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Effect of 35% sodium ascorbate on microtensile bond strength of composite resin immediately after bleaching

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THE EFFECT OF 35% SODIUM ASCORBATE ON MICROTENSILE BOND STRENGTH
OF COMPOSITE RESIN IMMEDIATELY AFTER BLEACHING

Thesis Presented

By

EMAN HANI ISMAIL, D.D.S.

Submitted to the College of Dental Medicine of Nova Southeastern University

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

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

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Dedication

I would like to dedicate this thesis to my parents, Hani Ismail; my dad, who taught me the value of hard work, and to be equally passionate and compassionate, and Maha Fatani; my mom, who taught me to always seek knowledge from its source, and to never stop questioning. Your love and generosity are endless.

I would also like to dedicate this work to my two wonderful boys, Khalid & Raed. I hope my hard work will be an inspiration for you both, to never stop learning, never stop dreaming, and to set no limit for your passion and ambition.

Last but not least, I dedicate this thesis to my knight in shining armor; Sami Khan. You have always been, and will always be, the most precious thing I have in life.

Acknowledgments

Abstract

THE EFFECT OF 35% SODIUM ASCORBATE ON MICROTENSILE BOND STRENGTH

OF COMPOSITE RESIN IMMEDIATELY AFTER BLEACHING

DEGREE DATE: JUNE 2015

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Background: Sodium ascorbate has been recently tested to reverse the temporary weakening of composite bonding when its application is done immediately after bleaching. This study compared the microtensile bond strength of immediate and delayed bonding of composite after bleaching, with and without the application of 35% sodium ascorbate. **Objective:** to assess the effect of 35% sodium ascorbate (SA) (Sigma-Aldrich Co.) on microtensile bond strength of dentin immediately after bleaching with 35% hydrogen peroxide (Pola Office/SDI). **Methods:** Twenty-five sound human 3rd molars were

collected in order to obtain one hundred twenty five specimens. Teeth were randomly divided into five groups: Grp1 (bleaching + immediate restoration), Grp2 (bleaching + delayed restoration), Grp3 (bleaching + SA + immediate restoration), Grp4 (bleaching + SA + delayed restoration), Grp5 (Control-no treatment). After bleaching, but prior to restoration, Grps 2 & 4 were stored for 1 week in deionized water at 37°C. All samples were restored using Optibond Solo Plus (Kerr) and Filtek Supreme Ultra (3M/ESPE) following manufacturers' instructions. Teeth were sectioned into 1x1 mm bars, obtaining around 10 bars from each tooth. Microtensile bond strength was measured with a universal testing machine (Instron model 8841) at a crosshead speed of 0.5 mm/min. **Results:** Descriptive statistics revealed that Grp1 (positive control) showed significant reduction in microtensile bond strength (34.17 ± 10.78). Grp5 (negative control) (45.83 ± 19.32), Grp2 (standard of care) (45.36± 16.57), Grp3 (45.06 ± 20.91) and Grp4 (44.73 ± 16.85) showed statistically similar microtensile bond strength. One-way ANOVA was used to determine if differences were evident between the five groups. Microtensile bond strength differed significantly across the five groups ($F_{(4, 1073.81)} = 3.54$, $p = .007$) Tukey's post-hoc comparisons of the five groups showed significant differences between Grp 1 and all other groups 2,3,4 and 5. **Conclusion:** Microtensile bond strength was improved significantly after the application of 35% sodium ascorbate when restored with resin composite immediately after bleaching. **Grants:** This study was funded by NSU, Health Profession Division.

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Chapter 1: Introduction

1.1. Overview

Patients are becoming more aware of their esthetics in general. Media plays a big role in marketing for esthetic dentistry, which has led to the dramatic growth of a dentally educated population, presenting increasing challenges to dentists to satisfy their demands.

For esthetic treatment planning, bleaching is usually recommended to patients as an initial step, especially when other anterior restorations such as direct/indirect composite or porcelain veneers are needed. Since indirect restorations do not respond to bleaching agents, mismatching of colors between the restoration and the bleached teeth will often be observed if bleaching was done after the restoration. For this reason, bleaching is always recommended before the restoration. However, the temporary weakening effect of the bleaching agent on the enamel and dentin bonding may render an immediate restoration suboptimal. Considerable time should be allowed for this effect to be reversed before initiating the restoration. Recently, researchers have attempted to test the effect of antioxidants in reversing the weakening effect of bleaching agents on composite bonding. The effect of bleaching on composite bonding and the use of antioxidants will be discussed in more depth in the following sections.

1.2. Bleaching

1.2.1. History

Since the late 1800s, esthetic dentistry has become more widespread. More recently, the effects of teeth bleaching have become a concern, as it has been widely accepted by patients and promoted by dentists as a very convenient, affordable, and as the most conservative esthetic treatment among other esthetic treatment modalities.[1] As early as 1848, reports showed evidence of chloride lime used as nonvital tooth bleaching.[1-3] Later, in 1864, the most effective technique for nonvital bleaching was introduced by Truman, which used chlorine from a solution of calcium hydrochlorite along with acetic acid.[1] By the late 1800s, other bleaching agents mentioned in the literature included aluminum chloride, oxalic acid, pyrozone, hydrogen dioxide, sodium peroxide, sulphorus acid, sodium hypophosphate, chloride of lime and cyanide of potassium. [1-3] In 1910, the use of 30% hydrogen peroxide was first reported,[1] and later in 1960, Klusmier reported in a study club a successful attempt to bleach teeth using 10% carbamide peroxide utilizing a customized tray.[1,4]

From the early 1800s until the early 1900s, approximately 40-60 articles were published per year in the area of bleaching, debating about the efficiency of bleaching, its indications, durability and above all, safety of the bleaching agent, and its effect on tooth structure.[1]

1.2.2. Materials used for bleaching

Peroxide compounds are readily available in the environment.[5] Peroxides are usually in the form of Hydrogen Peroxide H_2O_2, as they are normally dissolved in aqueous solution.[5] Hydrogen peroxides are widely used in many industries; processing of food and wine, and medications; and are detected naturally in human bodies.[5] In dentistry, peroxide has been used widely in many dental procedures, such as debridement of root canals during endodontic treatment[6], mouthwash treatment for periodontal diseases, and in tooth whitening (bleaching).[7] The most commonly used agents for dental bleaching are: hydrogen peroxide HP (H_2O_2) and carbamide peroxide CP ($CH_6N_4O_3$).[5]

Since the 1930s, HP has been accepted as an effective dental bleaching agent in dentistry was,[5] however, CP for dental bleaching was not described until 1989 by Haywood and Heymann[4]. It has been found that 10% of CP dissociates chemically into the active ingredient 3.35% HP (H_2O_2) and 6.65% urea (CH_4N_2O).[3,8] Carbopol containing bleaching materials is recommended for in-home use, because it adds viscosity, and extends the oxidation process.[8] It has been reported that the effect of HP is 2.76 times faster than CP,[9] yet, both agents appear safe and effective in teeth bleaching, and are approved by the American Dental Association ADA, however, bleaching under professional supervision is highly recommended. [10,11]

1.2.3. Mechanism of bleaching

The mechanisms of action of bleaching agents are not fully understood.[2,3,12] Evidence indicates that the "oxidation reaction" of the peroxide agents is the main attribute to the bleaching mechanism.[3,12] HP (H_2O_2) is usually unstable, and would dissociate easily into the following elements: water molecule (H_2O), hydrogen ion (H), highly reactive perhdroxyl (HO_2) and oxygen (O) radicals.[3] This reaction can be affected by concentration and time.[13] Other factors which may alter the reaction are: temperature (especially heat), pH, light and presence of metals.[13,14] Usually, perhydroxyl radicals are formed in a physiologic pH environment, rather than oxygen radicals, which tend to be formed in more acidic environment.[3,12,14] The highly reactive radicals can infiltrate the organic matrix of enamel and dentin via direct access through micropores, along a diffusion gradient, thus, reacting with the organic colored molecules.[2,13] By 1991, Albers had described the initial whitening process.[9] Once perhydroxyl radicals reach the organic matrix of the tooth structure, they convert the carbon-ring molecules into chains by breaking the double bonded carbon, resulting in lighter colored pigments.[3] As this reaction continues, the tooth will appear lighter in color, until the saturation point is reached.[3] At this point, the bleaching treatment should be discontinued to prevent some of the possible side effects of bleaching such as teeth hypersensitivity. Although verity of bleaching products has been in the market as over-the counter products, bleaching treatment should be done under the supervision of a dentist.[3,10,11]

1.2.4. Types of bleaching

Bleaching agents can be applied extracoronally; on the outer surface of the tooth and described as vital bleaching or external bleaching, or can be applied intracoronally; within the pulp chamber, and described as non-vital bleaching; or internal bleaching.[1,8,9,14] Several techniques and approaches for vital and non-vital bleaching have been described in the literature.[8,13] Methods of bleaching vary by bleaching agent concentration, exposure time, mode of application, length of treatment and light activation. [2,3,13,15]

Current available bleaching methods can be classified into 3 categories according to the mode of use:

- In-office bleaching or power bleaching: for professional use, which contains a high concentration of HP (30-40%) or CP (35%) that can be used extracoronally and intracoronally.[5,8,13]

- At-home bleaching, night guard bleaching, or walk-in bleaching: used by patients but dispensed by the dentist. These may consist of up to 10% HP or 16% CP. [5,8,13]

- Over the counter bleaching products: Used by patients and commercially available directly to consumers, and ranges from 2-6% HP. [5,8,13]

The choice of bleaching material depends on the dentist, the dental treatment plan, and patient preference.[3] Some factors that might affect the patient's decision are: cost, ease of application, flavor, and package design.[3] Bleaching is indicated for generalized staining, staining from aging, smoking, diet, fluorosis, tetracycline use, and traumatic pulpal changes.[14]

1.2.5. Effect of bleaching on teeth composition

The chemical effect of peroxide bleaching agents raised some questions and promoted further studies related to peroxide safety and its effect on teeth composition and sensitivity.[2,5] Ledoux et al. investigated the effect of 30% HP on rat teeth. They found that enamel and dentin had undergone some structural changes after exposure to the bleaching agent.[16] Titley et al. examined the smear layer of human dentin and enamel under scanning electron microscopy (SEM) after exposure to 35% HP agent for 60seconds and acid etching with m37% Phosphoric acid for 60 seconds before and after bleaching, in an attempt to determine if HP treatment would improve the surface characterization for better bonding. [17] An intact smear layer with dense amorphous precipitate was observed, which did not seem to alter the morphology of the dentin surface as much as it affected enamel.[17] Ruse et al. studied bovine enamel after exposure to 35-37% HP and found a significant increase in the nitrogen level. In addition, a thick organic rich layer was found, that was resistant to acid etching. [18] Shannon et al, studied enamel microhardness after the application of three kinds of 10% CP bleaching agents,[19] A significant decrease in microhardness was found after 2 weeks of exposure.[19] Additionally, enamel specimens were evaluated under scanning electron microscopy (SEM), where a significant alteration in enamel topography was found for the samples that underwent 4 weeks of bleaching; the severity of surface alteration was attributed to the pH of the CP bleaching agent (the lower the pH, the more damage observed).[19]

Sulieman and his colleagues conducted a study investigating the safety of 35% HP on enamel and dentin structure.[20] In this study, enamel and dentin structure were

meticulously examined, in an attempt to detect the slightest change that might have occurred after the exposure to a HP bleaching agent. Surface roughness was analyzed using a profilometer, and a Wallace indenter was used to determine the microhardness before and after exposure to the bleaching agent. Scanning electron microscopy (SEM) was also used to evaluate both enamel and dentin surfaces.[20] The researchers observed no evidence of damaging effects of 35% HP on enamel or dentin.[20] Cadenaro and others, in accordance with Suleiman et al, found no morphological changes on enamel surfaces when exposed to a high concentration of 38% HP and 35% CP.[21] Cadenaro et al., in one of their published articles, included a table of an extensive literature review of around thirty-nine articles, comparing and summarizing the lack of consensus of morphological changes occurring after the application of bleaching agents.[21] Cadenaro et al. explained that results of these studies varied since different methods of analysis, different methods of preparation, and different type and concentration of bleaching agents were utilized; [21] thus, the controversy of whether bleaching agents alter the morphology of tooth structure is not completely determined.

1.2.6. Effect of bleaching on composite bonding

While many studies have offered conflicting results with regard to the actual effect of bleaching agents on enamel and dentin structure and composition,[16,18,19,21-23] other studies have been in agreement on the effect of the bleaching agents on enamel and dentin bonding to composite.[24-28] Reduction of bond strength of composite to tooth structure has been suggested to be a result of the entrapment of a residual by-product of the bleaching process; oxygen and perhydroxyl, in the collagen

matrix and dentinal tubules.[29] SEM evaluation of bonded samples immediately after bleaching showed small bubbles of oxygen at the bonding interface.[30]   It has been suggested that residual oxygen interferes with resin infiltration into dentinal tubules, and inhibits complete polymerization of resin tags.[24,31,32] This would affect the retention of the composite restoration, causing staining, microleakage, recurrent caries, or even debonding of the restoration, thus, condemning the restorative procedure to failure.[27,33,34] Since this effect is only temporary, it has been recommended, for more reliable bond strength, to delay the bonding procedure until all the residual peroxides have leached. [35]

Tornock et al. observed decreased bond strength values of bonded composite to bleached bovine enamel, which were exposed to 30% HP.[36] They concluded that the weakening effect of bond strength after bleaching is time dependent.[36] Hence, their observation of the SEM evaluation of the failed samples suggested that the reason behind the temporary weakened bond strength is the presence of residual peroxides inside tooth structure.[36] Titley et al. conducted an SEM evaluation of bonded enamel immediately after bleaching; they found resin tags were shorter and less defined than those from unbleached enamel samples.[30] Dishman and his group, studied the effect of 25% HP in-office bleaching on composite bonding to enamel surfaces; composite was bonded to enamel at different time intervals after bleaching. They found a significant reduction in shear bond strength when composite was bonded immediately after bleaching, however, the bond strength values returned to control values after a delay in bonding for one day, one week and one month.[37]   Their SEM observations were in agreement with Titley et al. where SEM evaluation showed

a reduction in numbers of resin tags.[30] Dishman et al. explained that bleaching agents have an inhibition effect on composite polymerization, leading to weak bond strength.[37] Titley et al. and Torneck concluded that, to regain normal bond strength after bleaching, time is needed, and this time depends on the concentration of the bleaching agent as well as the exposure time to the bleaching agent.[36,38] For this reason, many researchers have attempted to identify the time needed in order for the hydrogen peroxide to be released completely in order to allow the bond strength to be recovered. Some authors confirmed that optimum bond strength could be regained after bleaching if bonding was delayed up to 3 weeks,[39,40] while, other studies have shown that 24 hours delayed boding is adequate.[37]

Spyrides et al. studied three different bleaching agents on dentin bond strength to composite.[28] A significant reduction in shear bond strength was observed when bonding was performed immediately after bleaching among the three different bleaching groups. Moreover, a significant increase in bond strength was observed after one week of delayed bonding, specifically with the 35% HP bleaching group.[28] Currently, there is no consensus on how long bonding of composite should be delayed after bleaching, however, the recommended time by most researchers to delay the bonding procedure after bleaching is one week.[8,25,28,41]

To avoid this delay, studies have been conducted to address the issue of the temporary weakened bond strength of composite bonded immediately after bleaching. Some studies have suggested a benefit from the removal of the superficial layer of enamel,[42] while others, have recommended the pretreatment of enamel with

alcohol prior to bonding,[43] or, the use of composite bonding agents containing organic solvents.[44]

1.3. The use of antioxidants

Antioxidants are substances that react with free radicals, such as oxygen, which are generated by degradation of HP, causing them to be neutralized.[45] Sodium ascorbate was evaluated and found to be free radical scavenger from a purely biological point of view by Rose & Bodein in 1993.[46] Lai et al. was among the first researchers who considered the use of sodium ascorbate to reverse the effect of the bleaching peroxide.[47] Lai and his research team hypothesized that if the decreased bonding strength was as a result of the oxidation reaction; then "it might be possible for the compromised bond strength to be reversed by reduction of the oxidized surface with a neutral, biocompatible anti-oxidant such as sodium ascorbate" as it was stated in their publication.[47]

Pursuing this approach, efforts have been made to identify antioxidant agents that work best for this purpose. Antioxidants mentioned in the literature are: Ascorbic Acid, Catalase, Butldydroxyanisole, Glutationperoxide, Sodium Ascorbyl Phosphate, Vitamin E, Sodium Bicarbonate, Proanthocyanidin, and Lycopene, however, Sodium Ascorbate has been the most studied antioxidant and has shown promising results in clinical applications.[48] Sodium ascorbate is a neutral and biocompatible anti-oxidant agent.[46,49] It is a bioavailable form of vitamin C, could be

used as an alternative to ascorbic acid supplements, and as a food preservative.[47] Due to its low pH (4), ascorbic acid has at times been avoided, for its potential of mild etching of tooth structure. [47]

In previous studies examining the use of sodium ascorbate as an antioxidant agent to reverse the weakening effect of bleaching on composite bonding, concentrations ranging from 10%-20% have been used, usually resulting in improved bonding of composite. A literature review revealed successful outcomes in 24 of 27 sodium ascorbate studies (Table 1). This table was adopted and modified from Garcia et al.[48] which summarizes the literature review on bond strength testing of composites after the utilization of an antioxidant treatment after bleaching from 2001 until 2015.

Table 1. Summary of literature review

Author	Bleaching Agent	Antioxidant agent	Concentration.	Time of application	Surface E or D	Method used to test composite bonding	Efficacy
Arumugam et al 2014[53]	CP 35%	Sodium Ascorbate solution Proanthocyanidin Lycopene	10% 6.5% 5%	10 mins 10 mins 10 mins	E Human	Shear bond strength	Yes No No
Briso et al 2014[65]	HP 35% CP 10%	Sodium Ascorbate solution	10%	10 mins	D Human	Microtensile bond strength Resin tag analysis	No Yes
Güler et al 2013[66]	HP 35% CP 16%	Sodium Ascorbate solution	10%	10 mins	E Bovine	Microtensile bond strength	Yes
Khouroushi & Saneie 2012[67]	CP 20%	Sodium Ascorbate gel	10%	6 hours	E Human	Shear bond strength	Yes
Briso et al 2012[68]	HP 35% CP 10%	Sodium Ascorbate solution	10%	10 mins	E Human	Resin tag analysis	Yes
Vidhya et al 2011[52]	HP 38%	Sodium Ascorbate solution Oligomeric Proanthocyanidine complex	10% 5%	10 mins 10 mins	E Human	Shear bond strength	Yes Yes
Lima et al 2011[69]	HP 35% CP 16%	Sodium Ascorbate solution	10%	1 min	E+D Bovine	Microshear bond test	Yes
Dabas et al 2011[70]	CP 17%	Sodium Ascorbate gel	10, 20%	30 mins 1, 2 hrs	E Human	Shear bond strength	Yes
Mazaheri et al 2011[64]	HP 9.5%	Sodium Ascorbate gel	10%	10 hrs	E Human	Shear bond strength	Yes
Comlekoglu 2010[72]	CP 10 %	Sodium Ascorbate solution	10%	10 mins	D Human	Shear bond strength SEM	Yes
Kimyai et al 2010[73]	CP 10%	Sodium Ascorbate solution & gel	10%	10 mins	E Human	Shear Bond strength Adhesive remnant index	No
				3 hrs			Yes
Uysal 2010[74]	CP 16%	Sodium Ascorbate solution	10%	10 mins	E Human	Shear Bond strength Adhesive remnant index	Yes
May 2010[75]	CP 35%	Sodium Ascorbate solution	10%	10 mins	D Bovine	Shear bond strength	No
Sasaki et al 2009[62]	CP 10 %	Sodium Ascorbate solution & gel 10% alpha-tocopherol solution & gel	10 % 10%	2 hrs	E+D Human	Shear bond strength	Yes
Türkün et al 2009[61]	CP 10%	Sodium Ascorbate solution & gel	10,2.5,5,10 %	10,120 mins	E Bovine	Shear bond strength	Yes
Kaya et al 2008[63]	CP 10%	Sodium Ascorbate gel	10%	10 mins 1, 2, 4, 8 hrs	E Bovine	Shear bond strength	Yes
Kimyai & Vilzadeh 2008[60]	CP 10%	Sodium Ascorbate solution & gel	10,20 %	3 hrs	D Human	Shear bond strength	Yes
Muraguchi et al 2007[58]	HP 30%	Ascorbic Acid solution	10 %	1 min	E+D Bovine	Shear bond strength SEM	Yes
Torres et al 2006[51]	HP 35%	Sodium Ascorbate solution	10%	20 mins	E Bovine	Shear bond strength	No
		Catalase	34 mg/ml				No
		Glutathione Peroxide	10 mg/ml				No
		Acetone	Pro analy.				No
		Ethanol	Pro analy.				No
		Sodium Bicarbonate	7%				No
Bulut et al 2006[56]	CP 10%	Sodium Ascorbate solution	10%	10 mins	E Human	Shear bond strength	Yes
Kimyai & Valizadeh 2006[59]	CP 10%	Sodium Ascorbate solution & gel	10, 20%	3 hrs	E Human	Shear bond strength	Yes
Arantes et al 2005[76]	CP 10%	Catalase	C-40 10mg/ml	3 mins	E+D Bovine	Tensile bond strength	Yes
Bulut et al 2005[55]	CP 10%	Sodium Ascorbate solution	10%	10 mins	E Human	Tensile bond test Adhesive remnant index	Yes
Türkün & Kaya 2004[54]	CP 10%, CP 16% CP 22%	Sodium Ascorbate solution	10%	10 mins	E Bovine	Shear bond strength SEM	Yes
Kum et al 2004[57]	HP 35% CP 10%	Catalase Ethanol	C-40 70%	3 mins 3 mins	E Human	Shear bond strength	Yes
Kaya & Türkün 2003[50]	HP 35%	Sodium Ascorbate solution Butylhydroxyanisole	10 % 10%	10 mins	D Human	Shear bond strength	No
Lai et al 2002[49]	CP 10%	Sodium Ascorbate	10%	3 hrs	E Human	Microtensile bond test Transmission electron microscopy	Yes
Lai et al 2001[47]	HP 10%	Sodium Ascorbate solution	10 %	10 mins	D Human	Tensile bond strength SEM Transmission electron microscopy	Yes

1.4. Sodium ascorbate

Sodium ascorbate has the ability to neutralize and reverse the oxidizing effect of peroxide containing agents.[47] It is capable of restoring the altered redox of the oxidized bonding substrate, hence allowing for the complete free-radical polymerization without interruption, therefore, reversing the impaired bonding of composite when performed immediately after bleaching.[47]

In 2003, Kaya and Türkün studied the effect of 10% butylhydroxyanisole and 10% sodium ascorbate after 35% HP bleaching.[50] Butylhydroxyanisole offered no improvement on the weakened bond strength of bleached samples. On the other hand, sodium ascorbate was found to restore the bonding strength when applied to samples, up to the level of bond strength of 7-days delayed bonding group with no application of sodium ascorbate, however, they were not to the same level of bond strength of unbleached group (negative control). [50] Butylhydroxyanisole has a tendency to decrease surface water since it is an alcohol based compound; however, it failed to improve the bonding strength.[50] Torres and others evaluated the effect of six different antioxidants including sodium ascorbate, after the application of 35% HP; none of the tested antioxidants were able to reverse the optimal bond strength. [51] In a recent study examining the efficiency of two potent antioxidants proanthocyanidin; which is a grape seed extract and Lycopene; which is a tomato extract, the authors claimed that these were twenty times more powerful as antioxidants than sodium ascorbate.[52] Vidhya et al. found a significant improvement of bond strength when proanthocyanidin was applied after bleaching,[52] while Arumugam et al. found that sodium ascorbate gave superior results over

proanthocyanidin.[53] Little information is available in the literature about grape seed and tomato extracts, therefore, more research is needed to investigate efficacy. Table 1 (See page 12), illustrates that throughout the years, many researchers have modified variables, in attempt to identify the best and most effective way to use sodium ascorbate. Those variables included form (gel or solution), concentration, and contact time.

1.4.1. Sodium ascorbate form

Sodium ascorbate in the solution (liquid) form was used in most studies,[49-51,54-58] as opposed to the gel form.[59-62] Kimyai and Valizadeh found no significant difference between the effectiveness of sodium ascorbate solution, and gel to reverse the weakened bond strength caused by a bleaching agent.[60] Hydrogel/gel form allows a steady and slower reaction of the antioxidant treatment, and, as a result, the suggested time of application this sodium ascorbate gel form is 3 hours.[63] Moreover, because of its high viscosity, it is easily applied by the dentist or by the patients before the bonding appointment, with the aid of custom-bleaching trays, especially if the patient is familiar with a home bleaching regimen. This in turn, will minimize the in-office wait time.[63,64]

1.4.2. Sodium ascorbate concentration

To date, the concentration of sodium ascorbate studied varies from 2.5-20%. The most studied concentration is 10%.[47,49-51,54,58-60,62-65,68,72-75] Türkün et al.[61] examined different concentrations of sodium ascorbate 2.5%, 5%, and 10% for 10 minutes and 2 hours; and observed that a concentration less than 10% of sodium

ascorbate was not adequate to reverse the compromised bonding.[61] A purely chemical reaction kinetics study was conducted by Freire et al[77] to determine the amount of sodium ascorbate needed to neutralize a bleaching agent. It was found that the amount of sodium ascorbate should be directly proportional to the concentration of hydrogen peroxide in order to efficiently deoxidize the bleaching agent. [77]

$$[Sodium\ Ascorbate\ Concentration\ (\%) \propto Hydrogen\ Peroxide\ Concentration(\%)]$$

Furthermore, Freire et al. investigated the effectiveness of 35% sodium ascorbate on bleached enamel by measuring the amount of residual hydrogen peroxide entrapped inside the tooth.[78] It was found that the application of 35% sodium ascorbate for 2 applications, one minute each, was sufficient to eliminate all the residual peroxide after a 35% HP bleaching procedure,[78] however, their findings have not yet been paired with a composite bond strength test.

1.4.3. Time of application of sodium ascorbate

The application time of sodium ascorbate ranges between 1 min and 8 hours. Lai et al. suggested applying sodium ascorbate at least 1/3 of the time in which teeth were exposed to the bleaching agent.[49] Dabas et al. suggested that sodium ascorbate application time should be directly proportional to the composite bond strength, rather than its sodium ascorbate concentration.[70] In other words, the longer the sodium ascorbate is applied after bleaching, the higher bond strength value of composite will be obtained. In contrast, the results of the previously mentioned study about the reaction kinetics of sodium ascorbate by Freire et al. [77] showed that the application time did not affect the reaction time of the de-oxidation, and when the

same amount of sodium ascorbate was used as the concentration of bleaching agent (which was 35%), a 5 minute application time was adequate for sodium ascorbate to exert its full effect.[77]

1.5. Composite bonding

Adhesive dentistry has been continuously developing since the concept was introduced by Buonocore.[79] The simplified concept of adhesive dentistry is based on an exchanging process; minerals are removed from the tooth and replaced by resin monomers, then, after resin polymerization, a micromechanical interlocking bond is created within the tooth structure.[21,80] Bonding to enamel provides the most reliable bond, which seals the margins of the restoration and provide protection to denting bonding which is more vulnerable to degradation.[81] Bonding to dentin, on the other hand, is considered less predictable due to its nature. Dentin is extremely hydrophilic in nature. It is composed of heterogeneous structure, which contains deposits of hydroxyapatite within collagen fibers mesh.[81] Dentin constitutes 50% of its volume inorganic materials, 30% of its volume organic material, and 20% of its volume fluids, while enamel constitutes 98% of its volume inorganic material and the rest is organic material and fluids.[82]

As a result, different bonding strategies have been developed to overcome those challenges in bonding.[81]

1.6. Microtensile bond strength:

Dentin bonding agents have been tested using several mechanical testing techniques; including microleakage, contraction gap size, shear bond strength, and tensile bond strength. [83] Most of the previous studies have utilized simple shear or tensile bond strength tests to evaluate the bond strength of new composites or to analyze different variables that could affect the bonding of composite to tooth structure.[84] Simple tests work well when the values of composite-dentin bond strength are relatively low (between 10-15 MPa),[84] however, the bond strength of newer composites has increased with the development of new bonding materials and techniques.[84] Utilizing simple shear and tensile strength with a strong bonding system usually results in cohesive failure within dentin. That does not mean that the resin-dentin bond is uniformly stronger, but indicates that the tensile or shear stress is not uniform, potentially leading to localized stress concentration areas, where cracks initiate within the dentin substrate.[84] Therefore, to avoid the cohesive failure during tensile bond testing, stress has to be distributed evenly. This can be achieved by either the single-plane lap-shear system or microtensile bond test.[84] Both are technique sensitive and require meticulous handling than conventional ways of bond testing.[84] Microtensile bond strength testing was developed in 1994.[85] The advantage of the microtensile bond test is that it allows multiple specimens to be obtained from each tooth, providing more data form each tooth. [84] Furthermore, because the bonding area is quite small (1.0 mm^2) which balance stress distribution during test loading so it facilitate less cohesive failure and more adhesive failure.[84]

1.7. Purpose

The purpose of this study was to investigate whether the application of 35% sodium ascorbate to bleached dentin increases the bond strength of composite when bonded immediately.

1.8. Specific Aims and Hypothesis

Specific aim 1:

- Aim: To compare the microtensile bond strength of bonded composite immediately after bleaching with and without the application of 35% sodium ascorbate.

- Hypothesis: The values of the microtensile bond strength will be greater for the immediately bonded composite group after the application of 35% sodium ascorbate.

- Null hypothesis: there will be no difference in the microtensile bond strength between the bonded composite immediately after bleaching with the application of 35% sodium ascorbate and with no sodium ascorbate.

Specific aim 2:

- Aim: To compare the microtensile bond strength of bonded composite immediately after bleaching with the application of sodium ascorbate vs. delayed bonding without application of sodium ascorbate.

- Hypothesis: The values of the microtensile bond strength of the immediate bonding of composite with the application of 35% sodium ascorbate will

be greater to or equivalent to the delayed bonded composite without the application of 35% sodium ascorbate.

- Null hypothesis: There will be no difference between the microtensile bond strength of immediately bonded composite with the application of 35% sodium ascorbate and delayed bonding without the application of 35% sodium ascorbate or it might have less microtensile bond strength values.

Specific aim 3:

- Aim: To compare the microtensile bond strength of bonded composite immediately after bleaching with the application of sodium ascorbate vs. the bonding of composite without bleaching.

- Hypothesis: The microtensile bond strength for immediately bonded composite with the application of 35% sodium ascorbate will be equivalent to the bonded composite to unbleached dentin.

- Null hypothesis: There will be no difference between the microtensile bond strength of immediately bonded composite after the application of sodium ascorbate and unbleached dentin.

1.9. Location of the study

The design, preparation, and data collection was held at:

Bioscience Research Center

College of Dental Medicine

Nova Southeastern University

3200 S University Drive

Fort Lauderdale (Davie) FL, 33328

Chapter 2: Material and methods

2.1. Sample Size

Sample size was calculated using a priori power analysis (GPower), $\alpha= 0.05$ and $\beta=80\%$. It was suggested to use 12 samples per group, however, using data from the work of Spyrides et al.[28] it was determined that a sample size of 225 specimens/bars would be obtained from approximately 25 teeth; about 10 bars from each tooth, 5 teeth per group.

After the approval of the Institutional Review Board (IRB) was obtained, (HPD-COM-2013-19) extracted human molars were collected from a de-identified bank of teeth at the College of Dental Medicine. Selection criteria included: 3rd molars with intact occlusal surface, with no significant enamel defect, decalcification, enamel wear, nor any restoration. Before any sample preparation was done, teeth were rinsed and cleansed to remove residual debris, then stored in distilled water at +4° C.

2.2. Groups

To test the effectiveness of the antioxidant agent sodium ascorbate (SA) on the bond strength of composite to enamel, the samples were randomly divided into the following five groups (see chart 1):

Group one: Served as the positive control, where bonding of composite was immediately after bleaching with 35% Hydrogen peroxide.

Group two: Received bonding of composite one week after bleaching process, which is the recommended wait time in clinical practice. [86]

Group three: Served as the experimental group. Received bonding of composite immediately after bleaching and the application of 35% SA.

Group four: Received bonding of composite one week after the bleaching process and the subsequent application of 35%SA.

Group five: Served as the negative control group where composite was applied with no bleaching process, nor further application of SA.

Chart 1. Sample distribution

```
                                          ┌────────────────────┐      ┌──────────┐
                                          │ 5 teeth: Immediate │──────│ 50 Bars  │
                                          │      bonding        │      └──────────┘
                     ┌──────────────────┐ └────────────────────┘
                     │ Group 1,2: 10    │ ┌────────────────────┐      ┌──────────┐
                     │ Teeth→ Bleaching+│ │ 5 teeth: One week  │──────│ 50 Bars  │
                     │ Bonding of       │ │ delayed bonding     │      └──────────┘
                     │ composite        │ └────────────────────┘
                     │ (Control)        │
                     └──────────────────┘ ┌────────────────────┐      ┌──────────┐
                                          │ 5 teeth: Immediate │──────│ 50 Bars  │
┌──────────────┐     ┌──────────────────┐ │      bonding        │      └──────────┘
│ Approximately│     │ Group 3,4 :10    │ └────────────────────┘
│ 25 human     │─────│ Teeth→ Bleaching │ ┌────────────────────┐      ┌──────────┐
│ teeth        │     │ + 35% Sodium     │ │ 5 teeth: One week  │──────│ 50 Bars  │
└──────────────┘     │ Ascorbate        │ │ delayed bonding     │      └──────────┘
                     │ application +    │ └────────────────────┘
                     │ bonding of       │
                     │ composite        │
                     └──────────────────┘
                     ┌──────────────────┐ ┌────────────────────┐      ┌──────────┐
                     │ Group 5: 5 Teeth→│ │ 5 teeth: Immediate │──────│ 50 Bars  │
                     │ bodning of       │─│      bonding        │      └──────────┘
                     │ composite to     │ └────────────────────┘
                     │ dentin           │
                     └──────────────────┘
```

2.3. Sample preparation

The microtensile bond test method has been described by Pashly et al. [84] Teeth were mounted in polymethyle methaacrltae PMMA (Jetacryl, Lang Dental Manufacturing Co., Inc. Wheeling, IL, USA) with the aid of a metal ring. Powder and liquid were mixed according to manufacturer's instructions. Teeth were placed inside the metal ring (root first) as the acrylic mix was poured. Teeth were covered with wet guaze to prevent heat dessication (Figure 1).

After acrlic plymerization, the coronal portion of the tooth was removed using an Isomet Low Speed Saw (Buehler, Illinois, USA) (Figure 2). Finishing and polishing were done using CarbiMet 2 silicon carbide (SiC) abrasive papers (Buehler, Illinois, USA) of 400 and 600 grit successively for 7 seconds each (Figure 3). The dentin surface was rinsed with water, and dried briefly with air then stored in distilled water. Teeth were randomly divided into five groups (Figure 4) and stored in a refrigerator at +4° C prior to testing.

2.4. Bleaching

A 35% HP bleaching gel (Polaoffice, SDI, Australia) was used with groups one, two, three and four in order to simulate the in-office bleaching procedure (Figure 5) went through bleaching process according to the. Bleaching gel was applied on dentinal surfaces for 8 minutes according to the manufacturer's instructions. Samples were then rinsed for 30 seconds and air-dried. This procedure was repeated two additional times, for a total of 3 cycles of bleaching. Then, samples went to the subsequent procedure according to its group.

2.5 Delayed bonding samples (storage)

Samples in the delayed bonding groups (two and four) were stored in 250 ml of deionized water at a temperature of 37° C and 100% humidity for one week in an Isotemp incubator (Fisher Scientific, Waltham, MA, US) (Figure 6).

2.6. Application of sodium ascorbate

For groups three and four, 3.5g of Sodium ascorbate (98% purity Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in 10 ml of distilled water at a pH of 7 with agitation using an SP Vortex Mixer (Scientific Products, Baxter, Greenbrier, Brighton) (Figure 7), resulting in a solution with a concentration of 35% sodium ascorbate. Before application, the solution was agitated and then applied to dentin using a micro brush. Two applications, one minute each, were performed with a subsequent 60-second dH_2O rinse, according to the method described by Freire et al.[78]

2.7. Bonding of composite

This procedure was identical for all groups. The Optibond Solo Plus adhesive two-step etch and rinse system (Kerr, Orange, CA, USA) was used for bonding according to the manufacturer's instructions. After a pumice prophylaxis, the exposed dentinal surface was etched using 37.5% of phosphoric acid (Best etch, Vista, Racine, Wisconsin, USA) for 15 seconds and then rinsed with water for 15 seconds and then air-dried for 5 seconds. The adhesive was applied in a circular motion to dentin surface for 15 seconds then air-dried for 3 seconds. Samples were light cured for 20 seconds using an Optilux 501 halogen LC unit (Kerr, Orange, CA,

USA) with an intensity output of 800mW/cm^2. A tofflemire matrix and metal band were used as the composite (Filtek Supreme Ultra A2 enamel shade, 3M ESPE) was applied in increments of 1.5 mm thickness, before curing, ultimately reaching a resin height of approximately 3-4 mm.[84] Samples composed of dentin and composite were the end result of this procedure (Figure 9). Samples were then immersed in deionized water and stored for 24 hours in an Isotemp incubator (Fisher Scientific, Waltham, MA, US) at a temperature of 37° C and 100% humidity to permit complete composite resin polymerization.

2.8. Sectioning

A 2 mm thick perimeter was marked on the top of the composite block to simulate the thickness of underlying enamel, so that marked specimens without the desired dentin/composite bond could be later excluded (Figure 9). Great care was taken to prevent excessive heat production and dehydration during cutting of all specimens by using copious amounts of water with the diamond blade of IsoMet low speed saw (Buehler, Illinois, USA) (Figure 10).[84] Each bonded tooth was sectioned vertically into a number of slabs of 1 mm thickness (Figure 11). The tooth was then rotated 90° and again sectioned lengthwise into multiple beam-shaped bars, each with a cross- sectional surface area of approximately 1mm (Figure 11). Length and width measurements of 1.0±0.3 mm was considered acceptable.[84] The lower half of the bar was composed of dentin, while the upper half was the composite resin build-up (Figure 12). Each tooth generated between 25 and 30 bars.[84] From each tooth, a total of 10 bars were selected for testing. The total numbers of specimens were 242

bars were obtained. Due to week bonding, many specimens from group one fractured during the sawing procedure.

2.9. Microtensile testing

Before testing of bond strength, each specimen was carefully checked under a stereomicroscope at 25X (Bausch&Lomb, Rochester, NY, USA), in order to confirm that the adhesive interface was perpendicular to the long axis of the specimen bar. This is important to ensure pure tensile forces are applied with no undesired torque component. Any specimen without a perpendicular interface relative to the long axis of the bar was discarded. The cross-sectional area of each specimen was recorded using a digital caliper Absolute IP66 (Mitutoyo, Aurora, Illinois) at the bonding interface between dentin and composite (Figure 13). This value was utilized in converting the force unit from Newton (N) to Megapascal (Mpa). The universal testing machine Instron model 8841 (Instron, Canton, MA, USA) was used (Figure 14) to record force to fracture values. A ruler and marker were used to align the bars vertically on the fixture at 90 degrees (Figure 15). The two ends of each specimen were carefully adhered to the flat stainless steel fixture with cyanoacrylate cement (Figure 15). The tensile load was applied at a crosshead speed of 0.5 mm/min, until the specimen fractured (Figure 16). At that point, the load at failure in Newtons (N) was recorded. Each fractured specimen was examined under a stereomicroscope (Bausch & Lomb) at 25X in order to check the failure mode. Only adhesive failure mode samples were used to calculate the bond strength. (See appendices A, B, C, D, and E for raw data).

2.10. Statistical analysis

The surface area of 242 specimens as well as the microtensile force in N were recorded on an excel spreadsheet with the resulting conversion from (N) to (Mpa) using the following formula[87]

$$\left[\frac{Bond\ Strength\ in\ Newton\ (N)}{Surface\ area\ (Width\ X\ Thickness)(\text{mm2})} = Bond\ strength\ in\ Megapascal\ (MPa) \right]$$

Table 2: Descriptive Statistics

	N	Mean	SD	Min	Max
Bleaching + Bonding of composite (Control) immediate	42	34.17	10.78	11.73	56.75
Bleaching + Bonding of composite (Control) delayed one week	50	45.36	16.57	18.27	86.54
Bleaching + 35% Sodium Ascorbate application + bonding of composite immediately	50	45.06	20.91	16.71	117.28
Bleaching + 35% Sodium Ascorbate application + bonding of composite delayed one week	50	44.73	16.85	14.50	95.74
Immediate bonding of composite with no bleaching (negative control)	50	45.83	19.32	14.54	95.39

Descriptive Statistics of mean microtensile bond strength (MPa)

There were minimal cohesive failures of the resin composite, and no cohesive failures were observed in dentin. Samples with cohesive failure were excluded, and only adhesive failure samples were included in the data sheath (Appendices A, B, C, D, E). Descriptive statistic means, and standard deviations were calculated for all 5 groups (Table 2).

Chapter 3: Results

Descriptive statistics are presented in (Table 2). To test our hypothesis, a one-way ANOVA was used to compare groups. In (Table 3) a significant difference in microtensile bond strength (MPa) was found between group one and groups two through five $F_{(4, 1073.81)} = 3.54$, $p = 0.07$ Tukey's post-hoc comparisons of the five groups are illustrated in (Chart 2).

Table 3: One-way ANOVA

Group		Group	Difference	Lower 95% CI	Upper 95% CI	P>t
2	vs	1	11.19	1.18	21.21	0.020
3	vs	1	10.90	0.88	20.92	0.025
4	vs	1	10.57	0.55	20.59	0.033
5	vs	1	11.67	1.65	21.69	0.013
3	vs	2	-0.30	-9.87	9.28	1.000
4	vs	2	-0.63	-10.20	8.95	1.000
5	vs	2	0.47	-9.10	10.05	1.000
4	vs	3	-0.33	-9.91	9.24	1.000
5	vs	3	0.77	-8.81	10.34	0.999
5	vs	4	1.10	-8.47	10.68	0.998

Comparison of mean bond strengths (MPa) among the five groups using one-way ANOVA

1 = Bleaching + Bonding of composite (Control) immediate
2 = Bleaching + Bonding of composite (Control) delayed one week
3 = Bleaching + 35% Sodium Ascorbate application + bonding of composite immediately
4 = Bleaching + 35% Sodium Ascorbate application + bonding of composite delayed one week
5 = Immediate bonding of composite with no bleaching (negative control)

Chart 2. Bar diagram
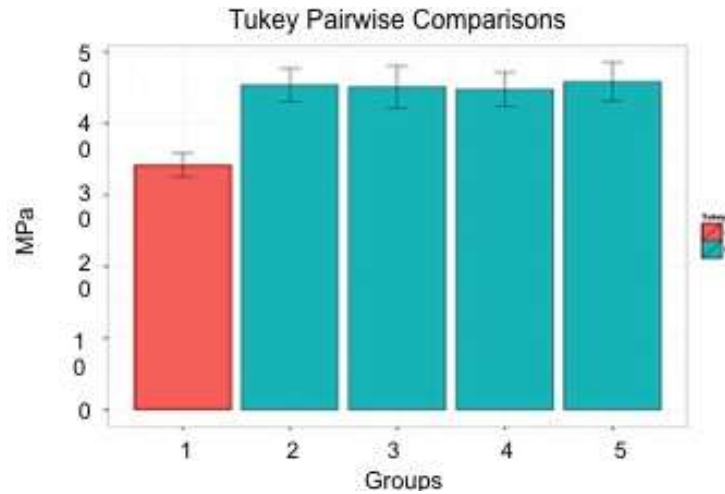
Bar diagram showing the comparison of the mean microtensile bond strength (MPa) among the five groups using Tukey's post-hoc comparisons.

* Groups not connected by the same color are significantly different
1 = Bleaching + Bonding of composite (Control) immediate
2 = Bleaching + Bonding of composite (Control) delayed one week
3 = Bleaching + 35% Sodium Ascorbate application + bonding of composite immediately
4 = Bleaching + 35% Sodium Ascorbate application + bonding of composite delayed one week
5 = Immediate bonding of composite with no bleaching (negative control)

The following results can be noted:

- Mean tensile bond strength of the positive control group; bleaching with immediate bonding (34.17±10.78), was significantly lower than all other groups ($P < 0.07$).

- The one-week delay of bonding showed similar values to the negative control group, (45.36±16.57) and (45.83±19.32) for group two and five respectively, with no statistical difference ($P \leq 1$).

- The mean microtensile bond strength values for the sodium ascorbate group bonded immediately after bleaching. Group three (45.06±20.91) showed

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significantly higher values than the immediate bonding after bleaching ($P<0.025$).

- Group three showed bond strength values that were almost identical difference ($P \leq 1$) to both group two, where bonding was delayed after 7 days of bleaching and group five, where bonding was performed with no bleaching.

- The application of sodium ascorbate after bleaching with 7 days delayed bonding showed no stronger bond strength value. It gave similar bond strength values to group two and five, (44.73±16.85) with no statistical difference ($P \leq 1$).

The application of 35% sodium ascorbate immediately after bleaching effectively improved the bond strength and was equal to the bond strength regained from the one week delayed bonding. Thus, the null hypothesis was rejected.

Chapter 4: Discussion

Patients who have a time constraint or issues with compliance are usually discouraged from receiving restorative esthetic treatments such as those requiring a delay of one week after bleaching. As a result, patients' enthusiasm toward more esthetic restorative treatments may be lower, and they may choose not to receive additional services. There may also be other situations in which a patient's personal schedule does not permit a delay in the bonding procedure after bleaching. An agent that can reverse the temporary weakening effect of the bleaching agent on bond strength would be a very practical solution, as it would help save time and be more convenient for both the patient and dentist.

Sodium ascorbate was used as an antioxidant treatment for its potential to neutralize and reverse the oxidizing effect of peroxide containing agents.[47] Our results, and those of multiple other studies, have verified its effectiveness in reversing the weakening effect of the bleaching agent on composite bonding. [47,49,50,54]

Our results also confirmed the finding of Freire et al. in their reaction kinetic study, that the effective concentration of the oxidant is directly proportional to the amount of the antioxidant.[88] This may explain why studies which have used 10% of sodium ascorbate to enhance the bonding strength after the application of 35% HP have failed to show the regain of optimal bonding strength,[50,51] while using 10% sodium ascorbate to reverse the effect of 10% HP was effective.[47,64] Briso et al. was the only exception to that observation as he found improved penetration of resin tags when 10% sodium ascorbate was applied after 35% HP.[68] Similarly, studies used 10%

sodium ascorbate to reverse the action of 10-17% CP showed improvement of the bond strength of composite.[49,54-56,59,60,62,63,70,73,74] As mentioned earlier, 10% CP will dissociate to provide 3.5% HP, [3,8] and 10% sodium ascorbate was more than enough to reverse this effect, confirming the finding of Freire et al.[88]

Previously, some authors have claimed that sodium ascorbate might have an enhancing effect on dentin bonding regardless of whether or not bleaching has been performed. Türkün et al. found that the application of 10% sodium ascorbate hydrogel to bleached enamel produced stronger shear bond strength of enamel when compared to the non-bleached teeth.[61] This result was attributed to the higher neutralizing ability of sodium ascorbate that diminished its effect to the normal atmospheric oxygen that is present in the normal enamel.[61] However, our findings failed to detect any significant improvement in bond strength after bleaching, among the two delayed bonding groups, either with or without the application of sodium ascorbate. This is not surprising when one considers the structural variation of enamel versus dentin; it is less porous than enamel and is rich in inorganic component and collagen.[82,89]

As previously mentioned, the concentration and application period of sodium ascorbate are vital factors in reversing the effect of bleaching.[50] In our study we have exposed bleached dentin to sodium ascorbate for a considerably short period of time, following the recommendation of Freire et al. 2011 i.e., two applications, one minute each.[78] This method was different than many previous studies, where contact time with the sodium ascorbate was believed to be the most important factor. Those studies utilized significantly longer periods of application that ranged between 10

minutes to 6 hours (Table 1). [48] Freire et al. stressed the importance of the number of applications rather than the contact time. They tested one application of sodium ascorbate for 5 and 10 minutes, compared with two applications of sodium ascorbate for 1, 2, 5, and 10 minutes, as well as three application of one minute each. They found that the 2-application group had no oxide residuals regardless the contact time. Their findings showed that the reaction between the oxide residuals and antioxidants usually reached its peak in about a minute, therefor; increasing the contact time will not eliminate more of the residual oxide.[78]

Our findings were in accordance with previous studies that showed a significant reduction in bond strength when composite was bonded immediately after 35% hydrogen peroxide bleaching.[36,38,41] Samples bonded immediately after bleaching showed significantly the lowest bond strength values ($P < 0.07$).

A common in-office bleaching method was used in our study (35% HP), which is the most commonly used agent for in-office bleaching method.[5,8,13] Patients who are undergoing an at-home bleaching treatment, where 2-5 weeks are needed for the outcome to be completed,[4] would most likely not be opposed to the delay in a bonding procedure which requires an additional 1-2 weeks. For that purpose, we did not find the necessity of testing CP nor the at-home bleaching regimen.

Similar findings were observed by Comlekoglu et al.[72], where immediately bonded groups after bleaching with 10% CP presented with the lowest bond strength values when compared to control groups. [72] A study by Briso et al. confirmed that the use of 35% HP is more harmful to resin tags than 10 % CP.[68] Therefore, it is expected to find lower bond strength values when hydrogen peroxide is used, which justifies

our utilization of an equal proportion of sodium ascorbate when hydrogen peroxide was used as a bleaching agent. Although sodium ascorbate is available as an over-the-counter vitamin supplement, it could cause some gastrointestinal discomfort, such as vomiting, diarrhea, and stomach cramps, when ingested in very high doses, however, topical usage is generally safe.[90]

Due to the variation in structure between coronal and radicular dentin, the microtensile bond strength varies within the same tooth,[84] which explains having different range values within the same tooth and among the same group of treatment . It has been reported in the literature that the bond strength value of dentin varies between 13-80 MPa for the total etch 2-step (fourth generation bonding systems)[91], however, with the advancement of bonding agents and improved techniques, a value of (34.17±10.78),MPa is considered low compared to our negative control groups (45.83±19.32) MPa.[91]

Previous studies have shown that one week of delayed bonding is enough to regain composite bonding strength after bleaching[25]. Spyrides et al. studied 3 different bleaching agents on dentin to composite bond strength.[28] They found that a significant reduction in shear bond strength was observed when bonding was performed immediately after bleaching with a 35%HP bleaching agent while a significant increase in bond strength was observed after one week of delayed bonding.[28]

Most of the previous published studies have investigated enamel, whether from a human or bovine source (Table 1), while in our study we investigated human dentin. Only six of twenty-five studies have investigated the effect of sodium

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ascorbate on human dentin, in which 2 of those studies concluded that sodium ascorbate did not improve bonding when applied immediately after bleaching.[50,65] It is well known that, enamel is more translucent, where dentin is opaque, offering the tooth its shade. The bleaching agent penetrates through enamel pores reaching dentin, where most of the chemical reaction of the bleaching agent takes place.[47] Dentin bonding was investigated in this study as it currently presents many challenges.[81]

Color stability is reached 7 days after bleaching[25], however, the influence of the tooth color change after the end of the bleaching treatment on esthetic restorations is still unknown.[69] Sodium ascorbate is unstable in solution and discolors after only a few hours, which could bring into question its stability inside the dentinal tubules, as well as the effect of the solution color change on the success of the bleaching procedure.

Further studies are needed to investigate the effect of sodium ascorbate on bonding of composites, and its effect of on shade stability after bleaching. Controlled clinical trials may provide more evidence of an agent effectiveness or technique.

Chapter 5: Limitations

The present study expanded upon the methods of Freire et al. in 2011, which utilized the application of 35% sodium ascorbate in 2 applications, one minute each after a 35% hydrogen peroxide treatment. Alternatively, we could have tested the validity of their suggestion by testing different modes of applications, but it was not feasible at that time because of the time and budget limitations. Also, we could have tested the efficacy of different kinds of bonding agents and different concentrations of hydrogen peroxide versus different amounts of sodium ascorbate to validate Freire's findings in 2009. Future studies may explore these other possible methodology design.

Chapter 6: Conclusions

- Bleaching with 35% HP immediately before bonding reduces the bond strength of composite resin to dentin.

- One-week delayed bonding after bleaching resulted in reversal of reduced bond strength.

- Sodium ascorbate has improved the bonding when applied immediately after bleaching.

- The clinical implication of this study would allow the clinician to bond composite to bleach teeth immediately without compromising the bonding strength, reducing appointments and chair time.

Figures

Figure 1. Sample preparation 1

Figure 2. Sample preparation 2: Removal of coronal section

IsoMet low speed saw
(Buehler, Illinois, USA)

Blade dressing

Coronal surface sectioning

Figure 3. Sample preparation 3: Samples polishing

400 and 600 grit abrasive

Figure 4. Sample division

Samples were divided randomly into 5 groups

Figure 5. Bleaching

35% hydrogen peroxide
(Polaoffice, SDI, Australia)

Figure 6. Delayed bonding samples (storage)

Isotemp incubator
(Fisher Scientific, Waltham, MA, US)
37° C and 100% humidity

Figure 7. Sodium ascorbate preparation

Sodium ascorbate
98% purity Sigma-
Aldrich Co., St.
Louis, MO, USA

3.5g of sodium
ascorbate and 10 ml
of distilled water
were mixed

SP Vortex Mixer (Scientific Products, Baxter,
Greenbrier, Brighton

Figure 8. Bonding of composite

Optibond Solo Plus
(Kerr, Orange, CA, USA),

Filtek Supreme Ultra A2 enamel shade
(3M ESPE)

Optilux 501 (Kerr, Orange, CA, USA

Figure 9. Composite build up

Composite
Dentin

2 mm margin was marked

Figure 10. Sample sectioning using IsoMet saw

IsoMet low speed saw
(Buehler, Illinois, USA)

Figure 11. Samples sectioning into bars

Figure 12. Bars dimension verification

Surface area≈ 1 x1 mm

Composite

Dentin
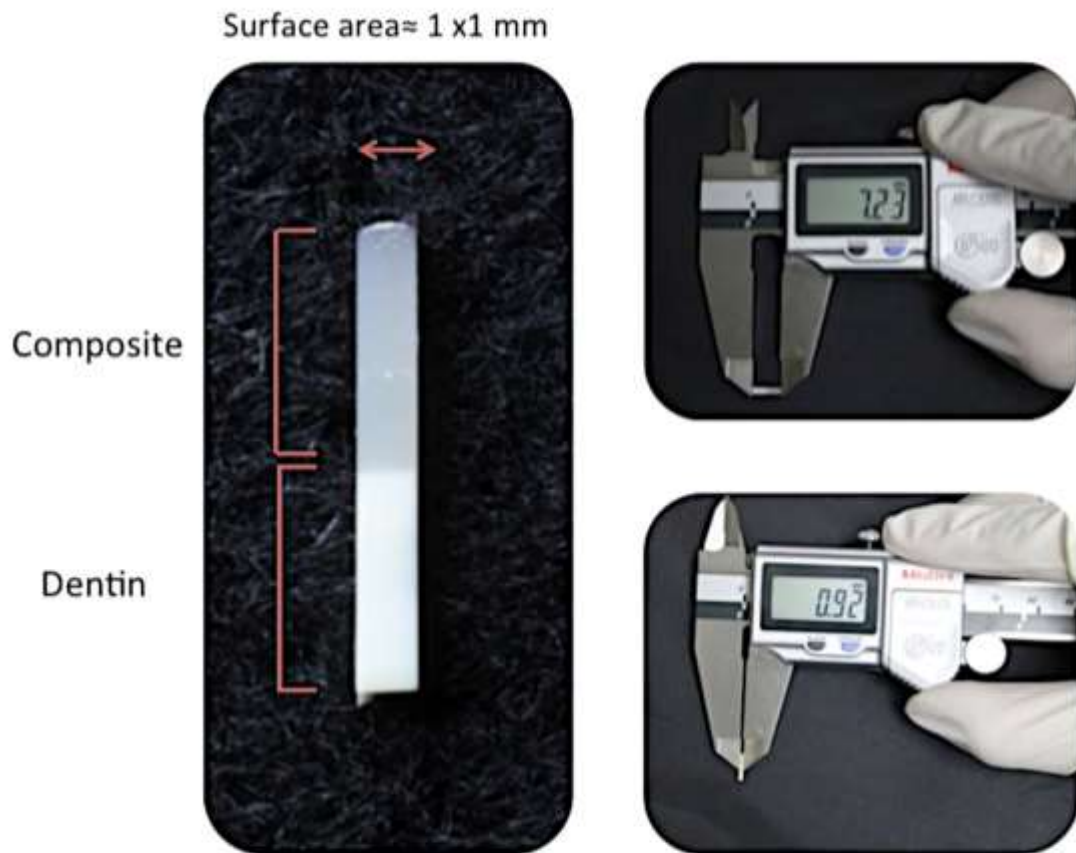
Sample dimension were checked using
Digital caliper Absuolute IP66
(Mitutoyo, Aurora, Illinois)

Figure 13. The universal loading machine (Instron)

The universal loading machine Instron model 8841
(Instron, Canton, MA, USA)

Figure 14. Bars measurements' recording before testing

Samples width and thickness measurement at the composite/dentin
interface using digital caliper Absuolute IP66
(Mitutoyo, Aurora, Illinois)

Figure 15. Sample attachment to the metal fixture using cyanoacrylate glue

Figure 16. Microtensile testing
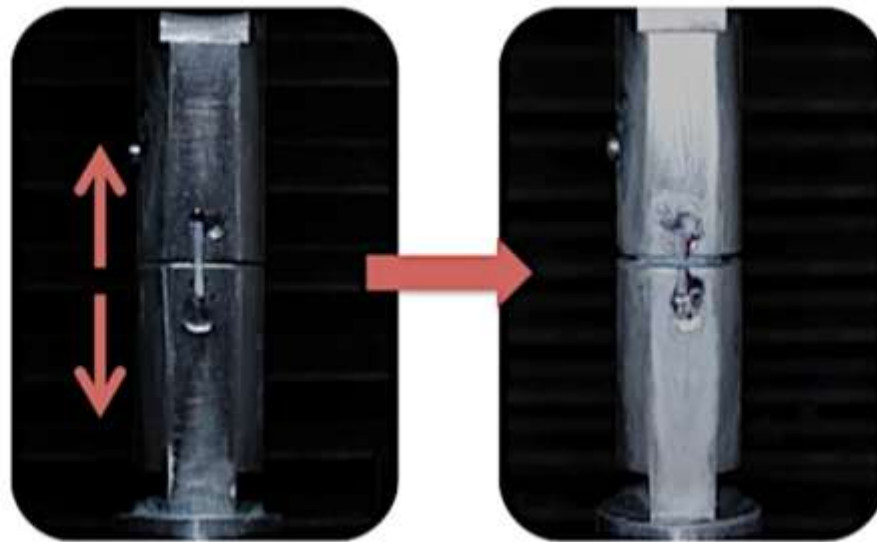
Crosshead speed of 0.5 mm/min
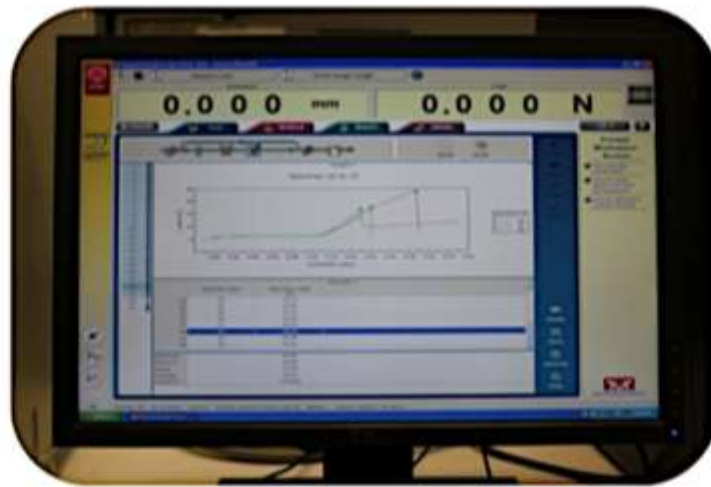
The load at failure in (Newton N) was recorded

Appendices

Appendix A. Raw data: Bleaching + Bonding of composite (Control) immediate

Group	Sample no.	Thickness	Width	Surface area	N	MPa
1	1	0.9	0.93	0.84	19.28	23.03
1	2	0.94	0.91	0.86	15.39	17.99
1	3	0.94	0.94	0.88	38.89	44.01
1	4	1.16	1.18	1.37	49.56	36.21
1	5	1.08	1.02	1.10	36.94	33.53
1	6	0.75	0.78	0.59	15.34	26.22
1	7	0.87	0.94	0.82	19.31	23.61
1	8	0.94	0.94	0.88	43.42	49.14
1	9	0.87	0.93	0.81	27.87	34.45
1	10	1.02	1.12	1.14	39.94	34.96
1	11	1.07	0.99	1.06	55	51.92
1	12	1.15	1.08	1.24	59.67	48.04
1	13	1.03	1.01	1.04	39.68	38.14
1	14	0.86	0.79	0.68	7.97	11.73
1	15	0.81	0.78	0.63	11.01	17.43
1	16	0.96	0.85	0.82	34.46	42.23
1	17	0.93	1	0.93	40.1	43.12
1	18	1.18	1.05	1.24	29.97	24.19
1	19	0.94	1	0.94	26.67	28.37
1	20	0.96	1.05	1.07	16.44	15.36
1	21	0.97	1.07	1.04	32.18	31.00
1	22	1.08	0.99	1.07	18.19	17.01
1	23	0.94	0.81	0.76	36.24	47.60
1	24	0.98	0.76	0.74	27.86	37.41
1	25	0.99	1.05	1.04	37.65	36.22
1	26	1.09	0.95	1.04	40.64	39.25
1	27	0.94	0.98	0.92	23.82	25.86
1	28	0.94	0.87	0.82	46.41	56.75
1	29	0.93	1.03	0.96	47.68	49.78
1	30	1.17	1.16	1.36	31.38	23.12
1	31	0.83	0.82	0.68	28.44	41.79
1	32	0.94	1	0.94	26.5	28.19
1	33	0.91	1	0.91	34.09	37.46
1	34	0.86	0.93	0.80	30.67	38.35

1	35	0.88	0.87	0.77	30.04	39.24
1	36	0.84	0.92	0.77	26.1	33.77
1	37	0.81	0.86	0.70	29.34	42.12
1	38	0.88	0.95	0.84	19.15	22.91
1	39	0.96	0.85	0.82	29.05	35.60
1	40	0.83	0.88	0.73	27.46	37.60
1	41	1.05	0.93	0.98	41.65	42.65
1	42	1.05	0.87	0.00	25.27	27.66

Appendix B. Raw data: Bleaching + Bonding of composite (Control) delayed one week

Group	Sample no.	Thickness	Width	Surface area	N	MPa
2	1	0.91	0.98	0.89	59.92	67.19
2	2	0.97	0.9	0.87	29.36	33.63
2	3	0.92	0.95	0.87	21.89	25.05
2	4	0.94	0.94	0.88	24.51	27.74
2	5	0.89	0.86	0.77	37.29	48.72
2	6	0.87	0.89	0.77	33.56	43.34
2	7	0.95	0.95	0.90	57.15	63.32
2	8	0.89	0.97	0.86	65.91	76.35
2	9	0.96	0.95	0.91	72.26	79.23
2	10	0.95	0.94	0.89	55.24	61.86
2	11	0.83	0.99	0.82	52.01	63.30
2	12	0.77	0.86	0.66	37.91	57.25
2	13	0.85	0.88	0.75	25.8	34.49
2	14	1	0.99	0.99	29.61	29.91
2	15	1.03	1.06	1.09	58.52	53.60
2	16	0.81	0.99	0.80	25.72	32.07
2	17	0.89	0.81	0.72	24.32	33.74
2	18	0.95	0.91	0.86	40.96	47.38
2	19	0.79	0.9	0.71	37.61	52.90
2	20	0.9	1.05	0.95	33.45	35.40
2	21	0.92	0.83	0.76	22.39	29.32
2	22	0.68	0.71	0.48	41.78	86.54
2	23	0.98	0.92	0.90	29.33	32.53
2	24	0.83	0.89	0.74	23.99	32.48
2	25	0.81	0.77	0.62	24.88	39.89
2	26	0.96	1.04	1.00	23.39	23.43
2	27	0.88	0.86	0.76	13.83	18.27
2	28	1.06	0.91	0.96	44.43	46.06
2	29	0.94	1.01	0.95	24.58	25.89
2	30	1.01	0.9	0.91	23.89	26.28
2	31	0.85	0.92	0.78	25.32	32.38
2	32	0.95	0.85	0.81	32.08	39.73
2	33	0.87	0.89	0.77	35.57	45.94
2	34	0.92	0.87	0.80	41.04	51.27
2	35	0.96	0.95	0.91	43.49	47.69
2	36	0.92	0.96	0.88	31.02	35.12
2	37	0.87	0.95	0.83	54.76	66.26
2	38	0.84	0.91	0.76	33.88	44.32
2	39	0.92	0.98	0.90	70.64	78.35

2	40	0.96	0.95	0.91	29.03	31.83
2	41	1.03	0.9	0.93	29.12	31.41
2	42	0.93	0.89	0.83	20.7	25.01
2	43	1.05	0.86	0.90	45.78	50.70
2	44	0.85	0.7	0.60	29.69	49.90
2	45	0.86	1.12	0.96	38.72	40.20
2	46	1.16	0.86	1.00	60.27	60.41
2	47	0.96	0.9	0.86	52.07	60.27
2	48	0.93	0.93	0.86	41.77	48.29
2	49	0.73	0.83	0.61	21.11	34.84
2	50	0.9	0.96	0.86	57.91	67.03

Appendix C. Raw data: Bleaching + 35% Sodium Ascorbate application + bonding of composite immediately

Group	Sample no.	Thickness	Width	Surface area	N	MPa
3	1	0.94	0.74	0.70	52.51	75.49
3	2	0.91	0.82	0.75	71.02	95.18
3	3	0.85	0.94	0.80	34.94	43.73
3	4	0.99	0.93	0.92	23.35	25.36
3	5	0.92	0.78	0.72	33.51	46.70
3	6	0.76	0.75	0.57	66.85	117.28
3	7	1.01	0.95	0.96	85.53	89.14
3	8	0.96	0.85	0.82	47.78	58.55
3	9	0.94	1.04	0.98	34.45	35.24
3	10	0.88	0.84	0.74	17.23	23.31
3	11	1	1.08	1.08	18.05	16.71
3	12	0.94	0.85	0.80	44.63	55.86
3	13	1.02	1.07	1.09	47.76	43.76
3	14	1.03	1.07	1.10	34.63	31.42
3	15	0.92	1.02	0.94	40.27	42.91
3	16	0.87	1	0.87	54.16	62.25
3	17	1.01	0.95	0.96	55.21	57.54
3	18	1.07	0.87	0.93	45.45	48.82
3	19	0.94	0.97	0.91	49.95	54.78
3	20	1.01	1.08	1.09	27.35	25.07
3	21	0.99	0.89	0.88	45.19	51.29
3	22	0.76	0.75	0.57	39.88	69.96
3	23	0.86	0.95	0.82	16.47	20.16
3	24	0.9	0.86	0.77	46.9	60.59
3	25	1.06	0.97	1.03	31.23	30.37
3	26	0.98	0.88	0.86	44.97	52.15
3	27	0.83	1	0.83	18.88	22.75
3	28	0.81	0.95	0.77	33.59	43.65
3	29	1	0.94	0.94	68.52	72.89
3	30	0.9	0.94	0.85	16.38	19.36
3	31	0.81	0.99	0.80	55.97	69.80
3	32	0.83	0.89	0.74	34.82	47.14
3	33	0.96	0.8	0.77	35.29	45.95
3	34	0.98	0.91	0.89	26.23	29.41
3	35	1.14	1.09	1.2	55.993	45.06
3	36	0.95	0.9	0.86	23.32	27.27
3	37	0.9	0.81	0.73	16.92	23.21
3	38	0.88	1.12	0.99	25.36	25.73

3	39	1.11	0.89	0.99	51.15	51.78
3	40	0.99	1.1	1.09	41.8	38.38
3	41	0.96	0.95	0.91	33.53	36.77
3	42	0.85	0.77	0.65	38.63	59.02
3	43	0.88	0.87	0.77	26.18	34.20
3	44	0.96	0.83	0.80	21.56	27.06
3	45	0.91	0.97	0.88	30.29	34.32
3	46	1	0.98	0.98	33.43	34.11
3	47	0.88	0.98	0.86	38	44.06
3	48	1.03	1.01	1.04	28.94	27.82
3	49	0.89	0.94	0.84	19.74	23.60
3	50	0.89	0.92	0.82	29.76	36.35

Appendix D. Raw Data: Bleaching + 35% Sodium Ascorbate application + bonding of composite delayed one week

Group	Sample no.	Thickness	Width	Surface area	N	MPa
4	1	0.98	0.97	0.95	48.19	50.69
4	2	0.92	0.95	0.87	44.35	50.74
4	3	0.83	0.94	0.78	26.86	34.43
4	4	0.85	0.85	0.72	29.56	40.91
4	5	0.93	1.07	1.00	51.6	51.85
4	6	0.93	0.84	0.78	38.12	48.80
4	7	1.01	0.93	0.94	34.25	36.46
4	8	0.91	0.82	0.75	47.22	63.28
4	9	0.94	0.81	0.76	28.75	37.76
4	10	0.88	0.89	0.78	31.11	39.72
4	11	0.83	0.92	0.76	39.41	51.61
4	12	0.87	0.82	0.71	10.35	14.51
4	13	0.92	0.83	0.76	49.24	64.48
4	14	0.97	1.03	1.00	39.05	39.09
4	15	1.04	1.07	1.11	45.43	40.82
4	16	0.82	0.92	0.75	59.15	78.41
4	17	0.89	0.83	0.74	26.79	36.27
4	18	0.95	0.93	0.88	48.26	54.62
4	19	0.85	0.99	0.84	32.82	39.00
4	20	0.93	0.82	0.76	22.22	29.14
4	21	0.93	1.06	0.99	28.09	28.49
4	22	1	0.92	0.92	53.33	57.97
4	23	0.97	0.9	0.87	20.98	24.03
4	24	0.99	0.84	0.83	48.63	58.48
4	25	0.91	0.89	0.81	23.76	29.34
4	26	0.84	0.85	0.71	27.41	38.39
4	27	0.79	0.79	0.62	43.92	70.37
4	28	0.82	0.78	0.64	32.63	51.02
4	29	0.73	0.83	0.61	58.01	95.74
4	30	0.78	0.82	0.64	58.88	92.06
4	31	0.81	0.87	0.70	39.67	56.29
4	32	0.76	1.11	0.84	36.86	43.69
4	33	0.86	1.14	0.98	30.92	31.54
4	34	0.73	1.09	0.80	40.89	51.39

4	35	1.03	1.15	1.18	42.11	35.55
4	36	0.9	1.03	0.93	36.55	39.43
4	37	0.72	1.03	0.74	33.75	45.51
4	38	0.85	0.71	0.60	29.13	48.27
4	39	0.93	0.85	0.79	16.46	20.82
4	40	0.92	0.93	0.86	42	49.09
4	41	1.16	1.03	1.19	31.12	26.05
4	42	0.9	1.03	0.93	25.23	27.22
4	43	0.85	1.11	0.94	32.33	34.27
4	44	1.02	0.91	0.93	29.92	32.23
4	45	0.7	0.86	0.60	37.17	61.74
4	46	0.94	1.03	0.97	23.95	24.74
4	47	1.09	1.01	1.10	38.07	34.58
4	48	0.93	1.11	1.03	25.1	24.31
4	49	1.15	0.84	0.97	44.4	45.96
4	50	0.84	0.92	0.77	42.87	55.47

Appendix E. Raw Data: Immediate bonding of composite with no bleaching (negative control)

Group	Sample no.	Thickness	Width	Surface area	N	MPa
5	1	0.89	0.92	0.82	15.17	18.53
5	2	0.99	0.93	0.92	42.97	46.67
5	3	0.98	0.94	0.92	47.24	51.28
5	4	0.98	0.95	0.93	14.12	15.17
5	5	0.73	0.76	0.55	26.48	47.73
5	6	0.93	0.94	0.87	27.11	31.01
5	7	0.84	0.96	0.81	17.87	22.16
5	8	0.87	0.87	0.76	47.37	62.58
5	9	0.94	1.01	0.95	26.87	28.30
5	10	0.98	0.85	0.83	39.69	47.65
5	11	0.86	0.87	0.75	10.88	14.54
5	12	0.97	1.07	1.04	36.99	35.64
5	13	0.85	0.96	0.82	33.71	41.31
5	14	1	1.09	1.09	25.22	23.14
5	15	0.89	0.89	0.79	42.58	53.76
5	16	1	0.99	0.99	31.84	32.16
5	17	0.96	0.89	0.85	22.06	25.82
5	18	0.99	0.94	0.93	57.28	61.55
5	19	0.87	0.89	0.77	27.97	36.12
5	20	0.97	0.89	0.86	29.99	34.74
5	21	0.96	0.97	0.93	57.86	62.13
5	22	0.98	0.99	0.97	23.17	23.88
5	23	0.84	0.9	0.76	22.26	29.44
5	24	1.01	0.82	0.83	36.48	44.05
5	25	0.99	0.99	0.98	22.19	22.64
5	26	0.97	0.93	0.90	41.56	46.07
5	27	0.85	0.93	0.79	35.51	44.92
5	28	0.99	0.91	0.90	61.23	67.97
5	29	0.97	0.85	0.82	14.17	17.19
5	30	0.88	1.08	0.95	31.69	33.34
5	31	0.88	0.9	0.79	43.38	54.77
5	32	0.73	0.74	0.54	47.54	88.00
5	33	0.8	0.89	0.71	25.94	36.43
5	34	1	0.83	0.83	53.99	65.05
5	35	0.99	1.03	1.02	54.45	53.40
5	36	0.84	0.94	0.79	37.38	47.34
5	37	1	0.82	0.82	38.26	46.66
5	38	0.82	0.89	0.73	33.88	46.42

5	39	0.9	0.95	0.86	39.4	46.08
5	40	0.91	1.13	1.03	50.1	48.72
5	41	0.93	0.7	0.65	42.93	65.94
5	42	0.86	0.96	0.83	24.95	30.22
5	43	0.99	1.05	1.04	80.57	77.51
5	44	1.03	0.95	0.98	44.39	45.37
5	45	0.9	1.07	0.96	68.79	71.43
5	46	0.75	1	0.75	44.49	59.32
5	47	1.04	0.9	0.94	48.72	52.05
5	48	0.93	1.03	0.96	46.59	48.64
5	49	0.95	0.82	0.78	74.31	95.39
5	50	0.85	0.96	0.82	74.63	91.46

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