PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (9) 2601-2613 September 2024

Exploring Carbon Nanotubes as Antimicrobial Agents: Efficacy, Toxicity, Challenges, and Future Prospects

JONATHAN, EM; AGBINI, OA

Benson Idahosa University, Benin City, Edo State, Nigeria
Delta State Polythetic, Otefe, Oghara, Delta State, Nigeria

**Corresponding Author Email: ejonathan@biu.edu.ng*
**ORCID: https://orcid.org/0009-0007-2153-2013*
**Tel: +2348066232465*

Co-Author Email: aslemagbini@gmail.com

ABSTRACT: Carbon nanotubes (CNTs), including single-walled carbon nanotubes (SWNTs), have been integrated into pharmaceutical and medical domains as drug delivery systems since the turn of the twenty-first century. Their capacity to effectively transport diverse therapeutics across membranes and into living cells has sparked interest in medicinal applications encompassing enhanced imaging, antimicrobial solutions, tissue regeneration, and targeted medication or gene delivery. Amidst the wealth of evidence showcasing the advantages of CNTs, such as heightened efficacy and reduced side effects, recent studies have unveiled unforeseen toxicities linked to their usage. Notably, the antimicrobial potential of CNTs has garnered significant attention, promising novel strategies for combating microbial infections. This mini review presents a synthesis and discussion of both the antimicrobial attributes and potential toxicity associated with carbon nanotubes. Despite their promising potential, several challenges stand as important considerations, deciphering the precise mechanisms through which CNTs exert their antibacterial effects remains a complex puzzle and ensuring the biocompatibility and safety of CNTs, both in terms of host cells and the environment, is paramount for clinical translation. Lastly, navigating regulatory pathways and standardization efforts will be integral for realizing the scope of CNTs as antimicrobial agents. The multifaceted nature of CNTs' antibacterial attributes underscores the complexity of their interactions within various systems. Addressing challenges through surface functionalization to enhance hydrophilicity and biocompatibility underscores a strategic approach for future exploration. As research progresses, carbon nanotubes hold the potential to emerge as a pivotal tool in the fight against microbial infections while offering innovative pathways for therapeutic advancements.

DOI: https://dx.doi.org/10.4314/jasem.v28i9.3

Cite this Article as: JONATHAN, EM; AGBINI, O. A (2024). Exploring Carbon Nanotubes as Antimicrobial Agents: Efficacy, Toxicity, Challenges, and Future Prospects. *J. Appl. Sci. Environ. Manage.* 28 (9) 2601-2613

Dates: Received: 04 June 2024; Revised: 27 June 2024; Accepted: 11 July 2024 Published: 05 August 2024

Keywords: Carbon nanotubes; antibacterial; toxicity; environment

In the pursuit of novel materials with versatile applications, the field of nanotechnology continues to expand (Ifijen *èt al.,* 2023); Jonathan *èt al.,* 2022); Maliki *èt al.,* 2022); Ifijen *èt al.,* 2022). Positioned as one of the most promising technologies of the 21st century, nanotechnology has emerged as a transformative force (Gnach *et al.,* 2015); Sharma *èt al.,* 2019); Allhoff 2007). Rooted in the ability to observe, measure, manipulate, assemble, and fabricate materials at the nanoscale, nanotechnology bridges the gap between theoretical nanoscience and practical applications (Yuan *èt al.,* 2019); Petersen *et al* 2018); Iqbal *et al* 2012); Bayda *et al.,* 2019). The National Nanotechnology Initiative (NNI) in the United States defines nanotechnology as "a science, engineering, and technology conducted at the nanoscale (1 to 100

**Corresponding Author Email: ejonathan@biu.edu.ng*
**ORCID: https://orcid.org/0009-0007-2153-2013*
**Tel: +2348066232465*

nm), where unique phenomena enable novel applications in a wide range of fields, from chemistry, physics, and biology to medicine, engineering, and electronics" (Malik *et al.,* 2023; Patra, Das and Fraceto *èt al.,* 2018); Swierczewska *et al.,* (2016); Wang, G., *et al* 2018). As scientific and technological advancements surge forward, the demand for new nanomaterials boasting advanced properties remains unrelenting (Wong and Choi (2015); Ifijen *et al.,* (2018); Ikhouria *et al.,* (2020); Ifijen *et al.,* 2020).

Among the array of nanostructures, carbon nanomaterials (CNMs) and their derivatives shine, showcasing exceptional properties and diverse applications (Ifijen *et al.,* 2020). The distinctive traits of carbon and its allotropes, attributed to their sp2 and sp3 bonds, underscore the uniqueness of these nanomaterials. Notably, carbon nanotubes (CNTs), graphite, graphene/graphene oxide (G/GO), and fullerenes exhibit robust interatomic interactions (Ifijen and Maliki, 2022). CNMs boast an array of attributes, including substantial surface area, mechanical resilience, thermal conductivity, photoluminescence, transparency, and structural durability, in addition to their remarkable antibacterial potential against pathogens and outstanding electrical conductivity (Eribe, *et al.,* 2022). These properties pave the way for CNMs to find utility in an array of applications, encompassing nanocomposites like thin-film transistors, transparent conducting electrodes, photovoltaics, supercapacitors, biosensors, drug delivery systems, tissue engineering, photothermal therapy, and antimicrobial food packaging.

Nonetheless, a key limitation of CNTs lies in their limited solubility in many solvents (Eribe, *et al.,* 2022), curtailing their potential applications. To surmount this challenge, researchers have explored surface modification techniques to enhance the utility of CNTs (Ifijen *et al.,* 2020); Ifijen and Maliki (2022); Eribe *et al.,* 2022). The antimicrobial efficacy of CNMs is influenced by factors including composition, surface modification, target bacteria, and the reaction environment (Salari and Jafari 2020). Mechanisms underlying the antibacterial effects of CNMs span from physically isolating microbial cells from their supportive milieu to penetrating the microbial cell wall/membrane and inducing structural damage (Su, Gan, Liu and Yang (2020); Abd-Elsalam 2020). A third category of processes involves the interaction of CNMs with bacteria, engendering conditions of oxidative stress through the generation of reactive oxygen species (ROS). These interactions trigger electron transfers, yielding ROS-independent oxidative stress and leading to cellular demise (Azizi-Lalabadi *et al.,* 2020).

Through a comprehensive exploration of carbon nanotubes' antibacterial attributes, toxicity concerns, challenges, and future prospects, this review aims to provide a holistic understanding of their potential as antimicrobial agents. By shedding light on both their remarkable capabilities and potential pitfalls, we strive to contribute to the informed advancement of CNTs' applications in the realm of infection control and medical therapeutics. As we navigate the complexities of harnessing CNTs' potential, we remain poised to leverage their unique properties for innovative solutions that address microbial challenges with precision and responsibility.

MATERIALS AND METHODS

The exploration of carbon nanotubes (CNTs) as antimicrobial agents and their associated attributes encompassing efficacy, toxicity, challenges, and future prospects was conducted through a systematic methodology involving comprehensive literature review and data analysis.

Literature Review: An extensive search was performed across reputable scientific databases, academic journals, and research repositories to gather relevant publications focused on CNTs as antimicrobial agents. The search encompassed articles discussing CNTs' antibacterial efficacy, potential toxicity concerns, challenges in their application, and future prospects.

Data Collection and Analysis: Selected articles were critically analyzed, and information pertaining to CNTs' antibacterial properties, toxicity evaluations, challenges, and potential applications was meticulously extracted. Data were organized, and relevant findings were categorized based on themes related to the main focus areas of the review.

Antibacterial Attributes: Studies emphasizing CNTs' antibacterial attributes were examined, with a focus on elucidating factors influencing their efficacy against various bacterial strains. Experimental methodologies utilized to assess antibacterial effects, including minimum inhibitory concentration (MIC) assays, disk diffusion tests, and bacterial growth inhibition assays, were documented.

Toxicity Evaluation: In-depth analysis of research investigating potential toxicity associated with CNTs' use was undertaken. Studies encompassing in vitro and in vivo assessments of cytotoxicity, genotoxicity, and immunological responses were considered. Various experimental techniques, such as cell viability assays, reactive oxygen species (ROS) measurements, and histopathological examinations, were noted.

*Challenges and Future Prospects***:** An examination of challenges in CNT application, such as mechanistic elucidation, biocompatibility assurance, and regulatory considerations, was conducted. Studies providing insights into the future prospects of CNTs, including strategies for surface modification, targeted delivery systems, and interdisciplinary collaborations, were synthesized.

Sources on Which the Analysis of The Study Is Based On: The analysis of the study is based on a comprehensive review of relevant scientific literature from reputable sources. The sources encompassed a wide range of research articles, academic journals, reviews, and studies that specifically addressed the attributes of carbon nanotubes (CNTs) as antimicrobial agents. These sources were selected from established scientific databases, academic journals, and research repositories, ensuring the accuracy and credibility of the information presented in the analysis.

Additionally, the analysis drew upon studies that explored the potential toxicity of CNTs, including investigations into in vitro and in vivo assessments of cytotoxicity, genotoxicity, and immunological responses. These studies provided valuable insights into the safety implications of CNT utilization.

Furthermore, the analysis integrated research that discussed challenges associated with the application of CNTs, such as deciphering mechanisms, ensuring biocompatibility, and navigating regulatory pathways. These sources shed light on the complexities and considerations involved in harnessing the potential of CNTs as antimicrobial agents.

Lastly, the analysis incorporated research that outlined the future prospects of CNTs, including strategies for surface modification, targeted delivery systems, and interdisciplinary collaborations. These sources offered valuable insights into the innovative directions that the field of CNT-based antimicrobial applications could take in the future.

Overall, the analysis is grounded in a diverse and comprehensive selection of credible sources that collectively contribute to a holistic understanding of CNTs as antimicrobial agents, their potential efficacy, challenges, and prospects.

Unveiling the Antimicrobial Potential of Carbon Nanotubes: Research has indicated that single-walled carbon nanotubes (SWCNTs) possess impressive antibacterial properties, and the size of these nanotubes plays a crucial role in their ability to deactivate microbes. Smaller carbon nanotomaterials (CNMs) exhibit a heightened surface-to-volume ratio, establishing stronger interactions with the cell walls or membranes of microorganisms, thus enhancing their efficacy (Eribe *et al.,* 2022). The antibacterial action of SWCNTs is rooted in their interaction with microbes and their disruption of key cellular components, including the cellular membrane, metabolic processes, and overall morphology (Dizaj *et al.,* 2015). This mechanism results in bacterial cell death due to the direct interaction between microorganisms and the SWCNTs, which damages the microbial cell membranes. Scanning electron microscope (SEM) analyses have unveiled noticeable changes in the shape of microorganisms following exposure to SWCNTs, indicative of compromised cellular integrity. Moreover, exposure to minute SWCNTs has demonstrated considerable increases in the release of plasmid DNA and RNA, alongside the leakage of cytoplasmic components (Ji, H., Sun, H., Qu, X 2016). The emerging understanding is that CNTs possess bacteriostatic qualities, primarily attributable to their substantial surface-to-volume ratio and considerable internal volume. These attributes render CNTs as promising carriers for targeted delivery, enhancing the bioavailability of antibiotics (Chong *et al.,* 2017).

In a nutshell, SWCNTs have been recognized for their remarkable antibacterial properties, with their size being a critical factor influencing their effectiveness. Their interaction with microbes and subsequent disruption of cellular elements underpin their bactericidal mechanism. The demonstrated increases in genetic material and cellular components after exposure to SWCNTs reinforce their potential antimicrobial efficacy. It is increasingly evident that CNTs' bacteriostatic attributes can be attributed to their unique structural properties, making them valuable not only for direct antibacterial action but also for facilitating drug delivery systems.

In a study by Hussan *et al*., (2021), multi-walled carbon nanotubes (MWCNTs) were subjected to chemical treatment involving a combination of acids to create functionalized MWCNTs. The researchers aimed to explore the antibacterial properties of these functionalized MWCNTs (Khan *et al*., 2016). They employed the well diffusion method, a commonly used approach for rapid antibiotic capacity testing, to assess the antibacterial activity of both raw MWCNTs (R-MWCNTs) and functionalized MWCNTs (FMWCNTs) against both gram-negative bacteria (E. coli) and gram-positive bacteria (S. aureus). The results of the antibacterial investigation indicated that FMWCNTs exhibited larger growth inhibition zones

(IZ) against E. coli and Pseudomonas aeruginosa compared to R-MWCNTs. This suggests that the functionalization process enhanced the antibacterial effects of the MWCNTs, particularly against these specific bacterial strains.

In another study conducted by Dong *et al.*, in 2012, the antibacterial activity of single-walled carbon nanotubes (SWCNTs) was investigated. These SWCNTs were dispersed in surfactant solutions containing sodium cholate, sodium dodecylbenzene sulfonate, and sodium dodecyl sulfate (Maksimova 2019). The researchers chose sodium cholate as a dispersing agent to evaluate the antibiotic activity of the nanotubes, given its relatively mild antibacterial effect on various bacterial strains, including Salmonella enterica, Escherichia coli, and Enterococcus faecalis. Notably, both S. enterica and E. coli displayed resistance to the antibacterial properties of SWCNTs. The growth curves of the bacteria reached a plateau at lower absorbance values as nanotube concentrations increased from 0.3 mg/mL to 1.5 mg/mL. However, changes in absorbance values were not visibly affected by varying incubation times between 5 minutes and 2 hours. The findings from this study suggested that the bactericidal activity of SWCNTs is primarily due to a physical mechanism, which raises the possibility of carbon nanotubes being utilized as a potent alternative to antibiotics in combating drug-resistant and multidrug-resistant bacterial strains. The authors emphasized the need for further research to validate the efficacy of a mixture involving SWCNTs and sodium cholate, as well as to delve deeper into the mechanisms that contribute to both minimal human toxicity and significant antibacterial efficacy. These studies collectively contribute to the growing understanding of carbon nanotubes' potential as antibacterial agents and highlight the importance of functionalization and dispersion methods in enhancing their effectiveness against specific bacterial strains.

Antimicrobial surfaces are of paramount importance in various applications within the healthcare industry. Single-walled carbon nanotubes (SWNTs) have emerged as potential candidates for antibacterial agents; however, concerns persist about the impact of tube bundling, a common consequence driven by the hydrophobic nature of these materials. A study conducted by Aslan *et al.*, in 2013 delved into the ramifications of bundling on the antibacterial properties of resulting films, along with the layer-by-layer (LbL) assembly of SWNTs with charged polymers (Mohammed *et al.*, 2019). The researchers employed a poly(ethylene glycol) functionalized phospholipid (PL-PEG) to disperse SWNTs in aqueous solutions. The study considered scenarios in which SWNTs were dispersed as both small bundles and nearly isolated entities.

Measurements utilizing quartz crystal microgravimetry with dissipation (QCMD) and ellipsometry unveiled that the adsorbed layers of the bundled SWNT system were twice as thick when hydrated and three times as thick when dried compared to those of solitary SWNTs. Molecular dynamics simulations indicated a decreased PL-PEG density and solution extension on bundled SWNTs, suggesting that larger adsorbed layers could be attributed to reduced steric repulsion among bundled nanotubes. The enhanced van der Waals attraction in the bundled system was also noted. Scanning electron micrographs showed that Escherichia coli on films with bundled SWNTs were essentially absorbed by the nanotubes, in contrast to bacteria resting on films with separated SWNTs. This observation suggested a swift-acting mechanism, as the bundled SWNT system exhibited a high inactivation rate within just one hour. Both systems achieved a 90% bacterial inactivation rate within 24 hours. The study offers an in-depth analysis of the molecular interactions between nanotubes, shedding light on the potential for rapidly acting SWNT-based antimicrobial coatings that envelop bacteria. Furthermore, it underscores the significant influence of SWNT bundling on LbL assembly and the resulting antimicrobial activity. This research provides valuable insights into harnessing the properties of SWNTs for developing effective antimicrobial surfaces in healthcare and other related fields.

A study conducted by Abo-Neima *et al*., in 2020 examined the potential of functionalized multiwall carbon nanotubes (F-MWNTs) as an alternative antibacterial material to commercial antibiotics (Hussan *et al.,* 2021). The researchers utilized Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) as model organisms to investigate the antibacterial activity of F-MWNTs. The study aimed to identify the optimal concentrations of F-MWNTs that would yield maximal inhibition and antibacterial effects. The findings of the study indicated that the optimal concentrations of F-MWNTs for achieving maximal inhibition and antibacterial activity were 80 µg/mL for E. coli and 60 µg/mL for S. aureus. To understand the underlying mechanisms, the researchers used transmission electron microscopy to reveal morphological changes that compromised the cellular integrity of these bacteria (Fig. 3-4). The presence of F-MWNTs appeared to disrupt the cell membrane, leading to a separation of the cell from its surroundings. This separation facilitated the

generation of harmful chemicals, induced oxidative stress within the cell, and ultimately led to cell death. Comparative analysis with conventional antibiotics showed that the effectiveness of F-MWNTs exhibited an improvement in inhibitory action, with percentages reaching up to 85%. The study also involved measurements of dielectric conductivity and bacterial growth to further elucidate the bactericidal performance of F-MWNTs against these pathogens.

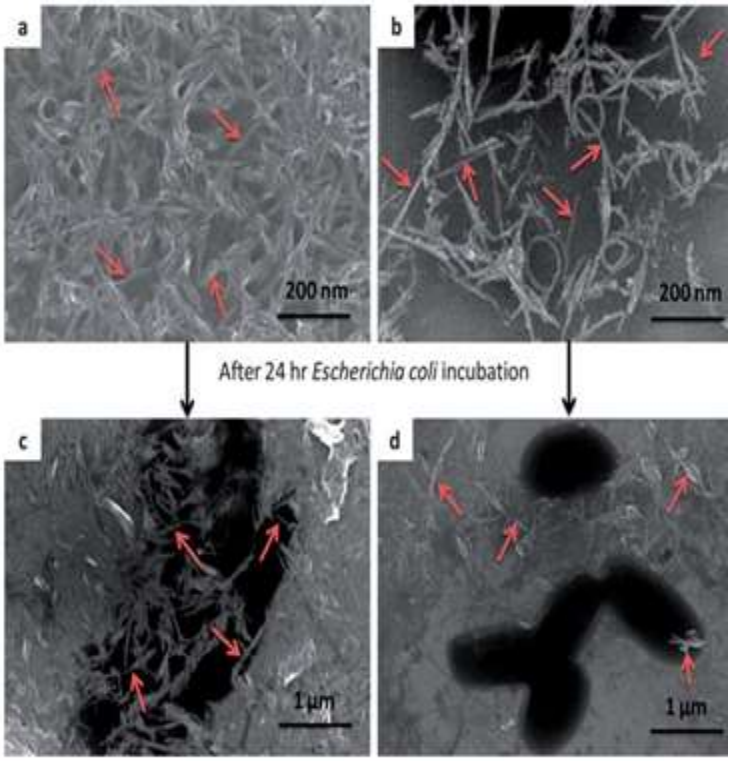

Fig. 1. Scanning electron microscopy (SEM) images of (a) (PLL/SWNT–PLPEG bundled/ PGA)4, (b) (PLL/SWNT–PL-PEG isolated/PGA)4, (c) sample (a) following 24 h Escherichia coli incubation, and (d) sample (b) following 24 h Escherichia coli incubation. Red arrows identify some of the SWNT present. Escherichia 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 (Mohammed MK., *et al* 2019).

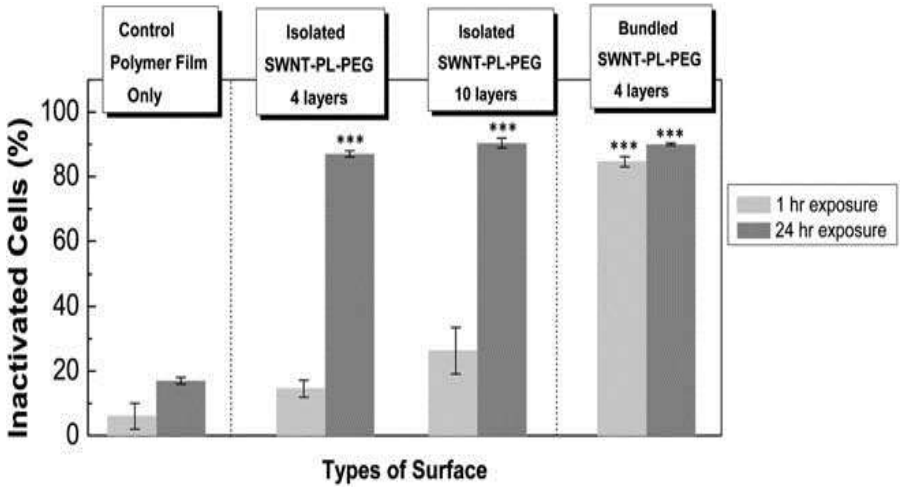

Fig. 2. Percent inactivation of Escherichia coli (K12) at 1 and 24 h on various substrates, as determined by LIVE/DEAD assay. Control polymer films (PLL/PGA)4 do not induce significant toxicity. At 1 h, films containing isolated SWNT–PL-PEG samples are less effective than those containing bundled SWNT–PL-PEG. 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 (Mohammed, MK., *et al* (2019).

The ultimate objective of this investigation was to assess the potential applicability of F-MWNTs in biological devices and systems, especially in the context of hospital and industrial cleaning applications. The results suggested that F-MWNTs could serve as promising antibacterial agents, potentially offering advantages over traditional antibiotics, and could contribute to the development of effective and innovative solutions for addressing bacterial contamination in various settings.

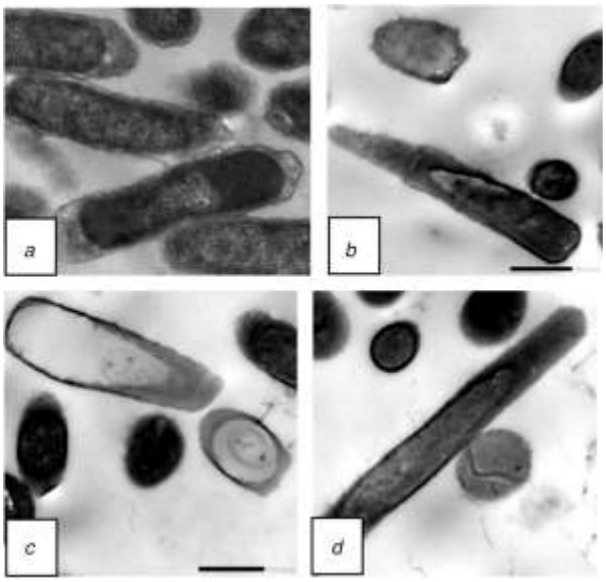

Fig. 3. TEM images of E. coli (a) Untreated cells of *E. coli,* (b)–(d) Treated samples with F-MWNTs at concentration of 8 μg/ml after 24 h of incubation (Hussan, NQA., *et al* 2021)

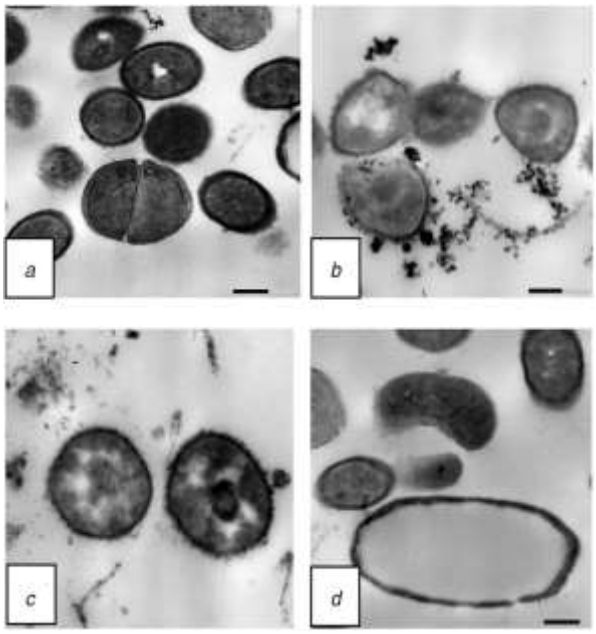

Fig. 4. TEM images of S. aureus (a) Untreated cells of S. aureus, (b)–(d) Compared to treated ones with F-MWNTs at concentration 6 μg/ml after 24 h of incubation (Hussan, NQA., 2021).

In a study conducted by Kang *et al.* in 2008, the mechanisms underlying carbon nanotube (CNT) cytotoxicity in Escherichia coli (E. coli) cells were investigated using well-purified single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) (Dong, L., Henderson, A., Field, C 2012). The researchers aimed to elucidate how direct contact with CNTs affects the cellular integrity, metabolic activity, and shape of E. coli. Molecular analysis of DNA microarrays revealed that E. coli experienced oxidative stress and stress associated with cell membrane damage upon exposure to CNTs. This was further supported by scanning electron microscope (SEM) observations of severe cell damage and the release of nucleic acids into the solution, which corresponded with the expression of genes related to cell damage. Notably, the detrimental effects observed were more pronounced with SWNTs compared to MWNTs. Several factors were proposed to contribute to the increased bacterial toxicity of SWNTs, including their smaller size, larger surface area for interaction, and unique chemical and electronic properties that enhance chemical reactivity. These factors collectively facilitate greater interaction with bacterial cells and penetration through the cell wall.

In a preceding study (Aslan, S., *et al* 2013), the antibacterial activity of SWCNTs in a deionized (DI) water solution was investigated in relation to their length. The research revealed that among three different lengths of SWCNTs (1 μm, 1-5 μm, and 5 μm), longer SWCNTs exhibited stronger antibacterial activity at the same weight concentration. The study demonstrated that SWCNT length influenced their direct interaction with bacterial cells, leading to the formation of aggregates. Fluorescence and SEM images indicated that shorter SWCNTs tended to self-aggregate without involving many bacterial cells, whereas longer SWCNTs efficiently aggregated with bacterial cells, especially when a greater number of bacterial cells were present.

The antibacterial activity of all tested SWCNTs was found to be dependent on both concentration and time. With longer SWCNTs, this dependence was more pronounced and intensified. These findings expanded the potential applications of SWNTs as antimicrobial agents by providing a fundamental understanding of the factors influencing interactions between SWCNTs and bacterial cells, as well as the mechanisms underlying SWCNTs' antimicrobial activity. This knowledge has significant implications for designing effective antimicrobial strategies using carbon nanotubes.

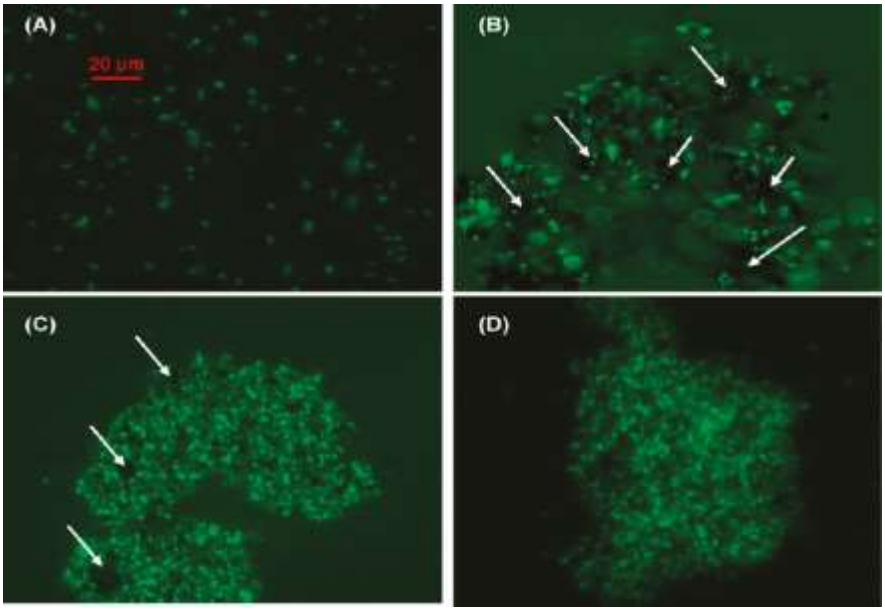

Fig. 5. Representative images of Salmonella cells in DI water suspension (A) without SWCNTs, and the aggregates formed by cells and SWCNTs of different lengths (B) <1 µm, (C) 1-5 µm, and (D) ~5 µm. Cells were stained with green fluorescence. Black spots in the aggregates (indicated by arrows in B and C) were clusters of SWCNTs (Aslan, S., *et al* 2013)

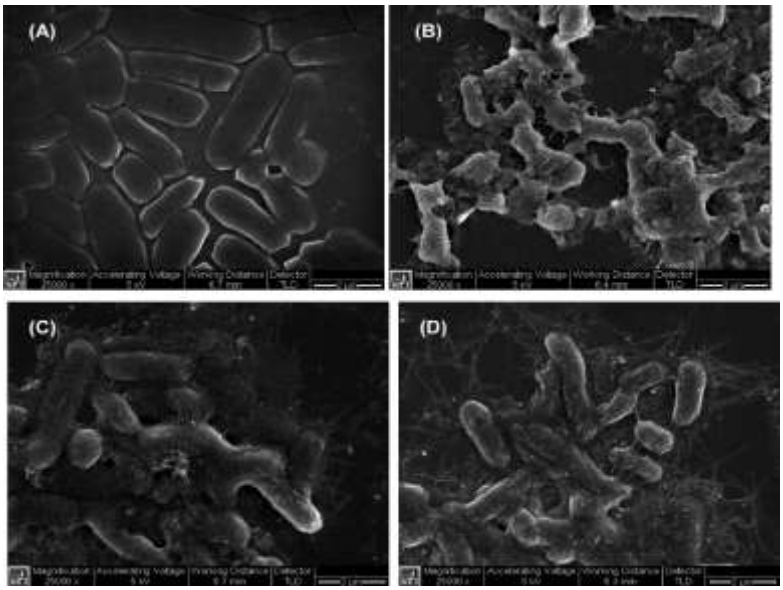

Fig. 6. SEM images of Salmonella cells (A) without SWCNTs, and the aggregates of cells-SWCNTs of (B) <1 µm, (C) 1-5 µm, and (D) ~5 µm (Aslan, S., *et al* 2013)

A new opportunity for direct comparison of single-walled carbon nanotube (SWNT) activity in media with varying effects on bacteria has emerged from the work of Sloan et al. (Abo-Neima *et al.,* 2020). They recently discovered that tryptic soy broth (TSB), a standard microbiological growth medium, can disperse SWNTs as individual units and small bundles. This discovery enables a fresh perspective on SWNT behavior in biological dispersants such as DNA, lysozyme, and TSB, in addition to the commonly used pure SWNT dispersants SDS and pluronic (Kang, S., *et al* 2008). To investigate the influence of these dispersants on SWNT activity, researchers used colony forming unit (CFU) counts, bacterial growth curve analysis, and optical density measurements at 600 nm. The study focused on Gram-positive Staphylococcus aureus and Gram-negative Salmonella enterica. Notably, synergistic interactions were highlighted as essential considerations in assessing antibacterial activity. The five selected dispersants were SDS, pluronic, lysozyme, DNA, and TSB. The experimental model organisms were

Staphylococcus aureus and Salmonella enterica, and measurements included CFU counts and optical density for activity assessment. Intriguingly, no activity was observed against Salmonella in any of the systems. However, Staph. aureus exhibited susceptibility to SDS, proving lethal in the presence of SWNTs. Furthermore, SWNTs enhanced the anti-Staph. aureus effects of pluronic and lysozyme. Conversely, regardless of SWNT presence, DNA and TSB dispersions exhibited no antibacterial effects. These results underscore the necessity for further research into the mechanisms through which interactions between SWNTs and dispersants lead to antibacterial activity. The findings suggest that the purported antibacterial activity of SWNTs might exclusively manifest against bacteria sensitized by the dispersant. In a distinct investigation, Jannati *et al.* (Yang, *et al.*, 2010) explored the impact of functionalized multi-walled carbon nanotubes nanofluid (F-MWCNTsN) on S. aureus. They functionalized multi-walled carbon nanotubes with COOH groups to create the nanofluid. The Microplate Alamar Blue Assay (MABA) method gauged bacterial growth post-treatment with F-MWCNTsN at concentrations ranging from 0.1 to 1%. Subsequently, TetM and TetO gene expression tests evaluated the Nanofluid's drug delivery potential containing F-MWCNTs. The outcomes revealed potential antibacterial effects of multi-wall functionalized carbon nanotubes on S. aureus (Fig. 7). Importantly, the researchers successfully overcame S. aureus's photogenic antibiotic resistance strain using nanofluid with functionalized carbon nanotubes. This innovative approach represents a novel avenue for nano medication therapy and delivery to combat antibiotic-resistant forms of bacteria like S. aureus, which contribute to various nosocomial infections.

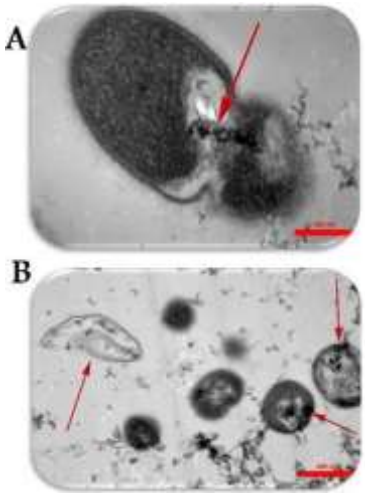

Fig. 7. A and B in different magnifications exhibit the impression mechanism from functionalized MWCNTs nanofluid on cell membrane demolition and antibiotic delivery to bacteria (Yang, C., *et al* 2010)

In pursuit of an efficient, secure, and rapid-acting nano-drug with minimal side effects, Hassani *et al.*, (Sloan, *et al.*, 2017) embarked on the synthesis of a novel nano-antibiotic by conjugating levofloxacin (LVX) with multi-walled carbon nanotubes (MWCNTs), resulting in MWCNT-LVX. This groundbreaking work not only investigated the in vivo antibacterial activity of the nano-antibiotic in a burn wound model but also marked the pioneering evaluation of its in vitro cell viability and antibacterial effects. The drug-loading and release profiles across various pH levels were determined using an ultraviolet-visible spectrometer. The synthesis of MWCNT-LVX was achieved through a simple, reproducible, and cost-effective procedure, followed by characterization using diverse techniques including scanning electron microscopy, transmission electron microscopy, Brunauer-Emmett-Teller analysis, among others.

In vitro, the noncytotoxic nano-antibiotic exhibited significantly enhanced antibacterial efficacy against Staphylococcus aureus compared to Pseudomonas aeruginosa. Notably, the synthetic nano-drug displayed pH-sensitive release behavior alongside a high loading capacity. This distinctive combination of features translated into a notably more potent bactericidal effect than LVX alone in a murine model of S. aureus wound infection. The synergy of antibacterial attributes was attributed to the surface-modified MWCNTs to which the drug was conjugated.

The innovation of this nano-antibiotic holds considerable promise for commercialization, facilitated by its facile production, lack of toxicity, appropriate drug loading and release profiles, low effective dosage requirement, and robust efficacy against wound infections. Leveraging the unique attributes of MWCNTs, such as controlled release and drug delivery capability, the nano-antibiotic can traverse biological barriers and membranes efficiently, potentially enabling lower dosages than what would be needed with the standalone active agent. This aspect, in turn, contributes to minimizing side effects. Thus, MWCNTs emerge as a promising nano-carrier for LVX, positioning them as a potential solution for treating skin infections.

Carbon nanotubes' toxicity: The issue of toxicity has presented a significant challenge for the incorporation of carbon nanomaterials (CNMs) into sectors like medicine and food, despite their diverse array of applications (Salari, S., Jafari, SM 2020). The literature on CNM toxicity has yielded somewhat

contradictory findings, adding complexity to the matter. Some studies have indicated that exposure to CNMs triggers apoptosis and inflammation (Noor *et al.,* 2022). Furthermore, these studies suggest that CNM exposure can disrupt cellular stress responses, as well as cellular transport and metabolism pathways. Reports highlighting the detrimental effects of CNMs, including nanotubes and nano-onions, have also emerged (Jannati H., *et al.,* (2021). Nanotubes, for instance, have been linked to immunological and inflammatory responses, while the effects of nano-onions appear to be influenced by external stimuli.

Specifically, single-walled carbon nanotubes (SWCNTs) have demonstrated cytotoxic effects in HaCaT cells, eliciting oxidative stress, generation of free radicals and peroxides, antioxidant scavenging activity, reduced cellular activity and viability, and alterations in cellular morphology (Hassani *et al.,* 2022). Despite this understanding of the toxic effects of CNMs like carbon nanotubes (CNTs) and fullerene on animal cells and organs, the underlying mechanisms of cellular toxicity remain incompletely elucidated (Yan *et al.,* 2011). Researchers have delved into the molecular toxicity of these materials in both human and animal contexts (Salari and Jafari 2020). At present, CNTs are generally considered more hazardous than fullerene (Noor *et al.,* 2022). In-depth gene expression studies have revealed that CNTs, particularly multi-walled carbon nanotubes (MWCNTs), can elevate both necrosis and apoptosis in cells. Remarkably, the toxicity of CNMs in cellular environments is influenced by factors such as their structural characteristics, size distribution, surface chemistry, concentration, charge, and aggregation state (Ding *et al.,* 2005) While certain experts have raised concerns about the safety of CNMs, several investigations have indicated that pure CNMs, including pure SWCNTs, exhibit minimal harm and have not demonstrated adverse effects in mouse models (Ding *et al.,* 2005).

*Challenges***:** The antimicrobial properties of carbon nanotubes (CNTs) have garnered considerable attention for their potential applications in various fields, from medicine to industry. These nanomaterials exhibit remarkable bactericidal capabilities, which offer promising avenues for combating microbial infections and enhancing hygiene (Dizaj *et al.,* 2015). However, the practical implementation of CNTs as antimicrobial agents is accompanied by several challenges that warrant careful consideration (Ji *et al.,* 2016).

One significant challenge lies in deciphering the mechanisms underlying the antibacterial activity of CNTs. While their effectiveness against bacteria is evident, the precise interactions and processes through which CNTs neutralize microorganisms remain intricate and not fully elucidated (Maksimova 2019). Understanding these mechanisms is pivotal for optimizing CNT-based antibacterial strategies and minimizing unintended consequences (Khan *et al.,* 2016).

Another crucial concern revolves around potential toxicity. While pure carbon nanomaterials (CNMs) have demonstrated safety in various contexts, the introduction of CNTs as antibacterial agents demands rigorous assessment of their impact on both microbial targets and host cells (Abo-Neima *et al.,* 2020). Ensuring that the antimicrobial properties of CNTs do not come at the expense of detrimental effects on human health is of paramount importance (Hussan *et al.,* 2021).

Furthermore, the practical application of CNTs as antimicrobial agents necessitates addressing challenges related to stability, dispersion, and delivery (Yang *et al.,* 2010). CNTs' insolubility in certain solvents and their tendency to agglomerate can limit their efficient dispersion within target environments, hindering their interaction with bacteria (Noor *et al.,* 2022). Developing effective strategies to ensure consistent dispersion and delivery of CNTs to infection sites is essential for realizing their full potential (Diza *et al.,* 2015).

Incorporating CNTs into existing medical and industrial practices also requires overcoming regulatory and standardization challenges. Ensuring the safe and effective deployment of CNT-based antimicrobial solutions demands adherence to rigorous regulatory frameworks and the establishment of standardized protocols for their synthesis, testing, and application (Hassani *et al.,* 2022).

In a nutshell, while the antimicrobial properties of carbon nanotubes offer promising solutions for various challenges, their practical utilization faces several hurdles. Addressing the intricate mechanisms, potential toxicity concerns, dispersion issues, and regulatory requirements is crucial for harnessing the full potential of CNTs as effective and safe antimicrobial agents. A comprehensive and holistic approach is essential to navigate these challenges and unlock the diverse benefits that CNTs can provide in the realm of antimicrobial applications.

Future Prospects: The potential of carbon nanotubes (CNTs) to revolutionize the field of antimicrobial agents is increasingly being recognized as a gateway

to innovative infection control strategies. As scientific understanding and technological capabilities continue to evolve, the future holds promising avenues for CNTs to emerge as potent and versatile tools in the fight against microbial infections.

Tailoring Antimicrobial Efficacy: The future of CNT-based antimicrobial applications lies in the capacity to engineer these nanomaterials to exhibit tailored and amplified antibacterial efficacy (Hussain, Kabir, Sood and 2009). By modifying factors such as size, surface functionalization, and structural composition, researchers can fine-tune the interactions between CNTs and microbial entities (Zhao and Liu 2012). This level of customization holds potential for targeting specific pathogens and optimizing treatment outcomes.

Multifaceted Approaches: Antimicrobial resistance remains a global health concern. In response, future strategies may integrate CNTs with traditional antibiotics or other therapeutic agents (Vankoningsloo *et al.,* 2010). Combining CNTs' inherent antimicrobial properties with complementary treatments could lead to synergistic effects, enhancing the overall efficacy and potentially overcoming resistance mechanisms (Vankoningsloo *et al.,* 2010).

*Precision Medicine***:** The development of personalized antimicrobial treatments is on the horizon, where CNTs could play a pivotal role. Leveraging advancements in nanotechnology and diagnostics, tailored CNT-based therapies could be designed to target infections with precision, minimizing collateral damage to healthy cells and tissues (Godoy-Gallardo *et al.,* 2021).

Targeted Delivery Systems: The unique physicochemical properties of CNTs make them excellent candidates for targeted drug delivery. Future endeavors could involve loading CNTs with antimicrobial agents and utilizing their high surface area and transport capabilities to deliver therapeutic payloads precisely to infection sites. This approach could minimize systemic exposure, side effects, and resistance development (Liu *et al.,* 2023).

Beyond Antibacterial Applications: CNTs' multifunctional nature extends their potential beyond antibacterial applications. With their demonstrated ability to modulate immune responses and promote tissue regeneration, CNTs could find utility in wound healing, implant coatings, and regenerative medicine, addressing a broader spectrum of medical challenges (Murugaiyan *et al.,* (2022).

Safety and Biocompatibility: The future outlook emphasizes rigorous assessment of CNT safety and biocompatibility. Advancements in toxicology studies and comprehensive evaluations will ensure that CNT-based therapies are both effective against infections and safe for human use. This facet is integral to gaining regulatory approvals and clinician confidence (Alghamdi *et al.,* 2022).

Sustainable and Eco-Friendly Approaches: As the world embraces sustainability, the development of eco-friendly CNT synthesis methods and environmentally responsible disposal strategies will be pivotal. Ensuring the lifecycle of CNT-based antimicrobial applications aligns with global environmental concerns remains an integral aspect of their future utilization (Murugaiyan *et al.,* 2022; Alghamdi *et al.,* 2022).

In general, the future prospects of CNTs as antimicrobial agents stand at the crossroads of scientific innovation and practical solutions for infection control. The coming years hold exciting possibilities for the customization, integration, and safe implementation of CNTs in diverse medical applications, underlining their potential to reshape the landscape of healthcare with cutting-edge strategies that tackle microbial challenges head-on.

Conclusion: The exploration of carbon nanotubes (CNTs) as potential antimicrobial agents has shed light on their remarkable attributes and potential challenges. This study has delved into their antibacterial properties, uncovering the intricate interplay of factors that determine their efficacy. The comprehensive analysis of existing literature underscores the promising bactericidal capabilities of CNTs, which can be tailored and harnessed for various medical applications.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the first author or corresponding author.

REFERENCES

Abd-Elsalam, KA (2020). Carbon nanomaterials: 30 years of research in agroecosystems, Carbon nanomaterials for agri-food and environmental applications. *Elsevier* 1–18.

Abo-Neima, SE; Motaweh, HE; Elsehly, EM (2020). Antimicrobial activity of functionalised carbonnanotubes against

pathogenicmicroorganisms. IET Nanobiotechnol. 14(6): 457-464

.

Alghamdi, MA; Fallica, AN; Virzì, N; Kesharwani, P; Pittalà, V; Greish, K (2022). The promise of nanotechnology in personalized medicine. *J. Personalized Med.* 12(5): 673.

Allhoff, F (2007). On the Autonomy and Justification of Nanoethics. *Nanoethics* 1: 185–210.

Aslan, S; Maatta, M; Haznedaroglu, BZ; Goodman, JPM; Pfefferle, LD; Elimelech, M; Pauthe, E; Sammalkorpi, M; Tassela, PRV (2013). Carbon nanotube bundling: influence on layer-by-layer assembly and antimicrobial activity. *Soft Matter*. 9: 2136.

Azizi-Lalabadi, M; Hashemi, H; Feng, J; Jafari, SM (2020). Carbon nanomaterials against pathogens; the antimicrobial activity of carbon nanotubes, graphene/graphene oxide, fullerenes, and their nanocomposites. *Adv. in Colloid and Interface Sci.* 284: 102250.

Bayda, S; Adeel, M; Tuccinardi, T; Cordani, M; Rizzolio, F (2019). The History of Nanoscience and Nanotechnology: From Chemical-Physical Applications to Nanomedicine. *Molécules* 25(1) : 112.

Chong, Y; Ge, C; Fang, G; Wu, R; Zhang, H; Chai, Z; Chen, C; Yin, J-J (2017). Light-enhanced antibacterial activity of graphene oxide, mainly via accelerated electron transfer. *Environ. Sci. Technol.* 51: 10154–61.

Ding, L; Stilwell, J; Zhang, T; Elboudwarej, O; Jiang, H; Selegue, JP; Cooke, PA; Gray, JW; Chen, FF (2005). Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano- onions on human skin fibroblast. *Nano Lett.* 5: 2448–64.

Dizaj, SM; Mennati, A; Jafari, S; Khezri, K; Adibkia, K (2015). Antimicrobial activity of carbonbased nanoparticles. *Adv. Pharm. Bull.* 5:19.

Dong, L; Henderson, A; Field, C (2012). Antimicrobial activity of single-walled carbon nanotubes suspended in different surfactants. *J. Nanotechnol.* 2012: 928924.

Eribe, MJ; Ikhazuagbe, HI; Kate EM; Okeke IE; Inono CO (2022). Review on the Heightened Mechanical Features of Nanosilica-Based Concrete and the Response of Human Fibroblasts to Nanosilica. *Biomed. Mater. Devices.* DOI: 10.1007/s44174-022-00013-4.

Gnach, A; Lipinski, T; Bednarkiewicz, A; Rybka, J; Capobianco, JA (2015). Upconverting nanoparticles: Assessing the toxicity. *Chemical Soc. Reviews* 44: 1561–1584.

Godoy-Gallardo, M; Eckhard, U; Delgado, L. M; de Roo Puente, YJ. D; Hoyos-Nogués, M; Gil, FJ; Perez, RA (2021). Antibacterial approaches in tissue engineering using metal ions and nanoparticles: From mechanisms to applications. *Bioactive Mater.* 6(12): 4470-4490.

Hamdan, S; Pastar, I; Drakulich, S; Dikici, E; Tomic-Canic, M; Deo, S; Daunert, S (2017). Nanotechnology-Driven Therapeutic Interventions in Wound Healing: Potential Uses and Applications. *ACS Central Sci.* 3(3): 163-175.

Hassani, M; Tahghighi, A; Rohani, M; Hekmati, M; Ahmadian, M; Ahmadvand, H (2022). Robust antibacterial activity of functionalized carbon nanotube levofloxacine conjugate based on in vitro and in vivo studies. Scientific Rep.12: 10064.

Hussain, M; Kabir, M; Sood, A (2009). On the cytotoxicity of carbon nanotubes. *Curr. Sci.* 96: 00113891.

Hussan, NQA; Taha, AA; Ahmed, DS (2021). Characterization of treated multi-walled carbon nanotubes and antibacterial properties. *J. Applied Sci. Nanotechnol.*1 (2): 1-9.

Ifijen IH; Itua AB; Maliki M; Ize-Iyamu CO; Omorogbe SO; Aigbodion AI; Ikhuoria EU (2020). The removal of nickel and lead ions from aqueous solutions using green synthesized silica microparticles. *Heliyon* 6(9): e04907.

Ifijen, IH; Ikhuoria, EU; Aigbodion, AI; Omorogbe, SO (2018). Impact of varying the concentration of tetraethyl-orthosilicate on the average particle diameter of monodisperse colloidal silica spheres. *Chem. Sci. J.* 9(1): 183-185.

Ifijen, IH; Ikhuoria, EU; Omorogbe, SO; Anegbe, B; Jonathan, EM; Chikaodili, DI (2023). Chemical, Plant and Microbial Mediated Synthesis of Tin oxide Nanoparticles: Antimicrobial and Anticancer Potency. *Braz. J. Chem. Eng.* DOI: 10.1007/s43153-023-00315-0.

Ifijen, IH; Maliki, M (2022). A comprehensive review on the synthesis and photothermal cancer therapy of titanium nitride nanostructures. *Inorg. Nano-Metal Chem.* DOI: 10.1080/24701556.2022.2068596.

Ifijen, IH; Maliki, M; Odiachi, IJ Omoruyi, IC; Aigbodion, AI; Ikhuoria, EU (2022). Performance of Metallic Based-Nanomaterials Doped with Strontium in Biomedical and Supercapacitor Electrodes: A Review. *Biomed. Mater. Dev*. https://doi.org/10.1007/s44174-022-00006-3.

Ifijen, IH; Maliki, M; Odiachi, IJ; Aghedo, ON; Ohiocheoya, EB (2022). Review on solvents-based alkyd resins and water borne alkyd resins: impacts of modification on their coating properties. *Chem. Afri.* 5: 211–225.

Ikhuoria, EU; Ifijen IH; Georgina OP; Ehigie AC; Omorogbe SO; Aigbodion AI (2020). The adsorption of heavy metals from aqueous solutions using silica microparticles synthesized from sodium silicate. Ni-Co 2021: *The 5th Int'l. Symposium on Ni and Co.* 195-205.

Iqbal, P; Preece, JA; Mendes, PM (2012). Nanotechnology: The "Top-Down" and "Bottom-Up" Approaches. In *Supramolecular Chem.* John Wiley & Sons, Ltd.: Chichester, UK.

Jannati H; Sheikhpour M; Siadat SD; Safarian P (2021). Antimicrobial activity and drug delivery ability of Functionalized Multi-Walled Carbon Nanotubes Nanofluid on staphylococcus aureus. *Nanomed. Res. J.* 6(3): 248-256.

Ji, H ; Sun, H ; Qu, X (2016). Antibacterial applications of graphene-based nanomaterials: recent achievements and challenges. *Adv. Drug Deliv. Rev.* 105: 176–89.

Jonathan, EM; Ifijen, IH; Mokobia, KE, Okeke, EI; Omoruyi, CI; Anegbe, B (2022). A Review on the Heightened Mechanical Features of Nanosilica-Based Concrete and the Response of Human Fibroblasts to Nanosilica. *Biomed. Mater. Devices*. https://doi.org/10.1007/s44174-022-00013-4.

Kang, S; Herzberg, M; Rodrigues, DF; Elimelech, M (2008). Antibacterial Effects of Carbon Nanotubes: Size Does Matter? *Langmuir* 24: 6409-6413.

Khan, AAP; Khan, A; Rahman, MM; Asiri, AM; Oves, M (2016). Lead sensors development and antimicrobial activities based on graphene oxide/carbon nanotube/poly (O-toluidine) nanocomposite. *Int. J. Biol. Macromol.* 89: 198–205.

Liu, H; Xing, F; Zhou, Y; Yu, P; Xu, J; Luo, R; Xiang, Z; Rommens, PM; Liu, M; Ritz, U (2023). Nanomaterials-based photothermal therapies for antibacterial applications. *Mater. Design* 233: 112231.

Mahima, KC; Johnson, AP; Hani, U; Ghazwani, M; Begum, MY; Alshehri, S; Ghoneim, MM; Shakeel, F; Gangadharappa, HV (2021). Carbon nanotubes: current perspectives on diverse applications in targeted drug delivery and therapies. *Mater* 14(21): 6707.

Maksimova, YG (2019). Microorganisms and carbon nanotubes: interaction and applications. *Appl. Biochem. Microbiol.* 55: 1–12.

Malik, S; Khan, M; Waheed, Y (2023). Nanotechnology: A Revolution in Modern Industry. *Molécules* 28(2): 661. doi:10.3390/molecules28020661.

Maliki, M; Ifijen, IH; Ikhuoria, EU; Jonathan, EM; Onaiwu, GE, Archibong, UD; Ighodaro, A (2022). Copper Nanoparticles and their oxides: Optical, Anticancer and *Antibacterial Properties. Int. Nano Lett.* 12: 379–398. https://doi.org/10.1007/s40089-022-00380-2.

Mohammed, MK; Ahmed, DS; Mohammad, MR (2019). Studying antimicrobial activity of carbon nanotubes decorated with metal-doped ZnO hybrid materials. *Mater. Res. Express* 6: 055404.

Murugaiyan, J; Kumar, PA; Rao, G S; Iskandar, K; Hawser, S; Hays, J P; Mohsen, Y; Adukkadukkam, S; Awuah, WA; Jose, RAM; et al (2022). Progress in alternative strategies to combat antimicrobial resistance: Focus on antibiotics. *Antibiotics* 11: 200.

Noor, MM; Santana-Pereira, ALR; Liles, MR; Davis, VA. (2022). Dispersant effects on single-walled carbon nanotube antibacterial activity. *Molecules* 27: 1606.

Patra, JK; Das, G; Fraceto, LF; et al. (2018). Nano based drug delivery systems: Recent developments and future prospects. *J. Nanobiotechnol.* 16: 71. doi:10.1186/s12951-018-0392-8.

Petersen, P; Tikhomirov, G; Qian, L (2018). Information-based autonomous reconfiguration in systems of interacting DNA nanostructures. *Nat. Communications* 9: 5362.

Salari, S; Jafari, SM (2020). Application of nanofluids for thermal processing of food products. *Trends. Food Sci. Technol.* 97: 100–13.

Sharma, N; Sharma, M; Sajid Jamal, QM; Kamal, MA; Akhtar, S (2019). Nanoinformatics and biomolecular nanomodeling: A novel move en route for effective cancer treatment. *Environ. Sci. Pollution Res*. Int. 1–15.

Sloan, AW; Santana-Pereira, AL; Goswami, J; Liles, MR; Davis, VA (2017). Single-walled carbon nanotube dispersion in tryptic soy broth. *ACS Macro Lett.* 6: 1228–1231.

Su, Q; Gan, L; Liu, J; Yang, X (2020). Carbon dots derived from pea for specifically binding with Cryptococcus neoformans. *Anal. Biochem.* 589:113476.

Swierczewska, M; Han, H; Kim, K; Park, J; Lee, S (2016). Polysaccharide-based nanoparticles for theranostic nanomedicine. *Adv. Drug Delivery Reviews* 99: 70–84.

Vankoningsloo, S; Piret, J-P; Saout, C; Noel, F; Mejia, J; Zouboulis, CC; Delhalle, J; Lucas, S; Toussaint, O (2010). Cytotoxicity of multi-walled carbon nanotubes in three skin cellular models: effects of sonication, dispersive agents and corneous layer of reconstructed epidermis. *Nanotoxicol.* 4: 84–97.

Wang, G ;Gao, S ; Tian, R ; Miller-Kleinhenz, J ;Qin, Z ; Liu, T ; Li, L ; Zhang, F ; Ma, Q ; Zhu, L (2018). Theranostic hyaluronic acid-iron micellar nanoparticles for magnetic-field-enhanced in vivo cancer chemotherapy. *Chem. Med. Chem.* 13: 78–86.

Wong, PT; Choi, SK (2015). Mechanisms of drug release in nanotherapeutic delivery systems. *Chemical Reviews* 115: 3388–3432.

Yan, L; Zhao, F; Li, S; Hu, Z; Zhao, Y (2011). Low-toxic and safe nanomaterials by surface- chemical design, carbon nanotubes, fullerenes, metallofullerenes, and graphenes. *Nanoscale* 3: 362–82.

Yang, C; Mamouni, J; Tang, Y; Yang, L (2010). Antimicrobial activity of single-walled carbon nanotubes: length effect. *Langmuir* 26(20): 16013–16019.

Yuan, Y; Gu, Z; Yao, C; Luo, D; Yang, D (2019). Nucleic Acid–Based Functional Nanomaterials as Advanced Cancer Therapeutics. *Small* 15: 1900172.

Zhao, X; Liu, R (2012). Recent progress and perspectives on the toxicity of carbon nanotubes at organism, organ, cell, and biomacromolecule levels. *Environ. Int.* 40: 244–55.