The Effects of Industrial Grade, Multi Walled Carbon Nanotubes on Saccharomyces Cerevisiae

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Abstract

Industrial applications of Carbon Nano-tubes(CNT) such as Multi-Walled Carbon Nano-tubes (MWCNT) has become a growing part of human lives in many interesting ways especially in delivering efficient healthcare treatment in hospitals and for biosensor studies in biomedical research institutes. This study is focused onevaluating the potential of CNT to cause harm to eukaryotic cells using a yeast cell, Saccharomyces cerevisiae. S. cerevisiaeis a microbial homologs of higher eukaryotes such as humans. Analyzing the effects of Industrial Multi-Walled Carbon Nano-tubes on S. cerevisiae cells with respect to cell viability and growth is evidently valuable for understanding the possible health and the genetic effects on humans. W303 S. cerevisiae wild-type was inoculated into yeast exract peptone dextrose(YPD) media containing 3mg of functionalized MWCNTs, and another media without a functionalized MWCNTs; and was incubated for a period of 36 hours. The cell culture, optical density and morphology were observed under a Genesys 20 Spectrophotometer, and a computer aided microscope aligned with a Nikon digital camera integrated into imaging software - Image-J. The study showed that W303 cells exposed to functionalize MWCNTs with a carboxyl (COOH), and hydroxyl (OH) functional groups grew with the same rateas the control and reached the stationary-phase of the cell growth cycle at the same time. Also, themorphological analysis of W303 cellsindicated that the functionalized MWCNTs had the greatest effect on cell morphology such as increase in size and diameter; whereas, a Non-functionalized MWCNTs was limited to no effect. Although the role of the functional groups, and the genetic effects of the CNT were not examined in this research, the study revealed acceptable level of eukaryotic cell viability as an indication that a well-managed use of CNT in biomedical applications such as in drug delivery for the treatment of cancer or biosensing may not have a single source of a damagingeffect on human health.

Keywords: Nanotubes, Nanomaterial, Saccharomyce cerevisiae, Reactive Oxidative Stress (ROS), Biosensing

Introduction

There are many forms of nanomaterial's that are vital to everyday applications. Especially members of the carbon nanotube which includes nanographites, nanoclays, Nitrogen Doped Carbon Nanotubes, nanopad, cup stacked peapod etc.Carbon nanotubes are tubes derived from carbon, having a diameter of 1 nm up to 50 nm on the nanometer scale; but recent technological advancements have made the nanotubes much longer such that it can be measured in centimeters.

There are presently three structurally distinct forms of CNTs that are of importance, they include the Multi-walled Carbon nanotubes (MWCNTs), Double-Walled Carbon Nanotubes (DWCNTs), and Single-Walled Carbon Nanotubes (SWCNTs).Among the numerous uses of CNT in industry ranging from engineering to medicine; studies focused on the evaluation of the dynamic applicationof CNT's in a therapeutic drug delivery, and biosensing of biological molecules; and its potential ability to damage cells viability and limit growth(Brown et al., 2004; Chen, 2002). Previous scientific research showed that CNTs can be specifically used to deliver drugs to targetedcells in-vivo in eukaryotes which make them efficient for the treatment of cancerous cell lines (Kewal and Jain 2005; Liko et al., 2006).

It has been observed through previous studies that CNTs are potentially Cytotoxic to cells causing problems like Apoptosis/necrosis, decrease in cell viability and DNA damage. CNT also affects the morphology of cells and often causes cell-cycle arrest, oxidative stress and metabolic effects. However, these effects are not observed in every cell that comes in contact with CNTs (Muller et al., 2005). A past toxicological study which analyzed the effects of CNTs on human lymphocyte cells in-vitro suggested that CNTs did not solely cause apoptosis (Oberdorster et al., 2011). In contrast with early studies which argued that CNTs do indeed cause apoptosis and necrosis. Nonetheless, the two studies are contradictory to one another, due to the fact that both cell types should have the same outcome of toxicity.

Historically,yeast cells such as Saccharomyces cerevisiae have been used in a broad range of applications from food processing to being a force in science and technological advancements. However, one of the greatest attributes of S. cerevisiae is that it shares similar metabolic functions and regulatory mechanisms to higher eukaryotes including humans. Thus, toxicity of MWCNTs on eukaryotic cells like S. cerevisiae could potentially mimic the toxic effects of carbon nanomaterial in human. Thus, this study examined the effects of MWCNTs in a dose and time dependent manner with respect to the viability and morphology of the cells.
The present study was carried out on functionalized and non-functionalized multi-walled carbon nanotubes using eukaryotic cells, such as Saccharomyces Cerevisiae, and then viability assay serve as an indicator to seewhether a well-managed use of CNT can be used for biomedical application such as drug delivery for the treatment of cancer or to show that biosensing may not be harmful to human health.

Abbreviations: MWCNTs, Multi-Walled Carbon Nanotubes; DWCNTs, Double-Walled Carbon Nanotubes; SWCNTs, Single-Walled Carbon Nanotubes; CNTs, Carbon Nanotubes.

Material and Methods

Cultivation of S. cerevisiae

The wild-type Saccharomyces cerevisiae species known as W 303 was obtained from the University of Texas Health Science Center (UTHSC)that was used in this experiment. 50 g of YPD placed into an autoclave at 121o C for 15 minutes in a laminar flow hood. Before, inoculating the medium into a petri-dish containing a stock culture of W 303 of S. cerevisiae, 45 colonies was acquired, then, sigma-Aldrich IKA® was used to vortex the S. cerevisiae cells for an amount of 1 minute to thoroughly disperse cells for culturing. S. cerevisiae has the ability to grow either anaerobically in oxygen poor conditions or aerobically in oxygen rich conditions. However, in this experiment it was essential to grow S. cerevisiae in an aerobic condition using the Erlenmeyer flask which was then placed into a 211 DS labnet incubator set at 30o C with a shaking range of 250 rpm.

Growth Analysis

The growth of S. cerevisiae was monitored through the use of a Thermo Fisher Scientific ® Genesys 20 Spectrophotometer, at a time interval of 36 hrs. After, the zero hours optical reading was taken, a 6 hour optical density reading was also taken from each of the four labeled Erlenmeyer flask making a total of 6 readings in 36 hours. However, to prevent confusion each of the four Erlenmeyer flask was numbered 1 - 12. It was important to prevent contamination of S. cerevisiae, so during the experiment, the Erlenmeyer flask was placed inside a horizontal laminar flow hood. While, the Erlenmeyer flask containing the S. cerevisiae culture was inside the horizontal laminar flow hood, four sterile 5mL pipettes were used to pipette 1mL from each of the four Erlenmeyer flask.

After, 1mL was extracted from an Erlenmeyer flask the pipette was disposed immediately and not used in another Erlenmeyer flask.

The 1mL extracted from each of the four Erlenmeyer flask was placed into four cuvettes and another 1mL of YPD medium void of S. cerevisiae was placed into a fifth cuvette that was used as the blank for optical density readings. After completing the 36 hour optical density readings of the four S. cerevisiae cultures the data obtained was placed in windows excel. To show Saccharomyces cerevisiae Exposure to Multi-Walled Carbon Nanotubes (MWCNTs)

In this, experiment S. cerevisiae was exposed to different types of MWCNTs, including functionalized and non-functionalized. The two functionalized MWCNTs used in this experiment were Hydroxyl (OH) and Carboxyl (COOH) functionalized MWCNTs. S. cerevisiae was exposed to MWCNTs by way of YPD medium, which is the medium used for culturing S. cerevisiae. Saccharomyces were grown in liquid medium and placed in Erlenmeyer flask that had a maximum volume of 250 ml. Before, placing MWCNTs into an Erlenmeyer flask an amount of 50 mL of YPD medium, one fifth of the volume capacity had to be placed into the flask to obtain an aerobic condition forS. cerevisiae. After, the medium was placed into the Erlenmeyer flask, 3mg MWCNTs were then weighed on a balance scale three times, so that the experiment could be in triplicates. Once the MWCNTs were weighed out and then added to Erlenmeyer flask containing media. Within the 36hour of growth period, optical density readings were taken for each of MWCNT containing samples. The readings were taken in the same manner as the S. cerevisiae samples that lacked MWCNTs in the growth medium. The optical density readings were taken every 6 hours beginning with a zero hour growth reading. A total amount of six readings was taken within a 36 hour time period. However, the readings were taken on the 0hr, 6hr, 12hr and 18th hour, along with the 24th hour, 30th hour and finally the 36th hour. The data and information obtained from the optical density readings were transferred to windows excel to illustrate the growth patterns of S. cerevisiae cultured with MWCNTs.

Morphological Studies of S. cerevisiae

The morphology of S. cerevisiae was analyzed in conditions void of SWCNTs and conditions that contained SWCNTs. It was essential to analyze the morphology of S. cerevisiae in normal conditions and stress conditions, such as the addition of MWCNTs to YPD liquid medium. However, the morphology of S. cerevisiae was analyzed through the use of a Nikon microscope, along with computer software known as image J. S. cerevisiae cultures were viewed under a Nikon microscope and pictures were taken from a digital camera. The pictures obtained from the digital camera were then, uploaded unto the computer software image J for the measure of diameter and size of S. cerevisiae cells. This was also done for S. cerevisiae grown in different concentrations of MWCNTs.

Results and Discussion

Several scholars have examined the toxicity of MWCNT based on size, concentration, dispersion, and time of exposure (Patlolla et al., 2011).Based on the previous study the functional groups of the functionalized MWCNTs has an effect on the growth W303 S. cerevisiae. The W303 cells were not affected by non-functionalized MWCNTs (Fig: 1). Additionally, MWCNTs functionalized with a hydroxyl group and carboxyl groupalso had no effects on cell growth (Fig: 2& 3). Figure 4 is functionalized as well as non-functionalized MWCNT comparison of all conditions.

Based on the data from the experiment the morphology of W303 S. cerevisiae was not affected when exposed to non-functionalized MWCNTs. When the W303 strain was exposed to MWCNTs functionalized with a hydroxyl group, the cells of the strain were larger in size. Nonetheless, W303 cells exposed to MWCNTs functionalized with a carboxyl group had similar sizes to W303 cells grown in conditions void of MWCNTs (fig: 5). Previous studies suggest that particles that are not degradable accumulate in lungs and increase the lung burden (Poland et al., 2008) and our study describes this fact in S. cerevisiae that the functionalized MWCNT increase the size of the cell making it difficult for the cell to clear nanotubes. In vivo and in vitro studies have shown that carbon nanotubes can createreactive oxygen species (ROS)(Risom et al., 2005). ROS have been shown to damage cells through any kind of oxidative stress e.g. peroxidizing lipids, altering proteins, disrupting DNA, interfering with signaling functions, andmodulating gene transcription (Sachar and Saxena, 2011).

We predict that the increase in size and effect on growth were indicators of oxidative stress to Sacchormyces cells which was a response to cell injury, and may be due disruption in cell respiration, metabolism or ischemia/reperfusion and inflammation as reported in different cells types (Stylianakis et al., 2010).

Conclusion

The Present study focused on growth and morphology of S. cerevisiae W303 strain exposed to functionalized and non-functionalized MWCNT.Based on the data from the experiment the morphology of W303 S. cerevisiae was affectedwhen exposed to functionalized MWCNTs.The data collected from this experiment is vital for understanding the phenotypic effects that MWCNTs has on W303 S. cerevisiae, which would give some idea of the genetic effects that MWCNTs may have on the species.Further study is necessary to elaborate on the effect of MWCNTs on W303 genome which was explored within this study. A study like this would prove to be valuable to institutions like NASA. Since, MWCNTs are used by NASA as biosensors and it will enhance the scientific community to have a better knowledge ofhow the two conditions may affect microorganisms. In addition, ifmore work on thegenetic analysis of the W303 strain were done when exposed to those conditions, it would reveal vital information.

Acknowledgement

Thisproject was accomplished by NASA CBER funding. Cooperative Agreement No: NNX10AQ16A

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W303 Lacking MWCNTs vs. W303 Containing MWCNTs

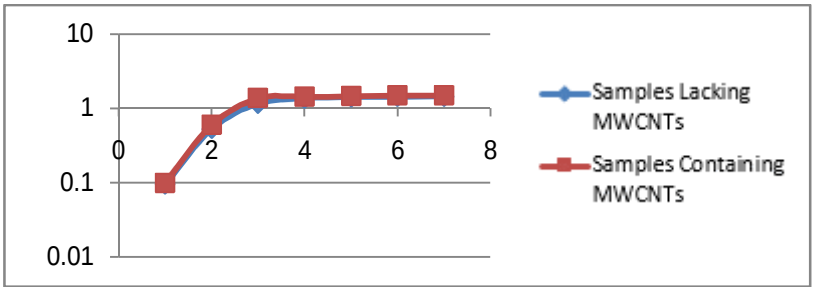

Figure 1: W303 Samples were similar in growth. However, W303 cells That Were Exposed to Non-Functionalized MWCNTsentered Stationary-Phase at the same time.

W303 Lacking MWCNTs vs. W303 Containing MWCNT-OH

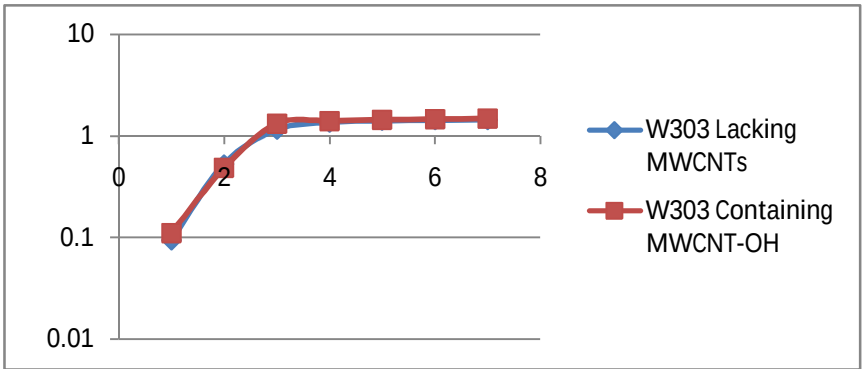

Figure 2: W303 Cells Grown in Media Containing MWCNTs Functionalized with a Hydroxyl Group (MWCNT-OH) Entered the Stationary-Phase of Growth at thesame time.

W303 Lacking MWCNTs vs. W303 Containing MWCNT-COOH

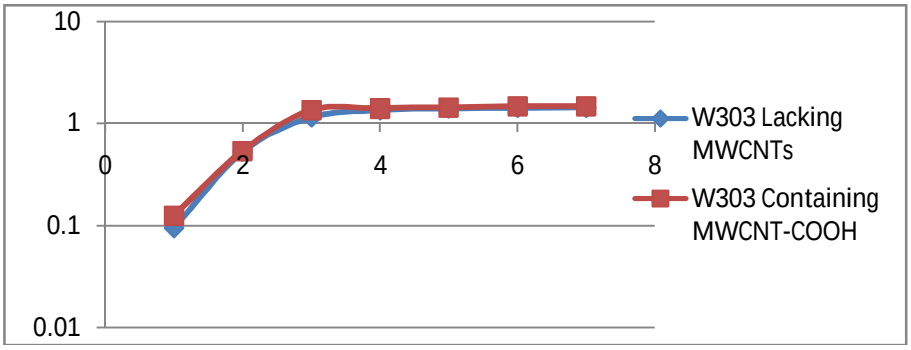

Figure 3: It was observed in the study that cells grown in media containing MWCNTs functionalized with a carboxyl group (MWCNT-COOH) grew at the same rate as W303 cells grown in media containing non-functionalized MWCNTs.

The Comparison of All Three Growth Conditions of W303 cells

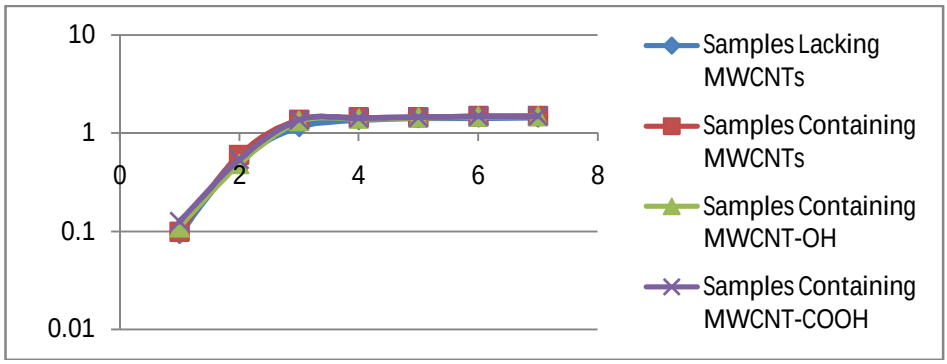

Figure 4: This figure correlates with the previous figures above, whereby media containing MWCNTs and lacking MWCNT W303 cells enter the stationary-phase of growth at thesame time.

Comparison of W303 *S.cerevisiae* size in Various Conditions

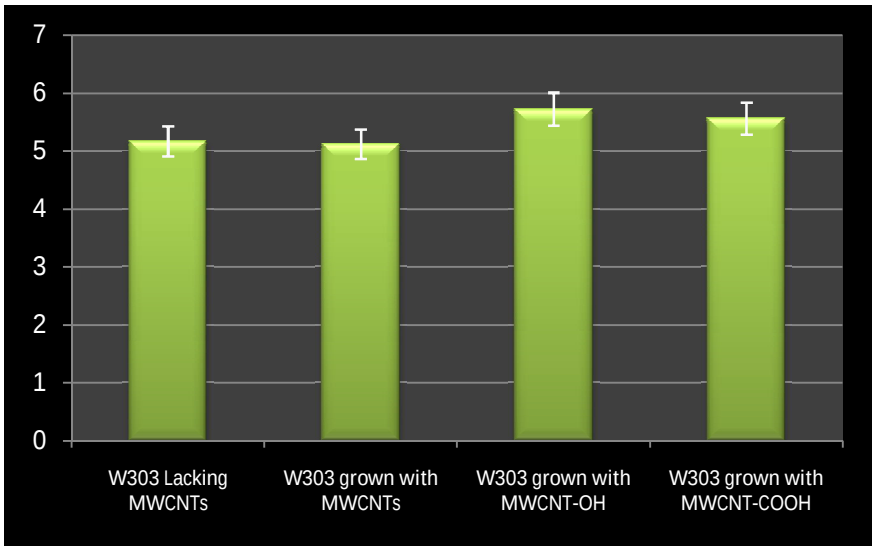

Figure 5: It can be observed from the study that W303 *S. cerevisiae* has a great size in diameter; when grown in functionalized MWCNTs. However, it was observed in the study that non-functionalized MWCNTs, do not have an effect on the size of W303 cells. A fascinating observation made in this, study was that MWCNTs functionalized with a hydroxyl group (MWCNT-OH), had the greatest effect on the size of W303 *S. cerevisiae*