A Randomized, Crossover Trial to Evaluate the Effect of Two Mouthrinses on Plaque Regrowth in the Absence of Brushing

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This study assessed the effects on plaque in the absence of brushing of two twice-daily mouthrinses, one with an enzymatic-based formulation (Biotène) and one with an antimicrobial chlorhexidine-based formulation (Peridex), and sterile water. Plaque levels were assessed in 23 participants using a 4-day, nonbrushing plaque regrowth model after twice-daily rinsing with sterile water (negative control), the enzyme-based mouthrinse, or the chlorhexidine-based mouthrinse (positive control). Peridex showed significantly greater prevention of plaque regrowth when compared with water and the enzyme-based Biotène mouthrinse. After 4 days, the enzyme-based mouthrinse was associated with a small but nonsignificant reduction in plaque regrowth compared with water. This study confirmed that Peridex is effective at prevention of plaque regrowth. Twice-daily rinsing with a Biotène formula that contained enzymes showed a small but nonsignificant trend toward prevention of plaque regrowth versus rinsing with water. (Int J Periodontics Restorative Dent 2015;35:387–393. doi: 10.11607/prd.2266)

Xerostomia is the medical term used to describe the subjective feeling of oral dryness. Although oral dryness is most commonly associated with a physical reduction in saliva output (hyposalivation), dry mouth may also be reported by patients who have little or no loss in salivary flow.1 Reported estimates of the prevalence of xerostomia vary: One study found that 21.3% of men and 27.3% of women in the general population suffer from dry mouth,2 but prevalence rates of up to 64.8% have been reported elsewhere.3 The disorder is strongly associated with polymedication and is most common in middle-aged and elderly individuals.1

As saliva is an essential component of the oral cavity, lack of this fluid can manifest with symptoms such as a sticky, dry, or burning feeling in the mouth; halitosis; increased risk of oral candidiasis and other oral infections; and/or dental caries.1,4 Patients with dry mouth may present with particularly severe forms of caries.4 The reduced antibacterial actions of saliva due to the lack of saliva in these individuals can lead to disruption of the oral pH, allowing cariogenic microorganisms to grow and colonize the oral cavity.3 For individuals with xerostomia, therefore, a mouthrinse that can be used daily to provide dry mouth relief as well as plaque prevention remains a key goal.
A variety of mouthrinse products exist on the market. Many are designed to inhibit plaque, whereas few are specifically designed to provide lubrication to dry mouth sufferers. Biotène mouthrinse (GlaxoSmithKline Consumer Healthcare) is a nonfluoridated mouthrinse that is specifically formulated to provide oral lubrication to dry mouth sufferers. The enzymatic-based Biotène product that was tested in this study—plaque biofilm loosening formula (PBF)—contained a lysozyme, lactoferrin, lactoperoxidase, salivary enzyme-protein system as well as two additional enzymes: dextranase and mutanase. Chlorhexidine (chlorhexidine digluconate) is a mouthrinse that is widely referred to as the gold standard for prevention of plaque regrowth, owing to its plaque-inhibitory effects, and is widely accepted as the positive control against which the efficacy of alternative anti-plaque agents should be measured. However, chlorhexidine mouthrinses are associated with adverse effects such as staining of the teeth, and, anecdotally, chlorhexidine can worsen xerostomia, which could be detrimental to those who already suffer from dry mouth.

This study was designed to examine the plaque-prevention efficacy of twice-daily use of an enzymatic-based Biotène mouthrinse alone (ie, in the absence of mechanical cleaning). This study employed a 4-day plaque regrowth model, an established methodology that has been used to evaluate agents that kill plaque bacteria and prevent them from multiplying and measure the effectiveness of anti-plaque chemical agents that aim to prohibit the adherence of plaque to the tooth surface. In the current crossover study, which involved healthy individuals, the plaque regrowth model was used to compare de novo plaque formation after twice-daily treatment with the Biotène PBF mouthrinse, Peridex (3M ESPE) mouthrinse (0.12% chlorhexidine digluconate: positive control), or sterile water (negative control) during a 4-day period of abstinence from brushing.

Method and materials

Trial design

Following initial screening, participants received a dental prophylaxis (scaling and polishing) and then used a wash-in toothbrush and toothpaste twice daily at home until 24 hours prior to day 1 of the first treatment period (minimum of 5 days after the screening visit). Participants used the same toothbrush and toothpaste during the washout periods between treatments (at least 5 days) and also abstained from brushing their teeth for 24 hours prior to day 1 of each treatment period.

On day 1 of the first treatment period only, subjects had a baseline plaque assessment, disclosed using a dye solution (Red Cote), and the level of plaque was assessed using the Turesky Plaque Index (TPI) at six sites per tooth according to Soparkar’s modification as described by Lobene et al. Only those with a mean plaque score ≥ 2.00 were randomized to study treatment. Subjects had their teeth polished at each day-1 visit by a dental professional using a conventional dental prophylaxis paste followed by flossing, and plaque removal was verified by an independent examiner; any residual plaque left was removed as required.

Study participants rinsed their mouths with the assigned treatment for the prescribed number of seconds under supervision and then returned 4 to 12 hours later for the second daily dosing. Participants returned to the site in the morning and evening of treatment days 2, 3, and 4 to receive two rinses per day. A 24-hour plaque assessment was conducted prior to the morning rinsing (third dose) on day 2. Participants refrained from any oral hygiene, including tooth brushing, flossing, or using mouthrinses during the 4-day treatment period (morning of day 1 to day 5, inclusive).

On the morning of day 5 (4 days after study initiation), participants returned to the clinical site and repeated the dental plaque assessment, after which they were allowed to brush their teeth. A minimum 5-day washout period was observed prior to the next crossover leg. Participants then repeated the twice-daily, 4-day treatment protocol followed by the minimum 5-day washout period sequence until they completed all three treatments.

Study population

Study participants were recruited to this randomized, single-center, examiner-blind, three-way crossover study by researchers at University Park Re-
search Center, Fort Wayne, Indiana, USA. The study was approved by a US institutional review board (6400 SW72 Court, Miami, Florida 33143). Participants were at least 18 years of age, in good general and oral health, with at least 20 natural gradable teeth and with a mean plaque score of ≥ 2.00 at the first baseline visit. A total of 23 subjects were randomized and 21 subjects completed all three treatment periods (Fig 1).

Main exclusion criteria were women who were pregnant or breastfeeding; people with poor dental condition (eg, gingivitis, periodontitis, severe recession, active caries lesions, diseases requiring treatment); wearers of partial dentures, orthodontic appliances, or fixed retainers; and/or people with an allergy or intolerance to the study materials, who use antimicrobial mouthrinses containing chlorhexidine or cetylpyridinium chloride, participated in another clinical study of an investigational drug within 30 days of screening, or with a medical history of psychiatric illness.

**Treatment regimens**

**Test mouthrinse**
A mouthrinse containing a lysozyme, lactoferrin, lactoperoxidase, salivary enzyme-protein system and dextranase and mutanase enzymes (Biotène PBF). Study participants were instructed to swirl 15 mL of this mouthrinse around the oral cavity for 60 timed seconds twice daily under the supervision of site staff, before expectorating. According to the product’s instructions, the PBF formulation can be used up to five times a day. For this clinical trial, however, it was decided that the number of uses should be standardized across the different tested products. Over the 4-day nonbrushing period, the test mouthrinse was used twice daily identically to the positive control. No rinsing with water followed treatment.

**Positive control**
Peridex mouthrinse (0.12% weight by volume chlorhexidine digluconate), batch no. 810-525. Participants were instructed to swirl 15 mL of mouthrinse around the oral cavity for 30 seconds (as per label instructions), timed under the supervision of site staff, before expectorating. No rinsing with water followed treatment.

**Negative control**
Sterile water, batch nos. G082586 and G083394. Participants were instructed to swirl with 15 mL of water around the oral cavity for 60 timed seconds under the supervision of site staff before expectorating. No rinsing with water followed treatment.

**Outcomes**

**Plaque scoring**
The primary objective of this study was to evaluate the effect of Biotène PBF on regrowth of plaque and to compare this product against two control products (Peridex and water) over a 4-day period. Plaque levels
were assessed and graded at baseline, 24 hours, and 4 days according to the TPI12 at six sites per tooth according to Soparkar’s modification as described by Lobene et al.13 The TPI scale is scored from 0 to 5 as follows: 0 (no plaque), 1 (slight flecks of plaque at the cervical margin of the tooth), 2 (a thin continuous band of plaque [1 mm or smaller] at the cervical margin of the tooth), 3 (a band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth), 4 (plaque covering at least one-third but less that two-thirds of the crown of the tooth), 5 (plaque covering two-thirds or more of the crown of the tooth).

The secondary objectives were to evaluate and compare the effect of Biotène PBF compared with two control mouthrinses (Peridex and water) on regrowth of plaque over a 24-hour period and to assess the efficacy of this plaque regrowth model for future studies.

Plaque mouthmap
To assess the topography of plaque regrowth, plaque scores were assigned a color and the average values across each group were applied to a “mouthmap.” This approach provided a visual representation of the plaque level on each tooth after each treatment at 24 hours and 4 days.

Data analysis
The variables analyzed in this study were overall plaque and interproximal plaque. Comparisons between treatments were carried out using analysis of covariance (ANCOVA). The ANCOVA model contained treatment and study period as a fixed effect. Subject was included as a random effect. The covariate was the baseline plaque score assessed at the first treatment visit. Pairwise comparisons between the treatments were carried out. All tests were two sided and performed at the 5% significance level. No adjustment for multiple comparisons was carried out. Examiners demonstrated good repeatability throughout the study.

Results
Population data and adverse events
A total of 31 people were screened, 23 were randomized, and 21 completed all three treatment periods (Fig 1). All 23 subjects were included in the safety population, with 22 subjects included in the intention-to-treat population. The majority of participants were female (60.9%) and white (95.7%), and their ages ranged from 19 to 69 years, with a mean age of 41.9 (SD ± 14.62) years (Table 1). Four participants reported four treatment-emergent adverse events (AEs): Three were mild and one was moderate. One person had to be withdrawn due to a “multiple sclerosis relapse.” None of the AEs was considered serious or related to study treatments.

Effect of mouthrinse on plaque regrowth after 4 days
In each round of the crossover study, participants continued to abstain from oral hygiene and received the twice-daily treatment for 3 further days (eight total rinses over a 4-day period). Final assessment of plaque regrowth for each regimen was made on the morning after the last dose (ie, on test day 5, 96 hours after the first treatment).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Study participant demographics (n = 23)</th>
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<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (60.9)</td>
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<tr>
<td>Race, n (%)</td>
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<tr>
<td>American Indian or Alaska Native</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>Native Hawaiian or other Pacific Islander</td>
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</tr>
<tr>
<td>White</td>
<td>22 (96.7)</td>
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<tr>
<td>Multiple</td>
<td>1 (4.3)</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Mean</td>
<td>41.9</td>
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<tr>
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Showing that the model worked, chlorhexidine treatment was associated with a lower level of overall plaque regrowth after 4 days compared with treatment with water (chlorhexidine plaque score [mean ± SE]: 1.36 ± 0.11, water plaque score: 3.23 ± 0.11; Tables 2 and 3; Fig 2).

Chlorhexidine treatment was associated with a statistically significant prevention of overall plaque regrowth compared with the PBF mouthrinse (Tables 2 and 3, Fig 2). Treatment with the PBF mouthrinse showed a trend toward reduced overall plaque regrowth at 4 days compared with water (Biotène PBF: 3.03 ± 0.10, water: 3.23 ± 0.11); however, this difference was not significant (Table 3). Similar outcomes were reported for interproximal plaque values.

**Effect of mouthrinses on plaque regrowth at 24 hours**

The secondary objective was to assess plaque regrowth after 24 hours of treatment. Similar to the 4-day outcomes, chlorhexidine treatment was associated with a significantly lower level of overall plaque regrowth at 24 hours compared with water (chlorhexidine plaque score: 0.58 ± 0.09, water plaque score: 1.29 ± 0.09; Table 2). Overall plaque regrowth with the Biotène PBF mouthrinse (1.33 ± 0.09) was not significantly different from that achieved with water. Again, outcomes for interproximal plaque regrowth were similar to the overall plaque results. Differences between treatments are presented in Table 3.

**Assessment of plaque regrowth topology**

To determine whether certain regions of the mouth were more susceptible to plaque regrowth, the TPI scores for each tooth were applied to a mouthmap. For this outcome measure, TPI scores were coded along a color spectrum to provide a clear visualization of the outcome (Fig 3).

The results showed that at both the 24-hour and 4-day time points, chlorhexidine treatment was clearly associated with lower plaque scores (Fig 2). Consequently, the 24-hour and 4-day mouthmaps for chlorhexidine treatment are populated with pale colors, indicating low-level plaque regrowth. Compared with chlorhexidine, treatment with either water or Biotène PBF was associated with reduced

<table>
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<th>Table 2</th>
<th>Effect of mouthrinse treatments on overall and interproximal plaque</th>
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<tr>
<td></td>
<td>Overall (adjusted mean ± SE)</td>
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<td>n</td>
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<tr>
<td>Day 4</td>
<td></td>
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<tr>
<td>Biotène PBF</td>
<td>22</td>
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<tr>
<td>Peridex</td>
<td>21</td>
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<td>Water</td>
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<tr>
<td>Day 1</td>
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<td>Biotène PBF</td>
<td>22</td>
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<tr>
<td>Peridex</td>
<td>21</td>
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<tr>
<td>Water</td>
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Fig 2  Overall plaque regrowth by treatment group. Plaque scores measured using Turesky Plaque Index after twice-daily rinsing with indicated treatment in the absence of brushing for 24 hours (day 1) and 4 days (day 4). Data show adjusted means ± SE.
prevention of plaque regrowth. Treatment with PBF or water led to similar levels of plaque regrowth at 24 hours, with the mouthmaps for these treatment regimens becoming increasingly darker (indicating high plaque scores) by day 4 (Fig 3).

In all three treatment groups, high levels of plaque regrowth were observed toward the rear of the mouth (Fig 3), confirming the authors’ previous observation that plaque regrowth is greatest in these harder to reach areas (unpublished data). These data show that...
the plaque mouthmap method is a useful tool for assessing the efficacy of plaque prevention agents, providing a visual representative of plaque regrowth geography.

Discussion

Biotène mouthrinses are designed for lubrication of a dry mouth. The Biotène formulation used in this study contained additional enzymes (dextranase and mutanase) that have been reported to aid with loosening (dextranase and mutanase) that have been reported to aid with loosening of the plaque biofilm and may, therefore, help with plaque removal. This study, which was conducted to assess the effect of using Biotène PBF alone on the prevention of plaque regrowth, found no significant benefit of the mouthrinse over water. This finding confirms that this enzyme-containing Biotène PBF formulation did not act via an antimicrobial chemotherapeutic mechanism or via a prevention of plaque build-up mechanism. However, further studies with mechanical plaque control are required to evaluate the hypothesis that the product has a plaque-disruption and plaque-loosening effect.

In the current study, the Biotène PBF mouthrinse was used twice a day to match the recommended frequency of use for Peridex mouthrinse, a frequency that is notably less than the on-label instructions for a dry mouth sufferer, which suggests use up to five times a day. As the PBF mouthrinse was not used in combination with brushing in this study, the efficacy of the product as part of a daily oral health routine was not examined.

This study demonstrated a significant difference between a positive control (chlorhexidine) and a negative control (water) with regard to plaque regrowth, demonstrating the success of the 4-day plaque regrowth model. In this exploratory proof-of-principle study, there was a small, nonsignificant benefit of the Biotène PBF mouthrinse over water in plaque regrowth after 4 days of treatment. No safety issues were seen with any of the products over the 4-day period of use. Using a plaque mouthmap, the authors confirmed previous observations that plaque regrowth is greatest in the posterior area of the mouth (unpublished data). This model provides a useful visual outcome for direct comparison of plaque-prevention treatment regimens that could be utilized in further plaque regrowth studies.

Acknowledgments

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