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Disinfection of waterborne viruses using silver nanoparticle-decorated silica hybrid composites in water environments

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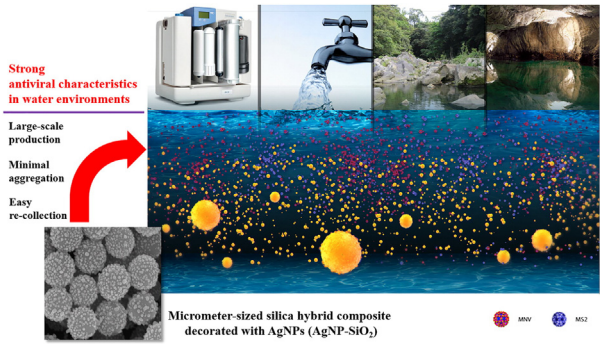
HIGHLIGHTS

- AgNP-SiO_2 can be synthesized using a high-yield, large-scale process.
- AgNP-SiO_2 maintained strong antiviral characteristics in different types of water.
- Modified Hom was the best model for murine norovirus disinfection using AgNP-SiO_2.
- AgNP-SiO_2 can be used without significant risk to human health and the environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Silver nanoparticles (AgNPs) have been reported as an effective alternative for controlling a broad-spectrum of pathogenic viruses. We developed a micrometer-sized silica hybrid composite decorated with AgNPs (AgNP-SiO_2) to prevent the inherent aggregation of AgNPs, and facilitated their recovery from environmental media after use. The production process had a high-yield, and fabrication was cost-effective. We evaluated the antiviral capabilities of Ag30-SiO_2 particles against two model viruses, bacteriophage MS2 and murine norovirus (MNV), in four different types of water (deionized, tap, surface, and ground). MNV was more susceptible to Ag30-SiO_2 particles in all four types of water compared to MS2. Furthermore, several water-related factors, including temperature and organic matter content, were shown to affect the antimicrobial capabilities of Ag30-SiO_2 particles. The modified Hom model was the best-fit disinfection model for MNV disinfection in the different types of water. Additionally, this study demonstrated that the effects of a certain level of physical obstacles in water were negligible in regards to the use of Ag30-SiO_2 particles. Thus, effective use of AgNPs in water disinfection processes can be achieved using our novel hybrid composites to inactivate various waterborne viruses.

1. Introduction

The capabilities of silver nanoparticles (AgNPs) to control various pathogenic microorganisms have been examined (Lara et al., 2011; Marambio-Jones and Hoek, 2010; Zhang et al., 2016). As previous

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studies have reported, AgNPs can effectively inhibit a broad spectrum of microorganisms, including various viruses (Cameron et al., 2015; Lara et al., 2011, 2009). Moreover, due to the extraordinary technological progress, it has become feasible to add functional materials, such as glycoprotein, curcumin and stabilizers, to AgNPs, to enhance their antimicrobial capability (Gahlawat et al., 2016; Yang et al., 2016; Zhang et al., 2016). Therefore, the use of AgNPs in water disinfection is an appealing alternative to conventional chemical disinfectants, which can form harmful byproducts after use (Ahmed et al., 2014; Li et al., 2008).

However, the use of AgNPs to control waterborne viruses faces several major obstacles. First, the agglomeration of AgNPs in water could decrease their antiviral effects (Lara et al., 2011). Second, as several studies have already found, the AgNPs remaining in water after the disinfection process could have severe cytotoxic and genotoxic effects on humans and damage ecosystems (AshaRani et al., 2009; Mukherjee et al., 2012; Nguyen et al., 2016; Soares et al., 2016). Therefore, the efficiency of the AgNPs in water disinfection and their effective removal from the environmental media are important factors to consider (Park et al., 2013, 2014). Third, the most appropriate use of AgNPs in water treatment is difficult to estimate because of the lack of studies undertaken to determine the best-fit disinfection model for each type of AgNP. Additionally, several factors, such as the cost-effectiveness of the use of AgNP in different types of water and the need for experts to synthesize and modify AgNPs, should be taken into consideration.

In this study, we performed a large-scale synthesis of novel silver nanoparticle-decorated silica hybrid composites (Ag30-SiO_2), that were suitable for mass-production (Ko et al., 2014). The antiviral capabilities of Ag30-SiO_2 particles were evaluated for different types of water using bacteriophage MS2 and murine norovirus (MNV), which is one of the major surrogates of human norovirus. Two well-known disinfection models were applied for the inactivation data of both viruses to suggest a best-fit model of Ag30-SiO_2 particles. Additionally, we evaluated the effects of turbidity-causing materials on the antiviral capabilities of Ag30-SiO_2 particles.

2. Materials and methods

2.1. Synthesis of ~30 nm-diameter silver nanoparticle-decorated silica hybrid composite (Ag30-SiO_2)

The Ag30-SiO_2 particles were fabricated on a large scale according to the reported method (Ko et al., 2014) with a minor modification for a stable dispersion of the material. Briefly, monodispersed silica particles with a diameter of 401 ± 13 nm (mean ± standard deviation [SD]) were prepared and then treated with 3-(aminopropyl)trimethoxysilane (97%, Sigma Aldrich Ltd., St Louis, MO, USA) to yield amine-functionalized silica particles (AP-SiO_2) by following the previously reported method (Ko et al., 2014). One hundred milliliters of 32.1% AP-SiO_2 aqueous solution was mixed with 530 mL of 0.1 M HCl to adjust the pH value to ~4. After stirring for homogeneous dispersion, the solution was equally divided into two bottles (solution A). Additionally, six sets of Ag seed solutions were prepared as follows: 0.34 mL of tetrakis(hydroxymethyl)phosphonium chloride (80% solution in water, Sigma Aldrich Korea Ltd.) and 32 mL of 1% $AgNO_3$ solution (Sigma Aldrich Ltd.) were consecutively added into 800 mL of 0.01 M NaOH solution and stirred for 15 min. Six sets of Ag seed solutions were combined and equally divided into two 5-L bottles. Each bottle of solution A was poured into each bottle of Ag seed solution. After gently swirling the mixtures intermittently for 2 h, the Ag-seeded silica was collected using centrifugation, and the combined solid from the two bottles was dispersed in 600 mL of deionized water (DW). This Ag-seeded silica solution was poured into a tailor-made reactor in which 21 g of $AgNO_3$ and 50 mL of NH_4OH (Junsei Chemical Ltd., Tokyo, Japan) were dissolved in 35 L of DW. The reaction temperature was kept at 12 °C using a chiller jacket. After stirring for 30 min to separate out relatively larger seeds, 10 mL of formaldehyde (Sigma Aldrich Ltd.) dissolved in 1 L of DW was added for 1 h; 27.5 mL of formaldehyde in 500 mL of DW was then added for 30 min as a reducing agent to grow the remaining seeds. The solution was further stirred for 1.5 h and left overnight without perturbation. The solid was washed with DW using centrifugation and dispersed in 850 mL of DW, producing a solution containing 4.9×10^{11} Ag30-SiO_2 composites per mL (0.0544 g/mL). It was found that increasing their molar ratio by 2–2.5 times consistently yielded highly stable dispersion for the final Ag30-SiO_2 solution. The Ag30-SiO_2 particles were characterized at the Advanced Analysis Center of Korea Institute of Science and Technology (KIST), as described previously (Park et al., 2013, 2014). A transmission electron microscope (CM30, Philips Inc., Amsterdam, The Netherlands) and an environmental scanning electron microscope (XL30, FEI Co., Hillsboro, OR, USA) were used to confirm homogeneous dispersion and that AgNPs have been robustly fixed onto the Ag30-SiO_2 particles. The naked metallic AgNPs with partial surface oxidation were identified using X-ray photoelectron spectroscopy (PHI 5000 VersaProbe, UlVAC-PHI, Chigasaki, Japan).

2.2. Sampling of various types of water

Four types of water (deionized, tap, surface, and ground) were collected to establish different test conditions. Surface water was sampled from August to September 2014 at Hangang Park Yeouido Area, located on the Han River in Korea. Groundwater was sampled in September 2014 at Haenam-gun, located in Jeollanam-do, Korea. All water samples were collected in 2-L sterilized bottles and stored at 4 °C until use. All physicochemical analyses of water samples were performed by a commercial company (Wendi-Bio Inc., Seongnam, Korea) that is accredited by the Ministry of Environment, Korea.

2.3. Propagation of tested viruses

Bacteriophages MS2 (ATCC 15597-B1) were propagated using the single-agar layer technique, according to our previous study (Lee et al., 2008; Park et al., 2014). First, MS2 were cultured overnight with *Escherichia coli* C3000 (ATCC 15597), the host bacteria, at 37 °C. The propagated phages were collected from the agar layer using phosphate-buffered saline and purified as previously described (Park et al., 2014). Briefly, an equal volume of chloroform was added to the collected lysates and the mixture was centrifuged at 5000 ×*g* for 20 min at 4 °C. Then, the supernatant, containing purified MS2 phages, was recovered and stored at −80 °C until use.

MNV was propagated in RAW264.7 cells as described previously (Park et al., 2014). RAW 264.7 cells were cultured in a sterilized flask using Dulbecco's modified Eagle's medium (Gibco, Waltham, MA, USA) containing 10% fetal bovine serum (Gibco), 10 mM HEPES (Gibco), 10 mM sodium bicarbonate (Gibco), 10 mM nonessential amino acids (Gibco), and 50 μg/μL gentamicin reagent (Gibco). Then, MNV stock was inoculated onto a monolayer of RAW 264.7 cells and cultivated for 4 days. MNV-infected cells were subjected to three times of freeze-thawing cycles to allow MNV to be easily released from damaged cells. An equal volume of chloroform was added to the collected lysate and the mixture was centrifuged at 5000 ×*g* for 20 min at 4 °C. The supernatant containing purified MNV, was concentrated using Amicon Ultra-15 centrifugal filter tubes (Merck Millipore Co., Billerica, MA, USA) and stored at −80 °C until use.

2.4. Antiviral capabilities of Ag30-SiO_2 particles in different types of water

Synthesized Ag30-SiO_2 particles were dispersed in samples of four different types of water for 30 min in a shaking incubator (150 rpm) under the shading condition. Then, approximately 1.0×10^6 plaque forming units (PFU)/mL of MS2 or MNV were exposed to 1.0×10^{10} particles/mL of Ag30-SiO_2 particles in the four types of water at two different temperatures (5 or 20 °C) in a shaking incubator (150 rpm) under the shading