



Effect of Adding nano titanium Dioxide and Chitosan On Antibacterial, Water Sorption and Solubility of Glass Ionomer Cement

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ABSTRACT

OBJECTIVE:

To evaluate the water sorption, solubility and antibacterial activity of glass ionomer cement incorporating nano-titanium dioxide as well as chitosan.

MATERIAL AND METHODS:

The samples were prepared in six groups, including non-modified GIGs (NMGIC, n = 12), chitosan incorporated GIGs (CHGIC, n = 12), 3% nanotitanium incorporated GIGs (nanoTiO₂GIC, n = 12), 5% nanotitanium incorporated GIGs (nanoTiO₂GIC, n = 12), chitosan/3% nanotitanium incorporated GIGs (CH/nanoTiO₂GIGs, n = 12), chitosan/5% nanotitanium incorporated GIGs (CH/nanoTiO₂GIGs, n = 12). The antibacterial activity was evaluated using a direct contact test against *Streptococcus mutans*. The water sorption and solubility of modified glass ionomer cement were evaluated. Data were analyzed using the ANOVA and Tukey tests. Statistical significance was set at the 0.05 probability level.

RESULTS:

With the dual-modification, a significant improvement in the antibacterial properties was found. However, no difference was found in water sorption.

CONCLUSION:

Under the limitations of the present investigation, the following conclusion can be drawn: 5% wt TiO₂ NP modified GIC powder with the chitosan modified GIC liquid (**Group 5**) exhibited the highest antibacterial activity against *S. mutans*.

I. Introduction:

Conventionally, glass ionomer cements (GICs) consist of two main components: a powder of fluoro-aluminosilicate glass and aqueous solution of a mixture of organic acids. Polyacrylic acid is the main constituent of the aqueous component. Less viscous polyacids such as maleic and itaconic acids may also be present in the solution to improve manipulation [1,2]. Tartaric acid is usually added to the liquid component to act as chelating agent, which allows setting reaction time control and improve the handling

properties. Conventional glass ionomer cements set via an acid-base reaction between the aqueous solution of polyacrylic acid and fluoro-aluminosilicate glass particles [3,4].

Glass ionomer cement (GIC) possesses certain properties of chemical adhesion to tooth structure, white color, low coefficient of thermal expansion similar to the tooth structure, biocompatibility, and fluoride releasing and their protective properties against tooth decay, which have led to be used for a wide range of applications in dentistry as luting, base, liners and restorative materials [5,6]. GICs also has a number of disadvantages such as brittleness, low wear resistance, inappropriate surface properties, low tensile and flexural strengths as well as high early moisture sensitivity. These drawbacks were limited its use for many clinical cases. So, many modifications have been applied to the conventional GICs to overcome the poor mechanical properties [7-9]. Nanotechnology involves the use of systems, modifications or materials ranging of 1 – 100 nm. Several ways have been attempted to improve the physical and mechanical properties of GIC using nano sized materials made by nano technology [10].

Titanium dioxide (TiO₂) is an inorganic additive which has some advantages such as its chemically stability, nontoxicity and biocompatibility. A recent previous studies has attempted to incorporate TiO₂ nanoparticles (NP) in the powder component of GICs resulting in significant improvement in the physical and mechanical properties [11,12]. The effectiveness of the antibacterial effect of conventional GICs against Streptococcus mutans, which have a major role in the formation of tooth decay, remains questionable and required. Alteration of GICs with different antibacterial agents is therefore important if it is not associated with adverse effects on physical or mechanical properties [13,14].

Chitosan (CH) is derived from chitin by deacetylation. It is a weak base, insoluble in water and organic solvents, but soluble in dilute aqueous acidic solution. It is a cationic, non-toxic, biodegradable, biocompatible and have various potential biological effects such as antibacterial and antifungal properties [15,16]. Chitosan displays a wide range of antibacterial activity against Gram-positive and Gram-negative bacteria. Liquid phase modification of GICs with chitosan was reported previously with significant improvement in antibacterial properties at optimum chitosan concentration of 10% (v/v) [17,18].

Glass ionomer cement (GIC) dually modified with chitosan (CH) in the liquid phase and titanium dioxide nanoparticles (TiO₂/NP) in the powder phase will be made to investigate both mechanical and antibacterial properties for dental restorative applications.

II. Material and methods:

The materials used in this study along with pertinent information (specification, manufacturers and batch numbers) are listed in Table [1]

Material	Specification	Manufacturer	Batch no.
Medifil	Glass ionomer filling cement containing: Al-Ca-La fluorosilicate glass and polyalkenoic acid, tartaric acid	Domagkstrasse 31 24537 Neumuenster Germany	1924439
Titanium dioxide(NP)	Mixture of rutile and anatase nanopowder <100 nm particle size MW:79.87	Sigma-Aldrich, St. Louis, MO, USA	634662

Chitosan	- C ₆ H ₁₁ NO ₄ X ₂ - Degree of Deacetylation: Min. 90%	Oxford Lab Fin CHEM LLP,India	4464
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2.1 Preparation of Chitosan Solution:

0.3N acetic acid was used as a solvent for chitosan. 1.8 ml of glacial acetic acid was made up to 100 ml with distilled water in a 100 ml standard flask to get 0.3N acetic acid. 20 mg of Chitosan were weighed separately and dissolved in 0.3 N acetic acid and made up to 100 ml with the same acetic acid in a 100 ml standard flask to get 0.2mg / ml Chitosan Solution. 0.1ml of 0.2mg/ml of Chitosan solution was added to 0.9ml of GIC liquid to get 10 v/v% Chitosan modified glass ionomer solution [19].

2.2 Preparation of Titanium Dioxide Nanoparticles Modified GIC:

TiO₂ nanotubes were weighed using a laboratory scale (Analytical balances KERN ABJ 220-4NM, KERN & SOHN GmbH, Balingen – Germany) (with weight range from 220g to 0.1mg) and adjusted to zero, then was added to the GIC's component powder prior to hand mix manipulation. Each GIC was blended with 3% or 5% (w/w) TiO₂ nanoparticles of rutile and anatase nanopowder <100 nm particle size (Sigma-Aldrich, St. Louis, MO, USA). GIC powder and TiO₂ NPs were mixed for one minute with vortex mixer (VM-300 Vortex Mixer, power: 220V / 50 Hz, Gemmy industrial corp., Taiwan) to obtain the most uniform distribution possible of the nanoparticles into GIC powder [11].

2.3 Antibacterial Activity Test:

The antibacterial activity of each group of GIC was evaluated using blood agar disc-diffusion test against *S. mutans*. Random samples of soft carious dentin were directly taken from carious cavities by a sterile excavator from randomly selected patients [20]. Specimens (10mm in diameter and 2 mm in thicknesses) of the different groups (n =12 for each group) was prepared and microscopic slides were pressed against teflon moulds on both sides to remove the cement excess and to assure a flat contact.

The antibacterial effect was evaluated using the disc diffusion method; and the culture medium was blood agar for *S. mutans*. The agar-based media were molten and poured over surface of 15 cm-diameter Petri dishes to the thickness of 5 mm and left to cool. When the medium become solid, a sterile disposable swab was used to evenly distribute the *S. mutans* microbial colonies over the medium surface. Specimens were set in direct contact over the agar containing the bacteria, where they were set on each plate at equal distances from each other then incubated at 37°C for 24 hours. 12 discs of each group were tested for microorganism and each Petri dish plate was labeled with the names of the tested groups [21]. After incubation period is completed, the diameters of inhibition zones were measured at three different points and sizes of inhibition zones were calculated by subtracting the diameter of the specimen from the average of the three measurements of the halo, for each tested GIC group.

2.4 Water Sorption and Solubility:

GIC specimens (n = 12 for each group) were prepared as previously described in (FS) testing, however split mold (9mm in diameter and 2 mm in thicknesses) was used. Then the specimens were weighed with precision weighing scale. The samples of each group were weighed in an electronic digital balance (Analytical balances KERN ABJ 220-4NM, KERN & SOHN GmbH, Balingen – Germany) with microgram readability to obtain the initial weight (m₁). Samples were then immersed in distilled water tubes and held in stand at 37 ± 1°C for 1 week in an incubator (Fisher scientific, 200 Series, Model 255D, U.S.A), removed and weighed again (m₂). The samples were then dehydrated in an oven at 37 ± 1°C for 24 h and weighed again (m₃). The water sorption was obtained from the difference between initial weighing and the wet weighing (m₂-m₁). The loss of material (solubility) was obtained from the difference between the initial and final drying mass of each sample (m₁-m₃).

The values of water sorption (W_{sp}) and solubility (W_{sol}) ratios for each sample were calculated using the following equations[22] :

$$W_{sp} = \frac{m_2 - m_1}{m_1} \times 100 \quad W_{sol} = \frac{m_1 - m_3}{m_1} \times 100$$

Statistical Analysis:

One-way ANOVA tests were used to compare antibacterial activity, water sorption and solubility of the different GIC groups. For all analyses, F - test was used for pair wise mean comparison among the tested groups. Calculations were handled by the software PASW Statistics 17 (SPSS Inc., Chicago, IL, USA), and all of the tests' accuracy was set at a significance level of 0.05.

III. Results:

3.1 Antibacterial Activity Examination:

The results of statistical analysis showed that; 5% wt TiO₂ NP modified GIC powder with the chitosan modified GIC liquid (**Group 5**) exhibited the highest antibacterial activity against *S. mutans*. while control group; unmodified glass ionomer cement (**Group 1**) recorded the lowest antibacterial activity means value Pair-wise comparisons among the groups revealed that; all groups were statistically significant difference as shown in table [2]

Table [2]: means ± SDs of inhibitory zone for all investigated groups

Antibacterial		G1	G2	G3	G4	G5	G6							
Range		0.2 – 0.8	7 – 10	11 – 14	18 – 21	20 – 25	15 – 18							
Mean ± SD		0.50 ± 0.23	8.70 ± 0.95	12.80 ± 1.03	19.20 ± 0.92	23.20 ± 1.48	16.30 ± 0.95							
F test		96.475												
P value		0.001*												
P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

3.2 Water Sorption and Solubility:

3.2.1 Water Sorption:

The results of statistical analysis showed that; 5% wt TiO₂ NP modified GIC powder with the non-modified GIC liquid (**Group 3**) showed the lowest statistically significantly water sorption mean value. Pair-wise comparisons among the groups revealed that; there was no statistically significant difference between (**Group 2 and Group 3**) and also between (**Group 4 and Group 5**).

Table [3]: Water Sorption results (Means ± SDs) for all tested groups

Water sorption	G1	G2	G3	G4	G5	G6
Range	10.56 – 11.9	8.77 – 10.53	8.53 – 9.58	9.33 – 11.73	9.03 – 11.05	11.02 – 12.5
Mean ± SD	11.23 ± 0.50	9.56 ± 0.60	9.06 ± 0.70	10.56 ± 0.74	10.25 ± 0.77	11.71 ± 0.53
F test	15.116					

P value					0.001*										
P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	
0.001*	0.001*	0.024*	0.001*	0.001*	0.110	0.001*	0.021*	0.001*	0.001*	0.001*	0.001*	0.281	0.001*	0.001*	

3.2.2 Water Solubility:

The results of statistical analysis showed that; 5% wt TiO₂ NP modified GIC powder with the chitosan modified GIC liquid (**Group 5**) showed the lowest statistically significantly water solubility mean value While; control group; unmodified glass ionomer cement (**Group 1**) recorded the highest water solubility mean value. Pair-wise comparisons among the groups revealed that; there was no statistically significant difference between (**Group 2 and Group 3**) and also between (**Group 4 and Group 5**).

Table [4]: Water Solubility results (Means ± SDs) for all tested groups

Water solubility					G1	G2	G3	G4	G5	G6				
Range					5.75 – 6.87	3.01 – 5.99	3.01 – 5.81	3.21 – 4.98	2.87 – 4.52	4.92 – 5.62				
Mean ± SD					6.31 ± 0.47	4.54 ± 1.10	4.41 ± 0.97	3.94 ± 0.64	3.19 ± 0.41	5.18 ± 0.39				
F test					12.069									
P value					0.001*									
P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
0.001*	0.001*	0.001*	0.001*	0.048*	0.191	0.019*	0.045*	0.001*	0.001*	0.001*	0.001*	0.706	0.001*	0.001*

IV. Discussion:

Glass ionomer cement was invented decades ago by Wilson and Kent in 1969. These materials form part of contemporary restorative dentistry largely due to their ability to chemically bond to tooth structure with insignificant heat formation or shrinkage, biocompatibility with pulp and periodontal tissues and fluoride releasing properties. They are used today in a variety of clinical situations such as restorative, lining, basing, luting and sealing materials[23]. Various modifications and the developments of glass powder and polymer liquid have been introduced to improve its antimicrobial effect in turn mechanical and physical properties of GICs. The nature of setting reaction of conventional glass ionomer is an acid base reaction between the acidic polyelectrolyte and the aluminosilicate glass[24]. Two concentration of titanium dioxide were selected to be studied after mixing with powder of glass ionomer cement at 3% and 5% by weight for efficiency and their effect on the mechanical and physical properties[25].

Chitosan, a widely used natural biocompatible linear biopolyaminosaccharide, has proven its potent antibacterial effect against oral biofilms, specifically *S. mutans*, thus paving the way for its use as a preventive and therapeutic agent to control dental caries and to effect on mechanical properties[18]. Chitosan can be considered a strong base as it possesses primary free amino groups (NH₃⁺) when dissolved with polyacrylic acid. The reaction had taken place between amino (-NH₂) group of CH and the functional group (OH group and C=O group) of GIC. Since CH possess hydroxyl and acetamide groups, they bind to hydroxyl group of powder particles and carboxylic groups of poly acrylic acid by hydrogen bonding [25].

Titanium dioxide (TiO₂) is an inorganic additive which has some advantages such as its chemically stability and biocompatibility, non toxicity, and improvement of mechanical properties in and hybrid materials. where they be used on assessing its effect on GICs' antibacterial, physical and mechanical performance[26]. The most common method for assessing the antibacterial property of dental

materials is agar diffusion test (ADT) which is based on placing samples on agar plates seeded with microorganisms and then evaluating antibacterial activity by measuring the inhibition zone around the disc which enables measurement of the activity of the tested material in the surrounding medium indicated by an inhibition halo[27]. The size of the inhibition zone depends on the antibacterial properties of the materials, the quantity used, and the diffusion potential across the culture medium. The greater the quantity and the higher the diffusion potential, the larger the inhibition zones which can be observed. This is agreement with our study where the inhibition zone increases significantly with adding chitosan and titanium dioxide nanoparticles[21].

Ebrahim et al., modified the liquid phase of a commercial GIC with 10% v/v chitosan and investigated the changes of antibacterial properties against *Streptococcus mutans*. The results showed significant improvement of the antibacterial properties of chitosan modified GIC (CH-GIC)[18]. *ArpanDebanth et al.*, found that modification the liquid phase of conventional GIC with 10% v/v CH solution improved the antibacterial properties of GIC against *S. mutans*. This is agreement with our results where for chitosan modified GIC (CH-GIC) as with (**Group 6**), there was an improvement in the antibacterial properties[19]. This is reflected by interaction of positively charged chitosan and negatively charged bacterial cell wall. This causes alteration in bacterial cell permeability, leading to leakage of proteinaceous and other intracellular constituents, other mechanism is based on that chitosan promotes displacement of Calcium of the anionic sites of the membrane resulting in cell damages[28]. Chitosan with low molecular weight and viscosity could possibly penetrate inside the bacteria cell to be linked to the microorganism DNA thereby inhibiting the transcription and consequently the translation. Low molecular weight and low viscosity chitosan was used, in this study, to prepare the chitosan solutions aiming to enhance the antibacterial effect of CH-modified GIC[19]. *Tayseer et al.*, evaluated antibacterial of modified glass ionomer cement (GIC) with titanium dioxide nanoparticles (TiO₂ NP) where recorded the higher diameters of growth inhibition zone against *streptococcus mutans* compared with the non-modified GIC[29]. *Esmi et al.*, studied the effect of adding titanium dioxide nanoparticles at 0.5, 1, 3, 5 weight percentage into glass ionomer cement on antibacterial properties against *streptococcus mutans*. Where results show decreasing bacteria's growth as additive ratio increased. This is agreement with our study where modifying GIC with TiO₂ NP (TiO₂-GIC) as with (**Group 2,3**) leads to decrease in the *S. mutans* compared with the non-modified GIC[21].

This improvement in antibacterial effect was attributed to the small size of the TiO₂NP, giving high surface area, which might enable the diffusion of the TiO₂ particles into the bacterial cell producing intracellular injury. The anatase phase of TiO₂ nanoparticles has a proven photocatalytic activity and high stability where the oxygen is activated in water, resulting in free hydroxyl radicals that have a very high oxidation capacity, and degrade organic matter effectively[30]. When bacteria adhere to the surface of anatase TiO₂, reactive oxygen ions and hydroxyl radicals can penetrate the walls of bacteria cells, cutting off their respiratory system and electronic transmission system, and effectively killing the bacteria. Another advantage of TiO₂ is that it degrades toxic substances released from bacteria when they are killed[31]. The current study showed that 5% wt TiO₂ NP modified GIC powder with the chitosan modified GIC liquid (**Group 5**) has the higher antimicrobial activity among all investigated groups with the dual modification of GIC liquid with CH and powder with TiO₂ NP. This could be explained, by the combined antibacterial effects of the nano-sized TiO₂ NP and the chitosan[32].

Elbahrawy et al., study was to investigate the effect of addition of chitosan to the liquid of GIC on water sorption, solubility. They found that the addition of chitosan to GIC increased water sorption of GIC and decreased its solubility. This is agreement with our results where chitosan modified GIC as with (**Group 6**) increased water sorption and decreased its solubility[22]. Chitosan modified GIC can absorb more water than conventional GIC because, whatever the type of structure, networks containing covalently cross linked chitosan are considered as porous. This term is used to describe networks containing free water that can diffuse through the hydrogel [33]. Chitosan has hydrophilic nature in polymerization linkages. In addition to that the chitosan polymeric chains form a network containing many pores that can be filled with small molecules such as water, which can be free or bound to the hydrophilic groups of the network[33]. In hydrogels formed by chitosan cross

linked with itself, release is mostly controlled by the cross linking density; consequently, the higher the cross linking density, the lower the release rate. This dissociation, together with increased hydrophilicity of chitosan polymer can explain the increase in water sorption and reduction in solubility of chitosan modified GIC groups[22]. *Howrah et al.*, studied the effect of adding 3 and 5 wt. % of TiO₂ particles additives to conventional GIC powder on sorption and solubility processes. they found that TiO₂ particles additives resulted in significant reduction of water sorption and solubility with increasing in the added ratio[34]. This is agreement with our results where modifying GIC with TiO₂ NP (TiO₂-GIC) resulted in significant reduction of water sorption and solubility as additive ratio increased as with (Group 2,3). TiO₂ particles fill the voids in between GIC particles and decrease the porosity and so reduce water sorption, in addition of TiO₂ NP makes the material more resistance to decompose in water and so reduce solubility[34]. The dual modification technique as with (Group 4,5) resulted in significant reduction in water sorption and solubility compared to the non-modified GIC, chitosan modified GIC. Accordingly, it could be postulated that the incorporation of TiO₂ nanoparticles with small size could be the main factor responsible for improving the water sorption of GIC.

V. Conclusion:

Under the limitations of the present study, 5% wt TiO₂ NP modified GIC powder with the chitosan modified GIC liquid (Group 5) exhibited the highest antibacterial activity against *S. mutans* with significant reduction in water sorption and solubility.

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