EFFECT OF GLASS IONOMER CEMENTS MODIFICATION WITH POLY(AMIDO-AMINE) AND BIOACTIVE GLASS ON THEIR COMPRESSIVE STRENGTH, SOLUBILITY AND SETTING TIME AN IN-VITRO STUDY

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ABSTRACT

Introduction: Secondary caries is one of the major factors responsible for clinical restorative failure. Dental restorative materials should be antibacterial to assist in long-lasting restoration. **Aim:** Evaluation of the effect of adding PAMAM liquid (with two concentrations) and bioactive glass powder (BAG) as antibacterial agents to glass ionomer cements (GICs) on their compressive strength, solubility, and setting time. **Materials and Method:** Four main groups were prepared as follows; Group (G) I: Samples of commercially available GIC (control), G II: Samples of GIC mixed with PAMAM, G III: Samples of GIC mixed with BAG, and GIV: Samples of GIC mixed with BAG and PAMAM. A total of 120 samples were prepared; 48 samples were prepared for the compressive strength test, 36 samples were used for the setting time test, 36 samples were prepared for the solubility test. **Results:** A significant decrease in the compressive strength of all groups compared to the control group was recorded. There was a significant increase in the solubility in G III compared to all other groups. For the final setting time test, the control group had the shortest final setting time, being significantly different from all other groups. **Conclusion:** This study showed that GIC modified with 12% V PAMAM has a reasonable compressive strength, which might help provide a modified GIC suitable for pediatric dentistry. Furthermore, the modification of GICs with PAMAM and BAGs worsens their compressive strength, and lengthens their setting time, while modification of GICs with PAMAM has no adverse effect on their solubility.

INTRODUCTION

Glass ionomer cements (GICs) have large-scale clinical applications as a consequence of the probable modification of their chemical formulations or physical properties. Additionally, the beneficial characteristics of GICs include their chemical bonding to enamel and dentin, good marginal seal, and fluoride (F) release. Furthermore, their coefficient of thermal expansion is close to that of the tooth structure[1].

Dental caries is one of the most popular dental diseases. Glass ionomer cements were considered to be anticaries and antibacterial due to their F release[2]. However, GIC failure by secondary caries still exists. This indicates that the F release from GICs is not effective enough to prevent bacterial destruction of the tooth structure[3].

Bioactive glass (BAG) has revealed antimicrobial intraoral activity[4]. Poly(amido-amine) (PAMAM), a dendrimer that is a particularly developed polymer with reactive end groups. The amino-terminated PAMAM dendrimers (PAMAM-NH$_2$) exhibited a potent antibacterial action[5]. Furthermore, the antibacterial mechanism of PAMAM dendrimers may significantly impact its evident lack of creating resistance[6].

In-vitro compressive strength tests revealed adequate for analyzing the mechanical properties of GIC[7,8], and are therefore utilized to evaluate their ability to withstand masticatory forces[9]. The clinical performance and durability of dental cements depend on many factors including the structural integrity and dimensional stability of the cement intraorally. On the other hand, structural integrity and dimensional changes, represent a function of the solubility properties[10]. Consequently, the solubility behavior of luting cements has been widely estimated both clinically trials and in the laboratory[11].

This study aimed to evaluate the effect of GICs modifications with antibacterial agents; polyamido-amine and bioactive glass on their compressive strength, solubility, and setting time properties. The setting time was measured to ensure the best functional efficacy. It is important to note that the properties evaluated were considered the most relevant to clinical success.

MATERIALS AND METHODS

I- Materials:

The materials used in this study are listed in **Table 1**.

Table (1) *The brands used, description, composition, presentation, manufacturers, and batch number.*

Brands	Description	Composition	Manufacturer	Batch no.
Glass ionomer filling material (GC Gold Label 9) High Strength Posterior Restorative	Conventional glass ionomer filling material	Powder: Fluoro-aliminosilicate glass Liquid: polyacrylic acid	GC Corporation Japan	1903071
Bioactive glass nanoparticles[1]	Bioactive glass nanoparticles	46.1% SiO$_2$, 26.9% CaO, 24.4% Na$_2$O, 2.6% P$_2$O$_5$	Chemistry laboratory, Faculty of Science, Suez Canal University.	
Generation 2 polyamido-amine (PAMAM-NH2)	Polyamido-amine		National Research Centre.	M27301

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1. *Siqueira RL, Peitl O, Zanotto ED. Gel-derived SiO$_2$–CaO–Na$_2$O–P$_2$O$_5$ bioactive powders: Synthesis and in vitro bioactivity. Mater Sci Eng.2011;C 31:983–991*

II- Methods:

Sample Grouping:

After sample size calculation, a total of 120 samples were prepared. These were divided into four main groups (G) G I, G II, G III, and G IV, according to the volume ratio (V) of PAMAM (L2) added to the GIC liquid (L1) and the weight ratio (wt) of BAG (P2) added to the GIC powder (P1). Groups II and IV were further subdivided into A and B according to liquid modification. Whereas subgroup A was (L1:L2; 87.5:12.48%) and subgroup B was (L1:L2; 75:25 %)

Four main groups were prepared as follows:

G I: Samples of commercially available GIC powder (P1) mixed with a liquid (L1) according to manufacturer instruction (3.6:1 g/ml) representing (100:100%)

   G II A: 100% P1/ L1: L2 (87.5:12.48%)

   G II B: 100% P1/ L1:L2 (75:25%),

   G III: P1:P2 (90:10%)/ 100%L1

   G IV A: P1:P2 (90:10%)/ L1:L2 (87.5:12.48%)

   G IV B: P1:P2 (90:10%)/ L1:L2 (75:25%).

Samples preparation:

The GIC samples were mixed according to the manufacturer's instructions, with a powder / liquid ratio of 3.6 to 1.0 (g/ml). The samples modified with BAG powders were added in 10% of the cement powder weight ratio, using a digital balance (Sartorius analytic, A 200 S, Germany). On the other hand, the samples modified with PAMAM liquid were added in two-volume ratios, at 8 and 16 multiple minimum bactericidal concentration (MBC) ratios of PAMAM (a pilot study was first made to select its effective antibacterial concentration). This ratio was equal to 12.2 %, and 24.9 % of the total volume of the liquid, respectively. A digital balance was used to weigh each sample separately. Each sample of different groups had the same powder/liquid ratio. Afterward, the cement powders were mixed with the cement liquid to form a paste set in teflon molds with particular dimensions as specified by each test.

Compressive strength test:

A total of 48 samples (8 samples/G and SG) were prepared in a cylindrical teflon mold (6 mm in height and 4 mm in diameter), following the specifications of ISO 7489:1986 (ISO 9917:1991 reference) for water-based dental cement[13].

After preparing the samples, they were stored in distilled water for 24 hours in an incubator (Heraeus, DIN 58945, Germany) at 37 °C and 100% humidity. The compressive strength was measured by a universal testing machine (Instron, 3345, England), recording the maximum load at failure, and calculating the compressive strength (δc) in MPa using the following formula:

$$\delta_c = \frac{\textit{Maximum load at failure}}{\textit{Cross-sectional area}}$$

The results were tabulated and statistically analyzed.

Solubility Test:

The solubility of the samples was assessed according to the ANSI/ADA specification No.66 for GICs with a slight modification in the sample dimensions as suggested by Carvalho-Junior[14].

A total of 36 samples (6 samples/G and SG) were prepared using a split teflon mold (internal diameter of 7.75 mm and a height of 1.5 mm). During the preparation of the samples, a convenient length of stainless steel orthodontic ligature wire was used

and inserted into the soft material to ensure proper handling of the sample and its complete immersion in the deionized water.

All samples were placed in a desiccator with freshly dried silica at 37°C for 22 hours. Subsequently, the samples were removed and stored in another desiccator for 2 hours at 23 °C. Later, they were accurately weighed with a 4-digit digital balancer (Fisher Scientific Balance UK Ltd). This cycle was repeated until a constant mass was achieved, with a mass loss of each specimen not exceeding 0.1 mg in 24 hours, to confirm the complete dehydration of the specimen. This record represents the initial dry weight (*m1*) of the specimen.

The diameter of each specimen was calculated from 2 perpendicular planes using a digital micrometer (Mitutoyo, USA). The thickness of the specimen was calculated at five points on its surface. These measurements were recorded to calculate the volume of each sample (V) in mm³ using the following equation:

$$V = \pi r^2 h$$

Where: *r =* the mean specimen radius, and *h=* the mean specimen thickness.

Samples were immersed separately in a 75 ml glass vial of deionized water for 7 days at 37 °C. After the planned storage period, the samples were reconditioned in a desiccator until gaining a constant weight (*m2*), applying the same procedure described for (*m1*). Solubility (*SL*) micrograms per cubic millimeter (μg/mm³) for one week of storage were calculated using the following formulae:

$$SL = \frac{M1 - M2}{V}$$

The results were tabulated and statistically analyzed.

Setting time test:

A total of 36 samples (6 samples/G and SG) were prepared to determine the final setting times. The prepared ring mold (5 mm hight and 10 mm in diameter) was placed on a flat glass plate and filled with the mixed cement.

The net setting times of the cement were measured according to the ISO method for water-based dental cement (ISO 9917- 1:2007)[15]. The final setting time was recorded as the time that elapsed between the start of mixing and the time when the indenter's flat-end needle (1.06 mm in diameter, and 453.6 g in weight) failed to make a complete circular indentation in the test material[16]. The results were tabulated and statistically analyzed.

Statistical analysis of data:

All values were shown as means ± standard deviation (SD). One-way ANOVA, and a Tukey post-hoc test for multiple comparisons were used to evaluate parametric data. While independent samples Kruskal-Wallis test was used to evaluate nonparametric data. P-value < 0.05 was statistically significant. The relation between parameters was analyzed by Pearson's Correlation and linear regression analysis tests.

RESULTS

Compressive strength test:

The means and SD values of the compressive strength test for the various investigated groups are listed in **Table (2).**

There was a significant reduction in the compressive strength value (p<0.05) for all experimental groups, compared to that of G I. While there was a significant increase in the compressive strength values for SG II A compared to G III, and G IV (A, and B).

Table (2) *Means, SD, and compressive strength results in MPa for different investigated groups.*

| Groups | | Mean ± SD | G I | G II | | G III | G IV | |
				G II A	G II B		G IV A	G IV B
G I		146±27.9[bcdef]		.000	.005	.000	.000	.000
G II	*G II A*	112.9±25.7[acdef]	.005		.000	.000	.000	.000
	G II B	71.2±[9]ab	.000	.000		.321	1.000	1.000
G III		53.3±9.9[ab]	.000	.000	.321		.323	.356
G IV	*G IV A*	71.2±5.1[ab]	.000	.000	1.000	.323		1.000
	G IV B	70.7±11.87[ab]	.000	.000	1.000	.356	1.000	

Means with different lowercase letters indicate statistically significant differences. The mean difference was significant at (p < 0.05). Letter a represents a statistically significant difference compared to G I, while letter b with G II A, letter c with G II B, letter d with G III, Letter e with G IV A, and letter f with G IV B.

Solubility test:

The means and SD values of the solubility test for the various investigated groups are listed in **Table (3).**

The results showed an insignificant difference ($p > 0.05$) in the solubility between the control G I and all other groups except G III, which had a significant increase ($p < 0.05$) in its solubility. There was a significant increase in the solubility for GIII compared to all other groups.

Table (3) *Means, SD, and solubility test results for different investigated groups.*

| Groups | | Mean ± SD | G I | G II | | G III | G IV | |
				G II A	G II B		G IV A	G IV B
G I		16.2±6.4[d]		1.000	1.000	.000	.908	.836
G II	*G II A*	17.6±6.6[d]	1.000		1.000	.000	.930	.867
	G II B	18.9±5.2[d]	1.000	1.000		.000	.946	.890
G III		125.6±8.5[abcef]	.000	.000	.000		.003	.005
G IV	*G IV A*	37.6±.13.6[d]	.908	.930	.946	.003		1.000
	G IV B	41.3±20.2[d]	.836	.867	.890	.005	1.000	

Means with different lowercase letters indicate statistically significant differences. The mean difference was significant at (p < 0.05). Letter a represents a statistically significant difference compared to G I, while letter b with G II A, letter c with G II B, letter d with G III, Letter e with G IV A, and letter f with G IV B.

Final setting time:

The means and SD values of the final setting time test for the various investigated groups are listed in Table (4).

The control group had the shortest final setting time, with a significant difference ($p < 0.05$) compared to other groups. The G II B had the longest final setting time with a significant difference ($p < 0.05$) with all groups except G IV B.

Table (4) *Means, SD, and results of the final setting time test (in minutes) for different investigated groups.*

Groups		Mean ± SD	G I	G II		G III	G IV	
				G II A	G II B		G IV A	G IV B
G I		5.44±.06[bcdef]		.000	.000	.002	.000	.000
G II	*G II A*	8.35±.18[acdef]	.000		.000	.000	.000	.000
	G II B	14.18±.88[abde]	.000	.000		.000	.000	.963
G III		6.67±.505[abcef]	.002	.000	.000		.000	.000
G IV	*G IV A*	10.32±.402[abcdf]	.000	.000	.000	.000		.000
	G IV B	13.95±.49[abde]	.000	.000	.963	.000	.000	

Means with different lowercase letters indicate statistically significant differences. The mean difference was significant at ($p < 0.05$). Letter a represents a statistically significant difference compared to G I, while letter b with G II A, letter c with G II B, letter d with G III, Letter e with G IV A, and letter f with G IV B.

DISCUSSION

Using an effective antibacterial lining material may solve possible problems such as the remnant cariogenic microorganisms after partial caries removal and consequently, avoidance of caries progression and pulp injuries[17]. Therefore, the aim of this study was to investigate the effect of modification of GICs with antibacterial agents such as PAMAM, and BAG, on their compressive strength, solubility, and setting time. It is important to note that the properties evaluated were considered the most relevant to clinical success.

Regarding the compressive strength, smaller specimen dimensions (6 mm x 4 mm) were used, according to ISO 7489:1986 specifications[13], to benefit from investigating the mechanical properties of GIC. The objective of this study was to minimize the discrepancy that may occur with large material specimens and reduce the variability that may result from the manipulation of larger amounts of material[18]. The compressive strength of different groups was measured following storage in deionized water for 24 hours since most of these materials reach their limit strength value within this period[19].

The addition of PAMAM decreased the compressive strength of the modified groups, this may be due to the high adsorption capacity of PAMAM dendrimer, which can absorb metal ions with highly tunable properties[20]. Furthermore, PAMAM-NH$_2$ has a high attraction ability to free calcium phosphate, as the amino-functional groups have a high affinity for binding to calcium ions. Therefore, PAMAM-NH$_2$ could attract more calcium phosphate, with a resultant weakening of the compressive strength due to the lower amount of Ca^{2+} ions crosslinked with a carboxylic acid[21].

Furthermore, the addition of the BAG particles decreased the compressive strength of modified groups, this could be attributed to the significant decrease in aluminum cations (Al^{3+}) following the partial substitution of the GIC powder by BAG particles. Al^{3+} is a principal constituent in improving the strength as it is essential in forming three-dimensional crosslinks, not Ca^{2+} or Sr^{2+}[22]. Moreover, Griffin *et al.*[23] explained such a decrease in the compressive strength following the addition of BAG by the competition of phosphate groups with carboxylate groups for aluminum ions, thereby inhibiting the crosslinking reaction in the cement matrix. Additionally, Mousavinasab *et al.*[24] and Lukowiak *et al.*[25] stated that additives increasing the antibacterial properties often have an adverse effect on their mechanical performance.

Regarding the solubility test, the higher values in G III compared to the control group may be attributed to many factors, such as the presence of BAG particles acting as a filler within GIC, separating the polymer chains from each other and making them more susceptible to dissolution. These results were in accordance with Gaber *et al.*(2019[26]).

On the other hand, G IV, modified by both PAMAM and BAG, showed a lower solubility than G III, modified by BAG alone. This may be due to the adsorption properties of PAMAM to ions, which may chelate with Ca^{++} ions of both BAG particles and glass ionomer particles. Therefore, a large number of crosslinking is established between the polymer chains, reducing the empty spaces and, thus, the water ingress into the material[27]. Also, this chelation may lower the solubility of BAG particles in G IV compared to G III.

Regarding the setting time test, the longer setting time of the modified groups may be due to a hindrance in the crosslinking of calcium ions with the polyacid chains, which may have delayed the initial matrix formation or gelation. Furthermore, PAMAM has an alkaline pH (9), which decreases the glass particles' solubility and hence delays their reaction with polyacrylic acid. According to our results, the longer the setting time, the higher the increased percentage of PAMAM concentration, confirming the results.

Furthermore, in G III and G IV, incorporating BAG may act as a physical obstacle, interfering with the setting reaction of cement by inhibiting the crosslinking between polyacid chains and calcium ions. Furthermore, BAG particles dissolved to give an alkaline medium, which hindered the solubility of glass particles and delayed the reaction. These results were in accordance with Saran *et al.*[28].

CONCLUSIONS

1. Modifications of GICs with 12% V PAMAM has a reasonable compressive strength which might help provide a modified GIC suitable for pediatric dentistry.

2. The modification of GICs with PAMAM and BAGs worsens their compressive strength, and lengthens their setting time, while modification of GICs with PAMAM has no adverse effect on their solubility.

REFERENCES

1. Kovarik RE, Haubenreich JE, Gore D. Glass ionomer cements: a review of composition, chemistry, and biocompatibility as a dental and medical implant material. J Long Term Eff Med Implants. 2005;15:655–671.

2. Svenson S TD. Dendrimers in biomedical applications-reflections on the field. Adv Drug Deliv Rev. 2005; 57(15):2106–2129.

3. Weng Y, Guo X, Zhao J, Gregory RL XD. A PQAS-containing glass-ionomer cement for improved antibacterial function. J Biomed Eng. 2010;3:955-962.

4. Salehi S, Davis HB, Ferracane JL MJ. Sol-gel-derived bioactive glasses demonstrate antimicrobial effects on common oral bacteria. Am J Dent. 2015;28(2):111-115.

5. Chen CZ CS. Interactions between dendrimer biocides and bacterial membranes. Biomaterials. 2002;23(16):3359–3368.

6. Xue X, Chen X, Mao X, Hou Z, Zhou Y, Bai H, et al. Amino-terminated generation 2 poly(amidoamine) dendrimer as a potential broad-spectrum, nonresistance-inducing antibacterial agent. AAPS J. 2013;15(1):132–142.

7. Lohbauer U. Dental glass ionomer cements as permanent filling materials? —properties, limitations future trends. Mater (Basel). 2010;3(1):76–96.

8. Naasan MA WT. Concentional glass ionomers as posterior restorations: A status report for the American Journal of Dentistry. Am J Dent. 1998;11:36–45.

9. Bonifacio CC, Kleverlaan CJ, Raggio DP, Werner A CR. Physical-mechanical properties of glass ionomer cements indicated for atraumatic restorative treatment. Aust Dent J. 2009;54:233–237.

10. Tuna SH KF. Water sorption and solubility of provisional and permanent luting cements. J Mater Sci. 2006;30(3):19–24.

11. Toledano M, Osorio R, Osorio E, Fuentes V, Prati C, Garcia F. Sorption and solubility of resin-based restorative dental materials. J Dent. 2003;31:43–50.

12. Charan J, Biswas T. How to calculate sample size for different study designs in medical research?. Indian J Psychol Med. 2013;35(2):121–126.

13. International Organization for Standardization. ISO 7489. Dental glass polyalkenoate cements. 1986.

14. Carvalho JR, Correr-Sobrinho L, Correr A, Sinhoreti M, Consani S, Sousa-neto M. Solubility and dimensional change after setting of root canal sealers : A proposal for smaller dimensions of test. J Endod. 2007;33(9):1110-1116.

15. International Organisation for Standardisation ISO 9917-1:2007. Dentistry – water-based cements Part 1. Powder/liquid acid–base cements. 2007.

16. Schauer J. Some physical properties of cements employed in the cementation of orthodontic bands. Master's Theses. 1971;2521:24-25.

17. Prabhakar AR, Prahlad D, Kumar S. Antibacterial activity, fluoride release, and physical properties of an antibiotic-modified glass ionomer cement. Pediatr Dent. 2013;35(5):411-415.

18. Mallmann A, Ataíde JC, Amoedo R, Rocha PV, Jacques L. Compressive strength of glass ionomer cements using different specimen dimensions. Braz Oral Res. 2007;21(3):204-208.

19. Amoedo R RP. Compressive strength of glass ionomer cements using different specimen dimensions. Dent Mater. 2007;21(3):204-208.

20. Kuhn JN, Woodcock HL, Barakat MA, Alghamdi MA, Alkhalaf A. Metal chelating composites, methods of using composites, and methods of making composites. USF Patents. 2019;1006:1-8.

21. Tao S, Fan M, Xu HHK, Li J, He L, Zhou X, Liang K LJ. The remineralization effectiveness of PAMAM dendrimer with different terminal groups on demineralized dentin in vitro. RSC Adv. 2017;7:54947–54955.

22. Ana ID, Matsuya S, Ohta M, Ishikawa K. Effects of added bioactive glass on the setting and mechanical properties of resin-modified glass ionomer cement. Biomaterials. 2003;24(18):3061-3067.

23. Griffin SG, Hill R. Influence of glass composition on the properties of glass polyalkenoate cements . Part I : influence of aluminium to silicon ratio. Biomaterials. 1999;20:1579-1586.

24. Mousavinasab SM, Khoroushi M, Keshani F HS. Flexural strength and morphological characteristics of resin-modified glass- ionomer containing bioactive glass. J Contemp Dent Pr. 2011;12(1):41-46.

25. Lukowiak A, Lao J, Lacroix J NJ. Bioactive glass nanoparticles obtained through sol–gel chemistry. R Soc Chem. 2013;49:6620-6622.

26. Gaber MA, Habib SI, Amin A. Effect of addition of nano-bioactive glass to resin modified glass ionomer in terms of solubility, ions release and bioactivity. EDJ. 2019;65(1):1–8.

27. Nicholson JW. Chemistry of glass-ionomer cements: A review. Biomaterial. 1998;19:485–494.

28. Saran R, Upadhya N P, Ginjupalli K, Amalan A, Rao B, Kumar S AA. Effect on physical and mechanical properties of conventional glass ionomer luting cements by incorporation of all-ceramic additives: An in vitro study. Int J Dent. 2020;2020:1–9.