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A Single-Walled-Carbon-Nanotube Filter for Removal of Viral and Bacterial Pathogens**

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Carbon nanotubes (CNTs) have been proposed for use in myriad applications, including electronics, composite materials, fuel cells, sensors, optical devices, and biomedicine.^[1] Their use in environmental applications, however, is still nascent, with few applications proposed or investigated so far. Previous studies in this field have focused on the use of CNTs or CNTs functionalized with inorganic nanoparticles for adsorption of inorganic contaminants and toxic metals from water.^[2–4] Other studies have explored the use of CNTs for adsorption of low-molecular-weight organic contaminants^[5] and toxins^[6] from water. Although reasonable contaminant adsorption has been demonstrated in some of these studies, the high levels of such contaminants in certain bodies of water would necessitate large quantities of CNTs for adequate contaminant removal.

A limited number of studies have explored the use of CNTs for filtration and separation applications. Srivastava et al.^[7] constructed a cylindrical membrane filter composed of radially aligned multi-walled carbon nanotubes (MWNTs) that formed a CNT layer several hundreds of micrometers thick. It was shown that the MWNT filter was effective in removing hydrocarbons from petroleum wastes as well as bacteria and viruses. Wang et al.^[8] developed a composite polymeric ultrafiltration membrane, with oxidized MWNTs incorporated into the top layer. The composite ultrafiltration membrane demonstrated high retention of oil/water emulsions. Lastly, it has recently been demonstrated that microfabricated membranes composed of aligned small-diameter MWNTs can produce high water flux and selective separation of low-molecular-weight solutes.^[9,10]

The primary separation mechanism of the MWNT filters in the studies discussed above is based on size exclusion or sieving. Such filters often require high pressure for operation and are prone to pore plugging and performance deterioration upon filtration of environmental samples. In this work we describe a novel, highly permeable, single-walled carbon nanotube (SWNT) filter and demonstrate its use for the effective removal of bacterial and viral pathogens from water

at low pressures. The filter utilizes several key properties of SWNTs: their small diameter and high surface area; their tendency to aggregate and form highly porous structures; and their antibacterial properties, as reported in our recent work.^[11] We demonstrate that bacteria are completely retained on the SWNT filter and are effectively inactivated upon contact with the SWNTs. We also show that viruses can be completely removed by a depth-filtration mechanism, that is, capture by nanotube bundles *inside* the SWNT layer. This work provides a basis for future development of a SWNT-hybrid filter, where the SWNTs are immobilized on a microporous ceramic filter. Such a hybrid filter would be exceptionally robust, permitting reuse, as the high thermal resistance of carbon nanotubes and ceramics would allow for simple thermal regeneration of the filter.

The filter was developed using a poly(vinylidene fluoride) (PVDF)-based microporous membrane (5 μm pore size) covered with a thin layer of SWNTs. Scanning electron microscopy (SEM) observations indicated that approximately 0.3 mg cm⁻² of SWNTs were necessary to ensure full coverage of the membrane surface. SWNT filters were then prepared at various SWNT loadings, ranging from 0.3 to 0.8 mg cm⁻², with corresponding depths of 2.0 to 6.0 μm. The use of a microporous base for the SWNT-hybrid filter allows for high water fluxes at low operating pressures while providing a sturdy support structure for the thin SWNT filtration layer.

Figure 1a presents a cross-section of a layer of SWNTs deposited onto the PVDF membrane base of the filter. SEM images were taken for each SWNT loading in order to determine the thickness and morphology of the resulting layer. The SWNT layers were made up of deposited SWNT bundles and displayed highly porous structures. Although the SWNTs were well dispersed for vacuum-assisted deposition onto the PVDF membrane, there were still small-scale heterogeneities in the overall layer depth owing to SWNT aggregation.

The average depth determined from SEM images of the SWNT layer cross-sections increased linearly with the SWNT loading (Fig. 1b). Water permeability values were inversely related to the SWNT layer depth, in agreement with the classical Kozeny–Carman equation^[12] for flow through porous filters (Fig. 1b). At 0.3 mg cm⁻² SWNT, a loading that allows full coverage of the filter, the permeability attained was 13 800 L m⁻² h⁻¹ bar⁻¹. Even at the highest SWNT loading (0.8 mg cm⁻²), the permeability (6500 L m⁻² h⁻¹ bar⁻¹) was still relatively high, in the range of typical microfiltration membranes.^[12]

The SWNT filter was then tested for its ability to remove microbial pathogens from water. *Escherichia coli* K12 was selected as a model bacterium. For the bacterial filtration experiments, an equal total number of *E. coli* cells (5 × 10⁵) were filtered through the SWNT filter (0.3 mg cm⁻²) and through the bare base filter (5 μm pore size) as a control. SEM images and analyses of permeate samples indicate that the *E. coli* cells were completely retained by the SWNT layer due to size exclusion (Fig. 2b), but passed readily through the base filter (Fig. 2a). Moreover, we found that the SWNT filter exhibited high antibacterial activity. A fluorescence-based viability kit (Live/Dead) was used to determine the percent of *E. coli* inactivated on the SWNT filter and, for comparison, a

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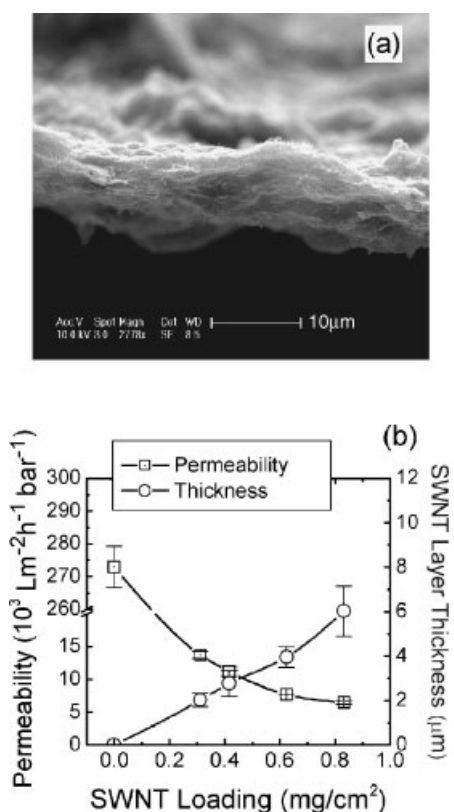


Figure 1. Properties of the SWNT filter. a) SEM image of the cross-section of SWNT layer. b) Variations of SWNT layer thickness and pure water permeability as a function of SWNT loading applied to filter. Water permeability experiments were carried out at 23 °C.

0.45 μm PVDF base filter. The level of cell inactivation measured was far higher on the SWNT filter, with (79 ± 9) % inactivation after a contact time of ca. 20 min, in comparison to less than 10% inactivation on the control PVDF membrane (Fig. 3). A parallel experiment for the measurement of metabolic activity with a tetrazolium salt (5-cyano-2,3-ditoly-tetrazolium chloride, CTC) indicated that only 6% of *E. coli* cells remained metabolically active after retention by the SWNT filter, in comparison to over 70% metabolically active cells on the control filter (Fig. 3). It is also noteworthy that the morphology of *E. coli* cells on the SWNT filter is strikingly different to that of the cells on the base filter (Fig. 2). Cells on the SWNT filter are flattened, with the original morphology and size of the cells significantly altered (Fig. 2b).

The SEM images and inactivation test results indicate that the SWNT filter is effective in both completely retaining the *E. coli* cells and promoting their inactivation. These observations are consistent with our recent work on the biotoxicity of highly purified SWNTs towards *E. coli* cells.^[11] In that study, it was shown that *E. coli* cells in direct contact with SWNT aggregates or an SWNT deposit layer underwent severe cell-membrane damage that resulted in their death. The antimicrobial properties of the SWNT layer of the filter may therefore be expected to inactivate pathogenic bacterial cells and retard biofilm growth.

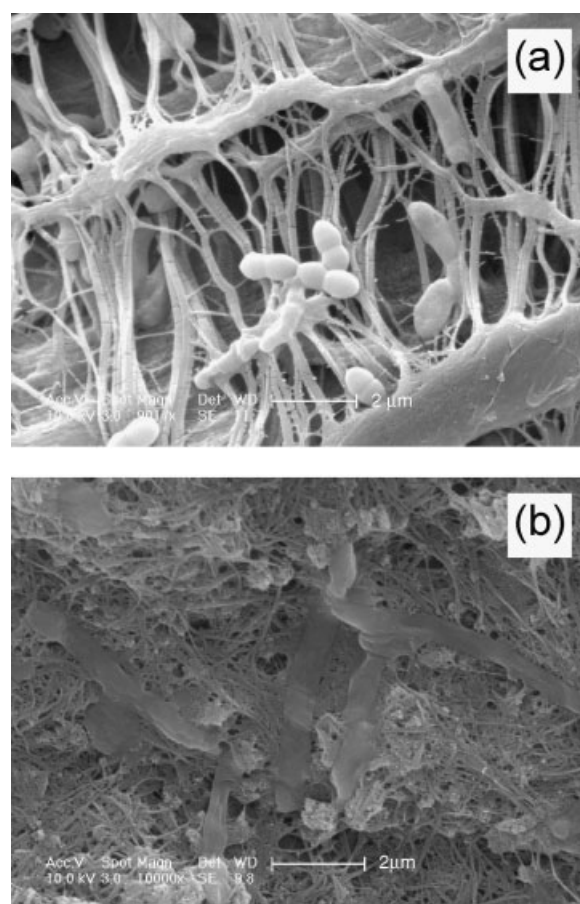


Figure 2. Retention of *E. coli* by control and SWNT filter. a) SEM image of *E. coli* cells on the base membrane (5 μm pore PVDF membrane). b) SEM image of *E. coli* cells retained on SWNT filter. The scale bars in both figures represent 2 μm.

The finding that the filter was capable of complete retention of *E. coli* was not surprising, since the bacterial cells (~2 μm) are much larger than the maximum gap between SWNT bundles (~0.3 μm) at the top of the SWNT layer. In

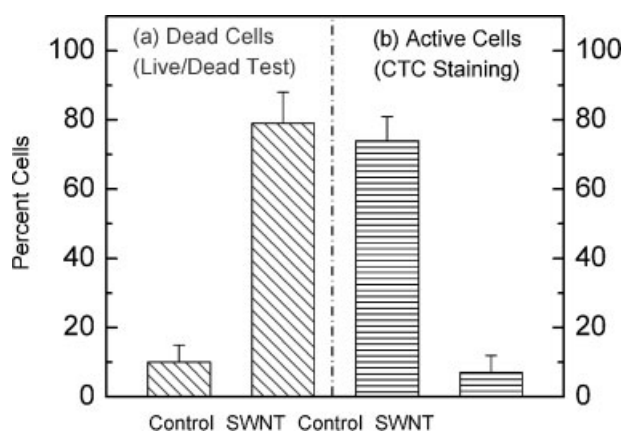


Figure 3. Inactivation and metabolic activity of *E. coli* cells retained on the SWNT filter and on the control PVDF membrane filter. a) Inactivation test results based on the Live/Dead test showing the percent of *E. coli* cells that are not viable. b) Metabolic activity test results based on CTC staining indicating the percent of metabolically active *E. coli* cells.

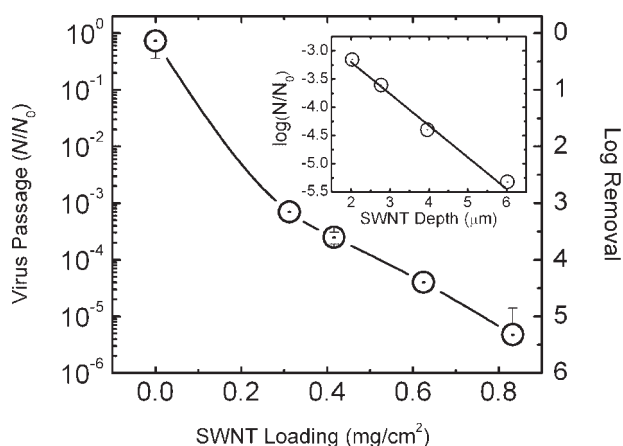


Figure 4. Virus removal by the SWNT filter at various SWNT loadings as filter coating. Initial virus concentration (N_0) was between 10^5 to 10^7 PFU mL⁻¹ for the various runs. Viral passage (N) was normalized by the initial concentration for that run (N_0). Experiments were carried out at a constant permeate water flux of $120 \text{ L m}^{-2} \text{ h}^{-1}$ and a temperature of 23°C . The inset graph describes the logarithm of the normalized residual virus concentration at the filter outlet (N/N_0) versus SWNT layer thickness. The SWNT layer thickness for each of the SWNT loadings is presented in Figure 1b.

light of these findings, however, we further sought to demonstrate the ability of the SWNT filter to remove viral particles, which are only tens of nanometers in diameter. A model virus particle, MS2 bacteriophage, diameter 27 nm, was selected. We hypothesized that convective–diffusive transport of the small virus particles would result in significant deposition of viruses on the nanotubes within the SWNT layer, as expected in depth filtration.^[13] We tested the MS2 viral removal with varying SWNT layer thicknesses. Figure 4 demonstrates that the relatively thin SWNT layers of the filter are very effective for virus removal. The removal rates range from 3.2 log removal at 0.3 mg cm^{-2} SWNT loading (a $2\text{-}\mu\text{m}$ -thick SWNT layer) to 5–7 log removal at 0.8 mg cm^{-2} SWNT loading ($6.0\text{-}\mu\text{m}$ thick SWNT layer). Several tests at an SWNT loading of 0.8 mg cm^{-2} showed that virus removal from the 10^7 virus particles per mL initial concentration was complete, without any viral particles detected by the PFU (plaque forming unit) method at the filter outlet.

The inset graph in Figure 4 presents the dependence of the logarithm of the normalized residual virus concentration at the filter outlet (N/N_0) on the SWNT layer thickness. The linear dependence is in accord with filtration theory,^[13] where $\log(N/N_0) \propto -L$, where L is the filter depth (thickness). This observation supports our conclusion that viruses are removed from the water inside the SWNT layer by attaching to the fibrous SWNTs. The small diameter of the nanotubes or nanotube bundles (of the order of several nanometers) compared to the SWNT layer thickness (a few micrometers) ensures effective depth filtration by the SWNT layer and therefore a very high removal efficiency.

The SWNT filter described offers several potential advantages for water purification—complete bacterial retention, exceptionally high viral removal (5–7 log), and high

antimicrobial activity—while operating at relatively low pressure. Because of the small amount of SWNTs per filter area, as well as the ease of preparation through simple SWNT dispersion and deposition on a base membrane, the cost of such filters should not be prohibitive. Furthermore, the SWNT filter would be expected to be reusable, as it could be regenerated by simple thermal or chemical treatment if a microporous ceramic layer is used as the membrane porous support structure. With these advantages, we envision the use of SWNT filters for point-of-use treatment of contaminated water in both developed and developing countries.

Experimental Section

SWNT filter preparation: Single-walled carbon nanotubes (SWNTs) were purchased from Stanford Materials (SWNT-90, lot #082106), and were purified to consist of more than 95% (w/w) SWNTs. The as-received SWNTs had an average outer diameter of 1.2 nm, lengths in the range of 10 to 20 μm , and a specific surface area of $407 \text{ m}^2 \text{ g}^{-1}$. Raman spectra (532 nm) and thermogravimetric analysis (TGA) showed that the SWNTs were highly ordered (G/D band ratio of 31), not functionalized, and contained 6% (w/w) metals. As-received SWNTs were uniformly coated on a $5\text{-}\mu\text{m}$ pore size PVDF membrane (Millipore, USA) by using a sonication/filtration procedure. Specified quantities of SWNTs (3–8 mg) were suspended in a dimethylsulfoxide (DMSO) solution to a concentration of 0.5 mg mL^{-1} . The suspension was sonicated for 15 min, and then vacuum-filtered through a PVDF membrane to achieve the various loadings of SWNTs on the base filters. 100 mL of ethanol followed by 200 mL of deionized water were then passed through the SWNT filters to remove residual DMSO and ethanol.

SWNT filter characterization: The permeability of the prepared SWNT filters (three freshly prepared SWNT filters were used for each SWNT loading) was evaluated by measurement of transmembrane pressure drops over a range of permeate water fluxes. The filtration system for these experiments consisted of a 47 mm plastic holder (Whatman, USA) to support the membranes and a pressurized tank system as described previously.^[14] The surface morphology of the SWNT filter was studied using scanning electron microscopy (SEM, FEI XL30) and public-domain image-analysis software (ImageJ, NIH). Cross-sectional images of the SWNT filters were taken, and the average thickness at each SWNT loading was determined by taking the average of 20 individual measurements.

Retention and viability of model bacteria: A model bacterium, *Escherichia coli* K12, was grown in LB medium and harvested at mid-exponential growth phase. Cells were rinsed twice using centrifugation, and then re-suspended by vortexing in 0.9% (w/v) NaCl solution. SWNT filters were freshly prepared and 50 mL of cell suspension (final cell concentration of 10^4 mL^{-1}) was then filtered through the SWNT filters for the retention and viability testing of the bacterial cells. Room temperature (23°C) was maintained during the bacterial-retention filtration experiments. The viability of *E. coli* on SWNT filters was examined by using two independent methods: a Live/Dead viability assay (Invitrogen, USA), and a metabolic activity test using 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC) (Sigma–Aldrich, USA). For the Live/Dead test, propidium iodide (PI)

was first applied to the SWNT filters, which were placed in the dark for 15 min, followed by counter staining with SYTO-9, in the dark, for 5 min. The viability of cells on the SWNT filters was taken to be the number of cells stained with PI (dead cells) divided by the number of cells stained with PI plus SYTO-9 (total cells). The viability of cells was further assessed by measurement of their metabolic activity with CTC.^[15] The detailed procedures for the use of CTC to determine the metabolic activity of *E. coli* retained on SWNT deposit layers are given in our previous publication.^[11] In both cases, bare Omnipore PVDF membranes (Millipore, USA) with a pore size of 0.45 μm (to ensure complete bacterial retention) were used as controls.

The retention and morphological changes of *E. coli* were investigated by SEM. Cell suspensions were filtered through the SWNT filters and PVDF membranes (5 μm pore) as explained above, and fixed with 2.5% glutaraldehyde and 1% osmium tetroxide.^[16] The samples were then sputter-coated with gold (30 s, 30 mA) and viewed at an accelerating voltage of 10 kV.

Retention of model virus: MS2 bacteriophage was used as a model virus particle. The MS2 virus was prepared following the method described previously.^[17] Viruses were suspended in 10 mL of a phosphate buffer solution (PBS; $\sim 10^6$ PFU mL^{-1}) and filtered through the SWNT filter and the PVDF membrane (5 μm pore, control) at a constant permeation flux (120 $\text{L m}^{-2} \text{h}^{-1}$) using a peristaltic pump. Filter permeate samples were collected in an autoclaved glass tube, and the virus concentration was determined by the PFU method (US Environmental Protection Agency (EPA) Method 1601).^[18] For PFU measurement, *E. coli* (American Type Culture Collection (ATCC) 15597) was used as a viral host bacterium and tryptic soy agar (TSA) plates were used to obtain 30–300 PFU/plate at the various dilution ratios of the permeate with molten soft agar (0.7% TSA). All experiments were, at a minimum, duplicated at each dilution. Room temperature (23 °C) was maintained during the virus filtration experiments.

Keywords:

adsorption · antibacterial materials · carbon nanotubes · filters · water purification

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