

EVALUATION OF CYTOTOXICITY OF MULTIWALLED CARBON NANOTUBES IN HEPATOCYTES AND ITS POTENTIAL IMPACT ON LIPID METABOLISM

Submitted in partial fulfilment for the award of the degree of
Bachelor of technology

By

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(DEEMED TO BE UNIVERSITY)
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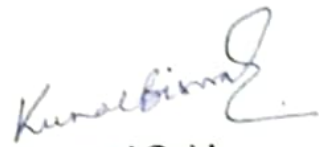
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Sandhya S (39230037) who carried out the project "EVALUATION OF CYTOTOXICITY OF MULTIWALLED CARBON NANOTUBES IN HEPATOCYTES AND ITS POTENTIAL IMPACT ON LIPID METABOLISM" under our supervision from January 2023 to May 2023.


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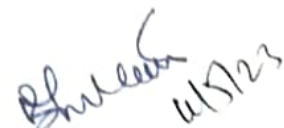
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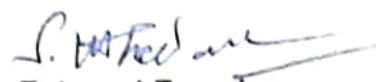


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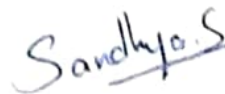

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DECLARATION

I, Sandhya S hereby declare that the Project Report entitled "**EVALUATION OF CYTOTOXICITY OF MULTIWALLED CARBON NANOTUBES IN HEPATOCYTES AND ITS POTENTIAL IMPACT ON LIPID METABOLISM**" done by me under the guidance of Dr. R. Thyagarajan and Dr. Kunal Biswas at International Research Centre, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology

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ABSTRACT

The increased volumes of carbon nanotubes (CNTs) being utilized in industrial and biomedical processes carries with it an increased risk of unintentional release into the environment, requiring a thorough hazard and risk assessment. Following exposure via inhalation, intratracheal instillation or ingestion CNTs have been shown to translocate to the liver. The liver has a crucial role in metabolic homeostasis, as it is responsible for the storage, synthesis, metabolism and redistribution of carbohydrates, fats and vitamins, and numerous essential proteins. Nanoparticle (NP) exposure might promote hepatic steatosis, but relatively few studies investigated the influence of multi-walled carbon nanotubes (MWCNTs) on lipid accumulation in hepatocytes *in vitro*. It's been observed that MWCNT showed hepatotoxicity *in vitro* at the concentration above 20µg/ml but its effect on liver cells at lower concentrations is not yet explored. Here, we performed integrated systems toxicology approach for detailed study to evaluate the dose-dependent response of hepatocytes against CNT and the possible role of lipophagy (autophagic degradation of lipid droplets) in MWCNT-exposed HepG2 cells. For this purpose, hepatocytes cell line was treated with MWCNT of different concentration ranging 100µg/ml to 5µg/ml. Pristine MWCNTs were internalized, accompanying cytotoxicity, lysosomal destabilization, and intracellular reactive oxygen species (ROS) production. DAPI staining revealed that MWCNT at higher concentration caused significant damage to the DNA and decreases the cell viability. Apoptosis was assessed using propidium iodide/acridine orange (PI/AO) staining, compared to nontreated controls. Furthermore, the propidium iodide/acridine orange (PI/AO) staining showed an increased apoptosis after treatment. The intracellular lipid accumulation was observed by Oil Red O staining methods. Combined, these results suggested that MWCNT exposure might promote lipid accumulation in hepatocytes probably through the modulation of lipophagy pathway.

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ABBREVIATIONS

AO	Acridine orange
ATG4	Autophagy-regulating protease 4
ATGL	adipose triglyceride lipase
ATP	Adenosine Triphosphate
BBB	Blood-brain barrier
CE	cholesteryl esters
CMA	chaperone mediated autophagy
CNTs	Carbon nanotubes
CO ₂	Carbon dioxide
COOH	Carboxylic acid
DAPI	4',6-diamidino-2-phenylindole,
DCFH-DA	Dichlorodihydrofluorescein diacetate
DGL	di-noglyceride lipase
DNA	Deoxyribonucleic acid
DNL	de novo lipogenesis
DMSO	Dimethyl sulfoxide
ED	Electron diffraction
EDTA	Ethylenediaminetetraacetic acid
EMT	Epithelial-mesenchymal transition;
ER	Endoplasmic Reticulum system
FAO	fatty acid oxidation

FBR	Foreign body response
FBS	Fetal bovine serum
FFA	Free Fatty Acids
FE-SEM	Field-Emission Scanning Electron Microscopy
FTIR	Fourier-transform infrared spectroscopy
FWHM	Full width at half maximum
GPa	Gigapascal
HEK	Human embryonic kidney
Hep G2	Hepatoblastoma cell line
HR-TEM	High-resolution transmission electron Microscopy
HSL	hormone sensitive lipase
IC ₅₀	Half maximal inhibitory concentration
IC ₉₀	90% of the maximum inhibition
LAL	lysosome acid lipase
LAMP	lysosomal-associated membrane protein
LD	lipid droplet
MAPK	Mitogen-activated protein kinase
MGL	Mono-glyceride lipase
MMC	Melanomacrophage centres
mTOR	mammalian target of rapamycin
MTT	3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
MWCNTs	Multi-walled carbon nanotube
NASH	Non-alcoholic steatohepatitis

NCCS	National Centre for Cell Science
NF-kB	Nuclear factor Kappa B
NIR	Near-infrared region
NLRP3	Nucleotide-binding oligomerization domain-like receptor: pyrin domain-containing 3
Nps	Nanoparticles
OD	Optical Density
PBS	Phosphate-buffered saline
PCA	Principle component analysis
PI	Propidium iodide
PLIN	perilipin
PSA	Polysialic acid
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RGR	relative growth rate
RSR	Cell relative survival rate
SEM	Scanning electron microscopy
SIRT1	signals via sirtuin-1
SWCNT	Single-walled carbon nanotube
TEM	Transmission electron microscopy
TFEB	transcription factor EB
TG	Triglyceride
TGF	Transforming growth factor

TPa	Terapascal
UV-vis	Ultraviolet-visible spectroscopy
VLDL	very low-density lipoprotein
V-ATPase	Vacuolar-type-H ⁺ -ATPase
XRD	X-Ray Diffraction

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CHAPTER 1

INTRODUCTION

Nanotechnology is the understanding, design, and development of new materials with interesting characteristics at the nanometric scale where the effects of quantum mechanics are noticeable. These nanomaterials have at least one dimension between 1 and 100 nm, and adapt the shape of nanoparticles, nanotubes, or nano surfaces. On the other side, nanobiotechnology refers to the intersection of nanotechnology and biology, combining the efficacy of biological materials and the rules and tools of basic sciences like physics and chemistry. It is also defined as the science that investigates, beyond the molecular level, the design and development of highly ordered structured materials that offer specific responses when exposed to stimuli. The development of these nanomaterials has reached a wide use in numerous scientific and technical fields, including water treatment electrocatalysis, catalysis, materials, and biomedical applications from cancer treatment to regenerative medicine. In fact, these structures have become increasingly useful in two main areas of biomedicine: (1) nanomedicine, with noteworthy applications in imaging, biosensors, drug delivery systems, and photo thermal therapy; and (2) tissue and implants engineering either as scaffold-based nanomaterials or as components of biomedical devices. These nano compounds can be subcategorized into four wide groups depending on their composition: carbon-based, inorganic-based, organic-based, and composite-based.

Among the carbon-based materials, carbon nanotubes (CNTs) found by Iijima in 1991—constitute a new allotrope of carbon that deserves special interest due to distinctive physical and chemical characteristics that make them especially suitable for preclinical applications. SWCNT (Single-walled carbon nanotube) and MWCNT (Multi-walled carbon nanotube) are two types of nanotubes. SWCNTs are carbon nanotubes that have a structural arrangement similar to that of a graphite sheet coiled up into a cylinder(Ohadi et al., 2022). In contrast, MWCNTs have a larger diameter than SWCNTs because they are made up of several single-walled tubes stacked one within the other in concentric layers(Johnston et al., 2010). While SWCNTs are recognized

for distinct electrical, optical, or chemical capabilities existing at the nanotube scale, the unique features of MWCNTs are mainly related to the bulk material(Jha et al., 2020; Khan et al., 2021). Post-synthesis chemical and thermal modification processes adjust aggregate features of MWCNTs such as large surface area, thermal conductivity, or tensile strength for specific applications(Shimizu et al., 2020),(Kang et al., 2008).

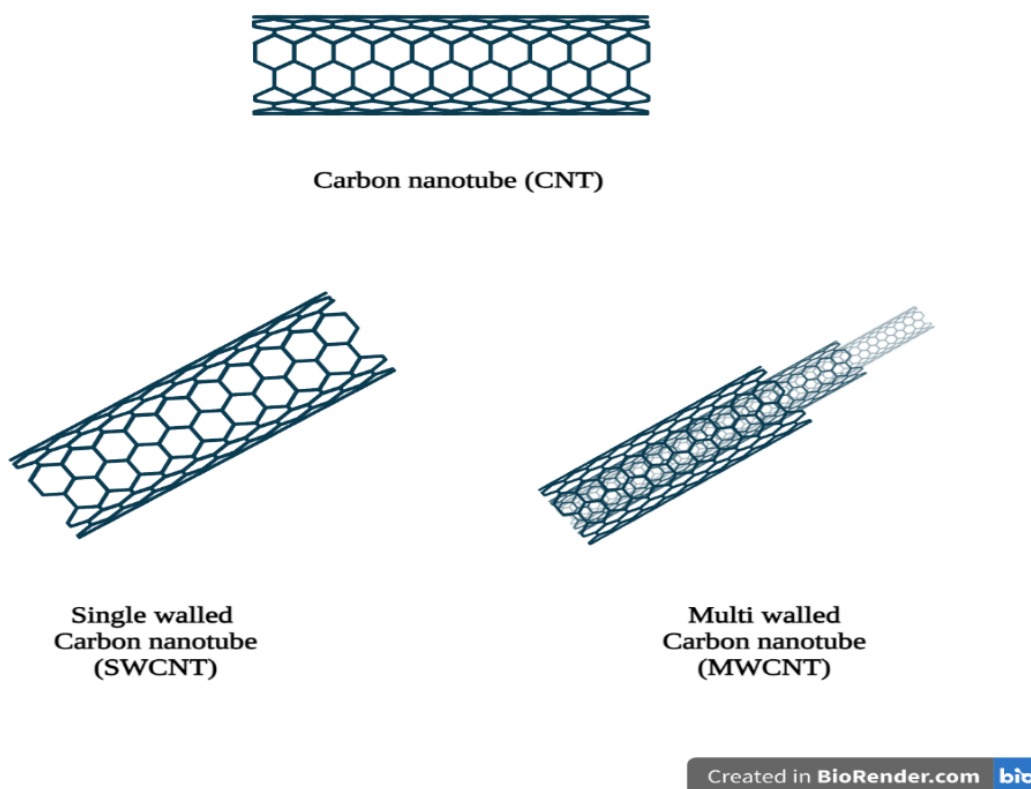


Fig 1.1: Structure of CNT, SWCNT and MWCNT (created using Biorender tool)

Multiple studies on the uses of carbon nanotubes in biology and medicine have been investigated in recent years(Sinha et al., 2021). CNTs (Carbon nanotubes) are currently used as drug delivery systems, membrane components in gene therapy, tissue engineering materials, neural scaffolds, and drug delivery systems. Carbon nanotubes can be utilised to simulate neural fibres for the formation of neurons, according to *in vitro* research. Additionally, carbon nanotube surfaces can be

chemically altered to provide electrically charged spots that can bind a variety of biomolecules(Sinha et al., 2016),(Fraczek et al., 2008). As the use of carbon nanotubes in increasing the quality and efficacy of drug delivery systems expands, so will their exposure to humans. As a result, it is critical to explore the difficulties related to CNT toxicity(Wu et al., 2020). Numerous data and research have been presented to assess the kind of toxicity introduced by the usage of CNTs in biological systems, particularly during the administration of medications (Mohanta et al., 2019a),(Yi & Chen, 2013). MWCNTs have previously been found to inhibit cell growth and produce oxidative damage, apoptosis, or necrosis *in vitro*. MWCNT inhalation causes lung injury or a systemic inflammatory response, oxidative damage, and genotoxicity. CNTs attached to proteins in systemic circulation are deposited in organs of the reticuloendothelial system, such as the liver and spleen via blood transport, where they might have therapeutic or potentially harmful effects. An in-depth investigation of CNT-protein interactions is critical for drug delivery applications and CNT biological safety concerns (T. Zhang, Tang, et al., 2019; Y. Zhang et al., 2016). Regardless of the dosing mode, almost all nanoparticles studied, including MWCNTs, accumulate predominantly in the liver. Furthermore, nanoparticles have been demonstrated to stay in the liver for a long time, usually months or years. A high concentration of nanoparticles in the liver induces inflammation and has the ability to impair various metabolic activities (Y. Zhang et al., 2016).

Moreover, carbon-based particle exposure could promote lipid accumulation in hepatocytes and consequently lead to the development of hepatic steatosis, which might further develop into non-alcoholic steatohepatitis (NASH) and liver cancers (Galassi et al., 2018). However, the mechanisms linking NP exposure and lipid accumulation remain poorly understood. In mammalian cells, neutral lipids are stored in lipid droplets for energy generation and membrane homeostasis, and the regulation of lipid droplets is heavily dependent on the proper function of the autophagy-endoplasmic reticulum (ER) system (Tsukahara & Haniu, 2011). Lipid droplets could be fused with lysosomes before the selective breakdown of the autophagic machinery, a process termed lipophagy. Lipophagy is a form of lipid metabolism in different types of mammalian cells, such as liver cells and macrophages. Lipophagy might also

influence lipid droplet formation since autophagy and ER stress are closely interconnected (H. Yang et al., 2019). ER is a major organelle involved in the regulation of de novo biogenesis of lipid droplets, and the perturbation of the normal function of ER could result in ER stress and consequently abnormal lipid droplet biogenesis (Henderson et al., 2015; Z. Liu et al., 2014; C. Zhao et al., 2018). However, relatively few studies investigated the possible involvement of lipophagy in NP induced lipid accumulation (Long et al., 2019). High concentrations of MWCNTs could modulate autophagy-ER stress gene expression. Given the importance of autophagy-ER stress in lipid metabolism, MWCNT exposure could also influence lipid droplets in liver cells (Jena et al., 2017).

In this study, we are planning investigated lipid accumulation in HepG2 cells after exposure to pristine MWCNTs and the potential role of the lipophagy pathway. Cytotoxicity is to be assessed by cell MTT assays. Lysosomal destabilization measurement by acridine orange staining, whereas oxidative stress is indicated by the measurement of intracellular reactive oxygen species (ROS). Lipid accumulation is determined by oil red staining, and lipid droplet formation is observed by confocal microscopy.

CHAPTER 2

LITERATURE SURVEY

2.1 PROPERTIES OF MWCNT

Because of their superior chemical and physical properties, including great chemical stability, thermal conductivity, mechanical strength, and electrical properties, MWCNTs, which are made up of an enrolled cylindrical graphitic sheet, have many uses in a variety of industries(Xu et al., 2010). Due in part to technical glitches in detecting and quantifying nanoparticles in biological settings, current research on the evaluation of the toxicity of nanomaterials mainly concentrates on one element of their properties at a time in order to identify the reasons of toxicity(Rezazadeh Azari & Mohammadian, 2020). In order to create prediction models for the safe design of nanomaterials and to comprehend nano biological interactions, it is essential to have a thorough grasp of the physicochemical properties of nanoparticles that influence their cytotoxicity.

2.1.1 Chirality

The chiral vector C , defined by the integers, completely describes the structure of a SWCNT (n,m) It is believed that the enantio-selective adsorption and intracellular distribution of chiral NPs will have an impact on their toxicity through their direct impact on NP biodistribution. Furthermore, variable surface chemistry, surface reactivity, and enantio-selective corona formation caused by chirality may impact the toxicity of NPs. It is also crucial to remember that chirality may modify exposure parameters like rates of aggregation, which are influenced by the relative interplay between attractive van der Waals forces and repulsive electrostatic interactions(Utembe, 2019). This means that chirality may not only affect NPs' toxicity by their direct interaction with biological systems.

2.1.2 Surface property

Large surface area caused band gap changes, a lower melting point, and increased reactivity. These characteristics have major consequences including pneumonitis, cytotoxicity, and toxicity *in vivo*. When employed as medication carriers, fillers, or cosmetics, a bigger surface area may result in increased reactivity with adjacent particles, perhaps having negative effects.

2.1.3 Mechanical property

The CNT is superior to other traditional reinforcing fibres in terms of strength and elastic modulus. About 1.2 TPa and 30 GPa (Gigapascal) were the elastic modulus and flexural/tensile strength of CNT, respectively. As a result, CNT is added to a range of nanocomposites during processing. Although solvents and water in general do not solubilize CNT, surface alterations like fluorination and acid treatment can provide more stable solubilization in solvents.

The experimental evidence demonstrates that small concentrations of COOH groups improve the strength of CNTs in comparison to that of defected CNTs, whereas higher concentrations result in tube degradation. carboxylation enhances the biodegradation of CNTs in comparison to other forms of functionalization (such as ozone treatment or aryl sulfonation)(Milowska, 2015).

COOH groups lead to the formation of active sites that serve as points of entry for further oxidative degradation. It reduces the possibility of long-term negative effects from drug delivery and selective therapeutic targeting of cancer cells using CNT. The impact of disorder on the stiffness of these nanotubes must be studied in order to appropriately simulate the impact of carboxylation on the mechanical characteristics of CNTs

2.2 INTRODUCTION OF MWCNT TOWARDS VARIOUS BIOLOGICAL SYSTEMS

2.2.1 *In prokaryotes*

In some recent studies conducted on the antibacterial effects of CNTs, it has been observed that MWCNTs have antibacterial effects on *E. coli* and *Streptococcus* and that SWCNTs have a more effective antibacterial effect than MWCNTs, due to the stronger Van der Waals forces (Nagajyothi et al., 2021). It has been revealed that CNTs adsorb bacteria, inhibit the growth of *E. coli* through Van der Waals forces and package them into blocks (Ertas & Dinc, 2022). Studies have been conducted showing that CNTs induce oxidative stress in bacteria and thus cause damage to the bacterial membrane. In some other reports, it was concluded that CNTs showed antibacterial effects as a result of mechanical interaction with bacteria.

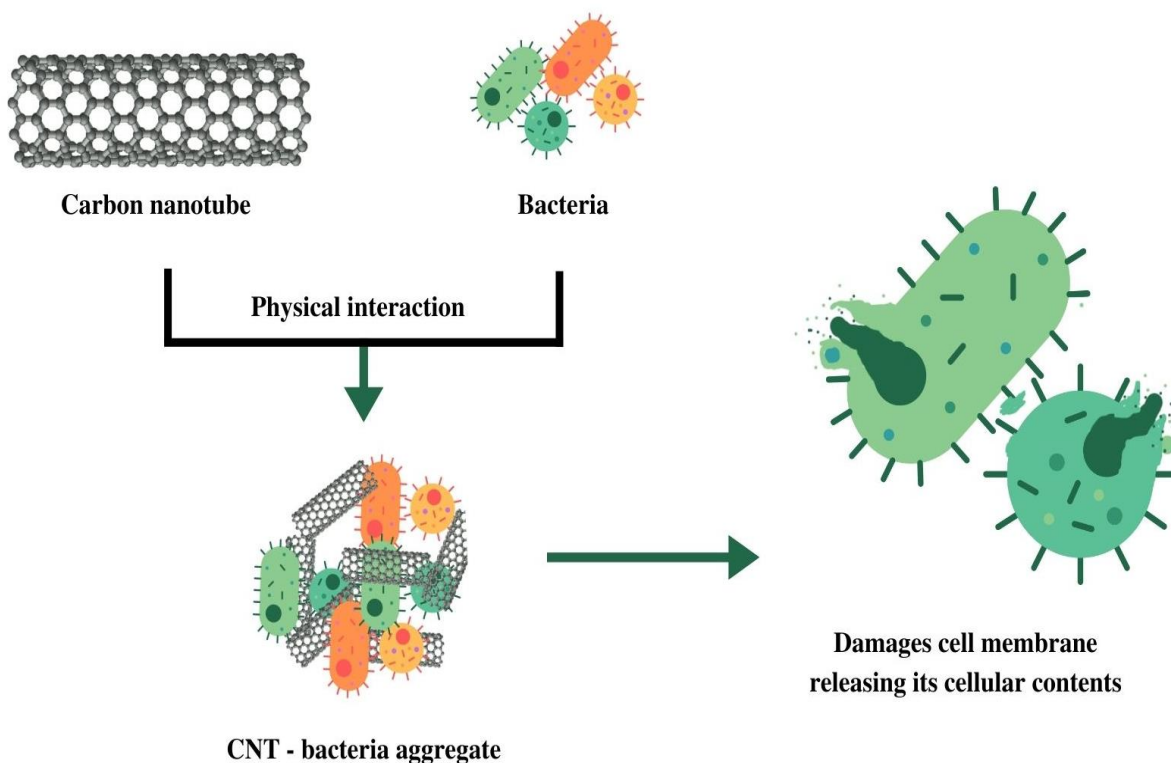


Fig 2.1: Bacterial interaction with CNTs (created using Canva tool)

The results and properties of CNTs used in these studies are different and they revealed bacterial inhibition results that may be due to many factors such as functional groups on CNT surface, concentration, shape, and size on antibacterial properties emerged(Ertas & Dinc, 2022; Hamouda et al., 2021; Hartono et al., 2018; Kang et al., 2008). Electron microscopy investigations of bacteria exposed to MWCNTs revealed that membrane integrity might be lost by piercing or entrapment. MWCNT toxicity appears to be increased in hypotonic and nutrient-free solutions(Atiyah et al., 2019; El Mrabate et al., 2021; Laganà et al., 2021). The incorporation of microorganisms with CNTs for the removal of pollutants would thus be dependent on the incubated medium and bacterial strain, with a high nutrient medium appearing to lower CNT toxicity(Hartono et al., 2018).

2.2.2 In aquatic organisms

Genotoxicity induced by carbon nanotubes (CNTs) can be attributed to a number of factors, involving acute interaction with DNA, an indirect effect resulting from ROS, ion release or interactions with cellular components. Despite this, only a few studies in aquatic toxicology have investigated the genotoxic consequences of carbon nanotubes. Water genotoxic impacts can have long-term consequences on aquatic ecosystems, and the existence of genotoxic substances in water can affect non-aquatic species through the food web or simply by potable water. Fish are frequently utilised as sentinel creatures in eco-toxicological investigations because they play various roles in the trophic web, can rapidly absorb toxic compounds, and respond to low xenobiotic concentrations(Seixas et al., 2018),(Song et al., 2019a). Nanocomposites may be absorbed by fish through the digestive system and gill surface before being delivered by the blood to target organs such as the spleen and liver. The liver has been employed in various toxicological investigations since it is an important organ in xenobiotic detoxification. The spleen, on the other hand, is an essential component of the immune system, acting as a selective filter of the vascular system and playing an important part in the response to pathogen invasion. MMC (Melanomacrophage centres) are

pigmented macrophage accumulations in the head, kidney, liver, and spleen of various fish species (Silva et al., 2021).

MMCs have been employed as biomarkers in multiple investigations involving teleost exposed to various xenobiotics. Thus, the action of stressors correlates with a rise in the number of MMCs, the area, and the kinds of pigments. However, research evaluating the deleterious effect of nanocomposite such as MWCNT on primary culture from fish melanomacrophages have yet to be established. Macrophage primary cultures from the cranial kidney and fish spleen have been generated to define this cell type morphologically and biochemically. As MWCNTs were exposed to melanomacrophages from *Nile tilapia* and *Oreochromis niloticus* the spleen and liver macrophages behaved differently, with the liver melanomacrophages being mainly lysed when compared to the spleen melanomacrophages. Furthermore, MWCNT inclusions were seen in the cytoplasm of macrophages, indicating phagocytic activity, and a concentration of 1 g mL^{-1} of the nanocomposite utilised was cytotoxic for both macrophages (Cimbaluk et al., 2018; Silva et al., 2021; Song et al., 2019a, 2019b).

Several studies investigated the possible toxicity of MWCNTs in *Xenopus* larvae. In larvae, growth inhibition was detected, resulting in acute and chronic toxicity. Among the harmful consequences, genotoxicity has the potential to have long-term impacts on aquatic ecosystems (J. Zhao et al., 2020, 2021). Initially, the association of genotoxic substances with DNA may cause morphological changes in the DNA molecule. Unrepaired damage might result in cell injuries which results in tumour formation (Mouchet et al., 2010).

2.2.3 In mammals

MWCNTs are important since they allow researchers to establish whether the genotoxic threat is consistent. MWCNT reference materials are also desirable since they are well-characterized and require little effort to specify (Møller et al., 2021). Data from cell culture research, which largely uses the comet test and the micronucleus assay to detect DNA strand breaking, dominate our understanding of the genotoxicity

of MWCNT. Some animal research examined genotoxic effects in extrapulmonary organs but found no link between exposure and levels of DNA damage. According to a literature review, around 1% of the CNTs deposits in the passageways trans locates to the liver, spleen, and bone marrow (with the greatest quantity found in the liver). As a result, the translocated dosage of MWCNTs in extra-pulmonary organs may be too low to produce noticeable DNA damage(Møller & Loft, 2010).

The results of a meta-analysis of strand breaks in DNA and a comprehensive analysis of all types of genotoxicity in cell cultures reveal a significant degree of response heterogeneity. It is always difficult to generalize data from basic *in vitro* systems and animal models to people. In particle toxicology, it is common practice to discover indicators of genotoxicity in humans by monitored doses, such as a randomized clinical trial with appropriate contrast in exposure, such as employees and a control population(Demir & Marcos, 2018; Joshi et al., 2018; Møller et al., 2021). Several human biological monitoring studies have looked at biomarkers that may be associated with degrees of DNA damage in particle-exposed individuals, implying that they are linked in the same causal network. Significant amounts of inflammatory markers in sputum and/or blood were discovered in a study of workers at a Russian MWCNT production factory(Fatkhutdinova et al., 2016).

2.3 TOXICOLOGICAL PROPERTY OF MWCNT

Before CNTs may be used safely for targeted medication delivery, a thorough understanding of their toxicological characteristics is also necessary. To fully comprehend nanotubes' potential for targeted drug administration, it is crucial to research their toxicological characteristics, preferably at a physiologically meaningful dosage. Studies utilizing both *in vivo* (using mice and rats) and *in vitro* (using cell lines) models are often used to evaluate the human toxicity of a wide range of nanomaterials. The following objectives have been addressed in research to date, including identifying the factors that contribute to CNT toxicity: 1. Physical parameters such as diameter and length (determined by the number of walls), 2. Agglomeration status, 3. Composition

which includes contaminants and surface modifications, and 4. The potential for unexpected outcomes due to an ineffective experimental design(Johnston et al., 2010).

2.3.1 *In vivo toxicity study*

CNTs are delivered into the body by a variety of methods, including inhalation, intra-peritoneal, oral, intravenous, subcutaneous, and transdermal. Because of the more apparent and definitive outcomes in the reported tests, the most commonly stated methods of administration *in vivo* are intratracheal and inhalation. According to several investigations, inhaling CNTs causes substantial irritation as compared to intravenous injection, oral, or dermal delivery(Mohanta et al., 2019b). When CNTs enter the body, they interact with proteins or cells, which may result in metabolism or the CNTs retaining their original shape. They can also be dispersed to other organs of the body, where they can either remain local, translocate, or be eliminated.

An increasing number of studies have shown that several signalling pathways are triggered by CNT and mediate the biological response to CNT, offering fresh molecular insights into the process by which CNT causes toxic outcomes. Numerous studies have demonstrated that CNT can have a positive or negative impact on a number of signalling pathways, including the NF-kB (Nuclear factor Kappa B), NLRP3 (nucleotide-binding oligomerization domain-like receptor: pyrin domain-containing 3), inflammasome, p53, TGF-b1 (transforming growth factor-b1), and MAPK (mitogen-activated protein kinase) pathways, which are important in both physiological and pathological circumstances. Dysregulation of these pathways results in aberrant protein function and gene expression, which in turn causes the onset and progression of disease.

The NF-kB and NLRP3 inflammasome activation play crucial roles in the inflammatory reactions brought on by CNT. Under the influence of CNTs, TGF-b1 signalling induces fibrosis via inducing EMT (epithelial-mesenchymal transition) and the transformation of fibroblasts into myofibroblasts. Through mis regulating cell proliferation and death, abnormal p53 function is crucial in the development of tumours

brought on by CNT. Although no *in vivo* investigations have been done, NF-kB, p53, and/or MAPK pathways may contribute to the development of fibrosis, and NF-kB, TGF-b1, and/or MAPK activation is anticipated to be implicated in carcinogenesis after CNT exposure. In the meantime, after exposure to CNT, inflammation may start and encourage both fibrosis and carcinogenesis as a crucial and quick immunological response.

2.3.1.1 *Effect of CNTs on liver*

One of the vital organs in the body, the liver is essential for carrying out the body's many metabolic pathways. Therefore, each substance administered to the body is either directly or indirectly metabolized by the liver. Studying and examining the harmful effects of CNTs on liver tissues is so crucial. It is crucial to research how these nanoparticles are digested in our biological systems to produce simpler molecules that can be quickly eliminated from the body. However, these metabolic processes are difficult to identify or track due to a lack of analytical equipment. The build-up of CNTs in liver cells was revealed by several *in vivo* scanning techniques including TEM (Transmission electron microscopy) and SEM (Scanning electron microscopy) analyses.

MWCNTs were orally administered to Swiss albino mice in an *in vivo* experiment, and the effects on the mice were then evaluated in toxicological tests (Mohanta et al., 2019a). The liver's histopathological analysis revealed coagulation of the blood, inflammation, and an elevated level of oxygen free radicals in addition to damaged macrophage cells (Kupffer cells) (Jin et al., 2021; T. Li et al., 2021). The experimental findings suggested that liver dysfunction resulted from either acute or long-term exposure to the toxicants in CNTs. Kupffer cells were activated in the mice's liver after MWCNTs treatment.

These cells are important for maintaining homeostasis and proper functioning. When harmful chemicals come into touch with liver cells, Kupffer cells are triggered. Toxic agents that can enter either directly or indirectly cause the release of growth

factors, inflammatory and pro-inflammatory responses, and ROS (Reactive oxygen species)(Roth et al., 2020; Sun et al., 2020). The increasing number of Kupffer cells implies that CNTs are the primary source of liver damage(Mohanta et al., 2019a). Effects of CNTs caused in liver cells are explained in Figure 2.2

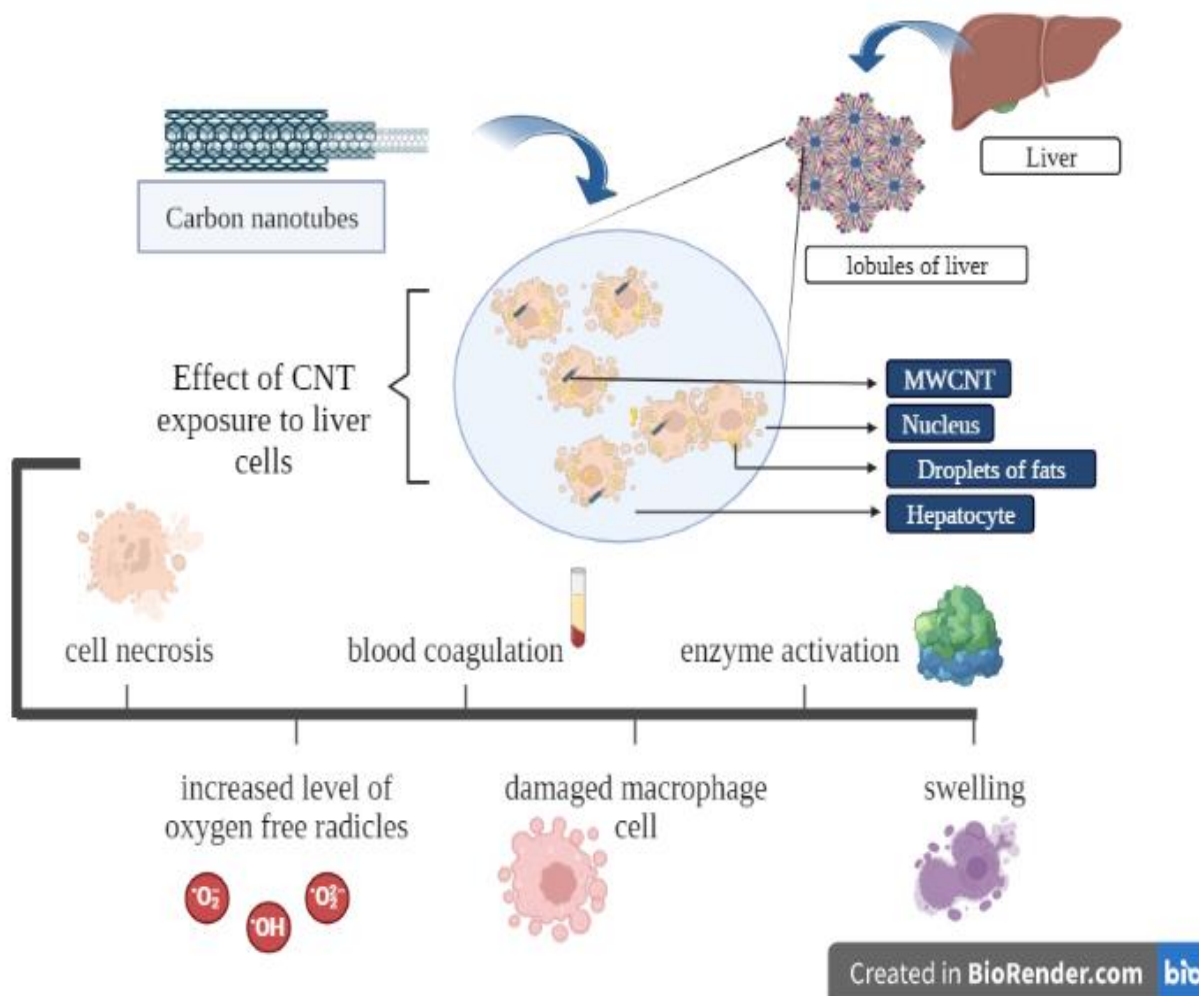


Fig 2.2: CNT toxicity in hepatocytes (created using biorender tool)

Table 2.1 *in vivo* toxicity assessment in various organism

Nanomaterials	Name of organism	Observation	Reference
MWCNTs	Mice	Liver function destruction and lipid droplet accumulation in hepatocytes	(H. Y. Zhang et al., 2018)
MWCNT	Rat	Pulmonary fibrosis	(Muller et al., 2005)
MWCNT	Mice	Inflammation and granulomas	(Poland et al., 2008)
MWCNT	Rat	Granuloma and collagen depositions	(Morimoto et al., 2012)
MWCNTs and SWCNTs	Guinea pigs	Pneumonitis	(Grubek-Jaworska et al., 2006)
MWCNT	Rat	Inflammation, granuloma, and lung fibrosis	(Francis et al., 2015)

2.3.2 *In vitro* toxicity studies

In vitro toxicity tests are a helpful tool for conducting well-controlled studies on the toxicity of MWCNTs under closely regulated environments. CNT toxicity has been investigated *in vitro* using cell types from the lung, skin, cardiovascular system, and immune system. The assessment of CNT toxicity to the lung *in vitro* has focused on the

impact of exposure on airway epithelium, as well as macrophages that reside in the lungs. A variety of cells present in the lung have been demonstrated to be susceptible to CNT toxicity, which manifests as inflammatory, reactive, and toxic. The poisonous reaction of CNTs, on the other hand, is not frequently replicated by various researchers, and could therefore be connected to the physicochemical features of the CNTs utilized, or the methodology used to test their hazardous potential(Davoren et al., 2007; Muller et al., 2005; Wick et al., 2007).

The *in vitro* evaluation of CNT cutaneous toxicity has primarily used cell lines and has mostly focused on the impact of CNTs on keratinocytes. MWCNTs may trigger an inflammatory response after dermal exposure. Two-dimensional gel electrophoresis and mass spectrometry were used to study protein expression changes associated with MWCNT exposure. Proteins involved in metabolism, cell signalling, the cytoskeleton, and vesicle trafficking were the most severely damaged(Monteiro-Riviere et al., 2005; Witzmann & Monteiro-Riviere, 2006). Several investigations have offered hypotheses for CNTs' harmful impact on heart muscles. Cells from the rat heart cell line H9c2 were treated with highly pure SWCNT in an *in vitro* study to examine cell proliferation and morphology.

Microscopic examination revealed that CNT attachment to cell walls resulted in a modest conformational shift in the cell shape. The investigation concluded that long-term CNT exposure caused obvious cardiovascular damage(Mohanta et al., 2019c).Further research is still needed to properly understand the possible toxicity of MWCNTs. The potential impact of nanoparticles (NPs) on the accumulation of lipids has recently gained research attention(Long et al., 2019). Lipids are stored in lipid droplets in mammalian cells to meet energy needs, and abnormal lipid metabolism has been linked to the emergence of chronic illnesses including atherosclerosis. MWCNT exposure has been linked to lipid buildup in macrophages, liver cells, and skeletal muscle cells, according to earlier research(Galassi et al., 2018).

Nevertheless, the chemical mechanism is still unclear, despite suggestions that MWCNT-induced oxidative stress may be involved. Although the regulation of lipid metabolism is a complicated process, it is generally acknowledged that the appropriate

functioning of the endoplasmic reticulum is crucial for the production of lipid droplets (ER)(Jena et al., 2017). Normal ER function is disturbed by ER stress, which causes lipid dysregulation and has been linked to the emergence of chronic illnesses. Interestingly, recent research has also indicated that NP exposure may be able to control ER stress. To the best of our knowledge, however, only a small number of studies have looked into whether NP can cause lipid accumulation through the modulation of ER stress, whereas many earlier studies have focused on ER stress as the mechanism for NP-induced cytotoxicity(H. Yang et al., 2019).

Given the importance of autophagy-ER stress in lipid metabolism, MWCNT exposure could also influence lipid droplets in liver cells. In a study lipid accumulation in HepG2 cells after exposure to pristine and carboxylated MWCNTs and the potential role of lipophagy pathway were investigated. carboxylation is one of the most commonly used surface functionalization of MWCNTs, particularly for bio medicinal purposes(Tsukahara & Haniu, 2011). Cytotoxicity of MWCNTs was investigated by cell counting kit-8 (CCK-8) and neutral red uptake assays, and the internalization was observed under transmission electron microscope (TEM).

Intracellular reactive oxygen MWCNT exposure may have an impact on lipid droplets in liver cells since autophagy-ER stress is crucial for lipid metabolism. In a study, the possible contribution of the lipophagy pathway and lipid accumulation in HepG2 cells following exposure to unmodified and carboxylated MWCNTs were examined. One of the most popular surfaces functionalization of MWCNTs, particularly for biomedical applications, is carboxylation(Z. Liu et al., 2014). Cell counting kit-8 (CCK-8) and neutral red uptake tests were used to study the cytotoxicity of MWCNTs, and transmission electron microscopy was used to analyse the internalisation (TEM).

Acridine orange staining was used to detect lysosomal instability, and the analysis of intracellular reactive oxygen species revealed oxidative stress (ROS). The expression of ER stress genes, including *ATG7* (autophagy related 7), *PLIN2* (perilipin 2), and *BECN1* (beclin 1), as well as lipophagic genes, including *ATG7* (autophagy related 7), *XBP-1s* (spliced X-box binding protein 1), and *HSPA5* (heat shock protein family A (Hsp70) member 5), *ATF4* (activating transcription factor 4), *ATF6* (activating

transcription factor). These genes serve as both important regulators of lipid droplet production and indicators for autophagy/ER stress(Henderson et al., 2015; C. Zhao et al., 2018).

Table 2.2 Cytotoxicity of CNTs in different cell lines

Nanomaterials	Cell lines	Observation	Reference
MWCNTs	HEK (Human embryonic kidney)	Decrease in cell viability and increase in IL-8 Cell-cycle inhibition	(Monteiro-Riviere & Inman, 2006)
SWCNTs and MWCNTs	Alveolar macrophages	SWCNTs showed higher toxicity than MWCNTs	(Jia et al., 2005)
Ground MWCNTs	Rat peritoneal macrophage	Cytotoxicity and overproduction of proinflammatory cytokines	(Muller et al., 2005)
MWCNTs	J774.1	Cytotoxic effects by the rupturing of plasma membrane	(Hirano et al., 2008)
MWCNTs	Alveolar macrophages	Cell death	(Murr et al., 2005)

Pristine MWCNTs and oxidized MWCNTs	T lymphocytes	Apoptosis	(Bottini et al., 2006)
MWCNTs	NHDF	Induction of cytotoxicity, genotoxicity, and apoptosis	(Patlolla et al., 2010)

2.3.3 Mechanisms involving with the toxicity aspects

CNTs are regarded as a potential drug delivery vehicle among the different reported vehicles (Maruyama et al., 2015). However, before they may be used for medicinal purposes, their impact on various organs must be studied. CNTs have been studied several times to understand the methods by which they infiltrate cells through the lipid bilayer of cellular walls. Several studies have suggested that endocytosis is the cellular absorption mechanism for CNTs (Jain et al., 2012; Witzmann & Monteiro-Riviere, 2006). CNT penetration into the plasma membrane of the cell generates oxidative stress, which can result in inflammation and cytotoxicity (Nikzamir et al., 2021; Saleemi et al., 2021). Because carbon nanotubes (CNTs) serve as a foreign entity to the host, the FBR (Foreign body response) is triggered, and substances are created to kill the CNTs. It has been discovered that carbon nanotubes cause oxidative stress in cells (Azizi-Lalabadi et al., 2020; Cao & Luo, 2019; Saleemi et al., 2021; Seo et al., 2018; Vijayalakshmi et al., 2022).

The key signalling mechanisms that govern cytokines in response to oxidative stress caused by CNTs are protein kinases and NF- κ B. One probable explanation for CNT cellular toxicity is the production of free radicals, which leads to oxidative stress (Malik et al., 2011; Valko et al., 2007). It is well recognised that excess free radical oxidises DNA (Deoxyribonucleic acid), proteins, and lipids in cells. They also regulate stimulating transcription and activator protein-1 that are involved in systemic inflammation (Malik et al., 2011). CNT length, metal content, ability to agglomerate, and

surface chemistry are among the characteristics of CNTs that have been identified as most likely to cause the observed toxicity. The involvement of the fibre framework in CNT toxicity is especially remarkable, as the length of nanotubes seems to be important to their hazardous potential(Johnston et al., 2010).

2.4 RECENT ADVANCES IN THE FUNCTIONALIZATION OF CARBON NANOTUBES

2.4.1 CNTs in drug administration and pharmacokinetics analysis

CNTs distinct physico-chemical properties, along with their capability of adaptation, have resulted in a wide range of applications in this interesting field. CNTs, in addition to other applications, are prospective drug carriers in target drug-delivery systems for oncology and other therapeutics(W. Zhang et al., 2011). Moreover, the capacity to functionalize their membrane with a wide diversity of biological and chemical species provides access to a wide range of medical uses. CNTs easily overcome numerous physiological barriers, slipping through the plasma membrane and entering the cytoplasm due to its nano needle structure method, allowing loaded molecules or medicines to be transported and delivered into the target region. CNTs are considered ideal candidates because of their satisfactory biocompatibility levels, threadlike structure, and large surface area, which allows for extensive modification, as well as a molecular ligand binding mechanism that allows for the transportation and distribution of drugs into the target tissue. In the medical area, CNT molecules conjugated with chitosan have very efficient drug-loading and -releasing properties that might be applied as a transdermal drug delivery system that responds to skin heat signals, sometimes known as a "patch"(Bhunia et al., 2013; Chakrabarti et al., 2015; Jhawar et al., 2013).

Since the safety concern regarding CNTs was first raised, the pharmacokinetics and bio-distribution of CNTs have been researched. The blood circulation and accumulation of CNTs in animals have an impact on their toxicity and biological applications. Long blood-circulated CNTs may accumulate more in tumors while acting

as a drug delivery system, making them effective for the detection and therapy of cancer(S. T. Yang et al., 2012). Aggregated CNTs, on the other hand, are quickly eliminated from the circulation by macrophage recognition. Their use in medication delivery is constrained by this quality. However, simply scattered CNTs might have a use in thermal therapy and diagnostics. CNTs can be identified by lymphocytes and can move via lymphatic channels. CNTs may therefore be potential materials for the essential cancer therapy technique of sentinel lymph node detection(S. T. Yang et al., 2012). Additionally, because CNTs migrate slowly, they are perfect for thermal therapy because once injected into a tumour, they remain there for a long time.

2.4.2 Tissue regeneration and artificial implantation

CNTs have remarkable thermal, mechanical, and electrical capabilities, allowing them to be used as reinforcements in a variety of materials to improve their qualities. The goal of tissue engineering is to replace damaged or diseased tissue with biological substitutes that can eventually re-establish the tissue's natural and original function(Bhunia et al., 2013). Significant advancements in this field have aided the anticipated growth of tissue regeneration engineering and medicine. The body typically rejects implants due to post-administration pain, however, carbon nanotubes easily attach to other protein molecules and can be used to implant prosthetic joints avoiding host rejection(Sahithi et al., 2010). Biomaterials containing polymers are frequently used in conjunction with osseous tissue. CNTs are often employed in osteo-biomaterials, mostly to promote better mechanical properties, and they are expected to work as scaffolds to assist and facilitate bone tissue regeneration(Gutiérrez-Hernández et al., 2017).

2.4.3 CNTs in genetic engineering

CNTs are employed in the creation of bioimaging genomes, proteomics, and tissue engineering to control genes and atoms. Gene therapy involves inserting a DNA

molecule into the cell nucleus to fix a faulty gene that causes some chronic or inherited disorders(Z. Liu et al., 2009),(He et al., 2013). Nanotubes crosslinked with DNA were revealed to liberate DNA before it was killed by a cell's defence system, resulting in a significant rise in transfection. Antiviral activity of nanostructures towards respiratory syncytial virus, a virus that causes severe bronchitis and asthma, has been discovered. Nanoparticles and gene-slicing technologies are typically used in therapy. Encapsulated within nanotubes, RNA snippets capable of blocking a protein are delivered as nasal sprays or drops. Nanotubes have been shown to aid in the helical crystallization of proteins and the formation of embryonic rat brain neurons.

2.4.4 Biomedical imaging

CNTs are fascinating nanomaterials that have a lot of potential for use in the biomedical sector. In contrast to spherical nanoparticles, needle like nanoparticles have superior flow dynamics and a greater ability to permeate cellular membranes in the field of biomedical imaging. From an imaging and therapeutic perspective, it is possible to take advantage of CNTs inherent features. SWCNTs can be employed for photoacoustic imaging and photothermal therapy because of the significant optical absorption in the near-infrared region (NIR). It is understood that owing to the emission range of SWCNTs (800-2000 nm), which falls well within the biological tissue window, it is thereafter capable of different biological imaging applications. It is because of their superior mechanical, electronic, optic, and chemical properties, carbon-based nanomaterials have become key agents for bio-imaging applications in recent years(Puett et al., 2018).

Functionalized SWCNTs coated with Ag Nps recently displayed an amazing surface-enhanced Raman scattering property, which was subsequently exploited for cell imaging(Puett et al., 2018; Ursu et al., 2017). Recent research has revealed that light with even longer wavelengths exhibits considerably lower tissue absorption and, more critically, noticeably lower scattering by biological tissues, enabling *in vivo* fluorescence imaging with increased tissue penetration and significantly higher spatial

resolutions. Principle component analysis (PCA) significantly improved the anatomic resolution of the organs. After PCA analysis of NIR-II fluorescence images of mice with SWCNTs injection, even the pancreas, which could not be resolved from real-time raw images, could be distinguished(Gong et al., 2013).

CHAPTER 3

AIM AND SCOPE

- To analyse the biophysical characteristics of MWCNTs.
- To study the cytotoxicity of MWCNT
- To understand the processes of lipid accumulation in hepatocytes.
- To investigate influence of MWCNT on the lipophagic pathway in hepatocytes
- To evaluate the biocompatibility of MWCNT on Liver cells.
- The goal of this study is to investigate the medicinal use in treatment of different health conditions.
- The purpose of toxicity testing is to develop data that can ensure appropriate protection of public health from the adverse effects of exposures to environmental agents.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

The chemicals used in the current experiments were: Pristine MWCNT (Code: 41335) were procured commercially from Sisco Research Laboratory, India of 99.99 % purity grade with no further purifications. Supplementary Table 4.1 lists their characteristics as reported by the vendor. The solvent used in this study was double-distilled water.

Table 4.1 Specifications of MWCNT provided by Sisco Research Laboratory

ITEMS	RESULT
Appearance (Colour)	Black
Appearance (Form)	Powder
Assay	min. 95%
OD	30 - 50nm
Length	10- 30 μ m

4.2 METHODS

4.2.1 MWCNT sample preparation

The pristine MWCNTs (Code: 41335) were acquired from Sisco Research Laboratories Pvt. Ltd., and Supplementary Table S1 lists their physico-chemical characteristics as reported by the vendor. MWCNTs were sonicated in MilliQ water containing utilizing an ultrasonic processor FS-250N three times for 40 minutes to create the suspensions. The suspensions were then diluted to the necessary concentrations in double distilled water

4.2.2 Cell culture and treatment

The tests employed HepG2 cells, which were purchased from NCCS, Pune. With 10% v/v fetal bovine serum and 100 U/mL penicillin, 100 µg/mL streptomycin added as supplements, Dulbecco's modified Eagle's medium/high glucose media with additives was used to sustain cell lines in tissue culture flasks. The cells were grown at 37 °C and 5% CO₂ in 95% air humidified incubator and divided when they reached high confluence (mostly twice a week). In the instance of the HepG2 cells, cells were seeded in several well plates using 8.5×10^4 cells/cm².

The cells were incubated with prepared pristine MWCNT solutions at various concentrations (5, 10, 20, 40, 80 or 100 µg/ml) for 24, 48, or 72 h as required. Control cells were incubated with double distilled water alone. The MWCNT exposure was estimated as µg/cm² growth area rather than µg/ml culture volume since culture containers of various volumes were employed during the studies.

4.2.3 Characterization studies

4.2.3.1 UV-vis spectroscopy

Ultraviolet-visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. This spectroscopy helps in identification of presence of Nanoparticles. The nanoparticles give unique absorption spectra over the range of wavelengths (Kumar, 2013; Pinheiro et al., 2004).

A double-beam UV-vis spectrophotometer (JascoV-630) in the 200–1200 nm range was used. The supernatant phase adsorption for water was measured in 1 cm quartz cell at 25 °C. These dispersions were diluted to keep their measured absorbance values in the 0.1–2 range throughout the entire wave-length range.

4.2.3.2 FTIR (Fourier Transform Infrared Spectroscopy)

FTIR is used to analyse the functional groups present in the surface of the sample. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. Fourier-transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many frequencies of light at once and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterward, a computer takes all this data and works backward to infer what the absorption is at each wavelength. In nano particle FTIR analysis will help to find out whichever chemical groups helped in the fabrication and stabilization of the nanoparticles (Latifi & Hayati, 2021).

In order to confirm any potential structural characteristics in the samples, Fourier FTIR spectrum studies of the pure MWCNT a were carried out. Using Fourier transform infrared spectroscopy, the surface of MWCNTs chemical functionalization by oxidation can be analysed. FTIR analysis was carried out on Agilent Cary 630 FTIR in mid-infrared range of $7000\text{--}350\text{ cm}^{-1}$. FTIR spectra of powdered MWCNT ground in KBr pellet were captured, with 200 scans averaged at a resolution of 1 cm^{-1}

4.2.3.3 Raman spectroscopy

In Raman spectroscopy, vibrational modes are identified by measuring the energy of scattered photons generated from a sample exposed to intense laser light. Raman spectra of MWCNTs typically consist of a graphitic or G-band, from highly ordered MWCNT sidewalls, while disorder in the sidewall structure results in a D-band. By determining the ratio between these two bands ($I_D:I_G$), a quantitative measure of defect density in the MWCNT sidewall can be determined. Consequently, $I_D:I_G$ band analysis can be used to obtain information regarding structural changes as a result of functionalization. Unfortunately, many MWCNTs can have amorphous carbon adsorbed on the sidewalls, which also contributes to the D-band. In the absence of

amorphous carbon, however, $I_D:I_G$ ratios can be very useful for determining changes in structure that occur as a result of functionalization strategies, such as those that involve the attachment of organic moieties (Costa et al., 2009; Curran et al., 2005; Kumar, 2012).

Prepared powder samples of MWCNT were subjected to Raman spectra and defect intensities of the nanomaterial were evaluated using a 785 nm Micro-Raman Spectrophotometer (Renishaw, UK, Invia0719-17) in spectral frequency 100 to 3,500 cm^{-1} with spectral resolution of 0.75 cm^{-1} (FWHM)

4.2.3.4 *Transmission electron microscope*

Transmission electron microscope (TEM) was used to investigate the internal structures of pristine MWCNTs by placing droplets of finely prepared ethanol-dispersed samples on the carbon-coated copper grids TEM uses a high-energy electron beam (up to 300 keV) to image MWCNTs based on electron transmission. The length and dispersion state of MWCNTs can be assessed by TEM at low magnification. At high magnifications, TEM can be used to count the number of walls in MWCNTs, measure inner and outer diameter, assess structural integrity, and identify structural changes caused by surface modification. Although TEM is capable of imaging the structure of MWCNTs, including the presence of sidewall damage, the electrons used for imaging can themselves also cause sidewall damage and structural modification. To avoid this problem, it has been recommended that analysis areas only be exposed to the electron beam for very short durations (<15 s), and that beam energies should be limited to 100 keV. As in many imaging techniques, verification that an observed structure remains unchanged with respect to irradiation time is the best way to ensure that the analysis technique is non-destructive (Ghasempour & Narei, 2018; Hassan et al., 2016; Hwang et al., 2007).

For the analyses, the powdered samples were deposited on carbon-coated, copper grids [300 mesh size, regular grip, spi 2030 C] and allowed to dry overnight.

The resulting samples were viewed in HR-TEM [Talos F200S G2 TEM] at a frequency of 80 Hz.

4.2.3.5 Field Emission Scanning Electron Microscopy

The surface topography of the membrane top-layer and pore shape of the powdered MWCNT was observed using FE-SEM [EVO-60, Zeiss company]. It is also used to analyses the distribution or dispersion of MWCNTs. The powdered samples of MWCNT were drop-casted on to the surface of a glass coverslip and dried overnight. The resulting samples were viewed under an FE-SEM at an operating voltage of 20 kV. The particle size of the MWCNT was calculated using a technique for determining the layer thickness and the particle diameter based on the use of a raster scanning mode (Daraei et al., 2013; Kapoor et al., 2017).

The analysis was done by FE-SEM EVO-60, Zeiss company]. The changes in membranes top-layer thickness were monitored by FESEM microphotographs in different casual locations of membrane cross section and the average measurements was reported.

4.2.3.6 X-Ray diffraction analysis

The X-Ray diffraction (XRD) was used to ascertain the crystallographic properties of the MWCNTs. The radiation used was Cu K α source at 1.8 kW power in a theta/theta scan mode with a wavelength of 0.15406 nm to determine the crystalline structure and lattice planes in the material. The samples were scanned at diffraction angle ranged from $2\theta = 5^\circ$ to 80° (the step size is 0.025 degree/step) with exposure time 0.1 s/step. The applied voltage and current were 40 kV and 40 mA, respectively. In order to avoid systematic errors from misalignments and thermal expansions of the sample holder, the 2θ scan data were corrected using the Nelson–Riley (NR) approximation (Latifi & Hayati, 2021). The Nelson–Riley method is an extrapolation procedure where the systematic error introduced by the eccentricity of the sample with

respect to the rotation axis in the diffractometer is corrected. This eccentricity has been found to be proportional to $\cos^2(\theta)$. For the correction, the calculated lattice parameter “a” from the raw data is plotted against $\cos^2(\theta)/\sin(\theta)$, which is a widely used function for cubic systems. The obtained points are later linearly fitted and the intersection with the y-axis provides the corrected lattice parameter (Suárez et al., 2013).

XRD analysis was carried out on ARL EQUINOX 3000. Different lattice planes against specific diffraction angles were calculated and used to determine the intrinsic details of the structure based on the diffractogram.

4.2.3.7 *PSA and Zeta potential*

Dispersion efficiency could be evaluated by zeta (ζ) potential analysis. Zeta potential is a convenient tool and gives valuable information about the charges (magnitude of electrical double layer) around particles which are accounting for repulsion between similarly charged particles and hence indicates degree of dispersion efficiency. Combining zeta potential measurements with UV–Vis spectroscopic results could yield valuable information in finding optimized dispersant to MWCNTs ratio (Tantra et al., 2010). The z-average hydrodynamic particle diameter $d_{h,z \text{ ave}}$ is obtained from the cumulant analysis of the normalized electric field autocorrelation function. It is calculated by the Stokes–Einstein equation with the diffusion coefficient determined from the decay time of autocorrelation function. In addition, there is certain probability that the light scattered by larger particles will swamp the light scattered by smaller particles since the light intensity of a scattered particle is proportional to the diameter in the sixth power. That means that larger particles are overestimated in the dispersions. For this reason, the change of $d_{h,z \text{ ave}}$ with ultrasonic treatment time shows only a trend in the change of particle size (Krause et al., 2010).

The particle size and zeta-potential of the powdered MWCN at room temperature were analysed using a Horiba nanoPartica SZ-100V2 [Horiba, Kyoto, Japan]. The dried samples were first adequately diluted in Phosphate Buffer Saline solution [0.15 M, pH 7.2]. Aliquots of the MWCNT, at a concentration of 40 $\mu\text{g/ml}$ from

a stock [1 mg/ml], were subjected to further analysis under nanoparticle analyser. Particle size distribution was determined at a scattering angle of ~90 °C.

4.2.4 Cytotoxicity analysis

4.2.4.1 MTT assay

The MTT assay was used to determine the cytotoxicity of pristine MWCNTs. Briefly, HepG2 cells (NCCS, Pune, India) were cultured in DMEM with glutamax and high-glucose medium supplemented with 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% FBS. The cells were cultured at 37 °C and 5% CO₂ in 95% air humidified incubator to near confluence and were deprived of serum for 18 h they were stimulated with agonists. The harvested cells are sub-cultured using 0.25% trypsin, 0.53 mM EDTA and then were seeded overnight into fat-bottomed 96-well plates at approximately 1×10⁶ cells/well. A series of dilution of pristine MWCNT solution (5, 10, 20, 40, 80, 100 µg/ml) in medium was added. After 24 h of incubation, medium was removed where 20 µl of MTT (5 mg/ml in PBS) was added to treat the cells in fresh medium and was kept further in dark for 2 h of incubation. After 2 h, formazan crystal was formed in each well and solubilized in 100 µl/well of DMSO and absorbance was read at 570 nm, using a (Spectramax M5, Sunnyvale, CA). Percent cytotoxicity was expressed as IC₅₀ (concentration of MWCNTs to induce 50% cell mortality).

Cell viability percentage was calculated and the result was expressed as percent mean of viability±standard error of the mean (Sharma et al., 2020; Vijayalakshmi et al., 2023)

Viability (%) = (Mean absorbance of treated cells/Mean absorbance of control) × 100

Cytotoxicity (%) = 100 – Viability (%).

Cell relative survival rate (RSR) and relative growth rate (RGR) were calculated according to the following equations:

$RSR = \{ \text{absorbance value (experimental group)} / \text{absorbance value (control group)} \} \times 100\%$

$RGR = \{ \text{average absorbance value (experimental group)} / \text{average absorbance value (control group)} \} \times 100\%$

4.2.4.2 *Acridine orange*

Acridine orange is a dye which, when irradiated with intense blue light, causes damage to the lysosome's membrane. Acridine orange is a weak base metachromatic dye capable of crossing plasma membranes and staining nucleic acids and lysosomes. At low concentrations, it can differentiate lysosomes (reddish-orange granules) from other cellular components (diffuse green). Acridine orange molecules become protonated under acidic conditions and hence get trapped within lysosomes. Accumulation of acridine orange molecules in lysosomes leads to a shift in excitation from green = 530 nm to red = 620 nm. When pH of the lysosomes rises or if their membranes are damaged, acridine orange molecules become deprotonated, and these molecules can then cross back into the cytoplasm. This shifts the emission back from red to green. Therefore, extended exposure (>1 min) of acridine orange loaded cells to blue light leads to lysosomal membrane damage and a shift in the lysosomes' colour from red to green (Hamilton et al., 2012; Sohaebuddin & Tang, 2013).

Acridine orange staining was used to assess the lysosomal destabilization. Briefly, 1×10^6 per well HepG2 cells were seeded in 96-well black plates and grew for 2 days prior to incubation with 40ug/ml concentration of MWCNTs. After 24 h, the cells were incubated with acridine orange (Sigma-Aldrich, USA) as described earlier.

4.2.4.3 *Intracellular ROS analysis*

Intracellular ROS production was measured using the oxidation-sensitive fluoroprobe 2',7'- dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich). DCFH-DA is

a nonfluorescent compound that is freely taken up by cells and hydrolysed by esterase that remove the DA group. DCFH is then oxidized to fluorescent dichlorofluorescein (DCF) by cellular oxidants, thereby indicating the level of intracellular ROS (Karlsson et al., 2008; X. ming Tan et al., 2009a).

The DMEM medium was removed from the wells in the 96-well plate, and particle suspensions were added to a final concentration of 20 or 40 $\mu\text{g}/\text{cm}^2$ (40 and 80 $\mu\text{g}/\text{mL}$). Briefly, 1×10^6 per well HepG2 cells were seeded on 96-well black plates and grew for 2 days. The cells were then exposed to the particles for 4 h in a humidified atmosphere at 37 °C and 5% CO_2 . After exposure, the particle suspensions were removed, and the cells were washed with DMEM medium. Thereafter, 40 μM DCFH-DA diluted in 37 °C DMEM was added to each well, and the cells were incubated for 30 min. The DCFH-DA was then removed, and each well was washed with 400 μL PBS (37 °C). Two hundred microliters of PBS were added, and fluorescence was measured in a computerized micro plate fluorometer. During the whole procedure with DCFH-DA, the plate was kept out of light to minimize the fading of the fluoroprobe.

4.2.4.4 *Oil red o staining*

The total intracellular lipid content was evaluated by Oil Red O staining. 1×10^6 per well HepG2 cells were seeded on 6-well plates and grew for 2 days before exposure to 40 $\mu\text{g}/\text{mL}$ pristine MWCNT for 24 h. As positive control, the cells were also exposed to 1 mM free fatty acids (FFA, oleate:palmitate 2:1; Sigma-Aldrich, USA) for 24 h. To figure out the possible role of lipophagy pathway, HepG2 cells were also pre-incubated with 10 mM NH_4Cl or 100 nM bafilomycin for 1 h before treatment by MWCNTs. The cells were thoroughly rinsed by using Hanks solution, and fixed by using 4% paraformaldehyde for 10 min at room temperature. The fixed cells were rinsed by using water and 60% isopropanol, respectively, followed by staining with 2.1 mg/mL oil red (prepared in 60% isopropanol; Sigma-Aldrich, USA) for 10 min. After rinsed twice, the oil red incorporated into cells was dissolved in isopropanol, and the OD values were read at 500 nm by an ELISA reader (Leng et al., 2018; J. F. Liu et al., 2011).

TG content was measured using a colorimetric assay. To remove glycerol, the cells were washed with PBS twice before being dissolved in standard diluent. The mixture was divided into two parts; 500 µl of the mixture was centrifuged at 2000 rpm for 10 min and the supernatant was collected. The absorbance of this dye is proportional to the concentration of TG present in each sample, and the absorbance was quantified at 550 nm following these reactions. The protein content of the remainder of the mixture was measured according to BCA methods. All samples were tested in duplicate, and the TG values were expressed as mmol of TG/g of protein (Bernardi et al., 2018; Jiang et al., 2016).

4.2.4.5 DAPI staining

Changes in cell morphology characteristic of apoptosis were examined by fluorescence microscopy of DAPI-stained cells (4',6-diamidino-2-phenylindole). Nuclear condensation and fragmentation can also be measured by nucleic acid staining with DAPI. HepG2 cells were cultured on a glass cover slip at a density of 1×10^6 cells per well in a 4-well dish. The cells were supplemented with 10% fetal bovine serum in RPMI-1640 medium-containing 1% penicillin, streptomycin, and incubated for 24 h at 37 °C under humidified 5% CO₂ atmosphere.

The cells were treated with MWCNTs samples at 40 µg/mL for 24 h. The cells grown on the cover slip were washed thrice with 1× phosphate-buffered saline (PBS) and fixed in 4% para formaldehyde for 20 min, and stained with DAPI after washing again with 1× PBS twice, and after rinsing it with 1×PBS, it was mounted on the glass slide using Vectashield mounting medium (Vector Labs). The images were captured on confocal microscope system (Zeiss LSM780NLO). The percentage of apoptotic nuclei was determined by [% of apoptotic cells / (total number of apoptotic nuclei/total number of cells counted) x100](R. H. Lee et al., 2015; Rohit Singh & Ezhilarasan, 2019).

4.2.4.6 *Fluorescent Microscope*

Fluorescent images were acquired using a Zeiss LSM 710 confocal laser scanning unit (Oberkochen, Germany) equipped with argon and an HeNe laser mounted on an Axio Observer Z1 Inverted Microscope. Hep2G cells or human hepatocytes from the suspension were briefly rinsed with PBS and fixed in 4% formaldehyde (pH 7) for 15 min. After three washing procedures in PBS for 15 min, the slides were covered for 60 min with a serum-free blocking buffer. The dyeing procedures were made in accordance with manufacturers' protocols. Specific visualization of cell structures was performed using DAPI (BP 385–470 nm emission), AO/PI (BP505–550 emission) and DCFH-DA (BP 590–650 nm emission)

4.2.4.7 *Statistical analysis*

Error limits cited and error bars plotted represent simple standard deviations of the mean using Microsoft excel. When comparing different samples, results were considered to be statistically significant when $P < 0.05$ (Student's t test for unpaired samples)

CHAPTER 5

RESULTS AND DISCUSSION

5.1 CHARACTERIZATION STUDIES

5.1.1 UV-visible spectroscopy

Powdered form of samples was used for analysis. The instrument used is JascoV-630, Japan, the commercially acquired MWCNT was analysed using UV Vis spectroscopy at the range of 200 to 1200nm. The MWCNT showed a maximum of absorbance (OD) at 1.4. Fig. 5.1 represents the UV-Vis absorption spectra of the pristine MWCNT nanoparticle. The nanoparticle gave a sharp absorption peak at 200nm. This indicates the presence of nanoparticle in the solution. Intensity of the peak in spectrum could be used to quantify the amount of individual MWCNTs in the solution. It could be observed that with increasing amount of MWCNTs in the solution, absorptivity peaks increased referring to larger number of individual CNTs.

Studies from (Zaman et al., 2020) stated that hypochromic shift is observed in the 200–250 nm spectral region of the absorbance spectrum of MWCNTs which is the result of π - π stacking interaction of dispersant with nanotube surface. CNTs exhibit broad absorptions at the UV region due to Plasmon resonances in the free electron cloud of π electrons and this adsorption has a long tail extending beyond near infrared region of the spectrum. Another study reported absorbance value at 253nm in which individual CNTs are active in the UV-visible region and exhibit characteristic bands corresponding to additional absorption due to 1D van Hove singularities(Goyanes et al., 2007). Therefore, it is possible to establish a relationship between the amounts of CNTs individually dispersed in the solution and the intensity of the corresponding absorption spectrum. Moreover, UV-visible spectroscopy can be used to monitor the dynamics of this CNT dispersion process (Hwang et al., 2007).

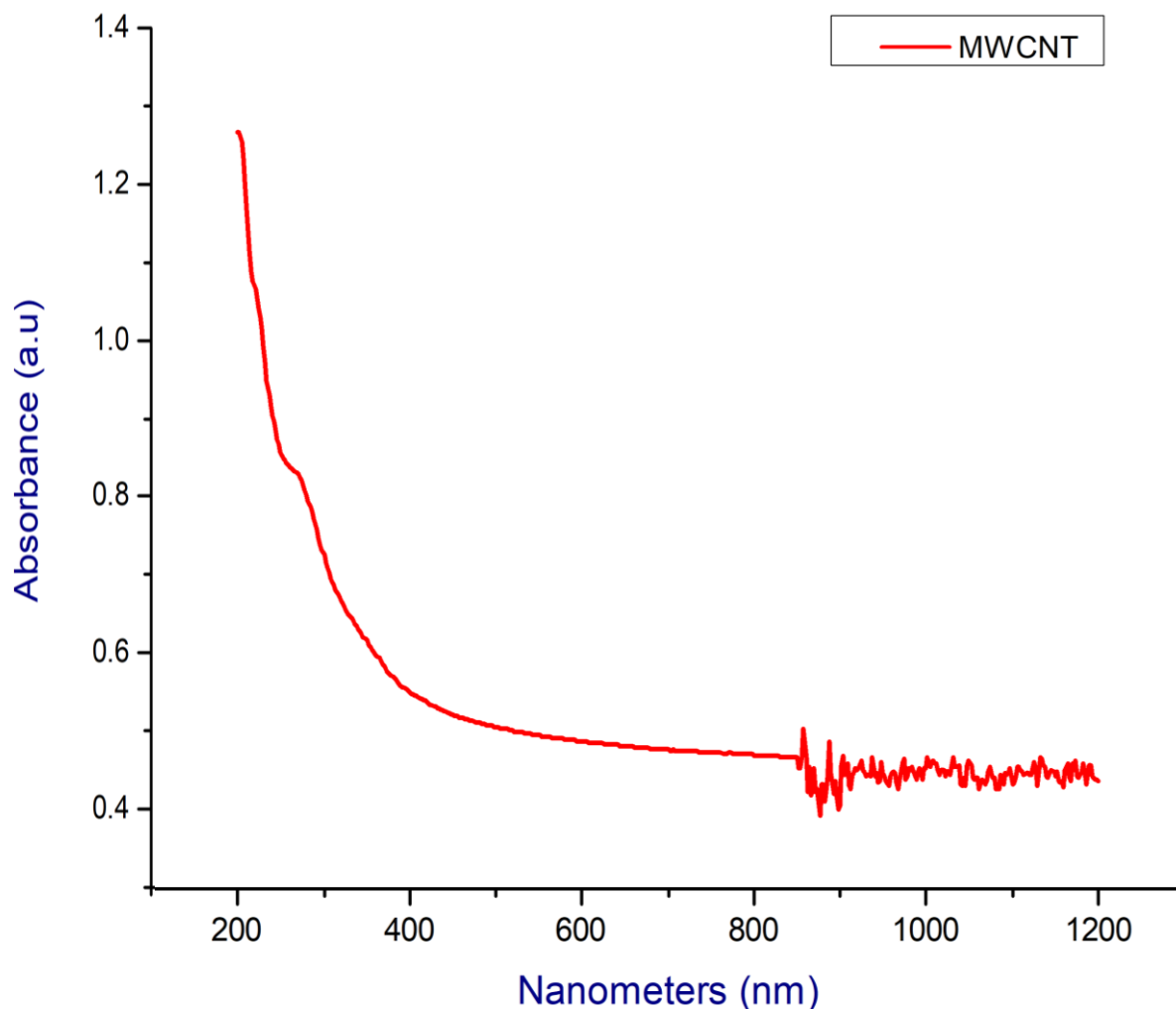


Fig 5.1: UV-visible spectroscopy of pristine MWCNT

5.1.2 FTIR (Fourier Transform Infrared Spectroscopy)

In nano particle FTIR analysis will help to find out whichever chemical groups helped in the fabrication and stabilization of the nanoparticles. For the analysis the sample is given in the powder form. The instrument used is Agilent Cary 630 FTIR in mid-infrared range of $7000\text{--}350\text{ cm}^{-1}$. Fig. 5.2 indicates the FTIR analysis of the pristine MWCNT. Powdered form of samples was used for analysis.

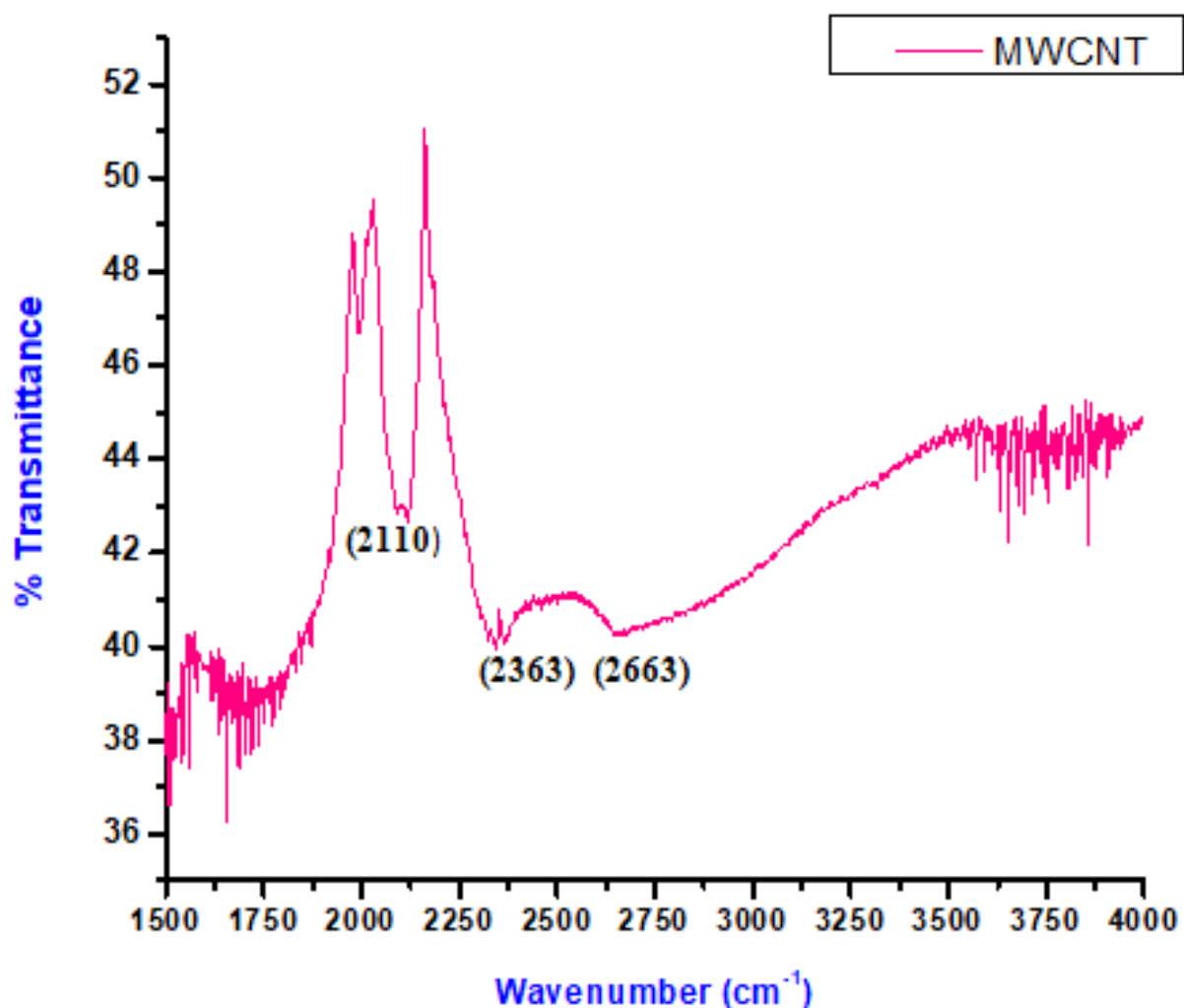


Fig 5.2: FTIR analysis of pristine MWCNT

The results show various chemical group involved in the formation of nanoparticles. The peaks at $\sim 2363\text{ cm}^{-1}$ and $\sim 2110\text{ cm}^{-1}$ indicates the presence of functional groups of C-O. In pristine MWCNTs, this stretching could be due to the partial oxidation of outer surface during their purification that led to the formation of carboxyl groups ($\text{O}=\text{C}-\text{OH}$ and $\text{C}-\text{OH}$) or due to the presence of unsaturated H_2O molecules in the KBr powder. The peak at $\sim 2663\text{ cm}^{-1}$ corresponds to C-H vibrations involved in stabilization of nanoparticle confirming the peak was either due to the presence of

unsaturated water molecules from KBr or due to the formation of carboxyl groups during their refinement by the manufacturer.

Studies have similar results in which a broad peak in the spectra of MWCNTs were observed around 2445 cm^{-1} . Another noticeable peak was observed at 2715 cm^{-1} . Small asymmetric stretching at 2260 cm^{-1} and 2320 cm^{-1} were observed due to methyl and methylene stretching band, respectively. Such bands are due to the presence of these groups on the sidewalls of MWCNTs as defects (Gaurav and Singh 2019).

5.1.3 Raman spectroscopy

Raman spectroscopy is an important analytical tool that provides both qualitative and quantitative data on nanomaterials based on its chemical composition and structure. The Raman plot in Fig. 5.3 contains the I_D/I_G ratio of ~ 1.17 , which is characteristic of powdered pristine MWCNT. The defect ratio represents the compromised proportion and lesser quantified nature of the available defects present in the MWCNTs. More specifically, it indicates the presence of a slightly lower number of dangling bond associations in the native structure of the MWCNTs, compared to the previously reported MWCNTs forms that have a value of 1.25.

Such observations indicate a more conductive nature for pristine MWCNT, relative to its other counterparts [COOH-MWCNT, etc.], making it suitable as an inhibitory anti-cancerous agent that can be used against a variety of cancer cells (Curran et al., 2005). Further, from the Raman analyses it is becoming evident the nature of the symmetry of the available D and G bands, which in return dictates the formation of the defects associated with the pristine MWCNT. Such defected symmetry is mainly occurring due to the presence of available sp^2 hybridization of the carbon architecture in the MWCNT. As it has been reported previously that G band peak refers to the symmetry of the carbon atoms whereas D band peak refers to the disorder induction in the available carbon atoms. This is in agreement with a previous work performed by Li

and co-workers in which the I_D/I_G ratio is 1.2. No significant shifts were observed in the peaks position (Y. Li & Cao, 2018).

However, the D band intensity has increased (Q. Li et al., 2008). A similar shape was found on the Raman spectra of synthesized MWCNT. In a recent work performed by Costa *et al.* the Raman spectra of CNTs with different stacking degrees were compared. An extra peak on the G_0 band profile was also observed and explained as a stacking effect in graphite. The two peaks observed in the G_0 band are assigned to the splitting in the p-electrons dispersion (Costa et al., 2009).

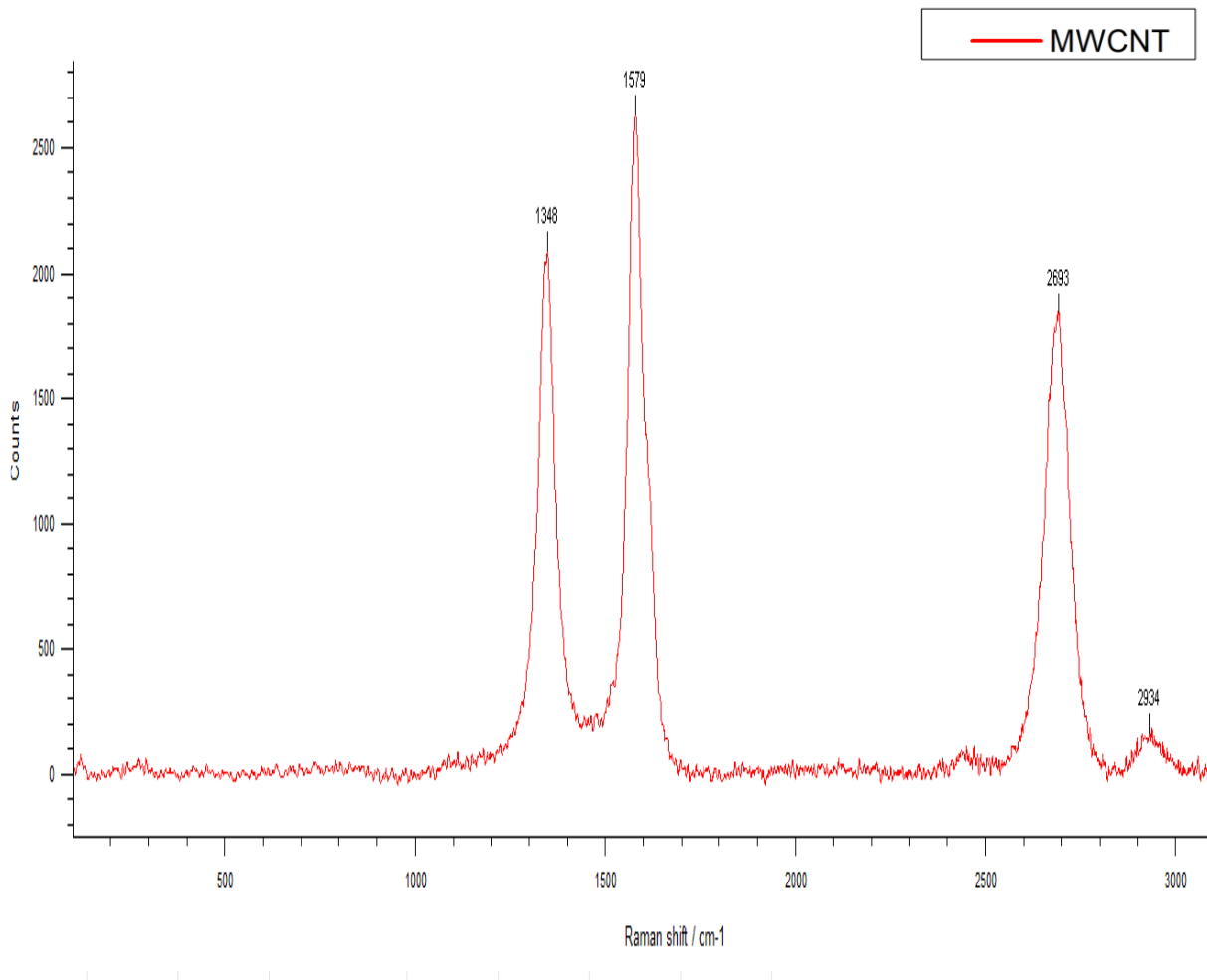
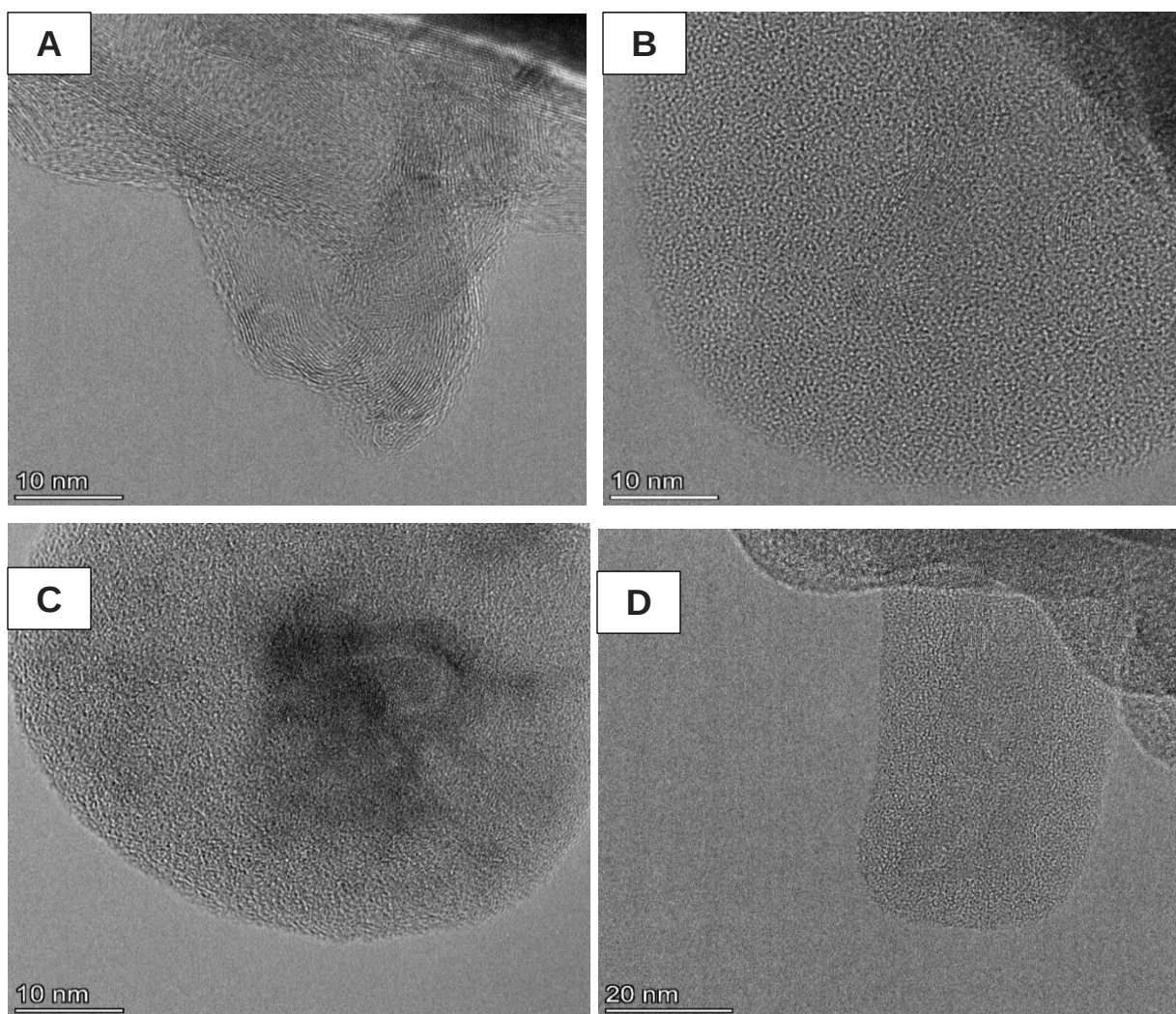


Fig 5.3: Raman spectroscopy of pristine MWCNT

5.1.4 Transmission electron microscope

The primary purpose of the High Resolution-Transmission Electron Microscopy (HR-TEM) analysis was to observe high magnification images of the internal structure of the MWCNT and gather information related to the crystalline structures, internal fractures, stress, and contamination of the test sample. Information on the internal structure allows one to have greater confidence in the potential application of the nanomaterial and predict potential effects on the target system. HR-TEM observations indicated that the internal structure of the prepared MWCNT possesses a tubular morphology.

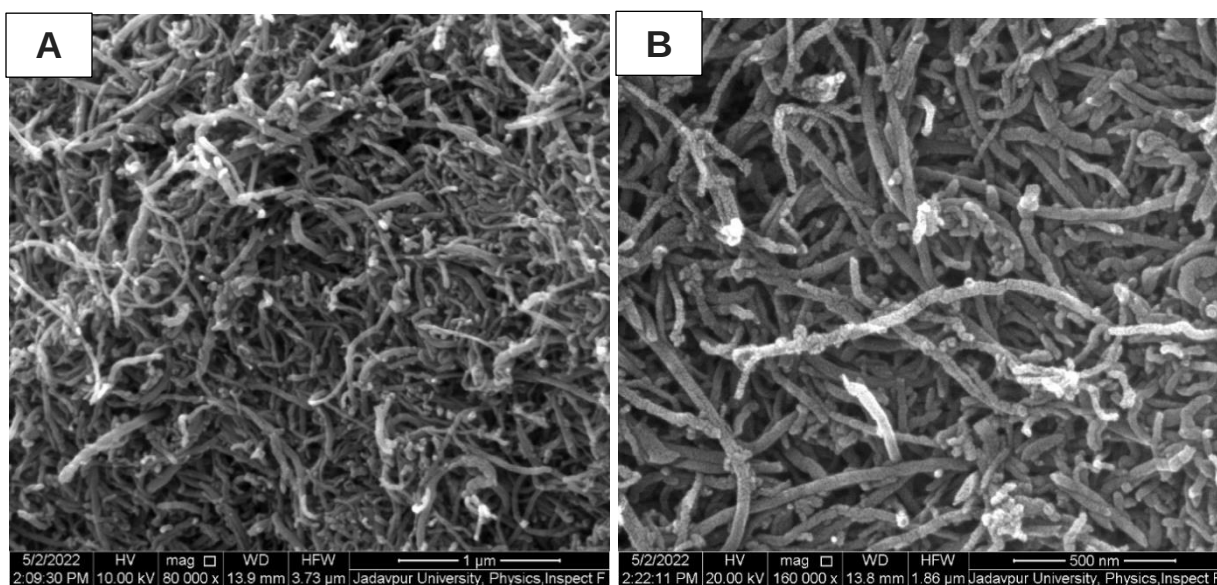


**Fig 5.4: TEM images of pristine MWCNT; A, B, C of scale bar = 10 nm;
D of scale bar = 20 nm**

The presence of undulating rolls and rough edges in the carbon nanotubes are evidence of the presents of MWCNT as previously noted by several authors. HR-TEM imaging enables one to visualize the internal, structural architecture of the prepared MWCNT, which is not possible by FE-SEM. In contrast, HR-TEM provides a clear picture of the internal structure of MWCNT at a resolution of 2 microns. The layers of the MWCNT can be readily seen in Fig. 5.4. The lateral thickness of the MWCNT was ~ 9 nm. (McClory *et al.*, 2010; Ruelle *et al.*, 2009)

5.1.5 Field emission scanning electron microscope

Scanning electron microscope is used to know the size, shape and morphology of the synthesized nanoparticle. The image shows the approximate size and the morphology of the nanoparticle. Data on the surface morphology is highly useful regarding the potential use of the nanomaterials in medical devices and for other applications. The typical surface topography of MWCNT was evident in the FESEM analysis (Fig. 5.5). Surface undulations were evident in MWCNT (Fig. 5.5), as well as rough edges. Undulations and rough edges are the result of reaction kinetic phenomenon of MWCNTs (S. H. Lee *et al.*, 2008; Park & Choi, 2009)



**Fig 5.5: FESEM images of pristine MWCNT; A of scale bar = 1 μ m;
D of scale bar = 500 nm**

5.1.6 X-Ray diffraction analysis

The X-Ray Diffraction [XRD] analysis (Fig. 5.6) revealed the presence of lattice planes that are characteristic for powdered MWCNT. Notably, (002), (100), (004) and (110) lattice planes at a diffraction angle of $\sim 10^\circ$ were evident. These characteristic lattice planes indicate the nature of the available crystallinity of MWCNT. The polycrystalline nature of MWCNT is evidence that such lattice planes are present and were corroborated by the TEM pattern. It is usually noticed from the X-Ray Diffraction studies that different phase purity of the synthesized MWCNT at the different amount of KMNO_4 reagent as oxidizing agents

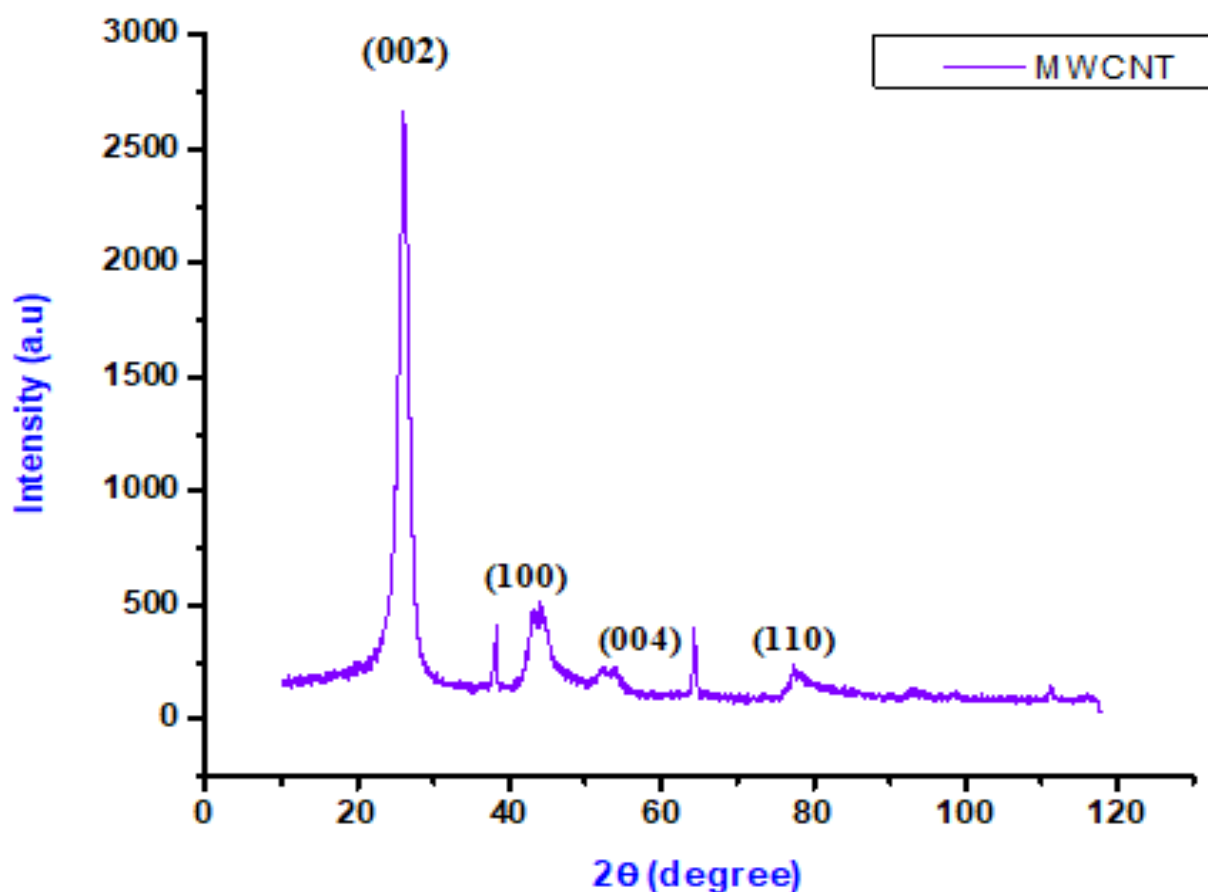


Fig 5.6: XRD pattern of pristine MWCNT

The differential peak of lattice planes as evident from the Fig. 5.6, is mainly because of pristine MWCNT. The crystalline lattice planes at around (002) demonstrates the formation of well-ordered crystalline nature of the. The studies explain the interlayer spacing distance of 0.19 nm, which is in parity with previous findings in which the strongest and sharpest diffraction peak for all samples are within $2\theta = 25.85^\circ$ which labelled as d (002). Compared to normal CNTs, $2\theta = 26.5^\circ$, this peak shows a downwards shift, which denoted an increase in the sp² C=C layer spacing. Besides, there is also a small diffraction at $2\theta = 43.0^\circ$ for all samples of MWCNTs (Pisal et al., 2014).

5.1.7 PSA and Zeta potential

MWCNT exhibits a ζ - potential of -14.9 mV, which is thought to be the most colloidal stable form (Fig. 5.7). An augmented cytotoxic effect against cancerous cells is observed by MWCNT due to its surface anionic charge. The percolation tendency of MWCNT may be attributed to the average size and overall tension occurring in the membranes of cancerous cells, which ruptures the membrane and allows entry of the material into the cell (Das et al., 2015).

Endocytosis appears to be the prime mechanism underlying the entry of MWCNT into cells, however, further validation is required to confirm the mechanism by which MWCNT enter and exit living cells. The interface of MWCNT with biological cells is also attributed to the electrical disruptions that occur at the material-bio interface. In this regard, the dimensions, size, shape, and intrinsic chemical composition of MWCNT play a significant role in the net cytotoxicity of the nanoparticles in biological systems. Colloidal stability of the MWCNT was observed, which determines the surface charge of the powdered nano tubes. The average colloidal stability of the material was determined to be ~29.3 mV.

Colloidal stability has been observed in this study which determines how much is the surface charge existing over the powdered sample when experimented. It is clearly understood that the MWCNT of differential particle distribution and colloidal

stability makes it a potential candidate for the net electrostatic interaction between the material and the cellular architecture. The colloidal stability also enables to understand the material's thermodynamical free energy associated with its intrinsic atomic configuration which makes it a suitable biomaterial when tested into different biological systems safely (Krause et al. 2010).

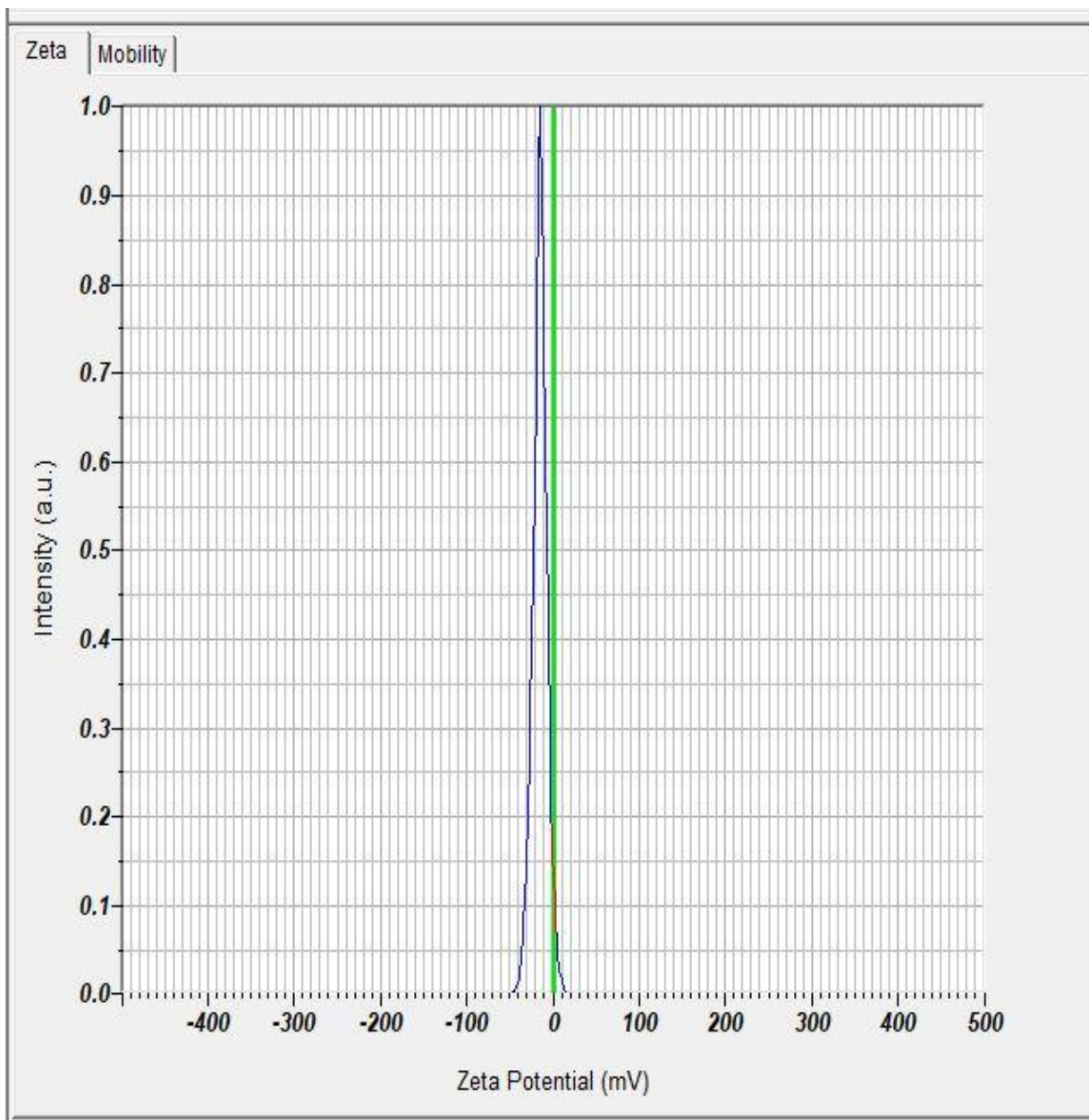


Fig 5.7: Zeta potential of pristine MWCNT

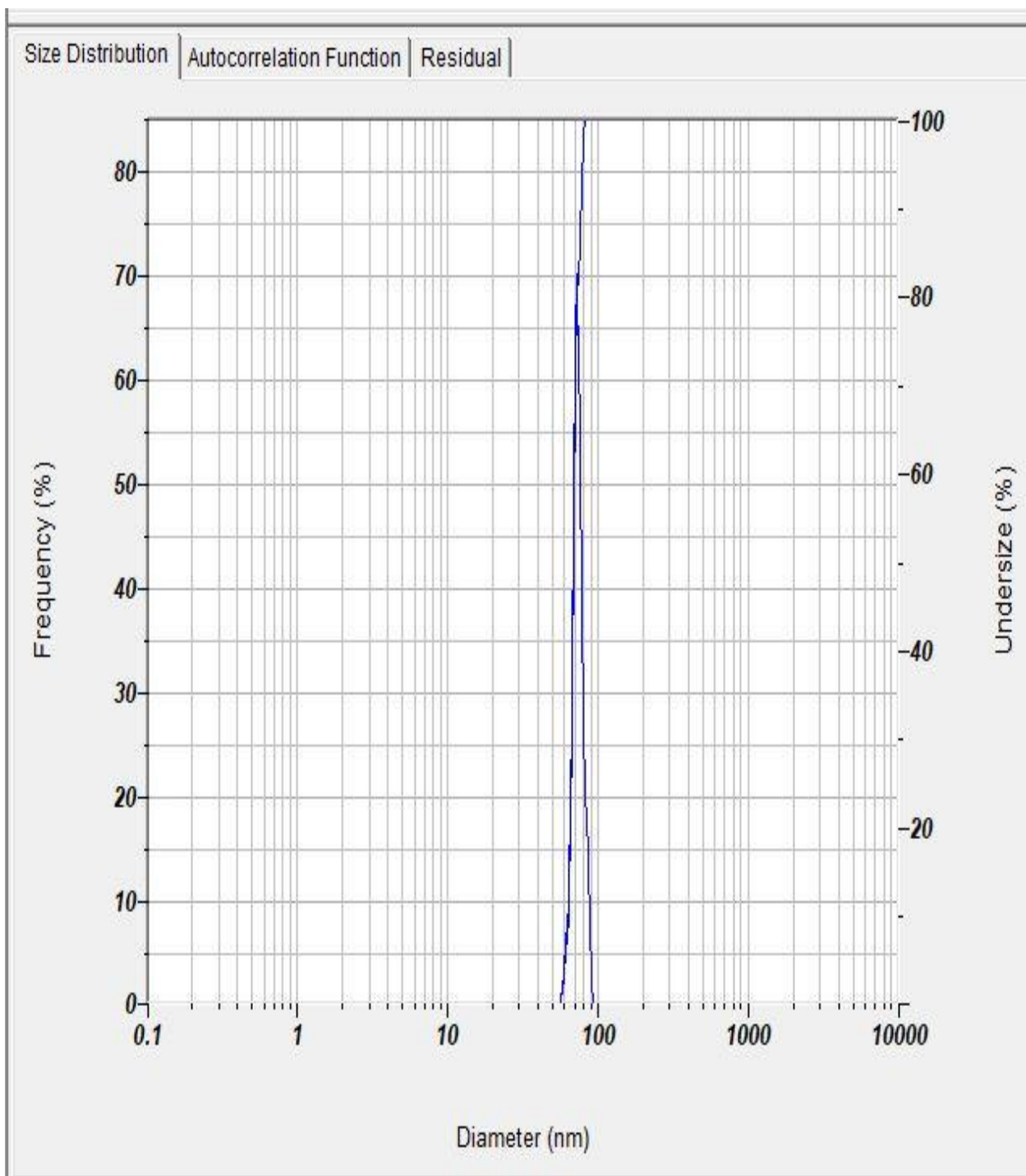


Fig 5.8: Particle size analysis of pristine MWCNT

5.2 CYTOTOXICITY ANALYSIS

5.2.1 MTT assay

After successful characterization of pristine MWCNTs, cytotoxicity has performed *in vitro* MTT assay study in HepG2 cell line by spectrophotometric absorption at 570 nm. The results showed that the viability of the HepG2 cells line was influenced by various concentrations (5, 10, 20, 40, 80, 100 µg/ml) of pristine MWCNTs drug solution. After incubation, cell viability was evaluated by applying the 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium.

The data obtained showed that there was statistically significant difference on cell viability at 5, 10, 20, 40, 80, 100 µg/ml doses compared to the control group as shown in Fig. 5.9 On the other hand, there was a statistically significant decrease in cell viability of the group exposed to the highest dose of 100 µg/ml. Fig. 5.10 shows the Dose-response curve of MWCNT against HepG2 cells that results in reduced the number of viable malignant HepG2 cells in a dose dependent manner. Fig. 5.11 is the microscopic images of HepG2 cells when treated with different concentrations of Pristine MWCNT. Furthermore, the prepared exhibited an IC₅₀ value of 69.82 µg/ml for 24 h as shown in Fig. 5.12. Similar observation was seen where IC₅₀ value was found to be 72.97 µg/ml (J. M. Tan et al., 2014; Yuan et al., 2011, 2012).

This observation is in accordance with the cytotoxicity studies of Lei *et al* performed in A549 and HepG2 using pristine MWCNTs at three concentrations (40, 200, and 400 µg/mL). Their findings suggested that that MWCNT demonstrated significant cell growth inhibitory to A549 cells and moderate cell growth inhibitory activity to HepG2 in a concentration-dependent manner. The IC₅₀ of MWCNT in A549 and HepG2 cell lines was 52.7 and 71.0 µg/mL, respectively. And results showed that the viable cells were reduced by 40% at a concentration of 40 µg/ml. However, their lactate dehydrogenase results demonstrated that there was no significant cellular lactate dehydrogenase release, indicating that the membrane of the cells was not damaged (Lei *et al.*, 2009)

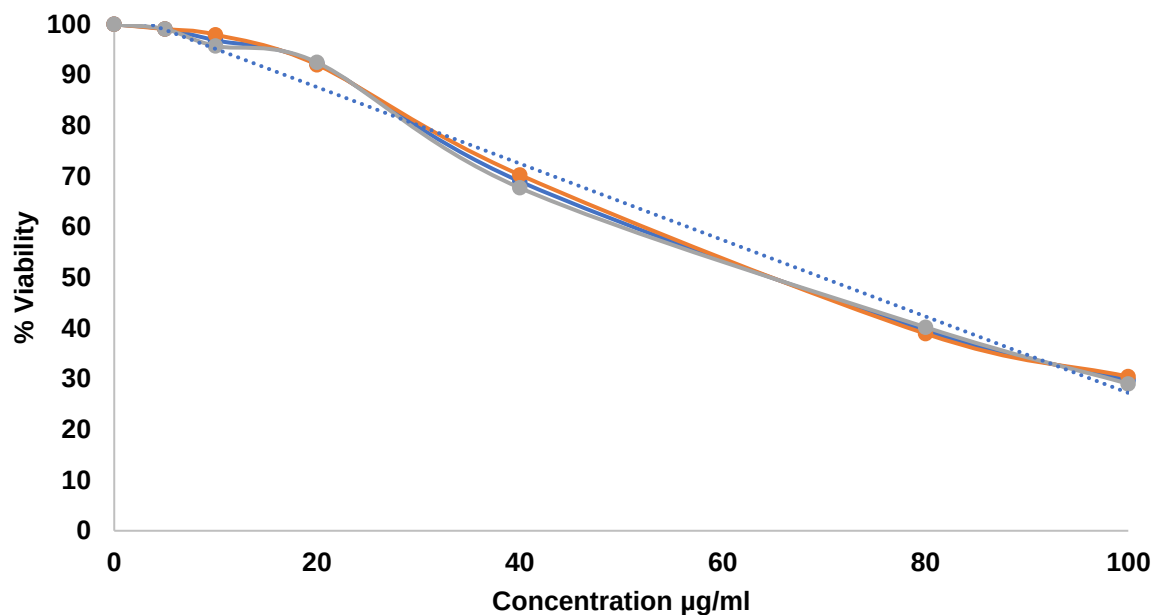


Fig 5.9: % Cell Viability calculated based on MTT absorbance value of HepG2 cell line cultures.

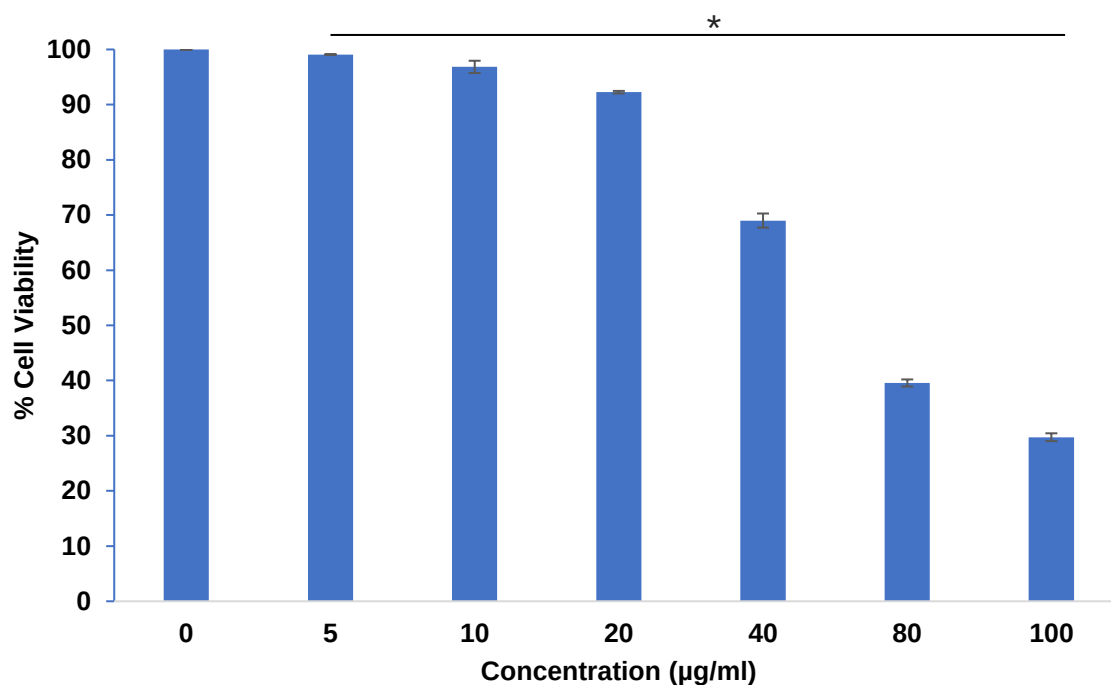


Fig 5.10: Cell Viability Dose-Response curve of MWCNT treated HepG2 cells. Values represent means $\pm$ SD (* $P < 0.05$ was considered significant compared to control) of three independent experiments carried out in triplicates

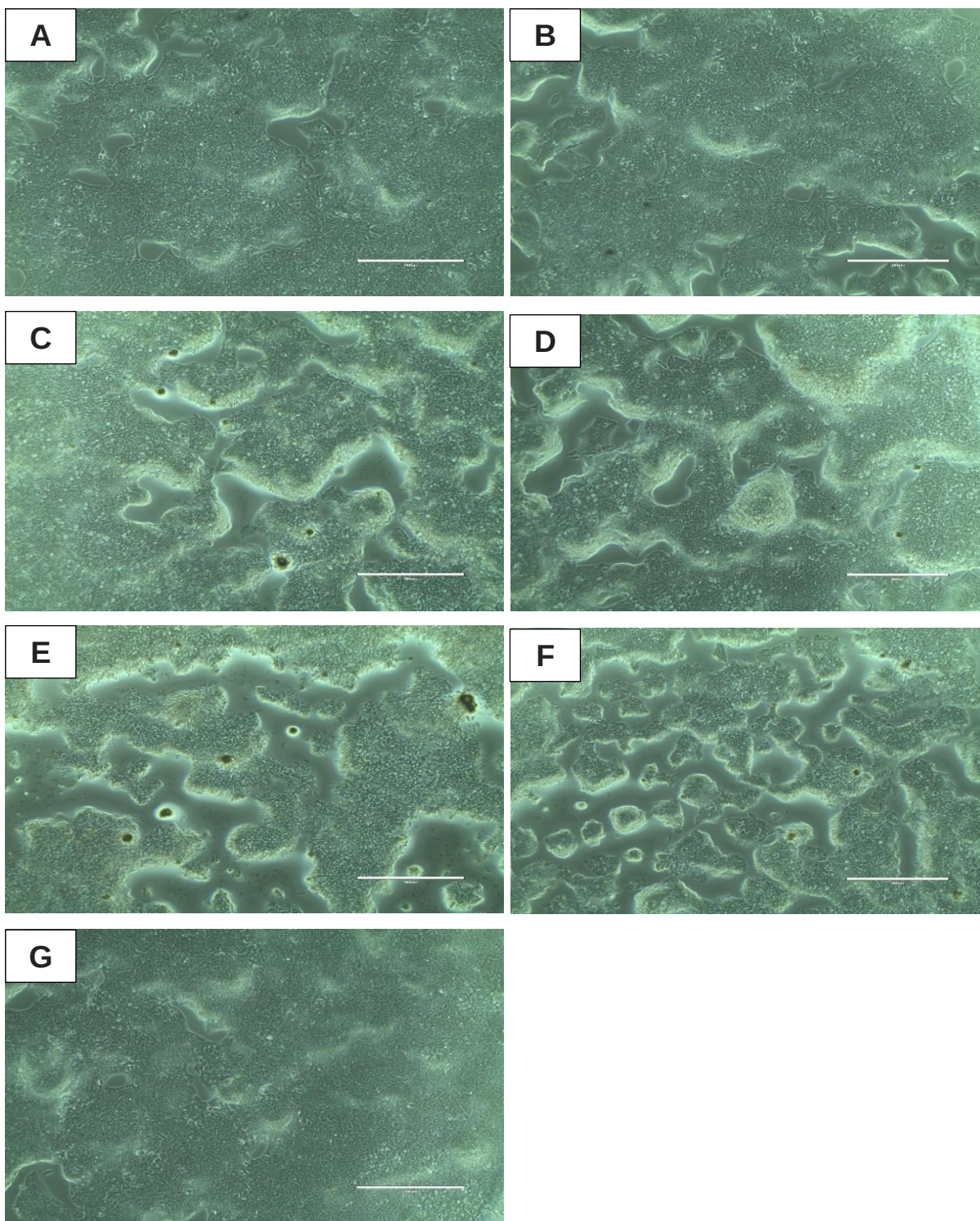


Fig 5.11: Morphology of HepG2 cells under phase contrast; after 24 h incubation with A) 5 µg/ml, B) 10 µg/ml, C) 20 µg/ml, D) 40 µg/ml, E) 80 µg/ml, F) 100 µg/ml, G) Represents control cells, with normal morphology.

Scale bar = 100 µm

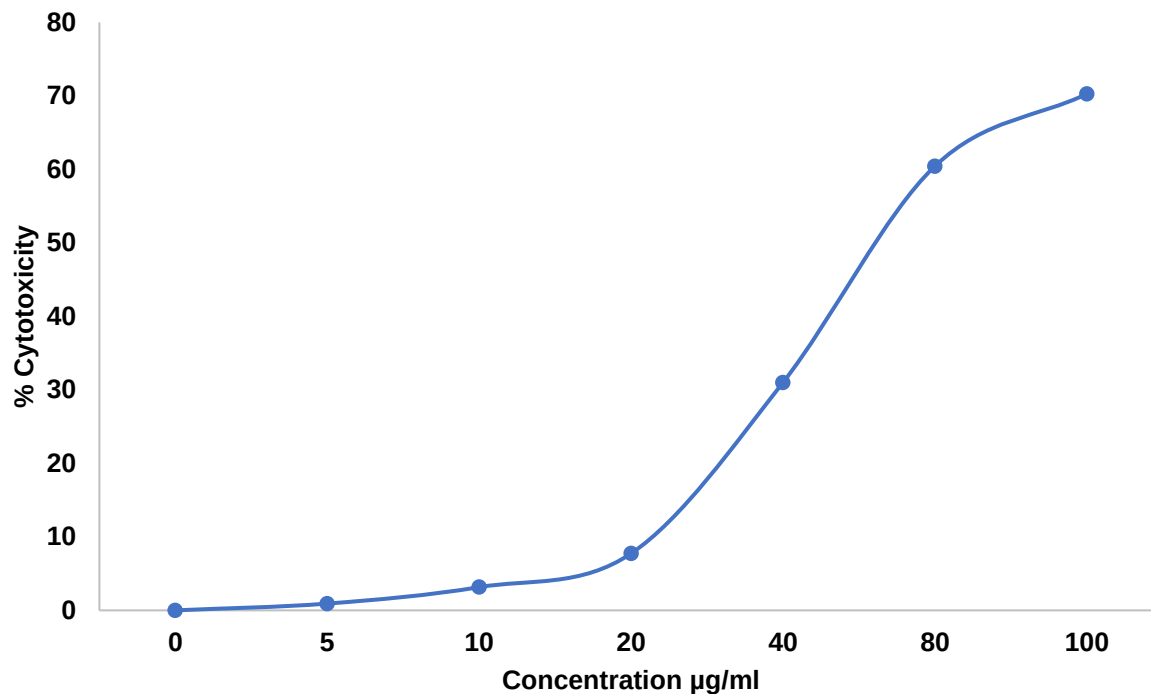


Fig 5.12: IC₅₀ calculated from dose vs. response graph; (MTT assay 24h In this graph, R square is 0.9891. (P < 0.05 and this difference is considered to be extremely statistically significant.)

The above graph is in the form of straight line (R^2 almost equals 1)

$$y = mx - c, \text{ where } m = 0.7548 \text{ and } c = 2.7.64$$

To calculate IC₅₀ value substitute y= 50 in the above equation

Where, **IC₅₀** is found to be **69.82 µg/ml**

To calculate IC₉₀ value substitute y= 90 in the above equation

Where, **IC₉₀** is found to be **122.82 µg/ml**

5.2.2 Acridine orange

AO/PI staining was based on the membrane integrity and incorporation of these dyes with cellular DNA. AO and PI intercalate with DNA once inside the cell, and emit green and red fluorescence, respectively. PI doesn't enter into viable cells with intact membrane so only viable cells will take only AO and appear green in fluorescent microscope. Non-viable cells will take both dyes and appear orange to red due to the dominance of PI. In treated groups, there were large number of cells-stained orange to red colour of PI due to membrane damage but untreated cells were almost uniform in size and shape, and emit green fluorescence of AO (Lei et al., 2009; Nagai & Toyokuni, 2012).

The effects on the endocytic compartment of exposed cells for 120 and 180 min were studied by AO. Conversely to PBS-treated cells which showed a rich set of intact lysosomes, the cells exposed both to pristine MWCNTs (80 ug/ml) clearly revealed lysosomal damage. After 180 min of exposure, we highlighted a diffuse green cytosolic fluorescence (Fig. 5.13) that was associated with the morphological changes of cell death, as pyknosis and karyorrhexis. Damage was more pronounced in p MWCNT-treated cells, several of which emitted only green fluorescence and showed blebs surrounding the cells. AO emission was also determined by quantitative cytofluorimetric analyses. Morphological investigations using this staining confirmed that cells showed typical characteristics of apoptosis such as cell shrinkage, membrane blebbing and ruffling when treated with MWCNT (Goepfert et al., 2011; Kntayya et al., 2018; Oberoi et al., 2018).

AO/PI staining is also used in morphological assessment of apoptotic cells. In a study after 24 h of incubation with MWCNT, there were clear indications of chromatin condensation of the HepG2 cells. Upon 72 h of incubation, small apoptotic bodies and dead cells were identified. The morphological changes as well as the shift from green to orange fluorescent cells that were observed from the AO/PI staining were sequential from 24 h up to 72 h of MWCNT treatment, indicating gradual cell death via apoptosis with increasing length of exposure (Hou et al., 2015; Tripathi et al., 2019).

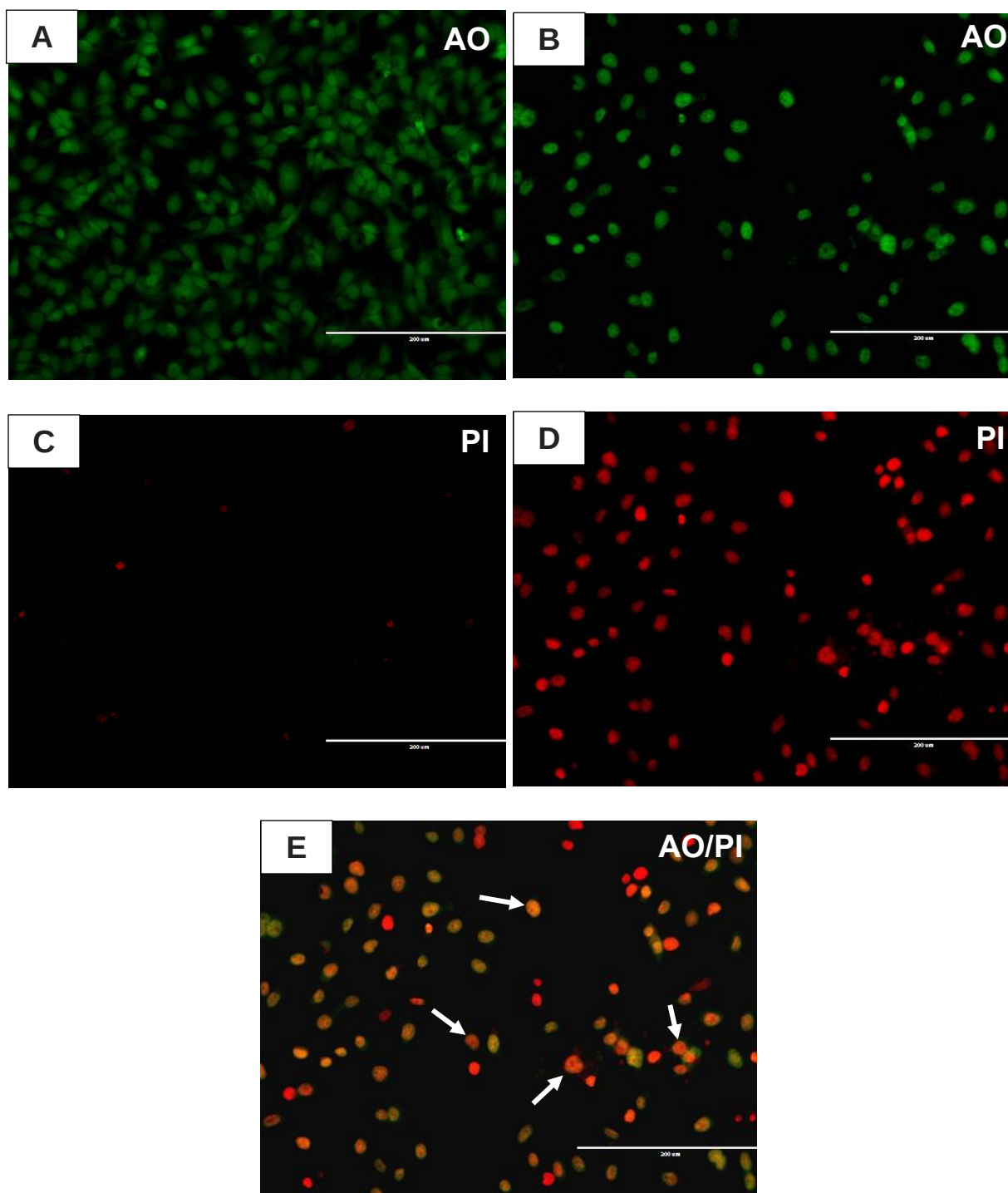


Fig 5.13: Acridine orange (AO, green) and propidium iodide (PI, red) double staining fluorescent micrographs of MWCNT treated HepG2; AO Control group (A), AO in MWCNT treated HepG2 cells (B), PI Control group (C), PI in MWCNT treated HepG2 cells (D), PI/AO in MWCNT treated HepG2 cells (E); Arrows indicate red/orange stained endosomal/lysosomal vesicles; Scale bar: 200 µm

5.2.3 Intracellular ROS

The disruption in the balance between ROS and the endogenous antioxidant systems contributes to oxidative damage of cellular macro-molecules ultimately leading to cell death. DCFH-DA is a dye molecule that can pass through the lipid membrane and is reduced to DCFH by esterase. Coming into contact with ROS such as OH^- or O_2^- , DCFH is converted to the fluorescent molecule DCF. The fluorescence intensity measured for each cell via flow cytometry is proportional to the amount of ROS generated inside the cell (Requardt et al., 2015; Shen et al., n.d.; Wan et al., 2015).

The maximum MWCNT concentration was decreased from 100 to 80 $\mu\text{g/ml}$ to ensure a sufficient MWCNT removal from the cell suspension, with a limited number of three centrifugation and resuspension steps. Results are shown after 24 h of exposure (results after 48 h are indicated in the supplement data) because of the short-lived nature and time-dependent occurrence of ROS (Iavicoli, n.d.; X. ming Tan et al., 2009b; Ye et al., 2009). Fig. 5.14 shows the results for cell lines after 24 h of MWCNT exposure. In the cell lines, the production of ROS was significantly increased when the cells were exposed to the MWCNTs at a concentration of 40 $\mu\text{g/cm}^2$.

Based on the physical and chemical properties of the MWCNTs, it could be expected that an increased number of delocalized electrons on their surface and, hence, an increased hydrophobicity could lead to an enhanced formation of ROS. This in turn could be attributed to an inductive effect as a result of a dense electron cloud. Our data using the DCFH-DA assay showed that 80 $\mu\text{g/ml}$ MWCNT led to a significantly increased intracellular production of ROS following a 24 and 48 h exposure. The increased production of ROS and the increase in oxidative stress following MWCNT exposure was also reported for various cell lines, including HepG2 cells, by several other research groups (Requardt et al., 2019; C. Zhao et al., 2018).

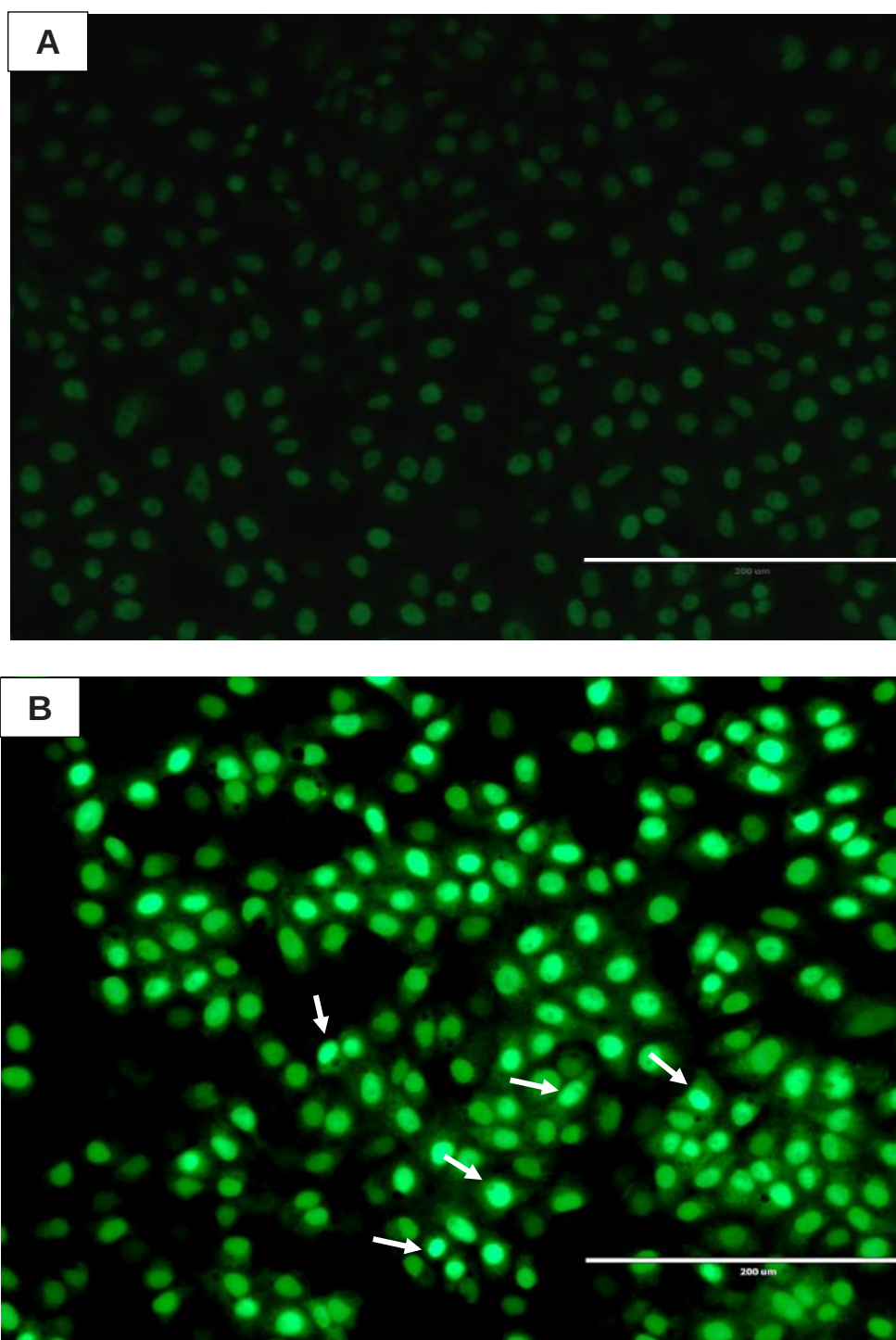


Fig 5.14: Confocal images of ROS generation in HepG2 cells; at 24 hrs after MWCNT treatments; Control group (A), MWCNT treated HepG2 cells (B); Arrows indicate DCFH stained cells; Scale bar: 200 μ m

5.2.4 Lipid accumulation

Four main pathways relating to hepatic lipid metabolism have been identified to contribute to hepatic TG accumulation (Fig. 5.15), namely 1) uptake of circulating fatty acids derived from the diet or adipose tissue; 2) hepatic fatty acid synthesis also known as de novo lipogenesis (DNL); 3) secretion of TGs in the form of very low-density lipoprotein (VLDL) particles; and 4) fatty acid oxidation (FAO). Recently, turnover of lipid droplets (LDs) by lipophagy, a form of autophagy specifically involved in the degradation of LDs has been identified as a novel pathway that also contributes to NAFLD (Han et al., 2021; Z. Li et al., 2020).

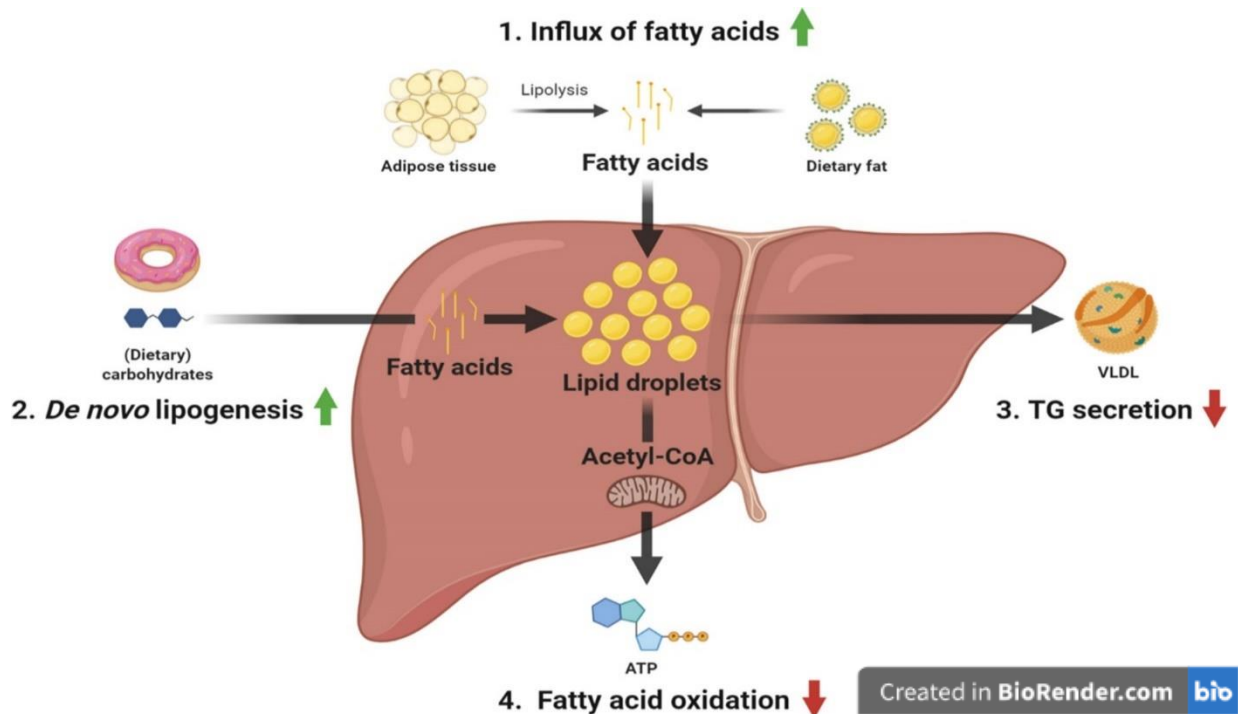


Fig 5.15: Overview of the main pathways contributing to TG accumulation in NAFLD (created using biorender tool)

A specific form of autophagy is lipophagy which mediates the liberation of lipids stored in LDs. Lipophagy is a relatively recent discovery compared to the four pathways. However, disturbances in lipophagy have been linked to NAFLD and

hepatic TG accumulation and this process can therefore be considered the fifth pathway controlling NAFLD development (Z. Li et al., 2020).

In order to protect against lipotoxicity, several organs including the liver store neutral lipids such as TGs in specialized single membrane vacuoles known as LDs. LDs are predominantly generated at the ER and increase in size by lipid transfer from the ER or by fusion with other LDs. LDs consist of a single phospholipid membrane filled with neutral lipids and harbour a variety of lipolytic enzymes and other LD regulatory proteins. Adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), di- and monoglyceride lipase (DGL and MGL, respectively) facilitate the hydrolysis of TGs into fatty acids (T. Zhang, Liu, et al., 2019).

Additionally, members of the perilipin family (PLIN1-PLIN5) are colocalized on the LD-membrane where they serve a regulatory function for lipolysis. PLIN2 and PLIN5 are highly expressed in the liver and contribute to the formation and regulation of lipolytic activity at the LDs membrane. The regulatory role of PLIN2 was demonstrated when Plin2 knockout mice did not develop hepatic steatosis in response to a high-fat diet. Their regulatory role was associated to an occupational function on the LDs, reducing the special availability for lipolytic enzymes.

Hepatic PLIN5 protein synthesis and activity were both induced in mice by fasting, and signals via sirtuin-1 (SIRT1) to promote autophagy and reduce hepatic inflammatory injury under starvation. In addition, PLIN5 was shown to regulated the phosphorolytic state of lipolytic enzymes (ATGL, HSL, and MGL), and thereby altering their lipolytic activity. These examples of dysfunction of LD-associated proteins elegantly illustrate the regulatory and inducible axis between LDs, cytosolic lipolysis, and lipophagy (Fukaya et al., 2021; T. Zhang, Liu, et al., 2019).

LDs and their content are among the structures that are degraded in the lipophagic process. As such, lipophagy is important to liberate fatty acids from the TGs stored in the LDs. A recent study reported that lipophagy-derived fatty acids undergo a cycle of efflux followed by reuptake before a subsequent reincorporation into cellular LDs. However, if lipophagy is associated with reduced hepatic lipid content, it is likely that some of the liberated fatty acids will be directed towards

FAO. In general, it is considered that lysosomal acid lipase (LAL) hydrolyses the TGs to generate fatty acids. The presence of a second lipolytic enzyme, e.g. ATGL, necessarily contributes to the degradation of the LD (Fukaya et al., 2021; Z. Li et al., 2020).

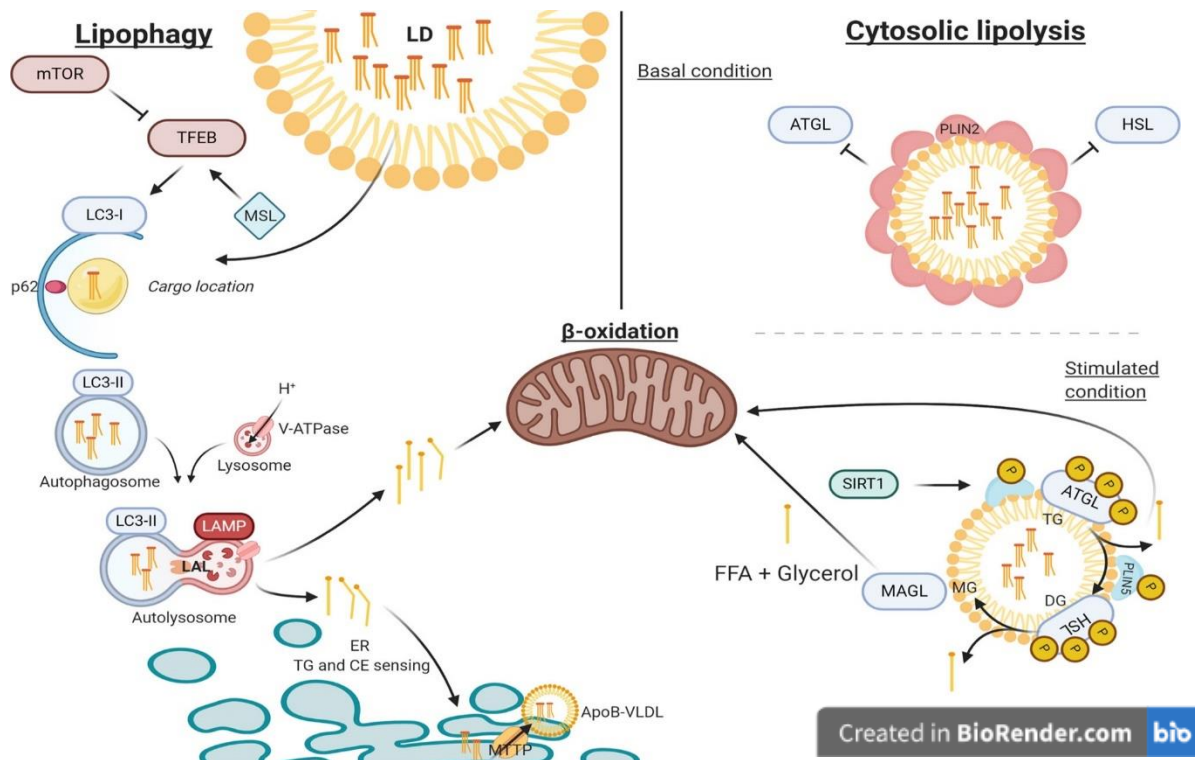


Fig 5.16: The process of lipophagy. (Created using biorender tool)

Lipophagy (Fig. 5.16) – Key regulator mTOR inhibits TFEB in autophagy induction. MSL induces TFEB that upregulates autophagy. LC3-I assembles at the autophagosome envelope elongating the bi-layer. P62 secures ‘chunks’ of the LD as lipid-cargo in the lumen of the autophagosome. After LC3-I is spliced by ATG4 to LC3-II, the autophagosome closes carrying its cargo. Upon fusion with the acidic lysosome (LAMP and V-ATPase driven), LAL can hydrolyse TG and CE into FFA and free cholesterol, respectively. FFA are directed towards either beta-oxidation or to the ER for further metabolism. Cytosolic Lipolysis – At basal conditions, LDs are covered in PLIN2 molecules that regulate the accessibility for lipolytic enzymes. When stimulated

PLIN2 releases and grants access at the membrane for ATGL and HSL (bound to PLIN5), which activate under phosphorylation. When stimulated, SIRT1 activates CMA to release PLIN2 and replace PLIN5 at the LD membrane, thereby mediating the lipolytic activity through autophagy (Y. Li et al., 2019; Schott et al., 2019).

Oil red O staining indicated that MWCNT exposure concentration-dependently promoted lipid accumulation in HepG2 cells. Significantly increased oil red staining was observed in cells following exposure to 69.82 $\mu\text{g/ml}$ (IC_{50} value) of p-MWCNT as shown in Fig. 5.17. At the concentration the pristine MWCNT induced significantly higher lipid accumulation in HepG2 cells. FFA as the positive control resulted in approximately two-fold increase of oil red staining. Under the confocal microscope (Fig. 5.18), control cells showed sparse lipid droplets, whereas MWCNT exposed cells appeared to have more lipid droplets. ImageJ analysis indicated increased fluorescence area from BODIPY staining in cells exposed to NPs (Iavicoli, n.d.; X. ming Tan et al., 2009b; Ye et al., 2009)

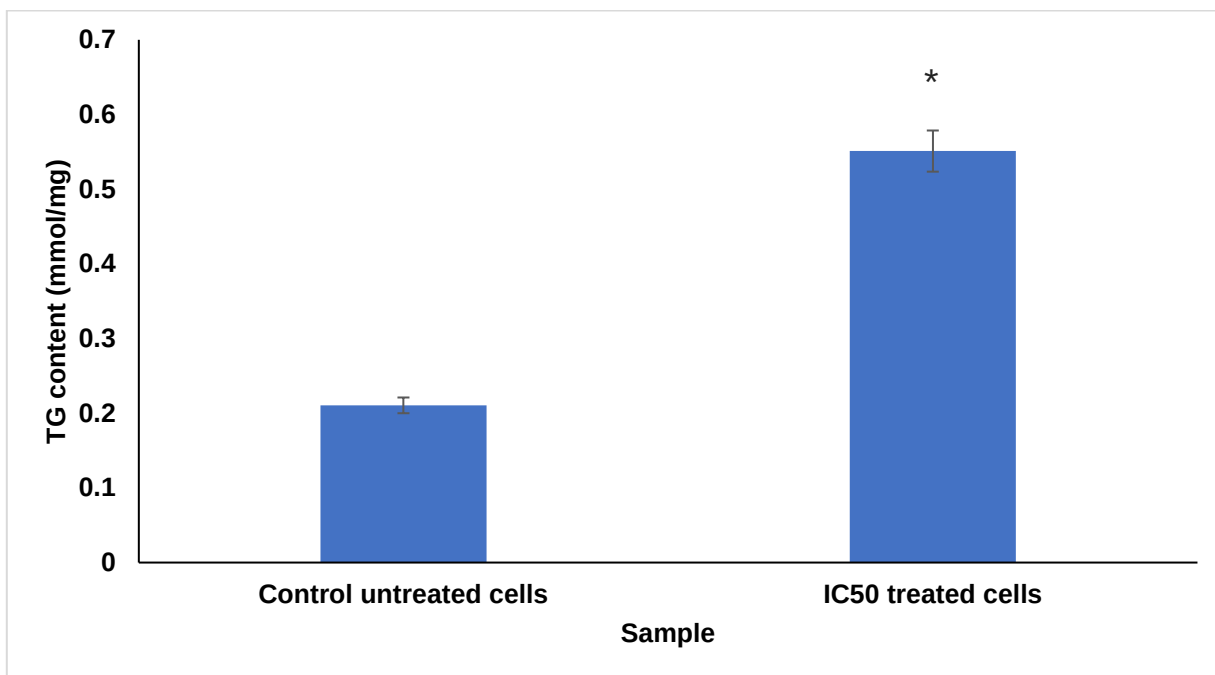


Fig 5.17: TG content assay of HepG2 cells treated with IC_{50} ; concentration of MWCNT for 24 h. Intracellular TG contents were normalized by total protein, and both originated from one sample. The data were presented as the mean $\pm$ SD. * $p < 0.05$ versus the control group

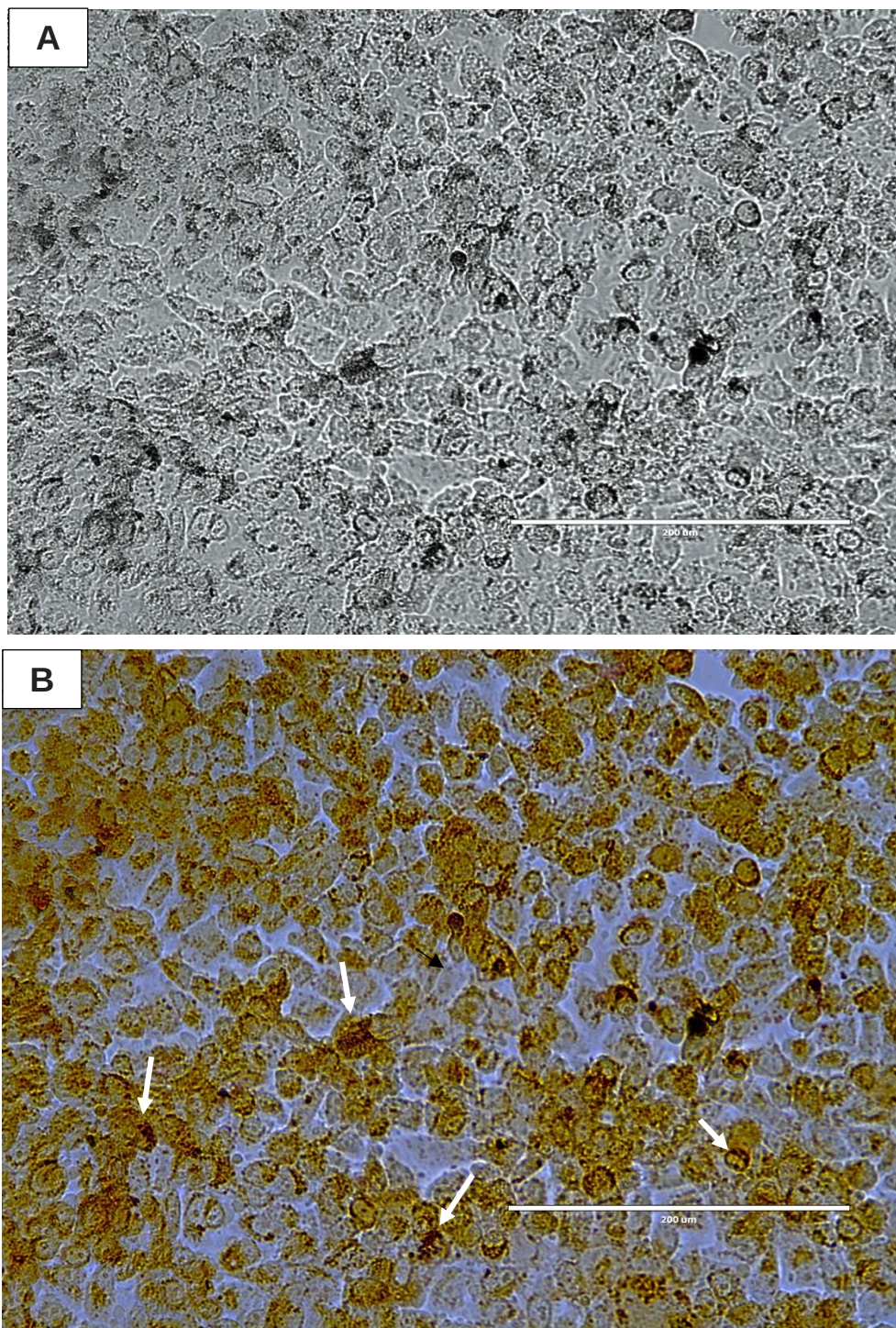


Fig 5.18: Intracellular lipid droplets in HepG2 cells stained with Oil Red O; Control group (A), MWCNT treated HepG2 cells (B); Arrows indicate lipid accumulated cells-stained cells; Scale bar: 200 μ m.

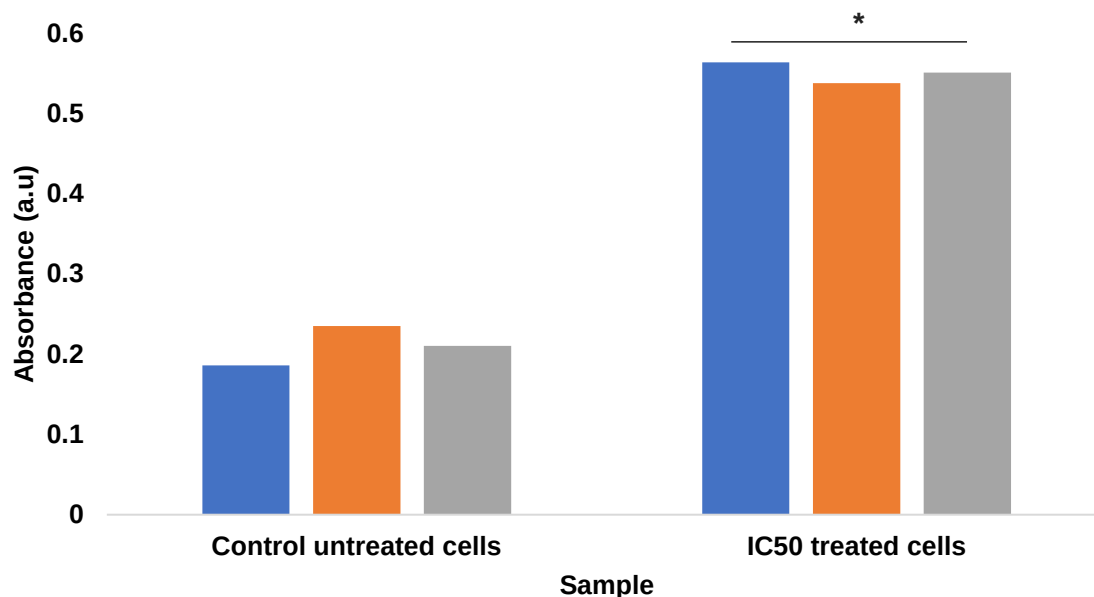


Fig 5.19: The accumulation of lipids. HepG2 were incubated with IC₅₀ concentration of MWCNT for 24 h, and then lipid accumulation was quantified by using Oil Red O staining and OD were measured, *P < 0.05, compared with control.

Oil Red O bases this technique on the staining of intracellular lipid droplet. Oil Red O staining showed that 69.82 µg/ml MWCNT treatment for 24 h induced the accumulation of lipid droplets in almost all HepG2 cells (Fig. 5.18B). There were no visible lipid droplets in HepG2 cells in the normal group (Fig. 5.18A).

Triglyceride assay showed that the triglyceride contents in HepG2 cells treated 69.82 µg/ml MWCNT treatment for 24 h were significantly increased compare with that in the normal group (P < 0.05, Fig. 5.19). These data suggested that 69.82 µg/ml MWCNT successfully induced steatosis in HepG2 cells. When treated with MWCNT the intra-cellular TG contents in differentiated HepG2 cells were increased by 38.2%, as shown in Fig. 5.19. These results indicated that the metabolism of TG in HepG2 cells may be regulated by MWCNT (Ikeda et al., 2014; Oberoi et al., 2018; Pei et al., 2019).

5.2.5 DAPI staining

To further understand the action mechanisms of the cytotoxic activity of the pristine MWCNTs, this study examines how the MWCNTs interact with HepG2 cells using fluorescence microscopy. Fluorescent dyes (DAPI), as an indicator of cell membrane damage, are used to assess the cytotoxic activity of all samples. Living cells are observed as blue. Fig. 5.20 shows a fluorescence microscopy image of the control sample where no MWCNTs are added. The cells are not aggregated and all the cells were alive (blue).

Fig. 5.20 shows images of the cells treated with the MWCNTs at 80 $\mu\text{g/mL}$ for 60 min. The formation of aggregates, including HepG2 cells and MWCNTs, is clearly observed, which is consistent with the results reported in other studies where MWCNTs have strong ability to capture hepatocytes and form aggregates. Interestingly, the blue-stained cells existed mainly in the external field of each aggregate. This is likely due to the tighter contact between the hepatocyte and MWCNTs in the central field than in the outer field of the aggregates. The tight contact provides more opportunity for the MWCNTs to interact with the cell membranes and destroy the cell membrane structure (Bai et al., 2012; Iancu et al., 2011; M. Wang et al., 2010)

DAPI staining is also performed to investigate if the drug induces apoptosis in the HepG2 cells is also use in identifying apoptotic cells. During apoptosis there is a loss of membrane asymmetry due to the translocation of phosphatidylserine from the inner to the outer layer of the cell membrane. This translocation occurs before nuclear breakdown and DNA fragmentation. The results showed in Fig 5.20 indicates that MWCNT causes nuclear fragmentation of the HepG2 cells, which is hallmark of apoptosis. Taken together, these results strongly indicate that MWCNT contains compounds that are able to induce apoptosis in a human hepatocyte (Vizetto-Duarte et al., 2016; J. Wang et al., 2017).

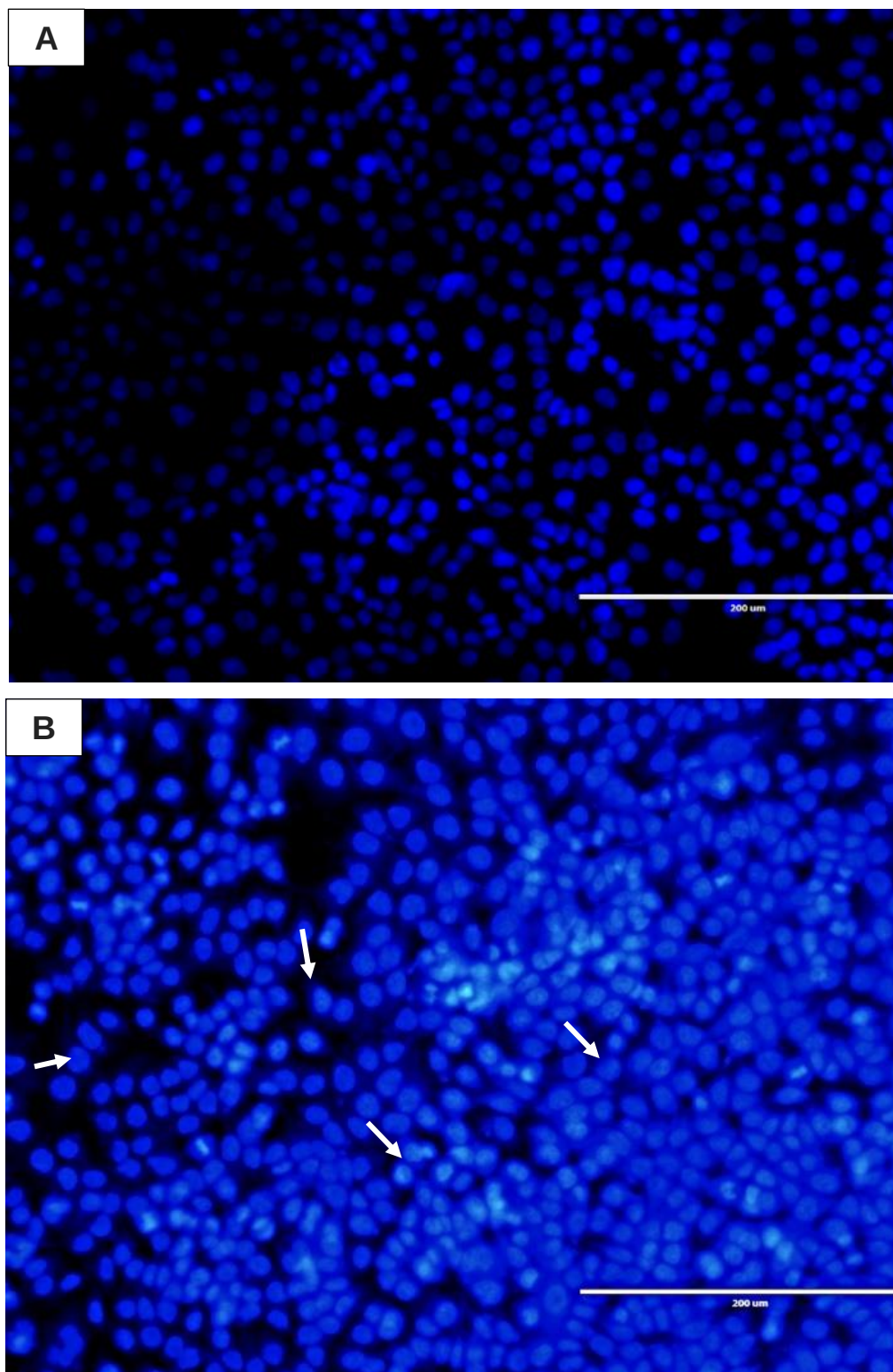


Fig 5.20: Fluorescent images of DAPI for the HepG2 cells exposed to MWCNT; Control group (A), MWCNT treated HepG2 cells (B); arrows depict apoptotic cell; Scale bar: 200 μ m.

CHAPTER 6

SUMMARY AND CONCLUSION

In this study, we investigated the Physical characterization of the commercially acquired pristine MWCNT. UV - Vis spectroscopy proves to be an effective tool for the assessment of dispersion state of MWCNTs in water, which were further backed by the FESEM images. The covalent linkage between the carbon nanotube and functional group was characterized by FTIR. The peaks indicate the presence of functional groups such as carboxyl groups ($\text{O}=\text{C}-\text{OH}$ and $\text{C}-\text{OH}$). In XRD analysis it was found that the p-MWCNT were crystalline in nature. In terms of CNT structure, Raman spectroscopy provides information about sidewall integrity in which the I_D/I_G ratio was found to be ~ 1.17 , although without TEM, $I_D:I_G$ band analysis can be misleading. TEM and Raman spectroscopy are among the techniques most widely used to provide chemical and structural information about CNTs. The maximum absorbance and high negative zeta potential of the suspension were measured as 1.485 abs and -14.9 mV, respectively for the best dispersibility of MWCNT.

Previous literatures showed that MWCNT could inhibit cell viability of human ocular cells and human keratinocyte cells. Similar to these studies, this study also found that the MWCNTs decreased cell viability of HepG2 in a concentration-dependent manner. Some literatures showed that the modification could increase the cytotoxicity of MWCNTs. The MWCNTs used in this study included p-MWCNTs, so the possible influence of surface modification could be investigated TEM images showed the presence of MWCNTs in single-layer membrane vesicles inside cells, which indicated the internalization of MWCNTs via endocytic pathways. The cytotoxicity assays indicated a modest but significant decrease of cellular viability after exposure to $40 \mu\text{g/ml}$ MWCNTs as shown by MTT. In this study, we also found that MWCNT significantly promoted intracellular ROS, which suggested that oxidative stress could be induced in MWCNT exposed hepatocytes. Interference on plasma membrane was also commonly used to assess the cytotoxic effects of CNTs. The size and shape of MWCNTs make them easily interact with cell surface. Thus, the damages in plasma

membrane induced by MWCNTs might more sensitive than cell viability and intracellular ROS generation. DAPI staining images showing induction of apoptosis by the introduced on the HepG2 cell images. Apoptosis was confirmed by DAPI staining which showed apparent changes in the nuclear morphology (chromatin condensation and nuclear fragmentation) of the MWCNT treated cells.

Morphological investigations using AO/PI staining confirmed that cells showed typical characteristics of apoptosis such as cell shrinkage, membrane blebbing and ruffling when treated with MWCNT. AO and PI intercalate with DNA inside the cell, and emit green and red fluorescence, respectively. PI doesn't enter into viable cells with intact membrane so only viable cells will take only AO and appear green in fluorescent microscope. Non-viable cells appeared orange to red because of Pi dominance. In treated groups, there were large number of cells-stained orange to red colour of PI due to membrane damage but untreated cells were almost uniform in size and shape, and emit green fluorescence of AO. Collectively, the results from this study showed that pristine could be internalized into HepG2 cells, leading to lipid accumulation without external lipid supply. MWCNT pre-incubation with autophagy inhibitors will also promote MWCNT induced lipid accumulation, which indicates a possible role of lipophagy. This also suggests that when using NPs for bio medicinal purposes, it may be necessary to consider the influence of NPs on lipophagy pathway and lipid accumulation. The achieved results are in full agreement with the literature.

Based on existing studies, it can be suggested that MWCNT Nps can act as nanocarrier of drug molecules for targeted drug delivery. However, so far, the scientific paradigm for the possible (adverse) reactivity of nanoparticles is lacking and we have little understanding of the basics of the interaction mechanism of nanoparticles within organisms. A conceptual understanding of biological responses to nanomaterials is needed to develop and apply safe nanomaterials in drug delivery in the future. Furthermore, a close collaboration between those working in drug delivery and particle production is necessary for the exchange of concepts, methods and know how to move this issue ahead.

REFERENCE

1. Atiyah, A. A., Haider, A. J., & Dhahi, R. M. (2019). Cytotoxicity properties of functionalised carbon nanotubes on pathogenic bacteria. *IET Nanobiotechnology*, 13(6), 597–601. <https://doi.org/10.1049/iet-nbt.2018.5394>
2. Azizi-Lalabadi, M., Hashemi, H., Feng, J., & Jafari, S. M. (2020). Carbon nanomaterials against pathogens; the antimicrobial activity of carbon nanotubes, graphene/graphene oxide, fullerenes, and their nanocomposites. *Advances in Colloid and Interface Science*, 284, 102250. <https://doi.org/10.1016/j.cis.2020.102250>
3. Bai, Y., Park, I. S., Lee, S. J., Wen, P. S., Bae, T. S., & Lee, M. H. (2012). Effect of AOT-assisted multi-walled carbon nanotubes on antibacterial activity. *Colloids and Surfaces B: Biointerfaces*, 89(1), 101–107. <https://doi.org/10.1016/J.COLSURFB.2011.09.001>
4. Bernardi, S., Toffoli, B., Tisato, V., Bossi, F., Biffi, S., Lorenzon, A., Zauli, G., Secchiero, P., & Fabris, B. (2018). TRAIL reduces impaired glucose tolerance and NAFLD in the high-fat diet fed mouse. *Clinical Science*, 132(1), 69–83. <https://doi.org/10.1042/CS20171221>
5. Bhunia, T., Giri, A., Nasim, T., Chattopadhyay, D., & Bandyopadhyay, A. (2013). Uniquely different PVA-xanthan gum irradiated membranes as transdermal diltiazem delivery device. *Carbohydrate Polymers*, 95(1), 252–261. <https://doi.org/10.1016/j.carbpol.2013.02.043>

6. Bottini, M., Bruckner, S., Nika, K., Bottini, N., Bellucci, S., Magrini, A., Bergamaschi, A., & Mustelin, T. (2006). Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicology Letters*, 160(2), 121–126.
<https://doi.org/10.1016/j.toxlet.2005.06.020>
7. Cao, Y., & Luo, Y. (2019). Pharmacological and toxicological aspects of carbon nanotubes (CNTs) to vascular system: A review. *Toxicology and Applied Pharmacology*, 385(November), 114801.
<https://doi.org/10.1016/j.taap.2019.114801>
8. Chakrabarti, M., Kiseleva, R., Vertegel, A., & Ray, S. K. (2015). Carbon nanomaterials for drug delivery and cancer therapy. *Journal of Nanoscience and Nanotechnology*, 15(8), 5501–5511.
<https://doi.org/10.1166/jnn.2015.10614>
9. Cimbaluk, G. V., Ramsdorf, W. A., Perussolo, M. C., Santos, H. K. F., Da Silva De Assis, H. C., Schnitzler, M. C., Schnitzler, D. C., Carneiro, P. G., & Cestari, M. M. (2018). Evaluation of multiwalled carbon nanotubes toxicity in two fish species. *Ecotoxicology and Environmental Safety*, 150(September 2017), 215–223.
<https://doi.org/10.1016/j.ecoenv.2017.12.034>
10. Costa, S., Scheibe, B., Rummeli, M., & Borowiak-Palen, E. (2009). Raman spectroscopy study on concentrated acid treated carbon nanotubes. *Physica Status Solidi (b)*, 246(11–12), 2717–2720.
<https://doi.org/10.1002/PSSB.200982297>
11. Curran, S. A., Talla, J. A., Zhang, D., & Carroll, D. L. (2005). Defect-induced vibrational response of multi-walled carbon nanotubes using resonance

- Raman spectroscopy. *Journal of Materials Research*, 20(12), 3368–3373.
<https://doi.org/10.1557/JMR.2005.0414>
12. Daraei, P., Madaeni, S. S., Ghaemi, N., Ahmadi Monfared, H., & Khadivi, M. A. (2013). Fabrication of PES nanofiltration membrane by simultaneous use of multi-walled carbon nanotube and surface graft polymerization method: Comparison of MWCNT and PAA modified MWCNT. *Separation and Purification Technology*, 104, 32–44.
<https://doi.org/10.1016/J.SEPPUR.2012.11.004>
 13. Das, R., Bee Abd Hamid, S., Eaqub Ali, Md., Ramakrishna, S., & Yongzhi, W. (2015). Carbon Nanotubes Characterization by X-ray Powder Diffraction – A Review. *Current Nanoscience*, 11(1), 23–35.
 14. Davoren, M., Herzog, E., Casey, A., Cottineau, B., Chambers, G., Byrne, H. J., & Lyng, F. M. (2007). In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicology in Vitro*, 21(3), 438–448.
<https://doi.org/10.1016/j.tiv.2006.10.007>
 15. Demir, E., & Marcos, R. (2018). Toxic and genotoxic effects of graphene and multi-walled carbon nanotubes. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, 81(14), 645–660.
<https://doi.org/10.1080/15287394.2018.1477314>
 16. El Mrabate, B., Szőri-Dorogházi, E., Ahmed Shehab, M., Chauhan, T., Muránszky, G., Sikora, E., Filep, Á., Sharma, N., Nánai, L., Hernadi, K., & Németh, Z. (2021). Widespread applicability of bacterial cellulose-ZnO-MWCNT hybrid membranes. *Arabian Journal of Chemistry*, 14(7), 0–11.
<https://doi.org/10.1016/j.arabjc.2021.103232>

17. Ertas, T., & Dinc, B. (2022). *EFFECT of SHORT MULTIWALLED CARBON NANOTUBES on ESCHERICHIA COLI K-12 STRAIN*. 90(June), 8–13.
<https://doi.org/1099944>
18. Fatkhutdinova, L. M., Khaliullin, T. O., Vasil'yeva, O. L., Zalyalov, R. R., Mustafin, I. G., Kisin, E. R., Birch, M. E., Yanamala, N., & Shvedova, A. A. (2016). Fibrosis biomarkers in workers exposed to MWCNTs. *Toxicology and Applied Pharmacology*, 299, 125–131.
<https://doi.org/10.1016/j.taap.2016.02.016>
19. Fraczek, A., Menaszek, E., Paluszkiewicz, C., & Blazewicz, M. (2008). Comparative in vivo biocompatibility study of single- and multi-wall carbon nanotubes. *Acta Biomaterialia*, 4(6), 1593–1602.
<https://doi.org/10.1016/j.actbio.2008.05.018>
20. Francis, A. P., Ganapathy, S., Palla, V. R., Murthy, P. B., Ramaprabhu, S., & Devasena, T. (2015). One time nose-only inhalation of MWCNTs: Exploring the mechanism of toxicity by intermittent sacrifice in Wistar rats. *Toxicology Reports*, 2, 111–120.
<https://doi.org/10.1016/j.toxrep.2015.02.003>
21. Fukaya, M., Sato, Y., Kondo, S., Adachi, S. ichi, Yoshizawa, F., & Sato, Y. (2021). Quercetin enhances fatty acid β -oxidation by inducing lipophagy in AML12 hepatocytes. *Heliyon*, 7(6), e07324.
<https://doi.org/10.1016/J.HELİYON.2021.E07324>
22. Galassi, T. V., Jena, P. V., Shah, J., Ao, G., Molitor, E., Bram, Y., Frankel, A., Park, J., Jessurun, J., Ory, D. S., Haimovitz-Friedman, A., Roxbury, D., Mittal, J., Zheng, M., Schwartz, R. E., & Heller, D. A. (2018). An optical

- nanoreporter of endolysosomal lipid accumulation reveals enduring effects of diet on hepatic macrophages in vivo. *Science Translational Medicine*, 10(461), 1–11. <https://doi.org/10.1126/scitranslmed.aar2680>
23. Gaurav, A., & Singh, K. K. (2019). Effect of pristine MWCNTs on the fatigue life of GFRP laminates-an experimental and statistical evaluation. *Composites Part B: Engineering*, 172, 83–96. <https://doi.org/10.1016/J.COMPOSITESB.2019.05.069>
24. Ghasempour, R., & Narei, H. (2018). CNT Basics and Characteristics. *Carbon Nanotube-Reinforced Polymers: From Nanoscale to Macroscale*, 1–24. <https://doi.org/10.1016/B978-0-323-48221-9.00001-7>
25. Goepfert, C., Scheurer, W., Rohn, S., Rathjen, B., Meyer, S., Dittmann, A., Wiegandt, K., Janßen, R., & Pörtner, R. (2011). 3D-Bioreactor culture of human hepatoma cell line HepG2 as a promising tool for in vitros substance testing. *BMC Proceedings* 2011 5:8, 5(8), 1–2. <https://doi.org/10.1186/1753-6561-5-S8-P61>
26. Gong, H., Peng, R., & Liu, Z. (2013). Carbon nanotubes for biomedical imaging: The recent advances. *Advanced Drug Delivery Reviews*, 65(15), 1951–1963. <https://doi.org/10.1016/j.addr.2013.10.002>
27. Goyanes, S., Rubiolo, G. R., Salazar, A., Jimeno, A., Corcuera, M. A., & Mondragon, I. (2007). Carboxylation treatment of multiwalled carbon nanotubes monitored by infrared and ultraviolet spectroscopies and scanning probe microscopy. *Diamond and Related Materials*, 16(2), 412–417. <https://doi.org/10.1016/J.DIAMOND.2006.08.021>
28. Grubek-Jaworska, H., Nejman, P., Czumińska, K., Przybyłowski, T.,

- Huczko, A., Lange, H., Bystrzejewski, M., Baranowski, P., & Chazan, R. (2006). Preliminary results on the pathogenic effects of intratracheal exposure to one-dimensional nanocarbons. *Carbon*, 44(6), 1057–1063. <https://doi.org/10.1016/j.carbon.2005.12.011>
29. Gutiérrez-Hernández, J. M., Escobar-García, D. M., Escalante, A., Flores, H., González, F. J., Gatenholm, P., & Toriz, G. (2017). In vitro evaluation of osteoblastic cells on bacterial cellulose modified with multi-walled carbon nanotubes as scaffold for bone regeneration. *Materials Science and Engineering C*, 75, 445–453. <https://doi.org/10.1016/j.msec.2017.02.074>
30. Hamilton, R. F., Buford, M., Xiang, C., Wu, N., & Holian, A. (2012). NLRP3 inflammasome activation in murine alveolar macrophages and related lung pathology is associated with MWCNT nickel contamination. *Http://Dx.Doi.Org/10.3109/08958378.2012.745633*, 24(14), 995–1008. <https://doi.org/10.3109/08958378.2012.745633>
31. Hamouda, H. I., Abdel-Ghafar, H. M., & Mahmoud, M. H. H. (2021). Multi-walled carbon nanotubes decorated with silver nanoparticles for antimicrobial applications. *Journal of Environmental Chemical Engineering*, 9(2), 105034. <https://doi.org/10.1016/j.jece.2021.105034>
32. Han, S. L., Qian, Y. C., Limbu, S. M., Wang, J., Chen, L. Q., Zhang, M. L., & Du, Z. Y. (2021). Lipolysis and lipophagy play individual and interactive roles in regulating triacylglycerol and cholesterol homeostasis and mitochondrial form in zebrafish. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1866(9), 158988. <https://doi.org/10.1016/J.BBALIP.2021.158988>

33. Hartono, M. R., Kushmaro, A., Chen, X., & Marks, R. S. (2018). Probing the toxicity mechanism of multiwalled carbon nanotubes on bacteria. *Environmental Science and Pollution Research*, 25(5), 5003–5012. <https://doi.org/10.1007/s11356-017-0782-8>
34. Hassan, S., Yasin, T., Imran, Z., & Batool, S. S. (2016). Silane Based Novel Crosslinked Chitosan/Poly(Vinyl Alcohol) Membrane: Structure, Characteristic and Adsorption Behaviour. *Journal of Inorganic and Organometallic Polymers and Materials*, 26(1), 208–218. <https://doi.org/10.1007/s10904-015-0309-z>
35. He, H., Pham-Huy, L. A., Dramou, P., Xiao, D., Zuo, P., & Pham-Huy, C. (2013). Carbon Nanotubes: Applications in Pharmacy and Medicine. *BioMed Research International*, 2013, 1–12. <https://doi.org/10.1155/2013/578290>
36. Henderson, W. M., Bouchard, D., Chang, X., Al-Abed, S. R., & Teng, Q. (2015). Biomarker analysis of liver cells exposed to surfactant-wrapped and oxidized multi-walled carbon nanotubes (MWCNTs). *Science of the Total Environment*, 565, 777–786. <https://doi.org/10.1016/j.scitotenv.2016.05.025>
37. Hirano, S., Kanno, S., & Furuyama, A. (2008). Multi-walled carbon nanotubes injure the plasma membrane of macrophages. *Toxicology and Applied Pharmacology*, 232(2), 244–251. <https://doi.org/10.1016/j.taap.2008.06.016>
38. Hou, Z., Imam, M. U., Ismail, M., Azmi, N. H., Ismail, N., Ideris, A., & Mahmud, R. (2015). Lactoferrin and ovotransferrin contribute toward

- antioxidative effects of Edible Bird's Nest against hydrogen peroxide-induced oxidative stress in human SH-SY5Y cells. *Bioscience, Biotechnology and Biochemistry*, 79(10), 1570–1578. <https://doi.org/10.1080/09168451.2015.1050989>
39. Hwang, Y., Lee, J. K., Lee, C. H., Jung, Y. M., Cheong, S. I., Lee, C. G., Ku, B. C., & Jang, S. P. (2007). Stability and thermal conductivity characteristics of nanofluids. *Thermochimica Acta*, 455(1–2), 70–74. <https://doi.org/10.1016/J.TCA.2006.11.036>
40. Iancu, C., Mocan, L., Bele, C., Orza, A. I., Tabaran, F. A., Catoi, C., Stiuftuc, R., Stir, A., Matea, C., Iancu, D., Agoston-Coldea, L., Zaharie, F., & Mocan, T. (2011). Enhanced laser thermal ablation for the in vitro treatment of liver cancer by specific delivery of multiwalled carbon nanotubes functionalized with human serum albumin. *International Journal of Nanomedicine*, 6(1), 129–141. <https://doi.org/10.2147/IJN.S15841>
41. Iavicoli, S. (n.d.). *Cyto-genotoxicity of engineered nanomaterials: Implications for occupational health*. Retrieved April 16, 2023, from <https://www.researchgate.net/publication/281773525>
42. Ikeda, Y., Zaima, N., Hirano, K. ichi, Mano, M., Kobayashi, K., Yamada, S., Yamaguchi, S., Suzuki, A., Kanzaki, H., Hamasaki, T., Kotani, J. ichi, Kato, S., Nagasaka, H., Setou, M., & Ishibashi-Ueda, H. (2014). Coronary triglyceride deposition in contemporary advanced diabetics. *Pathology International*, 64(7), 325–335. <https://doi.org/10.1111/PIN.12177/SUPPINFO>
43. Jain, S., Singh, S. R., & Pillai, S. (2012). Toxicity issues related to

- biomedical applications of carbon nanotubes. *Journal of Nanomedicine and Nanotechnology*, 3(5), 1–15. <https://doi.org/10.4172/2157-7439.1000140>
44. Jena, P. V., Roxbury, D., Galassi, T. V., Akkari, L., Horoszkó, C. P., Iaea, D. B., Budhathoki-Uprety, J., Pipalia, N., Haka, A. S., Harvey, J. D., Mittal, J., Maxfield, F. R., Joyce, J. A., & Heller, D. A. (2017). A Carbon Nanotube Optical Reporter Maps Endolysosomal Lipid Flux. *ACS Nano*, 11(11), 10689–10703. <https://doi.org/10.1021/acsnano.7b04743>
45. Jha, R., Singh, A., Sharma, P. K., & Fuloria, N. K. (2020). Smart carbon nanotubes for drug delivery system: A comprehensive study. *Journal of Drug Delivery Science and Technology*, 58(May), 101811. <https://doi.org/10.1016/j.jddst.2020.101811>
46. Jhawar, V. C., Saini, V., Kamboj, S., & Maggon, N. (2013). *Transdermal Drug Delivery Systems Approa*. 20(1), 47–56.
47. Jia, G., Wang, H., Yan, L., Wang, X., Pei, R., Yan, T., Zhao, Y., & Guo, X. (2005). Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. *Environmental Science and Technology*, 39(5), 1378–1383. <https://doi.org/10.1021/es048729l>
48. Jiang, D., Wang, D., Zhuang, X., Wang, Z., Ni, Y., Chen, S., & Sun, F. (2016). Berberine increases adipose triglyceride lipase in 3T3-L1 adipocytes through the AMPK pathway. *Lipids in Health and Disease*, 15(1). <https://doi.org/10.1186/S12944-016-0383-4>
49. Jin, Y., Wang, H., Yi, K., Lv, S., Hu, H., Li, M., & Tao, Y. (2021). Applications of Nanobiomaterials in the Therapy and Imaging of Acute Liver Failure. In *Nano-Micro Letters* (Vol. 13, Issue 1). <https://doi.org/10.1007/s40820-020->

50. Johnston, H. J., Hutchison, G. R., Christensen, F. M., Peters, S., Hankin, S., Aschberger, K., & Stone, V. (2010). A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: The contribution of physico-chemical characteristics. *Nanotoxicology*, 4(2), 207–246. <https://doi.org/10.3109/17435390903569639>
51. Joshi, A., Kaur, S., Singh, P., Dharamvir, K., Nayyar, H., & Verma, G. (2018). Tracking multi-walled carbon nanotubes inside oat (*Avena sativa* L.) plants and assessing their effect on growth, yield, and mammalian (human) cell viability. *Applied Nanoscience (Switzerland)*, 8(6), 1399–1414. <https://doi.org/10.1007/s13204-018-0801-1>
52. Kang, S., Mauter, M. S., & Elimelech, M. (2008). Physicochemical determinants of multiwalled carbon nanotube bacterial cytotoxicity. *Environmental Science and Technology*, 42(19), 7528–7534. <https://doi.org/10.1021/es8010173>
53. Kapoor, S., Goyal, M., & Jindal, P. (2017). Effect of Multi-Walled Carbon Nanotubes (MWCNT) on Mechanical Properties of Acrylonitrile Butadiene Styrene (ABS) Nano-Composite. *Indian Journal of Science and Technology*, 10(17). <https://doi.org/10.17485/ijst/2017/v10i17/114413>
54. Karlsson, H. L., Cronholm, P., Gustafsson, J., & Möller, L. (2008). Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. *Chemical Research in Toxicology*, 21(9), 1726–1732. <https://doi.org/10.1021/TX800064J/ASSET/IMAGES/MEDIUM/TX-2008->

55. Khan, F. S. A., Mubarak, N. M., Khalid, M., Khan, M. M., Tan, Y. H., Walvekar, R., Abdullah, E. C., Karri, R. R., & Rahman, M. E. (2021). Comprehensive review on carbon nanotubes embedded in different metal and polymer matrix: fabrications and applications. *Critical Reviews in Solid State and Materials Sciences*, 0(0), 1–28. <https://doi.org/10.1080/10408436.2021.1935713>
56. Kntayya, S. B., Ibrahim, M. D., Ain, N. M., Iori, R., Ioannides, C., & Abdull Razis, A. F. (2018). Induction of Apoptosis and Cytotoxicity by Isothiocyanate Sulforaphene in Human Hepatocarcinoma HepG2 Cells. *Nutrients* 2018, Vol. 10, Page 718, 10(6), 718. <https://doi.org/10.3390/NU10060718>
57. Krause, B., Mende, M., Pötschke, P., & Petzold, G. (2010). Dispersability and particle size distribution of CNTs in an aqueous surfactant dispersion as a function of ultrasonic treatment time. *Carbon*, 48(10), 2746–2754. <https://doi.org/10.1016/J.CARBON.2010.04.002>
58. Kumar, C. S. S. R. (2012). Raman spectroscopy for nanomaterials characterization. In *Raman Spectroscopy for Nanomaterials Characterization* (Vol. 9783642206). <https://doi.org/10.1007/978-3-642-20620-7>
59. Kumar, C. S. S. R. (2013). UV-VIS and photoluminescence spectroscopy for nanomaterials characterization. In *UV-VIS and Photoluminescence Spectroscopy for Nanomaterials Characterization*. <https://doi.org/10.1007/978-3-642-27594-4>

60. Laganà, P., Visalli, G., Facciola, A., Ciarello, M. P., Laganà, A., Iannazzo, D., & Di Pietro, A. (2021). Is the Antibacterial Activity of Multi-Walled Carbon Nanotubes (MWCNTs) Related to Antibiotic Resistance? An Assessment in Clinical Isolates. *International Journal of Environmental Research and Public Health*, 18(17), 9310. <https://doi.org/10.3390/ijerph18179310>
61. Latifi, H., & Hayati, P. (2021). *Effects of Nano-CuO, MWCNT and SBS on the Aging of Asphalt Binder: FTIR and XRD Analyses*. <https://www.researchgate.net/publication/352932909>
62. Lee, R. H., Cho, J. H., Jeon, Y. J., Bang, W., Cho, J. J., Choi, N. J., Seo, K. S., Shim, J. H., & Chae, J. Il. (2015). Quercetin Induces Antiproliferative Activity Against Human Hepatocellular Carcinoma (HepG2) Cells by Suppressing Specificity Protein 1 (Sp1). *Drug Development Research*, 76(1), 9–16. <https://doi.org/10.1002/DDR.21235>
63. Lee, S. H., Kim, M. W., Kim, S. H., & Youn, J. R. (2008). Rheological and electrical properties of polypropylene/MWCNT composites prepared with MWCNT masterbatch chips. *European Polymer Journal*, 44(6), 1620–1630. <https://doi.org/10.1016/J.EURPOLYMJ.2008.03.017>
64. Lei, H.-Y., Peng, C.-A., M.D., M.-J. T., Reindhart, K., & Szu, H. H. (2009). Con_A-carbone nanotube conjugate with short wave near-infrared laser ablation for tumor therapy. *Https://Doi.Org/10.1117/12.820838*, 7343, 221–229. <https://doi.org/10.1117/12.820838>
65. Leng, E., Xiao, Y., Mo, Z., Li, Y., Zhang, Y., Deng, X., Zhou, M., Zhou, C., He, Z., He, J., Xiao, L., Li, J., & Li, W. (2018). Synergistic effect of phytochemicals on cholesterol metabolism and lipid accumulation in HepG2

- cells. *BMC Complementary and Alternative Medicine*, 18(1), 1–10.
<https://doi.org/10.1186/S12906-018-2189-6/FIGURES/6>
66. Li, Q., Xue, Q., Hao, L., Gao, X., & Zheng, Q. (2008). Large dielectric constant of the chemically functionalized carbon nanotube/polymer composites. *Composites Science and Technology*, 68(10–11), 2290–2296.
<https://doi.org/10.1016/J.COMPSCITECH.2008.04.019>
67. Li, T., Cao, Y., Li, B., & Dai, R. (2021). The biological effects of radiation-induced liver damage and its natural protective medicine. *Progress in Biophysics and Molecular Biology*, 167(xxxx), 87–95.
<https://doi.org/10.1016/j.pbiomolbio.2021.06.012>
68. Li, Y., & Cao, J. (2018). The impact of multi-walled carbon nanotubes (MWCNTs) on macrophages: contribution of MWCNT characteristics. *Science China Life Sciences*, 61(11), 1333–1351.
<https://doi.org/10.1007/s11427-017-9242-3>
69. Li, Y., Yang, P., Zhao, L., Chen, Y., Zhang, X., Zeng, S., Wei, L., Varghese, Z., Moorhead, J. F., Chen, Y., & Ruan, X. Z. (2019). CD36 plays a negative role in the regulation of lipophagy in hepatocytes through an AMPK-dependent pathway. *Journal of Lipid Research*, 60(4), 844–855.
<https://doi.org/10.1194/jlr.M090969>
70. Li, Z., Weller, S. G., Drizyte-Miller, K., Chen, J., Krueger, E. W., Mehall, B., Casey, C. A., Cao, H., & McNiven, M. A. (2020). Maturation of Lipophagic Organelles in Hepatocytes Is Dependent Upon a Rab10/Dynamin-2 Complex. *Hepatology*, 72(2), 486–502. <https://doi.org/10.1002/HEP.31059>
71. Liu, J. F., Ma, Y., Wang, Y., Du, Z. Y., Shen, J. K., & Peng, H. L. (2011).

- Reduction of lipid accumulation in HepG2 Cells by luteolin is associated with activation of AMPK and Mitigation of oxidative stress. *Phytotherapy Research*, 25(4), 588–596. <https://doi.org/10.1002/PTR.3305>
72. Liu, Z., Dong, X., Song, L., Zhang, H., Liu, L., Zhu, D., Song, C., & Leng, X. (2014). Carboxylation of multiwalled carbon nanotube enhanced its biocompatibility with L02 cells through decreased activation of mitochondrial apoptotic pathway. *Journal of Biomedical Materials Research - Part A*, 102(3), 665–673. <https://doi.org/10.1002/jbm.a.34729>
73. Liu, Z., Tabakman, S., Welsher, K., & Dai, H. (2009). Carbon nanotubes in biology and medicine: In vitro and in vivo detection, imaging and drug delivery. *Nano Research*, 2(2), 85–120. <https://doi.org/10.1007/s12274-009-9009-8>
74. Long, J., Ma, W., Yu, Z., Liu, H., & Cao, Y. (2019). Multi-walled carbon nanotubes (MWCNTs) promoted lipid accumulation in THP-1 macrophages through modulation of endoplasmic reticulum (ER) stress. *Nanotoxicology*, 13(7), 938–951. <https://doi.org/10.1080/17435390.2019.1597204>
75. Malik, M. A., Wani, M. Y., Hashim, M. A., & Nabi, F. (2011). Nanotoxicity: Dimensional and morphological concerns. *Advances in Physical Chemistry*, 2011. <https://doi.org/10.1155/2011/450912>
76. Maruyama, K., Haniu, H., Saito, N., Matsuda, Y., Tsukahara, T., Kobayashi, S., Tanaka, M., Aoki, K., Takanashi, S., Okamoto, M., & Kato, H. (2015). Endocytosis of multiwalled carbon nanotubes in bronchial epithelial and mesothelial cells. *BioMed Research International*, 2015. <https://doi.org/10.1155/2015/793186>

77. Milowska, K. Z. (2015). Influence of Carboxylation on Structural and Mechanical Properties of Carbon Nanotubes: Composite Reinforcement and Toxicity Reduction Perspectives. *Journal of Physical Chemistry C*, 119(47), 26734–26746. <https://doi.org/10.1021/acs.jpcc.5b08402>
78. Mohanta, D., Patnaik, S., Sood, S., & Das, N. (2019a). Carbon nanotubes: Evaluation of toxicity at biointerfaces. *Journal of Pharmaceutical Analysis*, 9(5), 293–300. <https://doi.org/10.1016/j.jpha.2019.04.003>
79. Mohanta, D., Patnaik, S., Sood, S., & Das, N. (2019b). Carbon nanotubes: Evaluation of toxicity at biointerfaces. *Journal of Pharmaceutical Analysis*, 9(5), 293–300. <https://doi.org/10.1016/j.jpha.2019.04.003>
80. Mohanta, D., Patnaik, S., Sood, S., & Das, N. (2019c). Carbon nanotubes: Evaluation of toxicity at biointerfaces. *Journal of Pharmaceutical Analysis*, 9(5), 293–300. <https://doi.org/10.1016/j.jpha.2019.04.003>
81. Møller, P., & Loft, S. (2010). Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. *Environmental Health Perspectives*, 118(8), 1126–1136. <https://doi.org/10.1289/ehp.0901725>
82. Møller, P., Wils, R. S., Di Ianni, E., Gutierrez, C. A. T., Roursgaard, M., & Jacobsen, N. R. (2021). Genotoxicity of multi-walled carbon nanotube reference materials in mammalian cells and animals. *Mutation Research - Reviews in Mutation Research*, 788(August 2021). <https://doi.org/10.1016/j.mrrev.2021.108393>
83. Monteiro-Riviere, N. A., & Inman, A. O. (2006). Challenges for assessing carbon nanomaterial toxicity to the skin. *Carbon*, 44(6), 1070–1078. <https://doi.org/10.1016/j.carbon.2005.11.004>

84. Monteiro-Riviere, N. A., Nemanich, R. J., Inman, A. O., Wang, Y. Y., & Riviere, J. E. (2005). Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicology Letters*, 155(3), 377–384. <https://doi.org/10.1016/j.toxlet.2004.11.004>
85. Morimoto, Y., Hirohashi, M., Ogami, A., Oyabu, T., Myojo, T., Todoroki, M., Yamamoto, M., Hashiba, M., Mizuguchi, Y., Lee, B. W., Kuroda, E., Shimada, M., Wang, W. N., Yamamoto, K., Fujita, K., Endoh, S., Uchida, K., Kobayashi, N., Mizuno, K., ... Tanaka, I. (2012). Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation. *Nanotoxicology*, 6(6), 587–599. <https://doi.org/10.3109/17435390.2011.594912>
86. Mouchet, F., Landois, P., Puech, P., Pinelli, E., Flahaut, E., & Gauthier, L. (2010). Carbon nanotube ecotoxicity in amphibians: assessment of multiwalled carbon nanotubes and comparison with double-walled carbon nanotubes. *Nanomedicine*, 5(6), 963–974. <https://doi.org/10.2217/nnm.10.60>
87. Muller, J., Huaux, F., Moreau, N., Misson, P., Heilier, J. F., Delos, M., Arras, M., Fonseca, A., Nagy, J. B., & Lison, D. (2005). Respiratory toxicity of multi-wall carbon nanotubes. *Toxicology and Applied Pharmacology*, 207(3), 221–231. <https://doi.org/10.1016/j.taap.2005.01.008>
88. Murr, L., Garza, K., Soto, K., Carrasco, A., Powell, T., Ramirez, D., Guerrero, P., Lopez, D., & Venzor, J. (2005). Cytotoxicity Assessment of Some Carbon Nanotubes and Related Carbon Nanoparticle Aggregates and the Implications for Anthropogenic Carbon Nanotube Aggregates in the

- Environment. *International Journal of Environmental Research and Public Health*, 2(1), 31–42. <https://doi.org/10.3390/ijerph2005010031>
89. Nagai, H., & Toyokuni, S. (2012). Differences and similarities between carbon nanotubes and asbestos fibers during mesothelial carcinogenesis: Shedding light on fiber entry mechanism. *Cancer Science*, 103(8), 1378–1390. <https://doi.org/10.1111/J.1349-7006.2012.02326.X>
90. Nagajyothi, P. C., Veeranjaneey Reddy, L., Devarayapalli, K. C., Prabhakar Vattikuti, S. V., Wee, Y. J., & Shim, J. (2021). Environmentally Friendly Synthesis: Photocatalytic Dye Degradation and Bacteria Inactivation Using Ag/f-MWCNTs Composite. *Journal of Cluster Science*, 32(3), 711–718. <https://doi.org/10.1007/s10876-020-01821-8>
91. Nikzamir, M., Akbarzadeh, A., & Panahi, Y. (2021). An overview on nanoparticles used in biomedicine and their cytotoxicity. *Journal of Drug Delivery Science and Technology*, 61(December 2020), 102316. <https://doi.org/10.1016/j.jddst.2020.102316>
92. Oberoi, R., Vlacil, A. K., Schuett, J., Schösser, F., Schuett, H., Tietge, U. J. F., Schieffer, B., & Grote, K. (2018). Anti-tumor necrosis factor- α therapy increases plaque burden in a mouse model of experimental atherosclerosis. *Atherosclerosis*, 277, 80–89. <https://doi.org/10.1016/j.atherosclerosis.2018.08.030>
93. Ohadi, M., Rezaei, P., Mehrabani, M., Behnam, B., & Ansari, M. (2022). Synthesis, Characterization and Toxicity Assessment of the Novel Non covalent Functionalized Multi-walled Carbon Nanotubes with Glycyrrhizin, Curcumin and Rutin. *Journal of Cluster Science*, 33(3), 975–984.

<https://doi.org/10.1007/s10876-021-02026-3>

94. Park, S., & Choi, I. S. (2009). Production of Ultrahigh-Molecular-Weight Polyethylene/Pristine MWCNT Composites by Half-Titanocene Catalysts. *Advanced Materials*, 21(8), 902–905. <https://doi.org/10.1002/ADMA.200801674>
95. Patlolla, A., Patlolla, B., & Tchounwou, P. (2010). Evaluation of cell viability, DNA damage, and cell death in normal human dermal fibroblast cells induced by functionalized multiwalled carbon nanotube. *Molecular and Cellular Biochemistry*, 338(1–2), 225–232. <https://doi.org/10.1007/s11010-009-0356-2>
96. Pei, L., Yang, W., & Cao, Y. (2019). Influences of Unmodified and Carboxylated Carbon Nanotubes on Lipid Profiles in THP-1 Macrophages: A Lipidomics Study. *Original Article International Journal of Toxicology*, 2022(1), 16–25. <https://doi.org/10.1177/10915818211056633>
97. Pinheiro, H. M., Touraud, E., & Thomas, O. (2004). Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes and Pigments*, 61(2), 121–139. <https://doi.org/10.1016/j.dyepig.2003.10.009>
98. Pisal, S. H., Harale, N. S., Bhat, T. S., Deshmukh, H. P., & Patil, P. S. (2014). Functionalized Multi-Walled Carbon Nanotubes for Nitrogen Sensor. *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 7(11), 49–52. www.iosrjournals.org
99. Poland, C. A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W. A. H., Seaton, A., Stone, V., Brown, S., MacNee, W., & Donaldson, K. (2008). Carbon

- nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*, 3(7), 423–428. <https://doi.org/10.1038/nnano.2008.111>
100. Puett, C., Inscoe, C., Hartman, A., Calliste, J., Franceschi, D. K., Lu, J., Zhou, O., & Lee, Y. Z. (2018). An update on carbon nanotube-enabled X-ray sources for biomedical imaging. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 10(1), 1–11. <https://doi.org/10.1002/wnan.1475>
 101. Requardt, H., Braun, A., Steinberg, P., Hampel, S., & Hansen, T. (2019). Surface defects reduce Carbon Nanotube toxicity in vitro. *Toxicology in Vitro*, 60(March), 12–18. <https://doi.org/10.1016/j.tiv.2019.03.028>
 102. Requardt, H., Hansen, T., Eisenbarth, E., Hampel, S., & Dasenbrock, C. (2015). *In vitro toxicity testing of two multi-walled carbon nanotubes determined for the purpose of targeted drug delivery*. <https://publica.fraunhofer.de/handle/publica/240153>
 103. Rezazadeh Azari, M., & Mohammadian, Y. (2020). Comparing in vitro cytotoxicity of graphite, short multi-walled carbon nanotubes, and long multi-walled carbon nanotubes. *Environmental Science and Pollution Research*, 27(13), 15401–15406. <https://doi.org/10.1007/s11356-020-08036-4>
 104. Rohit Singh, T., & Ezhilarasan, D. (2019). Ethanolic Extract of Lagerstroemia Speciosa (L.) Pers., Induces Apoptosis and Cell Cycle Arrest in HepG2 Cells. <https://doi.org/10.1080/01635581.2019.1616780>, 72(1), 146–156. <https://doi.org/10.1080/01635581.2019.1616780>
 105. Roth, K., Strickland, J., & Copple, B. L. (2020). Regulation of macrophage

- activation in the liver after acute injury: Role of the fibrinolytic system. *World Journal of Gastroenterology*, 26(16), 1879–1887.
<https://doi.org/10.3748/wjg.v26.i16.1879>
106. Sahithi, K., Swetha, M., Ramasamy, K., Srinivasan, N., & Selvamurugan, N. (2010). Polymeric composites containing carbon nanotubes for bone tissue engineering. *International Journal of Biological Macromolecules*, 46(3), 281–283. <https://doi.org/10.1016/j.ijbiomac.2010.01.006>
 107. Saleemi, M. A., Hosseini Fouladi, M., Yong, P. V. C., Chinna, K., Palanisamy, N. K., & Wong, E. H. (2021). Toxicity of Carbon Nanotubes: Molecular Mechanisms, Signaling Cascades, and Remedies in Biomedical Applications. *Chemical Research in Toxicology*, 34(1), 24–46.
<https://doi.org/10.1021/acs.chemrestox.0c00172>
 108. Schott, M. B., Weller, S. G., Schulze, R. J., Krueger, E. W., Drizyte-Miller, K., Casey, C. A., & McNiven, M. A. (2019). Lipid droplet size directs lipolysis and lipophagy catabolism in hepatocytes. *Journal of Cell Biology*, 218(10), 3320–3335. <https://doi.org/10.1083/JCB.201803153>
 109. Seixas, A. L. R., Ferreira-Cravo, M., Kalb, A. C., Romano, L. A., Kaufmann, C. G. J. R., & Monserrat, J. M. (2018). Protein oxidation in the fish *Danio rerio* (Cyprinidae) fed with single- and multi-walled carbon nanotubes. *Energy, Ecology and Environment*, 3(2), 95–101.
<https://doi.org/10.1007/s40974-017-0080-9>
 110. Seo, Y., Hwang, J., Lee, E., Kim, Y. J., Lee, K., Park, C., Choi, Y., Jeon, H., & Choi, J. (2018). Engineering copper nanoparticles synthesized on the surface of carbon nanotubes for anti-microbial and anti-biofilm applications.

- Nanoscale*, 10(33), 15529–15544. <https://doi.org/10.1039/c8nr02768d>
111. Sharma, S., Naskar, S., & Kuotsu, K. (2020). Metronomic chemotherapy of carboplatin-loaded PEGylated MWCNTs: synthesis, characterization and in vitro toxicity in human breast cancer. *Carbon Letters*, 30(4), 435–447. <https://doi.org/10.1007/S42823-019-00113-0/METRICS>
 112. Shen, Z., Wu, J., Yu, Y., Liu, S., Jiang, W., Nurmamat, H., & Wu, B. (n.d.). *Comparison of cytotoxicity and membrane efflux pump inhibition in HepG2 cells induced by single-walled carbon nanotubes with different length and functional groups*. <https://doi.org/10.1038/s41598-019-43900-5>
 113. Shimizu, T., Ding, W., & Kameta, N. (2020). Soft-Matter Nanotubes: A Platform for Diverse Functions and Applications. *Chemical Reviews*, 120(4), 2347–2407. <https://doi.org/10.1021/acs.chemrev.9b00509>
 114. Silva, I. F., Papa, L. F., Carneiro, P. G., Schnitzler, M. C., Andrade, S. N., Ribeiro, R. I. M. A., Varotti, F. P., Thomé, R. G., & Santos, H. B. (2021). Primary culture macrophages from fish as potential experimental model in toxicity studies with multiwalled carbon nanotubes. *Acta Scientiarum - Biological Sciences*, 43(Mmc), 1–10. <https://doi.org/10.4025/actascibiolsoci.v43i1.52612>
 115. Sinha, S., Biswas, K., De, D., & Bandyopadhyay, J. (2016). Implications of multi wall carbon nanotube over living system using C. elegans as worm model. *Materials Today: Proceedings*, 3(10), 3310–3317. <https://doi.org/10.1016/j.matpr.2016.10.012>
 116. Sinha, S., Paul, T., Mishra, S., Shaw, S., Biswas, K., De, D., Sarkar, A., & Bandyopadhyay, J. (2021). In Vivo Biointeraction and Alleviation of

- Toxicity of MWCNTs upon Functionalization with ssDNA in a *Caenorhabditis elegans* Model. *Journal of Electronic Materials*, 50(8), 4974–4990. <https://doi.org/10.1007/s11664-021-09006-3>
117. Sohaebuddin, S. K., & Tang, L. (2013). A simple method to visualize and assess the integrity of lysosomal membrane in mammalian cells using a fluorescent dye. *Methods in Molecular Biology*, 991, 25–31. https://doi.org/10.1007/978-1-62703-336-7_3/COVER
 118. Song, B., Chen, M., Ye, S., Xu, P., Zeng, G., Gong, J., Li, J., Zhang, P., & Cao, W. (2019a). Effects of multi-walled carbon nanotubes on metabolic function of the microbial community in riverine sediment contaminated with phenanthrene. *Carbon*, 144, 1–7. <https://doi.org/10.1016/j.carbon.2018.12.016>
 119. Song, B., Chen, M., Ye, S., Xu, P., Zeng, G., Gong, J., Li, J., Zhang, P., & Cao, W. (2019b). Effects of multi-walled carbon nanotubes on metabolic function of the microbial community in riverine sediment contaminated with phenanthrene. *Carbon*, 144, 1–7. <https://doi.org/10.1016/j.carbon.2018.12.016>
 120. Suárez, S., Ramos-Moore, E., & Mücklich, F. (2013). A high temperature X-ray diffraction study of the influence of MWCNTs on the thermal expansion of MWCNT/Ni composites. *Carbon*, 51(1), 404–409. <https://doi.org/10.1016/J.CARBON.2012.09.002>
 121. Sun, H., Feng, J., & Tang, L. (2020). Function of TREM1 and TREM2 in Liver-Related Diseases. *Cells*, 9(12), 2626. <https://doi.org/10.3390/cells9122626>

122. Tan, J. M., Karthivashan, G., Arulselvan, P., Fakurazi, S., & Hussein, M. Z. (2014). Characterization and in vitro studies of the anticancer effect of oxidized carbon nanotubes functionalized with betulinic acid. *Drug Design, Development and Therapy*, 8, 2333–2343. <https://doi.org/10.2147/DDDT.S70650>
123. Tan, X. ming, Lin, C., & Fugetsu, B. (2009a). Studies on toxicity of multi-walled carbon nanotubes on suspension rice cells. *Carbon*, 47(15), 3479–3487. <https://doi.org/10.1016/J.CARBON.2009.08.018>
124. Tan, X. ming, Lin, C., & Fugetsu, B. (2009b). Studies on toxicity of multi-walled carbon nanotubes on suspension rice cells. *Carbon*, 47(15), 3479–3487. <https://doi.org/10.1016/J.CARBON.2009.08.018>
125. Tantra, R., Schulze, P., & Quincey, P. (2010). Effect of nanoparticle concentration on zeta-potential measurement results and reproducibility. *Particuology*, 8(3), 279–285. <https://doi.org/10.1016/J.PARTIC.2010.01.003>
126. Tripathi, A., Pandey, V., Sahu, A. N., Singh, A. K., & Dubey, P. K. (2019). Encircling granulosa cells protects against di-(2-ethylhexyl)phthalate-induced apoptosis in rat oocytes cultured in vitro. *Zygote*, 27(4), 203–213. <https://doi.org/10.1017/S0967199419000121>
127. Tsukahara, T., & Haniu, H. (2011). Nanoparticle-mediated intracellular lipid accumulation during C2C12 cell differentiation. *Biochemical and Biophysical Research Communications*, 406(4), 558–563. <https://doi.org/10.1016/j.bbrc.2011.02.090>
128. Ursu, E. L., Doroftei, F., Peptanariu, D., Pinteala, M., & Rotaru, A. (2017).

- DNA-assisted decoration of single-walled carbon nanotubes with gold nanoparticles for applications in surface-enhanced Raman scattering imaging of cells. *Journal of Nanoparticle Research*, 19(5), 1–9. <https://doi.org/10.1007/s11051-017-3876-9>
129. Utembe, W. (2019). Chirality, a neglected physico-chemical property of nanomaterials? A mini-review on the occurrence and importance of chirality on their toxicity. *Toxicology Letters*, 311(May), 58–65. <https://doi.org/10.1016/j.toxlet.2019.04.032>
 130. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 39(1), 44–84. <https://doi.org/10.1016/j.biocel.2006.07.001>
 131. Vijayalakshmi, V., Sadanandan, B., & Anjanapura, R. V. (2023). In vitro comparative cytotoxic assessment of pristine and carboxylic functionalized multiwalled carbon nanotubes on LN18 cells. *Journal of Biochemical and Molecular Toxicology*, 37(3), e23283. <https://doi.org/10.1002/JBT.23283>
 132. Vijayalakshmi, V., Sadanandan, B., & Venkataramanaiah Raghu, A. (2022). Single walled carbon nanotubes in high concentrations is cytotoxic to the human neuronal cell LN18. *Results in Chemistry*, 4(May), 100484. <https://doi.org/10.1016/j.rechem.2022.100484>
 133. Vizetto-Duarte, C., Custódio, L., Acosta, G., Lago, J. H. G., Morais, T. R., De Sousa, C. B., Gangadhar, K. N., Rodrigues, M. J., Pereira, H., Lima, R. T., Vasconcelos, M. H., Barreira, L., Rauter, A. P., Albericio, F., & Varela, J. (2016). Can macroalgae provide promising anti-tumoral compounds? A

- closer look at *Cystoseira tamariscifolia* as a source for antioxidant and anti-hepatocarcinoma compounds. *PeerJ*, 2016(2).
<https://doi.org/10.7717/PEERJ.1704>
134. Wan, Q., Tian, J., Liu, M., Zeng, G., Li, Z., Wang, K., Zhang, Q., Deng, F., Zhang, X., & Wei, Y. (2015). Mussel inspired preparation of highly dispersible and biocompatible carbon nanotubes. *RSC Advances*, 5(32), 25329–25336. <https://doi.org/10.1039/C4RA13408G>
 135. Wang, J., Li, W., Zhang, L., Ban, L., Chen, P., Du, W., Feng, X., & Liu, B. F. (2017). Chemically Edited Exosomes with Dual Ligand Purified by Microfluidic Device for Active Targeted Drug Delivery to Tumor Cells. *ACS Applied Materials and Interfaces*, 9(33), 27441–27452. https://doi.org/10.1021/ACSAMI.7B06464/SUPPL_FILE/AM7B06464_SI_001.PDF
 136. Wang, M., Yu, S., Wang, C., & Kong, J. (2010). Tracking the endocytic pathway of recombinant protein toxin delivered by multiwalled carbon nanotubes. *ACS Nano*, 4(11), 6483–6490. https://doi.org/10.1021/NN101445Y/SUPPL_FILE/NN101445Y_SI_001.AVI
 137. Wick, P., Manser, P., Limbach, L. K., Dettlaff-Weglikowska, U., Krumeich, F., Roth, S., Stark, W. J., & Bruinink, A. (2007). The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicology Letters*, 168(2), 121–131. <https://doi.org/10.1016/j.toxlet.2006.08.019>
 138. Witzmann, F. A., & Monteiro-Riviere, N. A. (2006). Multi-walled carbon nanotube exposure alters protein expression in human keratinocytes.

- Nanomedicine: Nanotechnology, Biology, and Medicine*, 2(3), 158–168.
<https://doi.org/10.1016/j.nano.2006.07.005>
139. Wu, B., Jiang, M., Liu, X., Huang, C., Gu, Z., & Cao, Y. (2020). Evaluation of toxicity of halloysite nanotubes and multi-walled carbon nanotubes to endothelial cells in vitro and blood vessels in vivo. *Nanotoxicology*, 14(8), 1017–1038. <https://doi.org/10.1080/17435390.2020.1780642>
 140. Xu, M., Fujita, D., Kajiwara, S., Minowa, T., Li, X., Takemura, T., Iwai, H., & Hanagata, N. (2010). Contribution of physicochemical characteristics of nano-oxides to cytotoxicity. *Biomaterials*, 31(31), 8022–8031. <https://doi.org/10.1016/j.biomaterials.2010.06.022>
 141. Yang, H., Li, J., Yang, C., Liu, H., & Cao, Y. (2019). Multi-walled carbon nanotubes promoted lipid accumulation in human aortic smooth muscle cells. *Toxicology and Applied Pharmacology*, 374(April), 11–19. <https://doi.org/10.1016/j.taap.2019.04.022>
 142. Yang, S. T., Luo, J., Zhou, Q., & Wang, H. (2012). Pharmacokinetics, metabolism and toxicity of carbon nanotubes for bio-medical purposes. *Theranostics*, 2(3), 271–282. <https://doi.org/10.7150/thno.3618>
 143. Ye, S. F., Wu, Y. H., Hou, Z. Q., & Zhang, Q. Q. (2009). ROS and NF- κ B are involved in upregulation of IL-8 in A549 cells exposed to multi-walled carbon nanotubes. *Biochemical and Biophysical Research Communications*, 379(2), 643–648. <https://doi.org/10.1016/J.BBRC.2008.12.137>
 144. Yi, P., & Chen, K. L. (2013). Interaction of multiwalled carbon nanotubes with supported lipid bilayers and vesicles as model biological membranes.

- Environmental Science and Technology*, 47(11), 5711–5719.
<https://doi.org/10.1021/es4002604>
145. Yuan, J., Gao, H., & Ching, C. B. (2011). Comparative protein profile of human hepatoma HepG2 cells treated with graphene and single-walled carbon nanotubes: An iTRAQ-coupled 2D LC–MS/MS proteome analysis. *Toxicology Letters*, 207(3), 213–221.
<https://doi.org/10.1016/J.TOXLET.2011.09.014>
146. Yuan, J., Gao, H., Sui, J., Duan, H., Chen, W. N., & Ching, C. B. (2012). Cytotoxicity Evaluation of Oxidized Single-Walled Carbon Nanotubes and Graphene Oxide on Human Hepatoma HepG2 cells: An iTRAQ-Coupled 2D LC-MS/MS Proteome Analysis. *Toxicological Sciences*, 126(1), 149–161.
<https://doi.org/10.1093/TOXSCI/KFR332>
147. Zaman, A. C., Kaya, F., & Kaya, C. (2020). A study on optimum surfactant to multiwalled carbon nanotube ratio in alcoholic stable suspensions via UV–Vis absorption spectroscopy and zeta potential analysis. *Ceramics International*, 46(18), 29120–29129.
<https://doi.org/10.1016/J.CERAMINT.2020.08.085>
148. Zhang, H. Y., Chen, R. L., Shao, Y., Wang, H. L., & Liu, Z. G. (2018). Effects of exposure of adult mice to multi-walled carbon nanotubes on the liver lipid metabolism of their offspring. *Toxicology Research*, 7(5), 809–816. <https://doi.org/10.1039/c8tx00032h>
149. Zhang, T., Liu, J., Shen, S., Tong, Q., Ma, X., & Lin, L. (2019). SIRT3 promotes lipophagy and chaperon-mediated autophagy to protect hepatocytes against lipotoxicity. *Cell Death & Differentiation* 2019 27:1,

- 27(1), 329–344. <https://doi.org/10.1038/s41418-019-0356-z>
150. Zhang, T., Tang, M., Yao, Y., Ma, Y., & Pu, Y. (2019). MWCNT interactions with protein: Surface-induced changes in protein adsorption and the impact of protein corona on cellular uptake and cytotoxicity. *International Journal of Nanomedicine*, 14, 993–1009. <https://doi.org/10.2147/IJN.S191689>
151. Zhang, W., Zhang, Z., & Zhang, Y. (2011). The application of carbon nanotubes in target drug delivery systems for cancer therapies. *Nanoscale Research Letters*, 6, 1–22. <https://doi.org/10.1186/1556-276X-6-555>
152. Zhang, Y., Wang, Y., Liu, A., Xu, S. L., Zhao, B., Zhang, Y., Zou, H., Wang, W., Zhu, H., & Yan, B. (2016). Modulation of Carbon Nanotubes' Perturbation to the Metabolic Activity of CYP3A4 in the Liver. *Advanced Functional Materials*, 26(6), 841–850. <https://doi.org/10.1002/adfm.201504182>
153. Zhao, C., Zhou, Y., Liu, L., Long, J., Liu, H., Li, J., & Cao, Y. (2018). Lipid accumulation in multi-walled carbon nanotube-exposed HepG2 cells: Possible role of lipophagy pathway. *Food and Chemical Toxicology*, 121, 65–71. <https://doi.org/10.1016/j.fct.2018.08.033>
154. Zhao, J., Luo, W., Xu, Y., Ling, J., & Deng, L. (2021). Potential reproductive toxicity of multi-walled carbon nanotubes and their chronic exposure effects on the growth and development of *Xenopus tropicalis*. *Science of the Total Environment*, 766(100), 142652. <https://doi.org/10.1016/j.scitotenv.2020.142652>
155. Zhao, J., Xie, G., Xu, Y., Zheng, L., & Ling, J. (2020). Accumulation and

toxicity of multi-walled carbon nanotubes in *Xenopus tropicalis* tadpoles.

Chemosphere, 257, 127205.

<https://doi.org/10.1016/j.chemosphere.2020.127205>

ANNEXURE

Submitted the book chapter “Emerging graphene-based nanomaterials as DNA biosensor(s) using lab-on-chip, computational approaches, and robotics” was submitted on 12th August to Elsevier, CRC press (Taylor & Francis) and it was accepted on 1st February, 2023.



Acceptance for the Contributed book chapter

1 message

Dr. Yugal Kishore Mohanta <ykmohanta@gmail.com>

Wed, 1 Feb, 2023 at 8:02 pm

To: Dr. Kunal Biswas <kunalbiswas.irc@sathyabama.ac.in>, Dr. Kunal Biswas <kunal.sapiens@gmail.com>

Dear Dr K Biswas
Greetings!

I am glad to inform you that the submitted chapter from your team EMERGING GRAPHENE-BASED NANOMATERIALS AS DNA BIOSENSOR(S) USING LAB-ON-CHIP, COMPUTATIONAL APPROACHES, AND ROBOTICS has been accepted for publication in the book Graphene Nanomaterials in Food, Health & Agriculture sectors published by CRC Press.

The final proof will be sent to you by the CRC publisher for your final corrections.

Best Wishes
Editorial Team

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Presented the article “**Multi-walled carbon nanotube-based therapy to cancer and the brain beyond the blood-brain barrier: A Review**” on 11th February in GCORT’23 conducted by Anna University and the abstract has been **published** in the GCORT proceedings by “Association of pharmacy professionals”

POSTER PRESENTATION (virtual mode)

PROSPECTS OF MULTI-WALLED CARBON NANOTUBE-BASED THERAPY TO CANCER AND THE BRAIN BEYOND THE BLOOD-BRAIN BARRIER: A REVIEW

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The most common type of central nervous system tumour that affects people of all ages is a brain tumour, which recently exceeded leukaemia as the leading cause of cancer-related mortality. There are currently few effective therapies for tumours and disorders related to the central nervous system. The lack of treatment is because of the challenges given by medication insolubility, poor distribution across cells, lack of selectivity, failure to pass cellular barriers, and blood-brain barrier (BBB). The blood-brain barrier (BBB) is a covert barrier that guards the brain, enables only a narrow range of substances to enter the brain, and contributes to the regulation of brain homeostasis. The BBB is made up of several molecular parts and transport mechanisms, which in turn provide facilities for drug efflux or blockages to drug entrance into the brain. Effective treatments for brain tumours are still difficult to find because of the BBB's low permeability and high selectivity to conventional anticancer medications.

Multi-walled carbon nanotubes (MWCNTs) have various unique properties that make them good candidates for drug delivery to the brain and cancer. Thus, to address this challenge in the treatment of brain tumours, effective drug delivery modification and unique therapeutic approaches are required in addition to the usual treatments. These characteristics include good electronic properties, a remarkable ability to penetrate the cell membrane, high drug-loading and pH-dependent therapeutic unloading capacities, thermal properties, a large surface area, and ease of molecular modification. MWCNTs might deliver drugs with great effectiveness, increased specificity, and reduced adverse effects. CNTs have mostly been used to treat cancer, although a few research have concentrated on employing MWCNTs to treat and diagnose disorders of the central nervous system.

Here, we evaluate the present status of *in vitro* and *in vivo* studies on CNT-based tumour-targeted drug delivery systems (DDS), photodynamic therapy (PDT), and photothermal therapy for the treatment of cancer (PTT). In the meanwhile, we examine the state of the art of *in vivo* and *in vitro* studies of CNT-based medication delivery to the brain.



DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

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GCORT'23
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Virtually / Physically organised by the Department of
Pharmaceutical Technology, BIT Campus, Anna University,
Tiruchirappalli, held on 11 & 12 February 2023.

Dr. S. Latha
Convener

Dr. P. Selvamani
Convener

Dr. A. Puratchikody
Prof & HOD

Dr. T. Senthilkumar
Prof & Dean

Presented the article “**Effect of Multi-Wall Carbon Nanotube on Hepatocyte and its impact on lipid metabolism**” on 21st March was submitted for ICONN’23 conducted by SRM University and the abstract has been **published** in the ICONN’23 proceedings.

7th International Conference on Nonoscience and Nanotechnology (ICONN 2023), SRM IST, Chennai.

Effect of Multi Walled Carbon Nanotube on various bio interphase and its impact on lipid metabolism

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Abstract

Multi-walled carbon nanotubes (MWCNTs) are carbon-based nanomaterials that have gained significant appeal in nanotechnology. They are highly coveted for usage in the industrial, environmental, and medicinal fields because of their remarkable one-dimensional hollow nanostructure and distinctive physicochemical features. Despite their extensive use, limited information is known about how they affect the environment and living organisms. The first stage in determining the biosafety and biocompatibility of nanoparticles is to determine their toxic concentration in humans. Therefore, it is critical to have precise knowledge about how MWCNTs affect specific organs in order to determine their effectiveness and potential implications on health. Numerous metabolic processes can be hindered by a high nanoparticle concentration in different organs. Recent research demonstrated that exposure to MWCNT could cause lipid accumulation in different biosystems. However, the mechanisms linking MWCNT exposure and lipid accumulation remain poorly understood. This study focuses on the recent research on the toxicity of MWCNT and its potential impact on lipid metabolism in various organ systems (bio-interphase).

References:

1. Y. Zhang, Y. Wang, A. Liu et al., *Advanced Functional Materials*, 2016.
2. H. Requardt, A. Braun, P. Steinberg et al., *Toxicology in Vitro*, 2019.
3. H. Yang, J. Li, C. Yang, et al. 2019. *Toxicology and Applied Pharmacology*, 2019.
4. C. Zhao, Y. Zhou, L. Liu et al. *Food and Chemical Toxicology*, 2018.
5. H. Y. Zhang, R. L. Chen, Y. Shao et al. 2018. *Toxicology Research*, 2018.
6. D Mohanta, S. Patnaik, S. Sood, and N. Das, *Journal of Pharmaceutical Analysis*, 2019.



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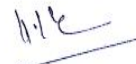
(Virtual Conference) March 27 - 29, 2023

Certificate of Participation

This is to certify that Mr. / Ms. / Dr. **Sandhya S** has participated and presented a paper entitled **Effect of Multi Walled Carbon Nanotube on various bio interphase and its impact on lipid metabolism** in the 7th International Conference on Nanoscience and Nanotechnology (ICONN-2023) organized by Department of Physics and Nanotechnology, SRM IST, India during March 27- 29, 2023, in association with Shizuoka University, Japan; National Yang Ming Chiao Tung University, Taiwan; GNS Science, New Zealand; University of Rome Tor Vergata, Italy; Asian Consortium on Computational Materials Science (ACCMS), Japan; Indian Ceramic Society; Indian Physics Association (IPA); Solar Energy Society of India (SESI); Innovation, Science & Technology Foundation - Tirupati (ISTF-T) and co-sponsored by Defence Research and Development Organization (DRDO), India; Council of Scientific & Industrial Research (CSIR), India; The Indian Science Congress Association (ISCA) and Springer Nature.


Head

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SRM IST, KTR


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School of Applied Sciences
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1672

Submitted the review article “**Current advances on biomedical applications and toxicity of MWCNTS: a review**” on 24th November in **Bionanoscience Journal, Springer**.

Received response for **acceptance** of paper on 19th April, 2023.



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Fwd: BioNanoScience: Decision on your manuscript

1 message

Dr. Kunal Biswas <kunal.sapiens@gmail.com>

19 April 2023 at 22:20

To: sandhya selvakumar <sandy1162105@gmail.com>, thyagarajen@gmail.com

Congratulations to Sandhya to get her web of science (WOS) , UGC care, Scopus indexed paper accepted.

Best Wishes,

Dr. Kunal Biswas
IRC

----- Forwarded message -----

From: **BioNanoScience** <joshua.manaba@springernature.com>

Date: Wed, 19 Apr, 2023, 9:53 pm

Subject: BioNanoScience: Decision on your manuscript

To: <kunal.sapiens@gmail.com>

Ref: Submission ID 7d438919-b36a-48bd-b2d4-8a7012a9d958

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CURRENT ADVANCES ON BIOMEDICAL APPLICATIONS AND TOXICITY OF MWCNTS: A REVIEW

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Abbreviations

BBB	Blood-brain barrier
CNTs	Carbon nanotubes
CTS-HNTs	Chitosan-functionalized-halloysite nanotubes
Cu	Copper
DNA	Deoxyribonucleic acid
ED	Electron Diffraction
EMT	Epithelial-mesenchymal transition
FBR	Foreign body response
F-HNT	Functionalized halloysite nanotubes
GPa	Gigapascal
HEK	Human Embryonic Kidney
HNTs	Halloysite nanotubes
Lac	Laccase
MAPK	Mitogen-activated protein kinase;
MMC	Melanomacrophage centres
MWCNTs	Multi-walled carbon nanotube
NASH	Non-alcoholic steatohepatitis
NF- κ B	Nuclear factor Kappa B
NLRP3	Nucleotide-binding oligomerization domain-like receptor: pyrin domain-containing 3
NMs	Nano materials

Nps	Nanoparticles
NIR	Near-infrared region
PCA	Principle component analysis
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SWCNTs	Single-walled carbon nanotubes
TEM	Transmission electron microscopy
TGF	Transforming growth factor
TPa	Terapascal

ABSTRACT

Recently, carbon nanotubes (CNTs) have become a possible new choice in variable disciplines such as gene therapy, bioengineering, cancer treatment and nanotechnology applications in devices, and optics. This is because MWCNTs are more durable and possess high electrical and thermal conductivity along with some of the significant unique physico-chemical characteristics. As the manufacturing and usage of CNTs rises, the likelihood of exposure to CNTs, whether directly or indirectly, is higher in the general population. As a result, it becomes crucial to examine the problems related to the toxicity of CNTs and identify the most effective solutions to address such concerns. Available data demonstrates unequivocally that nanotubes may, in some circumstances, pass membrane barriers and enter the organ which may lead to adverse consequences such as inflammatory and fibrotic responses. So, the first step in assessing the biosafety and biocompatibility of CNTs in living organisms is to quantify their abundance. This review article discusses the toxicity mechanisms of MWCNTs *in vitro* and *in vivo* in different bio-systems (specifically liver) and their interaction mechanism. In addition, it highlights the latest advances in carbon nanotubes in the healthcare and pharmaceutical sector from multiple perspectives.

Keywords: Multiwalled-carbon nanotubes, *in vivo* toxicity, *in vitro* toxicity, hepatocytes, bio-systems

1. INTRODUCTION

Engineering fields utilizing nanoparticles, structures with characteristic diameters ranging from 1 to 100 nm, have shown tremendous growth[1], [2]. When nanoparticles are properly engineered, they display a wide range of unique and adjustable chemical and physical characteristics. Because of these properties, synthesized nanoparticles have become essential components in a wide range of developing technologies. These distinct qualities may result in similarly diverse biological outcomes[3]. Iijima published the first description of carbon nanotubes over ten years ago[4]. SWCNTs (Single-walled carbon nanotubes) and MWCNTs (Multi-walled carbon nanotubes) are two types of nanotubes. SWCNTs are carbon nanotubes that have a structural arrangement similar to that of a graphite sheet coiled up into a cylinder[5]. In contrast,

MWCNTs have a larger diameter than SWCNTs because they are made up of several single-walled tubes stacked one within the other in concentric layers[6]. While SWCNTs are recognized for distinct electrical, optical, or chemical capabilities existing at the nanotube scale, the unique features of MWCNTs are mainly related to the bulk material[7], [8]. Post-synthesis chemical and thermal modification processes adjust aggregate features of MWCNTs such as large surface area, thermal conductivity, or tensile strength for specific applications[9]. The emphasis on targeted modification of the bulk properties of MWCNTs influences the interpretation of research demonstrating the effects of MWCNTs on human health or the environment[10].

Multiple studies on the uses of carbon nanotubes in biology and medicine have been investigated in recent years[11]. CNTs (Carbon nanotubes) are currently used as drug delivery systems, membrane components in gene therapy, tissue engineering

materials, neural scaffolds, and drug delivery systems. Carbon nanotubes can be utilized to simulate neural fibers for the formation of neurons, according to *in vitro* research[12]. Additionally, carbon nanotube surfaces can be chemically altered to provide electrically charged spots that can bind a variety of biomolecules[13]. CNTs easily diffuse through biological tissue and cellular membranes within the living body and migrate via the skin, hair follicles and the digestive and respiratory systems. CNTs are useful materials as medication and gene carriers due to these characteristics. Their adaptability to biological systems is a particularly beneficial quality in diagnostic, gene-delivery, and drug-delivery devices[14]. As the use of carbon nanotubes increases the quality and efficacy of drug delivery systems expands, so will their exposure to humans. As a result, it is critical to explore the difficulties related to CNT toxicity[15]. Numerous data and research have been presented to assess the kind of toxicity introduced by the usage of CNTs in biological systems, particularly during the administration of medications[16].

CNTs have been demonstrated in recent investigations to be harmful to microbes and mammalian cells[17]. Both SWCNTs and MWCNTs are poisonous to Gram-positive and Gram-negative bacteria, but to varying degrees depending on their physicochemical qualities. They revealed that when CNTs come into close contact with bacterial cells, they may severely damage the cell membranes, causing the cells to lose structural integrity and, as a result, inactivate them[18]. MWCNTs have previously been found to inhibit cell growth and produce oxidative damage, apoptosis, or necrosis *in vitro*. MWCNTs inhalation causes lung injury or a systemic inflammatory response, oxidative damage, and genotoxicity. However, few studies have been conducted on

the interactions of carbon nanotubes with biological macromolecules. CNTs attached to proteins in systemic circulation are deposited in organs of the reticulo-endothelial system, such as the liver and spleen via blood transport, where they might have therapeutic or potentially harmful effects. An in-depth investigation of CNT-protein interactions is critical for drug delivery applications and CNTs biological safety concerns[19], [20]. Regardless of the dosing mode, almost all nanoparticles studied, including MWCNTs, accumulate predominantly in the liver. Furthermore, nanoparticles have been demonstrated to stay in the liver for a long time, usually months or years. A high concentration of nanoparticles in the liver induces inflammation and has the ability to impair various metabolic activities[20].

In this review, the data published on the toxicity of CNTs that are likely to influence the cellular internalization process is addressed. Other parameters, such as electronic, electrical, surface mechanical, tribological property, nanotube diameter and chirality are also explained along with the interaction of MWCNTs with various potential bio-systems.

2. PHYSICO-CHEMICAL PROPERTIES OF MWCNTs

Because of their superior chemical and physical properties, including great chemical stability, thermal conductivity, mechanical strength, and electrical properties, MWCNTs, which are made up of a rolled cylindrical graphitic sheet, have many uses in a variety of industries[21]. Due in part to technical glitches in detecting and quantifying nanoparticles in biological settings, current research on the evaluation of the toxicity of nanomaterials mainly concentrates on one element of their properties at a time in order

to identify the reasons for toxicity[22].

In order to create prediction models for the safe design of nanomaterials and to comprehend nano-biological interactions, it is essential to have a thorough grasp of the physico-chemical properties of nanoparticles that influence their cytotoxicity.

2.1. CHIRALITY

The chiral vector C , defined by the integers, completely describes the structure of an SWCNT detailed in Figure 1[23]. This index, which controls the electrical characteristics of the tubes, may be discovered by scanning tunnelling microscopy or by ED (Electron Diffraction) of individual tubes[24]–[27]. An MWCNTs is more challenging to characterize since it requires knowledge of the walls' configuration, especially whether they are nested tubes (a "Russian doll" structure) or rolled-up carbon sheets, in addition to the chirality (scrolls)[28]–[30].

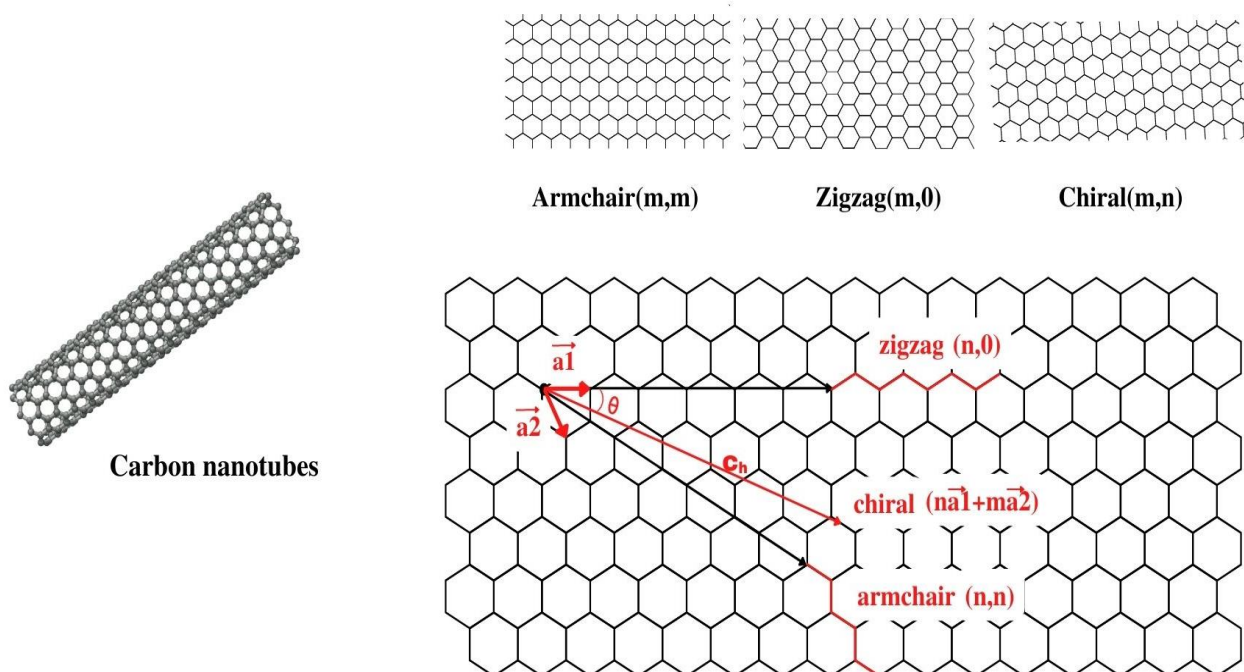


Fig:1 Chirality and geometry of CNTs. The lattice vectors $\vec{a}_1$ and $\vec{a}_2$ as well as the roll-up vector $\vec{C}_h = n\vec{a}_1 + m\vec{a}_2$ are shown in this schematic of a two-dimensional graphene sheet. The limiting examples of (n,0) zigzag and (n,n) armchair tubes are shown by black lines. Here, the zigzag design and $\vec{C}_h$ are at a negative angle. (Created using Canva tool)

It is believed that the enantio-selective adsorption and intracellular distribution of chiral Nps will have an impact on their toxicity through their direct impact on Nps bio-distribution. Furthermore, variable surface chemistry, surface reactivity, and enantio-selective corona formation caused by chirality may impact the toxicity of Nps[31]. It is also crucial to remember that chirality may modify exposure parameters like rates of aggregation, which are influenced by the relative interplay between attractive Van der

Waals forces and repulsive electrostatic interactions[32]. This means that chirality may not only affect Nps' toxicity by their direct interaction with biological systems. For instance, it has been demonstrated that chirality affects the aggregation kinetics of SWCNTs. In this instance, the stronger Van der Waals contacts in the armchair SWCNTs, which led to noticeably greater aggregation, might be attributed to the influence of chirality on SWCNTs[33]. The given dose of NMs can be considerably impacted by chirality by altering the aggregation kinetics, which contributes to the inconsistent results of numerous *in vitro* investigations.

There were also enantioselective rates of absorption, where CNTs with lower chiral indices (lower chirality) were found to enter lipid bilayer cell membranes more quickly than those with higher chirality[32]. Due to their reduced adherence to the membrane, CNTs with lower chirality needed less time to translocate through the membrane. Since creating CNTs with a specific chirality is a considerably more difficult task, chiral indices were used in this study. Targeting inflammation and liver fibrosis, studies showed that the technique is promising for the treatment of NASH (Non-alcoholic steatohepatitis) by using sorted chiral SWCNTs for multigene/multidrug delivery[33]. Different tissue cells can be penetrated by CNTs, which can then cause oxidative stress, inflammation, or even necrosis[34]. CNTs harm macrophages and cause the necrosis of hepatic cells in the liver[35].

2.2. SURFACE PROPERTIES

Large surface area causes band gap changes, a lower melting point, and increased reactivity[36]. These characteristics have major consequences including pneumonitis,

cytotoxicity, and toxicity *in vivo*. When employed as medication carriers, fillers, or cosmetics, a bigger surface area may result in increased reactivity with adjacent particles, perhaps having negative effects[37]. Surface hydrophobicity is recognized to have an impact on surface energy, tribological properties, and friction behaviors. As a result, CNT's surface characteristics may be changed to suit certain tribological and medicinal applications[38]. Reactive surfaces may function as efficient catalysts, but when used biologically, reactive groups on surfaces may have negative health effects. For this reason, surface chemistry at the interface or of the shell is a crucial factor to consider from the perspective of toxicity. Studies on lung cytotoxicity have shown that particle surface reactivity, as opposed to the core particle or just the particle size and surface area, has a significant impact in evoking an inflammatory response[39].

2.3. MECHANICAL PROPERTIES

The CNTs is superior to other traditional reinforcing fibers in terms of strength and elastic modulus. About 1.2 TPa (Terapascal) and 30 GPa (Gigapascal) were the elastic moduli and flexural/tensile strength of CNTs, respectively. As a result, CNTs is added to a range of nanocomposites during processing. Although solvents and water in general do not solubilize CNTs, surface alterations like fluorination and acid treatment can provide more stable solubilization in solvents. To get a more homogenous dispersion of CNTs in a polymer matrix, and therefore a greater reinforcing impact on the elastic modulus, it is frequently helpful to modify the surface of CNTs[40].

The experimental evidence demonstrates that small concentrations of COOH groups improve the strength of CNTs in comparison to that of defected CNTs, whereas

higher concentrations result in tube degradation. This improvement in strength is caused by the carboxylation of CNTs with different diameters, numbers of walls, and metallic and semiconducting characteristics[41]. The critical concentration above which the coexistence of COOH groups and vacancy defects will cause substantial changes in the structure of the nanotube, resulting in a considerable reduction in Young's modulus, rendering the CNTs unsuitable as reinforcement in composites. Above critical density, carboxylic group concentrations would begin to lessen the toxicity of CNTs.

Studies have shown that surface functionalization affects the SWCNTs' biodegradability. Particularly, carboxylation enhances the biodegradation of CNTs in comparison to other forms of functionalization (such as ozone treatment or aryl sulfonation)[42]. COOH groups lead to the formation of active sites that serve as points of entry for further oxidative degradation. It reduces the possibility of long-term negative effects from drug delivery and selective therapeutic targeting of cancer cells using CNTs. The impact of disorder on the stiffness of these nanotubes must be studied in order to appropriately simulate the impact of carboxylation on the mechanical characteristics of CNTs[43],[44].

3. RECENT ADVANCES IN THE FUNCTIONALIZATION OF CARBON NANOTUBES

3.1. CNTs IN DRUG ADMINISTRATION AND PHARMACOKINETICS ANALYSIS

CNTs distinct physico-chemical properties, along with their capability of adaptation, have resulted in a wide range of applications in this interesting field. CNTs, in addition to other applications, are prospective drug carriers in target drug-delivery systems for

oncology and other therapeutics[45]. Moreover, the capacity to functionalize their membrane with a wide diversity of biological and chemical species provides access to a wide range of medical uses. CNTs easily overcome numerous physiological barriers, slipping through the plasma membrane and entering the cytoplasm due to its nano needle structure method, allowing loaded molecules or medicines to be transported and delivered into the target region.

CNTs are considered ideal candidates because of their satisfactory biocompatibility levels, threadlike structure, and large surface area, which allows for extensive modification, as well as a molecular ligand binding mechanism that allows for the transportation and distribution of drugs into the target tissue. In the medical area, CNT molecules conjugated with chitosan have very efficient drug-loading and -releasing properties that might be applied as a transdermal drug delivery system that responds to skin heat signals, sometimes known as a "patch"[46]–[48]. Since the safety concern regarding CNTs was first raised, the pharmacokinetics and bio-distribution of CNTs have been researched. The blood circulation and accumulation of CNTs in animals have an impact on their toxicity and biological applications.

Long blood-circulated CNTs may accumulate more in tumors while acting as a drug delivery system, making them effective for the detection and therapy of cancer[49]. Aggregated CNTs, on the other hand, are quickly eliminated from the circulation by macrophage recognition. Their use in medication delivery is constrained by this quality. However, simply scattered CNTs might have a use in thermal therapy and diagnostics. CNTs may therefore be potential materials for the essential cancer therapy technique of sentinel lymph node detection[49]. Additionally, because CNTs

migrate slowly, they are perfect for thermal therapy because once injected into a tumor, they remain there for a long time.

3.2. TISSUE REGENERATION AND ARTIFICIAL IMPLANTATION

CNTs have remarkable thermal, mechanical, and electrical capabilities, allowing them to be used as reinforcements in a variety of materials to improve their qualities. The goal of tissue engineering is to replace damaged or diseased tissue with biological substitutes that can eventually reestablish the tissue's natural and original function[48]. Significant advancements in this field have aided the anticipated growth of tissue regeneration engineering and medicine. The body typically rejects implants due to post-administration pain, however, carbon nanotubes easily attach to other protein molecules and can be used to implant prosthetic joints avoiding host rejection[50]. Biomaterials containing polymers are frequently used in conjunction with osseous tissue. CNTs are often employed in osteo- biomaterials, mostly to promote better mechanical properties, and they are expected to work as scaffolds to assist and facilitate bone tissue regeneration[51].

3.3. CNTs IN GENETIC ENGINEERING

CNTs are employed in the creation of bioimaging genomes, proteomics, and tissue engineering to control genes and atoms. Gene therapy involves inserting a DNA molecule into the cell nucleus to fix a faulty gene that causes some chronic or inherited disorders[52],[53]. Nanotubes crosslinked with DNA were revealed to liberate DNA

before it was killed by a cell's defense system, resulting in a significant rise in transfection. Antiviral activity of nanostructures towards respiratory syncytial virus, a virus that causes severe bronchitis and asthma, has been discovered. Nanoparticles and gene-slicing technologies are typically used in therapy. Encapsulated within nanotubes, RNA snippets capable of blocking a protein are delivered as nasal sprays or drops. Nanotubes have been shown to aid in the helical crystallization of proteins and the formation of embryonic rat brain neurons.

3.4. BIOMEDICAL IMAGING

CNTs are fascinating nanomaterials that have a lot of potential for use in the biomedical sector. In contrast to spherical nanoparticles, needlelike nanoparticles have superior flow dynamics and a greater ability to permeate cellular membranes in the field of biomedical imaging. From an imaging and therapeutic perspective, it is possible to take advantage of CNTs inherent features. SWCNTs can be employed for photoacoustic imaging and photothermal therapy because of the significant optical absorption in the near-infrared region (NIR). It is understood that owing to the emission range of SWCNTs (800-2000 nm), which falls well within the biological tissue window, it is thereafter capable of different biological imaging applications[52]. It is because of their superior mechanical, electronic, optic, and chemical properties, carbon-based nanomaterials have become key agents for bio-imaging applications in recent years[54].

Roy *et al.* developed variable fluorescence quantum yield carbon nanoparticles and used them as high-brightness fluorescent probes for labelling human blood platelets

with excellent target specificity[55]. Functionalized SWCNTs coated with Ag Nps recently displayed an amazing surface-enhanced Raman scattering property, which was subsequently exploited for cell imaging[54], [56]. For scientific investigation and clinical applications, fluorescence imaging is essential. Recent research has revealed that light with even longer wavelengths exhibits considerably lower tissue absorption and, more critically, noticeably lower scattering by biological tissues, enabling *in vivo* fluorescence imaging with increased tissue penetration and significantly higher spatial resolutions. Principle component analysis (PCA) significantly improved the anatomic resolution of the organs. After PCA analysis of NIR-II fluorescence images of mice with SWCNTs injection, even the pancreas, which could not be resolved from real-time raw images, could be distinguished[57].

4. TOXICOLOGICAL PROPERTY OF MWCNTs

Before CNTs can be used safely for targeted medication delivery, a thorough understanding of their toxicological characteristics is also necessary. To fully comprehend nanotubes' potential for targeted drug administration, it is crucial to research their toxicological characteristics, preferably at a physiologically meaningful dosage. Different nanomaterials are tested for their human toxicity by testing different *in vitro* (cell lines) and *in vivo* (mice, rats etc.) models. Different parameters were analyzed for differential toxicity of CNTs in biological systems. Some of them are a) Agglomeration tendency of CNTs in the treating solutions; b) The inherent chirality and the diameter along with the aspect ratio of synthesized CNTs; c) Different forms of surface functionalization which involves chemical and biological; and d) The different

forms of biological toxicity are also seems to be attained by poor laboratory conditions and lack of followed protocol during synthesis of CNTs[6].

4.1. *IN VITRO*

In vitro toxicity tests are a helpful tool for conducting well-controlled studies on the toxicity of MWCNTs under closely regulated environments. Usually, *in vitro* systems like skin, cardiovascular systems, immune systems and lungs cell types have been studied by the treatment of CNTs toxicity. Different forms of CNTs toxicity have been studied rigorously in different forms of cell lines like immune system, skins, lungs etc. In the lungs cell types, specifically in the macrophages residing inside the lung airways epithelium were primarily targeted by CNTs treatment[57]. A variety of cells present in the lung have been demonstrated to be susceptible to CNTs toxicity, which manifests as inflammatory, reactive, and toxic. The poisonous reaction of CNTs, on the other hand, is not frequently replicated by various researchers, and could therefore be connected to the physico-chemical features of the CNTs utilized, or the methodology used to test their hazardous potential[58]–[60].

The *in vitro* evaluation of CNTs cutaneous toxicity has primarily used cell lines and has mostly focused on the impact of CNTs on keratinocytes. MWCNTs may trigger an inflammatory response after dermal exposure[61]. Two-dimensional gel electrophoresis and mass spectrometry were used to study protein expression changes associated with MWCNTs exposure. Proteins involved in metabolism, cell signaling, the cytoskeleton, and vesicle trafficking were the most severely damaged[3], [62]. Several investigations have offered hypotheses for CNTs harmful impact on heart

muscles.

Cells from the rat heart cell line H9c2 were treated with highly pure SWCNTs (0.2 mg/mL) in an *in vitro* study to examine cell proliferation and morphology. The comparative analysis (novelty) of the present and previous studies involving different types of CNTs and their toxicological implications with the different cell lines is shown in Table 1. Microscopic examination revealed that CNTs attachment to cell walls resulted in a modest conformational shift in the cell shape. The investigation concluded that long-term CNTs exposure caused obvious cardiovascular damage.

Table 1 Comparative analysis of the recent findings and previous discovery involving different types of CNTs and their toxicological implications with the different cell lines.

Nanomaterials	Cell lines	Recent findings in nanomaterial treated cells	Previous discovery in nanomaterial treated cells
MWCNTs	Keratinocytes	Increase epidermal thickness, mast cell and basophil degranulation ultimately leading to skin allergies[63]	Decrease in cell viability and increase in IL-8 Cell-cycle inhibition[64]
SWCNTs and	Alveolar	MWCNTs, causes	SWCNTs showed

MWCNTs	macrophages	inflammatory response, due to mitochondrial dysfunction and SWCNTs causes oxidative stress which leads to apoptosis through NF-kB induction[65]	higher toxicity than MWCNTs[66]
Ground MWCNTs	Rat peritoneal macrophage	Induce inflammation, apoptosis and impaired mitochondrial function[65]	Cytotoxicity and overproduction of proinflammatory cytokines[58]
MWCNTs	Reticulum cells	Induce ER stress which increases lipid accumulation level[67]	Cytotoxic effects by the rupturing of plasma membrane[68]
MWCNTs	Alveolar macrophages	MWCNTs alters ribosomal protein translation, cytoskeleton arrangement and induced pro-inflammatory response[69]	Cell death[70]
Pristine MWCNTs and oxidized	T lymphocytes	Causes fibrosis and induce adaptive immune response[71]	Apoptosis[72]

MWCNTs			
MWCNTs	NHDF (normal human dermal fibroblast)	Damages DNA by increase in the percent tail DNA causing genotoxicity[73]	Induction of cytotoxicity, genotoxicity, and apoptosis[74]

4.2. *IN VIVO*

CNTs are delivered into the body by a variety of methods, including inhalation, intra-peritoneal, oral, intravenous, subcutaneous, and transdermal. Because of the more apparent and definitive outcomes in the reported tests, the most commonly stated methods of administration *in vivo* are intra-tracheal and inhalation. According to several investigations, inhaling CNTs causes substantial irritation as compared to intravenous injection, oral, or dermal delivery[75]. When CNTs enter the body, they interact with proteins or cells, which may result in metabolism or the CNTs retaining their original shape.

They can also be dispersed to other organs of the body, where they can either remain local, translocate, or be eliminated. It has been studied and uncovered in detail that the molecular level of CNTs toxicological outcomes from the targeted biological systems emanated from different molecular signaling pathways associated with the treatment[63]. Numerous studies have demonstrated that CNTs can have a positive or negative impact on a number of signaling pathways, including the *NF-kB* (Nuclear factor Kappa B), *NLRP3* (nucleotide-binding oligomerization domain-like receptor: pyrin domain-containing 3), inflammasome, *p53*, *TGF-b1* (transforming growth factor-b1),

and *MAPK* (mitogen-activated protein kinase) pathways, which are important in both physiological and pathological circumstances.

Dysregulation of these pathways results in aberrant protein function and gene expression, which in turn causes the onset and progression of disease. The *NF- κ B* and *NLRP3* inflammasome activation play crucial roles in the inflammatory reactions brought on by CNTs. Under the influence of CNTs, *TGF- β 1* signaling induces fibrosis via inducing EMT (epithelial-mesenchymal transition) and the transformation of fibroblasts into myofibroblasts. Through mis-regulating cell proliferation and death, abnormal *p53* function is crucial in the development of tumors brought on by CNTs. It is understood from different toxicological studies pertaining to CNTs toxicity mechanism in biological systems, fibrosis may be induced by *MAPK*, *p53* and *NF- κ B* pathways, whereas carcinogenesis may be induced by the *TGF- β 1*, *MAPK* and *NF- κ B* activation after the CNTs introduction in cellular systems. On the other hand, after exposure to CNTs, inflammation may start and encourage both fibrosis and carcinogenesis as a crucial and quick immunological response.

4.2.1 PULMONARY EXPOSURE TO CNTs

With instillation, aspiration, and inhalation approaches being used to expose rats and mice, pulmonary models have by far been used in the majority of research that investigates CNTs toxicity[76]. Mice were exposed to MWCNTs in an experiment, and the findings indicated inflammation, increasing fibrosis, and granuloma development in the lung airways[16]. When MWCNTs are inhaled into the lungs, they are identified by

macrophages, resulting in failed phagocytosis. Failed phagocytosis triggers a severe inflammatory response, which raises oxidative stress through the production of pro-inflammatory cytokines detailed in Figure 2[65],[66].

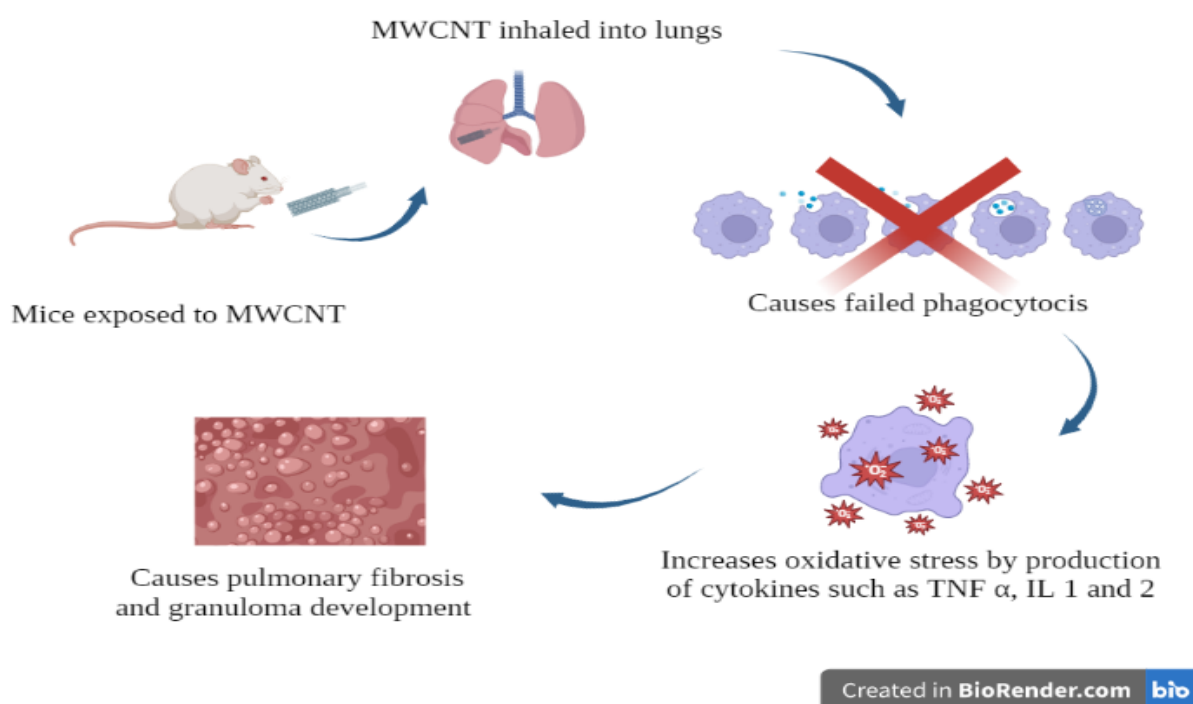


Fig:2 The degree of fibrogenesis, which is influenced by the physico-chemical properties of the nanotubes and genetic vulnerability, is decided by pulmonary exposure to carbon nanotubes, which can result in direct contact with lung cells and increase the population of myofibroblasts. (Created using Biorender.com)

Mitchell et.al. experimented on mice exposing them to MWCNTs and evaluating their pulmonary and general immune systems. It was noticed that alveolar macrophages swallowed MWCNTs. Despite the dispersion of MWCNTs across the

lung, there was no elevation in the infiltration of the inflammatory cell was discovered, and there was no inflammatory response, granuloma formation, scarring and tissue injury was verified through histological investigation[79]. Due to the suppressed T-cell dependent antibody responses as retrieved from the spleen-derived cells, pulmonary side effects seem to be minimized owing to compromission of the systemic immunity, also there is an occurrence of reduction in the proliferation of T-cells post stimulation, rise in the oxidative stress level along with the associated altered NK-cell killing rate, all resulting into vulnerability of infections.

4.2.2 CENTRAL NERVOUS SYSTEM (CNS) TOXICITY

Scientists have intensively investigated CNTs as drug delivery vehicles to the CNS due to their unique interaction with the component neural system[80]. Pristine CNTs are typically insoluble in aqueous solvents, but with some modification to the nanomaterial, they can even cross the BBB (Blood-brain barrier), which is the most difficult barrier for treatments[81]. Because of these unique features of CNTs, they are rapidly being investigated as a diagnostic for CNS illnesses such as Alzheimer's and Parkinson's[80]. As a result, the rising usage of nanomaterials has sparked a serious discussion over CNTs neurotoxicity. To establish CNTs toxicity, it is critical to understand how these molecules interact with the BBB. In an experiment conducted by Kafa *et al*, a neuro-inflammatory response was seen when MWCNTs were introduced into the brains of mice. Brain tissues take up the MWCNTs, resulting in a variety of neuro-inflammatory reactions[82]. The results of this investigation demonstrated elevated levels of cytokine and stimulation of glial cells, indicating oxidation of the CNTs

surface, which may result in an inflammatory response in neurons.

4.2.3 DERMAL AND SUBCUTANEOUS TOXICITY

CNTs enter the body through the skin, which is the most easily exposed surface to the environment. In a study, it was observed there was a development of granuloma with MWCNTs aggregation trapped within the subcutaneous tissues of mice[83]. Keratinocytes and dermal fibroblasts, which are primarily in charge of maintaining skin connective tissue, are abundant in the epidermis[84]. CNTs treatment of these keratinocytes reduces cell viability, produces genotoxicity and increases inflammation[85]. Inflammatory kind of skin reactions are usually noticed when exposed to CNTs. Post cutaneous exposure, systemic availabilities of CNTs should be taken care-off. It is argued that irrespective of the route of administration of CNTs, subcutaneous granulomas could be re-occurred irrespective of the tissue type infected with CNTs and is not restricted to only mesothelium or any respiratory systems.

4.2.4 EFFECT OF CNTs ON LIVER

One of the vital organs in the body, the liver is essential for carrying out the body's many metabolic pathways. Therefore, each substance administered to the body is either directly or indirectly metabolized by the liver. Studying and examining the harmful effects of CNTs on liver tissues is so crucial. It is crucial to research how these nanoparticles are digested in our biological systems to produce simpler molecules that can be quickly eliminated from the body. However, these metabolic processes are

difficult to identify or track due to a lack of analytical equipment. The buildup of CNTs in liver cells was revealed by several *in vivo* scanning techniques including TEM (Transmission electron microscopy) and SEM (Scanning electron microscopy) analyses. MWCNTs were orally administered to Swiss albino mice in an *in vivo* experiment, and the effects on the mice were then evaluated in toxicological tests[16]. The liver's histopathological analysis revealed coagulation of the blood, inflammation, and an elevated level of oxygen free radicals in addition to damaged macrophage cells (Kupffer cells)[86], [87]. The experimental findings suggested that liver dysfunction resulted from either acute or long-term exposure to the toxicants in CNTs. Kupffer cells were activated in the mice's liver after MWCNTs treatment.

These cells are important for maintaining homeostasis and proper functioning. When harmful chemicals come into touch with liver cells, Kupffer cells are triggered. Toxic agents that can enter either directly or indirectly cause the release of growth factors, inflammatory and pro-inflammatory responses, and ROS (Reactive oxygen species) [88], [89]. The increasing number of Kupffer cells implies that CNTs are the primary source of liver damage[16]. Effects of CNTs caused in liver cells are explained in Figure 3.

In a related study that looked at the potential of MWCNTs as drug delivery vehicles, the dispersion of radioactively labelled taurine-conjugated MWCNTs following exposure via a variety of routes was investigated. The MWCNTs surface was shown to benefit from a taurine attachment, increasing their water solubility, and TEM analysis showed that the MWCNTs structure was unaltered. Following intravenous administration, the liver was the site of MWCNTs accumulation with only little lung and

spleen localization[90]. The fact that MWCNTs were phagocytosed by Kupffer cells explained the MWCNTs accumulation in the liver, and serum markers and histopathological studies both showed that the high level of MWCNTs accumulation was not harmful. The MWCNTs rapidly dissipated after being injected intra-tracheally; at the end of the 28th day, only 20% of the initial dosage remained in the lung, confirming the evacuation of deposited MWCNTs. There has been no instance of obtaining MWCNTs in the blood, whereas majority of the orally administered MWCNTs have been seen to be found in the faces, guts and small and larger intestines, respectively. MWCNTs were also observed to remain intact in the tissues where they were found as well as in faces, indicating that they may be resistant to degradation or transformation and, if not expelled, may remain in the body[91].

Pure MWCNTs were created in mouse serum to improve their biocompatibility. To make MWCNTs more hydrophilic and hence water soluble, they were alternatively functionalized with ammonium[92]. It was noticed that reduction in the CNTs aggregation is more pronounced in case of the serum-dispersed MWCNTs, whereas the ammonium functionalized MWCNTs seems to be more personalized. Mice were injected with CNTs via their tail veins, and 24 hours later the lungs, kidney, spleen, and liver were investigated using the histological examination. It was shown that the propensity for tissue accumulation decreased as the functionalization of the CNTs surface increased. Quick excretion and minimized retention in the body are observed for the functionalized MWCNTs, when introduced into living biological systems. Different mice were adversely impacted by the serum-coated MWCNTs, as seen by their depressed behavior, slumped posture, decreased activity, and respiratory

distress. The Comparative analysis of present and previous studies involving MWCNTs and SWCNTs among *in vivo* toxicity assessment in various organisms is shown in Table 2. Large, black clusters of the MWCNTs that dispersed with serum gathered mostly in the liver and lungs. The buildup of CNTs in the pulmonary tissues was most likely the cause of the respiratory discomfort brought on by serum-dispersed MWCNTs[93].

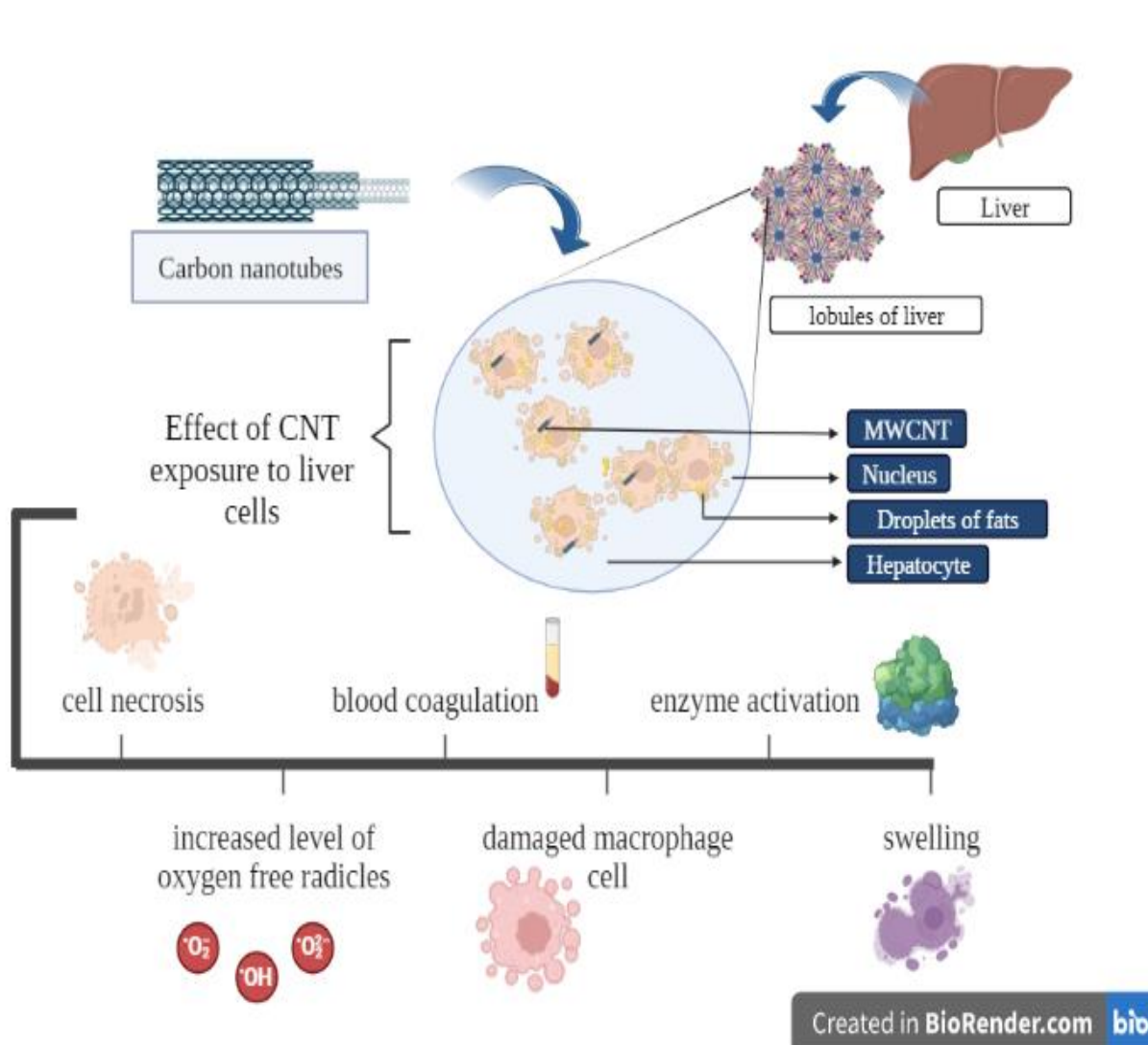


Fig:3 Toxic chemicals released due to CNT exposure enter either directly or indirectly and causes the release of growth factors, inflammatory and pro-

inflammatory responses, and ROS resulting in hepatotoxicity (Created with BioRender.com)

Table 2 Comparative analysis of recent findings and previous discovery involving MWCNTs and SWCNTs among in vivo toxicity assessment in various organisms

Nanomaterials	Name of organism	Recent findings in nanomaterial treated cells	Previous discovery in the nanomaterial treated cells
MWCNTs	Mice	Liver function destruction and lipid droplet accumulation in hepatocytes[94]	Causes low acute hepato-toxicity[95]
MWCNTs	Rat	Causes chronic lung fibrosis and act as carcinogen[96]	Pulmonary fibrosis[58]
MWCNTs	Mice	Induces oxidative stress damage, nephrotoxicity and neurotoxicity[97]	Inflammation and granulomas[98]
MWCNTs	Rat	Schwann cell matrix-coated MWCNTs nanofibers is a conduit for peripheral nerve regeneration[99]	Granuloma and collagen depositions[100]
MWCNTs and	Guinea pigs	Inhibit cell proliferation,	Pneumonitis[102]

SWCNTs		induce apoptosis[101]	
MWCNTs	Rat	MWCNT-induced carcinogenicity via cytokine expression by macrophages[103]	Inflammation, granuloma, and lung fibrosis[104]

4.3. MECHANISMS INVOLVING WITH THE TOXICITY ASPECTS

CNTs are regarded as a potential drug delivery vehicle among the different reported vehicles[105]. However, before they may be used for medicinal purposes, their impact on various organs must be studied. CNTs have been studied several times to understand the methods by which they infiltrate cells through the lipid bilayer of cellular walls. Several studies have suggested that endocytosis is the cellular absorption mechanism for CNTs[62], [106]. CNTs penetration into the plasma membrane of the cell generates oxidative stress, which can result in inflammation and cytotoxicity[107], [108]. Because carbon nanotubes (CNTs) serve as a foreign entity to the host, the FBR (Foreign body response) is triggered, and substances are created to kill the CNTs.

It has been discovered that carbon nanotubes cause oxidative stress in cells[108]–[112]. The key signaling mechanisms that govern cytokines in response to oxidative stress caused by CNTs are protein kinases and *NF-kB*. One probable explanation for CNTs cellular toxicity is the production of free radicals, which leads to oxidative stress[113], [114]. It is well recognized that excess free radical oxidizes DNA (Deoxyribonucleic acid), proteins, and lipids in cells. They also regulate stimulating

transcription and activator protein-1 that are involved in systemic inflammation[114]. Influential factors responsible for the observed toxicity of CNTs are mainly surface chemistry, length, metal content, ability to agglomerate etc. The involvement of the fiber framework in CNTs toxicity is especially remarkable, as the length of nanotubes seems to be important to their hazardous potential[6].

4.3.1 IN PROKARYOTES

In some recent studies conducted on the antibacterial effects of CNTs, it has been observed that MWCNTs have antibacterial effects on *E. coli* and *Streptococcus* and that SWCNTs have a more effective antibacterial effect than MWNTs, due to the stronger Van der Waals forces[115]. It has been revealed that CNTs adsorb bacteria, inhibit the growth of *E. coli* through Van der Waals forces and package them into blocks[116]. Studies have been conducted showing that CNTs induce oxidative stress in bacteria and thus cause damage to the bacterial membrane details in Figure 4.

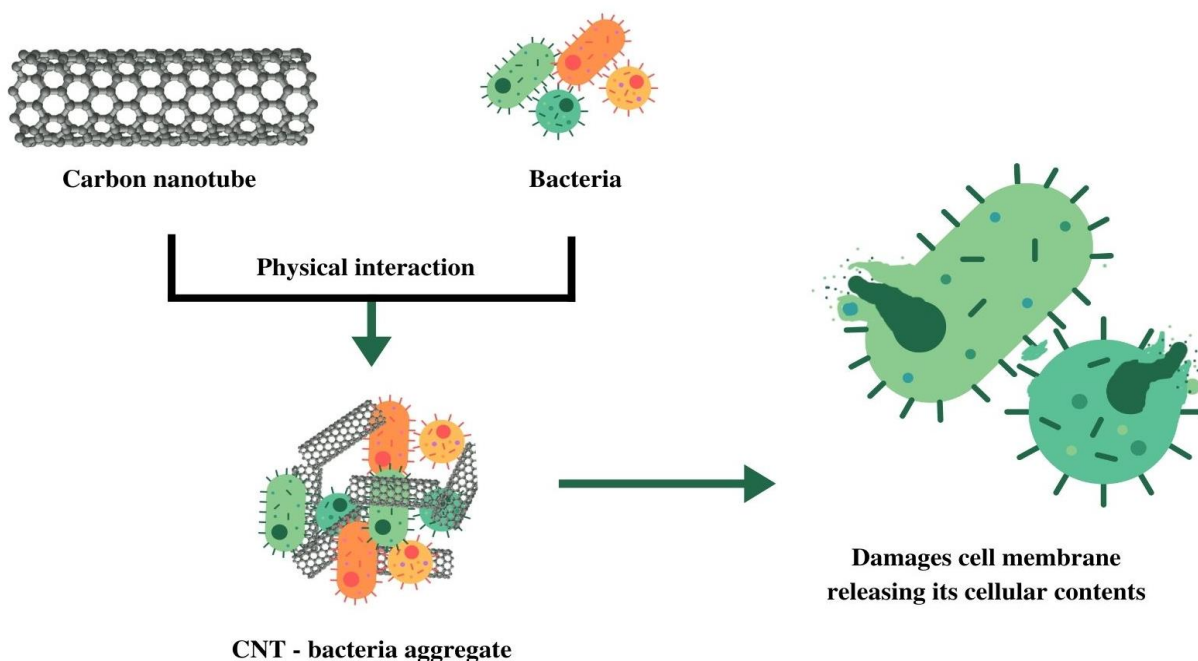


Fig:4 The piercing of the CNTs results in lysis and loss of bacterial membrane potential, trigger the release of internal components including DNA and RNA, leading to the full eradication of the bacterium. (Created using Canva tool)

In some other reports, it was concluded that CNTs showed antibacterial effects as a result of mechanical interaction with bacteria[117]. The results and properties of CNTs used in these studies are different and they revealed bacterial inhibition results that may be due to many factors such as functional groups on CNTs surface, concentration, shape, and size on antibacterial properties emerged[10, 80-82]. Electron microscopy investigations of bacteria exposed to MWCNTs revealed that membrane integrity might be lost by piercing or entrapment. MWCNTs toxicity appears to be increased in hypotonic and nutrient-free solutions[120]–[122]. The incorporation of microorganisms with CNTs for the removal of pollutants would thus be dependent on

the incubated medium and bacterial strain, with a high nutrient medium appearing to lower CNTs toxicity[118].

4.3.2 IN AQUATIC ORGANISMS

Genotoxicity induced by carbon nanotubes (CNTs) can be attributed to a number of factors, involving acute interaction with DNA, an indirect effect resulting from ROS, ion release or interactions with cellular components. Despite this, only a few studies in aquatic toxicology have investigated the genotoxic consequences of carbon nanotubes[123]. Water genotoxic impacts can have long-term consequences on aquatic ecosystems, and the existence of genotoxic substances in water can affect non-aquatic species through the food web or simply by potable water. Fish are frequently utilized as sentinel creatures in eco-toxicological investigations because they play various roles in the trophic web, can rapidly absorb toxic compounds, and respond to low xenobiotic concentrations[124],[125]. Nanocomposites may be absorbed by fish through the digestive system and gill surface before being delivered by the blood to target organs such as the spleen and liver. The liver has been employed in various toxicological investigations since it is an important organ in xenobiotic detoxification[126]. The spleen, on the other hand, is an essential component of the immune system, acting as a selective filter of the vascular system and playing an important part in the response to pathogen invasion. MMC (Melanomacrophage centers) are pigmented macrophage accumulations in the head, kidney, liver, and spleen of various fish species[127].

MMCs have been employed as biomarkers in multiple investigations involving

teleosts exposed to various xenobiotics. Thus, the action of stressors correlates with a rise in the number of MMCs, the area, and the kinds of pigments. However, research evaluating the deleterious effect of nanocomposites such as MWCNTs on primary culture from fish melanomacrophages have yet to be established. Macrophage primary cultures from the cranial kidney and fish spleen have been generated to define this cell type morphologically and biochemically. As MWCNTs were exposed to melanomacrophages from *Nile tilapia* and *Oreochromis niloticus* the spleen and liver macrophages behaved differently, with the liver melanomacrophages being mainly lysed when compared to the spleen melanomacrophages. Furthermore, MWCNTs inclusions were seen in the cytoplasm of macrophages, indicating phagocytic activity, and a concentration of 1 g mL^{-1} of the nanocomposite utilized was cytotoxic for both macrophages[125], [127]–[129].

Several studies investigated the possible toxicity of MWCNTs in *Xenopus* larvae. In larvae, growth inhibition was detected, resulting in acute and chronic toxicity. Among the harmful consequences, genotoxicity has the potential to have long-term impacts on aquatic ecosystems[130], [131]. Initially, the association of genotoxic substances with DNA may cause morphological changes in the DNA molecule. Unrepaired damage might result in cell injuries which results in tumor formation[132].

4.3.3 IN MAMMALS

MWCNTs are important since they allow researchers to establish whether the genotoxic threat is consistent. MWCNTs reference materials are also desirable since they are well-characterized and require little effort to specify[133]. Data from cell culture

research, which largely uses the comet test and the micronucleus assay to detect DNA strand breaking, dominates our understanding of the genotoxicity of MWCNTs. Some animal research examined genotoxic effects in extrapulmonary organs but found no link between exposure and levels of DNA damage. According to a literature review, around 1% of the CNTs deposits in the passageways translocates to the liver, spleen, and bone marrow (with the greatest quantity found in the liver). As a result, the translocated dosage of MWCNTs in extra-pulmonary organs may be too low to produce noticeable DNA damage[134].

The results of a meta-analysis of strand breaks in DNA and a comprehensive analysis of all types of genotoxicity in cell cultures reveal a significant degree of response heterogeneity. It is always difficult to generalize data from basic *in vitro* systems and animal models to people. In particle toxicology, it is common practice to discover indicators of genotoxicity in humans by monitored doses, such as a randomized clinical trial with appropriate contrast in exposure, such as employees and a control population[133], [135], [136]. Several human biological monitoring studies have looked at biomarkers that may be associated with degrees of DNA damage in particle-exposed individuals, implying that they are linked in the same causal network. Significant amounts of inflammatory markers in sputum and/or blood were discovered in a study of workers at a Russian MWCNTs production factory[137]

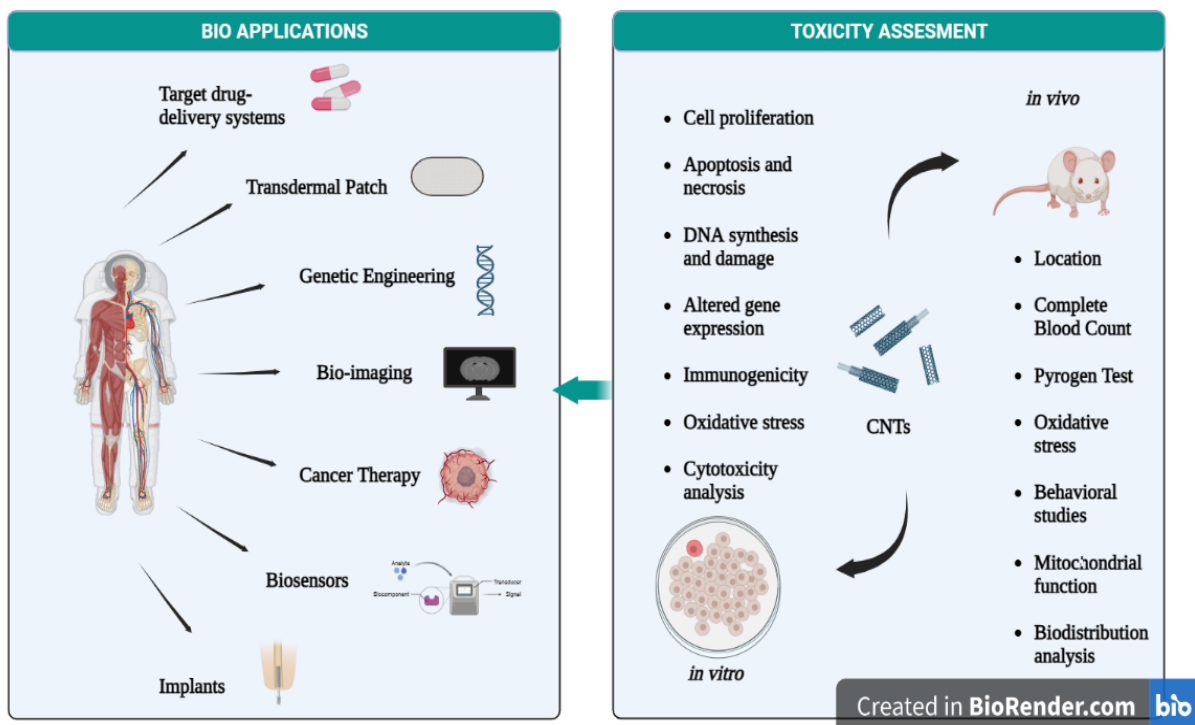


Fig:5 An overview of various applications of carbon nanotube in pharmaceutical and Biomedical sector along with toxicity assessment in various biosystems (Created with BioRender.com)

5. COMPARING CNTs AND HNTs

Halloysite nanotubes (HNTs) are tubing clay nanomaterials formed from aluminosilicate kaolin sheets that have been repeatedly rolled. The surface of HNT contains siloxane and aluminol groups, which help form hydrogen bonds with the biomaterials that are adhered to it. Owing to such unique properties, HNTs find applications ranging from forensic sciences, nanoelectronics, glass coatings, different catalytic studies etc[138]. Additionally, HNTs are enable in a wide range of biomedical and nanomedical applications, including bioimaging, tissue engineering, cancer and

stem cell isolation, medication administration, gene delivery, and tissue engineering.

HNTs are stratified aluminosilicates of tubular hollowed structures with high aspect ratios[138]. Typically, 10 to 15 aluminosilicate layers ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$) are wrapped into a hollow cylinder with multilayer walls and a frequency of 0.72 nm in pure state[139]. Halloysites have lengths between 0.4 and 1 micrometres, inner lumen diameters between 10 and 70 nm, and outside (gross) diameters between 20 and 200 nm. It is important to compare halloysite to CNTs due to the tubular structure. There are various uses for halloysite in biocompatible nano-containers. The chemistry of aluminium silicate is non-toxic and strong mechanically[140]. HNTs are more promising because of their non-toxic aqueous manufacturing, and even though carbon nanotubes have several engineering uses, they are unsafe for use in healthcare[141]. Also, the cost of halloysite is low, and the global availability exceeds a thousand tonnes annually (average of 4 dollars per one kilogram)[142].

In consideration of this, a real mass-scale may be utilised in industrial settings. Unlike the gram-scale CNTs producing at a very high cost (500 dollars per one kilogram)[143]. Moreover, HNT has a bigger mesoporous and macroporous structure than a number of artificial porous materials, including CNTs[144]. Lastly, it is known that CNTs have a very narrow, 1 nm-diameter lumen that can only load the tiniest molecules. An excellent nano reservoir for the absorption of active chemical agents, ranging from big and complex molecules like proteins and polymers to tiny molecules like medicines and corrosion inhibitors, is the empty lumen of HNT, in contrast[145]. These characteristics enable numerous potential applications, such as the ingestion of biomacromolecules by a nano-carrier.

Due to the biocompatibility attributes of HNTs, it finds applications in the anti-cancerous therapeutic materials along with the applications in the prolonged administration of biologically active ingredients in pharmaceuticals and biomedical fields[146], [147]. Toxicity towards living things must be carefully considered because HNT use has risen in recent years. To demonstrate the biocompatibility of the nanotube, various human cell lines, including epithelial adenocarcinoma cells and dermal fibroblasts, were subjected to toxicity tests using HNT. Animal cells including yeast, bacteria, algae, and micro worms were examined in a similar manner[148]. *In vitro* testing of HNT's cytotoxicity on human cell cultures and microbial cells has been documented in several papers. By altering the surface properties of iron oxide (Fe_3O_4) nanoparticles and decreasing their innate toxicity on bacterial cells, HNT decreased the cytotoxicity of iron oxide nanoparticles[149]. Several interesting and encouraging results have been exhibited by cell lines like MCF-7, NIH-3T3, HeLa and A549 upon treatment with HNTs[148]. Another *in vivo* eukaryotic nematode named *Caenorhabditis elegans* have exhibited a safety response of HNTs treatment even at the higher dosage concentration of 100 mg mL^{-1} [149].

On the other hand, significant cytogenetic toxicity at 100 mg mL^{-1} has been observed by HNTs by limiting the passage of cell cycle. Also, several important cell lines along with Human peripheral lymphocytes also exhibited a bio-compatibility nature of HNTs treatment at the *in vitro* conditions. Such studies propose the biocompatible HNTs as upcoming nano-scaled delivery agent in the biomedical arena which could be used in translational science as well[150]. Several researches have been performed including *Paramecium caudatum* toxicity, which has evaluated for the HNTs toxicity

when compared to similar different family nano-scaled materials like Graphene, kaolin, bentonite etc. It has been found that HNTs stand out from all the other nano and allied lower scaled materials, when introduced into biological systems in terms of bio-safety at different concentration dosages[151]. Hence, HNT has a wide range of useful applications in the fields of biology and other relevant sciences.

5.1.APPLICATION OF HNTs IN ENVIRONMENTAL SCIENCE

Nanomaterials differ from their bulk equivalents due to their nano size and high surface-to-volume ratio. They have a wide range of applications in the environmental sectors because of this feature. They offer exceptional adsorptive and catalytic capabilities, which are crucial for applications including nanofiltration, monitoring contaminant levels by sensing, and remediating pollutants from contaminated soil and water[150]. Eco-friendly HNTs in particular have emerged as an effective substitute for traditional materials based on nanotechnology for the detection and removal of many types of pesticides[151]. In the environmental sector, HNTs functionalized with various surface-modification agents can be used extremely effectively as an eco-friendly nanomaterial for detecting, degrading, or eliminating various pesticides present in soil and water. In a recent work, chitosan-functionalized HNT (CTS-HNT) was employed as a receptive nano-support for the grafting of copper (Cu) and laccase (Lac) for the breakdown of the pesticide chlorpyrifos[152].

In addition, HNTs exhibit outstanding adsorptive and catalytic capabilities, which are critical for applications including nanofiltration, contaminant level detection, and pollutant cleanup from polluted soil and water. Studies have revealed that

a functionalized halloysite nanotubes (f-HNT) containing 3-aminopropyltriethoxysilane (APTES) is extremely effective in removing the ions Fe^{2+} and Fe^{3+} from water[153]. The outer surface of HNTs has also been functionalized using polyphenols, which are predominantly sourced from plants. Tannic acid, a polyphenol, has zwitterionic qualities that not only enable the attachment of a certain functional group onto a surface but also provide the surface of HNTs with zwitterionic capabilities. HNTs can be employed as antifouling agents for the treatment of wastewater thanks to their qualities[154].

5.2.APPLICATION OF HNTs IN BIOMEDICINE

HNTs have been employed in several research and publications as nanocontainers or nanocarriers for both general medication delivery and targeted drug delivery. Adsorption, intercalation, and tubular entrapment are the three main techniques for loading medicines into the lumen or onto the surface of HNT (vacuum method)[151]. In research, the effects of alkali activation on HNT were examined before it was loaded with ofloxacin (OFL). The OFL that was adsorbed onto the surface of HNT released slowly using this approach. This provided a productive way to improve cationic medication adsorption and release. In order to study DNA damage and repair, HNT was bound to the DNA intercalator acridine orange[155]. As the inner lumen of HNT is positively charged at most pH conditions and the outside surface is negatively charged, hence are vulnerable to numerous modifications. Such unique attributes of HNTs makes it an ideal candidate in the applications of drug delivery, scaffolds for the tissue engineering and for the improved human cellular adhesion applications. This is possible due to inherent increased biocompatibility and lower cytotoxicity of HNTs.

The characteristics of HNTs, such as their wide surface area, tubular lumen, superior adsorption capacity, and changeable surface chemistry, might be advantageous in the field of forensic science when gathering evidence, such as fingerprints, and when detecting materials, such as DNA and explosives. The HNTs' nanoscale structure can facilitate their easy development and collection of fingerprints as well as their easy dispersion over a vast sample gathering area. These materials' mesoporous properties have been investigated for loading different dye molecules for fingerprinting purposes[156]. By immobilizing fluorescent dyes and agents on the surface and inside the lumen of HNTs, this capability may be applied during the creation of fingerprinting applications.

6. DISCUSSION

As a result of the fast growth of nanotechnology, humans will be exposed to nanoparticles by ingestion, inhalation, skin absorption, and vein injection. Although CNTs are very promising nanomaterials, very little is known about how they affect human health. Apart from that, variable outcomes have been noted as a result of using various CNT forms.

This review emphasizes the importance of toxicity and the interaction of MWCNTs with bio-systems. In order to design a safer and effective pharmaceutical material, more such molecular studies involving the CNTs, and its associated molecular toxicity profiles need to be researched extensively to derive its complete pharmacokinetics and pharmacodynamical analysis at the laboratory scale. It could be extremely beneficial in the expansion of biomedical CNTs applications and its associated implications.

Evaluation of the research previously reported on shown the challenges in determining the toxicity of CNTs materials. The state of aggregation, length, stiffness, concentration, time of exposure, and other factors are the key determinants of CNT toxicity. New types of carbon nanotubes have been created for biological applications uses, to reduce cytotoxicity. Nevertheless, several disadvantages of carbon nanotubes, such nonbiodegradability and intrinsic toxicity on the lungs, skin, and eyes, may restrict their utility. The results of several *in-vitro* and *in-vivo* toxicological investigations have been conducted, although they are not quite clear-cut to the point of being contentious. Because of the inherent toxicity of CNTs in various biological organ systems, they are not yet widely used in controlled medicine. An extensive study of CNTs toxicity at bio-interfaces should be performed at different lengths for efficient application in biomedical sciences and therapeutics.

The adoption of safety precautions in the manufacturing facilities and research laboratories would open the door for fabricating safer and newer nano scaled MWCNTs based products in the upcoming medical and clinical fields. Finally, CNTs are compared to HNT, a nanomaterial equivalent to CNTs due to similar tubular structure. HNTs are perceived to be of more biocompatible and less toxicological nature compared to CNTs owing to their unique physio-chemical properties and ease of formation of hydrogen bonding with biomaterials, paving the way into diverse biomedical applications.

7. CONCLUSION

Regardless of the numerous applications and relevance of carbon nanotubes, their toxicity levels and consequences in the human body continue to be a source of worry

across the world. Its biocompatibility challenge in the *in vivo* circumstances is the key barrier preventing it from being used widely in multiple treatments. Despite several animal and cell line studies, the precise cause of CNTs toxicity remains highly unclear. However, it has been noted that CNTs toxicity may be caused by the building up of CNTs particles and the subsequent formation of ROS in the biological systems. Furthermore, structural change, size distribution, surface charge, contaminants, and functionalization have all been linked to the increased CNTs toxicities in bio-systems. However, preliminary findings indicate that pharmaceutical applications are technically possible and may be done safely, but the current knowledge also cautions us about seriously considering the necessity for creation of strategies to avoid the toxicity provoked by the otherwise fantastic carbon nanotubes.

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9. REFERENCE

- [1] M. Goldberg, R. Langer, and X. Jia, "Nanostructured materials for applications in drug delivery and tissue engineering," *J Biomater Sci Polym Ed*, vol. 18, no. 3, pp. 241–268, 2007, doi: 10.1163/156856207779996931.
- [2] I. Khan, K. Saeed, and I. Khan, "Nanoparticles: Properties, applications and toxicities," *Arabian Journal of Chemistry*, vol. 12, no. 7, pp. 908–931, 2019, doi: 10.1016/j.arabjc.2017.05.011.
- [3] N. A. Monteiro-Riviere, R. J. Nemanich, A. O. Inman, Y. Y. Wang, and J. E. Riviere, "Multi-walled carbon nanotube interactions with human epidermal keratinocytes," *Toxicol Lett*, vol. 155, no. 3, pp. 377–384, 2005, doi: 10.1016/j.toxlet.2004.11.004.
- [4] W. Wei, A. Sethuraman, C. Jin, N. A. Monteiro-Riviere, and R. J. Narayan, "Biological properties of carbon nanotubes," *J Nanosci Nanotechnol*, vol. 7, no. 4–5, pp. 1284–1297, 2007, doi: 10.1166/jnn.2007.655.
- [5] M. Ohadi, P. Rezaei, M. Mehrabani, B. Behnam, and M. Ansari, "Synthesis, Characterization and Toxicity Assessment of the Novel Non covalent Functionalized Multi-walled Carbon Nanotubes with Glycyrrhizin, Curcumin and Rutin," *J Clust Sci*, vol. 33, no. 3, pp. 975–984, 2022, doi: 10.1007/s10876-021-02026-3.
- [6] H. J. Johnston *et al.*, "A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: The contribution of physico-chemical characteristics," *Nanotoxicology*, vol. 4, no. 2, pp. 207–246, 2010, doi: 10.3109/17435390903569639.

- [7] R. Jha, A. Singh, P. K. Sharma, and N. K. Fuloria, "Smart carbon nanotubes for drug delivery system: A comprehensive study," *J Drug Deliv Sci Technol*, vol. 58, no. May, p. 101811, 2020, doi: 10.1016/j.jddst.2020.101811.
- [8] F. S. A. Khan *et al.*, "Comprehensive review on carbon nanotubes embedded in different metal and polymer matrix: fabrications and applications," *Critical Reviews in Solid State and Materials Sciences*, vol. 0, no. 0, pp. 1–28, 2021, doi: 10.1080/10408436.2021.1935713.
- [9] T. Shimizu, W. Ding, and N. Kameta, "Soft-Matter Nanotubes: A Platform for Diverse Functions and Applications," *Chem Rev*, vol. 120, no. 4, pp. 2347–2407, 2020, doi: 10.1021/acs.chemrev.9b00509.
- [10] S. Kang, M. S. Mauter, and M. Elimelech, "Physicochemical determinants of multiwalled carbon nanotube bacterial cytotoxicity," *Environ Sci Technol*, vol. 42, no. 19, pp. 7528–7534, 2008, doi: 10.1021/es8010173.
- [11] S. Sinha *et al.*, "In Vivo Biointeraction and Alleviation of Toxicity of MWCNTs upon Functionalization with ssDNA in a *Caenorhabditis elegans* Model," *J Electron Mater*, vol. 50, no. 8, pp. 4974–4990, 2021, doi: 10.1007/s11664-021-09006-3.
- [12] A. Fraczek, E. Menaszek, C. Paluszkiwicz, and M. Blazewicz, "Comparative in vivo biocompatibility study of single- and multi-wall carbon nanotubes," *Acta Biomater*, vol. 4, no. 6, pp. 1593–1602, Nov. 2008, doi: 10.1016/J.ACTBIO.2008.05.018.
- [13] S. Song, Y. Qin, Y. He, Q. Huang, C. Fan, and H. Y. Chen, "Functional nanoprobe for ultrasensitive detection of biomolecules," *Chem Soc Rev*, vol. 39, no. 11, pp. 4234–4243, Oct. 2010, doi: 10.1039/C000682N.

- [14] A. Fraczek, E. Menaszek, C. Paluszkiewicz, and M. Blazewicz, "Comparative in vivo biocompatibility study of single- and multi-wall carbon nanotubes," *Acta Biomater*, vol. 4, no. 6, pp. 1593–1602, 2008, doi: 10.1016/j.actbio.2008.05.018.
- [15] B. Wu, M. Jiang, X. Liu, C. Huang, Z. Gu, and Y. Cao, "Evaluation of toxicity of halloysite nanotubes and multi-walled carbon nanotubes to endothelial cells in vitro and blood vessels in vivo," *Nanotoxicology*, vol. 14, no. 8, pp. 1017–1038, 2020, doi: 10.1080/17435390.2020.1780642.
- [16] D. Mohanta, S. Patnaik, S. Sood, and N. Das, "Carbon nanotubes: Evaluation of toxicity at biointerfaces," *J Pharm Anal*, vol. 9, no. 5, pp. 293–300, 2019, doi: 10.1016/j.jpha.2019.04.003.
- [17] L. Q. Chen, P. P. Hu, L. Zhang, S. Z. Huang, L. F. Luo, and C. Z. Huang, "Toxicity of graphene oxide and multi-walled carbon nanotubes against human cells and zebrafish," *Sci China Chem*, vol. 55, no. 10, pp. 2209–2216, Oct. 2012, doi: 10.1007/S11426-012-4620-Z/METRICS.
- [18] P. Yi and K. L. Chen, "Interaction of multiwalled carbon nanotubes with supported lipid bilayers and vesicles as model biological membranes," *Environ Sci Technol*, vol. 47, no. 11, pp. 5711–5719, 2013, doi: 10.1021/es4002604.
- [19] T. Zhang, M. Tang, Y. Yao, Y. Ma, and Y. Pu, "MWCNT interactions with protein: Surface-induced changes in protein adsorption and the impact of protein corona on cellular uptake and cytotoxicity," *Int J Nanomedicine*, vol. 14, pp. 993–1009, 2019, doi: 10.2147/IJN.S191689.
- [20] Y. Zhang *et al.*, "Modulation of Carbon Nanotubes' Perturbation to the Metabolic

Activity of CYP3A4 in the Liver,” *Adv Funct Mater*, vol. 26, no. 6, pp. 841–850, 2016, doi: 10.1002/adfm.201504182.

[21] M. Xu *et al.*, “Contribution of physicochemical characteristics of nano-oxides to cytotoxicity,” *Biomaterials*, vol. 31, no. 31, pp. 8022–8031, 2010, doi: 10.1016/j.biomaterials.2010.06.022.

[22] M. Rezazadeh Azari and Y. Mohammadian, “Comparing in vitro cytotoxicity of graphite, short multi-walled carbon nanotubes, and long multi-walled carbon nanotubes,” *Environmental Science and Pollution Research*, vol. 27, no. 13, pp. 15401–15406, 2020, doi: 10.1007/s11356-020-08036-4.

[23] W. Ruland, A. K. Schaper, H. Hou, and A. Greiner, “Multi-wall carbon nanotubes with uniform chirality: Evidence for scroll structures,” *Carbon N Y*, vol. 41, no. 3, pp. 423–427, 2003, doi: 10.1016/S0008-6223(02)00342-1.

[24] A. Hassanien *et al.*, “Geometrical structure and electronic properties of atomically resolved multiwall carbon nanotubes,” *Appl Phys Lett*, vol. 75, no. 18, pp. 2755–2757, 1999, doi: 10.1063/1.125139.

[25] T. W. Odom and J. Huang, “Atomic structure and electronic properties of swCNT,” vol. 391, no. January, pp. 1997–1999, 1998.

[26] P. Lambin and A. Lucas, “Quantitative theory of diffraction by carbon nanotubes,” *Phys Rev B Condens Matter Mater Phys*, vol. 56, no. 7, pp. 3571–3574, 1997, doi: 10.1103/PhysRevB.56.3571.

[27] S. Amelinckx, A. Lucas, and P. Lambin, “Electron diffraction and microscopy of nanotubes,” *Reports on Progress in Physics*, vol. 62, no. 11, pp. 1471–1524, 1999, doi:

10.1088/0034-4885/62/11/201.

[28] M. Liu and J. M. Cowley, "Structures of the helical carbon nanotubes," *Carbon N Y*, vol. 32, no. 3, pp. 393–403, 1994, doi: 10.1016/0008-6223(94)90159-7.

[29] G. Xu, Z. C. Feng, Z. Popovic, J. Y. Lin, and J. J. Vittal, "Nanotube structure revealed by high-resolution X-ray diffraction," *Advanced Materials*, vol. 13, no. 4, pp. 264–267, 2001, doi: 10.1002/1521-4095(200102)13:4<264::AID-ADMA264>3.0.CO;2-L.

[30] O. Zhou *et al.*, "Defects in carbon nanostructures," *Science (1979)*, vol. 263, no. 5154, pp. 1744–1747, 1994, doi: 10.1126/science.263.5154.1744.

[31] W. Utembe, "Chirality, a neglected physico-chemical property of nanomaterials? A mini-review on the occurrence and importance of chirality on their toxicity," *Toxicol Lett*, vol. 311, pp. 58–65, Sep. 2019, doi: 10.1016/J.TOXLET.2019.04.032.

[32] W. Utembe, "Chirality, a neglected physico-chemical property of nanomaterials? A mini-review on the occurrence and importance of chirality on their toxicity," *Toxicol Lett*, vol. 311, no. May, pp. 58–65, 2019, doi: 10.1016/j.toxlet.2019.04.032.

[33] Md. T. Hasan *et al.*, "Multi-Drug/Gene NASH Therapy Delivery and Selective Hyperspectral NIR Imaging Using Chirality-Sorted Single-Walled Carbon Nanotubes," *Cancers (Basel)*, vol. 11, no. 8, p. 1175, Aug. 2019, doi: 10.3390/cancers11081175.

[34] D. D. Z. Pop, D. R. Mitrea, S. Suci, and S. V. Clichici, "Nanostructure-based therapies for liver fibrosis," *Journal of Physiology and Pharmacology*, vol. 71, no. 6, 2020, doi: 10.26402/jpp.2020.6.01.

- [35] D. Mohanta, S. Patnaik, S. Sood, and N. Das, "Carbon nanotubes: Evaluation of toxicity at biointerfaces," *J Pharm Anal*, vol. 9, no. 5, pp. 293–300, 2019, doi: 10.1016/j.jpha.2019.04.003.
- [36] A. B. Asha and R. Narain, "Nanomaterials properties," *Polymer Science and Nanotechnology: Fundamentals and Applications*, pp. 343–359, Jan. 2020, doi: 10.1016/B978-0-12-816806-6.00015-7.
- [37] S. W. Shin, I. H. Song, and S. H. Um, "Role of Physicochemical Properties in Nanoparticle Toxicity," pp. 1351–1365, 2015, doi: 10.3390/nano5031351.
- [38] C. Wei, C. Wang, F. Tai, K. Ting, and R. Chang, "Diamond & Related Materials The effect of CNT content on the surface and mechanical properties of CNTs doped diamond like carbon fi lms," *Diam Relat Mater*, vol. 19, no. 5–6, pp. 562–566, 2010, doi: 10.1016/j.diamond.2010.01.024.
- [39] M. A. Hussain, M. A. Kabir, and A. K. Sood, "On the cytotoxicity of carbon nanotubes," *Curr Sci*, vol. 96, no. 5, pp. 664–673, 2009.
- [40] H. Miyagawa, M. Misra, and A. K. Mohanty, "Mechanical Properties of Carbon Nanotubes and Their Polymer Nanocomposites," vol. 5, no. 10, pp. 1593–1615, 2005, doi: 10.1166/jnn.2005.181.
- [41] J. W. Seo, A. Magrez, M. Milas, K. Lee, V. Lukovac, and L. Forró, "Catalytically grown carbon nanotubes: From synthesis to toxicity," *J Phys D Appl Phys*, vol. 40, no. 6, 2007, doi: 10.1088/0022-3727/40/6/R01.
- [42] K. Z. Milowska, "Influence of Carboxylation on Structural and Mechanical Properties of Carbon Nanotubes: Composite Reinforcement and Toxicity Reduction

Perspectives,” *Journal of Physical Chemistry C*, vol. 119, no. 47, pp. 26734–26746, 2015, doi: 10.1021/acs.jpcc.5b08402.

[43] Y. Liu, Y. Zhao, B. Sun, and C. Chen, “Understanding the toxicity of carbon nanotubes,” *Acc Chem Res*, vol. 46, no. 3, pp. 702–713, 2013, doi: 10.1021/ar300028m.

[44] A. D. Moghadam, E. Omrani, P. L. Menezes, and P. K. Rohatgi, “Mechanical and tribological properties of self-lubricating metal matrix nanocomposites reinforced by carbon nanotubes (CNTs) and graphene e A review,” *Composites Part B*, vol. 77, pp. 402–420, 2015, doi: 10.1016/j.compositesb.2015.03.014.

[45] W. Zhang, Z. Zhang, and Y. Zhang, “The application of carbon nanotubes in target drug delivery systems for cancer therapies,” *Nanoscale Res Lett*, vol. 6, pp. 1–22, 2011, doi: 10.1186/1556-276X-6-555.

[46] M. Chakrabarti, R. Kiseleva, A. Vertegel, and S. K. Ray, “Carbon nanomaterials for drug delivery and cancer therapy,” *J Nanosci Nanotechnol*, vol. 15, no. 8, pp. 5501–5511, 2015, doi: 10.1166/jnn.2015.10614.

[47] V. C. Jhawar, V. Saini, S. Kamboj, and N. Maggon, “Transdermal_Drug_Delivery_Systems_Approa,” vol. 20, no. 1, pp. 47–56, 2013.

[48] T. Bhunia, A. Giri, T. Nasim, D. Chattopadhyay, and A. Bandyopadhyay, “Uniquely different PVA-xanthan gum irradiated membranes as transdermal diltiazem delivery device,” *Carbohydr Polym*, vol. 95, no. 1, pp. 252–261, Jun. 2013, doi: 10.1016/j.carbpol.2013.02.043.

[49] S. T. Yang, J. Luo, Q. Zhou, and H. Wang, “Pharmacokinetics, metabolism and

toxicity of carbon nanotubes for bio-medical purposes,” *Theranostics*, vol. 2, no. 3, pp. 271–282, 2012, doi: 10.7150/thno.3618.

[50] K. Sahithi, M. Swetha, K. Ramasamy, N. Srinivasan, and N. Selvamurugan, “Polymeric composites containing carbon nanotubes for bone tissue engineering,” *Int J Biol Macromol*, vol. 46, no. 3, pp. 281–283, 2010, doi: 10.1016/j.ijbiomac.2010.01.006.

[51] J. M. Gutiérrez-Hernández *et al.*, “In vitro evaluation of osteoblastic cells on bacterial cellulose modified with multi-walled carbon nanotubes as scaffold for bone regeneration,” *Materials Science and Engineering C*, vol. 75, pp. 445–453, 2017, doi: 10.1016/j.msec.2017.02.074.

[52] Z. Liu, S. Tabakman, K. Welsher, and H. Dai, “Carbon nanotubes in biology and medicine: In vitro and in vivo detection, imaging and drug delivery,” *Nano Res*, vol. 2, no. 2, pp. 85–120, 2009, doi: 10.1007/s12274-009-9009-8.

[53] H. He, L. A. Pham-Huy, P. Dramou, D. Xiao, P. Zuo, and C. Pham-Huy, “Carbon Nanotubes: Applications in Pharmacy and Medicine,” *Biomed Res Int*, vol. 2013, pp. 1–12, 2013, doi: 10.1155/2013/578290.

[54] C. Puett *et al.*, “An update on carbon nanotube-enabled X-ray sources for biomedical imaging,” *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, vol. 10, no. 1, pp. 1–11, 2018, doi: 10.1002/wnan.1475.

[55] S. Roy, B. Korzeniowska, C. K. Dixit, G. Manickam, S. Daniels, and C. McDonagh, “Biocompatibility and bioimaging application of carbon nanoparticles synthesized by phosphorus pentoxide combustion method,” *J Nanomater*, vol. 2015,

pp. 14–17, 2015, doi: 10.1155/2015/761517.

[56] E. L. Ursu, F. Doroftei, D. Peptanariu, M. Pinteala, and A. Rotaru, “DNA-assisted decoration of single-walled carbon nanotubes with gold nanoparticles for applications in surface-enhanced Raman scattering imaging of cells,” *Journal of Nanoparticle Research*, vol. 19, no. 5, pp. 1–9, 2017, doi: 10.1007/s11051-017-3876-9.

[57] H. Gong, R. Peng, and Z. Liu, “Carbon nanotubes for biomedical imaging: The recent advances,” *Adv Drug Deliv Rev*, vol. 65, no. 15, pp. 1951–1963, 2013, doi: 10.1016/j.addr.2013.10.002.

[58] J. Muller *et al.*, “Respiratory toxicity of multi-wall carbon nanotubes,” *Toxicol Appl Pharmacol*, vol. 207, no. 3, pp. 221–231, 2005, doi: 10.1016/j.taap.2005.01.008.

[59] M. Davoren *et al.*, “In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells,” *Toxicology in Vitro*, vol. 21, no. 3, pp. 438–448, 2007, doi: 10.1016/j.tiv.2006.10.007.

[60] P. Wick *et al.*, “The degree and kind of agglomeration affect carbon nanotube cytotoxicity,” *Toxicol Lett*, vol. 168, no. 2, pp. 121–131, 2007, doi: 10.1016/j.toxlet.2006.08.019.

[61] B. C. Palmer, S. J. Phelan-Dickenson, and L. A. Delouise, “Multi-walled carbon nanotube oxidation dependent keratinocyte cytotoxicity and skin inflammation,” *Part Fibre Toxicol*, vol. 16, no. 1, pp. 1–15, Jan. 2019, doi: 10.1186/S12989-018-0285-X/FIGURES/7.

[62] F. A. Witzmann and N. A. Monteiro-Riviere, “Multi-walled carbon nanotube exposure alters protein expression in human keratinocytes,” *Nanomedicine*, vol. 2, no.

3, pp. 158–168, 2006, doi: 10.1016/j.nano.2006.07.005.

[63] B. C. Palmer, S. J. Phelan-Dickenson, and L. A. Delouise, “Multi-walled carbon nanotube oxidation dependent keratinocyte cytotoxicity and skin inflammation,” *Part Fibre Toxicol*, vol. 16, no. 1, pp. 1–15, Jan. 2019, doi: 10.1186/S12989-018-0285-X/FIGURES/7.

[64] N. A. Monteiro-Riviere and A. O. Inman, “Challenges for assessing carbon nanomaterial toxicity to the skin,” *Carbon N Y*, vol. 44, no. 6, pp. 1070–1078, 2006, doi: 10.1016/j.carbon.2005.11.004.

[65] S. Nahle *et al.*, “Single wall and multiwall carbon nanotubes induce different toxicological responses in rat alveolar macrophages,” *Journal of Applied Toxicology*, vol. 39, no. 5, pp. 764–772, May 2019, doi: 10.1002/JAT.3765.

[66] G. Jia *et al.*, “Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene,” *Environ Sci Technol*, vol. 39, no. 5, pp. 1378–1383, 2005, doi: 10.1021/es048729l.

[67] J. Long, W. Ma, Z. Yu, H. Liu, and Y. Cao, “Multi-walled carbon nanotubes (MWCNTs) promoted lipid accumulation in THP-1 macrophages through modulation of endoplasmic reticulum (ER) stress,” <https://doi.org/10.1080/17435390.2019.1597204>, vol. 13, no. 7, pp. 938–951, 2019, doi: 10.1080/17435390.2019.1597204.

[68] S. Hirano, S. Kanno, and A. Furuyama, “Multi-walled carbon nanotubes injure the plasma membrane of macrophages,” *Toxicol Appl Pharmacol*, vol. 232, no. 2, pp. 244–251, 2008, doi: 10.1016/j.taap.2008.06.016.

[69] S. Nahle *et al.*, “Genes expression profiling of alveolar macrophages exposed

to non-functionalized, anionic and cationic multi-walled carbon nanotubes shows three different mechanisms of toxicity,” *J Nanobiotechnology*, vol. 18, no. 1, pp. 1–18, Feb. 2020, doi: 10.1186/S12951-020-0587-7/FIGURES/9.

[70] L. Murr *et al.*, “Cytotoxicity Assessment of Some Carbon Nanotubes and Related Carbon Nanoparticle Aggregates and the Implications for Anthropogenic Carbon Nanotube Aggregates in the Environment,” *Int J Environ Res Public Health*, vol. 2, no. 1, pp. 31–42, Apr. 2005, doi: 10.3390/ijerph2005010031.

[71] A. Malur *et al.*, “PPARgamma Deficiency Promotes an Adaptive T Lymphocyte Response to Mycobacterial Antigen ESAT-6 in a Murine Model of Chronic Pulmonary Sarcoidosis,” *American Thoracic Society International Conference Meetings Abstracts American Thoracic Society International Conference Meetings Abstracts*, pp. A4505–A4505, May 2019, doi: 10.1164/AJRCCM-CONFERENCE.2019.199.1_MEETINGABSTRACTS.A4505.

[72] M. Bottini *et al.*, “Multi-walled carbon nanotubes induce T lymphocyte apoptosis,” *Toxicol Lett*, vol. 160, no. 2, pp. 121–126, 2006, doi: 10.1016/j.toxlet.2005.06.020.

[73] K. Aoki and N. Saito, “Biocompatibility and Carcinogenicity of Carbon Nanotubes as Biomaterials,” *Nanomaterials 2020, Vol. 10, Page 264*, vol. 10, no. 2, p. 264, Feb. 2020, doi: 10.3390/NANO10020264.

[74] A. Patlolla, B. Patlolla, and P. Tchounwou, “Evaluation of cell viability, DNA damage, and cell death in normal human dermal fibroblast cells induced by functionalized multiwalled carbon nanotube,” *Mol Cell Biochem*, vol. 338, no. 1–2, pp. 225–232, 2010, doi: 10.1007/s11010-009-0356-2.

- [75] D. Mohanta, S. Patnaik, S. Sood, and N. Das, "Carbon nanotubes: Evaluation of toxicity at biointerfaces," *J Pharm Anal*, vol. 9, no. 5, pp. 293–300, 2019, doi: 10.1016/j.jpha.2019.04.003.
- [76] M. Witkowska, E. Florek, and R. Mrówczyński, "Assessment of Pristine Carbon Nanotubes Toxicity in Rodent Models," *International Journal of Molecular Sciences* 2022, Vol. 23, Page 15343, vol. 23, no. 23, p. 15343, Dec. 2022, doi: 10.3390/IJMS232315343.
- [77] H. C. Nerl *et al.*, "Imaging methods for determining uptake and toxicity of carbon nanotubes in vitro and in vivo," *Nanomedicine*, vol. 6, no. 5, pp. 849–865, 2011, doi: 10.2217/nnm.11.87.
- [78] M. S. P. Boyles *et al.*, "Multi-walled carbon nanotube induced frustrated phagocytosis, cytotoxicity and pro-inflammatory conditions in macrophages are length dependent and greater than that of asbestos," *Toxicology in Vitro*, vol. 29, no. 7, pp. 1513–1528, 2015, doi: 10.1016/j.tiv.2015.06.012.
- [79] J. S. Kim, K. S. Song, H. J. Joo, J. H. Lee, and I. J. Yu, "Determination of cytotoxicity attributed to multiwall carbon nanotubes (MWCNT) in normal human embryonic lung cell (WI-38) line," *Journal of Toxicology and Environmental Health - Part A: Current Issues*, vol. 73, no. 21–22, pp. 1521–1529, 2010, doi: 10.1080/15287394.2010.511577.
- [80] L. Giri, S. R. Rout, K. Gowtham, M. A. S. Abourehab, P. Kesharwani, and R. Dandela, "Biomimetic carbon nanotubes for neurological disease therapeutic," *Emerging Applications of Carbon Nanotubes in Drug and Gene Delivery*, pp. 229–253,

Jan. 2023, doi: 10.1016/B978-0-323-85199-2.00001-7.

[81] A. Bhosale, G. Paul, F. Mazahir, and A. K. Yadav, "Theoretical and applied concepts of nanocarriers for the treatment of Parkinson's diseases," *OpenNano*, vol. 9, p. 100111, Jan. 2023, doi: 10.1016/J.ONANO.2022.100111.

[82] H. Kafa *et al.*, "The interaction of carbon nanotubes with an invitro blood-brain barrier model and mouse brain invivo," *Biomaterials*, vol. 53, pp. 437–452, 2015, doi: 10.1016/j.biomaterials.2015.02.083.

[83] S. Koyama *et al.*, "Role of systemic T-cells and histopathological aspects after subcutaneous implantation of various carbon nanotubes in mice," *Carbon N Y*, vol. 44, no. 6, pp. 1079–1092, 2006, doi: 10.1016/j.carbon.2005.08.006.

[84] M. Horie *et al.*, "Comparison of the effects of multiwall carbon nanotubes on the epithelial cells and macrophages," *Nanotoxicology*, vol. 13, no. 7, pp. 861–878, 2019, doi: 10.1080/17435390.2019.1592258.

[85] R. R. Mercer *et al.*, "Extrapulmonary transport of MWCNT following inhalation exposure," *Part Fibre Toxicol*, vol. 10, no. 1, 2013, doi: 10.1186/1743-8977-10-38.

[86] T. Li, Y. Cao, B. Li, and R. Dai, "The biological effects of radiation-induced liver damage and its natural protective medicine," *Prog Biophys Mol Biol*, vol. 167, no. xxxx, pp. 87–95, 2021, doi: 10.1016/j.pbiomolbio.2021.06.012.

[87] Y. Jin *et al.*, *Applications of Nanobiomaterials in the Therapy and Imaging of Acute Liver Failure*, vol. 13, no. 1. 2021. doi: 10.1007/s40820-020-00550-x.

[88] H. Sun, J. Feng, and L. Tang, "Function of TREM1 and TREM2 in Liver-Related

Diseases,” *Cells*, vol. 9, no. 12, p. 2626, Dec. 2020, doi: 10.3390/cells9122626.

[89] K. Roth, J. Strickland, and B. L. Copple, “Regulation of macrophage activation in the liver after acute injury: Role of the fibrinolytic system,” *World J Gastroenterol*, vol. 26, no. 16, pp. 1879–1887, Apr. 2020, doi: 10.3748/wjg.v26.i16.1879.

[90] S. Sharma, S. Naskar, and K. Kuotsu, “A review on carbon nanotubes: Influencing toxicity and emerging carrier for platinum based cytotoxic drug application,” *J Drug Deliv Sci Technol*, vol. 51, pp. 708–720, Jun. 2019, doi: 10.1016/J.JDDST.2019.02.028.

[91] X. Deng *et al.*, “Translocation and fate of multi-walled carbon nanotubes in vivo,” *Carbon N Y*, vol. 45, no. 7, pp. 1419–1424, 2007, doi: 10.1016/j.carbon.2007.03.035.

[92] R. Dubey, D. Dutta, A. Sarkar, and P. Chattopadhyay, “Functionalized carbon nanotubes: synthesis, properties and applications in water purification, drug delivery, and material and biomedical sciences,” *Nanoscale Adv*, vol. 3, no. 20, pp. 5722–5744, Oct. 2021, doi: 10.1039/D1NA00293G.

[93] L. Lacerda *et al.*, “Tissue histology and physiology following intravenous administration of different types of functionalized multiwalled carbon nanotubes,” *Nanomedicine*, vol. 3, no. 2, pp. 149–161, 2008, doi: 10.2217/17435889.3.2.149.

[94] H. Y. Zhang, R. L. Chen, Y. Shao, H. L. Wang, and Z. G. Liu, “Effects of exposure of adult mice to multi-walled carbon nanotubes on the liver lipid metabolism of their offspring,” *Toxicol Res (Camb)*, vol. 7, no. 5, pp. 809–816, 2018, doi: 10.1039/c8tx00032h.

[95] X. Deng *et al.*, “Translocation and fate of multi-walled carbon nanotubes in vivo,”

Carbon N Y, vol. 45, no. 7, pp. 1419–1424, Jun. 2007, doi: 10.1016/J.CARBON.2007.03.035.

[96] F. Murphy *et al.*, “Grouping MWCNTs based on their similar potential to cause pulmonary hazard after inhalation: a case-study,” *Part Fibre Toxicol*, vol. 19, no. 1, pp. 1–23, Dec. 2022, doi: 10.1186/S12989-022-00487-6/FIGURES/4.

[97] A. A. Sallam *et al.*, “Quercetin alleviated multi-walled carbon nanotubes-induced neurotoxicity in mice through inhibition of oxidation, inflammation, and pyroptosis,” *Biomedicine & Pharmacotherapy*, vol. 151, p. 113160, Jul. 2022, doi: 10.1016/J.BIOPHA.2022.113160.

[98] C. A. Poland *et al.*, “Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study,” *Nat Nanotechnol*, vol. 3, no. 7, pp. 423–428, 2008, doi: 10.1038/nnano.2008.111.

[99] Y. Al-Hadeethi *et al.*, “Schwann cell-matrix coated PCL-MWCNT multifunctional nanofibrous scaffolds for neural regeneration,” *RSC Adv*, vol. 13, no. 2, pp. 1392–1401, Jan. 2023, doi: 10.1039/D2RA05368C.

[100] Y. Morimoto *et al.*, “Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation,” *Nanotoxicology*, vol. 6, no. 6, pp. 587–599, 2012, doi: 10.3109/17435390.2011.594912.

[101] A. P. Francis and T. Devasena, “Toxicity of carbon nanotubes: A review,” <https://doi.org/10.1177/0748233717747472>, vol. 34, no. 3, pp. 200–210, Mar. 2018, doi: 10.1177/0748233717747472.

[102] H. Grubek-Jaworska *et al.*, “Preliminary results on the pathogenic effects of

intratracheal exposure to one-dimensional nanocarbons,” *Carbon N Y*, vol. 44, no. 6, pp. 1057–1063, 2006, doi: 10.1016/j.carbon.2005.12.011.

[103] N. Sultana, K. Fukamachi, J. Xu, H. Tsuda, and M. Suzui, “mRNA expression profile of cytokines in rat primary alveolar macrophages treated with multiwalled carbon nanotube (MWCNT),” *Fundam Toxicol Sci*, vol. 10, no. 1, pp. 27–30, 2023, doi: 10.2131/FTS.10.27.

[104] A. P. Francis, S. Ganapathy, V. R. Palla, P. B. Murthy, S. Ramaprabhu, and T. Devasena, “One time nose-only inhalation of MWCNTs: Exploring the mechanism of toxicity by intermittent sacrifice in Wistar rats,” *Toxicol Rep*, vol. 2, pp. 111–120, 2015, doi: 10.1016/j.toxrep.2015.02.003.

[105] K. Maruyama *et al.*, “Endocytosis of multiwalled carbon nanotubes in bronchial epithelial and mesothelial cells,” *Biomed Res Int*, vol. 2015, 2015, doi: 10.1155/2015/793186.

[106] S. Jain, S. R. Singh, and S. Pillai, “Toxicity issues related to biomedical applications of carbon nanotubes,” *J Nanomed Nanotechnol*, vol. 3, no. 5, pp. 1–15, 2012, doi: 10.4172/2157-7439.1000140.

[107] M. Nikzamir, A. Akbarzadeh, and Y. Panahi, “An overview on nanoparticles used in biomedicine and their cytotoxicity,” *J Drug Deliv Sci Technol*, vol. 61, no. December 2020, p. 102316, 2021, doi: 10.1016/j.jddst.2020.102316.

[108] M. A. Saleemi, M. Hosseini Fouladi, P. V. C. Yong, K. Chinna, N. K. Palanisamy, and E. H. Wong, “Toxicity of Carbon Nanotubes: Molecular Mechanisms, Signaling Cascades, and Remedies in Biomedical Applications,” *Chem Res Toxicol*, vol. 34, no.

1, pp. 24–46, 2021, doi: 10.1021/acs.chemrestox.0c00172.

[109] Y. Seo *et al.*, “Engineering copper nanoparticles synthesized on the surface of carbon nanotubes for anti-microbial and anti-biofilm applications,” *Nanoscale*, vol. 10, no. 33, pp. 15529–15544, 2018, doi: 10.1039/c8nr02768d.

[110] V. Vijayalakshmi, B. Sadanandan, and A. Venkataramanaiah Raghu, “Single walled carbon nanotubes in high concentrations is cytotoxic to the human neuronal cell LN18,” *Results Chem*, vol. 4, no. May, p. 100484, 2022, doi: 10.1016/j.rechem.2022.100484.

[111] M. Azizi-Lalabadi, H. Hashemi, J. Feng, and S. M. Jafari, “Carbon nanomaterials against pathogens; the antimicrobial activity of carbon nanotubes, graphene/graphene oxide, fullerenes, and their nanocomposites,” *Adv Colloid Interface Sci*, vol. 284, p. 102250, 2020, doi: 10.1016/j.cis.2020.102250.

[112] Y. Cao and Y. Luo, “Pharmacological and toxicological aspects of carbon nanotubes (CNTs) to vascular system: A review,” *Toxicol Appl Pharmacol*, vol. 385, no. November, p. 114801, 2019, doi: 10.1016/j.taap.2019.114801.

[113] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, “Free radicals and antioxidants in normal physiological functions and human disease,” *International Journal of Biochemistry and Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007, doi: 10.1016/j.biocel.2006.07.001.

[114] M. A. Malik, M. Y. Wani, M. A. Hashim, and F. Nabi, “Nanotoxicity: Dimensional and morphological concerns,” *Advances in Physical Chemistry*, vol. 2011, 2011, doi: 10.1155/2011/450912.

- [115] P. C. Nagajyothi, L. Veeranjanya Reddy, K. C. Devarayapalli, S. V. Prabhakar Vattikuti, Y. J. Wee, and J. Shim, "Environmentally Friendly Synthesis: Photocatalytic Dye Degradation and Bacteria Inactivation Using Ag/f-MWCNTs Composite," *J Clust Sci*, vol. 32, no. 3, pp. 711–718, 2021, doi: 10.1007/s10876-020-01821-8.
- [116] T. Ertas and B. Dinc, "EFFECT of SHORT MULTIWALLED CARBON NANOTUBES on ESCHERICHIA COLI K-12 STRAIN," vol. 90, no. June, pp. 8–13, 2022, doi: 1099944.
- [117] M. A. Saleemi, M. H. Fouladi, P. V. C. Yong, and E. H. Wong, "Elucidation of Antimicrobial Activity of Non-Covalently Dispersed Carbon Nanotubes," *Materials* 2020, Vol. 13, Page 1676, vol. 13, no. 7, p. 1676, Apr. 2020, doi: 10.3390/MA13071676.
- [118] M. R. Hartono, A. Kushmaro, X. Chen, and R. S. Marks, "Probing the toxicity mechanism of multiwalled carbon nanotubes on bacteria," *Environmental Science and Pollution Research*, vol. 25, no. 5, pp. 5003–5012, 2018, doi: 10.1007/s11356-017-0782-8.
- [119] H. I. Hamouda, H. M. Abdel-Ghafar, and M. H. H. Mahmoud, "Multi-walled carbon nanotubes decorated with silver nanoparticles for antimicrobial applications," *J Environ Chem Eng*, vol. 9, no. 2, p. 105034, 2021, doi: 10.1016/j.jece.2021.105034.
- [120] A. A. Atiyah, A. J. Haider, and R. M. Dhahi, "Cytotoxicity properties of functionalised carbon nanotubes on pathogenic bacteria," *IET Nanobiotechnol*, vol. 13, no. 6, pp. 597–601, Aug. 2019, doi: 10.1049/iet-nbt.2018.5394.
- [121] P. Laganà *et al.*, "Is the Antibacterial Activity of Multi-Walled Carbon Nanotubes (MWCNTs) Related to Antibiotic Resistance? An Assessment in Clinical Isolates," *Int J*

Environ Res Public Health, vol. 18, no. 17, p. 9310, Sep. 2021, doi: 10.3390/ijerph18179310.

[122] B. El Mrabate *et al.*, “Widespread applicability of bacterial cellulose-ZnO-MWCNT hybrid membranes,” *Arabian Journal of Chemistry*, vol. 14, no. 7, pp. 0–11, 2021, doi: 10.1016/j.arabjc.2021.103232.

[123] F. Pinto *et al.*, “Analysis of the In Vitro Toxicity of Nanocelluloses in Human Lung Cells as Compared to Multi-Walled Carbon Nanotubes,” *Nanomaterials 2022, Vol. 12, Page 1432*, vol. 12, no. 9, p. 1432, Apr. 2022, doi: 10.3390/NANO12091432.

[124] A. L. R. Seixas, M. Ferreira-Cravo, A. C. Kalb, L. A. Romano, C. G. J. R. Kaufmann, and J. M. Monserrat, “Protein oxidation in the fish *Danio rerio* (Cyprinidae) fed with single- and multi-walled carbon nanotubes,” *Energy Ecol Environ*, vol. 3, no. 2, pp. 95–101, 2018, doi: 10.1007/s40974-017-0080-9.

[125] B. Song *et al.*, “Effects of multi-walled carbon nanotubes on metabolic function of the microbial community in riverine sediment contaminated with phenanthrene,” *Carbon N Y*, vol. 144, pp. 1–7, 2019, doi: 10.1016/j.carbon.2018.12.016.

[126] H. Kan *et al.*, “Effects of carboxylated multi-walled carbon nanotubes on bioconcentration of pentachlorophenol and hepatic damages in goldfish,” *Ecotoxicology*, vol. 30, no. 7, pp. 1389–1398, Sep. 2021, doi: 10.1007/S10646-020-02328-8/METRICS.

[127] I. F. Silva *et al.*, “Primary culture macrophages from fish as potential experimental model in toxicity studies with multiwalled carbon nanotubes,” *Acta Sci Biol Sci*, vol. 43, no. Mmc, pp. 1–10, 2021, doi: 10.4025/actascibiolsoci.v43i1.52612.

- [128] B. Song *et al.*, “Effects of multi-walled carbon nanotubes on metabolic function of the microbial community in riverine sediment contaminated with phenanthrene,” *Carbon N Y*, vol. 144, pp. 1–7, Apr. 2019, doi: 10.1016/j.carbon.2018.12.016.
- [129] G. V. Cimbaluk *et al.*, “Evaluation of multiwalled carbon nanotubes toxicity in two fish species,” *Ecotoxicol Environ Saf*, vol. 150, no. September 2017, pp. 215–223, 2018, doi: 10.1016/j.ecoenv.2017.12.034.
- [130] J. Zhao, G. Xie, Y. Xu, L. Zheng, and J. Ling, “Accumulation and toxicity of multi-walled carbon nanotubes in *Xenopus tropicalis* tadpoles,” *Chemosphere*, vol. 257, p. 127205, 2020, doi: 10.1016/j.chemosphere.2020.127205.
- [131] J. Zhao, W. Luo, Y. Xu, J. Ling, and L. Deng, “Potential reproductive toxicity of multi-walled carbon nanotubes and their chronic exposure effects on the growth and development of *Xenopus tropicalis*,” *Science of the Total Environment*, vol. 766, no. 100, p. 142652, 2021, doi: 10.1016/j.scitotenv.2020.142652.
- [132] F. Mouchet, P. Landois, P. Puech, E. Pinelli, E. Flahaut, and L. Gauthier, “Carbon nanotube ecotoxicity in amphibians: assessment of multiwalled carbon nanotubes and comparison with double-walled carbon nanotubes,” *Nanomedicine*, vol. 5, no. 6, pp. 963–974, Aug. 2010, doi: 10.2217/nnm.10.60.
- [133] P. Møller, R. S. Wils, E. Di Ianni, C. A. T. Gutierrez, M. Roursgaard, and N. R. Jacobsen, “Genotoxicity of multi-walled carbon nanotube reference materials in mammalian cells and animals,” *Mutat Res Rev Mutat Res*, vol. 788, no. August 2021, 2021, doi: 10.1016/j.mrrev.2021.108393.
- [134] P. MØller and S. Loft, “Oxidative damage to DNA and lipids as biomarkers of

exposure to air pollution,” *Environ Health Perspect*, vol. 118, no. 8, pp. 1126–1136, 2010, doi: 10.1289/ehp.0901725.

[135] A. Joshi, S. Kaur, P. Singh, K. Dharamvir, H. Nayyar, and G. Verma, “Tracking multi-walled carbon nanotubes inside oat (*Avena sativa* L.) plants and assessing their effect on growth, yield, and mammalian (human) cell viability,” *Applied Nanoscience (Switzerland)*, vol. 8, no. 6, pp. 1399–1414, 2018, doi: 10.1007/s13204-018-0801-1.

[136] E. Demir and R. Marcos, “Toxic and genotoxic effects of graphene and multi-walled carbon nanotubes,” *Journal of Toxicology and Environmental Health - Part A: Current Issues*, vol. 81, no. 14, pp. 645–660, 2018, doi: 10.1080/15287394.2018.1477314.

[137] L. M. Fatkhutdinova *et al.*, “Fibrosis biomarkers in workers exposed to MWCNTs,” *Toxicol Appl Pharmacol*, vol. 299, pp. 125–131, 2016, doi: 10.1016/j.taap.2016.02.016.

[138] S. Satish, M. Tharmavaram, and D. Rawtani, “Halloysite nanotubes as a nature’s boon for biomedical applications”, doi: 10.1177/1849543519863625.

[139] Y. Lvov, A. Aerov, and R. Fakhrullin, “Clay nanotube encapsulation for functional biocomposites,” *Adv Colloid Interface Sci*, vol. 207, no. 1, pp. 189–198, May 2014, doi: 10.1016/J.CIS.2013.10.006.

[140] M. Liu *et al.*, “Polysaccharide-halloysite nanotube composites for biomedical applications: a review,” *Clay Miner*, vol. 51, no. 3, pp. 457–467, Jun. 2016, doi: 10.1180/CLAYMIN.2016.051.3.02.

[141] A. C. Santos *et al.*, “Evolution of Hair Treatment and Care: Prospects of

Nanotube-Based Formulations,” *Nanomaterials* 2019, Vol. 9, Page 903, vol. 9, no. 6, p. 903, Jun. 2019, doi: 10.3390/NANO9060903.

[142] R. Liu, B. Zhang, D. Mei, H. Zhang, and J. Liu, “Adsorption of methyl violet from aqueous solution by halloysite nanotubes,” *Desalination*, vol. 268, no. 1–3, pp. 111–116, Mar. 2011, doi: 10.1016/J.DESAL.2010.10.006.

[143] P. Yuan, D. Tan, and F. Annabi-Bergaya, “Properties and applications of halloysite nanotubes: recent research advances and future prospects,” *Appl Clay Sci*, vol. 112–113, pp. 75–93, Aug. 2015, doi: 10.1016/J.CLAY.2015.05.001.

[144] M. Fizir, P. Dramou, N. S. Dahiru, W. Ruya, T. Huang, and H. He, “Halloysite nanotubes in analytical sciences and in drug delivery: A review,” *Microchimica Acta* 2018 185:8, vol. 185, no. 8, pp. 1–33, Jul. 2018, doi: 10.1007/S00604-018-2908-1.

[145] J. Tully, R. Fakhrullin, and Y. Lvov, “Halloysite clay nanotube composites with sustained release of chemicals,” *Nanomaterials and Nanoarchitectures: A Complex Review of Current Hot Topics and their Applications*, pp. 87–118, Aug. 2015, doi: 10.1007/978-94-017-9921-8_5.

[146] A. C. Santos, I. Pereira, S. Reis, F. Veiga, M. Saleh, and Y. Lvov, “Biomedical potential of clay nanotube formulations and their toxicity assessment,” <https://doi.org/10.1080/17425247.2019.1665020>, vol. 16, no. 11, pp. 1169–1182, Nov. 2019, doi: 10.1080/17425247.2019.1665020.

[147] D. Peixoto *et al.*, “Emerging role of nanoclays in cancer research, diagnosis, and therapy,” *Coord Chem Rev*, vol. 440, p. 213956, Aug. 2021, doi: 10.1016/J.CCR.2021.213956.

- [148] A. C. Santos *et al.*, “Halloysite clay nanotubes for life sciences applications: From drug encapsulation to bioscaffold,” *Adv Colloid Interface Sci*, vol. 257, pp. 58–70, Jul. 2018, doi: 10.1016/J.CIS.2018.05.007.
- [149] R. Abhinayaa, G. Jeevitha, D. Mangalaraj, N. Ponpandian, K. Vidhya, and J. Angayarkanni, “Cytotoxic consequences of Halloysite nanotube/iron oxide nanocomposite and iron oxide nanoparticles upon interaction with bacterial, non-cancerous and cancerous cells,” *Colloids Surf B Biointerfaces*, vol. 169, pp. 395–403, Sep. 2018, doi: 10.1016/J.COLSURFB.2018.05.040.
- [150] G. Pandey, M. Tharmavaram, N. Khatri, and D. Rawtani, “Halloysite nanotubes as nano-support matrix to tailor cellulase and acetylcholinesterase-based ‘nano-biocatalysts’ for waste degradation and electrochemical sensing,” *Appl Clay Sci*, vol. 234, p. 106852, Mar. 2023, doi: 10.1016/J.CLAY.2023.106852.
- [151] G. Pandey, M. Tharmavaram, and D. Rawtan, “Functionalized halloysite nanotubes: an ‘ecofriendly’ nanomaterial in environmental industry,” *Handbook of Functionalized Nanomaterials for Industrial Applications*, pp. 417–433, Jan. 2020, doi: 10.1016/B978-0-12-816787-8.00015-6.
- [152] M. Tharmavaram *et al.*, “Chitosan functionalized Halloysite Nanotubes as a receptive surface for laccase and copper to perform degradation of chlorpyrifos in aqueous environment,” *Int J Biol Macromol*, vol. 191, pp. 1046–1055, Nov. 2021, doi: 10.1016/J.IJBIOMAC.2021.09.098.
- [153] G. Pandey, M. Tharmavaram, G. Phadke, D. Rawtani, M. Ranjan, and K. P. Sooraj, “Silanized halloysite nanotubes as ‘nano-platform’ for the complexation and

removal of Fe (II) and Fe (III) ions from aqueous environment,” *Sep Purif Technol*, vol. 293, p. 121141, Jul. 2022, doi: 10.1016/J.SEPPUR.2022.121141.

[154] M. Tharmavaram, G. Pandey, N. Khatri, and D. Rawtani, “L-arginine-grafted halloysite nanotubes as a sustainable excipient for antifouling composite coating,” *Mater Chem Phys*, vol. 293, p. 126937, Jan. 2023, doi: 10.1016/J.MATCHEMPHYS.2022.126937.

[155] D. Rawtani and Y. K. Agrawal, “A Study of the Behavior of HNT with DNA Intercalator Acridine Orange,” *Bionanoscience*, vol. 3, no. 1, pp. 52–57, Mar. 2013, doi: 10.1007/S12668-012-0066-1/METRICS.

[156] G. Pandey, M. Tharmavaram, N. Khatri, and D. Rawtani, “Mesoporous halloysite nanotubes as nano-support system for cationic dyes: An equilibrium, kinetic and thermodynamic study for latent fingerprinting,” *Microporous and Mesoporous Materials*, vol. 346, p. 112288, Dec. 2022, doi: 10.1016/J.MICROMESO.2022.112288.

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W	URL: https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-022-01483-w Fetched: 3/23/2023 9:36:02 PM	8
W	URL: https://www.spandidos-publications.com/10.3892/etm.2019.7510 Fetched: 7/23/2020 6:58:48 PM	2
W	URL: https://pubmed.ncbi.nlm.nih.gov/31678607/ Fetched: 12/22/2021 3:25:20 AM	2
W	URL: https://www2.mst.dk/Udgiv/publications/2015/12/978-87-93352-98-8.pdf Fetched: 12/10/2019 5:01:36 PM	6
W	URL: https://repositorio.ufscar.br/bitstream/handle/ufscar/11171/de%20Souza%2C%20B.F.S.%20-%20Disse... Fetched: 11/8/2021 3:35:10 PM	2
W	URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8708822/ Fetched: 1/3/2023 10:03:03 AM	4
W	URL: https://www.nature.com/articles/s41598-018-36531-9 Fetched: 3/15/2020 8:02:10 AM	2
W	URL: https://www.hindawi.com/journals/jnm/2021/4972770/ Fetched: 9/30/2021 10:33:06 PM	3
W	URL: https://koreascience.or.kr/article/JAKO201634864482835.page Fetched: 3/29/2023 11:49:08 AM	1
W	URL: https://mdpi-res.com/d_attachment/ijerph/ijerph-18-09310/article_deploy/ijerph-18-09310-v2.pdf... Fetched: 3/28/2023 12:11:52 PM	3
W	URL: https://www.frontiersin.org/articles/10.3389/fonc.2021.693814/full Fetched: 9/15/2021 6:07:49 AM	5
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