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Original Article

Dentistry Section

Evaluation of Setting Time, Compressive Strength, Microleakage and Antibacterial Properties of Nano Hydroxyapatite Incorporated GIC: An In-vitro Study

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ABSTRACT

Introduction: Glass Ionomer Cement (GIC) is widely used as a restorative material, but it still exhibits some limitations that pose significant drawbacks in clinical use. The composition of Hydroxyapatite (HA) mimics the inorganic fraction of mineralised tissues such as bones and teeth. HA possesses osteoconductive and bioactive properties, making it favourable for orthopaedic and dental applications.

Aim: The aim of this study was to evaluate and compare the setting time, compressive strength, microleakage and antibacterial properties of nano-HA incorporated GIC (NHa-GIC) at various concentrations with Type-II GIC.

Materials and Methods: This in-vitro study was conducted in the Department of Paediatric and Preventive Dentistry at Sri Aurobindo College of Dentistry in Indore, Madhya Pradesh, India, for a duration of nine months, from May 2023 to January 2024. The study comprised five study groups as follows: Group-1-Type-II GIC (control), Group-2-NHA-GIC 4%, Group-3-NHA-GIC 8%, Group-4-NHA-GIC 10% and Group-5-NHA-GIC 15%. A total of 50 acrylic moulds containing the test material and Type-II GIC, with 10 in each group (n=10), were prepared to record the setting time, which was tested using a Gillmore needle. The compressive strength was checked using a Universal Testing Machine. Additionally, 50 primary molars were selected according to inclusion and exclusion criteria and randomly divided into five groups (n=10) to evaluate microleakage. Microleakage was assessed using the dye penetration method under a stereomicroscope. For antibacterial properties, 50 samples (n=10) were taken for each strain, i.e., Strain A: *S. mutans* and Strain B: *L. fermentum* and the disk diffusion method was employed. Thus, a total sample size of 250 was used. The preparation of GIC-NHAp was done by adding nano- HA to GIC at selected concentrations by weight/weight percentage (w/w%). The results were analysed using a one-way Analysis of Variance (ANOVA) test.

Results: A statistically significant difference was observed ($p<0.001$) between Group-1 (GIC II) and Group-II (4% GIC-NHAp), III (8% GIC-NHAp), IV (10% GIC-NHAp) and V (15% GIC-NHAp) regarding setting time, compressive strength, microleakage and antibacterial properties.

Conclusion: It can be concluded that adding NHAp crystals to GIC enhances its properties, such as compressive strength and antimicrobial efficiency, as the concentration increases. The microleakage property showed a consistent decrease with increasing concentration. However, the setting time increased with higher concentrations of NHAp in GIC.

Keywords: Disk diffusion, Dye penetration, Glass Ionomer cement, Gillmore needle, Universal testing machine

INTRODUCTION

For many clinical applications, GIC is used as a restorative material due to its biocompatible properties [1]. Despite possessing beneficial traits such as good biocompatibility, fluoride release and adhesive characteristics, GIC still suffers from low mechanical properties [1]. To address these limitations, researchers have explored various modifications by incorporating different materials. For instance, to enhance the antibacterial properties, scholars have experimented with newly fabricated nano compounds, one of which is Nanoparticle Hydroxyapatite (NHA) [2].

The NHA is considered a promising bioceramic due to its superior osseointegration, biocompatibility and ability to promote enamel remineralisation [3]. Numerous studies have shown that NHA-modified GIC can substantially enhance bonding strength with teeth [4,5], reduce cytotoxicity and not impede the sustained release of fluoride [6].

Previous studies [7-9] have examined these parameters individually: some focused solely on compressive strength, others on microleakage and a few explored combinations of these parameters. However, none have assessed all these parameters together or tested antibacterial properties at three different incubation periods using two different bacterial strains.

Thus, the need and objective of the present study is to develop a novel GIC modified with NHA to improve the physical properties of GIC. The study aimed to investigate the setting time, compressive strength, microleakage and antibacterial properties of the modified GIC.

MATERIALS AND METHODS

The present in-vitro study was conducted in the Department of Paediatric and Preventive Dentistry at Sri Aurobindo College of Dentistry in Indore, Madhya Pradesh, India for a duration of nine months, from May 2023 to January 2024. The study was undertaken after receiving clearance from the institutional Ethical Committee at Sri Aurobindo College of Dentistry, Indore (IEC-SAIMS/IEC/46/22).

Inclusion criteria: Deciduous first and second molars must have an intact coronal portion and no deformities.

Exclusion criteria: Teeth showing fracture lines, teeth with carious lesions and teeth with deformities.

Sample size calculation: A sample size of 50 for each property was deemed feasible, with n=10. Therefore, a sample size of 50 was established for setting time, compressive strength and microleakage, as well as 50 for strain A and 50 for strain B for the antibacterial property. Consequently, a total sample size of 250 was considered.

Study Procedure

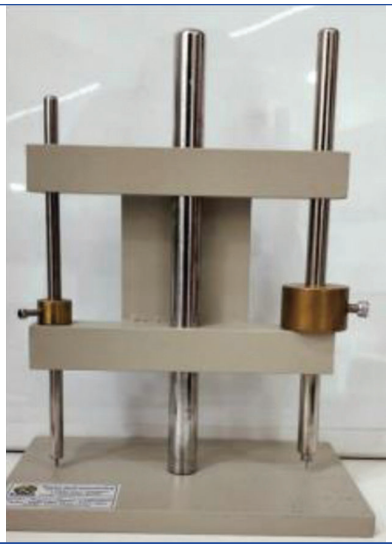
The study comprised five groups: a control group consisting of Type-II GIC (GC Tokyo, Japan) and four test groups that included NHA (IMCC, Nanotech) incorporated GIC (NHa-GIC) at concentrations of 4% [7], 8% [7], 10% [7] and 15% [10]. These were categorised as follows: Group-1- Type-II GIC, Group-2- NHa-GIC 4%, Group-3- NHa-GIC 8%, Group-4- NHa-GIC 10% and Group-5- NHa-GIC 15%.

Preparation of Nano Hydroxyapatite (NHA) Incorporated GIC: The addition of NHA to GIC powder was done by weight/weight (w/w) for concentrations of 4%, 8%, 10% and 15%. For example, in the 10% group, 10 mg of HA was mixed with 90 mg of GIC to achieve a GIC reinforced with 10% NHA [11]. The powder mixture was manually mixed using a mortar and pestle for 10 minutes.

Setting time: The samples used to determine the setting time were prepared following International Organisation for Standardisation (ISO) 9917-134 [12] (6 mm height, 4 mm diameter) using an acrylic-based resin mould [Table/Fig-1a,b]. The powder and liquid from each cement both with and without the determined concentrations of NHA particle powder, were mixed. The resulting mixture was then placed in cylindrical acrylic moulds with a diameter of 4 mm and a height of 6 mm.

[Table/Fig-1]: Showing preparation of acrylic based resin mould.

To determine the setting time, a Gilmore needle [Table/Fig-2] was used. The needle made indentations into the cement every 30 seconds. The surface was inspected for any indentations made by the needle and the time was recorded until the needle stopped making indentations. The first placement of the needle that did not make any indentation on the surface was recorded as the setting time [12].



[Table/Fig-2]: Gilmore needle apparatus.

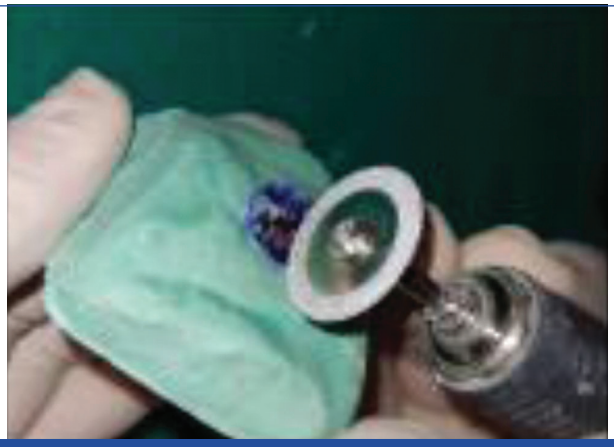
Compressive strength: The samples used to determine the compressive strength were prepared in the same manner as those used to determine the setting time. The specimens were polished with abrasive paper and then stored for 24 hours. After that, the compressive strength was measured using a universal testing machine [Table/Fig-3].

Microleakage: A total of 50 non carious extracted deciduous molars were collected. The teeth were then thoroughly cleaned and stored in distilled water. They were subsequently mounted in wax

[Table/Fig-3]: Showing sample for testing of compressive strength.

before cavity preparation. Standardised cavity slots measuring 3 mm×2 mm×2 mm were prepared on the molars using a round bur, a straight bur and an inverted cone bur. Following cavity preparation, the specimens were randomly divided into five experimental groups (n=10) and filled with test compounds. The specimens were then stored in distilled water for 24 hours. After this, the teeth were subjected to thermocycling (200 cycles) at 4°C±2 and 50°C±2 using a thermocycling machine.

Two coats of nail varnish were applied to all tooth surfaces except for 1 mm around the restoration. The teeth were then immersed in a 1% solution of methylene blue for 24 hours at room temperature. After removal from the solution, the teeth were rinsed under tap water until all the dye was removed from the surface. The teeth were then sectioned buccolingually using a diamond disc at a slow speed [Table/Fig-4].



[Table/Fig-4]: Sectioning of specimen.

The specimens were observed under a stereomicroscope with a magnification of 16x and the degree of microleakage was determined and scored according to the criteria established by Sharafeddin F and Feizi N, [Table/Fig-5a-e] [13].

0=no dye penetration

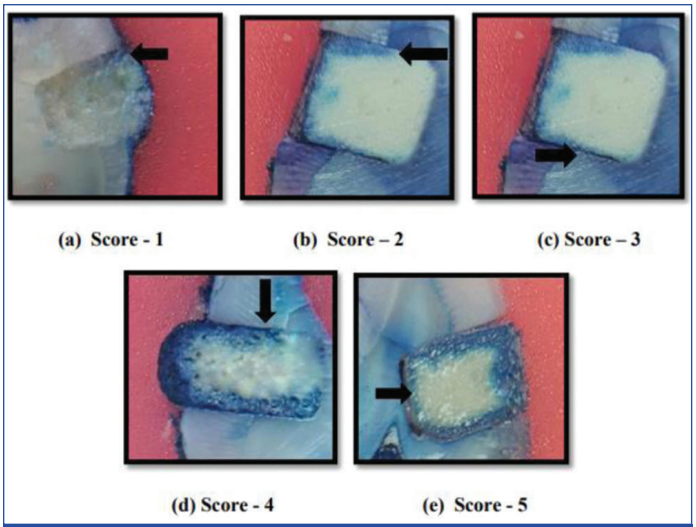
1=dye penetration between the restoration and the tooth up to one-third of the distance between the tooth surface and the axial wall

2=dye penetration extending beyond one-third of the distance up to two-thirds of the distance between the tooth surface and the axial wall

3=dye penetration extending up to two-thirds of the distance between the tooth surface and the axial wall

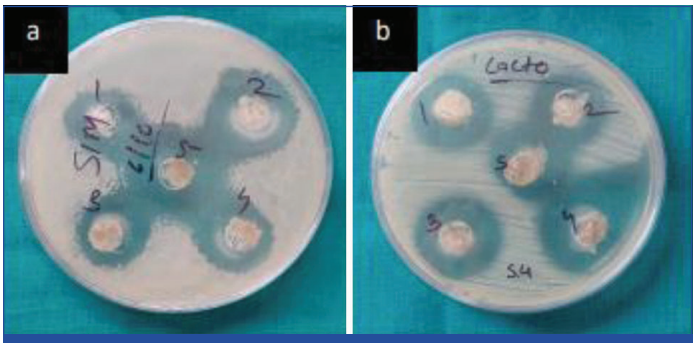
4=dye penetration reaching the axial wall

5=dye penetration reaching the entire axial wall.

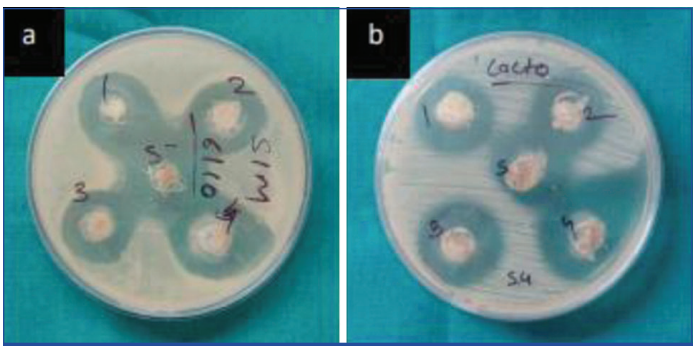


[Table/Fig-5]: Microleakage assessment showing varying depth of dye penetration.

Antibacterial property: To determine the antibacterial properties of NHA-GIC at various concentrations, agar plates were inoculated with a standardised inoculum of the test microorganism. Subsequently, agar was pounded with discs measuring approximately 6 mm in diameter. S sanguis agar was used for Streptococcus mutans and SL Rogosa agar was used for Lactobacillus fermentum. The test compounds were placed in the discs on the agar surface. The agar plates were then incubated at 37°C for 24, 48 and 72 hours and the diameters of the inhibition growth zones were recorded separately after each time period [Table/Fig-6-8a,b]. The test plates were held in front of a desk lamp and the zones were measured with a ruler, as this method was followed in several studies, including one conducted by Barry AL et al., [14].



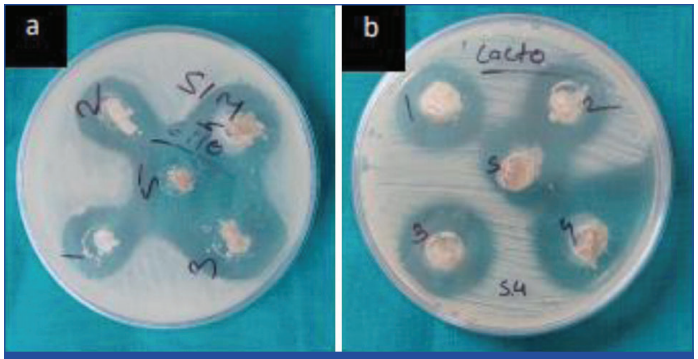
[Table/Fig-6]: Inhibition zones after incubation of 24 hours.



[Table/Fig-7]: Inhibition zones after incubation of 48 hours.

STATISTICAL ANALYSIS

The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software, version 22.0. The comparison of mean setting time, compressive strength, microleakage and diameter of inhibition zones for both strain A and strain B across different types of cement was calculated using one-way ANOVA. Mean±SD values were derived for all 50 samples (10 samples per group). A difference was considered significant when the p-value was below 0.05. Subsequently, a post-hoc test was applied to perform pair-wise comparisons.



[Table/Fig-8]: Inhibition zones after incubation of 48 hours.

RESULTS

The comparison of setting time among various types of cement, including GIC Type-II, NHAp-GIC 4%, NHAp-GIC 8%, NHAp-GIC 10% and NHAp-GIC 15%, is presented in [Table/Fig-9]. The results demonstrate a significant increase in setting time with higher concentrations of NHAp ($p<0.001$). Significant differences were observed between all the groups for setting time ($p<0.05$) as shown in [Table/Fig-10]. The longest setting time was noted in the NHAp-GIC 15% group.

Group	Setting_time				p-value
	Mean	Standard Deviation	Minimum	Maximum	
Type-II GIC	6:12	0:27	5:15	7:00	<0.001**
NHa-GIC 4%	7:21	0:20	7:00	8:00	
NHa-GIC 8%	8:11	0:10	7:55	8:30	
NHa-GIC 10%	13:07	0:10	12:55	13:25	
NHa-GIC 15%	16:19	1:04	15:00	19:00	

[Table/Fig-9]: Comparison of the setting time among various cements.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Type-II GIC	NHa-GIC 4%	-1:09*	0:14	<0.001	-1:39	-0:39
	NHa-GIC 8%	-1:59*	0:14	<0.001	-2:29	-1:28
	NHa-GIC 10%	-6:55*	0:14	<0.001	-7:25	-6:25
	NHa-GIC 15%	-10.07*	0:14	<0.001	-9.92	-9:36
NHa-GIC 4%	Type-II GIC	1:09*	0:14	<0.001	0:39	1:39
	NHa-GIC 8%	-0:49*	0:14	0.002	-1:19	-0:19
	NHa-GIC 10%	-5:46*	0:14	<0.001	-6:16	-5:15
	NHa-GIC 15%	-8:57*	0:14	<0.001	-9:27	-8:27
NHa-GIC 8%	Type-II GIC	1:59*	0:14	<0.001	1:28	2:29
	NHa-GIC 4%	0:49*	0:14	0.002	0:19	1:19
	NHa-GIC 10%	-4:56*	0:14	<0.001	-5:26	-4:26
	NHa-GIC 15%	-8:08*	0:14	<0.001	-8:38	-7:37
NHa-GIC 10%	Type-II GIC	6:55*	0:14	<0.001	6:25	7:25
	NHa-GIC 4%	5:46*	0:14	<0.001	5:15	6:16
	NHa-GIC 8%	4:56*	0:14	<0.001	4:26	5:26
	NHa-GIC 15%	-3:11*	0:14	<0.001	-3:41	-2:41
NHa-GIC 15%	Type-II GIC	10:07*	0:14	<0.001	9:36	10:37
	NHa-GIC 4%	8:57*	0:14	<0.001	8:27	9:27
	NHa-GIC 8%	8:08*	0:14	<0.001	7:37	8:38
	NHa-GIC 10%	3:11*	0:14	<0.001	2:41	3:41

[Table/Fig-10]: Pairwise comparison of the setting time between different groups.
*The mean difference is significant at the 0.05 level

The comparison of mean compressive strength among various types of cement is shown in [Table/Fig-11], indicating that compressive strength increases with higher concentrations of NHAp ($p<0.001$). A

significant difference was observed between all groups with different concentrations of NHAp when compared to Type-II GIC (p<0.001), as illustrated in [Table/Fig-12].

Group	Compressive_strength				p-value
	Mean	Standard Deviation	Minimum	Maximum	
Type-II GIC	163.62	22.11	129.00	190.19	<0.001**
NHa-GIC 4%	543.60	170.45	375.00	816.00	
NHa-GIC 8%	667.60	270.73	224.00	955.00	
NHa-GIC 10%	536.50	105.88	415.00	722.00	
NHa-GIC 15%	695.00	97.55	515.00	844.00	

[Table/Fig-11]: Comparison of the mean compressive strength among various cements.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Type-II GIC	NHa-GIC 4%	-379.98100*	70.30327	<0.001	-521.5791	-238.3829
	NHa-GIC 8%	-503.98100*	70.30327	<0.001	-645.5791	-362.3829
	NHa-GIC 10%	-372.88100*	70.30327	<0.001	-514.4791	-231.2829
	NHa-GIC 15%	-531.38100*	70.30327	<0.001	-672.9791	-389.7829
NHa-GIC 4%	Type-II GIC	379.98100*	70.30327	<0.001	238.3829	521.5791
	NHa-GIC 8%	-124.00000	70.30327	0.085	-265.5981	17.5981
	NHa-GIC 10%	7.10000	70.30327	0.920	-134.4981	148.6981
	NHa-GIC 15%	-151.40000*	70.30327	0.037	-292.9981	-9.8019
NHa-GIC 8%	Type-II GIC	503.98100*	70.30327	<0.001	362.3829	645.5791
	NHa-GIC 4%	124.00000	70.30327	0.085	-17.5981	265.5981
	NHa-GIC 10%	131.10000	70.30327	0.069	-10.4981	272.6981
	NHa-GIC 15%	-27.40000	70.30327	0.699	-168.9981	114.1981
NHa-GIC 10%	Type-II GIC	372.88100*	70.30327	<0.001	231.2829	514.4791
	NHa-GIC 4%	-7.10000	70.30327	0.920	-148.6981	134.4981
	NHa-GIC 8%	-131.10000	70.30327	0.069	-272.6981	10.4981
	NHa-GIC 15%	-158.50000*	70.30327	0.029	-300.0981	-16.9019
NHa-GIC 15%	Type-II GIC	531.38100*	70.30327	<0.001	389.7829	672.9791
	NHa-GIC 4%	151.40000*	70.30327	0.037	9.8019	292.9981
	NHa-GIC 8%	27.40000	70.30327	0.699	-114.1981	168.9981
	NHa-GIC 10%	158.50000*	70.30327	0.029	16.9019	300.0981

[Table/Fig-12]: Pairwise comparison of the compressive strength between different groups.
*The mean difference is significant at the 0.05 level

Microleakage gradually decreased with increasing concentrations of NHAp (p<0.001), except for Group-3, which contained 8% NHAp-GIC [Table/Fig-13]. A significant difference was noted between all the groups for microleakage (p<0.001), except when comparing 4% to 8% and 10% to 15%, as shown in [Table/Fig-14]. The distribution of samples based on the Sharfedin et al., scoring criteria is demonstrated in [Table/Fig-15]. In Group-2, 5 samples showed a score of 2, while another 5 samples showed a score of 5.

Group	Microleakage				p-value
	Mean	Standard Deviation	Minimum	Maximum	
Type-II GIC	4.30	0.67	3.00	5.00	<0.001**
NHa-GIC 4%	2.50	0.53	2.00	3.00	
NHa-GIC 8%	2.60	0.52	2.00	3.00	
NHa-GIC 10%	1.60	0.52	1.00	2.00	
NHa-GIC 15%	1.40	0.52	1.00	2.00	

[Table/Fig-13]: Comparison of the microleakage among various cements.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Type-II GIC	NHa-GIC 4%	1.80000*	0.24766	<0.001	1.3012	2.2988
	NHa-GIC 8%	1.70000*	0.24766	<0.001	1.2012	2.1988
	NHa-GIC 10%	2.70000*	0.24766	<0.001	2.2012	3.1988
	NHa-GIC 15%	2.90000*	0.24766	<0.001	2.4012	3.3988
NHa-GIC 4%	Type-II GIC	-1.80000*	0.24766	<0.001	-2.2988	-1.3012
	NHa-GIC 8%	-0.10000	0.24766	0.688	-0.5988	0.3988
	NHa-GIC 10%	0.90000*	0.24766	0.001	0.4012	1.3988
	NHa-GIC 15%	1.10000*	0.24766	<0.001	0.6012	1.5988
NHa-GIC 8%	Type-II GIC	-1.70000*	0.24766	<0.001	-2.1988	-1.2012
	NHa-GIC 4%	0.10000	0.24766	0.688	-0.3988	0.5988
	NHa-GIC 10%	1.00000*	0.24766	<0.001	0.5012	1.4988
	NHa-GIC 15%	1.20000*	0.24766	<0.001	0.7012	1.6988
NHa-GIC 10%	Type-II GIC	-2.70000*	0.24766	<0.001	-3.1988	-2.2012
	NHa-GIC 4%	-0.90000*	0.24766	0.001	-1.3988	-0.4012
	NHa-GIC 8%	-1.00000*	0.24766	<0.001	-1.4988	-0.5012
	NHa-GIC 15%	0.20000	0.24766	0.424	-0.2988	0.6988
NHa-GIC 15%	Type-II GIC	-2.90000*	0.24766	<0.001	-3.3988	-2.4012
	NHa-GIC 4%	-1.10000*	0.24766	<0.001	-1.5988	-0.6012
	NHa-GIC 8%	-1.20000*	0.24766	<0.001	-1.6988	-0.7012
	NHa-GIC 10%	-0.20000	0.24766	0.424	-0.6988	0.2988

[Table/Fig-14]: Pairwise comparison of the microleakage between different groups.
*The mean difference is significant at the 0.05 level

Score	C-GIC	4%	8%	10%	15%
0	-	-	-	-	-
1	-	-	-	40% (4)	60% (6)
2	-	50%(5)	40% (4)	60% (6)	40% (4)
3	10% (1)	50% (5)	60% (6)	-	-
4	50% (5)	-	-	-	-
5	40% (4)	-	-	-	-

[Table/Fig-15]: Microleakage percentage at different concentration of Nano Hydroxyapatite (NHA) incorporated GIC.

The comparison of inhibition zone diameter for Strain A at 24 hours, 48 hours and 72 hours among various cements is presented in [Table/Fig-16]. A significant difference was observed between the groups at each time point (p<0.001). Pairwise comparisons of the antibacterial properties of Strain A at 24 hours, 48 hours and 72 hours revealed significant differences between all groups at all three incubation periods, as shown in [Table/Fig-17].

Similarly, the comparison of inhibition zone diameter for Strain B at 24 hours, 48 hours and 72 hours among various cements indicated a significant difference between the groups at each time point (p<0.001) [Table/Fig-18]. Pair-wise comparison of the

Group	Inhibition zone diameter of strain A (mean±SD)		
	A_24 h	A_48 h	A_72 h
Type-II GIC	18.60±1.43	20.60±1.43	22.10±1.43
NHa-GIC 4%	21.60±1.07	23.60±1.07	25.10±1.07
NHa-GIC 8%	23.70±1.34	25.70±1.34	27.20±1.34
NHa-GIC 10%	25.80±1.23	27.80±1.23	29.30±1.23
NHa-GIC 15%	29.00±1.41	31.00±1.41	32.50±1.41
p-value	<0.001	<0.001	<0.001

[Table/Fig-16]: Comparison of the strain A inhibition zone diameter (24 hours, 48 hours and 72 hours), among various cements.

antibacterial properties of Strain B at 24 hours, 48 hours and 72 hours between different groups, demonstrating significant values for the inhibition zone diameter across all three incubation periods is presented in [Table/Fig-19].

The diameter of the zone of inhibition for all five different groups is illustrated in [Table/Fig-20,21], which show a gradual increase in the mean inhibition zone as the concentration of NHAp increases in GIC Type-II. This indicates enhanced antibacterial properties against Strain A and Strain B, specifically *S. mutans* and *L. fermentum*, after the incorporation of NHAp in GIC.

(I) Group	(J) Group	At 24 hours		At 48 hours		At 72 hours	
		Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.
Type-II GIC	NHa-GIC 4%	-3.00000*	<0.001	-3.00000*	<0.001	-3.00000*	<0.001
	NHa-GIC 8%	-5.10000*	<0.001	-5.10000*	<0.001	-5.10000*	<0.001
	NHa-GIC 10%	-7.20000*	<0.001	-7.20000*	<0.001	-7.20000*	<0.001
	NHa-GIC 15%	-10.40000*	<0.001	-10.40000*	<0.001	-10.40000*	<0.001
NHa-GIC 4%	Type-II GIC	3.00000*	<0.001	3.00000*	<0.001	3.00000*	<0.001
	NHa-GIC 8%	-2.10000*	<0.001	-2.10000*	0.001	-2.10000*	0.001
	NHa-GIC 10%	-4.20000*	<0.001	-4.20000*	<0.001	-4.20000*	<0.001
	NHa-GIC 15%	-7.40000*	<0.001	-7.40000*	<0.001	-7.40000*	<0.001
NHa-GIC 8%	Type-II GIC	5.10000*	<0.001	5.10000*	<0.001	5.10000*	<0.001
	NHa-GIC 4%	2.10000*	0.001	2.10000*	0.001	2.10000*	0.001
	NHa-GIC 10%	-2.10000*	0.001	-2.10000*	0.001	-2.10000*	0.001
	NHa-GIC 15%	-5.30000*	<0.001	-5.30000*	<0.001	-5.30000*	<0.001
NHa-GIC 10%	Type-II GIC	7.20000*	<0.001	7.20000*	<0.001	7.20000*	<0.001
	NHa-GIC 4%	4.20000*	<0.001	4.20000*	<0.001	4.20000*	<0.001
	NHa-GIC 8%	2.10000*	0.001	2.10000*	0.001	2.10000*	0.001
	NHa-GIC 15%	-3.20000*	<0.001	-3.20000*	<0.001	-3.20000*	<0.001
NHa-GIC 15%	Type-II GIC	10.40000*	<0.001	10.40000*	<0.001	10.40000*	<0.001
	NHa-GIC 4%	7.40000*	<0.001	7.40000*	<0.001	7.40000*	<0.001
	NHa-GIC 8%	5.30000*	<0.001	5.30000*	<0.001	5.30000*	<0.001
	NHa-GIC 10%	3.20000*	<0.001	3.20000*	<0.001	3.20000*	<0.001

[Table/Fig-17]: Pairwise comparison of the antibacterial properties of strain A-24 hours, 48 hours and 72 hours between different groups.

Group	Inhibition zone diameter of strain B (mean±SD)		
	A_2 4 h	A_48 h	A_72 h
Type-II GIC	26.00	27.50	28.10
NHa-GIC 4%	32.40	33.90	34.50
NHa-GIC 8%	36.40	37.90	38.50
NHa-GIC 10%	40.00	41.50	42.10
NHa-GIC 15%	45.40	46.90	47.50
p-value	<0.001	<0.001	<0.001

[Table/Fig-18]: Comparison of the strain A inhibition zone diameter (24 hours, 48 hours and 72 hours), among various cements.

DISCUSSION

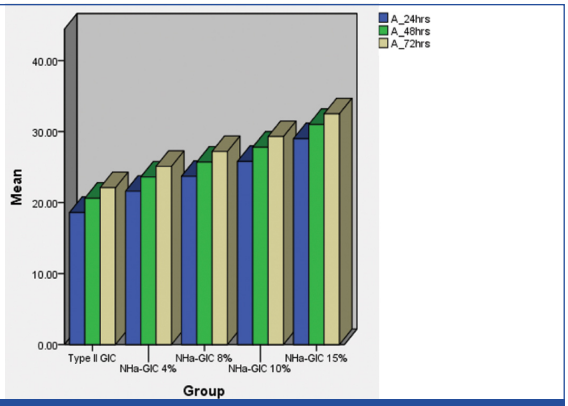
The current in-vitro study was conducted to evaluate the physical properties of GIC modified by incorporating NHAp at various concentrations. The results of the study revealed positive outcomes across all parameters, as the incorporation of NHAp enhanced the compressive strength of GIC, decreased the microleakage of the restoration and increased its antibacterial properties. However, the setting time was observed to increase with the addition of NHAp.

The concentrations of NHA used in the present study were chosen based on previous research. In the study conducted by Zhu K et al., NHAp was added at concentrations of 0%, 2%, 4%, 6%, 8%

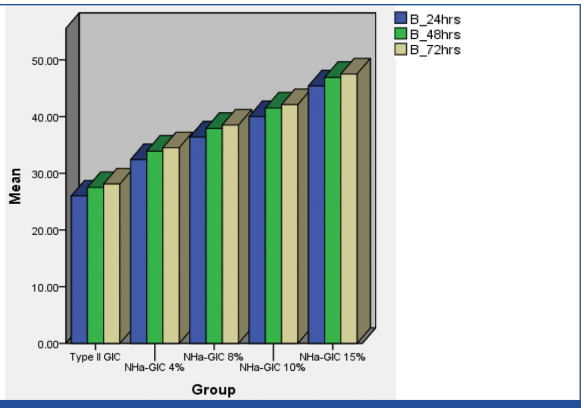
(I) Group	(J) Group	At 24 hours		At 48 hours		At 72 hours	
		Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.
Type-II GIC	NHa-GIC 4%	-6.40000*	<0.001	-6.40000*	<0.001	-6.40000*	<0.001
	NHa-GIC 8%	-10.40000*	<0.001	-10.40000*	<0.001	-10.40000*	<0.001
	NHa-GIC 10%	-14.00000*	<0.001	-14.00000*	<0.001	-14.00000*	<0.001
	NHa-GIC 15%	-19.40000*	<0.001	-19.40000*	<0.001	-19.40000*	<0.001
NHa-GIC 4%	Type-II GIC	6.40000*	<0.001	6.40000*	<0.001	6.40000*	<0.001
	NHa-GIC 8%	-4.00000*	0.001	-4.00000*	0.001	-4.00000*	0.001
	NHa-GIC 10%	-7.60000*	<0.001	-7.60000*	<0.001	-7.60000*	<0.001
	NHa-GIC 15%	-13.00000*	<0.001	-13.00000*	<0.001	-13.00000*	<0.001
NHa-GIC 8%	Type-II GIC	10.40000*	<0.001	10.40000*	<0.001	10.40000*	<0.001
	NHa-GIC 4%	4.00000*	0.001	4.00000*	0.001	4.00000*	0.001
	NHa-GIC 10%	-3.60000*	0.001	-3.60000*	0.001	-3.60000*	0.001

	NHa-GIC 15%	-9.00000*	<0.001	-9.00000*	<0.001	-9.00000*	<0.001
NHa-GIC 10%	Type-II GIC	14.00000*	<0.001	14.00000*	<0.001	14.00000*	<0.001
	NHa-GIC 4%	7.60000*	<0.001	7.60000*	<0.001	7.60000*	<0.001
	NHa-GIC 8%	3.60000*	0.001	3.60000*	0.001	3.60000*	0.001
	NHa-GIC 15%	-5.40000*	<0.001	-5.40000*	<0.001	-5.40000*	<0.001
NHa-GIC 15%	Type-II GIC	19.40000*	<0.001	19.40000*	<0.001	19.40000*	<0.001
	NHa-GIC 4%	13.00000*	<0.001	13.00000*	<0.001	13.00000*	<0.001
	NHa-GIC 8%	9.00000*	<0.001	9.00000*	<0.001	9.00000*	<0.001
	NHa-GIC 10%	5.40000*	<0.001	5.40000*	<0.001	5.40000*	<0.001

[Table/Fig-19]: Pairwise comparison of the antibacterial properties of strain B-24 hours, 48 hours and 72 hours between different groups.



[Table/Fig-20]: Comparison of the strain A inhibition zone diameter, among various cements.



[Table/Fig-21]: Comparison of the strain B inhibition zone diameter, among various cements.

and 10% (w/w) [7]. For compressive strength, no significant change was observed at 2%, whereas higher concentrations showed an increase in compressive strength. Other studies conducted at these concentrations also reported significant improvements in properties such as microleakage and antibacterial effects [15,16].

The selection of NHAp was based on its size specificity [17]. In the study by Bilić-Prcić M et al., one reason for the reduced mechanical properties in the HA-modified Fuji IX samples was the fracturing of HA particles due to their large size [17]. Many studies that considered a particle size of 20 nm found better results regarding mechanical properties after incorporation into GIC compared to other nano sizes and micron-sized particles [17,18].

According to ISO 9917-1:2007, the net setting time of a glass polyalkenoate cement suitable for dental applications should ideally fall within the range of 90 to 360 seconds. Incorporating NHA particles into the GIC could influence the setting reaction by promoting the formation of additional polysalt bridges within the network. In the present study, the setting time was determined using the Gillmore needle apparatus and the results showed an increase in setting time as the concentration of NHA in the GIC was increased. This phenomenon ultimately enhances the mechanical properties of the set GICs [19]. Studies have shown that the inclusion of NHA in GIC improves its mechanical properties, which has resulted in an extended setting time [7,20]. The 4% NHAp-GIC showed the highest mean difference in the present study. A study conducted by Zhu K et al., tested the setting time and showed results consistent with those of this study [7].

The GIC often demonstrates inferior mechanical properties, including low compressive strength, fracture strength, toughness and wear resistance [21]. GIC is frequently used as a restorative material in areas exposed to substantial masticatory forces, making the improvement of its compressive strength crucial. In the present study, the test for compressive strength was conducted using a universal testing machine. The addition of NHAp to GIC Type-II in this study produced higher values of compressive strength as the concentration of NHAp increased, up to 15%, when compared with the control group. This implies that the incorporation of NHAp in GIC increases its compressive strength. Notably, the 15% NHAp-GIC exhibited the highest compressive strength.

Upon the addition of HA into GIC, the hydrogen ions (H+) from the acid polymer initiate an attack on the ceramic particles during the formation of polysalt bridges and cross-linking. This interaction forms an intermediate layer that exhibits high resistance to acid and is challenging to break. Consequently, incorporating HA into GIC enhances the mechanical strength of the resulting material [21]. The results of the compressive strength test presented in the study conducted by Wan Jusoh WN et al., and other researchers [7,16,22-25] who performed similar studies with comparable concentrations of NHAp showed results consistent with those of this study.

Gladys S et al., previously suggested that microleakage is a common occurrence with all dental restorative materials [26]. Various techniques have been employed for this purpose, including dye penetration, fluid filtration and three-dimensional methods [27]. The dye penetration method is widely utilised for assessing the microleakage properties of dental restorative materials due to its cost-effectiveness and ease of implementation [28]. Several authors have followed the dye penetration method to evaluate microleakage [15,29-34] and this method was also employed in the current study. In this study, a 1% methylene blue dye was used for the dye penetration technique. Authors have commonly utilised a 1% methylene blue solution for 24 hours in their studies [29,30,31,34]. Methylene blue dye was chosen because it has a very small molecular size of 0.5-0.7 nm, which is smaller than bacteria (0.5-1 µm), allowing the dye to penetrate further compared to other dyes [35]. The dimensions for cavity slot preparation in this study were determined based on previous studies [31,34,36,37], with some modifications according to the primary molars. One of the causative factors for microleakage is the difference in the coefficients of thermal expansion between dentin and the restorative material [38]. Hence, thermocycling is the only method that simulates thermal stresses in the oral environment. In this study, thermocycling was performed for 200 cycles at 4°C±2 and 50°C±2 to simulate the oral environment. The present study showed microleakage of varying degrees. However, when compared among the study groups, Type-II GIC exhibited the highest microleakage score, while 15% GIC-NHAp showed the lowest microleakage and the highest mean difference among all other groups, as observed under a stereomicroscope at

16x magnification. This implies that the incorporation of NHAp in GIC reduced the microleakage of the restoration, thus preventing the chances of secondary caries. The reduced microleakage observed can be attributed to the formation of strong hydrogen and ionic bonds resulting from the ions released during the acid-base reaction between the GIC and HA [39]. Banu YN et al., investigated the microleakage properties of GIC incorporating HA and their findings revealed that the GIC enhanced with NHA exhibited significantly reduced microleakage compared to the GIC-only group [40].

Literature has highlighted a direct correlation between elevated levels of *Streptococcus mutans* bacteria and an increased incidence of dental caries [41]. Lactobacillus species were found to be overabundant in caries, with *L. fermentum* being the active group associated with dental plaque formation as well as caries [42]. Hence, *S. mutans* and *L. fermentum* were selected as test bacteria, as both are associated with caries progression and dental plaque formation.

The antibacterial properties in this study were assessed at three different incubation periods: 24 hours, 48 hours and 72 hours. The results for *S. mutans* and *L. fermentum* showed an increase in the diameter of the inhibition zone as the concentration increased when compared to the control group. This indicates that the incorporation of NHAp in GIC enhances its antibacterial properties, thus preventing bacterial activity to some extent. The reported mean inhibition zone sizes were 29.0 mm for S.mutans and 45.4 mm for *L. fermentum* after a 24-hour incubation period, representing significant improvements compared to the conventional GIC sample in the study. These findings align well with a previous study [43], confirming the enhanced antibacterial efficacy of GIC formulations incorporating nanoparticles.

The inhibition zone was also measured at 48 hours and 72 hours of incubation. The results at these incubation periods showed similar growth, with an increase in the diameter of the inhibition zone up to 15% for GIC-NHAp. The 15% NHAp-GIC showed the highest inhibition zone at all three incubation periods for both strains. The appearance of inhibition zones surrounding the cement samples is linked to the infiltration of nanoparticles into bacterial cells, which subsequently induces oxidative stress. This stress hampers bacterial growth and leads to bacterial cell death [44]. A previous study has shown similar results only for a 24-hour incubation period [43].

Shinonaga Y et al., investigated the antibacterial properties of HA-incorporated GIC. Their study reported an improvement in the antibacterial efficacy of GIC with the addition of HA compared to conventional GIC samples [45]. Furthermore, Pagano S et al., added 4% NHA into GIC and observed enhanced antibacterial activity against *S. mutans* bacteria [46]. To date, no study has been conducted on the antibacterial properties at 48 hours and 72 hours of incubation against *L. fermentum*.

Limitation(s)

The parameters examined in the study were evaluated in-vitro. However, the results would likely be different and more precise if evaluated in-vivo.

CONCLUSION(S)

The evaluation of setting time, compressive strength, microleakage and antibacterial properties by incorporating NHAp into GIC has shown promising results in improving these physical properties. However, an increase in setting time appears to be a drawback, as it increases with incorporation. The authors believe that the incorporation of NHAp can be a feasible way to optimise GIC. However, further research is needed to address the prolonged setting time by making adjustments to the test compound. Additionally, more experiments on microleakage using different restoration techniques, such as incremental filling, as well as studies on the coefficient of linear thermal expansion and some in-vivo research are still necessary for clinical application.

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