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Journal

The Journal of Clinical Dentistry, 29(2)

ISSN

0895-8831

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Publication Date

2018-06-01

Peer reviewed

Dental Plaque Removal and Re-Accumulation: A Clinical Randomized Pilot Study Evaluating a Gel Dentifrice Containing 2.6% Edathamil

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Abstract

- **Objective:** The goal of this clinical study was to determine the effects of a dental gel containing 2.6% edathamil on overnight plaque re-accumulation and plaque removal.
- **Methods:** In this double-blind, randomized crossover study, 10 subjects first brushed for one week with a washout toothpaste. On the evening of Day 7, prior to tooth brushing, Plaque Index (PI) was recorded, then plaque stained and photographed. Subsequently subjects were randomized to either brush with the test dental gel or the control. After overnight plaque accumulation, PI was recorded. Plaque was stained and photographed before and after subjects brushed with the same toothpaste as the previous night. Subsequently, the process was repeated with the second toothpaste. Image J software was used to quantify plaque presence.
- **Results:** Mean increase in PI overnight after brushing (1.78 versus 0.94) and final PI after tooth brushing the next morning (2.20 versus 1.31) were significantly ($p < 0.05$) better after use of the test gel. Tooth surface covered by plaque overnight was significantly higher after using the control gel (22.3%) than the test gel (11.8%; $p < 0.05$). After morning brushing, the residual area of plaque on the teeth was significantly higher for the control gel (9.2%) than for the test gel (3.6%; ($p< 0.05$).
- **Conclusions:** A test dental gel more effectively reduced overnight plaque re-accumulation and achieved better plaque removal than a control dentifrice.

Introduction

Oral biofilm consists of a community of microorganisms embedded in an extracellular polysaccharide matrix that develops on all surfaces in the oral cavity.[1] The oral cavity harbors one of the most diverse microbiomes in the human body, providing several distinct microbial habitats, such as the teeth and gingival sulcus. If left in situ, oral biofilm can be associated with the formation of dental calculus, dental demineralization and caries, gingival inflammation, and periodontal disease.[1-3] Some degree of gingivitis affects 50–90% of the adult population.[4] Furthermore, almost 50% of adults in the US suffer from periodontitis.[5] Oral biofilm can affect systemic health as well, providing added impetus for more effective approaches to oral hygiene.

Currently, daily oral biofilm control is primarily addressed by tooth brushing with dentifrices, many of which contain adjuncts to enhance and support the effectiveness of mechanical tooth cleaning.[6-8] If biofilm re-aggregation can be discouraged and its growth inhibited, this will improve gingival health. Conversely, ineffective plaque control is directly implicated in gingival inflammation and destructive chronic periodontitis.[9-11] Despite often considerable efforts at oral hygiene by patients, effective and stable plaque control remains elusive to many individuals.[9-12] Accordingly, a multitude of novel anti-plaque formulations are under investigation for their ability to remove oral biofilm and prevent its re-accumulation.

Adjuncts that may be contained in toothpaste formulations to enhance biofilm control and palatability include abrasives, detergents, flavors, colors, preservatives, and foaming agents. Many dentifrices contain abrasives, which can cause varying levels of abrasion in dentin, especially when it is eroded or demineralized. [13] Moreover, some nanomaterial microsphere abrasives may penetrate through the blood-brain barrier, gaining access to the central nervous system where their effects remain unknown.[14] When such nanoparticles enter the blood stream, they may travel throughout the body and accumulate in the heart, liver, lungs, and kidneys, potentially affecting biological behaviors at the cellular level.[14] Detergents such as sodium lauryl sulfate have been associated with adverse epithelial mucosal and other effects.[14-17] Artificial coloring agents added to toothpaste may cause sensitivity or allergic reactions in some patients.[17] Novel dentifrice formulations strive to avoid such ingredients in order to ensure that they are well tolerated and not potentially harmful in any way.

Cations, such as calcium and iron, are essential to microbial adherence, biofilm formation, and bacterial growth.[18] Recent studies have shown that by binding cations such as iron and calcium, the micro-chelator edathamil has the capability to inhibit biofilm formation and to disrupt its adherence to surfaces.[19] However, conventional edathamil formulations have a limited ability to penetrate biofilm, limiting their dental anti-plaque effect in previous studies.[20,21] The dental gel tested in this study (Livionex®Dental Gel, Livionex Dental, Los Gatos, CA, USA) contains 2.6% activated edathamil with an added carrier and permeability enhancer to promote biofilm penetration and enhance anti-plaque efficacy.[21] The primary goal of this clinical study was to determine the effects of this over-the-counter (OTC) dental gel on overnight re-accumulation of plaque. The secondary goal was to evaluate the effects of the test gel on plaque removal.

Materials and Methods

Study Population and Methodology

This project was performed at the University of California, Irvine, in full compliance with University of California at Irvine IRB-approved protocol #2013-9778. It was registered on Clinical trials.gov under the reference number NCT02271815.

Subject Inclusion and Exclusion Criteria

Study participants were recruited from students and staff of the University of California, Irvine by IRB-approved flyers and e-mails, and were not pregnant, non-lactating, and in a state of good general health as determined by verbal inquiry and yes/no answer. Subjects had no more than localized mild gingival inflammation as defined by a Gingival Index (GI) of less than 2 (Löe and Silness Gingival Index) [22,23] and pockets that did not exceed 4 mm depth on probing. Exclusion criteria included a known history of allergy to oral hygiene products, tobacco use, antibiotic therapy within the previous three months, and the presence of any evident oral abnormality at baseline.

Design and Protocol (Figure 1)

After obtaining informed written consent during the Baseline (Day 0) visit (PWS), standardized photographs as well as fullmouth Plaque Index (PI)24 were recorded for all teeth. Subjects were provided with a standard Oral-B® ProFlex toothbrush (Procter & Gamble, Cincinnati, OH, USA) and trained by the same clinician (TT) in the standard sulcular brushing technique using the tell-show-do method. For the first week of the study, all volunteers brushed with the washout toothpaste (Tom's of Maine Wholecare Toothpaste®, Kennebunkport, ME, USA) twice a day for seven days. On the evening of Day 7 of week 1, subjects abstained from oral hygiene measures, and intra-oral photographs and PI were recorded. Then plaque was stained using a plaque disclosing solution (2-Tone Disclosing Agent®, Young Dental, Earth City, MO,USA) and intra-oral photographs were again recorded to document the extent of the plaque staining. Next, subjects brushed with a new toothbrush and the first assigned toothpaste for two minutes. Subsequently, subjects abstained from any further oral hygiene until the next morning, when PI and intra-oral photographs were recorded again. Plaque was stained as described above, and intra-oral photographs were recorded yet again. Subsequently, subjects were provided with a new toothbrush and they brushed with the same toothpaste they had used the previous night; PI was recorded again and plaque was stained and photographed. After a washout period of one week, this entire process was repeated with the second assigned toothpaste.

Products

The washout toothpaste used was Tom's of Maine Wholecare Toothpaste. Ingredients: sodium monofluorophosphate, calcium fluoride, water, calcium carbonate, hydrated silica, xylitol, natural flavor, sodium lauryl sulfate, carrageenan, zinc citrate, sodium bicarbonate, benzyl alcohol.

The test dental gel used was Livionex Dental Gel. Ingredients: water, sulfonylbismethane, edathamil, stevia, peppermint, menthol essential oils, iota carrageenan gum, konjac gum and lecithin.

The control dental gel used was Colgate Total® Toothpaste (Colgate-Palmolive Company, Piscataway NJ USA). Ingredients: sodium fluoride, triclosan, silicon dioxide, water,

hydrated silica, glycerin, sorbitol, PVM/MA copolymer, sodium lauryl sulfate, cellulose gum, flavor, sodium hydroxide, propylene glycol, carrageenan, sodium saccharin, titanium dioxide.

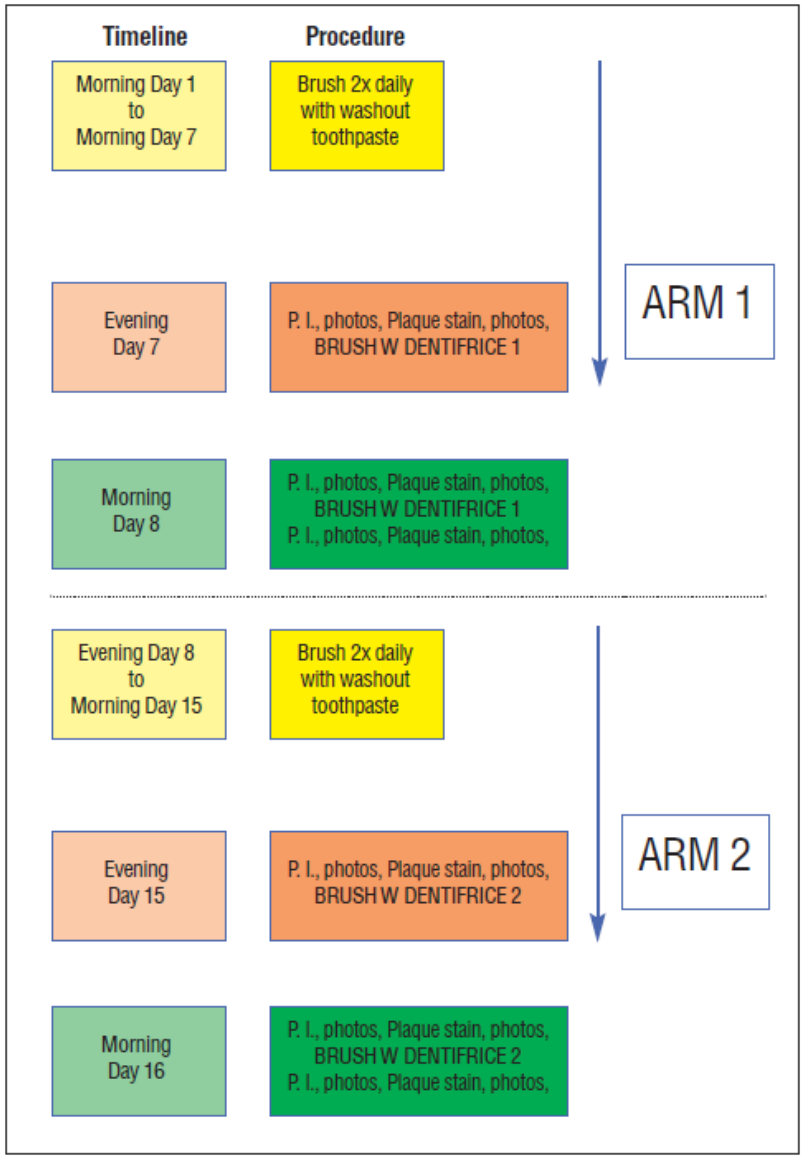

Figure 1. Study design overview.

Variables Recorded

Plaque Index. Throughout the study, the same clinician precalibrated to 95% consistency for clinical measurement of plaque index in 100 periodontal patients over the past six months, recorded the clinical plaque index (TT).

Clinical Photographs. The same clinician (TT) also recorded standardized photographs of the buccal/labial surfaces of all teeth using a Nikon D3200 camera with 18–55 mm lens and ring flash.

Dentifrice Allocation. Was pre-randomized using online randomizer software in a 1:1 ratio with regard to sequence of use (Research Randomizer software: https://www.randomizer.org/). Thus, five subjects brushed first with the test gel, and the remaining five subjects brushed first with the control gel. For the second leg of the study, toothpaste allocation was reversed. Subjects were provided with unidentified plain white, numbered tubes of toothpaste and new toothbrushes for each leg of the study. The entire study was conducted using a double-blind, randomized, crossover design, so that neither clinicians, study managers, outcome assessors, nor patients were aware of product allocation sequence.

Data Analysis

Image J software was used to process the digital intra-oral photographs of the buccal/labial surface of each tooth, subject, and time point. This was achieved using a standard technique whereby the borders of all plaque accumulations are visually delineated, and the areas thus mapped are expressed as percent coverage of each tooth surface. The clinical PI value for each tooth at the end of each washout period was used as baseline value for the subsequent leg of the study. The effects of each dentifrice on PI and plaque presence were tested using sums and differences of changes between study legs, calculated for each subject. A two-sample t-test was performed on the sums of the differences to determine whether there was a carryover effect from the crossover design in any of the indices. A two-sample t-test was also performed on the differences in the changes in each arm to see whether one treatment was more effective than the other.

Results

Subjects

Ten study participants were recruited by IRB-approved flyers and e-mails. Ten were recruited, 10 were enrolled, none were excluded, and none dropped out. All 10 subjects completed the study in full compliance with study parameters.

Study Design

A two-sample t-test identified no significant carryover effect from the crossover design in any of the indices ($p < 0.05$).

Plaque Index (Figure 2)

Mean Baseline PI after seven days of brushing with the washout toothpaste measured 2.44 (with a SD for control group of 0.29 and for test group of 0.36) for each leg. Mean increase in PI overnight was statistically significantly higher after the evening use of the control gel (1.78; SD 0.39) as compared to the test gel (0.94; SD 0.25; $p < 0.05$). After the morning brushing on Day 8, the PI was also statistically significantly better after using the test gel (1.31; SD 0.28) vs. the control gel (2.20; SD 0.36; $p < 0.05$).

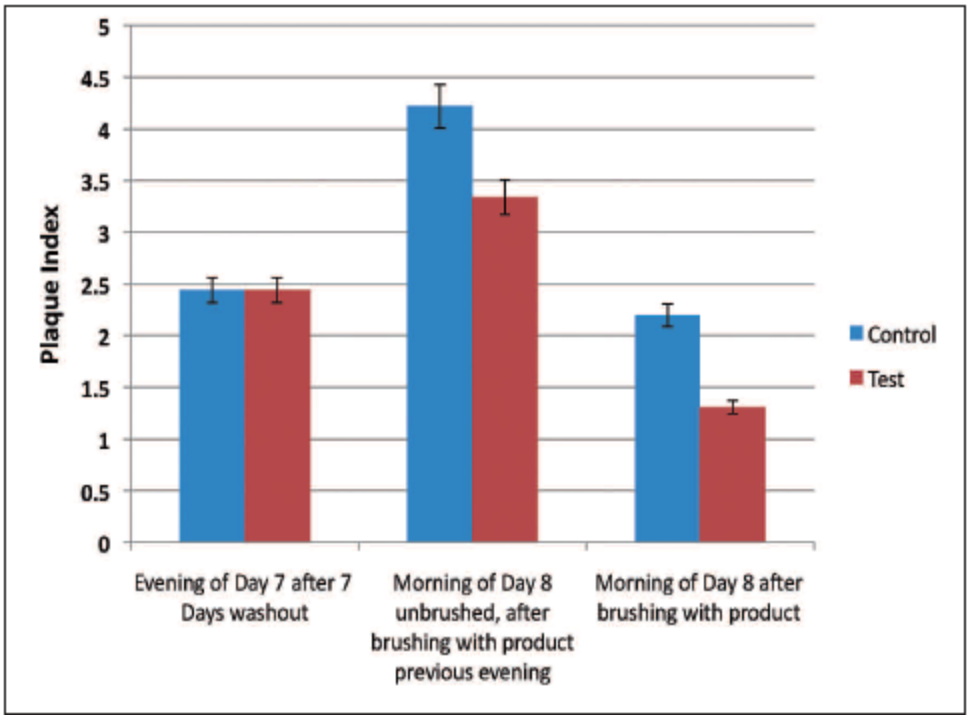

Figure 2. Mean Plaque Index (SD) (1) on Day 7 evening, after 7 days washout toothpaste; (2) Day 8 morning, after previously brushing with Product on the evening of Day 7 (3) Day 8 morning, directly after brushing with Product.

Area of Plaque Coverage (Figures 3 and 4)

The percentage of tooth surface that became covered by plaque overnight was significantly higher after using the control gel the previous evening (22.3%; SD 3.8%) than after using the test gel
the previous evening (11.8%; SD 2.4%; $p < 0.05$). After the morning brushing, the residual plaque area for the control gel (9.2%; SD 1.5%) was also significantly higher than for the test gel (3.6%; SD; 0.7%; $p < 0.05$).

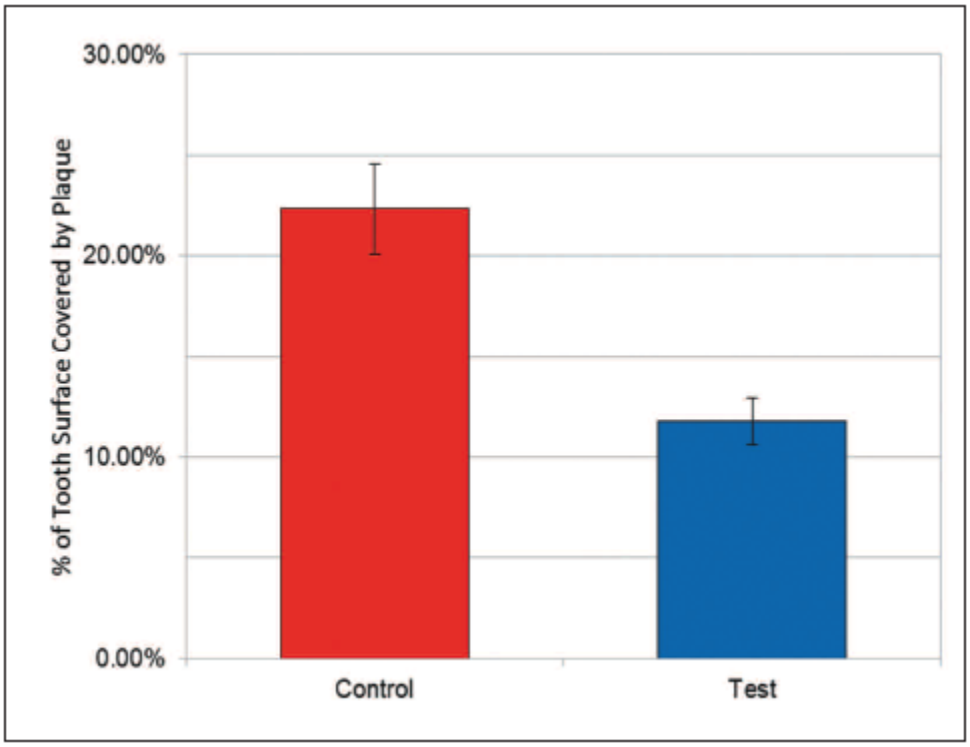

Figure 3. Tooth surface area coverage by plaque (SD) on Day 8 morning after brushing with Product on the evening of Day 7.

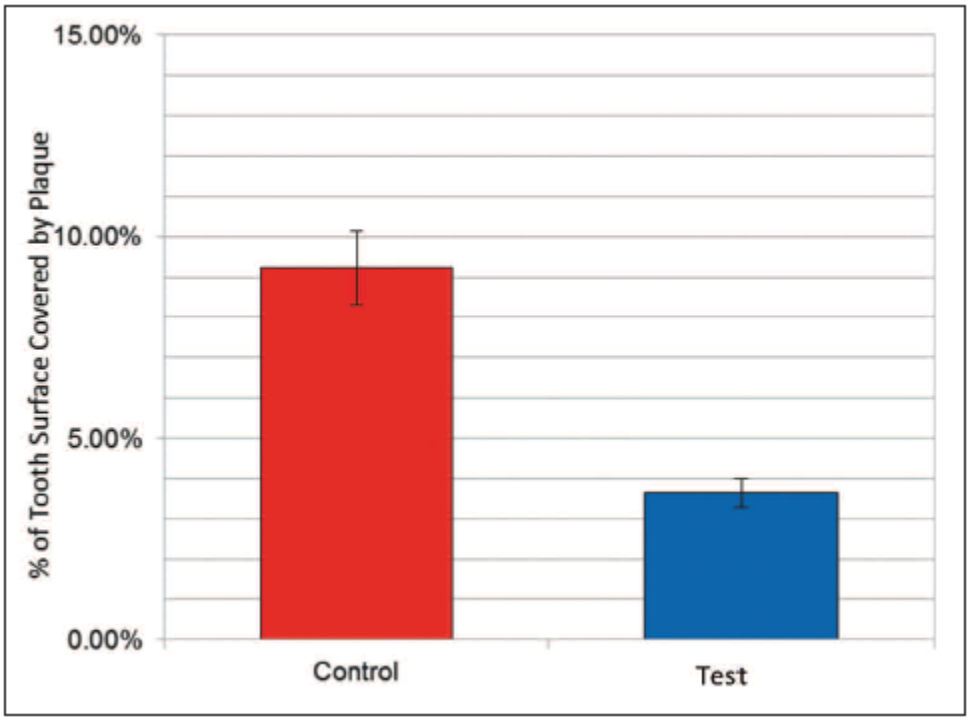

Figure 4. Tooth surface area coverage by plaque (SD) on Day 8 morning, directly after brushing with Product.

Discussion

The products that are currently available to control oral biofilm contain a wide range of active agents to augment mechanical plaque removal. Each has its strengths and weaknesses. For example, chlorhexidine preparations are very effective at combatting oral plaque. However, their side effects, such as staining and alterations in taste sensations, preclude long-term usage.[25] Dentifrices containing triclosan/copolymer, such as the Colgate Total formulation used as control product in this study, can improve oral biofilm reduction and reduce gingival inflammation. [26] However, in some studies the excellent short-term benefits of the broad-spectrum antibacterial agent were offset by a lack of evidence for any long-term benefits.[27] Moreover, there is an ongoing debate about potentially undesirable effects, including allergic sensitization[28] and antibiotic resistance.[29] Finally, long-term use of products that do not contain abrasives of any sort, such as the test formulation in this study, can be associated with a tendency to stain formation over time.[30]

This study is one in a series of projects to evaluate the effects of a novel formulation of dental gel that contains 2.6% edathamil on oral biofilm and gingival inflammation.[31-35] The findings of this study include superior plaque removal and prevention of biofilm re-accumulation as a result of tooth brushing with the test gel. These results are supported by data from previous research. In a double-blind study using twenty-five subjects over 21 days, those who brushed with the test gel showed significantly greater improvements in plaque levels, as well as gingival health, versus subjects who used a control gel.[32] A subsequent investigation, utilizing high-resolution in vivo oral biofilm imaging techniques to track the effects of the test gel versus a control gel over three weeks identified reduced biofilm levels associated with a macroscopic break-up of the dental plaque layer, and smaller, fragmented residual deposits in the test group versus the control group, with no apparent effects on the pellicle. Biofilm was also reduced in the control group, but to a lesser degree than the test group with regard to thickness, continuity and surface area. Paralleling these imaging results, clinical indices at the study end-point were significantly lower in the test group versus the control group.[31] Thus, that study confirmed directly with innovative in vivo imaging techniques the ability of the test formulation to break up biofilm cohesion and discourage plaque adhesion to the tooth surface, providing a direct imaging-based confirmation of the clinical results obtained in this study.

In an *in vivo* double-blind crossover study using sterilized enamel chips mounted onto removable intra-oral retainers, high-resolution multiphoton microscopy (MPM) and scanning electron microscopy (SEM) examinations showed no damage to the tooth surface from the metal chelation properties of the test formulation.[36] A subsequent in vivo study using previously demineralized enamel chips mounted onto an intra-oral retainer confirmed these findings, using standard microhardness measurements as well as SEM to document full remineralization of the chips after one week of intra-oral wear and twice-daily tooth brushing using the test formulation.[37] The use of controlled chelation formulations is not new to clinical dentistry as various components with microchelation action such as malic acid copolymer are commonly included in tartar control dentifrices without any demonstrated adverse effects.[38-40]

In summary, this study demonstrated effective dental plaque removal and reduced overnight plaque re-accumulation after brushing using a novel dental gel. Further studies are required that include greater patient numbers over longer periods of time.

Conclusions

A test dental gel more effectively reduced overnight plaque re-accumulation and achieved better plaque removal than a control dentifrice. In the future, larger studies over longer periods of time in subjects with diverse levels of periodontal health are needed to provide an indication of the short-, mid-, and long-term impact of the test product.

Acknowledgments: This research was supported by LAMMP NIH/NIBIB P41EB05890, the Arnold and Mabel Beckman Foundation, University of California School of Medicine Seed Grant, and Livionex Inc.

Conflict of Interest: The authors certify that they have no affiliation with or direct financial involvement in any organization or entity with direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, and honoraria). For correspondence with the authors of this paper, contact Dr. Petra Wilder-Smith – pwsmith@uci.edu.

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