# COMPARATIVE EVALUATION OF REVERSAL OF SHEAR BOND STRENGTH ON BLEACHED ENAMEL USING ANTIOXIDANTS – SODIUM ASCORBATE, SALICYLIC ACID & N-ACETYL CYSTEINE -AN IN VITRO STUDY

A Dissertation submitted in partial fulfilment of the requirements for the degree of

# **MASTER OF DENTAL SURGERY**

## **BRANCH – IV**

# CONSERVATIVE DENTISTRY AND ENDODONTICS



THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

# **CHENNAI – 600 032**

2010 - 2013

# CERTIFICATE



This is to certify that **Dr.MANJUNATH MANDHIRA DOSS M.R.**, Post Graduate student (2010 - 2013) in the Department of Conservative Dentistry and Endodontics, has done this dissertation titled —**COMPARATIVE EVALUATION OF REVERSAL OF SHEAR BOND STRENGTH ON BLEACHED ENAMEL USING ANTIOXIDANTS – SODIUM ASCORBATE, SALICYLIC ACID & N-ACETYL CYSTEINE -AN IN VITRO STUDY** under our direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai – 32** for **M.D.S.**, in Conservative Dentistry and Endodontics (Branch IV) Degree Examination.

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## DECLARATION

TITLE OF DISSERTATION	COMPARATIVE EVALUATION OF REVERSAL OF SHEAR BOND STRENGTH ON BLEACHED ENAMEL USING ANTIOXIDANTS – SODIUM ASCORBATE, SALICYLIC ACID & N- ACETYL CYSTEINE -IN VITRO STUDY
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And

**Mrs. Dr. B. RAMAPRABHA** aged 43 years working as **Professor** in Department of Conservtive Dentistry & Endodontics at the college, having residence address at 191/5, Green Fields Apts. R-30A, Ambattur, Thirumangalam High Road, Mugappair, Chennai –101 (herein after referred to as the 'Principal Investigator')

And

**Mr. Dr. M.R. MANJUNATH MANDHIRA DOSS** aged 28 years currently studying as **Post Graduate student** in Department of Conservtive Dentistry & Endodontics, Tamilnadu Government Dental College and Hospital, Chennai - 3 (herein after referred to as the 'PG student and co- investigator').

Whereas the PG student as part of his curriculum undertakes to research on "COMPARATIVE EVALUATION OF REVERSAL OF SHEAR BOND STRENGTH ON BLEACHED ENAMEL USING ANTIOXIDANTS – SODIUM ASCORBATE, SALICYLIC ACID & N-ACETYL CYSTEINE – AN IN VITRO STUDY" for which purpose the Principal Investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard.

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- 8. The Principal Investigator shall suitably guide the student research right from selection of the research topic and area till its completion. However the selection and conduct of research, topic and area of research by the student researcher under guidance from the Principal Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.
- 9. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the PG student, under guidance from the Principal Investigator, the decision of the college shall be binding and final.
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**PG Student** 

**Student Guide** 

Witnesses

1.

#### ABSTRACT

**Aim:** The purpose of the study was to compare the effect of antioxidants [sodium ascorbate, salicylic acid and N- acetyl cysteine] on enamel-resin nanocomposite shear bond strength after bleaching with 30% carbamide peroxide.

**Materials & methods:** 60 labial enamel surfaces of maxillary central incisors were taken, divided into 6 groups (n=10). Group I(without bleaching, without antioxidants), Group II(without antioxidant, bleaching with 30% carbamide peroxide, composite bonding immediately), Group III(without antioxidant, bleaching with 30% carbamide peroxide, composite bonding after 7 days of storage in artificial saliva), Group IV(Bleaching with 30% carbamide peroxide followed by antioxidant sodium ascorbate application for 10 minutes, composite was built and cured immediately), Group V(Bleaching with 30% carbamide peroxide followed by antioxidant salicylic acid application for 10 minutes composite was built and cured immediately),Group VI(Bleaching with 30% carbamide peroxide followed by antioxidant salicylic acid application for 10 minutes composite was built and cured immediately),Group VI(Bleaching with 30% carbamide peroxide followed by antioxidant N- acetyl cysteine application for 10 minutes composite was built and cured immediately). All the teeth were subjected to shear bond testing.

**Results:** The shear bond strength data was analysed by ANOVA test and multiple comparisons by Post Hoc tests at a significance level of P < 0.05. The result of this study showed that for shear bond strength, group I (normal enamel) showed statistically significant difference when compared to group II (immediate bleaching) (p<0.05) and no statistically significant difference was seen with group III (bleaching after 7 days), group IV (sodium ascorbate), group V (salicylic acid) and group VI (N-acetyl cysteine) (p>0.05).

**Conclusion:** 1 week delayed bonding procedure and 10% sodium ascorbate antioxidant after bleaching shows a good reversal of reduced bond strength which is nearly equal to control. Treatment of bleached enamel surface with salicylic acid and N-acetyl cysteine gives a good reversal of bond strength, but less when compared to Sodium ascorbate.

Keywords: Bleaching, Antioxidants, Shear Bond Strength, Nano Composites

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## **INTRODUCTION**

Bleaching of discoloured teeth has been recognized as a conservative and safe treatment in restorative dentistry<sup>1</sup>. Bleached teeth may require adhesive restorations for enhancing esthetics, diastema closure or for management of caries. Composite restoration is the most commonly used adhesive restoration for anterior esthetics<sup>2</sup>.

Hydrogen peroxide and carbamide peroxide are the commonly used bleaching agents in dentistry. Both are effective in lightening discolored teeth<sup>1</sup>. When bonding is performed immediately after bleaching, hydrogen peroxide and carbamide peroxide (CP) bleaching agents alter the bond strength of composites to acid-etched enamel. This reduction in bond strength was found due to the presence of free radicals that interferes with resin attachment and inhibits resin polymerization<sup>11</sup>.

Delay in bonding of 24hrs to 4 weeks is recommended following bleaching, to avoid the clinical problems related to bleaching-mediated compromised bond strength<sup>7</sup>. This period is important for the reduction of oxygen free radicals and stabilization of dental color in order to obtain better aesthetic results when composite resin is to be used. However, this waiting period makes it impossible to perform restorative procedures immediately after bleaching.

To overcome this inconvenience, several methods have been proposed to reverse the compromised bond strength after bleaching, such as removal of superficial layer of enamel, treatment of the bleached enamel with alcohol before the restoration, use of adhesives containing organic solvents, and application of antioxidant agents, with varying degree of success. Of these, application of antioxidant agents before bonding is the most accepted technique in the reversal of compromised bond strength after bleaching<sup>22</sup>.

Enzymatic antioxidant agents include catalase and peroxidase. Some of the non-enzymatic agents such as plant derivatives like polyphenols(ascorbic acid, tannic acid, gallic acid, salicylic acid, quercetin)and aminoacid derivative like n-acetyl cysteine are potent antioxidants<sup>53</sup>. Sparing sodium ascorbate, there is no study in the literature describing the effect of either salicylic acid or n-acetyl cysteine on the bond strength of adhesive restorations to bleached enamel.

Various conventional mechanical test methods, such as shear, tensile, and flexural tests, have been used to assess dental adhesion. Tensile and shear tests are the ones most commonly used. Even though the validity of the shear test has been questioned, this test is frequently used due to its reproducible and relatively uncomplicated method<sup>46</sup>.

Hence the purpose of this study was to evaluate the effect of 3 antioxidant agents [Sodium ascorbate, Salicylic acid, N-acetyl cysteine] on reversal of the shear bond strengths of composite to enamel bleached with 30% carbamide peroxide.

#### AIM

The aim of this study was to evaluate the effect of 3 antioxidant agents [Sodium ascorbate, Salicylic acid, N-acetyl cysteine] on reversal of the shear bond strengths of composite restorations to enamel bleached with 30% carbamide peroxide.

#### **OBJECTIVE**

- 1. To determine if the shear bond strength of composite is significantly reduced, when restored over enamel bleached with 30% carbamide peroxide immediately and after 7 days.
- 2. To determine whether antioxidants will reverse the shear bond strength of composite to bleached enamel.
- 3. To compare 3 antioxidants, Sodium ascorbate(10%), Salicylic acid(0.38%), and N-acetyl cysteine (0.25%), on reversal of the shear bond strength of composite to bleached enamel.

### **REVIEW OF LITERATURE**

**Howard Frysh** [1989]<sup>13</sup>: conducted studies on chemistry of bleaching. Bleaching is a chemical process for whitening materials which is widely used. The three most prominent commercial bleaching processes are using peroxide, chlorine or chloride. Among these peroxide bleaching requires the least time and is most commonly used. Although bleaching processes are complex, the vast majority work by oxidation and the chemical process by which organic materials are eventually converted into carbondioxide and water.

**Haywood and Heymann [1989]**<sup>17</sup>: offered night guard vital bleaching as an alternative technique to the vital bleaching techniques in use. Accomplishing majority of the bleaching outside the clinic, saving the cost and chair time. The apparent safety and effectiveness of the technique and the lack of necessity for etching of enamel or use of caustic substances or any post treatment polishing were sighted as advantages of this technique. The authors reported that optimal lightening of color took about 6 weeks, although slight initial effects were noted as early as 2 weeks. The author also reported the technique to be most effective when treating yellow, orange or slight brown teeth such as those darkened by aging or mild tetracycline staining while darker grey, blue and brown stains did not respond as well.

**Rueggeberg et al [1990]**<sup>40</sup> examined the outcome of the oxygen inhibition on the curing potential of a commercially unfilled resin and its bond strength to etched bovine enamel. Both high monomer conversion and shear strength values resulted when specimens were cured under all-argon conditions. SEM evaluation showed that the inhibited layer present in room-air curing was both physically displaced by and absorbed into the overlying filled composite.

**Cvitko et al [1991]<sup>9</sup>** Bleaching with 35 percent hydrogen peroxide causes enamel surface changes, which result in lower bond strengths of composite resin. Although a previous SEM study showed that home bleaching with 10 percent carbamide peroxide does not cause such surface changes, the results of the study indicate that carbamide peroxide bleaching reduces the shear bond strength of composite to etched enamel. Removal of surface enamel, however, restores bond strengths to normal levels.

**K.C. Titley et al [1993]**<sup>48</sup> evaluated the adhesion of a resin composite to bleached and unbleached human enamel. Cylinders of a visible light-cured microfil resin were formed on and bonded to the flattened enamel surface of 15 human hemisected premolar teeth which had previously been subjected to three different treatments: (a) immersion in 35% hydrogen peroxide (HP) for 60 min, (b) immersion in 35% HP for 60 min followed by storage in distilled water for 1 day prior to resin application, and (c) immersion in saline (S) for 60 min. Specimens were stored in distilled water at 37°C for 7 days prior to shear bond strength testing. A total of 30 specimens were tested. Statistical analysis of the data indicated that there was a highly significant reduction in shear bond strength between HP- and saline-treated specimens. Water storage of HP-treated specimens for 1 day prior to resin application appeared to restore the adhesiveness but not to a point that was statistically significant. Scanning electron microscopic examination of randomly selected, fractured test specimens indicated that the reduction in bond strength may be related to alterations in the

ability of the resin to attach itself to the HP-treated surface and to possible effects of the HP on the resin itself.

**Rose RC et al [1993]**<sup>37</sup>: Reactive free radical species (R.) are associated with several forms of tissue damage and disease, and also with the process of aging. Protection is thought to be available in the form of endogenous compounds that react with and thereby "scavenge" the free radical (R.). Because many R. are reactive forms of oxygen, an effective scavenger is often referred to as an antioxidant. To be an effective antioxidant physiologically, a substance must have certain chemical and biological properties: it must be present in adequate amounts in the body; it must react with a variety of R.; it must be suitable for compartmentation; it must be readily available; it might be suitable for regeneration; it must be conserved by the kidneys; and it must have tolerable toxicity. Several water-soluble candidates are mentioned, with most having no more than one or two of the attributes listed. Ascorbic acid is discussed in detail, and an analysis is made that it has the properties mentioned.

**Dishman MV et al [1994]**<sup>11</sup>: evaluated the effects of an in-office type of bleaching regimen on the composite to enamel bond. The enamel on forty extracted human teeth was subjected to a 25% hydrogen peroxide bleaching treatment. Ten additional unbleached specimens served as controls (Group A). The 40 bleached specimens were divided into four equal groups (Groups B-E). Composite cylinders were bonded to the prepared surfaces at various time intervals following bleaching. Group B was bonded immediately, Group C at 1 d, Group D at 1 wk, and Group E at 1 mon post-bleaching. Mean shear bond strength values showed a significant decrease in bond strength for Group B. However, the bond strength returned to normal values after 1 d and remained normal for at least 1 mon. Scanning electron microscope examination showed an apparent decrease in the number of resin tags present in the enamel/composite interface for Group B compared with the other groups including controls.Polymerization inhibition of the resin bonding agent is the likely mechanism for the effects of the bleaching on bond strength.

**Mc Craekan MS et al [1995]<sup>29</sup>:** evaluated the effect of three 10% carbamide peroxide home bleaching agents on the enamel surface hardness and on theresin to enamel tensile bond strength. Eighty extracted bicuspid crowns were divided into four groups (three bleaching agents and on control) and treated with the bleaching agents for 5 consecutive days for the durations specified by the manufacture each day.Specimens in control group were subjected to no treatment. A bonding site on the buccal surface of each crown was etched with phosphoric acid and orthodonticbracket bonded in place. Specimens were thermocycled and loaded to failure in a universal – testing machine. Five hardness specimens per group were measured prebleaching and after 5 days exposure. The authors found no significant differences in resin-enamel tensile bond strength between the study and control groups. There was also no difference in pre or post bleaching Knoop's hardness hardness values for the four groups. The authors concluded that short-term regimens of 10% carbamide peroxide did not significantly affect enamel surface hardness or bonding ability.

**Josey AL et al [1996]**<sup>21</sup>: examined the effect of a nightguard vital bleaching procedure on enamel surface morphology and the shear bond strength of a composite resin luting cement to enamel. Light microscopy investigation

suggested the bleaching process resulted in a loss of mineral from enamel which was evident 24 h after bleaching and was sustained following 12 weeks storage in artificial saliva. Scanning electron microscopy showed a definite change in the surface texture of the bleached enamel surface. Acid etching of the bleached enamel surface produced loss of prismatic form and the enamel appeared overetched. The mean shear bond strength between composite resin luting cement and etched enamel tended to be lower for bleached enamel surfaces, however no significant difference in shear bond strength was noted between control and experimental groups. The results of this study suggest that bleaching resulted in changes to the surface and subsurface layers of enamel. Although surface changes were observed in the etched enamel, the shear bond strength of composite resin luting cement to etched bleached enamel appeared to be clinically acceptable.

**Swift EJ** [1997]<sup>44</sup>: reviewed the implications of bleaching procedures on restorative dentistry. The effects of tooth whitening systems on the bond strength, marginal integrity, color and other properties of restorative materials are reviewed. Also, clinical considerations in combining bleaching and aesthetic restorative techniques are discussed.

**Pashley et al [1999]**<sup>34</sup>: reviewed the adhesion testing of bonding agents with the adhesion substrate, dentin, the variables involved in etching, priming and bonding, storage variables and testing variables. They concluded that dentin bond strength were often higher than resin bonds made to acid etched enamel. These higher dentin bond strengths develop non uniform stress distribution in dentin during in vivo testing, causing cohesive failures in the substrate than in the

bonded interface . Thus conventional bond testing methods can no longer be used. New standardized methods have to be developed for bonding procedures.

**Spyrides GM et al [2000]**<sup>43</sup>: evaluated the effect of three bleaching regimens: 35% hydrogen peroxide (HP), 35% carbamide peroxide (CP), and 10% CP, on dentin bond strengths. One hundred and twenty fresh bovine incisors were used in this study. For the groups bonded immediately after bleaching, one-way analysis of variance (ANOVA) followed by the Duncan's post hoc test revealed a statistically significant reduction in bond strengths in a range from 71% to 76%. For the groups bonded at 1 week, one-way ANOVA showed that group B (35% HP for 30 min) resulted in the highest bond strengths, whereas 10% CP resulted in the lowest bond strengths. Student's t-test showed that delayed bonding resulted in a significant increase in bond strengths for groups B (35% HP) and C (35% CP); whereas the group bleached with 10% CP (group D) remained in the same range obtained for immediate bonding. Storage in artificial saliva also affected the control group, reducing its bond strengths to 53% of the original.

**Morris et al** [2001]<sup>31</sup>: evaluated the effect of 5% NaOCl and RC-prep treatment on the bond strength of resin cement (C& B metabond), and to determine the effectiveness of ascorbic acid to restore bond strengths. Their results demonstrated that both 5% NaOCl and RC-prep produced significantly large reductions in resin-dentin bond strength and the reductions could be completely reversed by the application of either 10% ascorbic acid or 10% sodium ascorbate.

Yatabe M et al [2001]<sup>54</sup>: evaluated the effect of the reducing agent on the oxygen-inhibited layer of the cross-linked reline material. A commercial autopolymerizing reline resin containing 1,6-hexanediol dimethacrylate as crosslinking agent and 1 wt.% sodium sulphite solution as a reducing agent was prepared. The inhibited layer was observed using an optical transmission microscope, after application of sodium sulphite for 0, 1, 5 and 15 min and curing for 10 min in air. As a control, the reline material was cured on sealing from air. Moreover, the three-point flexural strength test was performed under the same conditions. The fracture was then observed using scanning electron microscopy (SEM). Although hardness of the inhibited layer was enhanced after the application of the reducing agent, the layer was still observed. The flexural strength of the control and the groups after application of the reducing agent was significantly higher than the group without reducing agent. SEM examination revealed many polymer beads on the group without reducing agent, whereas polymer beads could not be observed on the groups with reducing agent. These results indicated that the application of sodium sulphite was effective in hardening the surface unpolymerized zone.

**Cavalli V et al [2001]**<sup>7</sup>: concluded that carbamide peroxide bleaching agents significantly affect the bond strength of composite to bleached enamel. They evaluated the effects of bleaching regimen with different carbamide peroxide concentrations and post-treatment times on composite bond strength to enamel. Two hundred and four flat buccal and lingual enamel surfaces obtained from erupted sound third molars were randomly divided into 17 groups (n = 12). Sixteen experimental groups comprised the evaluation of four carbamide

peroxide home bleaching agents (Opalescence 10%-20% and Whiteness 10%-16%) and four time intervals after bleaching (one day, one, two and three weeks). Specimens of control group were not submitted to bleaching and were stored in artificial saliva at 37 degrees C for 10 days. The specimens of experimental groups were exposed to once daily application of carbamide peroxide for six hours for 10 consecutive days. After each daily treatment and post-bleaching, the specimens were stored in artificial saliva solution. Bonds were formed with Scotchbond MP and Z-100 composite resin, and shear bond test was carried out 24 hours after adhesive-composite application. Two-way ANOVA showed that the bond strengths were significantly different (p < 0.05). For the first two weeks post-bleaching, the bond strengths of resin to enamel were low. After a lapse of three weeks, the bond strength returned to that of the untreated control group.

Lai SC et al [2001]<sup>23</sup>: The mechanism responsible for hydrogen peroxide or sodium-hypochlorite induced reductions in dentin bond strength is unknown. They tested the hypothesis that these oxidizing agents were responsible for reduction in bond strength by attempting to reverse the effect with sodium ascorbate, a reducing agent. Human dentin was treated with these oxidants before or after being acid-etched and with or without post-treatment with sodium ascorbate. They were bonded with either Single Bond or Excite. Hydrogen peroxide reduced the bond strengths of both adhesives, while sodium hypochlorite produced reduction in adhesion of only Single Bond (p < 0.05). Following treatment with sodium ascorbate, reductions in bond strength were reversed. Transmission and scanning electron microscopy showed partial removal of the demineralized collagen matrix only by sodium hypochlorite. The observed compromised bond strengths cannot be attributed to incomplete

deproteinization and may be related to changes in the redox potential of the bonding substrates.

Lai SC et al  $[2002]^{24}$ : studied about oxygen inhibits polymerization of resinbased materials. They hypothesized that compromised bonding to bleached enamel can be reversed with sodium ascorbate, an anti-oxidant. Sandblasted human enamel specimens were treated with distilled water (control) and 10% carbamide peroxide gel with or without further treatment with 10% sodium ascorbate. They were bonded with Single Bond (3M-ESPE) or Prime&Bond NT (Dentsply DeTrey) and restored with a composite. Specimens were prepared for microtensile bond testing and transmission electron microscopy after immersion in ammoniacal silver nitrate for nanoleakage evaluation. Bond strengths of both adhesives were reduced after bleaching but were reversed following sodium ascorbate treatment (P < 0.001). Resin-enamel interfaces in bleached enamel exhibited more extensive nanoleakage in the form of isolated silver grains and bubble-like silver deposits. Reduction of resin-enamel bond strength in bleached etched enamel is likely to be caused by a delayed release of oxygen that affects the polymerization of resin components.

**Rwei-Fen S. Huang et al [2002]^{41}:** conducted study on the role of some radical scavengers (N-acetylcysteine, vitamin C, vitamin E) on the reduction of HcyT-induced apoptosis. Preincubation of cells with N-acetylcysteine (NAC; 5 mmol/L) for 2 h significantly reduced HcyT-promoted apoptosis measured by membrane phosphatidylserine exposure. The reduction of HcyT-induced apoptosis by NAC, Vit C or Vit E occurred simultaneously with a significant decrease in intracellular H<sub>2</sub>O<sub>2</sub> levels and reduced caspase-3 enzymatic activity.

This 1\*findings suggest that antioxidant pretreatment with NAC, Vit C or Vit E exerts more beneficial effects on reducing apoptotic cell damage induced by homocysteine thiolactone.

Kaya AD et al [2003]<sup>22</sup>: claimed that a certain waiting period is needed prior to restoration to reach the original bond strength values prior to bleaching. They determined the effect of anti-oxidant applications on the bond strength values of resin composites to bleached dentin. Ninety human teeth extracted for orthodontic purposes were used in the study. The labial surface of each tooth was ground and flattened until dentin appeared. The polished surfaces were subjected to nine different treatments: 1) bleaching with gel (35% Rembrandt Virtuoso); 2) bleaching with gel + 10% sodium ascorbate (SA); 3) bleaching with gel + 10%butylhydroxyanisole (BHA); 4) bleaching with sol (35% hydrogen peroxide); 5) bleaching with sol + 10% sodium ascorbate; 6) bleaching with sol + 10% BHA; 7) bleaching with gel + immersed in artificial saliva for seven days; 8) bleaching with sol + immersed in artificial saliva for seven days; 9) no treatment. After bonding application, the resin composite in standard dimensions was applied to all specimens. The teeth were stored in distilled water at 37 degrees C for 24 hours and a universal testing machine determined their resistance to shear bond strength. The data was evaluated using ANOVA and Duncan tests. Bond strength in the bleached dentin group significantly decreased compared to the control group. On the other hand, the antioxidant treatment had a reversal effect on the bond strength to dentin. After the bleaching treatment, the 10% sodium ascorbate application was effective in reversing bond strength. In the samples where antioxidant was applied after the bleaching process, bonding strength in dentin tissue was at the same level as those teeth kept in artificial saliva for seven days.

Y Shimada et al [2003]<sup>55</sup>: conducted studies on Shear Bond Strength of Current Adhesive Systems to Enamel, Dentin and Dentin-Enamel Junction Region. This study investigated the bonding of current -reSuT adhesives to the region approximating the dentin-enamel junction (DEJ), where the etch pattern to enamel or dentin may be different. Three kinds of tooth substrates were chosen for testing: enamel, dentin and the DEJ region. A self-etching primer system (Clearfil SE Bond) and two total-etch wet bonding systems (Single Bond and One-Step) were used. Each tooth region was bonded with one of the adhesive systems, and a resin composite and was subjected to a micro-shear bond test. In addition, morphological observations were performed on debonded specimens and etched surfaces using confocal laser scanning microscopy (CLSM). CLSM observations showed that the DEJ region was etched more deeply by phosphoric acid gel than enamel or dentin, suggesting that the action of acid etch seemed to be more intense on the DEJ. However, no statistically significant differences of shear bond strength values were observed between the DEJ region and enamel or dentin, or the adhesive systems used (p>0.05). Bonding to the DEJ was potentially as good as that of enamel or dentin.

**Thomas Attin et al [2004]**<sup>47</sup>: reviewed the available information concerning the effects of peroxide releasing bleaching agents on dental restorative materials and restorations. they revealed that bleaching therapies may have a negative effect on physical properties, marginal integrity, enamel and dentin bond strength, and color of restorative materials as investigated in numerous in vitro studies. However, there are no reports in literature indicating that bleaching may exert a negative impact on existing restorations requiring

renewal of the restorations under clinical conditions.and they concluded that bleaching may exert a negative influence on restorations and restorative materials.

**Luiz C et al [2004]**<sup>26</sup>: evaluated the effect of a 10% carbamide peroxide bleaching gel on the bond strength of a resin-based composite system to dentin. Dentin disks were obtained from human third molars. Two disks were exposed to a 10% carbamide peroxide bleaching gel for 2 h/d for 21 days, whereas two disks were not treated and served as controls. After the treatment phase, the dentin disks were retrieved and a resin-based composite system was applied to the specimens following manufacturer's instructions. Composite dentin "sticks" were obtained and tested in microtensile mode. Bond strength values were obtained for treated (n = 20) versus nontreated (n = 26) dentin and were analyzed statistically. Mean bond strengths values (SD) were 29.9 MPa (6.2) and 39.2 MPa (5.8) for treated and nontreated dentin specimens, respectively (p < .001). The results of their study suggest that nightguard (home) bleaching with 10% carbamide peroxide for 2 h/d for 21 days significantly affects resin-den tin bond strengths when dentin is exposed to bleaching material.

**Kaya AD et al [2004]**<sup>50</sup>: comparatively investigated the effect of antioxidant treatment and delayed bonding after bleaching with three different concentrations of carbamide peroxide (CP) on the shear bond strength of composite resin to enamel. One hundred flat buccal enamel surfaces obtained from bovine incisors were divided into three bleaching groups of 10, 16 and 22% CP (n = 30) and a control group. Each bleaching group was then divided into three subgroups (n = 10). Group 1 consisted of specimens bonded immediately after bleaching.

Group 2 specimens were treated with antioxidant agent, 10% sodium ascorbate, while Group 3 specimens were immersed in artificial saliva for 1 week after bleaching. Specimens in the control group were not bleached. After the specimens were bonded with Clearfil SE Bond and Clearfil AP-X, they were thermocycled and tested in shear until failure. Fracture analysis of the bonded enamel surface was performed using scanning electron microscope. The shear bond strength data was subjected to one-way analysis of variance followed by Duncan's multiple range test at a significance level of P < 0.05. Shear bond strength of composite resin to enamel that was bonded immediately after bleaching with 10, 16 and 22% CP was significantly lower than that of unbleached enamel (P < 0.05). For all three bleaching groups, when the antioxidant-treated and delayed bonding (1 week) subgroups were compared with the control group, no statistically significant differences in shear bond strength were noted (P < 0.05).

Hassimotto NMA et al [2005]<sup>16</sup>: Fruits, vegetables, and commercial frozen pulps (FP) consumed in the Brazilian diet were analyzed for antioxidant activities using two different methods, one that determines the inhibition of copper-induced peroxidation of liposome and another based on the inhibition of the co-oxidation of linoleic acid and beta-carotene. The anthocyanin-rich samples showed the highest, concentration-dependent, antioxidant activities in both systems. In the liposome system, at both 10 and 50 microM gallic acid equivalent (GAE) addition levels, the neutral and acidic flavonoids of red cabbage, red lettuce, black bean, mulberry, Gala apple peel, jambolao, acai FP, mulberry FP, and the acidic flavonoids of acerola FP showed the highest antioxidant activities (>85% inhibition). In the beta-carotene bleaching system, the samples cited

above plus red guava gave inhibition values >70%. On the other hand, some samples showed pro-oxidant activity in the liposome system coincident with a low antioxidant activity in the beta-carotene system. There was no relationship between total phenolics content, vitamin C, and antioxidant activity, suggesting that the antioxidant activity is a result of a combination of different compounds having synergic and antagonistic effects.

**Immerz I et al [2006]<sup>19</sup>:** there is a clear significant relationship for both concentration and duration of exposure for CP bleaching agents. The final shade change is independent of the concentration of bleaching agent, with time as the dominant variable. Higher concentrations of CP that have not been investigated previously may be a treatment option for esthetic improvement of shade where time is at a premium, but caution must be exercised in view of the possible increased incidence of sensitivity.

**Sulieman M et al [2006]**<sup>45</sup>: investigated the tooth whitening effects of various concentrations of carbamide peroxide (CP) gels and 6% hydrogen peroxide (HP) whitening strips used on an intrinsic, in vitro stain model in a simulated home-applied bleaching protocol. Extracted third molars were sectioned and stained to Vita shade C4 using a standardized tea solution. Stained specimens were then bleached with 10, 15, 20, 22, and 30% CP gels applied in custom-made trays for 8-hour sessions for 14 days. A 6% HP whitening strip product was also tested in a regimen of twice-daily 30-minute treatments for 14 days. Shades were assessed at baseline and at 2, 5, 7, 10, and 14 days of treatment using a shade guide (SG) and a shade vision system (SVS), recorded as shade guide unit (SGU) recordings using a chromometer. This in vitro study

supports the limited data available from the very few available randomized controlled clinical trials indicating that CP and HP home-use bleaching systems can achieve considerable tooth whitening outcomes, although at different rates, which appear to be concentration dependent. There is a clear significant relationship for both concentration and duration of exposure for CP bleaching agents. The final shade change is independent of the concentration of bleaching agent, with time as the dominant variable. Higher concentrations of CP that have not been investigated previously may be a treatment option for esthetic improvement of shade where time is at a premium, but caution must be exercised in view of the possible increased incidence of sensitivity.

**Bulut H, Turkun M, Kaya AD [2006]<sup>6</sup>:** The purpose of this study was to investigate the effect of antioxidant treatment and delayed bonding on the shear bond strength of metal brackets bonded with composite resin to human enamel after bleaching with carbamide peroxide (CP).Eighty recently extracted premolars were divided into an experimental group (n = 60), which was bleached with 10% CP, and a control group (n = 20), which was not bleached. The experimental group was further divided into 3 groups. Specimens in group 1 (n =20) were bonded immediately after bleaching; specimens in group 2 (n = 20) were bleached, then treated with 10% sodium ascorbate, an antioxidant agent, and then bonded; group 3 specimens (n = 20) were bleached, then immersed in artificial saliva and held for 1 week before bonding. The specimens were debonded, and the enamel surfaces and bracket bases were examined with a stereomicroscope. The adhesive remnant index was used to assess the amount of resin left on the enamel surfaces after debonding. The shear bond strength data were subjected to 1-way analysis of variance. Multiple comparisons were performed with the Bonferroni test. The level of significance was established at P < .05 for all statistical tests. Shear bond strength of brackets bonded immediately after bleaching with 10% CP was significantly lower than that of brackets bonded to unbleached enamel (P < .05). No statistically significant differences in shear bond strength were noted when the antioxidant-treated and delayed bonding groups were compared with the control group (P > .05).Bleaching with 10% CP immediately before bonding reduces the bond strength of composite resin to enamel. Treating the bleached enamel surface with 10% sodium ascorbate or waiting 1 week reverses the reduction.

**Elizabeth Cvitko et al [2007]<sup>9</sup>:** Bleaching with 35 percent hydrogen peroxide causes enamel surface changes, which result in lower bond strengths of composite resin. Although a previous SEM study showed that home bleaching with 10 percent carbamide peroxide does not cause such surface changes, the results of our study indicate that carbamide peroxide bleaching reduces the shear bond strength of composite to etched enamel. Removal of surface enamel, however, restores bond strengths to normal levels.

**Jason Jonghyuk Lee et al [2007]**<sup>20</sup>: conducted studies on the effect of storage medium and sterilization on dentin. One of the way in which clinicians select products for their practices is to compare the products performance both in invivo and invitro studies. One method used often to assess the performance of dental adhesives is to test how well they bond to dentin by measuring their composite-to-dentin bond strengths. When dental specimens are used for in vitro adhesive studies, one of the factors hat can affect the study outcome is how the specimens are stored and sterilized. The Centers for Disease Control and

Prevention (CDC) has adopted guidelines for infection control of extracted teeth used for research and teaching. These guidelines require that teeth not containing amalgam be heat-sterilized by an autoclave cycle for 40 minutes before use. Teeth that contain amalgam should be stored in 10 percent formalin for two weeks before use. Although autoclaving does not seem to alter the tooth's physical properties sufficiently for research purposes, it is unknown whether autoclaving affects the chemical and micromechanical relationship between dentin and dental. In this study, we investigated the effect of storage conditions and sterilization methods on the composite-to-dentin bond strength. Within the rotations of this study, we can draw the following conclusions. When storage solutions alone are compared, 0.9 percent NaCl and 5.25 percent NaClO resulted in significantly lower SBS values an those obtained with H<sub>2</sub> O<sub>2</sub>. Therefore, investigators should not use these solutions as the storage medium when teeth are to be used for dentin bonding studies. Sterilization with an autoclave negatively affected the SBS values of specimens stored initially in H<sub>2</sub> O<sub>2</sub> or 10 percent formalin, while sterilization with formalin alone had no significant effect. Although storage in H<sub>2</sub> O<sub>2</sub> resulted in the highest nominal SBS values among the sample studied, storage and simultaneous sterilization in 10 percent formalin appears to be the most logical treatment for extracted teeth that will be used in dentin bonding research.

**Gökçe B et al** [2008]<sup>14</sup>: comparatively investigated the effect of antioxidant treatment and delayed bonding after bleaching with carbamide peroxide on the shear bond strength (SBS) of a luting resin to enamel. Forty flat enamel surfaces were prepared from freshly extracted human molars using a low speed diamond saw, then divided into three bleaching groups (n = 10/group) and a control group

(n = 10). Group 1 consisted of specimens bonded immediately after bleaching. Group 2 specimens were treated with an antioxidant agent, 10% sodium ascorbate, while Group 3 specimens were immersed in artificial saliva for 1 week after bleaching. Specimens in Group 4 were not bleached, but immersed in artificial saliva for 1 week before bonding. Forty ceramic blocks (Empress 2, Ivoclar) were prepared and luted to teeth using a dual-curing resin cement (Variolink II, Ivoclar). The specimens were thermocycled and the SBS tests were performed using a universal testing machine (crosshead speed: 0.5 mm/min). Fracture analysis of the bonded surfaces was done using a scanning electron microscope. Statistical analysis was carried out by Kruskall-Wallis and Mann-Whitney U-tests.While the samples that were immediately bonded after bleaching (Group I) demonstrated significantly lower shear bond strengths and 10% sodium ascorbate group (Group II) demonstrated significantly higher bond strengths than control group samples (p < 0.05), no significant differences were found among delayed bonded group and control group (p > 0.05). Using sodium ascorbate with a concentration of 10% may be reliable for reversing the compromised bond strength.

**Varunraj Sendamangalam et al [2010]**<sup>52</sup>: examined the antimicrobial and antioxidant activity of phenolic compounds on Streptococcus mutans, and investigated the effect of the natural polyphenols on biofilm formation of S. mutans, and understand the anti-biofouling mechanisms of the natural polyphenols. Four natural sustainable polyphenols, gallic acid, tannic acid, quercetin and salicylic acid, were investigated for their antimicrobial and antioxidant activities against Streptococcus mutans. Ascorbic acid, well known for its strong antimicrobial and antioxidant activity, was used to compare the

results. First antimicrobial effect of the polyphenols was assessed using the plate dilution assay. Minimum inhibitory concentration (MIC) of each polyphenol has been determined. Salicylic acid showed the highest MIC, 3.8 mg/mL, and tannic acid showed the lowest, 0.4 mg/mL, indicating the weakest and strongest polyphenols respectively tested in our study. Antioxidant capacities were evaluated using the DMPD and ABTS decolorizing assays. Antioxidant capacity of each polyphenol was measured at its MIC concentration to see if there is a relationship between antimicrobial activity and antioxidant capacity. Results suggest that these polyphenols were good antimicrobials and also very good antioxidants. Although some conflicting results were observed between DMPD and ABTS methods, polyphenols with high antioxidant capacities also showed considerable antimicrobial activities suggesting that antioxidant capacity contributes to the antimicrobial effect of polyphenols.

**Turkun M, Kaya AD et al [2010]^{51}:** investigated the effect of antioxidant treatment and delayed bonding after bleaching with three different concentrations of carbamide peroxide (CP) on the shear bond strength of composite resin to enamel. One hundred flat buccal enamel surfaces obtained from bovine incisors were divided into three bleaching groups of 10, 16 and 22% CP (n = 30) and a control group. Each bleaching group was then divided into three subgroups (n = 10). Group 1 consisted of specimens bonded immediately after bleaching. Group 2 specimens were treated with antioxidant agent, 10% sodium ascorbate, while Group 3 specimens were immersed in artificial saliva for 1 week after bleaching. Specimens in the control group were not bleached. After the specimens were bonded with Clearfil SE Bond and Clearfil AP-X, they were thermocycled and tested in shear until failure. Fracture analysis of the bonded enamel surface was

performed using scanning electron microscope. The shear bond strength data was subjected to one-way analysis of variance followed by Duncan's multiple range test at a significance level of P < 0.05. Shear bond strength of composite resin to enamel that was bonded immediately after bleaching with 10, 16 and 22% CP was significantly lower than that of unbleached enamel (P < 0.05). For all three bleaching groups, when the antioxidant-treated and delayed bonding (1 week) subgroups were compared with the control group, no statistically significant differences in shear bond strength were noted (P < 0.05).

Masoomeh Hasani Tabatabaei et al [2011]<sup>28</sup>: investigated the effect of the antioxidant treatment on the micro-shear bond strength of a composite resin with a clinically acceptable antioxidant usage time taken into account. Using in vitro techniques, the effect of the antioxidant sodium ascorbate (SA) was evaluated on the micro-shear bond strength of a hybrid composite resin (Tetric® A2 Ivoclar Vivadent) to dentin, which was bleached with 35% carbamide peroxide (Opalescence Quick, Ultradent Products Inc). Thirty-five intact flat buccal dentin surfaces from bovine incisors were randomly assigned to five groups which were subjected to the following treatment protocols: group 1, bleached for 45 min and bonded immediately afterwards; groups 2 and 3, bleached and then treated with 10% SA for 10 and 5 min before bonding, respectively; group 4, stored in distilled water for seven days after bleaching and before bonding; group 5, received no bleaching or antioxidant treatment. After the bonding procedure, specimens were subjected to a micro-shear bonding test. Data were analyzed by ANOVA and a post-hoc Tukey's test. One-way ANOVA revealed significant differences in bond strength among the five groups. It was found that the shear bond strength was reduced by carbamide peroxide bleaching,

and that the antioxidant SA was ineffective at reversing the composite strength at the concentrations and treatment times examined.

Lima AF, Fonseca FM, et al [2011]<sup>25</sup>: evaluated the influence of bleaching on bond strength to enamel and subjacent dentin, and to determine whether a reduced application time (1 min) of the antioxidizing agent can obviate the compromised bond strength after bleaching. One hundred twelve bovine incisors were obtained, and the enamel and dentin surfaces were standardized to a thickness of 1 mm. The specimens were divided into two control groups (enamel and dentin without treatment) and 12 experimental groups each group contain (n=10), in which 6 groups bleached with the concentration of bleaching agent 16% carbamide peroxide and time interval between bleaching and restorative procedures is 24 hrs; 24 hrs + sodium ascorbate (SA) 10%/ for 1 min and 14 days and substrate (enamel or dentin). other 6 groups the concentration of bleaching agent is 35% hydrogen peroxide and time interval between bleaching and restorative procedures is 24 hrs; 24 hrs + sodium ascorbate (SA) 10%/ for 1 min and 14 days and substrate (enamel or dentin). All samples were submitted to the bleaching treatment on enamel. After the stipulated interval of time, a micro shear bond strength test was performed with universal testing machine. The bleaching treatment with 16% carbamide peroxide and 35% hydrogen peroxide compromised the bond strength to the enamel when the restoration was performed 24 h after the end of bleaching. The shear bond strength to dentin was not affected by bleaching when the restoration was performed 24 h after the end of bleaching. The Sodium ascorbate 10% application for 1 min obviates the

detrimental effect of bleaching on bond strength. The bleaching performed on enamel does not affect the bond strength to the subjacent dentin.

## MATERIALS AND METHODS

### **ARMAMENTARIUM (FIG. 2)**

- 60 freshly extracted maxillary central incisors
- Aluminium oxide abrasive paper ( 600 grit )
- 10% formalin
- Ultrasonic scaler
- O Diamond disk
- Self cure pink acylic material
- Metal mould (2\*4cm size)
- Polytetra fluroethylene mould with 2mm internal diameter and 1mm thickness
- Instron universal testing machine(5566 series, Instron, Canton, Mass., London, UK)
- Distilled water
- Teflon coated instrument
- Micro brush
- Visible light cure unit(Heraeus Kulzar Hulix model 2000)
- Artificial saliva

### **MATERIALS** (FIG. 2, 3 & 4)

- 30% carbamide peroxide(ivoclar vivadent)
- 10% Sodium ascorbate (Nutribiotic)
- 0.38% Salicylic acid (MEDISCA)
- 0.25% N-Acetyl cysteine (SRLchem Mumbai)
- Nanohybrid resin composite material (ivoclar vivadent)
- 37% phosphoric acid gel (ivoclar vivadent)
- Tetric N-bond 5<sup>th</sup> generation bonding agent(ivoclar vivadent)

### **METHODOLOGY**

60 freshly extracted, human central incisors due to periodontal reasons, which were free of caries, crack, fractures or other defects were collected (FIG. 1). Teeth were cleaned with ultrasonic scaler and stored in 10% formalin. The individual teeth enamel were polished with prophy paste and labial surfaces polished with aluminium oxide abrasive paper (600 grit) to creat a flat enamel surface. 60 teeth were embedded in acrylic blocks (2\*4cm) with labial surface exposed (FIG. 5) and divided into 6 groups of 10 specimens each (FIG. 12).

#### Group - I

Without bleaching and without antioxidant, composite was built (2mm diameter/1mm height) and cured - Normal enamel.

#### Group - II

Bleaching was done with 30% carbamide peroxide (half an hr) and without antioxidants application, composite was built and cured – Immediately after bleaching.

#### <u>Group – III</u>

Bleaching was done with 30% carbamide peroxide (half an hr) and specimens stored for 1 week in artificial saliva. After 1 week without anti oxidants application, composite was built and cured.

Artificial saliva (prepared in grams/litre) according to McKnight – Hanes, Whiteford[1992],

• Methyl – P- Hydroxybenzoate[2g],

- Sodium carboxy methylcellulose [10g],
- KCl [0.625g],
- MgCl<sub>2</sub>.6H<sub>2</sub>O [0.059g],
- K<sub>2</sub>HPO<sub>4</sub>[0.166g],
- KH<sub>2</sub>PO<sub>4</sub>[0.36g],
- Distilled water to make volume of 1 litre,
- The Ph of artificial saliva was adjusted to 6.75 with KOH

#### Group - IV

Bleaching was done with 30% carbamide peroxide (half an hr) followed by application of 10% sodium ascorbate for 10 minutes and then composite was built and cured immediately.

#### Group - V

Bleaching was done with 30% carbamide peroxide (half an hr) followed by application of 0.38% salicylic acid for 10 minutes and, then composite was built and cured immediately.

#### <u>Group – VI</u>

Bleaching was done with 30% carbamide peroxide (half an hr) followed by application of 0.25% N-acetyl cysteine for 10 minutes. Then, composite was built and cured immediately.

#### PREPARATION OF 10% SODIUM ASCORBATE SOLUTION (FIG. 7)

- 1. Sodium ascorbate is water soluble. Solution was prepared by mixing powder in distilled water.
- 2. 10% sodium ascorbate 10 gm/100ml distilled water

#### PREPARATION OF 0.38 %( 3.8mg/ml) SALICYLIC ACID (FIG. 7)

- Salicylic acid is insoluble in normal water however soluble in hot water. Solution was prepared by mixing powder with distilled water, kept in the hot water bath and stirred well every 5 sec until the powder become completely soluble in water.
- 2. 0.38% salicylic acid 3.8mg/ml
  - 380mg/100ml in distilled water

#### PREPARATION OF 0.25% N-ACETYL CYSTEINE (FIG. 7)

- N-acetyl cysteine is soluble in water. Solution was prepared by mixing powder in distilled water
- 2. 0.25% n-acetyl cysteine 250mg/100ml in distilled water

#### STANDARDIZATION OF BONDING SURFACE & COMPOSITE PLACEMENT

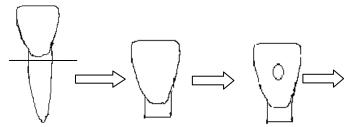
Prefabricated polytetrafluroethylene sheet mould with iris of 2mm internal diameter and 1mm thickness was placed on the prepared buccal surfaces of all the specimens with the help of double side adhesive sticker to get a standardized bonding surface of 2mm diameter on enamel before bonding procedure (FIG. 9).

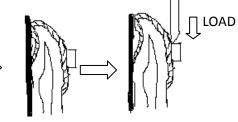
Composite build up of 2mm diameter and 1mm height was done in all the groups and bonded specimens were immersed in distilled water until testing (FIG. 10, 11).

#### SHEAR BOND STRENGTH EVALUATION (FIG. 13)

Each specimen with acrylic mould was attached to jig of universal testing machine. Shear testing was performed with a mono-angled chisel, with the edge closely aligned to the bonding interface and perpendicular to the longitudinal axis of the resin composite cylinder and gently held flush against the enamel composite interface and tested. Shear force was applied by Instron universal testing machine at a cross head speed of 1mm/ min.

# DIAGRAM FOR SHEAR BOND STRENGTH TEST



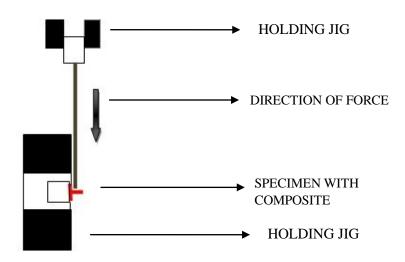


#### Central incisor

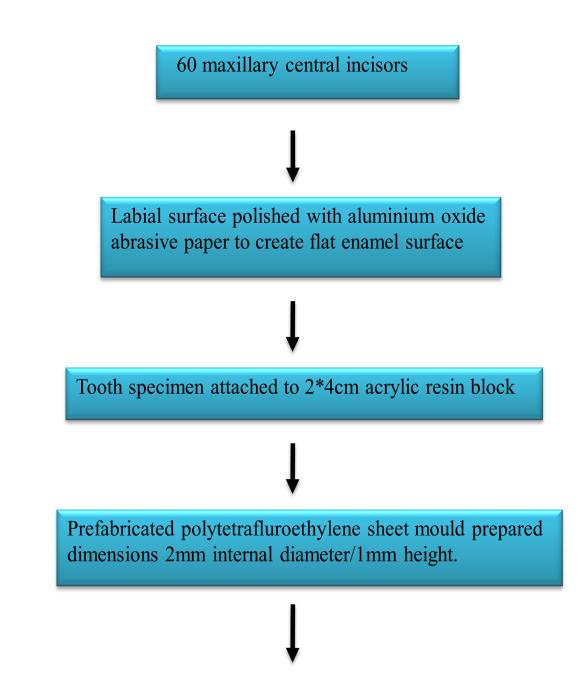
Crown part

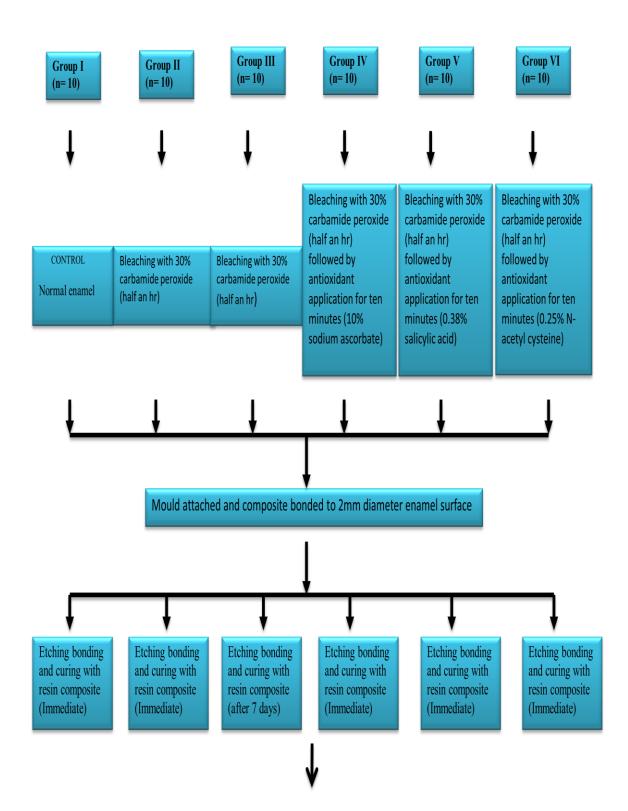
Resin composite with diameter 2mm bonded to flat enamel surface

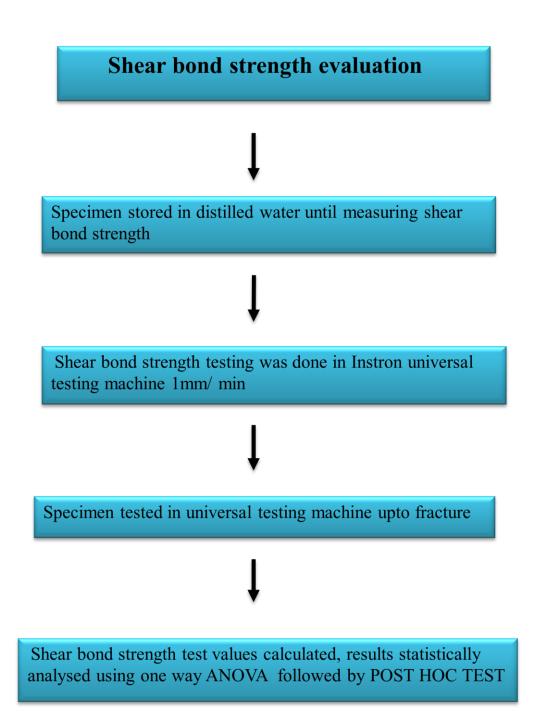
Shear load applied at the interface of enamel and composite



# PROCEDURAL FLOW CHART





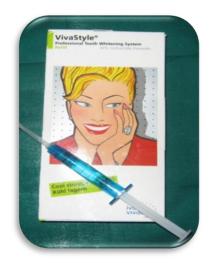




TEETH STORED IN 10% FORMALIN (FIG.1)



ARMAMENTARIUM (FIG.2)



30% CARBAMIDE PEROXIDE BLEACHING AGENT (FIG.3)



ETCHING, BONDING & COMPOSITE MATERIAL (IVOCLAR VIVADENT) (FIG.4)



TOOTH SPECIMEN ATTACHED TO THE RESIN BLOCK [2\*4cm] (FIG.5)



APPLICATION OF 30% CARBAMIDE PEROXIDE BLEACHING AGENT TO THE ENAMEL (FIG.6)



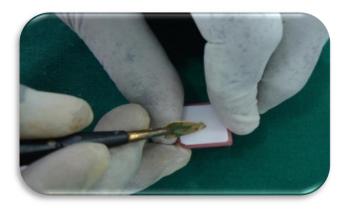
### PREPARATION OF ANTIOXIDANTS SOLUTION (FIG.7)



ANTIOXIDANT APPLICATION FOR 10 MINUTES (FIG.8)



PREFABRICATED POLYTETRAFLUROETHYLENE SHEET MOULD WITH IRIS OF 2MM INTERNAL DIAMETER AND 1MM THICKNESS WAS PLACED ON THE PREPARED BUCCAL SURFACES IN ALL THE SPECIMENS (FIG.9)



COMPOSITE BUILT UP OF 2MM DIAMETER AND 1MM HEIGHT WAS DONE (FIG.10)



FINAL SPECIMEN (FIG.11)



#### SPECIMENS STORED IN DISTILLED WATER STILL TESTING (FIG.12)



SHEAR BOND STRENGTH TESTING WAS DONE IN INSTRON UNIVERSAL TESTING MACHINE 1mm/ min (FIG.13)

## RESULTS

# FOR SHEAR BOND STRENGTH

In the present study the results of different groups observed were as follows:

Shear load at which the composite cylinder fracture (MPa) TABLE-1:

GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI
30.65	17.99	28.41	30.46	26.47	22.31
33.17	13.22	26.62	24.93	31.69	30.41
30.82	21.23	33.09	28.01	29.91	27.99
30.13	16.62	31.62	22.41	25.89	29.25
26.41	20.25	25.78	29.79	30.22	27.34
31.23	21.03	30.20	32.10	23.91	22.71
29.02	19.46	31.06	27.41	30.17	24.27
27.36	21.15	23.56	32.53	27.08	25.23
29.18	20.51	26.80	29.48	22.93	26.67
31.62	15.99	28.97	26.99	26.73	29.38

## STATISTICAL ANALYSIS FOR SHEAR BOND STRENGTH

Statistical analysis of the data recorded was performed using one way ANOVA test followed by post-hoc multiple comparisons by tukey HSD test.

#### **One way ANOVA (TABLE II)**

Shear load at which the composite cylinder fracture (MPa)

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Mini mum	Maxi mum
Control (GROUP I)	10	29.9636	2.01903	.63847	28.5193	31.4079	26.42	33.17
Bleaching Immediate	10	18.7512	2.71327	.85801	16.8102	20.6922	13.23	21.23
(GROUP II)								
Bleaching 1 week(GROUP III)	10	28.6672	3.04030	.96143	26.4923	30.8421	23.57	33.59
Sodium ascorbate(GROUP IV)	10	28.4159	3.14819	.99554	26.1638	30.6680	22.41	32.53
Salicylic acid (GROUP V)	10	27.5058	2.90769	.91949	25.4257	29.5858	22.93	31.70
NAC (GROUP VI)	10	26.5609	2.83855	.89763	24.5303	28.5915	22.32	30.42
Total	60	26.6441	4.57874	.59111	25.4613	27.8269	13.23	33.59

#### ShearStrength

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	812.984	5	162.597	20.711	.000
Within Groups	423.944	54	7.851		
Total	1236.929	59			

The mean difference for shear bond strength measured for all groups, range from minimum mean value of 18.7512Mpa (bleached enamel with immediate bonding of composite) and maximum mean value of 29.9636Mpa (control -normal enamel).

# **POST HOC TEST( TABLE III)**

# ShearStrength Tukey HSD

	-	Mean			95% Confidence Interval	
(I) Group	(J) Group	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
control	Bleaching Immediate	11.21238*	1.25306	.000	7.5102	14.9145
	Bleaching 1 week	1.29641	1.25306	.904	-2.4057	4.9986
	Salicylic acid	2.45780	1.25306	.378	-1.2443	6.1599
	Sodium ascorbate	1.54766	1.25306	.818	-2.1545	5.2498
	N-acetyl cysteine	3.40270	1.25306	.089	2994	7.1048
Bleaching	control	-11.21238*	1.25306	.000	-14.9145	-7.5102
Immediate	Bleaching 1 week	-9.91597*	1.25306	.000	-13.6181	-6.2138
	Salicylic acid	-8.75458*	1.25306	.000	-12.4567	-5.0524
	Sodium ascorbate	-9.66472*	1.25306	.000	-13.3669	-5.9626
	N-acetyl cysteine	-7.80968*	1.25306	.000	-11.5118	-4.1075
Bleaching 1	control	-1.29641	1.25306	.904	-4.9986	2.4057
week	Bleaching Immediate	9.91597*	1.25306	.000	6.2138	13.6181
	Salicylic acid	1.16139	1.25306	.938	-2.5408	4.8635
	Sodium ascorbate	.25125	1.25306	1.000	-3.4509	3.9534
	N-acetyl cysteine	2.10629	1.25306	.550	-1.5959	5.8084
Sodium	control	-1.54766	1.25306	.818	-5.2498	2.1545
ascorbate	Bleaching Immediate	9.66472*	1.25306	.000	5.9626	13.3669

	Bleaching 1 week	25125	1.25306	1.000	-3.9534	3.4509
	Salicylic acid	.91014	1.25306	.978	-2.7920	4.6123
	N-acetyl cysteine	1.85504	1.25306	.678	-1.8471	5.5572
Salicylic acid	l control	-2.45780	1.25306	.378	-6.1599	1.2443
	Bleaching Immediate	$8.75458^{*}$	1.25306	.000	5.0524	12.4567
	Bleaching 1 week	-1.16139	1.25306	.938	-4.8635	2.5408
	Sodium ascorbate	91014	1.25306	.978	-4.6123	2.7920
	N-acetyl cysteine	.94490	1.25306	.974	-2.7572	4.6470
N-acetyl	control	-3.40270	1.25306	.089	-7.1048	.2994
cysteine	Bleaching Immediate	7.80968*	1.25306	.000	4.1075	11.5118
	Bleaching 1 week	-2.10629	1.25306	.550	-5.8084	1.5959
	Salicylic acid	94490	1.25306	.974	-4.6470	2.7572
	Sodium ascorbate	-1.85504	1.25306	.678	-5.5572	1.8471

\*. The mean difference is significant at the 0.05 level.

One way ANOVA test followed by post hoc test were used to compare shear bond strength.

#### **GROUP I** (control normal enamel):

The result of this study showed that for shear bond strength, group I (normal enamel) showed statistically significant difference when compared to group II (immediate bleaching) (p<0.05) and no statistically significant difference was seen with group III (bleaching after 7 days), group IV (sodium ascorbate), group V (salicylic acid) and group VI (N- acetyl cysteine) (p>0.05).

#### **GROUP II** (bleached enamel with immediate bonding):

The shear bond strength of group II showed statistically significant difference with group I (control), group III (bleaching after 7 days), group IV (sodium ascorbate), group V (salicylic acid) and group VI (N- acetyl cysteine) (p<0.05).

#### **GROUP III** (bleached enamel with bonding after 7 days):

The shear bond strength of group III showed statistically significant difference with group II (immediate bonding) (p<0.05) and no statistically significant difference was seen with group I (control), group IV (sodium ascorbate), group V (salicylic acid) and group VI (N- acetyl cysteine) (p>0.05).

# **GROUP IV** (bleached enamel with sodium ascorbate application with immediate bonding):

The shear bond strength of group IV showed statistically significant difference with group II (immediate bonding) (p<0.05) and no statistically significant difference was seen with group I (control), group III (bleaching after 7 days), group V (salicylic acid) and group VI (N- acetyl cysteine) (p>0.05).

# **GROUP V** (bleached enamel with salicylic acid application with immediate bonding):

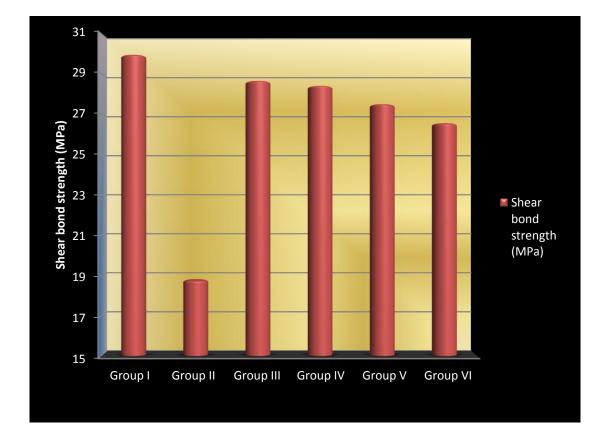
The shear bond strength of group V showed the statistically significant difference with group II (immediate bonding) (p<0.05) and no statistically significant difference was seen with group I (control), group III (bleaching after 7 days), group IV (sodium ascorbate), and group VI (N- acetyl cysteine) (p>0.05).

#### **GROUP VI** (bleached enamel with NAC application with immediate bonding):

The shear bond strength of group VI showed the statistically significant difference with group II (immediate bonding) (p<0.05) and no statistically significant difference was seen with group I (control), group III (bleaching after 7 days), group IV (sodium ascorbate), and group V (salicylic acid) (p>0.05).

Mean shear bond strength value of group II is 37% less than group I Mean shear bond strength value of group III is 4% less than group I Mean shear bond strength value of group IV is 5% less than group I Mean shear bond strength value of group V is 8% less than group I Mean shear bond strength value of group VI is 11% less than group I

Shear bond strength value between groups I, II, III, IV, V and VI showed that they are statistically significant at P Value < 0.001



Results of shear bond strength

Group I > Group III > Group IV > Group V > Group VI > Group II

#### DISCUSSION

Bleaching is the most common esthetic procedure performed in the dental office. It is the simplest, least invasive, and least expensive means available to whiten discolored teeth and diminish or eliminate several stains in both vital and pulpless teeth. In combination with other esthetic procedures such as micro abrasion, bleaching lightens a stained tooth before veneering, thus expanding the horizons of esthetic dentistry.

The earliest efforts to lighten teeth through bleaching dates back to more than a century ago, with **Chappel** describing the use of oxalic acid in 1877. **Harlan in 1884** was the first to use hydrogen peroxide, which he called hydrogen dioxide. Peroxide bleaching requires the least time and to date is the most commonly used<sup>56</sup>.

Historically, modern cosmetic teeth whitening or teeth bleaching began in **1989**, with the introduction of night guard bleaching by **Haywood and Heymann**, with the bleaching agent employed being carbamide peroxide. This is simple, apparently safe and comparatively inexpensive<sup>17</sup>.

Today, the teeth bleaching procedure is accomplished by placing a peroxidebased (either hydrogen or carbamide) bleaching gel, via various methods, into direct contact with the teeth. This contact of bleaching agent to teeth is made either by painting the gel directly onto the teeth or by using the custom tray method. It has been shown that these two modalities will result in noticeable whitening of the teeth. The finished degree of whitening ultimately achieved is dependent on the relative type, the strength or concentration of the peroxide-based gel that is used, as well as the amount of time that the active gel is left in contact with the surface of the teeth.

Chemistry of bleaching<sup>57</sup>

1)  $CH_2NCONH_2 \bullet H_2O_2 \longrightarrow in water \longrightarrow H_2NCONH_2 + 2H_2O_2$ Carbamide peroxide free carbamide hydrogen peroxide

Carbamide peroxide has two portions, the Carbamide portion and the Hydrogen portion(equation 1). In the presence of water (i.e. oral saliva) it dissociates into free carbamide and free hydrogen peroxide. Carbamide Peroxide must go through this initial step before any bleaching can occur because it is only the free HP that actually does the bleaching. Hydrogen peroxide is noted chemically as "H<sub>2</sub>O<sub>2</sub>", and is a relatively unstable molecule by itself, and will quickly dissociate into highly reactive, strong oxidizing agents through the formation of three types of free radicals (hydroxyl radicals, per-hydroxyl radicals, and superoxide anions), reactive oxygen molecules, and hydrogen peroxide anions.

2a.) 
$$H_2O_2 \longrightarrow 2HO^{\bullet}$$
  
2b.)  $HO + H_2O_2 \longrightarrow H_2O + HO^{\bullet}_2$   
2c.)  $HO^{\bullet}_2 \longrightarrow H^+ + O^{-\bullet}_2$   
3.)  $2H_2O_2 \longrightarrow 2H_2O + 2\{O\} \longrightarrow 2H_2O + O_2$   
4.)  $H_2O_2 \longrightarrow H^+ + HOO^-$ 

In equations 2, 3, and 4, we see the production of the different oxidizing agents that actually do the bleaching. In 2a, we see the formation of hydroxyl radicals (2HO•), in 2b, per-hydroxyl radicals (HO•<sub>2</sub>), and in 2c, the result is super oxide anions ( $O^{-}$ •<sub>2</sub>). In Equation 3, water (2H<sub>2</sub>O) and oxygen (O<sub>2</sub>) molecules are formed, and lastly, in equation 4 the final oxidizing agent products are hydrogen peroxide anions (HOO<sup>-</sup>).

In all of these free radicals, it is the highly unstable free oxygen component in each, that reacts with, oxidizes, or attacks the long-chained, dark-colored chromophore molecules (atoms or groups in a molecule that cause color) in the patient's tooth enamel. This attack splits the chromophore molecules into smaller, less colored and more diffusible (dissolvable) molecules, which results in whitening of the teeth. It does not really remove any stain, but it actually changes the nature and composition of the stains on the teeth. The final results of the bleaching treatment depends mostly on the concentration of the Hydrogen Peroxide available to produce the free radicals, or the amount of time that the produced radicals are in contact with the teeth, and the ease in which the radicals can reach the chromophore molecules.

Several studies have shown that hydrogen peroxide–and carbamide peroxidebased bleaching agents adversely affect the immediate bond strength of resins to enamel. A study by **Lai et al**<sup>24</sup> has shown reduction in bond strength of composite to enamel that was bleached with  $H_2O_2$ . There are several factors that affect the bond strength of the composite to the enamel after bleaching. Some authors have reported that residual oxygen after carbamide peroxide bleaching and its effect on polymerization of resin tags is the cause of decrease in the bond strength<sup>11</sup>.

Some in vitro studies reported alterations in the chemical and morphological structures of enamel (**Basting et al 2003**, **Josey et al 1996**, **Rodrigues et al 2001**)<sup>5,21,38</sup>. Such structural alterations resulted in diminished shear strength values after dental bleaching. **Rotstein et al**<sup>39</sup> indicated that bleaching agents cause changes in the levels of calcium and phosphorous that are present in the hydroxyapatite crystal, which are the main building blocks of dental hard tissue. **Perdiago et al 1998**<sup>35</sup> stated that the changes in proteins and minerals content of superficial layers of enamel may be responsible for reduced bond strength. Others attribute compromised shear bond strength of composite to bleached enamel to resin tags in bleached enamel being less numerous, shorter and less defined than those in unbleached enamel, in addition to presence and evidence of bubbling<sup>10</sup>.

For these reasons, they recommended that immediate bonding to bleached enamel should be avoided to allow delay in time (7 days) for the residual oxygen on the tooth surface to be eliminated (**Titley et al 1993**)<sup>48</sup>.

A study by **Chipault et al<sup>8</sup>** concluded that antioxidants can delay or inhibit the oxidation of lipids or other molecules by removing the free radicals that initiate or propagate oxidizing chain reaction. Bleached enamel treated with antioxidant before composite bonding is believed to reverse the reduction in bond strength of composite resin, thus eliminating the need to postpone bonding.

Several bond strength tests have been used to evaluate the adhesive performance of dentin adhesives. The most commonly used are the shear and tensile tests. **Oili et al (1988)**<sup>33</sup> demonstrated that both tensile and shear bond tests were equally adequate in providing information on the bonding efficiency. The shear bond strength tests are more frequently used due to its reproducibility and relative uncomplicated method. A universal testing machine (Instron 5566 series, Instron, Canton, Mass., London, UK) with a cross head speed of 1 mm/min was used. Shear testing was performed with a mono-angled chisel, with the edge closely aligned to the bonding interface and perpendicular to the longitudinal axis of the resin composite cylinder.

This study is aimed at evaluating the reversal of shear bond strength of enamel using various antioxidants (Sodium ascorbate, Salicylic acid and N-Acetyl cysteine).

Sodium ascorbate is a naturally occurring water soluble antioxidant. Green peppers, citrus fruits, strawberries and tomatoes are some of the sources. Ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives [Frei et al. 1989]<sup>12</sup>.

Salicylic acid, a polyphenol, is a strong antioxidant, found in most vegetables, fruits, herbs and in leaves of wintergreen and willow bark. It is a key ingredient in several skin-care products for the treatment of psoriasis, acne, corns, calluses, and warts [Grimes 1999<sup>15</sup>; Roberts 2004<sup>36</sup>]. It is also frequently used as a commercial cosmetic preservative [Himejima et al. 1991]<sup>18</sup>.

N-Acetyl cysteine is a pharmaceutical drug and is also a nutritional supplement used primarily as a mucolytic agent and in the treatment of paracetamol (acetaminophen) overdose. It also aids in sulfate repletion and hence used in autism, where cysteine and related sulfur amino acids may be depleted. N-Acetylcysteine (NAC), a sulfhydryl amino acid has several characteristics (scavenging of the hydroxyl radical, increased synthesis of reduced glutathione and diminished production of H<sub>2</sub>O<sub>2</sub>) favoring its usage as an antioxidant. NAC is also known as a thiol antioxidant due to its reactivity with oxidant species like  $O_2^{\bullet}$ , H<sub>2</sub>O<sub>2</sub>, and •OH<sup>3</sup>.

All these three agents are well known for their antioxidant properties in the field of medicine and are commonly used substances and easily available. Previous studies have already concluded that sodium ascorbate shows good antioxidant property and reversal of shear bond strength of bleached enamel<sup>50</sup>. However, till to date, there is no published study using salicylic acid and N-acetyl cysteine for the same. Hence, as a novel approach, we used Salicylic acid and N-Acetyl cysteine as antioxidant for the reversal of bond strength of bleached enamel.

Previous studies revealed that sodium ascorbate used at a concentration of 10% showed good reversal of bond strength to bleached enamel [Zhao et al & Lai et al] <sup>24, 57</sup>. Hence, the same concentration of sodium ascorbate was retained for our study as well.

In a study done by **Varunraj Sendamangalam et al [2010]**<sup>52</sup>, polyphenols (including salicylic acid) were tested for their antimicrobial and antioxidant properties against *Streptococcus mutans* and concluded that salicylic acid is good antioxidant at a concentration of 0.38%(3.8mg/ml). Hence we include this concentration for our study.

NAC is a pharmaceutical antioxidant that is used in various concentrations. NAC is commercially available as 250 - 500mg tablets (0.25% - 0.5%). However, the conc. to be used on bleached enamel for reversal of shear bond strength is unknown. In a study by **Rwei –Fen S.Huang et al (2002)<sup>41</sup>**, it was concluded that pretreatment with NAC at a conc of 0.1% (approx) reduced free radical induced apoptosis. Hence, we conducted a **pilot study** to find out the exact conc. at which NAC has good antioxidant property for reversal of shear bond strength of bleached enamel, with normal enamel as control group. We tested NAC at a conc of 0.1%, 0.25% and 0.5%. Of the three concentrations, 0.25% showed better results than 0.1%, with no significant difference between 0.25% and 0.5%. Hence, we selected the conc of 0.25% for our study.

A study by **M. Turkun and Kaya [2004, 2007]**<sup>50</sup> on the effect of different conc. of carbamide peroxide(10%, 16% and 20%) on the shear bond strength of composite resin to bleached enamel showed that higher conc. of carbamide peroxide produced greater reduction in bond strength than lower conc. Hence, we used 30% carbamide peroxide, which is the higher end of the conc. available for bleaching.

In our study, antioxidants 0.25% N-acetyl cysteine, 10% Sodium ascorbate and 0.38% salicylic acid were used for 10 minutes and were continuously replenished on the enamel surface using a sterile brush based on two recent studies<sup>23, 24, 57</sup>, where the duration of antioxidant treatment was 10 minutes.

Results of this study showed that Group III (Bond strength after 7 days) and Group IV (sodium ascorbate) have highest bond strength followed by Group V (Salicylic acid) and VI (N-acetyl cysteine). These findings are in accordance with the previous study of **M. Turkun and Kaya et al [2004, 2007]**<sup>50</sup> who stated that treating the bleached enamel surface with 10% sodium ascorbate or waiting 1 week reverses the reduction of bond strength of composite.

Sodium ascorbate showed higher reversal of bond strength when compared to salicylic acid and N-acetyl cysteine. **Zhao et al & Lai et al**<sup>23, 24, 57</sup> suggested that sodium ascorbate allows free radical polymerization of the adhesive resin to proceed without premature termination by restoring the altered redox potential of the oxidized bonding substrate and hence reverses the compromised bonding.

Salicylic acid showed good reversal of shear bond strength next to sodium ascorbate. Antioxidant property of salicylic acid is by donating hydrogen atoms from aromatic hydroxyl group to a free radical. This aromatic structure of salicylic acid provides support to the unpaired electron. Thus it can inhibit the oxidation by removing the free radicals that initiate or propagate oxidising chain reaction<sup>27</sup>.

NAC also showed good reversal of bond strength, but when compared to sodium ascorbate and salicylic acid it is less. As already stated, NAC is a amino acid derivative containing sulfhydryl group. The antioxidant property is mainly due to the hydrogen atom in the sulfhydryl group which can act as an electron for neutralizing free radicals. NAC can also act directly on reactive radicals. It is a powerful scavenger of HOCl and is capable of reducing HO and  $H_2O_2^{30}$ .

Within the limitation of the present study, salicylic acid and NAC gave appreciable reversal of bond strength compared to sodium ascorbate. Salicylic acid and NAC are non- toxic antioxidants present in cosmetics and food products and used in medications and these have been applied for the first time IN-VITRO for reversal of bond strength with favorable results. Hence, it provides newer avenues for further clinical research and application.

### SUMMARY

60 freshly extracted, human central incisors (free of caries, crack, fractures or other defects) were collected. Teeth were cleaned with ultrasonic scaler and stored in 10% formalin. The enamel was polished with prophy paste and labial surfaces polished with Aluminium Oxide abrasive paper to creat a flat enamel surface. 60 teeth were embedded in acrylic blocks (2\*4cm) with labial surface exposed and divided into 6 groups of 10 specimens each.

<u>Group - I</u> (Control) Normal enamel

- <u>Group II</u> Bleaching done with 30 % Carbamide Peroxide. Immediately composite was built (2mm diameter/1mm height) and cured without any antioxidants.
- <u>Group III</u> Bleaching done with 30 % Carbamide Peroxide. Composite was built and cured after 7 days.
- <u>Group IV</u> Bleaching with 30% Carbamide Peroxide was followed by application of 10% sodium ascorbate for 10 minutes with immediate bonding.
- <u>Group -V</u> Bleaching with 30% Carbamide Peroxide was followed by application of 0.38% Salicylic acid for 10 minutes with immediate bonding.
- <u>Group –VI</u> Bleaching with 30% Carbamide Peroxide was followed by application of 0.25% N-acetyl cysteine for 10 minutes with immediate bonding.

Teflon sheet of 2mm iris was placed in all specimens to expose 2mm enamel surface. A build up of 2mm diameter and 1mm height of composite resin is placed in all the specimens and were subjected to shear bond testing in universal testing machine. Values were calculated for each group, results were tabulated and values were statistically analyzed and discussed.

Results of this study showed that Group III (Bond strength after 7 days) and Group IV (sodium ascorbate) have highest reversal of bond strength followed by Group V (Salicylic acid) and VI (N-acetyl cysteine).

# CONCLUSION

Within the limitations of this study it is concluded that,

- Bleaching with 30% of carbamide peroxide immediately before bonding, reduce the bond strength of composite resin to enamel.
- 2. A 1 week delayed bonding procedure after bleaching results in a reversal of reduced bond strength which is nearly equal to control.
- 3. Treatment of bleached enamel surface with sodium ascorbate reverses the reduced bond strength, which is nearly equal to 1 week delay.
- Treatment of bleached enamel surface with salicylic acid and N-acetyl cysteine gives a good reversal of bond strength, but less when compared to Sodium ascorbate.
- 5. Treatment of bleached enamel surface with antioxidants may be an alternative to delay bonding especially when restoration is to be completed immediately after bleaching.

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