Science of the Total Environment 609 (2017) 289–296

Contents lists available at ScienceDirect

Science of the Total Environment

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

Disinfection of various bacterial pathogens using novel silver nanoparticle-decorated magnetic hybrid colloids

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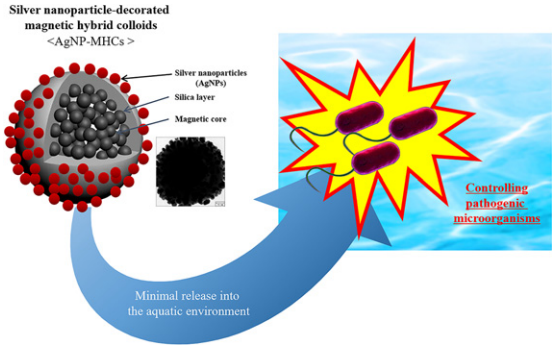
HIGHLIGHTS

- The use of AgNPs for controlling pathogens is hindered by several major obstacles.
- AgNP-MHCs can prevent the aggregation of AgNPs and be re-collected easily after use.
- AgNP-MHCs showed great antibacterial capabilities in various environmental conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 March 2017
Received in revised form 7 July 2017
Accepted 8 July 2017
Available online 25 July 2017

Editor: Simon Pollard

Keywords:
Silver nanoparticle
Magnetic hybrid colloid
Disinfection
Antibacterial agents
Aerobic
Anaerobic

ABSTRACT

Silver nanoparticles (AgNPs) have long been considered a powerful disinfectant for controlling pathogenic microorganisms. However, AgNPs might have adverse effects on both human health and our ecosystems due to their potential cytotoxicity and the difficulty in recovering them after their release into the environment. In this study, we characterized the antimicrobial efficacy caused by a novel micrometer-sized magnetic hybrid colloid (MHC) containing 7, 15, or 30 nm sized monodispersed AgNPs (AgNP-MHCs), which can be re-collected from the environment using simple procedures, such as a magnet or centrifugation. We evaluated the antibacterial capabilities of AgNP-MHCs against target bacteria (*Legionella pneumophila*, *Bacillus subtilis*, *Escherichia coli*, and *Clostridium perfringens*) and compared them with the inactivation efficacy of AgNPs ~30 nm in diameter (nAg30s). Among the different AgNP-MHCs composites evaluated, Ag30-MHCs had the greatest antibacterial effect. After 1 h of exposure, more than a 4-$\log_{10}$ reduction of *L. pneumophila* and 6-$\log_{10}$ reduction of *B. subtilis* was achieved by 4.6×10^9 particles/mL of Ag30-MHCs and Ag30-MHC-Ls. In addition, Ag30-MHC-Ls maintained their strong antibacterial capabilities under anaerobic conditions. Our results indicate that AgNP-MHCs can be considered excellent tools for controlling waterborne bacterial pathogens, with a minimal risk of release into the environment.

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http://dx.doi.org/10.1016/j.scitotenv.2017.07.071
0048-9697/

1. Introduction

The effective antimicrobial activity of silver has been known for centuries (Jung et al., 2008). It is widely used in cutlery, ornamentation, and to create medical instruments (Jung et al., 2008; Lara et al., 2011; Rai et al., 2009). Moreover, silver sulfadiazine cream has been used as a wound care product or for the treatment of burns because of its strong antimicrobial effect against a variety of microorganisms (Rai et al., 2009). Given the remarkable advances in nanotechnology, the practical uses of silver nanoparticles (AgNPs) for controlling pathogens have also been considered (Lara et al., 2011; Rai et al., 2009; Yin et al., 2011). A number of recent studies have indicated that AgNPs are effective agents for inactivating various microorganisms (Kim et al., 2007; Lara et al., 2010; Lu et al., 2008; Speshock et al., 2010). Therefore, AgNPs have recently been applied to various consumer products, such as detergents, plastics, toothpastes, and fabrics (Benn et al., 2010; Yin et al., 2011).

Various mechanisms could exist for the antimicrobial activity of AgNPs, depending on the type of microorganism and setting. Both Ag^+ ions and reactive oxygen species (ROS) released from AgNPs can damage microbial membranes and/or modify sulfur-containing biomolecules (He et al., 2011; Pal et al., 2007; Panacek et al., 2006; Park et al., 2009). The chemical abstraction of Mg^{2+} or Ca^{2+} from microbial membranes is also an important factor in the inhibition of various microorganisms, with the antimicrobial effect of AgNPs remaining controversial (He et al., 2011; Park et al., 2009, 2013a; Sotiriou and Pratsinis, 2010; Xiu et al., 2012; Yin et al., 2011). In addition, the shape, size, and concentration of AgNPs are crucial factors for determining the efficacy of their antimicrobial characteristics, and these parameters should be optimized prior to field applications (Kim et al., 2007; Morones et al., 2005; Pal et al., 2007; Sondi and Salopek-Sondi, 2004). The conjugation of AgNPs to particular functional materials, such as stabilizer or titanium dioxide, can enhance the efficacy of their antimicrobial capability (Liga et al., 2011; Schneid et al., 2014).

Despite the growing interest in AgNPs, they have not been widely applied in the field of water disinfection due to potential concerns about ecotoxicity. For example, the aggregation of AgNPs commonly occurs in water, which reduces their antimicrobial effectiveness (Lara et al., 2011). Furthermore, the release of AgNPs into aquatic environment could result in ecotoxicity. Several previous studies have revealed the various cytotoxic effects of AgNPs (Hsin et al., 2008; Mukherjee et al., 2012; Park et al., 2011; Yin et al., 2011). The release of AgNPs into aquatic environment should therefore be controlled, but the re-collection of AgNPs after disinfection is difficult due to their small particle sizes. To overcome these limitations of conventional AgNPs, novel micrometer-sized magnetic hybrid colloids (MHC) decorated with various sizes of AgNPs (AgNP-MHCs) were developed recently (Park et al., 2013a, 2013b). These AgNP-MHCs can prevent the aggregation of AgNPs and can be easily re-collected after the treatment. However, the efficacy of these particles against different bacterial pathogens under various field conditions has not yet been evaluated. In this study, we characterized the efficacy of AgNP-MHCs against various structural and physiological types of bacteria in different environmental conditions (i.e., various temperatures and oxygen contents). In addition, we compared the antibacterial effects of AgNP-MHCs and AgNPs ~30 nm in diameter (nAg30s) and evaluated the release of AgNPs from the AgNP-MHCs after use.

2. Materials and methods

2.1. Preparation of AgNP-MHCs and nAg30s

The MHCs were decorated with 7, 15, and 30 nm AgNPs (AgNP-MHCs; Ag07-MHCs, Ag15-MHCs, and Ag30-MHCs, respectively). The synthesis of nAg30s was undertaken at the Molecular Recognition Research Center of the Korea Institute of Science and Technology (KIST), Seoul, Korea. A version of Ag30-MHCs (Ag30-MHCs-Ls) was produced with a larger MHC core (~650 nm diameter) than that of AgNP-MHCs (~500 nm diameter). All of the AgNP-MHCs used in this study were synthesized following the seeding, coalescing, and growing strategy of AgNPs on the surface of the MHCs (Park et al., 2013a, 2013b, 2014). The characterization of AgNP-MHCs was conducted using a transmission electron microscope (TEM, CM30, Philips Inc., Amsterdam, The Netherlands) and an environmental scanning electron microscope (ESEM, XL30, FEI Co., Hillsboro, OR, USA) at the Advanced Analysis Center of KIST, as in our previous studies (Park et al., 2013a).

The synthesis of nAg30s occurred via a seed-mediated growth process. For the preparation of the Ag seeds, 1 mL 0.067 M tetrakis (hydroxymethyl) phosphonium chloride (Sigma Aldrich Ltd., St Louis, MO, USA) aqueous solution was added to 50 mL 0.01 M NaOH solution (Sigma Aldrich Ltd.), and the mixture was stirred for 1 min. Then, 1 mL 1% $AgNO_3$ (Sigma Aldrich Ltd.), by weight/volume (w/v) was rapidly injected into solution, followed by stirring for 5 min. To grow the seeds up to ~30 nm in size, 3.33 mL of the Ag seed solution were added to 1 L 0.01% $AgNO_3$ by w/v, containing 2 mL NH_4OH in an ice bath, followed by stirring for 5 min. Then, 0.13 mL formaldehyde (Sigma Aldrich Ltd.) was slowly injected, and the mixture was stirred for 1 min, with the subsequent addition of 50 mL 0.034 M trisodium citrate dihydrate (Sigma Aldrich Ltd.) aqueous solution. The mixture was stirred continuously for 1 h in an ice bath, and then stirred for a further 15 min without the ice bath. The resulting nAg30s solution (20 mL) was centrifuged after adding 20 mL ethanol (Sigma Aldrich Ltd.), and the solid was dispersed in 20 mL 0.01 M NaOH. The synthesized nAg30s were characterized by TEM. For TEM analysis, nAg30s solution was concentrated by centrifugation after mixing with ethanol. After removal of most of the supernatant, the resulting solution was dropped onto a carbon-coated copper grid.

2.2. Preparation of the bacteria used in tests

Legionella pneumophila (*L. pneumophila*) was isolated from a cooling tower in Jeju Island, Korea and cultured in *Legionella* charcoal yeast extract (CYE) agar base (Oxoid, Basingstoke, UK), with *Legionella* buffered CYE growth supplement (Oxoid) at 37 °C in a shaking incubator as described previously (Edelstein, 1981; Kim et al., 2012). In addition, *Bacillus subtilis* (*B. subtilis*, ATCC 6633) was cultured in a nutrient agar broth (BD Difco, Franklin Lakes, NJ, USA) at 37 °C (Reeve, 1974), and *Escherichia coli* K12 (*E.coli* K12, ATCC PTA-7555) was grown in tryptic soy broth (BD Bacto, Franklin Lakes, NJ, USA) at 37 °C as described previously (Clavero and Beuchat, 1996). *Clostridium perfringens* (*C. perfringens*, ATCC 13124) was cultivated on reinforced clostridial medium (BD Difco) at 37 °C in a vinyl anaerobic chamber (COY, Laboratory Products, Gass Lake, MI, USA), containing a mixture of 5% H_2 and CO_2 in N_2, as described previously, with some modification (Cocolin et al., 2004). After overnight cultivation of the bacteria described above, the bacterial concentration was measured by serial dilution and a subsequent cultivation method. All of the cultivated bacteria were stored at 4 °C until use.

2.3. Quantitative evaluation of the antibacterial efficacy of AgNP-MHCs and nAg30s against target bacteria

To evaluate the antibacterial efficacy of AgNP-MHCs, *L. pneumophila* and *B. subtilis* were exposed to 4.6×10^9 particles/mL of each type of AgNP-MHC (Ag07-MHCs, Ag15-MHCs, Ag30-MHCs, and Ag30-MHC-Ls) in a shaking incubator (150 rpm) at two different temperatures (5 or 20 °C) in deionized water (DW). The same amount (i.e., 4.6×10^9 particles/mL) of OH-MHCs, which was not decorated with any AgNPs, was used as a control. After 5, 10, 20, 30 min, or l h of exposure, the surviving bacteria were measured quantitatively by the serial dilution and cultivation method (Park et al., 2013a). To determine the antibacterial efficacy of Ag30-MHCs or Ag30-MHC-Ls, three disinfection kinetics models, the Chick-Watson, modified Chick-Watson (MCW), and modified Hom