates (Table 1).

Because preliminary experiments had shown that CC effectively adsorbed some of the oral bacteria, we examined the adsorption of the bacteria when these types of calcium carbonate were included in the chewing gum. The adsorption rates of *S. sobrinus*, *S. mutans* and *A. naeslundii* to PCC gum were significantly higher than those to CC gum ($p<0.01$). The adsorption rates of *S. sobrinus*, *S. mutans*, and *A. naeslundii* to PCC gum were about 1.5 times, 3.9 times and 4 times higher than those to CC beads (Fig. 2). These results demonstrated that both types of CC still absorbed some of these bacteria when they were mixed in the chewing gum and that this effect was more prominent when PCC was included.

It has been noted that the exclusion or reduction of pathogenic bacteria is beneficial in controlling oral infections such as dental caries and periodontal disease. We demonstrated that funoran extracted from seaweed inhibited colonization of cariogenic bacteria and periodontopathic bacteria and thus excluded them from human oral cavity. Saito et al. and Takatsuka et al. reported that OAIS (3-(trimethoxysilyl)-propyltrimethyloctadecyl ammonium chloride immobilized on silica) has bactericidal activity against gram-positive oral bacteria and adsorbs many other microorganisms that are highly hydrophobic. It is possible that adding OAIS to a toothpaste preparation might help exclusion of various bacterial species from tooth surfaces.

In the present study we demonstrated that the PCC chewing gum can absorb oral bacterial species effectively. Because the flow rate of parotid saliva increases during meals, the present experiments conducted in the presence of parotid saliva somewhat partly mimic the situation in the oral cavity. Therefore, the present results suggest that chewing gum containing PCC might exclude cariogenic and periodontopathic bacteria assisting in the prevention of dental caries and periodontal disease. It is also expected to exclude oral bacteria colonized on tooth surfaces, due that chewing gum will also increase salivary flow and remove bacteria mechanically.

REFERENCES


Reprint requests to:
Dr. Ayumi Yamanaka
Department of Microbiology,
Tokyo Dental College,
1-2-2 Masago, Mihama-ku,
Chiba 261-8502, Japan