The effect of chitosan derived silver nanoparticles on mechanical properties, color stability of glass ionomer luting cements

Syed Saad Bin Qasim 1,2, Dena Ali 1, Mahmoud Shahat Soliman 3 and Gregor-Georg Zafiropoulos 4

1 Department of Bioclinical Sciences, Faculty of Dentistry, Kuwait University, Kuwait City, Kuwait
2 Department of General Dental Practice, Faculty of Dentistry, Kuwait University, Kuwait City, Kuwait
3 Nanotechnology Research Facility, Faculty of Engineering and Petroleum, College of Engineering and Petroleum, Kuwait University, Kuwait City, Kuwait
4 Department of Surgical Sciences, Faculty of Dentistry, Kuwait University, Kuwait City, Kuwait

E-mail: sayed.binqasim@ku.edu.kw

Keywords: glass-ionomer cement, silver nanoparticles, microhardness, compressive strength, color stability, nano-computerized tomography

Abstract
Glass ionomer cements (GIC) also known as polyalkenoate cements have been used as dental luting material for decades. However, there are certain limitations with respect to its biomechanical properties. Therefore, the aim of current investigation was to synthesize and characterize silver nanoparticles (SNP) using a green approach and incorporating the synthesized SNP in commercially available GIC formulation. Methodology. SNP were synthesized using a green approach of chemical reduction and analysed by spectroscopy and Transmission Electron microscopy. SNP reinforced GIC in 10, 30 and 50% formulations were assessed for microhardness, compressive strength, color stability and Nano-computerized tomography was used for pore analysis. Results. Results from UV–Vis spectroscopy, Dynamic Light Scattering, Zeta potential, Transmission Electron Microscopy and Fourier Transform Infrared Spectroscopy revealed that the particles were spherical and polydisperse in nature with an average diameter of 122 nm. The synthesized particles had a positive surface charge of 74 mV. Their incorporation into the Glass ionomer cement (GIC) revealed non-significant results on microhardness and compressive strength. Significant color change was observed and Nano-CT revealed pores within the set cements. Conclusion. Nevertheless, the biosynthesized silver nanoparticles have much broader clinical application and can be used to reinforce properties of existing dental biomaterials. They can be conveniently synthesized by the biogenic route adapted in the current investigation. However, their addition to the luting cement still warrants further in-depth investigation.

1. Introduction

The invention of glass ionomer cements (GIC’S) in the late 1970’s served as a ray of light for many clinicians since this was a more aesthetic alternative to dental amalgam [1]. At the time of GIC’s invention, there was a significant amount of publicity, however, in retrospect, it is very likely that there was premature marketing aforesaid conducting solid amount of detailed clinical testing [2]. Unfortunately, this material had several drawbacks in terms of its overall quality and clinical longevity. Some of the limitations are its low wear resistance [3] and low compressive and flexural strength [4]. Investigators across the globe explored potential alternatives and possibilities to overcome existing issues and venture into novel formulations for enhancing the existing formulations [5, 6]. In order to enhance the strength, resin reinforced GIC were formulated [7]. Advancements in the field of nanotechnology led to addition of glass carbomer cement as filler particles as well [8]. A more recent investigation explored the addition of lithium coated Bioglass® in order to trigger tertiary dentine formation [9]. Their use as luting cements to link the dental prosthesis with the tooth structure has also been investigated. Luting cements not only provide a gap-free interface they also should prevent microleakage and...
secondary caries [10]. The original formula was water based made up of a fluoroalumino-silicate glass, usually a strontium or calcium salt and a homopolymer of acrylic acid [11, 12]. The presence of fluoride within the GIC formulation was reported to have a minimal effect on the carious activity as reports have suggested that the released amounts are below that required for antimicrobial and antibacterial effects [13].

The development of oral biomaterials with antibacterial potential and enhanced clinical properties has been always an area of interest for researchers [14]. Oral biomaterials that possess the tendency to prevent secondary caries, prolong the clinical service of oral restorative materials [13, 15] and decrease bacterial viability [16] has been an area of a major exploration. A number of antibacterial agents have been incorporated into either experimental or commercially available restorative [17] and luting cements [10]. These range from using chlorhexidine [18], glutaraldehyde [19] and or quaternary ammonium methacrylate (QAM) [20]. Other agents that have been added in the form of nanoparticles are silver [15], zinc oxide, titanium dioxide and calcium phosphate [16]. A drawback of incorporating these agents is the short lived burst release effect [13]. Nevertheless, different strategies are being experimented to prolong the release profile and acquire a sustained release [21].

Amongst the previously mentioned biomaterials silver ion (Ag+) in the form of nanoparticles have been exploited in a number of ways to achieve antibacterial and antimicrobial effect [22]. It has a long standing history for its ability to trigger antibacterial effect from its earliest reports of use from Hippocrates’s for treatment of Ulcers [23]. Reports from the past have shown that commercially available silver nanoparticles (SNP) have had positive effects on glass ionomer cements however their mode of interaction has not been clearly elucidated [21]. The high surface area to volume ratio of SNP provides greater presence of atoms on the surface and aids in penetration of the cell membrane, thereby inhibiting the intracellular process and eventually resulting in a greater reactivity and antibacterial potential [23]. A number of synthesis routes have been reported in the literature to achieve different particle size, shape and other morphological characteristics. Therefore, the aim of current investigation was (1) to synthesize silver nanoparticles (SNP) using a green approach and incorporate them at different concentrations in a commercially available glass ionomer cement. (2) To evaluate the effect of the SNP on microhardness, compressive strength, and color stability of GIC and (3) to analyse the pores present in GIC using nano nano-computerized tomography. The null hypothesis of this study is that biogenic SNP incorporation into GIC does not affect the mechanical properties and color stability of GIC.

2. Materials and methodology

First part of the investigation involved SNP synthesis and the second part involved incorporation of the SNP in commercially available GIC.

2.1. Materials used in the study

Chitosan (Ch) (ChitoClear® Iceland), (Molecular weight 133,760Da (Degree of Deacetylation, 96.6%), Glacial Acetic acid and sodium hydroxide (NaOH) (Sigma Aldrich), Silver nitrate ( Riedel-deHaën GmbH), Glass ionomer cement (Ketaç™ CEM, Easymix, 3M, GmbH, Germany. Lot No 7422644).

2.2. Preparation of SNP

A 30 ml solution of 0.1% chitosan (100 mg) in 1% Acetic acid was prepared. The chitosan was added before the dropwise addition of acetic acid. The reaction was conducted at 40 °C for 2 h (hrs) then 3 ml of 0.1 M silver nitrate was added dropwise whilst the stirrer was mixing at 90 °C and the reaction flask was covered with aluminium foil. Then 4 ml of NaOH was added drop wise and the reaction was left for mixing at Room Temperature (RT) 25° ± 1 °C for 24 h. Changes in the color of the solution from clear to yellowish were indicative of the silver nanoparticle formation.

2.3. Characterization

2.3.1. Ultra violet visible (UV–vis) Spectroscopy

The absorbance of synthesized SNP was scanned using a UV–vis spectrophotometer (Eppendorf, Biospectrometer® basic). Blank chitosan was used as a background and synthesized silver was measured from 200 to 500 nm.

2.3.2. Zeta potential and dynamic light scattering (DLS)

In order to evaluate the stability of the nano-particle suspension, the Zeta potential (ζ) in volts was measured with a laser Doppler electrophoresis (LDE) instrument (Malvern Instrument Ltd, UK, Nano ZS, Red badge). The instrument is able to automatically calculate the electrophoretic mobility and zeta potential according to Smoluchowski’s equation [24].
The particle size distribution and size distribution (dispersity) were analysed with a laser dynamic light scattering (DLS) instrument (Zetasizer Nano Series, Malvern Worcestershire, UK). The analysis was conducted at 25 °C and was equipped with a Standard Red Helium Neon laser, 4.0 mW and 632.8 nm. The measurement was conducted at a fixed scattering angle of 173° then the sample was loaded into a quartz cuvette.

2.3.3. Inductively coupled plasma- optical emission spectrometry (ICP-OES)
The concentration of the silver particles was determined by using ICP-OES. Analysis was performed on Varian ICP-OES (710-ES). The samples were then digested and sample volumes were increased to 35 ml with deionised water. Next, samples were analysed with an ICP-OES instrument (Model Varian 710-ES USA) using a low matrix tune to determine the metal concentration.

2.3.4. Transmission electron microscopy
The size and shape of the synthesized silver nanoparticles was characterized using a Transmission Electron Microscope (TEM) at a magnification of 40 k to 100 k and at an accelerating voltage of 80 kV. The TEM samples were prepared by dropping 10 μl of the solution onto the copper grids using a micropipette. The copper grids were placed on a filter paper that was kept inside a plastic petri dish. The solution was allowed to air dry in a fume hood for 30 min prior to analysing. Once the grid was completely dry, it was handled by tweezers and placed onto the specimen holder for examination under the TEM.

2.3.5. Fourier transform infrared spectroscopy
Fourier transform infrared (FTIR) spectroscopy was conducted in attenuated total reflectance (ATR) mode (Bruker, Tensor 27) that was equipped with a diamond ATR crystal. Spectra were collected for neat Chitosan, Silver nitrate and Silver nanoparticles in the mid infrared region from 400 to 4000 cm⁻¹ at a resolution of 4 cm⁻¹ by acquiring 16 scans. The obtained spectra were processed in OPUS software (version 6.5).

2.4. Preparation of SNP incorporated GIC’s
SNP incorporated GICs were prepared by adding increasing concentration of SNP to the liquid component of a commercially available GIC. For preparing 10, 30 and 50% SNP incorporated GIC formulations, 10% SNP incorporated GICs were prepared by adding increasing concentration of SNP to the liquid component of a GIC. For preparing 10, 30 and 50% SNP incorporated GIC formulations, 10% SNP incorporated GICs were prepared by adding increasing concentration of SNP to the liquid component of a GIC. For preparing 10, 30 and 50% SNP incorporated GIC formulations, 10% SNP incorporated GICs were prepared by adding increasing concentration of SNP to the liquid component of a GIC.

2.4.1. Microhardness
A polymethylmethacrylate mould was used to prepare disc shaped specimens with a 10 mm diameter and 2 mm thickness in accordance with ISO 9917-1:2007 specifications [25]. Powder liquid were mixed on a paper pad with a metal spatula for 25–30 s and placed inside the mould. The mould was covered with glass slides that had a cellophane strip stuck on them. The glass slides were compressed and the material was allowed to set for 10 min. All specimens were prepared at RT in 70% relative humidity and stored in distilled water at 37 °C for 24 h before testing. Five-disc shaped specimens from each SNP incorporated GIC groups and control were collected for microhardness evaluation. The test was conducted using a digital microhardness tester with a load of 50 g for 20 s on the surface of the specimen with a Vickers diamond indentor (CV Instruments 400DAT/3) at RT. The average value of 20 points randomly selected on each sample were taken into account for further analysis. The diagonal lengths of the indentations were measured by an objective lens at 40×. Microhardness in g μm² was calculated from the equation:

\[HV = \frac{1854.4P}{d^2}\]

Where HV is the Vickers Hardness, P is the load set in grams (g) and d is the diagonal’s length in μm.

2.4.2. Compressive strength
Compressive strength of SNP incorporated GIC was conducted using an ElectroPuls System (E300 Instron, Noorwood, MA, USA) for all groups equipped with a load cell of 1000 N at cross head speed of 0.5 mm min⁻¹. Six specimens were tested for each group. Specimens were mixed in the ratios described previously and packed in custom made acrylic split moulds with internal dimensions of 6.0 ± 0.1 mm in height and 4.0 ± 0.1 mm in diameter. The samples were allowed to set for 10 min and then stored in distilled water for 24 h. Just before loading, samples were slightly dried using a damp filter paper and compressive load was applied along the long axis of the specimen. The maximum recorded force occurred at fracture was recorded and the compressive strength of each specimen was calculated in MPa as indicated in ISO 9917:2007.
2.4.3. Color stability

Ten disk shaped specimens were prepared for each group (Diameter of 10 ± 1 mm and thickness of 2 ± 1 mm) using the polymethylmethacrylate mould in accordance with the manufacturer’s instructions. No polishing procedure was conducted after the samples were cured and they were stored in at RT 25°C ± 1°C. The color measurement was conducted immediately after curing within 1 h (Day 0), 24 h (hrs), 4, 7 and 14 days using an Easy shade spectrophotometer (VITA Easysahde® V, GmbH & Co.KG Bad Säckingen, Germany) which calculates CIELAB (Commission Internationale de léclairage) values. The aim of the instrument is to detect the amount of light illuminated from an object and the amount of light reflected from it using a 2° observation and D65 illumination curve. The handheld probe has a 5 mm measurement area that emits light using one halogen lamp. Calibration of the probe is performed by placing the probe tip in the calibration probe built in the holder stand for the device.

Each specimen is measured by placing the probe tip in the middle of the specimen at a 90° angle. Three readings were taken from each specimen. The instrument is comprised of an approximately uniform color space with color co-ordinates for white-black ($L^*$), redness-greenness ($a^*$) and yellowness-blueness ($b^*$). $L^*$ values refers to the lightness of the object and its values varies from 0 (black) to 100 (white). The $a^*$ and $b^*$ values measure the redness, greenness, blueness or yellowness of an object. These values reach 0 for neutral colors and increase in magnitude for saturated or intense colors. The mean $L^*$, $a^*$ and $b^*$ values were calculated at day 0, 1, 4, 7, 14, and $L_0$, $a_0$, $b_0$ were values of day 0. The color difference ($\Delta E$) was calculated using the mean $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ values using the formula

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2}$$

The clinical acceptability values were based at $\Delta E > 3.3$. The operators were blinded at the time of analysing the specimens for microhardness, compressive strength and color stability. This was done in order to eliminate the possibility of bias.

2.4.4. Nano-computerized tomography

The samples prepared for microhardness analysis were scanned using a Phoenix Nanotom® m (Nano CT GE, USA). The images were collected at a final isotropic resolution of 4.44 μm per voxel at 100 kV accelerating voltage and 100 μA current with a 0.5 mm aluminium filter that was kept in front of the camera. The specimens were rotated at 360° about its vertical axis. The total scan time was 2 h acquiring a total of 2000 images. Images were reconstructed using VGS Studio max (Version 3.3). The data analysis for porosity measurements was carried out using the same software. The diameter of the pores (μm) were then transferred to GraphPad Prism (Version 8) and frequency distribution graphs were plotted respectively.

2.5. Statistical analysis

Unless otherwise stated all experimental analysis was done in triplicates. The data shown refers to mean ± standard deviation (SD). Statistically significant differences were analysed using one-way ANOVA, followed by a Tukey’s post hoc test. All statistical data were analysed using GraphPad Prism Software (Version 8.0).

3. Results and discussion

Several chemical synthesis techniques have been adapted to prepare silver nanoparticles. These include microwave assisted [26], chemical reduction, ultrasonic assisted reduction, electrochemical, template methods, photo-irradiation [27], microemulsion and biochemical reduction [28]. These techniques are either top down or bottom up, however; the biogenic synthesis is classified under the bottom up approach. The SNP synthesized by the current methodology has adapted a chemical reduction process whereby chitosan was used as a stabilizing agent whilst simultaneously acting as a reducing agent. Where by sodium hydroxide acted as an accelerator, which is similar to the methodology adapted by Darroudi et al [29]. Although the production of silver nanoparticles was a slow process as compared to other techniques [30]. The process was efficient enough to synthesize SNP’s. The conventional reducing agents commonly reported in the literature commonly include sodium borohydride, formalin, hydrazine, ascorbic acid and trisodium citrate [31]. However, these synthetic agents contradicted with the green synthesis process. Green synthesis have been regarded as environmentally friendly, less biohazard and decreased cost of production process [32]. Consequently, Chitosan plays a critical role in biocompatible stabilizing and hence controls the formation of well dispersed SNP as observed in the current synthesis technique. The color change from pale to dark yellow is considered as a common indication of the nanoparticle synthesis, which suggests bioreduction of silver ion metallic silver nanoparticles.
3.1 UV–vis spectroscopy, zeta potential and dynamic light scattering

The biofabricated chitosan derived SNP suspension was characterized by UV–vis and FTIR spectroscopy. Initially, the chitosan solutions color was pale yellow then it was transformed to yellowish brown, which is indicative of nanoparticle formation. The light absorption pattern by UV–vis showed a characteristic peak at 420 nm accompanied by broadening of the band (figure 1(a)). Another spectral analysis conducted after 4 months showed the stability of the synthesized SNP. The size distribution and zeta potential of the synthesized particles, shown in figures 1(b) and (c), were achieved to be nanosized. The particles appeared well dispersed in the chitosan matrix. The size distribution analysis conducted by DLS showed two strong peaks where 93.7% of the distribution was denoted to 122 nm and only 5.7% were in the range of 723 nm. The zeta potential observed was 74.1 mV (pH-5.01), shown in figure 1(c). The ICP-OES revealed that the synthesized SNP were 663ppm. According to Susilowati et al this is due to the surface plasmon resonance phenomenon of SNP [30, 33]. Metal surface such as plasma have been reported to have free electrons in the conduction band and a positive charge on the core. This phenomenon triggers the collective oscillation of the conduction band electrons and eventually absorbs in the UV–vis due to the interaction of the photons and electrons [30]. Furthermore the broadening of this UV–vis peak (420 nm) is indicative of the presence of SNP [34]. Dynamic light scattering is known to be a reliable methodology adapted to statistically determine the particle size distribution of nanoscale materials dispersed in a solution or colloidal suspension. The results showed that the polydispersity index (PDI) was 1 which was representative of the fact that the sample was polydisperse with multiple size population particles in nature (122 nm at 93.7%). The size achieved was not only that of the metal core but also the size of the biomaterials absorbed onto the SNP surface and the dual electrical layer that moves in between the particles. Therefore, the size is reliant on the materials in the colloid suspension of SNP. Eventually the size of the particles is larger than the other physical techniques like TEM. Zeta potential is used to determine the potential stability of SNP. Moreover, electrical charge prevent agglomeration on the surface of nanoparticles. Interestingly the zeta potential was 74.9 mV which indicates that the solution had excellent stability with a cationic charge on them [32, 35].

3.2 Transmission electron microscopy and FTIR spectroscopy

Transmission electron microscopy showed that the synthesized particles had spherical shape which is consistent with previous findings on SNP [32]. TEM images of the chitosan derived SNP are shown in figure 2(a). The spectral data acquired by FTIR for neat chitosan depicted characteristic peaks correlated to glycosidic linkages.
At 1068 and 1030 cm$^{-1}$ along with Amide I and II peaks (figure 1(b)). The SNP spectra showed a broad band designated to O–H stretching vibration at 3256 and 2935 cm$^{-1}$. There were two sharp peaks at 1566 and 1404 cm$^{-1}$ are assigned to CO–NH bending. Furthermore, the peaks at 1042 and 1012 cm$^{-1}$ were attributed to Table 1. Of Peak identification of chitosan and silver nanoparticles (SNP).

| Wavenumber (cm$^{-1}$) | Identification                                      | References             |
|------------------------|------------------------------------------------------|------------------------|
| SNP                    |                                                      |                        |
| 3256                   | Broad−OH stretching band                             | Akmaz et al [36]       |
| 1566                   | CO–NH bending, Amino groups of Amide (NH$_2$)        | Qasim et al [37]       |
| 1404                   | CH$_3$ wagging                                       |                        |
| 1042                   | C–O–C antisymmetrical stretching linkages of polysaccharide moieties | Dara et al [38] |
| 1012                   | P=O stretching vibration from phosphate groups       |                        |
| 922, 644, 618          | CH group and −OH out of plane deformation            |                        |
| Chitosan               |                                                      |                        |
| 3361                   | NH$_2$, −OH group stretching vibrations              | Rwegasila et al [39]  |
| 1581                   | −NH$_2$ bending in the Amine group                   | Qasim et al [37]       |
| 1377                   | CH$_3$, in the amide group, CH bending, CH stretch   |                        |
| 1330                   |                                                      |                        |
| 1151                   | C–O–C glycosidic linkages                            |                        |
| 1068                   |                                                      |                        |
| 1030                   |                                                      |                        |
| 895                    | CH deformation of the β-glycosidic bond              | Antonio et al [40]     |

(C–O–C) at 1068 and 1030 cm$^{-1}$. Along with Amide I and II peaks (figure 1(b)). The SNP spectra showed a broad band designated to O–H stretching vibration at 3256 and 2935 cm$^{-1}$. There were two sharp peaks at 1566 and 1404 cm$^{-1}$ are assigned to CO–NH bending. Furthermore, the peaks at 1042 and 1012 cm$^{-1}$ were attributed to...
C–O–C groups (table 1). Spectroscopic reports from the literature suggested that a shift in the 1657 cm$^{-1}$ in chitosan is due to the binding of the SNP to N–H bond of chitosan. Whereby the amino groups act as capping sites for the SNP stabilization [34, 36]. In other reports the shifts in the positions of the bands and reduction in intensity of bands are pointing towards effective electronic interaction amongst silver ions and nitrogen and oxygen containing biomolecules in chitosan. Moreover, this biopolymer is held responsible for nucleation process, aggregation, determination of size and distribution of metal nanoparticles as well [32].

### 3.3. Microhardness and compressive strength

Mean and SEM values of (VHN) in g μm$^{-2}$ are shown in figure 3 for control 0% and SNP modified GIC specimens over a period of 7 days. The data for surface microhardness shows a gradual increment in hardness with values reaching 79 g μm$^{-2}$. The immediate microhardness within 1 h after setting (Day 0) was highest for 30% (43.3 ± 1.02), whereas on day 1 after 24 h it was highest for 50% (71.5 ± 1.5).

Compressive strength of control and SNP modified GIC are shown in figure 4. After 24 h, the compressive strength of control specimens was 27 MPa whereas 0% SNP modified GIC showed a higher strength of 37 MPa. This was comparatively higher than 30 and 50% of the specimens. A pivotal physical characteristic of restorative materials is their surface hardness which correlates with compressive strength which, on the other hand, is regarded as an indicator of masticatory forces resistance [41]. Investigations performed by Pavia et al [42] and Jowkar et al [43] have shown that SNP addition improved the compressive strength, enhanced the microhardness and micro-shear strength to dentine [44]. The original GIC formulation is known to show a progressive increment in mechanical strength over the first 24 h. Moreover, the maturation and strength continue to increase over a period of 2 years. Therefore, there are concern about the effect of SNP on this setting mechanism [41]. Although there have been several reports on the compressive strength to be between 80–85 MPa and even higher. The compressive strength of hand mixed and machine mixed GIC luting cements differ significantly [45, 46]. Although the relationship in between mixing methods and biomaterial property is not a simplistic one. There is disparity in the relationship in between mixing method and properties between materials within the same generic groups and the same manufacturer [46]. Also, The reported values, by the manufacturer, of hand mixed GIC have eliminated the presence of air bubbles or porosities from the specimen during the mechanical tests’ procedures. Such ideal condition is not coherent with the actual realistic clinical situations of hand mixed GIC for luting different dental prosthesis like single unit crown to multiunit bridges.

The authors feel that the current values obtained along with the data obtained from nano-computerized tomography reveal the effect of pores on the overall compressive strength. In contrast to the previous studies, the current results observed from compressive strength evaluation revealed statistically significant effect on the samples reinforced with SNP. The compressive strength of the specimens reinforced with 10% SNP showed higher values than the control group. Which could possibly be indicative of some polymer interaction during the setting mechanism at 10% addition. El-Wassefy et al reported similar results however, they adapted...
commercially available silver nano-powder. An interesting finding is the fact that the particle size was less than 100 nm which is also very close to the particles acquired by chemical reduction of chitosan in the current investigation [41]. Although the SNP were added to the liquid component of GIC, it seems that they were not able to achieve chemical interaction and interference with the cement matrix thereby causing a non-significant effect on the mechanical properties. Furthermore, another possible reason to such findings may be attributed to the nano size which triggered its dispersion within and around the polymer chains.

3.4. Color stability
Results from the color stability measurements are shown in Table 2. ΔE values of the control specimens were comparatively lower to the values of test groups (10, 30 and 50%). However, there was a significant difference in color change noticed in 50% SNP incorporation test group when compared to the values of the 10% test group. The color change observed with higher percentages is a commonly observed issue biomaterials reinforced with silver compounds. This could lead to an unpleasant appearance if the biomaterial is intended to be used in restoring anterior teeth. Similar findings were also noted by Pani et al [47] and El-Wassefy et al [41].

3.5. Nano-computerized tomography
High resolution Nano-CT allows a greater three-dimensional spatial visualization. Nano-CT of dental resin composites performed by Haugen et al [48] revealed similar pores as observed in the GIC and SNP reinforced specimens in the current investigation. They attributed the presence of these pores to have a detrimental effect on the longevity of such restorative materials. Moreover, these pores could also affect the mechanical properties such as wear, microhardness and compressive strength. The presence of voids and air bubbles during the process of mixing could be due to improper mixing technique and these can affect the physical properties of the dental biomaterial by acting as stress concentrators and points where fracture or cracks can propagate [49]. Nano CT analysis surprisingly revealed a mixture of homogenously distributed small and large pores (radiolucent zones)

![Figure 6. Frequency distribution histograms analysis of the pores in μm from (a) control, (b) 10%, (c) 30% and (d) 50% SNP reinforced specimens.](image)

Table 2. Color stability of GIC and SNP modified GIC over a time period of 14 days. Values shown are Mean ±SD of color change value ΔE.

| Time  | 0%    | 10%    | 30%    | 50%    |
|-------|-------|--------|--------|--------|
| 1 h   | 1.33±(0.21) | 4.03±(0.07) | 17.82±(0.77) | 30.47±(1.54) |
| 24 h  | 2.25±(0.81) | 6.86±(0.51) | 24.20±(2.86) | 33.20±(0.26) |
| 4 days | 4.77±(0.22) | 6.16±(1.38) | 12.05±(0.55) | 30.01±(1.86) |
| 7 days | 3.46±(0.95) | 17.41±(0.79) | 15.26±(0.53) | 34.30±(0.60) |
| 14 days | 3.31±(0.05) | 6.50±(0.74) | 19.69±(0.59) | 29.24±(1.67) |

* Indicates clinically unacceptable values (ΔE ≥ 3.3).
Similar superscripted letters in the same column indicate statistical significance (p < 0.05).
in the control group and the SNP reinforced test groups (figures 5(a) to (d)). SNP presence was also observed by shiny spots being evenly distributed in the form of radiopaque dots. The presence of micro cracks penetrating some specimens was also observed. The pore size distribution analysis showed that the pores varied in size from 40 to 180 μm. A dominance of pores at 70 μm was a common feature in all specimens (figures 6(a) to (d)).

Reports on the incorporation of synthetically or biogenic fabricated SNP are scarcely noted in available literature [50]. However, the addition of synthetically or commercially available SNP incorporation are readily observed [41, 42, 47]. Nevertheless, when GIC are reinforced with SNP they have been weakly characterized. The color change observed with higher percentages is a commonly observed issue biomaterials reinforced with silver compounds. This could lead to an unpleasant appearance if the biomaterial is intended to be used in restoring anterior teeth. Similar findings were also noted by Pani et al [47] and El-Wassefy et al [41].

The results from the current investigation reveal that the objective to synthesize biogenic SNP and their incorporation into commercially available GIC luting agent was successfully achieved. Furthermore, the null hypothesis can be rejected since the addition of these SNP had a significant effect on microhardness and color stability.

4. Conclusion

Biogenic silver nanoparticles can be conveniently synthesized using chitosan as a capping and reducing agent with a diameter of 122 nm. Its incorporation into the glass ionomer cement revealed significant effect on microhardness and color stability. At 10% the addition the experimental GIC showed highest compressive strength. Moreover, further investigations are required to study the effect of SNP with different morphological features like near spherical, cubic, nanoplates or even nanorods. Another aspect that needs further research could be to encapsulate nanoparticles in mesoporous silica and then incorporate them in experimental or commercially available GIC.

Acknowledgments

The authors would like to acknowledge Faculty of Dentistry, Kuwait University research facility grant number: SRUI01/14. The authors are grateful for analysis provided by KUNRF lab (GE 01 / 07) and Electron microscopy Unit of the Faculty of Medicine, Kuwait University. The authors would also like to thank Sreeja Saji for helping in compressive and microhardness evaluation.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

ORCID iDs

Syed Saad Bin Qasim  
https://orcid.org/0000-0001-8288-6279

References

[1] Kielbasa A M, Oehme E P, Shakavets N and Wolgin M 2021 In vitro wear of (resin-coated) high-viscosity glass ionomer cements and glass hybrid restorative systems J. Dent. 105 103554
[2] Mount G J 1997 Longevity in glass-ionomer restorations: review of a successful technique Quintessence Int. 28 643–50
[3] Knight G M 2016 The benefits and limitations of glass-ionomer cements and their use in contemporary dentistry Glass-Ionomers in Dentistry (Cham: Springer) pp 57–79
[4] Faridi M A, Khabert A and Haroon S 2018 Flexural strength of glass carborundum cement and conventional glass ionomer cement stored in different storage media over time Med. Princ. Pract. 27 372–7
[5] Saran R, Upadhyya N P, Ginipalli K, Amalan A, Rao B and Kumar S 2020 Effect on physical and mechanical properties of conventional glass ionomer luting cements by incorporation of all-ceramic additives: an in vitro study Int. J. Dent. 2020 8896225
[6] Mohheet I A, Luddin N, Rahman I A, Kannan T P, Ghani N R N A and Masudi S M 2019 Modifications of glass ionomer cement powder by addition of recently fabricated nano-fillers and their effect on the properties: a review Eur. J. Dent. 13 470–7
[7] Moraes J F, Gomes de Moraes T, Nunes F R S, Carvalho E M, Nunes G S, Carvalho C N, Ardenghi D M and Bauer J 2021 Formation of hydroxyapatite nanoprecursors by the addition of bioactive particles in resin-modified glass ionomer cements Int. J. Adhes. Adhes. 110 102913
[8] Cehreli S R, Tirali R E, Yalcinkaya Z and Cehreli Z C 2013 Microleakage of newly developed glasscarbomer cement in primary teeth Eur. J. Dent. 7 15–21
[9] Alaoahi A, Brauer D S, Gentleman E and Sharpe P T 2021 A modified glass ionomer cement to mediate dentine repair Dent. Mater. (https://doi.org/10.1016/j.dental.2021.05.003)
[10] Korkmaz F M, Tüzüner T, Baygin O, Buruk C K, Durkan R and Bagis B 2013 Antibacterial activity, surface roughness, flexural strength, and solubility of conventional luting cements containing chlorhexidine dicacetate / cetrimide mixtures J. Prosthet. Dent. 110 107–15
[11] Tyas M J 2006 Clinical evaluation of glass ionomer cement restorations J. Appl. Oral Sci. 14 10–3
[12] Sidhu S and Nicholson J 2016 A review of glass-ionomer cements for clinical dentistry J. Funct. Biomater. 7 1–16
[13] Porter G C, Tompkins G R, Schwass D R, Li K C, Waddell J N and Meledandri C J 2020 Anti-biofilm activity of silver nanoparticle-containing glass ionomer cements Dent. Mater. 36 1096–107
[14] Tüzüner T, Dimkov A and Nicholson J W 2019 The effect of antimicrobial additives on the properties of dental glass-ionomer cements: a review Acta Biomater. Odontol. Scand. 5 9–21
[15] Freire P L L, Stanford T C M, Albuquerque A J R, Sampaio F C, Cavalcante H M M, Macedo R O, Galemebeck A, Flores M A P and Rosenblatt A 2015 Action of silver nanoparticles towards biological systems: cytotoxicity evaluation using hen’s egg test and inhibition of Streptococcus mutans biofilm formation Int. J. Antimicrob. Agents 45 183–7
[16] Khan A S, Ur Rehman S, AlMaimouni Y K, Ahmad S, Khan M and Ashiq M 2020 Bibliometric analysis of literature published on antibacterial dental adhesive from 1996–2020 Polymers (Basel). 12 1–29
[17] Chen L, Sub B I and Yang J 2018 Antibacterial dental restorative materials: a review Am. J. Dent. 31 6B–2B
[18] Nakamura Y, Takahashi K, Shimetani A, Sakagami H and Nishikawa H 2005 Cytotoxicity of direct current with antibacterial agents against host cells in vitro J. Endod. 31 753–8
[19] Qasim S S B, Nogueira L P, Fawzy A S and Daood U 2020 The effect of cross-linking efficiency of drug-loaded novel freeze gelated chitosan templates for periodontal tissue regeneration AAPS Pharm. Sci. Tech. 21 173–9
[20] Ferreira C J, Leitune V C B, Balbinot G de S, Degrazia F W, Arakelyan M, Sauro S and Collares F M 2019 Antibacterial and remineralizing fillers in experimental orthodontic adhesives Materials (Basel) 12 652
[21] Melo M A S, Guedes S F F, Xu H H K and Rodrigues I. K. A 2013 Nanotechnology-based restorative dentals for caries caries AAMitrobiol. Technol. 31 459–67
[22] dos Santos Junior V E, Targino A G R, Flores M A P, Rodriguez-Diaz J M, Teixeira J A, Heimer M V, Pessoa H L F, Galemebeck A and Rosenblatt A 2017 Antimicrobial activity of silver nanoparticle colloids of different sizes and shapes against Streptococcus Mutans Res. Chem. Intermed. 43 5889–99
[23] Chaloupka K, Malam Y and Seifalian A M 2010 Nanosilver as a new generation of nanoproduct in biomedical applications Trends Biotechnol. 28 580–8
[24] Ferreyra Maillard A P V, Dalmasso P R, López de Mishima B A and Hollmann A 2018 Interaction of green silver nanoparticles with human fibroblasts Acta Biomater. 69 50–61
[25] Anon ISO - ISO 9917-1 2007 Dentistry — Powder — liquid acid-base cements (https://iso.org/standard/45818.html)
[26] Kempel D, Nguyen M T, Ishida Y and Yonezawa T 2018 L-arginine-stabilized highly uniform Ag nanoparticles prepared in a microwave-induced plasma-in-liquid process (MWPLP) Bull. Chem. Soc. Jpn. 91 162–7
[27] Dong Z Y, Rao M P N, Xiao M, Wang H F, Hozzein W N, Chen W and Li W J 2017 Antibacterial activity of silver nanoparticles against staphylococcus warneri synthesized using endophytic bacteria by photo-irradiation Front. Microbiol. 8 1090
[28] Firdhose M and Lilathi P 2015 Biosynthesis of silver nanoparticles and its applications J. Nanotechnol. 2015 1–18
[29] Darroudi M, Ahmad M B, Abdullah A H, Ibrahim N A and Shammeli K 2010 Effect of accelerator in green synthesis of silver nanoparticles Int. J. Mol. Sci. (https://doi.org/10.3390/ijms11105898)
[30] Susilowati E, Maryani and Ashadi 2019 Green synthesis of silver-chitosan nanocomposite and their application as antibacterial material J. Phys. Conf. Ser. 1153 012135
[31] Zhang Z, Shen W, Xue J, Liu Y, Liu Y, Yan P, Liu J and Tang J 2018 Recent advances in synthetic methods and applications of silver nanostructures Nanoscale Res. Lett. 13 1–18
[32] Rezazadeh N H, Buazar F and Matroodi S 2013 Synergistic effects of combinatorial chitosan and polyphenol biomolecules on enhanced antibacterial activity of biofunctionalized silver nanoparticles Sci. Rep. 10 1–13
[33] Mulvaney P 1996 Surface plasmon spectroscopy of nanosized metal particles Langmuir 12 788–800
[34] Kalavira R, Maruthupandy M, Munuswaran T, Hameedha Beevi A, Anand M, Ramakritinan C M and Kumaraguru A K 2018 Synthesis of chitosan mediated silver nanoparticles (AgNPs) for potential antimicrobial applications Front. Bioeng. Biotechnol. (https://doi.org/10.3389/fbioe.2018.00402)
[35] Shitomi K, Miyaji H, Miyata S, Nishida E, Mayumi K, Sugaya T and Kawasaki H 2019 Human dentin coated with silver noclusters exhibits antibacterial activity against streptococcus mutans Nano Biomed. 11 21–8
[36] Akmar S, Dilaver Akgul E, Yasar M and Ergevun O 2013 The effect of ag content of the chitosan–silver nanoparticle composite material on the structure and antibacterial activity Adv. Mater. Sci. Eng. 2013 690918
[37] Qasim S B, Delaine-Smith R M, Rawlinson A and Ur Rehman I 2015 Freeze gelated porous membranes for periodontal tissue regeneration Acta Biomater. 23 317–28
[38] Dara P K, Mahadevan R, Digita P, A, Visvavirayagam S, Kumar I R G, Mathew S, Ravishankar C N and Anandan R 2020 Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Ch–Ag–NPs): in vitro studies on antioxidant and antibacterial applications SN Appl. Sci. 2 1–12
[39] Rwegegasira E, Mubofu E B, Nyandoro S S, Erasto P and Munissi J J E 2016 Preparation, characterization and in vivo antimycobacterial studies of panchovillin-chitosan nanocomposites Int. J. Mol. Sci. 17 1559
[40] Antonino R S C M D Q, Fook B R P L, Lima V A D O, Rached R I D F, Lima E P N, Lima J R D S, Covas C A P and Fook M V L 2017 Preparation and characterization of chitosan obtained from shells of shrimp (Litopenaeus vannamei Boone) Mar. Drugs 15 141
[41] El-Wassefy N A, El-Mahdy H R, El-Holkany N R 2018 The impact of silver nanoparticles integration on biofilm formation and mechanical properties of glass ionomer cement J. Esthet. Restor. Dent. 30 146–52
[42] Paiva J, Fidalgo T K S, da Costa L P, Maia L C, Balan L, Anselme K, Ploux I and Thirion B M S M 2018 Antibacterial properties and compressive strength of new one-step preparation silver nanoparticles in glass ionomer cements (NanoAg–GIC) J. Dent. 69 102–9
[43] Jawkar Z, Jawkar M and Shafiei F 2019 Mechanical and dentin bond strength properties of the nanosilver enriched glass ionomer cement J. Clin. Exp. Dent. 11 e275–81
[44] Rai M, Yadav A and Gade A 2009 Silver nanoparticles as a new generation of antimicrobials Biotechnol. Adv. 27 76–83
[45] Oliveira G L, Carvalho C N, Carvalho E M, Bauer J and Leal A M A 2019 The influence of mixing methods on the compressive strength and fluoride release of conventional and resin-modified glass ionomer cements Int. J. Dent. 2019 6834931
[46] Nomoto R and McCabe J F 2001 Effect of mixing methods on the compressive strength of glass ionomer cements J. Dent. 29 205–10
[47] Pani S C, Aljummaa M T, Alriqui A M, Aljummaa A M, Alkahtani Y M and Alkhuwarif A 2020 Color stability of glass ionomer cement after reinforced with two different nanoparticles Int. J. Dent. 2020 7808535
[48] Haugen H J, Qasim S B, Matinlinna J P, Vallittu P and Nogueira L P 2020 Nano-CT as tool for characterization of dental resin composites Sci. Rep. 10 1–12

[49] Hirata R, Pacheco R R, Caceres E, Janal M N, Romero M F, Giannini M, Coelho P G and Rueggeberg F A 2018 Effect of sonic resin composite delivery on void formation assessed by micro-computed tomography Oper. Dent. 43 144–50

[50] Enan E T, Ashour A A, Basha S, Felemban N H and Gad El-Rab S M F 2021 Antimicrobial activity of biosynthesized silver nanoparticles, amoxicillin, and glass-ionomer cement against Streptococcus Mutans and Staphylococcus Aureus Nanotechnology 32 215101