Chapter 4
Carbon Nanotube-Based Antimicrobial and Antifouling Surfaces

R. Teixeira-Santos, M. Gomes, and F. J. Mergulhão

1 Introduction

Carbon nanotubes (CNTs) were first introduced in 1991 by Lijima [1]. These carbon nanomaterials have a small, thin, hollow, and concentric cylindrical structure which is closed at both ends [2]. Carbon nanotubes can be classified as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Single-walled carbon nanotubes consist of a single graphene layer wrapped in a seamless cylinder, whereas MWCNTs are composed by multiple graphene layers wrapped to form concentric tubes [2, 3].

Carbon nanotubes are attractive nanomaterials because of their outstanding properties, such as excellent electrical and thermal conductivity, high tensile strength, high hydrophobicity, microbial immobilization potential, and ability to blend with other materials to form nanocomposites (NC) [2–7]. Therefore, because of their unusual properties, there has been a vast interest in exploiting CNTs for several applications (Fig. 1).

In the last decade, CNTs were introduced in pharmaceutical and medical fields. The chemical stability of CNTs enables them to adsorb or conjugate with a wide variety of therapeutic molecules (proteins, antibodies, DNA, enzymes, drugs) acting as vehicles for drug delivery [8]. CNTs have also been used for the construction of biosensors for the detection of biomolecules and biological cells, tissue engineering, and neuronal interfaces [2, 8]. In addition, due to their antimicrobial activity, CNTs have been used in the fabrication of biomedical devices and prosthetic implants [9, 10].

Recently, the combination of CNTs and antimicrobial drugs or other bioactive molecules appears to be a promising strategy to fight antimicrobial resistance and develop new options in antimicrobial therapy [11–13].

R. Teixeira-Santos · M. Gomes · F. J. Mergulhão (✉)
LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
e-mail: filipem@fe.up.pt

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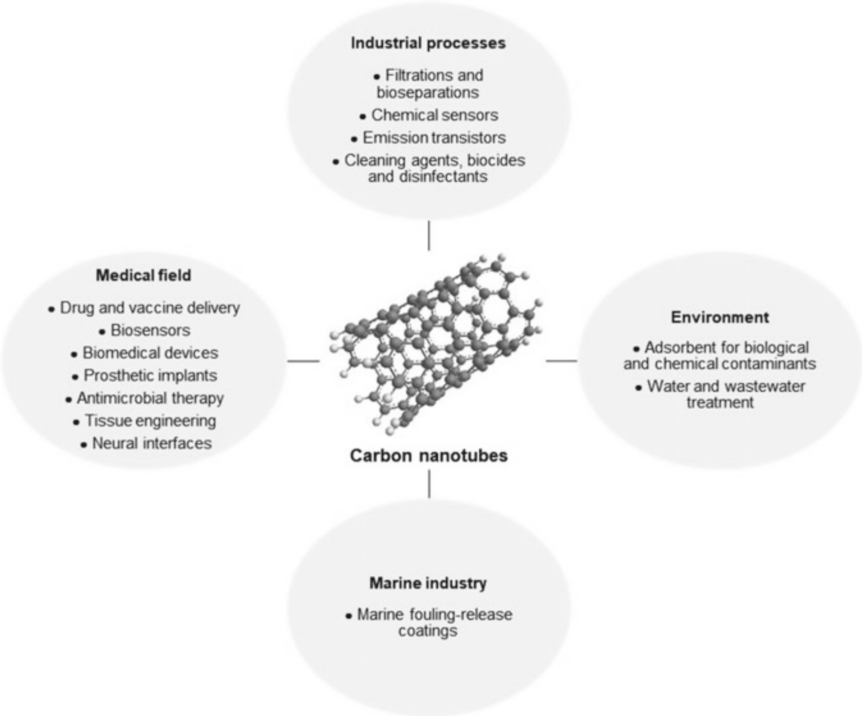

Fig. 1 Main applications of carbon nanotube-based surfaces

Prior to the scientific interest in the utilization of CNTs on biomedical applications, their mainstream use was in the industrial field. CNTs have been used to produce emission transistors and chemical sensors and to develop membranes for filtration and other separation processes [7, 14]. In addition, they have also been used to produce cleaning agents, biocides, and disinfectants for numerous industrial processes [15].

Moreover, the antibiofouling properties of CNTs allowed their application in the marine industry. Fouling on ship hulls decreases speed and increases fuel consumption. Up to date, several studies have proposed CNTs as good candidates for the development of fouling-release coatings against microalgae and barnacles [16–18].

Lastly, because of their antimicrobial and antifouling properties, CNTs have also been applied in water and wastewater treatment, and as absorbents for biological and chemical contaminants [19–22].

In this chapter, the antimicrobial and antifouling properties of CNTs will be reviewed using published studies. Additionally, based on collected data, the development of new CNT surfaces and their potential medical applications are discussed.

2 Antimicrobial and Antifouling Properties of Pristine CNTs

Carbon nanotubes are some of the most attractive nanomaterials for the development of antimicrobial and antifouling surfaces. The antimicrobial activity of CTNs depends on multiple factors related to their structure and composition such as (1) size and length; (2) physical disposition (aggregated or dispersed); and (3) the number of layers (single- or multi-walled) [6, 23, 24]. Table 1 lists several studies demonstrating the antimicrobial activity of pristine single- and multi-walled CNTs against different bacterial species.

In 2007, Kang et al. provided for the first time the evidence that pristine single-walled CNTs exhibit strong antimicrobial activity, inducing cell membrane damage by direct contact and, thus, reducing cell viability by 80% [25]. Since then, different mechanisms have been proposed to explain the toxicity of CNTs.

In 2008, a study involving gene expression analysis demonstrated that cell membrane damage is the main CNT-biocidal mechanism. According to the authors, bacteria exposed to CNTs suffer oxidative stress, followed by cell membrane damage and, ultimately, the release of intracellular content [6]. Nagai and Toyokuni considered that the cell membrane damage occurs through direct piercing of the bacterial surface [26]. Previously, Kang et al. reported that the length of CNTs plays a crucial role during their interactions with the cell membrane, where shorter tubes exhibit higher toxicity compared to longer tubes [6, 27]. Aslan et al. also demonstrated that shorter SWCNTs are more toxic due to higher density of open tube ends [9]. Similarly, smaller diameters were shown to induce accentuated cell membrane damage through the cell surface interaction [28]. On the other hand, studies have also postulated that bacterial death is caused by agglomerated nanotube networks trapping the cell surface, a phenomenon that triggers oxidative stress and inhibits bacterial growth [29, 30]. According to Arias and Yang, CNTs with large diameter (15–30 nm) mostly interact with bacteria through their sidewalls [4].

Likewise, several authors have demonstrated that SWCNTs exhibit more toxic effects in bacteria than MWCNTs [6, 24]. Indeed, SWCNTs showed a strong effectiveness in piercing the bacterial membrane [24]. In a study by Kang et al., it was shown with *Escherichia coli* that after incubation for 1 h with MWCNTs, most of the cells were still intact (Fig. 2a), whereas with SWCNTs, the majority of cells lost their integrity and became flattened (Fig. 2b) [6]. Additionally, the same authors also showed that in the presence of both MWCNTs and SWCNTs, *E. coli* expresses high levels of stress-related genes. Although most of the genes expressed in cells exposed to MWCNTs are also expressed in cells exposed to SWCNTs (Fig. 3), the quantity and magnitude of expression were much higher with SWCNTs [6].

Conversely, Young et al. described that MWCNTs have higher toxicity for bacteria than SWCNTs [31]. Despite the discrepant findings, the antimicrobial activity of single- and multi-walled CNTs have been demonstrated against a broad range of species including *Lactobacillus acidophilus*, *Bifidobacterium adolescentis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* [6, 24, 25]. However,

Table 1 Studies demonstrating the antimicrobial and antifouling activities of pristine carbon nanotubes

Property	Wall type	Species	Main conclusions	Refs.
Antimicrobial	Single	*E. coli*	Bacteria exposed to CNTs for 1 h exhibited a substantial loss in their viability (80%).	[25]
	Single and multi	*E. coli*	The percentage of inactivated cells attached to SWNT (80%) was higher than MWNTs (24%).	[6]
	Single and multi	*L. acidophilus* *B. adolescentis* *E. coli* *E. faecalis* *S. aureus*	CNTs demonstrated a significant and dose-dependent antibacterial activity against Gram-positive or Gram-negative bacteria when compared to the control ($p < 0.01$ or $p < 0.05$).	[24]
	Multi	*E. coli*	The MIC values obtained for MWCNTs were very high, indicating low toxicity for bacteria.	[15]
	Multi	*E. coli* *P. aeruginosa* *B. subtilis*	The viability study showed significant MWCNT toxicity (2-log reduction in cell density) against *E. coli*, *P. aeruginosa* and *B. subtilis*.	[33]
Antimicrobial and antifouling	Multi	*P. fluorescens*	The percentage of inactivated bacteria exposed to MWCNTs was 44%. Results showed that CNTs have a significant effect on the inhibition of bacterial adhesion under electrochemical potential.	[32]

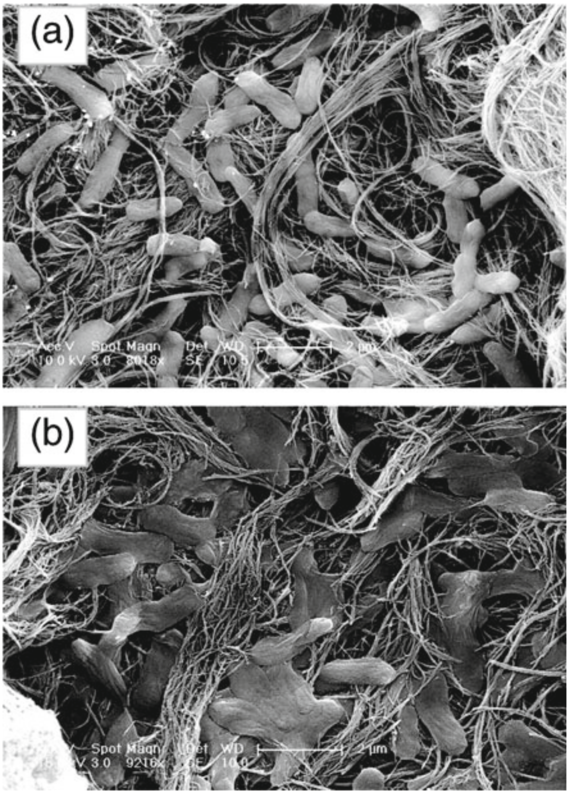

Fig. 2 Scanning electron microscopy (SEM) images of *E. coli* cells exposed to CNTs. **a** Cells incubated with MWCNTs for 60 min. **b** Cells incubated with SWCNTs for 60 min. The bars in both images represent 2 μm. Reprinted with permission from Kang et al. [6]. Copyright 2008 American Chemical Society

CNTs appear to display a selective activity, presenting lower toxicity against rod-like bacteria than spherical ones. This result suggests that their action may also depend on the shapes of bacteria [24].

Recently, the toxicity of MWCNTs was also evaluated for *Bacillus subtilis, Pseudomonas fluorescens* and *Pseudomonas aeruginosa* [32, 33]. The viability studies showed that the percentage of inactivated cells was significant and dependent on the concentration of CNTs [33]. In opposition, Vassallo et al. tested the antimicrobial activity of MWCNTs and obtained high MIC (minimal inhibitory concentration) values (> 100 mg/L), suggesting that MWCTNs display low toxicity for bacteria [15]. Thus, the antimicrobial activity of CNTs depends on a multiplicity of factors that may be modulated according to the desired application.

In the last decade, several studies have been developed with the purpose of improving the antimicrobial activity of CNTs. Their results will be further explored in the following sections.

The antibiofouling properties of CNTs also make them an attractive nanomaterial for a wide range of applications. Fouling can be inhibited by different mechanisms such as (1) increase of biocidal activity; (2) increase in resistance to protein adhesion; and (3) increase in resistance to other fouling components at the material surface [2].

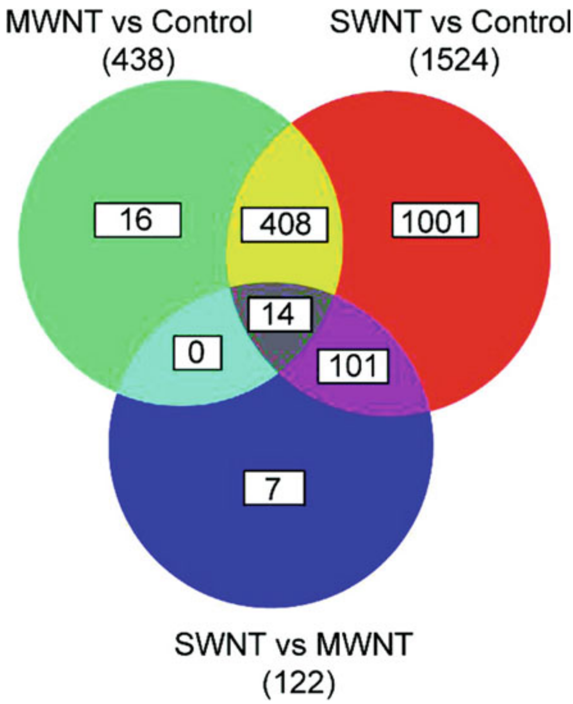

Fig. 3 Number of genes induced or repressed more than twofold for cells exposed to SWNTs and MWNTs compared to the culture without CNTs (control). Reprinted with permission from Kang et al. [6]. Copyright 2008 American Chemical Society

The effects of CNTs on biofilm formation have been addressed in many studies in order to evaluate their potential to inhibit microorganism attachment and proliferation at different stages (Table 1) [27]. Malek et al. reported that the biofilm inhibition increases with the increasing CNT length, proposing that longer CNTs are more flexible, which may prevent microbial attachment [34]. Zhang et al. showed that pristine multi-walled CNTs have a significant effect on the inhibition of *P. fluorescens* adhesion upon application of an electrochemical potential. However, this effect can be increased through surface modification of the MWCNTs [32]. Hence, the antifouling potential of CNTs may be improved by their modification or association with different materials.

3 Development of CNT-Based Antimicrobial and/or Antifouling Surfaces

Despite the promising antimicrobial and antifouling properties of pristine CNTs, their practical application is limited essentially due to their hydrophobic nature [2]. The modification of CNTs and/or their association with materials such as polymers, metals, or biomolecules results in a nanocomposite (NC) which may have improved activity. Simultaneously, the functionalization of CNTs can help in their dispersion in different matrices, increase biocompatibility, and decrease toxicity for human cells [2].

3.1 Single-Walled CNT Surfaces

The section above clearly demonstrated that SWNTs interact with microorganisms and exhibit strong antimicrobial properties. Likewise, their potential to inhibit the adhesion of organisms and other molecules should be highlighted. These observations point to the use of SWCNTs as building blocks for the development of antimicrobial and/or antifouling surfaces. Thus, the present section intends to explore the effect of functionalized SWCNTs or their nanocomposites on the improvement of these properties. Table 2 provides a description of surface modifications made to SWCNTs in order to increase their blending capacity in different materials, their antimicrobial and antifouling potential against several species.

As previously mentioned, CNTs can be functionalized, for instance, with acid or carboxyl moieties in order to increase their interaction with bacterial cells and the formation of bacterial–CNT aggregates [4]. Studies have reported that bacterial binding is facilitated upon CNT functionalization [2, 35]. Arias and Yang investigated the effects of different SWCNT surface functional groups (–OH, –COOH, and $–NH_2$) on their antimicrobial activity against both Gram-negative (*Salmonella typhimurium*) and Gram-positive bacteria (*B. subtilis and S. aureus*). Results showed that SWCNTs functionalized with –OH and –COOH groups exhibited a strong antimicrobial activity (7-log reduction), whereas the SWCNTs with $-NH_2$ groups only displayed antimicrobial activity at higher concentrations. Although functionalization of the SWCNTs promoted bacteria–SWCNTs interactions regardless of the surface group, the antimicrobial activity occurred in a selective way [4].

Several studies have reported the antimicrobial activity of silver and other noble metals and their potential to prevent and control healthcare-associated infections [2, 36]. Chaudhari et al. evaluated the antimicrobial activity of silver-coated SWCNTs functionalized with antimicrobial peptides (AMPs) against *S. aureus* using a skin model. In the skin treated with functionalized silver-CNTs, the bacterial proliferation was significantly inhibited (10^5 cfu/g) compared to non-treated skin (10^8 cfu/g) [12]. Silver nanoparticles (NP) have the capability to bind to the bacterial cell wall and penetrate it, causing changes in membrane permeability and, consequently, cell death. The production of reactive oxygen species may also be a consequence of silver NP action. [37]. Simultaneously, it is known that AMPs display a broad-spectrum antimicrobial activity toward bacteria, fungi, and viruses [36]. Thus, the synergic association of silver NP with AMPs enhanced the toxicity of SWCNTs. These observations may be helpful to develop new antimicrobial therapies [12].

Carbon nanotubes can be also functionalized with natural antimicrobial enzymes such as lysozyme (LSZ), increasing their toxicity to bacteria [38]. The antimicrobial activity of LSZ has been previously described with its mechanism of action consisting of the lyse of the cell wall by hydrolyzing the β-1,4 linkage between *N*-acetylmuramic

Table 2 Studies reporting the development of SWCNT-based surfaces and their interaction with different bacterial species

Property	Material blend	Species	Main conclusions	Refs.
Antimicrobial	**Functionalized CNTs** CNTs with different surface groups (–OH, –COOH, and $–NH_2$)	*S. typhimurium* *B. subtilis* *S. aureus*	SWNTs with -OH and –COOH surface groups exhibited strong antimicrobial activity to both Gram-positive and Gram-negative bacteria (7 log reduction). $SWNTs-NH_2$ only exhibited antimicrobial activity at higher concentrations.	[4]
	Silver Silver-coated CNTs functionalized with antimicrobial peptides (TP359, TP226 and TP557)	*S. aureus*	The bacterial viability increased 4 log in the non-treated skin model, whereas skin treated with functionalized silver-coated CNTs exhibited an increase of only 1 log (from 10^4 to 10^5 cfu/g).	[12]
	Enzymes CNTs with lysozyme (LSZ) and DNA (layer-by-layer)	*M. lysodeikticus* *S. aureus*	Coating terminating in a LSZ-SWCNT layer exhibited high antimicrobial activity (84% reduction in cell density).	[38]
	Antimicrobial peptides Antimicrobial peptides (TP359, TP226 and TP557)-functionalized silver-coated CNTs	*S. aureus*	Functionalized silver-coated CNTs inhibited *S. aureus* proliferation on a skin model.	[12]
	Polymers CNTs incorporated within poly(lactic-co-glycolic acid)	*E. coli* *S. epidermidis*	The bacterial metabolic activity was significantly diminished in the presence of SWNT-PLGA. Up to 98% of bacteria die within 1 h on SWNT-PLGA versus 15–20% on pure PGLA.	[9]

(continued)

Table 2 (continued)

Property	Material blend	Species	Main conclusions	Refs.
	Polymers Polyvinyl-*N*-carbazole (PVK, 97 wt%)/CNTs (3 wt%) composite	*E. coli* *B. subtilis*	PVK-SWNT composite induced high bacterial inactivation (94% for *E. coli* and 90% for *B. subtilis*) in planktonic cells. PVK-SWNT-coated surfaces demonstrated a significant reduction of biofilm growth.	[41]
	CNTs layer-by-layer assembled with the polyelectrolytes poly(L-lysine) (PLL) and poly(L-glutamic acid) (PGA)	*E. coli* *S. epidermidis*	SWNT/PLL/PGA films demonstrated higher inhibition rates (up to 90%) for *E. coli* and *S. epidermidis* compared to control films (PLL/PGA, 20%).	[42]
	Oxidized-CNT/Poly(vinyl alcohol) (PVOH) composite	*P. aeruginosa*	The viability of cells deposited on O-SWCNT/PVOH surfaces decreased exponentially with increasing CNT loading.	[43]
	CNTs/Porphyrin nanocomposite	*S. aureus*	CNTs/porphyrin nanocomposite induced cell membrane damage in the presence of visible light.	[44]
	Functionalized CNT copolymer of star-shaped poly(ε-caprolactone) (stPCL) and poly (ethylene glycol) (PEG) composite	*P. aeruginosa* *S. aureus*	The CNT/stPCL-PEG copolymer inhibited the proliferation of *S. aureus* and *P. aeruginosa* but to a lower extent than the pure polymer matrix.	[45]
Antimicrobial and antifouling	**Polymers** CNTs covalently bound to polyamide membranes	*E. coli*	SWNT membranes achieved up to 60% inactivation of the attached bacteria after 1 h of contact time. Additionally, SWNTs delayed the onset of membrane biofouling during operation.	[46]

acid (NAM) and *N*-acetylglucosamine (NAG) on peptidoglycan [2, 39, 40]. Nepal et al. evaluated the antimicrobial activity of LSZ-functionalized SWCNTs against Gram-positive bacteria (*Micrococcus lysodeikticus* and *S. aureus*). It was observed that this SWCNT composite exhibited a high biocidal activity toward the tested bacteria [38].

The association of SWCNTs with polymers to form nanocomposites has been vastly explored. Aslan et al. incorporated SWCNTs within poly(lactic-co-glycolic acid) (PLGA) matrix and evaluated its activity against *E. coli* and *Staphylococcus epidermidis*. Bacteria exposed to the SWCNTs-PLGA decreased their metabolic activity and viability (98% of cell reduction compared to 15–20% obtained for pure PLGA) [9]. The association of polyvinyl-N-carbazole with SWCNTs resulted in higher bacterial inactivation of planktonic cells (94% for *E. coli* and 90% for *B. subtilis*), and surfaces coated with this NC also demonstrated a significant reduction of biofilm formation [41]. Likewise, poly(L-lysine) and poly(L-glutamic acid) used to form SWNT-NC presented high inactivation values (up to 90%) for *E. coli* and *S. epidermidis* [42]. Goodwin and co-workers prepared a SWCNT-poly(vinyl alcohol) composite and investigated its activity against *P. aeruginosa.* The viability of bacteria adhered to this surface decreased exponentially with increasing SWCNT concentrations [43].

Recently, Sah et al. explored the potential of photosensitive molecules like porphyrins to produce a SWCNT-NC with biocidal activity against *S. aureus.* The bacteria–NC interaction in the presence of visible light induced cell membrane damage [44]. Conversely, the functionalized SWCNTs/copolymer of poly(ε-caprolactone) (stPCL) and poly (ethyleneglycol) (PEG) composite did not show antimicrobial activity [45].

While some SWCNT-composites display antimicrobial activity, others show a combination of antimicrobial and antifouling properties. Tiraferri et al. demonstrated that SWCNTs covalently bound to polyamide membranes inactivated 66% of attached bacteria and delayed the onset of membrane biofouling, which may be helpful in the filtration process [46].

Although SWCNTs have demonstrated promising results in antimicrobial and antifouling surfaces, the number of published studies is limited when compared to MWCNTs.

3.2 Multi-walled CNT Surfaces

Multi-walled carbon nanotubes have been vastly explored and applied in various sectors due to their favorable properties. Up to date, several studies about their antimicrobial potential were published. Table 3 describes the studies carried out during the last decade regarding the biocidal effect of MWCNTs and their interaction with a wide range of bacterial and fungal species.

In order to improve the interactions between CNTs and microorganisms, the functionalization of MWCNTs is a common procedure. Several studies reported on

Table 3 Studies reporting the development of MWCNT-based antimicrobial surfaces and their interaction with different bacteria and fungi

Material blend	Species	Main conclusions	Refs.
Functionalized CNTs CNTs functionalized with –OH, –COOH, and $–NH_2$ surface groups	*S. typhimurium* *B. subtilis* *S. aureus*	MWCNTs functionalized with –OH, –COOH, and NH_2 did not display significant antimicrobial activity against all tested bacteria.	[4]
	E. coli *P. aeruginosa* *S. aureus*	The bacterial inactivation percentage of MWCNT-COOH was 34.1, 26.9, and 22.8% for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively.	[47]
	E. coli *S. aureus* *B. subtilis*	The bacterial inactivation percentage of MWCNT-COOH was 30, 50, and 40% for *B. subtilis*, *S. aureus*, and *E. coli*, respectively.	[3]
	E. coli *P. aeruginosa* *S. aureus*	The bacterial inactivation percentage of MWCNT –COOH was 20 ± 0.8, 26.8 ± 1.1, and 14.7 ± 0.5% for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively.	[48]
	L. acidophilus *B. adolescentis* *E. coli* *E. faecalis* *S. aureus*	MWCNT-OH and MWCNT-COOH induced significant and dose-dependent antibacterial activity against all tested bacteria.	[24]
	V. parahaemolyticus	The antimicrobial activity of f-MWCNTs was time dependent. f-MWCNTs mostly wound around surfaces of *V. parahaemolyticus* cells instead of piercing into the bacterial cells.	[49]

(continued)

Table 3 (continued)

Material blend	Species	Main conclusions	Refs.
CNTs functionalized with ethanolamine (MEA, DEA, and TEA)	*E. coli* *K. pneumoniae* *P. aeruginosa* *S. typhimurium* *B. subtilis* *S. aureus* *B. cereus* *S. pneumoniae*	The results based on minimal inhibitory concentration (MIC) and radial diffusion assay demonstrated that the antimicrobial activity of MWCNT-TEA > MWCNT-DEA > MWCNT-MEA > pristine MWCNT.	[50]
CNTs functionalized with oxygen groups		The results suggested that the reduction of surface carboxyl groups and the redox activity of carbonyl groups enhanced the antimicrobial activity of MWNT.	[51]
Silver and other noble metals CNTs coated with silver nanoparticles	*E. coli* *P. aeruginosa* *S. aureus*	The antimicrobial activity of f-MWCNTs-Ag was 93.7, 69.7, and 56.7% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively.	[47]
	E. coli *S. epidermidis*	The viability percentage of bacteria exposed to Ag/CNTs-deposited filter was 0.1 and 0.9% for *E. coli* and *S. epidermidis*, respectively.	[52]
	Methylobacterium spp. *Sphingomonas* spp.	The inactivation percentage of Ag-MWCNTs (40 or 50 μg/mL) was 100% for all tested bacteria.	[54]
	E. coli	Ag-MWCNT inactivated 97% of bacteria.	[53]
Copper nanoparticles grafted on CNT surfaces	*E. coli*	Cu-MWCNT inactivated 75% of bacteria.	[53]
Silver nanoparticles (AgNPs)-deposited CNTs functionalized with an amphiphilic poly(propyleneimine) dendrimer (MWCNT-APPI-AgNPs)	*E. coli* *S. aureus* *B. subtilis*	The inactivation percentage of MWCNTs-APPI-AgNPs was 99.8, 99.7, and 93.1% for *B. subtilis*, *S. aureus*, and *E. coli*, respectively.	[3]

(continued)

Table 3 (continued)

Material blend	Species	Main conclusions	Refs.
Cadmium sulfide (CdS) and silver sulfide (Ag_2S) quantum dots immobilized on poly(amidoamine)-grafted carbon nanotubes	*E. coli* *P. aeruginosa* *S. aureus*	The antimicrobial activity of f-MWCNT-CdS was 87.2 ± 4.1, 68.9 ± 2.5 and 46.7 ± 1.4% for *E. coli*, *P. aeruginosa* and *S. aureus*, respectively. The efficacy of f-MWCNT-Ag_2S was of 97.8 ± 2.1, 78.5 ± 2.9 and 55.7 ± 1.5% for *E. coli*, *P. aeruginosa* and *S. aureus*.	[48]
Zinc Oxide (ZnO)-coated CNTs	*E. coli*	ZnO/MWCNTs exhibited a strong antibacterial activity for *E. coli* (3- to 6-log reduction) comparing with pristine MWCNTs.	[56]
Sandwiched type structure based on polymer colloids, CNTs, and silver nanoparticles	*E. coli* *S. aureus*	The polymer colloids/AgNPs/MWCNTs exhibited a good antimicrobial activity as demonstrated by disc inhibition zone (11.53 and 9.73 mm for *E. coli* and *S. aureus*, respectively, versus ≈7 mm obtained for the control).	[55]
CNTs coated with titanium alloy and impregnated with rifampicin	*S. epidermidis*	Coated surfaces induced a significant inhibition of biofilm formation for up to 5 days.	[10]
Carbon nanotubes/titanium oxide/gold nanocomposite (NC)	*S. dysenteriae* *P. vulgaris* *K. pneumoniae* *C. albicans* *B. subtilis* *S. pneumoniae* *S. aureus*	The new nanocomposite exhibited high antimicrobial activity when compared with controls.	[57]
Enzymes Laccase and chloroperoxidase (CPO) separately immobilized onto carbon nanotubes	*E. coli* *S. aureus* *B. cereus* *B. anthracis*	Laccase-CNTs displayed >99% bactericidal activity against *E. coli* and *S. aureus*, and >98% sporicidal activity against *B. cereus* and *B. anthracis*. The CPO-CNTs also showed >99% antimicrobial activity against *E. coli* and *S. aureus*.	[58]

(continued)

Table 3 (continued)

Material blend	Species	Main conclusions	Refs.
Polymers CNTs functionalized with amphiphilic poly(propyleneimine) dendrimer (APPI)	*E. coli* *S. aureus* *B. subtilis*	The inactivation percentages of MWCNT-APPI for *B. subtilis*, *S. aureus*, and *E. coli* were 96.6, 96.5, and 87%, respectively.	[3]
CNTs functionalized with aromatic polyamide dendrimer	*E. coli* *P. aeruginosa* *S. aureus*	The antimicrobial activity of f-MWCNTs was 72.6, 65.2, and 35.5% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively.	[47]
Poly(amidoamine) (PAMAM)-grafted CNTs	*E. coli* *P. aeruginosa* *S. aureus*	The bacteria killing ability of PAMAM-grafted CNTs was 34.1 ± 1.2, 60 ± 1.8, and $22.8 \pm 0.9\%$ for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively.	[48]
Oxidized CNTs/poly(vinyl alcohol) (PVOH) nanocomposite	*P. aeruginosa*	The percentage of viable cells deposited on O-MWCNT/PVOH surfaces decreased with increasing CNT concentration.	[43]
Hydrogels Chitosan-CNTs hydrogels	*E. coli* *S. aureus* *C. tropicalis*	Chitosan-CNT hydrogel exhibited strong antimicrobial activity toward *S. aureus* and *C. tropicalis* when compared to *E. coli.*	[59]
	B. subtilis *S. pneumoniae* *E. coli* *P. aeruginosa* *G. candidium* *C. albicans* *A. fumigatus* *S. racemosum*	CNT composites displayed a higher potency against Gram-positive than Gram-negative bacteria. Chitosan/MWCNT composites have equally or even higher activities than the reference bactericides or fungicides against some of the tested microbes.	[60]

the functionalization of MWCNTs with -COOH surface groups. MWCNT-COOH reduced the bacterial viability by 20–40% for *E. coli*, 27% for *P. aeruginosa*, 15–50% for *S. aureus*, and 30% for *B. subtilis* [3, 47, 48]. Chen et al. also demonstrated that MWCNT-COOH and MWCNT-OH showed a significant and dose-dependent antimicrobial activity against *L. acidophilus*, *B. adolescentis*, *E. coli*, *E. faecalis*, and *S. aureus* [24]. The same effect was detected against *Vibrio parahaemolyticus* [49]. However, Arias and Yang [4] observed that MWCNTs functionalized (f-MWCNTs) with –OH, –COOH, and –HN_2 did not have significant antimicrobial activity, contrary to what was found by the same authors for SWCNTs (Fig. 4).

Zardini et al. tested MWCNTs functionalized with ethanolamine against a broad range of species and verified that f-MWCNTs exhibited a higher antimicrobial activity than pristine MWCNTs [50]. Finally, it was shown that MWCNTs functionalized with oxygen groups can have enhanced antimicrobial activity [51].

Similarly to SWCNTs, MWCNTs coated with silver nanoparticles (AgNPs) exhibited excellent biocidal activity. Studies reported that the bacterial inactivation percentage of MWCNT-AgNPs was 93.7–99% for *E. coli* and *S. epidermidis*, 69.7%

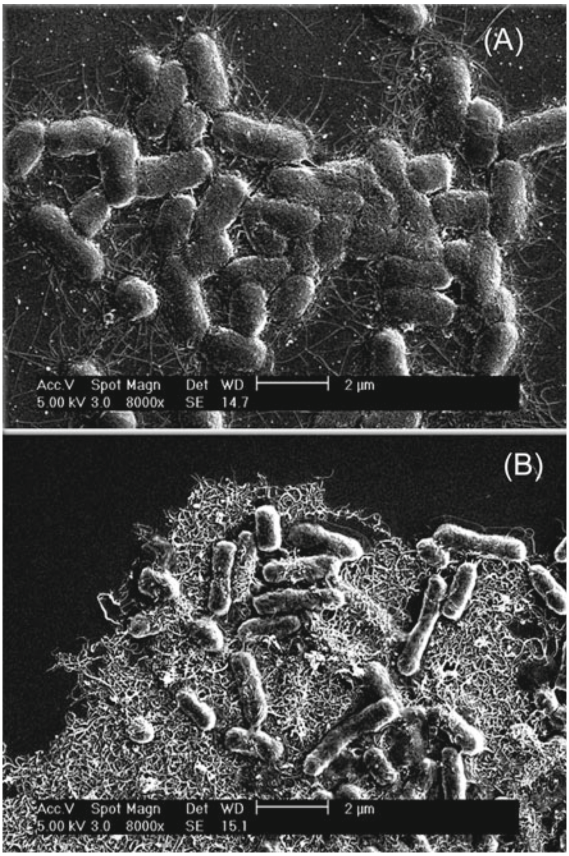

Fig. 4 SEM images of cell aggregates formed between *Salmonella* spp. cells and (**a**) SWNTs-COOH and (**b**) MWNTs-COOH. Reprinted with permission from Arias and Yang [4]. Copyright 2009 American Chemical Society

for *P. aeruginosa*, 56.7% for *S. aureus* and 100% for *Methylobacterium* spp. and *Sphingomonas* spp. [47, 52–54]. The association of MWCNT-AgNPs with amphiphilic poly(propyleneimine) dendrimers kept the inactivation percentages high for *E. coli*, *S. aureus,* and *B. subtilis* (>90%) [3]. Likewise, the immobilization of MWCNT-AgNPs with polymer colloids revealed a good antimicrobial activity against *E. coli* and *S. aureus* [55], and silver sulfide (Ag_2S) quantum dots immobilized on poly(amidoamine)-grafted MWCNTs were shown to reduce bacterial viability by 97.8, 78.5, and 55.7% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively. Moreover, Ag_2S-MWCNTs displayed better biocidal activity than MWCNTs coated with cadmium sulfide quantum dots [48].

The use of MWCNTs blended to other noble metals has also shown promising results. Bacterial cells exposed to MWCNTs coated with copper nanoparticles had their viability reduced by 75% [53]. Similarly, zinc oxide-coated MWCNTs showed strong antimicrobial activity against *E. coli* [56]. A nanocomposite constituted by MWCNTs, titanium, and gold exhibited high antibacterial activity against several species including *Shigella dysenteriae*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *B. subtilis*, *S. aureus*, and *Candida albicans* [57]. Lastly, multi-walled CNTs coated with a titanium alloy and impregnated with rifampicin were able to prevent biofilm formation for up five days [10].

Enzymes like laccase and chloroperoxidase (CPO) were immobilized onto MWCNTs. Laccase- and CPO-MWCNTs reduced more than 99% of bacterial viability for *E. coli* and *S. aureus*. MWCNTs combined with laccase also inhibited the *Bacillus cereus* and *Bacillus anthracis* spore formation by more than 98% [58].

The antimicrobial activity of nanocomposites formed by MWCNTs and different polymers has also been investigated. Murugan and Vimala evaluated the biocidal effect of MWCNTs functionalized with amphiphilic poly(propyleneimine) dendrimer (APPI). This NC was able to inactivate by 96.6% *B. subtilis*, 96.5% *S. aureus*, and 87% *E. coli* [3]. In another study conducted by Neelgund and Oki, MWCNTs functionalized with aromatic polyamide dendrimer presented a good antimicrobial activity against *E. coli* (72.6%) and *P. aeruginosa* (65.2%) [47]. On the other hand, poly(amidoamine)-grafted MWCNTs showed a reduced effect against all tested bacteria [48].

It is also possible that the antimicrobial activity of nanocomposites improves with increasing concentrations of CNTs. Goodwin et al. reported that the viability of bacteria deposited on MWCNT- poly(vinyl alcohol) surfaces decreased with increasing MWCNT concentration [43].

Recently, the interest in hydrogel-based materials has increased due to their physiological nature [59]. Some authors have explored MWCNT nanocomposites based on chitosan hydrogels. Venkatesan et al. reported the strong antimicrobial activity of chitosan-MWCNT hydrogel against *E. coli*, *S. aureus,* and *Candida tropicalis* [59]. Mohamed et al. also evaluated the biocidal activity of this kind of nanocomposites. Chitosan-MWCNT hydrogels showed a broad-spectrum antimicrobial activity [60]. Indeed, the use of chitosan as antimicrobial agent was previously reported by several authors [61, 62].

As described above, there are various material blends that may be applied aiming to develop effective antimicrobial surfaces.

Concerning the antifouling properties of MWCNTs, most studies relate to MWCNT-polymers nanocomposites. Indeed, the dispersion of MWCNTs can be increased by their addition to polymers. Thus, the bulk properties of CNTs may be extended through the nanocomposite improving their antifouling properties. Simultaneously, the addition of MWCNTs to polymers confers them certain properties such as resistance to protein interaction and higher biocompatibility [2].

Table 4 describes MWCNT modifications and/or associations with different polymers and their performance in increasing the fouling resistance. Only one study addressed the activity of oxidized MWCNTs on decreasing bacterial adhesion by eightfold to tenfold. [32].

The activity of MWCNTs incorporated into polydimethylsiloxane (PDMS) was reported in numerous studies. In all of them, the antifouling properties of PDMS were increased by the addition of MWCNTs, thus, decreasing biofouling [16–18, 63]. Moreover, MWCNT-PDMS coatings were applied to inhibit the microalga *Ulva linza* adhesion and decrease the removal stress for barnacles from ship hulls [16, 17].

Other studies have described the performance of MWCNT-polymer NCs to avoid bacterial adhesion and biofilm formation. Kim et al. showed that MWCNTs incorporated into poly(methyl methacrylate) inhibited *S. aureus*, *Streptococcus mutans,* and *C. albicans* adhesion by 35–95% [64]. Likewise, the biofilm growth on MWCNT-polyethylene composites decreased by 89.3 and 29% for *P. fluorescens* and *Mycobacterium smegmatis*, respectively [65]. *P. fluorescens* adhesion was also inhibited by the incorporation of MWCNTs into polyvinylidene fluoride membranes [66]. Lastly, Lin et al. demonstrate that tetraaniline covalently bonded to MWCNTs reduced the surface coverage percentage of *S. epidermidis* by more than 50% [67].

Protein adhesion to membranes during filtration processes can be reduced by the addition of MWCNTs. Liu et al. prepared a membrane composed of poly(sulfone), poly(sulfobetaine methacrylate), and MWCNTs that exhibited fouling resistance for bovine serum albumin (BSA) and fibrinogen in ultrafiltration processes [68]. The incorporation of MWCNTs into polyethersulfone (PES)-based membranes displayed higher flux and slower fouling rates than the usual PES membranes [69, 70]. Similar results were obtained for a study where PES membranes were incorporated with poly(citric acid)-grafted MWCNTs [71]. In another study performed by Takizawa et al., the presence of MWCNTs on polyamide reverse-osmosis membranes resulted in weaker interactions between the BSA molecules and membrane surface [72].

Polymer membranes are frequently used for water and wastewater treatments. In this context, several studies have been carried out to produce membranes with high fouling resistance and, consequently, high water flux. The combination of polyethyleneimine, MWCNTs, and trimesoyl chloride conferred to membranes high hydrophilicity, increasing their antifouling properties [73]. Also, the incorporation of CNTs into a polypropylene matrix showed high resistance to fouling deposition [74].

The polyamide membranes containing MWCNTs also demonstrated high fouling resistance rates against humic acid [20].

Table 4 Studies reporting the development of MWCNT-based antifouling surfaces

Material blend	Species	Main conclusions	Refs.
Functionalized CNTs Oxidized CNTs (O-CNT), oxidized-annealed CNTs (OA-CNT)	*P. fluorescens*	The rate of bacterial adhesion decreased eightfold to tenfold when an electric potential was applied.	[32]
Polymers CNTs incorporated into polydimethylsiloxane	*Zoospores* of *U. linza* *Barnacle cyprid*	Between 45 and 65% of the settled spores were removed from all coatings by exposure to a wall shear stress of 52 Pa. Adding 0.2% MWCNTs to the PDMS decreased the critical removal stress for barnacles significantly (70% compared to the control).	[16]
	U. linza	Addition of CNTs to amphiphilic block copolymers in PDMS caused a small reduction in the percentage of biomass released compared to the block copolymer without CNT (87% vs 76%).	[17]
		The antifouling properties of the PDMS matrix were improved with the incorporation of cMWCNT fillers, preventing biofouling for more than 14 week in marine environments.	[18]
		Nanocomposite surfaces only demonstrated weak modulating effects on the biological colonization.	[63]
CNTs with poly(sulfone) (PSF) and poly(sulfobetaine methacrylate) (PSBMA) (MWCNT-PSF/PSBMA)		The membrane made of PSF/MWCNT-PSF/PSBMA nanocomposite exhibited antifouling properties in BSA and fibrinogen ultrafiltration experiments.	[68]
CNTs incorporated into poly(methyl methacrylate) (PMMA)	*S. aureus* *S. mutans* *C. albicans*	Significant antiadhesive effects (35–95%) against all tested bacteria were verified for the 1% CNT/PMMA compared to the PMMA control group.	[64]

(continued)

Table 4 (continued)

Material blend	Species	Main conclusions	Refs.
CNTs with polyethersulfone (PES) blend membrane		CNT/PES blend membranes displayed a 15% higher flux and 7-fold slower fouling rate than the PES membranes.	[69]
CNTs incorporated into alumina/polyethersulfone hollow fiber membranes		CNT/alumina/PES membranes showed higher antifouling ability with the flux recoveries rates increasing by 84.1% for BSA and 53.2% for humic acid compared to the samples without CNTs.	[70]
Poly (citric acid)-grafted CNTs (PCA-g-MWCNT) incorporated as nanofiller in polyethersulfone (PES)		Compared to commercial PES hemodialysis membranes, the PES/PCA-g-MWCNT MMMs showed a lower flux decline (5-fold) and higher water flux recovery ratio (from $15.8 Lm^{-2}h^{-1}$ to $95.36 Lm^{-2}h^{-1}$)..	[71]
Polyethyleneimine/carbon nanotubes/trimesoyl chloride (PEI/CNT/TMC)		The high hydrophilicity and negatively charged PEI/CNT/TMC surface render membranes with good antifouling properties (90% more than PEI/CNT surface).	[73]
CNTs-Polyethylene (PE) composites	*P. fluorescens* *M. smegmatis*	Biofilm growth on PE-CNTs composites surface compared to PE decreased by 89.3% and 29% for *P. fluorescens* and *Mycobacterium smegmatis*, respectively.	[65]
CNTs with polypropylene (PP)		The present CNTs/PP nanocomposite showed a high resistance for fouling deposition in comparison with the typical PP matrix. After 24 h, the fluorescence intensity associated with the deposition of foulant was tenfold higher for the PP matrix.	[74]

(continued)

Table 4 (continued)

Material blend	Species	Main conclusions	Refs.
CNTs-polyamide nanocomposite (MWCNT-PA) reverse-osmosis (RO) membranes		MWCNTS-PA nanocomposite membranes had a flux reduction of 15% compared to 34–50% obtained for commercial membranes.	[72]
Carbon nanotube polyamide (CNT-PA) nanocomposite membrane		The fouling resistance against humic acid was constant for CNT-PA membranes. Conversely, the flow in commercial membranes decreased by 5%.	[20]
Interlaced CNT electrodes (ICE) on a polyvinylidene fluoride (PVDF) microfiltration membrane	*P. fluorescens*	The optimal operating conditions (2V alternating current) reduced the fouling rateby 75% versus the control and achieved up to 96% fouling resistance recovery.	[66]
Tetraaniline (TANI) covalently bonded to carbon nanotubes	*S. epidermidis*	Results revealed that the surface coverage percentage of *S. epidermidis* drops more than 50% from the unmodified to the modified film.	[67]
Polypyrrole (PPy)-coated CNTs nanocomposites		Results showed that the pure water flux increased from 152.8 L/m^2 h to 378.8 and 399.3 L/m^2 h for 0.1 and 1 wt% of PPy-coated raw and oxidized MWCNTs hybrid membranes, respectively.	[81]
Thermo-responsive *N*-isopropyle acryleamide (NIPAAm) polymerized on the surface of CNTs		The MWCNT-NIPAAm membranes demonstrated a flux recovery ratio of 78–99.9% compared to 47% of PES membranes.	[76]

Recently, Vatanpour et al. used polypyrrole, a natural polymer, to form MWCNTs-nanocomposite membranes, which demonstrated high water flux [75]. Finally, the combination of a thermo-responsive polymer, N-isopropyle acryleamide polymer, with MWCNTs also resulted in high water flux and high fouling resistance membranes [76].

It is important to highlight that there are MWCNT-based surfaces that combine both antimicrobial and antifouling properties. Table 5 summarizes the studies addressing these attractive MWCNT-based surfaces.

The application of silver and other noble metals on MWCNT-based coatings continues to yield excellent results. Various studies demonstrated that the association

Table 5 Studies reporting the development of MWCNT-based antifouling and antimicrobial surfaces

Coating	Species	Major conclusions	Refs.
Silver and other noble metals Silver nanoparticle/CNTs (Ag/MWNTs) coated on a polyacrylonitrile (PAN) hollow fiber membrane	*E. coli*	The relative flux drops over Ag/MWNTs/PAN was 6%, which was significantly lower than with pristine PAN (55%). The presence of the Ag/MWNTs inhibited bacterial growth and prevented biofilm formation.	[77]
Silver-CNT/poly(vinylidene fluoride-co-hexafluoropropene) membranes	*E. coli*	The 3 weight % Ag-MWCNTs/PVDF-HFP membrane showed a high fouling resistance rate and bactericidal activity (100% bacterial load reduction).	[78]
Silver nanoparticle with CNTs (Ag-CNT) on ceramic membrane under electrochemical assistance	*E. coli*	Viable cells on the CNT/ceramic membrane were reduced to 3.4 log while bacteria were completely inactivated by Ag-CNT/ceramic membrane.	[19]
Copper grafted on CNTs	*Methylobacterium* spp.	Cu/MWCNTs films were removed in more than 75% of the biofilm area.	[79]
Polyethersulfone (PES) membrane incorporated with zinc oxide (ZnO) and CNTs	*Enterobacter* spp.	ZnO/MWCNT/PES membrane demonstrated efficient antifouling properties with high flux ratios of 28–56 $Lm^{-2}h$ versus 7.8 $Lm^{-2}h$ obtained for the PES membrane. It also showed notable antibacterial properties with few bacteria attached to the membrane.	[80]
Enzymes CNTs with lysostaphin	*B. cereus* *E. coli* *S. aureus (MRSA)* *S. epidermidis*	Enzyme-based composites were highly efficient in killing MRSA (>99%) and inhibiting biofilm formation.	[81]

(continued)

of silver nanoparticles with MWCNT-polymer membranes conferred them a high antimicrobial activity and fouling resistance [19, 77, 78]. Simultaneously, copper grafted on MWCNT surfaces caused bacterial wall damage and inhibited biofilm formation [79]. Lastly, the association of zinc oxide and MWCNTs also demonstrated efficient antifouling and antibacterial properties [80].

Kang et al. described the combination of lysostaphin, an antibacterial enzyme with MWCNTs as a potent enzyme-based nanocomposite with high biocidal (<99%) and

Table 5 (continued)

Coating	Species	Major conclusions	Refs.
Antimicrobial peptides Immobilization of nisin on CNTs	*E. coli* *P. aeruginosa* *S. aureus* *B. subtilis*	The MWNT-nisin composite showed up to sevenfold higher antimicrobial property than pristine MWNTs. The MWNT-nisin deposit film exhibited a 100-fold higher anti-biofilm activity than the MWNT deposit film.	[82]
	B. anthracis	Nisin coating on MWCNT decreased surface hydrophobicity, reduced spore attachment, and reduced the germination of attached spores by 3.5-fold.	[83]
Polymers CNTs with epsilon-polylysine (MEPs)	*E. coli* *P. aeruginosa* *S. aureus*	MEPs nanocomposite killed 97.6, 91.5 and 88.5% of *E. coli*, *P. aeruginosa*, *S. aureus*, respectively. Results indicated that MEPs have also a stronger antiadhesive activity and can prevent biofilms formation.	[84]
N-halamine epoxide and siloxane grafted onto the CNTs (N-Si-MWNTs)	*E. coli* *S. aureus*	The films containing N–Si-MWNTs displayed a flux recovery ratio value above 96.5% and had excellent antibacterial efficacy (98.0 and 95.6% against *S. aureus* and *E. coli*, respectively).	[85]
CNT/poly(vinylidene fluoride-co-hexafluoropropene)	*E. coli*	The 1.5 weight % MWCNT/PVDF-HFP composite membrane showed high fouling resistance rate and bactericidal activity (100% bacterial load reduction).	[78]
Nanoporous solid-state membrane (NSSM) made by a two-step anodization method, and modified with CNTs	*E. coli* *S. aureus*	The BSA protein adsorption capacity reduced from 992 to 97 (μg mL^{-1} cm^{-2}). Results also showed that the percentage of inactivated bacteria was higher on the NSSM-MWCNT surface (98 and 99% for *E. coli* and *S. aureus*, respectively) than controls (8% for *E. coli* and 14% for *S. aureus*) as demonstrated by propidium iodide staining.	[86]

antifouling activities against methicillin-resistant *Staphylococcus aureus* (MRSA) [81].

Other studies also described the immobilization of nisin, an antimicrobial peptide, on MWCNTs. The MWCNT-nisin composite decreased surface hydrophobicity and exhibited higher antimicrobial and anti-biofilm activities than pristine MWCNTs [82, 83].

Multi-walled CNT-polymer composites continue to stand out due to their excellent properties and wide range of applications. Zhou J and Qi demonstrated that MWCNT-epsilon-polylysine killed 97.6, 91.5, and 88.5% of *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively. Additionally, this NC exhibited a stronger antiadhesive activity, preventing biofilm formation [84]. In another study, N-halamine epoxide/PDMS-grafted MWCNTs exhibited high antibacterial effect (98 and 95.6% reduction with *S. aureus* and *E. coli*, respectively). These films also displayed a high flux recovery rate [85]. MWCNT/poly(vinylidene fluoride-co-hexafluoropropene) composite exerted the same effect against *E. coli* [78]. Recently, Alizadeh et al. developed a nanoporous solid-state membrane made through a two-step anodization method and modified with MWCNTs. This new nanocomposite showed a promising effect upon *E. coli* and *S. aureus* and decreased protein absorption [86].

Although the reviewed studies demonstrated good results for both SWCNTs and MWCNTs, the latter seems to be more studied and applied for the development of antifouling and antimicrobial carbon-based nanomaterials.

4 Application of MWCNT-Based Surfaces in Urinary Tract Devices

The large number of published studies concerning MWCNTs confirmed their potential either for decreasing bacterial viability or inhibiting biofilm formation. Because of their outstanding properties, MWCNT-based surfaces have been widely applied in the medical field, in particular for the manufacture of medical devices.

Urinary catheters and ureteral stents are devices commonly used in clinical practice. However, their use often causes urinary tract infections (UTI). The UTIs correspond to about 17% of hospital-acquired bacteremias and have a prevalence of 36 and 27% in USA and Europe, respectively [87–89]. Therefore, these data act as a driving force for the development of new surfaces with antimicrobial/antifouling properties.

As noted above, MWCNTs have been successfully used in the production of hydrophilic silicone coatings. However, the employment of these nanomaterials in urinary tract devices remains understudied and further research is needed.

Recently, a study conducted by Vagos et al. under conditions that mimic the flow in the urinary tract devices demonstrated that bacterial adhesion can be modulated by the incorporation of different types of MWCNTs in PDMS composites. Results showed that the incorporation of small amounts (0.1%) of pristine MWCNTs can lead to a decrease of up to 20% on *E. coli* adhesion, whereas the use of oxidized MWCNTs (obtained by treatment with nitric acid) can increase bacterial adhesion also by 20% [90]. These results are corroborated by a previous study developed by Arias and Yang, where the MWCNTs-OH did not display significant antimicrobial activity against Gram-positive and Gram-negative bacteria [4]. Contrarily, Chen et al. demonstrated that MWCNTs-OH exhibit a significant and dose-dependent antimicrobial activity suggesting that assay conditions can have a great impact on surface performance [24].

Although these results are promising, further studies are needed to produce efficient MWCNTs/PDMS composites in order to prevent and reduce biofilm formation on device surfaces. A promising strategy may be to test different MWCNT loadings and also introduce chemical and textural variations on MWCNT/PDMS NCs.

5 Conclusions

Carbon nanotubes were described as excellent nanomaterials for numerous applications, particularly for the development of antimicrobial and antifouling surfaces.

Although the CNT mechanism of action is still being discussed by several authors, their antimicrobial and antifouling activities seem to depend on a multiplicity of factors, which may be modulated in order to improve their performance. The functionalization of CNTs surfaces is also essential to increase their hydrophilicity and, consequently, biocompatibility.

According to collected data, there are innumerous materials such as polymer, biomolecules, and metals, that may be blended in order to develop effective CNT-based nanocomposites.

The high antimicrobial activity of CNT-nanocomposites against a broad spectrum of microorganisms was reported. In addition, the significant fouling resistance of these nanocomposites was also proven at distinct levels. However, some studies suggested that MWCNTs are more effective than SWCNTs. Moreover, there are more studies using MWCNTs, which suggests that this type of CNTs is more promising for antimicrobial and antifouling activities. Nevertheless, further studies are needed to produce efficient MWCNT/PDMS composites aiming to develop new antimicrobial and antifouling surfaces.

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