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ANTIMICROBIAL PROPERTIES OF CARBON NANOTUBE: A SUCCINCT ASSESSMENT

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ABSTRACT

Carbon nanotubes (CNTs) particularly single walled carbon nanotubes (SWNT) have been used in pharmacy and medicine for drug delivery systems in therapeutics since the beginning of the twenty-first century. Because carbon nanotubes have demonstrated the ability to transport a wide range of chemicals across membranes and into living cells, they have piqued interest in medicinal applications such as improved imaging, antimicrobial agents, tissue regeneration, and medication or gene delivery. Despite the abundance of evidence demonstrating the benefits of CNTs in terms of higher efficacy and fewer side effects, numerous recent studies have revealed unanticipated toxicities caused by CNTs. CNTs have recently gained a lot of attention for their antibacterial properties. The antimicrobial properties of carbon nanotubes, as well as their toxicity, are summarized and discussed in this mini review.

Keywords: Carbon nanotubes; antibacterial; toxicity.

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1. INTRODUCTION

In an endeavor to develop innovative materials with useful uses, the discipline of nanotechnology is constantly growing [1-4]. One of the most exciting 21st-century technologies is nanotechnology [5-7]. It is the capacity to observe, measure, control, assemble, and manufacture materials at the nanoscale in order to translate the theory of nanoscience into practical applications [8-11]. Nanotechnology is described as "a science, engineering, and technology conducted at the nanoscale (1 to 100 nm), where unique phenomena enable novel applications in a wide range of fields, from chemistry, physics, and biology to medicine, engineering, and electronics" by the National Nanotechnology Initiative (NNI) in the United States [12-15]. New nanomaterials with cutting-edge and superior properties are required to keep up with the rate of advancements in science and technology [16-19]. Carbon nanomaterials (CNMs) and their derivatives stand out among the many nanostructures in terms of their special properties and uses [20]. Their sp2 and sp3 bonds are thought to be responsible for the unique characteristics of carbon and its allotropes. These nanomaterials, which include carbon nanotubes (CNTs), graphite, graphene/graphene oxide (G/GO), and fullerenes, have good interatomic interactions [21]. In particular, CNMs offer great surface area, mechanical resistance, thermal conductivity, photo-luminescence, transparency, and constructional durability in addition to antibacterial activities against pathogens and remarkable electrical conductivity [22]. These properties encourage CNM applications in nanocomposites such as thin-film transistors, transparent conducting electrodes, photovoltaics, supercapacitors, biosensors, drug delivery systems, tissue engineering, photothermal therapy, and antimicrobial food packaging. The main drawback of CNTs, however, is that they are not very soluble in most solvents [22], which limits their application. Several teams have used surface modification to enhance CNT uses [20–22].

The composition, surface modification, target bacteria, and reaction environment all affect the CNMs' antimicrobial activity [23]. In addition to the physical method of biological isolation of microbial cells from their supportive environment, the antibacterial actions of CNMs are based on penetration of the microbial cell wall/membrane and creation of structural damages [24–25]. The third class of processes relies on the interaction of CNMs with bacteria and the

creation of oxidative stress conditions through the generation of harmful substances such reactive oxygen species (ROS). The interactions between CNM and microorganisms result in an electron transfer, which induces ROS-independent oxidative stress and causes biological death [26]. In this brief review, the antibacterial characteristics and toxicity of carbon nanotubes are outlined.

2. ANTIMICROBIAL PROPERTIES OF CARBON NANOTUBES

SWCNTs can display excellent antibacterial action, according to certain research. In actuality, these chemicals' sizes play a significant part in the deactivation of microbes. In fact, as CNMs get smaller, their surface-to-volume ratio rises, creating a tighter bond with the microorganisms' cell wall or membrane and allowing them to exert their function more efficiently [22]. The interaction of CNTs with microbes and disruption of their cellular membrane, metabolic processes, and shape are the basis for the mechanism behind this activity [27]. According to an explanation, the bacteriostatic qualities of CNTs originate from bacterial cell death caused by microorganisms' direct interaction with CNTs, which damages their cell membranes. SEM patterns revealed that incubation with CNTs caused morphological alterations in microorganisms that were associated with a loss of cellular integrity. Additionally, 5-fold increases in plasmid DNA and RNA as well as the outflow of cytoplasmic components have been seen after exposure to tiny CNTs [28]. It is becoming more common knowledge that CNTs have bacteriostatic qualities, which are attributed to their high surface-to-volume ratio and huge interior volume. Targeted delivery is made easier and the antibiotics' bioavailability is increased when CNTs are used as carriers [29].

Multi-walled carbon nanotubes (MWCNTs) were chemically treated with a combination of acids to form functionalized MWNTs, and Hussan et al. (2021) studied the antibacterial characteristics of these functionalized MWNTs [30]. The well diffusion method, which is frequently used for fast antibiotic capacity testing, was used to investigate the antibacterial activity of R-MWCNTs and FMWCNTs against gram-negative (E. coli) and gram-positive (S. aureus) bacteria. When compared to R- MWCNTs, FMWCNTs had higher growth inhibition zones (IZ) against E. coli and Pseudomonas aeruginosa, according to the antibacterial

investigation.

Single-walled carbon nanotubes (SWCNTs) distributed in surfactant solutions of sodium cholate, sodium dodecylbenzene sulfonate, and sodium dodecyl sulfate were studied for their antibacterial activity by Dong *et al.* in 2012 [31]. Sodium cholate was employed to disperse bundled SWCNTs in order to evaluate the antibiotic activity of nanotubes since it had the smallest antibacterial effect on Salmonella enterica, Escherichia coli, and Enterococcus faecalis. Both S. enterica and E. coli were resistant to SWCNTs' antibacterial properties. The growth curves plateaued at lower absorbance values when nanotube concentrations increased from 0.3 mg/mL to 1.5 mg/mL, however the absorbance value was not visibly changed by the incubation times of 5 min to 2 h. The results of this work suggest that due to the physical method of bactericidal activity that SWCNTs exhibit, carbon nanotubes could replace antibiotics as a powerful option in treating drug-resistant and multidrug-resistant bacterial strains. The authors suggested that research be done to verify the validity of a SWCNT-SC mixture and learn more about the mechanisms that might account for both minimal human toxicity and significant antibacterial efficacy.

For many uses in the healthcare industry, antimicrobial surfaces are required. Single walled carbon nanotubes (SWNT) have demonstrated potential as antibacterial agents, but significant concerns remain over the effects of tube bundling, a frequent occurrence brought on by the materials' high hydrophobicity. Aslan et al. (2013) looked into how bundling affected the antibacterial qualities of the resulting films as well as the layer-by-layer (LbL) assembly of SWNT with charged polymers [32]. They use a poly(ethylene glycol) functionalized phospholipid (PL-PEG) to disperse SWNT in aqueous solution and take into account situations in which SWNT are dispersed both as tiny bundles and as virtually solitary objects. Measurements using quartz crystal microgravimetry with dissipation (QCMD) and ellipsometry reveal that the bundled SWNT system adsorbs with layers that are twice as thick while hydrated and three times as thick when dried as those of solo SWNT. A decreased PL-PEG density and degree of solution extension on bundled SWNT compared to solitary SWNT was revealed by molecular dynamics simulation, indicating larger adsorbed layers may be the result of less steric repulsion between bundled nanotubes. The bundled system's

improved van der Waals attraction might also be important. Escherichia coli on films with bundled SWNT are essentially absorbed by the nanotubes, in contrast to the bacteria resting on films with separated SWNT, according to scanning electron micrographs (Fig. 1). The bundled SWNT system is "fast-acting," reaching this inactivation rate in 1 h (Fig. 2), indicating a fast-acting mechanism that may be connected to elevated SWNT content and/or bacterial interaction. Both systems inactivate 90% of bacteria in 24 hours. This research analyzes the molecular underpinnings of nanotube-nanotube interactions, reveals the potential for bacteria-enveloping, swiftly acting SWNT-based antimicrobial coatings, and shows the major influence of SWNT bundling on LbL assembly and antimicrobial activity.

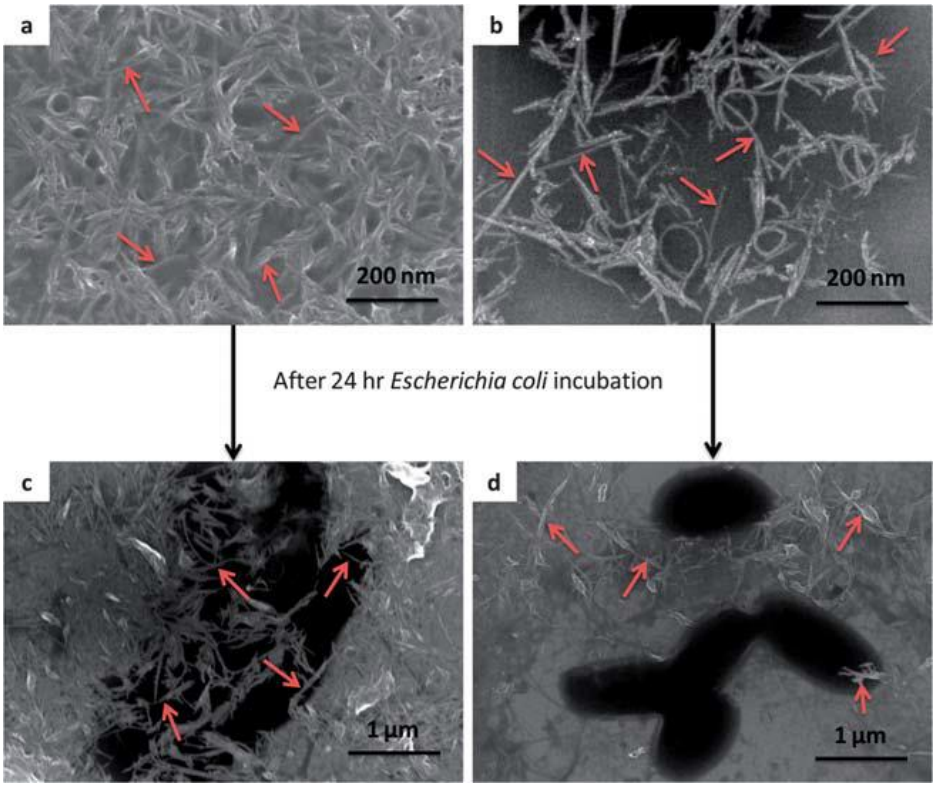

Fig.1. Scanning electron microscopy (SEM) images of (a) (PLL/SWNT–PLPEG bundled/ PGA)4, (b) (PLL/SWNT–PL-PEG isolated/PGA)4, (c) sample (a) following 24 h Escherichia coli incubation, and (d) sample (b) following 24 h Escherichia coli incubation. Red arrows identify some of the SWNT present. Escherichia coli are clearly visible as intact, black objects in (c) and (d). Bacteria appear to be engulfed by the bundled (c) but not isolated (d) SWNT–PL-PEG [32]

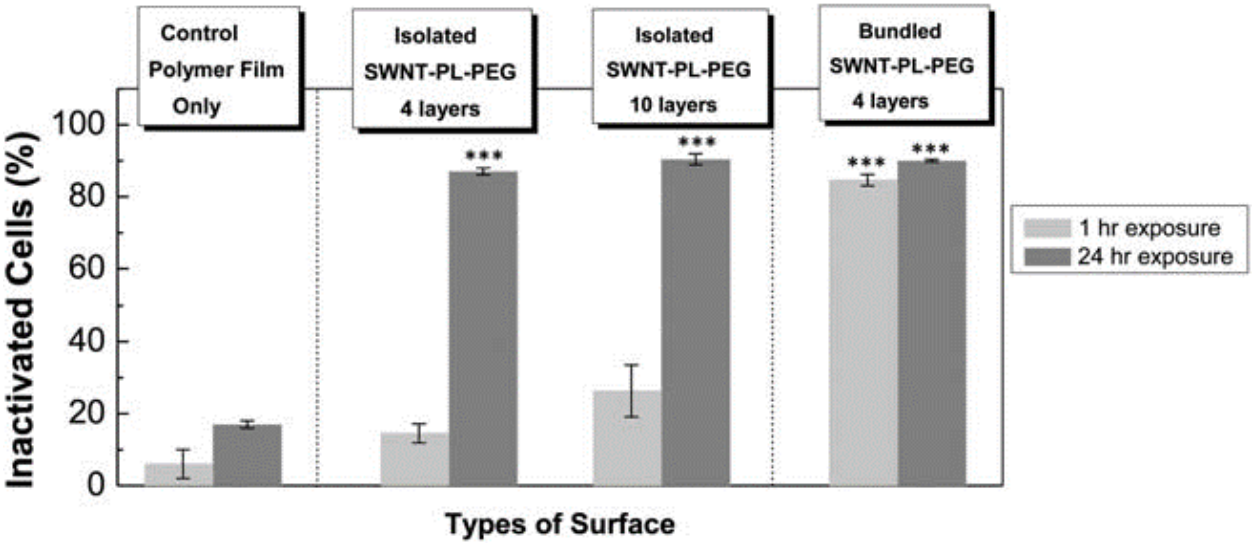

Fig.2. Percent inactivation of Escherichia coli (K12) at 1 and 24 h on various substrates, as determined by LIVE/DEAD assay. Control polymer films (PLL/PGA)4 do not induce significant toxicity. At 1 h, films containing isolated SWNT–PL-PEG samples are less effective than those containing bundled SWNT–PL-PEG. At 24 h, all SWNT–PL-PEG containing films inactivate about 90% of bacteria. Asterisks indicate statistical significance to $p < 0.001$, compared to the control film [32]

Functionalized multiwall carbon nanotubes (F-MWNTs) have been studied by Abo-Neima et al. (2020) as an alternative antibacterial material to commercial antibiotics [33]. E. coli and S. aureus are used as model organisms to study the antibacterial activity of F-MWNTs. The outcomes show that 80 and 60 g/ml for E. coli and S. aureus, respectively, are the optimal F-MWNT concentrations for maximal inhibition and antibacterial activity. The morphological modifications that cause the cellular dependability of these bacteria to deteriorate are revealed using the transmission electron microscope (Fig. 3-4). By biologically separating the cell from its milieu, F-MWNTs can promote the production of harmful chemicals, subject the cell to oxidative stress, and ultimately cause cellular death. When compared to conventional antibiotics, F-MWNT effectiveness exhibits an improvement in the inhibitory action with percentages reaching 85%. The dielectric conductivity and the bacterial growth measurements are carried out to account for the bactericidal performance of FMWNTs towards these pathogens. The goal of the current investigation is to determine whether F-MWNTs can be used in biological devices and systems that change for hospital and industrial cleaning

applications.

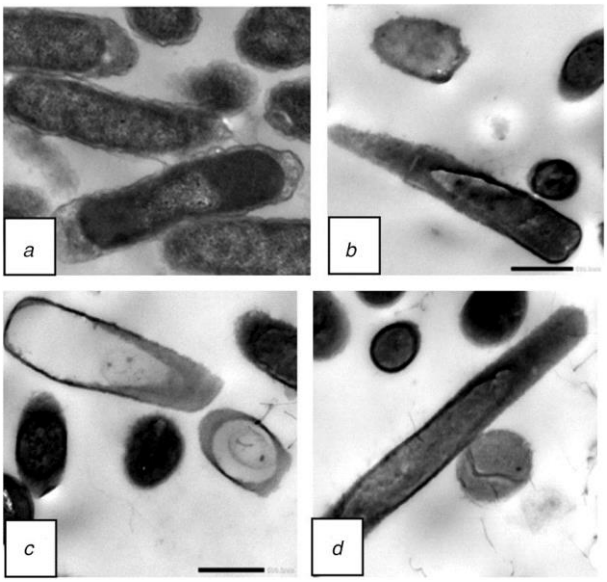

Fig.3. TEM images of E. coli (a) Untreated cells of *E. coli*, (b)–(d) Treated samples with F-MWNTs at concentration of 8 μg/ml after 24 h of incubation [33]

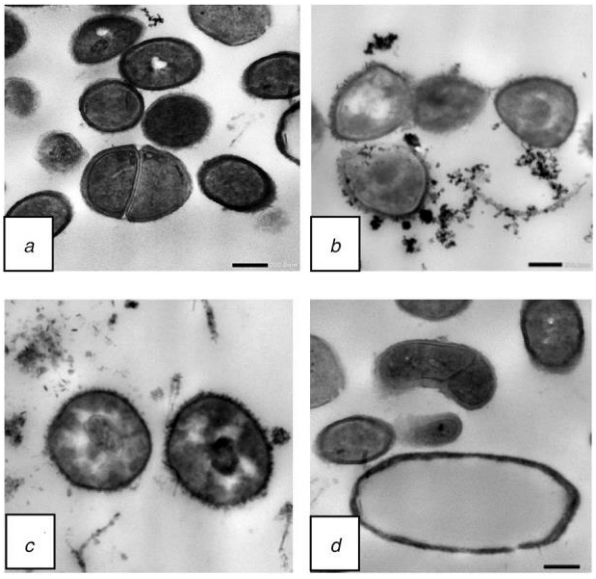

Fig.4. TEM images of S. aureus (a) Untreated cells of S. aureus, (b)–(d) Compared to treated ones with F-MWNTs at concentration 6 μg/ml after 24 h of incubation [33]

With the help of well-purified SWNTs and MWNTs, Kang et al. (2008) investigated the mechanisms underlying CNT cytotoxicity in E. coli cells [34]. They demonstrated how the integrity of the cellular membrane, metabolic activity, and shape of E. coli are adversely affected by direct cell contact with CNTs. E. coli experiences oxidative stress and stressors

related to cell membrane damage, according to a molecular analysis of DNA microarrays. Our SEM observations of severe cell damage and the release of nucleic acids into the solution are supported by the expression of genes relevant to cell damage. Results from SWNTs were significantly more pronounced than those from MWNTs in terms of bacterial cell damage and gene expression changes. SWNTs' increased bacterial toxicity may be attributed to several factors, including: (1) a relatively small nanotube size that makes it easier for nanotubes to partition and partially penetrate the cell wall; (2) a greater surface area for contact and interaction with cells; and/or (3) special chemical and electronic properties that promote greater chemical reactivity.

In a previous study [35], the antibacterial activity of bacterial cells in DI water solution was examined in relation to the length of SWCNTs. We found that, among the three tested SWCNTs of different lengths (1 m, 1 - 5 m, and 5 m), the longer SWCNTs had stronger antibacterial activity than the shorter ones at the same weight concentration. The experiment's findings demonstrated that SWCNT length had an impact on how well they interacted directly with bacterial cells by forming aggregates with them. The evidence for these various aggregate formations came from fluorescence and SEM images, which showed that shorter SWCNTs were more likely to self-aggregate without involving many bacterial cells while longer SWCNTs more efficiently aggregated with bacterial cells when more bacterial cells were involved. All of the studied SWCNTs' antibacterial activity was concentration- and time-dependent. With the longer SWCNTs, both the concentration and treatment time-dependence intensified. The findings of this work expand the prospective uses of SWNTs as antimicrobial agents by giving a fundamental understanding of the variables influencing SWCNT-bacterial cell interactions and SWCNTs' antimicrobial activity.

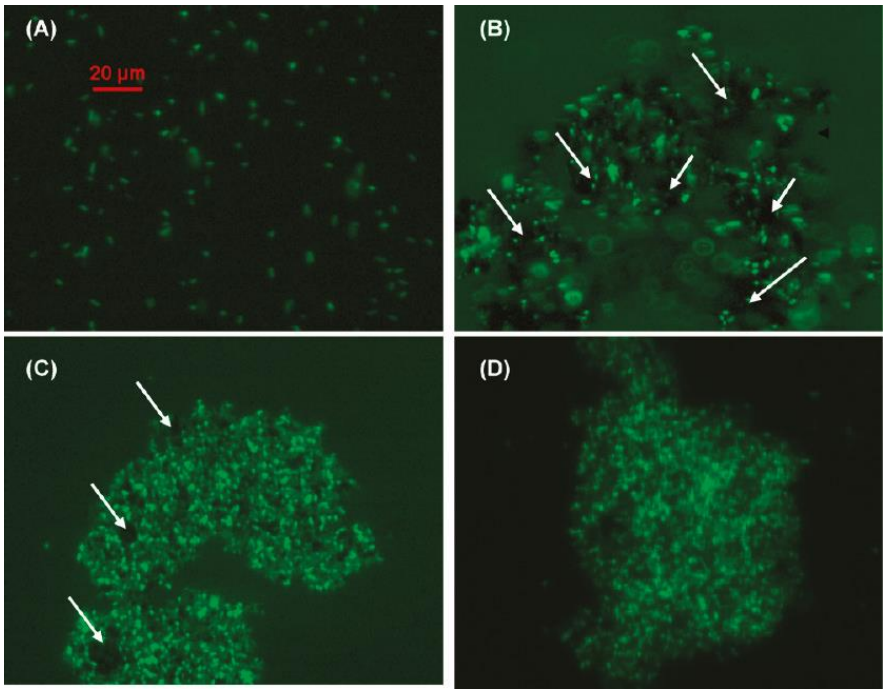

Fig.5. Representative images of Salmonella cells in DI water suspension (A) without SWCNTs, and the aggregates formed by cells and SWCNTs of different lengths (B) <1 µm, (C) 1-5 µm, and (D) ~5 µm. Cells were stained with green fluorescence. Black spots in the aggregates (indicated by arrows in B and C) were clusters of SWCNTs [35]

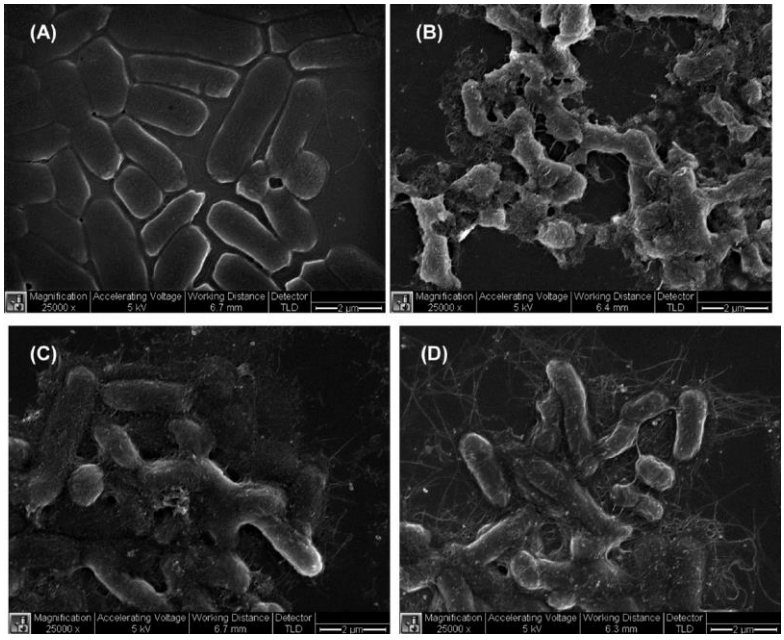

Fig.6. SEM images of Salmonella cells (A) without SWCNTs, and the aggregates of cells-SWCNTs of (B) <1 µm, (C) 1-5 µm, and (D) ~5 µm [35]

A new opportunity for direct comparison of SWNT activity in media ranging from mildly bactericidal to growth-supporting has emerged as a result of Sloan et alrelatively.'s recent discovery that a standard microbiological growth medium, tryptic soy broth, can disperse SWNTs as individuals and small bundles [36]. Therefore, in contrast to the current literature, a fairly recent work uses the same techniques to investigate the activity of SWNTs in the biological dispersants DNA, lysozyme, and tryptic soy broth (TSB), as well as the SDS and pluronic typically employed as pure SWNT dispersants [37]. Colony forming unit (CFU) counts, analyses of the bacterial growth curves, and optical density measurements at 600 nm were used to compare the effects of the dispersions on Gram-positive Staphylococcus aureus and Gram-negative Salmonella enterica (OD600). The findings emphasize the significance of taking into account synergistic interactions when evaluating antibacterial activity. SDS, pluronic, lysozyme, DNA, and tryptic soy broth were the five dispersants selected for this experiment. The model Gram-positive and Gram-negative microorganisms were Staphylococcus aureus and Salmonella enterica. Colony forming units (CFU) and optical density measurements were used to gauge activity. None of the systems showed any activity against Salmonella. Despite the presence of SWNTs, SDS proved lethal to Staph. aureus. The presence of SWNTs increased the anti-Staph. aureus action of pluronic and lysozyme. However, regardless of the presence of SWNTs, there was no action in the DNA and TSB dispersions. The need for more research on the processes by which SWNT-dispersant interactions can result in antibacterial activity is suggested by our results, which show that the allegedly antibacterial activity of SWNTs may only be effective against bacteria that have been sensitized by the dispersant.

In a different investigation, functionalized multi-walled carbon nanotubes nanofluid (F-MWCNTsN) on S. aureus was investigated by Jannati et al. (2021) [38]. The nanofluid was created after MWCNTs were functionalized with the COOH group. The Microplate Alamar Blue Assay (MABA) method was used to assess the bacterial growth following treatment with F-MWCNTsN at concentrations in the range of 0.1 to 1%. Then, tests of TetM and TetO gene expression were carried out to assess the drug delivery capacity of the Nanofluid containing F-MWCNTs. The outcomes demonstrated that multi-wall functionalized carbon nanotubes

may have antibacterial effects on S. aureus (Fig. 7). Additionally, the researchers were able to overcome the photogenic strain of S. aureus's resistance to antibiotics by employing nanofluid that contained functionalized carbon nanotubes. This study represents an innovative approach to nano medication therapy and delivery to S. aureus, an antibiotic-resistant form of bacterium that is responsible for a variety of nosocomial illnesses.

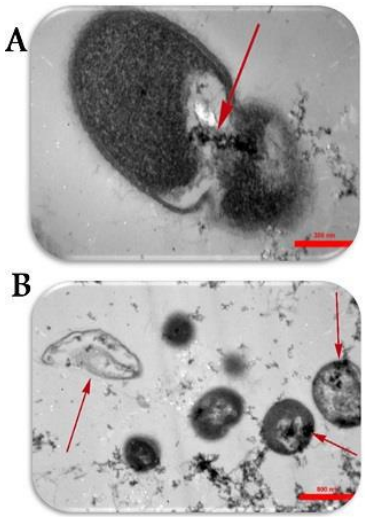

Fig.7. A and B in different magnifications exhibit the impression mechanism from functionalized MWCNTs nanofluid on cell membrane demolition and antibiotic delivery to bacteria [38]

In order to create an efficient, secure, and quickly acting nano-drug with the fewest side effects possible, Hassani et al. (2022) synthesized a new nano-antibiotic from the conjugation of multi-walled carbon nanotubes with levofloxacin (MWCNT-LVX) [39]. In addition to the in vivo antibacterial activity in a burn wound model, this work is the first to assess the in vitro cell viability and antibacterial activity of nano-antibiotics. An ultraviolet-visible spectrometer was used to determine the drug-loading and release profile at various pH levels. MWCNT-LVX was created for the first time utilizing a straightforward, repeatable, and affordable procedure, and it was then characterized using a variety of methods such scanning electron microscopy, transmission electron microscopy, Brunauer-Emmett-Teller analysis, and other methods. When compared to Pseudomona aeruginosa, the noncytotoxic nanoantibiotic demonstrated more effective in vitro antibacterial action against Staphylococcus aureus. The unique synthetic nano-drug had a high loading capacity and a pH-sensitive release profile,

which led to far stronger bactericidal action than LVX in a mouse model of S. aureus wound infection. As a result of conjugating with surface-modified MWCNTs, the drug's antibacterial capabilities improved. The nano-antibiotic has excellent commercialization potential due to its straightforward production, lack of toxicity, appropriate drug loading and release, low effective dose, and robust efficacy against wound infections. MWCNTs can be used as a controlled release and drug delivery device because of their special features. The ease with which the medicine can pass through biological membranes and barriers can also boost drug delivery at lower doses than the main agent alone, which can reduce side effects. As a result, MWCNTs are a promising nano-carrier of LVX for the treatment of skin infections.

3. CARBON NANOTUBES' TOXICITY

The problem of toxicity has made it difficult for carbon nanomaterials (CNMs) to be used in the medical and food industries, despite the fact that they have a wide range of uses [23]. There are apparently contradicting findings in the research of CNM toxicity. Apoptosis and inflammation are brought on by exposure to CNMs, according to some results [40], which also claim that it alters the stress response and the transport and metabolism pathways of cells. There have been reports of the harmful effects of CNMs in the form of nanotubes and nano-onions [41]; the impact of nanotubes is tied to the immunological and inflammatory responses, whilst that of nano-onions is related to external stimuli. SWCNTs also caused cytotoxic effects in the HaCaT cells, such as oxidative stress, the generation of free radicals and peroxides, antioxidant scavenging activity, decreased cell activity and viability, and changes to the morphological structure of the cell [42]. Even while CNMs like CNTs and fullerene have caused toxicity in animal organs and cells, the processes underlying this cellular toxicity are poorly understood [43]. The molecular toxicity of these materials for both people and animals has been studied by researchers [23]. According to what is known at this time, CNTs are more hazardous than fullerene [40]. Gene expression studies have shown that CNTs, particularly MWCNTs, can increase both necrosis and apoptosis in cells. In actuality, the CNMs' structure, size distribution, area, surface chemistry, concentration, charge, and aggregation conditions all affect in-vitro cellular toxicity [44]. Even while few experts have

expressed worries about the safety of CNMs, several studies have shown that pure CNMs, like pure SWCNTs, are harmless and have not been shown to have any harmful effects on mice [44].

4. CONCLUSION

This research described the antibacterial characteristics of carbon nanotubes and their possible toxicity. Literature reviews of several articles on the antibacterial potential of CNTs have indicated desirable bactericidal capabilities that can be modified for application as antibacterial agents. The research also showed that a variety of elements, like bacterial species, CNT concentration, CNT size, etc., affect how efficient carbon nanotubes are as an antibacterial agent. Although some scientists have expressed worries about the safety of carbon-based materials, several studies have shown that pure CNMs, such as pure single walled carbon nanotubes (SWNT), are safe and have not been associated with any harmful consequences in mice. Ultimately, due to their capacity to destroy bacteria, carbon nanotubes are attractive antibacterial prospects for a variety of medical applications. However, it is still unclear how these nanostructures work to render bacteria inactive. In order to properly define their specific mechanisms and toxicity, additional research, theoretical, and experimental studies are in fact necessary.

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