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Erosion of Teeth in Household Acids: A SEM Analysis

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To the Graduate Council:

I am submitting herewith a thesis written by Caroline Rebecca Amerson entitled "Erosion of Teeth in Household Acids: A SEM Analysis." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

Murray K. Marks, Major Professor

We have read this thesis and recommend its acceptance:

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(Original signatures are on file with official student records.)

Erosion of Teeth in Household Acids: A SEM Analysis

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Caroline Rebecca Amerson
August 2018

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ABSTRACT

Forensic dentistry attempts to identify unrecognizable decomposed, skeletonized, comingled human remains. A criminal act performed to purposefully dispose or disfigure a victim in order to conceal identity is acid erosion, rendering the dentition unrecognizable for antemortem and postmortem comparison. Despite current literature on the effects of household acids on teeth, there is a research deficiency in the microscopic appearance of acid-exposed teeth. The sample teeth evaluated in this study are non-restored roots, non-restored crowns, and crowns restored with amalgam and composite. The microscopic portion of this study utilized a dissecting scope at 6.3-12.5X magnification of the surface and scanning electron microscopy surface at 111X-1,082X. Data collected includes dental metrics of the mesiodistal and buccolingual dimensions, as well crown height, root length and width. Battery acid, toilet bowl cleaner, and drain unclogging liquids were used, as these are common acids that are readily available.

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CHAPTER ONE: INTRODUCTION

With the advent of forensic television shows such as “CSI”, “Bones”, and the non-fiction show “Forensic Files”, the average American has easy access to a basic knowledge of the forensic techniques that can identify an otherwise unidentifiable body. A common reliable and affordable method for making a human identification utilizes the dentition. Teeth are incredibly durable because enamel is the hardest substance in the body being 96% mineralized, compared to bone which is approximately 70% mineralized (Nanci, 2013). Additionally, most people visit the dentist annually or semi-annually and obtain diagnostic radiographs, intraoral photos, or charting of their teeth, thereby documenting the individual’s restorations and current condition of their dentition. Despite dental restorations being commonplace, once a tooth is prepared for filling and the dental restoration is placed, it often results in a unique radiographic appearance permitting comparison. Factoring in the combination of restorations, missing teeth, unique anatomical features and bony anatomy, no two people have the same features, making dental identification a useful tool (Senn and Weems).

A criminal may know that the teeth and bones are incredibly useful for identification. This makes the destruction of these mineralized tissues imperative to conceal the identity of their victim. One means of dental destruction is incineration or burning at high temperatures. Another method of destroying teeth and facial bones beyond recognition is the use of acid to dissolve them, thwarting identification. Dependent upon time of immersion and acid strength, teeth can degrade and erode to an

unrecognizable tissue. To understand how this process occurs, the mineral composition of teeth requires understanding.

Teeth are made up of four different and distinctive tissues: enamel, dentin, cementum, and the pulp (Brand and Isselhard, 2014). The enamel is the outer layer seen on the crown; it is the portion of the tooth one sees above the gingiva. Enamel is 96% mineralized with the inorganic portion consisting of hydroxyapatite, making it hard and durable (Suga, et. al. 1992). The layer beneath the enamel and cementum is dentin which comprises the majority of the crown and root. Dentin is not as hard as enamel, and is approximately 70% mineralized with the majority of its organic constituents consisting of collagen (Brand and Isselhard, 2014). The collagen gives teeth their ability to absorb and disperse pressure when chewing and eating. Cementum is the third hardest tooth tissue and is found only on the root surface of a healthy tooth. Cementum is 50-55% mineralized and permits attachment of the periodontal ligament to the root surface. (Brand and Isselhard, 2014). The periodontal ligament maintains the tooth within the bony socket. The dental pulp is the only soft tissue in the tooth and is located in the center of the crown and root. The pulp contains the nerve and blood vessels, making it a potential source for DNA. With the majority of a tooth structure being comprised of inorganic material, one can imagine how susceptible they are to acid dissolution.

If a tooth has a history of decay or caries, it may have a dental restoration. Due to cosmetic concerns, many patients choose to have their teeth restored with a tooth-colored filling called composite resin. Twenty years ago, the majority of posterior fillings were comprised of dental amalgam. Dental amalgam is still occasionally used today as a

restorative material. This material is an amalgamation of liquid elemental mercury, silver, tin and copper (Hilton et. al. 2013). When large restorations break down or dental decay is too large making a filling an inappropriate choice of treatment, a crown may be placed. A crown is a tooth shaped covering that fits over a prepared coronal portion of a tooth. Crowns are fabricated in different materials including porcelain, zirconia, gold, stainless steel, and porcelain-fused-to metal.

Dental professionals see on a daily basis the effects of acids during examination due to the prevalence of soft drinks and other acidic drinks. According to the American Dental Association the pH of an average carbonated soft drink is somewhere between 2.5-3 (Reddy et. al. 2016). Despite the buffering capabilities of saliva, if acidic drinks are sipped throughout the day they can still cause considerable damage to the structure and health of the tooth. Dental erosion is also seen in association with disorders such as acid-reflux and bulimia which cause stomach acid, largely hydrochloric acid, to enter from the esophagus into the oral cavity. An indication of dental erosion is a “smooth silky glazed appearance” (Amaechi, 2015). When erosion is severe, the enamel dissolves exposing the underlying yellow dentin. With extreme erosion, dentin can become so thin and translucent that the pulp chamber can be seen through the tooth. When teeth are dissolved in household acids, we expect to see the same characteristics with variation dependent on acid strength.

CHAPTER TWO: RELATIVE FORENSIC CASES

Dissolving a human body in acid to conceal victim identity has been chronicled in popular television documentaries. For example, in 2015, a young woman in France was murdered, her body placed in a plastic tub and dissolved with acid (Sims, 2015). The perpetrators were inspired by the show *Breaking Bad* where they dissolved a body in hydrofluoric acid. In Germany, two victims were killed in 2009 and 2011 and placed in hydrochloric acid to dissolve the bodies and the rest of the remains were flushed down the toilet. They were caught when a bad odor coming from the house alerted police to investigate (German house of horror: bodies dissolved in acid, 2012). The most famous forensic case involving acid dissolution of human remains was “The Acid Bath Murders” committed by John Haigh. He dubbed this the “perfect murder” because if there was no body, there is no crime (Holden and Simpson, 1950). There is an obvious need for research pertaining to how the body and teeth react to acid treatment in order to determine victim identity and timeline of a crime.

CHAPTER THREE: INTRODUCTION TO ACIDS

The acids used in this experiment were The Works Toilet Bowl Cleaner , Rooto Drain Cleaner, and Zoro Battery Acid. The Works Toilet Bowl Cleaner is hydrochloric acid, and Rooto Drain Cleaner and Zoro Battery Acid are both sulfuric acid. Table 3.1 displays information about the concentrations of each reagent.

Table 3.1. Concentration of Acids

Acid Name	Acid Type	Concentration
The Works Toilet Bowl Cleaner	Hydrochloric acid	9.5%
Rooto Drain Cleaner	Sulfuric Acid	93.2%
Zoro Battery Acid	Sulfuric Acid	35%

According to the Material Safety and Data sheets for each product, the pH of every acid listed is less than one (1). Acid strength can also be quantified by an acid dissolution (ionization) constant, or pK_A . The lower the pK_A , the stronger the acid (Perrin, 1981). For temperatures of 0-25 degrees Celsius, hydrochloric acid has a pK_A value of between -5.1 and -7 (Perrin, 1969). For temperatures of 0-25 degrees Celsius, sulfuric acid has a pK_A value of between 1.58 and 2.28 (Perrin, 1969).

CHAPTER FOUR: SCANNING ELECTRON MICROSCOPY

A scanning electron microscope is able to take a specimen and examine surface topography and features. Scanning is achieved by projecting a focused beam of electrons at the specimen and utilizes electrostatic and magnetic fields to change the direction of the electron beam. An image is created from the resulting secondary electrons emanating from the specimen in two perpendicular fields of scan (Egerton, 2016). Secondary electrons show the topography of the specimen and back-scatter electrons that come from within the tooth tell information about elemental atoms present.

Enamel's basic structure is made of enamel prisms or enamel rods. During tooth development, enamel rods are initially laid down by ameloblasts and are subsequently mineralized with hydroxyapatite. These rods create a geometric "keyhole" shape that interlock and the terminal ends create a smooth outer surface. In life, the enamel can become pitted from acid insult via bacterial byproducts. Figure 4.1 shows the shape of these enamel prisms or rods.

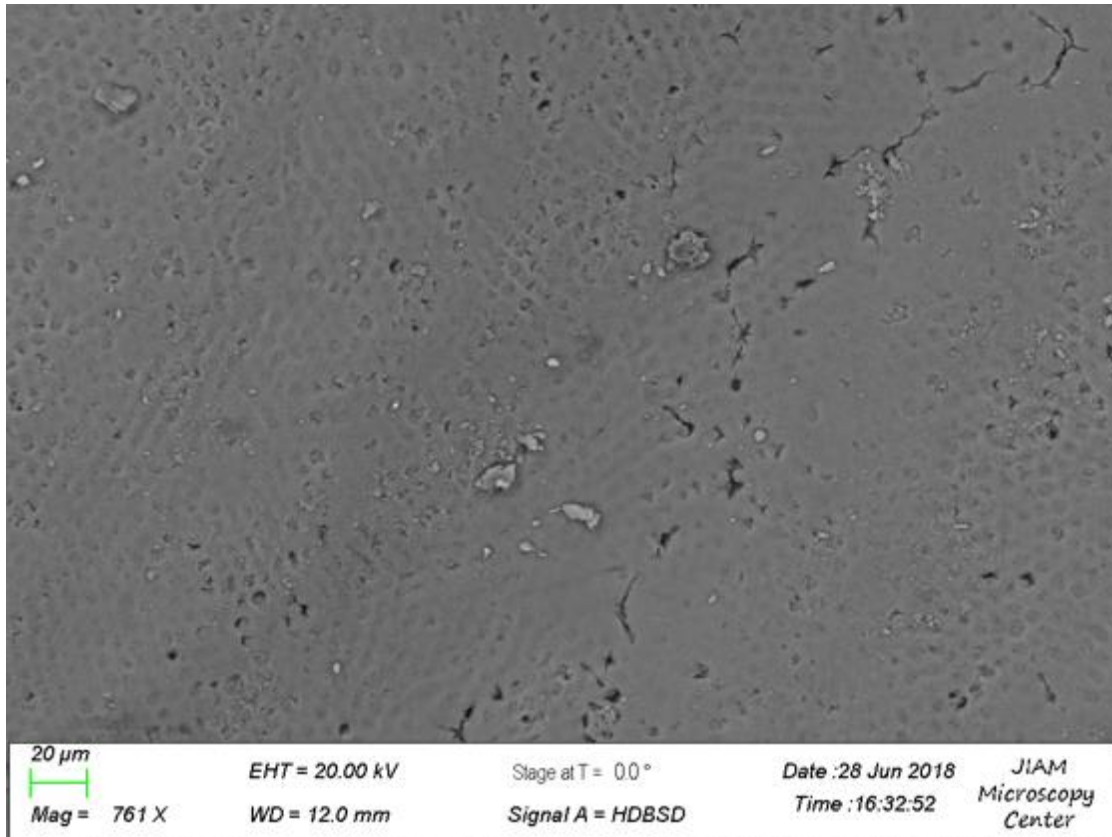


Figure 4.1. The appearance of enamel from a dried tooth on the crown surface at 761X. Note the geometric shape of the terminal portion of the enamel rods, and that the crown surface is relatively smooth

Dentin's basic structure consists of dentinal tubules. These tubules are laid down by the odontoblasts and like enamel, they are mineralized after the initial tubule is laid down. Unlike enamel, these tubules only become mineralized around the perimeter of the tubule, leaving behind a long communicating channel from the enamel to the pulp. This appearance is seen especially well on a tooth's cavity preparation surface. Figure 4.2 shows the opening and shape of the dentinal tubules.

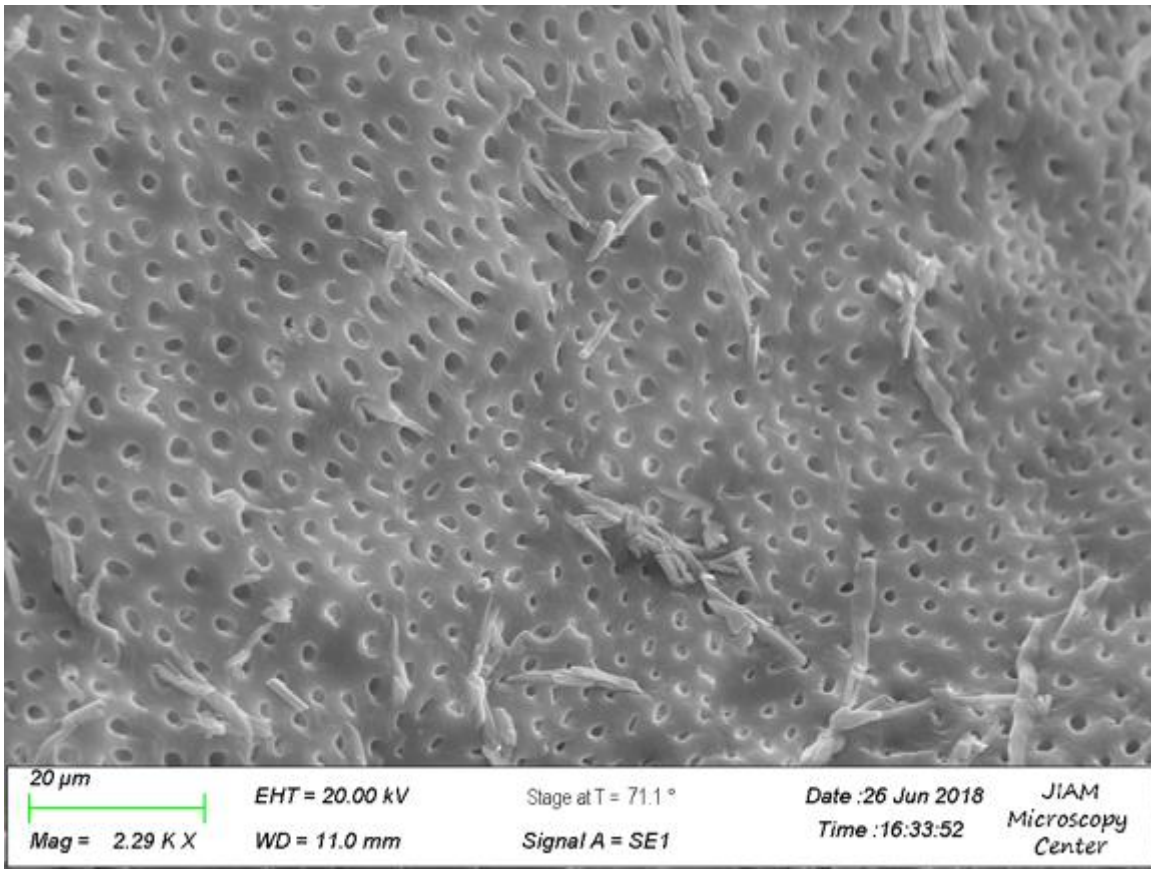


Figure 4.2. The appearance of acid-etched dentin at 2.29K X. Note the shape of the dentinal tubules.

As previously mentioned, amalgam is made up of tin, copper, silver and elemental mercury. The surface of an amalgam is relatively smooth. Any surface roughness is a result of differently shaped particles that make up the restorative material. Figure 4.3 shows the surface of an amalgam restoration.

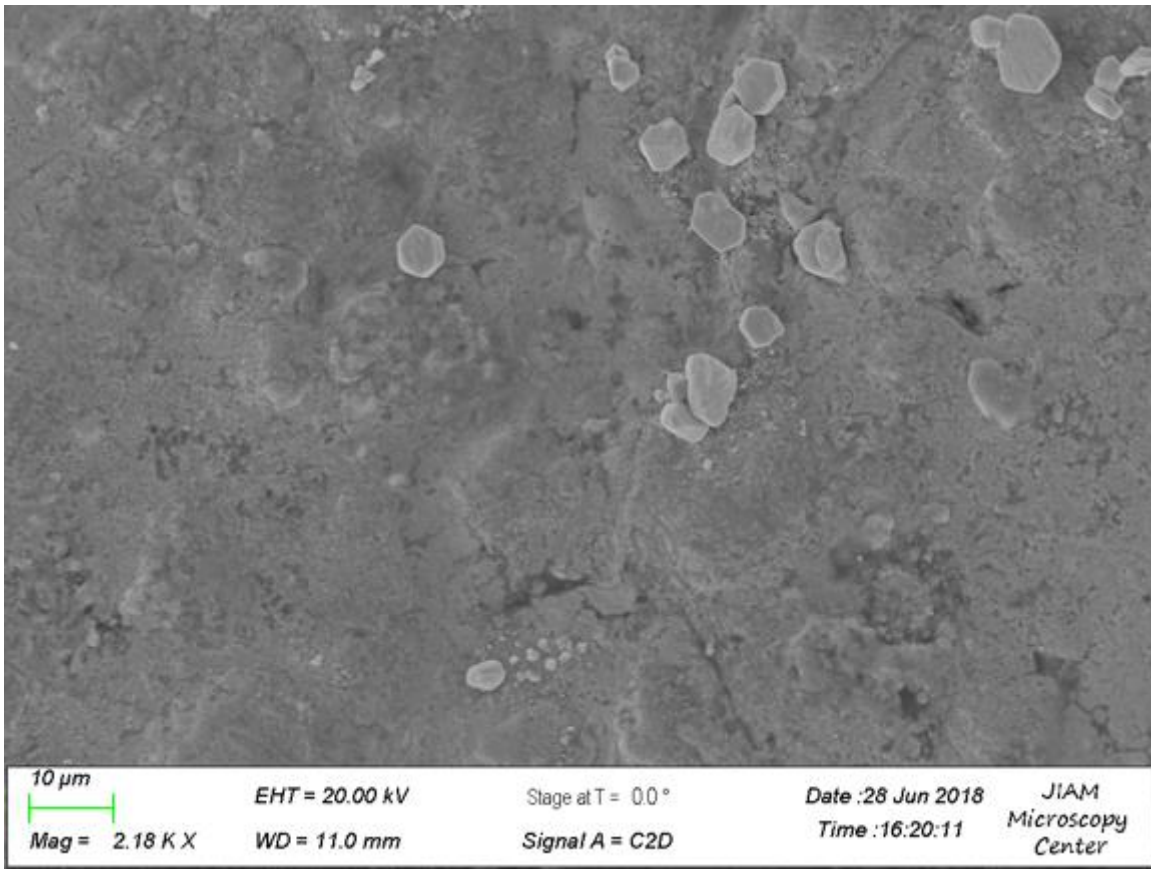


Figure 4.3. The appearance of amalgam on a dried tooth at 2.18 K X. Note that the overall surface is relatively smooth, with slight roughness contributing from the particulates of the amalgam

Composite is resin that bonds directly to an etched tooth surface upon curing with a light. Like amalgam, composite is relatively smooth demonstrating slight roughness due the shape of the material particulates. Figure 4.4 shows the surface of a composite restoration.

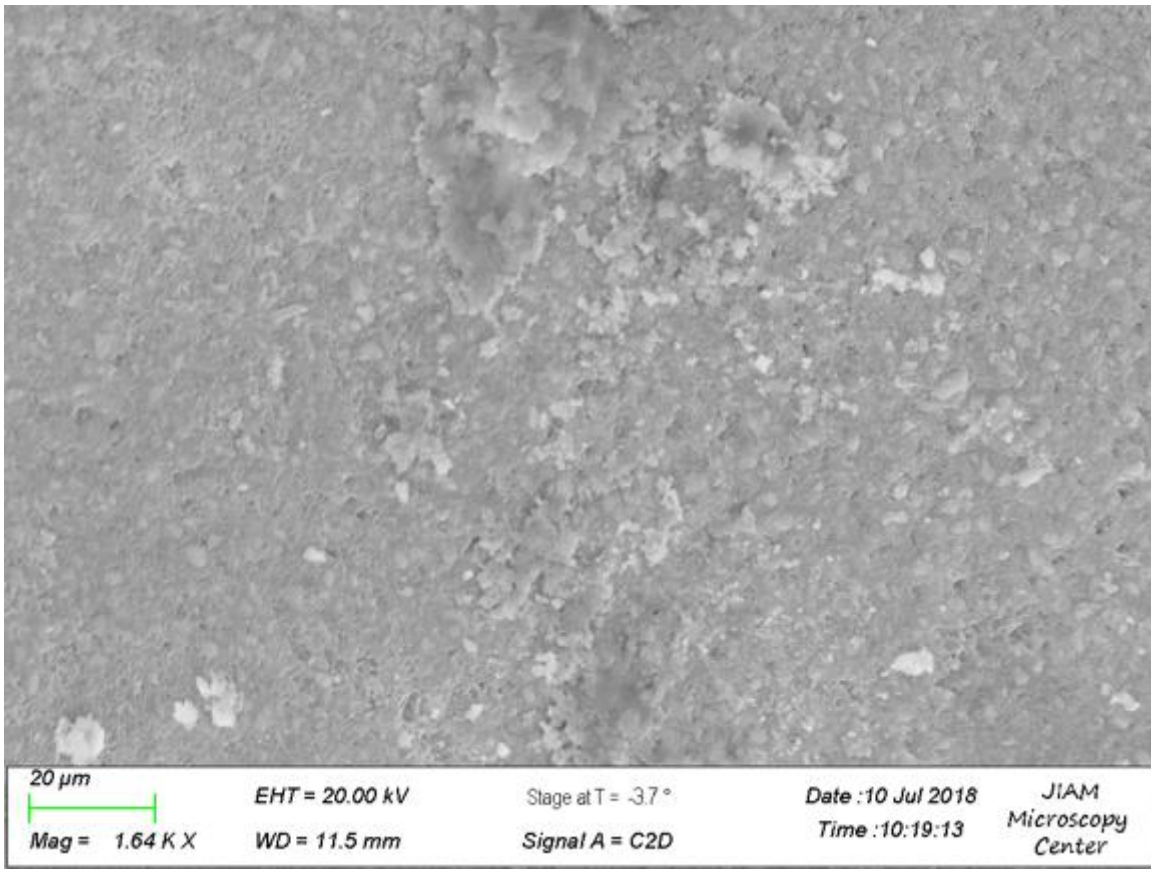


Figure 4.4. The appearance of a composite restoration at 1.64 K X. Note that overall the surface is relatively smooth with slight roughness from the particulates that make up the composite.

CHAPTER FIVE: LITERATURE REVIEW

Some early research was performed on the effect of acids on teeth in determining manufacturing safety hazards of industrial acids. Lynch (1947) observed changes in appearance of the teeth of workers who worked with dipping cotton waste into a mixture of “approximately 70 percent sulfuric acid, 22 percent nitric acid, and 8 percent water.” The fumes were so strong that their teeth eroded due to mouth-breathing, but could be avoided with a scarf over the mouth. Lynch observed that the incisors and the canines showed significant erosion starting on the incisal third extending up the labial surface. With increased exposure to these caustic agents, so did tooth destruction. Lynch noted that “when the enamel has been destroyed the dentine is attacked and there is brown or blackish discoloration of the affected teeth, which still retain their polished appearance.” (Lynch, 1947: 85). Within one year, nearly 40% of the workers had damage to their dentition, increasing to 62% if the workers were employed for one to three-and-a-half years.

Mazza et. al. (2005) conducted an experiment using four different acids to discern how long it took for teeth to dissolve to the point where dental identification would be impossible. They used hydrochloric acid in a 37% solution, 96% sulfuric acid, 65% nitric acid, and aqua regia (chloroazotic acid—hydrochloric/nitric acid 1:3). The samples placed in the 37% hydrochloric acid were dissolved completely at 14 hours while the samples placed in 96% sulfuric acid had not dissolved completely after 90 hours, but had a significant volume reduction. The teeth in 65% nitric acid had dissolved completely within 12 hours and the teeth in the aqua regia took 17 hours to completely dissolve. The

pictures taken for the study were considered “macro-images” documenting results seen with the naked eye.

In 2009 Cope and Dupras conducted dental research to see the effects of different easily accessible household acids. Their research design included eight different brands of acid classified as hydrochloric, sulfuric, phosphoric, and sodium hydroxide. They immersed teeth in these solutions for increments of 1, 2, 3, 4, 5, 6, 12, and 24 hours. The changes were quantified by measuring the crown width, tooth length, tooth weight, and by taking close-up pictures to document the qualitative surface changes. The products that contained hydrochloric acid did the most damage, followed by the products containing sulfuric acid, then the products containing phosphoric acid, while the products containing sodium hydroxide had little effect on the teeth.

Robino et. al. (2015) produced a study to see if DNA could be extracted from acid immersed teeth. They used 70% nitric acid reagent, 70%, sulfuric acid reagent, 95.0–98.0%, hydrochloric acid reagent, 37%, aqua regia (freshly prepared by mixing concentrated nitric acid and hydrochloric acid in a volumetric ratio of 1:3). Their results indicated that after two days the DNA was too degraded for analysis in all samples except for the sulfuric acid samples. After seven days, researchers were able to extract DNA from the sulfuric acid samples; however, after 28 days of immersion the DNA was too degraded to extract due to the dissolution of the dentin and enamel which protect the pulpal tissue.

Trapp (n.d.) measured the effects of household acids on teeth with four different acids to estimate the dissolution rate. Three of these acids were used for the current study.

Trapp took photographs, ordinal scores (before and after acid exposure), radiographic information, tooth mass, and buccolingual (BL) and mesiodistal (MD) measurements. She used non-restored crowns and roots, teeth with amalgam restorations, and porcelain fused to metal crowns (PFM). She noted that “86% of the teeth were identifiable via radiograph after their submergence in the household corrosive substances” (Trapp, n.d: 68) with degree of destruction based upon concentration and acid type. This study employs her research design with the modifications of evaluating the effects of acid on additional dental materials and microscopically documenting the structural changes to the tooth surface.

Previous research has not microscopically evaluated the structural changes of tooth structure based upon intervals of submersion in acids.

CHAPTER SIX: MATERIALS AND METHODS

The teeth used in this study were collected from oral surgery extractions over a time span of thirty years. A total of thirty-nine teeth were used: nine (9) teeth with non-restored roots (abbreviation of R), nine (9) with non-restored clinical crowns (abbreviation of V), nine (9) with composite filling(s) on the clinical crown (abbreviation of C), nine (9) with amalgam filling(s) on the clinical crown (abbreviation of A), one (1) tooth with a full-coverage porcelain-fused-to-metal crown (PFM), one (1) tooth with a stainless-steel crown (SC), and one (1) tooth with a porcelain crown (PC). No specifications were used regarding tooth type (incisor, molar, etc.) but rather the restorative material placed in each tooth.

Each category of restored/non-restored teeth was divided into three subgroups, submerging each subgroup into one of the three types of acids. Each subgroup was submerged in their specific acid type for either 24 hours straight, 120 hours straight, or for 264 hours, removing the tooth at intervals of 1, 2, 4, 15, 24, 72, 120, 168, and 264 hours for observation. When removed for observation, the teeth were rinsed, measured, photographed and then re-submerged. Teeth in the 24-hour and 120-hour groups were measured and photographed at the beginning of the experiment and then again at 24 and 120 hours respectively. The teeth in the 24-hour and 120-hour groups were photographed under the dissecting microscope, while the teeth in the 264-hour group were photographed under the dissecting microscope and analyzed using SEM. The porcelain fused to metal crown (PFM), stainless steel crown (SC) and porcelain crown (PC) samples were submerged in the toilet bowl cleaner for 264 hours.

Each tooth was labeled with two letters and a number: the first letter denotes restorative type (R, V, A, C), the second letter identifies the acid reagent (B-The Works® Toilet Bowl Cleaner, C- Roto Drain Cleaner, D- Zoro® Battery Acid) and the number denotes exposure time (24, 120, 264 hours). An additional acid was considered and labeled as A, but was ultimately excluded from this study. Table 6.1 organizes the teeth visually. Each letter in the chart signifies a single tooth submerged in its corresponding acid.

Table 6.1. Organization of Teeth in Acids

Treatment of Teeth in Acid							
	Root , R	Non-restored, V	Composite, C	Amalgam, A	PFM	Silver Crown, SC	Porcelain Crown, PC
24 Hours	B, C, D	B, C, D	B, C, D	B, C, D			
120 Hours	B, C, D	B, C, D	B, C, D	B, C, D			
264 Hours	B, C, D	B, C, D	B, C, D	B, C, D	B	B	B

Two measurements were taken on teeth with non-restored roots: a) at the widest bucco-lingual dimension, and b) root length, measuring from the most apical portion of the cemento-enamel junction (or CEJ) to the root apex. On teeth with non-restored crowns, clinical crowns containing composites or amalgams, and full-coverage restorative crowns, the measurements were taken buccolingually and mesiodistally at the widest part of the crown and the crown length measuring from the highest cusp to the most apical aspect of the CEJ.

Full personal protective equipment (PPE) including acid resistant gloves, an acid resistant apron and safety goggles were worn when in direct contact with acid.

Approximately 15 milliliters of acid were placed into a test tube using a pipette. The teeth

were placed in and removed from the acid using long serrated tweezers for safety. When removed from acid, the teeth were immediately placed in a stainless-steel ball tea infuser and rinsed under water. Next, the teeth were measured with Mitutoyo digital dial calipers, photographed, and placed back into the acid as outlined above.

After removal from acid for the final time, all teeth were viewed using a *Leica MZ6* dissecting microscope and photographs were taken of the gross findings at a magnification of 6.3-12.5 X. Teeth that were in acid for a total of 264 hours were also viewed using a *Zeiss EVO MA15* scanning electron microscope at 111X-1,082X.

CHAPTER SEVEN: RESULTS

The Works® Toilet Bowl Cleaner Quantitative Data

Fifteen (15) teeth were immersed in The Works® Toilet Bowl Cleaner: four of these teeth submerged in toilet bowl cleaner for 24 hours straight without removal, four (4) teeth submerged in toilet bowl cleaner for 120 hours straight without removal, and four (4) teeth submerged in toilet bowl cleaner for 264 hours with removal and re-submersion at the designated hourly intervals. The four (4) teeth in each time interval group consisted of a tooth with a non-restored root surface, a non-restored clinical crown, a clinical crown restored with an amalgam restoration, and a clinical crown restored with a composite restoration. Research conducted by Trapp (n.d.) showed that toilet bowl cleaner created the most tooth erosion. Therefore, teeth with the porcelain fused to metal, stainless steel and porcelain crowns were submerged in toilet bowl cleaner to observe the effects on the restorations. Tables 7.1- 7.3 report the difference in the initial measurement, post acid treatment measurement, and the percent difference.

Table 7.1. Measurement Differences for The Works® Toilet Bowl Cleaner for 264 Hours

Measurement differences for The Works® Toilet Bowl Cleaner (mm) for 264 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (maxillary premolar)	Root Length	3.54	22.10%
	Root Width	1.98	24.30%
Non-restored crown (canine or premolar)	Buccolingual	2.68	35.80%
	Mesiodistal	3.65	45.00%
	Crown Height	1.07	12.90%
Composite restored crown (maxillary incisor)	Buccolingual	3.36	43.10%
	Mesiodistal	3.74	44.30%
	Crown Height	5.93	55.80%
Amalgam restored crown (maxillary premolar)	Buccolingual	2.71	28.90%
	Mesiodistal	3.6	51.80%
	Crown Height	4.77	57.30%
PFM crown (molar)	Buccolingual	0.05	0.40%
	Mesiodistal	0.08	0.80%
	Crown Height	0.15	1.80%
Silver Crown (molar)	Buccolingual	0.15	1.50%
	Mesiodistal	0.01	0.09%
	Crown Height	0.22	0.03%
Porcelain Crown (premolar)	Buccolingual	0.3	3.20%
	Mesiodistal	0.48	6.10%
	Crown Height	0.03	0.50%

Table 7.2. Measurement Differences for The Works® Toilet Bowl Cleaner for 24 Hours

Measurement differences for The Works® Toilet Bowl Cleaner (mm) for 24 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (premolar)	Root Length	3.22	19.10%
	Root Width	1.15	14.20%
Non-restored crown (incisor)	Buccolingual	1.61	19.90%
	Mesiodistal	3.03	38.40%
	Crown Height	2.39	25.60%
Composite restored crown (molar)	Buccolingual	2.13	22.30%
	Mesiodistal	2.74	24.00%
	Crown Height	2.56	39.10%
Amalgam restored crown (molar)	Buccolingual	1.56	17.80%
	Mesiodistal	2.18	22.20%
	Crown Height	2.49	33.50%

Table 7.3. Measurement Differences for The Works® Toilet Bowl Cleaner for 120 Hours

Measurement differences for The Works® Toilet Bowl Cleaner (mm) for 120 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (incisor)	Root Length	5.59	33.60%
	Root Width	2.36	29.00%
Non-restored crown (premolar)	Buccolingual	1.32	16.00%
	Mesiodistal	3.44	54.10%
	Crown Height	2.41	28.20%
Composite restored crown (premolar)	Buccolingual	1.93	26.20%
	Mesiodistal	2.66	42.30%
	Crown Height	1.69	23.80%
Amalgam restored crown (molar)	Buccolingual	4.62	46.20%
	Mesiodistal	3.55	39.10%
	Crown Height	1.69	22.20%

The Works® Toilet Bowl Cleaner Qualitative Data

The teeth immersed in The Works® Toilet Bowl cleaner had one of the most dramatic changes in terms of tooth discoloration. Within one hour of immersion, the teeth began to turn blue from the dye used in the product, and the enamel on the cusp tips started to erode shortening the overall crown height. Within 24 hours, the teeth became slightly translucent. At the end of the experiment the teeth dried out, which caused them to darken and become more translucent. Figure 7.1 depicts differences in translucency.

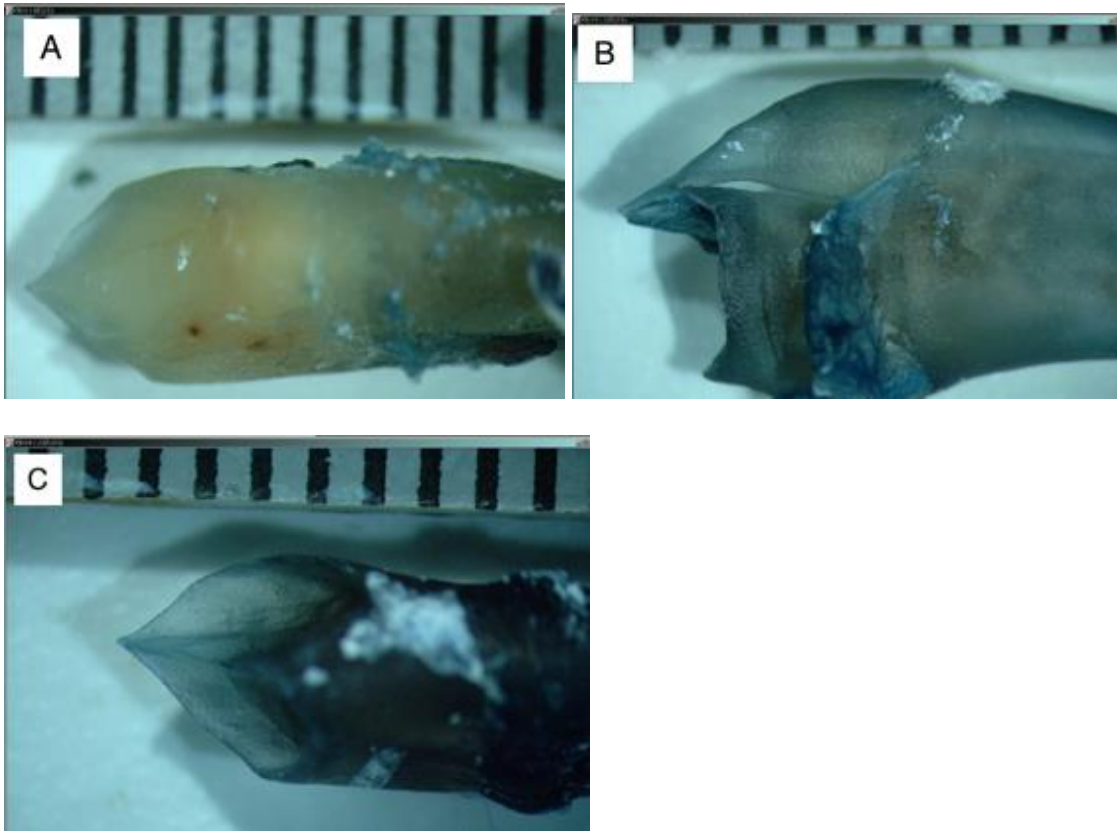


Figure 7.1. Degrees of Translucency of samples immersed in The Works® Toilet Bowl Cleaner taken at (A) 24 hours (B) 120 Hours (C) 264 Hours respectively

The toilet bowl cleaner had very little effect on the PFM, stainless steel or porcelain crowns with little to no change in appearance or measurements throughout the duration of the study. The toilet bowl cleaner created roughening of the outer surface of the amalgam starting at 24 hours, but created no changes in shape or dimension. Enamel eroded away from the margins of the restoration, and in some of the samples, the amalgam dislodged from the tooth due to severe erosion. There was no other significant change in the appearance of the amalgam for the duration of the experiment. Figure 7.2 shows how amalgam was affected by treatment with The Works® Toilet Bowl Cleaner.

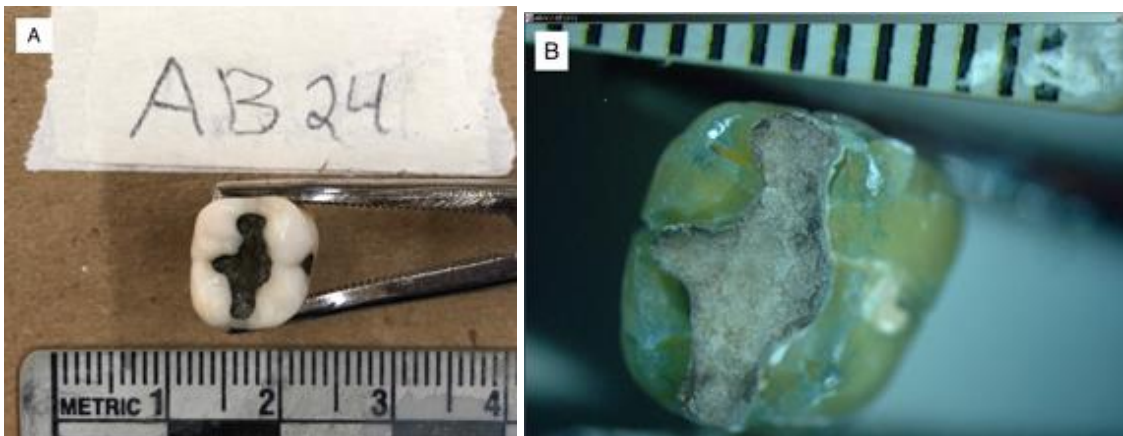


Figure 7.2. A) The initial appearance of an amalgam filling B) The appearance of an amalgam filling after immersion in The Works® Toilet Bowl Cleaner for 24 hours. Note the roughened look of the amalgam and the margins of the teeth eroding adjacent to the amalgam filling

The toilet bowl cleaner had very little effect on composite restorations in regards to shape and dimension and the enamel eroded around the restorations. Figure 7.3 shows how The Works® Toilet Bowl cleaner affects composite restorations.

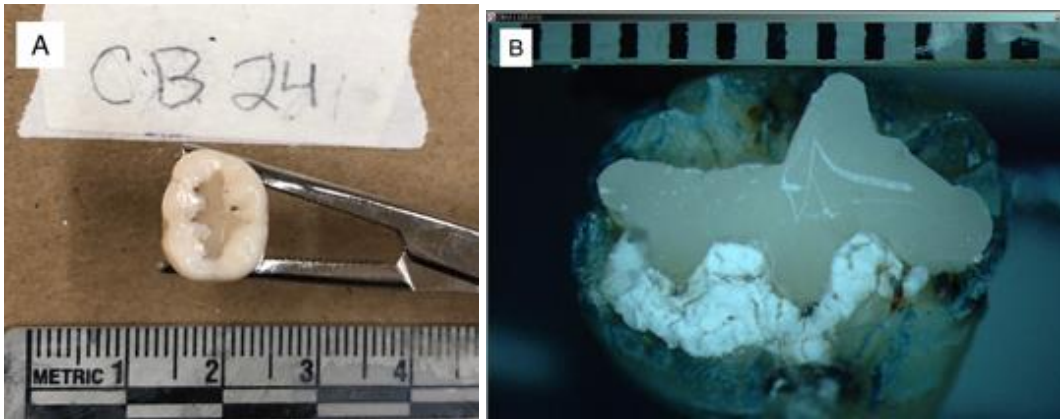


Figure 7.3. A) The initial appearance of a composite restoration B) The appearance of an composite restoration after immersion in The Works® Toilet Bowl Cleaner for 24 hours. Note there is very little difference in the shape or appearance of the composite restoration, but that the enamel around the filling has eroded.

In less than 24 hours and continuing for 3-5 days afterwards, multiple tubes containing the toilet bowl cleaner and a submerged tooth were found with their rubber stoppers off at each timed measurement interval.

Inside the test tube, the toilet bowl cleaner created complete dissolution and no enamel sludge or particles were noted at the bottom of the test tube.

Rooto Drain Cleaner Quantitative Data

Twelve teeth were immersed in Rooto Drain Cleaner: four (4) of these teeth submerged in drain cleaner for 24 hours straight without removal, four (4) teeth submerged in drain cleaner for 120 hours straight without removal, and four (4) teeth submerged in drain cleaner for 264 hours with removal and resubmersion at the designated hourly intervals. The four (4) teeth in each time interval group consisted of a tooth with a non-restored root surface, a non-restored clinical crown, a crown of a tooth

restored with an amalgam restoration, and a crown of a tooth restored with a composite restoration. Tables 7.4 - 7.6 report the difference in the initial measurement and post acid treatment measurement, and the percent difference.

Table 7.4. Measurement Differences for Rooto Drain Cleaner for 264 Hours

Measurement differences for Rooto Drain +A1:D13Cleaner (mm) for 264 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (canine)	Root Length	1.13	6.50%
	Root Width	2.83	41.00%
Non-restored crown (premolar)	Buccolingual	0.67	9.00%
	Mesiodistal	0.52	6.50%
	Crown Height	0.93	12.70%
Composite restored crown (maxillary incisor)	Buccolingual	1.97	26.50%
	Mesiodistal	0.94	10.50%
	Crown Height	4.52	35.20%
Amalgam restored crown (premolar)	Buccolingual	2.32	25.00%
	Mesiodistal	0.13	1.90%
	Crown Height	1.98	27.50%

Table 7.5. Measurement Differences for Rooto Drain Cleaner for 24 Hours

Measurement differences for Rooto Drain Cleaner (mm) for 24 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (canine)	Root Length	0.02	0.09%
	Root Width	0.22	2.40%
Non-restored crown (maxillary incisor)	Buccolingual	0.27	3.90%
	Mesiodistal	0.67	7.50%
	Crown Height	0.22	1.90%
Composite restored crown (incisor)	Buccolingual	0.06	0.90%
	Mesiodistal	0.69	10.80%
	Crown Height	0.23	2.40%
Amalgam restored crown (molar)	Buccolingual	0.4	4.20%
	Mesiodistal	0.17	1.50%
	Crown Height	0.02	0.35%

Table 7.6. Measurement Differences for Root Drain Cleaner for 120 Hours

Measurement differences for Rooto Drain Cleaner (mm) for 120 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (mandibular premolar)	Root Length	0.91	5.70%
	Root Width	0.17	2.60%
Non-restored crown (premolar)	Buccolingual	1.08	11.90%
	Mesiodistal	0.8	11.20%
Composite restored crown (mandibular molar)	Crown Height	0.24	2.80%
	Buccolingual	0.05	0.60%
	Mesiodistal	0.09	0.90%
Amalgam restored crown (maxillary molar)	Crown Height	0.02	0.30%
	Buccolingual	0.03	0.30%
	Mesiodistal	0.24	2.50%
	Crown Height	0.8	10.10%

Rooto Drain Cleaner Qualitative Data

Rooto Drain Cleaner produced a completely different tooth appearance than the toilet bowl cleaner. Rooto Drain Cleaner is a type of sulfuric acid. Significant changes in the teeth were not noted until between 24-72 hours following submersion. At this point, the enamel and dentin on the tooth surface took on a “curdled” appearance (i.e. – a curdled cheese appearance). One particular tooth (see Figure #7.4.B) began to turn pink at its apex. Initially, the pink color was vibrant but subsequently faded to a darker hue when immersed and left in the air before re-immersion. The drain cleaner roughened the outer amalgam surface, but created no significant changes in shape or dimension of the amalgam. Figure 7.4 shows how Rooto Drain Cleaner treatment affects teeth.



Figure 7.4: A) The initial appearance of a tooth B) The appearance of a tooth submerged in Rooto Drain Cleaner. Note the “curdling” of the calcified structures of the tooth, as well as the faded pink coloration at the apical end of the root. C) A tooth containing a mesio-occlusal-distal amalgam restoration submerged in Rooto Drain Cleaner for 264 hours. Note the roughened appearance of the amalgam that still retains its shape.

The drain cleaner had varied effects on the teeth with composite restorations. At 24 hours, the composites seemed unchanged, but “curdling” of the enamel was noted. In the tooth sample submerged and removed at hourly intervals for 264 hours, the composite changed to a dark reddish/brown color starting at 24 hours with the composite appearing to dissolve or break away from the tooth. This phenomenon is observed exceptionally well under the dissecting microscope after the tooth dried out. Figure 7.5 shows how Rooto Drain Cleaner affected composites.

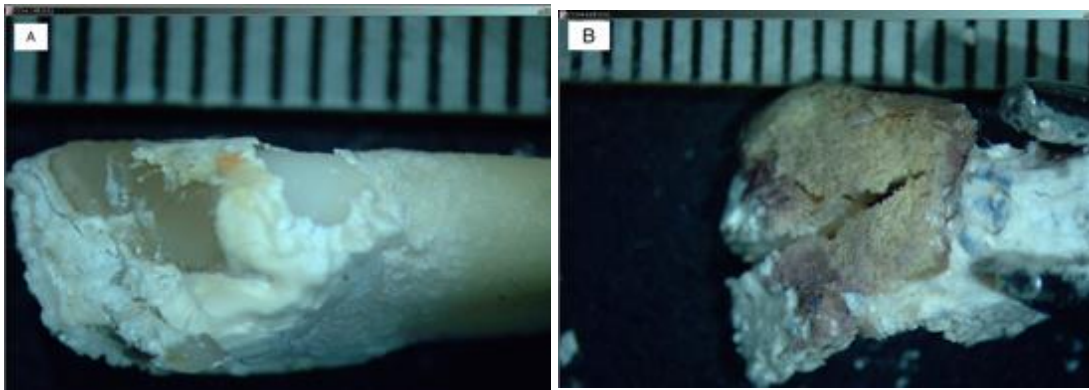


Figure 7.5. A) A tooth with composite fillings after 24 hours of immersion of tooth in Rooto Drain Cleaner. Note the lack of change in the appearance of the composites, but there is significant “curdling” around the filling. B) A tooth with a buccal filling that covers over $\frac{3}{4}$ of the tooth surface immersed in the drain cleaner for 264 hours. Note the brownish/reddish discoloration of the composite, as well as its porous appearance.

Inside the test tube, the drain cleaner created a sludge of tooth particles at the bottom of the test tube. This sludge remained and never fully dissolved, even months after the experiment ended.

Zoro® Battery Acid Quantitative Data

Twelve (12) teeth were immersed in Zoro® Battery Acid: four (4) of these teeth submerged in battery acid for 24 hours straight without removal, four (4) teeth submerged in battery acid for 120 hours straight without removal, and four (4) teeth submerged in battery acid for 264 hours with removal and re-submersion at the designated hourly intervals. The four (4) teeth in each time interval group consisted of a tooth with a non-restored root surface, a non-restored clinical crown, a crown of a tooth restored with an amalgam restoration, and a clinical crown of a tooth restored with a composite restoration. Tables 7.7 - 7.9 report the difference in the initial measurement and post the battery acid submersion measurement and the percentage of change.

Table 7.7. Measurement Differences for Zoro® Battery Acid for 264 Hours

Measurement differences for Zoro® Battery Acid (mm) for 264 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (molar)	Root Length	0.7	6.10%
	Root Width	1.18	11.80%
Non-restored crown (premolar)	Buccolingual	1.23	15.70%
	Mesiodistal	0.4	5.00%
	Crown Height	2.8	30.90%
Composite restored crown (incisor)	Buccolingual	0.76	9.20%
	Mesiodistal	1.81	24.30%
	Crown Height	2.53	25.50%
Amalgam restored crown (premolar)	Buccolingual	0.92	12.10%
	Mesiodistal	1.63	26.40%
	Crown Height	2.84	41.20%

Table 7.8. Measurement Differences for Zoro® Battery Acid for 24 Hours

Measurement differences for Zoro® Battery Acid (mm) for 24 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (incisor)	Root Length	0.29	1.80%
	Root Width	0.07	0.87%
Non-restored crown (premolar)	Buccolingual	0.27	3.40%
	Mesiodistal	0.18	2.50%
	Crown Height	1.67	26.10%
Composite restored crown (premolar)	Buccolingual	0.05	0.55%
	Mesiodistal	0.01	0.13%
	Crown Height	0.03	0.40%
Amalgam restored crown (mandibular molar)	Buccolingual	0.22	2.30%
	Mesiodistal	0.1	0.93%
	Crown Height	0.01	0.18%

Table 7.9. Measurement Differences for Zoro® Battery Acid for 120 Hours

Measurement differences for Zoro® Battery Acid (mm) for 120 hours			
Tooth Aspect Type, tooth type	Measurement Type	Pre/Post measurement difference (mm)	Percent Difference
Root (incisor)	Root Length	0.51	4.30%
	Root Width	0.16	2.40%
Non-restored crown (premolar)	Buccolingual	0.1	1.00%
	Mesiodistal	0.3	3.60%
	Crown Height	0.75	8.40%
Composite restored crown (maxillary incisor)	Buccolingual	0.01	0.15%
	Mesiodistal	1.09	12.30%
	Crown Height	3.91	36.10%
Amalgam restored crown (maxillary molar)	Buccolingual	1.48	13.10%
	Mesiodistal	0.19	1.90%
	Crown Height	0.28	3.90%

Zoro® Battery Acid Qualitative Data

Zoro® Battery Acid produced similar results to the drain cleaner, but took much longer to start dissolution. On average, it took between 72-264 hours to see any appreciable changes in appearance. At approximately 72 hours, the outer surface of the tooth began to become chalky, with evidence of tooth enamel and dentin demineralization. Eventually, the enamel eroded around the amalgam and composite restorations, with the restoration dislodging from the tooth. Some roughness of the amalgam restorations was noted with no changes in shape or dimension and there were no major changes to the composite restorations. Figure 7.6 shows how Zoro® Battery Acid treatment affects teeth.

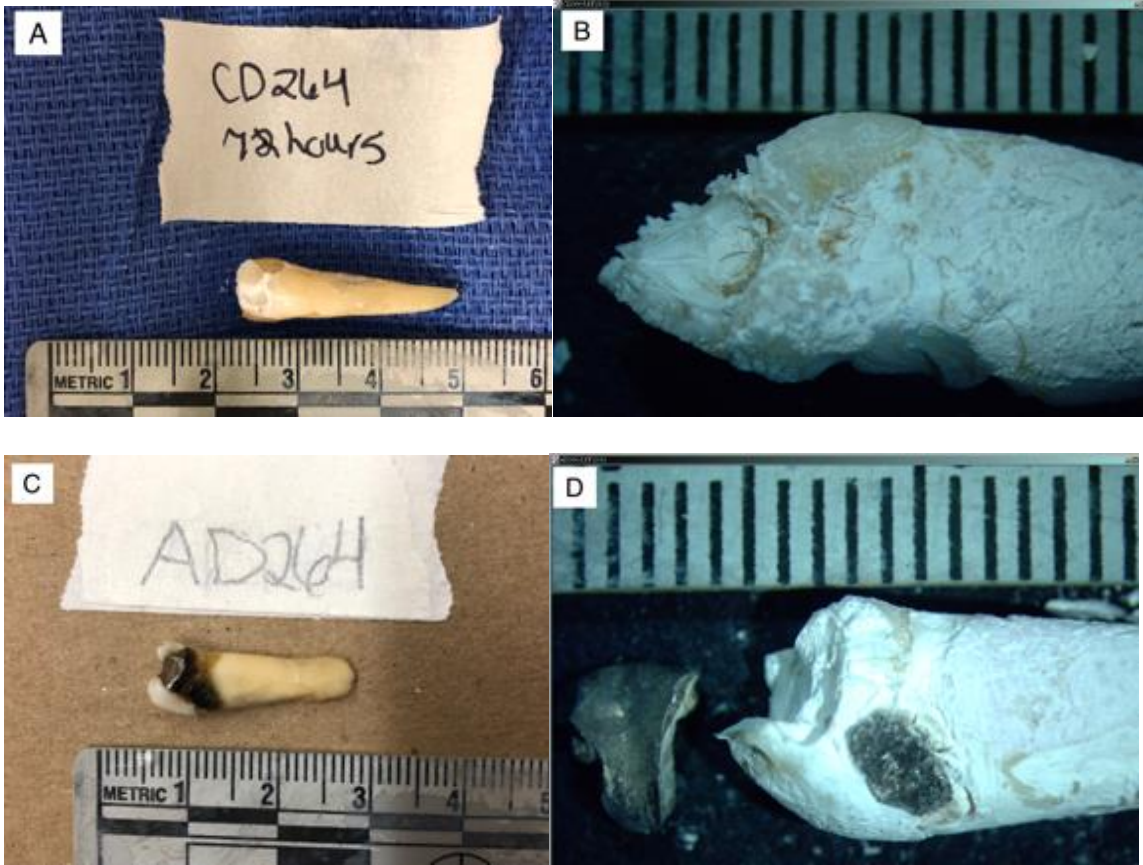


Figure 7.6. A) A tooth with composite restorations submerged in Zoro® Battery Acid for 72 hours. Note the chalkiness on the crown around the restoration. B) The same tooth seen in Figure A submerged in acid for a total of 264 hours. Note that the restoration is missing and the preparation is visible. C) The initial appearance of a tooth with two amalgam restorations, one on the crown surface and another one on the root surface. D) A tooth with two amalgam restorations submerged in Zoro® Battery Acid for 264 hours. One amalgam restoration has detached from the tooth while the other remains intact. Note the slight roughened appearance of the amalgams with no change in shape or dimension.

The battery acid created a sludge of tooth particles at the bottom of the test tube. This sludge remained and never full dissolved, even months after the experiment ended.

CHAPTER EIGHT: STATISTICAL ANALYSIS

In order to determine which acid created the greatest amount of tooth erosion, the overall percentage of tooth loss was calculated. This was accomplished by adding the initial measurements of one tooth, then subtracting the total from the final measurements, creating an overall measurement differential in millimeters from the initiation of acid submersion to the end. The overall measurement difference was then divided by the total of the initial measurements, to calculate overall percentage of tooth loss. The overall percentage of tooth loss was analyzed with a one-way ANOVA test to determine if there is a significant difference between the loss of tooth structure for the three different types of acids. An assumption of ANOVA is that the variances are homogenous; therefore, a test of variance homogeneity was calculated to make certain an ANOVA test is appropriate for this data set. If the variances are homogenous and the overall ANOVA F-test is significant, a post-hoc Tukey's test was performed to determine where the differences occur. Because the non-restored, composite restored and amalgam restored teeth all had three measurements and the roots had two measurements, a separate analysis was performed for the root measurements so that results will not be skewed. As there is only one value per tooth group for each amount of time submersed, the teeth measurements were evaluated individually.

The null and alternative hypotheses for the test of homogeneity, considering a 0.05 alpha, are:

Ho: all the variances are equal

H_A: the variances are not equal, with at least one being different

The null and alternative hypotheses for the one-way ANOVA test, considering a 0.05 alpha are:

H₀: all the means are equal

H_A: the means are not equal, with at least one being different

Table 8.1 summarizes the overall means of percent differences over time for all the measurements except the root measurements.

Table 8.1. Means of Percent Difference Over Time (No Roots)

Means of Percent Difference Over Time (No Roots)			
	24 Hours	120 Hours	264 Hours
The Works® Toilet Bowl Cleaner	26.233	32.7	43.167
Rooto Drain Cleaner	3.567	4.3667	17.867
Zoro® Battery Acid	3.867	10.1	21.422

Table 8.2 summarizes the root length percent difference over time.

Table 8.2. Root Length Percent Difference

Root Length Percent Difference			
	24 Hours	120 Hours	264 Hours
The Works® Toilet Bowl Cleaner	19.1	33.6	22.1
Rooto Drain Cleaner	0.09	5.7	6.5
Zoro® Battery Acid	1.8	4.3	6.1

Table 8.3 summarizes the root width percent differences over time.

Table 8.3. Root Width Percent Difference

Root Width Percent Difference			
	24 Hours	120 Hours	264 Hours
The Works® Toilet Bowl Cleaner	14.2	29	24.3
Rooto Drain Cleaner	2.4	2.6	41
Zoro® Battery Acid	0.87	2.4	11.8

Figure 8.1 shows the overall means of percent change over time for all the measurements except the root measurements.

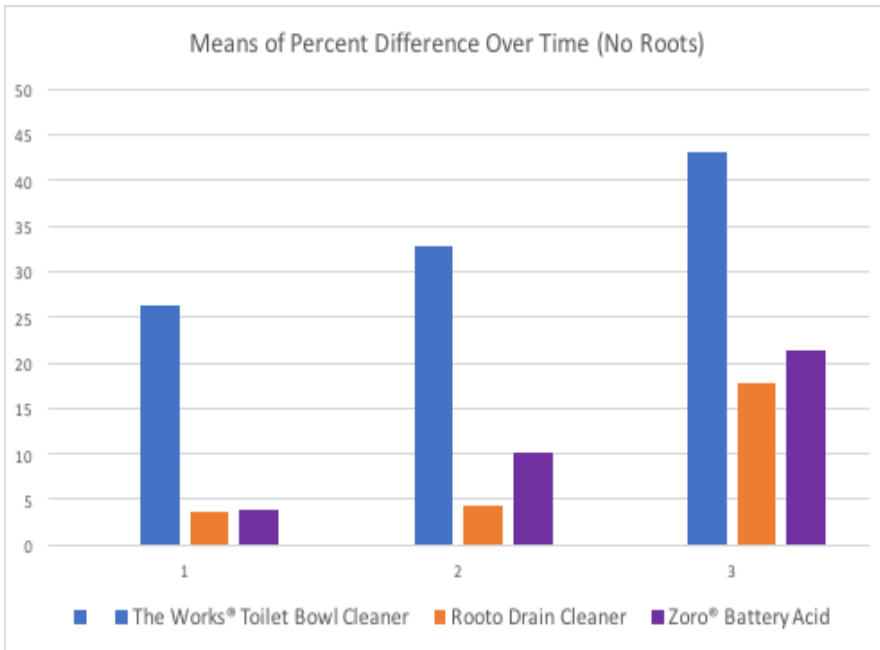


Figure 8.1. Means of Percent Difference Over Time (No Roots)

Figure 8.2 shows the root length percent differences change over time

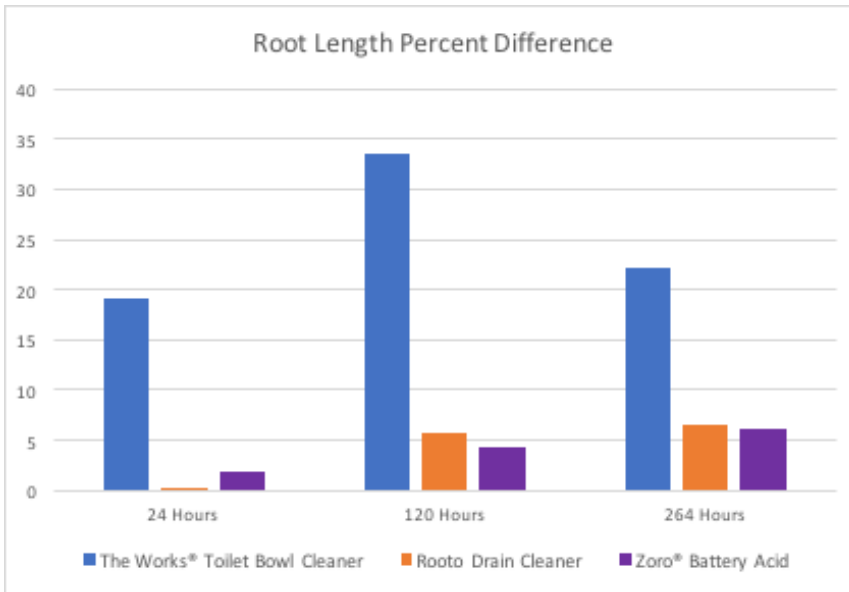


Figure 8.2. Root Length Percent Difference

Figure 8.3 shows the root width percent differences change over time.

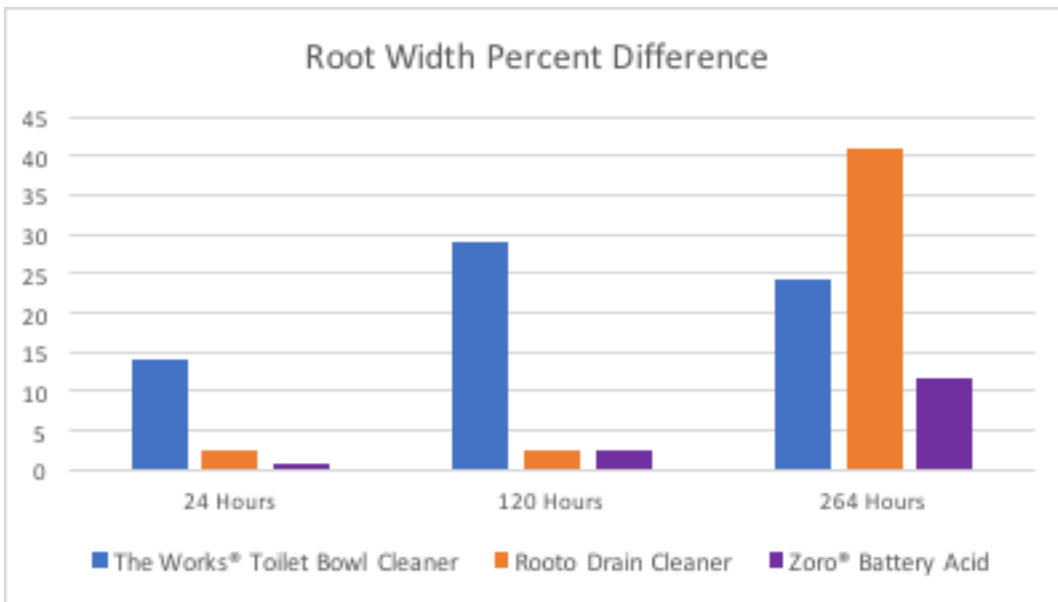


Figure 8.3. Root Width Percent Difference

For the teeth submersed in the three acids for 24 hours, the p-value for the homogeneity test was 0.034, which is below the alpha of 0.05. The conclusion reached is that the null hypothesis is rejected in favor of the alternative hypothesis, that the variances are not equal. Despite this assumption being negated, a Welch's test was performed to statistically confirm the results. The one-way ANOVA p-value for teeth submerged in the three acids is less than 0.001 and a Welch's test a p-value of 0.001 was calculated. Therefore, the null hypothesis is rejected in favor of the alternative hypothesis which states that the means between the groups of teeth in the three different acids are statistically different. To determine if there was not a statistically significant difference in means, a Tukey's post-hoc test was performed. This test indicated no statistically significant difference between the means of the drain cleaner and battery acid, but between the toilet bowl cleaner and battery acid and the toilet bowl cleaner and drain cleaner, the means were statistically significant. The root measurements for 24-hour treatment in the toilet bowl cleaner showed a variance in root length of 19.1% and a difference in root width of 14.2%. While the root measurements for 24-hour submergence in the drain cleaner showed a variance in root length percent of 0.09% and in root width 2.4%. Correspondingly, the root measurements for 24-hour treatment in the battery acid showed a root length percent variance of 1.8% and a root width variance of 0.87%.

Teeth submersed in the three acids for 120 hours had a homogeneity test p-value of 0.199, higher than the alpha of 0.05. Thus, the null hypothesis is not rejected, the variances are assumed to be homogeneous and a one-way ANOVA was performed. The one-way ANOVA p-value for teeth submerged in the three acids is 0.002 meaning the

null hypothesis is rejected in favor of the alternative hypothesis. The alternative hypothesis states that the means between the groups of teeth in the three different acids are statistically different. To analyze which difference in means was not statistically significant a Tukey's post-hoc test was performed and it demonstrated there was no statistically significant difference between the means of the drain cleaner and battery acid, but that between the toilet bowl cleaner and the battery acid and the toilet bowl cleaner and the drainer cleaner, the means were statistically significant. The root measurements for 120-hour submersion in the toilet bowl cleaner revealed a root length percent variance of 33.6% and a root width percent variance of 29%. The root measurements for 120-hour submersion in the drain cleaner showed a root length percent variance of 5.7% and a difference in root width of 2.6%. The root measurements for 120-hour treatment for the battery acid showed a root length percent variance of 4.3% and a difference in root width percent of 2.4%.

For the teeth submersed in the three acids for 264 hours, the homogeneity test p-value was 0.263 thereby failing to reject the null hypothesis. Therefore, since the variances are homogeneous, a one-way ANOVA was performed. The one-way ANOVA p-value for teeth submerged in the three acids for 264 hours was 0.017, confirming that the null hypothesis is rejected in favor of the alternative hypothesis, which states that the means between the groups of teeth in the three different acids are statistically different. To analyze which difference in means was not statistically significant, a Tukey's post-hoc test was performed indicating there was no statistically significant difference between the means of the drain cleaner and the battery acid, but between the toilet bowl

cleaner and the battery acid and the toilet bowl cleaner and the drain cleaner, the means were statistically significant. The root measurements for 264-hour treatment in the toilet bowl cleaner demonstrated a root length percent variance of 22.1% and a difference in root width percent of 24.3%. The root measurements for 264-hour submersion in the drain cleaner showed a root length percent variance of 6.1% and a difference in root width percent of 41%. The root measurements for 264-hour treatment in the battery acid demonstrated a root length percent variance of 6.1% and a difference in root width percent of 11.8%.

CHAPTER NINE: SEM RESULTS

The scanning electron microscope (SEM) permitted visualization of the tooth surface topography after acid submersion. As seen previously, enamel and dentin has a natural genetic organization. When the teeth were subjected to acid dissolution, there is a disorganization of the enamel prisms and dentinal tubules. The drain cleaner and battery acid created the most surface disorganization. The drain cleaner created smaller enamel particles than the battery acid, suggesting that the drain cleaner created more destruction of the enamel surface. Figure 9.1 shows the “curdling” of enamel produced by the battery acid and the drain cleaner.

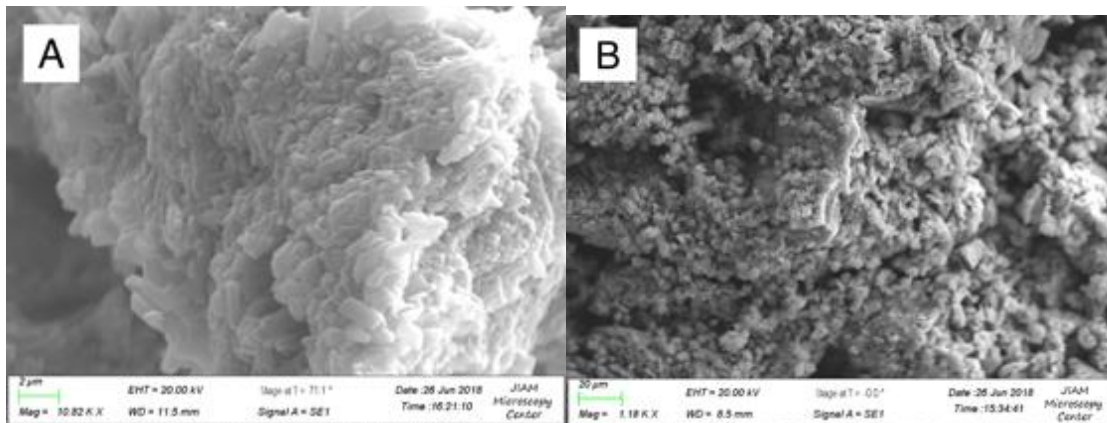


Figure 9.1. A) Enamel rods after 264 hours of Zoro® Battery Acid treatment at 10.82 K X. Note the disorganization of the enamel rods. B) Enamel rods after 264 hours in Rooto Drain Cleaner treatment at 1.18 K X. Note the size of the enamel rods compared to the battery acid treatment

Teeth subjected to the toilet bowl cleaner submersion demonstrated a smoother dentin surface, but the acid treatment created a rough enamel surface with flecking of the dentinal surface. Figure 9.2 shows the dentin surface of tooth AB264 after submersion in the toilet bowl cleaner.

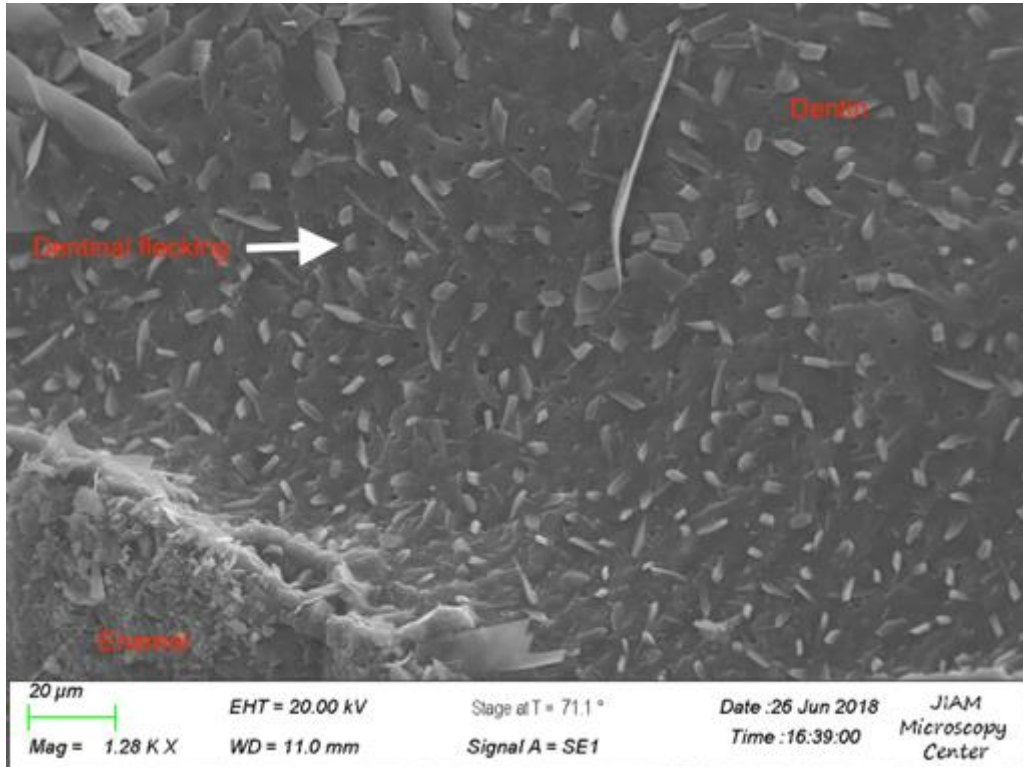


Figure 9.2. Tooth AB264 (submerged in The Works® Toilet Bowl Cleaner), the dentin surface with dentinal flecking. Note the roughness of the enamel, and the exposed dentinal tubules.

Overall, the appearance of amalgam fillings became roughened with an overall smooth appearance after treatment in the battery acid, but showed more roughness after treatment in the drain cleaner. Figure 9.3 shows the amalgam of specimen AD264 after battery acid treatment, as well as the amalgam of specimen AC264 after drain cleaner treatment.

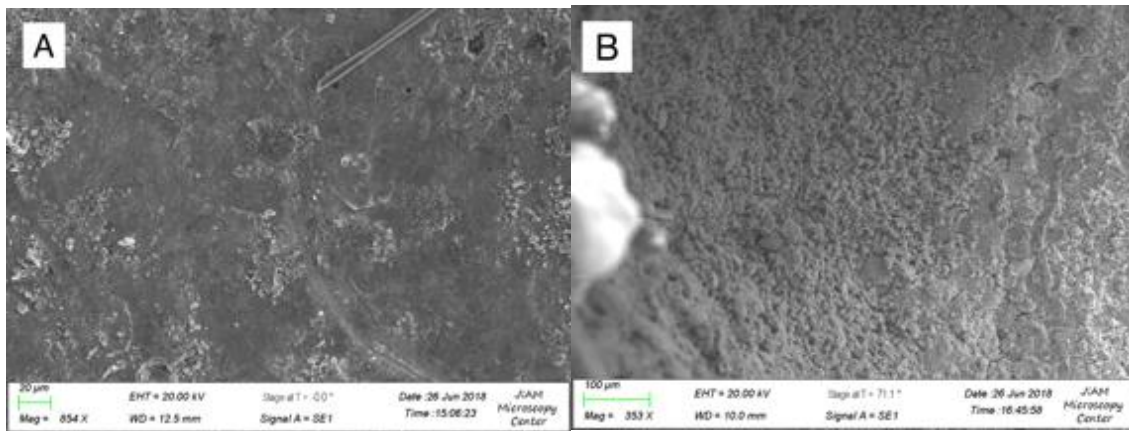


Figure 9.3. A) Amalgam after 264 hours of treatment in Zoro® Battery Acid at 856 X; note the rough appearance of the amalgam. B) Amalgam after 264 hours of treatment in Rooto Drain Cleaner at 353 X; note the increased roughness of the amalgam, suggesting further degradation of the amalgam.

Specimen CC264 was the tooth that demonstrated a reddish/brown change to the composite surface. Under SEM, the composite surface appears roughened and flaking, similarly to the photograph taken under the dissecting microscope. A view of the side and internal aspect of the composite where the acid had not penetrated shows composite roughness, but less flaking than the composite surface in direct contact with the drain cleaner. Figure 9.4 shows side by side the portion of composite directly in contact with the acid and the portion that was not in direct contact with the acid.

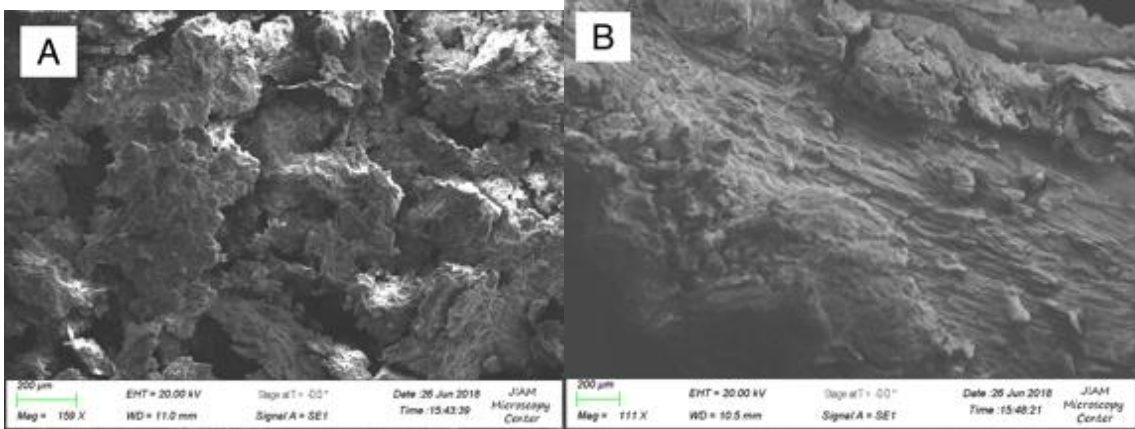


Figure 9.4. A) Specimen CC264 composite that had direct contact with Rooto Drain Cleaner at 159 X. Note the flaking of the composite. B) Specimen CC264 composite that is internal and was not in direct contact with the drain cleaner at 111X. Note some roughness but the composite surface is smoother than the composite that is directly in contact with the drain cleaner.

CHAPTER TEN: DISCUSSION

Previous research predicted reduction in tooth volume, and to an extent, the tooth appearance after acid submersion was as expected. The “curdling” that was seen throughout the whole experiment for the two sulfuric acids (Rooto Drain Cleaner and Zoro® Battery Acid), has been noted by Mazza et al. as “corpusculate deposits” and “corrosion” of the tooth. Cope and Dupras note in their study that the teeth immersed in hydrochloric acid took on a “jelly-like appearance” which describes the outer layer translucency observed in this study.

Hydrochloric acid exposure to teeth in the form of stomach acid is associated with patients diagnosed with Gastroesophageal reflux disease (GERD) and bulimia. Teeth in patients with these disorders can appear translucent with cupping of the posterior surfaces and severe erosion on the lingual surfaces (Chockattu et al. 2018). The tooth samples in the toilet bowl cleaner (hydrochloric acid) demonstrated significant translucency. The teeth submerged for 264 hours showed complete translucency down to the pulp chamber. The blue discoloration observed was caused by the blue dye in the toilet bowl cleaner.

Despite some of these expected results, there were some aspects that were not expected or well understood. As stated previously, between time zero and 3-5 days, some of the rubber stoppers on some of the test tubes containing the toilet bowl cleaner had come off by the next time measurements were taken. This observation leads to the hypothesis that there is a chemical reaction happening inside the test tube between the hydrochloric acid producing either an exothermic reaction, or a gas byproduct. This produced enough force to pop the rubber stoppers off the test tube. According to the first

law of thermodynamics “energy is neither created or destroyed” and outlines the definition of work defined as “motion against an opposing force” (Atkins, 2010). If it was an exothermic reaction the heat created from the chemical reaction inside the test tube would cause the air particles to move faster, creating pressure and causing the stopper to pop off. If the reaction noted was because of gas buildup in the test tube, the pressure from the gas would allow the stoppers to pop off. Unfortunately, since this was an unexpected observation, no official efforts were made to determine exactly how this force was created to pop the rubber stoppers off the test tubes.

Another confusing observation was the differing effects of the drain cleaner on the teeth with composite restorations. For example, a tooth submerged in the drain cleaner for 24 hours with a thick composite resin in the interproximal spaces demonstrated no effect or darkening of the composite. However, after 24 hours through the end of the 264 hours of submersion, a different tooth submerged in the drain cleaner with a large labial composite filling turned a darker red color over time. Eventually the outer layer of this tooth’s composite restoration dissolved leaving a porous and discolored surface. Figure 10.1 demonstrates these visual differences.

Figure 10.1. A) Initial appearance of a tooth with a large interproximal composite filling B) The same tooth after 24 hours of Rooto Drain Cleaner treatment C) initial appearance of a tooth with a large composite filling that covers the labial surface as well as interproximal coverage D) the same tooth after 24 hours of Rooto Drain Cleaner treatment. Note the initial darkening of the composite E) the same tooth after 264 hours of the drain cleaner treatment. Note that the composite filling has turned a dark reddish-brown and has a porous appearance.



Figure 10.

The difference in appearance may be due different types of composite used. Another difference noted is the technique of restoration placement. For interproximal composite restorations the tooth is prepped and then the composite material is packed in the prepped cavity space. Some layering may be done to be sure that the restoration is flush against the tooth surface. This technique was most likely utilized for tooth CC24. Tooth CC264 has a large labial composite that was most likely done to avoid restoring the tooth with a crown or veneer, while still creating an aesthetically acceptable option for restoring. The technique most likely used was multiple thin layers of flowable composite for restoration (Hilton et al., 2013). The drain cleaner may have been able to penetrate the restoration on CC264 better than CC24 because of this technique and the thinness of the composite layers. Unfortunately, with no information about the type of composite placed or absolute certainty about the technique used, the aforementioned hypothesis is impossible to test.

As one can see from the data, the toilet bowl cleaner outperformed the drain cleaner and the battery acid in every category, except one. The root width at 264 hours under the treatment of the drain cleaner had a 16.7% higher dissolution rate than the toilet bowl cleaner. There were no visible fractures noted on the root surface of this tooth before acid treatment, but it is possible that a large longitudinal fracture could have caused the root surface to break apart. Unfortunately this is only a speculation, and we can assume that despite this, overall the toilet bowl cleaner created more dissolution than the drain cleaner and battery acid and that this data point may be an outlier.

As mentioned above and shown by previous research, hydrochloric acid outperforms sulfuric acid. Despite that the concentration for the toilet bowl cleaner was 9.5% and the drain cleaner was 95%, the toilet bowl cleaner still outperformed the drain cleaner when analyzing the teeth measurements. This observation could possibly be due to the toilet bowl cleaner, which is a hydrochloric acid, having a smaller pK_a value than sulfuric acid, which means that the toilet bowl cleaner would technically be stronger, despite having a lower concentration. More research into acid chemistry is needed to confirm exactly what reactions take place between the acid and the tooth surface.

Following completion of the “curdling” phase seen on teeth submerged in the drain cleaner, the teeth soon thereafter crumbled, breaking apart into pieces. The majority of teeth submerged in the battery acid became chalky. In a few instances where the tooth’s structural integrity was compromised from decay the tooth would begin to break apart. This effect is explained by the fact that the drain cleaner is a 93.2% percent concentration of sulfuric acid and the battery acid is 35% and also explains why the battery acid took longer than the drain cleaner to create any appreciable differences in appearance.

The SEM results confirmed that the drain cleaner created more destruction than the battery acid. The surfaces analyzed between the teeth submerged in the drain cleaner consistently showed more surface roughness, smaller particles, and greater disorganization than the teeth submerged in the battery acid. The toilet bowl cleaner created roughness and disorganization of the particles, but overall showed a greater maintenance of the tooth’s structural integrity than the drain cleaner or battery acid

created. The dentin on teeth submerged in the toilet bowl cleaner showed some flecks on the dentinal surface, and the drain cleaner and battery acid showed disorganized dentin and enamel rods. In the drain cleaner and battery acid specimens, the enamel particles appeared to stack on top of one another. The drain cleaner and battery acid created the “curdled” effect and became sticky to handle suggesting that those enamel particles had coalesced and bound to one another. Additionally, the dentin consistently showed less disorganization and a smoother appearance than the enamel across all three acid reagents. This is explained by the fact that enamel is over 25% more mineralized than dentin, making it more susceptible to dissolution and disorganization of its structural integrity.

Limitations

Every experiment has limitations. Originally, this study was to include thin-sectioning of the teeth to determine how far the acid penetrated the tooth. When the teeth were embedded in epoxy in preparation for thin-sectioning, the epoxy did not harden due to the shelf life of the epoxy and hardener. The epoxy had to be melted back down in an oven, the teeth were pulled out of the epoxy and then reembedded in fresh epoxy. When the teeth were reembedded, some of them still never hardened. Because of this setback, thin-sectioning observation was removed from the experiment. Additionally, all of the teeth in the 24-hour and 120-hour groups were unable to be retrieved from the epoxy. Therefore, these teeth were not evaluated for surface changes using the SEM. However, these teeth still contributed to measurement analysis, and evaluation of qualitative changes following acid submersion because photographs were taken throughout the experiment.

Another limitation was the loss of anatomical landmarks as the teeth dissolved, making accurate measurements difficult. An example of this limitation was in estimating where the cemento-enamel junction (CEJ) was located on a tooth. A working knowledge of tooth anatomy is necessary in order to estimate where these anatomical features begin and end. Photographic documentation throughout the experiment permitted referencing to an earlier appearance aiding in the determination of the location of anatomical features -in order to produce accurate measurements.

An unexpected issue was that the teeth with “curdling” were sticky, and as a result, the teeth picked up any loose debris they contacted. For example, blue towels were used when photographing and subsequently some of the samples had blue fibers attached. Tooth surfaces that did not contain blue fibers were utilized for SEM analysis.

CHAPTER ELEVEN: CONCLUSION

Overall, the results showed that the toilet bowl cleaner out-performed the drain cleaner and the battery acid in terms of tooth dissolution for almost all of the time categories. For measurements pertaining to the clinical crown of the tooth, the toilet bowl cleaner produced more dissolution at every time interval. On the root surface, the toilet bowl cleaner produced more dissolution at almost every time interval; the only set of measurements that surpassed the toilet bowl cleaner was the drain cleaner at the 264-hour mark.

Most of the differences seen between the teeth observed in this study can be attributed to the specific class of acid and its concentration. As stated previously, the toilet bowl cleaner is a type of hydrochloric acid, and the drain cleaner and battery acid are forms of sulfuric acid, with the concentration of the drain cleaner being higher than that of the battery acid. The toilet bowl cleaner created more of a uniform tooth destruction, producing a generalized erosion and increased translucency over time. The anatomical features became smooth and less defined, and the teeth started to turn blue at 24 hours. The drain cleaner and battery acid dissolved the outer layer of the tooth which became chalky, dehydrated and took on a “curdled” appearance starting at 72 hours.

The scanning electron microscope revealed the extent of tooth destruction from acid reagent. Overall the acids disorganized the basic structures of the outer layers of the teeth by disorganizing the enamel rods and eroding the dentin surface. To the naked eye, amalgam and composite restorations roughened when placed in contact with acid and this roughness was noted to a greater extent under SEM. None of the composites or amalgams

changed their shape or dimension, except for sample CC264, which was previously discussed.

In conclusion, teeth in contact with acid can become unrecognizable to the naked eye. If teeth are outside of the jaw, forensic investigators may have a difficult time identifying whether their specimens are teeth or not. After 264 hours of acidic contact, many of the teeth became distorted and would not be useful for radiographic or photographic comparison. Despite showing what the tooth surface looks like at a microscopic level, more research is still needed to tell exactly what processes happen during acid treatment. Overall this experiment helped bridge the gap of how teeth respond to acid treatment.

Future Considerations

One consideration for future research would be whether during life a tooth had exposure to fluoride. When ingested during tooth development, fluoride creates a decay resistant tooth surface; therefore, a tooth without fluoride treatment may act differently to acid treatment versus a tooth that has been exposed to fluoride. Another consideration is how teeth that are freshly extracted react to acid treatment, versus teeth that have been dried out, like utilized in this study. Also, a criminal may not pull teeth out of the jaw to submerge in acid, so how does acid treatment affect the alveolar bone, or even soft tissue attached to the head? Future research should also expand on restorative crowns (such as including gold and zirconia crowns) and implants in different types of acid to see if any significant destruction can be seen to these commonplace restorative options. The

research attempted to answer some basic questions, but as one can see, future research is needed to complete the entire picture of acid treatment on teeth and facial features.

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