Chlorhexidine (CHX) rinses and stannous fluoride dentifrices have each been shown to reduce gingivitis (Perlich et al., J Clin Dent, Vi:54-58, 1995 & Mandel, J Clin Periodontol, 15:488-498, 1988). However, it has been reported that sequential use of SnF₂ and CHX solutions produced an antagonistic antiplaque effect which could potentially limit the antigingivitis efficacy of these agents (Waier & Rolla, Acta Odont Scand, 38:213-217, 1980). The aim of the present study was to evaluate the effects on plaque glycolysis and regrowth of a combined usage regimen of a SnF₂ dentifrice and CHX mouthrinse. The standard plaque glycolysis and regrowth model (PGRM-White et al., J Clin Dent, Vi:59-70, 1995) was used to assess glycolytic and regrowth activity of plaque following use of a CHX rinse (PerideX® Oral Rinse-Procter & Gamble:0.12% CHX) either alone or preceded by brushing with a stannous fluoride dentifrice (Crest® Gum Care-Procter & Gamble:0.454% SnF₂). On the morning of the test, subjects presented to the site having refrained from oral hygiene the previous 12 hrs. Baseline plaque samples were collected from the upper dentition. Subjects were then divided into two groups with one group (n=4) rinsing with CHX for 10 seconds, while the second group (n=7) first brushed their upper dentition for 30 seconds with the SnF₂ dentifrice then rinsed for 10 seconds with CHX. Plaque samples were collected from the lower left and lower right quadrants, 15 and 45 minutes post-product use. Normalized plaque biomasses, incubated in 0.03% TSB buffers, were then assayed for plaque glycolytic and regrowth activities as described by White (ibid). Results are presented as the area under the curve (AUC) over time. For glycolysis: (1) SnF₂ + CHX: 65.67a; (2) CHX alone: 45.67b (a>b:ANOVA, p=0.007). For re-growth: (1) SnF₂ + CHX: 233.23a (No sig. dif., ANOVA, p=0.437). These results indicate that the sequential use of a SnF₂ dentifrice and CHX rinse provides increased inhibition of plaque glycolytic activity relative to use of a CHX rinse alone.

**RESULTS**

The standard PGRM technique was used in this study (Special Issue J Clin Dent, 6:59-70, 1995). On the morning of the test, subjects presented to the site having refrained from oral hygiene the previous 12 hours. Subjects then self-collected baseline plaque samples from the maxillary dentition with a sterile polyester swab. Subjects were then divided into 2 groups with one group (n=4) rinsing with CHX for 10 seconds while the second group (n=7) first brushed their upper dentition with 1.5 grams of CGC for 30 seconds, then swished the resulting slurry throughout their mouth for an additional 30 seconds, then diluted the slurry with 10 ml of water and swished for 10 more seconds, expectorated, then rinsed with CHX for 10 seconds.

**INTRODUCTION**

Chlorhexidine (CHX) rinses and stannous fluoride dentifrices have each been shown to possess antimicrobial activity and to clinically reduce gingivitis. However, it has been reported that sequential use of SnF₂ and CHX solutions produced an antagonistic antiplaque effect which could potentially limit the antigingivitis efficacy of these agents when used in combination (Waier & Rolla, Acta Odont Scand, 38:213-217, 1980). The authors speculated that either the low pH (~3.2) of the SnF₂ solutions or competitive binding between the SnF₂ and CHX to the oral mucosa may have been responsible for the observed effects. With the recent marketing of an antagonistic stannous fluoride containing dentifrice and the availability of CHX mouthrinses through prescription, the effects of concomitant usage of these products on plaque virulence would be of interest to the dental profession.

**OBJECTIVE**

The purpose of this study was to evaluate the effects on plaque glycolysis and regrowth of a combined usage regimen of a SnF₂ dentifrice and CHX mouthrinse.

**MATERIALS AND METHODS**

**Products Tested**

- Crest® Gum Care (CGC) - 0.454% stannous fluoride/aqueous dentifrice base (Procter & Gamble Company)
- PerideX® (CHXR) - 0.12% chlorhexidine gluconate mouthrinse (Procter & Gamble Company)

**Test Product Application**

The standard PGRM technique was used in this study (Special Issue J Clin Dent, 6:59-70, 1995). On the morning of the test, subjects presented to the site having refrained from oral hygiene the previous 12 hours. Subjects then self-collected baseline plaque samples from the maxillary dentition with a sterile polyester swab. Subjects were then divided into 2 groups with one group (n=4) rinsing with CHX for 10 seconds while the second group (n=7) first brushed their upper dentition with 1.5 grams of CGC for 30 seconds, then swished the resulting slurry throughout their mouth for an additional 30 seconds, then diluted the slurry with 10 ml of water and swished for 10 more seconds, expectorated, then rinsed with CHX for 10 seconds.

**Plaque Collection**

Treated plaque samples were self-collected by subjects from the left and right quadrants of the mandibular dentition 15 and 45 minutes post product use, respectively.

**Plaque Sample Preparation**

Swabs containing plaque samples were placed in 1.75 ml of 0.03% BBL Trypticase Soy Broth and vortexed for 15 seconds. The resulting suspended plaque samples were then normalized for plaque biomass by adjusting to a constant optical density (OD) of 0.2 absorbance units with 0.03% TSB solution.

**For Glycolysis**

One ml of the normalized plaque/TSB solution was added to 50 µl of 40% sucrose solution in a 2 ml Eppendorf vial. The plaque samples (pH = 7.10) were then incubated for 2 hours at 37°C in an Eppendorf Thermomixer at 1200 rpm agitation. Acid production was determined by measuring the pH of the plaque samples following 2 hours of incubation. The lower the pH, the greater the glycolytic activity.

**For Regrowth**

300 µl of the normalized plaque/TSB solution was added to a 2 ml Eppendorf vial containing 0.5 ml of 6% (w/w) BBL TSB and 100 µl of sterile water. Bacterial growth was accelerated by the addition of 50 µl of a 40% sucrose solution. Prior to incubation, the OD of the plaque sample was measured at 600 nm.

Research presented at the ADA/FDI World Dental Congress, Orlando, September 28 - October 1, 1996
Samples were then incubated for 4 hours at 37°C in an Eppendorf Thermomixer at 1200 rpm agitation. After incubation, the OD of the plaque samples was again measured following homogenization with a pellet mixer. The extent of regrowth was determined by calculating the ratio of the post incubation OD to the pre-incubation OD. The greater the OD ratio, the greater the extent of plaque regrowth.

**Calculation of Area Under the Curve (AUC)**

The post incubation pH's and OD ratio's of the treated plaques at the various sampling points were compared graphically to the final pH's and OD ratio's of the baseline (pretreated) plaque samples. In this way, AUC's could be calculated from the area between the baseline plaque pH or OD ratio and the treated plaque pH or OD ratio. The greater the value of the AUC, the greater the inhibition of plaque glycolysis and regrowth activity.

**RESULTS**

**Table 1. Comparative Inhibition of Plaque Glycolysis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnF₂ + CHXR</td>
<td>65.7(5.9)a</td>
</tr>
<tr>
<td>CHXR</td>
<td>45.7(13.4)b</td>
</tr>
</tbody>
</table>

a>b; p = 0.007; Single Factor ANOVA

(parentheses enclose standard deviations)

**Table 2. Comparative Inhibition of Plaque Regrowth**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnF₂ + CHXR</td>
<td>233.2(97.6)a</td>
</tr>
<tr>
<td>CHXR</td>
<td>181.7(107.7)a</td>
</tr>
<tr>
<td>No Sig. Dif</td>
<td>p = 0.437; ANOVA</td>
</tr>
</tbody>
</table>

(parentheses enclose standard deviations)

**CONCLUSION**

- Sequential use of a stabilized stannous fluoride dentifrice and a CHX rinse resulted in increased inhibition of plaque glycolytic activity when compared to use of a CHX rinse alone.

- Enhanced inhibition of plaque acid production observed for the combined stannous fluoride/CHX regimen persisted for at least 45 minutes after product use.

- Sequential use of the stannous fluoride dentifrice and CHX rinse inhibited plaque regrowth to about the same extent as use of the CHX rinse alone.

- Results from this study indicate that brushing with a stannous fluoride dentifrice immediately before using a CHX rinse does not appear to reduce the biological activity of CHX. Rather, the results of this work suggest that a combined usage regimen of stannous fluoride and CHX might be expected to provide enhanced antibacterial effects.