

Carbon nanotubes as antimicrobial agents for water disinfection and pathogen control

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ABSTRACT

Waterborne diseases significantly affect human health and are responsible for high mortality rates worldwide. Antibiotics have been known for decades for treatment of bacterial strains and their overuse and irrational applications are causing increasing bacteria resistance. Therefore, there is a strong need to find alternative ways for efficient water disinfection and microbial control. Carbon nanotubes (CNTs) have demonstrated strong antimicrobial properties due to their remarkable structure. This paper reviews the antimicrobial properties of CNTs, discusses diverse mechanisms of action against microorganisms as well as their applicability for water disinfection and microbial control. Safety concerns, challenges of CNTs as antimicrobial agents and future opportunities for their application in the water remediation process are also highlighted.

Key words | antimicrobial, carbon nanotubes, mechanism of action, pathogen, toxicity, water disinfection

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INTRODUCTION

Due to rapid industrialization and environmental pollution, the contamination of water resources has occurred globally (Nemerow 1991). Waterborne diseases such as typhoid fever, dysentery, cholera, and diarrhea, etc., induced by microbial pathogens significantly affect human health and are responsible for high mortality rates worldwide (LeChevallier & Kwok-Keung 2004; Hamer *et al.* 2010). Clean drinking water free of pathogens is required for living organisms to sustain life. The removal of pathogens from contaminated water is an urgent need for the benefit of human health and environment. The removal process of pathogens from water is found to be difficult due to fluctuating concentration of pathogens and the type of pathogens present in the influent water. Conventional disinfectants, such as chlorine, ozone, and chlorine dioxide, can control the microbial growth, but they have short-lived reactivity and can be problematic due to formation of toxic disinfection byproducts. Therefore, it is important to develop an innovative alternative technique that can effectively improve the reliability of disinfection. Recent advancements in the field of nanotechnology can

contribute noticeable development and improvement to water disinfection processes (Qu *et al.* 2013; Santhosh *et al.* 2016). One of the explored applications is the utilization of carbon nanotubes (CNTs) as antimicrobial agents for water disinfection. CNTs are extensively studied due to their stability and efficient biological properties as promising antimicrobial agents (Maleki *et al.* 2015; Thines *et al.* 2017).

CNTs offer promise as antimicrobial agents against a broad spectrum of microorganisms in water. The first evidence of the antimicrobial activity of CNTs was reported for single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) against *Escherichia coli* by Kang *et al.* (2007, 2008a, 2008b, 2009). On the basis of their observations, several emerging applications of CNTs for microbial inactivation of pathogens *Micrococcus lysodeikticus*, *Streptococcus mutans*, *E. coli*, *Salmonella* spp. have been reported (Brady-Estevez *et al.* 2008, 2010; Arias & Yang 2009; Akasaka & Watari 2009; Liu *et al.* 2009). The cytotoxicity of CNTs to bacteria in aqueous solution is a complex function of solution chemistry, transport behavior, and physiochemical properties

of the nanomaterials (Kang *et al.* 2009). Researches have shown that CNTs of different size and diameters have significantly different effects on the antibacterial efficiency (Kang *et al.* 2008a; Yang *et al.* 2010). Some external factors such as CNTs' dispersing ability, culture medium, bacterial species, CNTs' dosage, reaction time, and the mode of action between bacteria and CNTs also influence the antibacterial activity (Kang *et al.* 2007; Arias & Yang 2009; Liu *et al.* 2009). In addition, functionalization of CNTs are realized to enhance the antimicrobial ability by improving the dispersing ability and stability of CNTs (Liu *et al.* 2007; Pasquini *et al.* 2012). Another potential application of CNTs for water disinfection is their use in the synthesis procedure or *in situ* generated for membrane formation process to remove bacteria and viruses in water system. Several studies have demonstrated that CNTs can be incorporated with polymers or nanomaterials to produce nanocomposite membranes and perform an effective inactivation of bacteria and viruses (Brady-Estévez *et al.* 2008, 2010).

The exact antimicrobial mechanism of CNTs for pathogen control in water is still not clear. Several researchers (Kang *et al.* 2007; Pulskamp *et al.* 2007; Arias & Yang 2009; Vecitis *et al.* 2010) have proposed different postulates regarding the role CNTs in microbial inactivation. The nanoparticles can either directly penetrate the cell envelope of the microbial cells or produce secondary products through altering cellular processes both at the biochemical and molecular levels.

Despite much scientific evidence throughout the past decade regarding the biological effects of CNTs, there are no updated reviews focusing on the antibacterial effects of CNTs for water disinfection and pathogen control. Since most literature is conducted on the CNTs' antimicrobial control of bacteria and viruses, only limited data are available on other pathogenic microorganisms. Thus, this review paper will focus on the antimicrobial activity and mechanisms of action of CNTs against bacteria. The promising applications of CNTs for water disinfection and bacteria control, their possible toxicological effects as well as the future challenges are discussed.

ANTIMICROBIAL ACTIVITY OF CNTS

The antimicrobial activity of CNTs in aqueous solution is a complex process, which is closely related to physicochemical

properties of CNTs (CNTs' size and surface area, dispersing ability, content of impurities) and some external factors (CNTs' dosage, treatment time, culture medium, and bacterial species).

Physicochemical properties

The size of CNTs plays an important role in the inactivation of bacteria cells. The study of Kang *et al.* (2008b) has shown that SWNTs exhibit stronger antibacterial activity to microbial cells, whereas MWNTs exhibit a milder antibacterial effect. Examples of scanning electron microscopy (SEM) images of cells exposed to SWNTs and MWNT are shown in Figure 1. Only a few *E. coli* cells exposed to MWNTs lost their cellular integrity (Figure 1(a)), with the majority of cells still intact, while the majority of *E. coli* cells incubated with SWNTs lost their cellular integrity and became flattened (Figure 1(b)). The difference in the

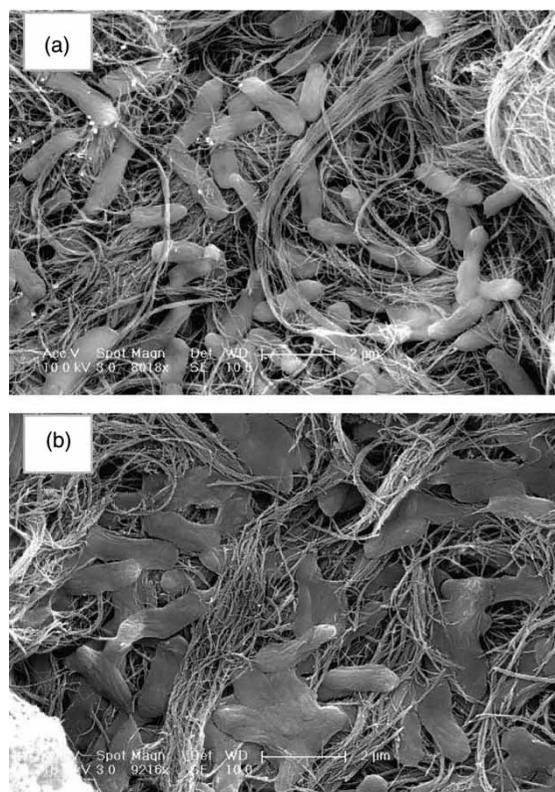


Figure 1 | SEM images of *E. coli* cells exposed to CNTs: (a) cells incubated with MWNTs for 60 min and (b) cells incubated with SWNTs for 60 min. Reprinted with permission from reference Kang *et al.* (2008b). Copyright from American Chemical Society.

antimicrobial performances of the two types of CNTs was probably due to the smaller diameter and larger specific surface area of SWNTs, which are more favorable for surface interaction between CNTs and bacteria cells, leading to stronger biological effect. The length of SWNTs is also proved to relate to the effectiveness of their antimicrobial activity (Yang *et al.* 2010). The survival of cell numbers in the samples treated with SWNTs of <1 μm , 1–5 μm , and $\sim 5 \mu\text{m}$ decreased with increasing length of SWNTs due to the higher efficiency of aggregation of longer SWNTs with *Salmonella* cells, whereas short-length SWNTs tended to aggregate without involving many bacterial cells. In addition, CNTs of different diameters also have different effects on the manners in which they capture the microbial cells. Bundles of SWNTs and thin MWNTs were easily wound around the curved surface of *S. mutans* (Akasaka & Watari 2009).

CNTs were found to have difficulty dispersing in water and this leads to an insufficient contact of CNT and microbes for the disinfection process. High dispersion can increase interaction opportunity of the CNTs with bacteria cells, and then promote the antibacterial activity of CNTs (Bai *et al.* 2011). Physicochemical modifications of MWNTs by dry oxidation and acid treatment were proved to alter their dispersing ability and demonstrated stronger antibacterial activity (Kang *et al.* 2008b). Liu *et al.* (2009) reported that individually dispersed SWNTs in Tween-20 saline solution possessed stronger antibacterial activity than SWNT aggregates. They assumed that individually dispersed SWNTs acted as numerous moving ‘nano darts’ in solution and constantly attacked bacterial cells, causing degradation of bacterial cell integrity that led to cell death.

Some researchers have hypothesized that the cytotoxicity of CNTs may also be related to the purity of CNTs. The impurities produced during the preparation of CNTs, such as transition metal residues, amorphous carbon, carbon nanoparticles, and graphite may cause CNTs to behave differently in microbial control (Liu *et al.* 2007a). Kang *et al.* (2007) provided direct evidence that highly purified SWNTs exhibit stronger antimicrobial activity than pristine SWNTs against *E. coli*. In another study (Kang *et al.* 2008a), they prepared MWNTs with different purities and found physical and chemical properties of MWNTs alter their cytotoxicity in bacterial systems. However,

Simon-Deckers *et al.* (2009) found that the bactericidal activity of purified MWNTs (removal of catalyst Fe under high temperature) and pristine MWNTs was not significantly different, which was probably due to capping of Fe inside CNTs with interaction of the bacteria. Liu *et al.* (2009) also found that the cobalt residue does not have an impact on the SWNTs’ antibacterial activity. Although these studies have demonstrated that the effects of impurities (amorphous carbon and metal catalysts, etc.) on antimicrobial activity of CNTs were not so obvious, the purification procedures still played an important role in alterations of the surface chemistry of CNTs by increasing their structural defects or changing the aggregation, dispersion, and length, thus affecting their antibacterial activity.

External factors

As already mentioned, not only physicochemical properties of CNTs are important concerning antimicrobial properties but also the external factors such as CNTs’ dosage, treatment time, culture medium, and bacterial species matter. The dosage and treatment time dependence of CNTs on microbial pathogen inactivation was investigated by several studies. SWNT filter exhibited higher antibacterial activity against *E. coli* with the increase of SWNT loadings as filter coating (Brady-Estevez *et al.* 2008). Liu *et al.* (2009) proved that the antibacterial activity of SWNTs can be remarkably improved by increasing SWNT concentration. Yang *et al.* (2010) also confirmed the concentration and treatment time dependence of SWNTs with different lengths on their antimicrobial activity. Furthermore, SWNTs exhibited a strong antibacterial activity against *Ralstonia solanacearum* cells, the higher the concentration of SWNTs, the lower the survival rates of bacterial cells (Wang *et al.* 2013). Besides treatment time, incubation time is also known to affect the cytotoxicity of CNTs in bacteria inactivation. Incubation time-dependent bactericidal behavior in Gram-positive *Bacillus subtilis* exposed to SWNTs was observed; after longer incubation with SWNTs, cell inactivation increased (Kang *et al.* 2009).

There also exists a strong dependence of antimicrobial activity of CNTs with the quality of the dispersant. Surfactants such as Tween-20 (Liu *et al.* 2009), sodium cholate

(Dong *et al.* 2012), or Triton-X (Alpatova *et al.* 2010) were used to ease the dispersion of CNTs in water for microbial attachment on CNTs' surface. The effects of dispersed agents on the antibacterial activities of SWNTs were explored, and stronger antimicrobial activity in DI water and 0.9% NaCl solution was observed, but no exhibition of antimicrobial activity in PBS buffer and brain heart infusion broth (Arias & Yang 2009). Furthermore, SWNTs were dispersed in water using a range of natural (gum arabic, amylose, Suwannee River natural organic matter) and synthetic (polyvinyl pyrrolidone, Triton X-100) dispersing agents. The cell viability of *E. coli* was affected only by SWNTs dispersed using cytotoxic dispersant Triton X-100 (Alpatova *et al.* 2010). Recently, sodium cholate surfactant was reported to efficiently disperse bundled nanotubes into suspensions of individual nanotubes and exhibited high antibacterial effect and low toxicity (Dong *et al.* 2012). In addition, the degree and kind of aggregation, the stabilization effects by organic matter, and the bioavailability of the CNTs also affect the CNTs' performance as a disinfectant.

The distinct difference in microbial cells may also be a factor affecting the effectiveness of CNTs' antimicrobial activity. Kang *et al.* (2009) made a systematic study on CNTs' cytotoxicity in Gram-negative and Gram-positive bacteria (Figure 2). SWNTs display the highest cytotoxicity toward Gram-negative bacteria. The antibacterial activity

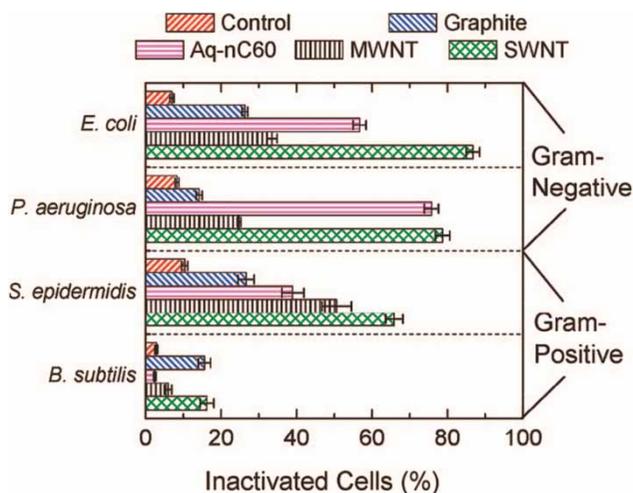


Figure 2 | Summary of fluorescence-based toxicity assays following bacterial contact with bare (control), MWNT-, or SWNT-coated filters. Reprinted with permission from reference Kang *et al.* (2009). Copyright from American Chemical Society.

of MWNTs was moderate with a wide distribution of toxicity between bacterial species. The different sensitivity of microbes to CNTs' exposure may be attributed to the difference in cell wall thickness and constituent of their cell membrane structure (Hajipour *et al.* 2012). In general, Gram-negative bacteria are more resistant to external stimuli than Gram-positive bacteria due to a complex outer membrane which enhanced their surface stiffness (Hossain *et al.* 2014).

ANTIMICROBIAL MECHANISMS OF CNTS

The antibacterial mechanism of CNTs is still not well explained. In the literature, several mechanisms have been postulated, as presented in Figure 3: (1) adhesion of CNTs to the surface of microbial cells to interrupt transmembrane electron transfer and cause disruption of membrane and cell wall; (2) CNTs penetrating inside bacterial cells, resulting in DNA damage and protein dysfunction; (3) formation of secondary products (e.g., reactive oxygen species (ROS) that cause damage).

Cell membrane damage

Cell membrane damage and release of intracellular contents were shown as a likely mechanism leading to bacterial cell death. Attachment of CNTs alters the structure, permeability, and proton motive force of the cell membrane. Several studies have shown that the contact of the bacteria with CNTs causes cell morphology distortion, damage to

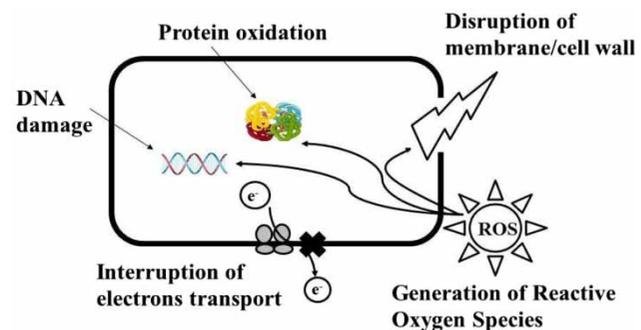


Figure 3 | Antimicrobial mechanisms exerted by CNTs. Reproduced with permission from reference Li *et al.* (2008). Copyright from Elsevier.

cell membrane integrity, and release of intracellular material. Kang *et al.* (2008a) observed the loss of cellular integrity of *E. coli* cells by SEM. They also proved the efflux of cytoplasmic materials by measurements of the concentrations of DNA and RNA. A five-fold increase of plasmid DNA and a two-fold increase of RNA in solutions in contact with SWNTs were observed, confirming the severe damage to cell membrane integrity. Liu *et al.* (2009) also found the phenomenon of cell membrane damage by CNTs. Dispersed SWNTs can be visualized as numerous moving 'nano darts' in the solution, constantly attacking both Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *B. subtilis*) bacteria; thereby degrading the bacterial cell integrity and causing cell death. The damage of bacterial membrane was further confirmed by the efflux of DNA and RNA and SEM study. A similar phenomenon after incubation with CNTs has been reported for *Ralstonia solanacearum* bacterial cells (Wang *et al.* 2013).

Production of ROS and oxidative stress

Oxidative stress is also a possible mechanism partially accounting for CNTs' toxicity in bacteria. Once CNTs enter into bacteria, they induce generation of harmful ROS including superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals (OH^{\bullet}), hydrogen peroxide (H_2O_2), and organic hydroperoxides. These free radicals produced in oxidative stress will initiate the peroxidation of unsaturated phospholipids in the membranes, thus producing more peroxy radical intermediates, leading to severe damage to lipoproteins and nucleic acids. Lipid peroxidation also causes damage to membrane properties and functions by inducing conformational changes in membrane proteins and altering of membrane fluidity and integrity, ultimately destroying the microbial cell (Raghunath & Perumal 2017).

Physicochemical properties of CNTs, such as surface area and electrophilic nature, determine the amount of ROS produced in microbial cells. After bacterial cells' exposure to SWNTs and MWNTs, several genes which are part of soxRS and oxyR systems linked to the bacterial oxidative stress response were expressed (Kang *et al.* 2008a). Moreover, Vecitis *et al.* (2010) investigated SWNT toxicity mechanism by *in vitro* study of SWNT-mediated oxidation

of glutathione, a non-enzymatic antioxidant and redox state mediator protecting bacteria from oxidative stress. The extent of glutathione oxidation was observed to increase with increasing fraction of metallic SWNTs, indicating an increase of lipid peroxidation in the bacterial membrane. This imbalance in oxidants and antioxidants causes elevated oxidative stress within the microbes. The antioxidant enzyme activities are disrupted by excessive generation of ROS within the bacterial cell leading to damage to bacterial cells.

Other mechanisms

Apart from the aforementioned two main mechanisms of action, some other mechanisms have been reported. Some researchers have shown that the adhesion of bacteria and CNTs was the cause of the antibacterial effect. CNTs can adhere to *S. mutans* surface only by entanglement and no cell membrane damage of *S. mutans* was observed (Akasaka & Watari 2009). In the study of Simon-Deckers *et al.* (2009), they observed bacteria *Cupriavidus metallidurans* or *E. coli* adsorbed onto MWNT by TEM. CNTs may also induce DNA damage and protein dysfunction. The direct interaction between CNTs and DNA could certainly cause DNA damage dominated by single-strand break. After contacting with CNTs, CNTs could undermine the power of base stacking of supercoiled DNA and make a conformational change of DNA. DNA had not only the supercoiled and relaxed forms but also others including the linear form and probably fragments. CNTs may also indirectly interact with DNA without entering the cell through secondary effects such as free radicals produced by the CNTs' interaction with the cell environment. The reactive oxygen could interact with DNA to cause changes in the DNA structure and inhibition of repairing mechanisms of DNA (Wang *et al.* 2016). In terms of the high surface area and the electrical properties, CNTs are capable of binding to the amino acid side chains and SH groups of many proteins and rendering them inactive (Lynch & Dawson 2008). In addition, since CNTs contained some nickel due to using nickel oxide as the catalyst in the synthesis, the nickel as a transition metal might be involved in the Fenton reaction to produce hydroxyl radicals to react with protein molecules.

In short, CNTs were proven to be good antimicrobial agents by employing diverse antibacterial mechanisms. The antibacterial activity of CNTs starts with an initial contact between the bacteria and CNTs, followed either by physical perturbation of cell membrane or by disruption of particular microbial processes through oxidizing of vital cellular structure/component such as DNA or proteins. Moreover, the physicochemical properties of CNTs (size, length, oxidation degree, metallic content, etc.) and bacteria features (bacteria cell envelope, age of bacteria cell, stress condition in the environment, metabolism) also have an important influence on bacterial mechanisms of CNTs.

APPLICATIONS OF CNTS FOR WATER DISINFECTION AND PATHOGEN CONTROL

CNTs are an ideal material for bacteria removal due to their strong antimicrobial activity. However, relevant study in water treatment and environmental remediation is limited. Generally, CNTs have been usually employed to be incorporated into membranes to remove bacteria and viruses in water. Brady-Estévez *et al.* (2008, 2010) developed a SWNT filter with PVDF microporous membrane to inactivate bacteria (i.e., *E. coli* K12) and virus (i.e., MS2 bacteriophage) from an aqueous matrix. They observed that an SWNT filter can effectively retain and inactivate bacteria and virus. In particular, Kang *et al.* (2009) evaluated the antimicrobial action of CNTs on *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. epidermidis*. SWNT inactivated all targeted microbial with the same efficiency for river water and wastewater effluent. Lilly *et al.* (2012) also investigated inactivation of *B. anthracis* spores by SWNTs alone and SWNTs coupled with oxidizing antimicrobial chemicals such as H₂O₂ and NaOCL. The obtained result indicates that SWNTs coupled with antimicrobial chemicals exhibited a stronger sporicidal effect on *B. anthracis* compared with untreated MWNTs or individual treatment with H₂O₂ or NaOCL at the same concentration, which is probably due to the synergistic effect contributed by the two individual antimicrobial mechanisms of SWNTs and the oxidizing antimicrobial chemicals.

Additional processes such as functionalization of CNTs can increase the antimicrobial efficiency of CNTs as a disinfectant. Surface function of CNTs can improve their limited

antibacterial properties. Nanocomposites formed by CNTs and metal particles are promising effective antibacterial agents with synergistic action which have been used in water treatments. Silver nanoparticle was successfully grafted on MWNT and achieved bacteria elimination without compromising the flux rate of membrane filtration for improved water decontamination and disinfection (Roy *et al.* 2015). A new nanofilter using a CNT–silver composite material was developed, which is capable of efficiently removing water-borne viruses and bacteria. No trace of viruses was found to flow through the nanofilter with this composite. Moreover, the surface of the filter has antibacterial properties to prevent bacterial clogging (Kim *et al.* 2016). Chang *et al.* (2016) have also shown that the antimicrobial performances of carbon–silver nanocomposites towards *E. coli* and *S. aureus* improved significantly, when loaded with silver nanoparticles. The SWNTs–Ag was found to be more effective towards *S. aureus* than *E. coli* due to enhanced accumulation of *S. aureus* on the SWNTs–Ag in water disinfection.

A number of metal oxides have also been proposed as coating on CNTs for inactivating microorganisms in water, such as zinc oxide, titanium dioxide, and ferric oxides. ZnO/MWNTs composite was designed and exhibited stronger antibacterial ability towards *E. coli*. The deposited ZnO was suggested to play an important role in the bactericidal action of ZnO/MWNTs (Sui *et al.* 2013). Sangari *et al.* (2015) demonstrated that MWNTs-fluorine-co-doped TiO₂ nanocomposites can enhance antimicrobial activity against *S. aureus* and *P. aeruginosa*, which may have environmental applications in the enhanced degradation of organic pollutants. Recently, Ali *et al.* (2017) reported a class of novel multifunctional nanocomposites composed of MWNTs with embedded iron oxide and silver nanoparticles to inhibit *E. coli* contamination in drinking water. The antibacterial properties of the synthesized composite materials have shown significant antibacterial activity against *E. coli*.

Polymers are another class of antimicrobial agents that can potentially be coated on CNTs to improve their antimicrobial properties in water disinfection. The addition of different polymer groups to SWNTs affect bacterial cell viability due to different molecular size, chemical composition, and physicochemical properties of the functional groups (Pasquini *et al.* 2012). Arias & Yang (2009) have explored the antimicrobial activities of both SWNTs and MWNTs

attached to different functional groups against bacterial pathogens. SWNTs with surface groups of -OH and -COOH exhibited extremely strong antimicrobial activity to both Gram-positive and Gram-negative bacterial cells, whereas SWNTs-NH₂ showed little toxicity. The presence of functional groups NH₂ in a long chain affect the interactions between the SWNTs-NH₂ and bacterial cells, in which the cylinder CNTs might not be in direct contact with the bacteria cell walls, leading to a decrease in antimicrobial activity. CNTs-polymer composites can also be used as additives in the synthesis procedure or *in situ* generated for membrane formation process (Kim & Van der Bruggen 2010). Ahmed *et al.* (2012) designed PVK-SWNT nanocomposite on solid surfaces, demonstrating significant antimicrobial effects against pathogen bacteria. In another study (Ahmed *et al.* 2013), they developed PVK-SWNT nanocomposite with only 3% SWNT content and achieved similar or better cell inactivation than 100% SWNT-coated membranes. The better dispersion and debundling of SWNT in the presence of PVK increase the contact probability of SWNT with bacterial cells. Moreover, Tiraferri *et al.* (2011) have reported that SWNTs were successfully attached to the surface of thin film membranes by covalent binding. This SWNTs-coated membrane was then employed to achieve up to 60% inactivation of *E. coli* bacteria within 1 hour of contact time. MWNTs can also cooperate with aromatic polyamide (Shawky *et al.* 2011) or polyethersulfone (Celik *et al.* 2011) to produce nanocomposite membranes for fouling control and microbial removal in water treatment.

TOXICOLOGICAL CONCERNS ABOUT CNTS

As we have already discussed, CNTs have potential applications in water disinfection and microbial control as an innovative antimicrobial agent. However, some safety concerns due to their toxicological effects on the environment and the human body should be considered. The high surface-to-volume ratio makes CNTs more reactive and potentially more toxic. They may cause new allergens, new toxic strains, and increased rates of CNTs absorption, interaction with other materials during disposal, and recycling of CNT-bearing composite materials by the environment (Albini *et al.* 2015). CNTs may be ingested inside the body from the mouth to the

final gastrointestinal tract, following absorption, distribution, metabolism, and excretion processes (Piperigkou *et al.* 2016). Interaction of CNTs with biological systems may give rise to cytotoxic effects, including chemical allergy, cytotoxicity, ROS generation, DNA damage, and protein dysfunction (Firme & Bandaru 2010; Shvedova *et al.* 2012). CNTs were tested using different cells and found to exhibit different levels of toxicity. The difference in toxicity depends on size, shape, reactivity, surface charges, types of coating, cell and tissue types, aggregation, and mode of interaction with cells (Fujita *et al.* 2015; Lee *et al.* 2015; Amiri *et al.* 2016). Therefore, before CNTs are commercially used as antimicrobial agents, a toxicity evaluation is important. Although there are a few studies on the assessment of toxicity of CNTs both *in vitro* and *in vivo*, more studies are needed to assess the risks associated with the presence of such extremely small particles in the human body or in the environment.

CONCLUSION, CHALLENGES, AND FUTURE PERSPECTIVES

The special emphasis in this review has been on antimicrobial properties of CNTs for bacteria control in water disinfection. As evident from the reviewed literature, interest in their usage to reduce the number of bacteria cells in water disinfection has increased significantly. Both the physico-chemical properties of CNTs, external factors, and bacteria cell wall structure influence the CNT-cell envelope interactions. The antimicrobial mechanism of CNTs is still a complex subject. CNTs can elicit antibacterial effects through adhesion to the surface of microbial cells, interruption of transmembrane electron transfer, disruption of cell membrane and cell wall, DNA damage, and oxidative stress.

Challenges exist for the use of CNTs for microbial control in water disinfection. One obvious challenge is the possible cytotoxicity effects of CNTs when used in practical applications. Cytotoxicity of these materials depends on the structure, manufacturing method, concentration, exposition time, surface coatings (active or passive), solubility, presence of other factors, and the kind of microbial cells. The release of CNTs into the environment will be a contributing factor to the ecological environment. CNTs may be transferred through the water to the human body, inducing a possible

negative impact on human health. Effective and reliable methods are needed to alleviate the (eco-)toxicological effects of CNTs. This includes developing better surface coating techniques, perhaps through changes of CNTs' surface, or better immobilization of CNTs in nanocomposites. Advances in these areas may allow incorporation of antimicrobial CNTs into existing water treatment systems. Another challenge is the cost-effectiveness. A great advantage of conventional antimicrobials is the low cost. The price of SWNTs and MWNTs are relatively high compared with conventional antimicrobials. Hence, the feasibility of the usage of nanomaterials as antimicrobial agents will only be able to compete with conventional antimicrobials by producing CNTs of lower price. Future research needs to address the scalability of CNTs' production as well as the reusability of these carbon nanomaterials.

After suitably addressing the toxic concerns and economic considerations of CNTs, their current and envisioned applications in environmental pollution control will reveal different possibilities. CNTs could be produced in a cost-effective way or combined with green technology for their synthesis, uses, and environmental friendly disposal. The increasing interest in microbial control by CNT-based nanomaterials will likely stimulate research activities in this area in the decades to come. Future research addressing scalability, economics, and safety of CNTs is likely to overcome many of the current limitations and create opportunities in environmental pollution protection (e.g., water treatment) and other applications, where control of microbe growth is essential.

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