Original Article

Effects of novel additives on the mechanical and Biological properties of glass ionomer cement: An *in vitro* study

Gupta Piyush, S. Sai Kalyan[1], U. Palekar Aparna[2], Gupta Khyati[3], Biradar Basawaraj[2]

Department of Conservative Dentistry and Endodontics, BhaBha Dental College, Bhopal, Madhya Pradesh, [1]Director, Prevest Research Institute, Prevest Denpro Limited, Jammu, [2]Department of Conservative Dentistry and Endodontics, Rural Dental College, PIMS loni, [3]Department of Orthodontics and Dentofacial Orthopedics, Sharad Pawar Dental College, DMIHER, Wardha, Maharashtra, India

Abstract

Aim: To evaluate the efficacy of incorporated novel additives in Glass Ionomer Cement to ameliorate biocompatibility and mechanical properties.

Introduction: Though Glass Ionomer Cement (GIC) has multiple advantages, it is not strong enough for medical applications, and its biocompatibility is questionable. To improve biocompatibility and its mechanical properties, a study was performed to investigate the potential benefits of adding graphene, carbon nanotubes, hydroxyapatite, and bioactive glass to GIC. The objective was to enhance both the mechanical properties and biocompatibility of GIC.

Material and Method: Modified Glass Ionomer Cement was prepared by creating five groups. Hydroxyapatite, multi-walled carbon nanotubes, graphene, and bioactive glass were incorporated in a 10:1 weight ratio, respectively. Group 5 was designated as the control group and used Fuji Type II GIC. After preparing 90 samples, they were kept in deionized water for a day and then evaluated their compressive strength, microhardness, and diametral tensile strength, and also checked their in vitro cytotoxicity by direct contact with L929 mammalian fibroblast cells.

Statistical Analysis: The data were examined using mean and standard deviation descriptive statistics. The comparative evaluation was done via Tukey HSD test and one-way ANOVA using S.P.S.S. software.

Result: It showed that Group 3 had better results in compressive strength (144.478+- 3.989), diametral tensile strength (20.29+- 0.8601), and microhardness (131+-3.536) when compared with other groups while in the biocompatibility (viability %) Group 1 [82.55], Group 3 [76.49], Group 4 [87.63], while Group 2[58.02].

Conclusion: Group 3 has better physical properties in microhardness, diametral tensile strength, and compressive strength, than the other groups. In Biocompatibility, Group 1, Group 3, Group 4, and Group 5 were noncytotoxic at the same time multi-walled carbon nanotubes group (Group 2) had cytotoxic potential.

Keywords: Bioactive glass; carbon nanotubes; glass ionomer cements; GRAPHENE; hydroxyapettite

INTRODUCTION

It is crucial for oral cavity materials to remain stable and passive, without adverse interactions. Amalgam, various cements, and composite resins exemplify such traits. Fluoride-releasing materials offer added benefits. In recent years, "smart" dental materials have gained traction for their potential in enhancing

Address for correspondence:
Dr. Gupta Piyush,
Department of Conservative Dentistry and Endodontics, BhaBha Dental College, Bhopal, Madhya Pradesh, India.
E-mail: dr.piyushgupta39@gmail.com

Date of submission : 12.02.2024
Review completed : 20.03.2024
Date of acceptance : 27.03.2024
Published : 10.05.2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcde

DOI: 10.4103/JCDE.JCDE_81_24

How to cite this article: Piyush G, Kalyan SS, Aparna UP, Khyati G, Basawaraj B. Effects of novel additives on the mechanical and Biological properties of glass ionomer cement: An *in vitro* study. J Conserv Dent Endod 2024;27:503-7.

dental health.[1] Hydroxyapatite (HAP) mimics bone minerals, sharing a similar composition and lattice structure with human dental and skeletal apatite. Its biocompatibility stems from a calcium–phosphorus ratio identical to human bone.[2] Bioactive glass (BAG) integrated into glass ionomer cement (GIC) aims to enhance tooth regeneration potential and bioactivity. Despite BAG incorporation reducing mechanical strength, combining it with GIC offers potential benefits.[3]

Graphene, a remarkable nanomaterial, boasts high elastic modulus, extensive surface area, and flexural strength. Incorporating graphene nanosheets enhances the hardness and mineralization of bioactive calcium silicate.[4,5] MWCNT feature cylindrical carbon structures arranged hexagonally. Their needle-like fibrous form may influence final product bioactivity.[6] Multiwalled carbon nanotubes (MWCNTs) exhibit excellent biological adaptability and bioactivity, expanding their potential applications in dentistry.[7]

MATERIALS AND METHODS

Modified GIC was prepared in an amalgamator for 30 s, as suggested by the manufacturer. The recommended P/L ratio for this particular substance is 2.7/1.

Five groups of 18 samples each were prepared, with Group 1 containing GIC (Fuji II) modified with HAP (ratio 10:1 by wt.), Group 2 with multiwall carbon nanotube (ratio 10:1 by wt.), Group 3 with graphene (ratio 10:1 by wt.), Group 4 with a BAG (ratio 10:1 by wt.), and Group 5 as the control group.

The compressive strength of the samples was measured by preparing a split mold of 4 mm in diameter and 6 mm in height, mixing cement for 30 s in an amalgamator, and applying a compressive load at 1.0 mm/min along the specimen's long axis after 24 h of mixing.

The diametral tensile strength was measured by positioning a cylindrical test specimen between the platens of a mechanical tester and applying a load along the long axis of the sample at a crosshead speed of 0.5 mm/min.

The microhardness of the samples was evaluated using the Vickers hardness test with a diamond indenter, a load of 100 g, and a dwell period of 10 s. The Vickers hardness values were calculated using the equation provided.

The biocompatibility of the samples was assessed using an *in vitro* cytotoxicity test with negative and positive control samples. The test materials were sterilized by autoclaving and placed on the cell layer of triplicate wells. The plates were then cultured for 24 h and examined under a phase contrast microscope to assess changes, detachment, vacuolization, cell lysis, and membrane integrity. The culture media was then replaced with fresh media and the MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity) solution was added to each well for incubation and measurement of absorbance at 570 nm concerning 690 nm on a microplate reader.

Statistical analysis

Descriptive statistics were used to calculate the mean and standard deviation. Tukey's honest significant difference and one-way analysis of variance tests were conducted using SPSS Statistical software (Acquired by IBM Chicago, IL) software for comparative evaluation.

RESULTS

Compressive strength comparison revealed Group 3 with the highest strength (144.478 ± 3.989), followed by Group 2 (138.756 ± 3.709), Group 5 (123.788 ± 7.442), Group 4 (103.214 ± 3.975), and Group 1 with the lowest strength (95.108 ± 20.765). Significant differences were observed between various groups: Group 1 versus Group 2 and Group 3 ($P < 0.001$, mean differences: −43.648 and −49.370), Group 1 versus Group 5 ($P < 0.01$, mean difference: −28.680), Group 2 versus Group 4 and Group 3 versus Group 4 ($P < 0.001$, mean differences: 35.542 and 41.264), Group 3 versus Group 5 and Group 4 versus Group 5 ($P < 0.05$, mean differences: 20.690 and − 20.574). Notably, some comparisons such as Group 1 versus Group 4, Group 2 versus Group 3, and Group 2 versus Group 5 were statistically nonsignificant ($P > 0.05$) [Table 2].

Diametral tensile strength analysis indicated that Group 3 exhibited the highest strength (20.29 ± 0.8601), followed by Group 2 (18.58 ± 2.206), Group 5 (17.928 ± 1.790), Group 1 (17.874 ± 4.766), and finally, Group 4 with the least strength (17.21 ± 1.571). Statistical analysis showed nonsignificant differences between various groups [Table 2].

Microhardness comparison displayed higher values for Group 3 (131 ± 3.536) compared to Group 2 (126.8 ± 2.950), Group 1 (109.6 ± 3.507), Group 5 (107 ± 3.742), and finally, Group 4 (105.2 ± 3.899). Significant differences were observed in certain comparisons such as between Group 1 versus Group 2 and Group 3 ($P < 0.001$, mean differences: −17.200 and −21.400) and Group 2 versus Group 4 and Group 5 ($P < 0.001$, mean differences: 21.600 and 19.800). Other comparisons were statistically nonsignificant [Table 2].

Biocompatibility assessments revealed varying reactivity levels among groups with microscopic observations indicating mild reactivity for Groups 1 and 4, moderate

reactivity for Groups 2 and 3, and no reactivity for Group 5. Quantitative cytotoxicity analysis using MMT assay showed varying cell viability percentages with Group 4 exhibiting the highest (87.63%), followed by Group 1 (82.55%), Group 5 (77.71%), Group 3 (76.49%), and Group 2 with the lowest percentage of cell viability (58.02%).

DISCUSSION

Novel additives aim to enhance GIC by improving its mechanical properties and biocompatibility. Incorporating nanoparticle fillers into GIC has shown promise in boosting its mechanical strength, biological compatibility, and antibacterial properties.[8,9] The interaction between polyacrylic acids carbonyl anion, Ca2+ ions, phosphoric acid, and hydroxyl groups in HAP, the tooth's main constituent, facilitates the binding of GIC to the tooth structure. HAP forms a macromolecular electrolyte chain, with polyacrylate replacing surface phosphate during adsorption, resulting in the formation of an "intermediate layer" of calcium and aluminum phosphates and polyacrylates at the GIC-HAP interface. Incorporating HAP into GIC can enhance both its biocompatibility and physical properties.[10,11]

Adding Micro-HAP to conventional GIC has been found not to affect diametral tensile strength.[12] Carbon nanotubes (CNTs), invented by Kroto have shown potential in improving the mechanical, biological, and structural properties of various materials, including biomaterials.[13-15] The composition of CNTs in biomaterials can significantly influence their bioactivity, with a higher CNT content offering improved mechanical and electrical characteristics.

In our study, multiwalled carbon nanotubes were incorporated into glass ionomer cement to enhance biocompatibility and mechanical properties.[16] Graphene nanoparticles have also been investigated for reinforcing glass ionomers, leading to significant improvements in their physio-mechanical characteristics when combined with poly (acrylic acid) glass ionomer or hydrothermally reacted with glass ionomer to create composite matrices. However, research on standard glass ionomer cement treated with graphene to enhance physical properties and biocompatibility is limited.[17] Graphene has been shown to improve the physical properties of various cementitious materials and composites, including BAG, resulting in increased Vickers hardness number (VHN) and fracture toughness.[18] Incorporating BAG into glass ionomer cement aims to enhance its mechanical and biological properties.

Specimen preparation was standardized using a brass split mold, and mechanical strength tests, including compressive strength and diametral tensile strength, were conducted. Microhardness testing indicated improvements in Group 3 compared to other groups, consistent with prior research. Cytotoxicity evaluation performed on L929 murine fibroblast cell lines revealed superior mechanical properties in Group 3 in terms of compressive strength, diametral tensile strength, and microhardness. However, the addition of BAG particles decreased the modulus of elasticity and compressive strength, suggesting loose attachment to the glass ionomer matrix.[19]

These findings are supported by previous studies conducted by Bresciani E (2004), Silva RC (2007), Ahmed HM (2011).[20-22] Fuji IX GIC exhibited lower microhardness compared to Bio dentine™ at 48 h, 7, and 14 days, but was equivalent at 21 days.[23]

Biocompatibility results indicated mild reactivity (Zone limited area under specimen) for Groups 1 and 4, while Groups 2 and 3 showed moderate reactivity (Zone extending specimen size up to 1 cm). Group 5 (control) exhibited nonreactivity (No detectable zone around or under the specimen). A grade >2 was considered cytotoxic [Table 1]. The MTT assay revealed that Group 4 had the highest cell viability (87.63%), followed by Group 1 (82.55%) and Group 5 (77.71%), while Group 3 exhibited 76.49% viability [Table 1]. However, Group 2 showed the lowest cell viability at 58.02%. Microscopic observation confirmed intact mitochondrial activity in [Figure 1c, e, g], indicating normal cell morphology [Figure 1]. Figure 1e displayed intact mitochondrial activity but with morphological changes, such as multiple granules and altered cell shapes. figure 1d exhibited more dead cells compared to the negative control [Figure 1a]. The MTT assay accurately assessed cytotoxic potential. Uneven sample surfaces, especially evident in the edges of groups 2 and 3, might have contributed to cell damage and

Table 1: Mean value of compressive strength, diametral tensile strength, microhardness, and reactivity grade and viability of cell for direct contact test by microscopic observation of Group 1–Group 5

Groups	Compressive strength (mean value) Mpa	Diametral tensile strength (mean value) Mpa	Microhardness (mean value) VHNV (kg/mm^2)	Grades	Reactivity	Quantitative measurements of cytotoxic effects by MTT assay Viability (%)
Group 1	95.108	17.874	109.6	2	Mild reactivity	82.55
Group 2	138.756	18.58	126.8	3	Moderate reactivity	58.02
Group 3	144.478	20.29	131	3	Moderate reactivity	76.49
Group 4	103.214	17.21	105.2	2	Mild reactivity	87.63
Group 5	123.788	17.928	107	0	No reactivity	77.71

The achievement of numerical grade >2 is considered cytotoxic viability and is reduced to <70% of the negative control, it has cytotoxic potential. VHNV: vickers hardness number value, MTT: (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity

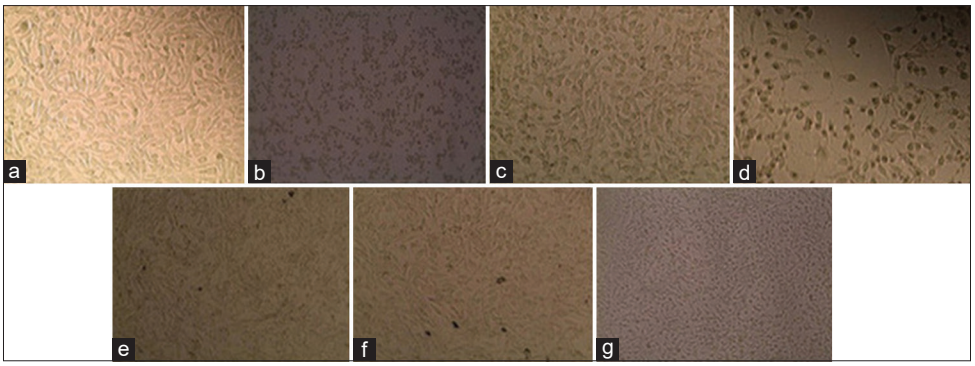

Figure 1: (a) Negative control shows normal mitochondrial activity and morphology of cells. (b) Positive control shows all dead cells. (c) Group 1 shows normal mitochondrial activity and morphology of cells. (d) Group 2 shows more dead cells with a smaller number of normal cells normal cells. (e) Group 3 shows some changes in morphology of cell with intact mitochondrial activity. (f) Group 4 shows normal mitochondrial activity and morphology of cells. (g) Group 5 shows normal mitochondrial activity and morphology of cells

Table 2: Mean difference and *P* value of compressive strength, diametral tensile strength, and microhardness of Group 1–Group 5

Comparison	Compressive strength		Diametral tensile strength		Microhardness	
	Mean difference	*P*	Mean difference	*P*	Mean difference	*P*
Group 1 versus Group 2	−43.648	<0.001***	−0.7060	>0.05 (NS)	−17.200	<0.001***
Group 1 versus Group 3	−49.370	<0.001***	−2.416	>0.05 (NS)	−21.400	<0.001***
Group 1 versus Group 4	−8.106	>0.05 (NS)	0.6640	>0.05 (NS)	4.400	>0.05 (NS)
Group 1 versus Group 5	−28.680	<0.01**	−0.05400	>0.05 (NS)	2.600	>0.05 (NS)
Group 2 versus Group 3	−5.722	>0.05 (NS)	−1.710	>0.05 (NS)	−4.200	>0.05 (NS)
Group 2 versus Group 4	35.542	<0.001***	1.370	>0.05 (NS)	21.600	<0.001***
Group 2 versus Group 5	14.968	>0.05 (NS)	0.6520	>0.05 (NS)	19.800	<0.001***
Group 3 versus Group 4	41.264	<0.001***	3.080	>0.05 (NS)	25.800	<0.001***
Group 3 versus Group 5	20.690	<0.05*	2.362	>0.05 (NS)	24.000	<0.001***
Group 4 versus Group 5	−20.574	<0.05*	−0.7180	>0.05 (NS)	−1.800	>0.05 (NS)

****P*<0.001 - highly significant, ***P*<0.01 - mild significant, **P*<0.05 - significant. NS: Not significant

cytotoxicity [Figure 1b and c]. Glass ionomer cement and nanohybrid composite demonstrated lower cytotoxicity compared to flowable and bulk-fill flowable composites.[24]

Regarding graphene biocompatibility, various factors influence its cytotoxicity, including surface functionalization, shape, dispersibility, size, and concentration. Graphene-based nanomaterials may induce cytotoxicity through reactive oxygen species production and cell membrane damage. Guazzo R. found that adding 1 wt.% Graphene oxide nanoparticles to Portland cement improved surface microhardness while maintaining biocompatibility.[25]

Li *et al*.[26] suggested that multiwalled carbon nanotubes (MWCNT) are more likely to interact with cells, with Group 3 showing the least cell contact. These findings were supported by Lulu Zhou *et al*. in 2017, who demonstrated that pristine MWCNTs are more harmful to cells, while functionalized MWCNTs cause more gene damage compared to the pristine form.[27]

CONCLUSION

Based on the study, it was concluded that the graphene-incorporated GIC group has better physical properties such as compressive strength, diametral tensile strength, and microhardness compared to other groups. In addition, in terms of biocompatibility, the HAP group, Graphene group, BAG group, and conventional GIC group did not show any cytotoxicity, while the multiwalled carbon nanotubes group was found to have cytotoxic potential.

Financial support and sponsorship

IACDE RESEARCH GRANT 2018.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Khoroushi M, Mousavinasab SM, Keshani F, Hashemi S. Effect of resin-modified glass ionomer containing bioactive glass on the flexural strength and morphology of demineralized dentin. Oper Dent 2013;38:E1-10.
2. Raedah AS, Nadia H, Wael S. Influence of hydroxyapatite nanoparticles on the properties of glass ionomer cement. J Mater Res Technol 2019;8:344-9. Available from: https://www.researchgate.net/publication/324513251_Influence_of_hydroxyapatite_nanoparticles_on_the_properties_of_glass_ionomer_cement/citation/download. [Last accessed on 2019 Jul 21].

3. De Caluwé T, Vercruysse CW, Ladik I, Convents R, Declercq H, Martens LC, *et al.* Addition of bioactive glass to glass ionomer cements: Effect on the physico-chemical properties and biocompatibility. Dent Mater 2017;33:e186-203.
4. Gong K, Pan Z, Korayem AH, Qiu L, Li D, Collins F, *et al.* Reinforcing effects of graphene oxide on Portland cement paste. J Mater Civ Eng 2015;27:A4014010.
5. Sun L, Yan Z, Duan Y, Zhang J, Liu B. Improvement of the mechanical, tribological and antibacterial properties of glass ionomer cements by fluorinated graphene. Dent Mater 2018;34:e115-27.
6. Foroughi MR, Khoroushi M, Nazem R, Tefaghi EA. The effect of carbon nanotubes/bioglass nanocomposite on mechanical and bioactivity properties of glass ionomer cement. Sci Iran 2016;23:3123-34.
7. Akasaka T, Nakata K, Uo M, Watari F. Modification of the dentin surface by using carbon nanotubes. Biomed Mater Eng 2009;19:179-85.
8. Panahandeh N, Torabzadeh H, Aghaee M, Hasani E, Safa S. Effect of incorporation of zinc oxide nanoparticles on mechanical properties of conventional glass ionomer cements. J Conserv Dent 2018;21:130-5.
9. Zandi Karimi A, Rezabeigi E, Drew RA. Glass ionomer cements with enhanced mechanical and remineralizing properties containing 45S5 bioglass-ceramic particles. J Mech Behav Biomed Mater 2019;97:396-405.
10. Wilson AD, Prosser HJ, Powis DM. Mechanism of adhesion of polyelectrolyte cements to hydroxyapatite. J Dent Res 1983;62:590-2.
11. Arita K, Yamamoto A, Shinonaga Y, Harada K, Abe Y, Nakagawa K, *et al.* Hydroxyapatite particle characteristics influence the enhancement of the mechanical and chemical properties of conventional restorative glass ionomer cement. Dent Mater J 2011;30:672-83.
12. Stout DA, Webster TJ. Carbon nanotubes for stem cell control. Mater Today 2012;15:312-8.
13. Sharafeddin F, Karimi S, Jowkar Z. Evaluation of the effect of micro-hydroxyapatite incorporation on the diametral tensile strength of glass ionomer cements. J Conserv Dent 2019;22:266-9.
14. Hirata E, Akasaka T, Uo M, Takita H, Watari F, Yokoyama A. Carbon nanotube-coating accelerated cell adhesion and proliferation on poly (L-lactide). Appl Surf Sci 2012;262:24-7.
15. Cicchetti R, Divizia M, Valentini F, Argentin G. Effects of single-wall carbon nanotubes in human cells of the oral cavity: Geno-cytotoxic risk. Toxicol *In Vitro* 2011;25:1811-9.
16. Martins-Júnior PA, Alcântara CE, Resende RR, Ferreira AJ. Carbon nanotubes: Directions and perspectives in oral regenerative medicine. J Dent Res 2013;92:575-83.
17. Malik S, Ruddock FM, Dowling AH, Byrne K, Schmitt W, Khalakhan I, *et al.* Graphene composites with dental and biomedical applicability. Beilstein J Nanotechnol 2018;9:801-8.
18. Gao C, Liu T, Shuai C, Peng S. Enhancement mechanisms of graphene in nano-58S bioactive glass scaffold: Mechanical and biological performance. Sci Rep 2014;4:4712.
19. Khoroushi M, Keshani F. A review of glass-ionomers: From conventional glass-ionomer to bioactive glass-ionomer. Dent Res J (Isfahan) 2013;10:411-20.
20. Bresciani E, Barata Tde J, Fagundes TC, Adachi A, Terrin MM, Navarro MF. Compressive and diametral tensile strength of glass ionomer cements. J Appl Oral Sci 2004;12:344-8.
21. Silva RC, Zuanon AC, Esberard RR, Candido MS, Machado JS. *In vitro* microhardness of glass ionomer cements. J Mater Sci Mater Med 2007;18:139-42.
22. Ahmed HM, Omar NS, Luddin N, Saini R, Saini D. Cytotoxicity evaluation of a new fast set highly viscous conventional glass ionomer cement with L929 fibroblast cell line. J Conserv Dent 2011;14:406-8.
23. Arnez MM, Castelo R, Ugarte D, Almeida LP, Dotta TC, Catirse AB. Microhardness and surface roughness of biodentine exposed to mouthwashes. J Conserv Dent 2021;24:379-83.
24. Hegde NN, Attavar SH, Hegde MN, Priya G. Antibacterial activity of dental restorative material: An *in vitro* study. J Conserv Dent 2018;21:42-6.
25. Guazzo R, Gardin C, Bellin G, Sbricoli L, Ferroni L, Ludovichetti FS, *et al.* Graphene-based nanomaterials for tissue engineering in the dental field. Nanomaterials (Basel) 2018;8:349.
26. Li Y, Cao J. The impact of multi-walled carbon nanotubes (MWCNTs) on macrophages: contribution of MWCNT characteristics. Sci China Life Sci. 2018;61:1333-51.
27. Zhou L, Forman HJ, Ge Y, Lunec J. Multi-walled carbon nanotubes: A cytotoxicity study in relation to functionalization, dose and dispersion. Toxicol *In Vitro* 2017;42:292-8.