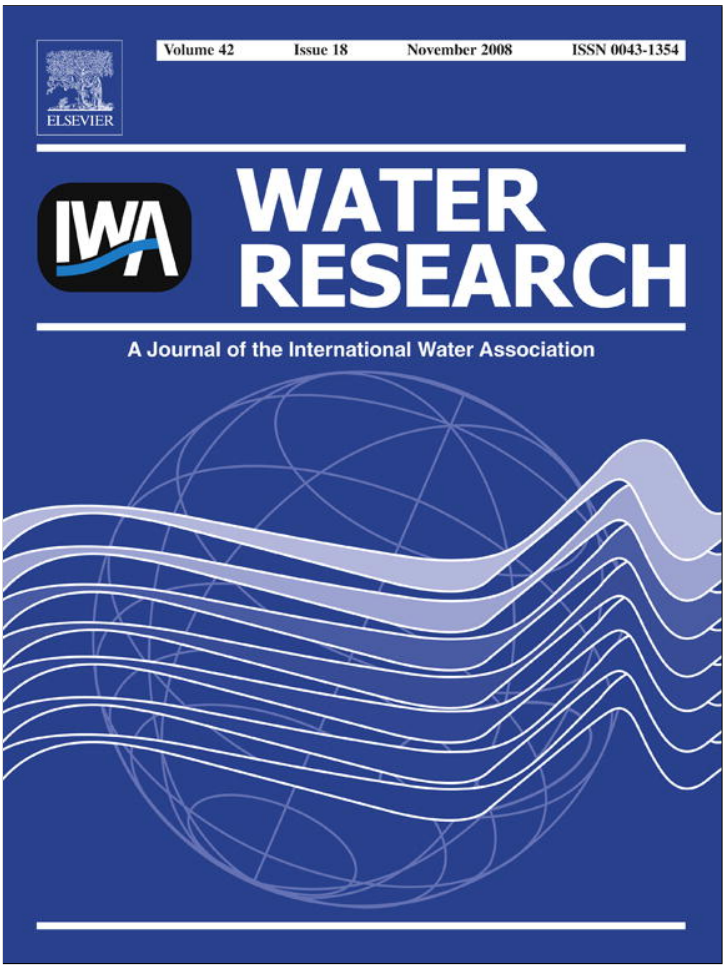

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Review

Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications

*Qilin Li, Shaily Mahendra, Delina Y. Lyon, Lena Brunet, Michael V. Liga, Dong Li, Pedro J.J. Alvarez**

Department of Civil and Environmental Engineering, Rice University, MS 317, 6100 Main Street, Houston, TX 77005, USA

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ABSTRACT

The challenge to achieve appropriate disinfection without forming harmful disinfection byproducts by conventional chemical disinfectants, as well as the growing demand for decentralized or point-of-use water treatment and recycling systems calls for new technologies for efficient disinfection and microbial control. Several natural and engineered nanomaterials have demonstrated strong antimicrobial properties through diverse mechanisms including photocatalytic production of reactive oxygen species that damage cell components and viruses (e.g. TiO_2, ZnO and fullerol), compromising the bacterial cell envelope (e.g. peptides, chitosan, carboxyfullerene, carbon nanotubes, ZnO and silver nanoparticles (*n*Ag)), interruption of energy transduction (e.g. *n*Ag and aqueous fullerene nanoparticles (nC_{60})), and inhibition of enzyme activity and DNA synthesis (e.g. chitosan). Although some nanomaterials have been used as antimicrobial agents in consumer products including home purification systems as antimicrobial agents, their potential for disinfection or microbial control in system level water treatment has not been carefully evaluated.

This paper reviews the antimicrobial mechanisms of several nanoparticles, discusses their merits, limitations and applicability for water disinfection and biofouling control, and highlights research needs to utilize novel nanomaterials for water treatment applications.

Contents

* *Corresponding author*. Tel.: +1 713 348 5903; fax: +1 713 348 2026.
E-mail address: alvarez@rice.edu (P.J.J. Alvarez).
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1. Introduction

The use of sand filtration and chlorine disinfection marked the end of waterborne epidemics in the developed world more than a century ago. However, outbreaks of water borne diseases continue to occur at unexpected high levels. According to the data compiled from Center of Disease Control Morbidity and Mortality Weekly Report, there were 155 outbreaks and 431,846 cases of illness in public and individual U.S. water systems from 1991 to 2000 (Chlorine Chemistry Division of the American Chemistry Council, 2003). Worldwide, waterborne diseases remain the leading cause of death in many developing nations. According to the 2004 WHO report, at least one-sixth of the world population (1.1 billion people) lack access to safe water (WHO, 2004). The consequences are daunting: diarrhea kills about 2.2 million people every year, mostly children under the age of 5. The importance of water disinfection and microbial control cannot be overstated.

Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, research in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection byproducts (DBPs). Chemical disinfectants commonly used by the water industry such as free chlorine, chloramines and ozone can react with various constituents in natural water to form DBPs, many of which are carcinogens. More than 600 DBPs have been reported in the literature (Krasner et al., 2006). Considering the mechanisms of DBP formation, it has been predicted that DBPs will be formed any time chemical oxidants are used in water treatment (Trussell, 1993). Furthermore, the resistance of some pathogens, such as *Cryptosporidium* and *Giardia*, to conventional chemical disinfectants requires extremely high disinfectant dosage, leading to aggravated DBP formation. Therefore, there is an urgent need to reevaluate conventional disinfection methods and to consider innovative approaches that enhance the reliability and robustness of disinfection while avoiding DBP formation.

The rapid growth in nanotechnology has spurred significant interest in the environmental applications of nanomaterials. In particular, its potential to revolutionize century-old conventional water treatment processes has been enunciated recently (USEPA, 2007; Shannon et al., 2008). Nanomaterials are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity. More recently, several natural and engineered nanomaterials have also been shown to have strong antimicrobial properties, including chitosan (Qi et al., 2004), silver nanoparticles (*n*Ag) (Morones et al., 2005), photocatalytic TiO_2 (Cho et al., 2005; Wei et al., 1994), fullerol (Badireddy et al., 2007), aqueous fullerene nanoparticles (nC_{60}) (Lyon et al., 2006), and carbon nanotubes (CNT) (Kang et al., 2007). Unlike conventional chemical disinfectants, these antimicrobial nanomaterials are not strong oxidants and are relatively inert in water. Therefore, they are not expected to produce harmful DBPs. If properly incorporated into treatment processes, they have the potential to replace or enhance conventional disinfection methods.

Another potential application of antimicrobial nanomaterials is their use in decentralized or point-of-use water treatment and reuse systems. The concept of decentralized or distributed water treatment systems has attracted much attention in recent years due to concerns on water loss and quality deterioration associated with aging distribution networks and the increasing energy cost to transport water, as well as the increasing need for alternative water sources and wastewater reuse for areas with water shortage problems and national security issues (NRC, 2006; Haas, 2000). It is envisioned that functional nanomaterials, including those with antimicrobial properties, can be used to build high-performance, small-scale or point-of-use systems to increase the robustness of water supply networks, for water systems not connected to a central network, and for emergency response following catastrophic events.

This paper reviews the antimicrobial mechanisms of several nanomaterials, discusses their applicability for water disinfection and microbial control as well as the limitations,

and highlights critical research needs to realize nanotechnology-enabled disinfection and microbial control.

2. Antimicrobial nanomaterials: microbial toxicity mechanisms

The antibacterial nanoparticles discussed in this paper fall into three general categories: naturally occurring antibacterial substances, metals and metal oxides, and novel engineered nanomaterials. These nanoparticles interact with microbial cells through a variety of mechanisms. The major antimicrobial mechanisms reported in the literature are summarized in Fig. 1. The nanoparticles can either directly interact with the microbial cells, e.g. interrupting transmembrane electron transfer, disrupting/penetrating the cell envelope, or oxidizing cell components, or produce secondary products (e.g. reactive oxygen species (ROS) or dissolved heavy metal ions) that cause damage.

2.1. *Antimicrobial peptides and chitosan*

Naturally occurring chitin and certain peptides have been long recognized for their antimicrobial properties. It is only recently that these materials have been engineered into nanoparticles (Qi et al., 2004; Gazit, 2007). They are promising for low-cost and low-tech disinfection applications, particularly in developing countries. The antibacterial mechanism of natural peptides, such as silk moth cecropins, is the formation of nano-scale channels in bacterial cell membranes, which causes osmotic collapse (Gazit, 2007). Antimicrobial peptides were synthesized initially based on the structures of known proteins (Ghadiri et al., 1994). The ability to synthesize these peptides allows the construction of tailored antimicrobial nanostructures (Gazit, 2007). Engineered peptides are being optimized with respect to size, morphology, coatings, derivatization, and other properties for specific antimicrobial applications.

Chitosan, obtained from chitin in arthropod shells, has long been noted for its antibacterial activity; it is only more recently that this polymer has been made into nanoparticles (Qi et al., 2004; Ye et al., 2006). Nano-scale chitosan as well as its derivatives exhibit antimicrobial effects towards bacteria, viruses, and fungi (Badawy et al., 2005; Qi et al., 2004; Rabea et al., 2003; No et al., 2002; Chirkov, 2002). It was found to be more effective for control of fungi and viruses than bacteria (Rabea et al., 2003); within bacteria, the antimicrobial activity of chitosan is higher for Gram-positive bacteria than Gram-negative bacteria (Don et al., 2005; No et al., 2002). Chitosan prevents the multiplication of bacteriophages in bacteria, and induces resistance towards viral diseases in plants (Chirkov, 2002). A wide range of minimum inhibitory concentrations (MICs) from 18 to 5000 ppm have been reported, depending upon the organism, pH, molecular weight, degree of polymerization, and the presence of lipids and proteins (Rabea et al., 2003; No et al., 2002).

Several antimicrobial mechanisms have been proposed for chitosan. One mechanism involves positively charged chitosan particles interacting with negatively charged cell membranes, causing an increase in membrane permeability and eventually rupture and leakage of intracellular components (Qi et al., 2004). This is consistent with the observation that natural chitosan macromolecules as well as engineered nanoparticles are not effective at pH values above 6 due to the absence of protonated amino groups (Qi et al., 2004). For the same reason, the antibacterial activities of chitosan derivatives containing quaternary ammonium groups, such as *N*,*N*,*N*-trimethyl chitosan, *N*-propyl-*N*,*N*-dimethyl chitosan and *N*-furfuryl-*N*,*N*-dimethyl chitosan are stronger than those of chitosan, and increase with decreasing pH (Jia et al., 2001). In another proposed mechanism, chitosan chelates trace metals, causing inhibition of enzyme activities (Rabea et al., 2003). In fungal cells, chitosan was hypothesized to penetrate the cell wall and nucleus to bind with DNA and inhibit RNA synthesis (Rabea et al., 2003).

2.2. *nAg*

The antimicrobial properties of silver compounds and silver ions have been historically recognized and applied in a wide range of applications from disinfecting medical devices and home appliances to water treatment (Bosetti et al., 2002; Chou et al., 2005; Gupta and Silver, 1998). However, the mechanism of toxicity is still only partially understood.

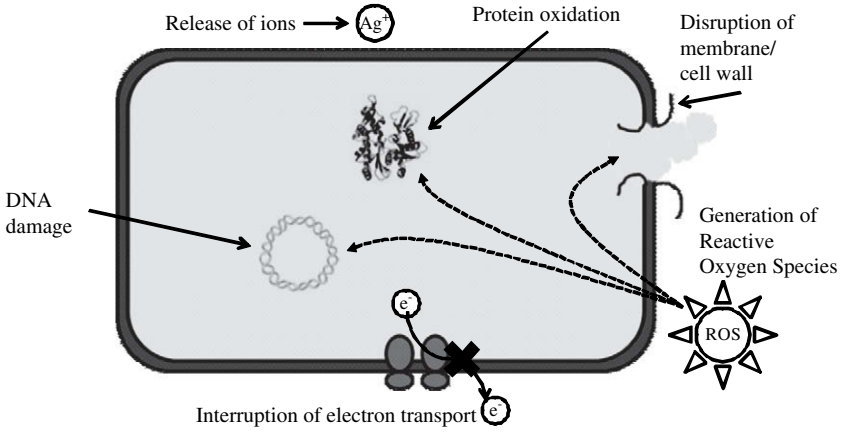

Fig. 1 – Various mechanisms of antimicrobial activities exerted by nanomaterials.

Silver ions interact with thiol groups in proteins, resulting in inactivation of respiratory enzymes and leading to the production of ROS (Matsumura et al., 2003). It was also shown that Ag^+ ions prevent DNA replication and affect the structure and permeability of the cell membrane (Feng et al., 2000). Silver ions are also photoactive in the presence of UV-A and UV-C irradiation, leading to enhanced UV inactivation of bacteria and viruses (Kim et al., 2008; Rahn et al., 1973). It is hypothesized that complexation of Ag^+ with cysteine accelerates photodimerization of viral DNA contributing to a synergistic effect observed in inactivation of *Haemophilus influenzae* phage (Rahn et al., 1973) and MS2 phage (Kim et al., 2008).

To date, several mechanisms have been postulated for the antimicrobial property of silver nanoparticles: (1) adhesion of nanoparticles to the surface altering the membrane properties. *n*Ag particles have been reported to degrade lipopolysaccharide molecules, accumulate inside the membrane by forming ''pits'', and cause large increases in membrane permeability (Sondi and Salopek-Sondi, 2004); (2) *n*Ag particle penetrating inside bacterial cell, resulting in DNA damage; (3) dissolution of *n*Ag releases antimicrobial Ag^+ ions (Morones et al., 2005).

Physicochemical properties play an important role in the antimicrobial activity of *n*Ag. In general, particles of less than 10 nm are more toxic to bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* (Xu et al., 2004; Gogoi et al., 2006). Silver nanoparticles ranging from 1 to 10 nm inhibit certain viruses from binding to host cells by preferentially binding to the virus' gp120 glycoproteins (Elichiguerra et al., 2005). Furthermore, triangular *n*Ag nanoplates containing more reactive <111> planes were found to be more toxic than *n*Ag rods, *n*Ag spheres, or Ag^+ ions (Pal et al., 2007).

2.3. TiO_2

TiO_2 is the most commonly used semiconductor photocatalyst. Among the different nanomaterials, it is the most studied. Activated by UV-A irradiation, its photocatalytic properties have been utilized in various environmental applications to remove contaminants from both water and air (Gelover et al., 2006; Murray et al., 2007; Salthammer and Fuhrmann, 2007). A wealth of information on TiO_2 photocatalytic inactivation of bacteria has been acquired over the last 20 years (Matsunaga et al., 1985; Wei et al., 1994). TiO_2 can kill both Gram-negative and Gram-positive bacteria, although Gram-positive bacteria are less sensitive due to their ability to form spores (Wei et al., 1994). More recently, nano-sized TiO_2 was also reported to kill viruses including poliovirus 1 (Watts et al., 1995), hepatitis B virus (Zan et al., 2007), Herpes simplex virus (Hajkova et al., 2007), and MS2 bacteriophage (Cho et al., 2005). The concentration of TiO_2 usually required to kill bacteria varies between 100 and 1000 ppm, depending on the size of the particles and the intensity and wavelength of the light used (Wei et al., 1994).

The antibacterial activity of TiO_2 is related to ROS production, especially hydroxyl free radicals and peroxide formed under UV-A irradiation via oxidative and reductive pathways, respectively (Kikuchi et al., 1997). Strong absorbance of UV-A renders activation of TiO_2 under solar irradiation, significantly enhancing solar disinfection. In a study by Gelover et al. (2006), complete inactivation of fecal coliforms was achieved in 15 min at an initial concentration of 3000 cfu/100 mL by exposing water in TiO_2-coated plastic containers to sunlight whereas the same inactivation required 60 min with uncoated containers. More importantly, the same study found that the bacteria exposed to TiO_2 photocatalytic disinfection do not self repair. However, bacterial death also occurred in the dark indicating that other unknown mechanisms may be involved (Adams et al., 2006).

An attractive feature of TiO_2 photocatalytic disinfection is its potential to be activated by visible light, e.g. sunlight. Metal doping has long been known to improve visible light absorbance of TiO_2 (Anpo et al., 2001; Williamson, 1939), and increase its photocatalytic activity under UV irradiation (Choi et al., 1994; Mu et al., 1989; Duonghong et al., 1981). Noble metals, especially silver, have received much attention for this purpose. Silver has been shown to enable visible light excitation of TiO_2 (Seery et al., 2007). Recently, it was demonstrated that doping TiO_2 with silver greatly improved photocatalytic inactivation of bacteria (Page et al., 2007; Reddy et al., 2007) and viruses (Kim et al., 2006). For example, Reddy et al. (2007) demonstrated that 1 wt% Ag(0) in TiO_2 reduced the reaction time required for complete removal of 10^7 cfu/mL *E. coli* from 65 to 16 min in UV-A light. Ag(0) is believed to enhance photoactivity by facilitating electron–hole separation and/or providing more surface area for adsorption (Sung-Suh et al., 2004; Sclafani et al., 1997). Visible light absorption by silver surface plasmons is thought to induce electron transfer to TiO_2 resulting in charge separation and thus activation by visible light (Tian and Tatsuma, 2004; Seery et al., 2007). Ag/TiO_2 therefore shows great promise as a photocatalytic material due to its photoreactivity and visible light response.

2.4. ZnO

Similar to TiO_2, nano-sized ZnO has been used in sunscreens, coatings, and paints because of its high UV absorption efficiency and transparency to visible light (Franklin et al., 2007). ZnO nanoparticles exhibit strong antibacterial activities on a broad spectrum of bacteria (Sawai, 2003; Adams et al., 2006; Jones et al., 2008; Huang et al., 2008a). The antibacterial mechanism of ZnO is still under investigation. The photocatalytic generation of hydrogen peroxide was suggested to be one of the primary mechanisms (Sawai, 2003). In addition, penetration of the cell envelope and disorganization of bacterial membrane upon contact with ZnO nanoparticles were also indicated to inhibit bacterial growth (Brayner et al., 2006; Huang et al., 2008a). However, the role of Zn^{2+} ion released from dissolution of ZnO is not clear (Franklin et al., 2007; Sawai, 2003). It has been suggested that Zn^{2+} ion binding to the membranes of microorganisms can prolong the lag phase of the microbial growth cycle (Atmaca et al., 1998). Contradictory results have been reported about the impact of particle size on the antibacterial activity of ZnO. Jones et al. (2008) observed that smaller ZnO particles were more toxic than bigger particles, but no size related effect was found in another study by Franklin et al. (2007).

2.5. Fullerenes

The discovery and subsequent ability to synthesize fullerenes introduced a new material with high electroconductivity, tensile strength, and unique thermal and optical properties (Kroto et al., 1985; Kratschmer et al., 1990). Current and future applications range from nanoelectronics to nanocomposites and drug delivery. Fullerenes (C_{60}, C_{70}, etc.) are highly insoluble in water (Heymann, 1994), but they can be made water-soluble by derivatization. Several C_{60} derivatives have shown antibacterial effects (Tsao et al., 2002; Spesia et al., 2007), but toxicity depends on the specific derivative (Sayes et al., 2004; Tang et al., 2007). C_{60} can also form stable aqueous suspensions of nanoparticles, termed nC_{60} (Fortner et al., 2005), with a large range of sizes (Brant et al., 2006). The nC_{60} nanoparticles are noted for their potent broad spectrum antibacterial activity (Lyon et al., 2005, 2006, 2008a).

The antibacterial mechanism for nC_{60} is still under debate. Studies observing nC_{60} cytotoxicity in eukaryotic systems have attributed the toxicity to ROS production (Oberdorster, 2004; Sayes et al., 2005; Isakovic et al., 2006; Markovic et al., 2007). However, studies observing nC_{60} in prokaryotic systems assert that antibacterial activity is mediated via direct oxidation of the cell (Fang et al., 2007; Lyon et al., 2008b). It is also debatable whether the antimicrobial activity observed is due to the organic solvent residual from the preparation (Brant et al., 2005; Zhang et al., 2008).

2.6. Carbon nanotubes

Carbon nanotubes are graphene sheets rolled into a tube and possibly capped by half a fullerene. They can be either single-walled (SWNTs), a single pipe with a diameter from 1 to 5 nm, or multi-walled (MWNTs), with several nested tubes, at lengths varying from 100 nm up to several tens of micrometers. Toxicity studies on CNTs indicate that they exert acute pulmonary toxicity and cytotoxicity to mammalian cells (Warheit et al., 2004; Wick et al., 2007), with the toxicity decreasing from SWNTs > MWNTs > quartz > C_{60} (Jia et al., 2005). Surprisingly, the effect of CNTs on bacteria and viruses has not received particular attention, probably due to the difficulty of dispersing CNTs in water. Surfactants or polymers such as sodium dodecyl benzenesulfate (SDBS), polyvinylpyrolidone (PVP) or Triton-X are generally used to facilitate the dispersion. The few studies available credited SWNTs with antimicrobial activity towards Gram-positive and Gram-negative bacteria, and the damages inflicted were attributed to either a physical interaction or oxidative stress that compromise cell membrane integrity (Kang et al., 2007, 2008; Narayan et al., 2005). Carbon nanotubes may therefore be useful for inhibiting microbial attachment and biofouling formation on surfaces. However, the degree of aggregation (Wick et al., 2007), stabilization effects by NOM (Hyung et al., 2007), and the bioavailability of the nanotubes (Brunet et al., 2008) will have to be considered with attention for these antimicrobial properties to be fully effective.

3. Current and potential applications for disinfection and microbial control

Several nanomaterials, such as *n*Ag, chitosan, and TiO_2, have found applications in diverse consumer products and industrial processes, including water treatment, as antimicrobial agents (Table 1).

Among all antimicrobial nanomaterials, *n*Ag is probably the most widely used. It is used as an antimicrobial agent in over 100 consumer products (Maynard, 2007), ranging from nutrition supplements to surface coating of kitchen appliances (Chen and Schluesenera, 2008; Maynard, 2007). Commercial home water purification systems such as Aquapure®, Kinetico®, and QSI-Nano®, which are reported to remove 99.99% pathogens, use membranes impregnated with silver or surfaces coated with silver (Maynard, 2007).

Nanoparticulate chitosan is used in cosmetics, food preservation, agriculture, and medical applications (Rabea et al., 2003). In addition to the antimicrobial function, it also confers hydrophobicity and mechanical strength to the products. Chitosan has been used in full scale water and wastewater treatment systems as a coagulant/flocculant (Zeng et al., 2008), and was recently used as a disinfectant (Denkbas and Ottenbrite, 2006; Rabea et al., 2003).

Because of its whiteness and high refractive index, TiO_2 nanoparticles, particularly in the anatase form, are already used in a large number of consumer products such as sunscreen, toothpaste, paints, and coatings. It is used in home air purifiers, e.g. 3Q™ Multi Stage Air Purifier and NanoBreeze® Room Air Purifier, to remove volatile organic compounds and kill bacteria. Industrial scale water purification systems using TiO_2 photocatalysis already exist (e.g. Purifics®), although its main application is organic contaminant degradation instead of disinfection.

To assess the potential of the antimicrobial nanomaterials as alternative disinfectants in drinking water treatment, the reported antimicrobial effectiveness of selected nanomaterials is compared to conventional water disinfectants in Table 2. It is demonstrated that these nanomaterials have similar or superior antimicrobial activities compared to conventional chemical disinfectants. They can potentially be used as alternative disinfectants or in conjunction with existing technologies, such as UV, to enhance disinfection efficacy. Another potential application is in fouling control of water filtration membranes and other surfaces in reactors and pipelines used in water treatment and distribution. Table 1 summarizes the potential antimicrobial applications of different nanomaterials, the form of which strongly depends on the properties of the specific nanomaterial, as discussed below.

3.1. Chitosan

Nano-scale chitosan has potential drinking water disinfection applications as an antimicrobial agent in membranes, sponges, or surface coatings of water storage tanks. It has advantages over other disinfectants because it has a higher antibacterial activity, a broader spectrum of activity against bacteria, viruses, and fungi, and a lower toxicity towards

Table 1 – Current and potential applications of antimicrobial nanomaterials

Nanomaterial	Antimicrobial mechanism	Current applications	Potential future applications
Chitosan	Membrane damage, chelation of trace metals	Personal care products, microbicide in agriculture and biomedical products, food wraps, biomedical, flocculants in water and wastewater treatment	Immobilizer of bacteria, enzymes and other biological molecules, biosorbents
*n*Ag	Release of Ag^+ ions, disruption of cell membrane and electron transport, DNA damage	Portable water filters, clothing, medical devices, coatings for washing machines, refrigerators, and food containers	Surface coatings, membranes
TiO_2	Production of ROS, cell membrane and cell wall damage.	Air purifiers, water treatment systems for organic contaminant degradation	Solar and UV disinfection of water and wastewater, reactive membranes, biofouling-resistant surfaces
CNT	Physically compromise cell envelope	None	Biofouling-resistant membranes, carbon hollow fibers, packed bed filters
ZnO	Intracellular accumulation of nanoparticles, cell membrane damage, H2O2 production, release of Zn^{2+} ions	Antibacterial creams, lotions and ointment, deodorant, self-cleaning glass and ceramics	Surface coating, mouthwash

higher order animals and humans. However, the effectiveness of microbial control depends upon the material preparation method and presence of organics. Chitosan is an effective disinfectant only at acidic pHs because of its solubility and the availability of charged amino groups (Rabea et al., 2003; No et al., 2002). The preparation of water-soluble derivatives of chitosan may eventually overcome this limitation.

3.2. TiO_2

TiO_2 is suitable for applications in water treatment because it is stable in water, non-toxic by ingestion and low-cost. A large number of studies on the photocatalytic disinfection efficiency of TiO_2 have demonstrated potential of TiO_2 for drinking water disinfection (Table 2). The photoactivity in the UV-A range and the potential visible light activity when doped with metals makes TiO_2 photocatalytic disinfection especially useful in developing countries where electricity is not available. However, TiO_2 based solar disinfection is in general a very slow process due to the small fraction of UV-A in solar radiation. Therefore, success in research on metal or nitrogen doping to improve visible light absorbance of TiO_2 or UV-A activity is critical to the application of TiO_2 solar disinfection. Recently, it was demonstrated that doping TiO_2 with silver greatly improved photocatalytic bacterial inactivation by UV-A activated TiO_2 (Page et al., 2007; Reddy et al.,

Table 2 – Relative effectiveness of various antimicrobial agents

Disinfectant	CT[a] (mg-min/L or mJ/cm^2)	References
Chitosan	7.5–144[b]	Don et al., 2005; Qi et al., 2004
nC_{60}	100	Lyon et al. (2007)
Chloramine	95–180	Hoff (1986)
Silver ion	0.075[c]–26	Huang et al. (2008b), Kim et al. (2008)
Free chlorine	0.03–0.05	Hoff (1986)
Ozone	0.0007–0.02	MWH (2005), Hunt and Marinas (1997), Sulzer et al. (1959), Farooq and Akhlaque (1982),
UV-C (254 nm)	3.8–4.8	Chang et al. (1985), Oguma et al. (2002)
UV-A (300–400 nm)	16,300–20,000	Choi et al. (2007), Mori et al. (2007)
TiO_2[d] + UV (300–400 nm)	700–5000	Benabbou et al. (2007), Liu and Yang (2003), Ibanez et al. (2003)
TiO_2[e] + UV (300–400 nm)	3600–8500	Kikuchi et al. (1997), Choi et al. (2007)
UV + H_2O_2 (315–400 nm)	66–89	Mamane et al. (2007)

a All CT values are for 99% disinfection of *E. coli* unless otherwise specified. Unit for CT values of all UV applications are mJ/cm^2.
b Minimum inhibition concentration multiplied by exposure time.
c CT for 99% disinfection of *Pseudomonas aeruginosa*.
d TiO_2 slurry in a UV reactor.
e TiO_2 thin film in a UV reactor.

2007). TiO_2 can be applied either as a suspension in a slurry UV reactor, a thin film coated on a reactor surface, or a membrane filter (Benabbou et al., 2007; Belhacova et al., 1999; Kwak et al., 2001).

3.3. Fullerenes and derivatives

Reports on fullerene antimicrobial properties are very recent and the understanding of these compounds is still limited. Therefore, it is premature to propose feasible applications. While certain C_{60} derivatives and nC_{60} have demonstrated strong antibacterial activity, fullerols did not exhibit toxicity in mammalian cells (Sayes et al., 2004) nor antibacterial activity (Lyon et al., 2005) despite exhibiting a stronger photochemical activity (Hotze et al., 2008). On the other hand, even though fullerenes' toxicity towards bacteria does not necessarily involve ROS (Lyon et al., 2008b), other microorganisms such as MS2 bacteriophage were efficiently inactivated by fullerol in UV-A light (Badireddy et al., 2007). An unpublished work by the same team revealed that the same concentration of TiO_2 had no impact on this same virus (Badireddy, 2007). This observation was consistent with the results of a recent study showing that carbonaceous nanoparticles produced more singlet oxygen than TiO_2 (Brunet et al., 2008). Moreover, fullerene derivatives offer functional groups on the carbon cage, which may facilitate its anchorage to a surface without compromising its antibacterial properties, a property desirable in disinfection applications requiring immobilization of the nanoparticles. Encapsulated C_{60} can still exert antimicrobial properties in water (Lyon et al., 2006), which might be more applicable due to the flexibility of the encapsulation. Both fullerols and encapsulated fullerenes can be considered for potential disinfection applications.

3.4. Carbon nanotubes

Antimicrobial activity of CNTs requires direct contact between CNTs and target microorganisms (Kang et al., 2007). Suspension of non-functionalized CNTs in water is extremely difficult and does not provide enough CNT-microbe contact for disinfection. Accordingly, the antibacterial activity of CNTs could be exploited by coating CNTs on a reactor surface in contact with the pathogen laden water. For example, Kang et al. (2007) immobilized SWNTs on a membrane filter surface and observed 87% killing of *E. coli* in 2 hours. Srivastava et al. (2004) showed that CNTs could be incorporated into hollow fibers and achieve effective inactivation of *E. coli* and poliovirus. Brady-Estévez et al. (2008) achieved complete retention and effective inactivation of *E. coli* as well as up to 5–7 log removal of MS2 bacteriophage using a PVDF microporous membrane coated with a thin layer of SWNTs. Bundles of non-aligned single or multi-walled nanotubes, easy to separate with a filter, could also be applied in a packed column/bed filter. Although the rate of bacterial inactivation by CNT is relatively low compared to conventional disinfectants, it may be sufficient to prevent biofilm formation and the subsequent biofouling of surfaces such as water filtration membranes.

3.5. ZnO

ZnO nanoparticles have already been used as an active ingredient in antibacterial cream, lotions and ointments (e.g. Sudocrem). Applications in mouthwashes and paints as an antimicrobial agent and in surface coatings to prevent biofilm growth are currently being considered (Jones et al., 2008). Although both Zn^{2+} ion and ZnO nanoparticles have antibacterial activity, aquatic organisms can be highly sensitive to dissolved zinc (Franklin et al., 2007). Since ZnO dissolves easily, its applications in drinking water treatment will be limited.

3.6. Combining current technologies with nanotechnology

One potential application of antimicrobial nanomaterials is in hybrid processes in combination with existing disinfection technologies. For example, photosensitive nanomaterials can be easily applied to UV reactors to improve UV disinfection. UV disinfection is increasingly used for drinking water treatment due to its effectiveness against cyst-forming protozoa such as *Giardia* and *Cryptosporidium*. However, some pathogenic viruses such as adenoviruses are highly resistant to UV disinfection, requiring very high dosages (Yates et al., 2006). A combination of UV with photocatalytic nanomaterials (e.g. fullerol and TiO_2) that provide additional inactivation mechanisms may be able to overcome this critical barrier. It has been shown that UV reactors internally coated with TiO_2 can enhance the disinfection rate (Sunada et al., 1998; Belhacova et al., 1999). This, in combination with its capability to degrade organic contaminants (Hoffmann et al., 1995) as well as natural organic matter (Huang et al., 2008b; Murray et al., 2007; Eggins et al., 1997), makes TiO_2 one of the most promising nanomaterials for application in large scale water treatment systems.

3.6.1. Enhancing membrane filtration with nanotechnology

The application of membranes for drinking water and wastewater treatment is rapidly growing (Marcucci et al., 2003). However, fouling of membrane materials, especially organic and biofouling, remains the greatest barrier to efficient application of membranes for water and wastewater treatment. In addition, the plethora of contaminants in water and the diversity in their properties usually requires multiple stages of treatment. Incorporation of antimicrobial or photoactive nanomaterials makes the membranes "reactive" instead of a simple physical barrier, achieving multiple treatment goals in one reactor while minimizing fouling. For example, polymeric and ceramic membranes containing TiO_2 were found to be highly efficient in removing a number of organic contaminants and pathogenic microorganisms in the presence of UV-A irradiation. The inactivation of bacteria and degradation of organic matter by TiO_2 make membranes less vulnerable to organic and biological fouling (Choi et al., 2007; Kim et al., 2003). Also, silver-loaded acetate hollow fibers have shown antibacterial activity against *E. coli* and *S. aureus* (Chou et al., 2005). Composite membranes containing a 50 nm thick chitosan layer on a poly(acrylic acid)/poly(ethylene glycol) diacrylate layer were found to exhibit potent antibacterial

activity towards Gram-positive and Gram-negative bacteria, and the antibacterial activity of the membrane improved with increasing chitosan content (Don et al., 2005). In a similar way, nano-composite membranes incorporating other functional (e.g. catalytic, photocatalytic and antimicrobial) nanoparticles into water treatment membranes can be developed.

When incorporating photocatalytic nanomaterials into membranes, a key consideration is introduction of UV light into the system. An outside-in submerged microfiltration (MF) or ultrafiltration (UF) membrane reactor configuration can be utilized to allow easy access to light using submerged UV sources. The potential use of optical fibers in these reactors is also promising.

4. Limitations of nanotechnology for water treatment

Several challenges exist for efficient application of antimicrobial nanomaterials in drinking water treatment, primarily concerning dispersion and retention of nanomaterials and the sustainability of antimicrobial activity. Although nanoparticles provide very high specific surface area, a primary reason for their high reactivity, aggregation in water negates this benefit. Nanoparticles such as TiO_2 aggregate severely when added to water. Although very stable in pure water, coagulation of nC_{60} can be induced by salt (Lyon et al., 2008a).

On the other hand, a well-dispersed nanoparticle suspension contains particles of extremely small sizes. If nanomaterials are applied in the form of slurry, an efficient separation process downstream such as membrane filtration is needed to retain and recycle the nanomaterials. Immobilization of nanomaterials on reactor surfaces or membrane filters eliminates the need for separation. However, the effective nanomaterial dose is then limited by the available surface area in the reactor, which in combination with reduced access to light source, lowers the overall disinfection efficiency compared to a slurry reactor. Nanoparticles may also escape from the treatment system and enter the product water. In addition, since all nanoparticles must be retained in the treatment system, no disinfectant residual is provided. Therefore, they must be used in conjunction with a secondary disinfectant that provides residual through the distribution system.

Retention of nanomaterials is critical not only because of the cost associated with loss of nanomaterials, but also, and more importantly, because of the potential impacts of nanomaterials on human health and ecosystems (Wiesner et al., 2006; U.K. Department for Environment Food and Rural Affairs, 2007; Powell and Kanarek, 2006; Moore, 2006; Service, 2003; Lyon et al., 2007; Benn and Westerhoff, 2008). Our understanding of the potential human health and environmental implication of nanomaterials is improving with the increasing number of nanotoxicity studies in the past few years. Bulk TiO_2 particles (>100 nm) are known to be harmless to humans and animals (Bernard et al., 1990; Chen and Fayerweather, 1988). Although nano-scale TiO_2 was classified recently as a possible carcinogen (if inhaled) by International Agency for Research on Cancer, IARC (2006), its potential ingestion via water is not expected to be a major concern, as reflected by its use in toothpastes and sunscreens. No evidence has been found on toxicity of *n*Ag to human. The only known negative health impact of Ag^+ is darkening of the skin and mucous membrane due to long-term exposure to high silver concentration. However, some nanomaterials such as nC_{60} have been shown to exhibits toxicity to mammalian cells (Sayes et al., 2004). ZnO nanoparticles have also been shown to reduce the viability of human T cells at an elevated concentration (≥5 mM) (Reddy, 2007). Nevertheless, available information is insufficient to determine the highest allowable concentration of a particular nanomaterial in drinking water. Until their human toxicity is thoroughly evaluated or technologies developed to retain the nanomaterials in the treatment system, application of nanomaterials in large-scale water treatment systems is unlikely in the near future.

Because most studies on antimicrobial activities of nanomaterials were conducted in relatively simple and clean solutions, the sustainability of their antimicrobial activities in natural or waste water, whose constituents may interfere with the nanomaterial–microbe interactions, is unclear. Adsorption onto geosorbents, and coating by natural organic matter have been shown to reduce toxicity of nC_{60} towards bacteria (Li et al., 2008) When coated on surfaces to prevent microbial attachment and biofilm formation, antimicrobial nanoparticle coatings may rapidly lose their effectiveness due to adsorption of extracellular polymeric material and occlusion by precipitating debris (Lyon et al., in press).

5. Critical research needs

As discussed above, significant limitations exist for the use of nanomaterials for disinfection or microbial control. This calls for more research to further assess their applicability and to address the limitations.

One obvious research need is better technologies to retain nanomaterials. Effective and reliable methods are needed to anchor the nanoparticles to reactor surfaces or the selective layer of filtration membranes, or to separate and retain suspended nanoparticles in order to reduce costs associated with premature material loss and to prevent potential human health and environmental impacts. This includes developing better surface coating techniques perhaps through nanoparticle surface functionalization, minimizing membrane fouling by the nanomaterial suspension, and impregnating nanoparticles into filter packing materials, e.g. granular activated carbon, or ion exchange resins. Advances in these areas may allow incorporation of antimicrobial nanomaterials into existing water treatment systems. Research on novel immobilization or separation technologies such as magnetic separation is needed. For example, mounting nanoparticles onto magnetic platforms such as nano-magnetite that were recently used to remove arsenic from water (Yavuz et al., 2006) can be explored. Nanoparticles of different properties can be mounted on the nano-magnetite core to create a multi-functional nano-composite material that could be retained and recycled via magnetic separation.

Thorough cost-benefit analyses are needed to further evaluate the applicability of nanotechnology for water treatment. Economic analyses must take into consideration the

benefit of lower DBP formation as well as the cost associated with the potential environmental impacts in the event that nanoparticles escape treatment systems. A great advantage of conventional disinfectants is the low-cost. Nanotechnology-based water treatment technologies will only be able to compete with conventional treatment if the cost of nanomaterials as well as the systems utilizing nanomaterials becomes comparable to the conventional methods. Future research needs to address the scalability of nanomaterial production as well as the nanomaterial-based treatment systems. Low-cost nanomaterials should be explored for potential applications in water treatment. For example, iron coated sands and micro-scale iron powder have been reported to adsorb viruses via electrostatic attraction and cause viruses to disintegrate or become noninfective (Ryan et al., 2002; You et al., 2005). Nano-sized iron particles may provide higher efficiency compared to their bulk or micro-scale counterparts.

6. Summary

Overall, several antimicrobial nanomaterials such as chitosan, *n*Ag, TiO_2, and CNTs show promise as alternatives to traditional chemical disinfectants that are prone to generate harmful disinfection byproducts. Although current economic consideration and undetermined human health and environmental impacts preclude the application of nanotechnology-based water treatment processes in the immediate future, the increasing interest in decentralized water treatment and reuse systems driven by concerns on stressed water distribution systems will likely stimulate research activities in this area in the decades to come. Future research addressing scalability, economics, and safety of these systems is likely to overcome many of the current limitations and create opportunities to revolutionize drinking water treatment.

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