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UTILIZING COMMERCIALLY AVAILABLE MOUTHRINSES AS EROSION PROTECTION AGAINST ACIDIC CHALLENGES TO ENAMEL

by

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DEDICATION

To my mother, Dr. Phuong Theresa Pham, who constantly warns me of the erosion potential acid of my choice beverages.

DISCLAIMER

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ABSTRACT

Utilizing commercially available mouthrinses as erosion protection against acidic challenges to enamel

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Carbonated soft drinks are one of the most common sources of dentinal acid erosion, and their consumption has been increasing in recent decades. Additionally, fluoride mouthrinses have been shown to be able to protect against acidic erosion. The purpose of this study is to examine the effectiveness of using OTC mouthrinses to counteract the demineralization effect of acidic challenges to enamel through evaluation of surface microhardness. Bovine teeth were polished and immersed in an acid soft drink, then underwent multiple cycles of rinsing with mouthrinses. Surface microhardness of the enamel were measured after the acidic challenge and after each rinsing event. The anticipated results are that rinsing with a fluoride mouthrinse have a significant protective effect on the surface microhardness of enamel that has been exposed to an acid challenge compared to water and non-fluoridated mouthrinses.

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LIST OF ABBREVIATONS

ADA	American Dental Association
AEP	Acquired enamel pellicle
Di-H2O	Deionized water
NAF	Sodium fluoride
SMH	Surface Microhardness
SnF2	Stannous fluoride
VH	Vickers pyramid number

CHAPTER 1: Introduction

Dentin Erosion and Soft Drink Consumption

Dental erosion is defined as the loss of dental hard tissue due to chemical dissolution by acids of non-bacterial origin. The etiology is multi-faceted involving behavioral, biological, and chemical factors.[1] The sources of the acid may be intrinsic, originating from endogenous acids (e.g., oral biofilm and gastroesophageal reflux disease) or extrinsic, usually coming from food or drink.[2] One of the most common sources of extrinsic acid is carbonated soft drinks. From around the 1950s to 2000s, per capita soda production has surged almost 10-fold.[3] From the late 1970's to 2001, there is an estimated two-fold increase in calories from sugar sweetened beverages.[4] Recent studies show that caloric intake from soft drinks have increased to 10% in adolescence[4, 5] and average of 1.66 carbonated soft drink consumed by adolescence in high-income countries per day.[6]

Tooth Demineralization and Remineralization

In addition to precipitating acidic factors that cause dental erosion, numerous other determinants mediate the extent at which the erosion will occur. The rate and extent of erosion is essentially a balancing system of remineralization and demineralization. Increases in the oral cavity acidity will shift the system towards erosion through demineralization. However, there are many factors such as salivary flow that can move the system back towards remineralization. Although the pH in the oral cavity depends on the specific site,[7] the average pH of saliva in the mouth has been found to be from 6.7[8] to 6.78.[7] Erosive acids are any substance that can lower pH of the oral environment to the

critical pH of enamel and dentin. A critical pH is the lowest pH that a solution can reach before it is undersaturated with minerals relative to a specified solid. At any level below the critical pH, the solution will begin to dissolve the solid and leach minerals out of it, such as hydroxyapatite, the predominant mineral in enamel and dentin.[9] For enamel, the critical pH has been found to range from 5.0 to 5.5, while for dentin the critical pH ranges from 6.0 to 6.9.[10]

EFFECTS OF FLUORIDE AND TIN

Application of fluoride and stannous ions to teeth has been widely reported to enhance remineralization and reduce demineralization. It has been shown that application of fluoride through acidulated phosphate fluoride gel, sodium fluoride varnish, and casein phosphopeptide-amorphous calcium phosphate fluoride paste to enamel prior to exposure to an acid can all reduce erosion, though at different extents.[11] Another study indicated that stannous fluoride (SnF2) is more protective against erosion than sodium fluoride (NaF), especially when applied before the acid challenge. NaF's protective effects were best observed when applied after acid immersion and only in the presence of saliva.[12] Algarni et al have reported that there is an additive effect in protection against enamel wear when stannous and fluoride ions are combined versus when used individually,[13] and studies have theorized that this is due to the enhanced resistance of the dental pellicle to acid.[13, 14] Recent results have confirmed SnF2 to be the most effective type of fluoride to protect enamel against demineralization, and have attributed this mainly to the presence of the stannous ions.[15]

Fluoride Mouthrinses

Along with stannous and fluoride content, mouthrinses have other ingredients and properties that change the way they affect dental erosion. Mouthrinses are most commonly composed of a mix of water, antimicrobials, coloring agents, essential oils, alcohol, and ion salts. Alcohol is often used as an antiseptic, and bleaching agents (i.e., teeth whitening) such as hydrogen peroxide are included in some over-the-counter consumer products.[16] Similar to carbonated soft drinks, the low pH of some fluoride mouthrinses can play a role in dental erosion, though research is mixed on its effect. Using carious enamel, Choi et al. have shown that fluoride mouthrinses with low pH can lead to surface microhardness loss.[17] Favaro et al. have demonstrated that acidic mouthrinses showed decrease in surface microhardness, even those with fluoride[16] whereas Dehghan et al. have found that using mouthrinses of low pH can raise salivary pH higher than rinsing with water.[18] The authors' theory is that the taste of the flavored mouthrinse stimulated more salivary flow compared to water. However, despite six tested mouthrinses having pH ranging from 3.87 to 6.20, only the one with lowest pH (3.87) caused dental erosion, and only after continuous use for 14 hours.[19] Gonzales-Cabezas et al. have demonstrated that 2% sodium fluoride solutions of lower pH (3.5) may in fact have enhanced fluoride uptake and rehardening effects compared to 2% sodium fluoride solutions of neutral pH (7.0).[20]

Saliva

In addition to fluoride mouthrinses, saliva itself can affect enamel erosion and remineralization. Saliva protects enamel by washing out carbohydrates from food sources and acid byproducts from oral bacteria, and while also neutralizing acids present in the

mouth, whether intrinsic or extrinsic. The pH of human saliva has a range of 6.2-7.6, with an average of 6.7, allowing it to act as the oral cavity's natural buffering system.[8] Mineral concentration also effects the physical properties of saliva, and its ability to act as a natural buffering agent.[21] Along with natural saliva, there are numerous artificial saliva formulations created as a human saliva alternative both for research and medicinal purposes. Saliva substitutes with high viscosity can protect against erosion, but products of low pH or those that contain citric acid can be damaging.[22]

Surface microhardness Testing

Various methods exist for measuring the change in enamel mineral content, including ultrasound microscopy,[23] ion chromatography,[24] transverse microradiography, quantitative light-induced fluorescence, and surface microhardness (SMH) testing.[25] Although transverse microradiography is considered the gold standard for mineral quantification, SMH is often used due to its relatively ease in experimental protocol. Indeed, SMH has been used to distinguish remineralization changes in enamel at different levels of fluoride treatment.[26] SMH testing involves using a pyramidal indenter at a predetermined load applied to the tested material. The distance of the resulting diagonal lines created by the pyramidal tip of the indenter corresponds to the hardness of the material – longer diagonal marks on the material indicate that the tip was able to penetrate more of the material, meaning the material is softer than if the diagonal marks were shorter. One specific type of SMH testing, the Vickers indenters, have been shown to be a suitable method of measure surface microhardness in shallow and deep demineralized tooth lesions up to 100 um.[25]

AIM OF STUDY

In recognition that over-the-counter consumer oral healthcare products can protect against dental erosion, the American Dental Association (ADA) created an erosion category in its ADA Seal program in 2017 for toothpastes. However, no mouthrinses have the ADA seal for protection against dental erosion. Although various solutions have been shown to remineralize enamel after an acidic challenge, many of these solutions are not readily available by over the counter, nor have these solutions been scientifically compared to each other especially for their effectiveness against erosion. Currently, patients and oral healthcare providers do not have comprehensive information to make an informed decision on using certain mouthrinses against harmful acidic challenges. Therefore, the objective of this in vitro study is to investigate the effectiveness of various OTC mouthrinses to mitigate the demineralization effect of acidic challenges to enamel through evaluation of surface microhardness. The null hypothesis is that rinsing with a fluoride mouthrinse will not have a significant effect on the surface microhardness of enamel that has been exposed to an acid challenge compared to a negative control (water).

CHAPTER 2: Materials and Methods

ENAMEL BLOCKS PREPARATION

Bovine enamel was used for this study due to the certain advantages over human enamel including, cost, availability, and larger surface area. Specimens were procured and prepared (mounted and polished) by Interek Group.

Although differences in morphology and composition of tooth structure of different species exist,[27] human and bovine enamel and dentin are most similar to each other compared to porcine and ovine sources.[28] White et al have reported that bovine enamel can serve as a substitute for human enamel in, finding that the amount and percentage of hardness reduction is the same between the two enamel species, despite bovine enamel eroding 30% faster than human enamel.[29] Arango-Santander et al also have found that human and bovine teeth may be interchanged when testing dental products in vitro, noting only a 7.89% lower microhardness of bovine enamel compared to that from humans.[30]

ACID SELECTION

Diet Coca Cola soda was used as the source of acidic challenge due to its common use and low pH of 3.1.[31] A pH electrode was used to measure the pH of the acid three times, then the reading will be averaged.

DEMINERALIZATION AND REMINERALIZATION CYCLE

For the initial acid challenge, samples were subject to a 10 minute bath of acid at 37ºC, then will be rinsed with remineralizing agent for 1 minute. Immediately after, samples were stored in a bath of pooled human saliva for 24 hours. The pooled human

saliva was procured from Innovative Research Inc, and each batch were collected fresh from at least two donors. A pH electrode was used to thrice measure and then average the pH of the saliva before use.

This completed the first cycle of demineralization and remineralization and subsequent cycles followed the same protocol. The cycles were repeated for 28 days for each of the remineralization interventions and as well as the three controls.

INTERVENTION SELECTION

The following OTC oral healthcare products were used as remineralization solutions due to their ease of availability to non-dental professionals: one high pH fluoride-free mouthrinse (Therabreath Oral Rinse – pH 8.5), one high pH sodium fluoride mouthrinse (Carifree CTx3 Maintenance Rinse – pH 8.1), one low pH sodium fluoride mouthrinse (Listerine Total Care Anticavitiy Mouthwash – pH 3.5), and one stannous fluoride mouthrinse (3M PerioMed 0.63% Stannous Fluoride Oral Rinse – pH to be determined).

Multiple scenarios were chosen as controls. A low pH fluoride-free mouthrinse (Tom's of Maine Wicked Fresh Mouthwash – pH 3.1) was selected as a comparison against the low pH sodium fluoride mouthrinse to determine whether fluoride can mitigate the effect of low pH. Di-H2O was used as a neutral solution rinse to compare against the therapeutic agents. The final control sample had no rinse after the acid challenge. A pH electrode was used to measure the pH of each experimental and control solution three times, then the reading will be averaged. For each solution, enamel blocks were placed on a rocking laboratory shaker set at 15 degrees and 30 rotations per minute.

MICROHARDNESS EVALUATION

Surface microhardness was evaluated using a HMV Micro Hardness Tester (Shimadzu, Kyoto, Japan) with a Vickers indenter with at a load of 50 g for 20 seconds and recorded as Vickers Pyramid Numbers (HV) Microhardness of enamel samples will be measured at the following time periods:

1) T-0: After sample preparation and prior to exposure to the acid challenge

2) T-A: Immediately after exposure to the acid challenge

3) T-7: After 7 days of the demineralization and remineralization challenge

4) T-14: After 14 days of the demineralization and remineralization challenge

5) T-28: After 28 days of the demineralization and remineralization challenge

In addition to the above protocol, another cycle of demineralization and remineralization was done without storage in saliva bath for 24 hours. Instead microhardness testing was performed immediately after 1 minute rinse in the interventions and controls. This cycle was repeated 10 times.

Indentations were made in the center of each sample and were separated by 100 μm linearly to the right relative to an arbitrary predetermined orientation. Indentations were made in triplicate and averaged for statistical analysis.

MODIFICATIONS FOR THESIS

Due to limited time and supply availabilities related to the COVID19 pandemic, not all proposed experiments were able to be accomplished. The results shown here are based on two enamel samples, one intervention (Carifree CTx3 Maintenance Rinse), one control (Tom's of Maine Wicked Fresh Mouthwash), and tap water (in lieu of pooled human saliva). The other mouthrinses and controls will be used for the full 28 day cycle

at a later date, along with pH testing of each solution. Finally, pH testing will be delayed until all supplies are available.

CHAPTER 3: Results

Microhardness values for surface microhardness testing with a demineralization and remineralization cycle that includes at 24 hour tap water bath are shown in **Table 1** and **Table 2**. It was found that there is an average 48.8% increase in hardness with from the post acid bath measurements to post rinse and water bath measurements with the high pH fluoride mouthrinse, whereas with the low pH fluoride free mouthrinse, there was a 24.7% increase in hardness (**Figure 1**).

Results for surface microhardness testing with a demineralization and remineralization cycle without a 24 hour tap water bath are summarized in **Table 3** and **Table 4**. Results from this round of testing show an average increase of 7.6% in hardness using the high pH fluoride mouthrinse and 0.38% with the Tom's low pH fluoride free mouthrinse (**Figure 2**).

CHAPTER 4: Discussion

INTERPRETATION OF RESULTS

This pilot study evaluated the effect of mouthrinse usage after acidic challenge, both direct after application of acid and after acid and a 24 hour bath in water. The results of this preliminary research seem to indicate a high pH fluoride solution (CTx3) has greater enamel rehardening potential than a low pH fluoride free (Tom's of Maine) solution.

With a 48.8% increase in hardness using the CTx3 mouthrinse compared to only 24.7% increase using the Tom's of Maine mouthrinse, the former displayed double the effectiveness of the latter, when used before a 24 hour bath in water (**Figure 1)**. The difference in effectiveness is even more pronounced when surface hardness measurements are recorded immediately after usage of the mouthrinses, without any bath in water. Here, with a 7.6% increase in hardness using the CTx3 mouthrinse compared to only 0.38% increase using the Tom's of Maine mouthrinse, a 20 times increase in hardening is seen with the CTx3 (**Figure 2)**.

Upon examination of the individual tests for the Tom's of Maine mouthwash in Table 4, it can be seen that the third measurement of testing had an increase in hardness whereas the other two had a decrease. This may indicate that the third instance could perhaps be an aberration. The negligible increase of 0.38% in hardness with the Tom's of Maine rinse makes sense due to the fact that its pH is exactly the same as the acid, Diet Coke, at 3.1.

The more pronounced difference in effectiveness between the high pH fluoride mouthrinse and the low pH fluoride free mouthrinse when the 24 hour water bath is

eliminated makes sense due to the presence of ions and minerals found in tap water. A 24 hour – or 1440 minute – bath in tap water is magnitudes longer compared to the 1 minute in the mouthwashes, and over that time, the minerals in the water would continuously deposit onto the enamel samples, which would somewhat mask the difference in effect from the mouthrinses.

LIMITATIONS

Although this research attempted to model the oral environment that enamel undergoes when individuals drink acidic beverages then rinse with a mouthwash, there are many variables that were not considered or controlled for, such as local enamel factors, enamel irregularities, and mouthrinse ingredient variability.

ACQUIRED ENAMEL PELLICLE

A local and individual factor that affects enamel erosion is the acquired enamel pellicle (AEP), a thin layer formed on all surfaces in the oral cavity through composed of salivary proteins, lipids, and other macromolecules.[32-34] This pellicle forms a natural barrier against acids as well as protecting against demineralization by decreasing ion diffusion from tooth surfaces.[35] Individuals have different concentrations of minerals and proteins in their AEP, and it has been shown that patients may be more prone to erosion due to reduced formation of AEP on tissue surfaces.[34] Studies have shown that application of acidulated phosphate fluoride changes the AEP composition,[36] as well as rinsing with fluoride and other stannous ions, which also reduces enamel surface loss.[13] As enamel samples must be polished in order to perform surface microhardness testing, the AEP is lost through the sample preparation process.

ENAMEL IRREGULARITIES

Even though the enamel samples were polished incrementally to 4000 grit silicon carbide paper, texture irregularities and scratches could still be seen under the microscope at 10 and 40 times magnification. Other irregularities noted in the sample were inconsistent color and inconsistent translucency along various regions of the enamel samples. Due to these variations in enamel samples, the absolute Vickers Pyramid Number (HV) of each instance in testing cannot be compared between samples. To compare measurements within a sample, testing had to be performed at 100 micron distance from previous tests within a sample, in order to minimize the effect of intra-sample variation. Thus although absolute HV numbers can be compared within a sample, these numbers were converted to percent change, as only the relative changes in microhardness within a sample could be compared to that of another sample.

FACTORS IN TOOTH WEAR

Tooth wear and enamel hardness is a multifactorial process that results from the interaction of three main mechanisms: attrition, abrasion, and erosion. Only erosion was evaluated in this study, and the effect of the mouthrinses may be reduced or pronounced in the presence of abrasive or attritional factors. Future studies can account for other factors by combining acid erosion and mouthwash remineraliztion cycles with brushing and or chewing simulation tests to more accurately model tooth wear in real life.

TIMING OF MOUTHRINSE

Although the purposes of this research was to examine the remineralization potential of mouthrinses used after enamel exposure to acid, research has shown rinsing with fluoride or stannous ion solutions prior to exposure to acidic soft drink exposure can

also reduce subsequent enamel softening.[37] It would be worthwhile to explore whether the mouthwashes used in this study are more effective if used before or after the acidic challenge.

The mouthrinse application can also be varied in length – for example from increasing the rinse from one minutes to two minutes could also greatly affect results, as well as other variations on time after the mouthwash bath. One approach would be to look at the hardness changes after immediate rinse, and hour intervals up to one day.

MOUTHRINSE VARIATION

The mouthrinses used in this study were formulated for a variety of purposes, including pH neutralization, halitosis, and prevention of dental caries. Additionally, mouthrinses have different pH and fluoride amounts, but they also contain other ingredients such as water, antimicrobials, coloring agents, essential oils, alcohol, preservatives, and flavoring agents.[38] which are not controlled for. Going beyond mouthrinses, there are many other modes of delivery for fluoride, including toothpaste, chewing gum, topical delivery systems, and systemic tablets.

CHAPTER 5: Conclusions

Utilizing mouthrinses for one minute after an acidic challenge to enamel demonstrates remineralization potential, even in cases of low pH and lack of fluoride. However, when using mouthwashes with high pH and fluoride, the remineralization effect may be enhanced.

Table 1. Surface microhardness measurement values using Carifree CTx3 Maintenance Rinse after 24 hour tap water bath.

	Pre-Acid (HV)	Post-Acid (HV)	Post-Rinse (HV)
Measurement 1	18.2	13	18.7
Measurement 2	18.6	12.2	20
Measurement 3	17.3	12.3	17.1
Average	18.0	12.5	18.6
Standard Deviation	0.54	0.36	1.19

Table 2. Surface microhardness measurement values using Tom's of Maine Wicked Fresh Mouthwash after 24 hour tap water bath.

	Pre-Acid (HV)	Post-Acid (HV)	Post-Rinse (HV)
Measurement 1	13.1	11.8	15.7
Measurement 2	15.3	13.5	16.9
Measurement 3	14.1	12.4	14.4
Average	14.2	12.6	15.7
Standard Deviation	0.90	0.70	1.02

Table 3. Surface microhardness measurement values immediately after Carifree CTx3 Maintenance Rinse.

	Post-Acid (HV)	Post-Rinse (HV)
Measurement 1	18.4	19.9
Measurement 2	19.9	21.6
Measurement 3	21.2	22.5
Average	18.6	21.3
Standard Deviation	1.30	1.08

Table 4. Surface measurement values immediately after Tom's of Maine Wicked Fresh Mouthwash.

	Post-Acid (HV)	**Post-Rinse (HV)**
Measurement 1	18.7	18.5
Measurement 2	17.6	17.3
Measurement 3	16.9	17.6
Average	17.8	17.8
Standard Deviation	0.74	0.51

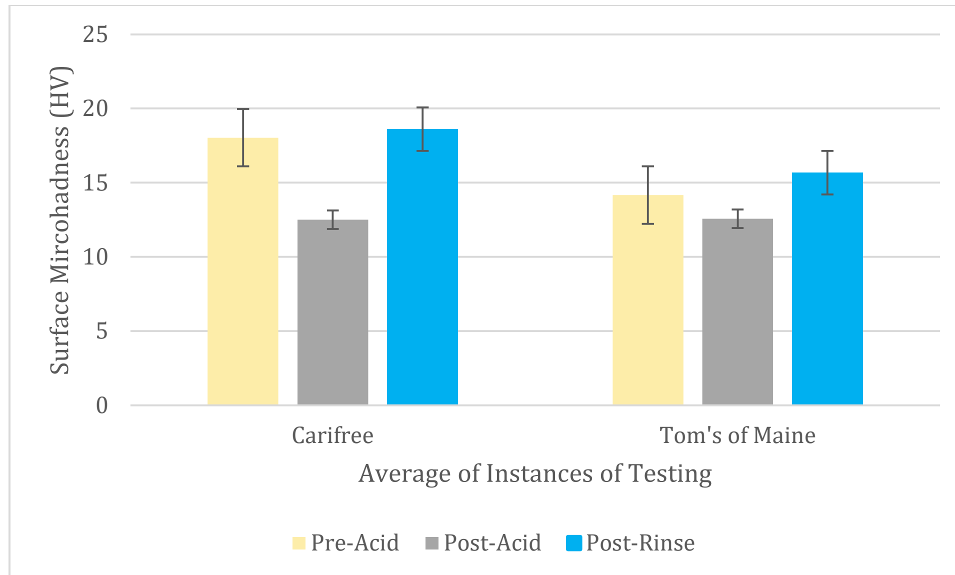

Figure 1: **Average of All Testing Results for Each Mouthrinse after 24 hour Tap Water Bath.** X-axis represents the average of surface microhardness values at three different measuring time points for each mouthrinse (pre-acid treatment before diet soda, post-acid treatment with diet soda, and post-rinse with mouthrinse). Y-axis represents surface microhardness in HV.

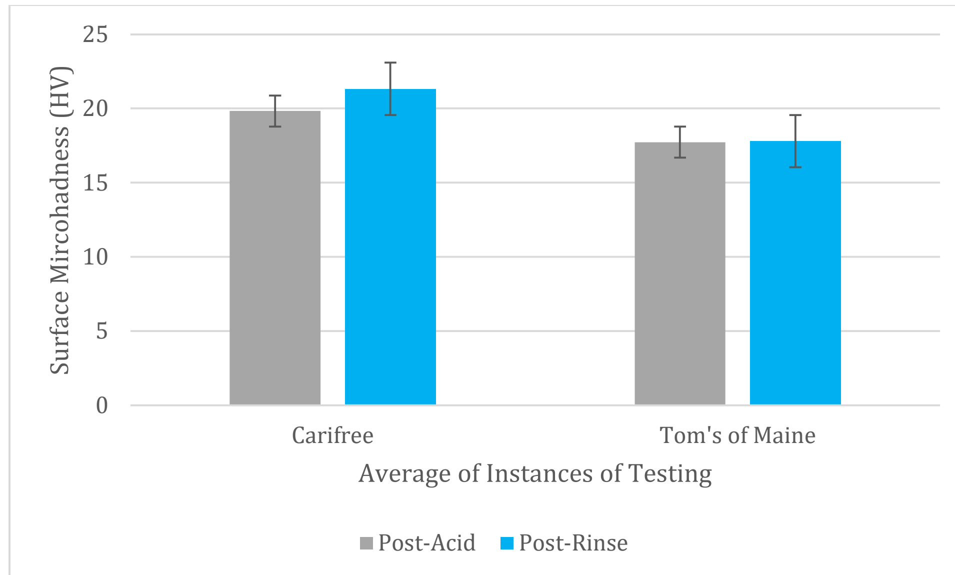

Figure 2: Average of All Testing Results after Immediate Use of Each Mouthrinse. X-axis represents the average of surface microhardness values at two different measuring time points for each mouthrinse (post-acid treatment with diet soda, and post-rinse with mouthrinse). Y-axis represents surface microhardness in HV.

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