ECRONICON OPEN ACCESS

EC DENTAL SCIENCE
Research Article

Antibacterial Activity and Setting Time of Glass Ionomer Cement Modified by Nano Gold and Nano Silver

Asmaa Abd-Elhakeem Metwally[1]*, Nour Habib[2], Yasser Fathi Gomaa[3] and Cherif Adel Mohsen[4]

[1]*Assistant Lecturer, Biomaterials Department, Faculty of Dentistry, Minia University, Egypt*

[2]*Professor of Dental Materials, Faculty of Dentistry, Cairo University, Egypt*

[3]*Professor of Dental Materials, Faculty of Dentistry, Minia University, Egypt*

[4]*Professor of Fixed Prosthodontics, Faculty of Dentistry, Minia University, Egypt*

***Corresponding Author:** Asmaa Abd-Elhakeem Metwally, Assistant Lecturer, Biomaterials Department, Faculty of Dentistry, Minia University, Egypt.

Received: January 16, 2018; **Published:** March 01, 2018

Abstract

Nano-structured materials have been receiving considerable attention as a result of their unique physical, chemical, biological properties.

Purpose: The purpose of this study was to evaluate the antibacterial activity and setting time of Glass Ionomer Cements modified by Nano Gold, Nano Silver and a mixture of both with different ratios.

Materials and Methods: For antibacterial activity: Agar diffusion test against *S. mutans* was done and areas of inhibition zones were measured using digital caliper. Setting time was measured according ISO 9917-1 standard. Statistical analysis was carried out by Kolmogorov-Smirnov and Shapiro-Wilk tests, One Way ANOVA and Tukey's post-hoc test. The significance level was set at $P \leq 0.05$.

Results: Modification of GIC with Nano Gold showed the highest antibacterial effect. Incorporation of Nano Silver showed non-significant increase in the antibacterial effect of GIC. Modification of GIC with a mixture of Nano Gold and Nano Silver with a ratio of 1:1 significantly decreased the antibacterial effect while ratios of 2:1 and 1:2 showed non-significant differences to the unmodified GIC. Setting time of all modified GIC was within the accepted range of ISO standard.

Conclusion: GIC modified by Nano Gold is a promising luting material because of its potential antibacterial activity and accepted setting time.

Keywords: *Nano Gold; Nano Silver; Antibacterial Activity; Setting Time; Glass Ionomer Cement*

Introduction

various types of cements are used as luting material for the permanent and temporary cementation of indirect restorations. These cements have different biological and mechanical characteristics and the most demanding characteristic is their stability in the oral environment that is their resistance to decomposition and degradation [1,2].

Glass-ionomer cement was developed from the desire to have a luting agent with the fluoride release/translucency of dental silicate cement and the adhesion to tooth of polycarboxylate cement [3]. The glass ionomer cement has a good biocompatibility and ability to adhere to both enamel and dentin, also known to inhibit demineralization and may even rematerialize adjacent tooth structure. However,

it has certain demerits, mainly, a low resistance to wear, low tensile and compression strengths, and an early susceptibility to moisture contamination [4,5].

Significant perfections have been developed since the invention of GIC, numerous filler components have been added including; silver-amalgam particles, spherical silica, zirconia, glass fiber, hydroxyapatite, bioactive glass particles as pre-reacted glass ionomer particles (PRG), giomer restorative material. The incorporation of these filler particles to GIC has significantly modified the mechanical properties of cements; however, fillers can interfere with metabolic activities for bacterial adhesion and inhibit the antibacterial activity of GIC [6].

The use of nanoparticles (NPs) has become a significant area of research in dentistry, the main use have been focused in increasing the mechanical properties and antibacterial effect; altering the hydrogen bonding, respiratory process, DNA unwinding, cell wall synthesis and division by making "pits" in the wall and increasing the permeability resulting in a bacterial death [7].

The metallic nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials. Among the important significance of nano-metals are gold and silver, they possess inherent antibacterial effect. Gold nanoparticles are chemically stable, inert and biocompatible so it can be used in dental materials easily [8]. silver nanoparticles have been proved to be most effective as they have good antimicrobial efficacy against bacteria, viruses, and other eukaryotic microorganisms. silver nanoparticles have been applied in several areas of dentistry, as endodontics, dental prostheses, implantology and restorative dentistry [9,10].

Due to the interesting properties of Nano Gold and Nano Silver and lack of studies on Nano Gold application in luting cements the purpose of this study was to evaluate the antibacterial activity and setting time of Glass Ionomer Cement modified by Nano Gold and Nano Silver.

Materials and Methods

Ketac cem radiopaque GIC (3M,ESP Germany), Nano Gold and Nano Silver (Purest Colloids, Inc U.SA) were used. Ketac cem radiopaque powder was divided into equal seven groups according to the liquid used for mixing: Group I (control group): Ketac cem radiopaque liquid; Group II: distilled water+15% tartaric acid; Group III: Nano Gold+15% tartaric acid; Group IV: Nano Silver+15% tartaric acid; Group V: Mixture of Nano Gold and Nano Silver with ratio of Au : Ag is 1: 1+15% tartaric acid; Group VI: Mixture of Nano Gold and Nano Silver with ratio of Au : Ag is 2: 1+15% tartaric acid; Group VI: Mixture of Nano Gold and Nano Silver with ratio of Au : Ag is 2: 1+15% tartaric acid; Group VII: Mixture of Nano Gold and Nano Silver with ratio of Au : Ag is 1: 2 +15% tartaric acid.

Antibacterial Activity

The Agar diffusion test was used to evaluate the antibacterial effect against *Streptococcus mutans*. All procedures were carried out under aseptic conditions in a laminar air flow cabinet. Bacterial strain from stock cultures was cultivated overnight in specific culture media: Trypticase-soy agar after incubation for 24h in an incubator at 37°C ± 1°C, two or three discrete representative overnight colonies of tested strain were inoculated into 2 ml sterile saline and diluted to obtain a turbidity equal to 107 CFU/ml equivalent to 0.5 McFarland turbidity standard solution [About 9.95 ml of solution A (1% (V/V) of sulfuric acid) was mixed with 0.05 ml of solution B (1.175% (W/V) aqueous solution of barium chloride dehydrate] slowly and with constant agitation in a clear glass test tube. The tube was sealed and stored in the dark at room temperature [11].

Petri dishes containing 30 ml agar to a thickness of 2 mm were seeded by 0.5 ml of microbial suspension using Automatic micropipette. After solidification of the agar, ten standardized wells with a diameter of 10 mm were punched into the agar in each Petri dish with the blunted end of a sterile Wither Man tube. The powder-liquid materials from each tested group were freshly mixed for 60 seconds with sterile metal spatulas according to manufacturer instructions and inserted in the wells (10 mm in diameter x 2 mm in thickness) within 1 minute with sterile dental instruments in each petri dish.

For monitoring the immediate antibacterial effect of the tested groups, the plates were incubated in an incubator at 37°C ± 1°C for 48h. Zones of bacterial growth inhibition were measured in millimeters with a digital caliper of 0.001 mm resolution. In order to measure the size of these zones; two diameters for each sample were recorded passing through the center of each disc one vertical diameter and one horizontal; the mean of the two diameters gave the final diameter that was used to calculate the net diameter of the inhibition zone. The size of the inhibition zones for each material was calculated from the diameters of the halo of inhibition produced and the disc's diameter as follows: Size of inhibition zone = diameter of halo - diameter of the disc.

Setting Time

21 specimens (n = 3/group) were used and measured according to ISO 9917-I: 2003 [12] in which an indenter of 400 ± 5g with a needle having a flat end of diameter 1 ± 0.1 mm was used. The mixed cement filled a mold of 10 mm diameter and 2 mm thickness placed

on a glass slap, 90s after end of mixing the indenter was lowered vertically onto the surface of cement and allowed to remain there for 5s repeating the indentations at 30s intervals until the needle failed to make a complete circular indentation when viewed with using x2 magnification and the setting time was recorded as the time elapsed between the end of mixing and the time when the needle fails to make a complete circular indentation in the cement.

Statistical analysis was performed with statistical package for social sciences IBM® SPSS® Statistics Version 22 for Windows. Data were presented as mean and standard deviation (SD). Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. All tested parameters showed a parametric distribution, so One Way ANOVA was used to compare tested groups. Tukey's post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. The significance level was set at $P \leq 0.05$.

Results

Antibacterial Test

The mean inhibition zone of the tested groups ranged between 2.35 and 3.89 mm. The highest was found in group III (3.89 mm) while the lowest was in group V (2.35 mm). There were statistical significant differences between groups where P value ≤ 0.05. Group III showed significantly higher mean value than that of groups I, V,VI and VII. Although groups II, IV and VII showed higher mean of inhibition zone compared to group I these differences were statistically non-significant. Group III showed statistically insignificant higher mean of inhibition zone compared to group IV. Group V showed the lowest statistically significant mean value compared to the other tested groups. There were no statistical significant differences between groups I, II, IV, VI and VII.

The mean inhibition zones and standard deviations of the tested groups are presented in table 1.

Groups	Inhibition Zone (mm)		p-value
	Mean	± SD	
I (KC)	3.10^{b}	± 0.62	≤ 0.001*
II (DW)	3.46^{ab}	± 0.29	
III (G)	3.89^{a}	± 0.27	
IV (S)	3.33^{ab}	± 0.76	
V (G: S)	2.35^{c}	± 0.39	
VI (2G:1S)	2.90^{b}	± 0.26	
VII (2S:1G)	3.24^{b}	± 0.59	

Table 1: *Mean and standard deviation (SD) of Inhibition Zone (mm) for different tested groups.*

Means with the same letter within each column are not significantly different at p = 0.05.

** = Significant.*

Setting Time

The mean setting time of the tested groups ranged between 3.93and 6.52 minutes. The highest was found in group IV (6.52 minutes) while the lowest was in group VII (3.93). There was statistical significant difference between groups where P value ≤ 0.05. Group IV showed the highest significant increase in mean setting time compared to all other tested materials. There was no statistical significant difference between groups II, III, V and VI compared to group I. Group VII showed significant decrease in mean value of setting time compared to group I.

The mean and standard deviation for setting time for different tested materials were presented in table 2.

Groups	Setting Tim (Min)		p-value
	Mean	± SD	
I (KC)	5.13^{bc}	± 0.15	≤ 0.001*
II (DW)	4.60^{cd}	± 0.40	
III (G)	5.67^{b}	± 0.34	
IV (S)	6.52^{a}	± 0.47	
V (G: S)	5.68^{b}	± 0.56	
VI (2G:1S)	4.68^{cd}	± 0.50	
VII (2S:1G)	3.93^{d}	± 0.44	

Table 2: *Mean and standard deviation (±SD) of setting time in Min for different tested groups.*

Means with the same letter within each column are not significantly different at p = 0.05.

**= Significant.*

Discussion

The idea of the work was incorporating nano gold, nano silver and a mixture of nano gold and nano silver in different ratios to GIC because according to Zhou., *et al*. in 2012 [13], Targino., *et al*. in 2013 [14], Umamaheswari., *et al*. in 2014 [15], Jegatha Christy., *et al*. in 2015 [16], Yu., *et al*. in 2016 [17], Parmar and Jangir, 2017 [18] nano gold and nano silver have antibacterial effect.

Antibacterial activity was evaluated in the current study because according to Daugela., *et al*. [19] antibacterial activity of dental luting cements is a very important property when applying dental crowns, bridges, inlays, onlays, or veneers, because bacteria may be still present on the walls of the preparation or gain access to the cavity if there is microleakage present after cementation. *Streptococcus mutans* is the most important organism in the initiation of dental caries. Experiments using strains of *S. mutans*, showed the important role of this microorganism in caries etiology [20].

The materials were investigated during setting with the agar diffusion method for the immediate antibacterial effects in which they were applied within the first minute after mixing according to Daugela., *et al*. [19] and Turkun., *et al*., [21] because the materials had significantly more antibacterial effect while setting than when tested completely set. This could be partially explained by the effect that most dental materials seem to be bactericidal while setting and their low pH during this period may also have an effect.

The results of antibacterial test revealed that all groups showed areas of inhibition zone which might be due to any of the components of the cement such as fluoride release, tartaric acid, preservatives, gold or silver nanoparticles.

There was no significant differences in the antibacterial effect between groups of distilled water+tartaric acid, nanosilver, a mixture of nanogold and nanosilver with ratios of 2:1, 1:2 and the control group while groups of GIC modified with nanogold and a mixture of nanogold and silver with ratio 1:1 there was significant differences as compared to control group. This significance difference between GIC modified with nanogold and the control group may be explained by the direct interaction of gold nanoparticles with bacteria cells to cause destructive effects, like penetration of cell membrane and disruption of cell function. The high surface to volume ratio provides more efficient means for enhanced antibacterial activity, another possible mechanism of antibacterial activity may be due to increased intracellular ROS generation. Such explanation in agreement with Azam., *et al.* in 2009 [22], Hayden., *et al.* in 2012 [23], Cui., *et al.* in 2012 [24], Shah., *et al.* in 2014 [25] and Umamaheswari., *et al.* in 2014 [15] who studied the antibacterial effect of gold nanoparticles.

The antibacterial effect of gold NPs may take place in two steps. First, they changed the membrane potential and reduced adenosine triphosphate (ATP) synthase activities, thus reducing the metabolism process. Secondly, they declined the subunit of the ribosome for tRNA binding, thus collapsing its biological mechanism. At the same time, they proved to be less toxic to mammal cells. Gold NPs with a small size and enhanced surface area produce some electronic effects which are beneficial for enhancing the surface reactivity of NPs. In addition, the high surface area percentage directly interacted with the microorganism to an enormous extent and hence provided an improved contact with the bacteria as explained by Shamaila., *et al*. [26]. The non-significant difference in antibacterial effect between GIC modified with nanosilver and control group was in disagreement with Magalhaes., *et al*. [27] who revealed that the addition of nanosilver to GIC cavity lining significantly increased the antibacterial effect. This could be explained by the difference in methodology. In our study GIC was mixed with nanosilver while in their study nanosilver was mixed to the already mixed GIC so the surface in direct contact with bacteria had higher concentration of nanosilver.

Although GIC modified with nanogold showed significant difference with control group while GIC modified with nanosilver showed insignificant difference with control group yet there was no significant difference in the antibacterial effect between them. This was in contrast to a study by Hernandez-Sierra., *et al*. [28] who revealed that nanosilver had higher antibacterial effect than nanogold against *S. mutans*. Such difference may be due to the difference in their methodology. In their study they use nanogolad and nanosilver separately without mixing with any powder and this will completely change the mechanism of releasing gold and silver ions from the mixed cement compared to absolute solutions.

The results also showed that the addition of gold and silver nanoparticles together with a ratio of 1:1 to GIC showed the significant lowest antibacterial effect as compared to all groups. This was surprising as it was expected that there would be synergistic effect of both nanoparticles together leading to increase the antibacterial effect as compared to GIC alone or with gold or silver nanoparticles. This may be due to different physicochemical interaction of both gold and silver nanoparticles together provided different properties from each one alone and change in electrochemical potential leading to reduction of silver ion release by increasing gold fraction as supported by Hann., *et al*. [29] and Alissiwy., *et al*. [30] who studied kinetics of ion release of different compositions of gold-silver nanoparticles.

The results showed that by decreasing the amount of gold nanoparticles from G group to 2G:1S and G:S groups there was gradual significant reduction in the antibacterial effect as gold nanoparticles had the highest antibacterial effect as revealed from the result of G group. On the other hand when ratio of silver nanoparticles was higher than gold nanoparticles in 2S:1G group there was higher signifi-

cant antibacterial effect than G:S group although the decreased amount of gold nanoparticles and this could be explained by higher release of silver ion which related to the amount of silver nanoparticles and the gold fraction as mentioned before.

As regard to setting time, modification of GIC with nanogold, nanogold with nanosilver with ratio of 1:1 and 2:1 showed similar setting time as unmodified GIC in group I.

On the other hand modification of GIC with nanosilver showed significant increase in setting time while a mixture of nanogold and nanosilver with a ratio of 1:2 showed significant decrease in setting time compared to control group. This might be explained by interference of silver nanoparticles with initial setting of GIC due to release of silver ions and affinity to form complex with carboxylate groups of poly acrylic acid and change in ions concentrations responsible for setting. Gold and silver nanoparticles together may provide energetic effect leading to faster release of calcium and aluminum ions thus decreasing setting time.

The results of setting time of all groups were in the accepted range provided by ISO 9917-1 for water- based cements which is from 1.5 to 8 minutes.

Conclusions

GIC modified by Nano Gold is a promising luting material because of its potential antibacterial activity and accepted setting time.

Bibliography

1. Phillips RW., *et al*. "In vivo disintegration of luting cements". *Journal of the American Dental Association* 114.4 (1987): 489-492.
2. Yoshida K., *et al*. "Properties of commercially available luting cements". *Japan Prosthodontic Society* 39.1 (1995): 35-40.
3. Wilson AD and Nicholson JW. "Acid-base cements, their biomedical and industrial applications". New York: Cambridge University Press (1993): 1-383.
4. Dewald P and Arcoria J. "Evaluation of glass Cement cores under cast crowns". *Dental Materials* 6.2 (1990): 129-132.
5. Anand C and Bhagwat SV. "Antibacterial effects of glass ionomers on carious dentin-An in vivo study". *Journal of Conservative Dentistry* 3.2 (2000): 65-74.
6. Dhull KS and Nandlal B. "Comparative evaluation of fluoride release from PGR-composites and compomer on application of topical fluoride: an in vitro study". *Journal of Indian Society of Pedodontics and Preventive Dentistry* 27.1 (2009): 27-32.
7. García-Contreras R., *et al*. "Perspectives for the use of silver nanoparticles in dental practice". *International Dental Journal* 61.6 (2011): 297-301.
8. Dadkana S., *et al*. "Mechanical Properties of Dental Adhesives Containing Gold Nano Particles". Proceedings of 5th International Congress on Nanoscience and Nanotechnology. Tehran, Iran (2014): 1-3.
9. Gong P., *et al*. "Preparation and antibacterial activity of Fe3O4@Ag nanoparticles". *Nanotechnology* 18.28 (2007): 604-611.
10. Corrêa JM., *et al*. "Silver Nanoparticles in Dental Biomaterials". International Journal of Biomaterials (2015).
11. Baron EJ and Finegold SM. "Baily and Scott's, Diagnostic microbiology, 8th Edition". Stephaine Manning (ed). The C.V. Mosby Company, St Louis, Missouri, USA (1990).
12. International Organization for Standardization. Dentistry-Water-based cements-Part 1: Powder/liquid acid-base cements. Geneva, Switzerland ISO 9917-1 (2003).
13. Zhou Y., *et al*. "Antibacterial activities of gold and silver nanoparticles against Escherichia coli and bacillus Calmette-Guérin". *Journal of Nanobiotechnology* 10 (2012): 19.
14. Targino AGR., *et al*. "Antimicrobial activity of silver nanoparticles in treating dental caries". *RFO, Passo Fundo* 18.3 (2013): 312-315.
15. Umamaheswari K., *et al*. "Antibacterial activity of gold nanoparticles and their toxicity assessment". *BMC Infectious Diseases* 14.3 (2014): 64.
16. Jegatha Christy A., *et al*. "Optical, Structural and morphological properties of Silver nanoparticles and their antimicrobial activity". *International Journal of ChemTech Research* 7.3 (2015): 1191-1197.
17. Yu Q., *et al*. "Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells". *Scientific Reports* 6 (2016): 26667.

18. Parmar K and Jangir OP. "Evaluation and Efficacy of the Antibacterial Activity of Silver and Gold Nanoparticles Synthesize from Camelus dromedarius (Camel) Milk against Oral Pathogenic Bacteria". *International Journal of Current Microbiology and Applied Sciences* 6.4 (2017): 600-605.

19. Daugela P., *et al*. "Antibacterial potential of contemporary dental luting cements". *Stomatologija* 10.1 (2008): 16-21.

20. El-Marakby AM., *et al*. "Evaluation of Anti-Cariogenic Properties among Four Types of Glass Ionomer Cements". *Journal of Dental and Oral Health* 1.1 (2017): 1-5.

21. Türkün LS., *et al*. "Long-Term Antibacterial Effects and Physical Properties of a Chlorhexidine-Containing Glass Ionomer Cement". *Journal of Esthetic and Restorative Dentistry* 20.1 (2008): 29-45.

22. Azam A., *et al*. "One step synthesis and characterization of gold nanoparticles and their antibacterial activities against E. coli (ATCC 2592 strain)". *International Journal of Theoretical and Applied Sciences* 1.2 (2009): 1-4.

23. Shah MR., *et al*. "Morphological analysis of the antimicrobial action of silver and gold nanoparticles stabilized with ceftriaxone on Escherichia coli using atomic force microscopy". *New Journal of Chemistry* 38.11 (2014): 5633-5640.

24. Cui Y Zhao., *et al*. "The molecular mechanism of action of bactericidal goldnanoparticles on Escherichia coli". *Biomaterials* 33.7 (2012): 2327-2333.

25. Hayden SC., *et al*. "Aggregation and Interaction of Cationic Nanoparticles on Bacterial Surfaces". *Journal of the American Chemical Society* 134.16 (2012): 6920-6923.

26. Shamaila S., *et al*. "Gold Nanoparticles: An Efficient Antimicrobial Agent against Enteric Bacterial Human Pathogen". *Nanomaterials* 6.4 (2016): E71.

27. Magalhaes APR., *et al*. "Nanosilver Application in Dental Cements". *ISRN Nanotechnology* (2012): 365438.

28. Hernández-Sierra JF., *et al*. "The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold". *Nanomedicine* 4.3 (2008): 237-240.

29. Hahn A., *et al*. "Electrochemistry controlled metal ion release from silicone elastomer nanocomposites through combination of different metal nanoparticles". *Journal of Materials Chemistry* 21.28 (2011): 10287-10289.

30. Alissawi N., *et al*. "Effect of gold alloying on stability of silver nanoparticles and control of silver ion release from vapor-deposited Ag-Au/polytetrafluoroethylene nanocomposites". *Gold Bulletin* 46.1 (2013): 3-11.

Volume 17 Issue 4 April 2018