Journal of Industrial and Engineering Chemistry 20 (2014) 1276–1279

Contents lists available at SciVerse ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

New routes to the preparation of silver-soft liner nanocomposites as an antibacterial agent

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ARTICLE INFO

Article history:
Received 21 January 2013
Accepted 2 July 2013
Available online 9 July 2013

Keywords:
Silver
Nanoparticles
Soft-liner
Antibacterial
E. coli

ABSTRACT

This study evaluated the antimicrobial and physical properties of silver-soft liner nanocomposites (Ag-SLN). Polymerized acrylic soft denture liner disk specimens containing 0 (control), 1500, 3000, or 6000 mg/L of silver nanoparticles were placed on the flat bottom of the separate 12-well cell culture plate dish and 100 μL samples of microbial suspensions of *Escherichia coli* strain were inoculated on each specimen and incubated at 37 °C, for 24 and or 72 h. The antimicrobial effects were determined according to the number of viable cells in the retrieved suspension. Characterization of silver nanoparticles was carried out based on UV–vis spectroscopy and transmission electron microscopy (TEM) analysis. The specimens were characterized by thermal gravimetric analysis (TGA), field emission scanning electron microscope and energy dispersive X-ray analysis (FE-SEM/EDAX), and atomic absorption spectroscopy (AAS). We successfully prepared Ag-SLN and identified the great excellent antimicrobial activity for *E coli*.

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1. Introduction

New technologies require new materials with special combinations of properties. Recently, the surface modification of polymer materials has been a major challenge in medical applications such as implants or other devices in the human body [1]. Particularly in the oral cavity, acrylic soft denture liners have been commonly used to enhance the recovery of denture-bearing tissues from trauma and damage, which are usually caused by ill-fitting dentures. However, lining materials have complicated molecular structures and are easily degradable, along with being susceptible to bacterial adhesion. The adhered bacteria can be released from denture plaque into salivary secretions and then aspirated into the lower respiratory tract, causing pneumonia, especially in elderly people or those with disabilities; such individuals have a diminished general immune response and pharyngeal reflex [2–6].

Systemic or local antibiotic agents have been prescribed to eliminate the bacterial population for treatment in cases of infection; however, as microbial resistance and healthcare costs are both increasing, the development of antimicrobial denture lining material could be necessary for the prevention and care of increased bacterial populations [7,8]. Although several in vitro and in vivo studies have shown the beneficial effects of antimicrobial agents combined in polymeric soft denture liners [9–11], no effective, commercial antimicrobial agent to be combined has yet been developed.

Silver nanoparticles are emerging as a new generation of antibacterial agent [12]; they have been used for hygiene [13], medical applications [14–16], and antibacterial water filters [17]. Recently, silver nanoparticles have shown superior antibacterial activity compared to that of other silver compounds, as well as bulk silver. Silver nanoparticles do not only possess strong antibacterial activity, but can also inhibit a broad spectrum of bacteria and fungi [13,18]. Although silver nanoparticles have been integrated with various materials, none of these works was carried out using soft liners (SLs). Therefore, the purposes of this study were to generate a new synthesis of silver-soft liner nanocomposites (Ag-SLN) and to assess its antimicrobial effects.

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http://dx.doi.org/10.1016/j.jiec.2013.07.004

2. Experimental

Silver nitrate ($AgNO_3$, 99.9%) was purchased from Kojima Chemicals Co. Ltd. (Japan). PVP (MW. avg. = 10,000) was obtained from Sigma–Aldrich Co. GC Soft-Liner was purchased from GC Co. Ltd. (Japan). All chemical reagents were used without further purification. Colloidal silver nanoparticles were prepared from the solutions of 0.3 M of $AgNO_3$ and 5 wt.% PVP in distilled water. The whole preparation procedure can be explained as follows: 12.50 g of PVP was dissolved in 250 mL of distilled water; following this, 12.74 g of silver nitrate was added to the solution. Then, the solution changed in color from yellow to dark olive green. The chemical process continued for 2 h at room temperature.

The size of the silver nanoparticles was measured using transmission electron microscopy (TEM; Hitachi H-7100). The UV–vis spectra of the silver nanoparticles were recorded with a SCINO UV S-2100 UV-Vis spectrophotometer.

Determination of each Ag-SLN sample (about 1.0 g) was dried under a vacuum until it maintained a constant weight and then placed in a flask. Distilled water (50 mL) was added to the flask and the mixture was shaken for about 24, 48, or 72 h. The concentration of silver nanoparticles in the obtained solution was determined using atomic absorption spectroscopy (AAs; Perkin Elmer AAnalyst 100, USA). A silver hollow cathode lamp (Perkin-Elmer, USA) with a 328.1 nm line and 0.7 nm spectral band pass operated at 12 mA was the radiation source.

GC Soft-Liner (GC cooperation, Tokyo, Japan) was selected as a soft denture liner in this study. Doses of Ag° added to the conditioner liquid are shown in Table 1. Colloidal Ag° was preliminarily combined and homogenized with the conditioner liquid in a sterile glass beaker at a concentration ranging from 0 (control) to 1500, 3000, and 6000 mg/L (vol/vol; colloidal Ag°/conditioner liquid). Acrylic powder was added and mixed for 30 s at a designated powder/liquid ratio following the manufacturer's instructions. To generate a uniform shape with a regular surface for samples, mixed conditioner paste was poured onto a custom-made brass mold with a hole (20 mm diameter × 3.0 mm height), which sandwiched the paste between glass slides until the SL was solidified. Sixty specimens were prepared and divided into four groups ($N = 15$) according to the concentration of Ag°. Before microbial assay, all samples were sterilized with ethylene oxide gas for 24 h to ensure the initial sterility of samples. The microstructure of obtained Ag-SLN was studied by field emission scanning electron microscope (FE-SEM; Philips XL30S FEG) equipped with EDAX (Sapphire detector with LEAP + Crystals). The Ag-SLN was gold sputtered under high vacuum before the analysis. EDAX studies were also performed for Ag-SLN to identify the silver nanoparticles of the surfaces.

Thermal gravimetric analyses (TGA) were carried out simultaneously using a TG-DTA92 instrument (SETARAM, France) with a heating rate of 10 °C min^{-1} from 30 °C to 600 °C. Each sample was analyzed in triplicate and mean values were estimated.

Microbial suspensions were obtained from a single colony (*Escherichia coli*; ATCC 6538) isolated on agar plates and then inoculated in appropriate broth for overnight cultures. Bacterial strains were grown in brain–heart infusion (BHI; Difco, Franklin Lakes, NJ, USA) broth and onto Mueller Hinton agar plates at 37 °C. After incubating microbial cells at 37 °C overnight, the optical density (OD) of the suspension at 600 nm was adjusted to 1.0 using a spectrophotometer (Milton Roy spectrophotometer 20D+, Milton Roy, Ivyland, USA). The suspension was diluted with phosphate-buffered saline (pH 7.4) to 1:100 and suspended to final concentration of 1.0×10^7 cells/mL [19,20].

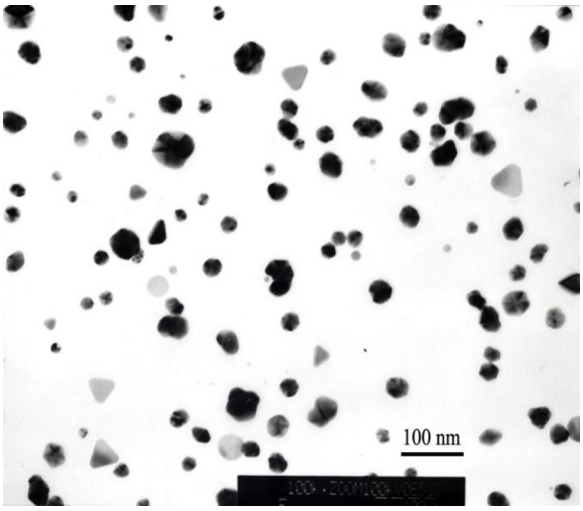

Fig. 1. TEM image of silver nanoparticles prepared by the in situ reduction method.

Samples of Ag-SLN and control were placed on the flat bottom of separate 12 well cell culture plates (Costa®, Corning, New York, USA), and then 100 μL of the initial microbial suspension in 1.0 mL of Sabouraud broth was inoculated in each well and incubated at 37 °C. After incubation for 24 h and 72 h for an extended contact period, the suspension (100 μL) was withdrawn through serial dilution; viable cells (colony forming units [CFU]) in the suspension were determined using the spread plate method at a level of detection within 500 CFU per plate. Assays were independently performed with three repetitive tests and data were recorded as means and standard deviations. According to conventional standards [21,22], the borderline of antimicrobial effect was determined to be 1500 mg/L viable cells (99.9% reduction of CFU) as the minimum bactericidal concentration (MBC) of antibiotics. A simple comparison of CFU means was carried out (t-test, $p < 0.05$ indicated a significant correlation).

3. Results and discussion

Fig. 1 gives a TEM image of silver nanoparticles prepared using the in situ reduction method. The average size of the silver nanoparticles was about 40 ± 8.0 nm. The UV–vis spectrum of the silver nanoparticles is presented in Fig. 2, where we identified that the peak at 410 nm was the surface plasmon band of the silver nanoparticles synthesized by the in situ reduction.

It is well known that a surface plasmon band of spherical silver nanoparticles appears at around the 400 nm region. The color

Table 1
Preparation recipe and mixing pattern of Ag-SLN.

Group	*N*	Sol Ag°/liquid (vol/vol, %)	Ag° dose (mg/L)	Mixing pattern	Strains tested
Control	15	0	0	GC Soft-Liner (GC Soft-Liner, GC cooperation)powder/ (sol Ag° + conditioner liquid) = 2.2/1.8	*E. coli*
I	15	0.5	1500		
II	15	1.0	3000		
III	15	2.0	6000		

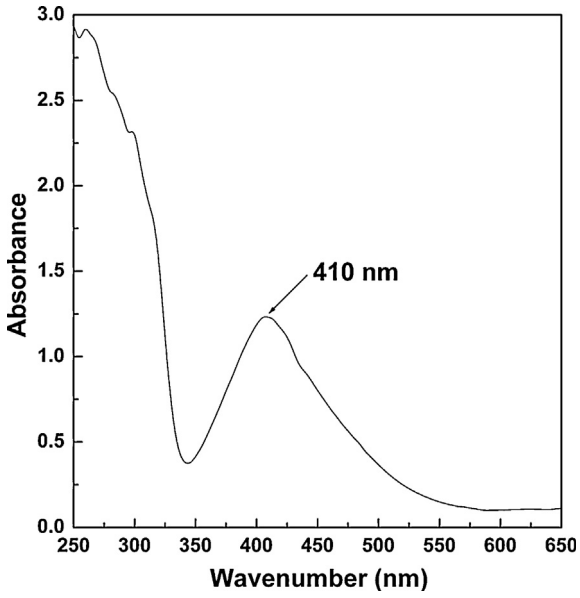

Fig. 2. UV–vis spectrum of silver nanoparticles prepared by the in situ reduction method.

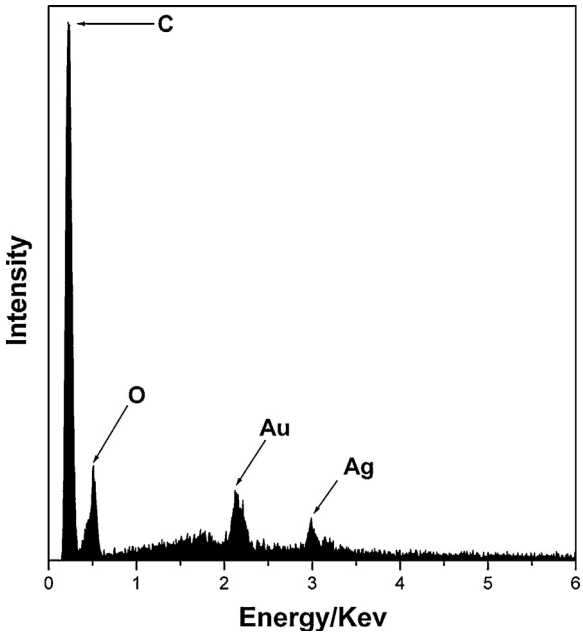

Fig. 4. Energy-dispersive X-ray analysis of Ag-SLN.

properties of silver nanoparticles are quite sensitive to the size of nanoparticles at a constant number density; at constant mass loading, they appear to be insensitive to small changes in the size of silver nanoparticles because of the inverse cubic coupling between the size of particles and number density at constant mass loading [23]. Therefore, the characteristics of aggregated silver particles are in good agreement with many previous studies [24].

In order to investigate the presence of silver nanoparticles on the surface of Ag-SLN, the FE-SEM and EDAX elemental analysis.

The SEM photographs in Fig. 3 were taken at 250× magnification to observe the surface morphology of Ag-SLN. Fig. 3 shows the Ag-SLN surface covered with silver particles; the diameter of a silver particle was about 5–50 μm. The EDAX spectrum for Ag-SLN is illustrated in Fig. 4. Only carbon, oxygen, and silver peaks can be observed. These have been identified as the principal elements of Ag-SLN. In this case, carbon and oxygen atoms were the main components of the SL. In addition, the peak of the silver atoms showed that silver nanoparticles were immobilized onto the SL surface. Therefore, we confirmed that the silver peak is clearly shown in Fig. 4, which indicates that the silver nanoparticles were successfully immobilized onto the SL surface.

SLs are used for short periods to improve the comfort and fit of an old denture until it can be remade or permanently relined. SLs are composed of powder containing poly ethyl methacrylate (PEMA) and a liquid containing an aromatic ester-ethyl alcohol mixture. SLs are very soft elastomers with a hardness of from 13 to 49 Shore A hardness units 24 h after mixing. The role of liquid is to speed up the polymerization of the monomer at room temperature.

Fig. 5 gives a comparative thermal decomposition pattern for (a) SL, (b) 1500 mg/L Ag-SLN, (c) 3000 mg/L Ag-SLN, and (d) 6000 mg/L Ag-SLN. SL gave two thermal degradation patterns corresponding to the degradation of the ethyl methacrylate and aromatic ester-ethyl alcohol. Fig. 5 shows similar thermograms for (b) 1500 mg/L Ag-SLN, (c) 3000 mg/L Ag-SLN and (d) 6000 mg/L Ag-SLN from 30 °C to 600 °C. A sharp weight reduction is observed between 250 °C and 350 °C.

Despite the use of silver nanoparticles, the onset of degradation for all samples appeared to start at about the same temperature. At the end of the degradation process, the amounts of residues were proportional to the concentrations of silver nanoparticles in SL, since the silver nanoparticles were stable at the experimental temperature. All the samples, including both SL

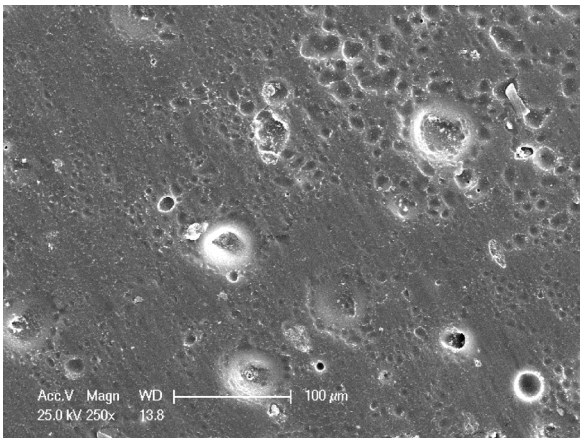

Fig. 3. SEM image of Ag-SLN.

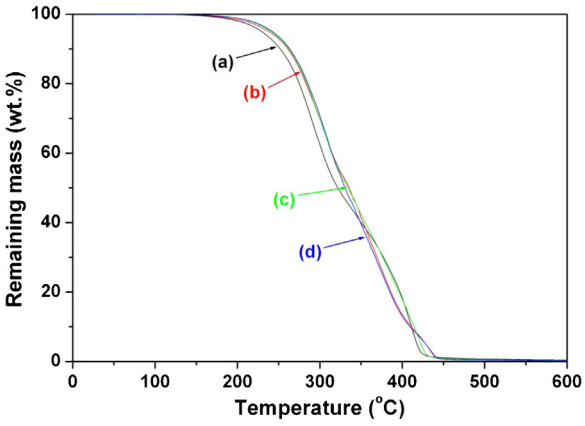

Fig. 5. TGA curves of (a) SL, (b) 1500 mg/L Ag-SLN, (c) 3000 mg/L Ag-SLN, and (d) 6000 mg/L Ag-SLN.

Table 2
Results for antimicrobial test of Ag-SLN.

Strain (CFU at 0 h)	Ag° dose (mg/L)				
	Incubated time (h)	Mean CFU values ± (s.d)			
		0 (Control)	1500 (I)	3000 (II)	6000 (III)
E. coli (10^7)	24	$2.4 \times 10^8 \pm 1.4 \times 10^7$	30 ± 10^2	0	0
	72	$1.7 \times 10^8 \pm 5.8 \times 10^7$	80 ± 10^3	0	0

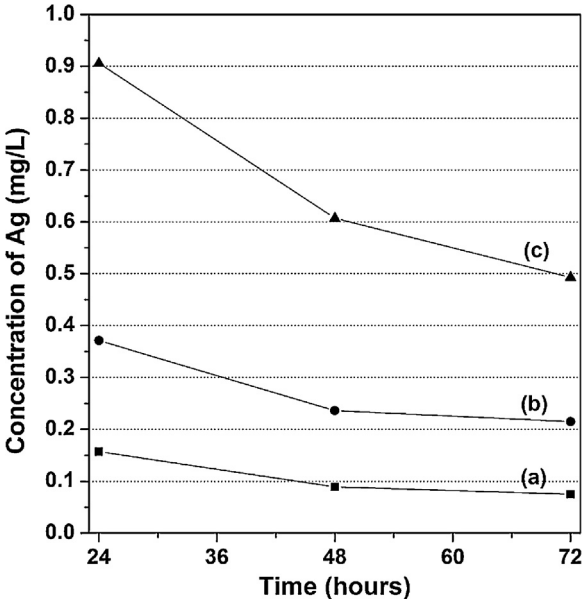

Fig. 6. Release rates of silver into (a) 1500 mg/L Ag-SLN, (b) 1500 mg/L Ag-SLN and (c) 1500 mg/L Ag-SLN.

and Ag-SLN, exhibited a rather similar pattern of degradation. This suggests that silver nanoparticles are uniformly immobilized with SL. In addition, there was a slight sign of thermal stability improvement obtained from the immobilization of the silver nanoparticles.

Fig. 6 shows the release of silver nanoparticles as a function of immersion time in water. It can be seen that for Ag-SLN, a small amount of silver nanoparticles was released. When (a) 1500 mg/L Ag-SLN, (b) 3000 mg/L Ag-SLN, or (c) 6000 mg/L Ag-SLN was shaken for 3 days, the remaining silver nanoparticles on the SL was reduced to 0.08 mg/L, 0.21 mg/L, and 0.49 mg/L, respectively. In addition, the average release rates of (a) 1500 mg/L Ag-SLN, (b) 3000 mg/L Ag-SLN, and (c) 6000 mg/L Ag-SLN were 0.0017, 0.0035, and 0.0088 mg/L for 1 h, respectively.

The silver nanoparticle release tests were combined with antimicrobial efficacy tests against *E. coli* in order to determine whether there was a direct correlation. The initial concentration of *E. coli* was 1.7×10^7 CFU mL^{-1}. After 24 h and 72 h, the concentration of living *E. coli* in the suspension was measured. The results are summarized in Table 2. When compared to the CFU at 0 h, the control group (0 mg/L Ag°) did not show any microbial inhibitory effect against the strains. For the *E. coli* bacterial strains, the MBC of the Ag°-incorporated samples was identified at doses above 1500 mg/L; no viable cells were detected (no CFU) at conditions of 3000 mg/L above.

The above results show that the *E. coli* were killed by silver nanoparticles released from the Ag-SLN (bactericidal effect). The *E. coli* survived, but could not grow into colonies on the Ag-SLN surfaces, because in the suspensions taken from the Ag-SLN surfaces silver nanoparticles were present. Therefore, we found that the silver nanoparticles inhibited the growth of *E. coli* cells.

4. Conclusion

This paper reported a new route for the preparation of Ag-ASDL composites as an antimicrobial agent. Our results illustrated the characteristics of Ag-SLN through UV–Vis spectroscopy, TEM, FE-SEM/EDAX, XRD, AAs, and antimicrobial efficacy tests. The Ag-SLN surface was covered with silver particles and the diameter of the silver particles was about 5–50 μm. As a result of thermal analysis, we found that the silver nanoparticles were uniformly immobilized with SL and the Ag-SLN showed a slight sign of thermal stability improvement obtained from the immobilization of the silver nanoparticles. The silver nanoparticle release tests were combined with antimicrobial efficacy tests against *E. coli*. As a result, we found that there was a direct correlation between the release amounts of silver nanoparticles and the antimicrobial effect on *E. coli*. Suspensions of 3000 mg/L Ag-SLN and 6000 mg/L Ag-SLN showed greater antimicrobial effects (99.9%) on the *E. coli* (ATCC 6538). Therefore, we demonstrated a new route for the preparation of Ag-SLN, and this could be proposed as an antimicrobial dental material.

Acknowledgement

This research was supported by Kyungpook National University Research Fund, 2011.

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