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Effects of sodium hypochlorite on enamel composition

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THE EFFECTS OF SODIUM HYPOCHLORITE ON ENAMEL COMPOSITION: A COMPARATIVE IN VITRO STUDY

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A Thesis Presented to the Faculty of the College of Dental Medicine of

Nova Southeastern University in Partial Fulfillment of the Requirements for the

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I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.S. degree and for this assignment.

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DEDICATION

To my mother who has always been my #1 fan and my inspiration, thank you for the countless pep talks and reminding me that “your education is something no one can ever take from you.” Without you, none of this would be possible. To my father whose hard work and attention to detail has been a perfect model to emulate in my career path, thank you for your selfless pursuit of achieving a better life for Matthew and I. To my brother whose sense of humor and support has been invaluable to me, thank you for teaching me to laugh at myself and reminding me of the true meaning of life. To my fiancé, Brian, who showed up at the perfect time, you have encouraged, pushed, and believed in me from Day 1. Your optimism and enthusiasm has been the ultimate blessing in seeing me through to the finish line. From the bottom of my heart, I love you and thank you all!

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Abstract

THE EFFECTS OF SODIUM HYPOCHLORITE ON ENAMEL COMPOSITION: A COMPARATIVE IN VITRO STUDY

DEGREE DATE: DECEMBER 6, 2015

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Objective: The purpose of this study was to evaluate the effects of sodium hypochlorite on the organic and inorganic composition of enamel. Background: With the advent of enamel bonding for orthodontic appliances in the late 1970s, it has been shown that traditional phosphoric acid etching affects the inorganic portion of the enamel.[1, 2] In an attempt to enhance the acid etching pattern and, furthermore, the bond strength, additional pretreatment techniques that target the organic components of the enamel biofilm have been proposed. One such method is the non-invasive enamel pretreatment with 5.25% sodium hypochlorite (NaOCl) prior to phosphoric acid etching.[3, 4] It has been suggested that the mechanism by which sodium hypochlorite enhances the etching pattern is enamel deproteinization, in which organic elements, including the acquired film, are removed from the enamel surface.[3, 5] This presumption is based on the multitude of endodontic literature supporting the use of NaOCl as an effective irrigant in root canal therapy[6-13]. In contrast to dentin and pulpal tissue, enamel is comprised of minimal organic matter.[14, 15] As a result of this fact and the limited amount of experimentation of the effect of NaOCl on the enamel surface, the true mechanism by which sodium hypochlorite enhances the etching pattern of enamel is questionable.[5, 16] The objective of this study was to determine the compositional effects of sodium hypochlorite on human enamel. Methods: Following IRB approval, 120 enamel sections from 22 extracted human premolar teeth were randomly divided into three experimental groups and one control group.[17] The control group (E = enamel) received no treatment. The first experimental group (A = phosphoric acid) received a 15-second treatment with

37% phosphoric acid, rinsed with distilled water and air sprayed for 20 seconds, then dried with oil free compressed air. The second experimental group (H = sodium hypochlorite) received a treatment of 5.25% sodium hypochlorite for 60 seconds, washed with distilled water for 10 seconds, and dried. The third experimental group (HA = sodium hypochlorite + phosphoric acid) received a treatment of 5.25% sodium hypochlorite for 60 seconds, washed with distilled water for 10 seconds, dried, then receive the 15-second treatment with 37% phosphoric acid as in Group A.[3] Following treatment preparations of the four groups, scanning electron microscopy (SEM)/energy-dispersive X-ray spectrometer (EDX) analysis was performed for all groups.[18] For elemental concentration, a one-way ANOVA and Tukey's post hoc statistical tests were applied.[17, 19, 20] ANOVA and Tukey tests were performed at a significance level of $p \leq 0.05$. Results: There were no significant effects of treatment on the enamel elements carbon (C), calcium (Ca) sodium (Na), oxygen (O), and phosphorous (P). There was a significant effect of treatment on the amount of chlorine (Cl) in enamel between groups acid (A) and hypochlorite + acid (HA) as well as between groups hypochlorite (H) and hypochlorite + acid (HA) ($p = 0.004$). The amount of variation of iodine (I) in the enamel composition between untreated enamel (E) and enamel treated with sodium hypochlorite + phosphoric acid (HA) was significant ($p = 0.004$). Additionally, there was a significant decrease in the quantity of antimony (Sb) found in the control group (E) versus the hypochlorite + acid (HA) experimental group ($p = 0.002$). Lastly, tin (Sn) was significantly reduced from the enamel surface (E) when treated with hypochlorite + acid (HA)

(p = 0.008). Conclusions: The various treatments minimally affected the elemental concentrations of C, Ca, Na, O, and P. The amount of chlorine present in enamel significantly increased following treatment with sodium hypochlorite (H) alone and even more so following treatment with phosphoric acid and sodium hypochlorite (AH). In contrast, elements I, Sb, and Sn demonstrated a congruent reduction in concentration after treatment with hypochlorite and acid (HA). Although it has been hypothesized that sodium hypochlorite targets the organic pellicle present on the surface of enamel via a process known as deproteinization, the findings presented here suggest that pre-treatment with NaOCl impacts the inorganic components of enamel more so than the organic constituents. These quantitative findings corroborate the enhanced etching pattern that can be visualized under scanning electron microscopy in this as well as previous studies.

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Chapter 1: Introduction

1.1. Enamel Composition

Dental enamel itself is the most highly mineralized extracellular tissue known, comprised of approximately 96% mineral content, by weight, and approximately 4% organic material and water.[14, 15] The inorganic portion of enamel is largely crystalline calcium phosphate, or hydroxyapatite, exchanged with carbonate ions, along with possible traces of strontium, magnesium, lead, and fluoride ions.[14, 15] The stages of mineralization of enamel culminate in a mature enamel layer that is most highly mineralized at the surface. [14] In the absence of water, the organic component, composed primarily of proteins and lipids, constitutes approximately 1% of mature enamel.[15] An adequate amount of organic nutrients and fundamental minerals, e.g., calcium (Ca) and magnesium (Mg) (hydroxyapatite and magnesium phosphate) is required for the proper functioning, structure, and resistance of these hard tissues.[21] Additionally, trace elements, i.e, zinc (Zn) and copper (Cu) are crucial for enamel integrity and flexibility.[21, 22] For instance, results from Brookes *et al.* reveal that Cu offers direct protection from enamel dissolution in an acidic environment.[21, 23, 24] Additionally, Zn has also shown it has positive effects as a strong deterrent of demineralization and a proponent of remineralization.[21, 25, 26]

1.2. Bonded Attachments in Orthodontics

Successful orthodontic treatment can only be rendered when proper tooth attachment is achieved. Historically, mechanical retention was employed, where the teeth were individually banded. It wasn't until 1955, when Buonocore developed the acid etch technique that a chemical preparation of the enamel surface was first presented.[1] In the 1970s, direct-bonding brackets were introduced, evaluated, and have since been subjected to innumerable studies in efforts to enhance the reliability and efficiency of orthodontic treatment. [1, 27-29]

Since its introduction over 50 years ago, enamel etching remains the most reliable method of surface preparation prior to bonding. [2] Phosphoric acid conditioning enhances bonding by dissolving hydroxyapatite crystals, which facilitates the penetration of the fluid adhesive components, forming resin tags, which provide micromechanical retention.[30] The etching quality depends upon four factors: etching agent, acid concentration, etching time, and enamel surface composition.[3, 31-40] In 1975, Silverstone described three different etching patterns, visible under scanning electron microscopy, resulting from phosphoric acid targeting the inorganic portion of enamel.[31] Type 1 etching pattern was characterized as phosphoric acid (H_3PO_4) dissolving the head of the prism, with peripheral material or interprismatic substance remaining intact.[3, 31] Conversely, type 2 results in a diluted peripheral zone with the prism head intact. [3, 31] In type 3, the dissolution is superficial and results in no specific features. [3, 31] Later, Silverstone revealed that etching types 1 and 2 yielded deeper levels of penetrability resulting in greater bonding retention.[3]

1.3. The Limitations of Phosphoric Acid Etching

Although phosphoric acid etching remains the gold standard for enamel conditioning, it has been found that as little as 2% of the treated surface is ideally etched.[16, 41, 42] In an attempt to maximize etching potential, various invasive and non-invasive procedures; such as enamel abrasion, air abrasion, and lasers have been studied with no appreciable results.[16,43] In 2008, Espinosa *et al.* explored the effects of non-invasive sodium hypochlorite (NaOCl) on enamel topography in comparison to traditional phosphoric acid etching.[3] They reported: double the enamel retentive surface area (relative to phosphoric acid etching alone) and significant increases in type 1 and 2 etch patterns on teeth treated with 5.25% NaOCl for 60 seconds prior to traditional H_3PO_4 etching.[3]

1.4. Development of White Spot Lesions in Orthodontics

In addition to attaining sufficient bond strength, orthodontists frequently find themselves concerned with patients' oral hygiene throughout the course of treatment. It is well documented that one of the principle risks of orthodontic treatment is enamel decalcification due to increased plaque retention sites in concordance with inadequate oral hygiene.[44-47] The incidence of demineralization during fixed appliance therapy has been reported to be as high as 50%.[47, 48] Since enamel translucency is directly related to the degree of mineralization, subsurface enamel porosity from demineralization is clinically manifested by a milky white opacity named a "white spot lesion" (WSL).[44, 49] It has been reported

that WSLs are detected on approximately 25% of patients undergoing treatment, as early as 4 weeks post-bonding.[50, 51] Fortunately, research has found strong evidence to support the usage of fluoride to prevent demineralization around orthodontic attachments.[44, 52-54] Thus, fluoride-releasing adhesive agents have been suggested as alternative bonding agents in orthodontic patients for their cariostatic effects.[44, 55-58] Glass ionomer cements were initially introduced as orthodontic adhesives namely for their ability to form a chemical bond with the tooth surface as well as their sustained fluoride release following bonding.[44] However, because of their lower bond strengths, their employment in bonding fixed attachments is fairly limited.[44, 59-62] If a stronger chemical bond between the glass ionomer cement and the enamel could be achieved, orthodontists could take advantage of the adhesive's fluoride-releasing potential.[44, 59-62] Justus *et al.* successfully demonstrated that pretreatment of enamel with 5.25% sodium hypochlorite prior to etching yields shear bond strengths with glass ionomer cement that are comparable to traditional composite adhesive.[4]

1.5. Importance of Study

With the universal obstacle of obtaining adequate oral hygiene to prevent the development of enamel demineralization during orthodontic treatment, the need for additional methods to minimize this risk is undisputedly justified.[3, 4, 44-46, 48, 50, 51] Although glass ionomer adhesives, with their fluoride releasing ability, have proved to be effective in reducing white spot lesion formation, historically they have not provided an adequate bond strength to be applied clinically to bond

fixed orthodontic attachments.[55-58, 60-62] Recent studies have yielded promising results by pretreating enamel with sodium hypochlorite prior to traditional phosphoric acid etching, providing clinically acceptable bond strengths with glass ionomer.[4, 5]

In the past, sodium hypochlorite's predominant role in dental treatment has been limited to the field of endodontics as an antimicrobial irrigant.[6-9, 12, 13, 63, 64] The usage of sodium hypochlorite as a pretreatment technique in the orthodontic specialty only exists in the literature.[3-5, 16] Currently, the majority of this research on NaOCl has focused on its effects on bond strength and enamel surface morphology.[3-5, 16] Prior to implementation in a clinical trial, its mechanism of action on enamel requires a deeper understanding. Until now, the effect of NaOCl on enamel content has not been examined. This study was the first one to propose the use of SEM in conjunction with energy-dispersive X-ray spectroscopy to investigate the potential NaOCl-induced alterations in the chemical composition of enamel.[18, 20] The findings of this study offered an insight view to the true mechanism by which sodium hypochlorite produces enhanced etching patterns and superior bonds. Moreover, the conclusions may have brought us one step closer to the eventual application of a novel clinical orthodontic bonding protocol that promotes the oral health of patients.[3, 4]

1.6. Purpose, Specific Aim and Hypothesis

1.6.1. Purpose

Although it has been shown that NaOCl as a conditioning agent achieves a more profound etching pattern resulting in superior bond strength, its mechanism of action has chiefly been speculation.[3-5] Lacking a deeper level of understanding, NaOCl has not been employed clinically as a pretreatment technique. If its mechanism of action is clinically acceptable, sodium hypochlorite could become a part of standard enamel surface preparation protocol, such that glass ionomer cement can be employed routinely for its cariostatic effects to minimize the ubiquitous demineralization present during fixed appliance therapy.[4, 44-46, 48, 50, 51, 55-62, 65] Therefore, the purpose of this *in vitro* study was to examine the effects of sodium hypochlorite on the composition of the enamel surface.

1.6.2. Specific Aim

1. To evaluate the effects of sodium hypochlorite conditioning on the organic and inorganic composition of enamel.

1.6.3. Hypothesis

H_o:

1. There are no statistically significant differences in the organic or the inorganic components of enamel when the enamel surface is conditioned with sodium hypochlorite versus those that will not be conditioned with sodium hypochlorite.

Chapter 2: Materials and Methods

2.1. Study

Based on a power analysis, one hundred and twenty ~1mm^2 enamel blocks from extracted human maxillary and mandibular premolars were included in this *in vitro* study.

2.1.1. IRB Approval

IRB approval to conduct research using extracted human teeth was obtained by Nova Southeastern University.

2.1.2. Ethical Issues

No potential ethical issues could be identified as part of this research study. All data collection complied with IRB and HIPAA regulations and all data was de-identified to ensure confidentiality.

2.1.3. Grant

This study was funded by a Nova Southeastern University's Health Profession Division grant.

2.2. Sample Size Estimate

Previous and similar studies, one, examining mineral changes in dental enamel by Soares *et al.* in 2013[17], and the other, evaluating the etching pattern of enamel treated with and without sodium hypochlorite by Ahuja *et al.* in 2010[16], were used as the mock "pilot studies" to determine the proper sample size required for the energy-dispersive x-ray spectroscopy mineral analysis in this

research study. G*Power 3.1 was used to determine the appropriate sample size. It was found that a total of 120 enamel blocks of 1mm^2 each were needed for testing, with 30 samples in each of the four groups.

a. Sample Size to be used = 30
b. Alpha = 0.05
c. Beta = 0.20
d. Effect Size = 50% (large)
e. Power = 80%

A total of twenty-two teeth were used to obtain 120 enamel sections, which were divided into 4 groups of 30 subjects each.

2.3. Sample Preparation

Following IRB approval, twenty-two unidentified extracted human premolar teeth, obtained from Nova Southeastern University College of Dental Medicine, were used in this research. The inclusion criteria was intact buccal and lingual enamel, no exposure to chemical agents, no surface cracks, and caries-free.[4, 16] Following extraction, all samples were stored in saline solution at 37°C.[3, 4]

The samples were randomized through a systematic method into four groups, three experimental and one control (no treatment).[3, 16] All groups underwent elemental analysis via scanning electron microscopy (SEM)/energy-dispersive x-ray spectroscopy (EDX) (Fig. 1).[17-20]

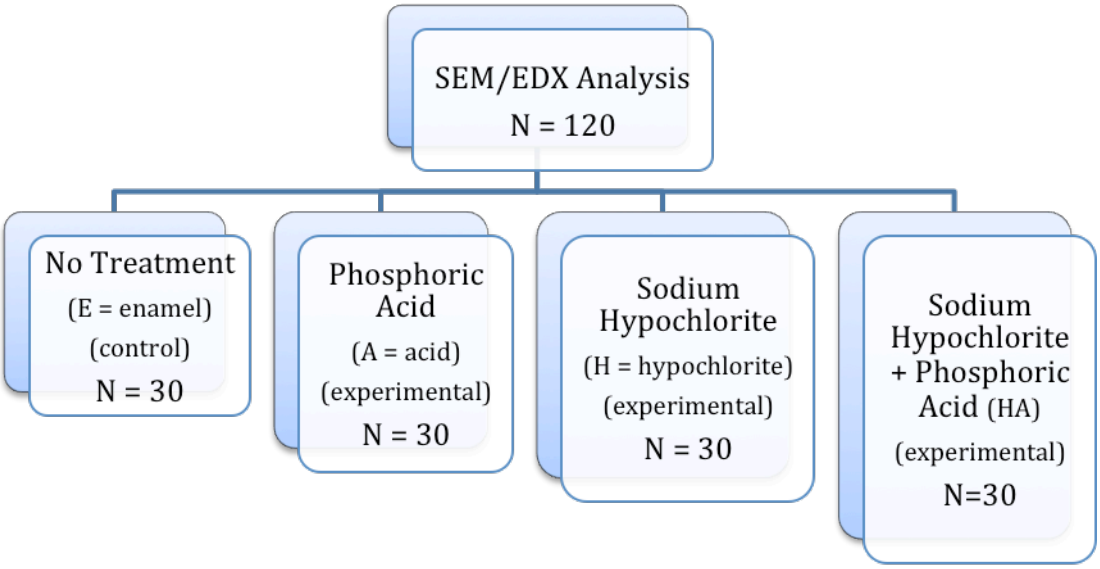


Figure 1. Flow chart of the division of the total sample into treatment groups by random assortment.

Prior to random sampling, the enamel surfaces of all samples were cleaned with a non-fluoridated prophylaxis paste and rinsed with distilled water for 10 seconds (Whip Mix, Louisville, KY, USA). To obtain comparable enamel surfaces with relatively uniform physical and chemical properties, the buccal and lingual surfaces of the crowns were marked with a horizontal line at the middle third, followed by three equidistant vertical lines. These demarcations designated where the cuts, made with a double-sided diamond disk, were to be made (Ortho Technology, Tampa, FL, USA) (Fig. 2). The blocks were trimmed to approximately a $1mm^2$ dimension. Thus, six $1mm^2$ enamel blocks were to be obtained per tooth.[16] Therefore, it was estimated that approximately 20 premolars would be necessary to satisfy the sample size of one hundred and twenty sections. Due to the loss of a few samples during sectioning, a total of 22 teeth were necessary to reach the required sample size.

Figure 2. Tooth sectioned using double-sided diamond disk.

Each enamel block was randomly assigned to and treated according to the protocol in one of the four treatment groups. Following treatment, samples were mounted in four rows, according to the corresponding treatment group, on SEM stubs with a conductive double-sided adhesive carbon tape previously on the analysis and inserted into a vacuum chamber (Fig. 3). Five points on each surface were selected for analysis. The system used in this study presents with one EDX detector used for qualitative and quantitative microanalysis.

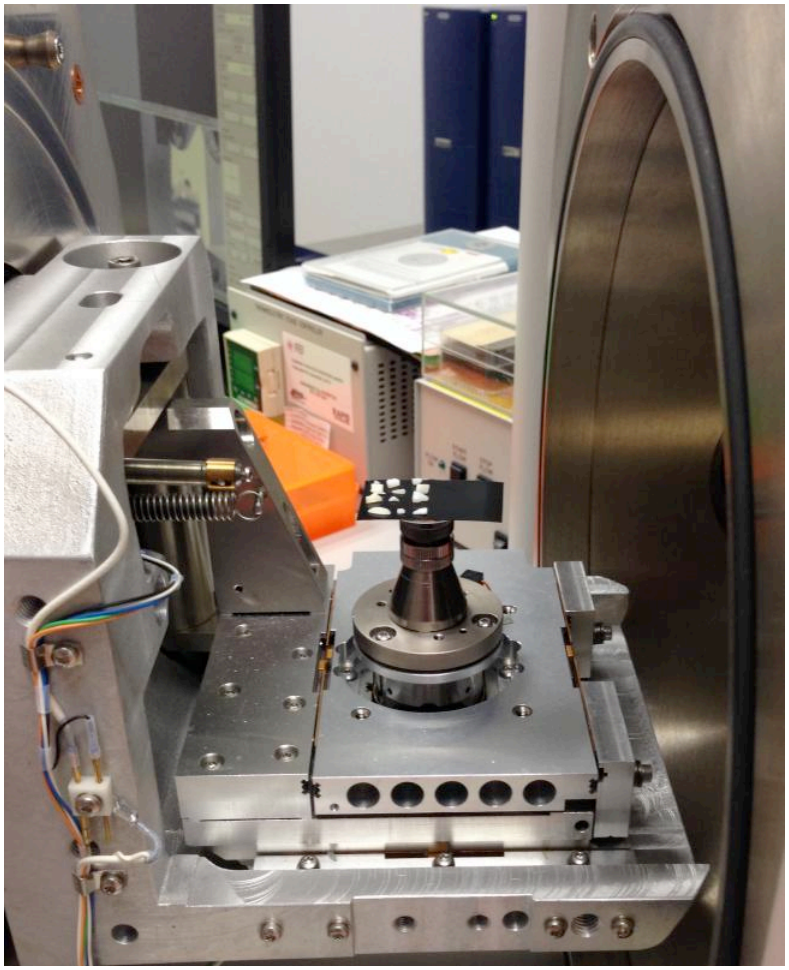


Figure 3. Samples mounted on a SEM stub with carbon tape about to be inserted into vacuum chamber.

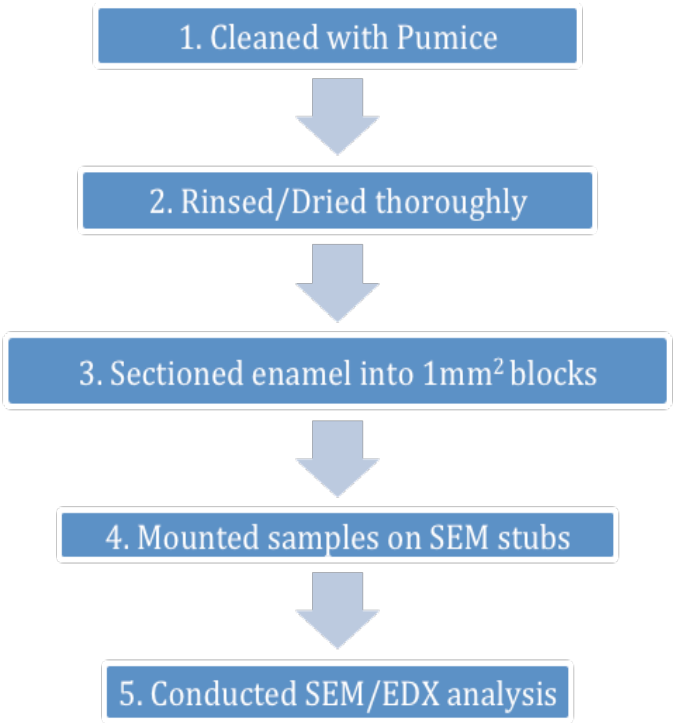
2.4. Methodology

The following treatment protocols were implemented in each testing group and were performed according to the manufacturer's instructions:

a. Group E (control) (Fig. 4): The buccal and lingual surfaces of all the extracted teeth were pumiced and rinsed with distilled water and air sprayed for 20 seconds, then dried with oil free compressed air. They received no other surface preparation. Following sectioning of the

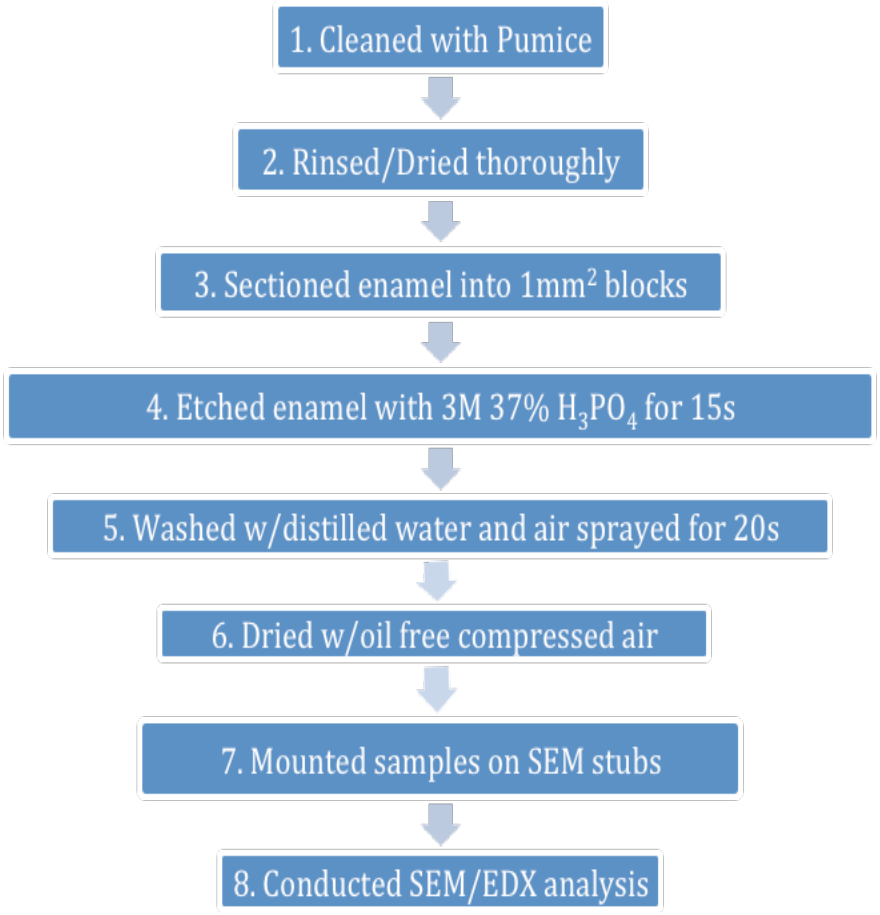
enamel surface into 1mm^2 dimensions, samples assigned to this control group were simply placed on the SEM stubs for SEM/EDX analysis.

Figure 4. Group E Protocol



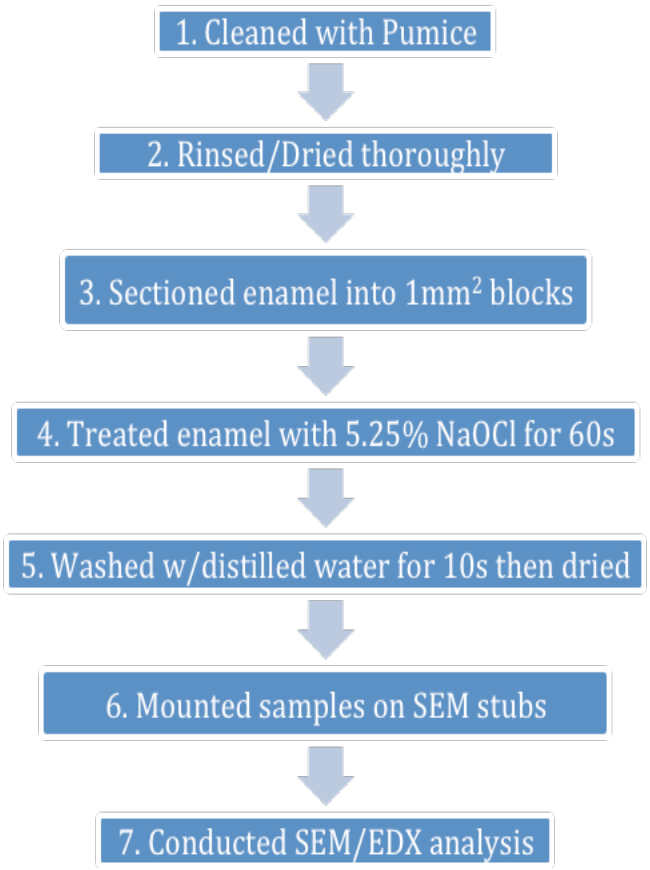
b. Group A (experimental) (Fig. 5): The enamel blocks randomly assigned to this treatment group were etched with 37% H_3PO_4 gel (3M ESPE Scotchbond etching gel, St Paul, MN) applied with a microbrush for 15 seconds, washed with distilled water and air sprayed for 20 seconds, then dried with oil free compressed air.[3]

Figure 5. Group A Protocol



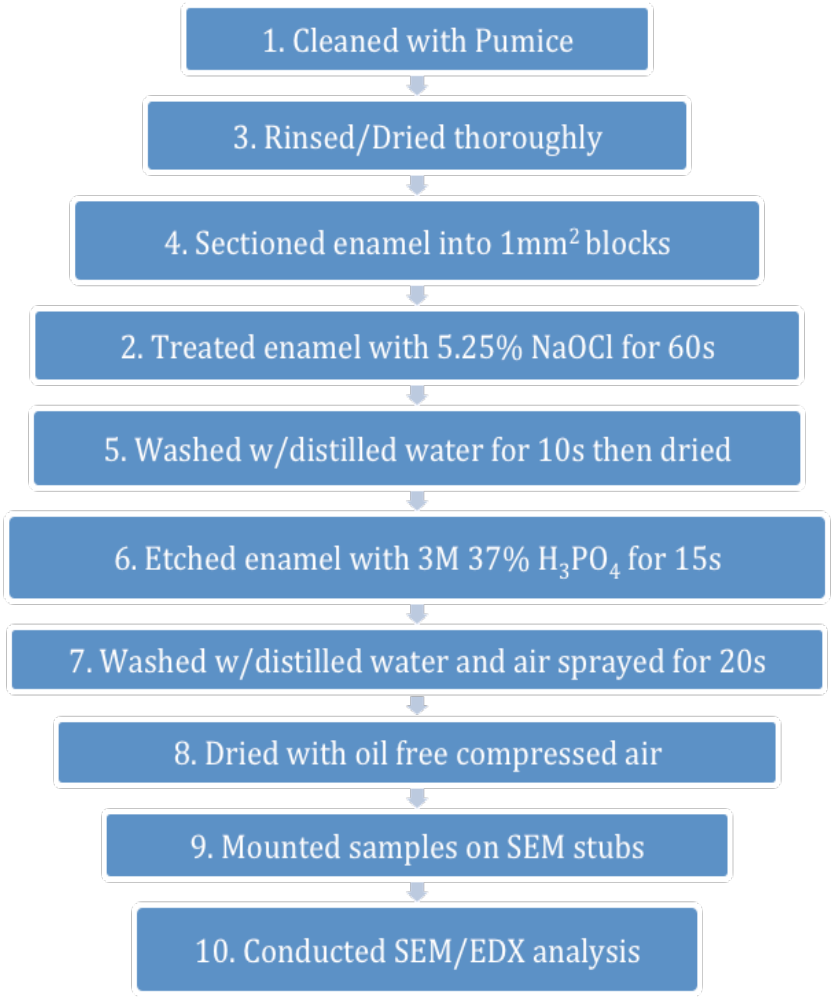
c. Group H (experimental) (Fig. 6): The enamel surface was treated with 5.25% sodium hypochlorite (NaOCl; Clorox, The Clorox Co., Oakland, CA) with a sterile cotton pellet for 60 seconds, washed with distilled water for 10 seconds, then dried.[3]

Figure 6. Group H Protocol



d. Group HA (experimental) (Fig. 7): The enamel surface was treated with 5.25% sodium hypochlorite (NaOCl; Clorox, The Clorox Co., Oakland, CA) with a sterile cotton pellet for 60 seconds, washed with distilled water for 10 seconds, dried, then etched as for Group A.[3]

Figure 7. Group HA Protocol



Of the 120 samples prepared, three samples per treatment group (for a total of 12 samples) were randomly selected to undergo SEM imaging. Each sample was photographed at a magnification of 500X and 1000X in order visualize the etching pattern achieved by means of the various surface preparations.

2.5. Data Storage

The de-identified data was entered and stored in a file on a password-protected computer.

2.6. Statistical analysis

Descriptive statistics, including means and standard deviations were calculated for each of the four groups.

A one-way analysis of variance test was used to determine statistically significant differences in the mineral content between the analyzed groups. To determine differences between groups, a Tukey's post hoc test was applied when statistically significant differences were found.[17, 19, 20] Statistical significance was predetermined at $p \leq 0.05$.

Chapter 3: Results

3.1. Quantitative Findings

For each specimen analyzed, elemental data was recorded that describes the atomic weight percentage contributed by each element detected. The nine elements that were consistently detected in all four treatment groups were carbon (C), calcium (Ca), chlorine (Cl), iodine (I), sodium (Na), oxygen (O), phosphorus (P), antimony (Sb), and tin (Sn) (Table. 1). Overall, SEM-EDX found oxygen, calcium, phosphorus, and carbon to be present in the greatest amounts, with O dominating at roughly 40% across all specimens.

Table 1.

Descriptive Statistics

		C	Ca	Cl	I	Na	O	P	Sb	Sn
Acid	N	30	30	30	30	30	30	30	30	30
	Mean	7.38	27.96	0.41	1.23	0.30	40.71	15.01	5.55	0.95
	SD	10.17	6.39	0.17	1.31	0.27	7.60	2.73	4.73	0.75
	Min	3.34	4.75	0.00	0.00	0.00	22.58	2.35	0.00	0.00
	Max	60.02	39.04	0.60	3.70	0.92	53.02	17.85	13.20	2.04
		C	Ca	Cl	I	Na	O	P	Sb	Sn
Enamel	N	30	30	30	30	30	30	30	30	30
	Mean	7.08	27.72	0.45	1.94	0.24	38.24	15.04	7.33	1.21
	SD	7.62	7.30	0.14	1.32	0.20	7.92	3.12	4.88	0.81
	Min	0.00	0.17	0.00	0.00	0.00	21.98	0.91	0.00	0.00
	Max	41.08	41.18	0.66	3.92	0.51	52.48	17.71	13.93	2.32
		C	Ca	Cl	I	Na	O	P	Sb	Sn
Hypochlorite	N	30	30	30	30	30	30	30	30	30
	Mean	5.48	28.29	0.44	1.40	0.37	41.39	15.44	5.45	0.92
	SD	1.08	4.38	0.14	1.24	0.21	7.58	0.93	4.26	0.70
	Min	3.62	22.97	0.00	0.00	0.00	25.28	13.43	0.00	0.00
	Max	7.60	39.36	0.62	3.27	0.71	50.95	17.30	11.88	2.07
		C	Ca	Cl	I	Na	O	P	Sb	Sn
Hypochlorite and Acid	N	30	30	30	30	30	30	30	30	30
	Mean	5.41	29.32	0.53	0.77	0.34	41.72	15.86	2.93	0.55
	SD	2.00	4.57	0.06	1.05	0.20	5.95	0.93	3.67	0.65
	Min	3.36	23.16	0.43	0.00	0.00	27.64	14.31	0.00	0.00
	Max	14.05	43.23	0.69	2.48	0.62	53.50	18.01	9.36	1.89

A one-way analysis of variance (ANOVA) was used to determine if there were significant differences in enamel content between the four groups – E (enamel/control), A (phosphoric acid), H (sodium hypochlorite), HA (sodium hypochlorite + phosphoric acid). Once a significant difference was discovered, a Tukey's post hoc test was performed in order to identify between which group/s this change occurred. The results are shown in Table 2.

The content of carbon [F(3, 116) = 0.78, p = 0.509], calcium [F(3, 116) = 0.45, p = 0.720], sodium [F(3, 116) = 1.78, p = 0.155], oxygen [F(3, 116) = 1.39, p = 0.249], and phosphorus [F(3, 116) = 1.01, p = 0.389] did not vary significantly between groups (Figs. 8-12). However, surface preparation significantly affected the weight percent of chlorine present on the enamel surface [F(3, 116) = 4.65, p = 0.004]. The amount of chlorine increased from surface preparation with acid alone to acid in combination with sodium hypochlorite as well as from sodium hypochlorite alone to acid in combination with sodium hypochlorite [difference = 0.12, 95% CI: 0.22, 0.03] [difference = 0.09, 95% CI: 0.19, 0.00] (Fig. 13). Whereas chlorine content increased with more surface preparation, iodine's presence showed a statistically significant decrease with more surface preparation [F(3, 116) = 4.63, p = 0.004]. There was more iodine detected in untreated enamel than on the enamel surface treated with NaOCl and H_3PO_4 [difference = -1.17, 95% CI: -0.32, -2.02] (Fig. 14). Similarly, the amount of antimony on untouched enamel significantly decreased once exposed to both sodium hypochlorite and phosphoric acid [F(3, 116) = 5.05, p = 0.002] [difference = -4.40, 95% CI: -1.35, -7.46] (Fig. 15). Lastly, the percent weight of tin significantly differed between treatment groups [F(3, 116) = 4.09, p = 0.008]. As was the case with iodine and antimony, group HA yielded a significantly lower amount of tin than group E [difference = -0.66, 95% CI: -0.15, -1.17] (Fig. 16). In summary, an ANOVA test showed that the various surface preparations examined in this study did not appreciably affect the enamel content of the elements C, Ca, Na, O, and P. The amount of chlorine, however, notably differed

when comparing treatment groups. Both group A and group H exhibited a lower percent weight of Cl when compared to group HA. In contrast, the concentrations of I, Sb, and Sn were higher in group E when compared to group HA.

Table 2

Tukey HSD Results

Cl	Contrast	Std. Err.	Lower 95% CI	Upper 95% CI	P-Value
Enamel vs. Acid	0.04	0.03	-0.05	0.13	1.00
Hypochlorite vs. Acid	0.03	0.03	-0.06	0.12	1.00
Hypochlorite and Acid vs. Acid	0.12	0.03	0.03	0.22	0.00
Hypochlorite vs. Enamel	-0.01	0.03	-0.11	0.08	1.00
Hypochlorite and Acid vs. Enamel	0.08	0.03	-0.01	0.17	0.12
Hypochlorite and Acid vs Hypochlorite	0.09	0.03	0.00	0.19	0.05
I	Contrast	Std. Err.	Lower 95% CI	Upper 95% CI	P-Value
Enamel vs. Acid	0.71	0.32	-0.14	1.57	0.16
Hypochlorite vs. Acid	0.17	0.32	-0.68	1.03	1.00
Hypochlorite and Acid vs. Acid	-0.46	0.32	-1.31	0.39	0.91
Hypochlorite vs. Enamel	-0.54	0.32	-1.39	0.31	0.56
Hypochlorite and Acid vs. Enamel	-1.17	0.32	-2.02	-0.32	0.00
Hypochlorite and Acid vs Hypochlorite	-0.63	0.32	-1.49	0.22	0.30
Sb	Contrast	Std. Err.	Lower 95% CI	Upper 95% CI	P-Value
Enamel vs. Acid	1.78	1.14	-1.28	4.84	0.73
Hypochlorite vs. Acid	-0.10	1.14	-3.16	2.96	1.00
Hypochlorite and Acid vs. Acid	-2.62	1.14	-5.68	0.43	0.14
Hypochlorite vs. Enamel	-1.88	1.14	-4.94	1.18	0.61
Hypochlorite and Acid vs. Enamel	-4.40	1.14	-7.46	-1.35	0.00
Hypochlorite and Acid vs Hypochlorite	-2.52	1.14	-5.58	0.53	0.17
Sn	Contrast	Std. Err.	Lower 95% CI	Upper 95% CI	P-Value
Enamel vs. Acid	0.26	0.19	-0.25	0.77	1.00
Hypochlorite vs. Acid	-0.03	0.19	-0.53	0.48	1.00
Hypochlorite and Acid vs. Acid	-0.40	0.19	-0.91	0.11	0.23
Hypochlorite vs. Enamel	-0.29	0.19	-0.79	0.22	0.80
Hypochlorite and Acid vs. Enamel	-0.66	0.19	-1.17	-0.15	0.00
Hypochlorite and Acid vs Hypochlorite	-0.37	0.19	-0.88	0.14	0.31

Figure 8. Mean Comparison for Carbon

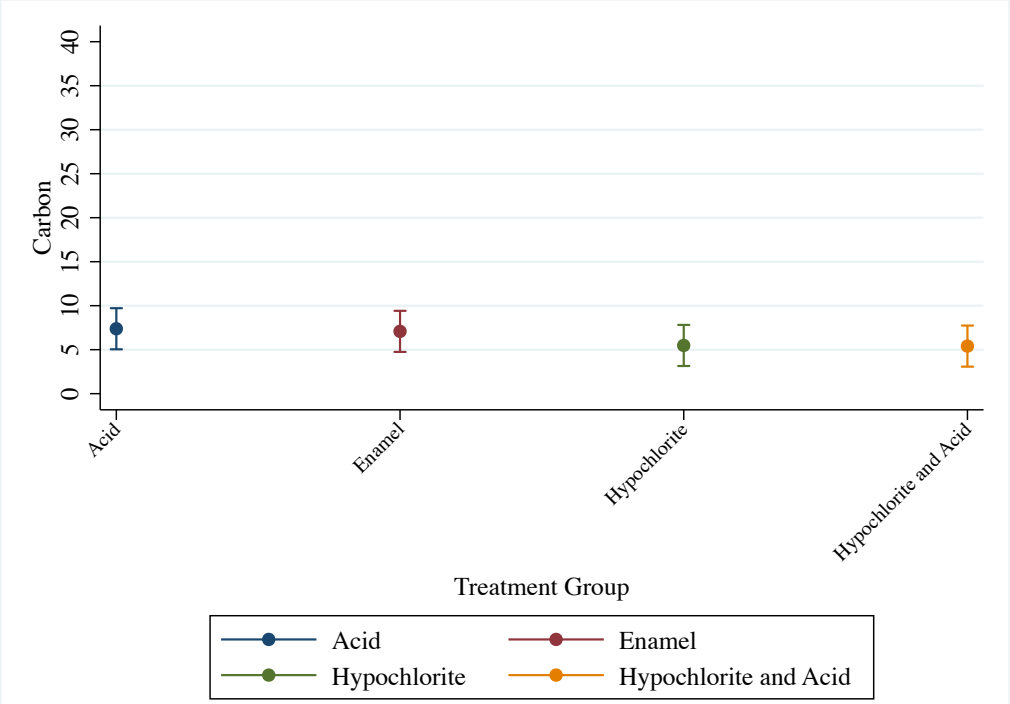


Figure 9. Mean Comparison for Calcium

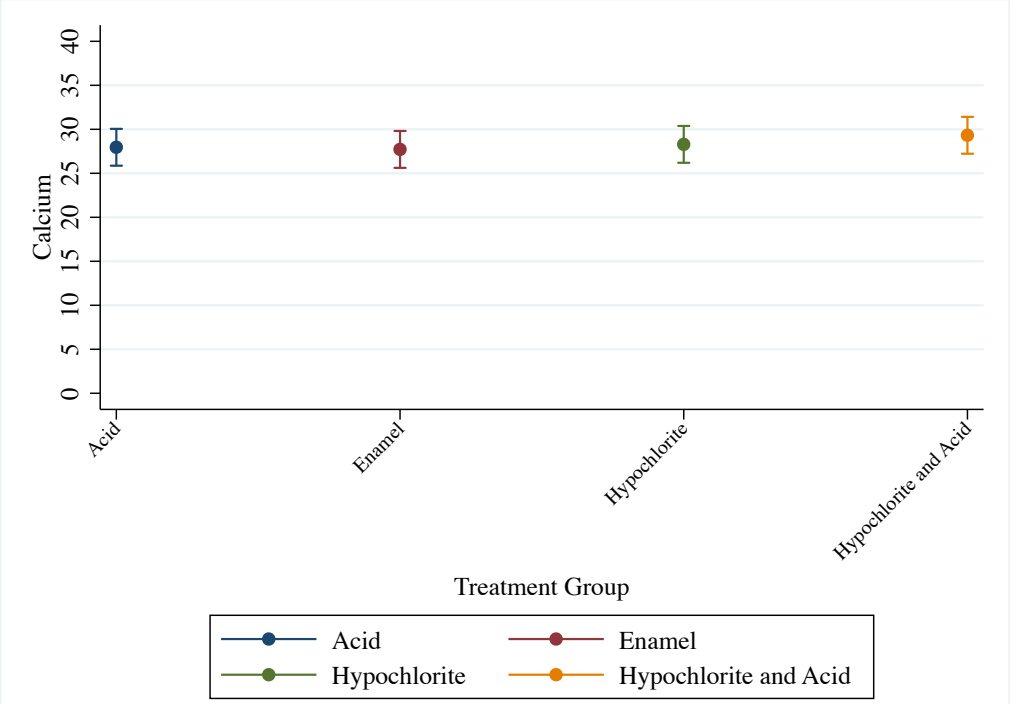


Figure 10. Mean Comparison for Sodium

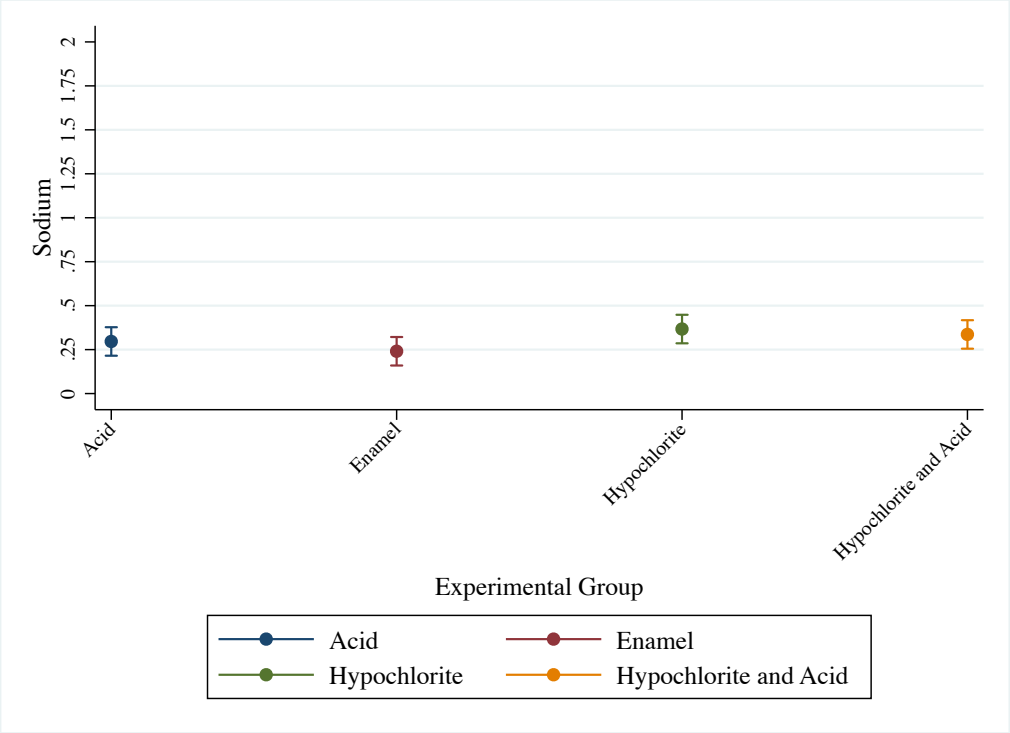


Figure 11. Mean Comparison for Oxygen

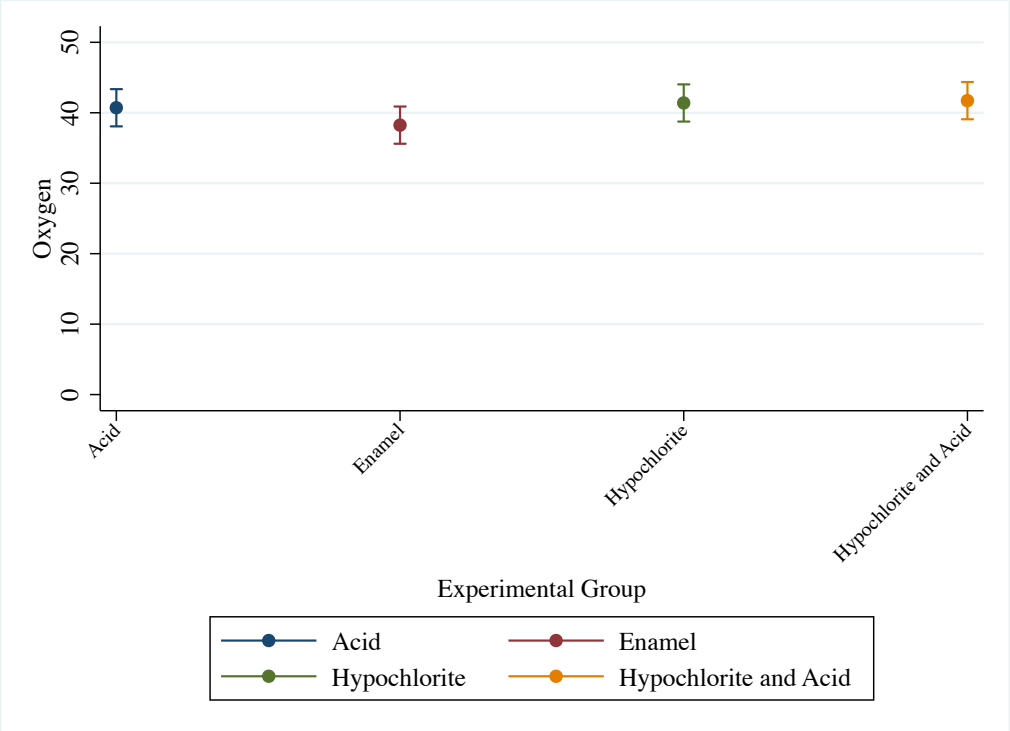


Figure 12. Mean Comparison for Phosphorus

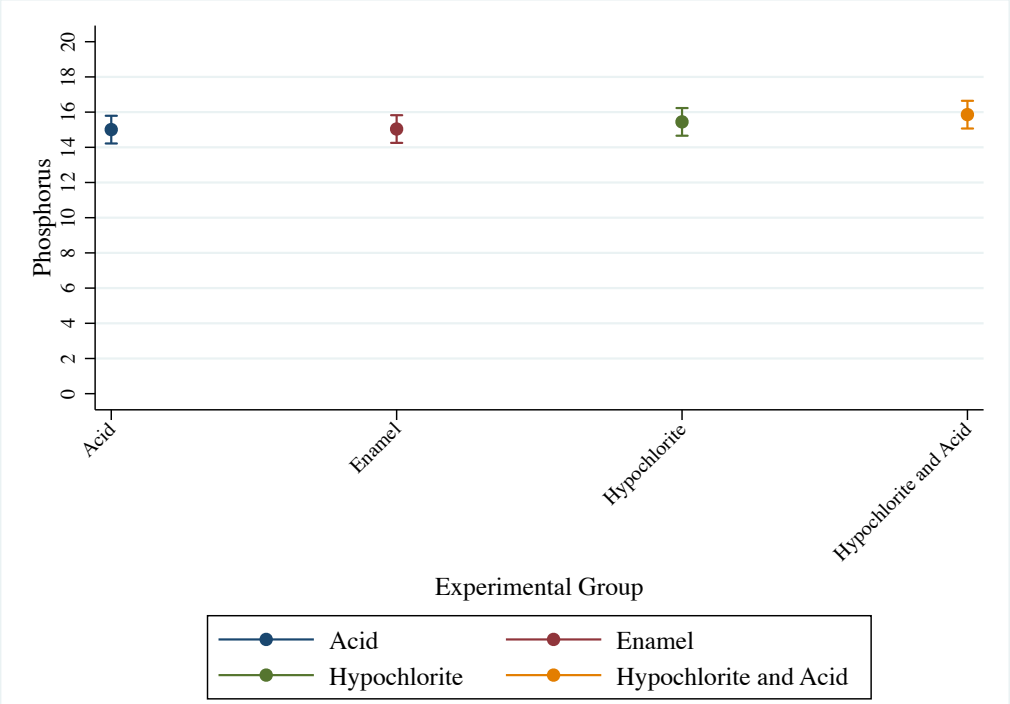


Figure 13. Mean Comparison for Chlorine

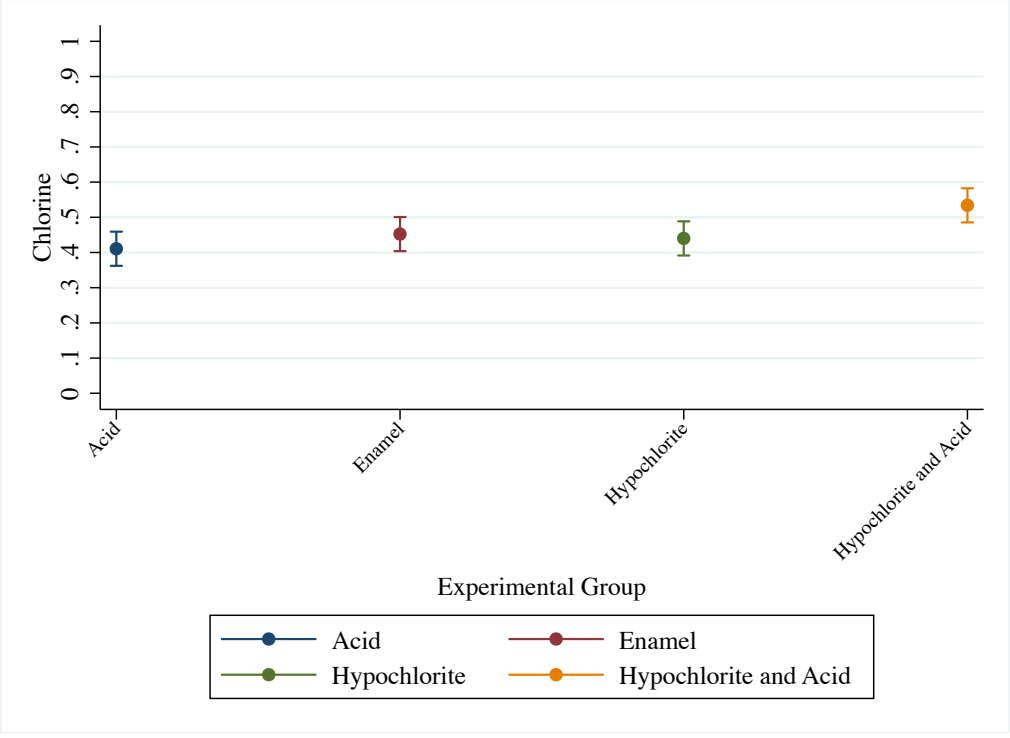


Figure 14. Iodine

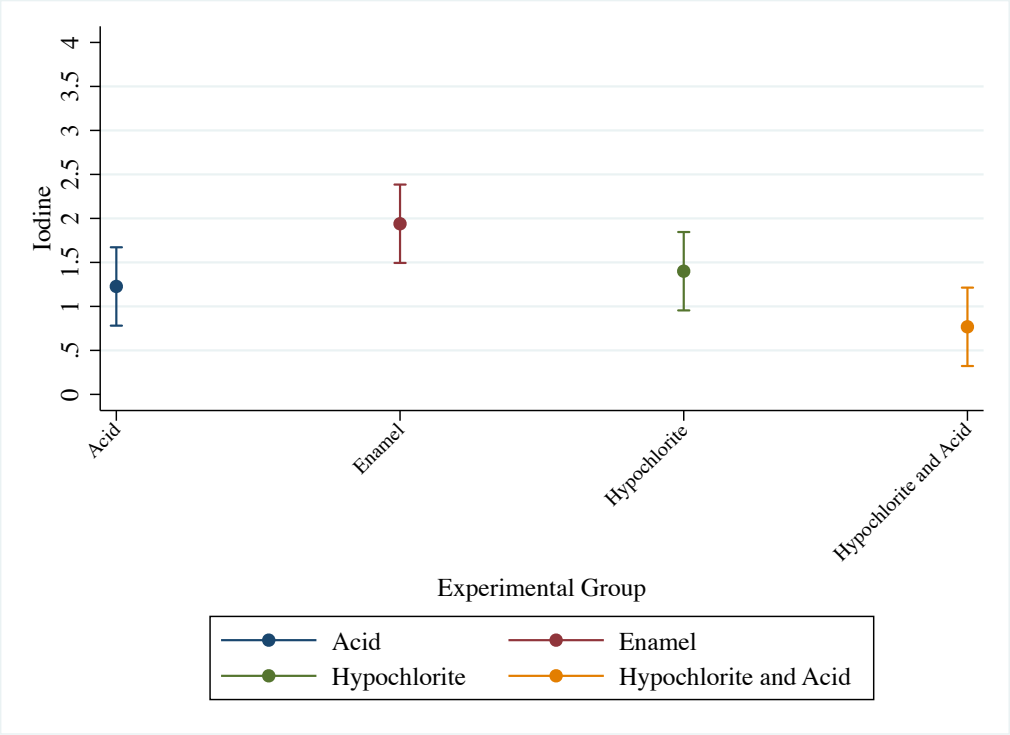


Figure 15. Mean Comparison for Antimony

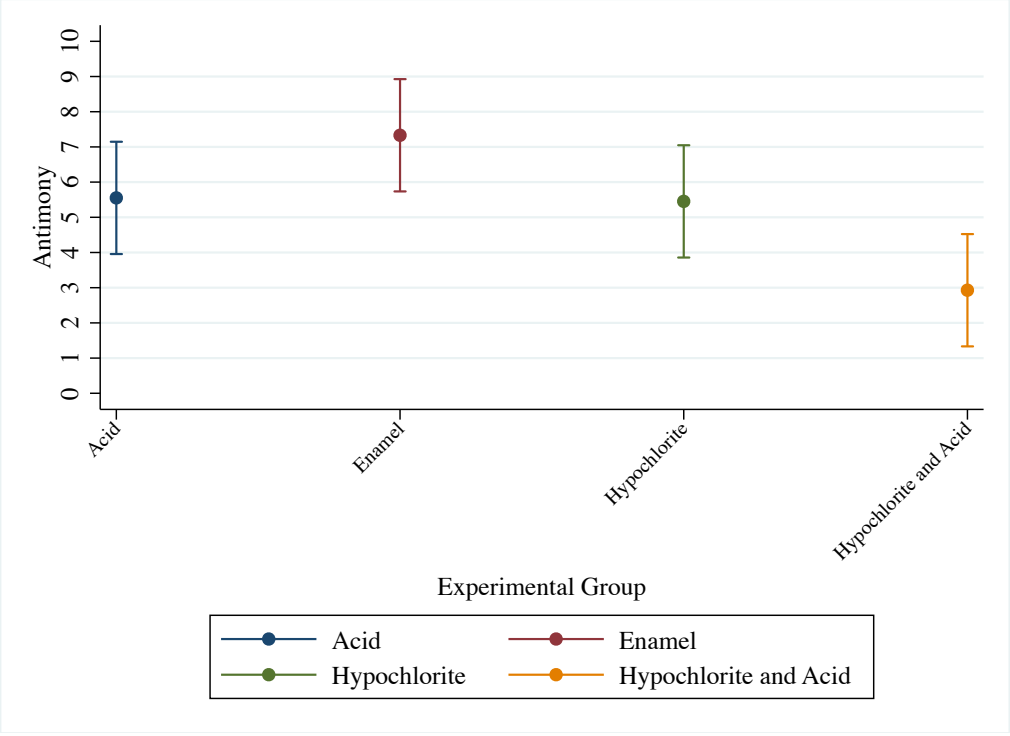
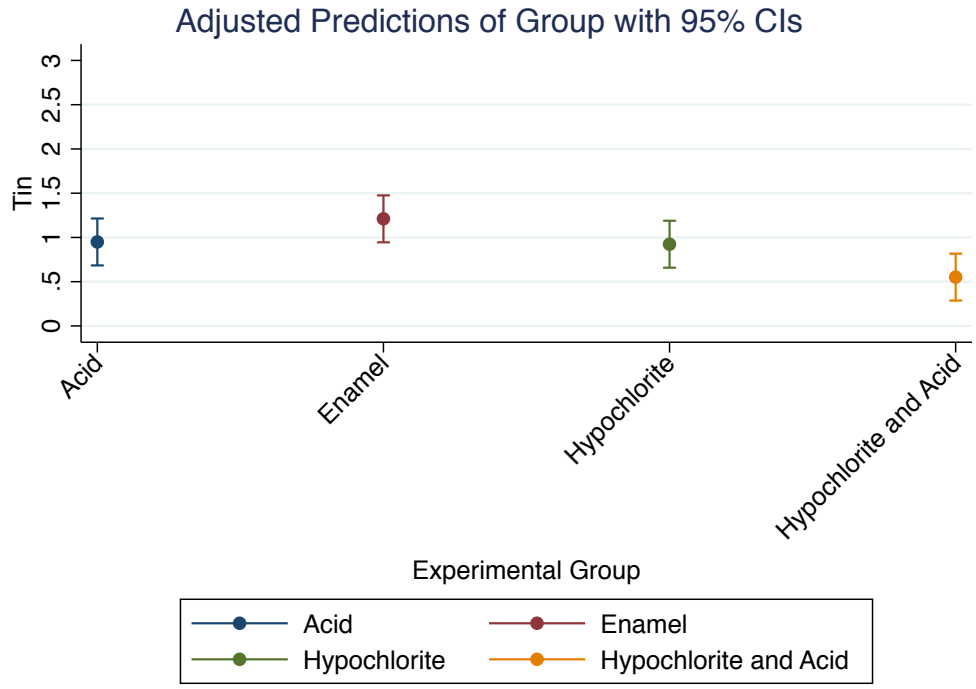


Figure 16. Mean Comparison for Tin



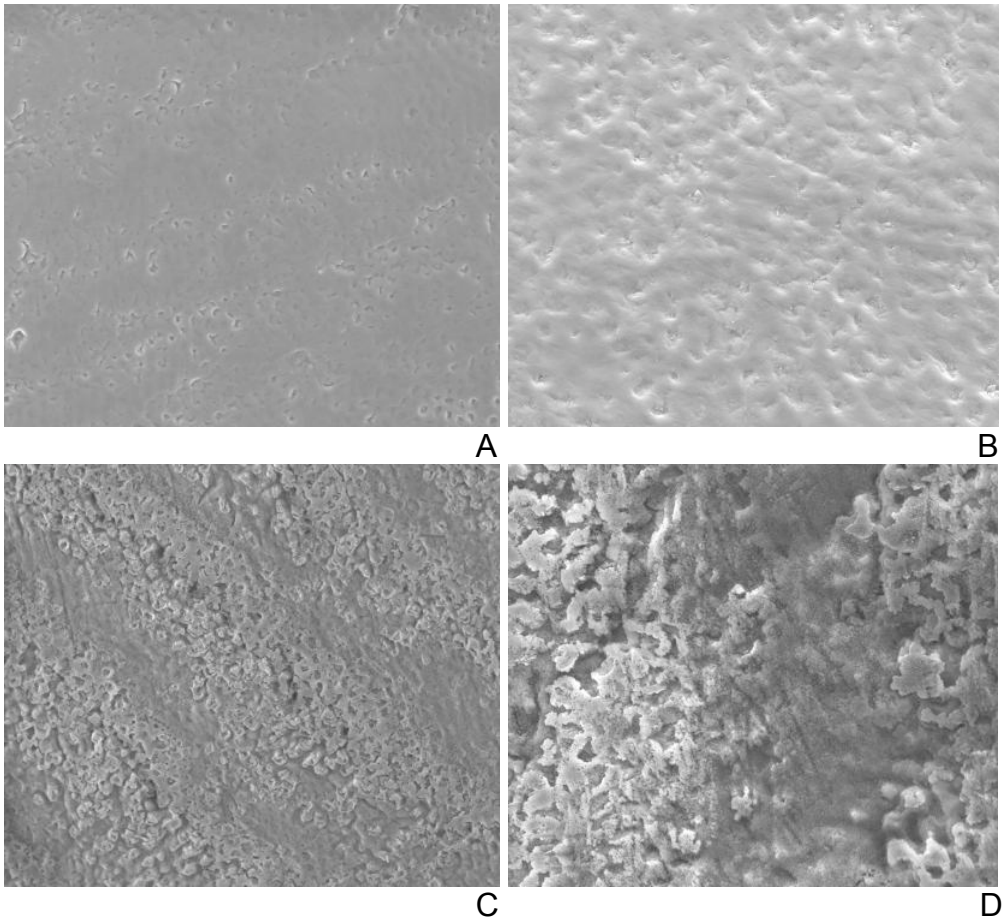
3.2. Qualitative Findings

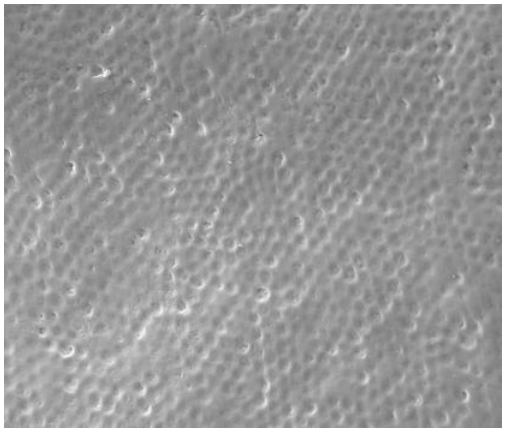
To visualize the virgin enamel surface relative to the etched enamel surfaces produced by the various treatment modalities evaluated in this study, three specimens per group were randomly selected to undergo SEM imaging. The visual assessment of the twelve randomly selected samples was conducted at a magnification of 500X and 1000X per the protocol of Espinosa *et al*.[3]

The murky complexion of the untreated enamel is the organic pellicle, which is not sufficiently removed with merely pumice and water (Fig. 17A-B). With phosphoric acid alone, the result visualized in *Figure 17C-D* is an erratic etching pattern consistent with type 3 etching as described by Silverstone *et al.*[31] Images of the samples from treatment group H (sodium hypochlorite for 60

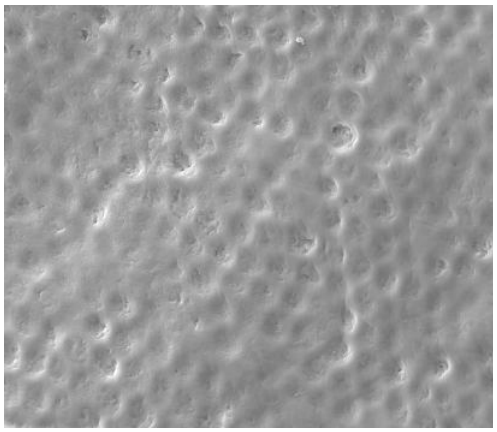
seconds) reveal a stark contrast in the profoundness of the etching pattern unlike those found in the first two groups (Fig. 17E-F). The organic pellicle is no longer present and the enamel prism heads are easily observed. Finally, the extent of the etching achieved by both phosphoric acid etching and sodium hypochlorite conditioning is irrefutably the most comprehensive when compared to the previous three groups as represented in *Figure 17G-H*.

Figure 17. Enamel following various surface preparations visualized under scanning electron microscopy at 500X (on left) and 1000X (on right) magnification. A. Group E (untreated enamel) under 500X magnification. B. Group E under 1000X magnification. C. Group A (phosphoric acid etch) at 500X. D. at 1000X. E. Group H (sodium hypochlorite) at 500X. F. at 1000X. G. Group HA (sodium hypochlorite and phosphoric acid etch) at 500X. H. at 1000X.

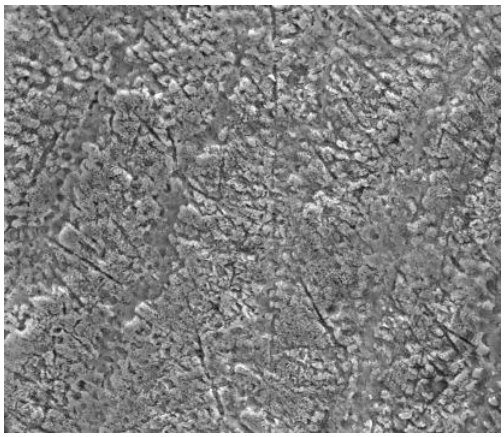




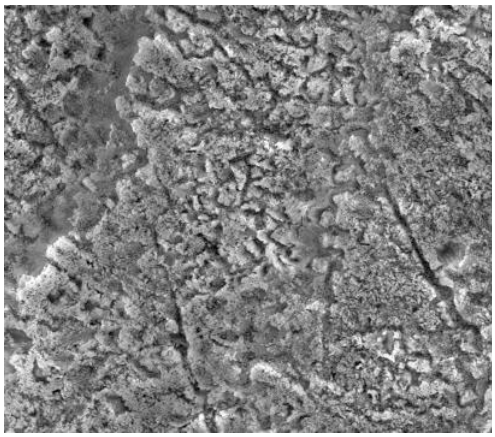
E



F



G



H

Chapter 4: Discussion

The purpose of this *in vitro* study was to evaluate the effect of a proposed pretreatment technique on the composition and structure of enamel. Enamel surface preparation with sodium hypochlorite has been suggested as a modality that enhances the etching pattern, consequently increasing bond strength, which, in turn, permits use of oral hygiene-promoting adhesives such as resin-modified glass ionomer cement.[3-5, 20, 55-57, 59-62, 66, 67] It has been upheld that the mechanism by which NaOCl acts is deproteinization of the enamel surface; thus, a systematic quantitative and qualitative evaluation of its apparent superficial effects was warranted. The effect of sodium hypochlorite on enamel content was examined using scanning electron microscopy/x-ray diffraction (SEM/EDX) and imaging. Sodium hypochlorite with and without phosphoric acid etching was compared to the gold standard bonding pretreatment technique of phosphoric acid etching alone as well as to a control group of untreated samples.

X-ray diffraction results indicated that the bulk of the enamel sections are comprised of the following nine elements: carbon, calcium, chlorine, iodine, sodium, oxygen, phosphorus, antimony, and tin. Enamel is 92-94% hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, therefore, calcium, phosphorus, and oxygen were expected findings.[68] Additionally, carbon present in lipids and in the form of carbonate as well as traces of sodium in human enamel were anticipated.[14, 15, 68] However, the detection of iodine, tin, and antimony across the sample pool was not. Although the sources of these elements cannot be definitively determined, there are some reasonable conjectures. Several studies such as that by Nixon *et*

al. have found antimony (Sb) present in human enamel.[69, 70] Detected Sb may be attributed to environmental exposure.[69] Antimony, in various compound forms, is used in the production of glass, ceramic, and polyethylene terephthalate (PET) bottles and fire retardants.[71] As a result of its usage in fabricating plastic bottles, Sb and its derivatives have the potential to penetrate into and have been detected in various consumer beverages.[71] Iodine is a critical element used by the thyroid to synthesize secretions, which regulate heart function, metabolism, and nerve responses, as examples. Most people obtain adequate iodine in their diet. Iodine is also known for its germicidal efficiency. Thus, its use as a disinfectant is fairly widespread. Iodine can be used in emergency situations to disinfect drinking water. Additionally, iodine is present in povidone-iodine, a mucosal antiseptic that has been utilized in medicine and periodically as a topical agent to control early childhood caries.[68, 72] Perhaps this can explain the incorporation of iodine into the enamel specimens. Finally, tin was an unforeseen elemental finding. However, stannous fluoride (SnF_2) is an anticaries agent used in dentrifices such as Crest® Pro-Health and Meridol®, which have proven to be clinically effective against gingivitis, sensitivity, and, more recently, dental erosion.[73] Tin's usage in this common fluoride-containing compound is most likely responsible for its detection in the enamel specimens. For all of these reasons, perhaps the trace elements of iodine, tin, and antimony were detected on the enamel surface.

Differences were found in the enamel constituents between the four treatment groups. The amount of chlorine present on the enamel surface was

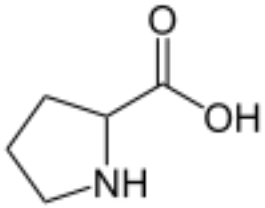
significantly higher in the HA group than both the A group and the H group. This finding is not surprising given the presence of Cl in NaOCl as well as its high electronegativity, the chemical property that describes the tendency of an atom to attract electrons.

In contrast to chlorine; iodine, antimony, and tin demonstrated a reduced concentration following treatment with sodium hypochlorite and phosphoric acid when compared to untreated enamel. It is possible that NaOCl and H_3PO_4 effectively removed these trace elements. However, there is another possible explanation. Since the amounts of all elements were presented as mass percentage (%), this could be a relative decrease that might be influenced by the increase of other elements. For example, the absolute amount of iodine might have remained unchanged, but if the true amount of chlorine present on the enamel surface increased due to treatment with sodium hypochlorite, then the amount of iodine would seemingly decrease as reflected by the lower weight percentage.

Statistically significant differences were found between groups for the elements chlorine, iodine, antimony, and tin. However, the content of carbon measured in weight percent was consistent across the board. If the mechanism by which sodium hypochlorite increases shear bond strength and enhances the etching pattern of enamel is removal of the enamel pellicle via deproteinization, then we would expect to see a decrease in the amount of organic material. An organic compound is a large class of chemical compounds composed of one or more carbon atoms that are covalently bonded. Other atoms present in organic

substances commonly include hydrogen, oxygen, and/or nitrogen. In fact, proline, an amino acid that predominates in the enamel pellicle, is made up entirely of carbon, hydrogen, oxygen and nitrogen (Fig. 18).[68] If sodium hypochlorite were acting, as suggested, by the removal of the organic enamel pellicle, then we would expect to see a distinct reduction in the amount of carbon as well as oxygen from the untreated enamel group to the groups treated with NaOCl. Therefore, based on the findings of this study, the organic component of enamel does not appear to be the primary target of sodium hypochlorite. In addition to carbon, the weight percent of calcium was relatively unaffected by the various treatment protocols. Hence, sodium hypochlorite does not seem to weaken the enamel structure fortified by calcium.

Figure 18. Diagram of proline.



Although, according to the findings in this study, neither the organic or the inorganic portion of enamel aside from some trace elements appear to be largely affected by treatment with sodium hypochlorite, visual assessment of images obtained from scanning electron microscopy reveal that a substantial transformation is occurring. As can be seen in Figure 17, with the addition of sodium hypochlorite to the surface preparation protocol, a tangible prism configuration is achieved. The superior retentive surface which results from treatment with NaOCl is comparable to that demonstrated by Espinosa *et al*.[3]

The action of sodium hypochlorite could, in fact, be reducing the surface tension via saponification, as suggested by Solera and Silva-Herzog[3], yielding a surface more responsive to phosphoric acid etching.

4.1. Limitations, Implications and Future Studies

The limitations in this study include the non-blind manner in which the study was conducted, the equipment used to carry out the experiment, as well as the *in vitro* nature of our study. Since data collection was not performed blind, confounding variables could have been introduced that may have affected our results. Use of the available equipment could have also affected the experiment. The SEM/EDX analysis that was used is considered quantitative with one study estimating that as great as 95% of the concentrations fall within ±5% relative of the correct value.[74] However, the protocol that is to be followed when using this tool is very technique sensitive, and it is vulnerable to measurement challenges that can significantly compromise analytical results.[74] One prime source of error could be from the automatic peak identification software.[74] Occasionally, peaks can be misidentified, with the probability of this increasing as the concentration level of an element decreases.[74] Thus, it is imperative to understand the limitations of this quantitative tool and for the analyst to interpret the findings.

The findings of this study suggest that pretreatment of enamel with sodium hypochlorite does not reduce the organic or the inorganic content, with the exception of the removal of some minor trace elements. Even so, the differences

found between treatment groups, although statistically significant, were minor. With differences as small as -0.66 for tin and -1.17 for iodine, the clinical merit of applying NaOCl to enamel may be negligible. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) would be a better instrument for elemental analysis of enamel composition. It can measure minute amounts of elements with small atomic sizes such as fluoride and is not affected by sample porosity or structure.[75, 76]

Ideally, a bonding protocol that would enable the use of a fluoride-releasing cement to aid in WSL prevention to benefit patients needs to be implemented. However, the exact mechanism of action of sodium hypochlorite on human enamel must first be uncovered prior to widespread *in vivo* application and acceptance. With the ultimate goal to improve the health and stability of orthodontic patients' dentitions, it is of supreme importance for the findings of future *in vitro* research studies to be better understood and the evidence to be implemented if clinical significance can be established.

Although useful findings are revealed by *in vitro* studies, these experimental conditions are limited. To most effectively evaluate the bond strength as well as the potential to inhibit enamel demineralization and formation of clinically visible WSLs, future studies should be conducted on patients actively undergoing fixed orthodontic treatment. Occasionally, the findings of *in vitro* studies have been found to conflict with the results of experiments performed *in vivo.* Thus, it is essential that future studies be conducted to test the effectiveness of sodium hypochlorite, phosphoric acid etch, and resin-modified

glass ionomer relative to phosphoric acid etch and composite cement in obtaining clinically-acceptable bond strengths and preventing enamel demineralization *in vivo* to evaluate the performance in a natural intra-oral environment.

Chapter 5: Conclusions

The weight percent of chlorine in enamel increased with the use of sodium hypochlorite and phosphoric acid in combination. Conversely, the iodine, tin, and antimony content decreased with the usage of sodium hypochlorite and acid compared to untreated controls.

Carbon content did not significantly differ between treatment groups. Because carbon is inherent in organic compounds, it can be inferred, based on our findings, that the organic portion of enamel, possibly in the form of the pellicle, is not notably affected by treatment with sodium hypochlorite.

Both calcium and phosphorus concentration were not influenced by surface preparation. Since calcium and phosphorus are key inorganic components contributing to the integrity of enamel composition, it can be postulated that sodium hypochlorite does not compromise enamel structure. For this reason, it has potential to be implemented clinically.

Based on our outcomes, the mechanism by which sodium hypochlorite increases bond strength and enhances the etching pattern may, in fact, not be deproteinization. Because these findings could be misleading due to relative increases/decreases in weight percent, error in peak identification software, and lack of light atomic weight detection capability; it is recommended that similar future *in vitro* studies be conducted utilizing secondary ion mass spectrometry.

Appendix

Group E Raw Data

	C	O	Na	P	Cl	Ca	Sn	Sb	I
E1	6.94	41.56	0.35	13.64	0.33	22.55	1.48	10.37	2.78
E2	3.79	37.66	0	15.49	0.53	27.12	1.78	10.79	2.85
E3	4.07	31.37	0	16.2	0.59	30.18	1.96	12.45	3.18
E4	3.74	36.46	0.43	15.13	0.52	26.24	2.05	12.05	3.38
E5	4.48	35.57	0.37	15.56	0.36	26.91	1.77	11.5	3.27
E6	0	38.89	0.38	15.8	0.47	27.93	1.97	11.57	3
E7	5.16	25.72	0	15.7	0.39	32.88	2.32	13.93	3.92
E8	5.12	31.41	0	16.13	0.47	29.96	1.83	11.8	3.27
E9	5.18	41.9	0.36	14.56	0.54	23.62	1.64	9.44	2.77
E10	4.02	28.57	0	16.84	0.59	37.29	1.95	10.74	0
E11	4.41	30.22	0.33	16.11	0.55	30.86	1.91	11.69	2.91
E12	20.93	45.48	0.37	9.42	0.31	15.75	0.97	5.23	1.54
E13	5.67	40.32	0	16.15	0.5	27.2	1.34	7.37	0
E14*	41.08	38.93	0	0.91	0.13	0.17	0	0	0
E15	6.1	48.33	0	16.14	0.66	27.85	0	0	0.92
E16	8.32	52.48	0.41	14.73	0.43	23.63	0	0	0
E17	5.54	42.06	0	17.71	0.55	32.8	0	0	1.34
E18	5.13	48.84	0.42	16.32	0.58	27.78	0	0	0.93
E19	4.61	48.01	0.51	15.35	0.54	25.84	0.8	3.49	0.85
E20	4.92	46.17	0.43	16.62	0.54	28.88	0	2.43	0
E21	4.25	25.63	0.3	16.38	0.39	34.55	2.09	12.89	3.52
E22	3.59	26.01	0	16.86	0.47	35.8	2.07	11.89	3.31
E23	4.57	40.38	0.41	14.99	0.45	25.91	1.36	8.69	2.67
E24	4.83	41.61	0.45	15.18	0.32	26.17	1.31	7.76	2.38
E25	4.88	32.01	0	16.88	0.61	32.48	1.44	9.07	2.62
E26	6.24	21.98	0	14.87	0.43	41.18	1.74	10.7	2.87
E27	4.5	42.71	0.43	15.22	0.42	26.3	1.27	7.15	2.01
E28	4.31	39.23	0.43	16.91	0.39	30.56	1.25	6.91	0
E29	6.85	41.2	0.37	17.32	0.51	32.61	0	0	1.13
E30	19.23	46.57	0.47	12.02	0	20.46	0	0	0.76

Group A Raw Data

	C	O	Na	P	Cl	Ca	Sn	Sb	I
A1	3.34	40.6	0.41	15.07	0.46	25.27	1.62	10.36	2.87
A2	12.63	43.59	0.65	11.56	0.16	19.76	1.31	8	2.09
A3	4.58	33.1	0	16.54	0.43	33.07	1.72	10.56	0
A4	4.69	37.82	0.42	16.33	0.36	28.85	1.86	9.67	0
A5	4.86	25.61	0	15.66	0.53	35.13	2.01	12.85	3.36
A6	5.27	22.58	0	15.16	0.47	37.88	1.75	13.2	3.7
A7	5.58	40.37	0	14.65	0.44	25.68	1.37	9.47	2.44
A8	4.81	22.64	0	14.8	0.46	39.04	2.04	12.85	3.36
A9	5.01	37.77	0.75	15.52	0.24	27.5	1.4	9.23	2.58
A10	4.12	38.33	0	15.46	0	28.06	1.67	9.64	2.6
A11	3.6	36.12	0.5	16.02	0.52	29.44	1.58	9.43	2.78
A12	4.17	38.91	0.59	16.11	0.41	28.78	1.31	7.64	2.07
A13	4.18	47.99	0.47	14.56	0.45	23.48	1.45	7.41	0
A14	4.86	45.72	0.62	14.23	0.37	23.3	1.33	7.51	2.06
A15	3.74	43.43	0.35	14.76	0.51	25.09	1.61	8.44	2.06
A16	3.58	42.65	0.43	15.77	0.4	26.82	1.21	7.35	1.8
A17*	60.02	30.81	0.35	2.35	0	4.75	0	1.23	0.3
A18	3.56	47.33	0.41	16.61	0.57	28.99	0.7	1.84	0
A19*	7.41	44.09	0.35	14.98	0.49	25.67	0	0	0
A20*	11.29	43.55	0.41	13.15	0.43	23.95	0	0	0
A21	4	45.46	0	15.62	0.47	27.46	1	4.96	1.02
A22	5.56	45.81	0	16.29	0.5	31.84	0	0	0
A23	6.2	44.23	0	16.65	0.49	32.43	0	0	0
A24	9.06	51.74	0.92	12.7	0	21.29	0.64	2.23	0.79
A25	5.52	43.6	0	17.2	0.46	33.22	0	0	0
A26	4.69	41.21	0.42	16.6	0.58	32.03	0.89	2.66	0.92
A27	7.27	42.14	0	16.86	0.55	33.18	0	0	0
A28	6.33	41.26	0.48	17.85	0.49	33.6	0	0	0
A29	6.1	53.02	0	15.08	0.48	25.32	0	0	0
A30	5.34	49.83	0.36	16.01	0.6	27.86	0	0	0

Group H Raw Data

	C	O	Na	P	Cl	Ca	Sn	Sb	I
H1	5.84	30.18	0.51	14.98	0.52	30.56	2.07	11.88	3.15
H2	4.33	46.27	0.36	14.14	0.39	23.05	1.26	8.11	2.08
H3	6.69	37.57	0.38	13.43	0.42	27.41	1.45	9.97	2.49
H4	4.55	25.28	0	16.13	0.6	36.83	1.77	11.55	3.27
H5	4.4	46.73	0	14.14	0.39	23.19	1.37	7.56	2.23
H6	6.99	36.6	0.4	15.62	0.45	27.54	1.41	9	2
H7	4.51	37.71	0.71	15.75	0	29.21	1.29	8.07	2.76
H8	3.62	46.04	0.36	14.72	0.54	24.15	1.48	7.27	1.84
H9	5.26	41.58	0	16.15	0.52	28.94	1.27	6.29	0
H10	7.6	34.74	0.44	15.59	0.38	29.39	1.58	7.87	2.41
H11	3.74	39.57	0.55	15.47	0.49	26.23	1.51	9.46	2.98
H12	4.64	45.1	0.54	14.38	0.44	23.69	1.36	7.95	1.91
H13	4.77	33.83	0.56	15.79	0	30.69	1.69	10.07	2.61
H14	5.84	27.09	0.44	15.8	0.24	35.63	1.65	10.35	2.97
H15	5.23	42.57	0	15.05	0.42	26.05	1.07	7.38	2.24
H16	4.02	37.09	0.46	16.54	0.51	30.18	1.23	7.82	2.15
H17	4.7	48.13	0.59	14.08	0.36	23.51	1.16	5.57	1.63
H18	5.78	26.66	0	15.58	0.51	39.36	1.25	8.48	2.15
H19	6.24	47.76	0.35	15.18	0.45	25.4	0.76	3.86	0
H20	3.9	47.92	0.36	15.17	0.42	24.97	1.07	5.05	1.13
H21	6.12	50.46	0.46	15.56	0.54	26.86	0	0	0
H22	6.84	46.26	0.39	16.86	0.49	29.15	0	0	0
H23	6.5	48.15	0.36	15.96	0.57	28.47	0	0	0
H24	6.79	35.77	0.33	17.3	0.62	37.61	0	0	0
H25	5.78	50.06	0.38	15.58	0.35	26.55	0	0	0
H26	6.08	47.12	0	16.12	0.52	28.66	0	0	0
H27	6.12	50.4	0.67	14.58	0.5	23.75	0	0	0
H28	6.42	40.99	0.47	16.79	0.58	29.88	0	0	0
H29	5.07	43.08	0.47	16.5	0.5	28.73	0	0	0
H30	6.1	50.95	0.47	14.39	0.48	22.97	0	0	0

Group HA Raw Data

	C	O	Na	P	Cl	Ca	Sn	Sb	I
HA1	4.1	39.32	0.37	15.84	0.46	27.63	1.28	8.52	2.48
HA2	3.36	39.75	0.35	15.75	0.57	27.87	1.58	8.49	2.28
HA3	4.94	32.74	0.33	16.42	0.45	32.61	1.36	8.69	2.47
HA4	4.23	33.04	0	16.34	0.51	31.98	1.89	9.36	2.4
HA5	4.38	45.62	0	14.61	0.47	25.17	1.19	6.59	1.97
HA6	4.63	32.49	0.37	16.68	0.61	32.98	1.56	8.42	2.27
HA7	4.22	43.55	0.4	15.34	0.51	26.37	1.4	6.49	1.73
HA8	4.15	41.54	0	16	0.58	27.19	1.13	7.21	2.22
HA9	4.44	42.78	0.5	15.39	0.52	26.96	1.21	6.31	1.89
HA10	3.8	40.99	0.53	16.15	0.58	28.16	0.99	6.76	2.04
HA11	3.5	37.67	0.32	16.43	0.58	33.58	1.05	5.58	1.29
HA12	5.97	45.36	0.39	16.84	0.61	30.83	0	0	0
HA13	6.65	46.43	0	16.28	0.57	30.08	0	0	0
HA14	4.19	46.05	0.54	16.24	0.52	28.69	0.77	3	0
HA15	5.13	49.3	0.57	15.3	0.47	26.24	0.58	2.42	0
HA16	5.47	37.71	0.36	17.49	0.69	38.28	0	0	0
HA17	4.48	46.73	0.53	16.87	0.66	30.73	0	0	0
HA18	14.05	27.64	0	14.62	0.46	43.23	0	0	0
HA19	7.06	53.5	0.41	14.72	0.54	23.76	0	0	0
HA20	5.84	48.14	0.44	16.43	0.61	28.55	0	0	0
HA21	3.66	34.62	0	17.18	0.54	35.32	0	0	0
HA22	5.45	46.59	0.47	15.13	0.43	25.06	0	0	0
HA23	4.71	37.53	0	18.01	0.5	34.12	0	0	0
HA24	8.26	44.66	0.57	14.31	0.56	24.27	0.56	0	0
HA25	5.25	45.21	0.38	14.96	0.47	25.53	0	0	0
HA26	5.47	34.63	0.33	15.88	0.5	32.32	0	0	0
HA27	5.6	43.21	0.4	15.15	0.56	26.01	0	0	0
HA28	6.43	47.93	0.62	14.55	0.52	23.16	0	0	0
HA29	6.67	43.34	0.46	15.49	0.5	25.29	0	0	0
HA30	6.09	43.44	0.45	15.26	0.47	27.61	0	0	0

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