EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 68, 3733:3740, OCTOBER, 2022
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
FIXED PROSTHODONTICS AND DENTAL MATERIALS
Submit Date : 28-08-2022 • Accept Date : 26-09-2022 • Available online: 01-10-2022 • DOI :10.21608/edj.2022.158532.2232

THE EFFECT OF INCORPORATING GRAPHENE OXIDE NANOPARTICLES WITHIN SELF-ETCH ADHESIVE ON THE ANTIBACTERIAL PROPERTIES AND SHEAR BOND STRENGTH

Wael Mohamed Gamal [*], Shaimaa Ahmed Alrafee[**] *and* Ahmed Magdy Sayed[***]

ABSTRACT

Objectives: This study assessed the antibacterial effect and the shear bond strength of self-etch adhesive after incorporating Graphene oxide nanoparticles.

Materials and methods: Graphene oxide nanoparticles was synthetized and incorporated within a self-etch adhesive (Quadrant Uni-SE-Bond) with 0% control group (without Graphene) (group I), 2% (group II) and 5% (group III). The antibacterial effect was evaluated against *S. Mutans* using agar well diffusion method. 24 holes (n=8) with a diameter of 6-8 mm were performed and the inhibition zone was evaluated in millimeters after 24 hrs. For shear bond strength, 12 premolars were sectioned horizontally to expose dentin and to obtain 24 specimens for assessing the bond strength (n=8). Composite cylinders (2x2 mm) were bonded to the dentin and subjected to shear bond strength using universal testing machine.

Results: The incorporation of Graphene oxide nanoparticles into self-etch adhesive showed a significantly dose-dependent antibacterial effect. On the other hand, the shear bond strength reported no significant difference between the three groups.

Conclusion: Addition of Graphene oxide NP to self-etch adhesive produced an antibacterial effect without affecting the bond strength.

KEYWORDS: Graphene oxide NP, self-etch adhesive, antibacterial, shear bond strength.

* Lecturer, Operative Department, Faculty of Dentistry, Modern University for Technology and Information
** Lecturer of Operative Dentistry, Faculty of Dental Medicine for Girl's, Al-Azhar Universit, Cairo, Egypt
*** Lecturer, Dental Biomaterials Department, Faculty of Oral and Dental Medicne, Modern University for Technology and Information, Cairo, Egypt

INTRODUCTION

Resin composite reserve its unique position as reliable solution for direct tooth restoration due to its superior aesthetics and enhanced mechanical properties [1]. However, their bonding with tooth structure requires using dentin adhesives to bond hydrophobic resin composites to hydrophilic dentin tissue, for preservation of dentin and increase the caries resistance [2]. Unfortunately, Adhesion with dentin showed more obstacles than adhesion to enamel due to lower mineralization of dentin and higher water content compared to enamel that makes dentin bonding procedure technique sensitivity [3]. Bonding with dentin depends on many factors such as ability of monomers to penetrate inter-collagen fiber spaces and stable resin tags that establishing a hybrid layer [3]. The failure of adhesive bond with tooth structure is considered one of the main causes of the composite restoration failure. This loss of bond leads to the formation of nano-gaps resulting in bond failure and development of secondary caries [3].

Dentin bonding agents play a major role in the bond durability. Therefore, many materials, techniques, concepts and classifications have been developed. The recent classification of dentin adhesives is based on clinical steps as etch-and-rinse and self-etch. The former approach involves application of phosphoric acid followed by its rinsing off then application of primer and adhesive either separately or in combination. Alternatively, self-etch approach evolves using of monomers with acidic functional groups to deal as conditioning and priming agent in two steps or only one step. The one-step, self-etch adhesives also known as seventh generation according to the chronological classification [4].

In order to increase durability of dentin adhesive bond, addition of inorganic fillers in adhesives is suggested to improve their interaction with the dentin surface and possibly minimizing dentin-adhesive degradation [5-7]. Previous studies have shown that addition of fillers in adhesives can reduce water sorption and solubility, in addition to enhancing its mechanical properties [5-9]. Therefore, addition of inorganic nano-materials as fillers to improve the physical and mechanical properties of dentin adhesives is highly desirable.

Graphene is one of the materials that attracted the attention of dental researchers in recent years. Graphene based materials own high surface area and a good chemical and thermal stability. Graphene family nanomaterials (GFNs) contain many members such as graphene, graphene oxide and reduced graphene oxide. Graphene oxide (GO) has a unique position within that family. It can be synthesized by graphite oxidation. Thanks to the occurrence of oxygen in GO as functional groups, its hydrophilic nature, when compared with other GFNs appeared. This hydrophilicity could be considered an advantageous property as it helps GO to form steady colloid dispersion and evade aggregation, thus making it more cyto-compatible [10,11].

One of the interesting properties of GO is their antibacterial effect. Previous studies were performed to assessing the efficacy of GO nanosheets in inhibiting the growth of *P. gingivalis*, *F. nucleatum*, *S. mutans*, *E coli*, *Candida albicans* and *S aureus*. It was reported that GO was able to inhibit the growth of these common dental pathogens effectively [12-13].

The notable features of thermal conductivity, superior mechanical properties, and enhanced electronic transport of GO enable it suitable for incorporation with adhesives for improving resin dentin bond formation [5]. Moreover, the presence of functional groups at GO, such as hydroxyl, epoxy, carboxylic, and ketonic groups enable it to interact by Van der Waals interaction with polymers [14].

The incorporation of the GO within dentin adhesives showed promising results in improving the mechanical properties and bonding with dentin [6,7,9]. However, the evidence related to the impact of GO nanoparticles on the mechanical and antimicrobial

properties of the adhesive is limited. Therefore, the study aimed to evaluate the effect of graphene oxide nanoparticles content on the bond strength and the antimicrobial properties of commercially available seventh generation adhesive system.

MATERIALS AND METHODS

Sample size calculation

The minimal sample size was calculated based on a study aimed to evaluate the effect of experimental dentin bonding agent loaded with different concentrations of graphene oxide on the micro-tensile bond strength [6]. The sample size was determined according to a one-way ANOVA study. It was calculated with a power of 95% and a significance level of 95%. A total sample size of 24 sound tooth (8 for each group) was required. Sample size was calculated using G power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).

Preparation of Graphene oxide

The preparation of graphene oxide was done by adding 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (360:40 mL) to a mixture of graphite flakes (3.0g, 1 wt. equiv.) and $KMnO_4$ (18.0 g, 6 wt. equiv.), in order to produce a slight exotherm to (35-40°C). After that, heating to 50°C and stirred for 12 h of the mixture was done then cooling to room temperature and pouring onto ice (-400 mL) with 30% H_2O_2 (3 mL). a centrifugation at 4000 rpm for 4 h was done to filtrate. The solid material washed with pure water, 30% HCl, and finally with 200 mL of ethanol. The mass was dried using oven at 90 °C for 24 hours.

Characterization of Graphene Oxide Nanoparticles

A) Visible Near Infra-Red (Vis-NIR) Spectroscopy

The absorption rate of the graphene oxide nanoparticles was assessed and drawn by a visible Near Infra-Red (Vis-NIR) with a special computer software **(Fathy** & **Riad** (2019) [15] and **Prabhu** & **Poulose** (2012) [16]**)**.

B) Transmission Electron Microscope Imaging (TEM Imaging)

Using a transmission electron microscope, the particles size, shape and distribution of the prepared GO NP were recorded **(Fathy** & **Riad** (2019) [15], **Pérez-Díaz** et al. (2015) [17] and **Espinosa-Cristóbal** et al (2009) [18]**)**.

C) X-Ray diffraction (XRD) analysis

The size of the crystallite was determined and calculated from the broadening of the reflection profile of the XRD pattern and the interlayer spacing from the corresponding position of the peaks **(Aidaros** & **Kamh** [19]**)**.

An XRD pattern has been performed using XPERT-PRO Powder Diffractometer system, with 2 theta (20^o - 80^o), with Minimum step size 2Theta: 0.001, and at wavelength (Kα) = 1.54614^o.

Preparation of graphene oxide in bonding agent

Graphene oxide were incorporated into self-etching bonding solution (Quadrant Uni-SE-Bond) in concentrations of 0, 0.02 and 0.05 gm of graphene oxide powder to 100 ml liter of the bonding agent to form three groups of bonding agent [6];

Group I: 0% (GO NP) (control group)

Group II: 0.02 (2 % GO NP)

Group III: 0.05 (5 % GO NP)

Antimicrobial activity test

Pure strains of *streptococcus mutans* (ATCC 25175) were obtained from a standard microbiology lab. The agar well diffusion method was used to determine the antimicrobial activity of the selected strains. Inoculation of the agar plate surface was done by spreading a volume of the microbial inoculum over the entire surface of the agar. Then,

TABLE (1): Material used, specification, composition, lot number and manufacturer.

Material/ Specification	Composition	Wt%	Manufacturer	Lot number
Quadrant Uni-SE-Bond	Methacrylate-based monomers	24 %	Cavex Holland BV,	K010925
Light curing self-etching	4-Metacryloxyethyl-trimellitic acid	19 %	https://www.cavex.nl/	
adhesive	Silica filler	~1 %		
	Polymerization catalysts	~1 %		
	Acetone	36 %		
	Water	19 %		

24 holes (8 holes for each group) with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100mL) of the different tested materials was introduced into the well. After incubation times of 24 h, the resulting inhibition zone diameters (in mm) surrounding the wells measured to the nearest whole millimeter at the point at which there was prominent reduction in growth [20].

Shear bond strength testing

A total of 12 premolars were extracted for orthodontic treatment was used for shear bond testing. The crowns were separated from the roots at about 1 mm apical to cemento-enamel junction then the crowns were sectioned into two halves in a buccolingual direction, so 24 samples were obtained (8 samples for each group n=8).

After removal of pulp remnants, the inner surfaces of the crowns were ground to produce a flat and smooth dentin surface. Specially fabricated rectangle two halves split Teflon mold of 8mm height, 2cm width and 2.8cm length were used. The specimens were mounted in self-cured acrylic resin inside the mold with their flat dentin surface upward and in the same line with the flat acrylic resin.

After curing of the acrylic resin, the samples were removed from the mold and the bonding agent with different graphene concentration were applied according to manufacture instruction. A transparent silicone cylinder (2mm in diameter and 2 mm in height) were used to produce composite cylinders. The transparent cylinders were filled with composite resin and applied onto the pretreated dentin surface then the composite resin was covered with a piece of clear matrix band and pressed with glass slap of 8ml thickness and the excess composite resin was removed with sharp instrument. After removal of glass slap, the composite material was cured for 20 sec. The silicone cylinders were removed and the specimens were stored in distilled water at 37°C for 24 hrs. [21].

The twenty-four samples were individually and horizontally mounted on a computer-controlled materials testing machine with a load cell of 5 kN. The samples were secured to the lower fixed compartment of testing machine by tightening screws. Shearing test was done by compressive mode of load applied at tooth- resin interface using a mono-beveled chisel shaped metallic rod attached to the upper movable compartment of the testing machine that travel with a cross-head speed of 0.5 mm/min. The load required to debonding was recorded in Newton and the data were recorded using computer software.

Statistical analysis

Data was analyzed with IBM® SPSS® Statistics Version 25 for Windows (Armonk, New York, USA). For each group, the mean and standard deviation values were calculated. Normality test was done using Kolmogorov-Smirnov test and clarified

normal distribution between values of each group. Homogeneity test was done using Levene's test and revealed that there was a homogenous distribution between all variables. Therefore, one-way ANOVA followed Tukey post-hoc tests were accomplished (with significance level set at $P \leq 0.05$) to reveal the statistically significant difference between the variables.

RESULTS

The antibacterial effect of the graphene oxide showed a significant difference between the three tested groups. The highest inhibition zones were recorded within samples incorporated with 5% GO NP (group III) = 35.67 mm ± 1.37, followed by inhibition zone formed around sampled incorporated with 2% GO NP (group II) = 32.17 mm ± 1.37. However, the least inhibition zones were reported at control group samples (group I) 28.33 mm ± 0.82. Moreover, there was a statistically significant difference between the results of the three tested groups (p-value = 0.000).

TABLE (2): Antibacterial effect (mm) and shear bond strength (MPa) of the three tested groups.

	Antibacterial effect (mm)		Shear bond strength (MPa)	
	Mean	S.D	Mean	S.D
Group I (Control)	28.33 [a]	0.82	32.18	6.58
Group II (0.2% GO NP)	32.17 [b]	0.75	24.34	2.68
Group III (0.5% GO NP)	35.67 [c]	1.37	25.76	6.52
P-value	0.000 *		0.161	

: indicates a significant difference within same column at p≤0.05

The results of shear bond strength of the present study showed that the highest shear bond strength was recorded at the control group (Group I) 32.18 MPa ± 6.58. The addition of 2% GO NP (group II) and 5% GO NP (group III) resulted in a decrease in the shear bond strength values 24.34 MPa ± 2.68 and 25.76 MPa ± 6.52 respectively. Moreover, the results of the shear bond strength of the three tested groups recorded a statistically non-significant difference (p-value = 0.161).

DISCUSSION

Achieving a long-standing aesthetic restoration is a main target for both the operator and the patient. Resin composite restorations offer a reliable solution [1]. However, resin composites can't bond to tooth structure. Therefore, the development of the adhesive systems became a mandatory and revolutionary process [3].

The development of dentin bonding agent over the years delivered to the market different bonding systems with different application steps and concepts. The evolution of the seventh generation, also called "all-in-one", carries to the operators bonding to dentin in a single step with clinical and laboratory performance approaching the superior performance of multistep adhesives that increased its popularity [4].

Bonding of resin composites to dentin, with the help of the adhesive system, performed mainly by forming a hybrid layer (HL). HL was firstly identified by **Nakabayashi** in 1982 as the mixture of demineralized dentin compounds and polymerized adhesive resin at molecular-level [3,4]. However, several studies showed that degradation of the HL happened in two patterns; the first one is disorganization and solubilization of the collagen fibrils while the other pattern is the hydrolytic degradation of the adhesive resin [3]. Reinforcing the adhesive resin with fillers was expected to improve the mechanical properties and reduces the water sensitivity and solubility which increased the performance of the HL [3,4]. Consequently, many authors investigated the effect of addition of different fillers to dentin adhesives such as hydroxyapatite

nanospheres, hydroxyapatite nanoparticles silica nanoparticles graphene oxide pre-reacted glass-ionomer filler [5-7,9,22-24].

Nevertheless, tooth-restoration interface is considered the weakest point of the restoration as the biofilms containing cariogenic bacteria tend to accumulate at this area. Therefore, incorporating an antibacterial nanofillers, such as silver, titanium oxide and curcumin, within adhesive resins reported an antibacterial effect. Moreover, many researchers reported the antibacterial effect of the graphene oxide [12,13]. Unfortunately, these nanofillers reported a decrease in the bond strength [25].

Therefore, the aim of the current study was to evaluate the antibacterial effect and the shear bond strength of commercially available seventh generation bonding system after incorporation of graphene oxide nanoparticles with two different concentrations.

Streptococcus mutans was chosen as the test organism because they are one of the predominant inhabitants of dental plaque and have been implicated in the formation of dental caries because of their acidogenic and aciduric properties [26].

Agar well diffusion method is one of the broadly used methods to evaluate the antimicrobial activity of plants or microbial extracts. The advantages of this test are simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided [27].

In vitro bond strength tests are beneficial and essential tests in predicting the performance of adhesive systems and possible correlation with clinical issues. Shear bond strength test is a simple evaluation procedure that has the advantages of easy specimen preparation and the ability at least qualitatively to rank different products according to bond strength values [28].

The results of the current study revealed that the addition of GO NP showed an inhibitory effect against *S. mutans* within group II and group III. This antibacterial effect of GO NP may occur due to three mechanisms; the first one is the 'nano-knives' action. The edges of the GO NP are sharp and can penetrate inside the bacterial cell wall causing its death. That is why it is also called "penetration mode" or "insertion mode". The second mechanism is the oxidative stress which implies distribution of the bacterial cell function and death either by reactive oxygen or by electric charge transfer from bacterial cell wall to GO. The last mechanism is wrapping effect where the graphene wraps around the bacterial cell and isolate it from surrounding environment [29,30].

Moreover, increasing the concentration of GO NP from 2% in group II to 5% in group III recorded a statistically significant antibacterial effect. **Liu** et al., [31] and **Pang** et al., [32] reported that the antibacterial effect of GO was recorded in a dose-dependent manner.

The results of the present study discovered that the incorporation of GO NP inside the bonding agent showed statistically non-significant difference with the control group. In accordance with the present study, **Alshahrani** et al., [9] reported non-significant change in micro-tensile bond strength after incorporation of 0.5% GO NP into an experimental dentin bonding agent. Moreover, **Bregnocchi** et al., [33] reported no significant difference in the micro-tensile bond strength after incorporation of graphene oxide nanoplates with 0.1%, 0.2% and 0.5%wt to an experimental dentin adhesive.

On the other hand, **Bin-Shuwaish** et al., [6] reported an increase in the micro-tensile bond strength after incorporation on graphene oxide and **Alfawaz** et al., [34] after addition of hydroxyapatite-graphene oxide fillers with percentages 0.5% and 2.0% to an experimental dentin adhesive. These differences may be claimed to the salinization of the filler particles that improves the interaction with the resinous matrix of the adhesive.

CONCLUSION

With the limitation of the present study, it can be concluded that incorporation of graphene oxide nanoparticles to the resin composite adhesive system can optimize the antibacterial effect without interfering with the bond strength.

REFERENCES

1. Aminoroaya A, Neisiany RE, Khorasani SN, Panahi P, Das O, Madry H, et al. A review of dental composites: challenges, chemistry aspects, filler influences, and future insights. Compos B Eng. 2021; 216:108852.
2. De Almeida Neves A, Coutinho E, Cardoso MV, Lambrechts P, Van Meerbeek B. Current concepts and techniques for caries excavation and adhesion to residual dentin. J. Adhes. Dent. 2011; 13,7–22.
3. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjaderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent. Mater. 2018; 34,78-96.
4. Van Meerbeek B, Yoshihara K, Van Landuyt K, Yoshida Y, Peumans M. From Buonocore's Pioneering Acid-Etch Technique to Self-Adhering Restoratives. A Status Perspective of Rapidly Advancing Dental Adhesive Technology. J Adhes Dent. 2020; 22(1):7-34.
5. Al-Hamdan RS, Almutairi B, Kattan HF, Alresayes S, Abduljabbar T, Vohra F. Assessment of Hydroxyapatite Nanospheres Incorporated Dentin Adhesive. A SEM/EDX, Micro-Raman, Microtensile and Micro-Indentation Study. Coatings. 2020; 10,1181.
6. Bin-Shuwaish MS, Maawadh AM, Alhamdan RS, Alresayes S, Almohareb T, Almutairi B, et al. 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. Mater. Res. Express. 2020; 7,11.
7. Mei L, Wei H, Wenjing C, Xiaokun H. Graphene oxide-silica composite fillers into the experimental dental adhesives for potential therapy. Medicine Research. 2017; 1(2):42-46.
8. Kalachandra S. Influence of fillers on the water sorption of composites. Dent. Mater. 1989; 5,283-288.
9. Alshahrani A, Bin-Shuwaish MS, Al-Hamdan RS, Almohareb T, Maawadh AM, Al Deeb M, et al. Graphene oxide nano-filler based experimental dentine adhesive. A SEM/EDX, Micro-Raman and microtensile bond strength analysis. J. Appl. Biomater. Fundam. Mater. 2020; 18:2280800020966936.
10. Ge Z, Yang L, Xiao F, Wu Y, Yu T, Chen J, et al. Y. Graphene Family Nanomaterials: Properties and Potential Applications in Dentistry. Int. J. Biomater. 2018; 2018,1539678.
11. Wei N, Lv C, Xu Z. Wetting of graphene oxide: A molecular dynamics study. Langmuir. 2014; 30,3572-3578.
12. He J, Zhu X, Qi Z, Wang C, Mao X, Zhu C, et al. Killing dental pathogens using antibacterial graphene oxide. ACS Appl. Mater. Interfaces. 2015; 7,5605-5611.
13. Lee JH, Jo JK, Kim DA, Patel KD, Kim HW, Lee HH. Nano-graphene oxide incorporated into PMMA resin to prevent microbial adhesion. Dental Materials. 2018; 34(4):e63-72.
14. Aradhana R, Mohanty S, Nayak SK. Comparison of mechanical, electrical and thermal properties in graphene oxide and reduced graphene oxide filled epoxy nanocomposite adhesives. Polymer. 2018; 141:109-123.
15. Fathy HM, Riad M. The effect of silver nanoparticles incorporation in the self-etch adhesive system on its antibacterial activity and degree of conversion: an in-vitro study. F1000Research 2019;18:1-8.
16. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2012;2:1–10.
17. Pérez-Díaz MA, Boegli L, James G, Velasquillo C, Sánchez-Sánchez R, Martínez-Martínez RE, et al. Silver nanoparticles with antimicrobial activities against Streptococcus mutans and their cytotoxic effect. Mater Sci Eng C Mater Biol Appl. 2015;55:360-366.
18. Espinosa-Cristóbal LF, Martínez-Castañón GA, Martínez-Martínez RE, Loyola-Rodríguez JP, Patiño-Marín N, Reyes-Macías JF, et al. Antibacterial effect of silver nanoparticles against Streptococcus mutans. Mater. Lett. 2009;63:2603-2606.
19. Aidaros N, Kamh R. Effect of incorporating nano-pearl and nano-seashell particles into fluoride-based pits and fissure sealant on enamel remineralization of permanent extracted molars: An In Vitro Study. ADJ, 2021; 3(4): 159-172.
20. Espinel-Ingroff A, Canton E, Fothergill A, Ghannoum M,

Johnson E, Jones RN, et al. Quality control guidelines for amphotericin B, Itraconazole, posaconazole, and voriconazole disk diffusion susceptibility tests with nonsupplemented Mueller-Hinton Agar (CLSI M51-A document) for nondermatophyte Filamentous Fungi. J Clin Microbiol. 2011; 49(7):2568-2571.

21. Chaharom MEE, Kahnamoii MA, Kimyai S, Moghaddam MH. Effect of sodium hypochlorite on the shear bond strength of fifth- and seventh-generation adhesives to coronal dentin. Afr J Biotechnol. 2011a; 10(59):12697-12701

22. Alhenaki AM, Attar EA, Alshahrani A, Farooq I, Vohra F, Abduljabbar T. Dentin Bond Integrity of Filled and Unfilled Resin Adhesive Enhanced with Silica Nanoparticles-An SEM, EDX, Micro-Raman, FTIR and Micro-Tensile Bond Strength Study. Polymers (Basel). 2021; 13(7):1093.

23. Ghyadh A, Ragab H, Osman E. Shear bond strength of a multi-mode universal adhesive containing hydroxy-apatite nanoparticles to dentin (In vitro study). EDJ. 2021; 67(3):2749-2758.

24. Kawashima S, Shinkai K, Suzuki M. The effect of multi-ion releasing filler contents on the dentin bond strength of an adhesive resin developed for direct pulp-capping. Dent Mater J. 2015; 34(6):841-846.

25. Makvandi P, Gu JT, Zare EN, Ashtari B, Moeini A, Tay FR, et al. Polymeric and inorganic nanoscopical antimicrobial fillers in dentistry. Acta biomaterialia. 2020; 101:69-101.

26. Devarasanahalli SV, Kurian B, Aswathanarayana RM, Khatib MS, Nadig RR. Comparison of the Antibacterial Efficiency of Herbal Extracts of Aloe Vera Leaves and Mushroom against *Streptococcus mutans* and *Lactobacillus*: An *In Vitro* Study. Int J Experiment Dent Sci 2020; 9(1):8-12.

27. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6:71-79

28. Armstrong S, Geraldeli S, Maia R, Raposo LHA, Soares CJ, Yamagawa J. Adhesion to tooth structure: A critical review of "micro" bond strength test methods. Dent Mater. 2010; 26(2):e50-e62.

29. Mohammed H, Kumar A, Bekyarova E, Al-Hadeethi Y, Zhang X, Chen M, et al. Antimicrobial Mechanisms and Effectiveness of Graphene and Graphene-Functionalized Biomaterials. A Scope review. Front Bioeng Biotechnol. 2020; 8:465.

30. Radhi A, Mohamad D, Abdul Rahman FS, Abdullah AM, Hasan H. Mechanism and factors influence of graphene-based nanomaterials antimicrobial activities and application in dentistry. Journal of Materials Research and Technology. 2021; 11:1290-1307.

31. Liu S, Zeng TH, Hofmann M, Burcombe E, Wei J, Jiang R, et al. Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. ACS Nano. 2011; 5(9):6971-6980.

32. Pang L, Dai C, Bi L, Guo Z, Fan J. Biosafety and Antibacterial Ability of Graphene and Graphene Oxide In Vitro and In Vivo. Nanoscale Res Lett. 2017; 12(1):564.

33. Bregnocchi A, Zanni E, Uccelletti D, Marra F, Cavallini D, De Angelis F, et al. Graphene-based dental adhesive with anti-biofilm activity. J. Nanobiotechnol. 2017; 15,89.

34. AlFawaz YF, Almutairi B, Kattan HF, Zafar MS, Farooq I, Naseem M, et al. Dentin Bond Integrity of Hydroxyapatite Containing Resin Adhesive Enhanced with Graphene Oxide Nano-Particles-An SEM, EDX, Micro-Raman, and Microtensile Bond Strength Study. Polymers (Basel). 2020; 12(12):2978.