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Study the Toxicity of Treated Multi Walled Carbon Anotubes (Mwcnts) on Escherichia Coli Bacteria and their Characterization

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ABSTRACT

In this study, we investigate the antimicrobial activity of treated Multi Walled Carbon Nanotubes (MWNTs) by oil toward gram negative bacteria Escherichia coli(*E*.coli) and treated MWNTs is performed according to a chemical procedure introducing -COOH groups on their surface . Structural and morphological characterizations of samples have been carried out using SEM and FT-IR spectroscopy. In addition, the SEM images prove the antimicrobial activities of treated MWNTs to bacterial cells by adhesion activity of *E*.coli on MWNTs surface. Since the functionalization of Multi Walled Carbon Nanotubes (MWCNT-COOH) can be used as effective antimicrobial materials and indicate that severe cell membrane damage by direct contact with MWNTs is the likely mechanism responsible for the toxicity to the model bacteria.

Keywords: Antibacterial, Multi Walled Carbon Nanotubes (MWCNTs), Antimicrobial activity, Functionalization

دراسة سمية الكاربون النانوي المتعدد الجدران المعامل على بكتريا Escherichia coli وخصائصه

الخلاصة

في هذه الدراسة يتم فحص الفعل المضاد للكاربون النانوي المتعدد المعامل بالزيت باتجاه بكتريا ذات شحنة سالبة Escherichia coli وان الكاربون النانوي المتعدد يكون مفضل لاحتواءه على جذور فعالة (COOH–) على سطحه.ان تركيب و طبوغرافية العينات يتم دراستها باستخدام كل من المجهر الماسح الالكتروني (SEM) و مطياف الاشعة تحت الحمراء (FTIR). بالاضافة الى ان صور المجهر الماسح الالكتروني يثبت فعالية الكاربون النانوي المتعدد المعامل للخلايا البكتيرية من خلال فعالية الالتصاق لبكتريا Escherichia coli على سطح MWNTs. اذا فان الكاربون النانوي المتعدد الموظف MWCNT-COOH يمكن استخدامه كمادة فعالة يمكن ان تدمر جدار الخلية بشكل حاد من خلال الاتصال المباشر مع MWNTs ويمكن اعتبارها الميكانيكية المسؤولة عن سمية موديل البكتريا.

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INTRODUCTION

Carbon nanotubes (CNTs), including multi-wall carbon nanoutubes (MWNTs) and single-wall carbon nanotubes (SWNTs), have attracted considerable attentions for their unique one dimensional tubular structure, outstanding mechanical, electronic, thermal, and chemical properties since their discovery in 1991 [1].The wide range of envisioned applications includes sensing elements , energy production and storage systems , drug delivery vehicles , cancer therapeutics , and antimicrobial agents. Although recently discovered, the antimicrobial nature of carbon annotates has attracted significant attention [2].

In recent years, the number of applications that require the dispersion of CNTs in aqueous solutions, particularly in the area of biomedical and environmental engineering, has been escalating. Since unfunctionalized CNTs are extremely hydrophobic and tend to aggregate quickly in aqueous solutions, one of the key strategies to enhance the colloidal stability of CNTs is to covalently attach either charged or hydrophilic functional groups on the surfaces of CNTs through chemical treatment. A popular approach is to expose the CNTs to strong oxidants or concentrated acids in order to create oxygen-containing functional groups, such as carboxyl and hydroxyl groups, on the sidewalls and tube ends of CNTs [3].

Antimicrobial materials are designed to kill, or at least prevent the growth of, microbial species (e.g. bacteria), and are beginning to make an impact on public health [4–11]. With the rapid development of new biomedical devices and implants, antimicrobial surfaces are particularly important [12]. The antimicrobial activity of carbon nanotubes was first reported by Kang et al., who considered aggregates of single walled carbon nanotubes (SWNT) in aqueous solution [13].

Taking into account that nanotubes are heterogenous materials and their dimensions, functionalization surface charge, and agglomeration have impact on reactivity we have to understand that is possible that in specific conditions nanotubes can cross membrane barriers inducing harmful effect such as inflammatory and fibrotic reactions [14]. There are studies showing that MWCNTs can enter human cells and accumulate in the cytoplasm, causing cell death [15].

The objective of this study was to investigate the antimicrobial activity of different concentrations of functionalization MWCNTs treatment with oxidizing agents (oil plant) improves dispersivity in aqueous solutions and mechanism of MWCNTs using samples inoculated with Escherichia coli (*E.coli*) and proved to destroy the bacteria successfully. The samples of functionalization MWCNTs were characterized by SEM, and FTIR and the results of antimicrobial activities to bacterial cells (Escherichia coli) showed that the F-MWNTs of different concentrations have antimicrobial activity.

EXPERIMENTAL WORK

1- Functionalized of MWCNT-COOH

MWCNTs (0.1 g) were dispersed in 100 ml of oil plants under ultrasonication technique for 1h to produce oxidized carbon nanotubes (MWCNT-COOH) as shown in Figure (1 a, b). Then the functionalized MWCNTs was collected via filtered method under vacuum with a 0.22 micro-aperture membrane and then washed thoroughly 100ml of $ChCl_3$ to remove oil from sample. After filtration step the sample is burn at 300 ^{0}C for 2h to obtain only MWNT without membrane.

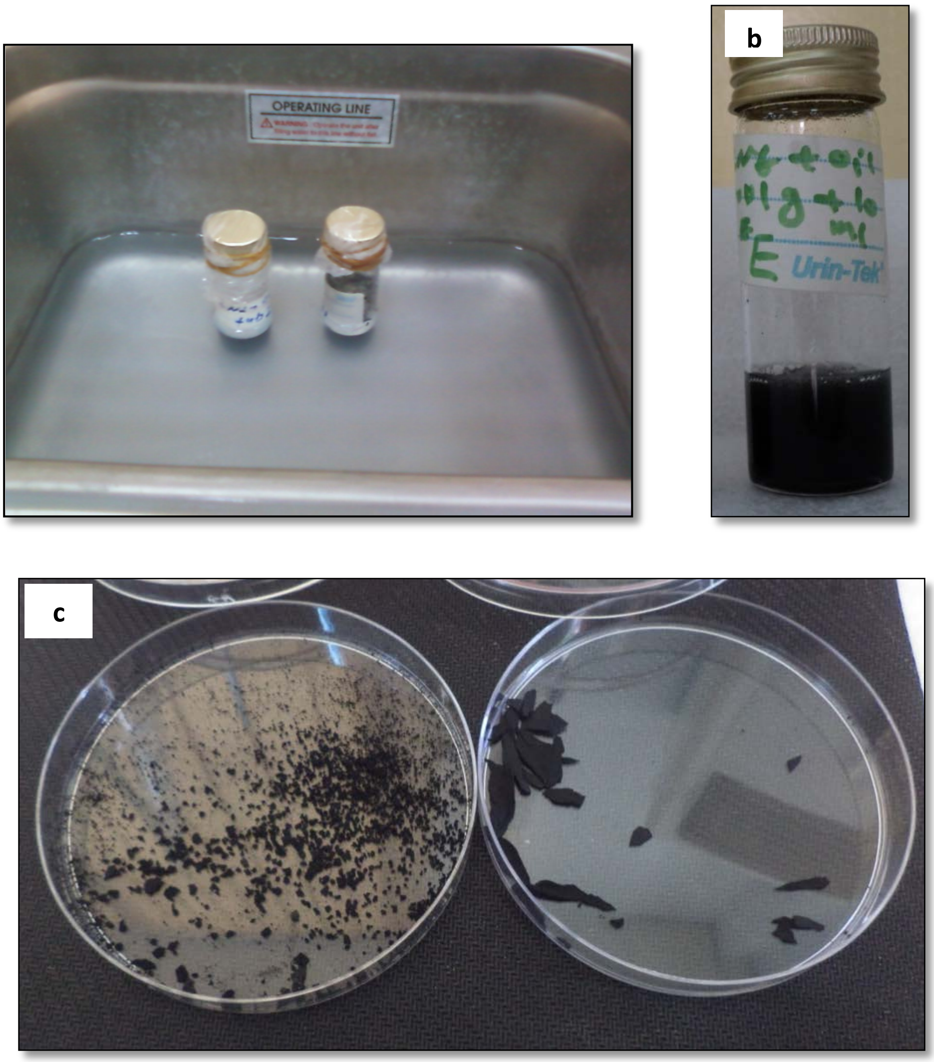

Figure (1 a) Photography of MWNTs with oil during sonicated in ultrasonic bath for 1h b) photography of MWNTs after 1h in ultrasonic bath c) MWNTs after 300 ^{0}C for 2h to obtain only MWNT without membrane.

CHARACTERIZATION OF FUNCTIONALIZED MWCNTS

Characterization of acid treated MWNTs (F-MWNTs) was performed by scanning electron microscopy (SEM) and Infrared spectroscopy (FTIR).

Antimicrobial activities of acid treated MWNTs and MWCNTs-COOAg.

The Escherichia coli strain was kindly provided by lab of biology/ department of applied science, Technology University / Iraq, by using Muller –Hinton agar plates were seeded with tenth ml of suspensions of activated bacterial isolated separately. The bacterial suspension should be equal to McFarland solution, and using as control that equal to 10^7 CFU/ml. Some concentrations of MWNTs (1,3,7 mg/ml) were dispersed in 9mL of 0.9% NaCl, and then 1mL of the diluted cell suspensions was added, after that the mixed solution were kept in a shaker incubator at 37 ◦C (150 rpm) for(10h).Then by using cell counting techniques, (100µl) of diluted samples solution, with different active material concentrations, was spread on solid agar plates and incubated overnight at 37 ◦C, which needed to be sterilized at 120 ◦C for 15 min before inoculation, and the number of colonies was counted. Antimicrobial material free solid agar plates were used as control.

RESULTS AND DISCUSSION

Characterization studies of functionalized MWCNTs

FTIR (FTIR-8400S, SHIMADZU CORPORATION) has been used to map the topographic distribution of treated multi-wall carbon nanoutubes (F-MWNTs) with oil and raw- MWCNTs as shown in Figure (2) From Figure(2), the MWNTs oil plant treatment show the broad peak at ~3437 cm^{-1}, which refers to the O-H stretch of the hydroxyl group. This band might have resulted due to chemical treatment during the functionalization process. FTIR spectra from the raw-MWCNTs show a broad peak at ~3412 cm^{-1}, which refers to the O−H stretch of the hydroxyl group, which can be ascribed to the oscillation of carboxyl groups. Carboxyl groups on the surfaces of raw- MWCNTs could be due to the partial oxidation of the surfaces of MWCNTs during functionalization by oil. While the peak at 2353 cm^{-1} can be associated with the O−H stretch from strongly hydrogen bonded –COOH. The peak at 1633 cm^{-1} is due to C=C stretching of the CNTs. The peak at 1533 cm−1 is related to the carboxylate anion stretch mode. The FT-IR data clearly confirms the attachment of the bacterial cells to the MWNT.

Figure (3 a) shows the SEM images of the raw annotates (a) as well as those treated with oil as shown in Figure (2 b, c). Thus, the treatment of MWNTs with oil agents leading to tubes shorter length and there is no MWCNTs structural damaged occurred. It is probably due to the mild condition of oil [9]. Analysis of several SEM images of treated MWCNTs allowed reliable length measurements of only a few nanotubes, and all of them were between 1 and 3µm, similar to pristine material [5].

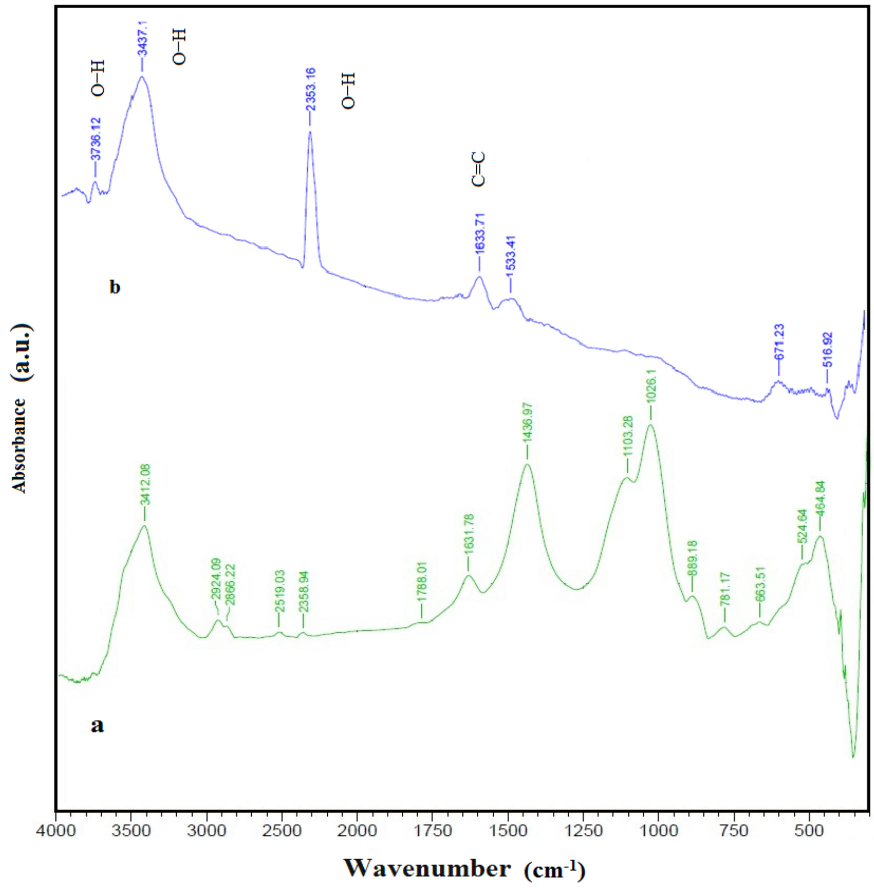

Figure (2) FTIR spectra of raw- MWCNTs (a) and oil treated MWCNT-COOH (b).

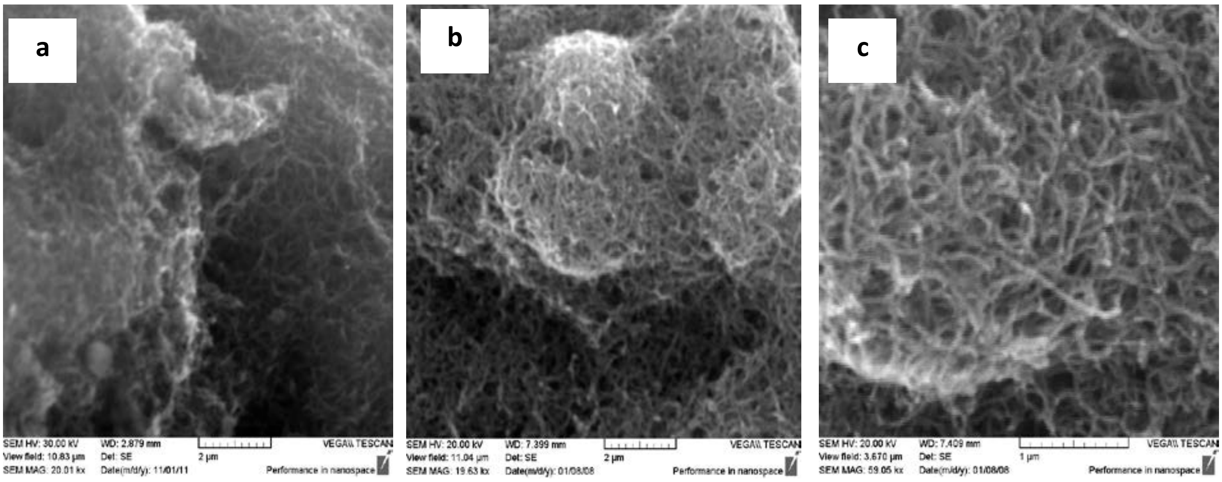

Figure (3) SEM images of MWCNTs: (a) raw-MWCNT and (B,C) functionalized-MWCNT at different magnifications.

Antibacterial tests

With the large scale production of carbon nanotubes and their wide applications, their biosafety studies have attracted much attention. Recent years the cytotoxicity studies of CNTs extend from human cells to single celled organisms (bacterial cells). To evaluate the biocompatiblity of the material, we construct the antimicrobial activity of functionalized carbon nanotubes by using a colony-forming unit (CFU) assay of samples.

Figure (4) shows the typical images of E. coli incubated for 24 h at 37 ◦C after treated with various concentrations (1, 3,7 mg/ml) of F-MWNTs was added to the solutions (shaker incubator for 10h) (b,c,d) and without any antimicrobial material (a).

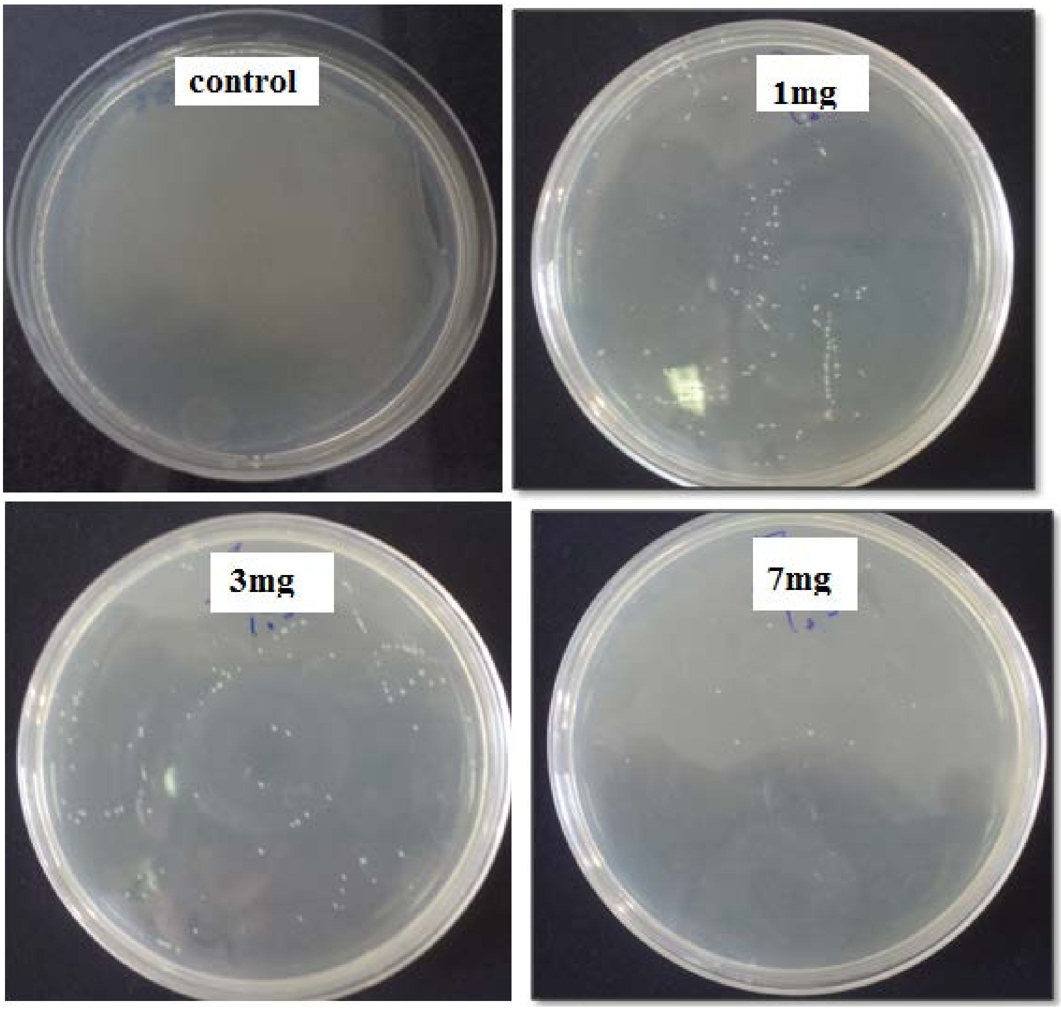

Figure (4) Typical images of E. coli incubated for 24 h at 37 ◦C: (a) without antimicrobial material, (b) after treated with 1mg, (c) 3mg and (d) 7mg of MWNTs.

Compared with control experiment (a), there are only limited colonies as shown in Figure (4d) and no bacteria growth on N. agar media which indicate that the MWNTs have obvious antimicrobial activity and represent suitable amounts of MWNTs that used to decrease a number of bacteria due to entrapped of cells in the cavities of carbon nanotubes during shaker incubator. The percentage of CFUs referenced to the control as shown in table 1 and Figure (5). The number of free bacteria decreased for all increasing carbon Wight, reaching reductions of 70-80% from the initial numbers of bacteria in the suspension and SEM images of treated MWCNTs by oil and its contact with E.coli incubated for 60 min with normal are shown in Figure (6).

Table (1) Number of *E*.coli decreased for increasing MWCNTs Wight.

Exposure time (h)	**Control sample (*E*.coli without MWNTs)**	**Sample 1 (*E*.coli +1mg MWCNTs)**	**Sample 2 (*E*.coli +3mg MWCNTs)**	**Sample 3 (E.coli +7mg /ml MWCNTs)**
10h	10^7 CFU/ml	$1.1x10^6$ CFU/ml	$1.0x10^6$ CFU/ml	$11x10^4$ CFU/ml

CFU: colony forming units
MWCNTs: Multi Walled Carbon Nanotubes

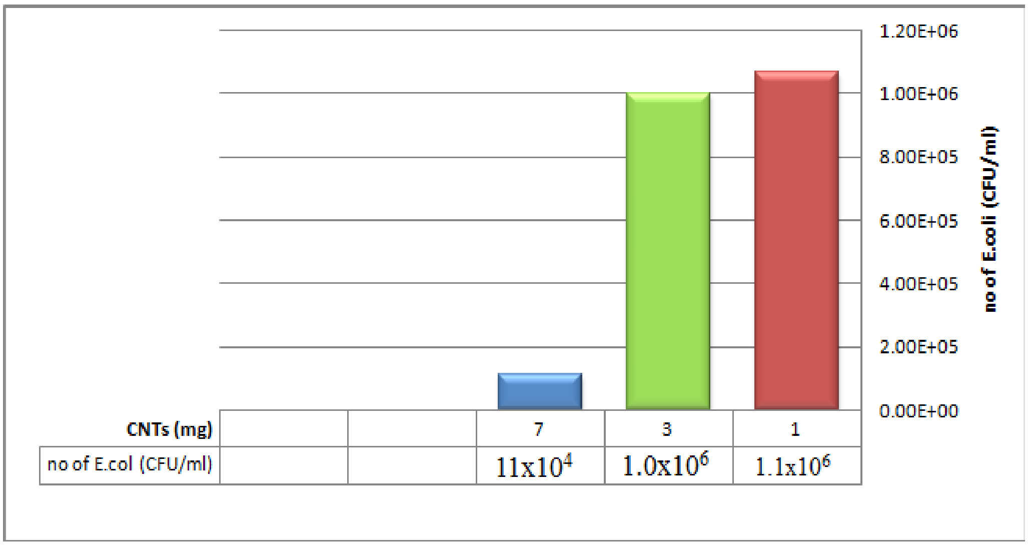

Figure (5) Typical diagram represented the number of E.coli decreased for all Increasing MWNTs Wight.

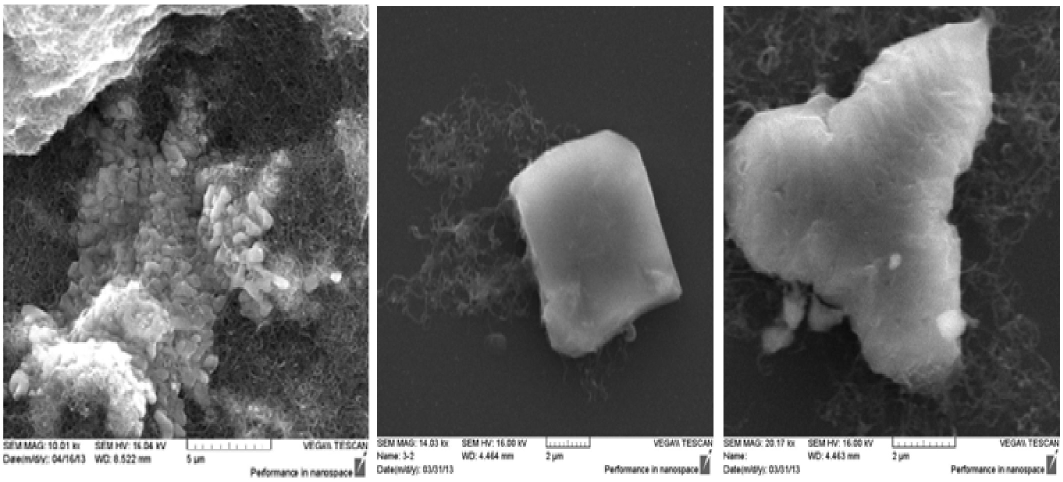

Figure (6) SEM images of treated MWCNTs and E.coli incubated for 60 min with normal saline prior to SEM imaging and after 10h of exposure time at different concentrations of treated-MWNTs and magnification a) at 5µm , b,c)2 µm.

CONCLUSIONS

In our study, data from both SEM images and infrared spectroscopy (FTIR) prove Functionalized multi walled carbon nanotubes (F-MWNTs) using concentrated sulfuric acid 95% H_2SO_4 and nitric acid 65%HNO_3 (3:1) under ultrasonication technique for 30 min by growing hydroxlic and carboxylic groups on the surface of the MWNTs. These functional groups improved the antimicrobial activity of F-MWNTs against Escherichia coli (*E.coli*) and proved to destroy the bacteria succesfully with increasing the concentration of MWNTs as shown in our study. Although all F-MWNTs materials show high affinity toward bacteria because of the generation of van der Waals forces, it has been shown that MWCNTs of different weights have significantly different effects on the precipitation efficiency and the manners in which they capture the cells are different.

Since the functionalization of carbon nanotubes may suggest that MWCNT-COOH can be used as effective antimicrobial materials and indicate that severe cell membrane damage by direct contact with MWNTs is the likely mechanism responsible for the toxicity to the model bacteria. These observations point to the potential use of MWNTs as building blocks for antimicrobial materials.

Finally, a certain *E*.coli bacteria is made to adsorb on the surface of functionalized carbon nanotubes and the corresponding activity of the adsorbed bacteria is monitored to detect the presence of toxic materials and leads to using multi-walled carbon nanotube (MWCNTs) as filters with water pretreatment to remove bacteria from raw seawater and thereby reduce biofouling problems of reverse osmosis membranes.

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