

EFFECTS OF ALPHA-TOCOPHEROL ANTIOXIDANT ON THE
BONDING PROPERTIES OF RESIN ADHESIVE TO DENTIN
BLEACHED WITH SODIUM PERBORATE

A THESIS IN
Oral and Craniofacial Sciences

Presented to the Faculty of the University
of Missouri-Kansas City in partial fulfillment of
the requirements for the degree

MASTER OF SCIENCE

by
M. STEPHEN HARRISON, JR.

B.S., University of Arkansas, 2001
D.D.S., University of Tennessee, 2005

Kansas City, Missouri
2017

EFFECTS OF ALPHA-TOCOPHEROL ANTIOXIDANT ON THE
BONDING PROPERTIES OF RESIN ADHESIVE TO DENTIN
BLEACHED WITH SODIUM PERBORATE

M. Stephen Harrison, Jr., Candidate for the Master of Science Degree

University of Missouri-Kansas City, 2017

ABSTRACT

Previous reports have shown diminished capacity for bonding after bleaching teeth. Reversal of the bond strengths back to pre-bleached levels can be obtained with application of 10% alpha-tocopherol in a 2-hour time frame, or by delaying bonding for two weeks. This study evaluated the effectiveness of a 5-minute application of 20% alpha-tocopherol to reverse the deleterious effect of nonvital bleaching on dentin bonding. Thirty third molars were prepared to obtain a flat dentin surface and assigned to three groups: unbleached, bleached, and bleached followed by 5-minute treatment with 20% alpha-tocopherol. The dentin surfaces of the bleached groups were exposed to sodium perborate (2 g/mL) for seven days. The post-bleach treatment group was subsequently treated with 20% alpha-tocopherol for 5 minutes, then all groups were restored with restorative composite. After 24-hour storage at 37°C and 100% humidity, restored tooth specimens were sectioned into 1-mm² dentin-composite beams. Four to six beams from each tooth were subjected to microtensile bond strength testing. Following microtensile testing, 2 beams from representative specimens were further evaluated with Raman microspectroscopy for depth of penetration and degree of conversion of adhesive resin. Mean bond strength values (MPa) for each group: unbleached control=26.2, bleached control=20.3, post-bleach treatment group=18.5. A 1-factor ANOVA and Tukey post hoc test ($\alpha=0.05$) indicated that

bleaching had a detrimental effect on bond strength and that short-term alpha-tocopherol treatments did not improve post-bleach bond strength. While Raman microspectroscopy revealed depth of penetration and degree of conversion for the post-bleach alpha-tocopherol group were similar to the bleached control, both values were markedly lower than the unbleached control group. Collectively, the results suggest that the application of 20% alpha-tocopherol as a post-bleach treatment in a clinically relevant time frame was not effective in counteracting the deleterious effect of bleaching on bond strength, and composite resin bonding procedures should be delayed following tooth bleaching.

APPROVAL PAGE

The faculty listed below, appointed by the Dean of the School of Dentistry have examined a thesis titled "Effects of Alpha-Tocopherol Antioxidant on the Bonding Properties of Resin Adhesive to Dentin Bleached with Sodium Perborate," presented by M. Stephen Harrison, Jr., candidate for the Master of Science degree, and certify that in their opinion it is worthy of acceptance.

Supervisory Committee

Mary P. Walker, D.D.S., Ph.D., Committee Co-Chair
Department of Oral and Craniofacial Sciences

Yong Wang, Ph.D., Committee Co-Chair
Department of Oral and Craniofacial Sciences

Kenneth F. Frick, D.D.S., M.S.
Department of Endodontics

CONTENTS

ABSTRACT.....	ii
LIST OF ILLUSTRATIONS.....	vi
LIST OF TABLES.....	vii
ACKNOWLEDGEMENTS.....	viii
Chapter	
1. INTRODUCTION 1	
Nonvital Bleaching Methods	1
Bleaching Agents' Negative Impact on Resin Adhesive Bond Strength.....	2
Dentin-Adhesive Interface Studies after Bleaching	3
Adhesive Raman Microspectroscopy Evaluations	3
Qualitative SEM Dentin-Adhesive Interface Characterizations	4
Antioxidant Agents to Reverse Bleaching on Dentin Bonding	4
Alpha-tocopherol to Counteract Negative Impact of Bleaching Agents on Dentin-Adhesive Bond	5
Problem Statement.....	7
Hypotheses.....	7
2. MATERIALS AND METHODS.....	8
Tooth Specimens.....	8
Alpha-tocopherol Antioxidant Gel.....	8
Specimen Preparation	8
Bleaching Application	8
Antioxidant Application	9
Adhesive Resin and Restorative Composite Application.....	9
Mechanical Testing.....	9

Preparation for Microtensile Testing.....	9
Microtensile Bond Strength Measurement of Dentin-Adhesive	10
Raman Microspectroscopy Evaluation of Dentin-Adhesive Interface	11
SEM Evaluation.....	14
Experimental Design.....	14
Sample Size	15
Data Analysis.....	15
3. RESULTS.....	16
Microtensile Bond Strength.....	16
Raman Microspectroscopy	17
Scanning Electron Microscopy.....	18
4. DISCUSSION.....	20
Microtensile Bond Strength.....	20
Depth of Penetration of Adhesive Resin	21
Degree of Conversion of Adhesive Resin	22
SEM Imaging.....	23
Incidental Observations	23
Clinical Implications	24
Study Limitations	24
Future Research Directions	26
5. CONCLUSIONS.....	27
LITERATURE CITED 28	
VITA	31

ILLUSTRATIONS

Figure	Page
1. Example of Specimen Preparation	10
2. Example of Beam Attached to Microtensile Tester Fixture.....	11
3. Example of Raman Microspectroscopy Spectra	12
4. Example Raman Spectra Close Up	13
5. SEM Imaging.....	19

TABLES

Table	Page
1. Experimental Design.....	15
2. Microtensile Bond Strength Mean and Standard Deviation Values	16
3. Depth of Adhesive Resin Intertubular Penetration	17
4. Adhesive Resin Degree of Conversion (%) Means and Standard Deviations.....	18

ACKNOWLEDGMENTS

Thank you to the following who made significant contributions to this project:

Dr. Mary Walker, for her guidance, experience, and professionalism.

Dr. Yong Wang, for his unmatched knowledge and insight.

Dr. Ken Frick, for his mentorship in the specialty of endodontics, and his passion for academia.

Dr. Xiaomei Yao, for her tutorials in using lab equipment and executing proper lab protocols.

Dr. Donggao Zhao, for his remarkable SEM expertise and interpretation.

Ms. Rachel Reed, for her kindness and helpfulness when in the lab.

Ms. Erin Liljequist and Ms. Karina Kearney, for their administrative attention to keep me on track.

Roy J. Rinehart Foundation and School of Dentistry Research and Graduate Programs Office for funding project costs.

DEDICATION

My lovely wife, Meritt, has put my desire for education and professional success before her personal ambitions time and time again. Her unwavering commitment to our children and family is unmatched. I dedicate this work to her for her consistent unselfishness and love throughout this process, and our life together.

CHAPTER 1

INTRODUCTION

Nonvital Bleaching Methods

Nonvital, root canal treated teeth will naturally darken with time due to the lack of blood and nutrient supply. The most conservative and effective way to combat this in natural tooth structure is to bleach the tooth internally via an access cavity into the pulp chamber. The most common internal bleaching agents for root canal treated teeth are through application of hydrogen peroxide, carbamide peroxide, and/or sodium perborate. Hydrogen peroxide is the active ingredient in all methods. It is either applied directly, or elucidated through a chemical reaction from carbamide peroxide or sodium perborate.

The most stable, safe, and suitable bleaching agent is sodium perborate mixed with distilled water (Holmstrup et al. 1988; Plotino et al. 2008; Sharma et al. 2011). Sodium perborate is also believed to be less likely to cause cervical external resorption (at the crown-root interface) as compared to hydrogen peroxide. This phenomenon is not well understood. The proposed explanation for this resorption is penetration of bleaching agent through the dentinal tubules to the external surface at the cervical margin of the tooth. Its presence externally may lead to an inflammatory response by the body causing the destructive effect. This is why a restorative seal is recommended at least one millimeter coronal to the cementum-enamel junction (CEJ). The hydrogen peroxide is not believed to penetrate the enamel. Even if it did, it would not likely cause an inflammatory response since it would be supragingival (Friedman et al. 1988; Kinomoto et al. 2001; Sharma et al. 2011).

Nonvital bleaching involves placement of the bleaching agent into the access cavity to the level of the CEJ. This where the crown of the tooth meets the root. The tooth is prepared internally by removing any decayed or decalcified tooth structure or debris

remaining from preparation. The orifice to the filled root canal is sealed by composite resin, glass ionomer, IRM, or other base (Hansen-Bayless and Davis 1992). Some advocate removal of the smear layer (inorganic and organic particle debris present on a microscopic level of a prepared tooth surface) with an acidic agent. This method is controversial, and may not be preferable to leaving the smear layer undisturbed. The additional chair time and possibility for increased cervical resorption are potential disadvantages (Casey et al. 1989). Sodium perborate is mixed with distilled water in a 2:1 (g/mL) ratio and sealed with a resin composite to avoid leakage into the oral cavity. The bleaching agent is changed every 3-7 days until an acceptable level of bleaching is achieved (Attin et al. 2003). The agent is then removed and replaced with composite resin (Plotino et al. 2008). This method is regularly referred to as “walking bleach” technique, as you send the patients on their way for a few days while the bleaching agent is in place.

Bleaching Agents’ Negative Impact on Resin Adhesive Bond Strength

The deleterious effect of bleaching agents on the bond strength of resin adhesive to enamel and dentin is well documented. Enamel bond strengths to resin adhesive were severely reduced after 24 hours of bleaching via carbamide peroxide gel immersion (Garcia-Godoy et al. 1993). Other studies have confirmed this finding of reduced bond strengths between tooth structure and composite resins (Crim 1992; Titley et al. 1993; Chng et al. 2002; Timpawat et al. 2005).

Oxygen created during the bleaching process remains in tooth structure for up to two weeks, and is believed to interfere with curing or setting of the bonding agents.

Polymerization of bonding agents is known to be inhibited by oxygen, leading to a diminished interface between resin and tooth structure (Dishman et al. 1994). While sodium perborate significantly decreased bond strength of resin to tooth structure, it was shown to

have less deleterious effect than hydrogen peroxide or hydrogen peroxide/sodium perborate mixture (Timpawat et al. 2005).

Microtensile testing of the bond strength between resin adhesive and dentin has been used to quantitatively examine the changes in this interface. Invariably, microtensile bond strength has been diminished after bleaching treatment (Lai et al. 2002; Timpawat et al. 2005; Hansen et al. 2014).

Dentin-Adhesive Interface Studies after Bleaching

The reduction in microtensile bond strength between dentin and resin post-bleach treatment is a well-documented outcome. While existing research provides qualitative evaluation of the bonding interface at enamel, a paucity of literature is available regarding dentin.

Adhesive Raman Microspectroscopy Evaluations

Studies using Raman microspectroscopy have shown a difference in the organic/inorganic component after treatment with hydrogen peroxide (Ubaladini et al. 2013; Lima et al. 2015). Degree of conversion (DC) is defined as the relative amount of C=C double bonds in monomer converted into C-C single bonds in polymer after the curing process. A high ratio of polymer to monomer would indicate a high degree of conversion, thereby successful curing of adhesive. A low ratio of polymer to monomer would indicate a low degree of conversion, indicating inhibition of the reaction. DC has been measured via Raman microspectroscopy after enamel bleaching and found it to be lower immediately and improved after 7-14 days, or after application of fluoride (Bittencourt et al. 2013). Reduced DC measured by Raman microspectroscopy and defects in the enamel-resin interface after SEM evaluation were discovered in immediately bleached enamel. DC and enamel-resin interface returned to normal after two weeks (Wilson et al. 2009).

Qualitative SEM Dentin-Adhesive Interface Characterizations

The scanning electron microscope (SEM) has been used to visualize the bonding interface between resin and tooth structure. A decrease in the number of resin tags has been found in the enamel-composite interface after bleaching treatment with 25% hydrogen peroxide (Dishman et al. 1994). Analysis of composite resin bonded to tooth structure immediately after bleaching with carbamide peroxide showed a granular, porous pattern. This was compared to a control group showing a uniform, pore-free appearance (Turkun and Kaya 2004).

Antioxidant Agents to Reverse Bleaching on Dentin Bonding

Dentin bonding after bleaching has been improved *in vitro* by using an antioxidant, most notably sodium ascorbate. The protocols for successfully reversing the effects of bleaching on enamel and dentin have been described, via a three hour method *in vitro* (Lai et al. 2002; Kimyai and Valizadeh 2006, 2008). This is not a clinically relevant period. Turkun and Kaya did show an improvement in enamel bonding to composite resin in bovine teeth after just a ten-minute application of sodium ascorbate, during which the antioxidant was continuously refreshed (Turkun and Kaya 2004). A case report showed clinical success a year later after applying sodium ascorbate for one hour to bleached enamel (Garcia et al. 2012a). Multiple studies have shown reversal in bond strength when waiting at least seven days after removal of bleaching material (Teixeira et al. 2004; Turkun and Kaya 2004; May et al. 2010). However, May and colleagues also reported sodium ascorbate application was necessary in conjunction with seven-day elapsed time to significantly improve bond strength (May et al. 2010).

Testing of a clinically relevant time frame for improvement of bleaching effects on dentin and enamel bonding has not been fruitful. Hansen and colleagues tested the ability of 35% sodium ascorbate to reverse the effects of 35% hydrogen peroxide bleaching on

nonvital teeth. Two one-minute applications and two five-minute applications had no significant reversal to pre-bleached bond strengths (Hansen et al. 2014). However, Muraguchi and colleagues found a reversal of deleterious effects using 10% ascorbic acid treatment for one minute in bovine teeth that were bleached using hydrogen peroxide/sodium perborate mixture. The investigators also explained that ascorbic acid might not be a clinically useful tool due to its short shelf life and possible impairment of the bleaching treatment (Muraguchi et al. 2007).

Alpha-tocopherol to Counteract Negative Impact of Bleaching Agents on Dentin-Adhesive Bond

The aforementioned success of Muraguchi and colleagues in reversal of lessened bond strengths via 10% ascorbic acid application suggests a more effective antioxidant is needed to achieve the desired improvement of bond strength after bleaching. Sasaki and colleagues tested the ability of 10% sodium ascorbate and 10% alpha-tocopherol to improve bond strength after bleaching with 10% carbamide peroxide. Only two-hour applications of antioxidant were measured. Alpha-tocopherol solution proved to be the only method that reversed the negative bleaching effect (Sasaki et al. 2009).

Another study tested the antioxidative capabilities of 15 compounds including ascorbic acid, sodium ascorbate, and alpha-tocopherol via DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) free radical method. This antioxidant assay is based on electron transfer that produces a violet solution in ethanol. An antioxidant will reduce the number of free radicals, and give rise to a colorless solution. These results are then analyzed by spectrophotometry and expressed as a percentage decrease in number of free radicals as compared to control values. The antioxidant activity of ascorbic acid and alpha-tocopherol was best at 95% and 92%, respectively. The next was sodium ascorbate gel at 76% and

sodium bicarbonate at 74%. All other substances tested fell at 51% and lower for antioxidant activity (Garcia et al. 2012b).

The ability to reverse diminished enamel and dentin bonding with a one minute application of 10% ascorbic acid treatment (Muraguchi et al. 2007) and diminished enamel bonding with two hour application of alpha-tocopherol (Sasaki et al. 2009), coupled with the antioxidant activity of ascorbic acid and alpha-tocopherol measured at near equal levels (Garcia et al. 2012b), suggests the possibility that the same reversal of bleaching effects on dentin-adhesive bonding may be obtained via alpha-tocopherol application in a clinically relevant time frame.

Problem Statement

No experimental research to date has investigated whether the application of alpha-tocopherol will lead to the reversal of the diminished dentin-adhesive microtensile bond strength after nonvital bleaching with sodium perborate in a clinically relevant time frame. Moreover, there have been no studies that included an associated evaluation of the quality of the dentin-adhesive interface that might mechanistically explain the reduced dentin-adhesive microtensile bond strength following nonvital bleaching. The ability to improve dentin bonding after nonvital bleaching is important for the longevity of the seal of the root canal system, and, therefore, the root canal treatment itself. The opportunity to place a dentin-bonded restoration at the same appointment after removing the bleach will improve efficiency for the practitioner and patient, saving both time and money.

The purpose of this study is to evaluate the effect of the antioxidant 20% alpha-tocopherol on dentin-adhesive microtensile bond strength after using sodium perborate in a nonvital bleaching procedure. In addition to microtensile bond strength, the quality of the dentin-adhesive interface will be evaluated via Raman microspectroscopy to assess the

degree of conversion of the resin adhesive. A complementary qualitative analysis to evaluate the dentin-adhesive interface will also be done via SEM.

Hypotheses

1. The microtensile bond strength will vary as a function of bleach treatment before or after alpha-tocopherol treatment.
2. The quality of the dentin-adhesive interface will vary as a function of bleach treatment before or after alpha-tocopherol treatment.

CHAPTER 2

MATERIALS AND METHODS

Tooth Specimens

Thirty previously extracted human third molar teeth with no subject identifiers were collected from private dental practices. Since the teeth were collected without any patient identifiers, they were exempt from IRB approval. The teeth were stored at 0.4°C in 0.9% phosphate buffered saline with 0.002% sodium azide to inhibit microbial growth.

Alpha-tocopherol Antioxidant Gel

The alpha-tocopherol formulation was compounded specifically for this project in a 20% solution to allow for maximum antioxidant effect.¹

Specimen Preparation

All procedures were done under normal ambient room light and humidity since clinical procedures will be done with rubber dam in place (Plasmans et al. 1994). The teeth were mounted into acrylic resin, leaving the coronal third of root structure and clinical crown exposed. The occlusal half of the crown was removed with a water-cooled saw² and diamond blade³ leaving a flat, dentinal surface devoid of enamel. The smear layer was then removed with a three-minute application of 17% EDTA to the dentinal surface. An air/water spray was administered for 15 seconds to remove any residual EDTA.

Bleaching Application

Two groups of specimens were placed inverted and suspended into a 5-mm layer of sodium perborate solution mixed with distilled water (2 g/mL), allowing for only the occlusal surface to be in contact with the bleaching agent. One group was not placed in bleaching

¹ Midwest Compounders Pharmacy, 13330 Santa Fe Trail Drive, Lenexa, KS 66215

² IsoMet 1000 Precision Cutter, Buehler, 41 Waukegan Road, Lake Bluff, IL 60044

³ Diamond Wafering Blade, No. 11-4246, Buehler, 42 Waukegan Road, Lake Bluff, IL 60044

solution. The specimens were stored at 37°C and 100% humidity for 7 days. After incubation, teeth were rinsed and dried with air/water syringe for 15 seconds.

Antioxidant Application

After 7 days of incubation, the teeth were sprayed with the air/water syringe for 15 seconds to remove any residual bleach and/or dry the specimens. Group 1 (unbleached control teeth) and group 2 (bleached control teeth) received no antioxidant application. Group 3 (bleached experimental teeth) received a 5-minute application of alpha-tocopherol that was re-applied with a microbrush every minute to the dentinal surface. In all groups, the dentinal surface was washed with a 15 second air/water syringe spray and subsequently dried.

Adhesive Resin and Restorative Composite Application

All three groups were treated the same moving forward. The specimens were dried with an air syringe. The entire prepared surface was etched with 35% phosphoric acid gel⁴ for 10 seconds. The surface was rinsed and dried with an air/water syringe. Adhesive resin⁵ was applied for 20 seconds using a microbrush. It was thinned with air and light cured⁶ for 20 seconds. The average light output was 1210 mW/cm² as measured by a radiometer⁷. Restorative composite⁸ was applied in increments of less than 2 mm, light curing for 40 seconds between layers. A total of at least 4 mm of composite was applied.

Mechanical Testing

Preparation for Microtensile Testing

The restored teeth were stored for 24 hours in a closed container with 100% humidity at 37°C and then serially sectioned into 1-mm thick vertical dentin-composite slabs with the

⁴ Ultra-Etch, Ultradent Products, Inc., 505 W 10200 South, South Jordan, UT 84095

⁵ Prime & Bond NT Dental Adhesive, Dentsply Caulk, 38 W Clarke Ave, Milford, DE 19963

⁶ Curing Light XL3000, 3M, 2501 Hudson Rd, Maplewood, MN 55144

⁷ Radiometer, 3H, 5 Zhushan Rd, Nanjing, Jiangsu, China

⁸ TPH, Dentsply Caulk, 38 W Clarke Ave, Milford, DE 19963

same water-cooled diamond blade as used previously. The specimens were rotated 90° and sectioned again with the diamond blade to obtain rectangular dentin-composite beams with a cross-sectional area of approximately 1 mm².



Figure 1. Example of Specimen Preparation. Beams before removing from tooth root.

The surface area was calculated for each specimen by measuring the narrowest portion with a digital caliper. The beams were inspected under light microscopy to evaluate the interface prior to testing. Four to six dentin-composite beams were obtained from each tooth for testing.

Microtensile Bond Strength Measurement of Dentin-Adhesive

The beams were attached to a microtensile tester fixture⁹ through application of cyanoacrylate to the composite and dentin ends of the beams, ensuring the dentin-adhesive interface is centered in the fixture.

⁹ Microtensile Tester, BISCO, Inc., 1100 W Irving Park Rd, Schaumburg, IL 60193



Figure 2. Example of Beam Attached to Microtensile Tester Fixture.

Adhered beam specimens were subjected to tensile forces at a crosshead speed of 1 mm/min. The force (N) at failure of the bond was recorded for each beam. Using that force value, the bond strength (MPa) was calculated according to the following formula:

$$\text{Bond Strength (MPa)} = \frac{\text{Force at bond failure (N)}}{\text{Beam cross sectional area (mm}^2\text{)}}$$

The bond strength values for all beams from a single tooth were averaged, providing a single bond strength measurement for that tooth.

Raman Microspectroscopy Evaluation of Dentin-Adhesive Interface

Two beams from each tooth were evaluated with Raman microspectroscopy. A Raman microspectrometer¹⁰ was utilized using a He-Ne laser at wavelength of 632.8 nm and excitation power of 20 mW. The laser was focused through a 50X Olympus objective. Data was collected in the Raman spectra range between 800 and 1800 cm⁻¹ with data acquired using a 30-second exposure time. Line maps starting at the composite were created to evaluate the adhesive and its penetration into intertubular dentin using two-micron interval measurements. Three line maps were generated per beam specimen.

¹⁰ LabRam HR 800, Horiba Scientific, 3880 Park Avenue Edison, NJ 08820

The collected line maps were analyzed with the aid of a computer program¹¹. Peaks corresponding to adhesive, mineralized and demineralized dentin were used to evaluate the depth of the intertubular penetration of the adhesive. Example spectra of line maps are below.

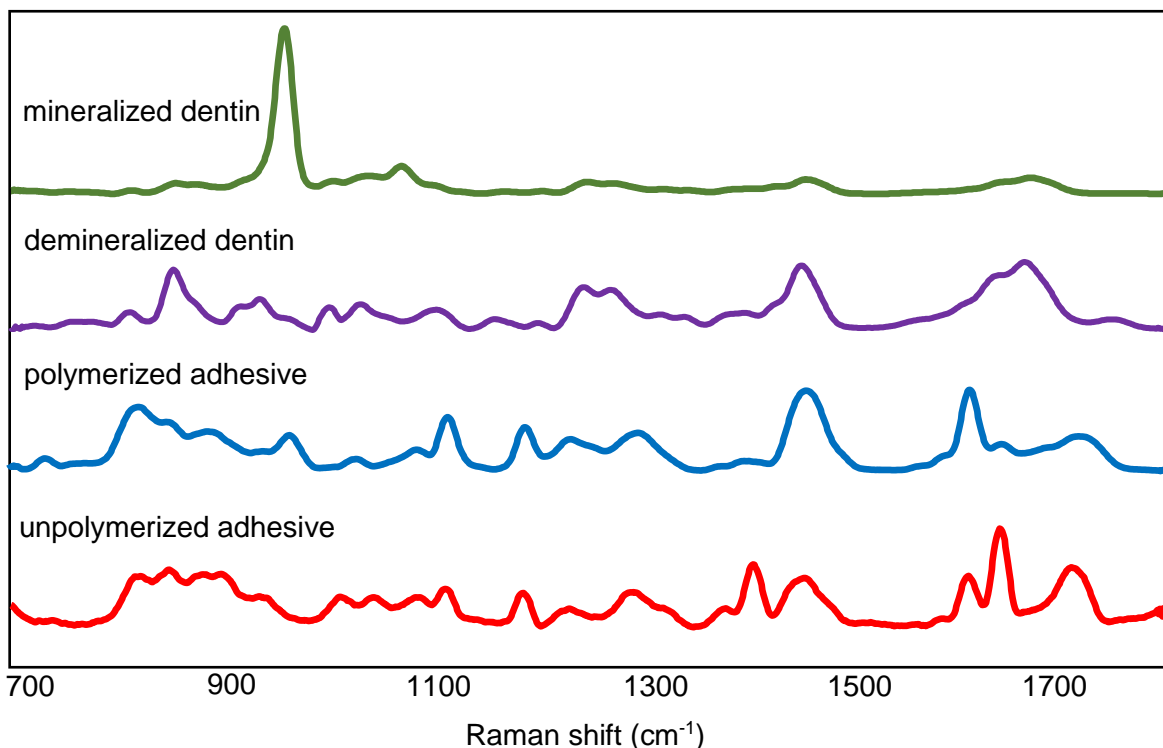


Figure 3. Example of Raman Microspectroscopy Spectra. Mineralized dentin, demineralized dentin, polymerized adhesive, and unpolymerized adhesive.

These line maps were used to evaluate degree of conversion of the adhesive within the dentin-adhesive interface. The following example spectra show a peak at 1637 cm⁻¹ for C=C double bond, and at peak at 1609 cm⁻¹ for phenyl C-C of both polymerized and unpolymerized adhesives. The spectra gathered were used to gain representative values for the degree of conversion at a standardized depth.

¹¹ NSG LabSpec 5, Horiba Scientific, 3880 Park Avenue Edison New Jersey NJ 08820

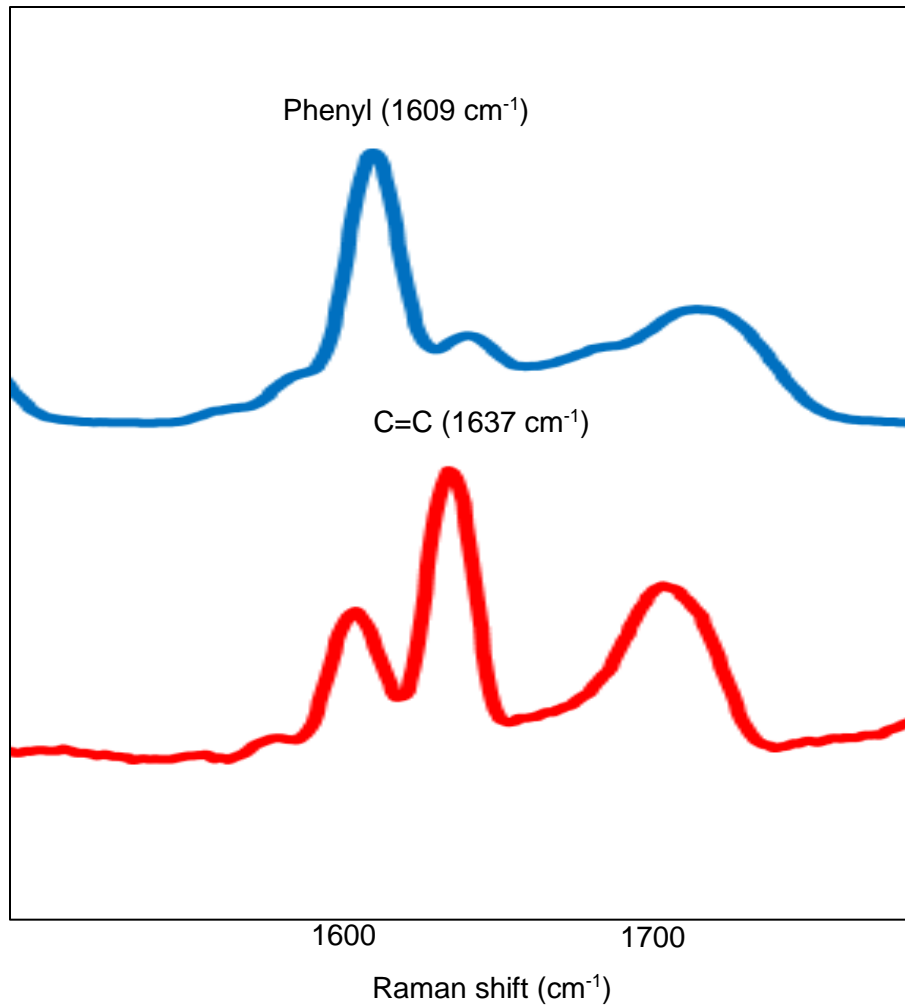


Figure 4. Example of Raman Spectra Close Up. Close up of spectra specific to polymerized (blue) and unpolymerized (red) adhesive

The degree of conversion calculations were made according to peak area ratios of 1637 cm^{-1} ($1625\text{-}1650\text{ cm}^{-1}$) and 1609 cm^{-1} ($1590\text{-}1625\text{ cm}^{-1}$). The following equation was used (Wu et al. 2010):

$$DC = \left(1 - \frac{\text{Intensity}_{1637\text{ cm}^{-1}}^{\text{Polymerized}} / \text{Intensity}_{1609\text{ cm}^{-1}}^{\text{Polymerized}}}{\text{Intensity}_{1637\text{ cm}^{-1}}^{\text{Unpolymerized}} / \text{Intensity}_{1609\text{ cm}^{-1}}^{\text{Unpolymerized}}} \right) \times 100$$

The same specimens used for dentin-adhesive interface quality were used to evaluate DC.

SEM Evaluation

After completion of Raman microspectroscopy evaluation, two representative specimens from each group were viewed with SEM¹². The mounted sections were each sputter coated with approximately 20 nm thick gold-palladium. The specimens were examined at 80X, 1000X, 2500X, 5000X, 10000X and 20000X magnification at 15 kV for both secondary electron emission analysis and backscattered electron emission analysis. These images provided a complimentary perspective on the dentin-adhesive hybrid layer previously analyzed using Raman microspectroscopy.

Experimental Design

This was a one-factor experimental study with one independent variable and four dependent variables. The independent variable was application of alpha-tocopherol antioxidant to bleached dentin. The dependent variables were the microtensile bond strength, the dentinal tubular penetration of the adhesive, degree of conversion of the resin adhesive, and visual inspection of the hybrid layer with SEM. Group 1 evaluated the dentin-adhesive bond to unbleached tooth structure with no antioxidant treatment and will serve as the negative control. Group 2 evaluated the dentin-adhesive bond to bleached tooth structure with no antioxidant treatment and will serve as the positive control. Group 3 evaluated the dentin-adhesive bond to bleached tooth structure after a 5-minute alpha-tocopherol treatment. Table 1 displays a schematic representation of the experimental design.

¹² XL30 ESEM-FEG, Philips, Amstelplein 2, 1096 BC Amsterdam, Netherlands.

TABLE 1
EXPERIMENTAL DESIGN

Group (n=10 teeth/group)	Alpha- tocopherol Application Time	Microtensile Bond Strength (MPa)	Degree of Conversion (%)	Depth of penetration of adhesive (μm)	SEM eval
1	0 min (unbleached control)				
2	0 min (bleached control)				
3	5 min				

Sample Size

The three groups each contained 10 specimens for a total experimental n of 30. Each tooth provided 6-10 dentin-composite beams for testing. As this is an exploratory study regarding the potential effects of alpha-tocopherol on post-bleach bonding, a convenience sample was used.

Data Analysis

Statistical analysis of the quantitative measures for microtensile strength was performed using a software statistical testing program¹³. It was via a one-factor (antioxidant application time) analysis of variance. If differences were detected, a Tukey post hoc test was used. All testing was done at $\alpha = 0.05$.

Data acquired from Raman microspectroscopy and SEM imaging were used to compliment other techniques and provide qualitative information regarding the dentin-adhesive interface. Due to the use of a representative sample, no statistical analyses were provided for this component.

¹³ IBM SPSS Statistics for Windows, Version 22.0, IBM Corp, Armonk, NY 10504

CHAPTER 3

RESULTS

Microtensile Bond Strength

Microtensile bond strength data were collected from 10 specimens per group. Six dentin-composite beams were tested from each specimen in all but one tooth. This specimen was tooth specimen #9 of the bleached control. Only 4 usable beams were collected from this specimen. This did not affect our statistical evaluation or study conclusions. The microtensile data were collected and analyzed from a total of 178 dentin-resin beams.

Based on the 1-factor ANOVA and Tukey's post hoc test, the unbleached control group exhibited significantly higher ($p \leq 0.05$) mean bond strength than the bleached control or post-bleach alpha-tocopherol groups, which were not significantly different ($p > 0.05$) from each other. The data are presented in Table 2.

TABLE 2

MICROTENSILE BOND STRENGTH MEAN AND STANDARD DEVIATION VALUES

Group*	Group Mean (MPa)	Standard Deviation
Unbleached Control ^a	26.2	±11.2
Bleached Control ^b	20.2	±10.0
Post-bleach Alpha-tocopherol ^b	18.5	±9.9

*Superscript letters indicate significant different subsets with the unbleached control group demonstrating significantly higher (≤ 0.05) mean bond strength than the bleached or post-bleach alpha-tocopherol groups, which were not significantly ($p > 0.05$) different from each other.

Raman Microspectroscopy

Raman microspectroscopy output provided line maps of spectra starting in resin adhesive and progressing into dentin in two-micron increments. These line maps were analyzed for depth of penetration and degree of conversion of adhesive. Two beams from representative specimens were selected for evaluation, three line maps per beam. No statistical evaluation was performed due to the size of the convenience sample. It is worth noting the similarity of the post-bleach alpha-tocopherol group to the bleached control, as compared to the dissimilarity to the unbleached control. These values are displayed in Tables 3 and 4.

TABLE 3
DEPTH OF ADHESIVE RESIN INTERTUBULAR PENETRATION

Group*	Group Mean (μm)	Standard Deviation
Unbleached Control	6.86	± 3.2
Bleached Control	4.00	± 0.0
Post-bleach Alpha-tocopherol	4.33	± 1.9

* Due to testing of a representative sample only, no statistical evaluation was performed. However, based on numerical value comparisons, there is a trend for higher resin penetration in the unbleached control group as compared to the bleached and post-bleach alpha-tocopherol groups.

Data for degree of conversion was reported at the point on the line map of pure adhesive closest to the hybrid layer, and at 2- μm into the hybrid layer from that pure adhesive point. No other points into the hybrid layer were reported because some specimens failed provide a hybrid layer measurement beyond the 2- μm mark. Therefore, an average could not be identified and compared across all specimens. See Table 4.

TABLE 4

ADHESIVE RESIN DEGREE OF CONVERSION (%)
MEANS AND STANDARD DEVIATIONS

Group*	Mean DC within Adhesive only (SD)	Mean DC 2 μ m into Hybrid Layer (SD)
Unbleached Control	82% (\pm 2.5%)	79% (\pm 6.4%)
Bleached Control	64% (\pm 14.1%)	53% (\pm 12.4%)
Post-bleach Alpha-tocopherol	67% (\pm 9.1%)	61% (\pm 10.5%)

*Due to testing of a representative sample only, no statistical evaluation was performed. However, based on numerical value comparisons, there is a trend for higher DC percentages in the unbleached control group as compared to the bleached and post-bleach alpha-tocopherol groups.

Scanning Electron Microscopy

The photomicrographs obtained via SEM (Figure 5) using secondary electron and backscattered electron emission imaging revealed a notable difference between the unbleached control specimen, and the bleached control and post-bleach alpha-tocopherol experimental specimen. . For example, colloidal spheres approximately 200-500 nm in diameter were noted on the bleached control and post-bleach alpha-tocopherol specimens (Fig. 5 E, H, K), but were not present on the unbleached control specimen (Fig. D, G, J). In addition, in the BSE images, it was noted that the spheres in the bleached control and bleach/alpha-tocopherol were a darker color suggesting a softer material such as adhesive resin.

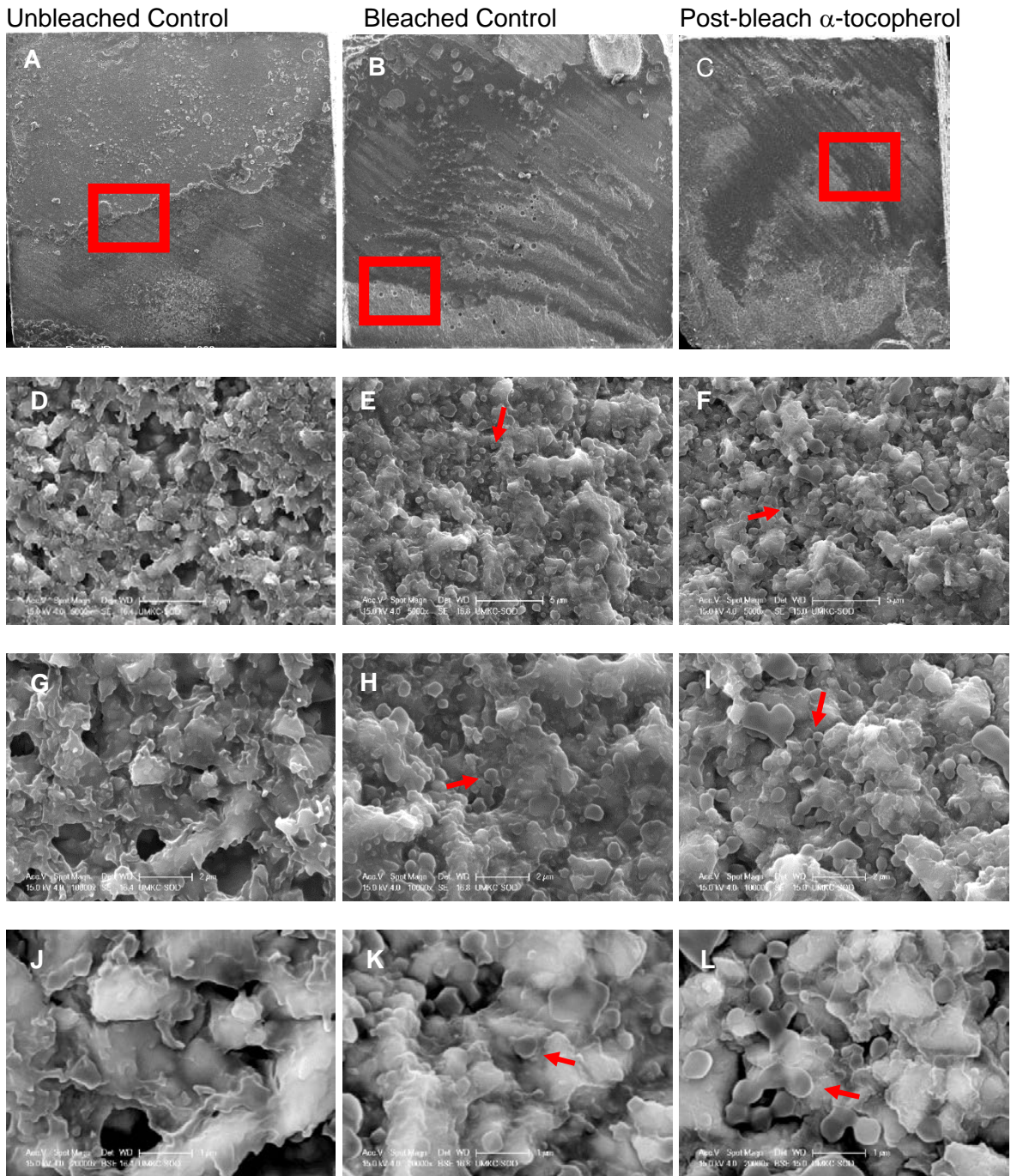


Figure 5. SEM Imaging. (A-I) secondary electron emission (SE) images; (J-L) Backscattered electron emission (BSE) images. Magnifications across image rows were: 80X, 5000X, 10000X, 20000X; Presence of colloidal spheres (arrows) was noted on both the bleached control (E, H, K) and the post-bleach alpha-tocopherol experimental group (F, I, L). However, no spheres were apparent on the unbleached control specimen (D, G, J). In the 20,000X BSE images of bleached and post-bleach alpha-tocopherol specimen (K, L, respectively), the spheres demonstrate a darker color suggesting they are composed of softer material such as adhesive resin.

CHAPTER 4

DISCUSSION

Streamlining of procedures, without diminishing the quality of the end-product, is useful in all industries. Removing an appointment from the process of nonvital bleaching would benefit both patients and care providers. The time savings could equate to less cost for the dentist, more savings for the patient, and higher levels of patient satisfaction.

This study sought to evaluate the effect of an antioxidant, alpha-tocopherol, to allow for composite bonding at the bleaching agent removal appointment. Due to the presence of residual oxygen, the current recommendation is to remove the bleach, seal the tooth temporarily for 7 or more days, and have the patient return for a definitive access repair (Dishman et al. 1994; Plotino et al. 2008). If the tooth is definitively restored with composite resin immediately after removal of bleach, the strength of the microtensile bond and the quality of the hybrid layer at the restorative margin are diminished (Crim 1992; Garcia-Godoy et al. 1993; Titley et al. 1993; Chng et al. 2002; Timpawat et al. 2005). The week-long window during temporization allows for dissipation of the oxygen from dentinal tubules, and the return of the subsequent microtensile bond strength and hybrid layer quality to pre-bleached levels (Teixeira et al. 2004).

The effect of an immediate 5-minute application of 20% alpha-tocopherol following the removal of sodium perborate (2 g/mL) after seven days of internal bleaching on microtensile bond strength and hybrid layer quality was evaluated in the current study.

Microtensile Bond Strength

Research to date has shown an improvement of bond strength back to pre-bleached levels 7 days after removal of bleaching agent (Teixeira et al. 2004). The same effect has been shown when bleached tooth structure was treated with sodium ascorbate (Vitamin C) and alpha-tocopherol (Vitamin E) for 2-3 hours (Turkun and Kaya 2004; Kimyai and

Valizadeh 2006; Sasaki et al. 2009). Despite these promising results, a major concern with these studies is that a 2-3 hour post-bleach application treatment is not realistic in the clinical environment.

In the current study, using post-bleach treatment with 20% alpha-tocopherol for a clinically relevant time, 5-min, the unbleached and bleached controls performed as expected, with the bleached control exhibiting significantly lower bond strengths. However, the alpha-tocopherol failed to provide an improvement of microtensile bond strengths following immediate application for 5 minutes to bleached dentin. The post-bleach alpha-tocopherol group exhibited bond strengths statistically similar to the bleached control (18.5-MPa and 20.2-MPa, respectively), and significantly less than the unbleached control (26.2-MPa). The results of the current study are similar to a previous study using 35% sodium ascorbate for a clinically-relevant time (10-min) in bleached human teeth with no improvement in bond strength (Hansen et al. 2014).

Depth of Penetration of Adhesive Resin

A literature search revealed no studies evaluating the depth of penetration of adhesive resin into bleached dentin via Raman microspectroscopy. Evaluating the hybrid layer for this measurement could provide some insight into what factors are leading to the diminished bond strengths following bleaching of tooth structure.

The results of our representative sample revealed consistently less penetration of adhesive resin in both the bleached control and the post-bleach alpha-tocopherol groups as compared to the unbleached control group. The depth of penetration of the unbleached group revealed a mean of 6.86- μm . This falls in line with previously reported hybrid layer thicknesses of 6-10 μm for etch-rinse adhesive systems (Hashimoto et al. 2000; Perdigao et al. 2000; Kenshima et al. 2006). However, the bleached control and the alpha-tocopherol group exhibited a mean depth of penetration of 4- μm and 4.33- μm , respectively. Mean

depths for the bleached groups of less than two-thirds that of the unbleached control could suggest this is one mechanism leading to lower microtensile bond strengths in the bleached groups. Little research could be located describing or evaluating this phenomenon. It has been hypothesized that less penetration may be due to an interaction between resin and residual peroxide near the tooth surface (Titley et al. 1991).

Degree of Conversion of Adhesive Resin

Degree of conversion was also measured to further evaluate the quality of the dentin-resin hybrid layer. DC values provide information regarding the percentage of carbon to carbon double bonds that are converted to carbon to carbon single bonds during resin polymerization. A higher degree of conversion indicates better polymerization and a higher quality cure. The literature recognized a lower degree of conversion of resin when immediately bonding to bleached tooth structure (Cadenaro et al. 2006). This is believed to be due to the presence of residual oxygen in the tooth structure from the bleach treatment inhibiting the polymerization of monomer (Dishman et al. 1994). This measurement can further provide insight as to the potential factors leading to diminished bond strengths in bleached tooth structure, and if the use of post-bleach alpha-tocopherol would improve DC.

Two specific points were reported from the Raman microspectroscopy data. The first point reported is in pure adhesive closest to the hybrid layer before presence of tooth structure. The second data point reported was 2- μm into the hybrid layer from that pure adhesive point. Because some specimens failed to provide a hybrid layer measurement beyond the 2- μm mark, no other data points were reported.

The unbleached control displayed mean adhesive conversion rates comparable to, or higher than, published expectations for this measure in both pure adhesive and 2- μm into the hybrid layer: 82% and 79%, respectively. The bleached control and alpha-tocopherol groups showed a marked drop in DC as compared to the unbleached control at both the

pure adhesive and 2 μm into the hybrid layer. The respective values for bleached control were 64% and 53%; the respective values for the alpha-tocopherol group were 67% and 61%. Due to the small representative sample, statistical evaluation was not performed on these data. However, the marked increase in DC for the unbleached control compared to both bleached groups, and the apparent similarities between the two bleached groups, are worth noting.

The lower levels of DC could potentially be a contributing factor in the diminished microtensile bond strengths reported in this study.

SEM Imaging

The SEM imaging of the hybrid layer following breakage revealed colloidal, spherical particles throughout the bleached specimens. The spheres were noted in both the bleached control and post-bleach treated specimens, suggesting the alpha-tocopherol treatment did not change this aspect. The lack of the spheres in the unbleached specimen is suggestive that these particles are a product of the bleaching treatment.

The 20000X BSE SEM image presented reveals more than just the spheres' presence. The darker color of the object under BSE, as compared to the surrounding hybrid layer, is suggestive that it has lower hardness. There is likely minimal to no filler in these spheres, suggesting they are largely composed of adhesive resin. The lack of continuity among the colloids could mean they are due to less than optimal polymerization of the adhesive layer. This observation could be consistent with the lower DC noted under evaluation with Raman microspectroscopy, and with the presence of residual oxygen following bleaching having an inhibitory effect on the polymerization reaction.

Incidental Observations

The 20% alpha-tocopherol solution had to be compounded by a local pharmacy. Very little was reported on this material in the dental literature. Information that was

available was at a concentration of 10%. The 20% concentration was used in an attempt to garner the highest antioxidant effect possible, without creating a solution that precipitates out of solution quickly. According to the pharmacy that compounded this material for our study, it needed to be used inside of 30 days of the compounding date. We followed that recommendation. More commonly, it was used within 3-7 days. An observation was made as the older bottles of alpha-tocopherol reached 45-60 days since their compounding date. The solution not only precipitated, but went from a clear fluid to a completely white foam-like solid. This solution, apparently, has a relatively short shelf life. This would make it difficult for clinicians to adapt to. Its use would likely require dissolving the alpha-tocopherol into an alcohol solution very shortly before application. Since this procedure is not that common, it would likely have to be compounded prior to each procedure. This creates another step that limits efficiency, and works against the efforts to streamline clinical practice. Many practitioners may be reluctant to adapt a system with such requirements.

Clinical Implications

The findings from this study will not lead to any changes in the current recommendations for restoration following nonvital bleaching. At this point, no literature supports the use of an antioxidant in a clinical setting to improve the diminished bond strengths following bleaching tooth structure. The best way to manage this concern is by waiting the requisite 7+ days between removing bleaching material and definitively bonding to the associated tooth structure.

Study Limitations

As with any *ex vivo* laboratory study, there limitations in translating the results to the clinical situation. However, to address some of the limitations, in the current study, composite placement was done in an environmental hood to simulate the oral environment temperature and humidity as much as possible.

Because a representative sample was used to generate the Raman microspectroscopy and SEM data, this approach did not allow for statistical evaluation. Nevertheless, differences in DC, depth of penetration, and SEM images are notable between the unbleached control and the bleached groups, these data cannot be extrapolated as statistically significant.

The large standard deviations reported for the microtensile bond strength data are not ideal. However, variability in tooth structure can be expected, as it can vary between patients depending on their age, nutrition, etc. (Atsu et al. 2005; Ligh et al. 2011). Moreover, differences in extraction procedures and storage protocol before these teeth are received by the lab could likewise contribute to the wide ranges. Also, preparation of the tooth structure for bonding by cutting a flat surface with a diamond saw is an inexact science. Removing the coronal third can reveal varying quality of dentin. If the pulp chamber is large in a given tooth, the dentinal tubules will be larger in diameter and increased in number. If the enamel fissures are particularly deep, it's possible a small portion of dentin-enamel junction and/or enamel are found at the flat occlusal surface following preparation. While every attempt was made to limit these concerns, human tissue provides a wealth of opportunity for variance.

Mounting the dentin-composite beams to the microtensile tester fixture with cyanoacrylate adhesive could have led error. If undetected cyanoacrylate came into contact with hybrid layer, it could potentially affect the measurement.

Preparation of the dentin-composite beams could have led to the creation or propagation of cracks and imperfections in the hybrid layer related to specimen preparation. For example, each beam is generated via several cuts using a diamond disc. These procedures could have led to changes in the dentin-composite bond strengths that were not related to the bleaching or bonding protocol.

Future Research Directions

Other antioxidants could be evaluated for their ability to rid the bleached tooth structure of residual oxygen prior to resin bonding. It would be recommended to have solutions created and observed for several weeks, or months, to evaluate the shelf life of potential products. The clinical usefulness of a product that has a short shelf life, and would require in-office preparation, would likely not be adapted by most practitioners. While ascorbic acid has been reported to counteract the negative impact of bleach on bond strength using a 1-min post-bleach application on bovine teeth, the authors had concerns about the low pH (2) and the potential for unacceptable over etching of dentin and enamel (Muraguchi et al. 2007). Perhaps, further investigation into the management of this antioxidant could lead to its clinical usefulness.

CHAPTER 5
CONCLUSIONS

The following conclusions can be made within the limitations of this *ex vivo* study:

1. There was no significant difference in the microtensile bond strength as a function of bleach treatment before or after alpha-tocopherol treatment.
2. There was no qualitative difference in the dentin-adhesive interface as a function of bleach treatment before or after alpha-tocopherol treatment.

LITERATURE CITED

- Atsu SS, Aka PS, Kucukesmen HC, Kilicarslan MA, Atakan C. Age-related changes in tooth enamel as measured by electron microscopy: Implications for porcelain laminate veneers. *J Prosthet Dent* 2005;94:336-41.
- Attin T, Paque F, Ajam F, Lennon AM. Review of the current status of tooth whitening with the walking bleach technique. *Int Endod J* 2003;36:313-29.
- Bittencourt BF, Dominguez JA, Loguercio AD, Gomes JC, Gomes OM. Influence of two different methods of delivering fluoride on bond strength and degree of conversion of an adhesive after bleaching. *J Adhes Dent* 2013;15:553-9.
- Cadenaro M, Breschi L, Antonioli F, Mazzoni A, Di Lenarda R. Influence of whitening on the degree of conversion of dental adhesives on dentin. *Eur J Oral Sci* 2006;114:257-62.
- Casey LJ, Schindler WG, Murata SM, Burgess JO. The use of dentinal etching with endodontic bleaching procedures. *J Endod* 1989;15:535-8.
- Chng HK, Palamara JE, Messer HH. Effect of hydrogen peroxide and sodium perborate on biomechanical properties of human dentin. *J Endod* 2002;28:62-7.
- Crim GA. Post-operative bleaching: Effect on microleakage. *Am J Dent* 1992;5:109-12.
- Dishman MV, Covey DA, Baughan LW. The effects of peroxide bleaching on composite to enamel bond strength. *Dent Mater* 1994;10:33-6.
- Friedman S, Rotstein I, Libfeld H, Stabholz A, Heling I. Incidence of external root resorption and esthetic results in 58 bleached pulpless teeth. *Endod Dent Traumatol* 1988;4:23-6.
- Garcia-Godoy F, Dodge WW, Donohue M, O'Quinn JA. Composite resin bond strength after enamel bleaching. *Oper Dent* 1993;18:144-7.
- Garcia EJ, Mena-Serrano A, de Andrade AM, Reis A, Grande RH, Loguercio AD. Immediate bonding to bleached enamel treated with 10% sodium ascorbate gel: A case report with one-year follow-up. *Eur J Esthet Dent* 2012a;7:154-62.
- Garcia EJ, Oldoni TL, Alencar SM, Reis A, Loguercio AD, Grande RH. Antioxidant activity by dpph assay of potential solutions to be applied on bleached teeth. *Braz Dent J* 2012b;23:22-7.
- Hansen-Bayless J, Davis R. Sealing ability of two intermediate restorative materials in bleached teeth. *Am J Dent* 1992;5:151-4.
- Hansen JR, Frick KJ, Walker MP. Effect of 35% sodium ascorbate treatment on microtensile bond strength after nonvital bleaching. *J Endod* 2014;40:1668-70.

- Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength: Demineralized dentin zone of the hybrid layer. *Dent Mater* 2000;16:406-11.
- Holmstrup G, Palm AM, Lambjerg-Hansen H. Bleaching of discoloured root-filled teeth. *Endod Dent Traumatol* 1988;4:197-201.
- Kenshima S, Francci C, Reis A, Loguercio AD, Filho LE. Conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer. *J Dent* 2006;34:775-83.
- Kimyai S, Valizadeh H. The effect of hydrogel and solution of sodium ascorbate on bond strength in bleached enamel. *Oper Dent* 2006;31:496-9.
- Kimyai S, Valizadeh H. Comparison of the effect of hydrogel and a solution of sodium ascorbate on dentin-composite bond strength after bleaching. *J Contemp Dent Pract* 2008;9:105-12.
- Kinomoto Y, Carnes DL, Jr., Ebisu S. Cytotoxicity of intracanal bleaching agents on periodontal ligament cells in vitro. *J Endod* 2001;27:574-7.
- Lai SC, Tay FR, Cheung GS, Mak YF, Carvalho RM, Wei SH, et al. Reversal of compromised bonding in bleached enamel. *J Dent Res* 2002;81:477-81.
- Ligh RQ, Fridgen J, Saxton C. The effect of nutrition and diet on dental structure integrity. *J Calif Dent Assoc* 2011;39:243-9.
- Lima DA, Aguiar FH, Pini NI, Soares LE, Martin AA, Liporoni PC, et al. In vitro effects of hydrogen peroxide combined with different activators for the in-office bleaching technique on enamel. *Acta Odontol Scand* 2015;73:516-21.
- May LG, Salvia AC, Souza RO, Michida SM, Valera MC, Takahashi FE, et al. Effect of sodium ascorbate and the time lapse before cementation after internal bleaching on bond strength between dentin and ceramic. *J Prosthodont* 2010;19:374-80.
- Muraguchi K, Shigenobu S, Suzuki S, Tanaka T. Improvement of bonding to bleached bovine tooth surfaces by ascorbic acid treatment. *Dent Mater J* 2007;26:875-81.
- Perdigao J, May KN, Jr., Wilder AD, Jr., Lopes M. The effect of depth of dentin demineralization on bond strengths and morphology of the hybrid layer. *Oper Dent* 2000;25:186-94.
- Plasmans PJ, Creugers NH, Hermsen RJ, Vrijhoef MM. Intraoral humidity during operative procedures. *J Dent* 1994;22:89-91.
- Plotino G, Buono L, Grande NM, Pameijer CH, Somma F. Nonvital tooth bleaching: A review of the literature and clinical procedures. *J Endod* 2008;34:394-407.

- Sasaki RT, Florio FM, Basting RT. Effect of 10% sodium ascorbate and 10% alpha-tocopherol in different formulations on the shear bond strength of enamel and dentin submitted to a home-use bleaching treatment. *Oper Dent* 2009;34:746-52.
- Sharma DS, Sharma S, Natu SM, Chandra S. An in vitro evaluation of radicular penetration of hydrogen peroxide from bleaching agents during intra-coronal tooth bleaching with an insight of biologic response. *J Clin Pediatr Dent* 2011;35:289-94.
- Teixeira FB, Teixeira EC, Thompson J, Leinfelder KF, Trope M. Dentinal bonding reaches the root canal system. *J Esthet Restor Dent* 2004;16:348-54; discussion 54.
- Timpawat S, Nipattamanon C, Kijssamanmith K, Messer HH. Effect of bleaching agents on bonding to pulp chamber dentine. *Int Endod J* 2005;38:211-7.
- Titley KC, Torneck CD, Smith DC, Chernecky R, Adibfar A. Scanning electron microscopy observations on the penetration and structure of resin tags in bleached and unbleached bovine enamel. *J Endod* 1991;17:72-5.
- Titley KC, Torneck CD, Ruse ND, Krmec D. Adhesion of a resin composite to bleached and unbleached human enamel. *J Endod* 1993;19:112-5.
- Turkun M, Kaya AD. Effect of 10% sodium ascorbate on the shear bond strength of composite resin to bleached bovine enamel. *J Oral Rehabil* 2004;31:1184-91.
- Ubal dini AL, Baesso ML, Medina Neto A, Sato F, Bento AC, Pascotto RC. Hydrogen peroxide diffusion dynamics in dental tissues. *J Dent Res* 2013;92:661-5.
- Wilson D, Xu C, Hong L, Wang Y. Effects of different preparation procedures during tooth whitening on enamel bonding. *J Mater Sci Mater Med* 2009;20:1001-7.
- Wu WC, Shrestha D, Wei X, Ling JQ, Zhang WH, Chen J. Degree of conversion of a methacrylate-based endodontic sealer: A micro-Raman spectroscopic study. *J Endod* 2010;36:329-33.

VITA

NAME:

Michael Stephen Harrison, Jr.

DATE AND PLACE OF BIRTH:

July 8, 1979; Hot Springs, Arkansas

MARITAL STATUS:

Married to Meritt Hargraves Harrison

EDUCATION:

5/1997	Diploma	Lakeside High School Hot Springs, Arkansas
5/2001	BS Microbiology	University of Arkansas Fayetteville, Arkansas
5/2005	DDS	University of Tennessee College of Dentistry Memphis, Tennessee
6/2006	AEGD Certificate	Lutheran Medical Center Brooklyn, NY; satellite in Memphis, TN
6/2017	Endodontic Certificate MS OCS	University of Missouri – Kansas City School of Dentistry Kansas City, Missouri

PUBLICATIONS:

Moore M, Harrison MS, Peterson EC, Henry R. *Chloroplast Oxa1p homolog albino3 is required for post-translational integration of the light harvesting chlorophyll-binding protein into thylakoid membranes.* J Biol Chem. 2000 Jan 21;275(3):1529-32.

Harrison MS, Gaber L, Haney L, Dabbous MK, *Matrix Metalloproteinase Expression in Kaposi's Sarcoma.* Abstract. American Association for Dental Research National Meeting, San Antonio, TX. March 12-15, 2003.

PROFESSIONAL ORGANIZATIONS:

American Association of Endodontists
America Dental Association
Arkansas State Dental Association
Garland County Dental Society

HONORS:

2005: Graduated from University of Tennessee School of Dentistry with Honors.

2003: Dean's List, University of Tennessee, Memphis, TN.

2002: Dr. R.O. and Fannie B. Ford Scholarship Award, University of Tennessee, Memphis, TN.

NIH Fellowship, University of Tennessee, Memphis, TN.

2001: First Ranked Senior Scholar, University of Arkansas, Fayetteville, AR.

Summa Cum Laude, University of Arkansas, Fayetteville, AR.

Outstanding Departmental Honors Scholar, University of Arkansas, Fayetteville, AR.

Microbiology Honors Scholar, University of Arkansas, Fayetteville, AR.

1997 to 2001: NSF Fellowship, U of Arkansas, Fayetteville, AR.

NIH Fellowship, U of Arkansas, Fayetteville, AR.

Howard Hughes Research Institute Internship, U of Arkansas, Fayetteville,

AR.

Barry Goldwater Honorable Mention, U of Arkansas, Fayetteville, AR.

University Scholarship, U of Arkansas, Fayetteville, AR.

Gilbert Premed Scholarship, U of Arkansas, Fayetteville, AR.

Microbiology Research Scholarship, U of Arkansas, Fayetteville, AR.

Chancellor's and Dean's Lists all four years, U of Arkansas, Fayetteville, AR.

National Dean's List all four years, Washington, DC.

Arkansas Distinguished Governor's Scholar, Little Rock, AR.

Academy of Model Aeronautics Scholarship, Muncie, IN.

#1 of 160 in Lakeside High School Class of 1997, Hot Springs, AR.

Knights of Columbus Scholarship, Hot Springs, AR.