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Carbon Nanotube Cluster Based Micro-Fluidic System for Bacteria Capture, Concentration, and Separation

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Abstract

Disease-causing pathogens continue presenting enormous global health problems, especially due to their easy transmittance to people via water supply systems. The detection, filtration, and purification of bacteria-contaminated water samples are complex activities, ones subject to considerable error. Here we present a new and highly effective micro-fluidic system with carbon nanotube (CNT) clusters for effective and efficient detection, filtration, and purification of bacteria-contaminated medium. The developed system is based upon two unique properties of CNT clusters: high bacterial affinity and magnetic susceptibility. The CNTs' high affinity to bacteria cells makes them a key candidate for the bacteria adsorption. The magnetic susceptibility of their clusters allows an effective way of separating as well as containing them in the system. In this study, we designed and tested a prototype CNT-cluster based micro-fluidic system by uniquely combining the two excellent properties of CNTs. The CNT-based micro-system consisted of a micro-channel, which positions CNT clusters evenly on the bottom surface using a strong Neodymium block (1" X 1" X 1") rare-earth magnet (surface magnetic field strength = 0.684 Tesla). When bacteria suspensions were introduced, the CNT clusters in the micro-fluidic system were shown to effectively serve as bacterial adsorbing centers, which led to spontaneous adsorption and concentration of bacteria to the clusters. This was shown to happen for both types of microorganisms, i.e., Gram-positive and Gram-negative bacteria. The results demonstrate the excellent potential of the CNT based micro-fluidic system for bacteria capture, concentration, and separation.

1. Background

The presence of bacteria in water systems is a danger to any operation; hence, the detection of bacterial contaminations has become a big issue. Current methods for determining the presence of bacteria include polymerase chain reaction (PCR), colony counting methods, and immunology-based methods, which have long waiting periods and are complicated processes [1]. Additionally, the problem still remains that bacteria in a dilute solution cannot be detected. In the following research, the beginnings of a method are suggested for determining the presence of particular pathogens and reducing the concentration requirements 107 – 104 colony forming units (CFU)/ml to close to 81 CFU/mL [1].

The advent of nanotechnology has opened many doors for sensing in the nano-scale. Nanotechnology has the ability to revolutionize molecular electronics, medical chemistry, and biomedical engineering [2]. Research has explored applications from electronics, composites, fuel cells, sensors, optics, and biomedicine [3]. Carbon nanotubes (CNTs) were first discovered in 1991 by Iijima and are cylindrical carbon rods with a diameter of 4 to 30 nm [4] and lengths up to millimeters in length [5]. The aspect ratio for such a material is enormously high at 1:28,000,000, making the CNT a very unique material with peculiar and novel properties. Multi-walled CNTs (MWNTs) are similar in structure with additional walls located around the central wall.

Recently, high affinity binding interactions between CNTs and bacteria have been demonstrated [6,7,8] and exploited for use as bacterial filters [7] and photothermal and photoacoustic antimicrobial contrast agents [8,9]. These studies demonstrate excellent potentials of CNTs as bacterial capturing agents. In addition, research by Brady-Estevez, et al. [3] explored the use of single-walled CNTs (SWNTs) as a size exclusion filter with Escherichia coli K12 (a model bacterium for E. coli 0157:H7). They also demonstrated a method by which the SWNT system can be reused. Further, CNTs have been shown to exhibit para-magnetic and di-magnetic properties under the presence of a strong magnetic field [10]. This previous research opens the door for continued exploration of the frontier of nanotechnology by testing specifically the feasibility of a MWNT system for filtering and concentrating bacteria in water solutions by adsorption and magnetic retainment.

Parallel to research being conducted on the applications of CNTs is the work that highlights the possible toxicity they may possess. This potential drawback needs to be addressed. Warheit, et al. [11] reported that CNTs could be harmful to the pulmonary system of rats and, by extrapolation, humans. If kept in solution, the CNTs will not cause pulmonary problems. In addition, Blaise et al. [12] reported that certain nano-materials are toxic to aquatic organisms. Their research emphasized that CNTs should be retained by any system that uses them. That is where the unique magnetic properties of CNTs can be used to increase the retention of CNTs inside a system. Systems using CNTs should incorporate this concept.

Such systems would have two very distinct potential applications. The first is bacteria "filtration," functioning by size exclusion or adsorption of the bacteria. This system would need to be reusable in order to make it cost effective. The second is bacteria "concentration". The concept is that the microbes in a dilute solution become concentrated inside

a micro channel, which would provide a mechanism for more efficient bacteria detection, in particular pathogen detection. This application likely will have the greatest impact on rapid water source analysis.

The goal of this study was to build and evaluate a MWNT based system for bacteria concentration and filtration. The specific objectives included designing and fabricating an apparatus to conduct tests, determining optimal operating parameters including flow rate and MWNT concentration, determining bacterial adsorption capacity of MWNT system, and finally imaging the bacteria and MWNTs to draw conclusions.

2. Materials and Methods

2.1 Bacteria Evaluation. E. coli were prepared from a frozen sample (-80oC) by creating a streak plate (Luria Bertani (LB) agar) and incubating for 24 hours at 37oC. A single colony was isolated and inoculated in LB medium and then incubated in a shaker (24 hours, 37oC, 150 rpm). It was considered important during this study to know the concentration of E. coli cells in a suspension before experiments begin. A spectrophotometer was used to evaluate dilutions of E. coli suspensions in parallel to spread platting (LB agar), which generated the relation of the optical density (OD) of an E. coli suspension with the concentration of the suspension. The OD of the E. coli samples was routinely measured and the cell concentration was estimated on the basis of the empirically determined relation.

2.2 Device Design. Figure 1 is a graphical representation of the designed device. The device consisted of a polydimethylsiloxane (PDMS) gasket (2.5 cm × 7.5 cm × 0.2 cm) with a channel cut into the center (1.0 cm × 5.5 cm). The channel was further encapsulated by two microscope slides. The top slide had 1-mm holes drilled into the surface at opposite ends with micro-fluidic fittings attached with epoxy glue. The fittings were connected to influent and effluent tubes, which transported the sample into and out of the system. The glass slides and PDMS were further encapsulated inside an ultra-high molecular weight polyethylene (UHMWPE) clamp, which was held together with nylon bolts and nuts. This provided even pressure maintaining the seal provided by the PDMS gasket. Finally, a strong Neodymium block (1" X 1" X 1") rare-earth magnet (surface magnetic field strength = 0.684 Tesla) was placed below the system to take advantage of the MWNT clusters' magnetic susceptibility.

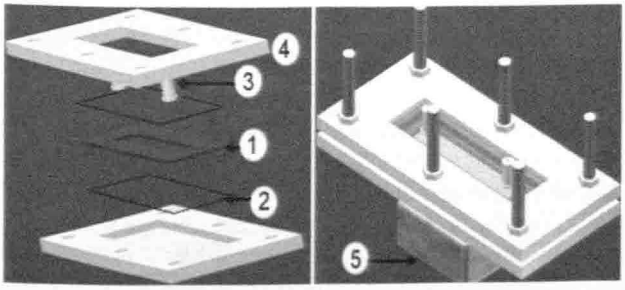

Figure 1: MWNT micro-fluidic device for experimentation 1-PDMS, 2-Glass Slides, 3-Micro-fluidic fittings, 4-UHMWPE clamp, 5-Rare-earth magnet

2.3 Channel MWNT Concentration Determination. MWNTs were injected into the system under presence of the rare-earth magnet, which caused the MWNTs to concentrate into the bottom of the channel. MWNTs were injected until the system was saturated. This was determined visually when, in the presence of the magnet, the MWNTs occupied the entire volume of the channel. The resulting MWNT concentration was selected as the optimal MWNT concentration for experimentation.

2.4 Maximal Flow Rate Determination. The MWNT channel retained the MWNT clusters on the basis of their magnetic susceptibility. This magnet susceptibility was of limited strength. A high flow rate could dislodge clusters from the channel. The maximal flow rate was determined on the basis of visible loss of MWNT clusters at varying flow rates.

2.5 Adsorption Capacity of MWNTs. To measure the capacity, a known concentration of E. coli was injected into the system. The effluent was collected and the concentration was measured to find the percent removal:

$$\%Removal = 1 - \frac{[E.coli]_{out}}{[E.coli]_{in}}$$

The test was conducted first by preparing a suspension of E. coli in LB medium. The optical density was measured to estimate the influent concentration and serial dilutions were made to the concentration that is to be tested. The system was injected with a pre-determined concentration of MWNT suspension (see section 2.3) and allowed to rest under the presence of the rare-earth magnet for 5-15 minutes. The system was then rinsed with 30 mL of water and the last few drops were collected as a negative control. Diluted cell suspensions were then injected into the system and rinsed into the collection tube. The collected samples were spread plated and incubated for 24 hours at 37°C. After incubation, the CFUs were counted and the effluent cell concentration was calculated.

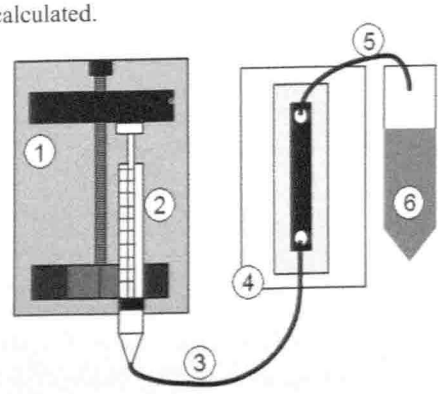

Figure 2: MWNT micro-fluidic system for estimating adsorption capacity 1- Syringe Pump, 2-10-mL Syringe, 3-Influent tube, 4-MWNT channel, 5- Effluent tube, 6-Collection for testing

2.6 Imaging. Epi-fluorescence microscopy was used by staining the E.coli with LIVE/DEAD BacLightTM Bacterial Viability Kit (Invitrogen, Carlsbad, CA). The kit contained two nucleic acid stains: green-florescent SYTO® 9 stain and a red-florescent propidium iodide stain. STYO 9 (green) labeled both live and dead bacteria when used alone. Propidium iodide (red) penetrated bacteria with damaged membranes (dead bacteria). The images were made with a Light Microscope (Axioskope 2 Plus, Carl Zeiss, Inc., Germany) equipped with 12-bit Color MicroImager II cooled digital camera (Qlmaging, Burnaby Canada). The light microscopy system was additionally equipped with filter sets consisting of band-pass filters covering 450 to 490 nm (fluorescein isothiocyanate (FITC)) and 512 to 546 nm (propidium iodide (PI)) for exciters and absorbance filters covering wavelengths beyond 515 nm (FITC) and 590 nm (PI) to acquire epi-fluorescence images for the stained cells. Imaging the MWNT system with infused E. coli showed the location relative to the MWNT clusters as well as their health.

3. Results

3.1 Bacteria Evaluation. The data collected from the spread plates was reduced by selecting the most accurate plate count (typically between 30 and 300 CFUs). At a dilution factor of 10-6 the colony count was 152, meaning that the culture contained 1.52 CFU/µL. This concentration data was related to the OD results from dilutions of 100, 5×10-1, 10-1, 5×10-2, 10-2, 5×10-3, 10-3. The data was plotted and a regression line completed the relationship (Figure 3). With an R2 value of 0.992, the experiment had a strong fit. The following equation relates the OD of E. coli to the concentration of the suspension:

$$Concentration\left(\frac{CFU}{\mu L}\right) = 97261(OD\ Measurment) - 16109$$

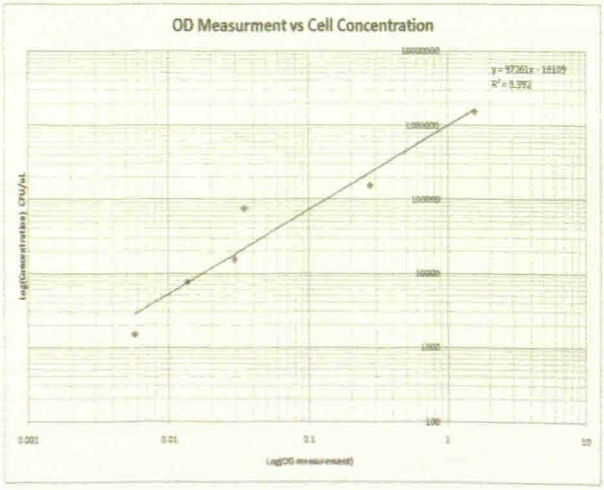

Figure 3: A standard linear regression graph relating OD and cell concentration in suspension

3.2 Device Design. The device worked as described in creating a channel for experiments to take place. The properties of MWNT clusters (magnetic susceptibility and bacteria adsorption) were utilized in the system.

3.3 MWNT Concentration Determination. After repeated injections, the ideal channel MWNT concentration was 4 mg/mL.

3.4 Maximum Flow Rate Determination. Flow rates of 2.0, 1.5, 1.0, 0.5, 0.2, and 0.1 mL/min were applied to a channel, which was saturated with MWNTs, for 1 minute and observed closely for MWNT cluster loss. On the basis of visual observations, 0.2 mL/min consistently had no loss of MWNT clusters. However, the 0.1 mL/min was selected for a safety factor of N=2. This prevents any MWNT loss during experimentation.

3.5 Adsorption Capacity of MWNTs. The experiments to determine the MWNT clusters' bacteria adsorption capacity were executed for varying cell concentrations in search for the threshold concentration, where MWNTs lose their ability to effectively filter E. coli, specifically the concentration where less than 95% are removed. The data below show important characteristics of the designed system. There was a strong correlation between the number of cells into the system and the % removal. Pearson's Correlation (r) relates two nonlinear parameters [13]. A Pearson's Correlation of 0.8 or above represents a good fit. In this case:

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{N}}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{N}\right)\left(\sum Y^2 - \frac{(\sum Y)^2}{N}\right)}} = -0.890$$

This represents a strong negative relationship. The variance in the system makes it difficult to quantify the system. As the graph shows, there are two distinct slopes (Figure 4). The intersection of the slopes will be defined as the capacity. Thus, the capacity for this system was estimated at 200,000 cells. The capacity per mg of MWNT was assessed on the basis of the volume of the system and the concentration of the MWNTs inside the channel:

$$Capacity = \frac{Number\ of\ Cells}{Number\ of\ Carbon\ Nanotubes} = \frac{[E.coli]}{[MWNT]}$$

$$Capacity = \frac{\frac{200{,}000\ CFU}{1.2\ mL}}{4\frac{mgMWNT}{ml}} = 41667\frac{CFU}{mgMWNT}$$

$$Capacity \cong 41{,}000\frac{CFU}{mgMWNT}$$

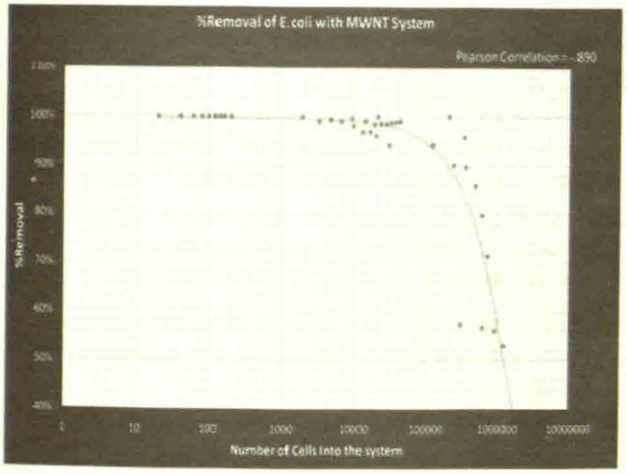

Figure 4: The percent removal of the MWNT system as a function of the number of cells injected

3.6 Imaging. Figures 5 to 7 show the results of the imaging experiment. Bacteria were infused into the system using the capacity testing procedure. MWNTs were isolated from the system and placed on a microscope slide and images were taken as described.

Figure 5: An image of MWNT alone taken with a light microscope at 40X optical zoom. Shows isolated MWNT clusters in solution

The images show that E. coli cells were adsorbed and retained by MWNT clusters. It appeared that the E. coli cells contacted MWNT clusters by diffusion or propelled by their own flagella mediated movement.

4. Discussion and Conclusions

Some previous research using CNTs as bacterial filters [3] suggests that the bacterial filtration is based on size exclusion. In contrast, research conducted by Kim et al. [8] suggests that bacteria are spontaneously adsorbed on to the CNT clusters. The results of this study firmly support the findings by Kim's group, showing cells attached to all surfaces of MWNT clusters. Also, a size exclusion filter would be either 100% effective or 0% effective; however, the filter in this study had varying effectiveness based on the input concentration.

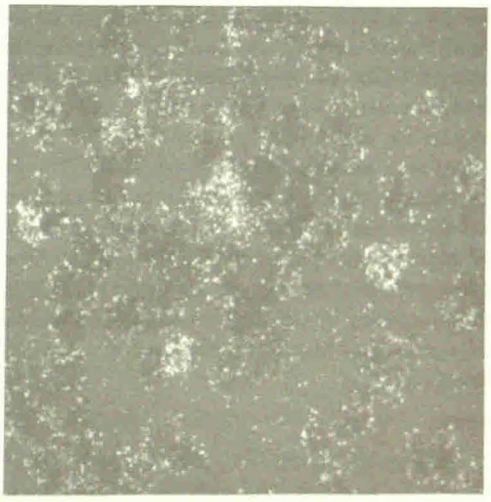

Figure 6: MWNT with epi-florescence microscopy (FITC filter) – showing live and dead cells as white dots. It is important to note that cells are highly concentrated around clusters of MWNTs

Figure 7: MWNT with epi-florescence microscopy (FITC and PI hybrid filters) showing live cells (blue-green) and dead cells (red). The cells are concentrated on the MWNT clusters. It appears that the number of dead bacteria outnumber the live. There is a MWNT cluster that shows live bacteria (circled)

Kim suggests that the specific chemistry, i.e., hydrophobic interactions and/or van der Waals interactions, between the MWNTs and the cell membranes may be responsible for the spontaneous cell adsorption [8].

The results from the study of the capacity experiment and imaging experiment show that the system was effectively filtering bacteria at 41,000 CFU/mg-MWNT. The two unique properties of the CNT clusters (i.e., bacteria adsorption and magnetic susceptibility) exploited in this study have proven a feasible system for the concentration of bacteria inside a micro-channel.

Further development of the CNT cluster-based process, however, is required through appropriate engineering designs

and optimizations to realize the unique advantages identified in this study. Future research may include redesigning the system using a Biological Micro-Elelctro-Mechanical Systems (Bio-MEMS) design, making a more efficient system that will maximize the capacity per quantity of MWNT. Future research may also quantify the toxicity of the MWNTs or test with other bacteria strains, viruses, and protozoa to determine their adsorption capacity. These would provide opportunities to extend the concepts demonstrated in this study to future applications, such as sampling units for environmental quality control or bio-sensing by offering ways to concentrate target biological agents, enabling us to accomplish the excellent properties of CNTs.

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Editor's note: Some of the figures in this study require full color imaging for maximum fidelity and accuracy. The black and white format of Inquiry reduces the quality of the images.

Mentor Comments:

In his letter of support for Chris Nelson's article, Jin-Woo Kim clarifies the way in which this research adds to our knowledge of carbon nanotubes and their potential in future work:

Mr. Chris Nelson's research with me involved a very exciting and challenging work to explore the unique properties of carbon nanotubes. The primary focus of the research was to study the possibility of developing an effective bacterial removal system by exploiting the unique properties of CNTs, in particular their high affinity to bacterial cells and magnetic property, which requires knowledge and tools of both biology and engineering. This cross-disciplinary research provided him an opportunity to strengthen his laboratory skills and broaden his knowledge in the fields of nanobiotechnology and biological engineering.

Chris has been very good at organizing his work. He also was an independent worker, requiring minimal supervision. While preparing a research proposal for the SILO Undergraduate Research Fellowship (SULF) a year ago, he excelled at working independently; however, he was not hesitant to ask questions when he had one. I was impressed by the level of the questions he asked regarding the research, indicative of his understanding of the topics of the research. As expected, he received the SURF grant. Furthermore, when getting started on the project, he not only sought to learn engineering and scientific concepts through literature search, but also sought out help to learn experimental techniques, including cell culturing, imaging, and analytical techniques. But then he just put his head down and practiced until he is very good at them. He has been very productive and also curious about the limits he believes the technology would undoubtedly face. In addition, he dealt with the frustrations of scientific experiments as well as writing with very mature equanimity while conducting the research and writing his thesis. He has not only successfully completed his research, but also he presented his

research results at the 2009 Annual Conference of Institute of Biological Engineering (IBE) on March 19 – 21, 2009. With my observation as his mentor for the last one and half years, I must say that I have found him to be a brilliant, very dedicated, and productive student.