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Impact of solution chemistry on the properties and bactericidal activity of silver nanoparticles decorated on superabsorbent cryogels

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Abstract

This study investigated the effects of dissolved organic matter (DOM) and various electrolytes commonly found in environmental aqueous matrices on the physicochemical properties and bactericidal efficacy of silver nanoparticles (AgNPs), which are immobilized on cryogels (or PSA/AgNP cryogel). The AgNPs in the PSA/AgNP cryogel that were exposed to different media underwent morphological transformation in terms of particle size and structure. In addition, the presence of DOM and electrolytes increased the release of dissolved Ag. The biological uptake of Ag species (determined as the total Ag in exposed cells) increased in the presence of DOM, but decreased in the presence of electrolytes. The presence of electrolytes did not result in any significant reduction in the bactericidal activity. Although an initial increase of the DOM to 2.5 mg-C L⁻¹ attenuated the bactericidal efficacy of the immobilized AgNPs, an increase in the DOM concentration beyond 5 mg-C L⁻¹

enhanced the bactericidal efficacy. This study found that the bactericidal activity of the immobilized AgNPs is less sensitive to the solution chemistry relative to the free AgNPs. This suggests that immobilizing the AgNPs in a supporting material is a good strategy to preserve their efficacy for disinfection in various aqueous matrices.

Keywords: Silver; Composite; Disinfection; Dissolution; Hydrogel; Environmental Matrix;

E. coli

1. Introduction

Risks associated with current disinfection techniques (e.g., formation of disinfection byproducts and multi-drug-resistant bacteria) have prompted the exploration of nanomaterials, particularly silver, as alternative disinfectants [1]. Furthermore, nanosilver-based disinfectants offer the potential to be developed into an effective, affordable, and low energy point-of-use (POU) means for water disinfection to increase the availability of clean water, especially in regions without a clean water supply or recovering from natural disasters [2,3]. There are various successful examples of the fabrication of novel nanosilver-based composites for POU disinfection (Fig S1, Supplementary Data). Among these composites, the PSA/AgNP cryogels have shown superior bactericidal efficacy whereby a 6-log disinfection of a range of typical bacteria can be achieved after 5-min of exposure [4,5]. In addition, these cryogels provide a simple means to recover the disinfected water; they can absorb water up to 200 times their mass in about 15 seconds, and can release this water by gentle squeezing [6].

There is still a debate as to whether the bactericidal mechanism of nanosilver is attributed to Ag^+ or to a nanoparticle effect [7]. Nonetheless, there is growing evidence that suggests localized interfacial interaction between silver nanoparticles (AgNPs) and bacteria

plays a key role in their toxicity mechanism; this is particularly the case for immobilized AgNPs [4,5,8]. Therefore, the composition of the exposure media can alter the surface chemistry of the native particles by influencing the species surrounding the cell-AgNP interface [9]. Indeed, solution parameters such as the dissolved oxygen [10,11], pH [11-13], and the presence of dissolved organic matter (e.g., humic acids, fulvic acids, alginate, albumin, etc.) [12-19] and electrolytes (e.g., Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , HCO_3^- , NO_3^- , SO_4^{2-}) [13,14,18,20-22] have been found to significantly affect the antimicrobial efficacy of free AgNPs. However, most prior studies on the impact of solution chemistry were conducted using free AgNP suspensions even though AgNPs are usually applied in the immobilized form to prevent their release into the treated water. The few studies that investigated the impact of solution chemistry on immobilized AgNPs have obtained results that contradict findings drawn from studies using free AgNPs [23,24]. However, no discussion was offered to consolidate the discrepancies. Thus, the understanding on the impact of solution chemistry on AgNPs, particularly in the immobilized form remains vague.

This study sought to gain a deeper understanding of the effects of environmental constituents (i.e., dissolved organic matter (DOM) and multiple ions of varying concentrations under circum-neutral conditions) on the physicochemical properties of immobilized AgNPs and their corresponding bactericidal efficacy using PSA/AgNP cryogels as the model system. Besides their excellent disinfection efficacy, the cryogels are a suitable model system to probe the effects of solution chemistry on the immobilized AgNPs because they are conducive to having the cell-matrix species-AgNP interaction occur within a confined environment that enables an amplified effect of the interaction to be manifest [5]. Unlike other support materials that are more commonly available (e.g., graphene, membranes, silica, zeolite, etc.), the cryogels can provide a dynamic driving force for the cells and matrix species to enter into the pore channels and be in close proximity to the immobilized AgNPs

due to their high and rapid water absorbency, and the presence of numerous well-interconnected pore channels. The underlying mechanisms for the different phenomena observed and discrepancies in earlier studies are also discussed in the present paper. Furthermore, plausible explanations were offered to resolve the discrepancies in observations reported for studies conducted using free AgNPs versus immobilized AgNPs. Although accidental release of large-sized composites such as the PSA/AgNP cryogels to the environment is unlikely to happen, they would be exposed to various environmental matrix species when applied for treatment of natural water samples. Therefore, these insights can help rationalizing changes in the properties of the immobilized AgNPs when applied for treatment of natural water samples with complex solution chemistry.

2. Experimental

2.1 Fabrication and characterization of PSA/AgNP cryogels

PSA cryogels were prepared by cryo-polymerizing sodium acrylate and *N,N'*-methylenebis(acrylamide); detailed description of the synthesis protocol can be found elsewhere [6]. To prepare the PSA/AgNP cryogels, 1 g of the dried PSA cryogels was immersed in 250 mL of 10 mM Ag⁺ solution for 24 h in the dark before they were reduced in a 250 mL 0.1 M NaBH₄ solution. The cryogels were thoroughly washed followed by dehydration in *t*-butanol and freeze-drying. The PSA/AgNP cryogel employed in this study had an Ag content of 166.7 ± 15.0 mg/g.

X-ray photoelectron spectroscopy (XPS) was conducted using a Theta Probe XPS spectrometer; the adventitious C 1s core level at 284.8 eV was used as a reference to calibrate the binding energies. An X-ray diffractometer (Bruker D8 Advanced) with monochromatic intensity Cu K α radiation ($\lambda = 1.5418$ Å) was used to acquire X-ray diffraction (XRD) spectra in a 2θ range of 30-80° at a scan rate of 1.5° min⁻¹. TEM samples were prepared by

drop casting a suspension of the powdered samples in ethanol on a carbon-coated Cu grid (300 mesh). After drying, the samples were studied using a transmission electron microscope (TEM, Carl Zeiss Libra 120) at an accelerating voltage of 120 kV. Image-analysis software (ImageJ) was used to analyze the TEM images to determine the AgNP-size distribution.

2.2 Preparation of test solutions for exposure studies

Two series of environmentally relevant freshwater matrices were used in this study: one contained varying concentrations of DOM, while the other contained different concentrations of multiple monovalent and divalent inorganic ions. The DOM-containing matrices were prepared by dissolving a well-characterized humic acid (with further details in Tables S1 and S2 in the Supplementary Data), Suwannee River Humic Acid (SRHA) Standard II obtained from International Humic Substances Society (IHSS), in deionized (DI) water followed by filtration through a 0.45 μm pore-size Millipore filter. The DOM concentration was varied from 0.5 to 25 mg-C L^{-1} (pH value range: 6-7). The total organic carbon (TOC) concentration in the filtered samples was verified using a TOC analyzer (TOC-V_{CSH}, Shimadzu). The concentration range was selected because DOM concentrations in surface water usually range from 0.1 mg-C L^{-1} (for springs and small mountain streams) to 25 mg-C L^{-1} (for water originating from moors and wetlands) [25,26]. Hereafter, the solutions containing DOM will be referred to as DOM water with their concentration specified, when necessary. Reconstituted water (pH 7-8) containing the major ions present in natural water (i.e., Cl^- , HCO_3^- , SO_4^{2-} , Na^+ , K^+ , Ca^{2+} , Mg^{2+}) [27] with varying concentrations (and hardness) were prepared according to a method described by USEPA [28]. Hereafter, the reconstituted water will be referred according to their hardness level, i.e., soft water (40-48 $\text{mg-CaCO}_3 \text{ L}^{-1}$); moderately hard water (80-100 $\text{mg-CaCO}_3 \text{ L}^{-1}$); and hard water (160-180 $\text{mg-CaCO}_3 \text{ L}^{-1}$).

2.3 Protocols for microbiological assays

Escherichia coli (*E. coli*; ATCC25922) was used as the model bacterium to evaluate the impact of solution chemistry on the bactericidal efficacy of the immobilized AgNPs. The test suspensions were prepared by re-suspending mid-exponential-phase cultures in the designated exposure medium forming a suspension of cell density 10^6 colony-forming units per mL (cfu mL⁻¹). A 0.02 g cryogel sample was allowed to swell in 10 mL of the bacterial suspension for 5 min before the absorbed water was recovered via squeezing. The spread-plate method was used for quantifying the number of viable cells. Note that the DOM and electrolytes added at the indicated concentrations did not cause any significant reduction of viable bacteria. At least five replicates were conducted.

E. coli before and after exposure to the PSA/AgNP cryogels in various media were observed using FESEM (JEOL JSM-7600F). The samples were fixed with 2% glutaraldehyde for 1 h followed by repeated washings in a 0.1 M sodium cacodylate buffer and dehydration in a series of aqueous ethanol solutions of increasing concentration for 15 min before they were freeze-dried for at least 24 h. The samples were coated with Pt for 30 s (20 mA) using an auto-fine coater (JEOL JFC-1600) prior to FESEM imaging.

The intracellular reactive oxygen species (ROS) buildup was monitored by measuring the enhancement of the fluorescence intensity of the exposed samples relative to that of the control. The *E. coli* were incubated in 2 $\mu\text{g mL}^{-1}$ of 2',7'-dichlorodihydrofluorescein diacetate for 30 min before they were exposed to the PSA/AgNP cryogels for 2 h. The fluorescence intensity of the water recovered by squeezing was measured using a microplate reader (Biotek Synergy 2) at excitation and emission wavelengths of 485 and 535 nm, respectively. A luciferin/luciferase assay using a BacTiter-Glo microbial cell viability assay kit (Promega) was employed to determine the intracellular ATP (adenosine triphosphate) content in the *E. coli*. The luminescence signal was measured in a microplate reader (Biotek Synergy 2).

Triplicate experiments were conducted for both assays. For both assays, abiotic control samples exposed to the PSA/AgNP cryogel samples were also prepared to account for possible interference due to the optical properties of the Ag species. Results for additional control experiments conducted using PSA cryogels can be found in the Supplementary Data (Figs. S2 and S3).

2.4 Analytical methods for quantification of Ag

The cellular fraction was separated from the suspensions via centrifugation at 10,000 rpm for 15 min. The supernatant of the bacterial suspension was further filtered through a 0.2 μm membrane to obtain the extracellular phase. All samples were digested in concentrated HNO_3 prior to total Ag analyses. A catalytic amount of H_2O_2 (30% v/v) was added to ensure the complete digestion of the samples with high organic content. Either an inductively coupled plasma-optical-emission spectrophotometer (ICP-OES, Perkin Elmer Optima 2000DV) or an inductively coupled plasma-mass spectrometer (ICP-MS, Elan DRC-e) was used to determine the total Ag concentration in the samples. All experiments were conducted in triplicate.

3. Results and discussion

3.1 Effect on the physicochemical properties of immobilized AgNPs

To assess the impact of matrix species on the particle morphology, powdered samples of the PSA/AgNP cryogels were dispersed in the test solutions for a prolonged duration before they were subjected to XPS and XRD analyses, and TEM imaging. While the XRD spectra of the exposed samples showed a negligible difference between them (Fig. S4, Supplementary Data), the XPS analyses revealed that most exposed samples showed different degrees of depletion in their surface Ag content (Table 1) and contained a higher amount of oxidized Ag species (Fig. 1). The presence of Ag_2O in the exposed samples is indicated by the presence of

a peak at 367.5 eV, while the predominance of Ag^0 is indicated by the intense peak centered at 368.2 eV [29]. Fresh PSA/AgNP cryogels contained 1.4% of Ag_2O that increased to 4.0% and 5.3% upon exposure to DI water and DOM water (25 mg-C L^{-1}), respectively (Fig. 1). In addition, the surface Ag composition of the PSA/AgNP cryogels was depleted by about 6% compared to the fresh samples (Table 1). However, in the presence of the electrolytes the amount of the Ag_2O layer was only 1.1% (Fig. 1d) and the extent of surface Ag depletion was lower compared to samples exposed to DI and DOM waters (Table 1). The significant enhancement in the surface oxidation of the AgNPs in the presence of DOM – even higher than that in DI water despite the low redox-potential of DOM – may be ascribed to the photoreactivity of SRHA that can generate reactive intermediates and reactive oxygen species (ROS) such as H_2O_2 , $\bullet\text{O}_2^-$, $\bullet\text{OH}$ and $^1\text{O}_2$ among others [30-33].

Besides changes in their chemical states, the AgNPs in samples exposed to the solutions also displayed morphological transformations (Fig. 2). In the fresh PSA/AgNP cryogel samples, AgNPs were well-dispersed in the PSA matrix with a relatively uniform particle diameter ranging from 5-15 nm with a few AgNP clusters of 20-30 nm diameter (Figs. 2a and 3a); the mean AgNP diameter for this sample was $8.6 \pm 3.8 \text{ nm}$. However, a prolonged exposure of the PSA/AgNP cryogels to various types of solutions resulted in the coarsening and broadening of the AgNP-size distribution (Figs. 2 and 3). For example, the mean AgNP diameters for samples exposed to DI water, DOM water, and hard water were 30.5 ± 24.5 , 21.7 ± 15.3 , and $89.9 \pm 28.9 \text{ nm}$, respectively. The broad particle-size distribution in the exposed samples indicates that the AgNP-size increase might be attributed to particle growth due to Ostwald ripening during which small AgNPs dissolve followed by re-deposition of the newly formed AgNPs on the large particles [34].

Despite the increase in the AgNP size, exposure to DOM water and DI water did not significantly change the shape of the particles retaining a somewhat quasi-spherical shape

(sphericity factor >0.88) (Fig. 2b and d). However, the AgNPs in the sample exposed to the hard water showed a significant change in shape to quasi-hexagonal structures with truncated edges (Fig. 2c). The hexagonally shaped particles are probably not due to precipitation of AgCl because there was no evidence of AgCl formation in both the XRD spectrum or the XPS survey scan (Figs. S4 and S5, Supplementary Data). Others have also observed such changes to spherical AgNPs in the presence of anions (e.g., chloride, sulfate, nitrate) [35-38]. It has been proposed that such a shape transformation is due to the selective removal of twinned particles through a chloride-mediated oxidative etching process [36]. This hypothesis is also supported by the fact that the TEM image shows the absence of any twinned particles in the sample (Fig. 2d).

Interestingly, a comparison of the samples exposed to DOM water (Fig. 2c) with those exposed to DI water (Fig. 2b) indicates that a large number of fine particles (mostly smaller than 10 nm) surrounding the larger clusters can be observed in the former. Although it can be argued that the fine AgNPs may well be the original AgNPs, this is unlikely because small AgNPs would have been preferentially oxidized especially in the presence of DOM, which were found to increase the extent of oxidation of the AgNP surface (Fig. 1). The smaller particles surrounding the large clusters might be the new particles formed from reduction of the dissolved Ag^+ . Although SRHAs enhances the oxidation of AgNPs due to photogeneration of ROS, the presence of functional groups such as phenolic $-\text{OH}$, quinones, hydroxyls, methoxyls, aldehydes, ketones, and enolic $-\text{OH}$ on SRHAs can also create an ideal reductive compartment for Ag^+ reduction to Ag^0 [39]. There is evidence that DOM in general can form AgNPs from Ag^+ , a process that can be accelerated in the presence of sunlight [40-42]. Because DOM can increase particle stability via electrosteric effects, the presence of DOM could have enhanced the mobility of the as-formed particles decreasing their propensity to deposit on the existing particles. This could have retarded AgNP growth

that explains the smaller mean AgNP size for samples exposed to DOM water (Fig. 2c) relative to those exposed to DI water (Fig. 2b) and hard water (Fig. 2d).

3.2 Effect on the Ag release behavior and subsequent cell-uptake

The presence of DOM and electrolytes generally increased the release of Ag from the PSA/AgNP cryogels into the dissolved phase (Fig. 4). Nonetheless, the total Ag loss remained less than 0.2% for all types of solutions. Despite the increase in dissolved Ag with solution hardness, the concentration of cell-bound Ag apparently showed the opposite trend (Fig. 4). The increase of dissolved Ag in the presence of electrolytes may be attributed to either (i) formation of dissolved Ag complexes with anions in the reconstituted water or (ii) sequestration of surface chemisorbed Ag^+ by the anions that can cause instability to the NP structure and induce further dissolution [43,44]. The reduced uptake of the Ag species might be due to the lower bioavailability of the Ag complexes with the inorganic anions [45,46]. Note that cell-bound Ag is thought to result from the uptake of dissolved Ag species rather than direct internalization of the AgNPs because passive diffusion across bacterial membranes is limited to solutes smaller than 600 Da [47]. Besides the lower bioavailability of the resultant Ag complexes with the inorganic anions, the reduction in cell-bound Ag could be also attributed to competitive sorption of cations (Ag^+ versus Mg^{2+} and Ca^{2+}) onto bacterial binding sites [48].

Increasing the DOM content in the solution increased the concentration of cell-bound Ag as well as the amount of dissolved Ag (Fig. 4). A similar observation has been made by other studies [16,49,50]. However, there are other studies that reported reduced Ag dissolution attributable to surface passivation by the DOM layer [14,15]. Notably, adsorption of SRHA on AgNP has been determined to follow a Langmuir adsorption pattern with a maximum adsorption capacity of 28.6 mg TOC per g of AgNPs. The authors further

postulated that SRHA adsorption on AgNPs involves interaction between the Ag^+ (formed on the AgNP surface due to oxidation) with carboxylate groups on the SRHA [51]. On the other hand, DOM-enhanced Ag dissolution has usually been ascribed to ligand-assisted Ag dissolution. It is also possible that the enhanced Ag dissolution in the presence of DOM be attributed to ROS generated by SRHA via photochemical reactions that can cause further oxidative dissolution of the AgNPs. The increase of cell-bound Ag with increasing DOM concentration suggests that Ag-SRHA complexes are sufficiently labile for bacterial uptake (Fig. 4). It has been determined that the interaction between SRHA and Ag^+ is weak [52]. Moreover, it has been found that the toxicity of Ag^+ in the presence of SRHA was not significantly reduced [12,17]. Even if the SRHA is initially biologically refractory, it may also be subsequently degraded into smaller molecules via the action of ROS or photolysis forming labile Ag-SRHA complexes that are more bio-available, especially in systems with high DOM levels [53].

3.3 Effect on the bactericidal efficacy of immobilized AgNPs

AgNPs disinfect by disrupting multiple cellular processes via the release of Ag^+ and particle-specific reactions. For example, uptake of Ag^+ ions can disrupt the bacterial respiratory chain that causes indirect generation of (intracellular) ROS leading to oxidative stress and cell damage as well as metabolic perturbation because of ATP loss. On the other hand, AgNPs can damage cell membranes via physical interactions or particle surface reactions that generate ROS [54]. In view of the multifaceted bactericidal action of AgNPs, the impact of solution chemistry on the toxicity was investigated by assessing the extent of cell topological destruction, metabolic perturbation through ATP depletion, and ROS-mediated damage as well as alterations in the disinfection efficacies to measure different biological outcomes.

The FESEM images revealed that *E. coli* cells exposed to PSA/AgNP cryogels under different solution chemistries sustained varying extents of cell-membrane lesions (Fig. 5). This suggests that the changes in the solution chemistry did not completely diminish the bactericidal effects of the cryogels. In fact, the presence of electrolytes did not cause a significant reduction in toxicity ($p > 0.05$; t -test; $n=6$) (Fig. 6a). This is in contrast to free AgNPs that usually showed a reduction in their bactericidal efficacy in the presence of elevated electrolyte concentration (and hence increased ionic strength) that induced particle aggregation [20,55,56]. The strong anchoring of the AgNPs on the PSA cryogel could have prevented such aggregation behavior; it has been determined that the majority of the Ag species released from PSA/AgNP cryogel was dissolved Ag^+ while the concentration of AgNPs in the solution phase was negligible ($< 10 \mu\text{g/L}$) [5]. Although there is no observable effect on the toxicity, the extent of intracellular ATP depletion decreased slightly with increasing concentration of ions (Fig. 6b). The observation that the disinfection efficacy is maintained in spite of a reduction in the ATP depletion indicates that there are other toxicity routes besides metabolic perturbation.

On the other hand, the DOM had a significant impact on the bactericidal activity of the AgNPs (Fig. 7a). Although the initial increase of DOM to 2.5 mg-C L^{-1} reduced the bactericidal efficacy of the AgNPs, a further increase in the DOM concentration beyond 5 mg-C L^{-1} enhanced the bactericidal efficacy (Fig. 7a). An FESEM image of the bacterial cells exposed to a high DOM concentration (25 mg-C L^{-1}) shows more severe damage (Fig. 5c-e). The observation that the cells appear more shriveled and apparently developed larger holes corroborates the bactericidal results (Figs. 5c-e and 7a). This is rather surprising because several prior studies found that DOM significantly attenuated the bactericidal efficacy of AgNPs [12,18,57]. However, the aforementioned studies conducted their bactericidal tests using free AgNPs. In contrast, the enhanced bactericidal activity of immobilized AgNPs in

the presence of DOM has also been observed by others [23,24]. These conflicting observations suggest that free and immobilized AgNPs may interact differently with DOM. For the toxicity attenuation it has been suggested that DOM may act as a physical barrier to cell-AgNP interactions (due to charge repulsion) by decreasing the AgNP binding to proteins, membrane pitting or decreasing the dissolution of Ag [12]. However, in this study, the presence of DOM enhanced the Ag dissolution and subsequent cell uptake of Ag leading to the enhanced depletion of the intracellular ATP level (Figs. 4 and 7b). Although the increased toxicity of AgNPs in the presence of DOM concentrations beyond 5 mg-C L⁻¹ may be ascribed to the reduction of intracellular ATP levels, the initial reduction of toxicity at low DOM concentrations and the dramatic enhancement in toxicity at high DOM concentrations cannot be solely explained by the depletion of intracellular ATP.

In our previous study, it was found that the formation of ROS plays a critical role in the bactericidal action of AgNPs that can subsequently initiate an autocidal process [5]. In view of the severity of the cell-membrane damage (at 25 mg-C L⁻¹) and the ability of DOM to form and consume radicals, ROS might have a role in modulating the toxicity. To test this hypothesis, the enhancement of intracellular ROS levels of the exposed bacterial cells relative to unexposed cells was determined. The experiment was conducted under illuminated (with ambient lighting) and non-illuminated conditions to discern the ROS formed as a result of the photoreactivity of the DOM. The data obtained under both non-illuminated and illuminated conditions showed that in the presence of DOM, the cells generally have a lower intracellular ROS except at a DOM concentration of 25 mg-C L⁻¹ (Fig. 8a). The initial reduction (for both illuminated and non-illuminated conditions) may be due to scavenging of ROS (and its intermediates) by DOM. The slightly higher intracellular ROS level at 25 mg-C L⁻¹ of DOM, in the absence of illumination, might be due to enhanced indirect ROS generation induced by increased cell-bound Ag. Interestingly, the results obtained under illuminated conditions

show a trend that is broadly consistent with the bactericidal data (Fig. 7a). Notably, a significant enhancement in the intracellular ROS level was observed at 25 mg-C L⁻¹. At such a high DOM concentration, the increased photochemical production of ROS may overcome the ROS scavenging effect on toxicity reduction.

As mentioned earlier, the photodecomposition of DOM produces several types of ROS, which are mostly short-lived with the exception of H₂O₂ whose half-life in water ranges from 1 to 8 h [58]. In this case the lifetime of the ROS is important because it exist as a diffusing species in the bulk solution whereby most of them are not in immediate vicinity of the bacterial cells. Because H₂O₂ is more stable, it may diffuse to the AgNP surface as the (extracellularly) generated H₂O₂ accumulates in the bulk solution (Fig. 9). H₂O₂ by itself is not very effective against microbes, but it has been shown to possess bactericidal properties, especially in the presence of a transition metal that could generate toxic •OH radicals [59]. Furthermore, there is growing evidence that AgNPs can induce the decomposition of H₂O₂, especially under acidic conditions [60-63]:

This hypothesis is consistent with the toxicity data in Fig. 7a that show no significant inactivation of bacteria in the presence of DOM (and illumination) without the AgNPs (i.e., control samples). Furthermore, Fig. 8b shows that the addition of H₂O₂ (up to 10 mM) without exposing the bacteria to PSA/AgNP cryogel did not result in any significant bacterial inactivation ($p > 0.05$; t -test; $n=6$). It should be noted that a narrow region outside the bacterial cell wall that is acidified due to proton extrusion under the influence of a proton motive force can provide the requisite acidic condition [64]. Therefore, it is postulated that the decomposition of H₂O₂ into •OH most likely occurs at the cell-AgNP interface. The immobilized AgNPs allow enhanced contact with bacterial cells [8], and this leads to a more efficient generation of •OH radicals that in turn have a more significant biological effect due

to the proximity of the cells to the as-formed short-lived radicals. This might explain the discrepancy in the influence of the DOM on the toxicity of AgNPs observed in free suspension versus those immobilized on support materials. Fig. 9 illustrates a summary of all the major processes that occur in an exposure media containing either electrolytes or DOM based on the findings of this study.

4. Conclusions

This study found that the exposure of immobilized AgNPs (using the PSA/AgNP cryogels as a model system) to media containing electrolytes and DOM showed an increase in the particle size and the amount of Ag₂O. In particular, immobilized AgNPs exposed to electrolytes transformed from a spherical to a quasi-hexagonal shape, while those exposed to DOM showed the formation of fine AgNPs surrounding larger clusters. Both the electrolytes and DOM resulted in the increased release of Ag into the dissolved phase. Interestingly, the biological uptake of Ag species increased in the presence of DOM, but decreased in the presence of electrolytes. In contrast to free AgNPs whose bactericidal efficacy is frequently reported to be severely affected by the presence of matrix species, little adverse effect was observed for the immobilized AgNPs. This implies that the prior understanding drawn from studies using free AgNPs may not be applicable to immobilized AgNPs. The discrepancies in this observation can in part be attributed to the immobilization of the AgNPs that can overcome physical changes such as aggregation (usually a problem for free AgNPs) when they are exposed to different environmental media. In addition, we propose that the lack of sensitivity of the bactericidal activity of immobilized AgNPs to matrix species may be attributed to the compensatory effect associated with the enhanced AgNP-cell contact that amplifies the toxic effect of biochemical reactions occurring at the interface. For example, the decomposition of H₂O₂, a by-product of the DOM photolysis, at the AgNP-cell interface to

form the toxic $\bullet\text{OH}$ radicals in close proximity to the cell. These new insights can help explain changes in the properties and efficacy of the immobilized AgNPs when used for treatment of natural water samples with complex solution chemistry that is currently lacking. Although the exposure media employed in this study were simulated natural water samples prepared in the laboratory, the principle and understanding generated from this study should also be valuable for future larger scale studies using actual environmental water samples whose compositions may be more complex. We believe that this work has brought us closer in developing this concept into actual devices for applications such as disaster relief.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcis>.

References

- [1] Lalley, J.; Dionysiou, D. D.; Varma, R. S.; Shankara, S.; Yang, D. J.; Nadagouda, M. N., Silver-based antibacterial surfaces for drinking water disinfection - an overview. *Current Opinion in Chemical Engineering* **2014**, *3*, (0), 25-29.
- [2] Loo, S.-L.; Fane, A. G.; Krantz, W. B.; Lim, T.-T., Emergency water supply: A review of potential technologies and selection criteria. *Water Research* **2012**, *46*, (10), 3125-3151.
- [3] Qu, X.; Alvarez, P. J. J.; Li, Q., Applications of nanotechnology in water and wastewater treatment. *Water Research* **2013**, *47*, (12), 3931-3946.
- [4] Loo, S.-L.; Fane, A. G.; Lim, T.-T.; Krantz, W. B.; Liang, Y.-N.; Liu, X.; Hu, X., Superabsorbent cryogels decorated with silver nanoparticles as a novel water technology for point-of-use disinfection. *Environmental Science & Technology* **2013**, *47*, (16), 9363-9371.
- [5] Loo, S.-L.; Krantz, W. B.; Fane, A. G.; Gao, Y.; Lim, T.-T.; Hu, X., Bactericidal mechanisms revealed for rapid water disinfection by superabsorbent cryogels decorated with silver nanoparticles. *Environmental Science & Technology* **2015**, *49*, (4), 2310-2318.
- [6] Loo, S.-L.; Krantz, W. B.; Lim, T.-T.; Fane, A. G.; Hu, X., Design and synthesis of ice-templated PSA cryogels for water purification: Towards tailored morphology and properties. *Soft Matter* **2013**, *9*, (1), 224-234.
- [7] Levard, C.; Hotze, E. M.; Lowry, G. V.; Brown, G. E., Environmental transformations of silver nanoparticles: Impact on stability and toxicity. *Environmental Science & Technology* **2012**, *46*, (13), 6900-6914.
- [8] Agnihotri, S.; Mukherji, S.; Mukherji, S., Immobilized silver nanoparticles enhance contact killing and show highest efficacy: Elucidation of the mechanism of bactericidal action of silver. *Nanoscale* **2013**, *5*, (16), 7328-7340.
- [9] Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M., Understanding biophysicochemical interactions at the nano-bio interface. *Nature Materials* **2009**, *8*, (7), 543-557.
- [10] Xiu, Z.-M.; Ma, J.; Alvarez, P. J. J., Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. *Environmental Science & Technology* **2011**, *45*, (20), 9003-9008.
- [11] Xiu, Z.-M.; Zhang, Q.-B.; Puppala, H. L.; Colvin, V. L.; Alvarez, P. J. J., Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Letters* **2012**, *12*, (8), 4271-4275.
- [12] Fabrega, J.; Fawcett, S. R.; Renshaw, J. C.; Lead, J. R., Silver nanoparticle impact on bacterial growth: Effect of pH, concentration, and organic matter. *Environmental Science & Technology* **2009**, *43*, (19), 7285-7290.
- [13] Fauss, E. K.; MacCuspie, R. I.; Oyanedel-Craver, V.; Smith, J. A.; Swami, N. S., Disinfection action of electrostatic versus steric-stabilized silver nanoparticles on *E. coli* under different water chemistries. *Colloids and Surfaces B: Biointerfaces* **2014**, *113*, (0), 77-84.
- [14] Gao, J.; Youn, S.; Hovsepyan, A.; Llaneza, V. L.; Wang, Y.; Bitton, G.; Bonzongo, J.-C. J., Dispersion and toxicity of selected manufactured nanomaterials in natural river water samples: Effects of water chemical composition. *Environmental Science & Technology* **2009**, *43*, (9), 3322-3328.

- [15] Ostermeyer, A.-K.; Kostigen Mumuper, C.; Semprini, L.; Radniecki, T., Influence of bovine serum albumin and alginate on silver nanoparticle dissolution and toxicity to *Nitrosomonas Europaea*. *Environmental Science & Technology* **2013**, *47*, (24), 14403-14410.
- [16] Pokhrel, L. R.; Dubey, B.; Scheuerman, P. R., Impacts of select organic ligands on the colloidal stability, dissolution dynamics, and toxicity of silver nanoparticles. *Environmental Science & Technology* **2013**, *47*, (22), 12877-12885.
- [17] Kennedy, A. J.; Chappell, M. A.; Bednar, A. J.; Ryan, A. C.; Laird, J. G.; Stanley, J. K.; Steevens, J. A., Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. *Environmental Science & Technology* **2012**, *46*, (19), 10772-10780.
- [18] Zhang, H.; Smith, J. A.; Oyanedel-Craver, V., The effect of natural water conditions on the anti-bacterial performance and stability of silver nanoparticles capped with different polymers. *Water Research* **2012**, *46*, (3), 691-699.
- [19] Yang, X.; Jiang, C.; Hsu-Kim, H.; Badireddy, A. R.; Dykstra, M.; Wiesner, M.; Hinton, D. E.; Meyer, J. N., Silver nanoparticle behavior, uptake, and toxicity in *Caenorhabditis Elegans*: Effects of natural organic matter. *Environmental Science & Technology* **2014**, *48*, (6), 3486-3495.
- [20] Jin, X.; Li, M.; Wang, J.; Marambio-Jones, C.; Peng, F.; Huang, X.; Damoiseaux, R.; Hoek, E. M. V., High-throughput screening of silver nanoparticle stability and bacterial inactivation in aquatic media: Influence of specific ions. *Environmental Science & Technology* **2010**, *44*, (19), 7321-7328.
- [21] Chambers, B. A.; Afrooz, A. R. M. N.; Bae, S.; Aich, N.; Katz, L.; Saleh, N. B.; Kirisits, M. J., Effects of chloride and ionic strength on physical morphology, dissolution, and bacterial toxicity of silver nanoparticles. *Environmental Science & Technology* **2013**, *48*, (1), 761-769.
- [22] Levard, C.; Mitra, S.; Yang, T.; Jew, A. D.; Badireddy, A. R.; Lowry, G. V.; Brown, G. E., Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to *E. coli*. *Environmental Science & Technology* **2013**, *47*, (11), 5738-5745.
- [23] Dankovich, T., Bactericidal paper containing silver nanoparticles for water treatment. Chapter 5: Impact of the influent water composition on bacterial inactivation by a silver nanoparticle paper. pp 127-149. Phd thesis, McGill University, 2012. Available from: http://digitool.library.ccgill.ca/webclient/streamgate?folder_id=0&dvs=1417413811669~411.
- [24] Diagne, F.; Malaisamy, R.; Boddie, V.; Holbrook, R. D.; Eribo, B.; Jones, K. L., Polyelectrolyte and silver nanoparticle modification of microfiltration membranes to mitigate organic and bacterial fouling. *Environmental Science & Technology* **2012**, *46*, (7), 4025-4033.
- [25] Thurman, E. M., Organic geochemistry of natural waters. Martinus Nijhoff/Junk Publishers, The Netherlands. **1985**.
- [26] Kramer, K. J. M.; Jak, R. G.; van Hattum, B.; Hoofman, R. N.; Zwolsman, J. J. G., Copper toxicity in relation to surface water-dissolved organic matter: Biological effects to daphnia magna. *Environmental Toxicology and Chemistry* **2004**, *23*, (12), 2971-2980.
- [27] A.M. Nikanorov; L.V. Brazhnikova, Water chemical composition of rivers, lakes and wetlands, in: M.G. Khublaryan (Ed.), Types and properties of water - volume 2, EOLSS Co Ltd **2009**, pp. 42–80. Available from: <http://www.eolss.net/sample-chapters/c07/e2-03.pdf>

- [28] United States Environmental Protection Agency, Dilution water, **2007**. Available from: http://water.epa.gov/scitech/methods/cwa/wet/upload/2007_07_10_methods_wet_disk2_atx7-10.pdf.
- [29] Hoflund, G.; Hazos, Z.; Salaita, G., Surface characterization study of Ag, AgO, and Ag₂O using X-ray photoelectron spectroscopy and electron energy-loss spectroscopy. *Physical Review B* **2000**, *62*, (16), 11126-11133.
- [30] Latch, D. E.; McNeill, K., Microheterogeneity of singlet oxygen distributions in irradiated humic acid solutions. *Science* **2006**, *311*, (5768), 1743-1747.
- [31] Aiken, G. R.; Hsu-Kim, H.; Ryan, J. N., Influence of dissolved organic matter on the environmental fate of metals, nanoparticles, and colloids. *Environmental Science & Technology* **2011**, *45*, (8), 3196-3201.
- [32] Mopper, K.; Zhou, X., Hydroxyl radical photoproduction in the sea and its potential impact on marine processes. *Science* **1990**, *250*, (4981), 661-664.
- [33] Scully, N. M.; Cooper, W. J.; Tranvik, L. J., Photochemical effects on microbial activity in natural waters: The interaction of reactive oxygen species and dissolved organic matter. *FEMS Microbiology Ecology* **2003**, *46*, (3), 353-357.
- [34] Peretyazhko, T. S.; Zhang, Q.; Colvin, V. L., Size-controlled dissolution of silver nanoparticles at neutral and acidic pH conditions: Kinetics and size changes. *Environmental Science & Technology* **2014**, *48*, (20), 11954-11961.
- [35] Tejamaya, M.; Römer, I.; Merrifield, R. C.; Lead, J. R., Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. *Environmental Science & Technology* **2012**, *46*, (13), 7011-7017.
- [36] Wiley, B.; Herricks, T.; Sun, Y.; Xia, Y., Polyol synthesis of silver nanoparticles: Use of chloride and oxygen to promote the formation of single-crystal, truncated cubes and tetrahedrons. *Nano Letters* **2004**, *4*, (9), 1733-1739.
- [37] Jin, R.; Cao, Y.; Mirkin, C. A.; Kelly, K. L.; Schatz, G. C.; Zheng, J. G., Photoinduced conversion of silver nanospheres to nanoprisms. *Science* **2001**, *294*, (5548), 1901-1903.
- [38] Yang, J.; Zhang, Q.; Lee, J. Y.; Too, H.-P., Dissolution–recrystallization mechanism for the conversion of silver nanospheres to triangular nanoplates. *Journal of Colloid and Interface Science* **2007**, *308*, (1), 157-161.
- [39] Stevenson, F. J., Humic chemistry: Genesis, composition, reactions. Wiley: New york, 1994.
- [40] Yin, Y.; Liu, J.; Jiang, G., Sunlight-induced reduction of ionic Ag and Au to metallic nanoparticles by dissolved organic matter. *ACS Nano* **2012**, *6*, (9), 7910-7919.
- [41] Hou, W.-C.; Stuart, B.; Howes, R.; Zepp, R. G., Sunlight-driven reduction of silver ions by natural organic matter: Formation and transformation of silver nanoparticles. *Environmental Science & Technology* **2013**, *47*, (14), 7713-7721.
- [42] Akaighe, N.; MacCusprie, R. I.; Navarro, D. A.; Aga, D. S.; Banerjee, S.; Sohn, M.; Sharma, V. K., Humic acid-induced silver nanoparticle formation under environmentally relevant conditions. *Environmental Science & Technology* **2011**, *45*, (9), 3895-3901.
- [43] Li, X.; Lenhart, J. J.; Walker, H. W., Dissolution-accompanied aggregation kinetics of silver nanoparticles. *Langmuir* **2010**, *26*, (22), 16690-16698.
- [44] Huynh, K. A.; Chen, K. L., Aggregation kinetics of citrate and polyvinylpyrrolidone coated silver nanoparticles in monovalent and divalent electrolyte solutions. *Environmental Science & Technology* **2011**, *45*, (13), 5564-5571.

- [45] Gupta, A.; Maynes, M.; Silver, S., Effects of halides on plasmid-mediated silver resistance in *Escherichia Coli*. *Applied and Environmental Microbiology* **1998**, *64*, (12), 5042-5045.
- [46] Behra, R.; Sigg, L.; Clift, M. J. D.; Herzog, F.; Minghetti, M.; Johnston, B.; Petri-Fink, A.; Rothen-Rutishauser, B., Bioavailability of silver nanoparticles and ions: From a chemical and biochemical perspective. *Journal of the Royal Society Interface* **2013**, *10*, (87), 20130396.
- [47] Denyer, S. P.; Stewart, G. S. A. B., Mechanisms of action of disinfectants. *International Biodeterioration & Biodegradation* **1998**, *41*, (3–4), 261-268.
- [48] Anderson, J. W.; Semprini, L.; Radniecki, T. S., Influence of water hardness on silver ion and silver nanoparticle fate and toxicity toward *Nitrosomonas Europaea*. *Environmental Engineering Science* **2014**, *31*, (7), 403-409.
- [49] Chappell, M. A.; Miller, L. F.; George, A. J.; Pettway, B. A.; Price, C. L.; Porter, B. E.; Bednar, A. J.; Seiter, J. M.; Kennedy, A. J.; Steevens, J. A., Simultaneous dispersion–dissolution behavior of concentrated silver nanoparticle suspensions in the presence of model organic solutes. *Chemosphere* **2011**, *84*, (8), 1108-1116.
- [50] Ivask, A.; ElBadawy, A.; Kaweeteerawat, C.; Boren, D.; Fischer, H.; Ji, Z.; Chang, C. H.; Liu, R.; Tolaymat, T.; Telesca, D.; Zink, J. I.; Cohen, Y.; Holden, P. A.; Godwin, H. A., Toxicity mechanisms in *Escherichia Coli* vary for silver nanoparticles and differ from ionic silver. *ACS Nano* **2013**, *8*, (1), 374-386.
- [51] Gao, J.; Powers, K.; Wang, Y.; Zhou, H.; Roberts, S. M.; Moudgil, B. M.; Koopman; B.; Barber, D. S., Influence of Suwannee River humic acid on particle properties and toxicity of silver nanoparticles. *Chemosphere* **2012**, *89*(1), 96-101.
- [52] Chen, Z.; Porcher, C.; Campbell, P. G. C.; Fortin, C., Influence of humic acid on algal uptake and toxicity of ionic silver. *Environmental Science & Technology* **2013**, *47*, (15), 8835-8842.
- [53] Mopper, K.; Zhou, X.; Kieber, R. J.; Kieber, D. J.; Sikorski, R. J.; Jones, R. D., Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature* **1991**, *353*, (6339), 60-62.
- [54] Rizzello, L.; Pompa, P. P., Nanosilver-based antibacterial drugs and devices: Mechanisms, methodological drawbacks, and guidelines. *Chemical Society Reviews* **2014**, *43*, (5), 1501-1518.
- [55] Liu, X.; Jin, X.; Cao, B.; Tang, C. Y., Bactericidal activity of silver nanoparticles in environmentally relevant freshwater matrices: Influences of organic matter and chelating agent. *Journal of Environmental Chemical Engineering* **2014**, *2*, (1), 525-531.
- [56] Zhang, H.; Oyanedel-Craver, V., Evaluation of the disinfectant performance of silver nanoparticles in different water chemistry conditions. *Journal of Environmental Engineering* **2012**, *138*, (1), 58-66.
- [57] Liu, J.; Hurt, R. H., Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environmental Science & Technology* **2010**, *44*, (6), 2169-2175.
- [58] Cooper, W. J.; Zepp, R. G., Hydrogen peroxide decay in waters with suspended soils: Evidence for biologically mediated processes. *Canadian Journal of Fisheries and Aquatic Sciences* **1990**, *47*, (5), 888-893.
- [59] Juven, B. J.; Pierson, M. D., Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation. *Journal of Food Protection* **1996**, *59*, (11), 1233-1241.
- [60] He, W.; Zhou, Y.-T.; Wamer, W. G.; Boudreau, M. D.; Yin, J.-J., Mechanisms of the pH dependent generation of hydroxyl radicals and oxygen induced by Ag nanoparticles. *Biomaterials* **2012**, *33*, (30), 7547-7555.

- [61] He, D.; Miller, C. J.; Waite, T. D., Fenton-like zero-valent silver nanoparticle-mediated hydroxyl radical production. *Journal of Catalysis* **2014**, *317*, (0), 198-205.
- [62] He, D.; Dorantes-Aranda, J. J.; Waite, T. D., Silver nanoparticle—algae interactions: Oxidative dissolution, reactive oxygen species generation and synergistic toxic effects. *Environmental Science & Technology* **2012**, *46*, (16), 8731-8738.
- [63] He, D.; Garg, S.; Waite, T. D., H₂O₂-mediated oxidation of zero-valent silver and resultant interactions among silver nanoparticles, silver ions, and reactive oxygen species. *Langmuir* **2012**, *28*, (27), 10266-10275.
- [64] Koch, A. L., The pH in the neighborhood of membranes generating a protonmotive force. *Journal of Theoretical Biology* **1986**, *120*, (1), 73-84.

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Table 1 Surface Ag content of PSA/AgNP cryogels as a function of the exposure solution

Samples	Ag3d atomic composition (%)
Fresh	15.7
DIW	9.7
DOM water (25 mg-C/L)	9.6
Hard water	11.3

Fig. 1 XPS spectra of the PSA/AgNP cryogel (a) before and after exposure to (b) DI water, (c) DOM water, and (d) reconstituted water for 168 h. Note that the insets in (c) and (d) provide a comparison of the XPS spectra of the control and cryogel samples exposed to varying concentrations of (c) DOM or (d) electrolytes.

Fig. 2 Representative TEM images of PSA/AgNP cryogels before exposure (a) and after prolonged exposure to deionized water for 168 h (b), hard water (c), and DOM water (25 mg-C L^{-1}) (d). Note that AgNPs in cryogel samples exposed to hard water transformed into hexagonal particles (c) while those exposed to DOM water show the formation of fine particles surrounding the larger AgNP clusters (d).

Fig. 3 AgNP-size distribution of the PSA/AgNP cryogels (a) before and after exposure to (b) DI water, (c) DOM water (25 mg-C L⁻¹), and (d) hard water for 168 h.

Fig. 4 Effect of DOM and electrolytes (as a mixture of Cl^- , HCO_3^- , SO_4^{2-} , Na^+ , K^+ , Ca^{2+} , Mg^{2+}) on the Ag release and cell-uptake behavior. Note: Statistically significant results compared to the control (i.e., samples exposed to DI water) at 95% and 99% confidence level are denoted by (*) and (**), respectively (Student's t test, $n = 3$). Note that soft water, moderately hard water, and hard water have hardness levels of 40-48 $\text{mg-CaCO}_3 \text{ L}^{-1}$, 80-100 $\text{mg-CaCO}_3 \text{ L}^{-1}$, and 160-180 $\text{mg-CaCO}_3 \text{ L}^{-1}$, respectively.

Fig. 5 FESEM images of *E. coli* cells (a) before and after exposure to the PSA/AgNP cryogels in (b) DI water, (c) 0.5 mg-C L⁻¹ DOM, (d) 5 mg-C L⁻¹ DOM, (e) 25 mg-C L⁻¹ DOM, (f) soft water, (g) moderately hard water, and (h) hard water. Note: all the scale bars represent 2 μ m, and soft water, moderately hard water, and hard water have hardness levels of 40-48 mg-CaCO₃ L⁻¹, 80-100 mg-CaCO₃ L⁻¹, and 160-180 mg-CaCO₃ L⁻¹, respectively.

Fig. 6 Effect of electrolytes (as a mixture of Cl^- , HCO_3^- , SO_4^{2-} , Na^+ , K^+ , Ca^{2+} , Mg^{2+}) on the (a) bactericidal efficacy of the PSA/AgNP cryogels and (b) extent of ATP depletion in the exposed cells. Note that soft water, moderately hard water, and hard water have hardness levels of 40-48 $\text{mg-CaCO}_3 \text{ L}^{-1}$, 80-100 $\text{mg-CaCO}_3 \text{ L}^{-1}$, and 160-180 $\text{mg-CaCO}_3 \text{ L}^{-1}$, respectively.

Fig. 7 Effect of DOM on the (a) bactericidal efficacy of the PSA/AgNP cryogels, (b) extent of ATP depletion in the exposed cells.

Fig. 8 (a) Effect of DOM concentration on the intracellular ROS level in the exposed cells. Note: RFU denotes relative fluorescence unit. (b) Effect of H₂O₂ addition on the viability of *E. coli* in DOM water (25 mg-C/L) without exposure to PSA/AgNP cryogels.

Fig. 9 Illustration summarizing the important processes that occur in exposure solutions due to the presence of the electrolyte (left panel) and DOM (right panel).