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RESEARCH ARTICLE

The Role of Chicken Egg-Shell Nano-Hydroxyapatite as Fillers on the Surface Hardness of Glass Ionomer Cement

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Abstract **Background**: In the last years, glass ionomer cement (GIC) has become an adhesive restorative material used to treat dental caries. However, GIC has relatively low surface hardness compared to other restorative materials that limit its performance. This study aimed to investigate the role of nano-hydroxyapatite (nHA) as fillers produced from chicken-eggshells to improve the GIC surface hardness. **Materials and methods**: The study was a laboratory experiment employing a post-test-only control group design. Chicken-eggshell nHA was synthesized using the precipitation method. Surface hardness was investigated for 25 samples in five groups, four treatment groups and one control group, each with five samples. For each treatment sample, 3%, 5%, 7%, and 9% of chicken-eggshell nHA powders were added to the GIC powders. The GIC surface hardness was characterized using a Vickers Microhardness Tester. The obtained data were analyzed using a linear regression model. **Results**: The chicken-eggshell nHA had a mean size of 39.15 nm. Mean surface hardness after adding chicken-eggshell nHA was 70.21–79.27 HV, higher than that of GIC before the addition (61.86 HV). The treatment groups containing 7% and 9% chicken-eggshell nHA filler showed significant differences from control group ($p < 0.01$). Interestingly, mean GIC surface hardness increased significantly by 61% with an increasing concentration of chicken-eggshell nHA. **Conclusion**: The chicken-eggshell nHA filler increases GIC surface hardness.

Keywords: Dental Caries, Egg Shell, Glass Ionomer Cement, Hydroxyapatite, Hardness

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Introduction

Globally, 60%–93% of school-age children and most adults suffer from dental caries [1,2], an infectious disease characterized by multifactorial etiology and progressive demineralization that affects dental tissues [3]. During the last years, glass ionomer cement (GIC) is an adhesive restorative material used to treat dental caries [4]. In general, GIC is a promising dental restorative material owing to its low thermal coefficient, biocompatibility, effective chemical bonding to the tooth structure, and ability to constantly release fluoride, which enables it to prevent recurrent caries by converting hydroxyapatite (HA) in the enamel group to acid-resistant fluorapatite [5,6]. However, relatively low surface hardness compared with restorative materials limits its application, but the higher surface hardness improving its ability to withstand abrasion [7,8,9].

Several methods have been developed to improve the mechanical properties of GIC, including the

modification of GIC powders by the addition of fillers such as glass fiber, polymer synthesis, bioactive glass, and HA [$Ca_{10}(PO_4)_6(OH)_2$] [8,10]. HA is a biocompatible bioceramic with homogenous size, morphology, chemical composition, and crystallinity to dentin, which enables the enamel remineralization properties of HA [11]. Furthermore, HA improves the mechanical properties of GIC through ionic interaction between polyacrylic acid in GIC and apatite crystals in HA [12].

Fascinatingly, in the term of nanoparticles, Nano HAs (nHAs; < 100 nm) exhibit high surface activities with an excellent structure, similar to the minerals that construct bones and teeth. Many experts in nanomaterials sciences have developed several synthesis methods to produce nHA powders. The precipitation method becomes one of the famous and simplest methods to produce nHA in specific forms and sizes because it is inexpensive, requires relatively low temperature, and yields a high purity product [13]. Moreover, in order to cut off the synthesis cost, it is also essential to use alternative precursors from natural resources that easy and inexpensive to be found, such as chicken-eggshell.

Chicken-eggshell contains approximately 11% of the total egg weight with 94% calcium carbonate ($CaCO_3$), 1% calcium phosphate, 4% organic matter, and 1% magnesium carbonate [14,15]. Based on such composition, $CaCO_3$ in chicken-eggshell opens new potency to be utilized as a calcium source precursor in synthesizing nHA [16]. The addition of HA-silica nanocomposites increases the hardness of GIC, with the highest hardness achieved by the addition of 5% HA-silica and the addition of HA-silica at a higher percentage resulting in a decrease in hardness [17]. However, they tended to produce materials with expensive precursors. Furthermore, the addition of 5% HA from inexpensive precursors from chicken-eggshells synthesized by precipitation method could increase the surface hardness of GIC. However, they were not able to produce HA in nanometric size [18]. Therefore, in this study, we hypothesized that GIC with chicken-eggshells nHA fillers and GIC without nHA fillers tend to have different effects on the surface hardness.

Materials and methods

Study Design

This study was a laboratory experiment, employing a post-test-only control group design. For the surface hardness investigation, a total of 25 samples were prepared and divided into five groups (four treatment groups and one control group) comprising five samples each. The number of samples was calculated using Federer's formula: (t-)(n-1) ≥ 15 [19]. The control group was made of GIC powder (Fuji IX GP, Japan) without chicken-eggshell nHA powder (0%). The other groups contained 3%, 5%, 7%, and 9% of chicken-eggshell nHA as fillers.

Materials and tools

The materials used in this study were chicken egg shells (Chicken Farm Malang, Indonesia), GIC powder (Fuji IX GP, Japan), aquadest (Rein Pure Water, Indonesia), 68% nitric acid solution (HNO_3), phosphoric acid solution (H_3PO_4) 85%, and a solution of ammonium hydroxide (NH_4OH). While the tools used are divided into 3 kinds, namely: 1). The tool for making nano hydroxyapatite consists of a digital scale, oven (BINDER Red Line Series RE-53, USA), ball-milling machine (Polinema Mechanical Engineering Laboratory, Malang), 200 mesh sieve (Faculty of Agricultural Technology, Brawijaya University, Malang), furnace (Linn Electro Therm LM 412.05, Germany), burette, erlenmeyer tube, magnetic stirrer, pH meter, glass breaker. 2). The sample making tool consists of a cylindrical mold made of aluminum, linear shaker (Scilogex SK-L330-Pro, UK), glass pad, paper pad, celluloid strip, GIC spatula, plastic filling instrument, tweezers, caliper, load 0.5 kg. 3). The tool for characterization of nano hydroxyapatite consists of X-Ray Power Diffraction (PANalytical X'pert3 Powder, UK), Scanning Electron Microscope (HITACHI FLEXSEM 1000, Japan), Particle Size Analyzer (Zetasizer Nano S90, UK). 4). Surface Hardness Testing Equipment consists of Vickers Microhardness Tester (Mitutoyo, Japan).

Synthesis of chicken-eggshell nHa via the precipitation method

First, 200 g of chicken-eggshells were washed and cleaned with aquadest to remove the mucous membrane; then, the eggshells were dried in an oven at 105 °C for 2 h. Subsequently, the dried eggshells were mashed using the ball milling method for 6 h, and then sieved using a 200-mesh sieve to obtain a fine eggshell powder [18,20]. The resulting powder was calcined at 1000°C for 5 h to convert $CaCO_3$ to calcium oxide (CaO), which was used as the ingredient for preparing the calcium precursor (Ca). This reaction can be written as the following equation: $CaCO_3 \rightarrow CaO + CO_2$ [16].

To obtain 100 mL of calcium nitrate 10 M $Ca(NO_3)_2$ solution, which was used as the Ca precursor, 56 g of the as-prepared CaO powder was dissolved in 81 mL of 68 % nitric acid solution (HNO_3) and 19 mL

of Aquadest to a total volume of 100 mL. The mixture was stirred with a magnetic stirrer until the suspension was homogeneous. This reaction can be written as the following equation: $CaO + 2HNO_3 \rightarrow Ca(NO_3)_2 + H_2O$ [21].

Chicken-eggshell nHA was then synthesized using the precipitation method. Briefly, 100 mL of phosphate solution (H_3PO_4) was added dropwise using a burette into an Erlenmeyer flask containing 100 mL of the as-prepared $Ca(NO_3)_2$ solution. Then, the solution was heated at 40°C at a stirring speed of 300 rpm. After the phosphate solution had completely reacted, stirring continued without heating for 30 min. The solution was maintained at pH 10 by adding ammonium hydroxide (NH_4OH). After the precipitation process, the solution was placed in an incubator for 24 h for aging until the precipitate was obtained. The precipitate was filtered using Whatman Grade 42 filter paper and then washed with Aquadest to remove the ammonium nitrate residue. The precipitate was then dried in an oven at 105 °C for 5 h. Subsequently, sintering or second calcination was performed by inserting the dry precipitate into a furnace at 900 °C for 5 h to increase the degree of crystallinity. The obtained chicken-eggshell nHA powder was then sieved again using a 200-mesh sieve to produce the nHA powder. The reaction can be written as the following equation: $10Ca(NO_3)_2 + 6H_3PO_4 + 20NH_4OH \rightarrow Ca10(PO_4)_6(OH_2) + 20NH_4NO_3 + 18H_2O$ [16,21,22].

Characterization of chicken-eggshell nHa

The characteristics of the chicken-eggshell nHA powder were investigated by X-ray diffraction (XRD; PANalytical X'pert3 Powder, UK) to determine the HA phase contained in the as-synthesized chicken-eggshell nHA powder and matched with XRD standards from the Joint Committee on Powder Diffraction Standards (JCPDS). A nano-particle size analyzer (Zetasizer Nano S90, UK) was then used to determine the size of the chicken-eggshell nHA powder particles produced by measuring the particle size on a nanometer scale (nm). Scanning electron microscopy (SEM; HITACHI FlexSEM 1000, Japan) was performed to observe the morphology of the chicken-eggshell nHA powder. The SEM analysis was performed at magnifications of 20,000× and 25,000×. Energy-dispersive X-ray spectroscopy (EDX) was performed to identify elements contained in the nHA powder. The EDX analysis confirmed the presence of calcium (Ca), phosphate (P), and oxygen (O) in the nHA powder.

Synthesis of chicken-eggshell nHa fillers in GIC

The GIC powder (Type II) used in the control group was manipulated according to the instructions of the manufacturer (3.6 g of GIC powder and 1 g of GIC liquid without chicken-eggshell nHA). Then GIC powder and hydroxyapatite were mixed in an erlenmeyer tube using a linear shaker for 15 seconds.The mixed GIC was placed in a cylindrical mold with a diameter of 5 mm and a height of 2 mm. The surface of the GIC was covered with celluloid strips and a load of 0.5 kg was applied. After the specimen was hardened, the celluloid strips were discarded, and the specimen was removed from the mold. For the first treatment group, 3.5 g of GIC powder was mixed with 3% or 0.1 g of chicken eggshell nHA powder using a shaker for 15 s. Subsequently, the mixed powder was mixed with 1 g of the GIC liquid. The second treatment group was prepared with 3.4 g of GIC powder mixed with 5% or 0.2 g of chicken-eggshell nHA powder and 1 g of the GIC liquid. The third treatment group was prepared using 3.3 g of GIC powder mixed with 7% or 0.3 g of chicken eggshell nHA powder and 1 g of the GIC liquid. The fourth treatment group was prepared using 3.2 g of GIC powder mixed with 9% or 0.4 g of chicken eggshell nHA powder and 1 g of the GIC liquid.

GIC surface hardness characterization

The GIC surface hardness was tested using a Vickers Microhardness Tester (Mitutoyo, Japan) with a force of 0.05 Newton (N) in the rhombic indentation area. Each specimen's hardness was tested at three indentation points at different locations and diagonal lengths. The mean of the three indentation points was determined. The surface hardness value was calculated using the formula: VHN = 1854.4 P/d2.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and a Posthoc-Tukey test to determine the differences between the five sample groups. The correlation between the concentration of the chicken eggshell nHA powder and the surface hardness of GIC were analyzed by Pearson's correlation, and linear regression were used to predict GIC surface hardness following the addition of chicken-eggshell nHA as fillers.

Results and discussion

Results

The Nano-PSA test revealed that the average size of the chicken-eggshell nHA powder particles was 39.15 nm (Figure 1). The XRD analysis revealed that the chicken-eggshell nHA powder comprised pure HA phase, with diffraction peaks of apatite and calcium-phosphate hydroxide $Ca10(PO_4)_6(OH_2)$ matching those of the JCPDS (Figure 2). [13]

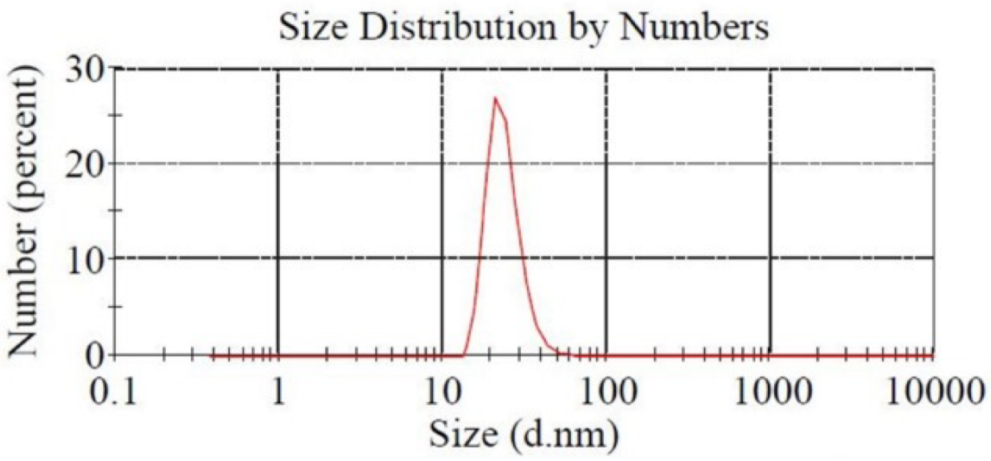


Figure 1. Nano-Particle Size Analyzer (Nano-PSA) results for the chicken-eggshell nano-hydroxyapatite (nHA) powder. The average size of the chicken-eggshell nHA powder is 39.15 nm.

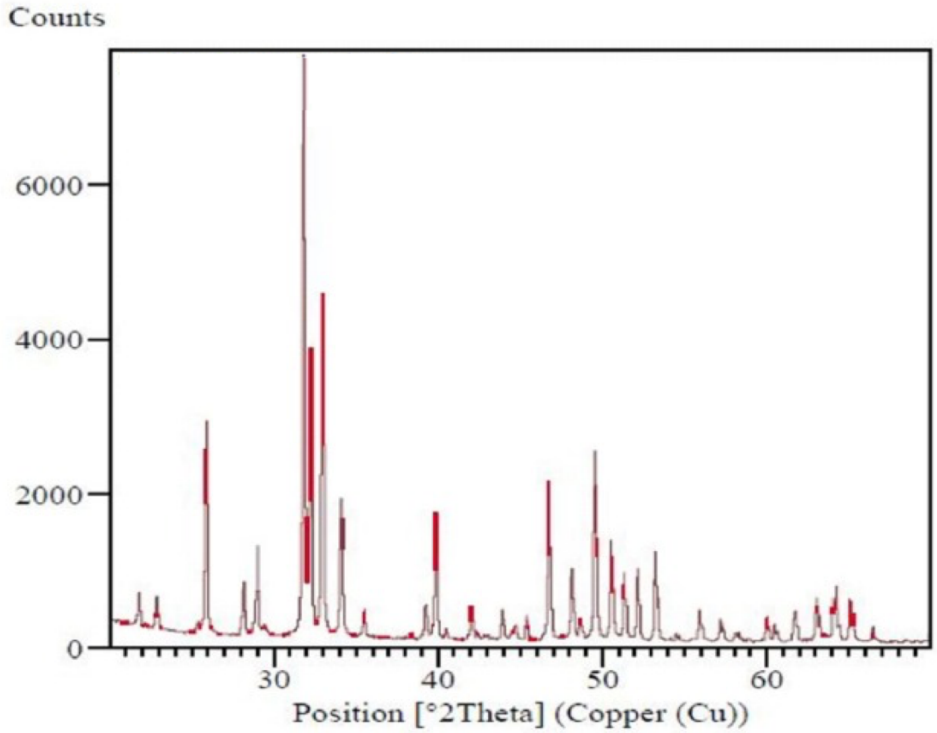
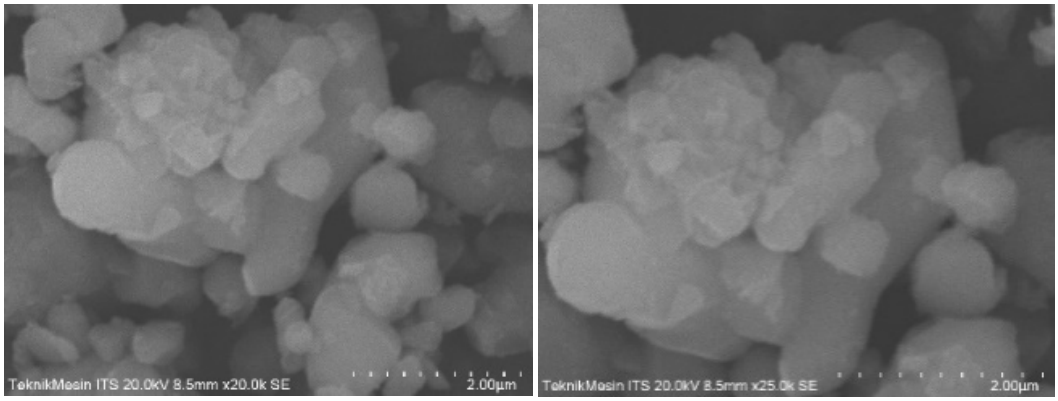


Figure 2. X-ray diffraction (XRD) result for the chicken-eggshell nano-hydroxyapatite (nHA) powder, showing 100% apatite or calcium-phosphate hydroxide [$Ca10(PO_4)_6(OH_2)$].



A B

Figure 3. SEM images of chicken-eggshell nHA at magnifications of (A) 20,000× and (B) 25,000×. Images indicate a powder size of 2 µm.

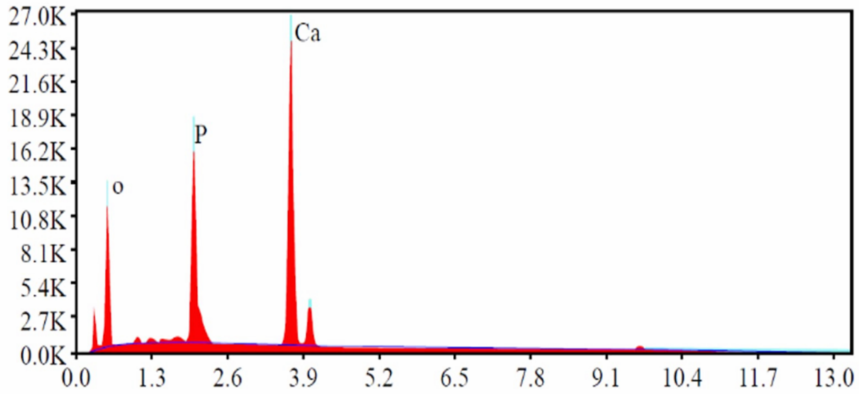


Figure 4. Energy-dispersive X-ray spectroscopy (EDX) of the chicken eggshell nano-hydroxyapatite (nHA).

The SEM images taken at magnifications of 20,000× and 25,000× revealed that the chicken-eggshell nHA powder particles were oval-shaped with a size of 2 µm. It is assumed that the shape of HA particles is strongly influenced by the CaO phase (Figure 3). The EDX analysis (map sum spectrum) revealed that the chicken-eggshell nHA powder was comprised of Ca, P, and O (Figure 4).

Table 1 EDX test results

Element	wt.%	at.%
Oxygen (O)	48.76	68.77
Phosphorus (P)	14.43	10.51
Calcium (Ca)	36.8	20.72
Matrix	Correction	ZAF

wt%: weight percentage; at%: atomic percentage

The percentage weight and atomic percent of the elements contained in the chicken-eggshell nHA are shown in Table 1. The EDX results were used to determine the Ca/P ratio of hydroxyapatite. The Ca/P ratio was obtained from the percentage weight of Calcium (wt%Ca) divided by the percentage weight of Phosphorus (wt%P). Table 1 shows that the Ca/P ratio is about 2.5, where the ideal Ca/P ratio for hydroxyapatite is 1.67. Furthermore, based on the Vickers Microhardness Test, the hardness values of the GIC samples prepared with a modified nHA composition varied: 3%, 5%, 7% and 9% are shown in Table 2.

Table 2 Vickers microhardness test results

Sample	Control Group (HV) GIC	Treatment Group (HV)			
		GIC + 3% nHA	GIC + 5% nHA	GIC + 7% nHA	GIC + 9% nHA
1	67.84	62.26	66.94	68.52	81.52
2	71.56	74.49	80.52	76.37	78.71
3	55.40	71.66	75.40	80.94	76.16
4	56.38	69.14	75.32	78.08	81.50
5	58.10	73.50	75.22	76.87	78.47
Mean	61.86	70.21	74.68	76.16	79.27

The one-way ANOVA test results (Figure 5) showed that the samples containing 3%, 5%, 7%, and 9% chicken-eggshell nHA filler showed no significant differences ($p > 0.05$). Samples containing 5% chicken-eggshell nHA filler showed significant difference from the control group ($p < 0.05$), samples containing 7% and 9% chicken-eggshell nHA filler showed significant difference from the control group ($p < 0.01$). However, samples containing 3% chicken-eggshell nHA filler showed no significant difference from the control group.

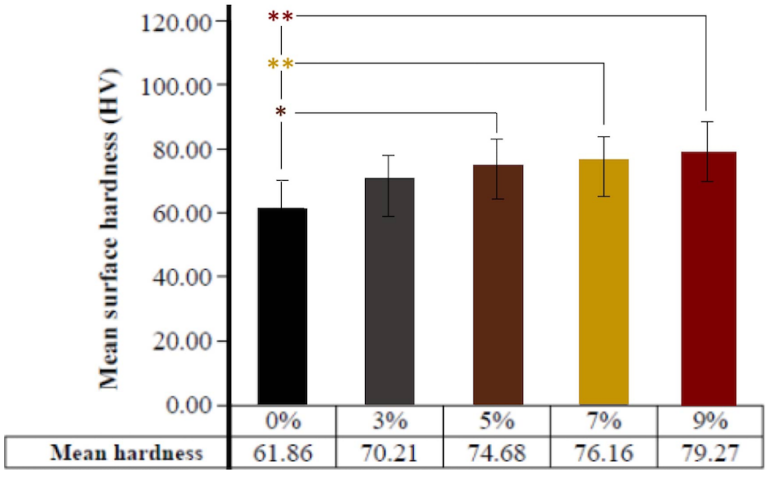


Figure 5. One-way analysis of variance (ANOVA) results for the mean hardness (HV) values of glass ionomer cement (GIC) containing 0%, 3%, 5%, 7%, and 9% chicken eggshell nano-hydroxyapatite (nHA) powder. * = $p < 0.05$; ** = $p < 0.01$.

We observed positive correlations between the concentration of the chicken eggshell nHA powder and the surface hardness of GIC (Table 3). Linear regression test results show an R square coefficient of 0.610, confirming that the addition of chicken eggshell nHA filler increased the surface hardness of the GIC by 61% (Figure 6).

Table 3 Correlations among chicken-eggshell nHA fillers concentration and surface hardness

Material test	Surface Hardness	
	R	*P*
GIC without nHA	r = 0.307 (weak)	*P* > 0.05
nHA 3%	r = 0.599 (strong enough)	*P* > 0.05
nHA 5%	r = 0.755 (strong)	*P* < 0.05
nHA 7%	r = 0.793 (strong)	*P* < 0.01
nHA 9%	r = 0.873 (strong)	*P* < 0.01

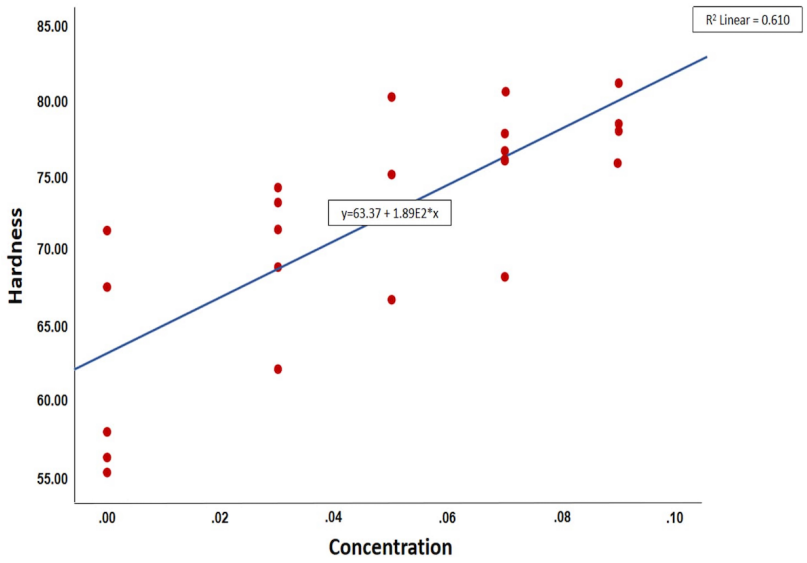


Figure 6. Linear regression of glass ionomer cement (GIC) hardness as a function of chicken-eggshell nano-hydroxyapatite (nHA) concentration.

Discussion

Our results confirm that chicken-eggshell nHA powders can be used as fillers in GIC powders to increase the surface hardness owing to the similarity between the composition of nHA and the primary minerals constructing bones and teeth [13]. The SEM and EDX results (chicken-eggshell nHA powder agglomerates with elliptical and irregular shapes, with constituent elements of Ca, P, and O) are consistent with the chemical composition and structure of apatite or $Ca10(PO_4)_6(OH_2)$. In addition, the Ca/P molar ratio was 2.5, which is higher than the ideal Ca/P molar ratio of HA (1.67) [23] indicating that

the Ca/P molar ratio of the chicken-eggshell nHA affected the strength of the synthesized chicken-eggshell nHA. The strength of the synthesized nHA increased with an increasing Ca/P molar ratio, and it reached a maximum Ca/P ratio of approximately 1.67 (HA stoichiometry). The strength is expected to decrease if the Ca/P molar ratio is greater than 1.67 [24] because calcium phosphate in the chicken-eggshell nHA powder may form other compounds such as calcium oxide (CaO).

The mean GIC surface hardness of the treatment group was significantly higher than that of the control group (Figure 5). However, there was no significant difference between the surface hardness values of samples containing 3% chicken-eggshell nHA filler (70.21 HV) and the samples of the control group (61.86 HV), indicating that the nHA content of the sample was low to induce any significant change. In contrast, there was a significant difference between the surface hardness of GIC samples containing 5% (74.86 HV), 7% (76.16 HV), and 9% (79.27 HV) chicken-eggshell nHA powder and that of the control group (61.86 HV), indicating that the surface hardness of GIC increased after the addition of 5% of chicken eggshell nHA (74.68 HV).

Based on Pearson's, a positive correlation was observed between the concentration of the chicken eggshell nHA powder and the surface hardness of GIC, wherein the hardness of GIC increased with an increase in the concentration of chicken-eggshell nHA powder (Table 3). The linear regression model predicted that the addition of the chicken-eggshell nHA powder increases the surface hardness of GIC by 61% (Figure 6). The remaining 39% can be attributed to the other materials that contribute to the surface hardness of GIC. This increase can be attributed to the formation of more salt bridges that form a cross-linking structure after the formation of the gel phase. With an increase in the concentration of the chicken-eggshell nHA filler in the GIC, the calcium content that can bind to GIC increases, allowing for the formation of more cross-linking structures. This makes the structure of the chain difficult to break, increasing the hardness [25].

The increase in the surface hardness of the GIC with the addition of 5% chicken eggshell nHA was also because the long-shaped HA particles filled the empty space between GIC particles and acted as a reinforcing material for GIC composition [17,26]. Moreover, previous studies have reported that the size and surface area of chicken-eggshell nHA powder significantly affects the mechanical properties of GIC [27]. In this study, the chicken-eggshell nHA powder had a small average size (39.15 nm), which enabled its even distribution in the GIC matrix, improving the mechanical properties of the GIC. In addition, the morphology of the chicken-eggshell nHA powder affected the hardness of the GIC. The SEM images revealed that the chicken-eggshell nHA powder particles had an oval shape. This is consistent with other studies that revealed that chicken eggshell nHA powder particles synthesized by the sol-gel method are oval-shaped and are embedded in the GIC matrix, improving the mechanical properties of GIC [17].

Conclusions

The addition of chicken-eggshell nHA as fillers into GIC powders significantly increased the surface hardness of the GIC. Correlation test results show that the mean hardness of GIC increased with an increase in the concentration of the chicken-eggshell nHA as fillers (positive correlation). In addition, a linear regression model predicted that the chicken eggshell nHA powder increased the hardness of GIC by 61%. The addition of 7%, and 9% chicken-eggshell nHA as fillers were the best concentration in increasing GIC surface hardness. The chicken-eggshell nHA as fillers tended to form an elliptical shape with a particle size of 39.15 nm. Furthermore, XRD results confirm that the nHA sample primarily comprised apatite or $Ca10(PO_4)_6(OH_2)$.

Conflicts of interest

The authors declare that they hold no competing interest.

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