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Authors

Anbarani, Afarin Golabgir
Ho, Jessica
Vu, Thinh Hung
et al.

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A Double-blinded, Randomized Study Evaluating the *In Vivo* Effects of a Novel Dental Gel on Enamel Surface Microstructure and Microhardness

Afarin Golabgir Anbarani, BS   **Jessica Ho, BS**   **Thinh Hung Vu, BS**   **Ali Forghany, BS**   **Tracie Lam, BS**   **Erisa Khashai, BS**
Karan Sahni, BS   **Thair Takesh, DDS, PhD**
Petra Wilder-Smith, DDS, PhD

Beckman Laser Institute, University of California, Irvine
Irvine, CA, USA

Abstract

- **Objective**: The objective of this study was to evaluate the *in vivo* effects of a 2.6% edathamil gel (Livionex® Dental Gel) on surface microhardness and microstructure in 180 pre-eroded enamel chips.

- **Methods**: This was a double-blind, randomized study. Two enamel chips each were cut from 90 healthy sterilized extracted teeth. One chip from each pair underwent microhardness testing and scanning electron microscopy (SEM) to establish baselines. The remaining 90 samples were demineralized, and then mounted onto intra-oral retainers worn by nine subjects, with five chips mounted on each retainer for each of the two study arms. In one two-week study arm subjects brushed with the control toothpaste; in the other they used the test gel. Study arms were separated by a two-week washout. Sequence of toothpaste use was randomized. At the end of each study arm, samples underwent microhardness measurements (Knoop) and SEM visualization.

- **Results**: After intraoral wear, enamel chips recovered fully from demineralization, with no significant difference in microhardness between the two treatments ($p > 0.05$). In SEM images, enamel surfaces at study's end also appeared comparable in the two groups.

- **Conclusion**: Pre-eroded enamel chips remineralized intra-orally to a similar level after using a control or a test toothpaste containing 2.6% edathamil.

Introduction

Most toothpastes achieve plaque control through a mechanical brushing mechanism, often aided by abrasives, and sometimes with added chemical anti-plaque mechanisms such as detergents, antibacterial, or plaque-disruptive ingredients. Mechanical plaque removal depends heavily on patient compliance, and sometimes it is difficult to establish daily habits for arduous, repetitive, and time-consuming habits like flossing and using interdental aids. Incorporating mild abrasives into a dentifrice can improve plaque removal through brushing, but, if used inexpertly, such ingredients can cause dental abrasion, sensitivity, or gingival lesions. Thus, there is interest in chemically supported, non-abrasive antiplaque modalities. To date, one of the most effective of these is chlorhexidine, but its side effects can include taste alteration and staining of teeth. Adding pyrophosphate to dentifrice formulations has been shown to reduce crystal formation in supragingival calculus, but it does not reduce subgingival calculus development. Clearly, there exists a need for novel, more effective approaches to oral plaque control.

Cations, such as calcium and iron, are essential to microbial adherence, biofilm formation, and bacterial growth. Recent studies have shown that by binding cations such as iron and calcium, the microchelator edathamil has the capability to inhibit biofilm formation and to disrupt its adhesion to surfaces. While some previous studies have demonstrated effective biofilm inhibition by edathamil, other publications describe only a small antiplaque effect, attributed to the limited ability of conventional edathamil formulations to penetrate biofilm. To overcome this hurdle, a dental gel formulation with 2.6% activated edathamil content (Livionex® Dental Gel; Livionex, Inc., Los Gatos, CA, USA) contains an added carrier and permeability enhancer with the goal of promoting biofilm penetration and enhancing antiplaque efficacy. Several clinical studies have evaluated the antiplaque effects of this approach, identifying reduced biofilm presence and gingival inflammation after test gel use versus a control.

Throughout each day, the tooth surface undergoes continuous cycles of de- and remineralization. Demineralization is paralleled by reduced enamel surface hardness, resulting in a heightened risk of abrasion and attrition. The rate of demineralization depends on various factors, including the pH and duration of the acid challenge. Prior to actual tissue loss, surface remineralization can occur through the replacement of lost mineral ions, typically from the salivary reservoir of calcium and phosphate ions. Dentifrices, especially those containing fluoride and mineral ion formulations, can be helpful in supporting dental recovery by promoting remineralization after acid attack. This raises the issue whether transient metal ion micro-chelating processes from the activated edathamil at the plaque surface might affect underlying enamel surface microstructure and mineralization, as well as its ability to remineralize after demineralization.

The goal of this clinical study was to compare the effects of *in vivo* use of a dental gel containing activated edathamil and no fluoride versus a sodium fluoride-containing dentifrice on enamel mineralization and microstructure after an erosive challenge.

Materials and Methods

This research was performed in full compliance with the University of California, Irvine-approved protocol #2013-9778. A total of 180 enamel samples were included in this study, 90 of which served as baseline controls, and 90 as test samples for intra-oral wear. Nine subjects wore custom-fabricated intra-oral retainers for two study arms of two weeks each, with five sterilized enamel chips attached

to the palatal area of the retainer. New chips were used for each arm of the study. In one study arm subjects used a control tooth-paste (Aquafresh® Extreme Clean, GlaxoSmithKline, Philadelphia, PA, USA); in another arm subjects used a test gel (LivionexDental Gel, Los Gatos, CA, USA). During the two-week washout period before the first study arm and between each arm of the study, sub-jects used Tom's of Maine® toothpaste (Tom's of Maine, Kennebunk, ME, USA). Subjects were supplied with a new Oral-B® toothbrush (Procter & Gamble, Cincinnati, OH, USA) at the beginning of each new arm and washout period of the study. The sequence of tooth-paste use by the subjects was randomized.

Dentifrices

Control Product — Aquafresh Extreme Clean. The active ingre-dients include sodium fluoride (0.15% w/v of fluoride ion). The inactive ingredients include water, hydrated silica, sorbitol, glyc-erin, PEG-8, flavor, sodium lauryl sulfate, xanthan gum, titanium dioxide, cocamidopropyl betaine, sodium saccharin, synthetic iron oxide, and D&C Red 30.

Washout Product — Tom's of Maine. Ingredients include propy-lene glycol (vegetable derived), water, sodium stearate, aloe bar-badensis leaf juice (organic), glyceryl laurate, natural fragrance, humulus lupulus (hops) CO2 extract, helianthus annuus (sunflower) seed oil.

Test Product – Livionex Dental Gel. Ingredients include aqua, sulfonylbismethane, edathamil, stevia, peppermint, menthol, FD&C Blue 1, natural gums and stabilizers.

Samples

Ninety extracted teeth, classified as healthy by an experienced dentist using a loupe and headlamp, were used in this study. They were sterilized using ethylene oxide (EtO) at 130°F for two hours and 10 minutes. This method was selected because it has been found to have no effect on the remineralization of enamel, unlike other methods of sterilization. Following gas sterilization, the specimens were placed in an aerator (AMSCO) for 12 hours at 120°F to remove any residual EtO products. Two enamel chips were cut from the same area of each extracted tooth (Figure 1). A total of 180 chips were prepared in this fashion. The chips were then polished under water cooling using a rotating polisher (Meta-Serv™ 3000 Grinder-Polisher; Buehler, Lake Bluff, IL, USA). The sequential polishing protocol used a 600-grit silica carbide disc for 10 seconds, 1200-grit for 20 seconds, 2400-grit for 30 seconds, and 4000-grit for 45 sec-onds. Finally, samples underwent one minute of ultrasonication to remove any residual polishing debris. From each chip "pair," one chip was held back as a control sample and subjected to standard Knoop microhardness testing (Figure 2) followed by standard SEM imaging. Knoop microhardness testing is an established and stan-dard technique for measuring enamel mineralization. These sam-ples were then stored in demineralized water at a temperature of 4C and 100% humidity, and protected from ambient light in a sealed and labeled double-walled container. A total of 90 control samples were evaluated in this way.

The remaining 90 chips were subjected to a standard demineral-ization protocol consisting of six hours of demineralization using an acetate/calcium/phosphate buffer. The buffer contained 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate
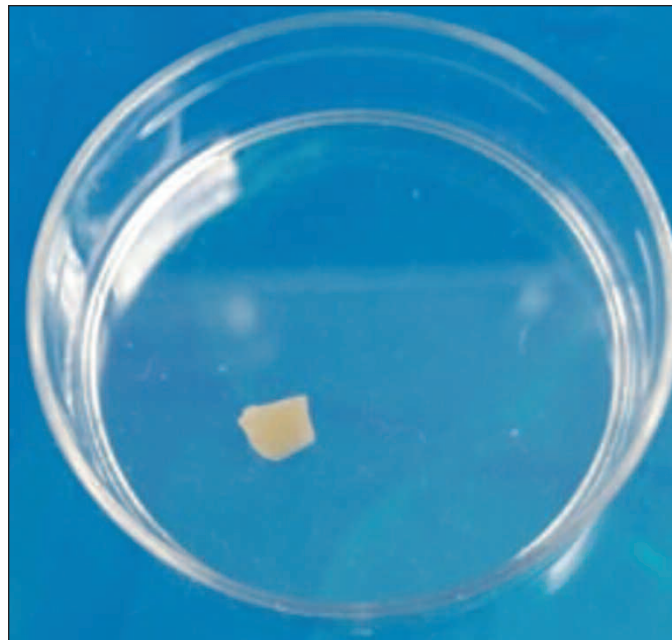

Figure 1. *Sterilized enamel chip ready for mounting on retainer.*

Figure 2. *Enamel chip embedded for microhardness testing.*

maintained at pH 4.5 with 40 ml per sample used individually. The demineralized chips were then attached to a custom-fabricated retain-er (Figure 3) for the duration of one arm of the study; *i.e.*, two weeks. Ninety samples were used intraorally, with five per retainer per study arm, in a total of nine patients, over two study arms. At the end of each study arm, samples were detached from the retainer and embed-ded in acrylic (SamplKwik™, Buehler, Lake Bluff, IL, USA) for micro-hardness measurements. Microhardness testing was performed using a Struers Duramin microhardness tester (Struers Ltd., Denmark) with a Knoop diamond indenter using a 50 g load and a 10-second indentation period. The indentations were imaged with a 40/0.65 NA objective. Knoop values were calculated using proprietary soft-ware supplied by the manufacturer. On each sample, three meas-

Figure 3. *Enamel chips mounted on retainer.*

urements were recorded at 500 μm intervals. The mean of these measurements was calculated to represent the surface microhardness of each sample. Finally, samples were mechanically removed from the acrylic investment and subsequently underwent processing for SEM that included dehydration in a graded series of aqueous ethanol (50, 70, 90, and 100% ethanol) for 10 minutes at each concentration. They were then mounted on stubs using colloidal silver liquid (Ted Pella, Redding, CA, USA), and gold-coated on a PAC-1 Pelco advanced coater 9500 (Ted Pella, Redding, CA, USA). Micrographs of the enamel surface were acquired utilizing a Philips 515 (Mohawk, NJ, USA) SEM at magnifications of 10x–10,000x.

Subjects
All subjects signed an informed consent form prior to enrollment in this study, which was performed in full compliance with UCI IRB approved protocol #2013-9778. Subjects consisted of nine healthy volunteers aged 18–30 (mean age of 26.4; five male, four female) with a minimum of 16 clinically and radiographically healthy teeth as defined by clinical examination, and with an absence of any pathological hard or soft tissue signs or symptoms.

Protocol
At the baseline visit, standard alginate impressions of the upper jaw were recorded. These were sent to an orthodontic laboratory for fabrication of a removable palatal appliance designed to hold five enamel blocks, a commonly used protocol for *in vivo* oral studies. Retainer fit and comfort were checked prior to adhering the enamel chips onto the retainer using yellow dental sticky wax.

During each arm of the study, subjects brushed their teeth twice daily and abstained from all other oral hygiene measures. Brushing proceeded with the retainer removed from the mouth; all surfaces of the teeth were brushed with a pre-moistened toothbrush and 1.5 g of dental gel for 120 seconds. Subjects did not expectorate. Then the retainer was replaced in the mouth, and the retained slurry was rinsed around the palatal area of the appliance where the chips were mounted for 60 seconds. Neither the appliance nor the enamel specimens were brushed. The subjects then expectorated

and rinsed gently with tap water (15 mL, 10 seconds) before again expectorating. Subjects wore the retainer for a minimum of 22 hours per day, removing it during meals and placing it in a sealed container during that time.

Since this was a double-blind, randomized study, neither subjects, clinicians, microhardness testers, SEM imagers, nor any other members of the study were aware of product allocation or treatment status of the samples.

Study Timeline
Day -14: Take impressions, begin two-week pre-study washout
Day 0:   Begin study arm 1
Day 14:   End study arm 1, begin washout
Day 28:   Washout completed, begin study arm 2
Day 42:   End study arm 2

Primary End Points
Data collected from each sample:
1. Microhardness (Knoop): Three indentation length measurements per sample; then the mean was computed for each sample
2. SEM images: Sample surfaces were scanned and photographed at magnifications from 10x–10,000x. Photomicrographs from each sample were recorded documenting:
   (a) Typical appearance
   (b) Areas with the most healthy (best) appearance
   (c) Areas with the most damaged (worst) appearance.

Results
All subjects completed the study in full compliance with the approved protocol.

Microhardness Measurements using Standard Knoop Indenter Technique
Microhardness results per sample and treatment are shown in Table I. Mean sample microhardness values after two weeks' expo-

Table I
Mean Microhardness Values Expressed as Percent of
the Original Hardness for Control and Test Dentifrice Groups

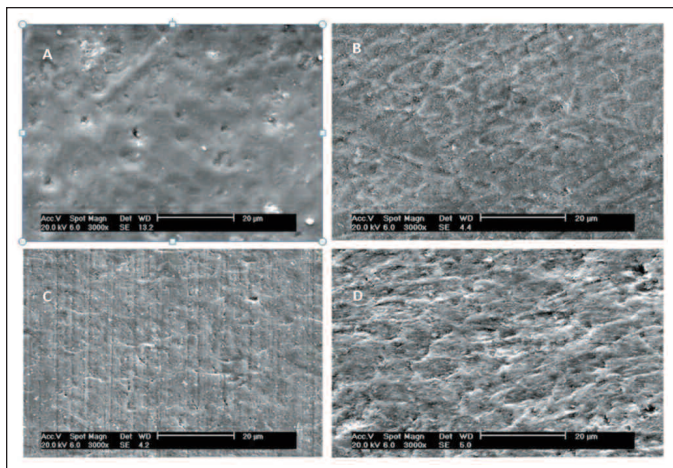| Control Dentifrice	Test Dentifrice
Mean Microhardness Ratio (Knoop) final microhardness/original microhardness (n=90; 3 measurements/sample)	Mean Microhardness Ratio (Knoop) final microhardness/original microhardness (n=90; 3 measurements/sample)
Mean (SD): 1.09 (0.24)	Mean (SD): 1.14 (0.19)

sure to either treatment were equal to or minimally greater than microhardness values prior to demineralization. This indicates that all enamel chips had re-hardened intra-orally since their initial *ex vivo* demineralization. Standard deviations in sample microhardness ranged from 19–24% within the study groups. This is not unusual given the varied and undefined history of the different source teeth used to generate the enamel slab samples. Using a two-tailed t-test, the "final to initial" microhardness ratios were comparable for the samples exposed to the two toothpastes under the same conditions ($p > 0.05$). The data indicate that enamel surface remineral-
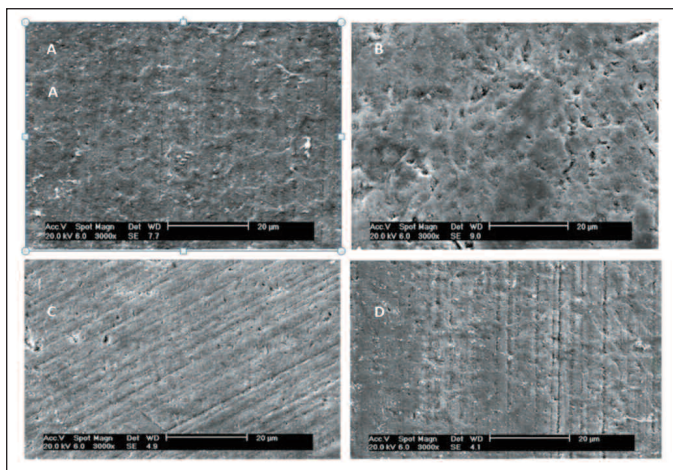
ization over a two-week period was similar using the test product and the control product.

Scanning Electron Microscopy

Samples appeared unaltered to the naked eye at the culmination of this study. Overall, the samples from both treatment groups had a similar appearance in the SEM (Figures 4 and 5). Figure 4

Figure 4. *Photomicrographs (x3,000) of samples treated with control dentifrice. Scratches from the polishing process prior to microhardness measurement are visible in 4(C). 4(B) and 4(D) show evidence of mild demineralization.*

Figure 5. *Photomicrographs (x3,000) of samples treated with test dentifrice. In 4(C) and 4(D) scratches from the polishing process prior to microhardness measurement are visible. 5(A) and 5(B) show evidence of mild demineralization.*

shows representative photomicrographs of samples in the control group at a magnification of 3,000x. In Figure 4(C), scratches resulting from the polishing process performed prior to microhardness measurement are visible. Figures 4(B) and 4(D) show evidence of mild demineralization. Some small areas of enamel defects or localized roughening were evident in most images of samples from the control group. Typically, these changes extended over 10–15% of each sample's total surface area. Figure 5 shows representative photomicrographs of samples in the test group at a magnification of 3,000x. In Figures 5(B) and 5(C), scratches resulting from the polishing process performed prior to microhardness measurement are visible. In Figures 5(A) and 5(B), the surface area appears roughened and mildly demineralized. Figure 5(B) also reveals some minor pitting and cracking. The cracking may be artefactual from the

SEM preparation process. Overall, the sample surfaces appeared somewhat more homogeneous in appearance in the test group than the control group.

Discussion

The goal of this clinical study was to compare the effects of *in vivo* use of a dental gel containing 2.6% activated edathamil and no fluoride versus a control dentifrice containing 0.15% w/v of fluoride ion on enamel mineralization and microstructure after an erosive challenge.

Samples were eroded by means of a standard technique through exposure to demineralization using an acetate/calcium/phosphate buffer. This technique was developed by the Featherstone laboratory and has been used as standard procedure for many years.

Dental erosion is a multi-factorial condition wherein an initial softening of the enamel surface in response to an erosive challenge can eventually be followed by permanent loss of the demineralized tooth structure. Partial loss of mineral on the surface is accompanied by a reduction in microhardness, leaving eroded enamel more prone to abrasion and wear. Additional factors contributing to the erosive properties of specific agents include their mineral content and their buffering capacity, as well as the composition and flow rate of saliva in the mouth. The degree of saliva and plaque saturation with regard to dental minerals such as hydroxyapatite and fluorapatite also affect outcomes of the erosive challenge.

Mounting enamel slabs onto a removable retainer for intra-oral wear permits intra-oral exposure of samples that can then be removed from the oral cavity for *ex vivo* analysis. In this study, pre-eroded enamel samples were exposed intra-orally to dental gel slurry produced by brushing the natural teeth. This model was chosen to avoid the potentially confounding effects of variables in tooth brushing techniques, and in differing levels of abrasiveness of the two dental gels used. While several studies have demonstrated comparable effects of a slurry versus a tooth brushing technique, other investigations have identified differences in the outcomes from the two application techniques. Further studies are required to identify the best techniques for accurately reproducing the clinical *in vivo* effects of specific toothpastes.

In this study, the enamel surface recovery from erosion appeared similar in SEM images after use of a control or test dental gel. The SEM images show isolated circumscribed patches of surface enamel deficiencies. A few areas of typical erosive damage are visible, paralleling the results of other studies investigating enamel erosion followed by remineralization. Comparable results were recently reported from another study that used SEM to compare the intra-oral effects of the same two dentifrices on pre-eroded samples.

Because SEM is not suitable for quantifying actual mineralization changes, enamel surface sample microhardness was also measured prior to erosion and after intra-oral sample wear. Mean sample microhardness at the study endpoint did not differ significantly between the enamel chips exposed to the control versus the test agent. Ideally, sample microhardness should also have been determined directly after erosion. However, the enamel chips were individually embedded and mounted in acrylic for microhardness indentation measurements. After microhardness measurements, samples were mechanically removed from the acrylic to allow sample preparation for SEM, a process during which samples are prone to frac-

ture and damage. Adding a second cycle of mounting and subsequent extraction from the acrylic investment to the protocol could have resulted in breakage of a considerable number of the samples, based on our previous experience. For this reason, the investigators decided to use a well-established erosion model and exclude post-demineralization microhardness measurements from the protocol.

The control gel used in this study contains fluoride, whereas the test gel does not. This study determined similar levels of recovery from erosion as determined by SEM and by microhardness testing in the test and the control groups. A large number of *in vitro* and *in situ* studies have reported the beneficial effects of fluoride dentifrices for the prevention and management of dental erosion. Moreover, dentifrices containing NaF have been demonstrated to remineralize acid-softened enamel. However, there is increasing interest in alternative dentifrice formulations that avoid fluoride content.

Interventional effectiveness appears to depend not only on the dentifrice formulation and application mode, but also on the erosion model used. Exposure to saliva and some dietary products can support remineralization. The postulated mechanism for this effect is that the deposition of salivary calcium and phosphate onto the softened tooth surface once the erosive agent is neutralized will cause re-hardening of the enamel. In an *ex vivo* study using citric acid erosion, immersion of the samples in artificial saliva caused partial re-hardening after one to four hours and complete remineralization after six to 24 hours. In another study, tooth samples underwent acid erosion with grapefruit juice for 20 minutes followed by remineralization using casein phosphopeptide amorphous calcium phosphate (CPP-ACP) paste. SEM images of the samples suggested a remineralization-supportive effect by this dentifrice formulation. CPP-ACP contains inorganic components that can potentially act as remineralizing agents on the enamel. Indeed, a wide range of studies involving a plethora of toothpastes have reported varying degrees of remineralizing efficacy for many calcium and/or phosphate and/or fluoride-containing formulations.

This study is one in a series of projects to evaluate the effects on oral biofilm and gingival inflammation of a dental gel that contains 2.6% edathamil. The OTC dental gel contains no soaps, abrasives, or antibiotic agents. Instead, it disrupts surface adherence of biofilm and prevents biofilm cohesion through metal-binding chelators. In a double-blind study using twenty-five subjects over 21 days, those who brushed with the test gel showed significantly greater improvement in plaque levels as well as gingival health versus subjects who used a control gel. Recent clinical and high-resolution imaging studies have confirmed these findings, demonstrating significantly reduced biofilm formation, re-accumulation and persistence after clinical use of this formulation. Utilizing high-resolution multiphoton microscopy oral biofilm imaging techniques to track the effects of the test gel versus a control gel over three weeks, significantly lower clinical plaque levels were associated with a macroscopic break-up of the dental biofilm layer and smaller, fragmented residual deposits in the test group versus the control group. Metallic cations are essential to microbial adherence, biofilm formation, and bacterial growth, and their presence can disrupt surface attachment and prevent biofilm production. Moreover, calcium and iron also play critical roles in the inflammatory process, so that the use of a metal-binding agent such as edathamil may also have a benefi-

cial effect on mitigating inflammation. Recent studies have determined that metal chelation inhibits the formation of cytotoxic 4-Hydroxynonenal (HNE) and the initiation of apoptotic/inflammatory events. Several pilot clinical studies support these findings, demonstrating lower levels of gingival inflammation in subjects after using the test gel versus a conventional control dentifrice.

In summary, pre-eroded enamel samples recovered equally after two weeks of intra-oral test or control gel use, as determined by microhardness measurements and SEM imaging. Limitations of the study include the use of an intra-oral slurry rinse rather than actual tooth brushing on enamel, and the use of a one-time *ex vivo* demineralizing event rather than controlled ongoing cycles of intra-oral de- and remineralization. Moreover, subjects' intra-oral parameters, such as eating habits, intra-oral pH, and salivary function, were not recorded nor taken into account. Additional, more extensive and better controlled studies over longer periods of time are currently under way to provide additional information regarding the mid- and long-term effects of this dentifrice on oral health, and to evaluate mechanistic models for the test gel's effects.

Conclusion

Two-weeks' use of a 2.6% edathamil test gel produced a similar level of recovery from demineralization as a fluoride-containing control gel in enamel samples that were worn intra-orally.

For correspondence with the authors of this paper, contact Petra Wilder-Smith, DDS, PhD – pwsmith@uci.edu.

References

1. Van Der Weijden G A, Hioe, K P . A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. *J Clin Periodontol* 2005;32: 214-28.
2. Ganss C, Schulze K, Schlueter N. Toothpaste and erosion. *Monogr Oral Sci* 2013;23:88-99.
3. Moore C, Addy M. Wear of dentine in vitro by toothpaste abrasives and detergents alone and combined. *J Clin Periodontol* 2005;32:1242-6.
4. Absi EG, Addy M, Adams D. Dentine hypersensitivity: uptake of toothpastes onto dentine and effects of brushing, washing and dietary acid—SEM in vitro study. *J Oral Rehabil* 1995;22:175-82.
5. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. *J Am Dent Assoc* 2006;137:1649-57.
6. Schiff T, Delgado E, DeVizio W, Proskin HM. A clinical investigation of the efficacy of two dentifrices for the reduction of supragingival calculus formation. *J Clin Dent* 2008;19:102-5.
7. Jin Y, Yip HK. Supragingival calculus: formation and control. *Crit Rev Oral Biol Med* 2002;13:426-41.
8. Wu H, Moser C, Wang H , Høiby, N, and Song Z J. Strategies for combating bacterial biofilm infections. *Int J Oral Sci* 2015;7:1-7.
9. Fang X, Keutgen XM, Jiang Y, Sherertz R, Hachem R. The role of chelators in preventing biofilm formation and catheter-related bloodstream infections. *Curr Opin Infect Dis* 2008;21:385-92.
10. Dadkhah M, Chung NE, Ajdaharian J, Wink C, Klokkevold P, Wilder-Smith P. Effects of a novel dental gel on plaque and gingivitis: A Comparative study. *Dentistry* 2014;4:239.
11. Nayudu A, Lam T, Ho J, Forghany A, Vu T, Ngo W, Ajdaharian J, Wilder-

Smith P. Plaque removal and gingival health after use of a novel dental gel: a clinical study. *Dentistry* 2016;6:10.

12. Ajdaharian J, Dadkhah M, Sabokpey S, Biren-Fetz J, Chung NE, Wink C, Wilder-Smith P. Multimodality imaging of the effects of a novel dentifrice on oral biofilm. *Lasers Surg Med* 2014;46:546-52.

13. Chang Y, Gu W, McLandsborough L. Low concentration of ethylenediaminetetraacetic acid (EDTA) affects biofilm formation of Listeria monocytogenes by inhibiting its initial adherence. *Food Microbiol* 2012;29:10-7.

14. Bryce G, O'Donnell D, Ready D, Ng YL, Pratten J, Gulabivala K. Contemporary root canal irrigants are able to disrupt and eradicate single- and dual-species biofilms. *J Endod* 2009;35:1243-8.

15. Zhang M, Wong IG, Gin JB, Ansari NH. Assessment of methylsulfonylmethane as a permeability enhancer for regional EDTA chelation therapy. *Drug Deliv* 2009;16:243-48.

16. Zero DT, Hara AT, Kelly SA, González-Cabezas C, Eckert GJ, Barlow AP, Mason SC . Evaluation of a desensitizing test dentifrice using an in situ erosion remineralization model. *J Clin Dent* 2006;17:112-6.

17. Barbour ME, Rees GD. The role of erosion, abrasion and attrition in tooth wear. *J Clin Dent* 2006;17:88-93.

18. Fowler C, Willson R, Rees GD. In vitro microhardness studies on a new anti-erosion desensitizing toothpaste. *J Clin Dent* 2006;17:100-5.

19. Margolis HC, Zhang YP, Lee CY, Kent RL Jr, Moreno EC. Kinetics of enamel demineralization in vitro. *J Dent Res* 1999;78: 1326-35.

20. Theuns HM, Van Dijk JWE, Driessens FCM, Groeneveld A. Effect of the pH of buffer solutions on artificial carious lesion formation in human tooth enamel. *Caries Res* 1984;18:7-11.

21. Theuns HM, Van Dijk JWE, Driessens FCM, Groeneveld A. Effect of time and degree of saturation of buffer solutions on artificial carious lesion formation in human tooth enamel. *Caries Res* 1983;17:503-12.

22. Amaechi BT, Higham SM. In vitro remineralisation of eroded enamel lesions by saliva. *J Dent* 2001;29:371-6.

23. Attin T, Knofel S, Buchalla W, Tutuncu R. In situ evaluation of different remineralization periods to decrease brushing abrasion of demineralised enamel. *Caries Res* 2001;5:216-22.

24. Maggio B, Guibert RG, Mason SC, Karwal R, Rees GD, Kelly S, Zero DT. Evaluation of mouthrinse and dentifrice regimens in an in situ erosion remineralisation model. *J Dent* 2010;38: S37-44.

25. Featherstone JD. Remineralization, the natural caries repair process—the need for new approaches. *Adv Dent Res* 2009;21:4-7.

26. Moretto MJ, Magalhães AC, Sassaki KT, Delbem AC, Martinhon CC. Effect of different fluoride concentrations of experimental dentifrices on enamel erosion and abrasion. *Caries Res* 2010;44:135-40.

27. Addy M, Shellis RP. Interaction between attrition, abrasion and erosion in tooth wear. *Monogr Oral Sci* 2006;20:17-31.

28. Lussi A. Erosive Tooth wear- a multifactorial condition of growing concern and increasing knowledge. *Monogr Oral Sci* 2006;20:1-8.

29. Ganss C, Schulze K, Schlueter N. Toothpaste and erosion. *Monogr Oral Sci* 2013;23:88-99.

30. https://www.google.com/search?rlz=1C1AFAB_enUS481US496&q= AquafreshR+Extreme+Clean%2C+ingredients&oq=AquafreshR+Extreme+C lean%2C+ingredients&gs_l=psy-ab.3..0i13k1j0i8i13i30k1l2. 3550.10573.0.10785.14.14.0.0.0.0.161.1159.13j1.14.0....0...1.1.64.psy-ab..0.13.998...0i13i30k1.Hk9dAtzskeY Accessed 9.13.2017

31. https://www.google.com/search?rlz=1C1AFAB_enUS481US496&q=Toms+ of+Maine%2C+ingredients&oq=Toms+of+Maine%2C+ingredients&gs_l=psy -ab.3..0i7i10i30k1l3j0i10k1.59548.61981.0.64510.13.13.0.0.0.0.152.1072.9j3. 2.0....0...1.1.64.psy-ab..1.12.1071...0i7i30k1j0i8i13i30k1.RMchzCR4BaM Accessed 9.13.2017

32. https://www.google.com/search?rlz=1C1AFAB_enUS481US496&q= Livionex+Dental+Gel%2C+ingredients&oq=Livionex+Dental+Gel%2C+ingr edients&gs_l=psy-ab.3..64157.67779.0.69564.19.19.0.0.0.0.164.1535.17j2.19.0.... 0...1.1.64.psy-ab..0.9.820...0j0i7i30k1j0i13k1j0i8i13i30k1.lpXVIt5ganQ Accessed 9.13.2017

33. Toro MJ, Lukantsova LL, Williamson M, Hinesley R, Eckert GJ, Dunipace AJ. In vitro fluoride dose–response study of sterilized enamel lesions. *Caries Res* 2000;34:246-53

34. Craig RG, Peyton FA. The microhardness of enamel and dentin. *J Dent Res* 1958;37:661-8

35. Stookey GK, Featherstone JD, Rapozo-Hilo M, Schemehorn BR, Williams RA, Baker RA, Barker ML, Kaminski MA, McQueen CM, Amburgey JS, Casey K, Faller RV. The Featherstone laboratory pH cycling model: a prospec-

36. Sullivan R, Rege A, Corby P, Klaczany G, Allen K, Hershkowitz D, Goldder B, Wolff M. Evaluation of a dentifrice containing 8% arginine, calcium carbonate, and sodium monofluorophosphate to repair acid-softened enamel using an intra-oral remineralization model. *J Clin Dent* 2014;25(Spec No A):A14-9.

37. Cantore R, Petrou I, Lavender S, Santarpia P, Liu Z, Gittins E, Vandeven M, Cummins D, Sullivan R, Utgikar N. In situ clinical effects of new dentifrices containing 1.5% arginine and fluoride on enamel de- and remineralization and plaque metabolism. *J Clin Dent*. 2013;24(Spec No A):A32-44.

38. Featherstone J, Lussi A. Understanding the chemistry of dental erosion. *Monogr Oral Sci* 2006;20:66-76.

39. Huang Y, Duan Y, Qian Y, Huang R, Yang Z, Li Y, Zhou Z. Remineralization efficacy of a toothpaste containing 8% arginine and calcium carbonate on enamel surface. *Am J Dent* 2013;26: 291-7.

40. Ganss C, Lussi A, Grunau O, Klimek J, Schlueter N. Conventional and anti-erosion fluoride toothpastes: effect on enamel erosion and erosion-abrasion. *Caries Res* 2011;45:581-9.

41. Rochel ID, Souza JG, Silva TC, Pereira AF, Rios D, Buzalaf MA, Magalhães AC. Effect of experimental xylitol and fluoride-containing dentifrices on enamel erosion with or without abrasion in vitro. *J Oral Sci* 2011;53:163-8.

42. Braga SRM, De Faria DLA, De Oliveira E, Sobral MAP. Morphological and mineral analysis of dental enamel after erosive challenge in gastric juice and orange juice. *Microsc Res Tech* 2011;74:1083-7.

43. Kensche A, Pötschke S, Hannig C, Richter G, Hoth-Hannig W, Hannig M. Influence of calcium phosphate and apatite containing products on enamel erosion. *Sci World J* 2016;7959273.

44. Lam T, Ho J, Anbarani AG, Liaw LH, Takesh T, Wilder-Smith P. Effects of a novel dental gel on enamel surface recovery from acid challenge. *Dentistry* 2016;6:397.

45. Sabokpey S, Biren-Fetz J, Dadkhah M, Lam T, Ho J, Anbarani AG, Liaw LH, Takesh T, Wilder-Smith P. In vivo study of the effects of a novel dental gel on enamel re-mineralization after acid erosion. Paper presented at: 33rd Annual Session of the American Society for Laser Medicine and Surgery, April 2, 2013, Boston, MA, USA.

46. Zero DT, Hara AT, Kelly SA, González-Cabezas C, Eckert GJ, Barlow AP, Mason SC: Evaluation of a desensitizing test dentifrice using an in situ erosion remineralization model. *J Clin Dent* 2006;17:112-6.

47. Schleuter N, Klimek J, Ganss C: Efficacy of an experimental tin-F-containing solution in erosive tissue loss in enamel and dentine in situ. *Caries Res* 2009;43:415-21

48. Fowler CE, Gracia L, Edwards MI, Rees GD, Willson R, Brown A: Inhibition of enamel erosion and promotion of lesion rehardening by fluoride: a white light interferometry and microindentation study. *J Clin Dent* 2009;20:178-85

49. Ganss C, Klimek J, Schaffer U, Spall T: Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. *Caries Res* 2001;35:325-30

50. Nehme M, Jeffery P, Mason S, Lippert F, Zero DT, Hara AT. Erosion remineralization efficacy of gel-to-foam fluoride toothpastes in situ: a randomized clinical trial. *Caries Res* 2016;50:62-70.

51. Patil N, Choudhari S, Kulkarni S, Joshi SR. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. *J Conserv Dent* 2013;16:116-20.

52. Vashisht R, Kumar A, Indira R, Srinivasan MR, Ramachandran S. Remineralization of early enamel lesions using casein phosphopeptide amorphous calcium phosphate: An ex-vivo study. *Contemp Clin Dent* 2010;1: 210-3

53. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study. *J Oral Sci* 2007;49:115-20.

54. Somasundaram P, Vimala N, Mandke LG. Protective potential of casein phosphopeptide amorphous calcium phosphate containing paste on enamel surfaces. *J Conserv Dent* 2013;16:152-6.

55. Lynch RJ, Smith SR. Remineralization agents - new and effective or just marketing hype? *Adv Dent Res* 2012;24:63-7.

56. Ren YF, Liu X, Fadel N, Malmstrom H, Barnes V, Xu T. Preventive effects of dentifrice containing 5000 ppm fluoride against dental erosion in situ. *J Dent* 2011;39:672-8.

57. Anbarani AG, Vu T, Ho J, Forghany A. Mapping dental plaque re-accumulation: an imaging approach. Paper presented at: Annual Session of the American Association for Dental Research, March 18, 2016, Los Angeles,

CA, USA.

58. Chung ME, Dadkhah M, Sarraf A. An imaging-based approach to evaluating dentifrice. Paper presented at: Annual Session of the American Society for Laser Medicine and Surgery, April 6, 2013, Boston, MA, USA.

59. Ajdaharian J, Dadkhah M, Sarraf A. Imaging gingivitis response to a novel dentifrice. Paper presented at: Annual Session of the American Society for Laser Medicine and Surgery, April 6, 2013, Boston, MA, USA.

60. Sabokpey S, Biren-Fetz J, Krasieva TB. Optical approach to evaluating the effects of a novel dental gel on oral biofilm. Paper presented at: Annual Session of the American Society for Laser Medicine and Surgery, April 6, 2013, Phoenix, AZ, USA.

61. Lam T, Zadmehr S, Khashai F. Effects of toothpaste on surface enamel: an in vivo imaging study. Paper presented at: Annual Session of the American Association for Dental Research, March 18, 2016, Los Angeles, CA, USA.

62. Sabokpey S, Biren-Fetz J, Wink C, Wilder-Smith P. Imaging in vivo the effects of dentifrices on oral biofilm. Paper presented at: Annual Session of the American Association for Dental Research, March 21, 2014, Charlotte, NC, USA.

63. Kell D. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Arch Toxicol* 2010;84:825–89.

64. Ansari NH, Campbell G, Shoeb M, Xiao TL, Zhang M. Inhibition of endotoxin-induced uveitis by a metal chelator. *ARVO* 2007;E-5156.

65. Zhang M, Shoeb M, Liu P, Xiao T, Hogan D, Wong IG, Campbell GA, Ansari NH. Topical metal chelation therapy ameliorates oxidation-induced toxicity in diabetic cataract. *J Toxicol Environ Health A* 2011;74:380–91.