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# The Effect of Protective Agents on the Demineralization of Human Tooth Enamel

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## ABSTRACT

Enamel demineralization is caused by food and beverages reducing the mineral phase of the enamel. Protective agents, such as fluoride, are believed to minimize enamel demineralization. This *in vitro* study focused on the effectiveness of commercial protective agents against demineralization. Seventy-two human teeth and five protective agents were used in the study with soda as the demineralization agent. The change in calcium concentration of soda solutions before and after was used as the indication of demineralization. The protective agents were applied to the teeth in the treatment groups, while the control group did not receive any protective agents. The analysis of the resultant soda solutions using atomic absorption spectroscopy showed a significant difference in the concentration of calcium ions between the treatment and control groups. Protective agents Voco, MI Paste Plus, and Sparkle V showed a significant difference in calcium concentration between the control group and the treatment group. Orthowash and Prevident did not show a significant difference but showed a decreased amount of calcium concentration in the treatment group between the first and second treatments. These results therefore suggest that protective agents could prevent enamel demineralization by reducing the loss of calcium ions.

Keywords: *enamel demineralization, erosion, fluoride, remineralization agent, tooth enamel.*

## INTRODUCTION

The enamel of human teeth is composed of more than 96% inorganic mineral (Zhihong, et al., 2011). The main component of this inorganic mineral is calcium phosphate in the form of hydroxyapatite crystals. These crystals are large and arranged nicely with each other and are perpendicular to the dentino-enamel junction (Zhihong, et al., 2011). Dentine is the hard, dense, bony tissue under the tooth enamel and the crystals on the dentine are weakly arranged (Zhihong, et al., 2011). Over time, food and beverages constantly disturb tooth enamel and the mineral phase of the enamel begins to disintegrate, in a process called enamel demineralization (Zhihong, et al., 2011). In the early stages of enamel demineralization, oral saliva is able to re-mineralize and counteract the loss of minerals because saliva is saturated with calcium and phosphate ions (Lata, et al., 2010).

Over the last twenty years, the sale and consumption of commercial soft drinks has increased drastically. These soft drinks are suggested to cause damage to tooth enamel in two ways. First, soft drinks tend to have a low pH and high titratable acidity that causes erosion of enamel (Tahmassebi, 2006). Second, sugars in the soft drinks can be metabolized by plaque microorganisms that generate organic acids, which cause demineralization (Tahmassebi, 2006). Acidic beverages cause a major problem because the beverages have a low concentration of calcium and phosphate (Larsen, Nyvad, 1999). At pH 5.5 or below, the $H^+$ ions from the metabolism of food, performed by the bacteria in the mouth, reacts with the phosphate group in the hydroxyapatite crystals of the enamel (Lata et al , 2010). This reaction converts $PO_4^{2-}$ into $HPO_4^{2-}$which can no longer form the crystal lattice of the enamel (Lata, et al., 2010). When the demineralization is too high, oral saliva cannot fully remineralize the enamel, which leads to caries and white-spot lesions (Lata, et al., 2010). In order to fix these problems, the infected area must be treated by drilling and removal of the cavity followed filling with a material such as a composite or amalgam. Treatments can be costly for the patient and timely for the dentist when the problem could have been avoided by using a remineralization agent.

Protective agents counteract demineralization by balancing the pH and strengthening the enamel (Lata, et al., 2010). Fluoride is a protective agent because when acid approaches the fluoride protected enamel, the pH begins to rise and new, larger crystals containing fluoride begin to form (Lata, et al., 2010). Fluoride binds to several calcium ions at the surface of the tooth, forming fluorhydroxyapatite crystals, which secures the ions together and cuts down the rate of demineralization (Peplow, 2004). The fluorhydroxyapatite crystals form a stronger surface layer that forms an opposition to future demineralization (Lata, et al., 2010). Another remineralization agent is amorphous calcium phosphate- Casein phosphopeptide (ACP-CPP), which is a phosphopeptide based on the milk protein casein (Lata, et al., 2010). ACP-CPP hinders the dissociation of calcium and phosphate ions (Lata, et al., 2010). ACP-CPP provides the enamel with a supersaturated solution of calcium and phosphate

that promotes remineralization (Poggio, 2009).

This *in vitro* study compares the protective potential of five commercial protective agents demineralized by an acidic soft drink. This study is beneficial to dentists and patients because the experiments were performed on actual human teeth and shows which products work the best in an *in vitro* study. The study shows patients the benefits of using a protectant or remineralization agent.

## MATERIALS AND METHODS

*Tooth Preparation*

Extracted human teeth were collected from dental offices and visually assessed for major issues including extreme decay and crowns. Seventy-two teeth were chosen to be used in the study. Molars and pre-molars were cut longitudinally and the roots were removed from all teeth used as suggested by other studies (Gjorgievska, Nicholson, 2011). The newly exposed parts of the teeth were covered with clear nail polish to decrease the chance of accelerated demineralization caused by exposure of the dentin (Savarino, et al., 2002.) Dentin has a weak arrangement of apatite crystals and is not as strong as enamel (Zhihong, et al., 2011). The teeth were divided into groups consisting of two teeth divided in half (molars) and two full teeth of equal size (anteriors). Each group consisted of a properly labeled experimental tube and control tube. Two half-teeth and one whole tooth were placed in each tube. Vinegar was then added to the tubes to disinfect for 7 days (Tijare, et al., 2011). After the disinfectant period, the vinegar was decanted and 1X concentration phosphate saline buffer was added to all of the tubes to act as saliva.

*Pre-Experimental Research*

The pre-experimental research was conducted to discover the necessary time for teeth exposure to the soft drink. Twelve teeth were divided in halves and put into six Erlenmeyer flasks, four half teeth per flask. The flasks were labeled with a time period including 10 min, 20 min, 30 min, 40min, 50 min, and 60 min. The nail polish layer was checked before placing the divided teeth into the Erlenmeyer flasks. The flasks were placed on the rotary shaker table and 30 ml of soft drink was added to each flask. A stopwatch was started as soon as the soft drink was added, then the speed of the rotary shaker was set to 200 RPM. This speed allowed the teeth to have a slight shimmy. After the specified amount of time, the soft drink was decanted and set aside for AAS testing. The results showed the 10 min soft drink had detectable amounts of calcium. The time of 10 minutes would be equated to sipping a soda over the period of one hour.

*Application of Experimental Material*

The five protective agents used in the study were CrossTex Sparkle V, 3M ESPE Orthowash, GC MI Paste Plus, Colgate PreviDent 5000, and Voco Profluorid Varnish.

CrossTex Sparkle V was applied to each tooth using the included brush. The teeth were allowed five minutes to dry then placed back into the original labeled tube with new phosphate saline buffer for at least 30 minutes as suggested on the package.

3M ESPE Orthowash was applied by decanting the phosphate saline buffer from the tube, then adding 10 ml of Orthowash to the tube. The tube was then capped and agitated vigorously for one minute. The Orthowash was decanted and new phosphate saline buffer was added to the tube and allowed to sit for at least 30 minutes as suggested by the package.

GC MI Paste Plus was applied by placing a small amount of the paste on a brush and applying it to the tooth. After each tooth was completely covered, the teeth were allowed to sit undisturbed for five minutes. The teeth were then placed back in the original labeled tube with new phosphate saline buffer for 30 minutes as suggested by the package.

Colgate PreviDent 5000 was applied by brushing each tooth with a toothbrush and a pea size drop of PreviDent for ten to twelve seconds. The teeth were then placed back into the original labeled tube with new phosphate saline buffer and allowed to sit for at least 30 minutes as suggested by the package.

Voco Profluorid Varnish was applied to the teeth using the enclosed brush. Each tooth was completely covered in varnish then placed back into the original tube. New phosphate saline buffer was added to the tube and allowed to sit for at least 30 minutes as suggested by the package.

The control groups did not receive a protective agent.

*Demineralization of Teeth*

Each tube of teeth was checked to ensure the nail polish layer of each tooth was intact. The phosphate saline buffer was decanted and the teeth were placed in a labeled Erlenmeyer flask. The flasks were placed on the rotary shaker table and 30ml of soft drink was added to each flask. A stopwatch was started as soon as the soft drink was added and the speed of the rotary shaker was set to 200 RPM. After the specified amount of time, 10 minutes, the soft drink was decanted and set aside for AAS testing.

*Soft Drink Analysis*

The soda mixture was analyzed using a Varian Spectra AA 55, which is the atomic absorption spectroscopy (AAS) machine at Wichita State University. The instrumental parameters were adjusted to the manufacturer's recommendations. The soda mixture was aspirated into a flame that is

lined up with a light beam that is generated by a lamp specific for calcium. A detector measured the intensity of the beam of light and calculated the absorbance. Calcium standard solutions were prepared and the absorption was recorded. The absorption of all samples was recorded and compared to the standard solutions to find the parts per million of calcium in each solution.

## RESULTS

Calcium absorption was measured and converted to concentration measured in parts per million (PPM). Statistical analysis was performed using JASP for Mac (JASP, JASP Team, 2016, 0.7.5.5).

Statistical analysis was first performed using a Paired T-Test to determine a statistical significance at $p \leq 0.05$ between each experimental group and its corresponding control group. Two Treatments were performed and data was collected for each treatment.

In the first treatment, MI Paste Plus and Sparkle V showed a significant difference between the control and treatment groups, p=0.001 and p=0.03 respectively. Orthowash and Voco did not show significant differences between the treatment and control groups but figure 1 shows that the control group has a higher concentration of calcium compared to the treatment group. Figure 1 shows that PreviDent has a higher concentration of calcium in the treatment group compared to the control group. An ANOVA test followed by a multiple comparisons test was used to detect differences at $p \leq 0.05$ between the five protective agents. The ANOVA test showed a significant difference between Sparkle V and the four other protective agents. None of the other protective agents showed a significant difference between each other.

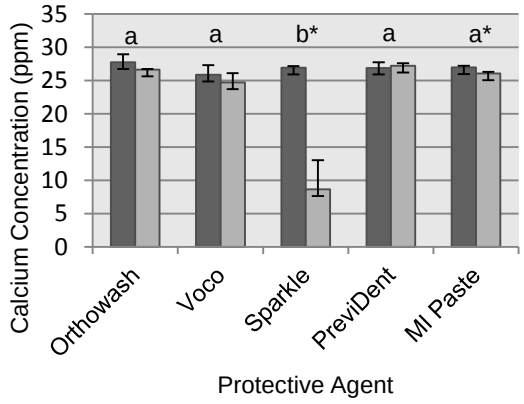


**Figure 1.** Calcium concentration (ppm) in soda after the first treatment.

The darker band on the left for each protective agent is the control group and the lighter band on the right is the treatment group. The error bars are ±SE. An asterisk next to the letter indicates a significant difference between the control and treatment group. The letter a indicates no significant difference between the protective agents in the ANOVA test. The letter b indicates a significant difference between Sparkle V and the other protective agents.

In the second treatment, Voco and Sparkle V showed a significant difference between the control and treatment groups, p=0.005 and p=0.001 respectively. Orthowash, Prevident, and MI Paste Plus did not show significant differences between the control and treatment group but figure 2 shows that the control group has a higher concentration of calcium compared to the treatment group. The ANOVA test showed a significant difference between Sparkle V and the four other protective agents. Voco showed a significant difference between the four other protective agents.

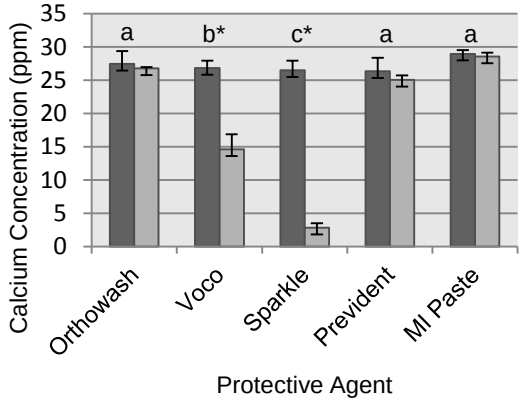


**Figure 2.** Calcium concentration (ppm) in soda after the second treatment.

The darker band on the left of each protective agent is the control group and the lighter band on the right is the treatment group. The error bars are ±SE. An asterisk next to the letter indicates a significant difference between the control and treatment group. The letter a indicates no significant difference between the protective agents in the ANOVA test. The letter b indicates a significant difference between Voco and the other protective agents. The letter c indicates a significant difference between Sparkle V and the other protective agents.

## DISCUSSION

The statistical data analysis showed a significant difference in two of the five protective agents and the respective control group in both treatments. However, the two protective agents with a significant

difference were not consistent between both treatments.

In the first treatment, Sparkle V and MI Paste Plus showed a significant difference between the treatment and control group. The sample size (3) was adequate to show that the treatment group had a significantly lower concentration of calcium compared to the control group. Orthowash and Voco did not show a significant difference but the control group had a higher calcium concentration compared to the treatment group. With a larger sample size both Orthowash and Voco would show a significant difference. Prevident showed a slightly higher calcium concentration in the treatment group compared to the control group.

In the second treatment Sparkle V and Voco showed a significant difference between the treatment group and the control group. The sample size was adequate to show that the treatment group had a significantly lower calcium concentration. Orthowash, MI Paste Plus, and Prevident did not show a significant difference but the control group had a higher calcium concentration compared to the treatment group. With a larger sample size, these three protective agents would show a significant difference.

Sparkle V stayed consistent between the two treatments by showing a significant difference in both. Voco improved from the first treatment to the second treatment and showed a significant difference in the second treatment. MI Paste Plus appears to have declined between the two treatments. Orthowash and Prevident did not show a significant difference in either treatment, but did show improvement from the first treatment to the second treatment.

Sparkle V and Voco are fluoride varnishes that are recommended by dentists to be applied every six months at a cleaning. Fluoride in the varnish binds to the calcium ions on the surface of the tooth and binds the calcium ions together. Binding the ions together forms a stronger surface layer which in turn decreases the rate of demineralization (Lata, et al., 2010). The results show that the treatment group has a lower concentration of calcium compared to the control group. The concentration of calcium in the treatment group decreases from the first treatment to the second treatment.

Orthowash and Prevident contain a lower concentration of fluoride compared to a varnish. These two protective agents are intended to be used by a patient daily. Orthowash is used by patients with orthodontia to prevent decalcification and caries. A mouthwash is used to ensure that fluoride is reaching all areas of the teeth. Prevident is a fluoride toothpaste. Both protective agents showed a decrease in calcium concentration between the first treatment and the second treatment. A significant difference between the control group and the treatment group would be expected with a larger sample size or an increased number of treatments.

MI Paste Plus increases the number of calcium and phosphate ions and adds a small amount of fluoride. MI Paste Plus is a topical crème that can be applied to the teeth multiple times each day. The CPP in MI Paste Plus is able to stabilize Amorphous Calcium Phosphate. The results show that MI Paste plus increases in calcium concentration between the first treatment and the second treatment. However, this increase in calcium concentration is not caused by enamel demineralization. Using MI Paste Plus results in enamel that is supersaturated with calcium and phosphate ions (Poggio, 2009). In the second treatment, there is an abundant amount of calcium and phosphate ions on the enamel surface. The surplus calcium is collected by the soda and appears in the calcium concentration.

Overall, the results prove that some protective agents show a significant difference after two treatments. Fluoride varnishes did and are expected to show this result. Other protective agents such as mouthwash or fluoride toothpaste are not expected to show a significant difference after two treatments because these protective agents are used daily. We can see in these results that the concentration of calcium in the control is higher than the treatment and there is a decrease in calcium concentration from the first treatment to the second treatment. In conclusion, teeth protected by protective agents show a lower concentration of calcium released compared to teeth not protected by protective agents.

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## LITERATURE CITED

Gjorgievska E and J Nicholson. 2011. Prevention of enamel demineralization after tooth bleaching by bioactive glass incorporated into toothpaste. Australian Dental Journal 56:193-200.

Larsen M and B Nyvad. 1999. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries Research 33(1):81-87.

Lata S, NO Varghese, and JM Varughese. 2010. Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho

peptide on enamel lesions: An in vitro comparative evaluation. J Conserv Dent 13:42-6. http://www.jcd.org.in/text.asp?2010/13/1/42/62634 (14 Oct. 2014).

Peplow M. 2004. How fluoride firms up teeth. Nature News. doi:10.1038/news0401198.

Poggio C, M Lombardini, A Dagna, M Chiesa, and S Bianchi. 2009. Protective effect on enamel demineralization of a CPP-ACP paste: an AFM in vitro study. Journal of Dentistry 37(12):949-954.

Savarino L, A Saponara Teutonico, C Tarabusi, L Breschi, and C Prati. 2002. Enamel microhardness after in vitro demineralization and role of different restorative materials. Journal of Biomaterials Science- Polymer Edition (3):349-357.

Tahmassebi, J, M Duggal, G Malik-Kotru, and M Curzon. 2006. Soft Drinks and Dental Health: A Review of the Current Literature. Journal of Dentistry 34.1: 2-11.

Tijare M, D Smitha, S Kasetty, S Kallianpur, S Gupta, and H Amith. 2011. Vinegar as a disinfectant of extracted human teeth for dental educational use. Journal of Oral and Macillofacial Pathology 18.1:14-18.

Zhihong D, C Jiang, Z Yue, and L Kaili. 2011. *In vitro* remineralization of human dental enamel by bioactive glasses. Journal of Materials Science 46(6):1591-1596.