Antibacterial Activity of Epoxy Resins Mixed with Polyelectrolyte/Silver Nanoparticle Composite Filler

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In this study, antibacterial polyelectrolyte/silver nanoparticle (Ag NP) composite powder was mixed with epoxy resins as fillers to impart antibacterial activity. Either an anhydride-type or a polyamideamine-type curing agent was applied to a bisphenol A-type base compound to prepare the epoxy resins. Antibacterial assays of these resins against Escherichia coli were performed using the shake tube technique; suppression of antibacterial activity was found for the anhydride-type resin, although the polyamideamine-type resin did exhibit antibacterial activity. The anhydride-type resin is considered to have suppressed antibacterial activity because carboxylate ions derived from unreacted anhydride in aqueous medium can induce chemical adsorption of silver ions and/or charge repulsion of E. coli.

Key words : Nanocomposite / Thermosetting resin / Escherichia coli.

Silver nanoparticles (Ag NPs) have broad spectrum antimicrobial activity, affecting bacteria (Pal et al., 2007), fungi (Gajbhiye et al., 2009), and viruses (Elechiguerra et al., 2005). Antimicrobial activity of Ag NPs is usually determined by size and shape of the particles. Pal et al. (2007) reported that truncated triangular AgNPs displayed stronger antibacterial activity against Escherichia coli than spherical and rod-shaped Ag NPs. Elechiguerra et al. (2005) showed that Ag NPs in a range of 1–10 nm exclusively inhibited the viral infectivity for the MT-2 cells and cMAGI cells in human immunodeficiency virus 1 (HIV-1) studies.

Although fundamental research on antimicrobial activity of Ag NPs is usually carried out with particle suspension, practical applications of Ag NPs as a suspension are difficult because Ag NPs may diffuse into the environment and thus reach living organisms, with harmful effects (Braydich-Stolle et al., 2010; Wise et al., 2010). Thus, it is preferable to apply Ag NPs by fixing to suitable materials such as polymer resins. However, when Ag NPs are mixed with polymer resins, they tend to aggregate because of their large specific surface area, causing functional reduction in antimicrobial activity (Agnihotri et al., 2014). Avoiding aggregation of Ag NPs in resins could contribute to express their proper antimicrobial activity.

Previously, we investigated polymer/Ag NP composites in which Ag NPs were homogeneously dispersed without aggregation (Mori et al., 2013; Nguyen et al., 2013). These composites were water-insoluble brown powders displaying broad-spectrum antimicrobial activity against bacteria, fungi (Nguyen et al., 2013), and viruses (Mori et al., 2013), similar to suspensions of Ag NPs. It is expected that antimicrobial activity derived from Ag NPs can be conveniently transferred to general polymer resins by mixing with polymer/Ag NP composites. In this paper, we briefly report on the antibacterial activity of thermosetting epoxy resins mixed with polymer/Ag NP composite powder as filler. Two types of epoxy resin were investigated to consider the effect of chemical structure of the resin on the antibacterial activity of the filler.

Synthesis of Ag NPs was carried out by reduction of Ag⁺ in water with glucose as a reductant and soluble starch as a stabilizing agent (Vigneshwaran et al., 2006). An aqueous mixture of 0.2 M silver nitrate, 2 wt% glucose, and 20 wt% soluble starch was heated in an autoclave at 121°C for 20 min for aqueous dispersion of Ag NPs. These reagents were obtained from Wako.
Two types of epoxy resins were used in this study. Type "AH" resin was prepared from Epikote 828 resin base and an anhydride-type curing agent HN-2200 (Hitachi chemical Co., Ltd., Tokyo, Japan). Epikote 828 and HN-2200 were combined at a ratio of 5:4 (wt:wt). Before curing, a drop of 1-(2-cyanoethyl)-2-ethyl-4-methylimidazole was added as a curing promoter. The mixture was preheated at 150°C for 90 min and then cured at 180°C for 2 h. Type "PA" resin was prepared from ThreeBond 2022 resin base and a polyamideamine-type curing agent, ThreeBond 2105C (ThreeBond Co., Ltd., Tokyo, Japan) at a ratio of 2:1 (wt:wt). This was pre-cured at room temperature overnight and then cured at 100°C for 1 h. The composite was mixed with the resin base prior to applying the curing agent. It should be noted that both Epikote 828 and ThreeBond 2022 are bisphenol A-type base compounds.

Specimens for transmission electron microscopy (TEM) were prepared by casting a drop of Ag NPs suspension or the composites onto a carbon-coated copper grid. Observations were carried out with JEOL JEM-2100 (Tokyo, Japan) at 200kV.

Antibacterial activity of the composite was assayed using the shake tube technique as follows: E. coli (NBRC3301) was pre-incubated at 30°C in lysogeny broth (LB) medium consisting of 1 wt% polypepton (hipolypepton, Nihon Pharmaceutical Co., Ltd., Osaka, Japan), 0.5 wt% yeast extract (Difco, NJ), and 1 wt% of NaCl overnight until reaching saturation. The initial working dilution of E. coli was prepared as concentration of 5 x 10^7 cells/cm³ in 10-fold diluted NB medium with sterilized water. All resin squares (surface area: ca. 2 cm²) were then soaked in 5 ml of working dilution in test tubes which were then shaken at 30°C and 80 rpm for 24 h. Bacterial concentrations of the incubated dilution were evaluated by measuring optical density (OD) at 600 nm, which of 0.1 corresponds to a concentration of ca. 10^5 E. coli cells per cm³ (Sondi and Salopek-Sondi, 2004). A ratio of OD of solution treated with the composite to untreated control indicates a survival rate of E. coli cells by treatment and was used as an index of antibacterial activity in this study. Elution of Ag from resins containing the composite to water was assayed with inductively coupled plasma optical emission spectrometry (ICP-OES, Varian 720-ES, CA). Elution test was carried out in 5 ml of distilled water which was shaken at 30°C and 80 rpm for 24 h. Test solutions for ICP-OES were prepared as 10-fold dilution of original solutions in 0.1 M of HNO₃.

The CHP800/Ag NP composite powder prepared in this study was dark brown in color with an average particle size of 1.00 μm (measured with a laser diffraction particle size analyzer SALD-2200, Shimadzu, Japan). As similar as our previous work (Nguyen et al., 2013), the Ag NPs were strongly bound to CHP800 matrices and Ag NPs in the composite did not elute in water. Figure 1 shows TEM micrographs of Ag NPs (indicated by arrows) against E. coli displayed on an lysogeny broth (LB) agar medium.

![FIG. 1. TEM micrographs of Ag NPs (left) and the CHP800/Ag NPs composite (right). Scale bar = 100 nm.](image)

![FIG. 2. Antibacterial activity of the CHP800/Ag NPs composite powder (indicated by arrows) against E. coli displayed on an lysogeny broth (LB) agar medium.](image)
Ag NP composite powder placed on LB agar plate, indicating expression of antibacterial activity against *E. coli*. Despite the Ag NPs in the matrix tending to be aggregated compared with chitosan/Ag NP composites previously studied, levels of antibacterial activity of the CHP800/Ag NPs composite against *E. coli* were similar to those of other chitosan/Ag NPs composites, which could release silver ions to water (Nguyen et al., 2013).

For both AH- and PA-type resins, CHP800/Ag NP composite powder could be combined by mixing before curing. To determine the effects of combining the composite with the resins on antibacterial activity, we assayed their activity against *E. coli*. Figure 3 shows antibacterial activity of the resins with and without the composite against *E. coli* assayed by the shake tube technique. OD of untreated control solution during incubation was increased from < 0.01 to around 0.1–0.2, which indicated normal cell growth of *E. coli*. Both AH- and PA-type resins without the composite did not show antibacterial activity. For PA-type resins with the composite, antibacterial activity was enhanced with inclusion of the composite. The ratios of OD of solution treated with the PA resin containing 2 and 5 wt% of the composite to untreated control at 24 h were 0.513 and 0.056, respectively, suggesting antibacterial activity of the composite. PA-type resins did not show antibacterial activity. For PA-type resins with the composite, antibacterial activity was enhanced with inclusion of the composite. The ratios of OD of solution treated with the PA resin containing 2 and 5 wt% of the composite to untreated control at 24 h were 0.513 and 0.056, respectively, suggesting antibacterial activity of the composite.

Another hypothesis is by repulsion between AH-type resins and *E. coli*. It is known that surfaces of general *E. coli* strains were negatively charged in wide ranges of pH (2 – 11.2) and ionic strength (1.5 – 150 mM of PBS) (Li and McLandsborough, 1999). Thus, it is difficult for *E. coli* to adhere onto negatively charged surfaces (Gottenbos et al., 2001). Radovic-Moreno et al. (2012) developed nanoparticle-type antibacterial materials which could be controlled their antibacterial activities against *E. coli* by varying surface charge of the particles; their positively charged surface could bind to bacteria and enhanced antibacterial activity, whereas negatively charged surface could not bind to bacteria and suppressed the activity. Because AH-type resins were considered to be charged negatively by carboxylate ions, we also deduced that antibacterial activity of the composite in AH-type resin was suppressed by adsorption of silver ions by carboxylate ions derived from unreacted anhydride.

In summary, we present the preparation of antibacterial epoxy resins using polycation/silver nanoparticle composite. Cationic coagulant CHP800 was suitable for synthesis of well-segregated polymer/Ag NP composite with antibacterial activity against *E. coli*. By binding the composite to the resin, antibacterial activity of the composite was suppressed in AH-type resin; however, PA-type resin could express this activity. We consider that adsorption of silver ions and/or charge repulsion of *E. coli* by carboxylate ions derived from unreacted anhydride in the AH-type resin suppressed the antibacterial activity of the composite against *E. coli*.

![FIG. 3. Antibacterial activity of the epoxy resins assayed by the shake tube technique. AH: anhydride-type curing agent; PA: polyamideamine-type curing agent.](image-url)
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