

EVALUATION OF FLUORIDE ION RELEASE AND COLOR STABILITY OF NANO-HYDROXYAPATITE-SILICA ADDED GLASS IONOMER CEMENT FOR DENTAL APPLICATION

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ABSTRACT: The aim of this study was to synthesize nano-hydroxyapatite-silica (nano-HA-Si) particles, followed by the evaluation of fluoride ion releasing property and color stability of conventional glass ionomer cement (cGIC) with the addition of nano-hydroxyapatite-silica. Nano-HA-Si was synthesized by one-pot sol-gel technique. Experimental nano-hydroxyapatite-silica added GIC (nano-HA-Si-GIC) was prepared by adding 10% by weight ratio of nano-HA-Si to cGIC powder using manual mixing. Color measurements were measured according to Commission Internationale de l'Éclairage (CIE) L*a*b* system. Fluoride ion release was measured by fluoride ion selective electrode method. The 10% nano-HA-Si-GIC samples were successfully fabricated. Nano-HA-Si-GIC reported "slight - noticeable" change in color as compared to cGIC that displayed "noticeable to appreciable". Additionally, a highly significant increase in fluoride ion release was observed for nano-HA-Si-GIC samples as compared to cGIC ($p \leq 0.05$). Incorporation of nano-HA-Si into the formulation of cGIC enhances the physical properties of the glass ionomer cement and provides a better-sustained fluoride release. Nano-HA-Si-GIC has the ability to enhance the characteristics of glass ionomer dental restorative materials. Hence, it can be suggested as a potential future restorative material in dentistry.

Keywords: Colour stability; Fluoride release; Glass ionomer cement; Nano-hydroxyapatite.

1. INTRODUCTION

Dental caries is a sugar-dependent disease that damages tooth structure. It is the most prevalent disease worldwide and is considered the most important burden of oral health by WHO.¹ A number of studies have been done on the risk factors, etiology, preventive modalities, and treatment options involved in dental caries.²⁻⁷ In general, the management of caries using dental restorative treatment requires expensive materials, an electricity supply, specialized equipment, and highly trained dental health personnel.⁷ Consequently, these factors may limit the access of dental treatment to patients in underdeveloped regions.

Glass ionomer cement (GIC) is a very discrete direct restorative material. The main differentiating factor between GIC and other dental restorative materials is the chemistry. The chemistry and the acid-base chemical reaction by which it sets is the reason for its various clinical applications. GIC is a bio-compatible,⁸ translucent,⁹

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tooth-colored,¹⁰ self-adhesive to tooth,^{11,12} and anti-cariogenic,¹³ restorative material. Based on these properties, along with its tolerance to moisture, GIC is being used for many dental applications such as lining, luting, and filling.^{14,15} It is particularly utilized in Atraumatic Restorative Treatment (ART), where resources and facilities such as dental chair, hand drill, light curing machine, electricity and trained manpower are limited.¹⁶

Inside the oral cavity, the interaction between oral fluids and dental restorative materials is unavoidable. As a result of this interaction, there is fluid uptake by the resin-based dental restorations. This fluid uptake occurs as a result of a combination of absorption and adsorption processes. Absorption is the process by which a liquid molecule penetrates into a solid structure through diffusion.¹⁷ On the contrary, adsorption involves adherence of a liquid molecule to the external surface of the solid structure.¹⁷ The water uptake occurs mainly within the resin matrix, thus increasing the volume of the polymeric restorative material.¹⁸ The sorption process has an adverse effect on the restorative material. It affects bond strength, color stability, and physicomaterial properties, thus reducing the material's overall durability.¹⁹

Glass-ionomer cement is generally indicated as a material of choice to treat high caries-risk patients and hence will be in direct contact with the oral fluids.²⁰⁻²² Conventional glass ionomer cement (cGIC) has been shown to impede both initiation and progression of recurrent caries through its ability to release and recharge fluoride.²³ Direct dental restorative materials such as polyacid-modified resin composites, cGIC, resin-modified GIC (RMGIC), and pre-reacted glass-ionomers or giomers have different fluoride quantities and release rates.²³

Historically, cGICs are mechanically weak and thus are not indicated for restoring stress-bearing areas inside the oral cavity.^{20,24} Ideally, ART requires a material with enhanced mechanical properties and bonding ability. Various types of GICs have been purposely formulated for ART restorations. *In vitro* studies have compared the performance of cGICs to high-density GICs.^{20,24} In 2018, Moheet et al. evaluated the mechanical properties and shear bond strength of nano-hydroxyapatite-silica (nano-HA-Si) added GIC.²⁵ The authors reported an improvement in mechanical properties with the addition of nano-HA-Si to cGIC. This addition has resulted in an increase in Vickers hardness, compressive strength, and flexural strength of ~36%, ~19.7%, and ~53.4% respectively. Shear bond strength was also increased by ~17.34% in comparison to cGIC (Fuji IX GP).²⁵ These enhanced properties are suggested to be due to better packing of the nano-HA-Si crystals into glass ionomer cement matrix. Even though nano-HA-Si added GIC has demonstrated an improvement in mechanical properties, the data with regards to color stability and fluoride ion are yet to be reported. Hence, the current study was carried out to evaluate the effects on color stability and fluoride ion release of the addition of nano-HA-Si to cGIC.

Therefore, the objective of this study was to synthesize and characterize nano-HA-Si particles, followed by an assessment of the color stability and the fluoride ion release of the nano-HA-Si added GIC in comparison to cGIC.

2. MATERIALS AND METHODS

2.1. Materials: Commercialized glass ionomer cement (Fuji IX GP, GC International, Japan), in powder and liquid state, was used in this study. The rest of

the chemicals used were of analytical grade. The chemicals used in the current study were calcium hydroxide ($\geq 98\%$, RM Chemicals, India), phosphoric acid ($\geq 99\%$, Sigma-Aldrich, Germany), tetraethyl orthosilicate (TEOS, 99%, Fluka, Germany), ethanol (99%, System, Malaysia), ammonia (99%, Sigma-Aldrich, Germany) and total ionic strength buffer III (TISAB, Sigma-Aldrich, Germany).

2.2. Synthesis of nano-hydroxyapatite-silica: Nano-hydroxyapatite-silica powder was synthesized by a one-pot sol-gel technique.^{25,26} 7.408 g of calcium hydroxide was dissolved in 100 mL of distilled water. This suspension was mixed with a magnetic stirrer for 30 min. 4.104 mL of phosphoric acid was added drop-wise to calcium hydroxide suspension.²⁵ This suspension was stirred for 48 hr. Liquid ammonia was used to maintain the pH of the suspension between 11–12. A quantity of 20 mL TEOS was dissolved in 10 mL of absolute ethanol and was added drop-wise to calcium hydroxide suspension after 12 hr. After 48 hr, the sol produced was centrifuged (Eppendorf Centrifuge 5804, Germany) followed by freeze-drying (ScanVac CoolSafe, Denmark), and calcined (WiseTherm, Germany) at 600°C. The calcined powder was grounded manually using a mortar and pestle for 10 min.²⁵

2.3. Preparation of nano-hydroxyapatite-silica added GIC samples: Glass ionomer cement samples were hand mixed at 1:1 powder/liquid ratio following manufacturer's instructions. Nano-hydroxyapatite-silica-GIC (nano-HA-Si-GIC) samples were prepared following the same powder/liquid proportions. Nano-HA-Si powder was weighed and added to cGIC powder at a percentage by weight of 10%. The powder mixture of nano-HA-Si and cGIC was hand mixed by mortar and pestle for 10 min.²⁵ 40 specimens were prepared in total. Sample size for each group was 10 ($n = 10$). The details of the experimental groups for different investigations are shown in Table 1. The specimens were then tested for color stability and fluoride ion release.

Table 1. Testing groups with their corresponding sample size

Parameter	Sample size of groups	
	GIC Fuji IX (Group 1) (control group)	Nano-HA-Si-GIC (Group 2)
Color stability	10	10
Fluoride release	10	10
Total	20	20
Total for both group 1 and group 2	40	

*Indicates a significant difference between the GIC Fuji IX (control) group and the nano-HA-Si-GIC group ($p \leq 0.05$).

2.3.1. Color stability: Ten disc-like, plastic ring moulds measuring 10 mm in diameter and 2 mm thick (10 × 2 mm) were used for color stability analysis. The

moulds were filled with cement and covered by cellophane strip. A glass slide was placed on the top and bottom of the mould, and gentle hand pressure was applied for 30 sec to remove the excess material and air bubbles as well as to smooth the uncured cement. A baseline color measurement was recorded using a digital spectrophotometer (Vita Easyshade, Compact, Vita, Zahnfabrik, Bad Sackingen, Germany) before storing the samples in distilled water. The specimens were removed from the moulds after the material was set and kept in distilled water for 24 hr at $37\pm 1^\circ\text{C}$ in an incubator.

Color measurements were measured according to the Commission Internationale de l'Eclairage (CIE) $L^*a^*b^*$ system., where L^* represents the lightness (varying from white to black), and a^* and b^* represent the chroma (red to green and yellow to blue, respectively) of the samples. The CIE $L^*a^*b^*$ values of the samples were recorded against a white background to prevent any absorption effects. For each color measurement, three L^* , a^* and b^* values were taken from each sample and the mean of the values was recorded. The color measurements were acquired at different time intervals; after 7, 14, and 28 days of storage in distilled water at $37\pm 1^\circ\text{C}$. Before every color measurement interval, the digital spectrometer was calibrated according to the manufacturer's recommendations. The color change (ΔE) was calculated using the following formula:

$$\Delta E^* = \{(L_2^* - L_1^*) + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2\}^{1/2}$$

To quantify the results obtained for color changes the ΔE values were presented in reference to National Bureau of Standards (NBS) by applying the following formula:

$$\text{NBS} = \Delta E \times 0.92$$

2.3.2. Fluoride release: Ten stainless steel split moulds measuring 6 mm height and 4 mm in diameter (6×4 mm) were used to prepare the specimens for fluoride release assessment. The cement was poured into the metallic mould. The mould was covered by cellophane strip supported by glass slides on both sides. Gentle pressure was applied by hand to remove the excess material and air bubbles. The specimen pellets were allowed to set in room temperature before storing them in 5 mL of de-ionized water at $37\pm 1^\circ\text{C}$.

After 24 hr of storage, the vials were shaken properly and the water was removed from the vials and analyzed for fluoride concentration. The 5 mL elute was buffered with 1mL of TISAB III. The samples were then re-immersed in 5 mL of fresh de-ionized water. The measurements for fluoride release were recorded at different time intervals. The readings for fluoride concentration were taken each day for one week, followed by readings on days 14 and 28 of storage in distilled water at $37\pm 1^\circ\text{C}$. Fluoride concentrations for the two groups were measured with a fluoride ion specific electrode (ISE) using a digital ion analyzer (Orion™ Versa Star Pro™, Thermo Scientific™, USA). The electrode was recalibrated after every 10 measurements with standard solutions of sodium fluoride (NaF) with 1 and 10 ppm F.

2.4 Statistical analysis: All the data were analyzed using SPSS version 23 (IBM Corp., USA). Repeated measure analysis of variance (ANOVA) and Bonferroni correction were used to determine the inter-group significant differences between nano-HA-Si-GIC and cGIC for color stability and fluoride ion release. A value of $p \leq 0.05$ was considered statistically significant.

3. RESULTS

3.1 Colour stability: Table 2, Figure 1, and Figure 2 show the ΔE values for cGIC and nano-HA-Si-GIC recorded at different time intervals.

Table 2. Mean and standard deviation of the ΔE value for various time intervals related to the previous time and the baseline (day 1). (NBS=National Bureau of Standards)

Color change (ΔE) in relation to the previous time					
Time interval	Groups				p value
	cGIC		Nano-HA-Si-GIC		
	ΔE	NBS	ΔE	NBS	
Day 1 – day 7 (ΔE_1)	2.4 ± 0.9	2.2	2.2 ± 0.6	1.9	0.501
Day 7 – day 14 (ΔE_2)	2.2 ± 1.5	2	1.9 ± 0.8	1.7	0.59
Day 14 – day 28 (ΔE_3)	2.7 ± 1.3	2.5	1.5 ± 0.5	1.4	0.016*
Color change (ΔE) in relation to the baseline (day 1)					
Time interval	Groups				p value
	cGIC		Nano-HA-Si-GIC		
	ΔE	NBS	ΔE	NBS	
Day 1 – day 7 (ΔE_4)	2.4 ± 0.9	2.2	2.2 ± 0.6	1.9	0.501
Day 1 – day 14 (ΔE_5)	2.6 ± 0.8	2.4	1.8 ± 0.8	1.6	0.04*
Day 1 – day 28 (ΔE_6)	3.6 ± 1.1	3.3	1.3 ± 0.8	1.2	0.000*

*Indicates a significant difference between the cGIC (GIC Fuji IX, control) group and the nano-HA-Si-GIC group ($p \leq 0.05$).

Color change of the tested materials were evaluated in relation to the previous time interval measurements and in comparison to baseline (day 1) measurements. Repeated measure ANOVA was used to find a significant difference between cGIC and nano-HA-Si-GIC for each ΔE recorded. Overall the ΔE values (ΔE_1 , ΔE_2 , and ΔE_3) for nano-HA-Si-GIC at every time interval were lower when compared to cGIC and the values for ΔE_3 (day 14 – day 28) for nano-HA-Si-GIC were found to be significantly lower compared to cGIC ($p \leq 0.05$). The NBS units for the cGIC corresponding to ΔE_1 , ΔE_2 , and ΔE_3 were all in the range of 2 – 2.5, signifying a noticeable change. Meanwhile, the NBS units suggestive of ΔE_1 and ΔE_2 for nano-

HA-Si-GIC were 1.7 and 1.9 showing noticeable change, while for ΔE_3 it was less than 1.5, which demonstrated slight color change.

As for baseline measurements, ΔE_4 , ΔE_5 , and ΔE_6 for nano-HA-Si-GIC were observed to be lower as compared to cGIC (Table 2). Significantly lower color change was observed at ΔE_5 (day 1 – day 14) and ΔE_6 (day 1 – day 28) for nano-HA-Si-GIC when compared to cGIC ($p \leq 0.05$). The NBS unit corresponding to ΔE_6 for cGIC was 3.3 indicative of appreciable change. On the contrary, the NBS unit for nano-HA-Si-GIC for ΔE_6 was 1.2 indicating a slight change that shows that nano-HA-Si-GIC was more color stable as compared to cGIC after 28 days of immersion in distilled water.

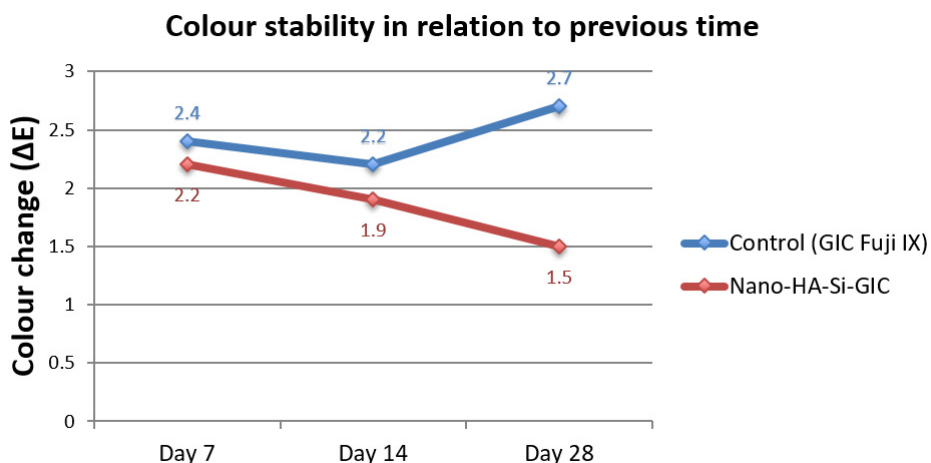


Figure 1. ΔE in relation to previous time measurements

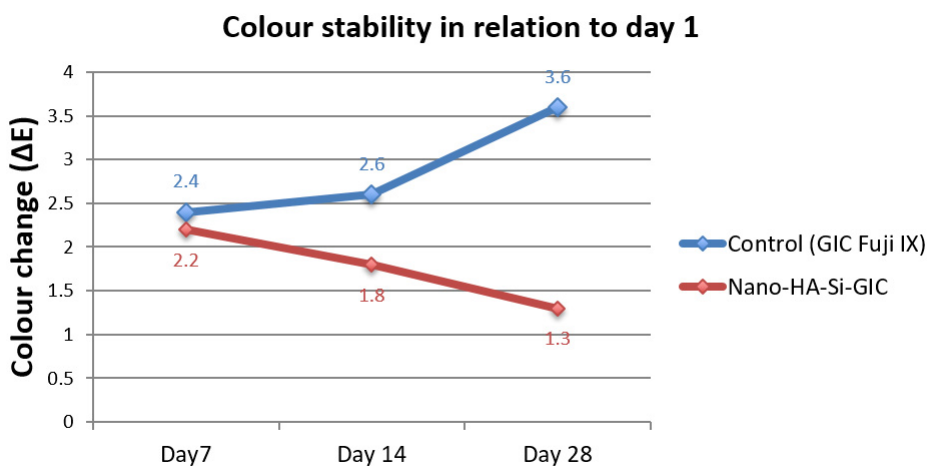


Figure 2. ΔE in relation to baseline (day 1) measurements

3.2 Fluoride release: Table 3 shows the mean (\pm SD) fluoride release daily from days 1, 2, 3, 4, 5, 6, 14, 21, and 28. A highly significant difference ($p=0.002$) in amount of mean fluoride release was observed from both the groups for all the time intervals. Both cGIC and nano-HA-Si-GIC showed an identical graphical pattern of fluoride release during the course of the study (Figure 3). Repeated measures ANOVA demonstrated that for each time interval, fluoride ion release for nano-HA-Si-GIC were highly significant when compared to fluoride ion release for cGIC.

Table 3. Mean and standard deviation of fluoride release for cGIC and nano-HA-Si-GIC

	Fluoride release (ppm)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 14	Day 21	Day 28	
cGIC	3.682 \pm 0.69	0.947 \pm 0.13	0.512 \pm 0.07	0.428 \pm 0.15	0.395 \pm 0.11	0.280 \pm 0.03	0.194 \pm 0.04	0.111 \pm 0.02	0.092 \pm 0.03	
Nano-HA-Si-GIC	4.692 \pm 0.54	1.475 \pm 0.17	0.897 \pm 0.15	0.832 \pm 0.07	0.826 \pm 0.09	0.527 \pm 0.09	0.398 \pm 0.05	0.301 \pm 0.06	0.216 \pm 0.06	
p value	0.002*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	

*Indicates a significant difference between the cGIC (GIC Fuji IX, control) group and the nano-HA-Si-GIC group ($p \leq 0.05$).

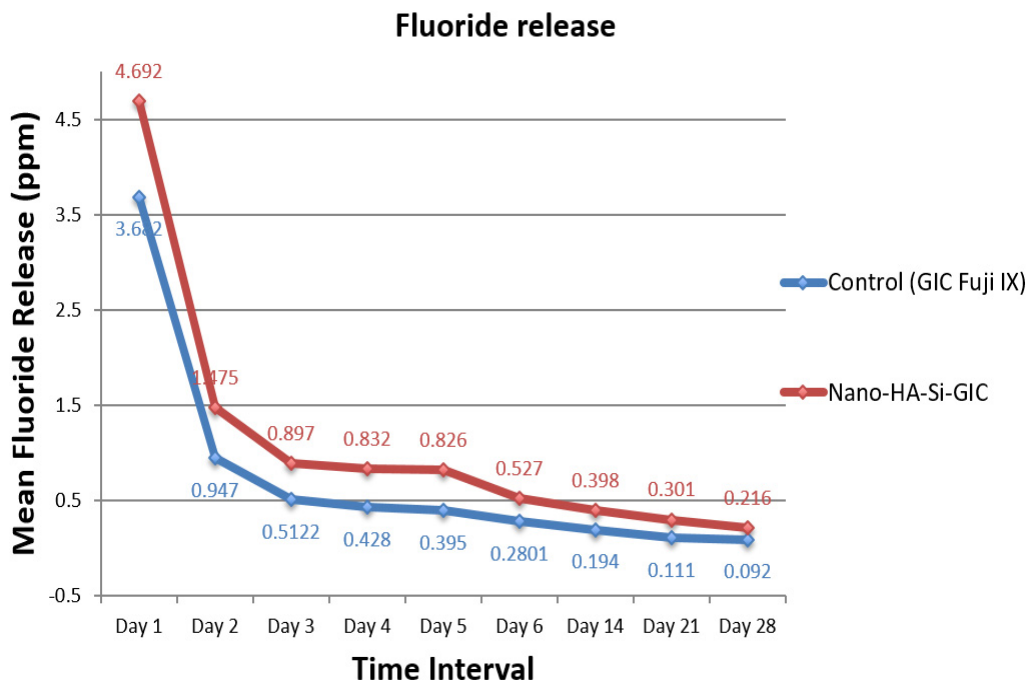


Figure 3. Fluoride ion release from cGIC and nano-HA-Si-GIC

The greatest fluoride ion release was recorded on day 1 ($p \leq 0.002$) for both nano-HA-Si-GIC (4.692 ± 0.54 ppm) and cGIC (3.682 ± 0.69 ppm). The lowest value for fluoride release, for both nano-HA-Si-GIC (0.216 ± 0.06 ppm) and cGIC (0.092 ± 0.03 ppm), was recorded on day 28 ($p=0.000$). Both materials presented with the classical fluoride ion release pattern, a higher fluoride ion release on day 1 that continued to decrease until reaching a plateau phase which continued until day 28 (Figure 3).

4. DISCUSSION

4.1 Color stability: Esthetic success is an imperative feature of a restorative material. In clinical dentistry, the long-term color stability of restorative materials is pivotal because poor esthetics not only lead to additional costs incurred by the replacement of restorations but also could lead to psycho-social problems.²⁷

Color is one of the fundamental properties of any aesthetic restoration. Matrix, filler composition, filler content, filler coupling agents, minor pigment addition, and initiation components are factors that may affect the color of cosmetic materials. The interaction between any of these factors may have a critical role in the color stability of the restorative material.²⁸

In the present study, nano-hydroxyapatite-silica-GIC was synthesized by addition of ten percent by weight of nano-hydroxyapatite-silica powder to the glass ionomer powder as this proportion of nano-hydroxyapatite-silica powder has led to the highest increase in mechanical properties and shear bond strength.²⁵ In the current study, the color change was evaluated using the CIELAB color system. The highest color change for nano-HA-Si-GIC was observed for ΔE_1 (2.2 ± 0.6). This result is in agreement with the findings reported by Prabharkar et al.²⁹ The author also reported a higher ΔE value for modified GIC on day 1. Conventional GIC also recorded a high color change for ΔE_1 (2.4 ± 0.9), higher than the nano-HA-Si-GIC. After ΔE_1 , cGIC generally recorded higher color change values (Figures 1 and 2). On the contrary, color change values for nano-HA-Si-GIC decreased signifying a color stable material (Figures 1 and 2). A limit of $\Delta E = 3.3$ has been taken as a clinically acceptable color change by many authors.³⁰ This study considered color change values (ΔE) ranging from 1.0 to 3.3 to be clinically acceptable. ΔE for both cGIC and nano-HA-Si-GIC were less than 3 which is in the clinically acceptable range and we can conclude that both materials have satisfactory color stability. Overall, the ΔE value for nano-HA-Si-GIC was lower than the cGIC indicating nano-HA-Si-GIC has better color stability compared to cGIC.

Based on NBS standards (Table 4) that evaluate the color change on the basis of visual perception, nano-hydroxyapatite-silica glass ionomer cement recorded very promising results. With regards to color change (ΔE) in relation to previous time measurement and baseline (day 1), nano-HA-Si-GIC displayed “noticeable - slight change” showing a downward trend, signifying stability in color change over a period of 28 days. On the contrary, cGIC (GIC Fuji IX) showed a “noticeable to appreciable” change in color, demonstrating that cGIC changes its color over the period of time.

Table 4. National Bureau of Standards system for color difference expression ³¹

NBS units	Critical remark on color differences
0.0 to <0.5	Trace: Excessively mere change
0.5 to <1.5	Slight: Slight change
1.5 to <3	Noticeable: Perceivable change
3 to <6	Appreciable: Marked change
6 to <12	Much: Excessively marked change
≥12	Very much: change to other color

4.2 Fluoride release: Fluoride is released as a result of the acid-base reaction in cGIC. This acid-base reaction is responsible for the initial burst of fluoride release from the material in which a high amount of fluoride is released to the surrounding environment.^{23,32,33} Fluoride release by a material is proportional to the concentration of fluoride present in the material.^{23,32} *In vitro* studies have reported that fluoride release declines rapidly during the first few days and within three to four weeks it stabilizes and reaches a plateau phase.^{33,34} Recurrent caries or carious lesions next to the restorations are likely to take several months to several years to develop depending on several factors. As a preventive measure to halt these lesions from developing, fluoride must be released over an extended period of time.

Different mediums like artificial saliva, lactic acid, and deionized water have been used to determine fluoride release for restorative materials.³² Since there are no existing ions in deionized water, the use of deionized water as a medium to determine fluoride release has been advocated by numerous investigators.^{35,36} Based on this fact, deionized water gives a precise estimate of the fluoride ions released from experimental material.³⁵

The current study demonstrated that nano-HA-Si-GIC released a significantly higher amount of fluoride ion compared to cGIC (GIC Fuji IX). The highest value of fluoride released for both cGIC and nano-HA-Si-GIC was recorded on day 1. There was a sharp decline of fluoride release from both materials on day 2 which then gradually decreased to a nearly constant level on day 28. This trend is an agreement with the results reported by in 2011 by Neelakantan et al., who demonstrated a similar fluoride release pattern from different glass ionomer products.³⁶ This display of initial rapid fluoride release may be explained as a result of the acid-base chemical reaction, which takes place on the surface of the glass particles and is more rapid in the first few hours of the setting time.³⁶ In the current study, the fluoride release from the nano-HA-Si-GIC at day 1 was recorded at 4.692 ± 0.54 ppm and the lowest value

recorded was 0.216 ± 0.06 ppm at day 28, which is significantly higher than the cGIC at both time intervals as shown in Figure 3. In 2016, Panigrahi et al. reported the highest value of 0.3319 ± 0.42 ppm from HA added GIC,³⁷ which is significantly lower than the fluoride release reported for the current study. It can be suggested that the nano-HA-Si particles provide a larger surface area, which might increase the overall acid-base reactivity of the cement, hence increasing the capacity of the cement to release fluoride from the surface of glass particles more rapidly. In another *in vitro* study, Tiwari and Nandlal reported a higher fluoride release for HA added GIC on day 1. However, fluoride release value on day 21 decreased to 0.0055 ± 0.0009 ppm³⁸ which is very low as compared to the value recorded for nano-HA-Si-GIC in the current study. In comparison, nano-HA-Si-GIC in the current study has shown better-sustained fluoride release until day 28.

5. CONCLUSION

Within the limitations of this *in vitro* study, the addition of nano-HA-Si to cGIC (Fuji IX GP) made the dental restorative material more color stable. In addition, fluoride ion releasing property was enhanced. ΔE recorded for nano-HA-Si-GIC was lower than cGIC for all time intervals. Overall ΔE for nano-HA-Si-GIC after 28 days of immersion in distilled water was less than 3, which falls in the clinically acceptable range. Additionally, nano-HA-Si-GIC reported a significant increase in fluoride ion releasing property on day 1, with a significantly higher and sustained fluoride release over a period of 28 days. In conclusion, the application of nano-HA-Si-GIC, in conjunction with the ART, may be an excellent alternative to cGIC (Fuji IX) in order to bring dentistry to the bottom billion as well as a conservative approach for the management of early childhood caries, because it can be used without the use of complex instrumentation.

DECLARATION OF INTERESTS

The authors declare that they have no conflict of interest.

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