Antibacterial Effects of a New Dental Acrylic Resin Containing Silver Nanoparticles

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Received 20 December 2007; accepted 14 May 2008
DOI 10.1002/app.28762
Published online 28 July 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The addition of silver nanoparticles (Ag NPs) to an acrylic resin rendered a dental composite that exhibited strong antibacterial activity against *Escherichia coli* and showed rather improved mechanical properties. Such antibacterial effects were mainly attributed to the release of silver ions upon immersion of the composite in water, which appeared to be fairly nontoxic to humans. Furthermore, an acrylic liquid was used as a new reducing agent for the conversion of silver ions into pure elemental Ag NPs in quantitative yields. The structure, morphology, average size, and size distribution of the as-prepared Ag NPs were determined by scanning electron microscopy, transmission electron microscopy, and X-ray diffraction, which confirmed the fabrication of rather dispersed, face-centered cubic nanoparticles with a mean average diameter of 38 nm. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 1699–1703, 2008

Key words: biomaterials; dental polymers; nanocomposites

INTRODUCTION

Resin composites are widely used in dental clinics for the replacement of hard tissues.[1] Although the mechanical properties and wear resistance of these materials have been improved substantially,[2] their antibacterial properties are still of great interest.[3,4] This is despite a number of antimicrobial agents, such as antibiotics[5] and organic[6] and inorganic biocides,[7] that are being used for the preparation of antimicrobial medical devices. Among them, silver exhibits the highest antimicrobial efficacy in combination with fairly low toxicity against human tissues.[8] Both elemental silver and silver compounds are used as antimicrobial coating materials or fillers for catheters,[9,10] wound dressings,[11] bone cements,[12] and dental materials.[13] Polymers filled with silver-ion compounds exhibit a strong and fast Ag^+ release, which limits their efficacy to short-term applications, whereas elemental silver particles can provide a large reservoir of silver ions that can be released gradually and result in long-term antimicrobial activity.[14,15] Polymers filled with elemental silver nanoparticles (Ag NPs) release silver ions even more effectively than materials filled with conventional silver particles, which have sizes in the micrometer range.[16]

Today, acrylic resins are the most common used polymers used in denture-base applications for their relative simplicity of fabrication. Typically, a polymer powder of methyl methacrylate and a liquid monomer of methyl methacrylate are mixed in a certain ratio. This gives a doughy mixture, which is subsequently put into molds and is allowed to polymerize and cure.[17] After our recent works on different nanostructures,[18–20] in this article, we present a simple and effective method for the preparation of a dental acrylic resin containing elemental Ag NPs. In the first step, elemental Ag NPs were prepared via the chemical reduction of silver ions by an acrylic liquid as a new reducing agent. In the second step, the prepared Ag NPs were added to a commercial acrylic resin, and the initiation of polymerization was accomplished at room temperature. Consequently, the silver-ion release and antibacterial and mechanical properties of the silver-containing composites were evaluated.

EXPERIMENTAL

Materials

A self-curing acryl resin system consisting of a powder portion and an acrylic liquid part was purchased from Denstply Co. Silver nitrate was provided by Aldrich. Analytical-grade isopropyl alcohol was

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supplied by Merck (Germany). Deionized water for the sample preparation was made with a water purification system from GFL Co. (Germany). All chemicals were used without further purification.

Preparation of the Ag NPs

Aqueous silver nitrate (100 μL, 0.1*M*) was added to isopropyl alcohol (2 mL). With vigorous stirring, an acryl liquid (1 mL) was added all at once at room temperature. After about 30 min, the transparent solution was converted into a characteristic pale gray color, which indicated the formation of Ag NPs. The suspension was carefully centrifuged and washed with ethanol before further characterization.

Preparation of the acrylic resin/Ag NP composite

The acrylic resin composite, loaded with 0.5% (w/w) Ag NPs, was prepared as follows. The synthesized Ag NPs were added to the acryl liquid and sonicated for 15 min. Then, the solution (liquid portion) was mixed with the powder portion of the system. This produced a doughy mixture, which was subsequently put into molds to create specimens with the desired shapes. A neat acrylic resin composite without Ag NPs was also prepared for comparison.

Structure and morphology

The X-ray diffraction (XRD) pattern of the Ag NPs was recorded with a Philips Xpert X-ray diffractometer (Cu Kα) (Holland). Scanning electron microscopy (SEM) images were taken on a Philips XL30 microscope. The transmission electron microscopy (TEM) micrographs were obtained with a Zeiss EM 900 microscope operating at 120 kV. The TEM sample was prepared via the drying of a drop of the suspension on a TEM copper grid. The flexural strength test was performed on a Santam Co. (Iran) testing machine according to the ISO 1567 standard.

Silver-ion release

Sample sheets with a surface area of 2 cm^2/sheet were immersed in distilled water. After 1, 2, 4, 7, 11, and 15 days, water samples were taken and analyzed via anodic stripping voltammetry.[16,21]

Antimicrobial assays

Escherichia coli ATCC 25922 was used as a silver-sensitive bacterium to study the antibacterial efficiency of the prepared composites. The bacteria were grown overnight in Luria broth medium (1% tryptone, 0.5% yeast extract, 1% sodium chloride, and 1.5% agar) at 35°C. The resulting suspension of the bacteria was adjusted to a concentration of 1.5×10^8 CFU/mL and then diluted to about 1.5×10^5 CFU/mL. The exact initial concentration of the bacteria was determined through the counting of the living cells in the suspension as described next. One milliliter of the suspension was diluted in a 1/4 Ringer solution (2.25 g of NaCl, 0.11 g of KCl, 0.12 g of $CaCl_2 \cdot H_2O$, 0.05 g of $NaHCO_3$, and 1000 mL of distilled water) and inoculated in Luria broth solid agar by the pour-plate method. The agar plates were incubated for 24 h at 37°C. During this time, each living bacterial cell grew to a colony with a diameter of 1–2 mm. These colonies were counted. From the number of colonies and the dilution factors, the concentrations of bacteria were calculated. The concentrations of bacteria mentioned in this article are the mean values of four trials.

RESULTS AND DISCUSSION

Ag NPs

Our interdisciplinary quest for producing clinically relevant antibacterial dental resin composites led us to the employment of the liquid portion of our acryl resin system (Denstply) for the reduction of $AgNO_3$ into elemental Ag NPs. Actually, this commercial self-curing acrylic resin system consists of a liquid and a powder portion. The liquid portion contains a tertiary amine (or other typical organic redox accelerators) after being mixed with the powder portion to initiate polymerization.[17] Hence, these redox accelerators appear as our reducing agents for the reduction of $AgNO_3$ into a fair yield of pure Ag NPs, whose XRD pattern shows three distinct diffraction peaks at 2θ values of 38.1, 44.3, and 64.4°, respectively (Fig. 1). These peaks correspond to the (111), (200), and (220) crystalline planes of face-centered cubic silver (JCPDS card no. 04-0783). The strong and sharp peaks with a broadening form suggest the formation of highly crystalline silver particles in the nanoscale structure. The mean crystallite size, calculated from the (111) diffraction peak with Scherrer's formula,[22] is about 38 nm. It seems that the interaction of silver atoms with oxygen atoms in methyl methacrylate (the main component of the

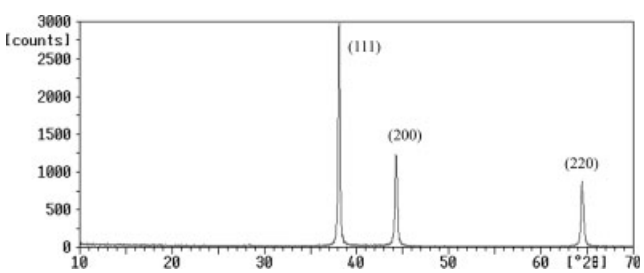

Figure 1 XRD pattern of the Ag NPs.

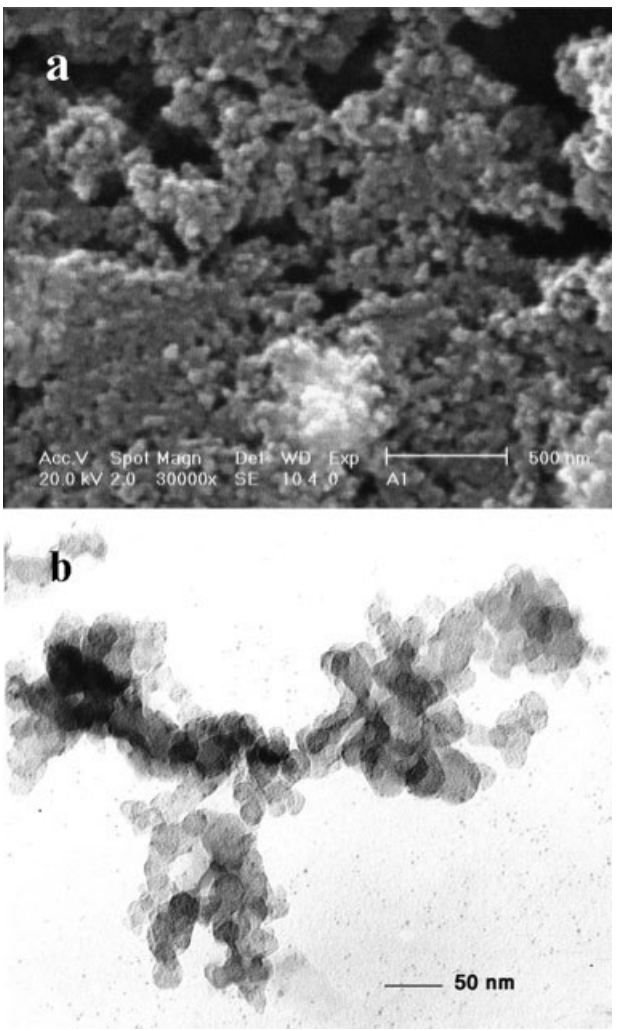

Figure 2 (a) SEM and (b) TEM images of the Ag NPs.

acryl liquid) prevents excessive aggregation of Ag NPs. This is illustrated by the SEM and TEM micrographs, which show the dominant formation of spherical Ag NPs with an average diameter lower than 50 nm and with little aggregation (Fig. 2).

Acrylic resin/Ag NP composite

Evidently, compared with that of conventional composites, the preparation of polymer/silver nanocomposites is much more complicated because of the easy agglomeration of Ag NPs. To reduce the agglomeration, we well dispersed the prepared Ag NPs in an acryl liquid and then mixed it homogeneously with the powder part according to the method mentioned in the Experimental section.

Silver-ion release

The concentration of silver ions released from the acrylic resin/Ag NP composite to the aqueous medium was measured by anodic stripping voltammetry, which is a widely used electrochemical technique for trace element detection in aqueous and nonaqueous media. Plotting the silver-ion concentrations in water samples against the immersion times shows a relatively sharp increase in the Ag^+ concentration in the first 2 days, which is followed by a gradual leveling to 0.175 mg/L thereafter (Fig. 3). Nevertheless, silver ions at such concentrations, while potent against *E. coli*, are fairly safe for humans.[23] The composite filled with Ag NPs releases silver ions more effectively than that filled with conventional silver particles, which have sizes in micrometer ranges.

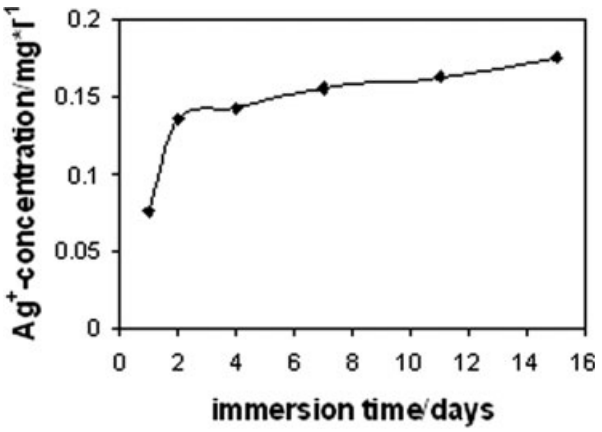

Figure 3 Silver-ion release as a function of the immersion time for the acryl resin/Ag NP composite containing 0.5 wt % Ag NPs with a surface area of 2 cm^2.

Surface morphology

The SEM analysis of the acrylic resin/Ag NP composite (Fig. 4) showed a fairly good dispersion of Ag NPs in the polymer matrix, with little aggregation, which facilitated a higher release of silver ions and consequently higher antibacterial activity.

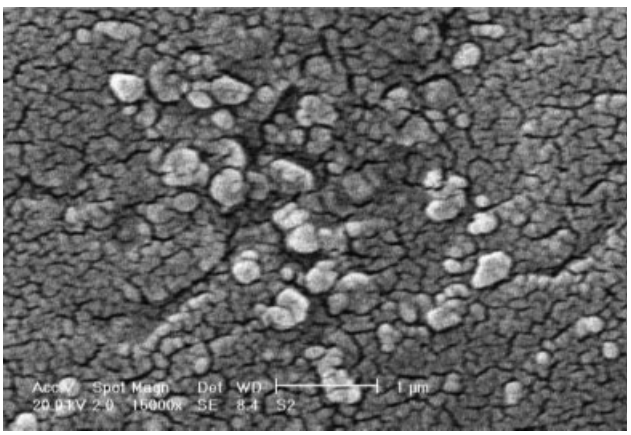

Figure 4 SEM image of the acryl resin/Ag NP composite.

Antimicrobial efficiency

The antimicrobial efficiency of the neat composite (containing no Ag NPs) and acryl resin/Ag NP composite was studied against *E. coli*. For this purpose, two sets of specimens, with a surface area of 2 cm^2 for each one, were prepared. They were sterilized with ethanol and transferred into sterile vials. Two vials containing the specimens as well as a control vial (without any sample) were incubated with 2 mL of the diluted bacterial suspension for 24 h at room temperature. The concentration of living bacterial cells that survived after 24 h of contact with the samples was determined as mentioned in the Experimental section. After 24 h, the concentration of bacteria was not reduced in the control sample (Table I). This means that the bacteria survived in the suspension under the conditions used and did not adhere to the walls of the vials. The small increase in bacteria could be attributed to the Ringer effect. Similarly, no loss of bacteria was observed, with respect to the control sample, in the suspension in contact with the neat composite (with no Ag NPs) for 24 h. Thus, it can be concluded that the neat composite did not show measurable antibacterial efficiency. In contrast, no bacteria could be detected in the suspension that was in contact with the composite containing Ag NPs for 24 h. Because our test method was able to record bacterial cells in a suspension only, any disappearance of the bacteria from the suspension containing acrylic resin/Ag NPs samples could be caused by either adherence of the cells to the specimens or death of the bacteria due to the bactericidal efficiency of the samples. Therefore, after 24 h of incubation, the samples were removed from the bacterial suspensions and rinsed carefully with a "Ringer solution" to remove loosely bound bacteria. Then, they were immersed in a Luria broth agar medium and incubated at 35°C overnight. Also (the rinsing Ringer solution) was filtered through 0.22-μm-pore-size filters. The filters were placed on the surface of the plate count agar medium and incubated at 35°C, overnight. No bacterial colonies could be detected on, under, or besides the samples and the rinsing solutions. This means that the composite containing Ag NPs was not colonized and that the disappearance of the bacteria in the suspension was caused only by the antimicrobial efficacy of the sample. Generally, the biological tests confirmed that the acrylic resin/Ag NP composites containing small amounts of Ag NPs exhibited strong antimicrobial efficiency against *E. coli*, regardless of the neat commercial composite to which they were added.

TABLE I
Initial Concentration of *E. coli* and Concentrations of the Bacteria in Suspensions After 24 h of Contact with Differently Modified Samples

	Concentration after 24 h (CFU/mL)		
Initial concentration (CFU/mL)	In the absence of the neat acrylic resin and acrylic resin/Ag NP composites (control)	In contact with the neat composite	In contact with the acrylic resin/Ag NP composite
$(1.9 \pm 0.1) \times 10^5$	$(7.2 \pm 0.9) \times 10^6$	$(7.4 \pm 1.0) \times 10^6$	0

TABLE II
Flexural Strength and Modulus Values of the Tested Materials

Material	Flexural strength (MPa)	Flexural modulus (MPa)
Neat composite	61.05 ± 1.2	1115 ± 107
Acrylic resin/Ag NP composite	64.83 ± 1.3	1554 ± 22

Mechanical properties

Flexural strength was chosen as a screening mechanical test to investigate the effect of Ag NPs incorporated into the neat acrylic resin composite. Five specimens ($50 \times 10 \times 3.5$ mm^3) from each test group were prepared and tested according to the ISO 1567 standard. The flexural strength and modulus were calculated in megapascals with the following equations:

$$\text{Flexural strength} = (3Fl/2bh^2)$$

$$\text{Flexural modulus} = (Fl^3/4bh^3d)$$

where F is the maximum load, l is the distance between the specimen supports, b is the width, h is the height of the specimen, and d is the deflection (mm). The flexural strength and modulus were determined, and the mean and standard deviations were calculated for the experimental groups (Table II). The results indicated that the addition of a small amount of Ag NPs partially improved both the flexural strength and modulus of the acrylic resin/Ag NP composite.

CONCLUSIONS

Pure elemental Ag NPs with a mean average diameter less than 50 nm were synthesized by the acryl liquid reduction of silver ions. These Ag NPs were incorporated into the commercial acrylic resin with little aggregation. The amount of silver ions released,

in addition to the antibacterial tests, showed that the acryl resin composite containing about 0.5% Ag NPs had a strong antibacterial effect against *E. coli* with low toxicity to humans. The addition of this small amount of Ag NPs partially improved the mechanical properties of the acrylic resin/Ag NP composite. Further *in vivo* experiments are necessary before the clinical implementation of this technique.

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