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Silver Distribution and Release from an Antimicrobial Denture Base Resin Containing Silver Colloidal Nanoparticles

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Abstract

Purpose: The aim of this study was to evaluate a denture base resin containing silver colloidal nanoparticles through morphological analysis to check the distribution and dispersion of these particles in the polymer and by testing the silver release in deionized water at different time periods.
Materials and Methods: A Lucitone 550 denture resin was used, and silver nanoparticles were synthesized by reduction of silver nitrate with sodium citrate. The acrylic resin was prepared in accordance with the manufacturers' instructions, and silver nanoparticle suspension was added to the acrylic resin monomer in different concentrations (0.05, 0.5, and 5 vol% silver colloidal). Controls devoid of silver nanoparticles were included. The specimens were stored in deionized water at 37°C for 7, 15, 30, 60, and 120 days, and each solution was analyzed using atomic absorption spectroscopy.
Results: Silver was not detected in deionized water regardless of the silver nanoparticles added to the resin and of the storage period. Micrographs showed that with lower concentrations, the distribution of silver nanoparticles was reduced, whereas their dispersion was improved in the polymer. Moreover, after 120 days of storage, nanoparticles were mainly located on the surface of the nanocomposite specimens.
Conclusions: Incorporation of silver nanoparticles in the acrylic resin was evidenced. Moreover, silver was not detected by the detection limit of the atomic absorption spectrophotometer used in this study, even after 120 days of storage in deionized water. Silver nanoparticles are incorporated in the PMMA denture resin to attain an effective antimicrobial material to help control common infections involving oral mucosal tissues in complete denture wearers.

About 50–70% of complete denture wearers present a pathogenic state known as denture stomatitis.[1,2] This pathology is an inflammatory process characterized by homogeneous erythema or red focal areas, especially in the palatal mucosa and usually associated with *Candida* species, particularly *Candida albicans*.[3,4] The treatment of denture stomatitis is problematic since recurrent infection is frequently encountered after the end of treatment. In addition, several *Candida* species exhibit acquired or inherent resistance to several of the antifungal drugs frequently used to treat this condition.[5] Stomatitis represents a challenge for the dental field, and methods for prevention of denture stomatitis, such as the incorporation of antimicrobial agents into polymers used as denture base and as tissue conditioners, have been developed.[6-11] In contrast, silver (Ag) has been widely used in medical and life-care polymers[12-29] and exhibits antimicrobial action against Gram-positive and Gram-negative bacteria[30-34] and fungi.[7,8,35-37] Silver ions (Ag^+) bind to electron donor groups in biological molecules containing sulfur or nitrogen, resulting in defects in the bacteria cell membrane and leading to loss of their cell contents and to the death of bacteria.[24] Furthermore, Ag^+ can interact with the DNA chain, preventing cell reproduction.[24] However, the use of polymers containing silver must be undertaken with caution, since a concentration-dependent toxicity has been demonstrated.[38] For instance, concentrations of silver between 5 μg/mL and 10 μg/mL induced necrosis or apoptosis of mouse spermatogonial stem cells.[38]

The use of silver nanoparticles as antimicrobial agents has received a great deal of attention. Due to their small size, nanoparticles present a large surface area and therefore larger available

area for oxidation. Furthermore, the incorporation of silver nanoparticles into polymer can be useful in drugs,[18] wound dressing,[18] venous,[27] and urinary catheters,[15] wound sutures,[24] artificial tendons,[24] orthodontic adhesives,[23] surgical masks,[20] and endotracheal tubes.[39]

Furno et al[17] used organometallic precursors dissolved in supercritical carbon dioxide to synthesize in situ and incorporated silver nanoparticles into a silicone matrix used in implantable devices. They verified that the release of Ag^+ in protein plasma was almost 15 times higher than in deionized water during the first 3 days and that a significant amount of Ag^+ was still being released during subsequent days. The study showed the avidity of Ag for proteins that could compromise the clinical efficacy of Ag-coated implantable devices, since the device surfaces become coated with glycoproteins from the tissue and plasma.[28] Thus, there must be enough Ag^+ available over a sufficient period to exceed those lost to protein binding.

Several studies[12,19,22,24-26] have evaluated the Ag^+ release from polyamide polymers (nylon) and morphologically characterized these materials by scanning (SEM) and transmission electron microscopy (TEM). Distribution and dispersion of silver particles seem to depend on the specific surface area of the particles. The smaller the diameter of the particle, the better the particle distribution and dispersion in the polymer mass.[12] Silver ion release was higher in hydrophilic polymers[19] and in polymers with a lower degree of crystallinity.[26] The Ag^+ release rate increased with Ag concentration in the polymer and with the immersion time in water.[12] Finally, Damm et al[24] reported that Ag^+ release from nanocomposites was more effective than that from microcomposites.

Yu et al[11] incorporated silver nanoparticles in polymethylmethacrylate (PMMA) denture base, and noticed that the release of these nanoparticles was extremely slow during the test, with a very small fraction of Ag^+ released in artificial saliva after 54 days. In PMMA nanofibers containing silver nanoparticles, Kong and Jang[21] observed that after 6 months of immersion in distilled water, some nanoparticles leached out into the supernatant. Photographs of TEM confirmed the release, showing the nanofiber surface completely covered by nanoparticles before storage in water, and, after that, only a few particles remained adhered to the surface of the material.

Another factor that may influence the release of silver nanoparticles is their specific surface area.[12] Irzh et al[29] observed by SEM that the best way to achieve a coating on PMMA surfaces with small silver particles was by using poly(ethylene glycol) as a reducing agent and solvent simultaneously. Silver acetate was found to be a better Ag precursor than silver nitrate, because acetate ions can act as surfactants preventing aggregation of nanoparticles.

Although the literature reports various studies related to silver nanocomposites with antimicrobial application in the medical field,[15,17-22,24,27,39] very few studies about addition of silver particles to denture base resins have been published.[8,11] Despite this, the aim of this in vitro study was to evaluate a denture base resin containing silver colloidal nanoparticles through morphological analysis by checking the distribution and dispersion of these particles in the polymer, and by testing the silver release in deionized water at different time periods. The hypothesis tested was that the silver nanoparticle concentration added to the PMMA/Ag nanocomposite would influence (1) the silver distribution and dispersion in the polymer and (2) the amount of silver released, and this amount could change according to the immersion period in deionized water. Silver nanoparticles are incorporated into a PMMA denture resin to attain an effective antimicrobial material to control common infections involving oral mucosal tissues in complete denture wearers.

Materials and methods

Synthesis and characterization of silver colloidal nanoparticles

Silver nanoparticles were synthesized by means of the Turkevich method[40] through the reduction of silver nitrate ($AgNO_3$) (Merck KGaA, Darmstadt, Hesse, Germany) with sodium citrate ($Na_3C_6H_5O_7$) (Merck KGaA), as detailed elsewhere.[41,42] The aqueous solution of silver nitrate and sodium citrate was kept at boiling temperature for a couple of minutes until the solution turned amber yellow, indicating the formation of colloidal silver nanoparticles, confirmed by UV/Visible spectroscopy (Spectrophotometer Shimadzu MultSpec-1501, Shimadzu Corporation, Tokyo, Japan) and, later, by X-ray diffraction (XRD) (Diffractometer Rigaku DMax-2000PC, Rigaku Corporation, Tokyo, Japan). TEM (Electron Microscope FEG-VP Supra 35, Carl Zeiss, Jena, Thüringen, Germany) was used to record the micrograph images of synthesized silver nanoparticles.

Preparation of PMMA/Ag nanocomposite

For standardization of acrylic resin specimens, silicone rubber (Zetalabor, Zhermack S.p.A., Badia Polesine, Rovigo, Italy) molds were prepared from a metallic round pattern with compatible measures to the future specimen (diameter: 10 mm; height: 3 mm). The denture base resin (Lucitone 550, Dentsply Ind. e Com. Ltda, Petrópolis, Brazil) was mixed according to manufacturer's instructions. For each specimen, 4 g of powder was mixed with 1.9 mL of liquid. The aqueous colloidal dispersion containing silver nanoparticles was added to the monomer of the acrylic resin at 0.05%, 0.5%, and 5%, based on the polymer mass. Specimens devoid of silver nanoparticles were included as controls. Five specimens were fabricated for each concentration. The nanocomposites were packed directly into the silicone molds invested in dental stone (Herodent, Vigodent S/A Ind. e Com., Rio de Janeiro, Brazil). Specimens were pressed using a hydraulic press (Delta, Vipi-Delta Máquinas Especiais, Pirassununga, Brazil), and they were polymerized in conventional water bath for 1.5 hours at 73°C and then 30 minutes at 100°C (Thermal tank, Solab Equipamentos para Laboratório Ltd., Piracicaba, Brazil). After cooling, the specimens were removed from the flask.

Silver nanoparticles release test

All specimens were immersed in 10 mL of deionized water inside of polypropylene tubes (Injeplast, São Paulo, Brazil) and kept at 37°C under agitation for 6 hours a day (Shaking incubator, Tecnal TE 420, Tecnal Equipamentos para Laboratórios Ltd., Piracicaba, Brazil) for different periods: $t_1 = 7$,

$t_2 = 15$, $t_3 = 30$, $t_4 = 60$, and $t_5 = 120$ days. The water was reconstituted every 10 days to account for evaporation. During each period, 1 mL of solution of each tube was collected, and Ag dosage was analyzed by atomic absorption spectroscopy (Varian AA240FS, Varian Inc., Palo Alto, CA), and the amount of Ag^+ released was calculated using a linear calibration curve in the equipment prepared from standard $AgNO_3$ solutions at different concentrations. The evaluations for detection of Ag released were carried out in a spectrophotometer with a limit for detection of Ag of 0.003 mg/L, which included the nanocomposites with 5% and 0.5% silver nanoparticles. Therefore, the specimens with 0.05% of silver colloidal solution (i.e., 0.00162 mg of Ag/L by specimen) were not analyzed.

Organic reagent test

Rhodanine (Sigma–Aldrich Brazil Ltda, São Paulo, Brazil), a selective and sensitive reagent for the detection of Ag^+,[21] was used as an organic reagent test to monitor Ag release. The solution of PMMA/Ag nanocomposite with higher amounts of silver nanoparticles (5%) stored for 120 days was evaluated. Rhodanine (20 mM) was added to this solution, and the color changes were monitored and recorded by a digital camera (Sony Cyber-shot DSC-W130 8.1Mp, New York, NY). A solution of silver nitrate (1 mg/L) was used as control.[21]

Morphologic assessment of the PMMA/Ag nanocomposite

SEM (Electron Microscope FEG-VP Supra 35) was used to investigate the morphology of the PMMA/Ag nanocomposites. This analysis was performed before and after storage in deionized water for 120 days. Some of the specimens were frozen with liquid nitrogen and fractions used to analyze the bulk sample.

Preliminary antimicrobial and mechanical tests

The preliminary antimicrobial efficacy of the silver nanoparticle colloidal suspension and of the PMMA/Ag nanocomposites against *Candida albicans* (ATCC 10231) was evaluated by zone of growth inhibition and number of *C. albicans* colonies in agar plates, respectively. For contact biocidal property of silver nanoparticles, *C.albicans* diluted in 0.9% NaCl ($1 \pm 0.2 \times 10^7$ CFU/mL) was plated onto Sabouraud dextrose agar (Sabouraud Dextrose Agar, Becton Dickinson France SAS, Le Pont de Claix, France). Sterile paper disks were placed on the agar and wetted with 10 μL of silver colloidal nanoparticles. These agar plates were incubated for 48 hours at 37°C ± 2°C. The zones of inhibition were visualized and compared with deionized water and nystatin.

To examine the antimicrobial efficacy of the PMMA/Ag nanocomposites, *C. albicans* was diluted in 0.9% NaCl, and a yeast suspension of approximately 10^7 CFU/mL was prepared using a spectrophotometer (at 530 nm). Each specimen was placed in a test tube containing 9.9 mL of Sabouraud dextrose broth (Sabouraud Dextrose Broth, Becton Dickinson France SAS), into which were dispensed 100 μL of the yeast suspension. The final concentration of cells was 10^5 CFU/mL. After incubation for 24 hours at 37°C, the specimens were collected and washed five times in a standard manner by dipping in sterile deionized water to remove loosely attached cells. Viable cells on the surface of the specimens were counted in agar plates. Counting was repeated three times per group, and data were recorded as means.

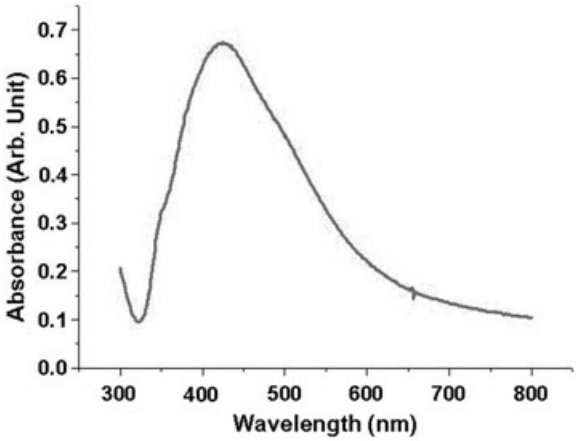

Figure 1 UV/Vis spectrum of silver nanoparticle aqueous dispersion prepared by reduction of silver nitrate with sodium citrate.

The flexural strength test was performed in accordance with International Standards Organization (ISO) specification no. 1567.[43] Twelve specimens were fabricated and assigned to four groups (n = 3) according to their percentage of silver colloidal nanoparticles: 0% (control), 0.05%, 0.5%, and 5%. The specimens were subjected to the flexural strength test in a Universal testing machine (EMIC-DL 3000; EMIC Equip. e Sist. de Ensaios Ltd., Curitiba, Brazil), using three-point loading. A 1 mm/min crosshead speed was used and the load was applied until fracture. Mean flexural strengths were calculated in MPa.

Results

Synthesis and characterization of silver colloidal nanoparticles

Silver nanoparticles are known to exhibit a characteristic surface plasmon resonance band that can be measured by UV-Vis spectroscopy. Figure 1 shows the plasmon band of the silver nanoparticle suspensions, showing a typical absorbance peak for nanoparticles centered at 430 nm. The symmetrical shape of the plasmon band can indicate a relative sharp particle size distribution. To confirm it, SEM and TEM images were collected. When the nanoparticles were observed by SEM on a silicon holder (Fig 2A), it was possible to conclude that the particles were uniform with an average diameter of 60 nm. Moreover, by TEM analysis (Fig 2B) some agglomerates were observed, probably due to the specimen preparation procedure; however, if the images from SEM and TEM are analyzed together, it is possible to conclude that the particles are well formed and nonagglomerated in the aqueous dispersion. If not, agglomerates would be observed in both images. It was also interesting to note that using the two different approaches, silver nanoparticles showed a spherical shape of the particles and sharp size

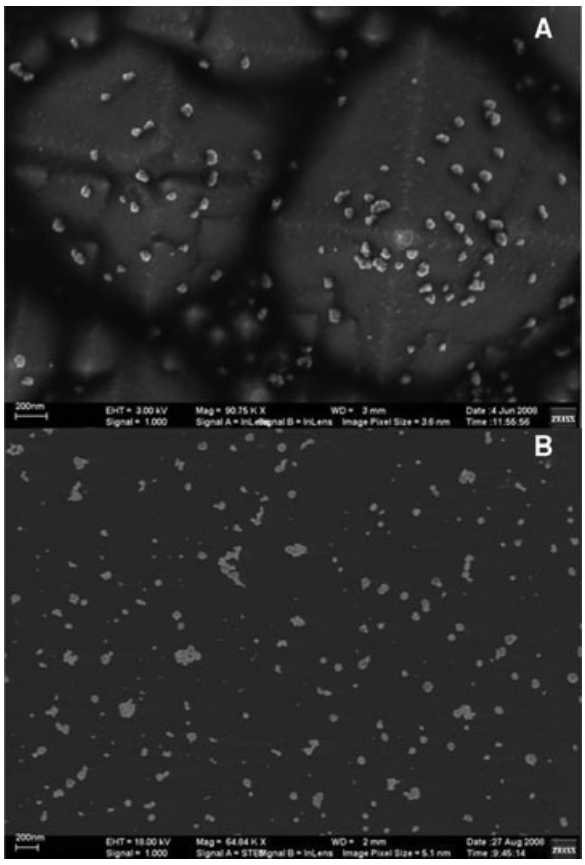

Figure 2 (A) SEM image of silver nanoparticle on silicon substrate degraded by KOH for 2 hours. Silver colloidal was deposited and dried to obtain the image. This technique illustrates more clearly the shape and size of the silver nanoparticles (white particles ca. 60 nm; dark, citrate salt precipitated to the deeper part of the silicious substrate). (B) TEM images of silver nanoparticles. Note the small size of the silver particles. Magnification: (A) 90,750× (B) 64,840×.

distribution, in agreement with the UV/Vis information. It is important note that the silver nanoparticles have uniform morphologies because of an intrinsic correlation between size and antimicrobial activity. The characteristic XRD pattern (Fig 3) also confirmed the presence of metallic silver nanoparticles, with a cubic crystalline structure (Joint Committee on Powder Diffraction Standards 04-0783). The diffraction peaks assigned with Ag at 38.5°, 44.5°, 64.8°, and 78° can be attributed to the (111), (200), (220), and (311) crystallographic planes of metallic Ag, respectively. The diffraction peak (111) at 28.4° can be attributed to the silicon specimen holder.

Release of silver nanoparticles

PMMA/Ag nanocomposites with 0.5% and 5% silver nanoparticles were analyzed. Despite the high sensitivity of the analytical technique used, no Ag^+ was detected in the deionized water under all storage conditions and type of nanoparticle used. These findings were in contrast to those of Furno et al,[17] who observed Ag^+ release, even in distilled water. Figure 4 shows the silver absorption spectrum for the silver nitrate standard (A)

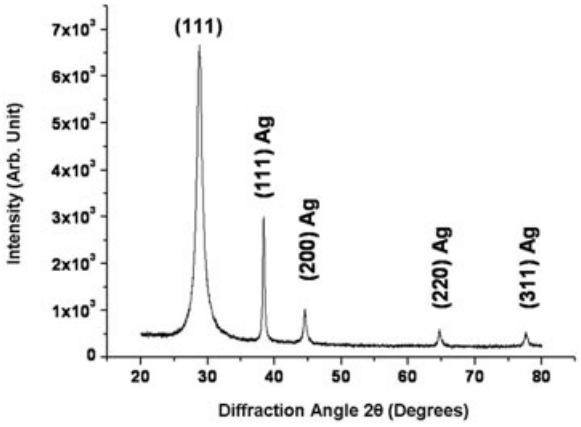

Figure 3 X-ray diffraction pattern of silver nanoparticle on silicon substrate. The (111) diffraction peak at 28.4 belongs to the substrate.

and the result found for one of the solutions (B) with no absorption level for Ag. All the aqueous media where the PMMA/Ag nanocomposites were immersed showed similar results, indicating that the method used to incorporate the nanoparticles into the polymeric matrix was effective to retain the nanoparticles.

Organic reagent test

The rhodanine test to identify the presence of free Ag^+ is based on the formation of an insoluble Ag^+-rhodanine complex. The presence of these complexes can be easily identified by the yellow color in aqueous media, followed by the segregation of brown precipitates. Figure 5A, for instance, shows

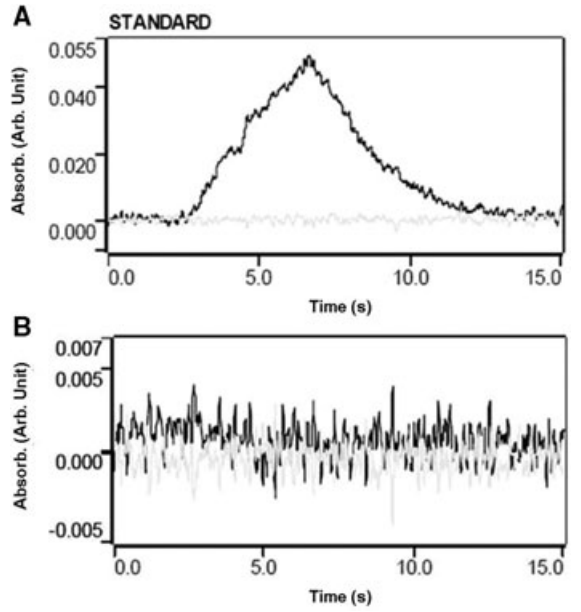

Figure 4 Silver absorption spectrum found for (A) standard solution containing 0.5 mg $AgNO_3$/L; (B) spectrum of one of the specimens, which was similar for all the others. Spectrum clearly shows the absorption of deionized water.

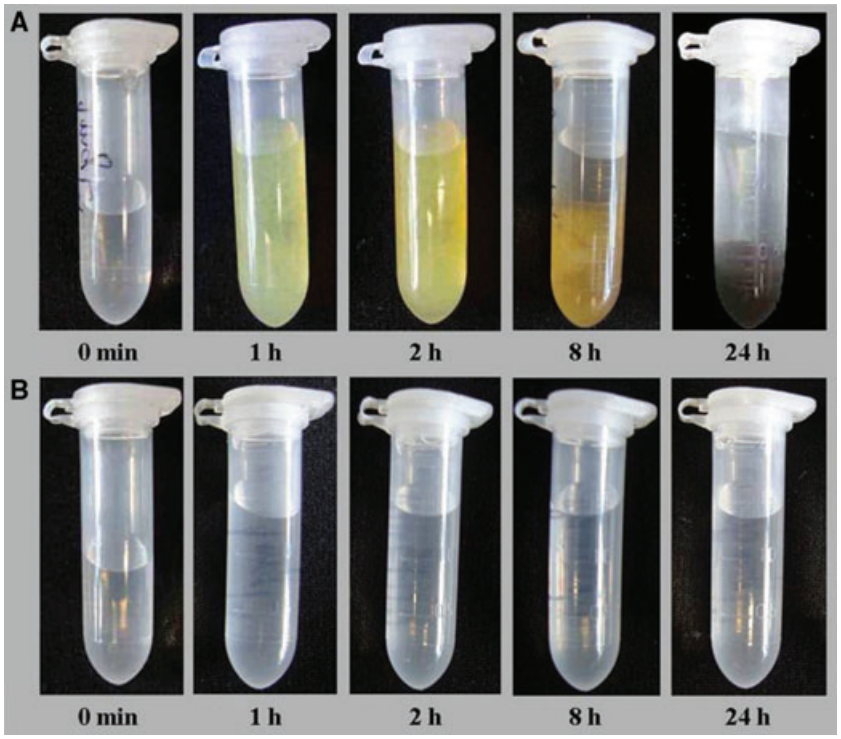

Figure 5 Rhodanine test for 24 hours: (A) $AgNO_3$ solution, showing the presence of Ag^+, and (B) liquid carried away from the specimen in which the nanocomposite at 5% silver colloidal nanoparticles was stored for 120 days, indicating the absence of Ag^+ in this solution.

the color change that occurs when rhodanine is added to an $AgNO_3$ aqueous solution, first becoming yellow after a couple hours and gradually forming the typical brown–yellow precipitates. However, when rhodanine was added to media where the PMMA/Ag nanocomposite with the highest concentration of silver nanoparticles was kept for 120 days (Fig 5B), a yellow color does not appear, nor was the brown precipitate observed, with the color remaining unchanged for the first 24 hours; definitive to conclude that the PMMA/Ag nanocomposites do not release Ag^+, in agreement with the atomic absorption results previously discussed.

Morphologic assessment of the PMMA/Ag nanocomposites

One of the most important characteristics of polymeric-based nanocomposites designed to be used as antimicrobials is the ability to keep the active nanoparticles on the surface well distributed and fixed. The analysis of silver nanoparticle release and the organic reagent test showed that the method used to impregnate the PMMA matrixes with silver nanoparticles was successful. Furthermore, SEM images of the nanocomposite surfaces showed how these nanoparticles are dispersed on the polymer surface. Figure 6 represents two SEM images of pure PMMA used as control—one of the surface of the specimen (A), and another (B), the fractured surface showing inside the bulk of the specimen. These two images should be used as reference when the nanocomposites are analyzed. It was interesting to observe the characteristic pattern of the fracture surface.

Figure 7 shows the surface of the PMMA/Ag nanocomposite with only 0.05% of nanoparticles before (A) and after (B) 120 days of storage in deionized water, and of the fractured surface of the nanocomposite (C to F), showing four images of the interior of the material. The white dots uniformly distributed on the surface of the polymer, identified as silver nanoparticles, are strong evidence of the homogeneous distribution of the nanoparticles on the surface. On the other hand, few nanoparticles were observed, indicating that the particles migrate from the bulk to the surface during the polymerizing process. Considering that Ag was not identified by absorption spectroscopy or by the complex reaction with rhodanine, it is improbable that these particles were eliminated from the specimen bulk to the solution. Instead, the most plausible hypothesis is that the nanoparticles migrate to the interface between polymer and ambient, where the energy is high enough to accommodate a large concentration of them.

Preliminary antimicrobial and mechanical tests

The zones of inhibition of silver colloidal nanoparticles are shown in Fig 8A and B. The results in Fig 8C showed that the nanocomposites had good efficacy against *C. albicans*, especially the PMMA/Ag nanocomposites containing 5% silver colloidal nanoparticles. In addition, similar flexural strength values between the nanocomposites and the control group were observed (Table 1).

Discussion

This study evaluated the incorporation of silver nanoparticles into an acrylic denture base resin through SEM images and the silver release by means of atomic absorption spectroscopy and

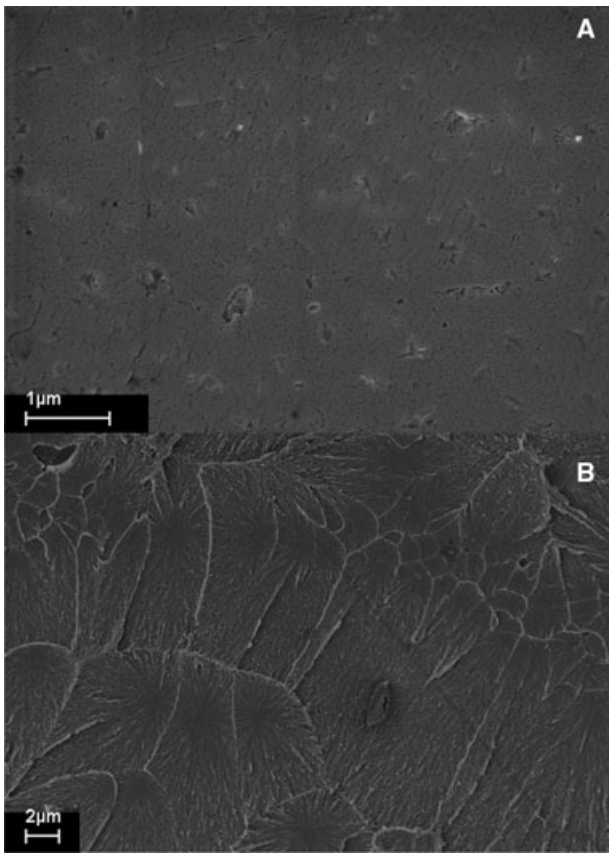

Figure 6 SEM images of the Lucitone 550 denture base resin with no silver nanoparticle. (A) polymer surface and (B) polymer fracture surface. Magnification (A) 25,000× (B) 5000×.

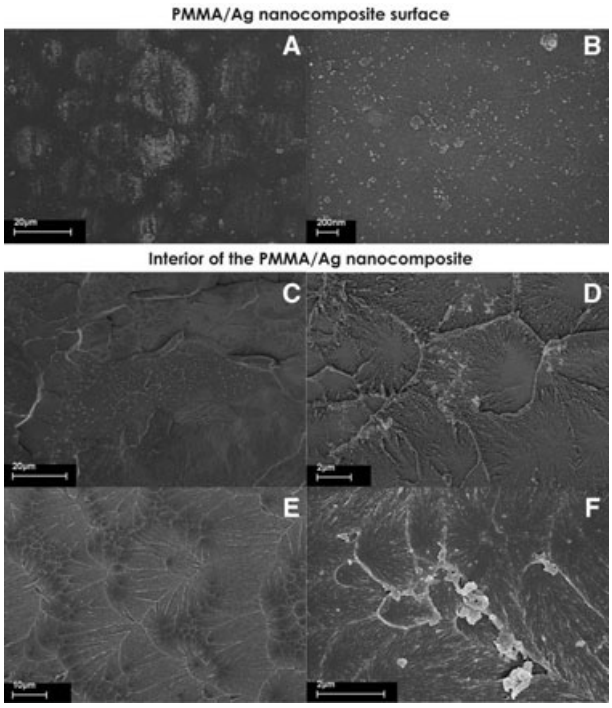

Figure 7 SEM of the PMMA/Ag 0.05% nanocomposite surface, before (A) and after (B) 120 days storage in deionized water. The nanocomposite surface before the storage period shows both fewer particles well distributed and dispersed than the surface after storage for 120 days. Interior of the PMMA/Ag 0.05% nanocomposite, before (C, D) and after (E, F) 120 days of storage in deionized water. Most of the particles are agglomerated and scarcely distributed in C and D, and very few particles are visualized in E and F. Magnification (A) 1690× (B) 61,890× (C) 1610× (D) 10,010× (E) 2000× (F) 20,000×.

chemical analysis. The research hypothesis was partly accepted, since the silver nanoparticle concentration added to the acrylic resin influenced the silver distribution and dispersion in the polymer, but did not influence the silver release, regardless of the immersion period in deionized water.

The nanocomposites with high contents of silver nanoparticles were kept in deionized water, and aliquots of the media were collected after 7, 15, 30, 60, and 120 days of storage, aiming to assess the influence of time on the release of silver particles. In accordance with a previous study,[22] the particle release from the surface of the nanocomposites should occur during the initial period. Thus, the rate of release should decrease with the necessity of water diffusion into the polymeric mass. Usually, water diffusion into the PMMA body could result in the plasticizing of the material, allowing the migration of the particles or Ag^+ toward the surface, which would be released into the water.[12] However, the results of the atomic absorption spectroscopy, which are very sensitive, did not indicate the presence of Ag in liquid media, even after the PMMA/Ag nanocomposites had been immersed for the longest period. Additional tests were carried out to quantify the Ag released, especially in the surface of the polymer. Ten specimens containing the maximum concentration of silver colloid (5%) were stored in deionized water in the same polypropylene tube under agitation at 37°C for 7 days. Nevertheless, again, no Ag was detected in that media. Furthermore, other tests were also conducted to evaluate if the Ag release would occur in a different medium of storage such as artificial saliva. Considering that artificial saliva presents substances with potential ionic charge ($Ca(NO_3)_2$; KCl; NaH_2PO_4; methyl parahydroxybenzoate of sodium), an attraction between inorganic particles in this liquid and Ag^+ in the PMMA would possibly occur; however, Ag was also not detected by the atomic absorption spectroscopy analysis. This finding was in disagreement with Furno et al[17] who evaluated silicones impregnated with silver nanoparticles, finding a higher release of Ag^+ in a human plasma protein solution than in deionized water. The difference between the results may be explained by differences in the nanoparticle synthesis method. Furno et al.[17] used an in situ route that has low yield to prepare silver nanoparticles, and therefore a high excess of Ag^+ ions that were free to migrate to the solution media. But when the Turkvich method[40] was used to prepare silver nanoparticles in a separate flask, their transference to the PMMA base to prepare the nanocomposite eliminated the interference of free Ag^+. Secondly, from the SEM images of the nanocomposite specimens before and after storage in water, it is clear that silver nanoparticles are well adhered on the surface of the PMMA

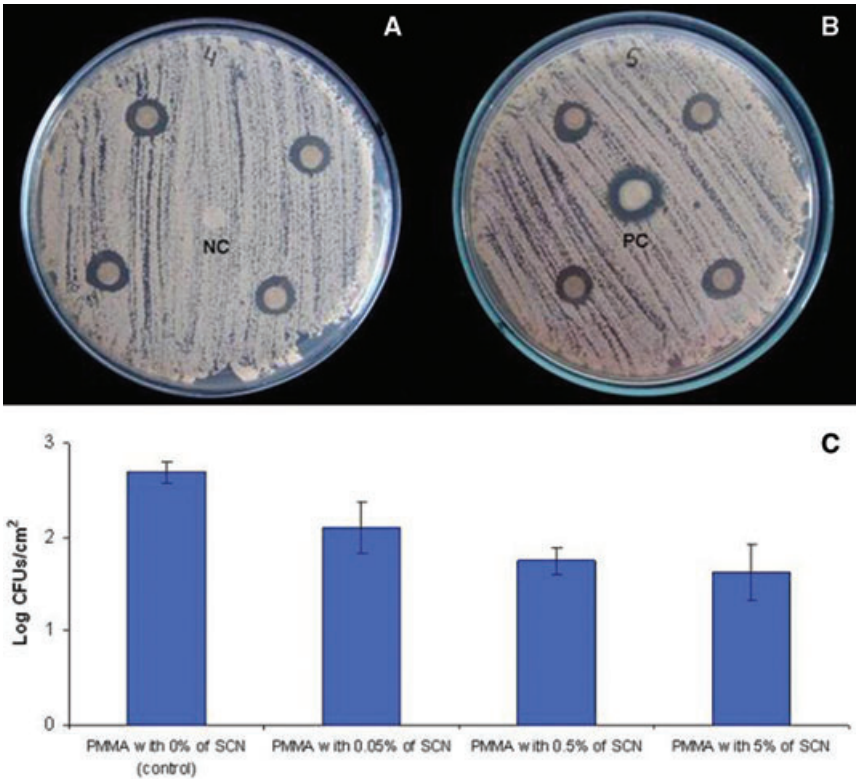

Figure 8 (A) and (B) Photograph images of the zone of inhibition of silver colloidal nanoparticles with 60 nm mean diameter. The zones of inhibition were compared with deionized water (negative control, NC) and nystatin (positive control, PC). (C) Logarithm of number of cells of *C. albicans* per cm^2 on PMMA/Ag nanocomposite surface. Error bars represent standard deviation.

matrix. Tests with rhodanine performed in water aliquots from the tubes with nanocomposites with the highest concentration (5%) immersed for 120 days also confirmed that the PMMA/Ag nanocomposites do not release Ag. For a blank test, rhodanine was also added to the aqueous colloidal suspensions of silver nanoparticles, indicating that Ag^+ in this colloid occurred only after 2 weeks, confirming that the nanoparticle itself does not react with rhodanine, but the test is only positive for free Ag^+.

Some studies have demonstrated that factors such as storage time in aqueous solution,[12,22] gradient of concentration between the Ag in the polymer and in the immersion liquid,[24] concentration and specific surface area of silver particles added to the polymer,[24] and the characteristics of the polymer base[19,26] may influence the release of Ag in nano- or microcomposites. In this study, Ag release was not identified independent of the nanoparticle amount in the PMMA/Ag nanocomposite and of the immersion period in deionized water. For instance, Fig 4B illustrates typical atomic absorption spectrums of a standard solution of $AgNO_3$ and a sample of water in which the specimen was stored, which is similar in all specimens regardless of the nanoparticle amount (5 or 0.5%) and storage period (up to 120 days); however, some authors[19,22] have reported that nanocomposites of Ag and polyamide immersed for 100 days in deionized water released Ag^+ proportional to the storage period. Obviously, these polymers have different properties, since polyamide is a more hydrophilic polymer than PMMA and for this reason, allows plasticizing by the action of water. PMMA, on the other hand, is a more hydrophobic material than polyamide, which may have generated a barrier for water diffusion and consequently Ag^+ release.[19] In addition, the cross-linking agent ethylene glycol dimethacrylate present in the Lucitone 550 resin may have restricted the rotation of polymeric chains, decreasing the velocity of water diffusion to the polymer.[44] However, even considering all of the effects on the polymer matrix, the experiments with rhodanine showed that only free Ag^+ could react with this organic molecule; therefore, the results regarding the Ag release (Figs 4 and 5) are conclusive about the absence of Ag^+ in the PMMA/Ag nanocomposite.

Table 1 Mean values (±SD) for the flexural strength assessment according to different silver colloidal nanoparticle concentrations added to the denture base resin

Groups	Flexural strength (MPa)
0% (Control)	77.63 ± 7.9
0.05%	82.35 ± 4.9
0.5%	78.11 ± 9.7
5%	75.89 ± 3.3

1 MPa = 10.19 kgf/cm^2.

The glass transition temperature (T_g) is another physical property involved in the diffusion of liquid substances in polymeric materials. The T_g may be influenced by the polymer thickness, and, for PMMA, it ranges from 97°C to 125°C.[21,45] In Kong and Jang's study,[21] a PMMA nanofiber, with approximately 20 nm of diameter, containing silver nanoparticles surrounded by polyvinyl alcohol allowed a release rate of 0.43 μg

silver nanoparticle/mL/h, but they also observed color change of the aqueous media because of the nanoparticle release. The higher the polymer thickness, the higher the T_g, and the lower the plasticization effect. In addition, the velocity of water sorption and the migration of the particles to the polymeric surface would be reduced, but the SEM images showed that the amount of silver nanoparticles on the specimen surface is much higher than that in the bulk. Therefore, the first material released probably migrated from the surface, not from the bulk, and if Ag was not detected, it means the silver nanoparticles are strongly fixed on the polymer base. It was demonstrated that, before 120 days of storage, the higher the amount of Ag in the PMMA/Ag nanocomposite, the higher the distribution of the nanoparticles and the lower their dispersion into the polymeric mass. The reduced dispersion in those specimens could be associated with the 100°C temperature during acrylic resin polymerization that may have contributed to the particle coalescence,[18] increasing their specific surface area, and reducing their release to the liquid medium. After 120 days of storage in water, silver nanoparticles were located on the polymer surface, regardless of the amount of Ag added to the PMMA. It may indicate the migration of silver nanoparticles from the interior to the surface of the polymer. Furthermore, with water diffusion, polar groups present in the polymeric chains are separated, and the water dipoles are able to attach to those of the polymer.[12] Thus, the polymeric structure is re-established with water incorporation.[12] This effect generates a free volume in the polymer that could allow molecular flexibility[12] and ion and particle migration. Another important characteristic of the specimens is the presence of pores in the nanocomposite surface, increasing the contact area between the nanocomposite and the aqueous media. These irregularities may have been caused by the colloidal suspension that was not accurately dispersed in the polymeric mass, perhaps because of the difference between the polarity of the polymer and the colloidal suspension. It is also important to consider that, clinically, tongue and chin muscle action could favor the nanoparticle release from the denture base surface.

Clinical and in situ studies are required to investigate the mechanism of Ag release from PMMA/Ag nanocomposite and their antimicrobial action. Moreover, studies using instrumental color analysis could be suggested, once the specimens containing the higher concentration of silver colloidal nanoparticles showed light alteration of denture base resin color.

Conclusion

After adding a silver nanoparticle suspension to the denture base resin, it was observed that the lower the volume of this suspension added, the lower the distribution and the higher the dispersion of the nanoparticles in the PMMA matrix. There was a tendency for silver nanoparticles to be located on the nanocomposite surface after 120 days of storage in deionized water. Moreover, silver was not detected by the detection limit of the atomic absorption spectrophotometer used in this study, even after 120 days of storage in deionized water. Regarding clinical implications, a gradual silver release over a long period through plasticization of PMMA would be desirable, considering 5 years of wear time for removable dentures. Further studies with storage periods over 120 days are indicated to evaluate the influence of time on the silver release. These studies would stimulate the development of PMMA/Ag nanocomposites, working to prevent denture stomatitis.

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