Impact Of CNT Ingestion On In-Vitro Cells

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ABSTRACT

In this paper we describe our results of CNT ingestion on in-vitro cell cultures. We present AFM pictures of the observed impact of CNT on cell growth. We describe how cells react and how CNT affect the cell division process of the bacteria. We describe the bio-nano interaction and describe the implications for biological systems. Our methods specifically apply to CNTs obtained by the HiPCO process. However, we assume the method can be extended to other cases of cells and bacteria agents. We expect this approach will lead to finding novel ways to fight diseases, cell manipulation and creation of bio-structures for future nano-engineered devices that could be investigated further for use in nano-scale electronics, implants and bio-materials.

Keywords: Cells, HiPCO CNT, cytokinesis , bacteria, purification, segregation.

1 INTRODUTION

The structural and electronic properties of SWNTs lend themselves to a variety of biomedical applications involving the detection and treatment of diseases, most notably cancer. For example, the structural change in DNA upon interacting with CNTs sufficiently perturbs the electronic structure of SWNTs such that the change can be detected, so that CNTs can be used as sensors in living cells. Finally, there are a number of reports that CNTs facilitate the transport of bound oligonucleotides, peptides, and proteins across the plasma membrane. However, despite these and other intracellular applications not listed here, there remain technical challenges towards realizing the potential benefits of CNTs in bio-medicine. Namely, CNTs are extremely hydrophobic, bundle together, and are insoluble in water. Covalently attaching the materials to CNTs solves the dispersion problem, however it introduces defects in the surface of the CNTs that often interfere with the electronic and optical properties that make CNTs so useful. Another challenge in the field is assessing whether CNTs are inherently cytotoxic At present, there are roughly as many publications reporting no apparent cytotoxicity [1, and references therein, 2] as there are reporting varying degrees of significant cytotoxicity. Two major considerations in this area are how the CNTs are presented to the organism and the purity and concentration of the CNTs.

Our work is based on the CNTs obtained by HipCo process. This process results in primarily iron as the metallic impurity in the CNTs. We have used the bacteria cells that “eat” iron, as a purifying and cleaning agent. In the course of this work we have observed that there is significant interference with cell mechanisms, and in fact, cytokinesis is interrupted by the CNTs.

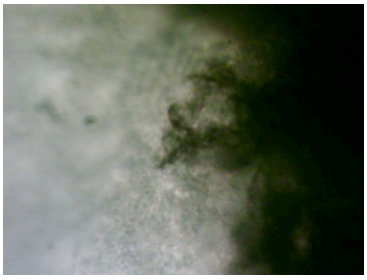
2 EXPERIMENTS

The main purpose of our research was to create thin films for use in electronic applications. After experimenting with several methods already researched to clean the same type of material from their impurities, we concluded that these methods caused structural damage of the SWCNT which will also lead to a change in the electrical properties. We created a more passive process for the purification that will prevent damage in the SWCNT structure, but at the same time eliminate over 90% of the contaminants such as carbonaceous and iron impurities, leaving a pristine material to be used in the creation of thin films. We devised the use of a biological agent for the purification process. We found that by using bacteria we achieve the cleaning task without affecting the condition and/or structure of the CNTs; thus eliminating most of the mineral contaminants in the process and also affecting the cell division properties of the bacteria itself*.

After several attempts to formulate a procedure to expose the CNT dust to biological cleaning agents, we

finally created a working protocol in which the biological agents "ingest" the CNT material looking for their nutrient supply of Iron*. Therefore, after the intake of the CNT material, the bacteria agent started to show a change in their biological structure. Those

(a)



(b)

Figure 1: (a) Optical image of CNT dust when bacteria are just applied; (b) Optical Image of CNT dust after 4h once the bacteria start the cleaning process. The red area is the Iron oxide from the Iron being pulled out from the inside of the CNTs.

changes not only affect the cell mitosis process of the bacteria, but also the bacteria show adaptation changes to compensate for the weight of the material ingested. Figure 2 shows an adaptation change in the bacteria such the growth of extra pili to manage is mobility due to the change in weight.

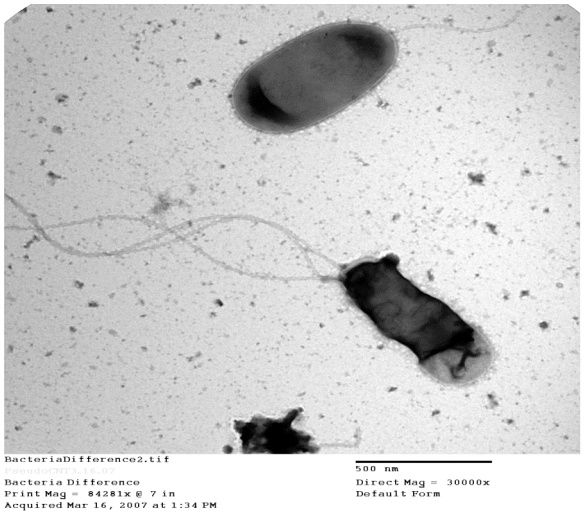
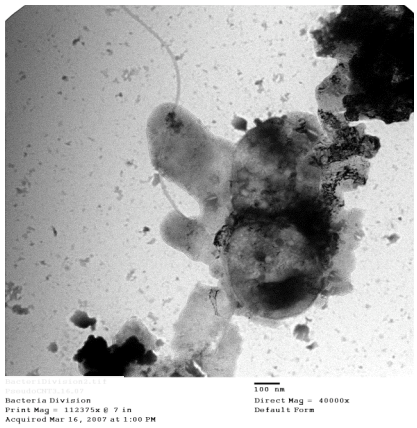


Figure 2: Bacteria Difference (number of pili) one without SWCNT and one with SWCNT inside.

In the biological cleaning process one of the bacterium main nutrients is Iron which is needed for the production of nitrogen. Using this to our advantage the bacterium is grown in a starving environment (lack of Iron nutrients) and then is released in an environment containing only the raw CNT. Once the bacteria colony reaches the raw material the absorption process starts. During this process each bacterium works as a "biological cleaning machine" absorbing the raw CNT to extract the Iron from the SWCNT. In Figure 2 (a) TEM micrograph shows the difference between a normal bacterium and one after "ingesting" the raw CNT material. After the CNT are absorbed the bacterium breaks down the carbonaceous cocoons were the iron is enclosed leaving only clean SWCNT.



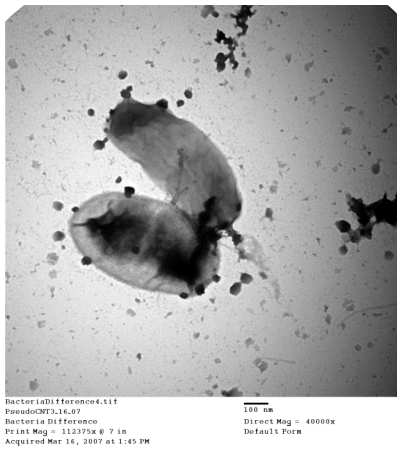


Figure 3: TEM Micrographs showing CNT effect on cell division

In Figure 3, we observe that the CNTs create internal electrical "wires" That is affecting the mitosis process and interferes with the cell division.

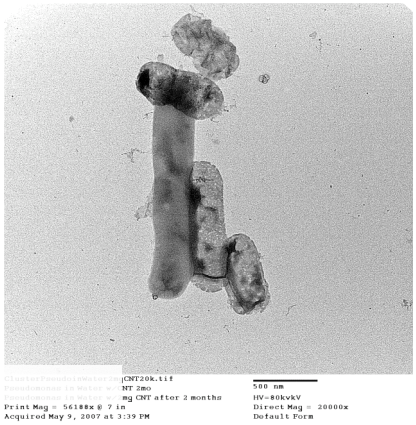


Figure 4 (continued)

In Figure 4, we show the effect of the CNT ingestion on bacterial cell division. In this picture, the mitosis of the bacteria is stopped as the amount of CNT ingested by the bacteria is interfering in the cytokinesis process of the bacterial division. In the red circles it can be observed how the division was blocked because of CNT. We are currently investigating the implications and explanations for this process.

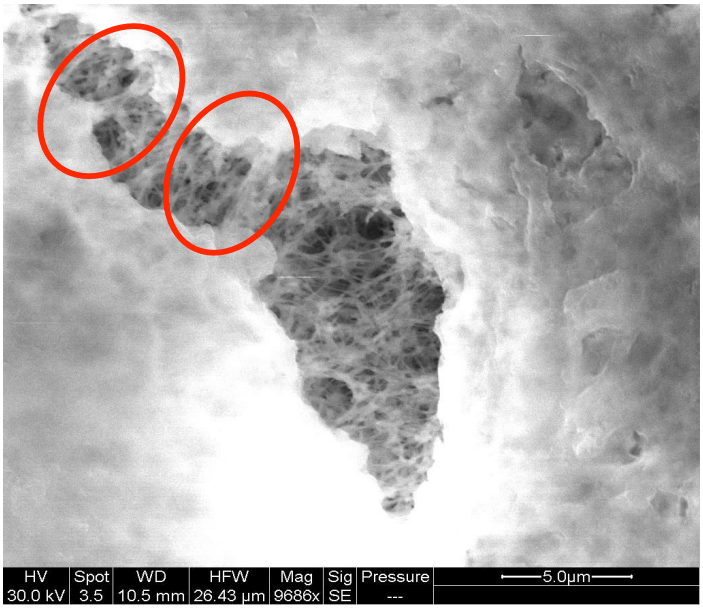


Figure 5: (a) SEM micrograph of the SWCNT thin film inside the bacterium.

We carried on the AFM and TEM studies of the bacteria ingesting CNT and observed the amount of CNT inside the bacteria at the time of cytokinesis. As stated before this was a surprising finding of this approach creating thin films.

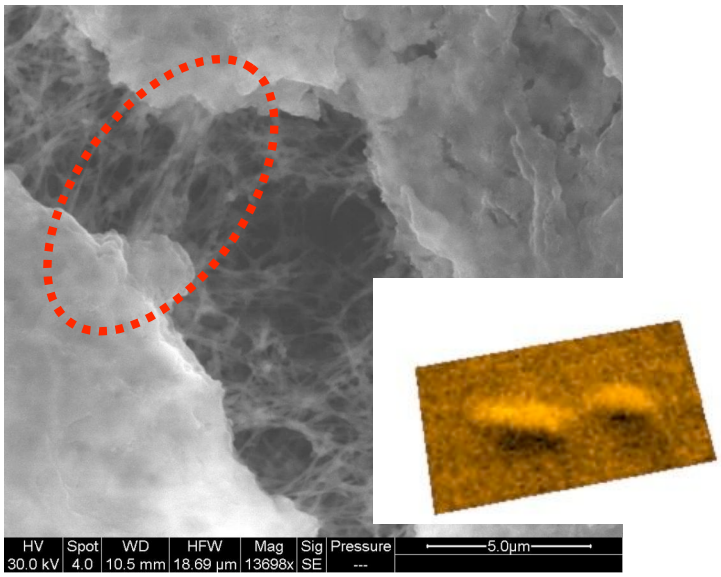


Figure 6: A closer approach of the bacteria shell reveals the amount of CNT inside the bacteria and the blockage of the cytokinesis process for the CNT.

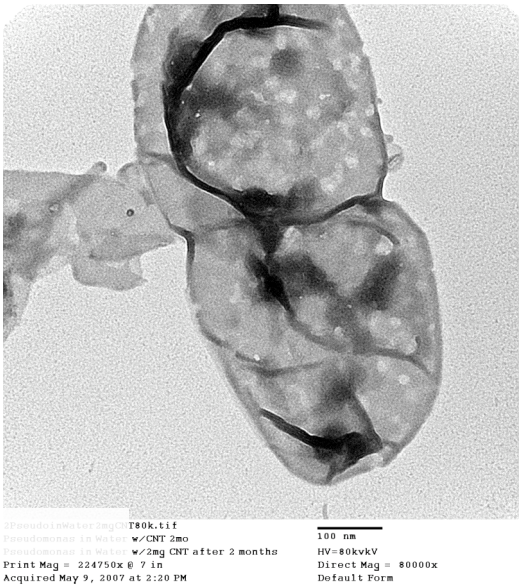


Figure 7: Closer look in this TEM image of CNT interfering with the cytokinesis process of the bacteria.

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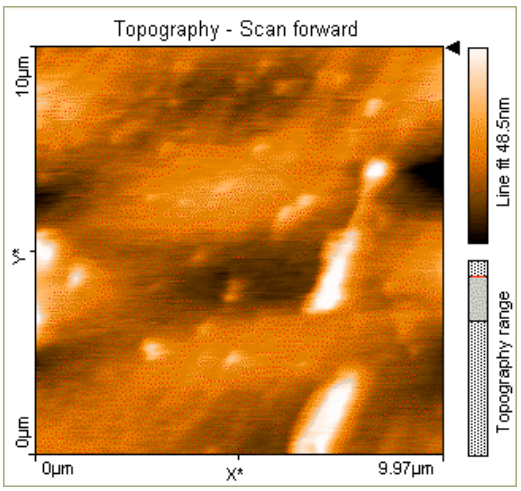

Figure 8: AFM Image of bacteria with unsuccessful cytokinesis process.

3 DISCUSSIONS AND CONCLUSION

Our work is based on the CNTs obtained by HipCo process. This process results in primarily iron as the metallic impurity in the CNTs. We have used the bacteria cells that "eat" iron, as a purifying and cleaning agent We created a working protocol in which the biological agents "ingest" the CNT material looking for their nutrient supply of Iron*. Therefore, after the intake of the CNT material, the bacteria started to show a change in their biological structure.. In the course of this work we have observed that there is significant interference with cell mechanisms, and in fact, cytokinesis is interrupted by the CNTs.

Those changes not only affect the cell mitosis process of the bacteria, but also the bacteria show adaptation changes to compensate for the weight of the material ingested. An adaptation change we have observed resulted the in the bacteria growing extra pili to manage its mobility due to the change in weight in the iron rich environment.

We are currently investigating the details of the mechanisms and the bio-nano interface and implications for toxicity, drug delivery and other applications of this process. We will also try the techniques described in [4].

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* **Patent Pending. Some details are omitted from this paper.**

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