Clinical performance of nano-hydroxyapatite-modified glass ionomer cements in class V cavities: split mouth, randomized controlled trial

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Abstract
Background: To evaluate the effect of modifying conventional glass ionomer (CGIC) and resin-modified glass ionomer (RMGIC) with nano-hydroxyapatite (Nano-HA) on their clinical performance as restorations for class V cavities.

Methods: The wet-chemical precipitation method was utilized to prepare the nano-hydroxyapatite particles. Nano-HA was then characterized using Scanning Electron Microscopy, Elemental Dispersive X-ray and thermal gravimetric analysis. Five wt% of the nano-hydroxyapatite (Nano-HA) was then incorporated into the powder portion of the CGIC and RMGIC. Sixty Class V cavities were prepared in thirty patients with at least two cervical caries lesions. Prepared cavities were restored using the tested materials. Modified United States Public Health Service (USPHS) criteria were used to evaluate the restorations at baseline, after three, six and nine months.

Results: Regarding the investigated modified USPHS criteria, the tested restorative materials showed no statistically significant difference throughout the study period. While color match, surface texture and marginal integrity criteria showed a statistically significant change in nano-HA-GIC. Surface texture and marginal integrity showed a significant change with CGIC. A significant change in surface texture was only found with nano-HA-RMGIC.

Conclusions: The investigated restorative materials showed an equivalent clinical performance at the nine months follow-up.

Keywords: Nano-hydroxyapatite, Modified USPHS criteria, Class V, Glass Ionomer

Background
Nowadays, a diversity of adhesive systems and tooth colored restorative materials are recommended as class V restorations. Resin composite (RC) or glass ionomer cements (GICs) are suggested as a restoration for these lesions. Though resin composite has been reported many problems when used to restore such cervical lesions, like marginal sealing insufficiency, polymerization shrinkage, and degradation of the tooth-resin interface with time (Boing et al. 2018).

Conventional and resin-modified glass ionomers have been recommended for class V restorations specially in patients categorized as high caries risk. The clinical performance of GICs was evaluated in a number of clinical trials in restoring cervical lesions, which showed an adequate clinical outcome (Priyadarshini et al. 2017; Mahn et al. 2015). GICs have exceptional properties as chemical bonding to the tooth substrates, perfect cavity walls...
sealing, remineralization capability due to fluoride discharge, and same elastic modules and coefficient of thermal expansion to the tooth structure (Singh et al. 2011).

In spite of GICs numerous benefits, unfortunately it has many shortcomings as low physical and mechanical characteristics, brittleness, low fracture toughness, poor wear resistance, imperfect surface characteristics, sensitivity to moisture as well as poor aesthetics in relation to resin composite restorations (Khoroushi and Keshani 2013).

Lately, studies have advocated integration of nanoclusters and/or nano-sized particles such as zinc, titanium dioxide, silver, gold and zirconia to enhance the low physical and mechanical properties of conventional GIC and resin-modified glass ionomer (RMGIC) (Najeeb et al. 2016; Khurshid et al. 2015). Najeeb et al. 2016 suggested that these nanoparticles enhanced the strength properties and decrease abrasion, they exhibited inferior aesthetics, cause possible risks and hazards effect which restrains their usage. Thus, chemical similarity of nano-hydroxyapatite particles (Nano-HA) to dental tissues are believed to be an excellent applicant as filler for both GICs and resin-modified glass ionomer (RMGICs) and are much chosen for clinical application (Kantharia et al. 2014).

Therefore, this study was conducted to evaluate the effect of nano-HA addition adding nano-HA on CGI and RMGII restorations clinical performance in class V prepared cavities at different time intervals. The null hypotheses studied were that there is a difference in CGIC and RMGIC clinical performance with or without nano-HA in class V cavities.

Methods

Trial design and setting

This double-blinded randomized, controlled, multicenter, split-mouth clinical trial with 1:1 allocation ratio was performed at the National Research Centre dental clinic, Egypt and at the restorative dental clinic, faculty of Oral and Dental Medicine, Girl’s branch, Al-Azhar University, Egypt. The experimental design was following the CONSORT 2010 guidelines (Consolidated Standards of Reporting Trial).

Trial registration and ethical approval

Registration of this clinical trial was done in Pan African Clinical Trial Registry (PACTR) (www.pactr.org) under the identification number (PACTR201908519911054). Ethical approval was gained from the Ethical Research Committee—National Research Centre (NRC), Cairo, Egypt (Ref number: 16039) and from the Faculty of Dental Medicine, Girls branch, Al-Azhar University, Cairo, Egypt (approval code: OPDEN-108-1-i). This clinical study was performed in agreement with the 1964 Declaration of Helsinki and its subsequent amendments.

Sample size calculation

The sample size estimation was calculated using a priori by G*power 3.1.2 software based on the previous studies (Jyothi et al. 2011; Medeiros et al. 2015). The minimum size of each group was calculated, following these input conditions into software: α (allowable type I error) 0.05 (5%), β (allowable type II error) 0.20 (20%), and power of the study (1-β) = 80%. The minimum sample size arrived 12 teeth per group. Yet the number was increased to a total 15 teeth in each group to allow for losses of around 25%. The final restorations were placed in 15 teeth per group which was obtained from 30 participants. Each participant received two class V restorations, totaling 60 dental restorations.

Eligibility criteria

Eligibility criteria for patients: Thirty participants with ages ranged from (25–45) years were selected to fulfil the following inclusion and exclusion criteria (Fagundes et al. 2014). The inclusion criteria for patients were: (a) Patient able to read and sign the informed consent form, (b) Cooperative patients who are willing to participate, (c) have no medical or behavioral problems preventing from attending recall treatments, and (d) patient with moderate and high caries risk index. The exclusion criteria were: (a) rampant uncontrolled caries, (b) parafunctional habit, bruxism or abnormal occlusion, (c) pregnant or breast feeding females, (d) Heavy smokers, (e) xerostomia, and (f) abnormal oral soft tissue.

Eligibility criteria for teeth: The inclusion criteria for teeth were: (a) presence of contra-lateral matched pair of cavitated buccal cervical carious lesion, (b) small to medium size cavities that extended into dentin, while maintain natural tooth contour, (c) Absence of tooth mobility, tenderness, extensive carious lesion, sever pain or pre-operative sensitivity, and (d) Easily accessible gingival margins during tooth restoration. While the exclusion criteria were: (a) teeth with periapical pathology, internal or external resorption, (b) questionable pulp vitality or have root canal therapy, (c) teeth have been pulp capped, (d) teeth with periodontal pocket and bleeding on probing, (e) cracked teeth.

Participants’ recruitment

The patients were selected from outpatient clinic of National Research Centre (NRC), Cairo; Egypt and Faculty of Dental Medicine, Girl’s branch, Al-Azhar University, Cairo; Egypt. Eligible patients who agreed to participate in this trial signed on written informed consents.
Patient risk assessment using American Dental Association® (ADA) caries risk assessment form (Ages > 6) were carried out for all the selected patients to be categorized into the medium and high risk category. Radiographical evaluation was also carried to dismiss any patient with any sign of periapical pathosis.

Randomization, allocation and blinding
Each participant had two contra-lateral class V carious lesions and received the experimental restorative material (Nano-HA-GIC or Nano-HA-RMGIC) in one cavity and the corresponding control restorative material (CGIC or RMGIC) in the other cavity. Thus, a total of 60 restorations were placed in the 60 teeth of 30 patients.

The method used to generate the random allocation sequence of the participants and arch side was a computer-generated list of random numbers using Microsoft® Excel program. Each participant occupied a sequence no (ID) from "1 to 30" to randomly assigned to one of the two control groups either CGIC or RMGIC. Each participant was then given another randomized letter either "R or L", to determine which side was to be restored with the control material and finally the other side was restored with the experimental material.

Generation of random allocation sequence was done by a person who was not involved in this clinical trial. Neither the participants nor the outcome assessor knew which tested restorative materials was used, while the primary investigator was not blinded, thus result in a double bind study.

Preparation and characterization of Nano-hydroxyapatite particles (Nano-HA)
The wet-chemical precipitation method was utilized to prepare the nano-hydroxyapatite particles (Nano-HA). Preparation was carried out at the National Research Centre, Egypt. Synthesized nano-HA was characterized using Scanning Electron Microscopy (SEM) (Philips XL, 30; Japan), Elemental Dispersive X-ray (EDX) (Model Quanta 250, FEI company, Netherlands) and thermal gravimetric analysis (Elmer thermogravimetric, USA).

The nano-HA were examined by SEM to investigate the morphology, particles size and porosity within the prepared nanoparticles. The samples were prepared by dispersing a thin layer of the powders on aluminum stubs and dried in vacuum. The dried powders were then sputter coated with gold to overcome the effect of samples charging in the electron beam. Finally, coated specimens were inspected under SEM at different magnification (2000 × & 80,000 ×) and H.V (20.000 kV). Representative SEM photomicrographs were selected from several images made of each specimen.

Energy-Dispersive X-ray (EDX) was used to detect mineral element composition (Ca/P) of the synthesized nano-HA, by estimation of calcium (Ca) and phosphate (P) weight content (%).

The thermal behavior of the prepared nano-HA powder was performed by Thermogravimetric (TG) and Differential thermal analysis (DTA) using thermal gravimetric analysis in a temperature range up to 1200°C.

Preparation and characterization of NHA-modified glass ionomer cements
CGIC (Fuji II, GC gold label 2) and RMGIC (Fuji II LC, improved, GC) were modified by replacing 5% by weight (5% w/w) of their powder component by the prepared nano-HA and then mixed using ball mill machine (LFJS, Hunan, China) with 200 rpm for 30 min to produce a homogenous mixed powder (Baranadehfar et al. 2016; Poorzandpoush et al. 2017). Materials brand name, manufacturers and their composition are presented in (Table 1).

Characterization of the modified cements (Nano-HA-GIC and Nano-HA-RMGIC) was carried out by SEM and TEM (Philips XL, 30; Japan) to examine the dispersal of nano-HA into CGIC and RMGIC powder.

Cavities preparation, materials application, intervention and outcome
Recording the participants’ demographic data and the teeth numbers, types and arch distribution was done (Table 3). Scaling and polishing of the teeth were done to all the participants to clean the teeth surfaces. Preoperatively, 1 mg dental local anesthesia (Mepecaine-L) had been injected. Field isolation was done using rubber dam (OptraDam® Plus) (Ivoclar Vivadent, size S & M AG; USA) and saliva ejector. Carbide bur #330 (FG, Dentsply Midwest®) fixed to a high-speed hand piece with water coolant system was used to prepare conservative class V cavities on the buccal surface of each tooth. Cavity preparation was restricted to only remove the caries tissue. A new bur was used after five cavity preparation (Nassar et al. 2014). A total of 60 cavities were prepared on 44 maxillary anterior teeth and 16 mandibular teeth.

After cavity preparation, Ketac™ dentin conditioner was applied for 10 s, then washed and gently dried with a cotton pellet. Control and experimental restorative materials were mixed according to manufacturer instructions and then packed into the prepared cavities on the contra-lateral side of each tooth.

Finishing and polishing procedures for CGIC and nano-HA-GIC/C restorations were carried out immediately after their initial setting, as recommended by the manufacturer’s instructions. One the other hand, RMGIC and nano-HA-RMGIC restorations were light cured for
20 s before finishing and polishing procedures. Finishing of the restorations were carried out using super fine diamond burs under copious water coolant and restorations were polished using a SoF-Lex abrasive disk.

A thin layer of EQUIA® Coat was finally coated on the restorations, then photo-polymerized 20 s. Patients were given oral hygiene instructions and the value of periodic follow-up and recall visits were emphasized.

The clinical performance of the tested restorative materials using modified (USPHS) criteria (Kharma et al. 2018) (Table 2) were assessed at baseline (immediately), after three, six and nine months interval. Restorations were assessed visually using mirror and probe under good operating light for all criteria. Assessment was done by single independent experienced examiner who was blinded to the aim of the study and tooth allocation. Figure 1, CONSORT flow diagram 2010, showed data about patient's enrollment, intervention, follow-up and data analysis.

### Statistical analysis
Qualitative data were presented as frequencies and percentages. Wilcoxon signed-rank test was used to compare between each group and its contra-lateral side. Friedman’s test was used to study the changes by time within each group. Chi-square test or Fisher’s Exact test (when applicable) were used to compare between the groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

### Results
**Synthesized Nano-HA characterization results**

*Scanning electron microscope (SEM) observation*

Figure 2 represents scanning electron microscope (SEM) photomicrographs for the prepared nano-HA at magnification 2000 × and 8000 ×. It shows that the synthesized HA has tendency to agglomerate. The shapes of the synthesized nanoparticles were almost rod shape with the same average size. Crystalline size estimated was (10–25) nm in width and (20–80) nm in length. Also, individual fine particles with cuboids and hexagonal shapes were observed.
Table 2  Primary outcome, measuring devices and measuring units for in-vivo study using modified (USBHS) criteria:

| Primary outcome               | Measuring devices                          | Measuring units                      |
|-------------------------------|--------------------------------------------|---------------------------------------|
| Retention                     | Visual inspection with mirror at 18 inches | Complete retention                    |
|                               |                                            | Mobilization of the restoration       |
|                               |                                            | Loss of the restoration               |
| Color match                   | Visual inspection with mirror at 18 inches | Perfectly matched color shade         |
|                               |                                            | Unperfected color match               |
|                               |                                            | Unacceptable color match              |
| Marginal integrity            | Visual inspection with explorer and mirror, if needed | Absence of discrepancy at probing |
|                               |                                            | Presence of discrepancy at probing without dentin exposure |
|                               |                                            | Probe penetrates in the discrepancy at probing with dentin exposure |
| Marginal discoloration        | Visual inspection with mirror at 18 inches | Absence of marginal discoloration     |
|                               |                                            | Presence of marginal discoloration limited and not extended |
|                               |                                            | Evident marginal discoloration penetrated toward the pulp chamber |
| Surface texture               | Visual inspection with explorer and mirror, if needed | Surface is not rough                  |
|                               |                                            | Surface is slightly rough             |
|                               |                                            | Surface is highly rough               |
| Surface staining              | Visual inspection with explorer and mirror, if needed | Surface is not staining               |
|                               |                                            | Surface is slightly staining          |
|                               |                                            | Surface is highly staining            |
| Postoperative sensitivity     | Ask the patient. "Questionnaire"         | Absence of hypersensitivity           |
|                               |                                            | Mild hypersensitivity                 |
|                               |                                            | Presence of strong and intolerable hypersensitivity |
| Secondary caries              | Visual inspection with explorer and mirror, if needed | No evidence of caries                 |
|                               |                                            | Evidence of caries along the margin of the restoration |

Energy dispersive X-ray (EDX)
Elemental analysis of nano-HA is shown in Fig. 3. The EDX spectra showed that the weight percentages (wt %) of phosphorous (P) and calcium (Ca) elements were 19.82% and 31.14%, respectively.

Thermal analysis (TG/DTA)
Figure 4 represents Thermogravimetric (TG) and Differential thermal analysis (DTA) graph of the prepared nano-HA. TGA curve showed slight slopping indicating thermal stability of the prepared nanoparticles. Heating up the nano-HA to 1200 °C, caused the material to undergo a 16% weight loss, which was attributed to CO₂ and water molecules desorption. Moreover, the changes in TGA resulted in changes in DTA as well as presence of endothermic peaks that could be owed to the breakdown of the residual CaCO₃ and removal of the hydroxyl group.

Results of SEM and TEM analysis for Nano-HA-modified glass ionomer cements
Figures 5 and 6 represent SEM and TEM photomicrographs of the prepared nano-HA-GIC and nano-HA-RMGIC powder, respectively. Nano-HA particles showed rod shape and were even distributed within the cements’ powder.

In vivo study results
Table 3 shows percentages (%), frequencies, and Chi-square test results for the comparisons of demographic data of (CGIC, nano-HA-GIC) & (RMGIC, nano-HA-RMGIC) groups. No statistically significant difference between age categories, gender distributions, marital status, arch and tooth types in the two groups were shown.

Tables 4, 5, 6, 7, 8, 9 and 10 present data for USPHS criteria evaluated for the four restorative materials.

All restorations in the four groups showed (Alpha) score at base line and after three months follow up period in all criteria of evaluated apart from postoperative sensitivity, surface texture, and color match.

There was one case dropped out (6.7%) in all groups at six and nine months follow up periods. No statistically significant difference was found between the four groups at each follow up period in all evaluation criteria.
In GIC group, a statistically significant change in the marginal integrity and surface was found throughout the period of the study. In nano-HA-GIC group, a statistically significant change in color match, marginal integrity, and surface texture was revealed throughout the different follow up periods.

On the other hand, RMGIC group showed a statistically significant change in color match and surface texture criteria, while nano-HA-RMGIC group, revealed a statistically significant change in surface texture criterion only through the study follow up periods.

A 100% overall cumulative survival rate was obtained, as the four tested restorative materials showed (Alpha) and (Bravo) scores which was considered success.

Discussion

In spite of all advances and modifications evolved for improvement of the poor mechanical properties associated with glass ionomer restorative materials, a significant improvement has not yet been approached. Thus, this randomized clinical trial was carried out to investigate the clinical performance of 5 wt% nano-HA-incorporated GIC and RMGIC cements in class V cavities at
different follow up periods using the modified USPHS criteria.

The reason behind the selection of nano-HA as an additive for the GICs were their verified bioactivity, biocompatibility, and low solubility in water (Pepla et al. 2014). Beside, their high colloidal stability and crystallinity (Khurshid et al. 2015).

The five percent by weight (5% w/w) of CGIC or RMGI powder that was replaced by nano-HA was chosen as it was recommended by previous studies (Basir et al. 2013; Bali et al. 2015; Barandehfard et al. 2016). They found a significant enhancement in the mechanical properties and the bond strength for the tested glass ionomer cements. Moreover, it was revealed that agglomeration of nanoparticles in the cement matrix
took place when percentage higher than 5 wt% of the nanoparticles was utilized. Resulting in decreasing the mechanical properties and the bond strength by acting as a weak points within the cement matrix (Basir et al. 2013).

Split mouth design (SMD) was selected for the current study, as it was considered a unique self-controlled randomized trial. SMD anatomically splits the oral cavity into left and right halves through the mid-sagittal plane, thus allow for easy evaluation and comparing of an experimental material on one side with its contra-lateral control one. Moreover, SMD eliminates variations in age, general systemic health and oral hygiene condition.
that might make the trial results less valid and unreliable (Pozos-Guillén et al. 2017).

Results of the clinical performance regarding the retention (Table 4), showed that all tested restorative materials either; control or experimental had excellent retention 93.3% (Alpha) rating at 9-m follow-up period, which agreed with further studies conducted by Franco et al. (2006), Jyothi et al. (2011) and Fagundes et al. (2014).

GICs ability for chemical adhesion to the tooth structure throughout the ionic bond formed between the tooth hydroxyapatite and the carboxyl groups of poly-alkenoic acid in GICs might be the reason for the high retention rate of these materials. In addition, RMGIC had added adhesion mechanism to the tooth structure through the micro-mechanical interlocking of its polymer, thus improving its retention rate (Shikumar et al. 2016).

Moreover, the modulus of elasticity of the tooth structure was close to that of the tested CGIC and RMGIC (Shikumar et al. 2016), leading to an increase in the restorations’ strain capability under occlusal loading thus avoiding its deformation and preserve adhesion to the cavity walls (Abdalla et al. 1997).

Results of the comparing the experimental cements (Nano-HA-GIC and Nano-HA-RMGIC) to their corresponding control cements (GIC and RMGIC) regarding the retention rates showed no statistically significant difference between them at the tested follow up periods. This might be related to the same main composition of the control and the experimental cements.

Regarding the color match criterion results (Table 5), no statistically significant difference was found between the tested restoration materials at different study periods. Only the color matching criterion showed a significant change in the nano-HA-GIC and RMGIC groups along the nine months study period. The prevalence of Bravo score was increased and the prevalence of Alpha score was decreased from: three to six months in

Table 3  Percentages (%), frequencies, and Chi-square test results for the comparison of the demographic data of the two tested main groups

|                | CGIC nano-HA-GIC (n = 15) patients | RMGIC nano-HA-RMGIC (n = 15) patients | P value |
|----------------|-----------------------------------|-------------------------------------|---------|
| Age            |                                   |                                     |         |
| 25–35 y        | 6 (40%)                           | 5 (33.3%)                           | 0.635   |
| 35–45 y        | 9 (60%)                           | 10 (66.7%)                          |         |
| Gender         |                                   |                                     |         |
| Male           | 7 (46.7)                          | 6 (40%)                             | 0.615   |
| Female         | 8 (53.3)                          | 9 (60%)                             |         |
| Marital status |                                   |                                     |         |
| Single         | 5 (33.3%)                         | 1 (6.7%)                            | 0.179   |
| Married        | 10 (66.7%)                        | 14 (93.3%)                          |         |
| Arch           |                                   |                                     |         |
| Upper          | 20 (66.7%)                        | 24 (80%)                            | 0.244   |
| Lower          | 10 (33.3%)                        | 6 (20%)                             |         |
| Tooth type     |                                   |                                     |         |
| Central incisor| 8 (26.7%)                         | 8 (26.7%)                           | 0.477   |
| Lateral incisor| 8 (26.7%)                         | 12 (40%)                            |         |
| Canine         | 14 (46.7%)                        | 10 (33.3%)                          |         |

*Significant at P ≤ 0.05

Table 4  Fisher’s exact test results and descriptive statistics for retention and recurrent caries criteria in the four groups

| Time       | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|------------|--------------|----------------------|----------------|------------------------|---------|-----------------|
|            | N | %   | N | %   | N | %   | N | %   |              |         |                 |
| Base line  | 15 | 100 | 15 | 100 | 15 | 100 | 15 | 100 | NC†         |         |                 |
| Alpha      | 15 | 100 | 15 | 100 | 15 | 100 | 15 | 100 | NC†         |         |                 |
| 3 months   | 15 | 100 | 15 | 100 | 15 | 100 | 15 | 100 | 1.000       | 0.000   |                 |
| 6 months   | 15 | 100 | 15 | 100 | 15 | 100 | 15 | 100 | 1.000       | 0.000   |                 |
| Alpha      | 14 | 93.3| 14 | 93.3| 14 | 93.3| 14 | 93.3| 1.000       | 0.000   |                 |
| Drop out   | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1.000       | 0.000   |                 |
| 9 months   | 14 | 93.3| 14 | 93.3| 14 | 93.3| 14 | 93.3| 1.000       | 0.000   |                 |
| Alpha      | 14 | 93.3| 14 | 93.3| 14 | 93.3| 14 | 93.3| 1.000       | 0.000   |                 |
| Drop out   | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1.000       | 0.000   |                 |
| P-value    | 0.392 | 0.392 | 0.392 | 0.392 | 0.392 | 0.392 | 0.392 | 0.392 |                 |         |                 |
| Effect size (v) | 0.067 | 0.067 | 0.067 | 0.067 | 0.067 | 0.067 | 0.067 | 0.067 |                 |         |                 |

*Significant at P ≤ 0.05

NC†: Not computed because the variable is constant
nano-HA-GIC and RMGIC groups, in addition to six to nine months in in nano-HA-GIC group. These results were in consistence with clinical trials of Sidhu (2010), Lee et al. (2010) and Priyadarshini et al. (2017).

Color matching deficiency of the tested material might be related to the "chalking phenomenon" of glass ionomers that makes its surface appear weak and opaque under dry conditions (Perdigão et al. 2012). Furthermore, the color stability of the tested materials could be

Table 5  Fisher's exact test results and descriptive statistics for comparison between color match in the four groups

| Time     | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|----------|--------------|----------------------|----------------|------------------------|---------|-----------------|
|          | N | %  | N | %  | N | %  | N | %  |                 |          |                 |
| Base line|   |    |   |    |   |    |   |    |                 | 0.969    | 0.118           |
| Alpha    | 12 | 80 | 12 | 80 | 11 | 73.3 | 11 | 73.3 |                 |          |                 |
| Bravo    | 3  | 20 | 2  | 13.3 | 3  | 20 | 4  | 26.7 |                 |          |                 |
| 3 months |   |    |   |    |   |    |   |    |                 | 1.000    | 0.070           |
| Alpha    | 12 | 80 | 12 | 80 | 11 | 73.3 | 11 | 73.3 |                 |          |                 |
| Bravo    | 3  | 20 | 3  | 20 | 4  | 26.7 | 4  | 26.7 |                 |          |                 |
| 6 months |   |    |   |    |   |    |   |    |                 | 1.000    | 0.048           |
| Alpha    | 11 | 73.3 | 10 | 66.7 | 10 | 66.7 | 10 | 66.7 |                 |          |                 |
| Bravo    | 3  | 20 | 4  | 26.7 | 4  | 26.7 | 4  | 26.7 |                 |          |                 |
| Drop out | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 |                 |          |                 |
| 9 months |   |    |   |    |   |    |   |    |                 | 1.000    | 0.052           |
| Alpha    | 10 | 66.7 | 9  | 60 | 10 | 66.7 | 9  | 60 |                 |          |                 |
| Bravo    | 4  | 26.7 | 5  | 33.3 | 4  | 26.7 | 5  | 33.3 |                 |          |                 |
| Drop out | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 |                 |          |                 |
| P-value  | 0.061 | 0.010* | 0.029* | 0.061 |        |        |        |        |                 |          |                 |
| Effect size (v) | 0.164 | 0.252 | 0.200 | 0.164 |        |        |        |        |                 |          |                 |

*Significant at P ≤ 0.05

Table 6  Fisher's exact test results and descriptive statistics for comparison between surface texture in the four groups

| Time     | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|----------|--------------|----------------------|----------------|------------------------|---------|-----------------|
|          | N | %  | N | %  | N | %  | N | %  |                 |          |                 |
| Base line|   |    |   |    |   |    |   |    |                 | NC†       |                 |
| Alpha    | 15 | 100 | 15 | 100 | 15 | 100 | 15 | 100 |                 | 1.000    | 0.225           |
| Bravo    | 0  | 0  | 14 | 93.3 | 15 | 100 | 15 | 100 |                 |          |                 |
| 3 months |   |    |   |    |   |    |   |    |                 | 0.997    | 0.084           |
| Alpha    | 12 | 80 | 11 | 73.3 | 11 | 73.3 | 10 | 66.7 |                 |          |                 |
| Bravo    | 2  | 13.3 | 3  | 20 | 3  | 20 | 4  | 26.7 |                 |          |                 |
| Drop out | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 |                 |          |                 |
| 6 months |   |    |   |    |   |    |   |    |                 | 0.992    | 0.085           |
| Alpha    | 10 | 66.7 | 10 | 66.7 | 9  | 60  | 8  | 53.3 |                 |          |                 |
| Bravo    | 4  | 26.7 | 4  | 26.7 | 5  | 33.3 | 6  | 40  |                 |          |                 |
| Drop out | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 | 1  | 6.7 |                 |          |                 |
| P-value  | 0.007* | 0.005* | 0.002* | 0.001* |        |        |        |        |                 |          |                 |
| Effect size (v) | 0.267 | 0.285 | 0.327 | 0.390 |        |        |        |        |                 |          |                 |

*Significant at P ≤ 0.05

NC†: Not Computed because the variable is constant
influenced by the existence of microcracks or porosity in the microstructure of the glass ionomers. This might lead to adsorption of stains from the oral fluid and subsequent restorations discoloration (Priyadarshini et al. 2017).

Moreover, degradation of the metal polycrylate salts content in GICs could be another reason for the poor color stability of the material. In the patients’ oral environment GICs could expose to acid attack where diffusion of H\(^+\) ions in GICs took place replacing the metal cations in its matrix. The release of these cations on the cement surface made the restorations rough with voids, leading to more water and food stains absorption (Cardoso et al. 2010).

Table 7  Fisher’s exact test results and descriptive statistics for comparison between surface staining in the four groups

| Time        | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|-------------|--------------|----------------------|----------------|-------------------------|---------|-----------------|
| N           | %            | N                    | %              | N                       |         |                 |
| Base line   |              |                       |                |                         |         |                 |
| Alpha       | 15           | 100                   | 15             | 100                     | 15      | 100             |
| 3 months    |              |                       |                |                         |         |                 |
| Alpha       | 15           | 100                   | 15             | 100                     | 15      | 100             |
| 6 months    |              |                       |                |                         |         |                 |
| Alpha       | 13           | 86.7                  | 14             | 93.3                    | 14      | 93.3            |
| Bravo       | 1            | 6.7                   | 0              | 0                       | 0       | 0               |
| Drop out    | 1            | 6.7                   | 1              | 6.7                     | 1       | 6.7             |
| 9 months    |              |                       |                |                         |         |                 |
| Alpha       | 13           | 86.7                  | 12             | 80                      | 14      | 93.3            |
| Bravo       | 1            | 6.7                   | 2              | 13.3                    | 0       | 0               |
| Drop out    | 1            | 6.7                   | 1              | 6.7                     | 1       | 6.7             |
| P-value     | 0.112        | 0.066                 | 0.392          | 0.112                   |         |                 |
| Effect size (v) | 0.133  | 0.160                 | 0.067          | 0.133                   |         |                 |

*Significant at $P \leq 0.05$
NC*: Not Computed because the variable is constant

Table 8  Fisher’s exact test results and descriptive statistics for comparison between marginal discoloration in the four groups

| Time        | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|-------------|--------------|----------------------|----------------|-------------------------|---------|-----------------|
| N           | %            | N                    | %              | N                       |         |                 |
| Base line   |              |                       |                |                         |         |                 |
| Alpha       | 15           | 100                   | 15             | 100                     | 15      | 100             |
| 3 months    |              |                       |                |                         |         |                 |
| Alpha       | 15           | 100                   | 15             | 100                     | 15      | 100             |
| 6 months    |              |                       |                |                         |         |                 |
| Alpha       | 13           | 86.7                  | 14             | 93.3                    | 14      | 93.3            |
| Bravo       | 1            | 6.7                   | 0              | 0                       | 0       | 0               |
| Drop out    | 1            | 6.7                   | 1              | 6.7                     | 1       | 6.7             |
| 9 months    |              |                       |                |                         |         |                 |
| Alpha       | 13           | 86.7                  | 12             | 80                      | 14      | 93.3            |
| Bravo       | 1            | 6.7                   | 2              | 13.3                    | 0       | 0               |
| Drop out    | 1            | 6.7                   | 1              | 6.7                     | 1       | 6.7             |
| P-value     | 0.112        | 0.066                 | 0.392          | 0.112                   |         |                 |
| Effect size (v) | 0.133  | 0.160                 | 0.067          | 0.133                   |         |                 |

*Significant at $P \leq 0.05$
NC*: Not Computed because the variable is constant
Additionally, the RMGIC undergoes post-polymerization color change. This is due to the retarded acid–base reaction during polymerization of the resin components of the cement (Adusumilli et al. 2016). Moreover, water sorption of their hydrophilic monomers, incomplete polymerization, and residual HEMA molecules after light activation were all more factors that could influence RMGIC color stability and raise its surface and body discoloration, according to Cardoso et al. (2010).

On the contrary, Hussainy et al. (2018) revealed that RMGIC "Fuji II LC, improved" showed Alpha rating (100%) and (95%) after six months and one year follow-up period, respectively. This result difference might be related to the selection of carious class V lesions in the current study in contrast to the non-carious cervical

### Table 9
Fisher’s exact test results and descriptive statistics for comparison between marginal integrity in the four groups

| Time      | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|-----------|--------------|----------------------|----------------|------------------------|---------|-----------------|
|           | N %          | N %                  | N %            | N %                    |         |                 |
| Base line |              |                      |                |                        |         |                 |
| Alpha     | 15 100       | 15 100               | 15 100         | 15 100                 | NC      |                 |
| 3 months  |              |                      |                |                        |         |                 |
| Alpha     | 15 100       | 15 100               | 15 100         | 15 100                 | NC      |                 |
| 6 months  |              |                      |                |                        |         |                 |
| Alpha     | 12 80        | 13 86.7              | 14 93.3        | 13 86.7                | 0.989   | 0.134           |
| Bravo     | 2 13.3       | 1 6.7                | 0 0            | 1 6.7                  |         |                 |
| Drop out  | 1 6.7        | 1 6.7                | 1 6.7          | 1 6.7                  |         |                 |
| 9 months  |              |                      |                |                        |         |                 |
| Alpha     | 10 66.7      | 11 73.3              | 13 86.7        | 12 80                  | 0.890   | 0.142           |
| Bravo     | 4 26.7       | 3 20                 | 1 6.7          | 2 13.3                 |         |                 |
| Drop out  | 1 6.7        | 1 6.7                | 1 6.7          | 1 6.7                  |         |                 |
| P-value   | 0.007*       | 0.024*               | 0.194          | 0.061                  |         |                 |
| Effect size (v) | 0.267 | 0.210 | 0.105 | 0.164 |

*Significant at P ≤ 0.05

NC*: Not computed because the variable is constant

### Table 10
Fisher’s Exact test results and Descriptive statistics for comparison between post-operative sensitivity in the four groups

| Time      | GIC (n = 15) | Nano-HA GIC (n = 15) | RMGIC (n = 15) | Nano-HA RMGIC (n = 15) | P-value | Effect size (v) |
|-----------|--------------|----------------------|----------------|------------------------|---------|-----------------|
|           | N %          | N %                  | N %            | N %                    |         |                 |
| Base line |              |                      |                |                        |         |                 |
| Alpha     | 14 93.3      | 15 100               | 12 80          | 14 93.3                | 0.306   | 0.261           |
| Bravo     | 1 6.7        | 0 0                  | 3 20           | 1 6.7                  |         |                 |
| 3 months  |              |                      |                |                        |         |                 |
| Alpha     | 15 100       | 15 100               | 15 100         | 15 100                 | NC      |                 |
| 6 months  |              |                      |                |                        |         |                 |
| Alpha     | 14 93.3      | 14 93.3              | 14 93.3        | 14 93.3                | 1.000   | 0.000           |
| Drop out  | 1 6.7        | 1 6.7                | 1 6.7          | 1 6.7                  |         |                 |
| 9 months  |              |                      |                |                        |         |                 |
| Alpha     | 14 93.3      | 14 93.3              | 14 93.3        | 14 93.3                | 1.000   | 0.000           |
| Drop out  | 1 6.7        | 1 6.7                | 1 6.7          | 1 6.7                  |         |                 |
| P-value   | 0.392        | 0.392                | 0.312          | 0.392                  |         |                 |
| Effect size (v) | 0.067 | 0.079 | 0.079 | 0.067 |

*Significant at P ≤ 0.05

NC*: Not computed because the variable is constant
lesions utilized in the studies conducted by Priyadarshini et al. (2017) and Hussainy et al. (2018). Deeper carious cavities were expected to increase restorations’ volume leading to increased water sorption rate and marked change in color as explained by de Oliveira et al. (2012).

Results of the tested restorative materials surface texture (Table 6) showed no significant difference between them throughout the study period. There was decrease in the quality of surface texture for all tested restorative materials from: base line to three, three to six as well as six to nine months. This was in accordance to previous studies conducted by Sooraparaju et al. (2014) and Hussainy et al. (2018).

Surface texture declined results might be related to the effect of the filler particle size and the microstructure of the tested materials on its abrasion resistance and physical characteristics. The tested RMGIC (Fuji II LC, improved) had large (4–5 µ) and irregular filler particles, that might negatively influence its surface roughness (Fagundes et al. 2014).

Additionally, this particles’ size was considered larger than the light wavelength (350–750 nm), thus scattering of light occurred producing opaque appearance (Konde et al. 2012). Additionally, the conventional GIC (Fuji II gold label 2) had bigger mean particles size, longer setting time and was more sensitive to water sorption compared to RMGIC; this could be the reason for the higher values of surface roughness of the CGIC (Pacifici et al. 2013).

Moreover, the utilized glass ionomer cements in the current study were in the form of “powder and liquid” to allow the incorporation of the nano-HA. This might lead to the incorporation of air bubbles during the manual mixing process, thus decreasing the degree of polymer conversion as a result of oxygen inhibitory effect on the setting reaction, resulting in rough surface that was retentive to plaque and stains (Priyadarshini et al. 2017).

In the current study a positive statistical correlation between color match and surface texture was found. That could be explained by increasing the restoration surface roughness, favors dental plaque and stains accumulation from the oral environment which result in surface gloss and color stability decline of the restorations. In addition, increasing the surface roughness lead to more random light reflection with consequent decline in color. That was in agreement with Pacifici et al. (2013) and Nassar et al. (2014).

Results revealed no statistically significant change regarding the surface staining criterion (Table 7) through the study period for all tested restorative materials at different study periods. Besides, no statistically significant difference was found between the four restorative materials through the 9-m follow up period. It could be explained by the short follow-up period tested in the current study, as surface staining is usually recorded after long period (Jyothi et al. 2011).

Regarding the marginal integrity (adaption) (Table 9), results revealed statistically significant changes in the marginal integrity throughout the study for CGIC and nano-HA-GIC groups. There was a rise in the prevalence of Bravo score and a decline in Alpha score from; three to six as well as six to nine months for these two groups. Simultaneously, no significant change in marginal integrity criterion for RMGIC and nano-HA -RMGIC groups through the study period were observed. This agreed with previous studies of Adusumilli et al. (2016) and Hussainy et al. (2018).

This difference in the marginal integrity results between CGIC and RMGIC might be due to more solubility of CGIC as a result of its sensitive to humidity in the early setting period. On the contrary, RMGIC was more resistance for moisture contamination due to its instant setting by resin polymerization, resulting in a better marginal adaptation of the evaluated restorations (Bapna et al. 2002).

Immediate finishing/polishing procedures that was done as per manufacturers’ instructions might be another cause for the inadequate marginal adaptation in CGIC groups. Consequently, this might have caused a significant deterioration in the marginal seal of the CGIC to the tooth structure, as the materials could have been still soft during their initial setting (Fagundes et al. 2014).

On the contrary to the current study results, Sidhu SK, 2010 observed inadequate marginal integrity of the RMGIC restorations. They owed these results to the hygroscopic expansion of the constitutes of glass ionomer, resulting in slight marginal fractures at the restoration-tooth interface.

The results of the marginal discoloration (Table 8), showed no statistically significant change in marginal discoloration throughout the study for all tested materials. This was in accordance with Shikumar et al. (2016) and Priyadarshini et al. (2017). Microleakage could be indicated by marginal discoloration due to gap formation at the cavo-surface margins. There was no marginal discoloration with CGIC and RMGIC restorations which could be a sign of good bonding of these materials to tooth structure as well as absence of microleakage (Jyothi et al. 2011).

Results of the current study revealed no secondary caries (Table 4) with all tested materials throughout the study. This might be due to the cariostatic action of GICs due to the fluoride release property that inhibit tooth demineralization (Fagundes et al. 2014; Dias et al. 2018).

Post-operative sensitivity results (Table 10) showed no statistically significant difference among all tested group along the study period. Some patients showed minor
Immediate hypersensitivity (at baseline) with RMGIC, GIC and nano-HA-RMGIC restorations. This finding was in accordance to Gurgan et al. (2015) and Hussainy et al. (2018). This baseline post-operative sensitivity could be due to mechanical irritation during the cavity preparation and restorations finishing and polishing procedures. Initial hypersensitivity was declined with time and vanished totally at the three months follow-up. Which might be related to the absence of marginal leakage that reduce the hydrostatic fluid movement inside the cut dentinal tubules (Hussainy et al. 2018).

Finally, the cumulative survival rate for the four tested materials at nine months follow-up interval was 100%. This was in agreement with other short-term studies of Yap et al. (2006) and Nassar et al. (2014) and long-term studies of Jyothi et al. (2011) and Fagundes et al. (2014) who utilized similar materials.

Finally, in the present study the null hypothesis was rejected, as the clinical performance of the modified nano-HA-GIC and nano-HA-RMGIC over the nine months study period was not statistically different from that of the conventional GIC and RMGIC.

Conclusions
Under limitations of the present clinical trial, it could be inferred that adding 5% nano-HA to CGIC and RMGIC had no beneficial effect on their clinical performance as restorations in class V cavities.

Recommendations
Long-term clinical studies are further required for better evaluation of the clinical performance of the modified nano-HA-GIC and nano-HA-RMGIC.

Abbreviations
RC: Resin composite; GICs: Glass ionomer cements; RMGIC: Resin-modified glass ionomer cement; USPHS: United States Public Health Service; NHA: Nano-hydroxyapatite particles; TEM: Transmission electron microscopy; FTIR: Fourier transform infrared spectroscopy; XRD: X-ray diffraction; EDX: Elemental dispersive X-ray; NHA-GIC: Nano-hydroxyapatite glass ionomer; NHA-RMGIC: Nano-hydroxyapatite resin-modified glass ionomer; hEMA: Hydroxyethyl-methacrylate; TEGDMA: Triethlène-glycol dimethacrylate; TMHMDCA: Trimethyl-hexamethylenediacarbonate; MMA: Methyl-methacrylate.

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Author contributions
ZMZ, MAN, MHZ and DWE performed the study design. AAH prepared the nano-AH. ZMZ performed the whole methodology. ZMZ, SMN, MAN, MHZ, AE and DWE analyzed the data. ZMZ and SMN were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The authors announce that the data supporting the results of this study are existing within the article.

Declarations

Ethics approval and consent to participate
The ethical approval was obtained from the Ethical Research Committee—National Research Centre (NRC), Cairo, Egypt (Ref number: 16039) and from the Faculty of Dental Medicine, Girls branch, Al-Azhar University, Cairo, Egypt (approval code: OPDEN-108-1-i).

Consent for publication
Consent for publication was obtained from the ethical committee and patients.

Competing interests
The authors declare that they have no competing interests.

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