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Influence of graphene oxide filler content on the dentin bond integrity, degree of conversion and bond strength of experimental adhesive. A SEM, micro-Raman, FTIR and microtensile study

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Abstract

The study aimed to evaluate the effect of graphene oxide (GO) nano-filler content in experimental dental adhesive on its degree of conversion (DC), microtensile bond strength ($\mu$TBS) and structural reliability, using Fourier transform infrared spectroscopy (FTIR), Micro-Raman spectroscopy and Scanning electron microscopy (SEM). A resin adhesive was fabricated (control adhesive - CA) and fabricated GO nano-particles were added at 0.5% and 2.0% (m/m) to produce adhesives GOA1 and GOA2 respectively. One hundred and two teeth (specimens) were prepared for dentin exposure and conditioned with 36% phosphoric acid. Specimens in each group (n = 34) were treated with formulated adhesives (CA, GOA1 & GOA2) and photo-polymerized for 20 s followed by composite build up. Sixty specimens were used for $\mu$TBS testing in the adhesive groups (CA, GOA1 & GOA2), with half exposed to thermocycling (TC) whereas the remaining half (n = 10) stored in distilled water. Seven specimens each were assessed using SEM and Micro-Raman spectroscopy, in each adhesive group (n = 7). DC for the adhesives was assessed using FTIR. The means of $\mu$TBS and DC were analyzed using ANOVA and post hoc Tukey multiple comparisons test. GO nano-filler content showed significant influence on the adhesive $\mu$TBS in comparison to controls (p < 0.01). Ageing (TC) revealed significant reduction in the $\mu$TBS, except in GOA2 specimens, which showed comparable outcomes among TC and non-TC specimens (p > 0.05). DC was significantly higher in control adhesive [46.8 (3.6%)] compared to GOA2 [37.7 (4.2%)] specimens, however DC was comparable among GOA1 [42.3 (2.9%)] and GOA2 [37.7 (4.2%)] specimens (p > 0.05) respectively. GO exhibited interaction within adhesive and tooth dentin comparable to control adhesive. Increasing GO content showed increase in $\mu$TBS of adhesive to dentin, but a decrease in degree of conversion. Under ideal conditions, experimental adhesive with 2% GO content showed acceptable bond strength and DC; and should be further assessed under dynamic conditions to recommend clinical use.

Introduction

To advance the clinical consistency of dental restorations, major advances have been made in the field of adhesive dentistry [1]. The hybrid layer formation is a recognized theory in the establishment of a resin dentine bond [2]. The collagen fibrils have demonstrated to play an important role in the mechanical and chemical stability of the bond. Regardless of the contents used in the adhesives, the hybrid layer acts as a permeable membrane for the water sorption that reduces bond stability and deteriorates over time due to microleakage.

Research has pointed out that the average strength of collagen matrix is approximate 18000 MPa; however, chemical surface treatment reduces the bond strength value to 1–3 MPa. This indicates low modulus of elasticity
and permits ease in rotational and lateral movements of the adjacent collagen peptides, allowing matrix metalloproteinases (MMPs) for degradation of the fibrils [3]. Thus, strengthening of collagen fibrils can be established through effective cross-linking using specific agents to reduce the risk of enzymatic degradation [3, 4]. Therefore, it is hypothesized that alteration of resin adhesive composition improves its interaction with dentine and provides physical stability to resin dentine bond.

Conventionally dental adhesives are formulated using 2, 20-bis-[4-(methacryloxypropoxy)-phenyl]-propane (Bisphenol A glycerolate dimethacrylate, Bis-GMA) because of known mechanical strength and low polymerization shrinkage. However, to reduce its viscosity and increase the flow within the hybrid layer, triethyleneglycol dimethacrylate (TEGDMA) was incorporated [4, 5]. The advent of graphene-modified resins has the potential to develop adhesive resins for improved bond integrity. Graphene has remarkable features of thermal conductivity, superior mechanical properties, and enhanced electronic transport, that makes it suitable for integration with composite and adhesive for improved resin dentine bond formation [6]. On the contrary, the graphene oxide (GO) is an oxidized form of graphene that comprises of diverse set of functional group including hydroxyl, epoxy, carboxylic, and ketonic groups, having the capacity to interact with Vander Waal interaction with organic polymers [7]. This increases the compatibility of the organic polymer to interact with the functional group of GO. Thus, the variance in the GO content acts upon the chemical compatibility between adhesives and adherent surfaces that influence the tensile bond strength compared to unfilled adherents.

For the development of the new improved materials, researchers often evaluate the effectiveness of polymerization. The clinical application has shown that the severe light attenuation due to filler content and variation in polymerization time presents with a low degree of conversion and limits mechanical property developments [8]. In addition, reduced polymerization increases the risk of corresponding problems such as polymerization shrinkage, microleakage, secondary caries, pulp irritation, post-operative sensitivity, and gap formation [6, 9]. Moreover, the mechanical strength and adhesive failure of the adherents often are strongly influenced by the degree of conversion (DC), which represents aliphatic C–C bonds conversion during the polymerization of resins [8]. It is suggested that a high percentage of non-reacted C–C bonds tend to leave an open structure, which is more susceptible to degradation possibly compromising the mechanical strength and physical behavior of GO modified adhesive resins [9, 10].

Previous studies have employed several techniques in the characterization of reduced graphene modified resins [4, 10]. GO with different functional groups has demonstrated positive interactions with organic polymers, supporting its structure and enhancing tensile strength. Investigations on the influence of GO nano-filler quantity on mechanical properties of the restoration have shown positive outcomes [6, 8]. However, the evidence related to the influence of GO nano-filler content on the degree of conversion and adhesive bond integrity is limited. It is hypothesized that incorporation of GO nano-particles and increase in its content will improve the bond strength and degree of conversion of experimental resin adhesive. Therefore, the study aimed to evaluate the effect of different GO nano-filler content on the degree of conversion, microtensile bond strength and structural reliability of the formulated dental adhesive using Fourier transform infrared spectroscopy (FTIR), Micro-Raman spectroscopy and Scanning electron microscopy (SEM).

**Methods**

**Graphene oxide synthesis (GO)**

GO was synthesized from natural graphite powder using the Hummers method [4]. The stepwise process for the synthesis was adopted from the study of Deshmukh et al [11]. 3 gm. of Graphite powder and Sodium Nitrate (NaNO₃) each, was charged in a three-neck flask and added to 150ml of Sulphuric acid (H₂SO₄) with stirring. The solution was cooled at 5 °C for 5 h followed by addition of 9 gm. of Potassium permanganate (KMnO₄) with continuous stirring. Graphite oxidation was performed for 48 h at 40 °C and dilutions for the mixture included mixing with 150 ml distilled water and 30 ml of 30% Hydrogen peroxide (H₂O₂). Finally, the contents were washed, filtered and centrifuged for de-acidification. GO powder was produced by vacuum drying for 60 °C for 13 h. Silanization of GO nanoparticles was performed to improve its interaction and adhesion to resin matrix, by adding 5% silane to the solvent (95% acetone) [12].

**Preparation of control and experimental adhesives**

The process for adhesive fabrication was adopted from the study by Ye et al [13]. The ingredient portions were selected accurately using a precision balance (The Precisa 1600C, MN, USA). The adhesive was formulated by mixing 25% HEMA, 25% TEGDMA and 50% Bis-GMA by weight with ethanol as a solvent (30% - m/m). With respect to monomer amount, 0.5% (n/n) ethyl 4- dimethylamino benzoate and 0.5% camphorquinone as photo-initiators, and 1.0% (n/n) diphenyliodonium hexafluorophosphate (DIIPHP) as an electron initiator were added [14]. A mixture was produced with a magnetic stirrer and condenser (SUS304 REXIM; MonotaRO,
Tokyo, Japan) in a flask. The mixture was mixed in a dark box with red light and was kept in isolation under foil to prevent exposure to ambient light and avoid photo-polymerization. The adhesive produced was considered control adhesive (CA) in the study.

GO powder was added in 2 ml ethanol in microvial and sonicated for 10 min at 37 °C in an ultrasonicator (VWR USC-TH sonicator bath, Tokyo, Japan). GO powder was introduced in the ethanol solvent of the synthesized control adhesive at 0.5% and 2.0% for producing experimental adhesives GOA1 and GOA2 respectively (table 1). For a homogenous mix of the GO particles with other components, the nanoparticles were initially mixed in resin and sonicated in an ultrasonic bath (VWR USC-TH sonicator bath, Tokyo, Japan) for 10 min. Later, the mixture was homogenized in an ultrasonic homogenizer (Q500 Sonica) at pulse on/off for 60 s at room temperature. To ensure GO was homogenously dispersed after storage, the mixture was re-homogenized in the ultrasonic homogenizer on every use. The weight of the nanoparticles was calculated in milligrams and the volume of resin in milliliters. In order to calculate 0.5 wt.vol % and 2.0 wt.vol %, the following calculations were used. The adhesives produced were stored at 4 °C with 2 weeks to use.

\[
\text{Weight} / \text{Volume} \% = \text{weight of solute/volume of solution} \times 100
\]

Table 1. Components of the adhesive produced.

| Categories     | CA Resin adhesive (volume in ml) | Nanoparticles (weight in mg) |
|----------------|----------------------------------|------------------------------|
| GOA1–0.5 wt.vol% | 2 ml                            | 10 mg                        |
| GOA2–2.0 wt.vol% | 2 ml                            | 40 mg                        |

CA Resin adhesive composition- wt% (24% HEMA, 24% TEGDMA, 50% bis-GMA, 1% DPIHP, 0.5% EDB & 0.5% camphorquinone).

Appraisal of experimental adhesive

Five samples each for experimental adhesives, (GOA1 and GOA2) were produced after photo-polymerization with a dental light curing source (Curing Light, Eliphar S10; 3M ESPE, St. Paul, MN, USA) with 600 mW.cm\(^{-2}\) output for 20 s at 10 mm distance. They were assessed for GO presence and interaction within the resin adhesive using a scanning electron microscopy (SEM). Specimens were sputter coated with gold for 120 s (Baltec SCD sputter, Scotia, NY, USA) and employing a carbon conductive tape, specimens were mounted on aluminum stubs. Analysis was performed using SEM (JEOL, JSM-6513, SEM, Tokyo, Japan) at 30 kV accelerating voltage at multiple magnifications of convenience.

Specimen preparation and bonding procedure

One hundred and two extracted non-carious maxillary premolars were obtained from department of clinical dentistry. Teeth were cleaned off inorganic tissues using a ultrasonic scaler (Superior Instruments Co, Newyork, USA). Teeth were disinfected with chloramine Trihydrate (Merck, Germany) immersion and stored in distilled water at 4 °C. Teeth were embedded vertically using acrylic resin (Opti-Cryl, South Carolina, Columbia) in sections of polyvinyl tubes (4 mm diameter) at the cemento enamel junction (CEJ).

Occlusal surface of all teeth were removed with a diamond saw (Buehler Isomet 2000 Precision saw, IL, USA) to expose dentin 1 mm below the dentino enamel junction (DEJ). An area of sound dentin (5 mm) was identified in the middle of the occlusal surface and conditioned with 36% phosphoric acid (DeTrey conditioner, Dentsply, PA, USA) for 10 s followed by washing and drying with cotton pellets. 34 teeth each were treated with formulated adhesives (CA, GOA1 & GOA2) using standard protocol, resulting in three study groups. The adhesives are dispensed on a disposable mixing pad and smeared on the conditioned dentin with a micro-brush for 15 s followed by air thinning for 5 s. A second application in a similar way was also applied to all specimens. This followed photo-polymerization for 20 s at 10 mm distance using a curing device (Curing Light Eliphar S10; 3M ESPE, St. Paul, MN, USA).

Adhesive applied specimens were covered with resin composite (Filtrek Supreme; 3M ESPE, St. Paul, MN, USA) build-ups of 4mm height in increments (2 mm) using a acrylic jig, plastic instrument and condenser. Excess was removed and the interface was light cured from all sides for 20 s each. These bonded tooth specimens were stored in distilled water at 37 °C for 1 week. 60 specimens were used for microtensile bond testing (μTBS) in the three adhesive groups (n = 10) (CA, GOA1 & GOA2). Seven bonded specimens in each adhesive group (n = 7) were used for assessment of bond integrity using SEM and similarly seven more bonded teeth were assessed using Micro-Raman spectroscopy within each adhesive group. All bonded tooth specimens were sectioned to produce bonded beams of 1 mm × 1 mm.
Microtensile bond test (μTBS)

Half of the bonded specimen in each adhesive group (CA, GOA1, & GOA2) (n = 10) were exposed to 10,000 thermo cycles in distilled water baths at 5°C and 55°C for 30 s each and 5 s dwelling time (THE-1100, SD Mechatronik GmbH, Germany). The remaining half specimens (n = 10) were stored in distilled water for 24 h prior to sectioning. The bonded specimens within each adhesive group (n = 40) [CA, GOA1 (0.5%) & GOA2 (2.0%)] were sectioned to fabricate 1 mm x 1 mm beams of composite–adhesive-dentin using a diamond slow speed saw (Buehler Isomet 2000 Precision saw, IL, USA). Each group included 20 teeth and each tooth produced 6 beams, however beams, which fractured or did not fit the dimensions, were discarded. A minimum of five bonded beams from each tooth was assessed for μTBS in respective adhesive groups. Beams were secured to the jaws of micro tensile tester (Bisco Inc., Virginia, USA) with cyanoacrylate (Zapit, Dental ventures Inc., CA, USA) and loaded under tension at 0.5 mm min \(^{-1}\) crosshead speed until fracture. Obtained μTBS in each group was compared using ANOVA and multiple comparisons test.

Micro-Raman spectroscopy of dentin-adhesive bonded specimen

Twenty-one bonded teeth were sectioned to produce beams for interfacial assessment of specimens within three study groups [CA, GOA1 & GOA2 (n = 7)] using Micro-Raman spectroscopy analysis. A Micro-Raman spectrophotometer (ProRaman-L Analyzer; TSI, Shoreview, MN, USA) along with its software (Raman reader) was utilized to identify Raman spectra. Corrections for dark counts were undertaken with sample positioning adjustments to obtain adequate signals. The laser beam was focused using a 0.9 objective lens and 600 mW power on the adhesive interface. The computer operated stage allowed for sample movements for Raman scatter at desired locations. Specimens were divided into sections of 1 mm and a 60 s scan was performed thrice for selected specimen area. The details of the spectrum were obtained at a laser beam wavelength of 785 nm between at 800 cm \(^{-1}\) to 1800 cm \(^{-1}\) with noise filtration.

SEM observation of adhesive-dentin interface

Twenty-one bonded teeth among study groups [CA, GOA1 & GOA2 (n = 7)] were sectioned (explained previously) to produce bonded beams (1 mm x 1 mm) for SEM and energy dispersive x-ray spectroscopy (EDX). The beams were wet-polished (Buehler Polisher, Lake Bluff, IL, USA), washed and placed in ultrasonic bath (distilled water for 5 min) (Bandelin Digital- Sigma-Aldrich Darmstadt, Germany) with distilled water. This followed conditioning with 36% phosphoric acid (DeTrey conditioner, Dentsply, PA, USA) followed by washing and immersion in NaOCl solution (5.25%, 15 min) and washing with water again. The specimens were dehydrated by placement in ethanol solution (80%, 90% and 100%). Specimen mounting and gold coating was performed as explained earlier. Observations of the interface for the specimens in groups CA, GOA1 and GOA2 were performed using SEM (JEOL, JSM-6513, SEM, Tokyo, Japan) operating at 30 kV voltage at multiple magnifications and line EDX.

Degree of conversion (DC)

Assessment of DC of the adhesives included in the study was performed using FTIR. The adhesives including CA, GOA1 and GOA2 were tested before and after photo-polymerization by smearing a drop on the Potassium bromide disc (Shimadzu, Kyoto, Japan). The C–C double bonds were assessed and the absorbance peaks were recorded. For analysis of spectrum, the adhesive remain in contact with the sensors and FTIR (Thermo Scientific Nicolet iS20 FTIR spectrometer, MA USA) provided absorbance peaks. DC was determined from spectrum between 400 to 4000 cm \(^{-1}\). After recording peaks for uncured resin, the adhesive resin was photo-polymerized for 40 s and FTIR was recorded again. The C=C absorbance peak (1638 cm \(^{-1}\)) and C–C reference peaks (1607 cm \(^{-1}\)) were obtained using baseline methods \([15, 16]\). The ratios of (C=C & C–C) absorbance intensities (percentage of unreacted double bonds) before and after polymerization was identified employing the below equation \([17]\),

\[
DC = \left[1 - \frac{\text{Aliphatic}}{\text{Aromatic}}\right] \times 100%
\]

Where,

\(\frac{\text{C} \text{aliphatic}}{\text{C} \text{aromatic}}\) is 1638 cm \(^{-1}\) absorption peak of polymerized resin,

\(\frac{\text{U} \text{aliphatic}}{\text{U} \text{aromatic}}\) is 1607 cm \(^{-1}\) absorption peak of polymerized resin,

\(\frac{\text{C} \text{aromatic}}{\text{U} \text{aromatic}}\) is 1638 cm \(^{-1}\) absorption peak of unpolymerized resin and

\(\frac{\text{C} \text{aliphatic}}{\text{U} \text{aliphatic}}\) is 1607 cm \(^{-1}\) absorption peak of unpolymerized resin.
Results

SEM and EDX analysis of synthesized adhesives
SEM analysis of synthesized GO nano-filler particles showed presence of numerous layers of both sheets stacked together and agglomerated structures (figure 1). In addition, formulated GOA1 showing (A) 0.5 wt.% and GOA2 (B) 2 wt.% graphene in resin matrix (figures 2(A) and (B)). In addition, figure 2(C) shows EDX Electron image with elemental mapping showing distribution of C = carbon attributing to the added graphene and Si = silica attributing to the main filler additive from the dentin adhesive.

SEM, EDX and Raman spectroscopy assessment of adhesive interface
SEM examination of graphene modified dentin-adhesive interface revealed numerous resin tags from well-filled dentinal tubules (figure 3(A)). A clear demarcated hybrid layer was observed between GO experimental adhesive and dentin surface. In addition several graphene nanoparticles were widely dispersed within resin matrix of dentine adhesive that forms coupling interaction between graphene nanoparticles and resin matrix (figure 3(A)). Figure 3(B) presents SEM images of GO (0.5%) adhesive (GOA1) interface, revealing significant thickness of hybrid layer, with formation of resin tags of GOA1 as its integral part. Presence of patent dentinal tubules was also observed. GO nanoparticles were dispersed in the adhesive resin and dentin indicating positive interaction between graphene nanoparticles, adhesive resin matrix and dentin. EDX line analysis (GOA 2–2.0 wt%) of the bonded interface displayed evidence of high amount of carbon (C) and oxygen (O) which was due to the graphene oxide nanoparticles, with the dispersion of Si in relation to the main filler of the dentin adhesive (figure 3(A)).

Micro-Raman spectra of GO showed characteristic D and G bands with peaks at 1341 and 1607 cm⁻¹ (figure 4(A)). Where the G mode is assigned to the vibration of sp²-hybridized carbon, while the D mode is related to the sp³-hybridized carbon and deficiencies relevant with grain boundaries and vacancies in GO. In addition, Micro-Raman spectrum of dentin at the interface was observed between 700 cm⁻¹ and 1800 cm⁻¹ (figure 4(B)). The peaks at 1661 cm⁻¹ and 1246 cm⁻¹ were associated with organic components such as Amide I and Amide III for dentin collagen (figure 4(B)). Moreover, the spectra of 0.5 wt.% and 2.0% GOA1 and GOA2 bonded specimens showed the presence of similar peaks at 1341 and 1607 cm⁻¹, representative of GO in the adhesive (figure 4(C)). The figure 4(C), also presents characteristic dentin peaks at 1661 cm⁻¹ and 1246 cm⁻¹.

Microtensile bond strength (μTBS)
Outcomes for microtensile bond strength among the study groups are presented in table 2. The present study compared μTBS for experimental adhesive (CA-control) and GOA1 and GOA2, with and without thermocycling (TC). Incorporation of GO nano-filler content showed a significant difference in the adhesive μTBS with dentin in comparison to controls (p < 0.01). Furthermore, the ageing process revealed a significant
reduction in the $\mu$TBS, except in GOA2 specimens who showed comparable $\mu$TBS outcomes among TC and non-TC specimens ($p > 0.05$).

The highest mean $\mu$TBS was observed in non-TC GOA2 $[33.20 \pm 3.55 \text{ MPa}]$ specimens, whereas the lowest $\mu$TBS mean value was observed in thermocycled GOA1 $[23.71 \pm 3.10 \text{ MPa}]$. Comparatively, the non-TC specimens showed significant difference in the $\mu$TBS among the three groups (CA, GOA1 & GOA2) indicating an increase in the $\mu$TBS with increase incorporation of graphene content in the experimental adhesive. However, TC specimens revealed a significant difference only among the GO containing adhesives; with GOA2 $[31.17 \pm 3.15 \text{ MPa}]$ exhibiting higher $\mu$TBS compared to GOA1 $[25.68 \pm 3.80 \text{ MPa}]$.

Majority of the specimens displayed adhesive failure in the bond strength test as shown in the table 2. The adhesive failure ranged between 70%–80% among the non-TC groups whereas 100% adhesive failure was observed only in TC control specimens. GOA1 and GOA2 showed 70% adhesive failures; with 20 and 40% mixed failures respectively. Regardless of the ageing, comparable bond failure types were observed among study groups.
Degree of conversion (DC)
FITR analysis was employed to assess the DC in the experimental adhesives as shown in table 3. Three experimental adhesives were compared; 0.5% GOA1, 2.0% GOA2 and control (CA) to assess the influence of graphene content on the DC. Study groups were assessed on two peak levels; the \(C=\)C absorbance peak (1638 cm\(^{-1}\)) and C–C reference peaks (1607 cm\(^{-1}\)) to calculate and compare the DC.

The figures for the 0.5% and 2.0% GO represented symmetric and asymmetric stretching of \(C=\)C and C–C at 1638 cm\(^{-1}\) and 1607 cm\(^{-1}\) respectively, implying a high degree of reduction of GO with an increase in GO content level whereas peaks levels observed prior and after to 1000 cm\(^{-1}\) and 1638 cm\(^{-1}\) corresponds to the degree of C–O bending and C=O stretching vibration, respectively (figure 5).

Tukey-Kramer test revealed comparable outcomes \((p > 0.05)\) of DC between GOA2 [37.7 (4.2)\%] and GOA1 [42.3 (2.9)\%] adhesives; however, a significant difference was noted between the control [46.8 (3.6)\%] and GOA2 [37.7 (4.2)\%] adhesives (table 3). Thus, it was observed that increasing the graphene oxide content presented with a decrease in the DC mainly due to limited light absorbance as a result of overfilling of the adhesive.

Discussion
The present study aimed to compare the formulated experimental GO adhesive with varying percentage of filler content; GOA1 (0.5\%) and GOA2 (2.0\%), to observe the influence on the dentine interaction, bond strength and structural reliability of the material using Fourier transform infrared spectroscopy (FTIR), Micro-Raman spectroscopy, DC and SEM.

The longevity of the restoration depends upon the bond strength, which remains a challenge to accomplish that relies partially on the development of the adhesive properties. Thus, the study intended to demonstrate the effectiveness of introducing an inorganic nanofiller in the adhesive for augmented mechanical properties and longevity of the restoration. The study revealed a significant difference in the tensile strength among the GO adhesives compared to the control \((p < 0.01)\). However, with an increase in the filler content, the effect of the aging process was comparable among GOA2 adhesives \((p > 0.05)\). Likewise, SEM analysis and Micro-Raman analysis revealed that increasing the GO nanofiller content displayed a well-dispersed demarcated hybrid layer with signs of patent tubules in GOA2. On the contrary, increasing the graphene oxide content negatively affected the degree of conversion, which is bound to influence the strength of the hybrid layer. Thus, the hypothesis can be partially accepted. To corroborate the findings, a multiplicity of explanation will be discussed including the comparison among the experimental adhesives (GOA1 & GOA2) with different nano-filler content, bond strength and DC.
To establish the clinical application of the present study, the aging process was employed on the experimental specimens. TC is a process employed to simulate oral conditions that adversely affect bond integrity due to difference in thermal expansion coefficients between the tooth structure and the adhesive [18]. Thus, the thermal stress generated under TC weakens the bond strength and enables adhesive failures due to the hydrolysis of the bond. Studies have shown that the impact of thermocycling varies with the number of thermal cycles and the type of adhesive used [19, 20]. Hence, the increased number of thermocycles reduces the bond strength due to increased absorption of water. The present study revealed a significant difference between non-thermocycled (NTC) and thermocycled (TC) specimens; however, increasing filler content in the adhesive reduced the effect of aging process probably due to GO amphiphilic nature where water channels from hydrophilic sites to hydrophobic carbon sites [21]. Thus, GOA2 presented with comparable outcomes for bond strength with and without TC. It was further reported that the thermocycling influenced the fracture modes, thus varying filler content and thermocycling reduces cohesive failures and increases the mixed failure among the specimens [20, 22]. Hence, the findings of the study are in agreement with reported results, which presented a reduction in adhesive failure with an increase in filler content.

A standard universal adhesive mostly comprises of two major components, Bis GMA and HEMA, which enhances the flow properties of the material for deeper resin tags formations [14]. A plausible explanation for the observed difference in μTBS between the experimental adhesive and control, was due to the resin blends and the dentinal interaction. BisGMA has high molecular weight compared to the HEMA, which provides stiffness to the materials whereas HEMA low weight property enables it to flow deep to form the resin tags [14]. Thus, adding the filler to the adhesive to improve the mechanical properties raises the concern for influencing the viscosity of the material and deeper resin tags formation. Furthermore, the SEM analysis provided a detailed

![Figure 3](A) SEM (50X) examination of graphene modified dentin-adhesive (GOA2%) interface revealed resin tags from well-filled dentinal tubules. A clear demarcated hybrid layer exists between graphene modified adhesive and dentin surface in longitudinal section. Note several graphene nanoparticles widely dispersed within resin matrix of dentine adhesive that forms coupling interaction between graphene nanoparticles and resin matrix. The EDX line analysis (GOA 2–2.0 wt%) of electron image showing high amount of carbon (C) and oxygen (O) which is due to the graphene oxide nanoparticles, with the dispersion of Si in relation to the main filler of the dentin adhesive. (B) SEM examination of graphene oxide (0.5%) adhesive (GOA1) interface revealing significant thickness of hybrid layer (HL). Formation of resin tags (white arrows) from the GOA1 are clearly forming a part of hybrid layer. Dentin tubules are identified (DT). Graphene oxide nanoparticles are well dispersed in the adhesive resin and dentin indicating positive interaction between graphene nanoparticles, adhesive resin matrix and dentin.

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examination of the two types of GOA indicating GOA2 occupying a higher degree of tubules compared to the GOA 1 that left some patent tubules (figure 3). Nevertheless, the study showed that the graphene oxide incorporation enhanced bond strength with deeper dentin penetration with an increase in the filler content. This is attributed to its hydrophilic nature that assimilated the water content to improve flow and interaction, providing mechanical support to the hybrid layer. In addition, as the percentage of coupling agent in the system is not very significant, GO particles were silanized, to improve the interaction at the resin matrix and filler interface. As dental resins are exposed to mechanical and thermal stresses, treatment with coupling agents improves material performance and long-term stability [23]. The quality of the interface also affects the mechanical properties of the material [24], as shown by adequate μTBS outcomes in the present study. This is also supported by the fact that adequate coupling of the matrix and filler, improves compatibility and reduces leaching of particles, therefore diminishing the possible hydrolytic degradation of the dental resins [25].

Incorporation of the GO has presented with an increase in the bond strength; however, the developing strength depends upon the DC. Studies have shown that the DC varied inversely to the filler percentage in the material [26]. The addition of the filler to the material has shown to reduce the space for transmission of light as the filler acts as a barrier for deep penetration and scatter the light, especially when the filler size is equivalent to

![Figure 4](image-url)

**Figure 4.** Raman spectra of (A) GO particles showing characteristic D and G bands, as two prominent peaks at 1341 and 1607 cm$^{-1}$. The G mode is assigned to the vibration of sp2-hybridized carbon, while the D mode is related to the sp3-hybridized carbon and deficiencies relevant with grain boundaries and vacancies in GO. (B) Raman spectrum of dentin samples recorded between 700 cm$^{-1}$ and 1800 cm$^{-1}$. The peaks at 1661 cm$^{-1}$ and 1246 cm$^{-1}$ are associated with organic components such as amide I and amide III for dentin collagen. (C) Raman spectra of 0.5 wt.% and 2.0 wt.% GO experimental adhesive bonded specimens to tooth dentin. The presence of same peaks at 1341 and 1607 cm$^{-1}$ is representative of GO in the adhesive resin bonded with tooth dentin showing their own characteristic peaks at 1661 cm$^{-1}$ and 1246 cm$^{-1}$.
the output wavelength of the light-curing device [5]. Likewise, a significant effect was observed in the present study between the control and GO adhesive, as the percentage of added filler was increased, numerous layers of both sheets were stacked together and created agglomerated structures that hindered the pathway of light. Furthermore, in accord with previous studies, the increasing filler content from 0.5% to 2.0% did not show an evident effect on the degree of conversion [5, 27]. Scientist has inferred that the particle size and percentage influence the rheological properties of the polymer-based materials [28]. The higher percentage has been shown to affect the viscosity of the material due to alteration in surface area interaction. Thus, the concentration of the filler in the material should be optimized to avoid cluster creations, which leaves voids within the hybrid layer due to insufficient inter-tubular space occupation thereby reducing the bond strength. Therefore further studies correlating material viscosity and rheological properties with regards to adhesive bond integrity are recommended.

For characterization of graphene-based materials, Micro-Raman microscopy and FTIR analysis are a powerful technique employed, as it discriminates between the layers of graphene. Micro-Raman spectroscopy highlighted the two peak bands G and D, which indicate in-plane vibrations of sp2-bonded carbon atoms and single phonon lattice vibrations representing the sp3-bonded carbon atom, respectively [6, 7]. According to Aguiar et al [5], 0.5% loaded nanocomposite displayed D band at 1360 cm\(^{-1}\) and a G band at 1627 cm\(^{-1}\) whereas for 2.5% loaded nanocomposite, the D band and the G band was observed at 1345 cm\(^{-1}\) and 1601 cm\(^{-1}\), respectively, due to decrease resonance of isolated double bonds. However, the present study demonstrated comparable peaks for the G and D band for both the experimental adhesives due to the difference in the type of material that influenced the carbon double bond presences and interactions. On the other hand, the FTIR study analysed the complex interaction between the compounds in a material [29]. FTIR studies have shown that the oxidation of the graphite shifts the C–C bond value from 1586 cm\(^{-1}\) to 1618 cm\(^{-1}\) indicating the stretching vibration of the C=C bond; however, different values were obtained from various studies indicating changes in the oxygen functional group [3, 17]. The present study displayed similar changes in the findings. Both experimental GOA represented symmetric and asymmetric stretching of C=C and C–C at 1638 cm\(^{-1}\) and 1607 cm\(^{-1}\) respectively; nevertheless, an increase in GO content level implied a high degree of reduction of GO.

According to Yousefi et al [30], filler size plays an important role in modifying the material behavior and interaction with the dentine. Lowering the average size has shown nuance in regulating the viscosity of the material that increases the surface area for interaction among the particles even in the state of lower filler concentration. Thus, this enables them to create clusters and provides resistance for deeper penetration into the

| Table 2. Mean and SD of Microtensile bond strength and failure modes among the tested study groups. |
|-----------------------------------------------|
| Group (n=10) | $\mu TBS$ (MPa) (Mean $\pm$ SD) | Failure mode analysis (%) |
|-----------------|-----------------|-----------------|
| NTC TC | P value$^a$ | Adhesive | Cohesive | Mixed |
| CA | 26.73 ± 2.51$^{bA}$ | — | < 0.01 | 80 | 0 | 20 |
| GOA1 (0.5%) | 30.87 ± 3.32$^{bA}$ | 23.71 ± 3.10$^{bB}$ | 100 | 0 | 0 |
| GOA2 (2.0%) | 33.20 ± 3.55$^{bA}$ | — | 70 | 10 | 20 |
| — | 31.17 ± 3.15$^{bA}$ | 60 | 0 | 40 |

TC: Thermocycling, NTC: No Thermocycling, GOA: Graphene oxide adhesive, CA: Control adhesive.

$^a$ ANOVA. Dissimilar small alphabets in same column indicate statistical significance. Dissimilar capital alphabets in same row indicate statistical significance.

| Table 3. Degree of conversion in percentage of the Graphene-modified experimental adhesives and control. |
|-----------------|-----------------|-----------------|
| Adhesive Groups | Degree of conversion (mean $\pm$ SD) | Tukey$^a$ |
|-----------------|-----------------|-----------------|
| Control | 46.8 ± 3.6 | A |
| GOA1 (0.5 wt% GO) | 42.3 ± 2.9 | AB |
| GOA2 (2.0 wt% GO) | 37.7 ± 4.2 | B |

GO: Graphene oxide.

$^a$ Tukey Kramer multiple comparisons test.

Dissimilar letters indicate statistical significance.
tubules. However, the present study does not account for the filler size, which should be a mere focus in future studies to evaluate the effects on their long-term degradation on bond strength. Moreover, the in-vitro experiment was under a controlled environment; however, variables such as dentine humidity and material shrinkage over a period might alter the mechanical properties of the material in vivo conditions. Therefore, clinical prospective studies are required, that reflect variations of the clinical environments.

Conclusions

Within the experimental conditions, GO exhibited interaction within adhesive and tooth dentin comparable to control adhesive. Increasing GO content showed increase in $\mu$TBS of adhesive to dentin, but a decrease in DC. Under ideal conditions, experimental adhesive with 2% GO content showed acceptable bond strength and DC should be further assessed under dynamic conditions to recommend clinical use.

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Figure 5. FTIR spectra of the (A) 0.5 wt% GO uncured (B) 0.5 wt% GO cured (C) 2.0 wt% GO uncured (D) 2.0 wt% GO cured (E) control nanoparticle free uncured and (F) control nanoparticle free cured adhesive.
Declarations

Conflicting interests
The authors declare that they have no conflict of interest and all authors have read and approved the final draft.

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