INTRODUCTION

Dental caries represents a multifactorial chronic infectious disease. Tooth decay is a very common disease; although its prevalence has decreased in most developed countries, caries remains a major public health problem. Susceptible teeth, bacterial plaques, and the substrate are three main factors that determined the development of a caries lesion. A goal of modern dentistry is to manage non-cavitated carious lesions non-invasively in an attempt to prevent further disease progression and preserve the integrity of healthy tooth substrate.

The processes of demineralization and remineralization is regulated by the degree of saturation of the oral cavity (saliva and plaque) with apatite minerals. The development of a caries lesion starts when the saliva/plaque pH at the enamel surface reaches the critical value of 5.5 and the organic acid from cariogenic bacteria diffuses into the enamel. At low pH values, the phosphate group exhibits protonation, releasing calcium phosphate from the enamel’s surface and decreasing the enamel microhardness. Visually, the demineralized enamel appears as white spot lesions. These lesions can either develop into cavities or be remineralized. Given an appropriate change in conditions, remineralization can become the predominant process, thus leading to lesion repair. Remineralization of hard dental tissues is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel, producing a net mineral gain.

Hydroxyapatite (HA; (Ca₁₀(PO₄)₆(OH)₂), represents the calcium phosphate family, which is a major component of human bones and teeth and is generally added to medical procedures in orthopedics, dental, and maxillofacial applications. In the microscopic structure of tooth enamel, HA fills the fine pores of the tooth surface in almost the entire tooth enamel, with the result that teeth are not brittle. The development of restorative and preventive dentistry materials by adding HA is a currently attracting a great deal of attention. The addition of HA to teeth is expected to increase the remineralization process in teeth.

The effect of remineralization is expected to be more pronounced if the HA particle size can be reduced to smaller than micron size. With the introduction of nanotechnology, several researchers have tested the use of nanoparticles in restorative and preventive dentistry. One type of nanoparticles used in dentistry is nano-HA. Nano-HA is considered promising because of its similarity to bone and the mineral structure of teeth, as well as because of its biocompatibility, and bioactivity. In addition, nano-HA acts as filler by repairing small holes and depressions on the enamel surface — a function enhanced by the small size of the particles that compose it. The remineralization characteristics of nano-HA particles have been reported in studies in which nanoparticles were added to a glass ionomer or other restorative materials.

Various techniques have been developed to synthesize nano-HA, such as the sol-gel procedure.
precipitation from an aqueous solution\textsuperscript{[10,11,17-20]}, and hydrothermal\textsuperscript{[11,20]} and solid-state reactions\textsuperscript{[20]}.

In this study, the precipitation method was selected to synthesize nano-HA per several considerations. Specifically, nano-HA is synthesized without the use of organic solvents (at relatively low cost). This is a simple process with a large output (87%), making the method suitable for large-scale (i.e., industrial) production.

Nano-HA made by chemical synthesis is called synthetic nano-HA. Synthetic nano-HA can be obtained via the reaction of either synthetic or natural compounds that are high in calcium. Some such natural materials include cow bones, fish bones, cuttlefish, and mussel shells\textsuperscript{[10]}. In this study, abalone mussel shells (Haliotis asinina) from Indonesia were used as the natural compound for chemical synthesis; they are 90–95% calcium carbonate\textsuperscript{[26]}. Abalone mussel shells have been developed as the basic material to fabricate nano-HA. In previous research\textsuperscript{[25]}, it was found that abalone meat is a rare ingredient of traditional Chinese food and one of the necessary dishes for Chinese banquets because of its delicious taste and high nutritional values. However, thousands of tons of shells were found abandoned around a town. This resulted in a waste of natural resources and polluted the environment. In a contrasting case in Indonesia, the cultivation of abalone mussel shells has been carried out at the Center for Marine Cultivation Research and Fisheries Extension in Bali, Indonesia\textsuperscript{[26]}. The process of cultivating abalone mussel shells is applied for research needs; moreover, the shells are sold to craftspeople and the production of abalone shells is usually 200 kg/month.

Mouthwash, toothpaste, gum, and gel are common preparations as nano-HA carriers. A gel formulation was chosen in this study because it also increased the contact time between the active ingredients and the tooth enamel\textsuperscript{[19]}. The absorption process of a substance in the body is influenced by the preparation and the concentration of the materials. A high ion concentration increases the remineralization potential many times compared with saliva\textsuperscript{[19]}. In this study, carbomer-based gel preparations were used because they can easily flow and enter the tooth enamel surface.

In this study, nano-HA was synthesized via the co-precipitation method, using calcium carbonate (CaCO$_3$) from abalone mussel shells and a calcination temperature of 1,000°C to obtain calcium oxide; this approach was adopted following a previous study\textsuperscript{[27]}. The characteristics of nano-HA made from abalone mussel shells were observed. As mentioned at the beginning of the introduction, the addition of HA to teeth is expected to enhance the remineralization process. In addition, nano-HA acts as a filler by repairing small holes and depressions on the enamel surface. In this study, nanocomposite HA with carbomer-based gel is developed for the enamel remineralization process because it can release active ingredients and diffuse into tooth enamel tissue quickly. The synthesized nano-HA was mixed with the carbomer for the gel fabrication process, with concentrations of carbomer to nano-HA of 0, 10, 20, 30, and 40 wt%. The specimens used were 25 freshly extracted caries-free premolar teeth, following the inclusion criteria. The physicochemical properties of the gel HA-Abalone were characterized using scanning electron microscopy (SEM), X-ray diffractionmetry (XRD), and Fourier transform infrared spectroscopy (FTIR). Evaluation based on enamel remineralization parameters, including an enamel surface microhardness test, was performed using a Vickers microhardness (VHN) tester.

**MATERIALS AND METHODS**

The fabrication was divided into four main stages, which were as follows: synthesizing nano-HA from abalone mussel shells, fabrication of gel HA-Abalone, preparation of freshly extracted caries-free premolar teeth, and conducting the enamel remineralization procedure. The schematic methods for this study are shown in Fig. 1.

**Materials**

The abalone mussel shells used as a source of CaCO$_3$ were taken from Bali, Indonesia. The precursors of diammonium hydrogen phosphate ([NH$_4$]$_2$HPO$_4$)≥99.5% and ammonium hydroxide (NH$_4$OH) 25% solution were purchased from Merck (NJ, USA). The gel HA-Abalone was fabricated in the pharmacy laboratory of Universitas Islam Agung Semarang, Indonesia. Bovine calf serum 10% and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Penicillin-streptomycin, fungizone, and DMEM high-glucose medium were purchased from Gibco (New York, USA), whereas 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Biobasic (New York, USA), and dimethyl sulfoxide (DMSO) was purchased from Merck KGaA (Darmstadt, Germany). The specimens used were 25 freshly extracted caries-free premolar teeth that were removed for orthodontics reasons, following the inclusion criteria. These samples obtained clearance from the Human Ethics Review Committee of the Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Indonesia.

![Fig. 1 Schematic of methods to fabricate and characterize of nano-HA and gel HA-abalone, and the enamel remineralization procedure.](image-url)
Research (No. 264/B.1-KEPK/SA-FKG/1/2021). Written informed consent was obtained from all participants. Saline solution, glycerin, propylene glycol, and demineralization water were also obtained from the Faculty of Dentistry, Universitas Islam Sultan Agung, Indonesia.

Preparation of calcium oxide (CaO) from abalone mussel shells and synthesis of nano HA
The CaO and nano-HA were fabricated in previous research, so this study used those samples.

Fabrication of gel HA-Abalone
Fabrication of the gel was carried out using carbomer as acrylic acid polymers materials, with concentrations of carbomer to nano-HA of 0, 10, 20, 30, and 40 wt%. Nano-HA powder was dissolved in 100 mL of distilled water that had been heated to 50°C. Carbomer was added to the nano-HA powder solution and distilled water and stirred until homogeneous. Then, 10 mL of glycerin and 5 mL of propylene glycol were added to the nano-HA and carbomer solution mixture and stirred until the solution turned into a gel. The gel that formed was kept at room temperature for 24 h.

Preparation of freshly extracted caries-free premolar teeth
The specimens used were 25 freshly extracted caries-free premolar teeth that had been removed for orthodontics reasons, following the inclusion criteria. First premolars, also called bicuspid, are the permanent teeth located at upper jaw between first molars in the back of mouth and canine teeth (cuspids) in the front. They are transitional teeth, displaying some of the features of both canines and molars. They have two cups on the buccal and palatal parts, so they are called bicuspid. Caries-free premolar teeth can be selected via visual selection; such teeth have no white spots, no carious cavities, and attrition, abrasion, erosion, or enamel structure anomalies. The tooth cutting was done in the cementoenamel junction area with a diamond bur, so the crown was left intact. The cut teeth were planted in self-curing acrylic beams measuring of 2×2 cm. The surface of the tooth enamel was sanded using sandpaper (1,000 and 1,500 numbers). The surface thickness of the sample was 0.5 mm, and the samples were polished with a polishing tool bur with alumina coating until smooth, flat, and shiny. The border of the acrylic beam and the surface of the tooth enamel were stained with the red nail polish. The surface of the samples was rinsed in running water.

Enamel remineralization procedure
The specimens were randomly divided into the five following groups: gel HA-Abalone 0 wt%, gel HA-Abalone 10 wt%, gel HA-Abalone 20 wt%, gel HA-Abalone 30 wt%, and gel HA-Abalone 40 wt%. Each gel was applied to the tooth enamel surface for 10 min. The determined time of 10 min was the average time for a person to eat. The samples were rinsed with distilled water and soaked with saline solution for 10 min. Then, the samples were incubated for 10 min. At the same time, each gel was also applied to the tooth enamel surface for 10 min. The samples were rinsed with distilled water and soaked with demineralization water for 10 min. Then, the samples were incubated for 10 min. This treatment was done twice a day for 14 days.

Characterization of gel HA-Abalone and enamel surface
1. Morphology, particle grain size, and composition analysis
SEM (JSM-6510LA-1400, JOEL, Tokyo, Japan) was used to observe the morphology of the gel HA-Abalone. The particle grain size distribution of the gel HA-Abalone was calculated according to the measurements of 100 randomly selected particles using ImageJ software.

2. Crystallographic analysis
The crystallographic properties of the gel HA-Abalone were determined by XRD (PAN analytical Type X’Pert Pro, Tokyo, Japan). The XRD data were recorded in the range of 2θ: 10–80° using Cu-Kα radiation at λ=0.154 nm.

3. FTIR analysis
The analysis of the functional groups of the gel HA-Abalone were conducted using FTIR (Thermo Nicolet iS10, Tokyo, Japan). Separately, the powder and gel were ground and mixed with potassium bromide and then passed into compact tablets. The FTIR instrument was operated in the range of 400–1,000 cm⁻¹.

4. Enamel surface microhardness test
Evaluation based on enamel remineralization parameters, including an enamel surface microhardness test (measuring baseline, after demineralization, and after gel treatment), was conducted using a VHN tester (ASTM E92, Buehler, IL, USA) by evaluating the Vickers hardness number (VHN). The test on the 25 samples was set with a load of 100 gf. Data analysis was performed using one-way analysis of variance (ANOVA). A p-value less than 0.05 was considered statistically significant.

5. Cell viability assay of the gel HA-Abalone
1) Extraction solution of gel HA-Abalone
The gel HA-Abalone 20 wt% had the best results in terms of physicochemical properties, so it was used in the cell viability assay. An amount of 0.377 g of gel HA-Abalone 20 wt% was mixed with 94.2 mL of distilled water for analysis to reach a concentration of 2,000 µg/mL. The solution was then stirred at a temperature of 60°C at a velocity of 350 rpm until it turned into a homogeneous solution. It was sonicated at a temperature of 60°C for 1 h before the gel HA-Abalone solution was stored in the refrigerator.

2) Cell culture and seeding
Mouse fibroblast (NIH/3T3) cells were cultured in DMEM high-glucose (Gibco)+10% Bovine Calf Serum (Sigma) 2% Penicillin-Streptomycin (Gibco)+0.5% Fungizone (Gibco). The NIH/3T3 were seeded on the bottom of a
96-well plate at a density of \(2 \times 10^4\) cells/well. The cell was incubated at 37°C in 5% \(\text{CO}_2\) for 24 h. A 100 \(\mu\text{L}\) amount of scaffold solution was added to the cells. The cell seeded on the scaffold was incubated at 37°C in 5% \(\text{CO}_2\) for 24 h. Prior to cell seeding, the gel HA-Abalone 20 wt% solution was stored in the refrigerator.

3) MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

MTT assay was performed to measure the cell viability of NIH/3T3 cells and estimated through a color change phenomenon from yellow tetrazolium salt to purple formazan\(^{28,29}\). Cell viability was studied for an incubation period of 48 h. The measurement was taken for the gel HA-Abalone 20 wt% and a control (well without gel). Then, the medium was discarded, 100 \(\mu\text{L}\) of MTT solution with a concentration of 0.5 mg/mL was added to the well, and the gel was incubated for 4 h. Then, DMSO was added to the well at 100 \(\mu\text{L/well}\). The absorbance was recorded by Tecan Spark\(^{8}\) (Tecan Trading, Zurich, Switzerland) at 570 nm\(^{27}\). The cell viability was calculated using the following equation:

\[
\text{Cell Viability (\%) = } \frac{\text{absorbance of scaffold} - \text{absorbance of control media}}{\text{absorbance of control} - \text{absorbance of control media}} \times 100. \tag{2.1}
\]

Based on Eq. (2.1), cell viability was determined according to the absorption value of the test cultures, expressed as a percentage of absorption for unstimulated control cultures\(^{27,30}\). Then, the \(IC_{50}\) value was analyzed via nonlinear regression using GraphPad Prism software version 7 (GraphPad Software, CA, USA).

6. Statistical analysis

All enamel surface microhardness and cell viability assay data were presented as the mean±standard deviation (SD) and one-way ANOVA was used to analyze the obtained results, followed by Tukey’s test. \(p\)-Values<0.05 were considered statistically significant. These data were statistically analyzed using OriginPro software version 2018 (OriginLab, Northampton, MA, USA).

RESULTS

Nano-HA synthesis from abalone mussel shells

The psychochemical properties of nano-HA from abalone mussel shells were determined using SEM-EDS, XRD, and FTIR, as shown in Fig. 2. The results of these analyses have been studied in previous research\(^{27}\), where nano-HA based on abalone mussel shells had a Ca/P molar ratio of 1.67. The crystallite size, microstrain, and X-ray density of the synthesized nano-HA were 33.91±7.5 nm, 0.00373, and 10.46 g/cm\(^3\), respectively. The distance between the crystal planes of the nano-HA was determined using the Scherrer equation and calculated to be 2.81Å\(^27\). This result is close to the crystal
plane of the nano-HA at 2.88Å, making it appropriate by international standards (ISO 13779-3, ISO 13175-3) for HA implants\textsuperscript{10,31}).

Gel HA-Abalone

1. Morphology analysis

The morphology of the gel HA-Abalone is shown in Fig. 3 (a1–d1). Particle grain size distribution for all gel HA-Abalone at a size of ~500 nm is also shown by Fig. 3 (a2–c2) and Table 1. According to SEM results, gel HA-Abalone 10 wt% (Fig. 3a) had a form with large and uneven clumps. As the concentration increased, gel HA-Abalone with concentrations of 20, 30, and 40 wt% showed a small agglomerate shape and solid structure. The lumpy and even structure on the bottom and in the middle was presented in gel HA-Abalone 20 wt% (Fig. 3b) and gel HA-Abalone 30 wt% (Fig. 3c). As shown in Fig. 3d, gel HA-Abalone 40 wt% had granules with uniform grain morphology.

2. Crystallography analysis of gel HA-Abalone

The results of the XRD data (Fig. 4) show that the diffraction pattern formed was equivalent to the XRD pattern of HA. Determined using the Scherrer equation, the crystallite size and microstrain of the gel HA-Abalone with concentrations of 10, 20, 30, and 40 wt% can be seen in Table 2. This was supported by the decreasing microstrain of the gel HA-Abalone caused by the addition of the HA. According to these data, the addition of nano-HA caused the crystallite size of gel

Table 1  Particle grain size distribution analysis of gel HA-Abalone

| No | Gel with concentration variation | Particle grain size (nm) |
|----|---------------------------------|--------------------------|
| 1  | Gel HA-Abalone 10 wt%           | 502.12±13.07             |
| 2  | Gel HA-Abalone 20 wt%           | 487.60±13.35             |
| 3  | Gel HA-Abalone 30 wt%           | 498.68±12.65             |
| 4  | Gel HA-Abalone 40 wt%           | 574.00±27.62             |

Fig. 3  Morphology and particle grain size distribution of (a) gel HA-Abalone 10 wt%, (b) gel HA-Abalone 20 wt%, (c) gel HA-Abalone 30 wt%, and (d) gel HA-Abalone 40 wt%.

Fig. 4  XRD pattern of (a) gel HA-Abalone 0 wt%, (b) gel HA-Abalone 10 wt%, (c) gel HA-Abalone 20 wt%, (d) gel HA-Abalone 30 wt%, (e) gel HA-Abalone 40 wt%.
Table 2  Crystallography analysis of gel HA-Abalone

| No | Gel type               | Crystallite size (nm) | Microstrain |
|----|------------------------|-----------------------|-------------|
| 1  | Gel HA-Abalone 10 wt%  | 28.37±3.03            | 0.0045      |
| 2  | Gel HA-Abalone 20 wt%  | 14.70±1.21            | 0.0086      |
| 3  | Gel HA-Abalone 30 wt%  | 26.54±2.66            | 0.0048      |
| 4  | Gel HA-Abalone 40 wt%  | 31.65±3.40            | 0.0040      |

Fig. 5 FTIR spectra of (a) gel HA-Abalone 0 wt%, (b) gel HA-Abalone 10 wt%, (c) gel HA-Abalone 20 wt%, (d) gel HA-Abalone 30 wt%, and (e) gel HA-Abalone 40 wt%.

Fig. 6 Average enamel surface microhardness values after demineralization and after remineralization for 14 days ($p<0.05$).

Table 3  Average enamel surface microhardness

| No | Group                     | Mean±SD      | Mean±SD      | $p$-value |
|----|---------------------------|--------------|--------------|-----------|
|    |                           | After |
|    |                           | demineralization | remineralization |          |
| 1  | Gel HA Abalone 0 wt%      | 568.25±15.89 | 875.01±139.95 |           |
| 2  | Gel HA Abalone 10 wt%     | 580.63±26.59 | 855.13±75.03 |           |
| 3  | Gel HA Abalone 20 wt%     | 560.20±29.41 | 863.32±64.49 | 0.000     |
| 4  | Gel HA Abalone 30 wt%     | 564.64±32.58 | 838.81±113.92|           |
| 5  | Gel HA Abalone 40 wt%     | 580.92±16.44 | 855.05±89.34 |           |

HA-Abalone to increase.

3. FTIR analysis
The FTIR spectra data (Fig. 5) show that nano-HA without carborner material contained the functional groups of B-type CO$_3^{2-}$ at 1,476.66 cm$^{-1}$, and displayed PO$_4^{3-}$ absorption at 963.28, 1,020.31, and 1,085.81 cm$^{-1}$; the absorption band was attributed to the hydroxyl at 3,571.66 cm$^{-1}$. The gel HA-Abalone with concentrations of 10, 20, 30, and 40 wt% exhibited absorption of the functional groups of PO$_4^{3-}$ at 570–569 cm$^{-1}$ and 1,091–963 cm$^{-1}$ for all concentrations of gel HA-Abalone. On the spectrum of all variations in gel HA-Abalone preparation, the absorption band attributed to hydroxyl was observed at 631 cm$^{-1}$.

Enamel remineralization
As shown in Fig. 6 and Tables 3, 4, the increase in the enamel surface microhardness value of the free premolar teeth with the addition of the gel HA-Abalone 20 wt% was the highest compared with the other groups. The enamel surface microhardness value of
Table 4  Increase in enamel surface microhardness

| No | Group                | Increase in enamel surface microhardness (VHN) | p-value |
|----|----------------------|-----------------------------------------------|---------|
| 1  | Gel HA Abalone 0 wt% | 306.76±134.92                                 |         |
| 2  | Gel HA Abalone 10 wt%| 274.50±64.27                                  | 0.000   |
| 3  | Gel HA Abalone 20 wt%| 303.12±38.88                                  |         |
| 4  | Gel HA Abalone 30 wt%| 274.17±84.11                                  |         |
| 5  | Gel HA Abalone 40 wt%| 274.12±87.41                                  |         |

Fig. 7  (a) Cell viability of Gel HA-Abalone 20 wt% (*: p<0.05) and (b) the IC50 analysis of Gel HA-Abalone 20 wt% (log dose vs. response). The sample was incubated for 48 h.

Table 5  Average cell viability of gel HA-Abalone 20 wt%

| No | Concentration serial of gel HA-Abalone (µg/mL) | Cell Viability (%) | p-value |
|----|-----------------------------------------------|-------------------|---------|
| 1  | 31.25                                         | 102.99±2.73       |         |
| 2  | 62.5                                          | 101.63±8.19       |         |
| 3  | 125                                           | 93.17±19.11       |         |
| 4  | 250                                           | 92.55±7.20        | 0.000   |
| 5  | 500                                           | 87.68±6.63        |         |
| 6  | 1,000                                         | 66.68±6.37        |         |
| 7  | 2,000                                         | 43.14±9.08        |         |

1. Cell viability of Gel HA-Abalone 20 wt%
In this study, gel HA-Abalone 20 wt% was demonstrated to be the best gel because of its physicochemical characteristics (particle grain size distribution and crystallography properties) and enamel surface microhardness analysis. The cell viability assay study was carried out using MTT assay. The results of the MTT assay on gel HA-Abalone 20 wt% showed that all serial doses of scaffold concentrations to NIH/3T3 cells were safe. However, the growth of NIH/3T3 cells began
to be inhibited by gel HA-Abalone 20 wt% at a dose concentration of 1,000 µg/mL because the percentage of viability decreased to ~66%, as shown in Fig. 7a and Table 5. As demonstrated in Table 5 and according to the one-way ANOVA to determine the effect of concentration serial on the cell viability value, the p-value was 0.008 (p<0.05). This result reflected a significant difference in the average of cell viability value in the six groups. These results were also supported by the analysis of the IC50 value of NIH/3T3 in the gel HA-Abalone 20 wt% at 1,497 µg/mL, as shown in Fig. 7b.

The NIH/3T3 cells mostly clustered and formed several sub confluent structures to ~80%, as shown in Fig. 8a. The fibroblast cells had the morphology with cell-to-cell contacts and filopodia extension33. The morphology of NIH/3T3 cells attached to the gel HA-Abalone 20 wt% surface that had formed after 48 h of incubation is shown in Fig. 8b.

**DISCUSSION**

**Gel HA-Abalone**

The morphology of the gel HA-Abalone is shown in SEM results. Some fingerlike crystals were distributed on this gel in a disorderly manner (Fig. 3). According to the analysis of the SEM results using ImageJ software, as shown in inset of Fig. 3 and Table 1, the addition of a higher gel concentration tended to generate a gel HA-Abalone with a higher particle size distribution.

In the results of the XRD data (Fig. 4), the diffraction pattern formed was equivalent to the XRD pattern of HA. The carbomer gel formed a cross-link gel after a hydrolysis reaction with glycerin and propylene glycol. Based on the results, gel HA-Abalone may influence the deposition or absorption of nano-HA on the demineralized enamel surface during remineralization34,35.

In recent years, nano-HA particles have been widely studied as one of materials to reconstruct tooth enamel that is suffering from mineral loss and discussed as an effective anticaries agent because of its unique potential of remineralization. Some studies reported that a suspension containing 10 wt% nano-HA particles (10–20 nm in size) enhanced remineralization of the superficial layer in initial caries lesions to a depth of 20–40 µm. However, little remineralization was seen in the body of the lesion3,32. Based on XRD results, gel HA-Abalone 20 wt% was the best concentration to achieve the best remineralization of the superficial layer because the crystallite size of this sample was 1.470±1.21 nm.

FTIR spectroscopy was also used to observe the chemical changes during the fabrication process of gel HA-Abalone. The gel HA-Abalone with concentrations of 10, 20, 30, and 40 wt% exhibited the functional groups of carboxylic acids vibration at 3,419–3,381 cm⁻¹, and C=O was observed at 1,642–1,641 cm⁻¹ for all concentrations of gel HA-Abalone (Fig. 5). Based on the FTIR results, there was a significant difference in the peaks of the synthesized nano-HA and the gel HA-Abalone.

**Enamel remineralization**

As shown in Fig. 6 and according to the one-way ANOVA to determine the effect of gel HA-Abalone on the enamel surface microhardness value (Tables 3 and 4), the p-value was 0.000 (p<0.05). This result reflects a significant difference in the average of enamel surface microhardness value in the four groups. After the demineralization process (Fig. 6 and Table 4), there is a residual protein matrix, including an organic part making up of ~1% of the mature enamel, which is useful in the process of ionic conduction and deposition in interprismatic space nanogap34,35. The enamel protein located in the interprismatic space is capable for capturing mineral solutions and permitting penetration of minerals along the sides of these crystallites34,36,37. In addition, isomorphic and isionic exchange processes on the diffusion of calcium and phosphate into HA crystals via interprismatic space occur in enamel crystals. It is possible that these proteins act as ion exchange scaffolds in the enamel remineralization process.

This result is related to the ability of the gel in the remineralization process34,39. Thus, nano-HA particles in the gel can increase the penetration of the crystals through the enamel interprismatic space. The increase in enamel surface microhardness occurred because there were deposits of ion crystals, including calcium and phosphate, in the HA. Enamel mineral deposition could be affected by many factors, such as contact time, preparation, contamination, treatment procedure, storage, and laboratory transfer30. Overall, the enamel content of microelements, including contents of Ca and P in various layers of the enamel, microelement composition of intact enamel, and enamel covered with dental plaque, must be assessed using an electron probe micro-analyzer (EPMA) for further research.

The enamel surface microhardness value of the free premolar teeth tended to decrease with increasing gel HA-Abalone concentration, as shown in Table 5. This was caused by the application of demineralization water. It was used in this experiment as an alternative to artificial saliva to remove confounding factors, such as amino acids in salivary protein that could affect the natural enamel matrix protein of tooth specimens34,39. In addition, minerals in artificial saliva can affect confounding factors during the remineralization process,
because enamel deproteination causes a significant decrease in enamel characteristics\textsuperscript{34,39}.

Cell viability of gel HA-Abalone 20 wt%  
The mouse fibroblast NIH/3T3 cell line is one of the widely employed cell lines most sensitive to chemical-induced cytotoxicity in toxicity tests of dental materials\textsuperscript{40}. Cell-based cytototoxicity tests are a widely accepted methodology for testing dental materials’ cytotoxicity, with fibroblast cells being a convenient and reproducible model, as listed by ISO/EN10993-5\textsuperscript{41-43}. From the morphology of NIH/3T3 cells, it was evident that the gel HA-Abalone 20 wt% was cytocompatible with the fibroblast cell line.

CONCLUSION  
This study presents the successful fabrication of a nano-HA-based gel using carborber materials with concentrations of carborber to nano-HA of 0, 10, 20, 30, and 40 wt%. The specimens used were 25 freshly extracted caries-free premolars teeth. Gel HA-Abalone 20 wt% was the best concentration to achieve the best remineralization (∼863 VHN) of the superficial layer. Overall, the cell viability assay and morphology of the gel HA-Abalone 20 wt% showed that the gel could facilitate the attachment of NIH/3T3 cells to its surface. The increase in enamel surface microhardness occurred because there were deposits of ion crystals of HA. The enamel surface microhardness value of the free premolar teeth decreased with increasing gel HA-Abalone concentration.

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CONFLICT OF INTEREST  
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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