

Original Article



Innovation in Glass-Ionomer Cement Modified with Fibroin Nanoparticles: Evaluation of Mechanical, Surface, and Microbiological Properties

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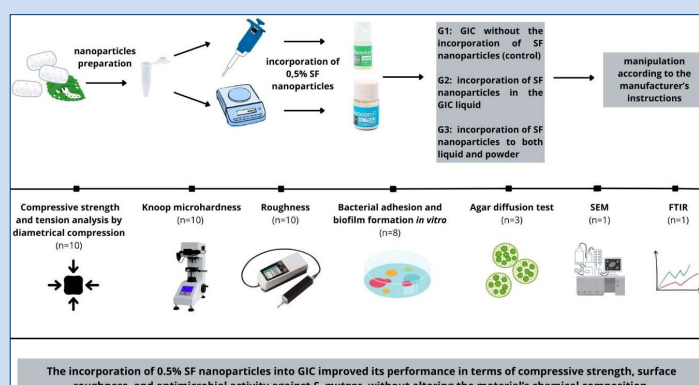
Abstract

Introduction: Silk fibroin (SF), due to its biocompatibility and strength, can act as an additive to optimize glass-ionomer cement (GIC). This study aimed to incorporate 0.5% SF nanoparticles into restorative GIC (Maxxion® R) to improve its mechanical, surface, and microbiological properties.

Methods: Compression, diametral tensile strength, microhardness, surface roughness, and microbiological tests involving *Streptococcus mutans* (bacterial adhesion, in vitro biofilm formation, and inhibition halo) were conducted, along with scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). The specimens were randomly divided into three groups: G1: GIC without SF nanoparticle incorporation; G2: GIC with SF nanoparticles incorporated only into the liquid; and G3: GIC with SF nanoparticles incorporated into both the liquid and powder.

Results: Incorporating nanoparticles into GIC resulted in favorable outcomes, with statistically significant differences in compression tests ($P=0.048$), surface roughness ($P=0.03$), and microbiological evaluation. CFU counts differed significantly between groups ($P=0.049$), while differences in inhibition halo (IH) measurements were descriptive without statistical comparison. Diametral tensile strength tests ($P=0.598$) and microhardness ($P=0.241$) showed no statistically significant differences, although values increased numerically with SF incorporation. SEM analysis suggested reduced biofilm formation on the surfaces of groups G2 and G3. FTIR indicated that the incorporation did not interfere with the acid-base setting reaction of the GIC.

Conclusion: Incorporating SF nanoparticles improved GIC performance in terms of compressive strength, surface roughness, and antimicrobial activity against *S. mutans*.



Introduction

Glass-ionomer cement (GIC) is an innovative material composed of a liquid and a powder component that has revolutionized several areas of dentistry since its

introduction.¹ This versatile compound is widely used in procedures such as conventional restorations, atraumatic restorative treatments,² and cementation. It is also effective as a sealant for pits and fissures.³ Its continuous use is

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notable due to its reliability and the exceptional benefits it provides to professionals and patients.

GIC exhibits satisfactory characteristics, including fluoride release, biocompatibility, and clinical versatility.³ However, limitations such as low mechanical resistance to abrasion,⁴ reduced translucency, friability,⁵ low fracture toughness, low wear resistance, and high water sorption⁶ have made the material the target of numerous studies aimed at improving its properties. These disadvantages make the material susceptible to fracture, limiting its indication for areas subject to intense masticatory stress.⁷ The common reasons for GIC failure were the breakdown of restoration and secondary caries.⁸

In this context, several elements have been incorporated into the GIC to enhance its properties. Among them are titanium dioxide,⁹ chlorhexidine,¹⁰ seashell nanoparticles,¹¹ silver diamine fluoride,¹² hydroxyapatite nanoparticles,¹³ silver nanoparticles,^{14,15} silica nanoparticles,¹⁶ chitosan,^{17–19} and cellulose.^{20–22} These innovations aim to enhance the performance of the GIC further, providing more efficient and long-lasting solutions for dental treatments.

Silk fibroin (SF), which originates from the silkworm (*Bombyx mori*), has a long history of use in the textile industry.²³ It is a natural protein polymer that can be mass-produced, composed of disulfide bond-linked light and heavy chains.²⁴ Silk I and silk II are the dominant crystalline structures of SF, where silk I is a metastable crystalline structure that includes bound water molecules, and silk II is the most stable state due to strong hydrogen bonding between adjacent peptide blocks, resulting in increased mechanical properties, including rigidity and tensile strength.²⁵

SF has emerged as a promising nanoparticle in both medicine and dentistry²⁶ due to its remarkable properties in biomedical and biotechnological applications, such as high strength, mechanical toughness,²⁷ biocompatibility, processing versatility, and resistance to chemical substances and microorganisms.^{23,28}

SF has gained prominence through its incorporation into dental materials, demonstrating benefits in oral health. Its performance is notable in regenerative endodontics,²⁹ under laboratory conditions simulating the oral environment³⁰ and pulp tissue engineering,³¹ proving to be a valuable tool for health promotion and the treatment of dental conditions.

Thus, this study proposed to investigate in depth the mechanical, surface, and microbiological properties of an innovative GIC, enriched with SF nanoparticles. For this purpose, detailed analyses were carried out, including compressive strength and diametrical tension tests, evaluation of microhardness, measurement of surface roughness, and microbiological analysis.^{32–38} In addition, the characteristics of the materials were thoroughly examined by scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR) to understand the interactions and resulting properties from this modification.^{39–41}

The null hypothesis was that incorporating 0.5% SF

nanoparticles would not improve the performance of GIC in terms of mechanical, surface, and microbiological properties.

Methods

The Maxxion® R GIC (FGM, Joinville, SC, Brazil), indicated for restorative procedures, and the SF nanoparticles extracted from the silkworm cocoon (*Bombyx mori*) were used.

The SF nanoparticles were prepared using 5 g of shredded silkworm cocoons, following the exact, validated protocol described in another study.²⁶ The process of degumming ensures the removal of the external sericin layer. The material was immersed for 30 min in a solution containing 5 g of anhydrous sodium carbonate (Batch: 88682, Dinâmica, Química contemporânea Ltda., SP, Brazil) and 1L of deionized water at 100°C. This procedure was repeated twice, and the degummed silk was rinsed with deionized water and oven-dried at 40°C (SL-104/30, Solab, SP, Brazil) until constant weight was achieved.

The process of silk dissolution was performed by mixing 5 g of dried degummed silk in a solution containing 1-mol anhydrous calcium chloride (Batch: 212377, Synth, Labsynth, SP, Brazil), 2-mol ethanol (Batch: 140548, Dinâmica, Química contemporânea Ltda., SP, Brazil), and 8-mol deionized water. This mixture was heated to 90°C using a thermal mantle (TE-0851, Tecnal, SP, Brazil) for 2 hours. A reflux trap was used to prevent solvent loss by evaporation. The final solution was centrifuged (Sorvall Legend Mach 1.6R, Thermo Scientific, MA, USA) for 20 min at 4000 rpm.

For dialysis, the solution was distributed into small flasks containing a semi-permeable cellulose acetate membrane (Unifil, Filtering Membrane, PR, Brazil). These flasks were submerged in deionized water for 96 hours, and the water was changed each 24 hours. This process removes ions while retaining the nanoparticles.

The solution containing approximately 5% silk (w/v) was slowly pipetted into an aqueous solution of acetone (Batch: AC0224, Dinâmica, Química Contemporânea Ltda., SP, Brazil) at 75% (v/v). The suspension was centrifuged for 1 hour at 4000 rpm, and the supernatant was discarded. A small aliquot of water was added to the precipitate. To obtain a complete dispersion of the nanoparticles, the suspension was stirred with a Vortex stirrer (VX-28, Warmnest, SP, Brazil) for 20 seconds and subjected twice to a 55-W ultrasonic mixer (Q55 Sonicator, Qsonica, CT, USA) for 30 seconds at 40% amplitude. The suspension was then centrifuged for 1 hour at 4000 rpm. The centrifugation and resuspension steps were repeated. Finally, the material was lyophilized to remove water.

The silk fibroin (SF) nanoparticles synthesized for this study were characterized by dynamic light scattering (DLS) and zeta potential measurements to confirm their size and colloidal stability. The DLS analysis revealed a multimodal size distribution by number, confirming that the vast majority of the particles were within the nanometric range: peak 1 at 42.91 nm (33.2%) and peak 2 at 106.0 nm

(64.7%), with only a minor fraction represented by peak 3 at 540.7 nm (2.1%). The recorded Z-average was 1297 nm with a high polydispersity index (PDI = 0.854), reflecting particle agglomerates in the aqueous suspension, a behavior frequently reported in the literature for SF nanoparticles due to their inherent tendency for self-assembly. The zeta potential was measured at -31.8 mV (with a conductivity of 0.0135 mS/cm), indicating robust colloidal stability. These findings confirmed the nanometric dimensions of the individual particles in the current batch and were consistent with the morphology and size range previously reported by Torres et al.²⁶ using scanning electron microscopy (SEM).

Incorporation of SF Nanoparticles into GIC

The SF nanoparticles were incorporated into the liquid and powder components in a proportion of 0.5% by weight. This concentration was based on preliminary studies conducted in our laboratory.³⁰ These values were measured using an analytical precision balance (Urano - UA 220/0.0001) for powder dosing and an automatic pipette (HTL Lab. Solutions, Barra Funda, SP, Brazil) for liquid dosing. After weighing, the mixture was placed in a plastic Eppendorf tube and stirred with a Digital Amalgamator YG-100 stirrer (Kondentech) for 30 seconds to obtain a well-mixed particle dispersion. The materials were handled under controlled temperature ($23 \pm 1^\circ\text{C}$) and relative humidity ($50 \pm 5\%$) conditions. After mixing, the materials were stored in the powder and liquid flasks provided by the manufacturer and then kept at room temperature.

Preparation of the Specimens

The specimens were prepared according to the following groups: G1 (control): GIC without the incorporation of SF nanoparticles; G2: incorporation of SF nanoparticles in the proportion of 0.5% by weight in the GIC liquid; and G3: incorporation of SF nanoparticles, totaling 0.5% by weight in the final GIC mixture, equally distributed between the two components (0.25% by weight in the liquid and 0.25% by weight in the powder).

After preparing the GIC according to the manufacturer's instructions, the material was inserted into two Teflon molds (3 mm in diameter and 6 mm in depth, or 6 mm in diameter and 2 mm in depth) using a Centrix syringe and a plastic spatula. The material was covered with a polyester strip and a glass plate, on which a 100-g load was applied for 40 seconds to flatten the surface and extrude excess material. After the setting reaction, the specimens were removed from the molds, incubated at 37°C , and immersed in 20 mL of distilled water for 24 hours. The smaller-diameter specimens were used for compressive strength and diametral tensile strength tests, while the larger-diameter specimens were utilized for microhardness, roughness, microbiological analysis, SEM, and FTIR tests. Immediately before the mechanical tests, excess surface moisture was removed from the specimens with a paper towel after 24 h of water storage. No surface finishing or polishing was performed before Knoop microhardness and roughness measurements, as the specimens had a flat,

smooth surface after preparation with a polyester strip and glass plate under a 100-g load for 40 seconds, which also extruded excess material.

Compressive Strength and Tension Analysis by Diametrical Compression

The specimens ($n = 10$) from the three GIC groups were obtained. The compressive strength tests were conducted using a universal testing machine (EMIC-DL, São José dos Pinhais, PR, Brazil) equipped with a 50-kgf load cell, at a speed of 0.5 mm/min. The specimens were positioned vertically, with the force applied along their long axis. The samples were tested under the same load cell and speed, but positioned horizontally for the diametral tensile strength tests. The results were expressed in megapascals (MPa).

Knoop Microhardness

The specimens ($n = 10$) were also subjected to the Knoop microhardness test using a microhardness tester (HMV-2, Shimadzu, Kyoto, Japan) under a static vertical load of 50 g applied for 30 s. Three indentations were performed per specimen, and the average of these readings was considered the microhardness value.

Roughness

Surface roughness (R_a) was evaluated using a profilometer (Surftest SJ-201P; Mitutoyo Corp., Kawasaki, Japan). Three measurements were obtained from the surface of each specimen ($n = 10$ per group), and the arithmetic mean roughness (R_a , μm) was calculated. The analyses were performed using a cutoff length of 0.8 mm, an evaluation length of 4.0 mm, and a scanning speed of 0.5 mm/s.

Bacterial Adhesion and Biofilm Formation in Vitro

Four specimens were prepared for each GIC group: two for biofilm formation and two as negative controls (without bacterial inoculum), ensuring experimental asepsis. The microbiological experiment was performed as two independent experiments conducted on separate occasions under identical experimental conditions. In each independent experiment, each condition was tested in quadruplicate, yielding four technical replicates per experiment and eight measurements per condition across the two independent experiments. The specimens were disinfected in 70% (v/v) ethanol and then exposed to UV light for 20 minutes on each surface, for a total of 40 minutes per specimen. A standard strain of *Streptococcus mutans* (ATCC 25175) was used for biofilm formation under aseptic conditions.

The specimens were placed in a 48-well cell culture plate (Kasvi, São José dos Pinhais, PR, Brazil). They received 600 μL of brain heart infusion (BHI) broth (Kasvi Ltda., Pinhais, PR, Brazil) containing the standardized bacterial inoculum ($\sim 10^7$ CFU/mL). The plates were incubated at 37°C for 24 h under microaerophilic conditions with shaking at 75 rpm. After this period, half of the culture medium was replaced with freshly prepared medium, and the plates were incubated again at 37°C for 24 h.

After incubation, the culture medium was removed from the wells, and the specimens were rinsed with PBS. They were then carefully transferred, using sterilized surgical forceps, into microtubes containing 1 mL of PBS. The microtubes were placed in an ultrasonic bath (Alt Equipamentos, Ribeirão Preto, SP, Brazil) at 40 kHz and 200 W for 20 minutes. They were then agitated for 30 s using a tube shaker, and serial decimal dilutions from 10^{-1} to 10^{-7} were prepared and seeded onto Petri dishes (90×15 mm) containing BHI Agar (Kasvi Ltda., Pinhais, PR, Brazil). The plates were incubated at 37°C for 48 h under microaerophilic conditions. The bacterial load of *S. mutans* was determined and expressed in $\log_{10}$ CFU/mL.

Agar Diffusion Test

The specimens were disinfected with 70% (v/v) ethanol and exposed to UV radiation for 20 minutes on each surface, for a total of 40 minutes per specimen. The test was performed in triplicate ($n=3$). Petri dishes (90×15 mm) containing BHI Agar were inoculated with a standardized bacterial suspension ($\sim 10^8$ CFU/mL) of *S. mutans* (ATCC 25175) in PBS. After inoculation, the plates were incubated in a microbiological incubator (DeLeo Equipamentos Laboratoriais, Bento Gonçalves, Porto Alegre, RS, Brazil) at 37°C for 48 h under microaerophilic conditions, following a protocol described in a previous study.³³ After incubation, the diameters of the bacterial growth inhibition halos around the specimens were measured in millimeters at three different points, and the mean values were calculated using ImageJ® software (National Institutes of Health, Madison, WI, USA).

Scanning Electron Microscopy

For the morphological characterization of isolated SF nanoparticles, a diluted aqueous suspension of SF nanoparticles was carefully deposited onto an aluminum stub and allowed to air-dry. For the GIC samples (G1, G2, G3, and GIC powder mixed with SF), the specimens were prepared as described previously, dehydrated in an alcohol solution, and dried in a vacuum desiccator. All the samples were subsequently metalized with gold film using a Bal-Tec SCD 050 Sputter Coater (Fürstentum, Liechtenstein) for 240 s. Morphological analysis was performed using a scanning electron microscope (Shimadzu model SS-550, Kyoto, Japan) operating at an accelerating voltage of 30 kV, in secondary electron contrast mode. Micrographs were captured at $\times 1000$, $\times 5000$, and $\times 10000$ magnification. One representative specimen of each GIC group (G1, G2, and G3) was examined after the biofilm formation assay, in addition to one specimen of GIC powder mixed with SF nanoparticles and one specimen of isolated SF nanoparticles.³⁹

Fourier-Transform Infrared Spectroscopy (FTIR)

One representative specimen from each group was analyzed using FTIR. The FTIR analysis was performed as qualitative characterization to verify whether incorporating SF nanoparticles interfered with the acid–base setting reaction

and the formation of the ionic cross-linked GIC matrix by comparing characteristic absorption bands among the groups. The FTIR absorption spectra were recorded using an FTIR spectrometer (Agilent Cary 600) equipped with a DTGS detector, covering a spectral range of $4000\text{--}500$ cm^{-1} with a resolution of 4 cm^{-1} . A total of 32 scans were performed for each spectrum, with a corresponding background spectrum collected under identical conditions (32 scans of air), using the Attenuated Total Reflection (ATR) accessory from PIKE Technologies. Data acquisition and processing were performed using Agilent MicroLab PC software.

Statistical Analysis

Statistical analyses were performed using SPSS 20.0. Data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was verified with Levene's test. For the results of microhardness, bacterial adhesion, and in vitro biofilm formation, which showed normal distribution, analysis of variance (ANOVA) was applied, followed by Duncan's post hoc test to identify differences between groups. For roughness, compressive strength, and diametral tensile strength data, non-parametric Kruskal–Wallis tests were used, followed by the Mann–Whitney test, with a significance level of 0.05.

Results

Table 1 presents the mean values and standard deviations of the compressive and diametral tensile strength tests. A statistically significant difference was observed in compressive strength, particularly in group G3, which showed a notable increase. Regarding diametral tensile strength, no significant differences were found between the groups, although group G3 presented higher mean values.

Incorporating SF nanoparticles into GIC significantly increased compressive strength. Group G1 showed the lowest values, which were significantly different from those of group G3. Group G2 presented intermediate values, statistically similar to both G1 and G3 in the compressive strength analysis.

Table 2 shows the mean and standard deviation of microhardness values. Although no statistically significant differences were observed, microhardness increased numerically when SF nanoparticles were incorporated into the GIC.

In the surface roughness test, the data were not homogeneous and did not follow a normal distribution. As shown in Table 3, the groups differed significantly. Group

Table 1. Mean and standard deviation for compression and tension by diametral compression

Groups	Compression (MPa)	Diametral tensile strength (MPa)
G1	8.05 ± 3.80^a	4.17 ± 3.09^a
G2	12.53 ± 6.63^{ab}	4.31 ± 1.67^a
G3	18.32 ± 15.10^b	7.72 ± 1.27^a
P-value	0.048	0.598

Different lowercase letters indicate statistically significant differences among groups ($P < 0.05$)

G1 had the highest mean roughness, with substantial differences from groups G2 and G3, which did not differ. These results suggest that adding SF nanoparticles to GIC reduced surface roughness.

The results of bacterial adhesion and in vitro biofilm formation (expressed in $\log_{10}$ CFU/mL) followed a normal distribution. The means and standard deviations are presented in Table 4.

Incorporating SF nanoparticles only in the liquid component (G2) resulted in a lower bacterial load ($\log_{10}$ CFU/mL) and a larger inhibition halo. Incorporating nanoparticles into both the powder and liquid (G3) also led to lower bacterial load and larger inhibition zones compared to the control (G1), although without statistically significant differences from the other groups.

Figure 1a shows the GIC powder mixed with SF nanoparticles, illustrating how these particles integrate into the cement matrix. The image reveals a homogeneous distribution of SF, forming a porous, interconnected network of granular aggregates that supports effective incorporation without significant larger-scale agglomeration. This organizational pattern within the matrix is consistent with the morphology observed for isolated nanoparticles. For comparison, the SEM image of

the G1 group, which does not exhibit biofilm formation (Figure 1b), shown at a lower magnification ($\times 1000$), clearly demonstrates that the GIC has inherent voids and microcracks on its surface.

The SEM images obtained after the in vitro microbiological experiment confirmed the presence of biofilm in all three GIC groups (Figure 2). A denser accumulation was observed in G1, where no SF nanoparticles were incorporated. At magnification $\times 1000$, the biofilm in G1 appears to completely cover the material's surface, making the underlying microcracks barely visible. In contrast, in G2 and G3, the material's surface texture remained distinguishable, indicating reduced biofilm presence. Furthermore, the agar diffusion test revealed a larger inhibition halo in the groups modified with SF nanoparticles, demonstrating enhanced antibacterial activity.

The FTIR results (Figure 3) were similar across the three groups, indicating comparable chemical composition. The absorption peaks appeared in two intervals: 1093–1029 cm^{-1} , corresponding to siloxane (Si–O–Si), and 1697–1650 cm^{-1} , related to carbonyl groups (C–O and C=O) in polyacrylic acid. The similarity in the spectra suggests that adding 0.5% SF nanoparticles, both in the liquid and in the powder, did not interfere with the acid–base reaction or the formation of the ionic cross-linked matrix of the Maxxion® R GIC.

Furthermore, the intensity of the characteristic peaks remained consistent across all groups, indicating that incorporating SF nanoparticles did not lead to significant formation of new functional groups, especially in the most intense band region (between 1300 and 900 cm^{-1}). The

Table 2. Mean and standard deviation of microhardness values after 24 h

Groups	Microhardness (KHN)
G1	7.82±3.88 ^a
G2	8.64±5.09 ^a
G3	11.32±5.07 ^a
P-value	0.241

Different lowercase letters indicate statistically significant differences among groups ($P < 0.05$)

Table 3. Mean and standard deviation of roughness values

Groups	Ra (μm)
G1	2.50±1.01 ^a
G2	1.27±0.76 ^b
G3	1.39±0.47 ^b
P-value	0.03

Different lowercase letters indicate statistically significant differences among groups ($P < 0.05$)

Table 4. Means and standard deviations for bacterial adhesion and biofilm formation ($\log_{10}$ CFU/mL)

Groups	$\log_{10}$ UFC/mL
G1	4.72±1.27 ^a
G2	3.02±0.38 ^b
G3	4.10±1.67 ^{ab}
P-value	0.049

Different lowercase letters indicate statistically significant differences among groups ($P < 0.05$)

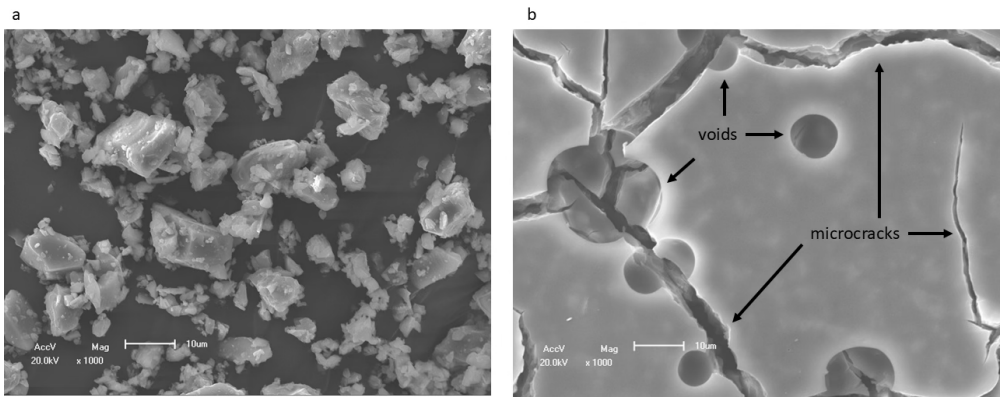


Figure 1. SEM images of SF nanoparticles and GIC surface characterization. (a) GIC powder mixed with SF nanoparticles (magnification $\times 1000$), illustrating the homogeneous distribution and organization of the SF within the cement matrix, forming a porous and interconnected network. (b) Control group (G1) specimen without biofilm formation (magnification $\times 1000$), demonstrating inherent voids and microcracks on the GIC surface

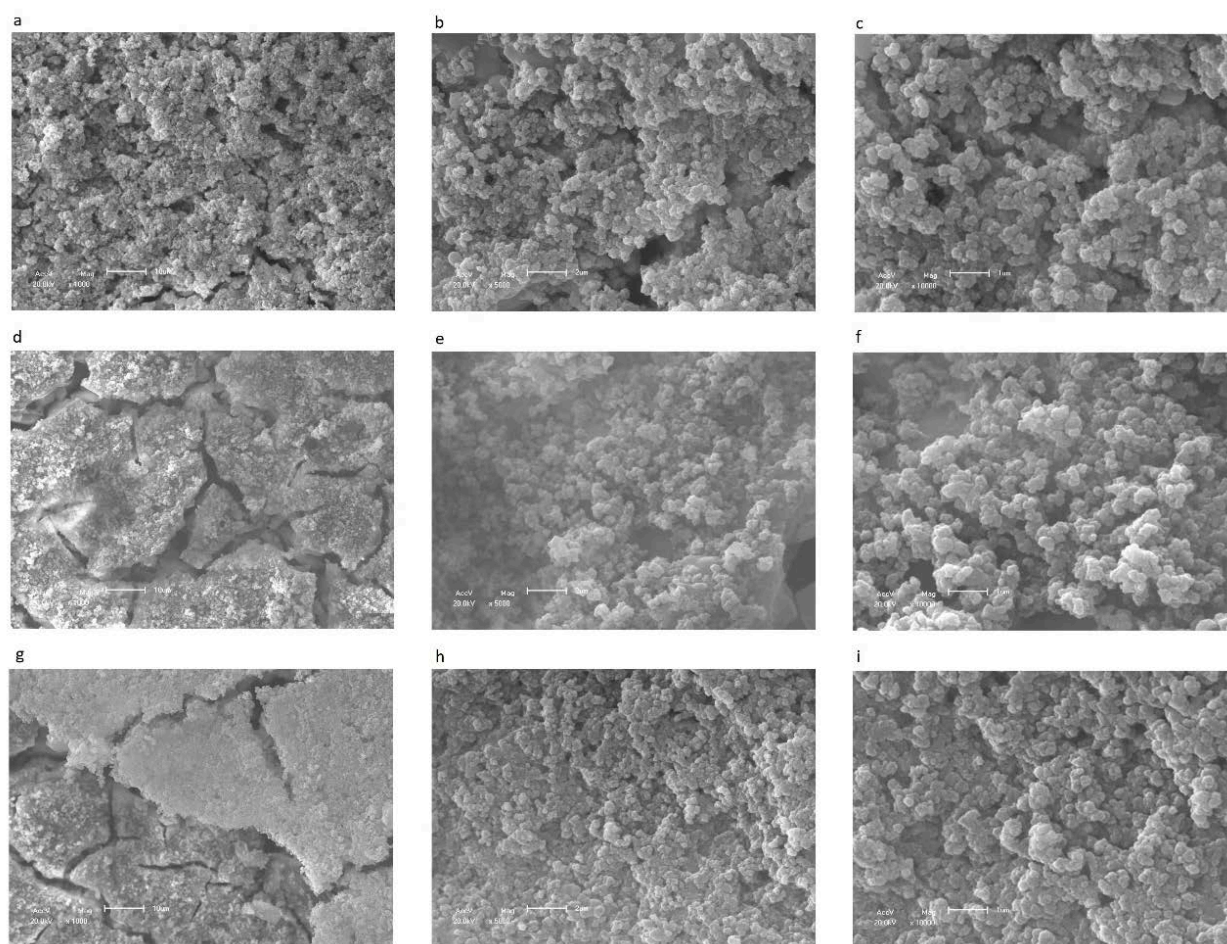


Figure 2. SEM images of GIC specimens after *S. mutans* biofilm formation at three magnifications ($\times 1000$, $\times 5000$, and $\times 10000$). (a, b, and c) Control group (G1). (d, e, and f) GIC with incorporation of SF nanoparticles only in the liquid (G2). (g, h, and i) GIC with incorporation of SF nanoparticles in the liquid and powder (G3). At low magnification, the biofilm covers the entire surface in G1 (a), making the underlying microcracks barely visible, whereas in G2 (d) and G3 (g), the material and microcracks are clearly visible, suggesting a lower amount of biofilm covering the surface

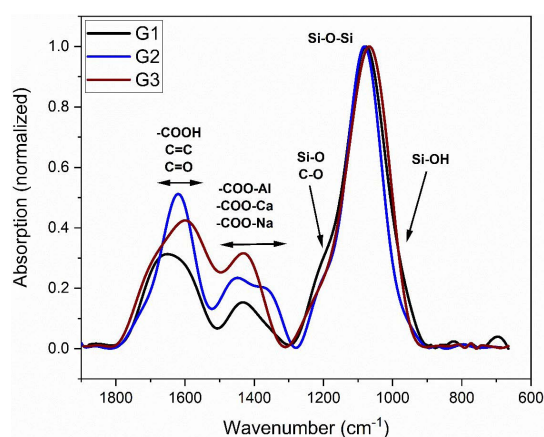


Figure 3. FTIR spectra. Control group (G1), GIC with incorporation of SF nanoparticles only in the liquid (G2), GIC with incorporation of SF nanoparticles in the liquid and powder (G3). The results were similar between the three groups, indicating a comparable chemical composition

presence of slight changes in the lower-intensity bands of the spectra (between 1800 and 1300 cm^{-1}) is observable in Figure 3. The intensity of the band attributed to -COOH of polyacrylic acid at 1715 cm^{-1} tended to decrease, possibly converting to COO- (polycarboxylate salt) present between 1400–1600 cm^{-1} , as well as contributing to the increase of the bands related to C=C and C=O at 1620 and 1580 cm^{-1} ,

respectively.

Discussion

GIC is one of the main dental materials that receives additives to improve its properties.^{11,12,14,16,20,21} However, studies investigating SF as a reinforcement option for this material remain limited, highlighting a promising area of research.

As restorative materials are intended to replace lost tooth structure, they must possess sufficient strength to withstand masticatory forces.³² This study showed a significant increase in compressive strength after adding SF nanoparticles, although the diametral tensile strength values did not differ significantly. This improvement can be attributed to silk fibroin's high mechanical strength and toughness.²⁷

When incorporated into the GIC (Figure 1a), these nanoparticles formed a highly porous, interconnected network of granular aggregates. This visual evidence of the nanometric scale and intricate morphology supports understanding of how SF contributes to improved GIC properties. The extensive surface area provided by this porous nanostructure, coupled with the inherent mechanical strength and toughness of SF,²⁷ may contribute

to the observed increase in compressive strength. This aligns with previous reports in which the incorporation of other nanoparticles, such as zirconium oxide, titanium dioxide,⁹ silver nanoparticles,^{14,15} and cellulose nanocrystals,^{20–22} also led to enhanced compressive strength in GICs, often attributed to a more homogeneous surface and reduced microcracks. The homogeneous appearance and good distribution of SF within the GIC matrix, as also suggested by Figure 1a, suggest successful incorporation without significant agglomeration that would compromise the material's integrity or performance.

Previous studies have also reported enhanced compressive strength in conventional GICs following the incorporation of zirconium oxide nanoparticles, titanium dioxide,⁹ silver nanoparticles,^{14,15} and cellulose nanocrystals.^{20–22} These enhancements have been associated with a more homogeneous surface, containing fewer voids and microcracks, which contributes to the improved consistency and mechanical performance of the GIC.

In this study, compressive strength values were higher than those observed in the diametral tensile strength test, consistent with previous studies involving different types of GICs.^{7,9} This discrepancy can be attributed to the nature of cohesive forces acting in different directions in the two tests, despite their presence in both.⁷

The surface microhardness test is widely used to assess the degradation and long-term durability of dental materials.³⁶ In the present study, microhardness values did not show statistically significant differences between groups, although a numerical increase was observed following the incorporation of SF nanoparticles. In this work, microhardness was measured at only one time point, 24 hours after specimen preparation.

Studies have reported varying outcomes regarding the effect of nanoparticle incorporation on GICs. For instance, a study¹⁴ showed an increase in microhardness after incorporating silver nanoparticles. In contrast, another study¹⁸ demonstrated a decrease in hardness when chitosan was added to Fuji IX GIC. Furthermore, other studies^{36,37} have shown that the microhardness of dental materials can change over time, reinforcing the importance of longitudinal evaluations to understand their behavior in clinical use.

The reduction in surface roughness observed after adding SF nanoparticles aligns with their morphological characteristics. The nanometric size and granular nature of SF, along with their ability to form an interconnected network, allow them to act as effective fillers.^{18,22} This integration likely fills microscopic irregularities and voids on the GIC surface, resulting in a more homogeneous and compact topography, which is consistent with improved consistency and mechanical performance, as observed with other nanofillers.^{9,14,22}

The smoothing effect, driven by the physical presence and optimal dispersion of these nanometric structures, results in significantly lower surface roughness values, making the material less conducive to bacterial adhesion. This is particularly important, considering the critical

mean surface roughness (Ra) threshold for bacterial colonization of 0.2 μm , as defined by one study.³⁸ While all GIC groups in this study exhibited roughness values above this limit, the addition of SF nanoparticles still provided a significant improvement in surface smoothness compared to the control group. Other studies^{2,18} evaluating GICs reported Ra values below the threshold;³⁸ however, none used the Maxxion® brand, suggesting material-specific characteristics may influence this parameter. GIC exhibits antimicrobial activity against *Streptococcus*, *Lactobacillus*, and *Actinomyces*, significantly inhibiting bacterial growth.¹⁰ The antibacterial effect in conventional GICs is likely associated with the continuous release of fluoride.^{34,35}

In parallel, biofilm and agar diffusion experiments in this study revealed the antimicrobial potential of SF nanoparticles against *S. mutans*. This was evident from the numerical results of microbiological tests and SEM images. In the control group, the biofilm layer covered the entire material surface, obscuring microcracks. In contrast, in the SF nanoparticle groups, microcracks were clearly visible, suggesting reduced biofilm coverage. The agar diffusion test also demonstrated a larger inhibition halo in GICs containing SF nanoparticles.

The agar diffusion assay is a well-established microbiological method widely used for the initial screening of antimicrobial activity. In dental research, it has been extensively applied to evaluate restorative materials, dental cements, endodontic materials, adhesive systems, and other biomaterials^{10,33} with potential antimicrobial properties, supporting its use in the present study and facilitating comparison with previous findings. However, for solid materials, inhibition zone formation depends on the release, solubility, and diffusion of antimicrobial components through the agar and therefore does not necessarily reflect the material's intrinsic antimicrobial activity. Although this inherent limitation does not preclude the use of agar diffusion as an initial screening method, it should be considered when interpreting the findings. Accordingly, the inhibition zone results were interpreted in conjunction with the complementary microbiological findings of the present study.

SEM images revealed the presence of voids and microcracks on the surfaces of all GIC groups. Such surface irregularities are characteristic of GIC and have been consistently reported in the literature, regardless of the commercial brand.^{2,18}

Regarding the chemical composition of the GIC, the FTIR results showed that incorporating SF nanoparticles did not modify the material's chemical structure. The spectra were consistent with other FTIR findings on GICs reported in the literature.^{22,34,40,41} Other studies have shown that adding cellulose²² and chitosan¹⁷ also did not alter the material's acid–base setting reaction.

Although fluoride release was not measured in this study, it is an important parameter to be addressed in future investigations. The FTIR results showed that incorporating SF nanoparticles did not interfere with the acid–base setting reaction or the ionic cross-linked matrix

of the GIC, suggesting that the fluoride release mechanism is likely preserved. This interpretation is consistent with previous reports showing that the incorporation of other nanoparticles, such as hydroxyapatite, cellulose nanocrystals, and chitosan, did not significantly alter fluoride release from GICs.^{6,17,22}

Similarly, the pH and viscosity of the GIC liquid were not measured directly after SF incorporation. However, the FTIR results indicated that the acid–base setting reaction remained unchanged, suggesting that the polyacrylic acid solution's intrinsic acidity was not significantly altered. In addition, the low concentration of SF used (0.5 wt%) and the homogeneous dispersion achieved during mixing suggest that any viscosity changes were minimal and did not compromise the material's handling characteristics. Future studies should address these parameters to confirm this assumption.

These findings are favorable and refute the null hypothesis, as the incorporation of 0.5% contributes to the improved performance of GIC in terms of compressive strength, surface roughness, and antimicrobial activity against *S. mutans*, without altering the material's chemical composition.

Conclusion

The incorporation of 0.5% SF nanoparticles into GIC improved its performance in terms of compressive strength, surface roughness, and antimicrobial activity against *S. mutans*, without altering the material's chemical composition. Among the tested groups, G3 (SF incorporated into both powder and liquid) showed the best mechanical properties, while G2 (SF in liquid only) and G3 demonstrated superior bacterial inhibition compared with G1 (control). These findings indicate that incorporating SF into both powder and liquid represents the most promising approach for enhancing GIC performance. Further studies are warranted to confirm the long-term effects of this composition in restorative dentistry.

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Competing Interests

The authors declare no potential conflicts of interest concerning the authorship or publication of this work. The authors do not have any financial interest in the companies whose materials are included in this article.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Ethical Approval

We clarify that our work did not require ethics committee approval, since all tests were laboratory-based and the silk fibroin nanoparticle was obtained from silkworm cocoons after the animal's life cycle, without interfering with the species' way of life. For this reason, we did not include an ethical approval code.

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