Evaluation of Candida albicans adhesion and biofilm formation on a denture base acrylic resin containing silver nanoparticles

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EVALUATION OF CANDIDA ALBICANS ADHESION AND BIOFILM FORMATION ON A DENTURE BASE ACRYLIC RESIN CONTAINING SILVER NANOPARTICLES

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INTRODUCTION

Candida species are found in the oral cavity of 60–100% of denture wearers (Dagistan et al. 2009) and are among the most common aetiologic agents causing fungal infections (Ramage et al. 2009). These micro-organisms are able to colonize the denture surfaces forming a biofilm (Samaranayake and Nair 1995), which is critical in the development of denture stomatitis (Chandra et al. 2001). Among the various Candida species, Candida albicans is still considered the most important fungal pathogen responsible for this disease (Budtz-Jørgensen 1990; He et al. 2006). Many factors that affect adhesion and biofilm formation of Candida on acrylic surfaces have been described, among them surface roughness and hydrophobic interactions. The characteristics of surface roughness affect the initial formation and development of C. albicans biofilms probably because it provides a greater surface area...
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and protected sites for colonization (Lamfon et al. 2003). The surface hydrophobicity, which can be determined by the water contact angle, also plays an important role in the adhesion of fungal cells (Luo and Samaranayake 2002).

The current antifungal therapy is topical or systemic antifungal drugs, including fluconazole and nystatin (Perezous et al. 2005; Sims et al. 2005; Rowan et al. 2010). Despite the use of antifungal drugs to treat denture stomatitis, infection is often persistent and antifungal resistance has been observed in Candida biofilms (Chandra et al. 2001). In addition, studies have shown that biofilms are significantly less susceptible to antimicrobial agents than planktonic cells (Douglas 2003; Seneviratne et al. 2008; Monteiro et al. 2009).

Silver is known to have a wide antibacterial spectrum (Panáček et al. 2006; Kim et al. 2007, 2008; Pal et al. 2007) and has been studied in the development of alternative antibacterial agents (Sondi and Salopek-Sondi 2004; Morones et al. 2005; Choi et al. 2008; Kim et al. 2009; Panáček et al. 2009). Currently, nanotechnology provides a broad range of opportunities to develop new bactericidal materials (Sondi and Salopek-Sondi 2004). Therefore, the antimicrobial effect of silver nanoparticles (AgNPs) has been investigated (Panáček et al. 2006; Kim et al. 2007; Pal et al. 2007; Choi et al. 2008; Kim et al. 2008, 2009; Panáček et al. 2009). While some authors have suggested that the antimicrobial efficiency of AgNPs-incorporated polymers is related to the release of silver ions or AgNPs themselves (Kassae et al. 2008; Kong and Jang 2008), others have reported that the inhibitory effect was probably due to the direct contact between the micro-organisms and the materials (Ahn et al. 2009).

AgNPs have been used in some applications such as topical antimicrobial gel or dressing for burns and wounds (Jain et al. 2009; Madhumathi et al. 2010), cotton fabric (Zhang et al. 2009), catheters (Roe et al. 2008) and as a safe preservative for use in cosmetics (Kokura et al. 2010). On the other hand, up to now there are few applications of AgNPs in dental materials (Ahn et al. 2009), particularly those used for denture bases (Kassae et al. 2008; Fan et al. 2011). Thus, this study firstly evaluated the activity of a AgNPs solution against C. albicans and then the effect of incorporation of AgNPs into a denture base acrylic resin on the material’s hydrophobicity, C. albicans adhesion and biofilm formation.

Materials and methods

Preparation and characterization of AgNPs

All reagents, including silver nitrate (AgNO₃) and polyvinyl alcohol (PVA), were obtained from Aldrich and used without further purification. AgNPs were synthesized by chemical reduction (Khanna et al. 2005) of silver nitrate (AgNO₃, 90 mg ml⁻¹) using sodium borohydride (NaBH₄, 540 mg ml⁻¹). The reaction occurred in the presence of PVA (230 mg ml⁻¹) to control the growth and the agglomeration of nanoparticles (Kim et al. 2007), and the molar ratio of AgNO₃ to PVA was maintained at 1 : 4. The solution was characterized by UV-visible spectroscopy (UV-vis) (Kim et al. 2007; Pal et al. 2007; Radziuk et al. 2007; Choi et al. 2008) (U-2800 spectrophotometer; Hitachi High Technologies Corporation, Tokyo, Japan), Fourier transform infrared (FTIR) spectroscopy (Khanna et al. 2005) (Nicolet 6700 FT-IR, Madison, WI, USA) and Dynamic Light Scattering (DLS, Zetasizer Nano ZS; Malvern Co., Irvine, CA, USA).

Micro-organisms and culture conditions

Candida albicans strain ATCC 90028 was used. Stock cultures were maintained at −70°C. After recovery, this was maintained on Sabouraud dextrose agar (SDA) stored at 4–6°C during the experimental period. To prepare the yeast inoculum, a loopful of the stock culture was streaked onto SDA and incubated at 37°C for 48 h. Two loopfuls of this young culture were transferred to 20 ml of yeast nitrogen base (YNB) medium with 50 mmol l⁻¹ glucose and incubated at 37°C for 24 h. Cells of the resultant culture were harvested, washed twice with phosphate-buffered saline (PBS) (pH 7.2) at 3220 g for 5 min and resuspended in YNB with 100 mmol l⁻¹ glucose. Candida suspensions were spectrophotometrically standardized to 1 × 10⁵ cells ml⁻¹ (Chandra et al. 2001).

Minimum inhibitory (MIC) and fungicidal (MFC) concentrations

The antifungal activity of AgNPs solution was evaluated by estimation of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) to planktonic cells, using a broth microdilution assay, as described in the Clinical and Laboratory Standards Institute document M27-A3 (CLSI, 2008). MIC and MFC were determined by incubating the C. albicans in 96-well microtitrater plate for 48 h at 35°C exposed to serial 2-fold dilution in RPMI-1640 culture medium of the AgNPs solution (from 1000 to 0·49 μg ml⁻¹). The MIC was defined as the lowest concentration at which there was no visible growth by visual inspection. To establish MFC, aliquots from each well were removed, and 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ dilutions were performed and inoculated (10 μl), in duplicate, on SDA. After 48 h of incubation at 37°C, the colony-forming units per millilitre (CFU ml⁻¹) were then calculated and log 10 trans-
formed. The MFC value was defined as the lowest concentration of the AgNPs solution resulting in no growth. All assays were performed in triplicate on three different occasions.

The antifungal activity of AgNPs solution was also evaluated by estimation of minimum fungicidal concentration to sessile cells (MFCs). Candida albicans was grown in SDA medium at 37°C for 48 h and then inoculated into 10 ml of RPMI-1640 medium and incubated at 37°C for 24 h, in an orbital shaker, at 75 rev min⁻¹. After incubation, C. albicans were washed twice with 10 ml of PBS. Candida albicans suspensions were spectrophotometrically standardized to a concentration of 1 x 10⁷ cells ml⁻¹ (Chandra et al. 2001). Aliquots of 200 µl of the standardized cell suspension were transferred into each well of polystyrene 96-well microtiter plate. The plate was incubated for 90 min at 37°C, in an orbital shaker, at 75 rev min⁻¹ for the adhesion phase. After this period, the wells were washed with 200 µl of PBS to remove loosely adhered cells. Each of the wells was filled with 200 µl of RPMI-1640 medium, and the plates were incubated at 37°C, in an orbital shaker, at 75 rev min⁻¹ for 48 h. Thereafter, the wells were carefully washed twice with 200 µl of PBS to remove nonadherent cells.

After biofilm formation, MFCs were determined by incubating C. albicans biofilms for 48 h exposed to serial twofold dilution in culture medium of the AgNPs solution (from 1000 to 0.49 µg ml⁻¹). To establish MFCs, the biofilms were scraped out of the wells of the 96-well microtiter plate and suspended in 1000 µl of PBS with vigorous vortex mixing for 1 min. Then, 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ dilutions were performed, and aliquots of 10 µl were inoculated, in duplicate, on SDA plates (Ramage et al. 2001). After 48 h of incubation at 37°C, the number of the CFU ml⁻¹ was determined. The MFCs was defined as the lowest concentration at which no cells were grown on plates. All assays were performed in triplicate on three different occasions.

Cell surface hydrophobicity

Candida albicans were grown in SDA medium at 37°C for 48 h and then inoculated into 10 ml of RPMI-1640 medium at 37°C, overnight in an orbital shaker, at 75 rev min⁻¹. Cells were harvested by centrifugation at 9000 g for 5 min and washed twice with PBS. Then, the cells were resuspended in 5 ml of PBS to a concentration of 10⁶ cells ml⁻¹. The cell suspension was filtered through membrane filter under vacuum. The membrane was dried for 3-5 h in a Petri plate containing 1% (w/v) agar in water containing 10% (v/v) glycerol to obtain a constant moisture content (Silva et al. 2010). The filter was cut and fixed on glass slides with double-sided adhesive tape.

The water contact angles were measured at room temperature by the sessile drop technique on the cell lawns, using an automated goniometer (Rame-Hart 200-00 Rame-Hart Instrument Co., Succasunna, NJ, USA). Five measurements were performed in three different occasions.

Preparation of the silver-incorporated acrylic resin

A microwave denture base acrylic resin (Vipi Wave; VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda Pirassununga, SP, Brazil) was used in this study. Forty millilitres of prepared AgNPs solutions were mixed with 75 g of the acrylic powder to produce silver-incorporated acrylic resins at the final concentrations of 1000, 750, 500, 250 and 30 ppm. Specimens without AgNPs (0 ppm) were used as control. The mixture was dried in an oven at 37°C for 48 h, passed through a sieve (60 mesh, 250 µm) and homogenized in a ball mill for 4 h.

Preparation of silver-incorporated acrylic resin specimens

The control and silver-incorporated acrylic resin specimens of each concentration (n = 72) were randomly fabricated using a conventional flasking and pressure-pack technique. Initially, a metal mould was used to make disc-shaped silicone patterns (Zetaplus/Indurent; Zhermack, Badia Polesine, Rovigo, Italy) measuring 13.8 x 2 mm. The patterns were sandwiched between two glass slides before investing. This technique was used to obtain smooth and standardized specimens (Mutluay et al. 2010; Vural et al. 2010). The flasks were separated, silicone patterns removed, and for each specimen, 1 g of powder and 0.47 ml of monomer liquid were mixed and processed according to the manufacturer’s instructions. The mixture was packed into the moulds, a trial pack was completed and excess material was removed. A final pack was performed and held for 15 min. The denture base acrylic resin was processed in a 500 W domestic microwave oven (Brastemp; Brastemp da Amazônia SA, Manaus, AM, Brazil) for 20 min at 20% power, followed by 5 min at 90% power. The flasks were allowed to bench cool at room temperature, the specimens were deflasked and excess flash was removed using a sterile bur (Maxi-Cut; Lesfils de August Malleifer SA, Ballaigues, Switzerland).

Surface roughness measurements

The surface roughness (Ra-µm) of each specimen was measured using a profilometer (Mitutoyo SJ 400; Mitutoyo Corp., Tokyo, Japan). Three measurements were made for each specimen, and the average reading was
designated as the Ra (µm) value of that specimen. The resolution was 0.01 µm, the interval (cut-off length) 0.8 mm, the transverse length 2.4 mm, the stylus speed 0.5 mm s⁻¹ and the diamond stylus tip radius 5 µm. All measurements were recorded by one operator. Only specimens with median surface roughness values of 0.27 µm were selected.

The control and silver-incorporated acrylic resin specimens of each concentration were randomly assigned to one of four storage periods of 0, 7, 90 and 180 days (n = 18) in sterile PBS at 37°C.

Contact angle measurements

The contact angle of all specimens was then determined after each period. The water contact angle has been measured to characterize the surface hydrophobicity. This angle is defined as the angle at the intercept of a plane tangent to the drop and the plane containing the substrate–liquid interface. The measurements were performed in an automated goniometer (Rame-Hart, 200-00) using deionized water as test liquid. The goniometer comprises a CCD camera to record the image of a droplet placed onto the surface using a microsyringe and a dedicated image processing software to determine the contact angle. Measurements in two different positions were made for each specimen, and the average was calculated.

Adherence and biofilm formation assays

The adherence and biofilm formation assays were performed according to da Silva et al. (2010). After the storage periods, nine silver-incorporated acrylic resin specimens of each concentration were used for the adherence assay (90 min of incubation), and nine specimens were used for the biofilm formation assay (48 h of incubation). Prior to the tests, the specimens were ultrasonically cleaned in distilled water for 20 min and then exposed to ultraviolet light in dry conditions at room temperature for another 20 min to kill any micro-organisms that may have contaminated the specimens during fabrication or storage (Sheridan et al. 1997). Three millilitres of the standardized C. albicans suspension was added to each well of a 12-well microplate containing the specimens. The cells were incubated for 90 min (adherence assay) and 48 h (biofilm formation) at 37°C in a shaker at 75 rev min⁻¹. The non-adherent cells were removed from the specimens by gently washing twice with 3 ml PBS after 90 min and 24 h of incubation. For all experiments, the negative controls were acrylic specimens (with or without AgNPs) to which no cells were added. All experiments were performed in triplicate on three independent occasions.

Measurement of adherent Candida albicans and biofilm formation

Cell adhesion and biofilm formation were evaluated using the XTT reduction assay, which is based on the metabolic activity of the cells (Ramage et al. 2011). Although XTT is a semiquantitative colorimetric assay, it has been demonstrated that this method correlates well with other quantitative techniques such as ATP and CFU assays (Ramage et al. 2002; Jin et al. 2004). XTT (Sigma, St Louis, MO, USA) was prepared in ultrapure water at a final concentration of 1 mg ml⁻¹. The solution was filter sterilized and stored at −70°C until use. Menadione (Sigma) solution was prepared in acetone at 0.4 mmol l⁻¹ immediately before each assay. After washing, the specimens were transferred to a 12-well microplate containing each well 2370 µl of PBS supplemented with 200 mmol l⁻¹ glucose, 600 µl of XTT and 30 µl of menadione (da Silva et al. 2010). The plates were incubated for 3 h in the dark at 37°C. The whole content of each well was transferred to a tube and centrifuged at 5000 g for 1 min. The colorimetric change of the supernatant was measured using a 96-well microtitre plate reader (Thermo Plate – TP Reader) at 492 nm.

Statistical analysis

The effects of incorporation of AgNPs and storage time on material’s hydrophobicity, C. albicans adhesion and biofilm formation were evaluated by two-way analyses of variance, followed by Tukey’s test. A significance level of 0.05 was used for all statistical tests.

Results

Characterization of the synthesized AgNPs

UV-visible spectroscopy was used for structural characterization of the AgNPs. The absorption spectrum of aqueous solution of AgNPs showed an absorption peak near 400 nm as shown in Fig. 1, indicating the presence of AgNPs.

FTIR spectroscopy was used to characterize the AgNPs-PVA nanocomplexes. From Fig. 2, it can be observed the following vibration bands for neat PVA: OH stretching (3200–3400 cm⁻¹) and symmetric/asymmetric stretching of aliphatic CH₂ groups (2900 and 1420 cm⁻¹). In the spectra corresponding to the AgNPs-PVA complex, the broadening observed in the band from OH (3200–3400 cm⁻¹) suggests the interactions between Ag and the OH groups from PVA. The latter interaction may also have been inferred based upon the significant decrease observed in the band at 840 cm⁻¹ (out-of-plane CH
vibration in PVA), which was probably related to the OH/Ag interactions. Figure 3 presents the results of analysis of the size of the AgNPs obtained from DLS. It can be observed that the highest percentage of AgNPs showed size of c. 9 nm with sodium borohydride as the reducing agent.

Determination of antifungal susceptibility of the Candida albicans to the AgNPs solution

The results showed that the AgNPs solution was fungicidal against the C. albicans tested in the planktonic state at low concentrations, with MIC and MFC values of 3.98 and 15.63 ppm, respectively. For sessile (biofilm) cells, a higher value was recorded (MFCs = 1000 ppm). All negative controls exhibited no metabolic activity (data not shown).

Cell surface hydrophobicity

The average value of the water contact angle of the C. albicans evaluated was 39° (±2.7). The low (<50°) contact angle value indicated hydrophilic surface.

Contact angle

The effect of the storage periods on the hydrophobicity of the specimens is shown in Table 1. There was no significant difference between either 0 and 7 days or 90- and 180-day storage periods. In the 90- and 180-day storage periods, the contact angle values were significantly higher than those observed at the 0 and 7 days. Table 1 also shows that at AgNPs concentrations of 30 ppm or higher, the contact angle values were significantly lower than those of the R0, regardless of the storage period.

Biofilm formation and adherence assay on the acrylic resin specimens

The two-way analyses of variance showed that for both the adherence and biofilm formation assays, there was a significant effect of the storage period (P = 0.000), but no significant effect of acrylic resin or the interaction between the two factors. The absorbance values obtained with the adherence and biofilm formation assays, in all storage periods evaluated, are presented in Figs 4 and 5, respectively. There was no significant difference between periods 0 and 7 days and the same result was observed.
### Table 1

Means and standard deviations (SD) of water contact angles of the acrylic resin in the storage periods evaluated.

<table>
<thead>
<tr>
<th>Acrylic resin with AgNPs (ppm)</th>
<th>Storage period (days)</th>
<th>0</th>
<th>7</th>
<th>90</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>51·17 (4·17)&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>50·89 (5·20)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>56·94 (5·39)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>56·10 (4·29)&lt;sup&gt;BA&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>48·51 (6·42)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>47·33 (6·55)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>54·13 (4·45)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>53·20 (4·74)&lt;sup&gt;BA&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>49·86 (4·65)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>46·08 (5·01)&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>55·68 (4·80)&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>52·28 (4·01)&lt;sup&gt;BA&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>46·74 (4·95)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>44·14 (4·30)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>51·04 (4·53)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>52·70 (3·83)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>47·44 (6·04)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>44·43 (4·92)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>53·10 (4·78)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>51·86 (4·03)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>46·60 (4·95)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>43·96 (5·22)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>52·11 (5·39)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>50·65 (4·56)&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

AgNPs, silver nanoparticles.  
Horizontally, identical superscript capital letters denote means that are not statistically different at a level of $P > 0.05$.  
Vertically, identical superscript small letters denote means that are not statistically different at a level of $P > 0.05$.

**Figure 4** Mean absorbance (OD at 492 nm) and 95% confidence intervals obtained in the adherence assay (90 min) for all experimental conditions evaluated. *Different from the corresponding group at 0 and 7 days. (□) 0; (■) 30; (△) 250; (▲) 500; (□) 750 and (▲) 1000.

**Figure 5** Mean absorbance (OD at 492 nm) and 95% confidence intervals obtained in the biofilm formation assay (48 h) for all experimental conditions evaluated. *Different from the corresponding group at 0 and 7 days. (□) 0; (■) 30; (△) 250; (▲) 500; (□) 750 and (▲) 1000.
between 90 and 180 days. Significantly higher absorbance values were observed for the periods 90 and 180 days when compared to 0 and 7 days. There were no significant differences between the mean absorbance values of the unmodified acrylic resin (R0 – control) and those of the resins containing the AgNPs, regardless the concentration used.

**Discussion**

In this study, an AgNPs solution was synthesized by chemical reduction method and characterized. UV-vis spectra of the AgNPs solution showed absorption band at around 400 nm, which indicates the presence of spherical AgNPs (Kim et al. 2007; Pal et al. 2007; Radziuk et al. 2007; Choi et al. 2008; Panáček et al. 2009; Fan et al. 2011). FTIR spectroscopy data confirmed the presence of PVA, which was used as the stabilizing agent to prevent agglomeration of nanoparticles (Choi et al. 2008; Kong and Jang 2008). In addition, the absence of the band at about 1596 cm\(^{-1}\) may be an indication that the AgNPs influence the vibration of the atoms of the polymer chains. The DLS analysis (Panáček et al. 2006, 2009) showed that the size of the particles was 9 nm. It has been reported that the smaller the particles, the greater the antimicrobial effect (Morones et al. 2005; Panáček et al. 2006), because of the higher surface area for interacting with the micro-organisms (Morones et al. 2005). Nanoparticles smaller than 10 nm are not only found on the surface of the cell membrane but also inside the bacteria (Morones et al. 2005). Thus, the size of the AgNPs synthesized in this study may have accounted for the activity of the prepared solution against *C. albicans*. The growth inhibition of planktonic cells was complete in the presence of AgNPs at concentration of 3.98 ppm, and the fungicidal effect was observed at the concentration of 15.63 ppm. The synthesized AgNPs solution was also effective against sessile cells at the highest concentration evaluated. Other studies (Kim et al. 2007; Choi et al. 2008) have also evaluated spherical AgNPs and found that they were effective in inhibiting the growth of bacteria (*Escherichia coli*) and yeast (isolated from bovine mastitis). The mechanism of the inhibitory effects of AgNPs on micro-organisms has been investigated. Studies with bacteria have shown that AgNPs may be incorporated into the bacterial membrane (Sondi and Salopek-Sondi 2004; Morones et al. 2005; Pal et al. 2007) causing structural changes, increased membrane permeability and, finally, cell death (Sondi and Salopek-Sondi 2004; Pal et al. 2007). Moreover, phosphorus-containing elements like DNA can be preferential sites for AgNPs binding, because silver has a high tendency to react with such compounds (Morones et al. 2005; Panáček et al. 2006; Pal et al. 2007; Monteiro et al. 2009). On the other hand, few studies have evaluated the effect of AgNPs against *C. albicans* and their antifungal mode of action is not completely clear (Kim et al. 2008, 2009; Roe et al. 2008; Panáček et al. 2009). Similar to our findings, it has been observed that AgNPs exhibited antifungal activity against *C. albicans* (Kim et al. 2009; Panáček et al. 2009), causing fungal membrane depolarization and disruption with an increase in the amounts of intracellular and released glucose and trehalose, damage in the envelope structure, and inhibition of the normal budding process (Kim et al. 2009). Although the microbiological effects underlying the activity against *C. albicans* observed in this study was not explored, it is likely that the AgNPs solution inhibited the growth through destruction of membrane integrity.

After the AgNPs were incorporated into the denture base acrylic resin, no effect was observed against *C. albicans*. It should be noted that cell adhesion results were highly variable even within control groups. It could be that the differences among the groups were not detected because of this variability. The literature reveals that although many studies have investigated the antimicrobial activity of AgNPs solutions against several bacteria, information on the effect of these nanoparticles after their incorporation on denture base polymers is limited (Kassae et al. 2008; Fan et al. 2011). Moreover, the effect of AgNPs-incorporated denture base acrylic resins on *C. albicans* adherence and biofilm formation has not, to the authors’ knowledge, been reported. The antimicrobial efficacy of AgNPs-incorporated polymers has been related to the diffusion of water molecules into the material and the migration of the silver ions or AgNPs through the polymer to the aqueous medium (Kumar and Münstedt 2005; Morones et al. 2005; Kassae et al. 2008; Kong and Jang 2008; Fan et al. 2011). Thus, the properties of the matrix polymer and its water diffusion characteristics play a role for the release process (Kumar and Münstedt 2005; Damm et al. 2007). It was found that the PMMA/Ag nanocomposite released much fewer silver ions than the PA6/Ag nanocomposite (Damm et al. 2007). These findings were explained by the fact that PMMA, in comparison with PA6, is a rather hydrophobic polymer, and its water uptake may not be sufficient for a silver ion release from particles situated in the bulk of the specimen (Damm et al. 2007). Therefore, the absence of antifungal properties of the AgNPs-incorporated PMMA denture base acrylic resin observed in the present investigation could be related to poor release of the silver ions or AgNPs from the specimens. The results from this study may also be attributed to the structure of the polymer network. The denture base acrylic resin VIPI Wave contains a cross-linking agent (ethylene glycol dimethacrylate) in
the liquid. Thus, it could be that the resulting network of the specimens may be of such configuration that the silver ions or AgNPs were probably trapped into the cross-linked polymer structure and their release into the aqueous medium was restricted. Recently, AgNPs were synthesized in situ in acrylic dental resins (Fan et al. 2011) and an inhibitory effect of AgNPs-loaded resin discs on the growth of Streptococcus mutans was found. The differences between results could be related to the fact that in the present study the effects of the AgNPs-incorporated acrylic resin on C. albicans were evaluated using adhesion and biofilm assays, while in the study of Fan et al. (2011) the efficacy was evaluated by identifying zones of inhibition of bacterial growth.

In the present investigation, the effect of AgNPs incorporation and storage periods on the hydrophobicity of the specimens was also evaluated. It has been reported that polymers and cell surface hydrophobicity correlated well with the adhesion of Candida spp. (Klotz et al. 1985; Samaranayake et al. 1994; Luo and Samaranayake 2002). Yoshijima et al. (2010) also observed that hydrophilic coatings of denture acrylic surfaces reduced the adhesion of the hydrophobic C. albicans hyphae. In the present study, the storage for 90 and 180 days resulted in a small but significant increase in the hydrophobicity of both control and silver-incorporated specimens, regardless the concentration of AgNPs. Accordingly, a significant increase in the adherence and biofilm formation values was found for these periods, although the C. albicans strain evaluated was hydrophilic. These results suggest that the higher hydrophobicity of the surfaces might have been the major factor accounting for the results observed.

This study has limitations because only one strain of C. albicans and one heat-polymerized denture base were used. Although no effect of the AgNPs incorporated into the denture base acrylic resin was observed, the results demonstrated that prepared AgNPs aqueous solution showed antifungal activity against C. albicans, justifying further investigation. In addition, studies must be carried out to examine the cytotoxicity of tested AgNPs towards human cells.

**Conclusion**

Within the limitations of this in vitro study, the following conclusions can be drawn:

1. The prepared AgNPs solution showed antifungal activity against planktonic and sessile C. albicans cells.
2. After the AgNPs were incorporated into the denture base acrylic resin, no effect on the C. albicans adherence and biofilm formation was detected, regardless of the concentrations.

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