# Antibacterial Activity of Nanoclay - Modified Glass Ionomer versus Nanosilver - Modified Glass Ionomer

Marwa Mohamed Temirek[1]*, Monaliza Maher Abdelaziz[2], Walaa Alshareef[3]

1. October 6 University Faculty of Dentistry, Conservative Dentistry, October 6 city. EG.
2. October 6 University Faculty of Dentistry, Conservative Dentistry, October 6 city. EG.
3. October 6 University Faculty of Pharmacy, Microbiology and Immunology, October 6 city. EG.

## Abstract

To assess the antibacterial activity of nanoclay-modified glass ionomer versus nanosilver-modified glass ionomer on Streptococcus mutans (in vitro).

24 samples were used in the study, divided into three groups according to the materials used (n=8). Group (1) conventional glass ionomer Medifill, Group (2) nanoclay-modified glass ionomer, and Group (3) nanosilver-modified glass ionomer. The antibacterial effect of each group was assessed by disc diffusion methods, at two time intervals: 24 and 48 hours.

There was a statistically significant difference between the effects of the used materials on the inhibition zone diameter after 24 and 48 hours. Nanoclay-modified glass ionomer showed the highest statified glass ionomer. The conventional glass ionomer group showed the lowest mean inhibition statistically significant mean inhibition zone diameter, followed by nanosilver-mod zone diameter, with a statistically significant difference.

The addition of nanoscale and nanosilver enhanced the antibacterial activity of conventional glass ionomer restoration.

**Experimental article (J Int Dent Med Res 2022; 15(4): 1442-1449)**

**Keywords:** Glass ionomer, nanoclay, nanosilver, antibacterial activity, streptococcus mutans.

**Received date:** 09 August 2022 **Accept date:** 13 September 2022

## Introduction

Dental caries is a chronic infectious disease produced by the interaction of oral microbes in dental plaque, diet, and a wide variety of host factors ranging from social and environmental factors to genetic and biochemical/immunologic host responses. The principal agents of enamel caries are two species of the mutants streptococci: *Streptococcus mutans* and *Streptococcus sobrinus* [1]. *Streptococcus mutans* metabolite a variety of sugars and glycosides, including glucose, fructose, sucrose, lactose, galactose, mannose, cellobiose, glucosides, trehalose, and maltose, as well as alcohol. *Streptococcus mutans* synthesize intracellular glycogen, such as polysaccharides in extracellular glucose and sucrose, decreasing salivary pH, and continuously causing the demineralization of the tooth surface and, eventually, dental caries[1 2]. One of the essential methods for preventing secondary caries is applying restorative materials to prevent bacterial growth[3]. Glass ionomer restorations (GICs) are interesting dental materials that catch the attention for their clinical applications. They have particular characteristics that make them functional adhesive materials for restoring carious cavities in low stress bearing areas[4]. These properties include chemical bonding to different tooth tissues, acceptable pulp reaction, low coefficient of thermal expansion, low toxicity, and ability to release fluoride.[5]

Despite incorporating fluoride in GICs, secondary caries is still an unsolved problem since the amount of released fluoride is not enough to guarantee its anticarcinogenic activity. Therefore, antibacterial additives have been proposed as an alternative to modify GIC formulations, including different types of nanoparticles, metals, antiseptics, etc.[6]. The incorporation of nanoparticles with antibacterial activity into dental care materials is being investigated, in order to generate restorative

***Corresponding author:***
Dr. Marwa Mohamed Temirek,
Fayoum University, October 6 University, Faculty of Dentistry, Conservative Dentistry,
October 6 city 6th of October City,12563,EG
E-mail: marwa.dent@o6u.edu.eg;
mma25@fayoum.edu.eg

materials that can prevent recurrent dental caries, and prolong the effectiveness of treatment[7].

Among various nanoparticles, silver nanoparticles have been one of the most popular objects of study in recent decades. Nanoparticles of silver include 20 to 15,000 atoms of silver, and their diameters are typically less than 100 nm. Even at low concentrations, silver nanoparticles demonstrate extraordinary antibacterial activity due to their high surface-to-volume ratio. In addition, they are low in cost, and demonstrate little cytotoxicity and immune response[8].

Moreover, nanoclay (montmorillonite) is a crystalline structure formed of clay mineral layers. Each layer of clay consists of an octahedral alumina sheet in the center, between two tetrahedral silica plates. Through getting over the forces among the layers, small molecules may penetrate and polymerize inside clay architecture. Nanoclays have become one of the most interesting research materials. Because of the large interface of the polymer-nanoclay, reciprocal action considers an attractive material for high dispersion ability in polymers[9]. The integration of a small quantity of montmorillonite in the polymer matrix improves the characteristics of the resulting compound when compared to the classical one[10]. MMT is used as an inorganic carrier for antibacterial materials because of its good adsorption ability, drug-carrying capability, high cation-exchange capacity, high surface area, and chemical inertness[11]. Although clay nanoparticles have been used for various purposes, till now, no study has been conducted to investigate their effect after incorporation into dental restorative materials, such as glass ionomer restoration. Therefore, this in vitro study evaluates the antibacterial effect of nanoclay-modified glass ionomer and nanosilver-modified glass ionomer on *Streptococcus mutans*.

## Materials and methods

Table (1) lists the glass ionomer restorative material, in addition to the specification, composition, and manufacturers of the nanoparticles used in the current study.

Sample size calculation

A power analysis was designed to have the adequate power to apply the statistical test of the null hypothesis that no difference would be found between the antibacterial effect of the different groups. By adopting an alpha level of (0.05), a beta of (0.2), i.e. power=80%, and an effect size (f) of (0.68), the predicted sample size (n) was a total of (24) samples. Sample size calculation was performed using G*Power version (3.1.9.7)[12]

| Material | Specification | Composition | Manufacturer |
|---|---|---|---|
| **Medifil®** | A radiopaque conventional glass ionomer restorative material with chemical adhesive abitilty to enamel and dentin.<br>Shade A2 | *Powder:* fluoroaluminosilicate glass containing Si, Al, Sr, and Na.<br>*Liquid:* polyacrylic acid, copolymers of carboxylic acid and tartaric acid | Promedica (*dental material*), Germany www.promedica.de |
| **Dellite® 43B** | An off-white nanoclay of 7-9 um derived from a naturally occurring montmorillonite with organic modifier | Pure nano montmorillonite (nanoclay) consists of a layer made of an inner octahedral sheet of alumina or magnesia sandwiched between two tetrahedral sheets of silica modified with a quaternary ammonium salt | Laviosa (*advanced mineral solution*), Italy www.laviosa.com |
| **Silver nanoparticles** | liquid form with yellowish color | 10 ml of 1% ethanolic solution of Polyvinyl pyrrolidine (PVP) and 0.2 ml of 0.1 M silver nitrate powder (AgNO3) in a closed test tube containing 25 ml distilled water | Prepared at National Center of Research. (Giza,Egypt) |

**Table 1.** Materials' composition, specification, and manufacturer.

Sample grouping

A total of twenty-four samples were used in the study, divided into three groups according to the materials used (n=8): Group1 is the conventional glass ionomer (G1); Group 2 is nanoclay-modified glass ionomer (G2); and Group 3 is nanosilver-modified glass ionomer (G3). The antibacterial effect of each group was assessed after two time intervals: 24 and 48 hours.

Characterization of Nanoclay

The characterization of nanoclay (montmorillonite) was done by X-ray diffraction, and Fourier transform infrared spectroscopy. The analysis was done in the laboratories of the conservation center at the Grand Egyptian Museum (GEM).

X-ray Diffraction: the samples were analyzed using an analytical X-pert pro with copper anode, working at 40 mA / 45 kV. The samples were analyzed after grounding in an agate mortar. The sample holder was then used to attach a flat surface sample to the XRD instrument. The data were interpreted using the software of the XRD machine. The long scan was used between 2θ angles of 7°–75° at a step size of 0.03° and time per step(s): 100, scan speed (2θ /S): 0.076°, No. of steps (2266)[10 13].

Fourier Transform Infra-Red Spectroscopy: Infrared spectra were obtained in the mid-range using JASCO-6100 FTIR spectroscopy. The 1 to 3mg from the samples were ground with 99-97mg of potassium bromide (KBr) in an agate mortar, forming a 3mm diameter KBr pellet. Transmittance data were gathered between 4000 and 400 cm1, with a 4 cm1 resolution[13].

Preparation of nanoclay-modified glass ionomer

Nanoclay powder was dispersed in Medifill™ liquid by the exfoliation-adsorption method[10]. Dellite ® 43B weights were measured by a balance (0.375 grams = 2.0 wt. %). Then, the mixing was done on a hot tray for 24 hours at 75 °C, with a speed of 100 rpm using a magnetic stirrer.

Preparation and characterization of nanoparticles of silver

To synthesize silver nanoparticles, 10 ml of 1% ethanolic solution of Polyvinyl pyrrolidine (PVP), and 0.2 ml of 0.1 M silver nitrate powder ($AgNO_3$) were added in a closed test tube containing 25 ml distilled water, and placed in a microwave oven for 5 seconds. Instantaneously, the colorless solution changed to its typical yellow hue, indicating the creation of silver nanoparticles that were spherical in shape, and 5.55-8.77 nm in size[14]. Visible Near Infra-Red (Vis-NIR) Spectroscopy monitored the absorption rate of the silver nanoparticles. It consisted of a Tungsten halogen light source [Versatile white light source optimized for Vis-NIR (360-2500 nm)]. A sensor was connected to the computer to detect the change in the amount of emitted light passed through the silver nanoparticle solution. The light source and detector were linked together through two optical fibers. The cuvette containing the silver nanoparticle solution was placed between these fibers. The first optical fiber was connected to the light source through which the emitted light was carried to the cuvette containing the silver nanoparticle solution. The second optical fiber was connected to the sensor to which the light that passed through the cuvette was transferred. Special computer software was used for recording data, and an absorption rate curve was drawn.

Preparation of nanosilver-modified glass ionomer

The concentration of the resultant nanosilver solution was 107µg/ml. It was taken at 1.1µg/µl, and applied to 0.5 ml polyacrylic acid, till it reached the minimum concentration that provided the most effective antibacterial action (0.5µg/ml).

Preparation of glass ionomer specimens

Glass ionomer specimens were made using a sterilized custom split Teflon mold (5mm in diameter x 2mm in height). A sterilized glass slap covered with a Mylar strip was used for the preparation of the specimens. The glass ionomer powder and the polyacrylic acid (G1) were proportioned according to the manufacturer's instructions, and mixed until a homogenous mix was obtained. The materials were packed by a condenser of suitable size into the mold, as one increment, then covered with another Mylar strip and a glass slide, and pressed for a few seconds in order to expel the excess material, and achieve a uniform smooth surface. The glass ionomer mix was left into the mold till complete setting. Regarding G2 and G3, the glass ionomer specimens were prepared according to the same procedures, except that the nanoclay and nanosilver particles were first added to the glass ionomer liquid (polyacrylic acid), as aforementioned , and mixed with glass ionomer powder.

Testing antibacterial activity

For the purposes of this in vitro study, *Streptococcus mutans* ATCC 25175 standard type strain (16S rRNA gene, serotype c. carious dentin) was obtained from the Microbiology and Immunology Department, at the Faculty of Pharmacy, the 6th of October University, Giza, Egypt. According to the manufacturer's instructions, bacteria were inoculated overnight at 37 °C in Mitis Salivarius Agar (Becton, Dickinson, and Company; Sparks, MD, USA) supplemented with potassium tellurite. The turbidity of the suspension was determined using the McFarland 0.5 turbidity standard (Densimat, BioMerieux, France). The bacteria concentration was standardized to approximately 1.5 × 108 CFU/ml, and used as a working microbial solution based on such absorbance. At 37 degrees Celsius, the plates were incubated in a bacteriological incubator. After 24 and 48 hours, the antibacterial effect of each group was evaluated by measuring the diameter of bacterial growth inhibition zones. The diameter of the bacterial growth inhibition zones was measured in millimeters at three different points on each specimen, using an electronic digital caliper (STECO, Germany).

Assessment of minimum inhibitory concentration (MIC) by Microtitre Broth Dilution Method

Ten tubes/materials were filled with 100 µL of Brain Heart Infusion broth using the Microtitre Broth Dilution Method (BHI, Sigma-Aldrich, St. Louis, USA). One hundred µL of the antibacterial agent under investigation was added to the first well of a sterile 96-well microtiter plate, in order to achieve a concentration of 53.5 µL. This tube was left alone for one minute before transferring 100 µL to the second tube, to achieve a 26.75 µL concentration. Serial dilution was obtained by repeating this procedure with the subsequent tubes until dilution of 0.1045 µL was obtained. A bacterial suspension was prepared by mixing 0.5 µL *Streptococcus mutans* from stock cultures (ATCC 25175) with 9.5 µL BHI. Each serially diluted tube received one droplet of the *Streptococcus mutans* suspension. The dilution resulted in an 80% reduction of visible bacterial growth compared to the control.

The solution remained clear and was selected as the minimum inhibitory concentration (MIC)[15] to be used as the study's starting point. Additional turbid samples with lower concentrations were discarded. The test was administered three times. For each antibacterial agent investigated, a positive control was used. *Streptococcus mutans* was added to BHI without any agents. A negative control was used in which the agents were added to BHI without the bacteria.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism 9.3.1 (GraphPad Software Inc., CA, United States). The results were presented as median and error interquartile range. The normality of the numerical data was determined by inspecting the distribution of the data, and performing normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests). The data demonstrated a parametric distribution. The results were presented as mean, standard deviation (SD), and 95% Confidence Interval (CI) values for the mean. The effect of material, time (24 and 48 hours), and their interaction on the mean inhibition zone diameter was investigated using a two-way analysis of variance (ANOVA). Bonferroni's post-hoc test was used for pairwise comparisons when the ANOVA test was significant. P 0.05 was chosen as the level of significance.

Approval or consent is not required; the work done didn't include patients or other individuals.

## Results

Characterization of Nanoclay:

The samples Dellite 43B was subjected to XRD analysis. Both pieces indicated the presence of the montmorillonite (Na, Ca) (Al, Mg)$_2$(Si$_4$O10) (OH)$_2$. nH$_2$O in the sample, as well as mica and cristobalite, proving that the main structure of the inorganic crystalline component of the filling material composed most of the smectite group. The FTIR analysis of the sample of Dellite 43B montmorillonite indicated broadband at 3419 cm-1, and adsorbed OH stretching of the clay mineral, followed by two large doublets at 1627 and 1589 cm-1 OH bending band, and adsorption bands resulting from the organic-modified that was added to the clay mineral to enhance the properties of the montmorillonite. On the other hand, the Si-O-Si stretching could be observed by large broad and at 1062 cm-1, while the two peaks at 516 cm-1 and 457 cm-1, respectively, indicated the Si-O-Al and Al-OH adsorption bands.

Characterization of Nanosilver

Vis-NIR Spectroscopy is based on the idea of Surface Plasmon Resonance (the collective excitation of electrons in the conduction band). Silver nanoparticles have positively charged nuclei in the center, and a surface-like plasma has free electrons (Conduction Band). The Plasmon Absorption Rate (measured in nanometer) is a characteristic optical absorption spectrum representing the movement of free electrons in the conduction band, after the absorption of emitted light. The amount of emitted light absorbed differs according to particle size and shape.

The results of a two-way ANOVA revealed that Glass Ionomer Groups had a statistically significant effect on the mean diameter of the inhibition zone Table (2). Since the time and interaction between the variables were not statistically significant, the variables were independent of one another. The descriptive statistics of the inhibition zone diameter of Glass Ionomer Groups, regardless of time, are represented by mean, standard deviation (SD), and 95% Confidence Interval values in Table (3). There was an extremely statistically significant difference between the three groups of treatment (P-value <0.001). Comparing the time periods pairwise revealed that the 48-hour period was statistically insignificant. *Streptococcus mutans* were around the group treatments. Figure (1) shows a statistically significant difference between the materials used on inhibition zone diameter after 24 and 48 hours. Pairwise comparisons between the materials revealed that the nanoclay-modified glass ionomer showed the highest statistically significant mean inhibition zone diameter. Nanosilver-modified glass ionomer showed a lower statistically significant mean inhibition zone diameter, followed by Conventional Glass Ionomer group. Regarding the effect of time, there was no statistically significant difference between the two follow-up times. The mean inhibition zone diameter after 24 and 48 hours was statistically insignificant.

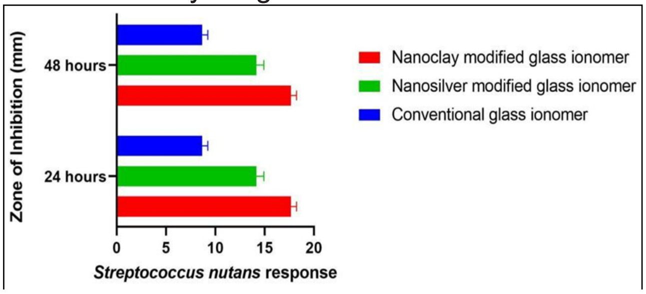


**Figure 1.** Bar chart illustrating the mean and standard deviation of inhibition zone diameters with various Glass Ionomer Groups.

| Source of variation | SS | Df | Mean Square | *F*-value | *P*-value | Significance |
|---|---|---|---|---|---|---|
| Glass Ionomer Groups | 658.7 | 2 | 329.3 | 1153 | <0.001* | Yes |
| Time | 0.000 | 1 | 0.000 | 0.000 | P>0.9999 | NO |
| Glass Ionomer Groups x Time interaction | 0.000 | 2 | 0.000 | 0.000 | P>0.9999 | NO |

df: degrees of freedom = (n-1), *: Significant at P ≤ 0.05

**Table 2.** Two-way ANOVA results for the effect of different variables on mean inhibition zone diameter.

## Discussion

The present study evaluated the antibacterial effect of nanoclay-modified glass ionomer and nanosilver-modified glass ionomer on *Streptococcus mutans*. Glass Ionomer Cements (GICs) are clinically attractive and advantageous restorative dental materials with unique properties[9]. However, their antimicrobial activity is still not efficient against cariogenic bacteria, since they cannot prevent adherence, cell proliferation, and biofilm formation where *Streptococcus mutans* are present[16]. ProMedica (Midifil™) conventional glass ionomer was selected in this study because it is the most used restorative material for ART technique, class III, V cavities, and posterior teeth in pediatric patients, due to its adequate strength, excellent biocompatibility, low acidity, self-adhesion to tooth tissue, high fluoride release, and with no temperature rise during setting. GICs inhibit caries' initiation or progression through their inherent ability to release and reuptake fluoride ions. However, there is no clear scientific evidence to fully support this caries' inhibitory potential. Many investigations have evaluated the possibility of incorporating a direct antimicrobial agent into GICs to innovate an esthetically pleasing, self-adhesive dental material that can arrest residual caries, decrease the incidence of recurrent caries, and provide a tight seal under restorations. Adding a different antimicrobial compound to glass ionomers, such as antibiotics, metal ions, oxides, iodine, and chlorhexidine, is considered an attempt to enhance their antibacterial activity. However, these attempts are still far from offering the optimal effect of antibacterial properties against cariogenic bacteria[17 18].

| | Conventional Glass Ionomer | Nano clay Modified Glass Ionomer | Nanosilver Modified glass Ionomer | *P*-value |
|---|---|---|---|---|
| Mean (SD) | 8.571 (0.5345) | 17.71 (0.4880) | 14.29 (0.7559) | P<0.0001*** |
| Lower 95% CI | 8.077 | 17.26 | 13.59 | |
| Upper 95% CI | 9.066 | 18.17 | 14.98 | |

***: Extremely Significant at P ≤ 0.05

**Table 3.** The mean, standard deviation (SD), 95% Confidence Interval values, and the results of comparison between the inhibition zone diameters of Glass Ionomer Groups.

Nanoparticles have emerged as an alternative to high reactivity in dentistry owing to their superior properties, such as their smaller size, shape, and high surface area to volume ratio. Nanoparticles exhibited solid antimicrobial activity due to their multidisciplinary application, and extraordinary defense against a broad spectrum of bacteria, including Gram-negative and Gram-positive strains[19]. Montmorillonite (MMT, $Na_{0.7}(Al_{33}Mg_{0.7}) Si_8O_{20} (OH)_4 - nH_2O$) was selected as a carrier owing to its excellent biocompatibility, and extensive uses in a broad range of medical and pharmaceutical applications[20]. MMT is a type of natural clay, and a safe carrier material with a layered silicate structure, wide surface area, high adsorption capacity, and ion exchanging properties[21 22].

In particular, nano sized MMT has been successfully used as a drug carrier, catalyst, food additives[20], and tissue engineering material[23]. Metals mixed with many biomaterials have been documented to improve antibacterial performance[6]. Nanosilver shows great activity against many bacteria, and is widely used in medical devices, food packaging, bandages, and clinical applications[24]. The AgNPs are the most extensively tested inorganic antimicrobial agents due to their long-term antibacterial activity, low bacterial resistance, low volatility, high surface-to-volume ratio, and high thermal stability[25].

In the present study, all the tested materials showed a statistically significant effect on mean inhibition zone diameter after 24 and 48 hours. The nanoclay-modified glass ionomer showed the highest statistically significant mean inhibition zone diameter. This may be because Montmorillonite (MMT) is smectite-type clay composed of an expandable type of aluminosilicate clay mineral that has a layered structure, and relatively high cation exchange capacity. By replacing the natural inorganic exchange cations with an organic compound, the MMT surface was converted from being primarily hydrophilic to hydrophobic, enabling its strong interaction with organic compounds dissolved in water[26]. This agrees with other studies[13 27], which found that Polymer-nanoclay composites involved the interaction of polymer matrix of polyacrylic acid with the nanoplates of clay. The composites were formed by the dispersion of low weight percentages of nanoclay into polymers, improving physical, biological, and chemical properties of the resulting composite, when compared to conventional materials.

In the current in vitro study, nanosilver-modified glass ionomer showed a statistically significant effect on the mean inhibition zone diameter. This may be because silver ions cause bactericidal effects by interacting with bacterial cell walls, plasma membranes, bacterial DNA, proteins, and ribosomes[28]. Moreover, with the release of silver ions, the formation of reactive oxygen species (ROS), or both processes, silver broke down the bacterial cell walls, dimerized their DNA, and blocked the respiratory chains that caused the death of the microorganism[24]. According to another study, Moshfeghi and his team[29], none of the samples showed a bacterial growth inhibition zone, including nanosilver, after 24 and 48 hours. There was a reduced bacterial growth for *Streptococcus mutans* bacteria by increasing nanosilver. This finding also corresponds with other studies, which reported that the number of silver ions released from the cement surface inactivated the growth of bacteria, and affected the viability of *Streptococcus mutans* biofilm[30]. In this in vitro study, nanosilver-modified glass ionomer showed a lower statistically significant mean inhibition zone diameter than nanoclay-modified glass ionomer. This may be attributed to the numerous substances that relatively easily intercalated montmorillonite (MMT), due to their crystal structure unit cell, consisting of one octahedral alumina sheet sandwiched between two tetrahedral silica sheets. Cations and/or water molecules are present in the interlayers. In addition, both octahedral and tetrahedral replacements were feasible, which allowed for significant cation exchange capacities[31], and led to strong interaction with certain antimicrobials, affecting their bioavailability[32].

GI group showed the lowest statistically significant mean inhibition zone diameter. Although the antibacterial activity of glass cement is due to its fluoride release potential, there is no published evidence for the inhibiting effects of GI cement on the occurrence of secondary caries, which are mainly caused by the accumulation of *Streptococcus mutans*. Fluoride release may be influenced by many factors, such as the released fluoride and other ions, the chemical composition of glass ionomer, and low pH value during setting[12]. In other words, GI cement had antibacterial effects on a narrow spectrum of microorganisms and had insignificant bactericidal activity[33]. This is in agreement with another study that evaluated the antibacterial activity of conventional Glass Ionomer Cement (GIC II), and a zirconia-reinforced glass ionomer cement (Zirconomer™) against *Streptococcus mutans* [34], and reported that Zirconomer™ had the most antibacterial effect against *Streptococcus mutans*. This result is also in agreement with another study, which revealed that the modified GIC with Chloroxylenol, Boric acid, and Thymol was more effective against *Streptococcus mutans* than unmodified GIC powder[35].

## Conclusions

The incorporation of nanoparticles in glass ionomer improved its antibacterial activity. Montmorillonite exhibited promising antibacterial activity. Further investigations may be required to assess the antibacterial activity of glass ionomer with a different modification on *Streptococcus mutans*.

## Acknowledgements

T. M. and M. A. contributed to the conception, design, data acquisition and interpretation, and manuscript drafting and revision. A. W. contributed to conception, design, and manuscript revision, and performed all statistical analyses. All authors gave their final approval, and agreed to be accountable for all aspects of the work.

All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.

## Declaration of Interest

The authors have no conflict of interest to declare. The authors did not receive support from any organization for the submitted work.

## References

1. Colombo AP V, Tanner ACR. The role of bacterial biofilms in dental caries and periodontal and peri-implant diseases: a historical perspective. J Dent Res. 2019;98(4):373-385.
2. Nonong YH, Mieke HS, Ratna I, Selly P. Antibacterial test between Aloe vera and chlorhexidine based on the number of colony of Streptococcus mutans ATCC 25 175 in vitro. IJSR. 2016;5(1):1379-1385.
3. Ezzeldin N, Elshishtawy H, El-Borady O, Mohamed M. Comparative evaluation of the antibacterial activity of Glass ionomer restoration incorporated with different metal oxides Nanoparticles on oral Streptococcus mutans. Egypt Dent J. 2019;65(4-October (Orthodontics, Pediatric & Preventive Dentistry)):3213-3220.
4. Ilie N, Hickel R, Valceanu AS, Huth KC. Fracture toughness of dental restorative materials. Clin Oral Investig. 2012;16(2):489-498.
5. Moshaverinia A, Roohpour N, Chee WWL, Schricker SR. A review of polyelectrolyte modifications in conventional glass-ionomer dental cements. J Mater Chem. 2012;22(7):2824-2833.
6. Aguilar-Perez D, Vargas-Coronado R, Cervantes-Uc JM, et al. Antibacterial activity of a glass ionomer cement doped with copper nanoparticles. Dent Mater J. 2020:2019-2046.
7. Garcia PPNS, Cardia MFB, Francisconi RS, et al. Antibacterial activity of glass ionomer cement modified by zinc oxide nanoparticles. Microsc Res Tech. 2017;80(5):456-461.
8. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The antibacterial mechanism of silver nanoparticles and its application in dentistry. Int J Nanomedicine. 2020;15:2555.
9. Abdelaziz MM, Niazy MA, Taher HM. The Effect of pH Cycling on Surface Microhardness and Fluoride Release of Two Modified Nanoclay Glass Ionomer Restorations In Class V Cavities. Al-Azhar Dent J Girls. 2020;7(4 October-Restorative Dentistry issue (Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)):511-520.
10. Fareed MA, Stamboulis A. Nanoclays reinforced glass ionomer cements: dispersion and interaction of polymer grade (PG) montmorillonite with poly (acrylic acid). J Mater Sci Mater Med. 2014;25(1):91-99.
11. Ma X, Gao L, Ma Y, Zhang X. Antibacterial Activity and Mechanism of ZnO/Cu2+-Chitosan/Montmorillonite. J Wuhan Univ Technol Sci Ed. 2021;36(4):510-516.
12. Sultan MS. Antibacterial effect of aloe Vera and glass ionomer modified by aloe Vera on streptococcus mutans. Egypt Dent J. 2019;65(3-July (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):2607-2616.
13. Fareed MA, Stamboulis A. Effect of nanoclay dispersion on the properties of a commercial glass ionomer cement. Int J Biomater. 2014;2014.
14. Riad M, Harhash AY, Elhiny OA, Salem GA. Evaluation of the shear bond strength of orthodontic adhesive system containing antimicrobial silver nano particles on bonding of metal brackets to enamel. Life Sci J. 2015;12(12):27-34.
15. dos Santos AO, Ueda-Nakamura T, Dias Filho BP, da Veiga Junior VF, da Cunha Pinto Â, Nakamura CV. Antimicrobial activity of copaiba oils obtained from different species of Copaifera in Brazil. Planta Med. 2007;73(09):P_120.
16. Bayoumi RE, Habib SI. Assessment of Antibacterial Properties and Compressive Strength of Copaiba Oil–modified Glass Ionomer Cement versus Nanosilver or Nanogold-modified Glass Ionomer Cements: An In-Vitro Study. Futur Dent J. 2019;5(1):3.
17. Sun X, Yang X, Xue P, Zhang Z, Ren G. Improved antibacterial effects of alkali-transformed saponin from quinoa husks against halitosis-related bacteria. BMC Complement Altern Med. 2019;19(1):1-10.
18. Singer L, Bourauel CP. Shear Bond Strength and Film Thickness of a Naturally Antimicrobial Modified Dental Luting Cement. Molecules. 2021;26(5):1276.
19. Salayová A, Bedlovičová Z, Daneu N, et al. Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: Morphology and antibacterial efficacy. Nanomaterials. 2021;11(4):1005.
20. Zou Y-H, Wang J, Cui L-Y, et al. Corrosion resistance and antibacterial activity of zinc-loaded montmorillonite coatings on biodegradable magnesium alloy AZ31. Acta Biomater. 2019;98:196-214.
21. Xia M, Jiang Y, Zhao L, et al. Wet grinding of montmorillonite and its effect on the properties of mesoporous montmorillonite. Colloids Surfaces A Physicochem Eng Asp. 2010;356(1-3):1-9.
22. Drelich J, Li B, Bowen P, Hwang J-Y, Mills O, Hoffman D. Vermiculite decorated with copper nanoparticles: Novel antibacterial hybrid material. Appl Surf Sci. 2011;257(22):9435-9443.
23. Mohd SS, Abdullah MAA, Mat Amin KA. Gellan gum/clay hydrogels for tissue engineering application: Mechanical, thermal behavior, cell viability, and antibacterial properties. J Bioact Compat Polym. 2016;31(6):648-666.
24. Beyler Çiğil A, Şen F, Birtane H, Kahraman MV. Covalently

bonded nanosilver-hydroxyethyl cellulose/polyacrylic acid/sorbitol hybrid matrix: thermal, morphological and antibacterial properties. Polym Bull. 2022:1-16.
25. Enan ET, Ashour AA, Basha S, Felemban NH, El-Rab SMFG. Antimicrobial activity of biosynthesized silver nanoparticles, amoxicillin, and glass-ionomer cement against Streptococcus mutans and Staphylococcus aureus. Nanotechnology. 2021;32(21):215101.
26. Kokabi M, Sirousazar M, Hassan ZM. PVA–clay nanocomposite hydrogels for wound dressing. Eur Polym J. 2007;43(3):773-781.
27. Munhoz T, Fredholm Y, Rivory P, et al. Effect of nanoclay addition on physical, chemical, optical and biological properties of experimental dental resin composites. Dent Mater. 2017;33(3):271-279.
28. Li H, Huang D, Ren K, Ji J. Inorganic-polymer composite coatings for biomedical devices. Smart Mater Med. 2021;2:1-14.
29. Moshfeghi H, Haghgoo R, Sadeghi R, Niakan M, Rezvani MB. Antibacterial activity of a glass ionomer containing silver nanoparticles against Streptococcus mutans and Streptococcus sanguinis. Indian J Dent Res. 2020;31(4):589.
30. Paiva L, Fidalgo TKS, Da Costa LP, et al. Antibacterial properties and compressive strength of new one-step preparation silver nanoparticles in glass ionomer cements (NanoAg-GIC). J Dent. 2018;69:102-109.
31. Viseras C, Aguzzi C, Cerezo P, Lopez-Galindo A. Uses of clay minerals in semisolid health care and therapeutic products. Appl Clay Sci. 2007;36(1-3):37-50.
32. Rapacz-Kmita A, Bućko MM, Stodolak-Zych E, Mikołajczyk M, Dudek P, Trybus M. Characterisation, in vitro release study, and antibacterial activity of montmorillonite-gentamicin complex material. Mater Sci Eng C. 2017;70:471-478.
33. Panahandeh N, Adinehlou F, Sheikh-Al-Eslamian SM, Torabzadeh H. Extract of Propolis on Resin-Modified Glass Ionomer Cement: Effect on Mechanical and Antimicrobial Properties and Dentin Bonding Strength. Int J Biomater. 2021;2021.
34. Tiwari S, Kenchappa M, Bhayya D, et al. Antibacterial activity and fluoride release of glass-ionomer cement, compomer and zirconia reinforced glass-ionomer cement. J Clin diagnostic Res JCDR. 2016;10(4):ZC90.
35. Prasad MP, Maradia MA. Antibacterial activity of conventional and modified glass ionomer cement against Streptococcus mutans. J Appl Biol Biotechnol. 2014;2(3):0-2.