

Antimicrobial Activity of Single-Walled Carbon Nanotubes (Swcnts) Against Marine Bacteria *Staphylococcus aureus*

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Abstract

Nanotechnology is a global investment that makes the structures, materials, devices, and the systems whose size ranges from approximately 1nm to 100 nm. Based on physiochemical properties of SWCNTs, there are possible adverse effects of their presence on biological systems by using SWCNTs as biosensors or drug delivery. The increasing use of SWCNTs through anthropogenic activity is maybe indicator to increase the release it into the environment. The antibacterial activity and mechanism of SWCNTs (Ag- on *Staphylococcus aureus* ATCC 6538P) were investigated. *S. aureus* was exposed to nominal concentration of SWCNTs (1.5, 2.5, 5, 10 and 20µg/ml). The experiment results showed that, 1.5 and 2.5 20µg/ml did not either effect on *S. aureus* growth inhibition and enzymatic activity. While the growth of *S. aureus* was inhibited when exposed to 5, 10 and 20µg/ml SWCNTs, and then, the enzymatic activity of respiratory chain dehydrogenase was significantly decreased. It concludes that, SWCNTs are able to inhibit the bacterial cells growth.

Keywords: *Staphylococcus aureus*; Single-Walled

Introduction

The spread production of nanometre-diameter particles (NMs, NPs) may increase their inhalation through air borne, which may cause effect on biological system. A lot of attention by scientists, practically to understand the toxicity effect of nanomaterials (NMs) on environmental health, small numbers are known about possible effect of carbon nanotubes (CNTs) on aquatic organisms, and also their potential environmental risk and fate are still unknown. In general, the toxicological research of carbon nanomaterials (CNTs) on aquatic biota *in vivo* and single walled carbon nanotubes (SWCNTs) in particular are essentially need to be well understand, probably because of the lack information mechanisms of their complicated physiochemical properties behavior (surface charge, stabilization and agglomeration) in water. In marine environment, aquatic biota can take up Carbon nanotubes, *Staphylo-*

coccus aureus (*S. aureus*) is distributed in varied habitats, starting from humans to sea mammals [1]. Although many species of *S. aureus* are harmless but some pathogenic species are always a threat, thus there is a need for discovery of new antimicrobial drugs [2]. Many different antibiotics and herbal medicines have been used to combat the major problem of antimicrobial resistance but it's not completely eliminated yet [3]. There are various potential virulence factors such as efflux, quorum sensing and antibody-mediated immune responses which play a major role in development of resistance against antibiotics [4]. Apart from this the strains of *S. aureus* has multiple mechanisms for evading both innate immunity mediated by polymorphonuclear leukocytes and the immunity induced by both T cells and B cells [5]. The organism also expresses a number of super antigen toxins and membrane-damaging toxins that can cause symptoms of septic shock and the tissue damage re-

spectively [6]. Few of the virulence factors are as a result of variations in gene expression by some of the genes located on mobile genetic elements called pathogenicity islands [7].

Research work is being carried out on both beneficial and harmful effects of single walled carbon nanotubes (SWCNTs) on the biological environment is amongst the most emerging fields in the world of biomedical applications of nanoparticles [8]. The exceptional chemical and physical properties of SWCNTs are responsible for these nanostructures fascinating is their multifaceted behavior towards human cellular [9]. The possibility for tuning the properties of these nanoparticles through chemical modifications and their capability to be internalized into various cellular organelles and also its positive interaction with cellular systems such as neuronal and bone cells [10]. Nanomaterials with required size, structure and shape are the major building blocks in various electronic, magnetic and biomedical applications [11]. Thus nanomaterials for therapeutic and diagnostic uses.

Materials and Methods

Materials

A SWCNT stock (1 mg L⁻¹; Sigma-Aldrich; catalog 704121; manufacturer's specifications, diameter 1.1 nm - length 0.5-100 nm; G/D Ratio: ≥ 15 (Raman 633nm); $\geq 90\%$ carbon basis ($\geq 77\%$ as carbon nanotubes) was prepared in distilled water using 0.02% Suwannee River natural organic matter. Prior to use, SWCNT stock was dispersed using an ultrasonic bath for 2 hours.

Microbial strains and media

The *Staphylococcus aureus* ATCC6538P strain was purchased from American Type Culture Collection (ATCC). The bacteria were sub cultured and Mueller-Hinton medium pH7.4 \pm 0.2 (25 °C) (purchased from Sigma-Aldrich; contains: 2.0 g/l of beef extract, 1.5 g/l soluble starch, and 17.5 g/l of casein hydrolysate. The medium was used for testing the efficacy of single walled carbon nanotubes (SWCNTs) in liquid culture. Simultaneously the CNTs were tested for the antibacterial activity against *S. aureus* on Muller Hinton agar plates at various concentrations. The bacteria were then subjected for centrifugation at 6000 rpm for 10 minutes. After centrifugation the pellets were washed 3 times using saline. This bacterial broth was then diluted using saline and adjusted to 0.5 McFarland units which is at optical density 0.08 to 0.1 O.D and the bacterial count is estimated to be 10⁷ CFU per ml [12].

1. Agar well assay: The CNTs were subjected to Muller Hinton agar plates and bacteria were spread on agar plates, 6mm wells were bored on agar and the CNTs suspension was poured into it.
2. Liquid Broth assay: 1 ml of bacteria with a concentration of about 10⁷ cells are poured into multiple tubes and different concentrations of CNTs were poured in the tubes with bacteria ranging from 5 μ g/ml to 20 μ g/ml. Then positive and negative controls were set with only bacteria and only nanotubes in separate tubes. These tubes were then kept in a shaking incubator at 200rpm and 37°C for 2 hours. After incubation the Optical density of each tube was measured in a spectrophotometer at 600 nm. The bacteria and control tubes were diluted and cultured on separate petri plates for colony counting assay.

Minimum inhibitory concentration and minimum bactericidal concentration of SWCNTs

To examine the growth curves effect, *Staphylococcus aureus* were exposed to SWCNTs at nominal concentrations (1.5, 2.5, 5, 10, and 20 μ g/ml) for five days respectively. Medium, SWCNTs at different concentrations, and *S. aureus* cells were placed separately to 20 ml cultures, resulting in final concentrations of SWCNTs from 0 to 20 μ g/ml and 10⁷cfu/ml *S. aureus* cells. The incubating and determining methods were according to [13].

Effect of SWCNTs on enzymatic activity of respiratory chain dehydrogenase

As a negative control, *S. aureus* cells were boiled for 15 to 20 minutes to gain completely inactivated enzymes, while the *S. aureus* cells that were not boiled were considered as positive control, which their enzymes remained activity. Dehydrogenase activity was determined according to the iodinitrotetrazolium chloride method [13-15].

Results

Growth of *S. aureus* exposed to SWCNTs

Antibacterial activity of SWCNTs was evaluated by using Agar well assay and Liquid broth assay. SWCNTs were used at different concentrations ranging from 5 to 20 μ g/ml against. when *Staphylococcus aureus* exposed to of 1.25 and 2.5 μ g/ml SWCNTS did not show effect on their growth over 5 days, however, the 5,10 and 20 μ g/ml of SWCNTS did show effect on cells growth significantly

above that of the control group ($p = 0.991$). The *S. aureus* growth at 5, 10 and 20 $\mu\text{g/ml}$ concentrations of SWCNTS was clearly shown inhibited by day 3 (Figure 1).

Figure 1: Growth curves of *S. aureus* cells exposed to different concentrations of Ag-NPs.

Effect of Ag-NPs on enzymatic activity of respiratory chain dehydrogenase

The respiratory chain dehydrogenase activity (RCD) was shown increased in a positive control cells incubation in 30 minutes. 2.5 $\mu\text{g/ml}$ of SWCNTs was shown as high as positive control in the enzymatic activity of bacterial cells (Figure 2), however, there is a significant decrease in the enzymatic activity of bacterial cells at 5 and 20 $\mu\text{g/ml}$ SWCNTs. It suggested that 2.5 $\mu\text{g/ml}$ SWCNTs did not inhibit the 10^7cfu/ml *S. aureus* cells. But in the enzymatic activity of bacterial cells exposed to 50 $\mu\text{g/ml}$ SWCNTs did incur inhibition significantly above that of the positive control group ($p = 0.996$) and 2.5 $\mu\text{g/ml}$ SWCNTs ($p = 0.984$).

Figure 2: Effect of SWCNTs on respiration chain dehydrogenases in *S. aureus* cells. Error bars represent standard deviations of duplicate incubations. The negative and positive controls represent the boiled and not boiled *S. aureus* cells respectively.

Data analysis

The statistical analysis was performed with Sigma Stat. Differences between treatments were analysed with a One Way ANOVA, followed by a Tukey all pairwise multiple comparisons procedure (Sparks, 2000).

Discussion

The antibacterial activity of 5 $\mu\text{g/ml}$ to 20 $\mu\text{g/ml}$ of SWCNTs against *Staphylococcus aureus* bacteria was observed and found to be effective. In one of the studies carried out by Seo., et al. 2014, the silver coupled carbon nanotubes were effective against *Methylobacterium* spp. CNTs are well known in possessing an excellent conductivity with strong dielectric breakdown capacity and hold an outstanding field electron emission properties [16]. The Marine Species of *Staphylococcus aureus* is found to be inhibited at high concentrations 5, 10 and 20 $\mu\text{g/ml}$ SWCNTs. At this high concentrations of SWCNTs the highest number of bacteria were inhibited leading to increase in the density of the solution and simultaneously the optical density. For the first time in 2007, Kang., et al. have reported the study about antimicrobial activity of single wall carbon nanotubes (SWCNTs) against bacterial species *E. coli* [17]. *E. coli* cells and *S aureus* were effected on their growth inhibiotion and RCD when they exposed to Ag-NPs [13,18]. Similarly, in the presence study The RCD was effected by SWCNTs at high concentrations (5, 10, 20 $\mu\text{g/ml}$), SWCNTs showed to destroy the RCD and accordingly inhibited the respiration to interfere with normal growth and metabolism of *Staphylococcus aureus* cells.

Conclusion

In conclusion, the above experiments demonstrated a possible antibacterial process of SWCNTs on *S. aureus* cells: SWCNTs maybe caused cell wall breakage, and then internalized into the bacteria cell. Ag-NPs internalized into *S. aureus* cells and caused DNA damage [17,18-21]. SWNTs can exhibit strong antimicrobial activity. Our finding suggests that single walled carbon nanotubes can be useful as building blocks for antimicrobial product.

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