

Characterization of a new material based on silver nanoclusters synthesized in polymethacrylic acid for the infiltration of dental structures affected by caries*

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ABSTRACT

Objective: To characterize the physical and biological properties of a solution of silver nanoclusters (AgNCs) synthesized in polymethacrylic acid (PMAA) for the infiltration of dental structures affected by caries. **Materials and methods:** AgNCs were synthesized in PMAA by photoreduction of AgNO₃. The physical properties evaluated were: color change (by spectrophotometer), shear bond strength (SBS) of glass ionomer cement (GIC) to demineralized dentin, surface hardness (measured using the Vickers hardness test), and depth of silver ion penetration (tracked using scanning electron microscopy with energy-dispersive X-ray spectroscopy [SEM-EDS]). The biological properties evaluated included antibacterial effect (minimum inhibitory concentration [MIC], minimum bactericidal concentration [MBC], and colony-forming unit [CFU] count) against *Streptococcus mutans* and *Lactobacillus acidophilus*, and cytotoxicity in pulp cells (via MTT assay). 38% silver diamine fluoride (SDF) was used as a reference, and specific statistical analyses were applied. **Results:** Surfaces treated with AgNCs in PMAA showed color stability and high values of microhardness and GIC SBS. A consistent presence of silver was detected throughout the depth of the lesion. The antibacterial effect was significant for MIC and MBC, starting at dilutions of 1.25% and 2.5%, respectively. Cell viability remained high, between 92% and 89% (from 24 to 72 hours). **Conclusions:** The AgNCs solution in PMAA did not cause staining, improved GIC SBS, restored surface hardness, showed acceptable cytotoxicity, and had an excellent antibacterial effect, with silver ion penetration throughout the depth of caries-affected dental structure lesions.

Keywords: non-invasive treatment; dental caries; polymethacrylic acids; medical nanotechnology.

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INTRODUCTION

Dental caries is the most prevalent noncommunicable chronic disease worldwide (1). Contemporary approaches to the management of caries lesions prioritize minimally invasive strategies aimed at preserving tooth structure and promoting remineralization processes. In this context, silver diamine fluoride (SDF) has demonstrated efficacy as a cariostatic agent for arresting the progression of caries lesions (2). The increase in surface hardness of caries-affected dentin and the antibacterial activity against cariogenic biofilms are among the key clinical benefits of this material, particularly when used at a concentration of 38%.

Since 2013, scientific interest in SDF has increased substantially. In 2014, the U.S. Food and Drug Administration (FDA) approved its use as a Class II medical device for the treatment of dentin hypersensitivity. In addition, its efficacy as a nonrestorative treatment alternative for caries lesions in both primary and permanent dentitions has been extensively documented in high-impact publications (3).

However, SDF has important limitations, most notably the intense dark discoloration of the treated surface. This adverse effect compromises dental esthetics and is often unacceptable to patients (4). Various strategies have been proposed to mitigate discoloration, including the application of potassium iodide (KI), potassium fluoride (KF), or subsequent coverage with restorative materials; however, these interventions have not consistently eliminated residual discoloration, which remains a significant clinical disadvantage (5,6). These limitations have prompted the search for innovative technologies capable of preserving and restoring caries-affected dental structures without causing color alterations. In this regard, the incorporation of nanoscale silver combined with fluoride compounds has shown potential to reduce the undesirable esthetic effects associated with the formation of metal oxides resulting from the photoreduction of silver.

In the present study, silver nanoclusters (AgNCs) were synthesized using polymethacrylic acid (PMAA) as a stabilizing matrix, with the aim of reducing the esthetic drawbacks associated with SDF without compromising its antibacterial and remineralizing properties. Furthermore, as an added benefit, it was hypothesized that the use of a polycarboxylic acid as a vehicle for the nanoparticles could promote adhesion of glass ionomer restorative cements, in a manner analogous to the conditioners used clinically.

Therefore, the primary objective of this study was to characterize the physical and biological properties of a solution of AgNCs synthesized in PMAA. To this end, changes in the color and surface microhardness of the

treated surfaces, the depth of penetration of the solution into demineralized dentin, and the shear bond strength (SBS) of restorative glass ionomer cements (GIC) applied to these surfaces were evaluated. In addition, the antibacterial activity of the compound against cariogenic pathogens and its cytotoxicity in pulp cells were determined.

MATERIALS AND METHODS

Study type

An *in vitro* study was conducted. The study consisted of an initial stage aimed at describing the synthesis, characterization, and stability of the AgNCs/PMAA complex, followed by an evaluation of its efficacy based on variables of its physical and biological properties.

Inclusion and exclusion criteria

To evaluate the physical properties, sound third molars obtained from the Human Tooth Bank of the Faculty of Dentistry of Universidad Nacional de Córdoba (FO-UNC) were used, whereas pulp cell cultures and bacterial strains of *Streptococcus mutans* and *Lactobacillus acidophilus* were used to evaluate the biological properties.

As exclusion criteria, third molars with caries lesions, structural cracks, or defects identified after the cutting and standardization process were excluded.

Sample size determination

To evaluate the physical properties, sample size was determined using a two-sided test based on the proportion comparison formula, considering a significance level of 5% and a statistical power of 80%. For this purpose, the results of a previous study reporting the shear bond strength (SBS) of glass ionomer cement (GIC) to dentin surfaces treated with SDF were used as a reference (5), which led to the conclusion that each group needed to have at least 7 specimens.

Study variables

Synthesis

The AgNCs/PMAA complex was characterized using fluorescence and absorption spectroscopy, and its stability over time was evaluated.

Physical variables

1. **Color changes:** Color stability was evaluated using the CIE Lab* system, which interprets measurements in three dimensions: L (lightness), a (red-green),

and b (yellow–blue), determining the total color change (ΔE).

2. *Shear bond strength (SBS)*: The bond strength of the restorative material to treated dentin was quantified and expressed in megapascals (MPa).
3. *Surface hardness*: Surface hardness was determined using the Vickers microhardness test (VHN) and expressed in pressure units (MPa) to compare the structural recovery of dentin.
4. *Penetration depth*: Penetration depth was quantified based on the silver concentration expressed as weight/volume percentage (% w/v) at different depths.

Biological variables

1. *Antibacterial activity*: Antibacterial activity was evaluated based on the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and colony-forming unit count (CFU/mL).
2. *Cytotoxicity*: Cytotoxicity was determined based on the percentage of cell viability (%) using the colorimetric MTT assay in pulp cell cultures.

Procedures and techniques

All experimental protocols were approved by the Research and Development Office of Universidad Católica de Córdoba, Argentina (SI-UCC research grants), and by the National Agency for the Promotion of Research, under research grants FONCYT-PICT2020 Series A No. 00539 and PICT2019 No. 241, CONICET-PIP, and PRIMAR2017 (SeCyT-UNC).

All methods were conducted in accordance with the relevant guidelines and regulations. In addition, to ensure the linguistic quality of the manuscript, the artificial intelligence tool Google Gemini was used for grammar and spelling review of the text. All resulting content was supervised, edited, and critically reviewed by the authors before its final inclusion.

Synthesis, characterization and stability of the AgNCIs/PMAA complex

The AgNCIs/PMAA solution was synthesized at room temperature by photoreduction of AgNO_3 in the presence of PMAA using a 355-nm lamp, as described in the literature (7,8). For this purpose, aqueous solutions were prepared using Milli-Q®-grade distilled water. PMAA was used as a 30% aqueous solution of its sodium salt (Sigma-Aldrich®, Mw = 9,500), along with silver nitrate (Sigma-Aldrich®, >99.8%). These components were mixed to obtain a final Ag^+ concentration of 5×10^{-4} M, with a molar ratio of PMAA polymer of 5:1 ($\text{Ag}^+:\text{MAA}$). This ratio corresponds to five Ag^+ ions per MAA unit. Throughout the procedure, pH conditions were monitored and adjusted, maintaining them within the optimal range of 5.5–6.5.

The mixture was prepared in a completely sealed flask fitted with a Teflon septum. It was subsequently exposed to 355-nm UV light for 1 hour. After irradiation, the flask was wrapped in aluminum foil and stored at 4 °C. This composition was used as a comparative reference against the commercial 38% SDF solution (Fagamin®, lot #8252 08/24, Tedequim; Cordoba, Argentina).

The AgNCIs/PMAA solution, with a total silver concentration of 54 ppm, was used as a comparative reference against the commercial 38% SDF solution (248,400 ppm silver). The stability of this complex was evaluated using fluorescence spectroscopy by analyzing the excitation-emission matrix (EEM), recording emission intensity over a period of 9 months following synthesis (9–12).

Physical properties

Sample preparation

Twenty-four sound third molars were obtained from the Human Tooth Bank (FO-UNC, Argentina), under Ordinance 3/16 HCD and Resolution 333/17 HCD. The teeth were sterilized by gamma irradiation for 24 hours before sectioning. Four-millimeter-thick dentin blocks were prepared after removal of the occlusal enamel and using a low-speed cutting machine (Buehler®, Germany) with copious water cooling. Sections were made perpendicular to the longitudinal axis of the tooth to obtain flat dentin surfaces. The specimens were subsequently polished with 400-grit silicon carbide paper and coated with nail polish (Revlon®, New York, NY, USA), leaving a 5×5 mm window exposed on the occlusal dentin surface for the production of demineralized dentin to simulate dental caries. The specimens were immersed for 66 hours in a solution containing 0.05 M acetate buffer and 2.2 mM calcium phosphate, adjusted to pH 5.0, to generate an approximately 150- μm -deep demineralized layer simulating a caries lesion.

Color changes in treated surfaces

Color change represents the adverse effect that the new formulation is intended to improve. The CIE $L^*a^*b^*$ color system was used. Measurements were repeated three times to obtain a mean value. In this regard, a ΔE value perceptible to the human eye in dental restorations is generally considered to occur when it exceeds 1.0–1.2 in this system, although values below 3.3 are often considered clinically acceptable. Values above 3.7 are commonly detected as a color mismatch by untrained observers.

The specimens were divided into two treatment groups (A and B) and one untreated control group (C) (n = 8 per group):

- Group A: Treated with AgNCl/PMAA. The solution was applied to the exposed demineralized surface using a microbrush for 10 seconds, followed by incubation at 37 °C and 100% relative humidity for 24 hours.
- Group B: Treated with 38% SDF. The solution was applied to the exposed demineralized surface using a microbrush for 10 seconds, followed by incubation at 37 °C and 100% relative humidity for 24 hours.
- Group C: Control (untreated). The exposed demineralized surfaces were left untreated and then incubated for 24 hours at 37 °C and 100% relative humidity.

After 24 hours of incubation, the specimens were analyzed for initial color changes following application of the different treatments and were then immersed again for an additional 6 days at 37 °C and 100% relative humidity. Measurements were performed using a spectrophotometer (CM-600D Konica Minolta®), which had been previously calibrated. To ensure data reproducibility and avoid interference caused by dentin translucency, all measurements were performed with the specimens placed against a matte white background.

Differences in color coordinates (ΔL , Δa , and Δb) were recorded at four time points (R0, R1, R2, and R3). Total ΔE was calculated using the following formula:

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{\frac{1}{2}}$$

For interpretation of results, a clinical acceptability threshold of $\Delta E = 3.7$ was established. Values above this threshold were considered perceptible and clinically significant color changes. Finally, the effect of the treatments was evaluated by comparing the results of groups A and B with those of the untreated control group (group C).

Shear bond strength

SBS was evaluated to determine the effect of surface treatment on the adhesion of glass ionomer cement (GIC) to dentin. Accordingly, the shear bond strength of cement bonded to dentin surfaces treated with the different study products was evaluated.

Groups A and B received a second application of AgNCl/PMAA and 38% SDF, respectively, using a microbrush for 10 seconds. Immediately afterward, a conventional high-viscosity GIC (Fuji IX Gold Label®) was placed. The material was manually mixed on a glass slab for 20–30 seconds, following the manufacturer's instructions. The material was subsequently inserted into a cylindrical mold (4 mm in diameter × 3 mm in height) placed on the demineralized dentin surfaces, which had been treated or left untreated according to the experimental group. During GIC setting, a glass slab coated

with petroleum jelly (Vaseline®) was placed over the mold and maintained in position for 5 minutes. After this period, the specimens were stored at 37 °C and 100% relative humidity for 24 hours before mechanical testing.

SBS was evaluated using a universal testing machine (Digimess®), applying a shear load with a beveled blade at a crosshead speed of 1 mm/min until failure occurred. SBS values were calculated in megapascals using the formula $\text{MPa} = \text{N}/12.6 \text{ mm}^2$. Finally, the failure mode (adhesive, cohesive, or mixed) was analyzed using a confocal laser microscope (Olympus LEXT® OLS 4000) at 100x magnification.

Surface hardness

The aim was to determine whether application of the different treatments to the surface of caries-affected dentin could restore or exceed the hardness of sound dentin. For this purpose, four 2-mm-thick sections were obtained from each specimen, extending from the occlusal to the apical region, resulting in four treatment groups:

- Group A: Treated with AgNCl/PMAA. The solution was applied to the exposed demineralized surface using a microbrush for 10 seconds, followed by incubation at 37 °C and 100% relative humidity for 24 hours.
- Group B: Treated with 38% SDF. The solution was applied to the exposed demineralized surface using a microbrush for 10 seconds, followed by incubation at 37 °C and 100% relative humidity for 24 hours.
- Group C: This group consisted of a section of sound dentin protected with nail polish during artificial caries formation and incubated for 24 hours at 37 °C and 100% relative humidity.
- Group D: Control (untreated). The exposed demineralized surface was left untreated and incubated for 24 hours at 37 °C and 100% relative humidity.

Surface hardness was evaluated using the Vickers microhardness test (VHN) at room temperature with a Microhardness Tester FM-300 (Future-Tech Corp.). Five indentations were made per specimen, applying a 100-g load for 10 seconds for each indentation. The mean values for each specimen were recorded in Excel for statistical analysis.

Penetration depth

Penetration depth was evaluated to determine the distribution of silver throughout the demineralized dentin lesion. For this purpose, the penetration of metal ions was assessed to a minimum depth of 200 μm , which corresponded to the mean depth of demineralization obtained through the generation of artificial caries lesions.

The elemental composition of the specimens was determined using energy-dispersive X-ray spectroscopy (EDS) coupled to a scanning electron microscope (SEM) (JSM 7800F, JEOL Ltd.).^c The specimens were previously gold-coated by sputter coating (23 mA for 45 seconds), and the surfaces were examined using SEM (5 kV voltage and magnifications ranging from 2,500x to 20,000x).

To track the penetration of silver ions, 50- μ m bands were defined from the surface to the base of the lesion. Measurements were performed in three areas per band (20,000x and 5 kV), recording a mean elemental silver value (% w/v). An incremental quantitative analysis was performed at 50- μ m intervals until a depth of 400 μ m was reached (eight subsegments).

Biological properties

Antibacterial activity

The bactericidal and bacteriostatic activity of different concentrations of the developed product was investigated and compared with the well-established effects of SDF to evaluate its comparative efficacy. For this purpose, strains of *S. mutans* and *L. acidophilus* were cultured, and the following biological properties were determined: minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and colony-forming units (CFU).

1. *Microorganism culture*: Frozen strains of *S. mutans* and *L. acidophilus* were plated on blood agar and incubated at 37 °C for 24 hours in an atmosphere composed of 85% N₂, 5% CO₂, and 10% H₂. A single colony was inoculated into 10 mL of BHI broth and incubated for 24 hours under the same conditions. Cells were collected by centrifugation, washed with phosphate-buffered saline (PBS; pH 7.2), and their optical density (OD) was measured at 660 nm. Serial dilutions were then prepared in PBS up to 10⁻⁸.
2. *Minimum inhibitory concentration (MIC)*: Serial dilutions of AgNCl/PMAA (54 to 0.1 ppm silver) and SDF (248,400 to 485 ppm silver) were prepared. In 96-well plates, 100 μ L of each dilution, 100 μ L of bacterial culture, and 100 μ L of BHI broth were added. Positive and negative controls were included. The MIC was determined by recording the initial OD and measuring bacterial growth at 660 nm after 24 hours of anaerobic incubation at 37 °C.
3. *Minimum bactericidal concentration (MBC)*: MBC was determined using the *Agar Diffusion Test* (ADT). Aliquots of 10 μ L were taken separately from the treated wells after 24 hours of anaerobic incubation and plated on BHI agar. The plates were incubated

for 48 hours at 37 °C to determine the presence or absence of bacteria.

4. *Colony-forming units (CFU)*: A 10- μ L aliquot of the AgNCl/PMAA dilution was removed, plated on BHI agar, and uniformly distributed over the plate. The plates were incubated for 48 hours at 37 °C. CFU/mL counts for both bacterial strains were quantified using a digital counter, and the measurements were repeated three times.

Cytotoxicity

Cytotoxicity was assessed because, for the product to be suitable for clinical application, it must demonstrate that it is neither toxic nor irritating to the surrounding tissues and/or structures. For this purpose, pulp cell cultures were used because these cells are more susceptible to irritation due to their proximity to the pulpal floor in caries lesions. The MTT assay was selected to evaluate this property.

1. *Cell culture*: Dental pulp stem cells (DPSC) were obtained from a commercial supplier and cultured in alpha-minimum essential medium (α -MEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin (P/S). The cells were incubated at 37 °C and 5% CO₂. To ensure optimal growth, the culture medium was changed every 3–4 days until the cells reached confluence.
2. *MTT assay*: DPSC were seeded in 96-well plates (7,000 cells/well) and incubated for 24 hours. The medium was then replaced with 200 μ L of fresh medium containing different concentrations of AgNCl/PMAA (20% to 0.03%, prepared by serial dilution). SDF at 38% was used as a positive control. The plates were incubated for 24 and 72 hours. Following morphological examination by inverted microscopy, mitochondrial function was assessed using the MTT assay. Twenty μ L of MTT (5 mg/mL) were added, and the samples were incubated for 4 hours. Subsequently, 100 μ L of dimethyl sulfoxide (DMSO) were added, and the plates were kept in the dark. Optical density (OD) was measured at 570 nm using an ELISA reader. The assay was performed in triplicate in three independent experiments. Cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{\text{OD of treated cells}}{\text{OD of untreated cells}} \times 100$$

Analysis plan

Data were analyzed using SPSS version 20.0. Statistical significance was set at $p < 0.05$, with a 95% confidence level. The analysis was structured as follows:

Physical properties

1. **Color changes:** Color stability was assessed by comparing the color coordinates at four time points: baseline (R0), after demineralization (R1), at 24 hours (R2), and at 7 days of treatment (R3). Statistical analysis was performed using the paired Student's t-test, with the R1 measurement used as the reference value to determine the significance of the changes produced by the therapeutic agents (AgNClS/PMAA and SDF).
2. **Shear bond strength:** ANOVA followed by Scheffé's post hoc test was used, with a significance level of 95% ($p < 0.05$).
3. **Surface hardness:** Differences among the treatment groups were evaluated using ANOVA, followed by Bonferroni's post hoc test to identify groups with statistically significant differences ($p < 0.05$).
4. **Penetration depth:** To compare the treatment options at each penetration depth, Student's t-test was applied ($p < 0.05$). To analyze differences in silver concentration across all penetration depths, Student-Newman-Keuls (SNK) test was used ($\alpha = 0.05$).

Biological properties

1. **Antibacterial activity:** The respective treatments were compared with an untreated control group using ANOVA ($p < 0.05$).

2. **Cytotoxicity:** The groups were compared using the Mann-Whitney and chi-square tests. Within-group comparisons were performed using the Wilcoxon and McNemar tests, with a significance level of 95% ($p < 0.05$).

RESULTS

Synthesis, characterization, and stability of the AgNClS/PMAA Complex

AgNClS/PMAA consisted of clusters containing 3–5 silver atoms, with sizes below 1 nm and therefore below the resolution limit of atomic force microscopy; consequently, only substrate roughness was observed (14,15).

Figure 1A summarizes the synthesis and spectroscopic characterization. The EEM map and UV-Vis spectra confirmed a single signal ($\lambda_{exc}/\lambda_{em} = 520 \text{ nm}/630 \text{ nm}$), indicating that these 3–5-atom AgNClS were the only fluorescent particles present in the solution.

Regarding stability, the positions of the absorption and emission bands, as well as fluorescence intensity, remained unchanged over the 9-month observation period (Figure 1B). These results indicate that the nanoclusters retained their original size and structure, demonstrating high temporal stability and resistance to coalescence, even in the presence of oxygen.

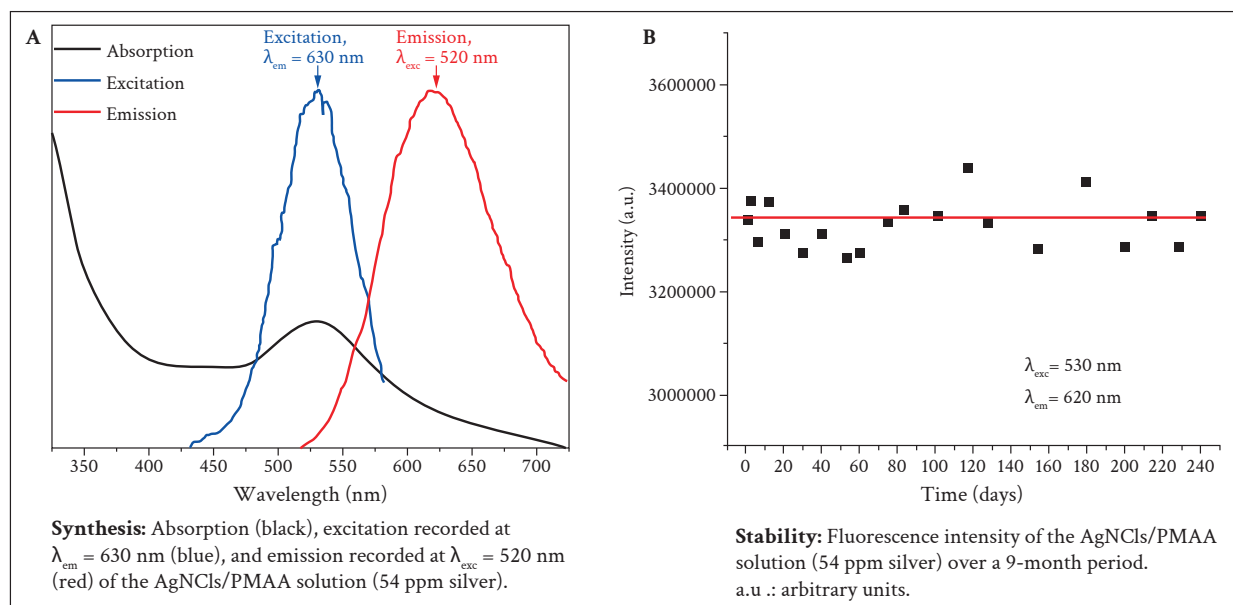


Figure 1. Synthesis and stability of AgNClS/PMAA.

Physical properties

Color changes

Group A (AgNClS/PMAA) exhibited color stability ($p = 0.24$) in demineralized dentin at both 24 hours and 7 days. In contrast, significant color changes were

observed in group B (SDF) ($p = 0.02$) following its application to the demineralized dentin surfaces (Table 1).

Shear bond strength

SBS values were significantly higher ($p < 0.01$) in group A (AgNClS/PMAA) than in groups B (SDF) and

C (control) ($p < 0.05$). No significant differences were found between groups B and C ($p > 0.05$) (Table 1). The control and SDF-treated specimens exhibited adhesive

failures, whereas mixed failures were observed in 3 of the 8 specimens in the AgNClS/PMAA group.

Table 1. Color changes and shear bond strength of the treatment groups.

Group	n	Color changes*			Shear bond strength**	
		ΔE 1-2	ΔE 1-3	p (t-test)	SBS (MPa)	ANOVA (p-value)
A	8	4.4 ± 2.9	2.9 ± 1.7	0.24	10.4 ± 2.7^a	0.003
B	8	8.3 ± 2.6	12.6 ± 3.8	0.02	3.3 ± 1.3^b	0.05
C	8	2.2 ± 1.3	2.7 ± 1.6	0.68	4.0 ± 0.4^b	0.05

* Mean color differences and standard deviations of the treatment groups between application of the surface treatment and subsequent measurements.

** Different superscript letters indicate statistically significant differences. ΔE : color difference.

Surface hardness

Vickers microhardness analysis revealed statistically significant differences among the groups evaluated (ANOVA: $F(3, 28) = 114.73$; $p = 0.0001$). Multiple comparisons using Bonferroni's test showed that group A (AgNClS/PMAA: 111.2 ± 8.2 MPa) and group C (sound dentin: 103.3 ± 7.2 MPa) exhibited the highest hardness values, with no significant differences between them ($p > 0.05$). However, both groups showed significantly higher values ($p = 0.001$) than group B (SDF: 72.3 ± 6.4 MPa) and group D (untreated artificial lesion: 50.5 ± 3.0 MPa). These results confirm that treatment with AgNClS/PMAA achieves microhardness levels comparable to those of sound dentin.

Penetration depth

Energy-dispersive X-ray spectroscopy (EDS) analysis confirmed the absence of silver in sound dentin (group C) and in the control group consisting of untreated demineralized dentin (group D). In groups A and B, silver was detected up to a depth of 400 μm and quantified as weight percentage. Figure 2 shows that group B (38% SDF) exhibited the highest silver content at the surface (0–50 μm), whereas group A (AgNClS/PMAA) maintained significant and consistent amounts of silver throughout the entire evaluated depth (0–400 μm).

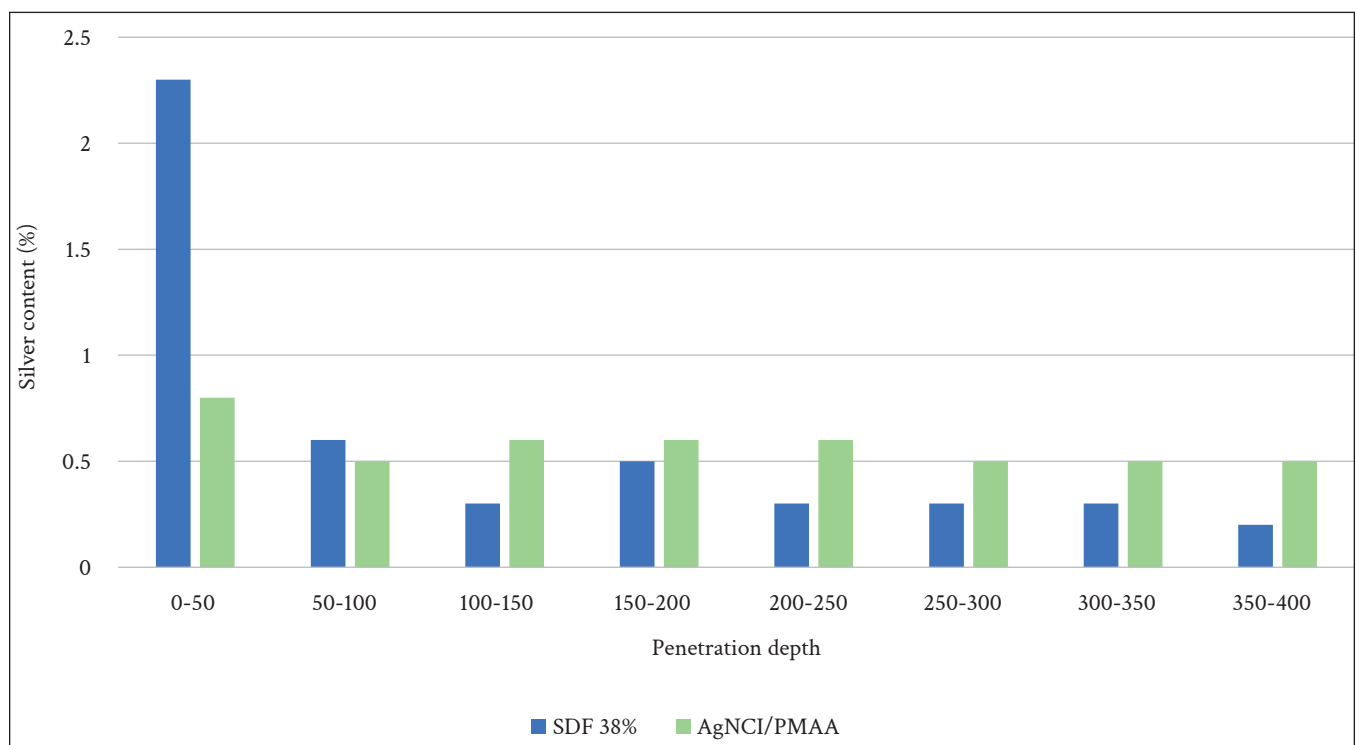


Figure 2. Penetration of silver ions at different depths of treated dentin, as measured by EDS.

Biological properties

Antibacterial activity

1. **Minimum inhibitory concentration:** Figure 3 shows that inhibition of bacterial growth increases with AgNCl/PMAA concentration up to the 1:16 dilution (1.25%; 3.4 ppm silver), at which point a plateau is reached. This low MIC value confirms the strong antibacterial activity of AgNCl/PMAA against both strains tested (*S. mutans* and *L. acidophilus*).
2. **Minimum bactericidal concentration:** Based on bacterial growth observed on the agar plates, the MBC for both cariogenic bacteria was determined to correspond to a 1:8 dilution (2.5%). This value represents

the lowest concentration required to eliminate most of the bacterial inoculum.

3. **Colony-forming units:** CFU counts for *S. mutans* and *L. acidophilus* were determined for AgNCl/PMAA and SDF at a comparative dilution of 1:16. The CFU/mL count in the AgNCl/PMAA group (4.3 ± 0.4 for *S. mutans*; 6.3 ± 0.3 for *L. acidophilus*) was significantly lower than that in the controls (13.7 ± 1.6 and 15.9 ± 0.8 , respectively; $p < 0.01$). For SDF, at the same dilution, counts of 8.6 CFU/mL for *S. mutans* and 9.5 CFU/mL for *L. acidophilus* were obtained. The AgNCl/PMAA solution was more effective than SDF, despite having a silver concentration of 6.75 ppm, which is 4,600 times lower than that of SDF (15,525 ppm) at the same dilution.

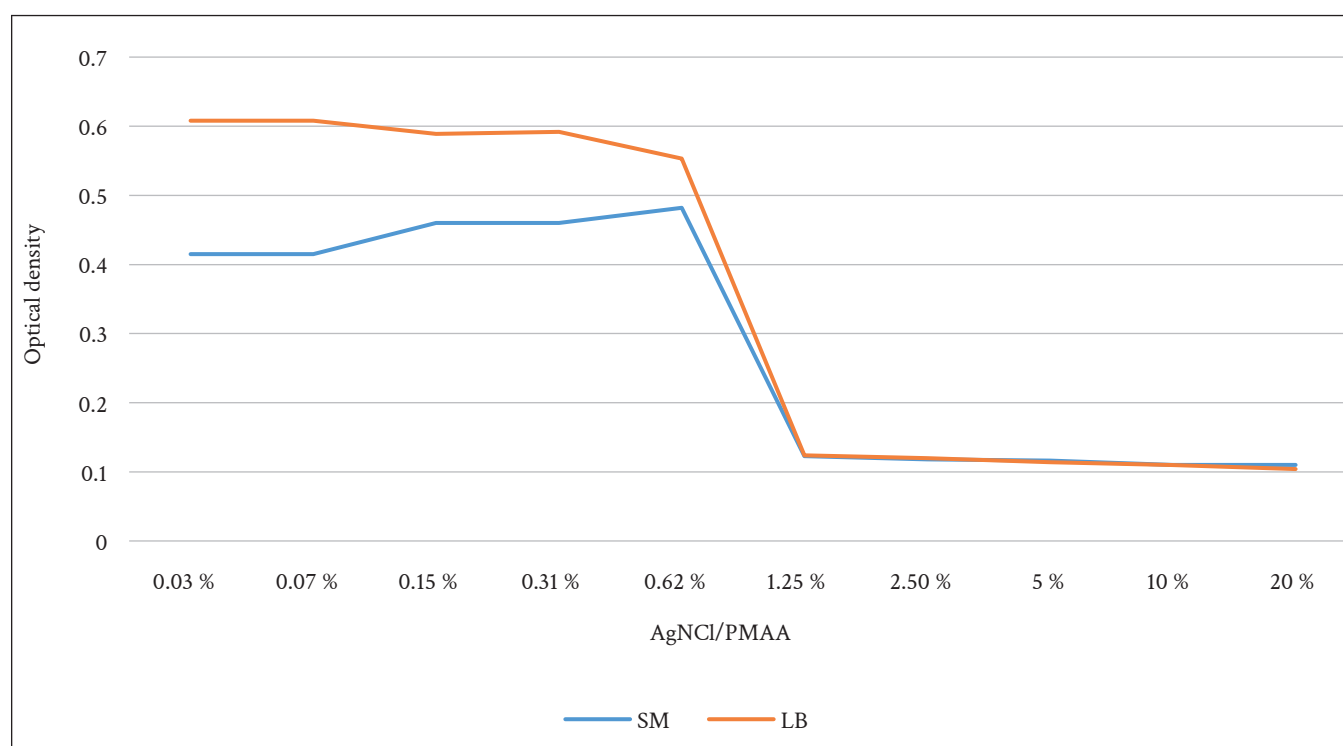


Figure 3. Line graph showing the optical density of *S. mutans* and *L. acidophilus* at different concentrations of AgNCl/PMAA. Solution dilution factors: 20% (1); 10% (1:2); 5% (1:4); 2.5% (1:8); 1.25% (1:16); 0.03% (1:512).

Cytotoxicity

The results of the cell viability experiments at different concentrations of AgNCl/PMAA and SDF after 24 and 48 hours of incubation are presented in Table 2. Cell density increased as the concentration of the material extract decreased.

DISCUSSION

The comprehensive evaluation of the physical and biological properties positions AgNCl/PMAA as a potentially superior alternative to SDF. First, its color stability is particularly noteworthy: unlike SDF, it did

not produce clinically significant color changes in demineralized dentin. This represents a relevant clinical advantage, as it addresses one of the main esthetic barriers associated with the use of SDF.

Regarding adhesive properties, GIC SBS values were significantly higher in the AgNCl/PMAA group than in the SDF-treated group. Furthermore, the predominance of mixed failures in the experimental group suggests better integration between the material and the dentin substrate. This improvement could be attributed to the vehicle used, the polymeric precursor PMAA, which has previously demonstrated its ability to interact chemically with collagen fibers in demineralized dentin.

Table 2. MTT assay results after 24 and 48 hours of incubation for AgNCls/PMAA and SDF at different concentrations.

Dilution	Ag concentration (ppm)		24 h (mean $\pm$ SD)		48 h (mean $\pm$ SD)	
	AgNCs/PMAA	SDF	AgNCs/PMAA	SDF	AgNCs/PMAA	SDF
1	54.00	248.400	40.7 $\pm$ 0.01	19.2 $\pm$ 0.09	38.6 $\pm$ 0.05	21.4 $\pm$ 0.07
1:2	27.00	124.200	40.5 $\pm$ 0.01	45.3 $\pm$ 0.03	37.5 $\pm$ 0.06	40.4 $\pm$ 0.03
1:4	13.50	62.100	47.7 $\pm$ 0.07	46.3 $\pm$ 0.03	46.4 $\pm$ 0.05	39.9 $\pm$ 0.04
1:8	6.75	31.050	46.6 $\pm$ 0.05	49.3 $\pm$ 0.05	43.7 $\pm$ 0.06	43.7 $\pm$ 0.07
1:16	3.40	15.525	49.2 $\pm$ 0.06	52.9 $\pm$ 0.07	47.4 $\pm$ 0.04	44.2 $\pm$ 0.05
1:32	1.70	7762	53.3 $\pm$ 0.08	55.5 $\pm$ 0.09	50.3 $\pm$ 0.01	49.5 $\pm$ 0.02
1:64	0.85	3882	55.0 $\pm$ 0.03	57.8 $\pm$ 0.06	53.8 $\pm$ 0.07	52.7 $\pm$ 0.03
1:128	0.42	1941	63.0 $\pm$ 0.05	59.6 $\pm$ 0.01	61.2 $\pm$ 0.01	58.5 $\pm$ 0.05
1:256	0.21	970	78.7 $\pm$ 0.09	67.8 $\pm$ 0.07	75.6 $\pm$ 0.05	64.4 $\pm$ 0.03
1:512	0.10	485	87.9 $\pm$ 0.01	80.0 $\pm$ 0.07	88.1 $\pm$ 0.09	78.3 $\pm$ 0.03

SD: standard deviation.

This behavior is also reflected in the recovery of surface hardness, with the AgNCls/PMAA group achieving values similar to or even higher than those of sound dentin. In this regard, the hypothesis that PMAA may induce modifications in the organization or arrangement of collagen fibers opens an interesting avenue for future research. Conditioning with this polycarboxylic acid could not only increase the availability of calcium ions capable of binding to carboxyl groups but could also contribute to the formation of a more compact and denser structural network with improved mechanical properties.

SEM-EDS analysis also confirmed the material's penetration capacity. Whereas SDF showed a higher silver concentration primarily restricted to the surface (0–50 μ m), AgNCls/PMAA maintained significant and relatively constant amounts of silver throughout the entire depth analyzed. This finding is particularly relevant because it overcomes the penetration limitations reported in previous studies that used higher-viscosity polymeric precursors, which were unable to infiltrate beyond 20 μ m, thereby potentially favoring recovery throughout the depth of the carious lesion (16).

From a biological standpoint, results are encouraging in terms of efficacy and safety. *In vitro* assays showed that the solution acts as a potent antibacterial agent against cariogenic bacteria such as *S. mutans* and *L. acidophilus*, even at silver concentrations substantially lower than those used in SDF (17,18). This suggests that cariogenic activity could be controlled without compromising biocompatibility.

The safety of the compound is further supported by the observation that AgNCls/PMAA cytotoxicity decreases

based on concentration. This profile is particularly favorable for the development of clinically applicable biomaterials and could be attributed to the smaller size of the nanoclusters compared with conventional silver nanoparticles, suggesting a more controlled biological behavior (19–21).

An inherent limitation of this study is its *in vitro* design, which does not fully reproduce the complexity of the oral environment. The static caries model used does not account for the dynamic cycles of demineralization and remineralization modulated by fluctuations in salivary pH and the buffering capacity of saliva *in vivo*. Similarly, the absence of acquired biofilm and salivary proteins constitutes an additional limitation, as these components regulate ion exchange and surface energy, factors that could influence both the effectiveness of AgNCls/PMAA and its interaction with restorative materials.

Furthermore, bond strength was evaluated using a single type of high-viscosity glass ionomer cement. Considering that the chemical interactions between PMAA and the substrate may vary according to the composition of the restorative material, the results cannot be broadly extrapolated to other formulations. In the same way, the absence of cyclic mechanical loading reflects behavior under ideal conditions that may differ from the actual clinical environment. Therefore, *in vivo* studies will be necessary to validate the clinical safety and efficacy of this solution.

Despite these limitations, the findings obtained in this controlled model provide a solid foundation supporting the therapeutic potential of the material. The physicochemical, mechanical, and biological properties observed suggest that the AgNCls/PMAA complex

could overcome several of the limitations associated with SDF-based treatments and represent a significant advance in the field of minimally invasive dentistry.

CONCLUSIONS

This *in vitro* study confirms the feasibility and high potential of the AgNCl/PMAA formulation as a promising and superior alternative to 38% SDF for the noninvasive treatment of dental caries. The formula-

tion not only eliminated the esthetic drawback of dentin staining but also improved the bonding of restorative cements to the treated surface and restored dentin surface hardness to levels comparable to or higher than those of sound dentin. At the biological level, it demonstrated lower cytotoxicity in pulp cells and potential antibacterial activity against *S. mutans* and *L. acidophilus*. In addition, the high long-term stability of the nanoclusters could allow their storage without compromising their properties.

Conflict of interest:

The authors declare no conflicts of interest.

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Authorship contribution:

CMB: Investigation, data collection, formal analysis, Investigation, writing of original draft.

PGA: conceptualization, methodology, Investigation. **AJP:** resources. **MGF:** conceptualization, methodology, supervision, project administration, writing - review & editing.

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All experimental protocols were approved by the Research and Development Office of Universidad Católica de Córdoba, Argentina, through its SI-UCC research grants, and were conducted in accordance with the relevant guidelines and regulations.

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Google Gemini was used for grammar, spelling, and punctuation correction, as well as for editorial support to improve the clarity and fluency of the text. The authors reviewed and edited the resulting content and assume full responsibility for the integrity and accuracy of the manuscript.

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