7-1-2015

Microtensile bond strength of resin-dentin bonds following application of a chemical collagen cross-linker using different dentin bonding systems

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COURSE DESCRIPTION: Master of Science with specialization in

Operative Dentistry

TITLE OF SUBMISSION: Microtensile bond strength of resin-dentin bonds following application of a chemical collagen cross-linker using different dentin bonding systems

DATE SUBMITED: July, 2015

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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A Thesis Presented

By

BASSAM NAORALDEAN ZIDANE, B.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

July 2015

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Dedication

To my wife, Taraji Komies, for her constant support and encouragement during the challenges I faced through my postgraduate education at NOVA Southeastern University. Also, I would like to dedicate this work to my parents; Dr. Naoraldean Zidane and Dr. Afaf Nassar, whom without their unconditional love and guidance, I would not have been able to pursue my dreams. Last but not least, I would also like to dedicate this work to my family and friends.

Acknowledgements

I would like to acknowledge the following individuals:

Dr. Luana Oliveira-Haas for chairing my thesis committee. Thank you for all of your guidance throughout this process. I want to say thank you for challenging me to look further into the literature and for helping me to realize what was possible.

Dr. Mario Ramos for serving on my committee. You have been an integral part of this process for me and I can't thank you enough for all of your guidance and support. I could not have completed this project and thesis without you.

Dr. Evren Kilinc for serving on my committee. I truly appreciated your insight during the thesis writing process. You have inspired me more than you know.

Dr. Rashondia Gaines for all of your knowledge and suggestions for this research.

Dr. Patrick Hardigan for providing your assistance to determine the appropriate statistical analysis and for your guidance throughout this process.

Dr. Saulo Geradeli research dental laboratory at University of Florida. Thank you for taking me in and helping me with the laboratory part of my research, thank you for dedicating your time and effort.

ABSTRACT

Microtensile bond strength of resin-dentin bonds following application of a chemical collagen cross-linker using different dentin bonding systems

DEGREE DATE: June 2015

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Introduction: The stabilization of dentinal collagen fibers against enzymatic degradation by the use of biocompatible cross-linker agents is of clinical importance for effective dentin bonding to surpass the test of time.

Objective: The present study aims to evaluate and compare the effect of the application of two versions of a desensitizer solution to sound coronal dentin, on the microtensile bond strength (μTBS) of the resin-sound coronal dentin using 4th and 6th generation dentin bonding systems.

Materials and Methods: Extracted human third molars were collected from an unidentified bank of teeth followed by IRB approval. A flat surface of all 12 teeth was prepared utilizing a water-cooled high-speed diamond disc, leaving an entire hard sound dentinal area for testing. Subsequently, according to the assigned group, specimens followed specific manufacturer's instructions for application of dentin bonding systems: specimens were subdivided into 6 groups ($n=20$). Group 1 (G1) First positive control group. Specimens received an application of a 4th generation dentin bonding system (DBS). Group 2 (G2) Second positive control group. Specimens received an application of a 6th generation DBS. Group 3 (G3) Specimens were exposed to Gluma Desensitizer agent, blot-dried and followed by application of a 4th generation DBS. Group 4 (G4)

Specimens were exposed to Gluma Desensitizer agent, blot-dried and followed by application of a 6th generation DBS. Group 5 (G5) Specimens were exposed to Gluma Desensitizer PowerGel agent, blot-dried and followed by application of a 4th generation DBS. Group 6 (G6) Specimens were be exposed to Gluma Desensitizer PowerGel agent, blot-dried and received an application of a 6th generation DBS. After application of the adhesive systems, all specimens were restored using a microhybrid resin composite. The root portion was sectioned 1mm below the CEJ, and discarded. All specimens were thermocycled at 5-55 Cº for 7000 cycles on distilled water. Then each restored tooth was sectioned perpendicular to the bonding interface into 1mm x 1mm x 8mm beams with a slow speed diamond wafering blade under thorough irrigation. Then specimens were subjected to μTBS testing at a crosshead speed of 1mm/min. Subsequently; specimens were subjected to fracture analysis and SEM evaluation of the different failure's mode of the involved surfaces. Statistical analysis was performed by using one- way ANOVA, two-way ANOVA and Fisher's PLSD test ($p<0.05$).

Results: For the first aim of the study and after obtaining the μTBS in MPa: Group G1: 15.50 ± 6.28, Group G2: 13.06 ± 11.53, Group G3: 19.20 ± 9.43, Group G4: 12.76 ± 4.61, Group G5: 14.38 ± 5.95, Group G6: 18.54 ± 9.49. Statistical analysis showed that there is no significant influence of variables on the μTBS (Welch ANOVA [F (5,114) =2.21, p=0.057]). Treatment with Gluma desensitizing agent and Gluma desensitizing PowerGel has no significant influence on the bond strength. For the second aim of the study and to analyze group differences for type of fracture data was first recoded into two groups: (1) Adhesive failure and (2) Cohesive failure. Group differences were analyzed by type of fracture using a Fisher's exact test. No difference was found between the groups by type of fracture (5, N = 120) = 8.62, p = 0.090

Conclusion: Within the limitations of this *in vitro* study it can be concluded that Gluma desensitizing agent and Gluma desensitizing PowerGel did not significantly affect the μTBS of both 4th and 6th generation bonding system using extracted human teeth.

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Chapter 1: Introduction

1.1 Literature Review

Restorative dentistry continues to evolve through the improvement in the properties of the dentin bonding systems.[1,2] Although significant improvements have been made since 1955 when the prophetic work of Dr. Buonocore laid the foundation for adhesive dentistry, the weakest area of restorative dentistry still remains the stability of the resin-dentin bonds.[2] The premature loss of bond strength is one of the complications that still disturbs direct and indirect adhesive restorations, and significantly decreases their durability over time.[3] Hence, it had been commonly accepted that resin-dentin bonds achieved with contemporary adhesive systems deteriorate over time, and the durability of the bond between the dentinal substrate and bonding systems may not be as durable as was previously anticipated.[4,5] Dentin is a complex structure formed by odontoblasts which synthetize and set up the characteristic apatite crystals particles in a collagen matrix, the composition of the human dentin is approximately 70w% inorganic material, 18w% organic material which is largely composed of collagen fibers type I, and 12w% of water.[6] Among the organic matrix, collagen accounts for almost 90%[7] while 10% is constituted of non-proteinaceous components,[8] lipids[9] and small groups of noncollagenous proteins such as (phosphoprotein and proteoglycans) type I collagen provides tissues and organs with its characteristic tensile strength, form and cohesiveness.[8] Naturally, type I collagen is stabilized by lysil oxidase-mediated covalent intermolecular cross-linkers.[10,11] The formation of covalent intermolecular crosslinks between collagen molecules in macromolecular fibrils with appropriate biocoMPatible molecules is an effective process to provide opposition against enzymatic degradation, to improve the tissue's internal and external integrity as well as to enhance its tensile properties.[8]

During restorative procedures, enamel and dentin are etched prior to or concomitant to the application of a primer/adhesive monomer that penetrate into the collagen network, forming a hybrid layer *in situ* that is believed to be crucial for dentin bonding.[2] Since bonding is created by the impregnation of the dentin substrate by blends of resin monomers, the stability of the resin- dentin bonds relies on the creation of a coMPact and homogenous hybrid layer. The concept of hybrid layer or hybridization is referred as the replacement of minerals removed from the hard dental tissue by resin monomers.[12]In order for bonding to dentin to be effective, elimination of the smear layer (a 2-5 *u*m layer of debris produced on dentin by instrumentation) is achieved by using the total-etch adhesive system (4th and 5th DBS generation); were an acid is applied and rinsed off, followed by a priming phase and application of the adhesive resin.[4] This three-step procedure creates effective moistening of the exposed collagen

fibrils due to shifting any residual surface moisture, altering a hydrophilic into a hydrophobic surface condition and carrying monomers into the inter fibrillar networks to envelop individual collagen fibrils.[13] On the contrary, self-etch DBS (6th generation) which composed of self-etch primer and bond or primer and self-etch bonding simultaneously dissolves the smear layer; nevertheless it does not eliminate it, as there is no rinse phase; it inserts the dissolved product inside the interfacial transition zone.[14] This self-etch method was introduced on demand for a simplified, user friendly, and less technique-sensitive system.[4] The 4th generation dentin bonding system is considered the gold standard in adhesive dentistry due to its proven clinical success as well as its adequate behavior on the laboratory setting. Recently, the 6th generation dentin bonding system has been getting more attention due to a simplification on the adhesive protocol, and the significant reduction of post-operative sensitivity.

Dentine hypersensitivity which is characterized by short, sharp pain rising from exposed dentine in response to different stimuli and which cannot be related to any other dental disease or pathology.[15,16] there are many theories about the dentin hypersensitivity but the most accepted theory is the hydrodynamic theory by Brännström in which the stimuli will result in rapid movement in the dentinal tubules resulting in activating mechanoreceptors at the pulp-dentin interface leading to pain.[17] Collagen cross linkers such as Gluma desensitizing and Gluma desensitizing PowerGel has been used in dentistry to treat dentin hypersensitivity by blocking the exposed dentinal tubules which will result in reduction of the movement of the fluid inside the dentinal tubules.[18]

1.2 Cross-linkers in dentistry

The two main methods to promote collagen crosslinking are the physical and the chemical methods.[19] The physical method such as (exposure to ultraviolet radiation and dehydrothermal treatment among others) are used to avoid the incorporation of potentially cytotoxic chemical residues in the process; however it does not result in a high degree conversion of cross-linkers. The chemical crosslinking method is based on the utilization of a network of structures that binds free amine groups of lysine and hydroxylysine or free carboxylic acids residues of glutamic and aspartic acid of the protein molecules.[20,21]

Glutaraldehyde (Fig.1) – one of the most popular chemical collagen cross-linker has been widely used in many *in vitro* and *in vivo* studies; is generally considered the cross-linker of choice when fixing biological collagenous materials;[6,20] however, it has disadvantages related with causing ectopic calcification and producing high levels of cytotoxicity.[20] Latest research efforts have aimed to develop an alternative chemical cross-linker that has better cytocompatibility over time.[21,22]

Among the new chemical cross-linker agents introduced and tested in dentistry, genipin[23,19,24,25] and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide – (EDC)[22,26] have shown less cytotoxicity as well as adequate mechanical strength, and moderate and long-term stability.[27]

Admittedly, biomodification of the collagen provides the collagen matrix with enhanced mechanical properties and lower rates of enzymatic degradation.[22] Carbodiimide (Fig.2) is characterized as a urea derivative zero-length chemical crosslinking agent which does not present an aldehyde residue.[21,22,26] Carbodiimide cross-link all proteins by activating the carboxylic acid groups of glutamic and aspartic acids to form an O -acylisourea intermediate. The latter reacts with the ϵ-amino groups of lysine or hydroxylysine to form an amide cross-link, leaving urea as the terminal by-product.[26,21]

Additionally, it seems that one of the main factors affecting the durability of the resin- dentin bonds is the degradation of the collagen matrices by specific enzymes called matrix metalloproteinases (MMPs). The MMPs are present in inactive form in the bone and dentin, and upon activation of these enzymes by mild acidic adhesive resin components, they causes slow hydrolysis of the exposed collagen fibrils jeopardizing the integrity of the interface resin composite/underlying mineralized dentin.[26,28] Gluma Desensitizing (Heraeus Kulzer, South Bend, IN) and Gluma PowerGel Desensitizing (Heraeus Kulzer, South Bend, IN) agents, are two of the most popular desensitizing agents used in dentistry today. Gluma Desensitizing agent contained 2-hydroxyethyl methacrylate (HEMA), and glutardialdehyde. It is recommended to be utilized prior to direct restorations to minimize post-operative sensitivity,[29] whereas Gluma PowerGel Desensitizing agent contained 2-hydroxyethyl methacrylate (HEMA), glutardialdehyde, and pyrogenic acid. It's recommended to be used prior to bleaching, and on the dentinal surface prior to receiving an indirect restoration.[30] One of the main components of Gluma is the hydroxyethylmethacrylate (HEMA), which contain both hydrophilic and hydrophobic groups.[31] Among different properties, HEMA has been shown to retain the collagen and intertubular spaces as a framework for subsequent infiltration of the monomers.[29] Therefore, Walter and colleagues, have concluded that HEMA can return the bond strengths to a similar level of moist sound dentin.[1] There is scarce evidence on the literature on the comparison of both proposed desensitizing agents and its effects on the strength and durability of the resin-dentin bonds. It can be postulated that the ultimate goal of adhesive dentistry is to achieve an intimate adaptation between the restorative material and the dental substrate; which surpasses the test of time.[32] The stability of such intimate adaptation varies as a function of anatomical location since the profile and tensile strength of the collagen fibers varies within the same dentin substrate having an impact on the ultimate tensile strength of the resin-dentin bonds.[33-35]

1.3 Innovation

Although the 4th and 6th generation dentin bonding systems OptiBond FL (Kerr Corporation, Orange, CA) and Clearfil SE (Kuraray Noritake Dental Inc. Okayama, Japan) proposed to be used on this project have been extensively utilized in the clinical and laboratory trials, the present project distant from other studies on the inclusion of two desensitizer agents. Additionally, there is scarce evidence on the use of the two proposed solutions (Gluma Desensitizer agent and Gluma PowerGel Desensitizer agent) when used in conjunction with a 4th and 6th generation dentin bonding systems on sound dentin. The use of novel agents for the strengthening of collagen fibrils within the dentin to provide resistance against enzymatic degradation may improve the integrity and enhance physical properties on the resin-dentin bonds over time; which may have a critical positive impact in adhesive dentistry. The purpose of the present study is to evaluate and compare the effect of the application of a chemical collagen cross-linker and two versions of a desensitizer solution to sound coronal dentin, on the microtensile bond strength of the resin-sound coronal dentin using 4th and 6th generation dentin bonding systems under thermocycling.

2- Material and methods

2.1 Selection

Extracted human non-carious third molars were collected from an unidentified bank of teeth. Teeth cannot be traced to their source, carious teeth were excluded from this study, and no patient records were used.

2.2 Sample size

The G Power Statistics Software was used to calculate the sample size. A power analysis was conducted using data from Bedran-Russo and collaborators.[23] The G power analysis was obtained to compare the difference in-between groups for the differences in materials with the effect size of 9.27, α 0.5, power of 80%. After using the two-way ANOVA option in G power software, the total sample size for each group was determined as 20 beams per group.[36]

2.3 Storage

Selected teeth will be stored for one month or less after extraction in 0.1% Thymol in distilled water, which has been proven that it does not affect the bond strength.[37]

2.4 Preparation of specimens

All procedures were be performed by a single operator (BZ) after a training session in order to achieve adequate handling of materials and procedures. All specifications including composition and mode of use are specified on (Tables 1 and 2).

Occlusal enamel and dentin were removed horizontally (perpendicular to the long axis of the tooth) mesio-distally, using a Buehler IsoMet diamond wafering blades 15 LC (Buehler Ltd, Lake Bluff, IL) in order to remove the occlusal enamel and superficial dentin, exposing a flat surface of middle deep sound dentin (Fig 3) .[1,38] Flattening of the occlusal surface by removal of the cusps allows accurate sectioning of samples in beam shape.[1] Specimens that show visible pulp exposure would be excluded from the study. The entire dentin surface of every specimen was ground flat with a Buehler Isomet 600-grit SiC paper (Buehler Ltd, Lake Bluff, IL) under running water.[39]

2.5 Bonding procedure

Concentration of the chemical crosslinker and the desensitizer agents were chosen according to previous reports.[21,23,33,38,40] Teeth were subdivided according to dentin treatment (chemical crosslinker agent) and DBS (4th and 6th generation dentin bonding systems). All DBS were used according to manufacturer's instructions (Fig 4).

All of the teeth that were assigned to the 4th generation DBS will be etched with 35% phosphoric acid gel (Ultradent Products, Inc. South jordan, UT) for 15 seconds, rinsed for 15 seconds with tap water, dried with oil/water-free air and leaving a visible moisture surface.[41] All of the teeth that were assigned to the 6th generation DBS will not receive any pre-treatment. All teeth will be randomly allocated to the groups:

Group 1 (G1) First positive control group. Specimens obtained only from coronal occlusal caries-free teeth. Teeth assigned to this group were re-hydrated with distilled water, gently blot-dried and will receive an application of the 4th generation DBS, according to the manufacturer's instructions.

Group 2 (G2) Second positive control group. Specimens obtained only from coronal occlusal caries-free teeth. Teeth assigned to this control group received an application of the 6th generation DBS, according to the manufacturer's instructions.

Group 3 (G3) Specimens were exposed to Gluma Desensitizer agent, blot- dried, rinsed and will receive an application of a 4th generation DBS, according to the manufacturer's instructions.

Group 4 (G4) Specimens were exposed to Gluma Desensitizer agent, blot- dried, rinsed and will receive an application of a 6th generation DBS, according to the manufacturer's instructions.

Group 5 (G5) Specimens were exposed to Gluma Desensitizer PowerGel agent, blot-dried, rinsed and will receive an application of a 4th generation DBS, according to the manufacturer's instructions.

Group 6 (G6) Specimens were exposed to Gluma Desensitizer PowerGel agent, blot-dried, rinsed and will receive an application of a 6th generation DBS, according to the manufacturer's instructions.

For the teeth assigned to the 4th generation DBS, the bonding protocol followed the three-step total-etch adhesive system OptiBond FL according to the manufacturer's instructions: After application of the etchant on the dentinal surface for 15 seconds (Ultradent Products, Inc. South jordan, UT), the surface was rinsed thoroughly for 15 seconds followed by air dry for 3 seconds, subsequently OptiBond FL primer is applied by light brushing motion for 15 seconds followed by air dry for 5 seconds. Lastly, the OptiBond FL adhesive was applied with light brushing for 15 seconds, followed by air dry for 3 seconds. All surface was light cured for 10 seconds using the XL 3000 quartz-tungsten-halogen curing-light unit (3M ESPE, Irvin, CA) with an output of 700 mW/cm².

For the teeth assigned to the 6th generation DBS, the bonding protocol followed the two-step self-etch adhesive system Clearfil SE (Kuraray Noritake Dental Inc. Okayama, Japan) according to the manufacturer's instructions: Application of the primer (first bottle) for 20 seconds followed by air dry; subsequently application of the Clearfil SE Bond bottle followed by air dry. All surface was light-cured for 10 seconds using the XL 3000 quartz-tungsten-halogen curing-light unit (3M ESPE, Irvin, CA) with an output of 700 mW/cm², The light output of the halogen curing light was checked using a radiometer at each use of light cure.[42]

Following dental adhesive protocols, teeth were restored with resin composite. Three- 2.0 mm thick layers of the Filtek Z250 microhybrid resin composite (3M ESPE, Irvin, CA)[1,31] were incrementally placed over the bonded dentin surfaces and individually light-cured for 20 seconds using the XL 3000 quartz-tungsten-halogen curing-light unit (3M ESPE, Irvin, CA) with an output of 700 mW/cm². All specimens were stored in distilled water at 37ºC for 24h prior to any further preparation (Fig 5).

2.6 Thermocycling

Specimens were subjected to thermocycling (Chewing Simulator, SD Mechatronik, Feldkirchen-Westerham, Germany) distilled water between 5-55ºC for 7000 cycles, each group was placed in a labeled container inside the thermocyclin simulator (Fig 6).[3,4]

2.7 Microtensile Bond Strength Test (Fig. 3)

The roots were sectioned 1mm below the CEJ and discarded; each restored tooth was sectioned perpendicular to the bonded interface into 1mm x 1mm x 8mm beams[1,3] by using a Buehler Series 15LC Diamond low speed diamond wafering blade (Buehler Ltd. Lake Bluff, IL) under irrigation. The beams were visually examined and subdivided in all different groups (n=20) (Fig 7, 10).

The mean microtensile bond strength of the beams originated from each tooth was used for statistical analysis. The cross-sectional area of each specimen was measured with digital calipers (Salvin Dental Specialties, Charlotte, NC) to confirm adequate dimensions of the beams. Each beam was glued with a cyanoacrylate adhesive (Zapit Dental Ventures of America, Corona, CA) to Microtensile Geradeli Jig 2 (Odeme Dental research, São Francisco, brazil) (Fig 8) , these jigs were mounted in INSTRON (Fig 9) (universal testing machine, EZ Test, Shimadzu, Tokyo, Japan) device which consist of two stainless-steel components which slide away from each other when the apparatus is subjected to tensile force, thus pulling the specimen apart and were subjected to microtensile testing at a crosshead speed of 1mm/min. The values were expressed in Newton and transferred to Mega Pascals (MPa) by using the following equation "Microtensile bond strength (MPa) = Force (Newton) / Area (mm^2) ".[36]

The microtensile bond strength test was adevised to be a more clinically relevant test. It is claimed that the test reduces the probability of crack initiation and propagation within individual specimens because of the small bonded area. Additionally, it produces less coefficient variation coMPared with the shear bond strength test.[43,44]

Data were collected using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA).

2.8 Fracture analysis

After testing the microtensile bond strength, the failure mode of each debonded specimen were analyzed under a steromicroscope (Olympus, Center Valley, PA) at 40% magnification. The slices were rinsed in 95% alcohol solution then air dried. Then each slice was mounted in a metallic stub. The failure mode were classified into the following five categories:

1. Adhesive failure between dentin and DBS.

2. Adhesive failure between composite resin and DBS.

3. Cohesive failure within dentin.

4. Cohesive failure within composite resin.

5. Mixed failure - combination of failure that occurred both at the interface between dentin/DBS and composite resin/DBS.

2.9 SEM analysis

One representative slice from each of the five failure modes was randomly selected for scanning electron microscope (SEM) examination of the surface morphology using a the SEM model Quanta 200 (FEI, Hillsboro, OR); in order to obtain SEM images of the failure patterns (Fig 11). The specimens were prepared and the interface between dentin, adhesive agent and resin composite were analyzed.

The failure modes were classified into the next five categories:

1. Adhesive failure between dentin and DBS.

2. Adhesive failure between composite resin and DBS.

3. Cohesive failure within dentin.

4. Cohesive failure within composite resin.

5. Mixed failure - combination of failure that occurred both at the interface between dentin/DBS and composite resin/DBS.

2.10 Statistical Analysis

1-To compare the microtensile bond strength between two test groups (Gluma Desensitizer and Gluma Desensitizer PowerGel) on sound coronal dentin, when using a 4th and 6th generation DBS under thermocycling, there was no significant difference between the groups.

2-To compare the modes of failure among two test groups (Gluma Desensitizer, Gluma Desensitizer PowerGel) resulted from the microtensile bond strength test when using a 4th generation and a 6th generation DBS under thermocycling, adhesive failure was predominant.

A Welch ANOVA to compare the groups for maximum load. Also, Welch ANOVA used to compensate for unequal variances as seen in (Table 3) [F (5,114) =2.21, p=0.057].

3 Result:

Raw data was saved in excel sheet for statistical analysis (Table 6), for the first aim of the study, there was no significant difference between the groups regarding the microtensile bond strength shown in (chart 1) and in the Pairwise Comparisons for Microtensile bond strength (Table 3), also for the second aim of the study there was no significant difference between the six groups in this study regarding the mode of failure

4 Discussion:

In the present study extracted non-carious third molars were collected from unidentified bank of teeth for this study, thymol 0.1mg/L was used to disinfect these teeth, which is know that it has no influence on bond strength,[37] Low Speed Diamond Wafering Blade from Buehler Ltd. was used to create the samples by making two parallel surface the first cut in the occlusal surface and the second cut to separate the root from the crown. Following that, for the teeth assigned to the 4th generation DBS, the bonding protocol followed the three-step total-etch adhesive system OptiBond FL according to the manufacturer's instructions: The desensitizing agent was applied after application of the etchant, after that the prime and bond was applied according the manufacture instruction. And for the self-etchant prime and bond the desensitizing agent was applied before the application of the self-etchant primer.

The same shade A2 of the Filtek Z250 microhybrid resin composite from 3M used for all of the groups using the incremental layer technique, each layer consist of 2mm in thickness, and All surface was light-cured for 10 seconds using the XL 3000 quartz-tungsten-halogen curing-light unit (3M ESPE, Irvin, CA) with an output of 700 mW/cm², and we notice that there was no significant difference between the group.

Studies has shown that Gluma desensitizer and Gluma PowerGel can be used as desensitizing agent to treat low and moderate hypersensitvity [18] Gluma Desensitizer, contain both HEMA and glutaraldehyde considered to be one of the most successful desensitizing agent. Glutaraldehyde reacts with part of the serum albumin in dentinal fluid, which induces a precipitation of serum albumin; and, second, the reaction of Glutaraldehyde with serum albumin induces the polymerization of HEMA.[45] According to the literature, HEMA and glutaraldehyde together have shown to achieve dentine tubule occlusion to depths of 50-200 μm.[46,47]

The result of this study reveal that the Gluma desensitizer and Gluma PowerGel didn't affect the microtensile bond strength of both the 4th and 6th generation. Both Gluma desensitizer and Gluma PowerGel desensitizer contains Water, 35% HEMA, and 5% glutardialdehyde, but Gluma PowerGel desensitizer characterized by having pyrogenic acid and expressed as green gel unlike the Gluma desensitizing agent which doesn't contain pyrogenic acid and expressed as clear liquid, according to literature 6th generation bonding system showed better post-operative sensitivity when compared with 4th generation of total etch technique because of the partial removal of the smear layer unlike the 4th generation,[48] using Gluma desensitizer agent showed a statistically significant result in reducing dentinal permeability in 6th generation bonding system [46] and a reduction in the post-operative sensitivity especially in class V restoration.[49]

35% HEMA which found in both Gluma desensitizer and Gluma PowerGel desensitizer didn't affect the microtensile bond strength in this study, but according to the literature using 50% of HEMA or more will result in a negative effect by stiffen the collapsed collagen fibers instead of expand it.[50,51]

This study coincide with Kobler et al study which was done in vitro setting using extracted third molars,[52] and they found that there was no significant difference when using the Gluma desensitizing agent with 4th or 6th generation bonding system.

An increase in the mean value was noticed when using HEMA-based cross-linker especially Optibond Fl with Gluma desensitizing agent and Clearfil SE with Gluma PowerGel without noticing any significant difference statistically.

It was observed that 4th generation bonding system has wider opening of the dentinal tubules, and longer tags in the hybrid layer than the one observed in the 6th generation (figure 12) due to completely removal of the smear layer in the 4th generation vs. partially removal or dissolving of the smear layer in the 6th generation.

This is a vitro study which provide us with the platform to create, compare and check dental material before their clinical application. Also, it helps understanding the physical, mechanical and biological properties of the dental material. Also, in this study third molars were used to make the beams for the microtensile bond strength which as reported by other studies.[53]

5 Conclusion:

Within the limitations of an in vitro study it can be concluded that Gluma desensitizing agent and Gluma desensitizing PowerGel did not significantly affect the tensile bond strength of both 4th and 6th generation bonding system using extracted human teeth.

6 Acknowledgment:

This research is supported by HPD committee at NOVA Southeastern University (account #335930)

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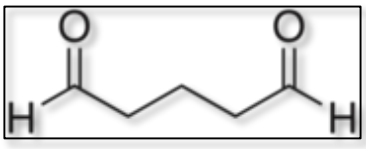


Figure 1. Chemical structure of Glutaraldehyde

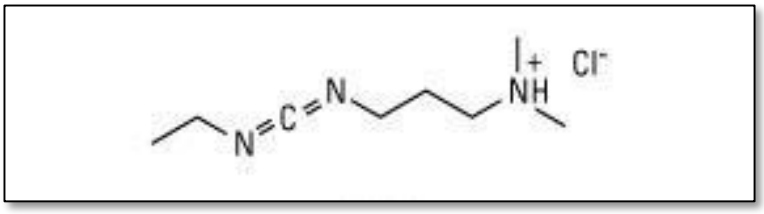


Figure 2. Chemical structure of EDC

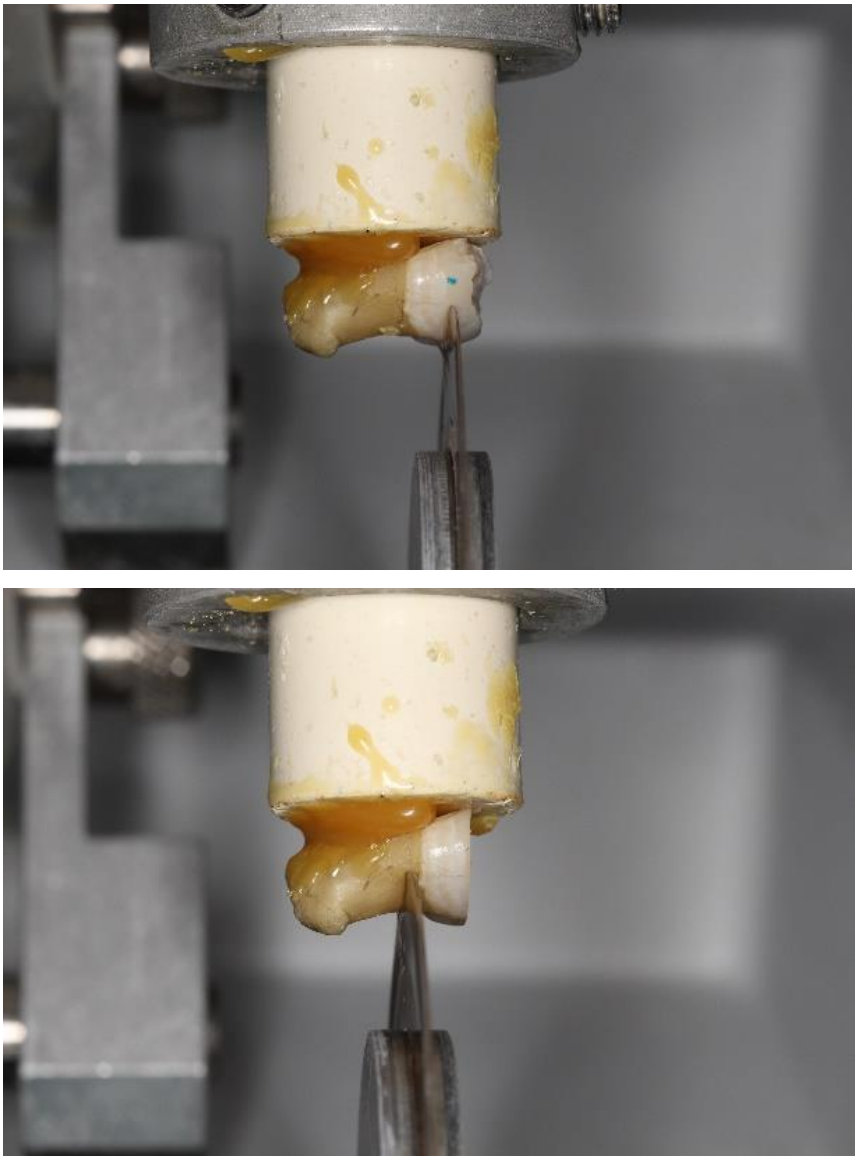


Figure 3. Removal of the occlusal enamel and dentin horizontally Buehler IsoMet diamond wafering blades 15 LC

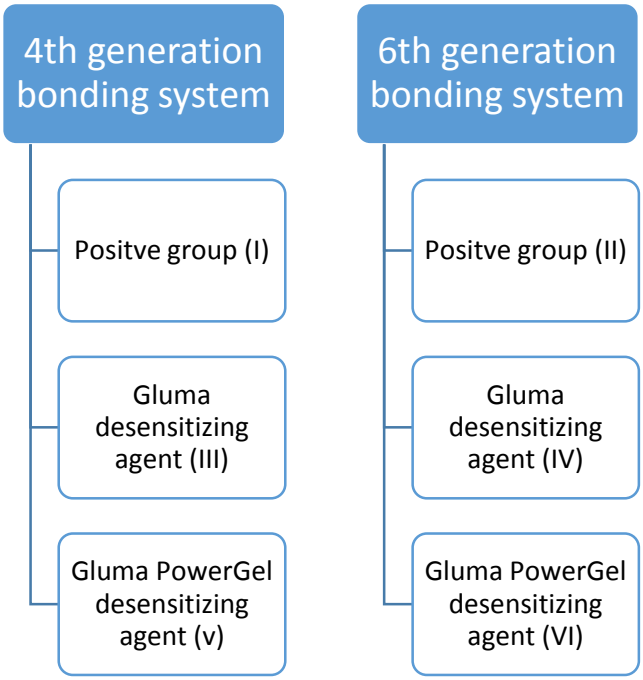


Figure 4. Dentin bonding systems with different cross linker agents

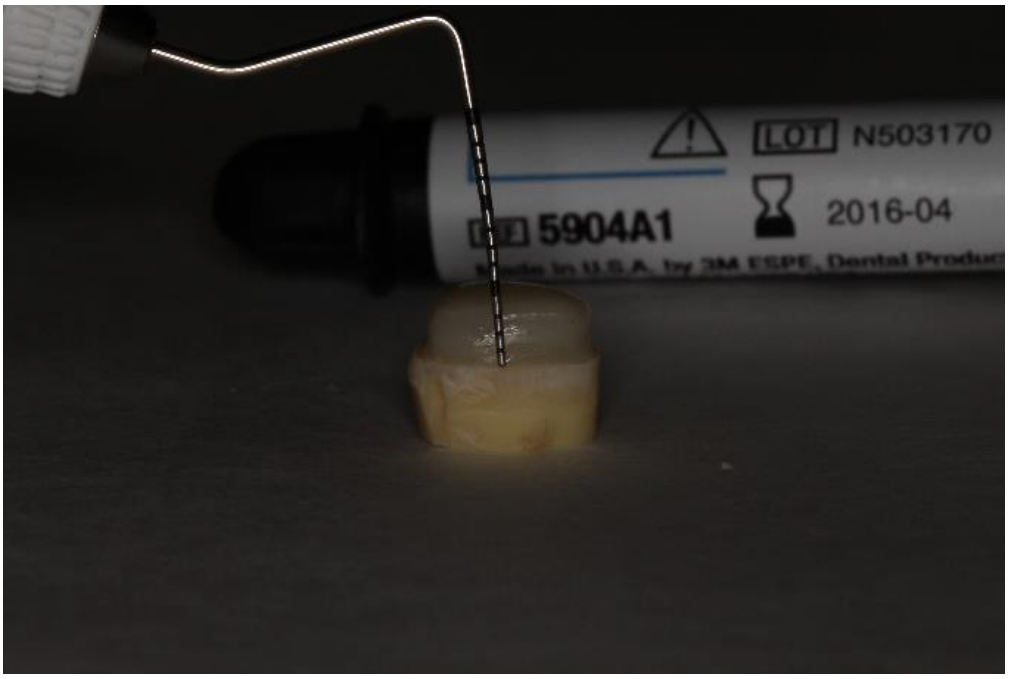


Figure 5. Composite build up

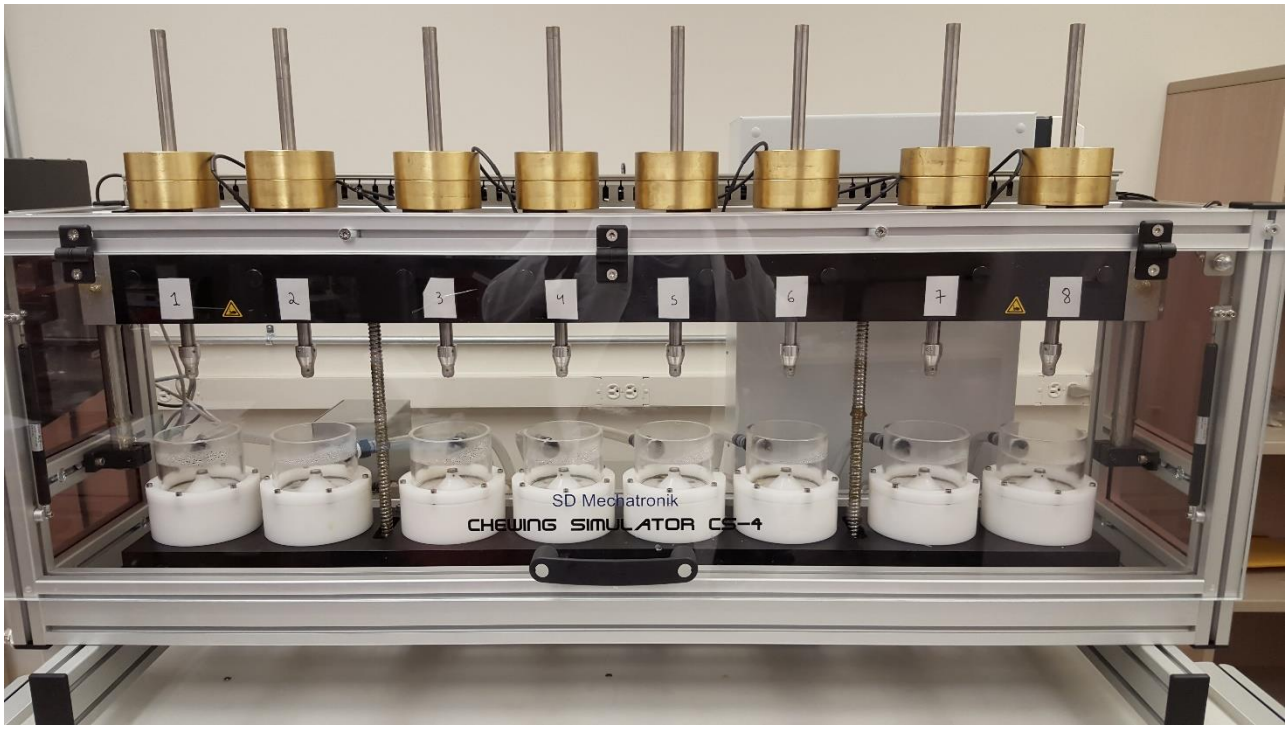
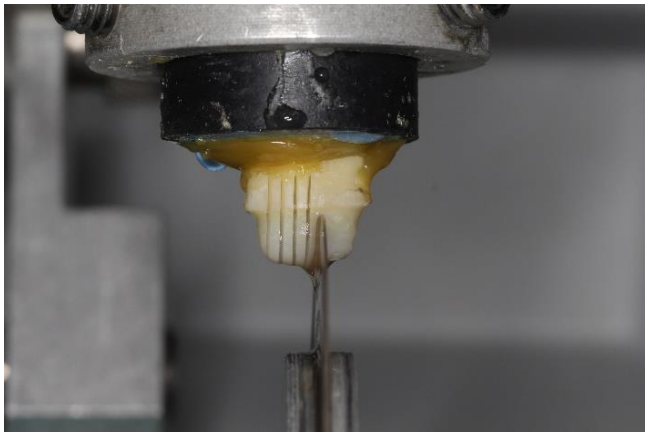
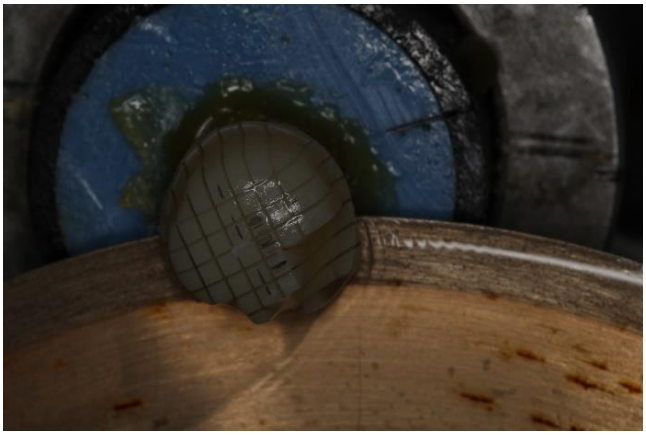


Figure 6. Thermocycling (Chewing Simulator, SD Mechatronik, Feldkirchen-Westerham, Germany)



(A) First cut in the tooth sectioning



(B) Separating the beams from the base of the tooth

(C)The beams

Figure 7. Pictures show the sequence of cutting of the beams using Diamond low speed diamond wafering blade (Buehler Ltd. Lake Bluff, IL)

Figure 8. Beams were glued with a cyanoacrylate adhesive (Zapit Dental Ventures of America, Corona, CA) to Microtensile Geradeli Jig 2

Figure 9. INSTRON (universal testing machine, EZ Test, Shimadzu, Tokyo, Japan) with Geradeli jigs 2 mounted

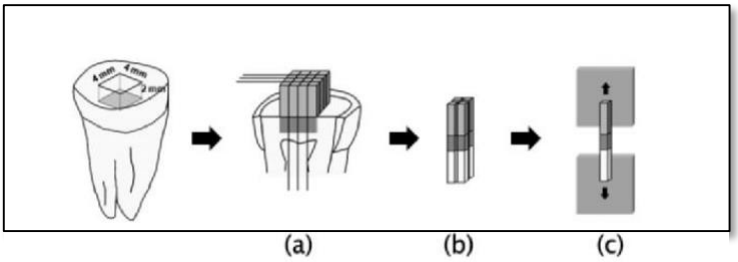
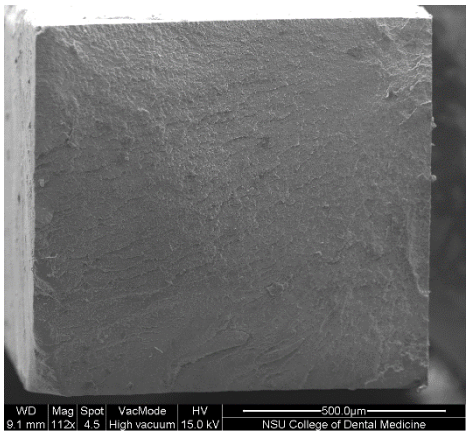
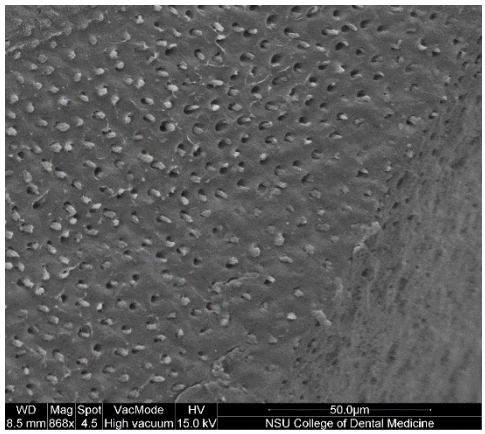


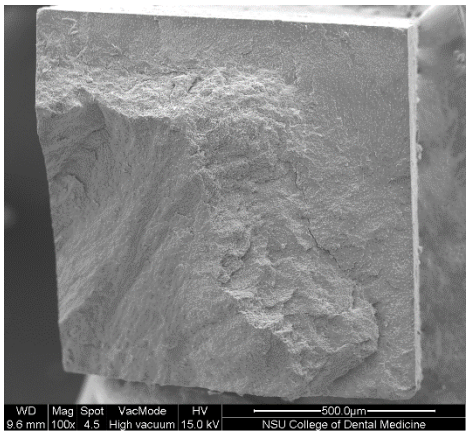
Figure 10. Microtensile bond strength test



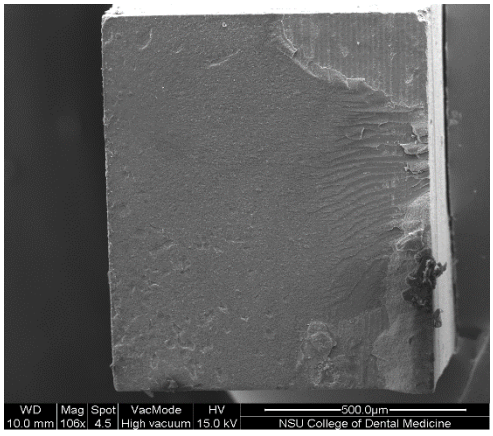
Type I failure



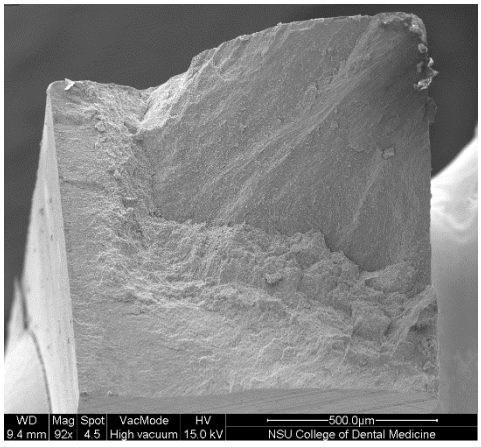
Type II failure



Type III failure



Type IV failure



Type V failure

Figure 11. Different types of failure under SEM

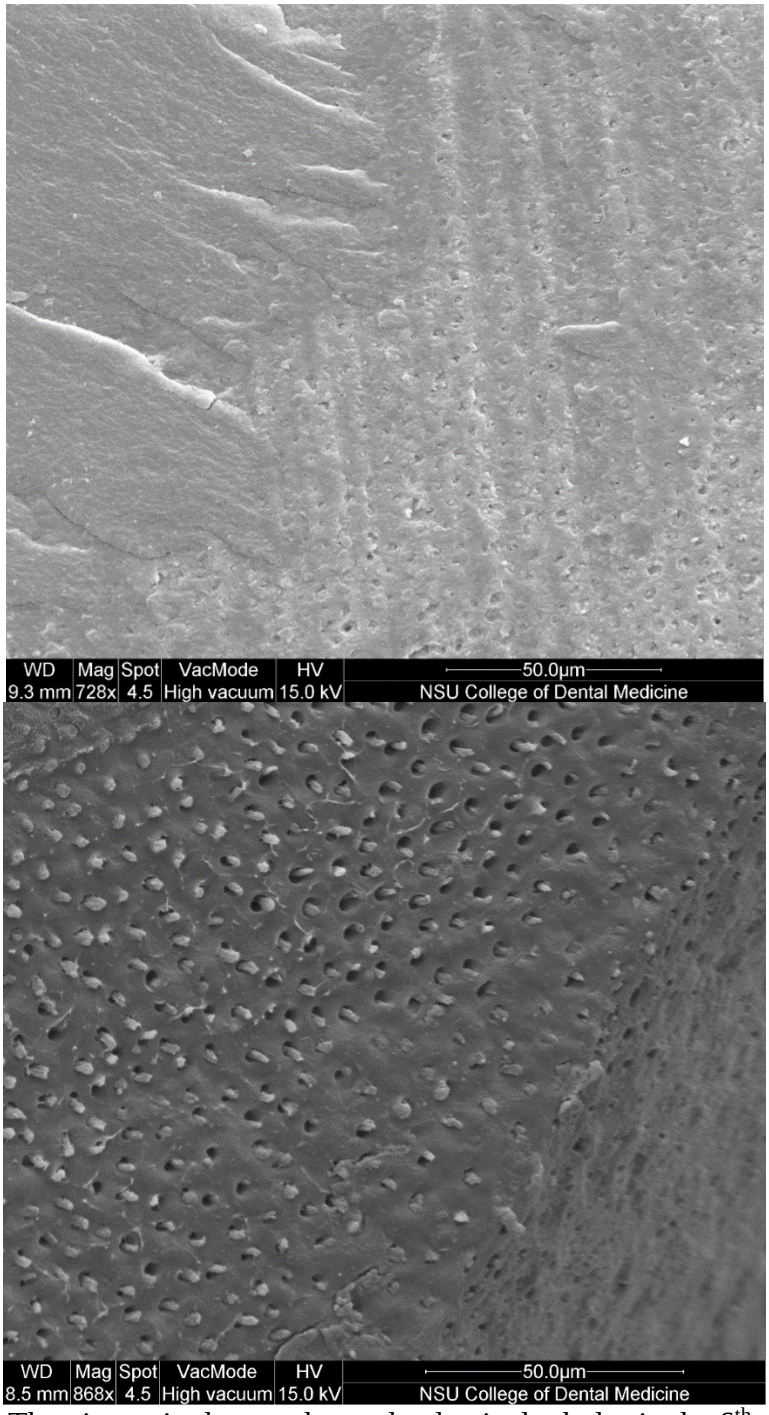


Figure 12. The picture in the top shows the dentinal tubules in the 6th generation bonding system while the picture below shows the dentinal tubules in the 4th generation

Tab1e 1. Dental materials used in the study

Dental material	Generation	Composition	Treatment	Manufacturer
OptiBond FL	4th: Three-step total-etch	Primer: 40% HEMA, 13% polyalkenoic acid copolymer with methacrylate groups, water 50 vol% Bonding resin: HEMA, Bis-GMA, hexafluorphosphate, photoinitiator	1. Apply primer leave 15 s. 2. Blot-dry for 5 s. 3. Apply bond for 15s 4. Blot-dry for 3 s. Light cure for 10 seconds.	Kerr Corporation Orange, CA, USA
Clearfil SE Bond	6th: Two-step self-etch primer	Primer: 10-MDP, HEMA, hydrophilic DMA, tertiary amine, water, photo-initiator Bonding resin: 10-MDP, HEMA, bis-GMA, hydrophilic DMA, tertiary amine, silanated colloidal silica, photo-initiator	1. Apply etch+ primer 2. Dry with mild air flow. 3. Apply bond, blot-dry. 4. Light cure for 10 seconds.	Kuraray Noritake Dental Inc. Okayama, Japan
Gluma	desensitizing Agent	2-hydroxyethyl methacrylate (HEMA), and glutardialdehyde.	1. Apply for 30s. 2. Blot dry 3. Rinse	Heraeus, South Bend, IN, USA
Gluma PowerGel	desensitizing Agent	2-hydroxyethyl methacrylate (HEMA), glutardialdehyde, and pyrogenic acid	1. Apply for 30s. 2. Rinse thoroughly	Heraeus, South Bend, IN, USA
Z250 Composite resin 3M ESPE	Microhybrid composite resin	Filler: 60% volume and 77.6% wt of zircon silicate particles.	**8** Application of 2mm increments. **9** Light-curing according to	St. Paul, MN, USA

		Organic matrix: Bis-GMA, Bis-EMA, TEGDMA, UDMA.	manufacturer's instructions.	
Ultra-etch	Conditioner	35% phosphoric acid	Apply for 15 s then rinse for 15 s.	Ultradent Products, Inc. South jordan, UT, USA

Tab1e 2. Additional materials used in the study

Product	Company	Origin
0.1% Thymol in distilled water	Sigma-Aldrich Co. LLC.	St. St. Louis, MO, USA
#320 Silicon Carbide Paper	Buehler Isomet, Buehler Ltd.	Lake Bluff, IL, USA
Paper with 600-Grit SiC	Buehler Isomet, Buehler Ltd.	Lake Bluff, IL, USA
Low Speed Diamond Wafering Blade	Buehler Isomet, Buehler Ltd.	Lake Bluff, IL, USA
Cyanocrylate Adhesive	Zapit Dental Ventures of America	Corona, CA, USA
Isomet 1000	Buehler Isomet, Buehler Ltd.	Lake Bluff, IL, USA
INSTRON universal testing machine	EZ Test, Shimadzu	Tokyo, Japan
Microtensile Geradeli Jig2	Odeme Dental research	São Francisco, Brazil
Chewing Simulator	SD Mechatronik	Feldkirchen-Westerham, Germany
Quartz-Tungsten-Halogen Curing-Light Unit: XL 3000	3M ESPE	St. Paul, MN, USA

Table 3. Pairwise Comparisons for Microtensile Bond strength

Group	Difference	Lower 95% CI	Upper 95% CI	P-Value
G2 vs. G1	2.43	-5.12	9.99	0.937
G3 vs. G1	6.14	-1.42	13.70	0.181
G4 vs. G1	-0.31	-7.86	7.25	1.000
G5 vs. G1	1.32	-6.24	8.88	0.996
G6 vs. G1	5.48	-2.08	13.03	0.294
G3 vs. G2	3.71	-3.85	11.26	0.714
G4 vs. G2	-2.74	-10.30	4.82	0.900
G5 vs. G2	-1.11	-8.67	6.44	0.998
G6 vs. G2	3.04	-4.51	10.60	0.851
G4 vs. G3	-6.44	-14.00	1.11	0.141
G5 vs. G3	-4.82	-12.38	2.74	0.439
G6 vs. G3	-0.66	-8.22	6.90	1.000
G5 vs. G4	1.63	-5.93	9.18	0.989
G6 vs. G4	5.78	-1.77	13.34	0.238
G6 vs. G5	4.16	-3.40	11.71	0.604

Table 4. Recoded Frequency Table for Type of Fracture by Group

	Adhesive failure between dentin and DBS	Adhesive failure between composite resin and DBS	Cohesive failure within dentin	Cohesive failure within composite resin	Mixed failure
G1	1 (5%)	14 (70%)	2 (10%)	0 (0%)	3 (15%)
G2	0 (0%)	16 (80%)	0 (0%)	3 (15%)	1 (5%)
G3	0 (0%)	14 (70%)	0 (0%)	0 (0%)	6 (30%)
G4	0 (0%)	15 (75%)	1 (5%)	2 (10%)	2 (10%)
G5	0 (0%)	15 (75%)	0 (0%)	1 (5%)	4 (20%)
G6	0 (0%)	16 (80%)	3 (15%)	0 (0%)	1 (5%)

Group	N	M	SD	Min	Max
G1	20	15.50	6.28	1.41	25.00
G2	20	13.06	11.53	1.21	39.55
G3	20	19.20	9.43	4.47	39.38
G4	20	12.76	4.61	5.37	19.85
G5	20	14.38	5.95	1.98	24.93
G6	20	18.54	9.49	1.00	34.07

Table 5. Descriptive Statistics for Microtensile Bond Strength in MPa

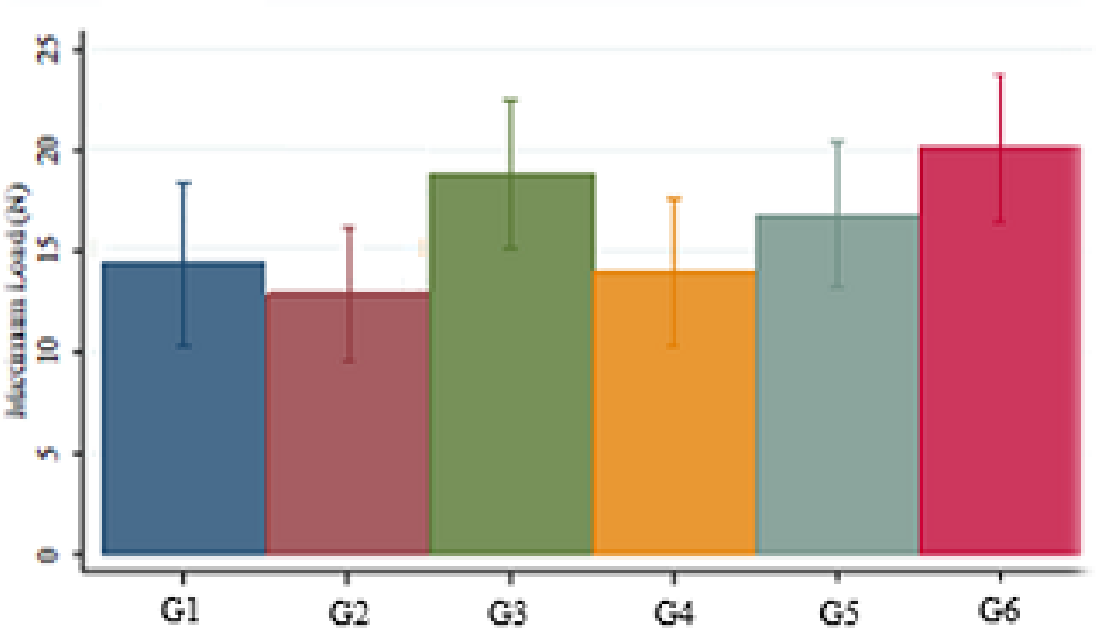


Chart 1. Microtensile bond strength in MPa

Table 6. Raw data

Name of the group	Specimen label	Maximum Load (N)	Side A	Side B	MTS by area (MPa)	Type of fracture
G1	1	11.44	1	0.92	12.44	2
G1	2	14.69	1.12	0.95	13.81	1
G1	3	11.05	0.95	0.96	12.11	2
G1	4	17.13	0.99	1.01	17.13	2
G1	5	15.66	0.98	1	15.98	2
G1	6	8.9	0.97	0.96	9.55	2
G1	7	12.24	0.87	0.9	15.64	2
G1	8	7.62	0.87	0.94	9.32	2
G1	9	10.85	0.88	0.91	13.54	5
G1	10	14.09	1.5	1.2	7.83	2
G1	11	13.05	1.2	1.03	10.56	2
G1	12	2.1	0.95	1.01	2.19	5
G1	13	12.78	0.97	1.14	11.56	2
G1	14	24.14	1.01	1.02	23.43	2
G1	15	20.88	0.9	0.95	24.42	2
G1	16	1.53	1.18	0.92	1.41	3
G1	17	20.24	0.88	0.92	25	3
G1	18	10.83	0.94	0.9	12.81	2
G1	19	7.46	0.94	0.91	8.72	2
G1	20	21.55	1.3	1.2	13.81	5
G2	21	22.69	1.14	0.8	24.88	2
G2	22	17.45	1	0.85	20.55	4

G2	23	22.53	0.84	1.04	25.78	2
G2	24	5.86	1.13	0.84	6.17	2
G2	25	19.69	0.99	0.9	22.09	4
G2	26	37.57	0.95	1	39.55	2
G2	27	7.41	1.11	0.87	7.67	2
G2	28	6.96	1.12	1.06	5.86	4
G2	29	1.04	0.85	1.01	1.21	2
G2	30	4.5	0.94	1.05	4.56	2
G2	31	4.9	1.02	0.86	5.59	2
G2	32	31.95	0.99	1.08	29.88	2
G2	33	15.46	1.08	0.94	15.23	2
G2	34	18.07	1.01	0.81	22.09	2
G2	35	3.83	0.96	1.02	3.91	2
G2	36	27.89	1.05	0.82	32.39	5
G2	37	3.14	0.95	1.12	2.95	2
G2	38	4.47	1.02	1.06	4.13	2
G2	39	18.2	0.78	1.02	22.88	2
G2	40	13.96	1.03	1.08	12.55	2
G3	41	12.99	1.06	0.96	12.76	2
G3	42	22.65	1.06	0.96	22.26	2
G3	43	24.57	1	1.01	24.32	2
G3	44	7.49	0.88	1.08	7.88	2
G3	45	40.96	1.04	1	39.38	5
G3	46	15.28	1.09	1.01	13.88	2
G3	47	2.92	0.86	0.76	4.47	2
G3	48	16.95	0.94	1.1	16.39	2

G3	49	18.46	1.03	0.83	21.6	2
G3	50	32.24	1.05	0.82	37.44	5
G3	51	23.16	1.15	0.82	24.56	5
G3	52	28.97	1.05	0.95	29.05	2
G3	53	28.17	0.93	1	30.29	2
G3	54	14.9	1.08	0.8	17.25	2
G3	55	9.64	1.08	0.98	9.1	5
G3	56	20.09	1.01	1.09	18.25	2
G3	57	15.27	1.04	0.97	15.14	2
G3	58	10.72	0.97	0.8	13.81	2
G3	59	18.29	1.1	1.1	15.12	5
G3	60	13.64	1.11	1.11	11.07	5
G4	61	14.38	1.05	1.05	13.05	2
G4	62	7.29	1.07	1.06	6.43	3
G4	63	6.6	1.01	1.02	6.41	4
G4	64	18.14	1.01	1.03	17.44	2
G4	65	13.6	1.06	1.03	12.46	2
G4	66	7.44	1.07	1.05	6.62	2
G4	67	21.67	1.07	1.06	19.11	2
G4	68	20.02	1.03	1	19.43	2
G4	69	12.74	1.06	1.03	11.67	2
G4	70	5.86	1.03	1.06	5.37	2
G4	71	18.78	1.11	1.15	14.71	4
G4	72	17.87	1.04	1.03	16.68	2
G4	73	10	1.08	1.06	8.73	2
G4	74	18.11	1.03	1.09	16.13	2

G4	75	9.26	1.03	0.98	9.17	5
G4	76	12.05	1.02	1.02	11.58	2
G4	77	17.49	1.03	1.06	16.02	2
G4	78	22.09	1.05	1.06	19.85	2
G4	79	12.82	1	1.04	12.33	5
G4	80	13.04	1.02	1.07	11.95	2
G5	81	25.64	0.97	1.06	24.93	2
G5	82	15.77	0.98	1.03	15.62	4
G5	83	6.64	1.02	0.98	6.64	2
G5	84	12.51	1.06	1.03	11.46	2
G5	85	14.22	1.18	1.08	11.16	2
G5	86	23.18	1.1	1.17	18.01	2
G5	87	18.38	1.13	1.21	13.44	2
G5	88	25.97	1.08	1.08	22.26	5
G5	89	4.54	1.07	1.08	3.93	2
G5	90	26.68	1.07	1.21	20.61	2
G5	91	18.29	1.07	1.05	16.28	5
G5	92	14.39	1.19	1.05	11.52	2
G5	93	22.43	1.08	1.06	19.59	2
G5	94	13.76	1.06	1.08	12.02	2
G5	95	24.73	1.06	1.07	21.81	2
G5	96	15.65	1.07	1.13	12.95	2
G5	97	17.53	1.19	1.08	13.64	2
G5	98	2.12	1.06	1.01	1.98	5
G5	99	16.81	0.96	1.17	14.96	5
G5	100	17.82	1.2	1	14.85	2

G6	101	11.82	0.97	0.93	13.11	2
G6	102	16.69	0.91	0.98	18.71	2
G6	103	36.09	0.99	1.07	34.07	2
G6	104	37.79	1.08	1.06	33.01	2
G6	105	1.15	1.06	1.09	1	3
G6	106	13.32	1.11	1.01	11.88	2
G6	107	30.08	1.01	1.02	29.2	2
G6	108	31.4	1.06	1.08	27.43	2
G6	109	10.11	1.03	1.15	8.53	5
G6	110	34.26	1.03	1.06	31.38	2
G6	111	13.91	1.03	1.03	13.11	3
G6	112	28.66	1.03	1.08	25.76	2
G6	113	15.81	1.03	1.3	11.8	2
G6	114	24.69	1.09	1.19	19.04	2
G6	115	17.47	1.04	1	16.8	2
G6	116	14.27	1.2	1.05	11.32	3
G6	117	19.14	1.01	1.02	18.58	2
G6	118	19.84	1	1.04	19.07	2
G6	119	3.87	1	1.02	3.8	2
G6	120	22.72	0.98	1	23.19	2