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Research Article

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Investigation of Toxicological and Functionalized Formulation of Carbon Nanotubes

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Abstract

Carbon nanotubes (CNTs) are emerging technology for biomedical application, including facilitating feasible drug delivery, non-invasive treatment modalities, scaffold for regenerative medicine and more. However, impeding the clinical translation of such products is CNT toxicity, which has unfavourable consequences at the cellular level. CNT toxicity is onset by factors including CNT nanoscale size, which is associated with increased reactivity, its fibrous nature which promotes inflammatory responses, and its graphitic structure, linked to poor physiological elimination and toxic buildup. To assess how CNT toxicity could be minimised in-vitro, this study analysed two different lengths of single-walled carbon nanotubes (SWCNTs), altering their concentration, functionalization levels, and exposure period to cell lines MCF7 and HT29. Toxicity was assessed by analysing cell viability and DNA concentration using Alamar Blue and DNA assays, respectivel reduction of the metabolic actitivity and DNA concentration following the exposure of both MCF7 and HT29 to short and long pristine SWCNT was observed.

In this experiment the thickness of both short and long SWCNT was in the similar range. However, functionalization resulted in the SWCNT to become ticker, which could be one possible reason of reducing the CNT's toxicity. This is because the CNT functionalization leads to lower chance of SWCNT entering the nucleus and interrupting normal DNA functions. With regards to the length of the SWCNT, the result shows that the long SWCNT is more toxic than the short SWCNT. It was demonstrated that short SWCNTs functionalized for a longer duration were significantly linked to less toxicity ($P<0.05$). Implementing this strategy in reduction of SWCNT's toxicity is one way of improving the potentional application of CNT in clinical area. The application of nanoparticles for drugs, genes and vaccines is a multi-billion-dollar industry with great interest to clinicians and pharmaceutical industries, being considered as an emerging technology.

Keywords: Carbon nanotube, Functionalization, Toxicity, Drug Delivery, Vaccine Delivery, Gene Delivery, Nanotechnology, Nanoparticles, Nanomaterials.

1. Introduction

Carbon nanotubes (CNTs) have significant potential in the field of medical science, ranging from cancer treatment through targeted drug delivery and thermal treatment methods, as well as cancer cell localization [1,2,3]. Although CNTs have proven beneficial in-vitro, they have likewise been linked to various toxicological effects, which continue to delay their use in the clinical setting.

Toxicity of CNTs remains a major concern. The route of exposure, surface chemistry, and dimensions are all key factors to the toxicity and fate of SWCNTs in the body. Overall, it is seen that CNTs have a tendency to be taken up via the reticuloendothelial system (RES) -mediated pathway following with distribution to the systemic organs. Accumulation in these organs have been reported and potential long-term effects shouldn't be underestimated. Understanding the kinetics of SWCNTs when entering the body will help researchers gain further knowledge about potential tissue-specific impacts[4 . There are conflicting reports on the toxicity of SWCNTs, varying from reports of toxic effects to very low or even insignificant cellular responses. These inconsistencies seem to be

caused by experimental differences and external/intrinsic factors such as modifications, length, shape, and purity of the CNTs [4,5]. Systemic distribution and toxicokinetic profiling of CNTs is ongoing and further complicated by the amount of variables affecting CNTs' toxicity.

CNTs' toxicity mechanisms have been proposed to be linked to inflammation, inhibition of cell proliferation, oxidative stress, and finally cell death [6]. One studied mechanism of toxicity is ROS formation, leading to oxidative stress. This is regarded as one of the most common mechanisms, where increased ROS levels can lead to inflammation and apoptosis or necrosis [7,8]. Oxidative stress has also been seen to trigger mitochondrial impairment, where the Institute of Health and Environmental Medicine identified in their study that ROS production activates mitochondria-dependant apoptosis in aorta endothelial cells in rats [9]. In another investigation conducted by Northwest A&F University in China, significant ROS production as well as antioxidant enzymes were detected during SWCNT biocompatibility testing with Saccharomyces cerevisiae as the model organism. After exposure of SWCNTs (376.4 mg/L), 20.8% of cells had undergone apoptosis, as well as a reduction in mitochondrial transmembrane potential (MTP). A MTP decrease is linked to release of apoptogenic factors, and so an early event in the apoptosis pathway7. In another study the lung epithelia cells were exposed to SWCNT. The results show increased ROS on exposure to SWCNT in a dose and time dependent manner. Measurment of the ROS is one way of determining the toxicity of the SWCNT [10].

The main concern regarding nanoparticles, particularly CNTs, is that irreversible cellular damage may result once they interact with biological tissue [11,12]. Such outcomes are an obvious impediment to their use in human clinical trials. For this reason, various research groups have focused on developing methods to reduce nanoparticle toxicity, especially that associated with CNTs [13]. Factors including length, purity, dosage, production methods, and functionalization techniques have been shown to affect the toxicity of CNTs [14]. According to some research papers large CNTs may be more challenging to eliminate by phagocytosis and are associated with increased levels of toxic outcomes Purity also plays an important role in contributing to the toxicity of CNTs [15]. Pristine CNTs are not as pure, and various metals, including Co, Fe, Ni have been used as catalysts to promote CNT growth during synthesis Following synthesis, residual metal is encapsulated within a layer of carbon, accounting for the toxicity [16]. Various toxicologic studies have also shown that impurities promote cell death through mitochondrial destruction [15].

Functionalization is a crucial procedure performed prior to using CNTs for cell studies. It is defined as the process of CNT surface modification by coating with new materials, which are not biologically hazardous [17]. Several studies also support that following the successful functionalization of CNTs, CNT toxicity is significantly reduced [2]. Optimal material functionalization techniques, as well material concentrations necessary for minimal CNT toxicity were studied by several groups [18].

The aim of this study was to investigate, will OctaAmmonium-POSS as a functionalization agent, to reduce the toxicity of SWCNT. The effect of various concentrations of OctaAmmonium-POSS on cellular metabolism and DNA concentration was also investigated.

2. Materials and Methods

2.1 Experimental agents

SWCNT (75% purity), with a diameter in the range of 0.7 nm-1.3nm and a length from 0.4 μm-1μm and also, SWNCT (75% purity) with the diameter of 0.7nm-1.3nm and length from 1.5μm-2μm were purchased from Nanothinx (Greece). 3-dimethylaminopropyl-N-ethylcarbodiimide hydrochloride (EDC) (99%), H2SO4 (95%), HNO3 (70%), 4% formaldehyde, N-hydroxysuccinimide (NHS), and L-Glutamine-penicillin-streptomycin solution were purchased from Sigma Aldrich (Dorset, UK). Phosphate buffered saline (PBS), Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA, AlamarBlue® reagent, Quant-iT™ Pico Green® DNA assay, To-Pro®-3 were purchased from Invitrogen™. Human colonic adenocarcinoma cell line (HT-29) and SW480 human breast cancer cell line (MCF-7) were purchased from Abcam® (Cambridge, UK). OctaAmmonium polyhedral oligomeric silsesquioxane (OctaAmmonium-POSS) was purchased from Hybrid Plastics® (Hattiesburg, USA). HPA lectin-fluorescein isothiocyanate (FITC) (20 μg/ml) was supplied by Westminster University.

Preparation of pristine SWCNT:- 10 mg of Pristine SWCNT was measured. 10ml of water was added to 10 mg of pristine SWCNT to make a 1 mg/ml SWCNT mixture, which was vortexed for 5 min and sonicated for 1 hr. Afterward, the pristine SWCNT was diluted to obtain solutions of 0.125 mg/ml, 0.250 mg/ml, 0.5 mg/ml, and 1 mg/ml. The choices of the SWCNT concentrations were based on the prelimanry experiemnt performed by the same research group.

Preparation of carboxyl-functionalized SWCNTs:- SWCNT (short) and (long) in powder form were purchased from Sigma Aldrich. 10mg of each SWCNT was measured and functionalized, as previously described. Both short and long SWCNT mixtures were centrifuged for 30 min at 2400 x g. The supernatant was discarded thereafter. 5ml of PBS was added to the pellet and mixed. The mixture was filtered with a vacuum filter flask and washed extensively in deionized water until a constant pH value ranging from 5-6 was attained. The resultant neutralised acid functionalized SWCNT on the filter paper, which was then collected by placing the paper in a pre-weighted centrifuge tube and adding 1ml of deionized water. Upon the full removal of SWCNT from the paper, the paper was removed. The SWCNT mixture was then centrifuged for 30 min at 2400 x g. The supernatant was removed, and the tubes were placed in the oven to dry. The tube was then weighed to determine the total dry weight of functionalized SWCNT. The

dry acid functionalized SWCNT powder was then dissolved in deionized water to produce a 1 mg/ml working solution. The above procedure was then conducted under conditions of 2 hrs and 5 hrs reflux time intervals for both SWCNT (short) and SWCNT (long). Following the formation of 1 mg/ml SWCNT-COOH, the functionalized SWCNT was then diluted in differing volumes of water (refer to Table 1), which formed SWCNT-COOH of concentrations 0.125 mg/ml, 0.250 mg/ml, and 0.5 mg/ml.

Concentration of SWCNT (mg/ml)	Volume of 1mg/ml COOH-SWCNT (ml)	Volume of water (ml)	Total volume (ml)
0.125	0.500	3.500	4
0.250	1.000	3.000	4
0.500	2.000	2.000	4
1.000	4.000	0.000	4

Table 1: Preparation of Different SWCNT-COOH Concentrations

Synthesis of pristine SWCNT and OctaAmmonium-POSS conjugate:- 0.125, 0.250, 0.5 and 1.00 mg/ml OctaAmmonium-POSS concentrations were prepared as explained. 1 ml of each concentration of OctaAmmonium-POSS was added to 1 ml of short and long pristine SWCNT (1 mg/ml). Please refer to Table 1 for the dilution strategy. The mixture was then vortexed for 5 min and sonicated for 1 hr. This was then washed five times with water to remove non-adsorbed OctaAmmonium-POSS followed by vacuum-drying at room temperature for 24 hrs.

Synthesis of SWCNT-COOH and OctaAmmonium-POSS conjugate:- First, OctaAmmonium-POSS concentrations of 0.125 mg/ml, 0.250 mg/ml, 0.500 mg/ml, and 1 mg/ml were prepared using –NH2 as the functional group. 1 mg of OctaAmmonium-POSS was measured and dissolved in 1 ml of NaOH to convert the ammonium groups on the POSS molecule into amine groups. This conversion provided 8 functional groups suitable for linkage to COOH groups on the oxidized SWCNT. A 1 mg/ml working solution was vortexed for 5 min and then sonicated for 1 hr until fully dissolved. The OctaAmmonium-POSS (1 mg/ml) was then diluted in water accordingly to obtain OctaAmmonium-POSS in a solution of 0.125 mg/ml, 0.250 mg/ml, 0.500 mg/ml, and 1 mg/ml concentration (refer to Table 2). Next, 1 ml of short and long SWCNT-COOH (1 mg/ml) was mixed with 5 mg EDC and 5 mg NHS and stirred at room temperature for 2 hours, then ultrasonicated for an additional 2 hours. Then 1 ml of each concentration of OctaAmmonium-POSS (0.125, 0.250, 0.500 and 1mg/ml) was added to 1 ml of short SWCNT-COOH (1 mg/ml) and long SWCNT-COOH (1 mg/ml) solution as prepared in the previous stage. The mixture was then vortexed for 5 min and sonicated for 1 hr until fully dissolved. The mixtures were washed with water at least five times to remove non-adsorbed OctaAmmonium-POSS and then vacuum- dried at room temperature for 24 hrs.

Concentration of OctaAmmonium-POSS (mg/ml)	Volume of 1mg/ml OCTA-POSS dispersion (ml)	Volume of water (ml)	Total volume (ml)
0.125	0.500	3.500	4
0.250	1.000	3.000	4
0.500	2.000	2.000	4
1.000	4.000	0.000	4

Table 2. Preparation of different OctaAmmonium- POSS Concentrations

2.2 Evaluation of toxicity using human cells

Human breast cancer cell line (MCF7) and Human colon adenocarcinoma cell line (HT29) were used as cell culture. 96 well plates were used with each well containing 10,000 cells of HT29 cells and MCF7 cells. The cells were seeded for 24hrs. They were then incubated with different treatment groups for 24 hrs, 48 hrs, and 72 hrs. The cell viability and the DNA concentration of each cell was analysed using Alamar Blue and DNA assay, respectively after each of the three-time intervals.

2.2.1 Metabolic activity

Cellular metabolic activity was measured by the Alamar Blue assay. Initially, a 10% (v/v) dilution of stock Alamar Blue was prepared by diluting 4.5 ml of Alamar Blue with 45ml of growth medium, which was then stored at 4°C. After preparation of diluted Alamar Blue, all culture medium and treatment groups were aspirated from the wells via a multi-channel pipette and 50 µl of diluted Alamar Blue (10%) was added into each well plate. The plates were then wrapped in aluminium foil and incubated for 4 hr at 37°C with 5% CO2. Following incubation, 50 µl from each well plate was transferred to a 96 well black microplate, which was thereafter placed in the fluoroskan Ascent FL (Thermo labsystem) plate reader. Fluorescence was measured at excitation and emission wavelengths of 530 nm and 620 nm and recorded using the Ascent Software package. Wells containing medium and Alamar Blue without cells were used as blanks (controls). The mean blank value was subtracted from each reading. This experiment was conducted independently three times; therefore "n" is equal to 3.

2.2.2 DNA assay

Following the Alamar Blue assay, all culture media, treatment solution, and Alamar Blue reagent were removed. 100 µl of distilled water was then added to each well. The well plates were then freeze-thawed for three cycles. Each cycle involved freezing at -80°C for 30 min followed by 30min of incubation at 37°C. 100 µl of sample DNA (previously freeze-thawed in 100 µl of distilled water per well) was transferred from each well to the corresponding wells in a black 96-well plate. 100 µl of aqueous working solution of the Quant-It Pico Green reagent was then added to every well, producing a total volume of 200 µl per well. The well plates were then incubated at room temperature for 5 min. Plates were placed in the fluoroskan ascent machine to measure fluorescence. The fluorescence value of the reagent blank was subtracted from each of the samples and the standard curve was generated. All assay components were prepared as per the Invitrogen™ protocol for Quant-iT™ Pico Green® DNA assay. Cell viability using a DNA assay was assessed on three consecutive days. Sample fluorescence was then measured using a fluorometer (wavelength of excitation and emission being 485 nm and 538 nm, respectively), and the data was statistically processed. This experiment was conducted independently three times; therefore "n" is equal to 3.

2.3 Characterization of nanoparticles

2.3.1FTIR

FTIR spectroscopy analysis was carried out to detect carboxylic acid groups (-COOH) and OctaAmmonium-POSS on both SWCNTs, the full descriptions of which are included in the following paper: Application of OctaAmmonium-POSS functionalized single-walled carbon nanotubes for thermal treatment of cancer (Madani et al.)19

2.3.2 UV-VIS spectroscopy

UV-VIS spectrometry was used to indicate changes in optical absorption following the functionalization of SWCNT with carboxylic acid and OctaAmmonium-POSS19

2.3.3 Morphological study

The images derived from TEM illustrate the interconnected and clustered appearance of tubes of pristine SWCNT. Upon functionalization with acid, however, the tubes appear separate from each other and a larger diameter of SWCNT is observed. As demonstrated in Figure 1, in the case of OctaAmmonium-POSS added to carboxylic-acid coated SWCNT, a thick, black layer appears on the surface of the SWCNT due the presence of OctaAmmonium-POSS.

2.3.4 Confocal microscopy experiementLectin is carbohydrate-binding proteins that show specificity for particular glycan moieties. They are known to be of utility in the recognition of cancer cells, as changes in glycosylation are a feature of tumour genesis and relate to the metastatic behaviour of cancer cells. EDC and NHS were used as coupling agents to attach FITC-labelled HPA lectin to the SWCNTs. Following the addition of the lectin-FITC, the SWCNTs were viewed using the confocal microscope. The SWCNTs emitted light in the green spectra consistent with attachment of FITC-labelled material (Figure 1).

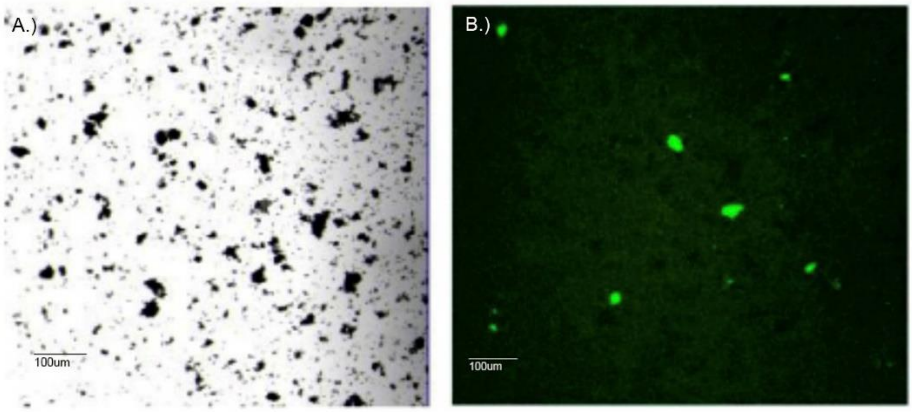

Figure 1: Confocal microscopy image taken from HPA-lectin-FITC-SWCNTs [2]. A sample of functionalized SWCNTs (2hrs) conjugated to HPA-FITC was viewed under the confocal microscope. In transmission mode (A) and in fluorescence mode, following excitation at 488nm (B). The results show that a proportion of the SWCNTs had been successfully conjugated to the FITC labelled lectin.

3. Results

3.1 FTIR and UV-VIS spectroscopy

In the FTIR experiment, results illustrated the presence of the -COOH group on the functionalized SWCNTs. For further information, please reference study by Madani et ., 2012[19]

3.2 Morphological study

SWCNTs functionalzed with different functional groups and control were deposited on copper grid, coated with Piloform (TAAB, Aldermaston, UK) and then TEM images (Phillips CM 120, Amsterdam, The Netherlands) were taken to investigate the efficiency of functionalised SWCNT.

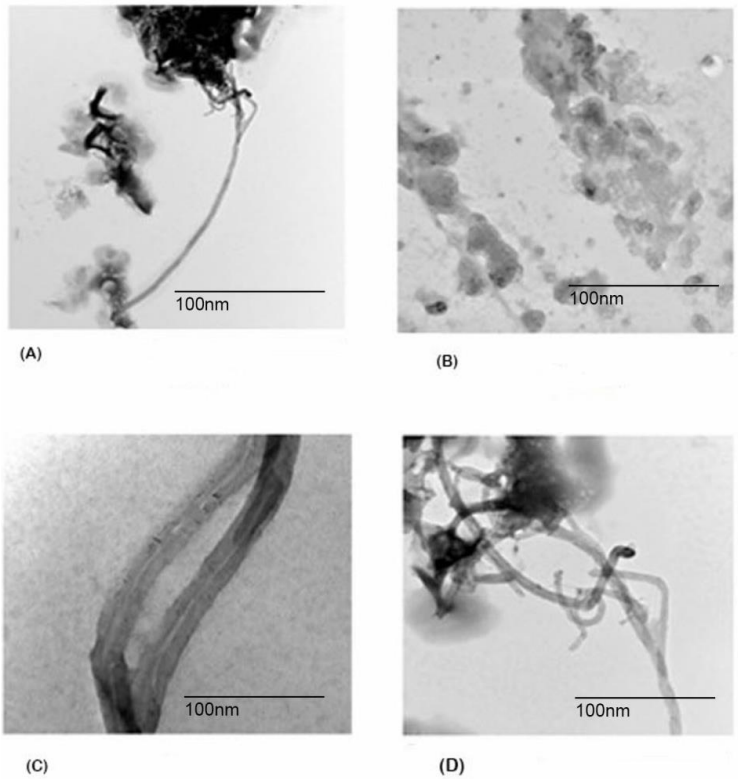

Figure 2: TEM Images of Short SWCNT with different functionalization groups. A) Short pristine SWCNT, B) OctaAmmonium-POSS, C) Short SWCNT-COOH OctaAmmonium-POSS and D) Short SWCNT-COOH.

3.3 Metabolic activity and DNA assay

3.3.1 Cell only

Results indicated that in both cell lines, cellular metabolic activity increased over 72 hrs. This phenomenon was as a pattern, shown to be more pronounced in cell line HT29 in comparison to MCF7. Likewise, the DNA assay also demonstrated a greater increase of DNA concentration in the HT29 cell line in comparison to cell line MCF7.

3.3.2 OctaAmmonium-POSS

In both cell lines, results did not demonstrate significant alteration of cellular metabolic activity following the addition of all four concentrations of OctaAmmonium-POSS (0.125, 0.250, 0.5, 1 mg/ml). This pattern was observed for all three time intervals (24 hrs, 4 8hrs, and 72 hrs). Likewise, the DNA assay showed no significant changes in DNA concentration following addition of different concentrations of OctaAmmonium-POSS to both cell lines at all three time intervals.

3.3.3 Pristine SWCNT

Following the addition of all four concentrations of pristine SWCNT, the metabolic activity of both cell lines significantly declined in comparison to the cell-only control. This pattern was consistent at all three time intervals. Figures indicate that cellular metabolic activity decreases after increasing the concentration of pristine SWCNT in both cell lines from 0.125 mg/ml to 1.00 mg/ml. In the case of cell line HT29, with respect to the short SWCNT, it was determined that following the addition of all four concentrations of SWCNT, the metabolic activity significantly decreased in comparison to the cell only control ($P<0.05$). This pattern was observed at all three time intervals (Figure 3).

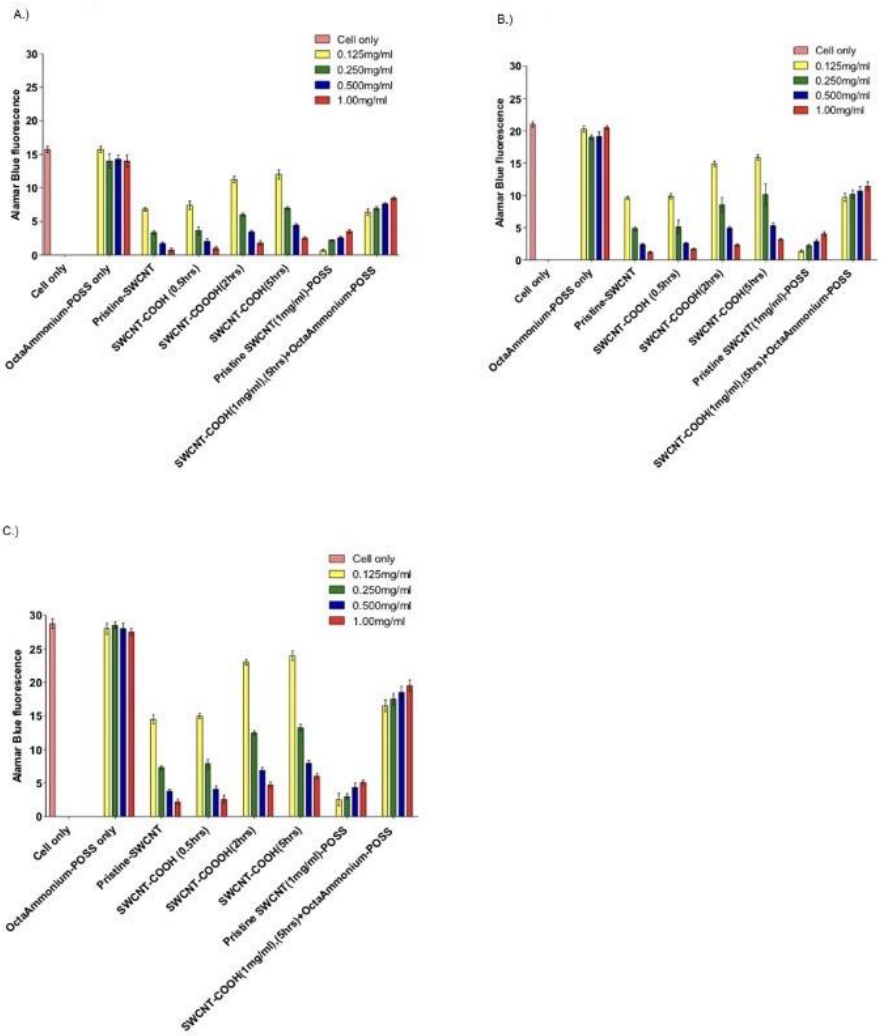

Figure 3: The data shows the effect of functionalisation of short SWCNT with COOH or OctAmmonium-POSS on toxicology (HT29) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time (2hrs, 5hrs) of SWCNT resulted in greater cells viability in comparison to pristine SWNCT ($P<0.05$). Addition of all POSS concentrations to SWCNT (1mg/ml) except the 0.125 mg/ml resulted in a significant increase in emission when compared to pristine SWCNT. Conjugation of all POSS concentrations to SWCNT-COOH (1 mg/ml) resulted in a significant increase in emission compared with SWCNT-COOH (1 mg/ml) ($P<0.05$).

In the MCF7 cell line, similar results were observed, as depicted in figures. Following the addition of all four concentrations of pristine short SWCNT to the MCF7 cell line, the metabolic activity significantly decreased in comparison to cell-only controls. The results show that as the concentration of short SWCNT increases from 0.125 mg/ml to 1 mg/ml, the metabolism significantly declines ($P<0.05$). Moreover, in the case of MCF7 cell line, addition of pristine SWCNT decreases the cell's metabolic activity with a greater magnitude at all time intervals in comparison to the HT29 cell line. For example, after adding 0.125 mg/ml of pristine SWCNT to MCF7, results show that at 24 hrs, 48 hrs, and 72 hrs, the metabolic activity decreased by approximately 64%, 72%, and 78%, respectively (Figure 4). However, in the case of HT29, following the addition of short pristine SWCNT (0.125 mg/ml), the metabolic activity of the HT29 cell line decreased by approximately 57%, 55%, and 50% at 24 hrs, 48 hrs, and 72 hrs, respectively. Regarding the long SWCNT (1.5-2nm), with respect to HT29 and MCF7 cell lines, results show that as SWCNT concentration increases, cellular metabolic activity decreases (Figure 5).

At all three time intervals in the HT29 cell line, it was determined that at all four concentrations of long SWCNT, metabolic activity was reduced significantly when compared to short SWCNT. P is determined to be less than 0.05 when the metabolic activity of the HT29 cell line was compared following the addition of each concentration of long SWCNT compared to short SWCNT (Figure 6). This was also observed in the MCF7 cell line. A similar trend in metabolic activity was observed when analysing the results of the DNA assay. Upon the addition of the short SWCNT to the HT29 cell line, the DNA concentration significantly decreased. Following the addition of all four SWCNT's concentrations, the DNA concentration was observed to significantly decrease in comparison to the cell-only control. This was illustrated at all three time intervals. By increasing the concentration of pristine SWCNT, DNA concentrations significantly declined (Figure 7).

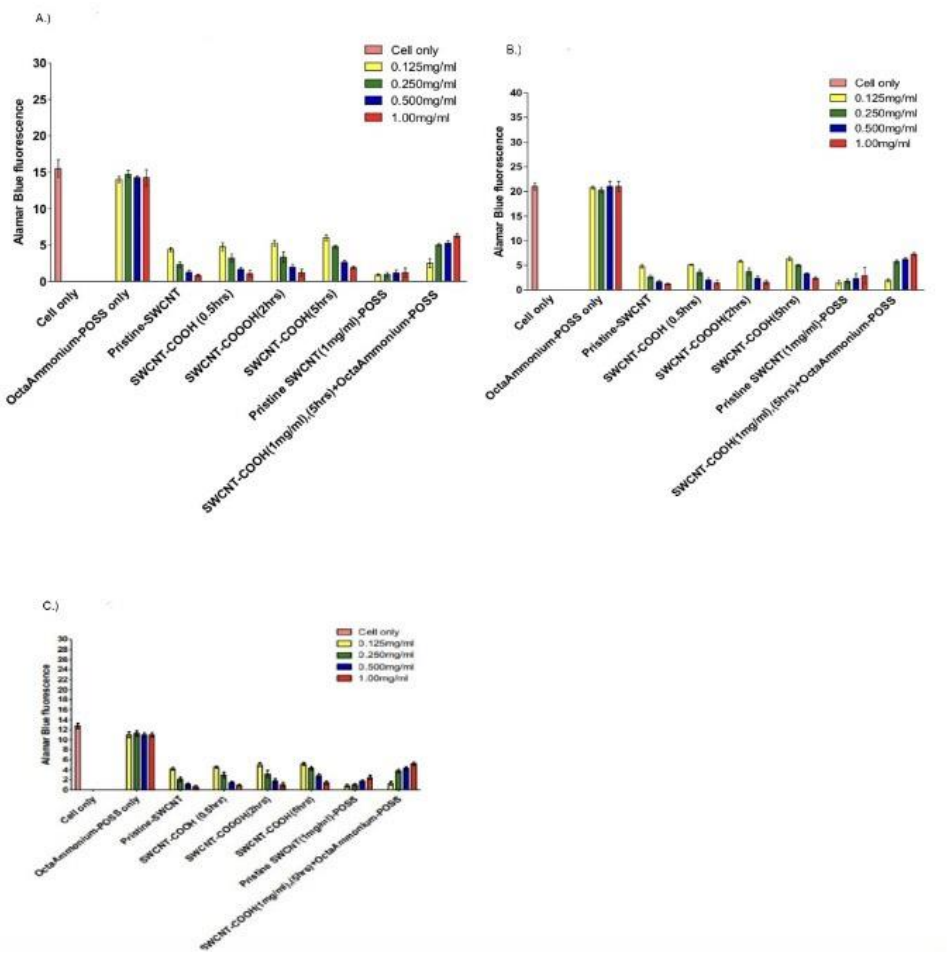

Figure 4. The data shows the effect of functionalisation of short SWCNT with COOH or OctAmmonium-POSS on toxicology (MCF7) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time (5 hrs only) of SWCNT resulted in greater fluorescence emission in comparison to pristine SWNCT ($P<0.05$). Addition of 0.5 and 1 mg/ml of POSS concentrations to SWCNT (1 mg/ml) resulted in a significant increase in emission when compared with pristine SWCNT. Conjugation of all POSS concentrations (except 0.125 mg/ml) to SWCNT-COOH (1mg/ml) resulted in a significant difference in emission when compared with SWCNT-COOH (1 mg/ml) ($P<0.05$).

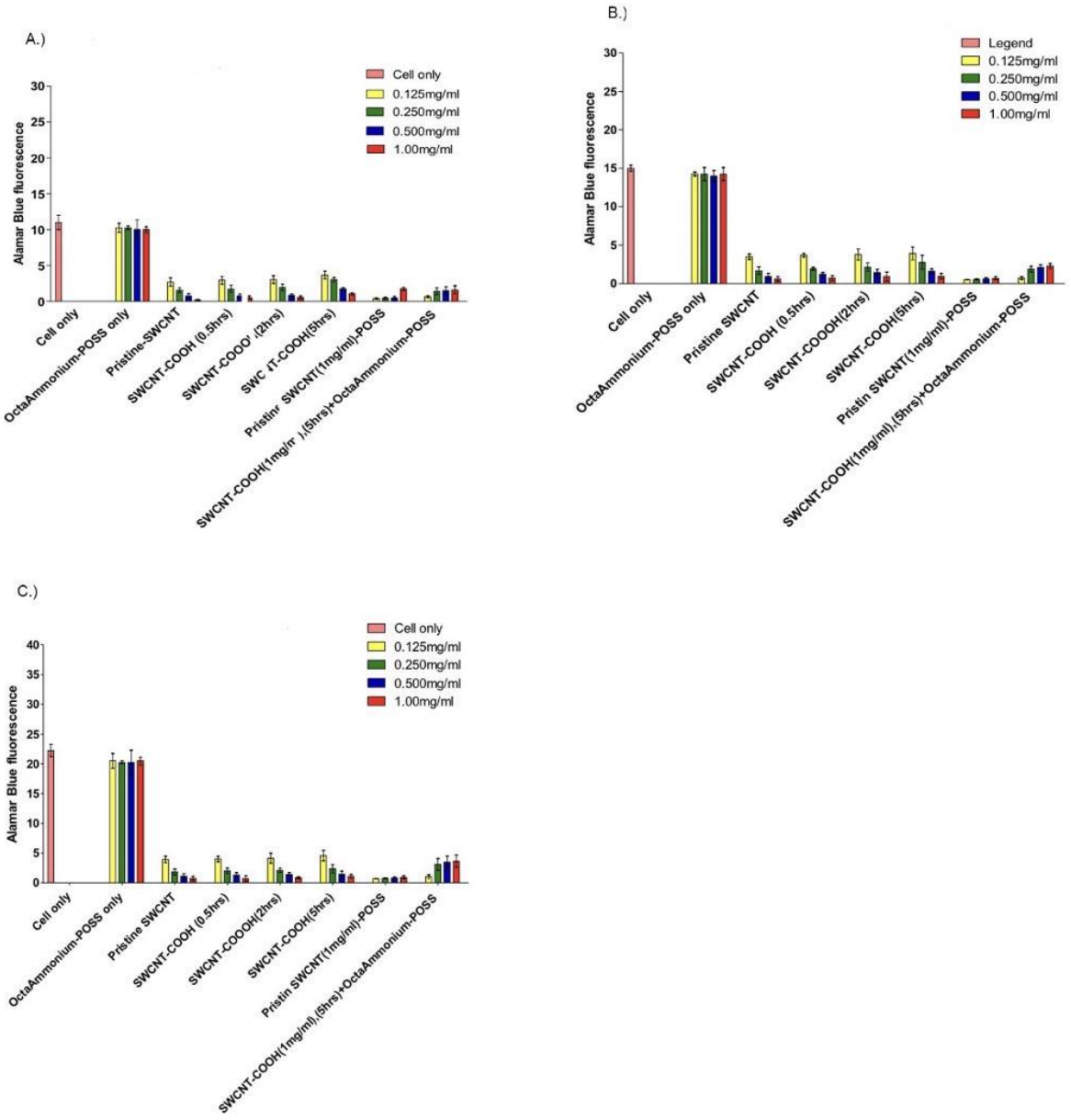

Figure 5: The data shows the effect of functionalisation of long SWCNT with COOH or OctAmmonium-POSS on toxicology (MCF7) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time (5 hrs only) of SWCNT resulted in greater fluorescence emission in comparison to pristine SWNCT ($P<0.05$). Conjugation of 1 mg/ml POSS concentrations only to SWCNT (1 mg/ml) resulted in a significant increase in emission in comparison to pristine SWCNT. Conjugation of all POSS concentrations (except 0.125 mg/ml) to SWCNT-COOH (1 mg/ml) resulted in a significant change in emission when compared with SWCNT-COOH (1 mg/ml) ($P<0.05$).

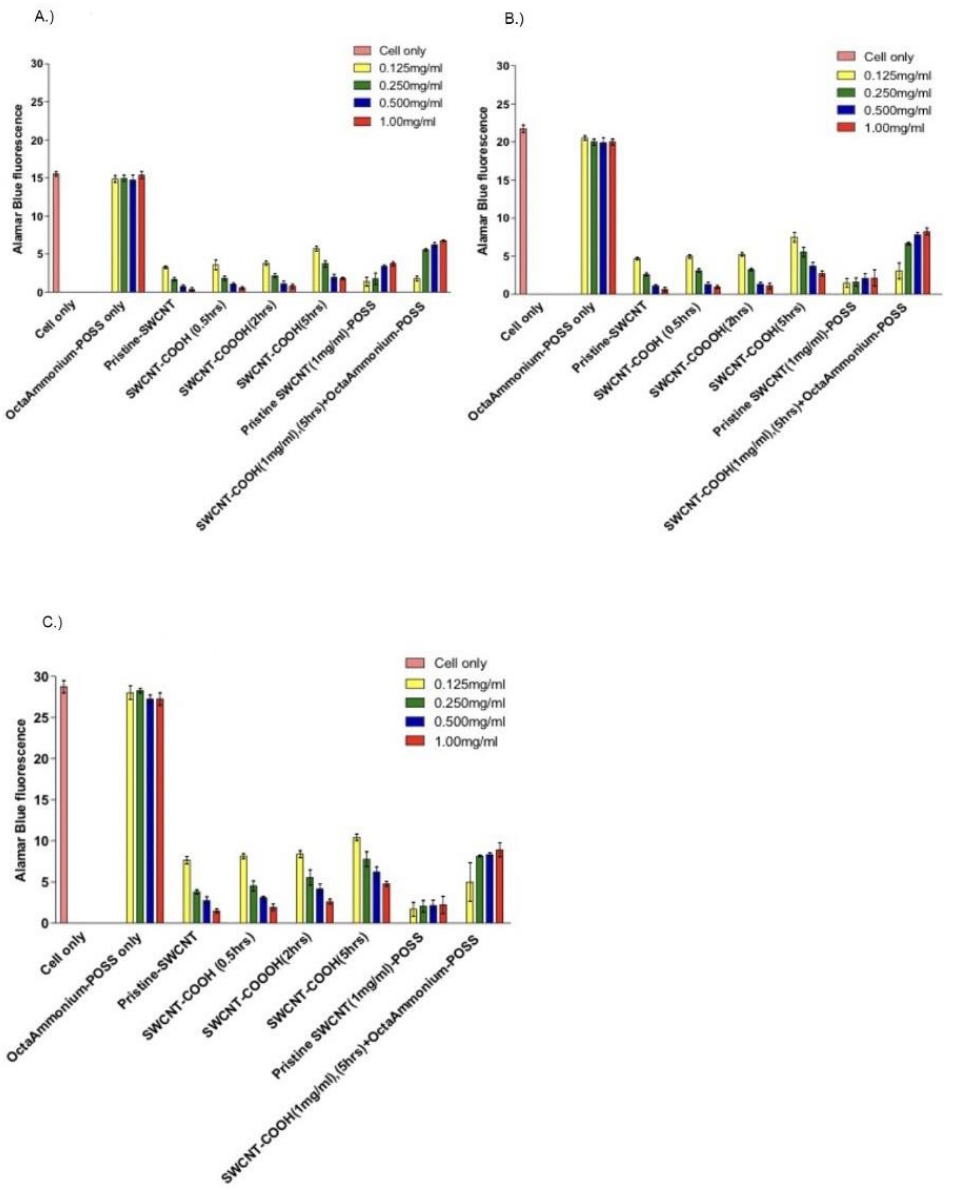

Figure 6: The data shows the effect of functionalisation of long SWCNT with COOH or OctAmmonium-POSS on toxicology (HT29) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time (5 hrs only) of SWCNT resulted in greater fluorescence emission in comparison to pristine SWNCT ($P<0.05$). Addition of POSS (0.500 and 1 mg/ml) concentrations to SWCNT (1 mg/ml) resulted in a significant increase in emission in comparison to pristine SWCNT. Conjugation of all POSS concentrations (except 0.125 mg/ml) to SWCNT-COOH (1 mg/ml) resulted in a significant difference in emission when compared with SWCNT-COOH (1 mg/ml) ($P<0.05$).

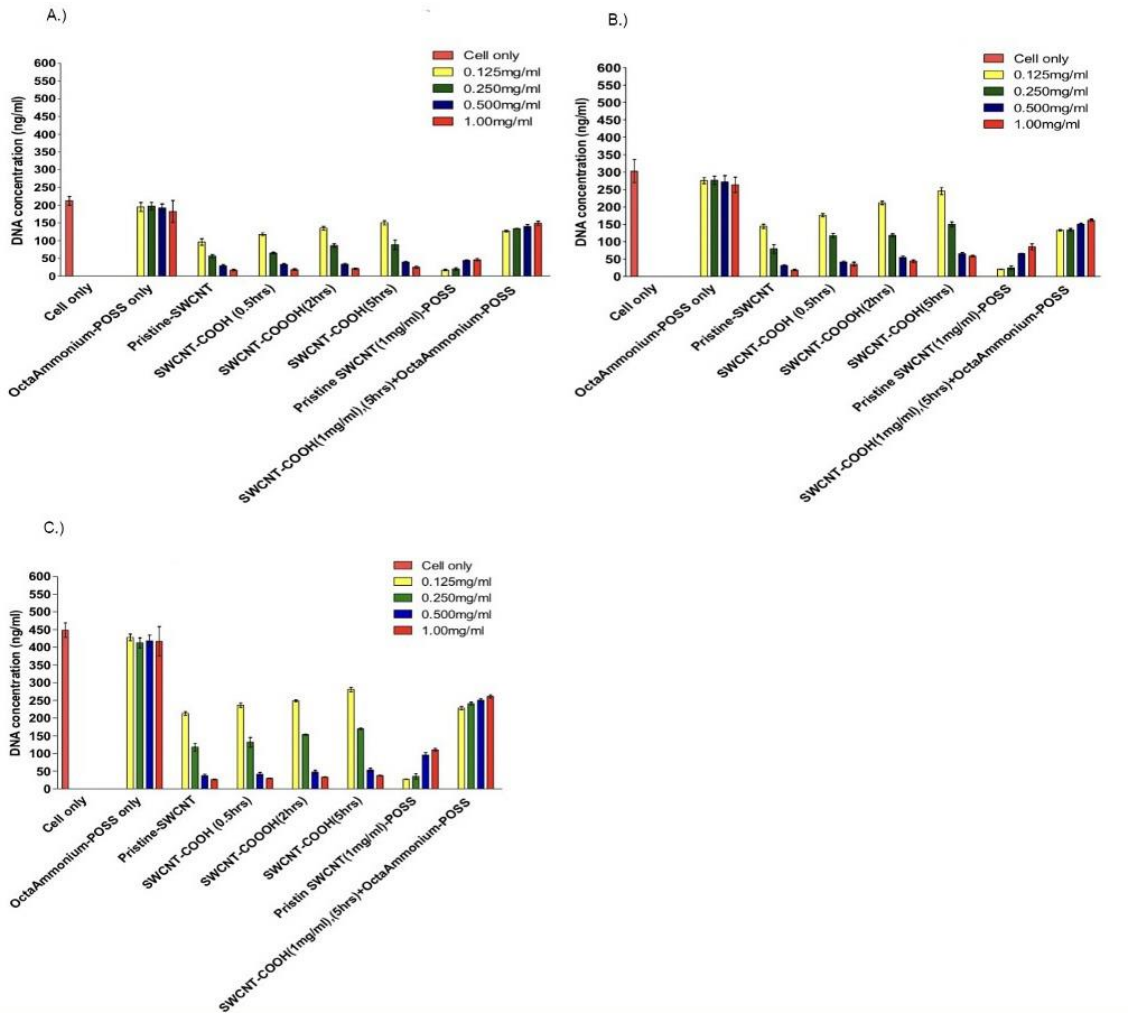

Figure 7: The data shows the effect of functionalisation of short SWCNT with COOH or OctAmmonium-POSS on toxicology (HT29) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time of SWCNT led to a significant increase in DNA concentration in comparison to pristine SWNCT ($P<0.05$). Conjugation of 0.5 mg/ml and 1 mg/ml of POSS to SWCNT (1 mg/ml) resulted in a significant increase in DNA concentration when compared to pristine SWCNT. Conjugation of all POSS concentrations to SWCNT-COOH (1 mg/ml) resulted in a significant increase in DNA concentration in comparison to SWCNT-COOH (1 mg/ml) ($P<0.05$).

In the case of pristine long SWCNT, a similar trend was observed at all four concentrations and all three time intervals. The decrease in DNA concentrations following addition of pristine long SWCNT appeared to be more significant in comparison to that of short SWCNT (Figure 8). Both instances, however, demonstrated that a higher concentration of SWCNT (i.e., 0.5 mg/ml and 1 mg/ml), proceeding from 24 hrs to 72 hrs, does not increase DNA concentration with a magnitude as high as the addition of lower concentration (i.e., 0.125 mg and 0.250 mg/ml). For example, addition of 0.125 mg/ml of short pristine SWCNT to the HT29 cell line demonstrated that the DNA concentration from 24 hrs to 48 hrs increased by approximately 27% and by 32% from 48 hrs to 72 hrs. However, following the addition of 1mg/ml of short pristine SWCNT to the cell line from 24 hrs to 48 hrs, the DNA concentration increased by 19% and from 48 hrs to 72 hrs, the DNA concentration increased by 24%. In the case of pristine short SWCNT with respect to MCF7, results showed that as the concentration of pristine short SWCNT increases, DNA concentration decreases. Moreover, this decreased percentage of DNA concentration in the MCF7 cell line is more significant than the DNA concentration decline seen in the HT29 cell line at all three time intervals. Also in the MCF7 cell line, addition of short SWCNT significantly decreased DNA concentration. It was illustrated that the decrease in DNA concentration following the addition of 0.5 mg/ml and 1.0 mg/ml short SWCNT was at the higher end in most cases in comparison to 0.125 mg/ml and 0.250 mg/ml (Figure 9). In the case of long SWCNT, addition of all four concentrations to the MCF7 cell line has shown a significant decrease in DNA concentration (Figure 10).

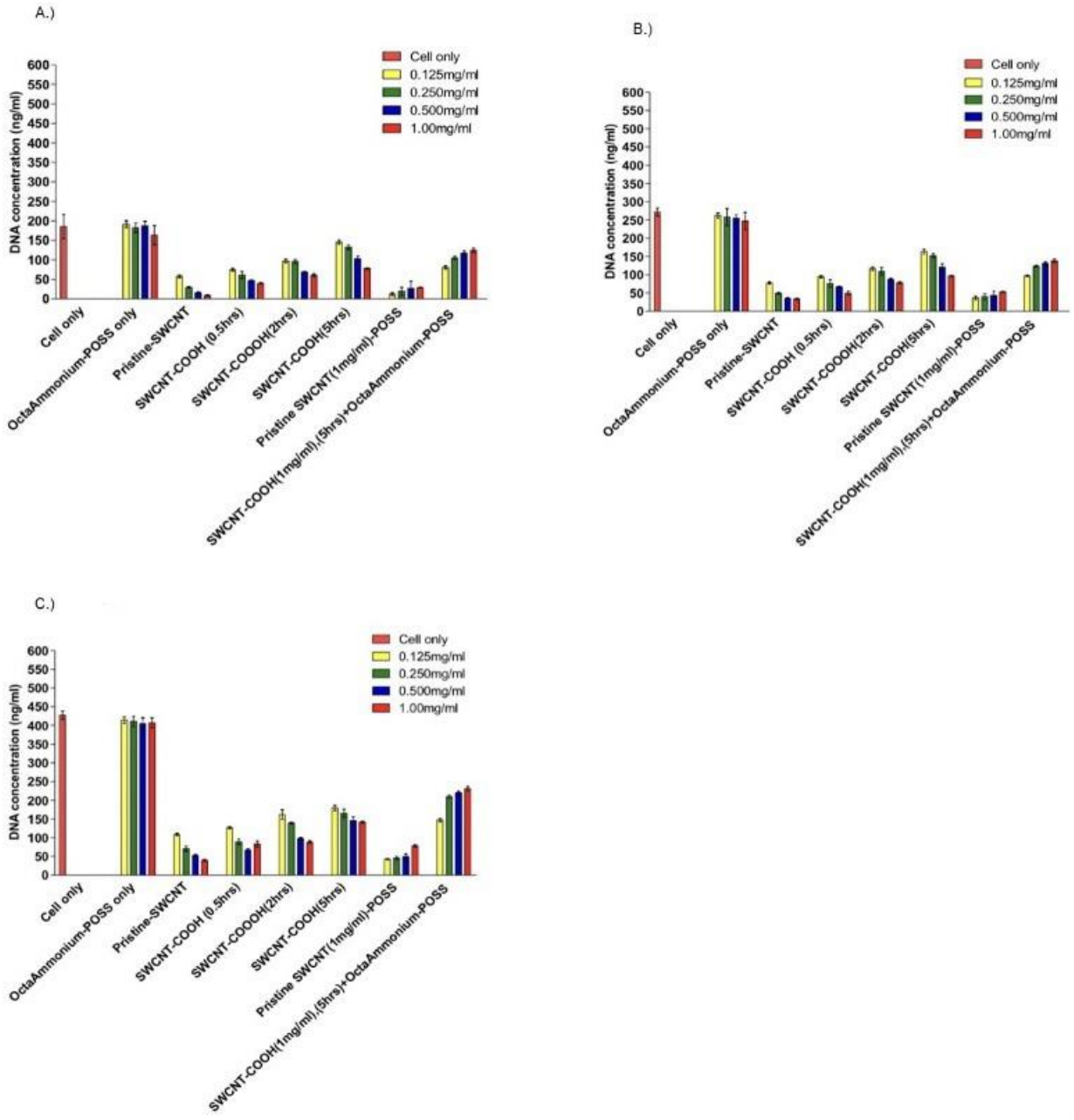

Figure 8: The data shows the effect of functionalisation of long SWCNT with COOH or OctAmmonium-POSS on toxicology (HT29) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time of SWCNT led to a significant increase in DNA concentration in comparison to pristine SWNCT ($P<0.05$). Conjugation of 1 mg/ml of POSS only to SWCNT (1 mg/ml) resulted in a significant increase in DNA concentration in comparison to pristine SWCNT. Conjugation of all POSS concentrations except 0.125 mg/ml to SWCNT-COOH (1 mg/ml) resulted in a significant increase in DNA concentration when compared to SWCNT-COOH (1 mg/ml) ($P<0.05$).

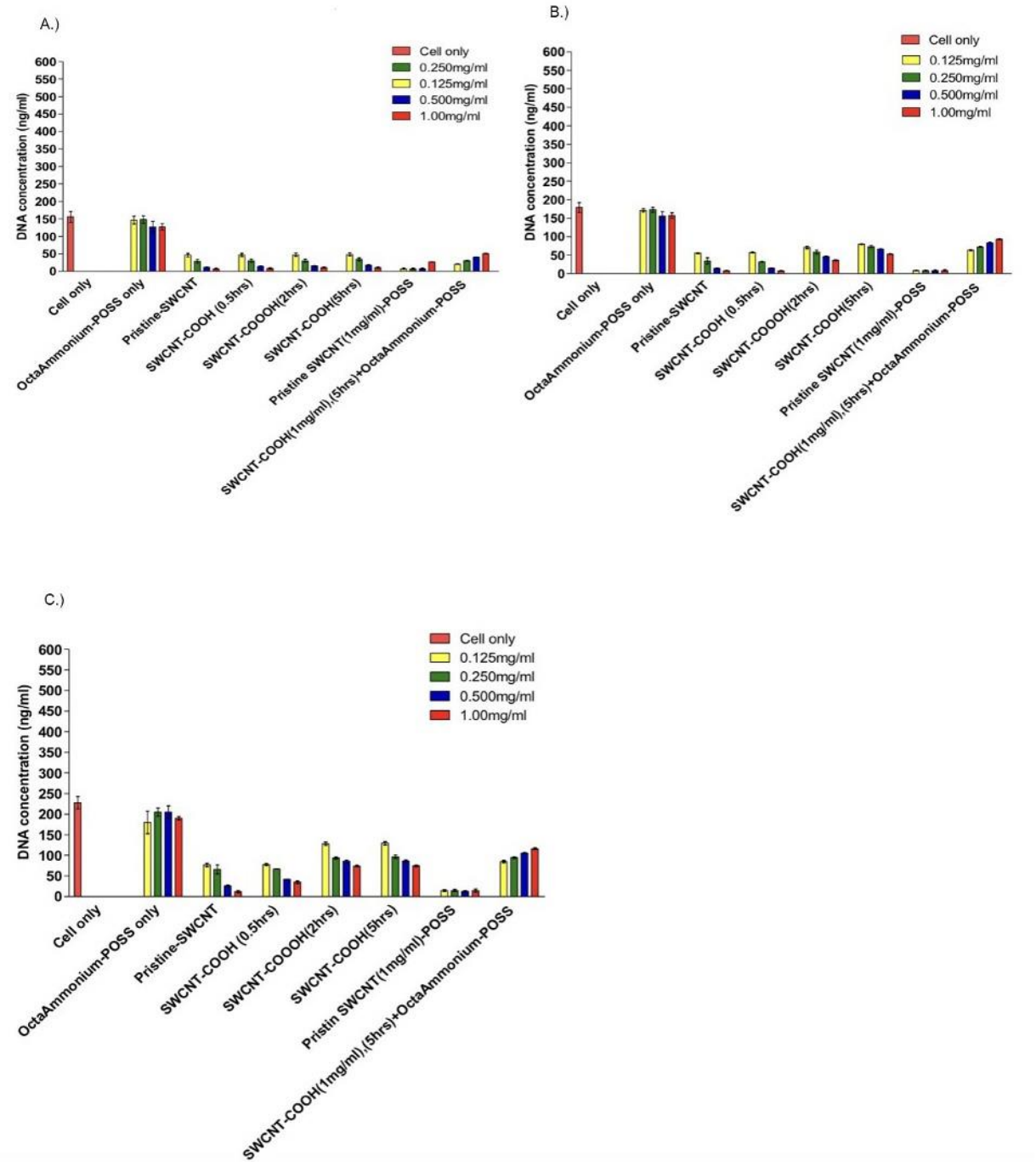

Figure 9: The data shows the effect of functionalisation of short SWCNT with COOH or OctAmmonium-POSS on toxicology (MCF7) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time of SWCNT showed a non-significant increase in DNA concentration in comparison to pristine SWNCT ($P>0.05$). Conjugation of only 1 mg/ml POSS to SWCNT (1 mg/ml) resulted in significant increase in DNA concentration in comparison to pristine SWCNT. Conjugation of all POSS concentrations to SWCNT-COOH (1 mg/ml) resulted in a significant increase in DNA concentration in comparison to SWCNT-COOH (1 mg/ml) ($P<0.05$).

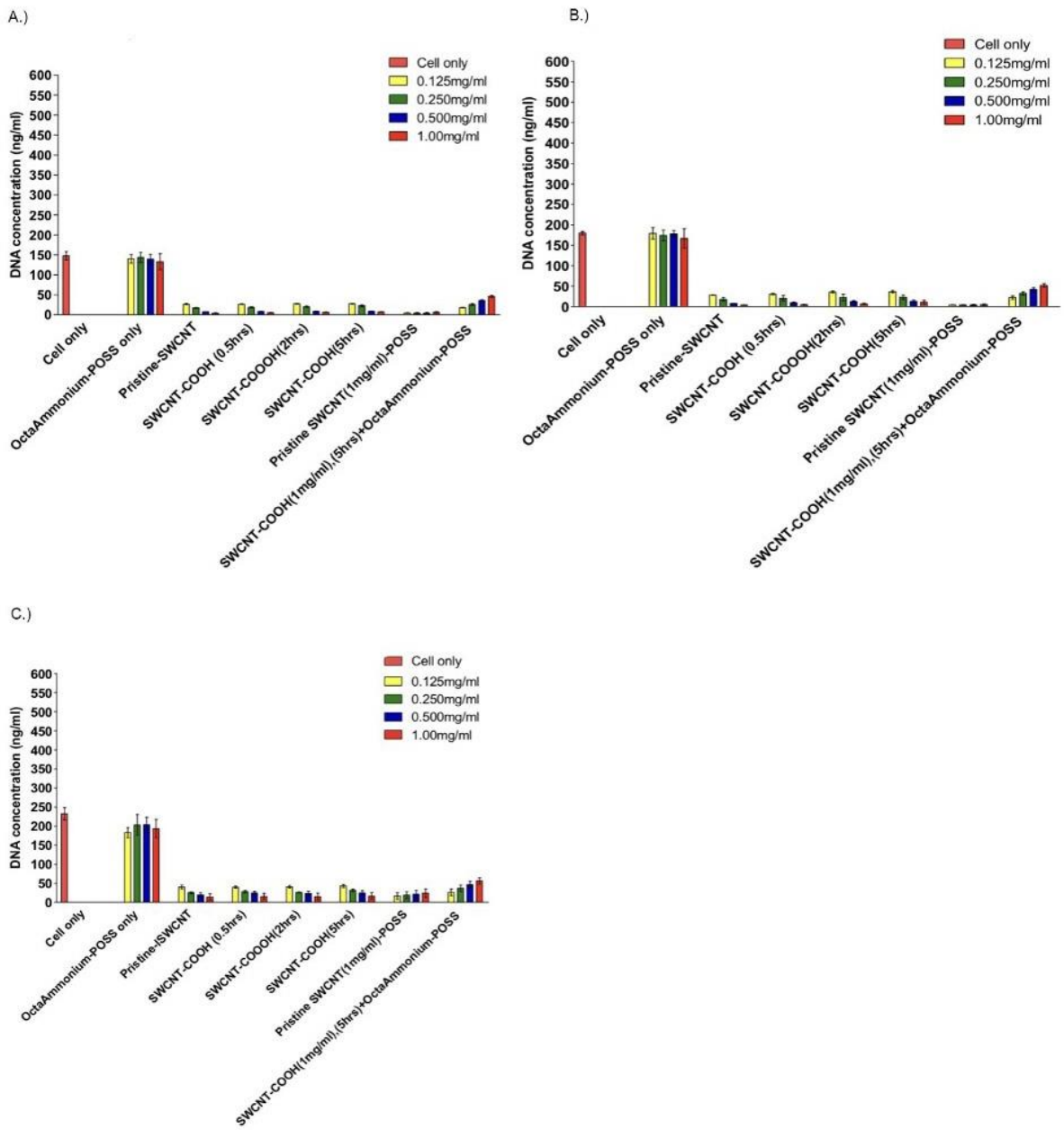

Figure 10: The data shows the effect of functionalisation of long SWCNT with COOH or OctAmmonium-POSS on toxicology (MCF7) after 24 hrs (A), 48 hrs (B) and 72 hrs (C). Increasing the functionalization time of SWCNT did not show a significant increase in DNA concentration in comparison to pristine SWNCT ($P>0.05$). Conjugation of POSS to the pristine SWCNT did not result in a significant change in DNA concentration in comparison to pristine concentration. Conjugation of all POSS concentrations to the SWCNT-COOH resulted in a significant increase in DNA concentration in comparison to SWCNT-COOH ($P<0.05$).

Functionalized SWCNT:-

As depicted by figures, as the exposure time of acid to short SWCNT increased from 0.5 hrs to 5 hrs (the process of functionalization), the metabolic activity of HT29 also increased in comparison to pristine short SWCNT. It was observed that following incubation of short SWCNT, functionalized for a period of 2 hrs and 5 hrs with the cells, there was a significant increase in metabolic activity ($P<0.05$). This trend was noted at all three time intervals (24 hrs, 48 hrs, and 72 hrs). Results show no significant increase in metabolic activity of the HT29 cell line following incubation with all four concentrations of short SWCNT functionalized for 0.5 hrs in comparison to pristine short SWCNT. Results demonstrate a p value higher than 0.05 when the metabolic activity of HT29 cells following the addition of pristine SWCNT and SWCNT functionalized for 0.5 hrs were compared. This result was consistent at all concentrations of SWCNT. This pattern appeared to be the same at all three time intervals (24 hrs, 48 hrs, and 72 hrs) as well. With respect to long SWCNT, results show that following the incubation of all four concentrations of SWCNT functionalized for 5 hrs, the metabolic activity of HT29 cell line significantly increased in comparison to that incubated with pristine SWCNT. This was also observed at all three time intervals. In the case of MCF7, with respect to both short and long SWCNT following 0.5 hrs and 2 hrs of functionalization, the metabolic activity of the cells increased, but this increase was only significant 5 hours after functionalization. With respect to the short SWCNT, this trend was observed for all three time intervals. Long SWCNT resulted in a significant increase of DNA concentration following 5 hrs of functionalization at the 24 hr interval. No further significant changes of metabolic activity were reported with other functionalization periods. With respect to the DNA assay, results also illustrated that functionalization had a direct effect on increasing DNA concentration. According to our results, increasing the functionalization time from 0.5 hrs to 5 hrs led to the increase in DNA concentration in both cell lines. In the case of the HT29 cell line, increasing the functionalization time of short SWCNT resulted in an increase of DNA concentration. This result showed that at all three time intervals (24 hrs, 48 hrs, and 72 hrs), the concentration of DNA significantly increased following functionalization for as short a time as 0.5 hrs. With respect to long SWCNT, a similar result was obtained. It was also indicated that by increasing functionalization time from 0.5 hrs to 5.0 hrs, the DNA concentration significantly increased in comparison to that of pristine long SWCNT. The graphs demonstrate that the increase in DNA concentration at all functionalization times of long SWCNT is lower than those exhibited with short SWCNT. With respect to the MCF7 cell line, similar results were obtained. However, in the case of incubation of short SWCNT, it was illustrated that only following the 2 hrs and 5 hrs of functionalization of SWCNT and its incubation with the cells does the DNA concentration significantly increase in comparison to pristine SWCNT. This result was only illustrated at 48 hrs and 72 hrs. At 24 hrs, no change in metabolism was detected following acid treatment.

Pristine SWNCT (1 mg/ml) + OctaAmmonium-POSS:-

Following incubation of pristine SWCNT (1 mg/ml), and conjugation with 0.250 mg/ml, 0.5 mg/ml, and 1 mg/ml concentration of OctaAmmonium-POSS, the metabolic activity of the MCF7 cells significantly increased in comparison to incubation with pristine SWCNT (1 mg/ml) alone. In the case of a 0.125 mg/ml concentration of OctaAmmonium-POSS, results indicated that at all three time intervals (24 hrs, 48 hrs, and 72 hrs), the cell's metabolic activity did not significantly increase following the conjugation of OctaAmmonium-POSS to the short SWCNT-COOH in comparison to pristine short SWCNT. With regards to long SWCNT, results indicated that increasing OctaAmmonium-POSS's concentration would result in increased metabolic activity of HT29 cell line. At 24 hours, after exposure to long SWCNT, it was shown that only at 0.5 mg/ml and 1.00 mg/ml OctaAmmonium-POSS does the metabolic activity of cells significantly increase. Additionally, varying the concentration of OctaAmmonium-POSS after 48 and 72 hr does not affect cellular metabolic activity. With respect to the MCF7 cell line, results have also shown a significant increase in the cell's metabolic activity following the conjugation of OctaAmmonium-POSS to short SWCNT-COOH (1 mg/ml) in comparison to unconjugated SWCNT (1 mg/ml). This was only observed following the addition of 0.5 mg and 1 mg of OctaAmmonium-POSS at a 24 hr interval. No significant changes were observed following the conjugation of 0.125 mg/ml and 0.250 mg/ml OctaAmmonium-POSS. Moreover, after incubation of long SWCNT conjugated with OctaAmmonium-POSS, metabolic activity increased. This increase is present only at 1 mg/ml OctaAmmonium-POSS at 24 hrs. DNA assay has also demonstrated results similar to an Alamar Blue test. In this assay, results show that at all time intervals, short SWCNT is associated with increased DNA concentration in the HT29 cell line, following the conjugation of 0.5 mg/ml and 1 mg/ml OctaAmmonium-POSS to SWCNT. With respect to long SWCNT, however, only 1 mg/ml POSS at all three time intervals appeared to increase the DNA concentration significantly. Lastly, in the case of the MCF7, conjugation of 1mg/ml OctaAmmonium-POSS to short SWCNT increased the concentration of DNA significantly at 24 hrs. No change was observed in the case of other concentrations at other time intervals. For long SWCNT, irrespective of the concentration of POSS it was conjugated to, no increase in DNA concentration was observed.

SWCNT-COOH (1 mg/ml) + OctaAmmonium-POSS:- As observed with HT29 cancer cells, by increasing the concentration of OctaAmmonium-POSS, the metabolic activity of the cell also increased and after conjugation of all four concentrations (0.125 mg/ml, 0.250 mg/ml, 0.500 mg/ml and 1 mg/ml) of OctaAmmonium-POSS to short SWCNT-COOH (1 mg/ml), there was a significant increase in metabolic activity in comparison to short SWCNT-COOH (1 mg/ml). This pattern appeared to be consistent across all three time intervals. With respect to long SWCNT-COOH, it was demonstrated that apart from the conjugation of 0.125 mg/ml OctaAmmonium-POSS to SWCNT-COOH, the remaining three concentrations were associated with

an increase in metabolic activity in the HT29 cell line at all three time intervals. In the MCF7 cell line, it was determined that the conjugation of OctaAmmonium-POSS to short SWCNT-COOH at 0.250 mg/ml, 0.5 mg/ml and 1.0 mg/ml results in a significant increase in metabolic activity when compared to that achieved using 0.125 mg/ml POSS. This trend was observed at all three time intervals. OctaAmmonium-POSS conjugation with long SWCNT-COOH (1 mg/ml) also demonstrated an increase in metabolic activity. Results showed that identical data to that observed with short OctaAmmonium-POSS conjugated SWCNT was observed. Excluding the 0.125 mg/ml concentration, results showed that the addition of all OctaAmmonium-POSS concentrations at all three time intervals resulted in a significant increase in metabolic activity. A DNA assay was conducted in all cases. With respect to HT29 cells, results showed that conjugation of all four concentrations of OctaAmmonium-POSS to short SWCNT significantly increased the metabolic activity of the HT29 at all three-time intervals.

With respect to conjugation of OctaAmmonium-POSS to long SWCNT-COOH, the trend was similar to that exhibited with OctaAmmonium-POSS conjugated to pristine long SWCNT, however no significant increase of DNA concentration was observed following conjugation with 0.125 mg/ml OctaAmmonium-POSS. In the case of MCF7 cells, the DNA assay produced results similar to the HT29 cancer cell line. Results show that following the addition of all OctaAmmonium-POSS concentrations to both the short and long SWCNT-COOH, DNA concentration significantly increases. It was demonstrated that this increase in the case of long SWCNT-COOH occurs with lower magnitude than seen in the short SWCNT-COOH.

In summary, the graphs depict the metabolic activity of HT29 and MCF7 cell lines at 24 hrs, 48 hrs, and 72 hrs time intervals. Different concentrations of OctaAmmonium-POSS added to both short and long length SWCNTs affected metabolic activity of the two cell lines. This was followed by an observation into the effect of increasing the functionalization time on the metabolic activity of the cell lines. The effect of conjugation of OctaAmmonium-POSS both covalently and non-covalently on the metabolic activity of the cell lines were also observed. The DNA concentration of both HT29 and MCF7 cell lines were investigated at 24 hrs, 48 hrs, and 72 hrs. The graph clearly demonstrates the effect of different concentrations of short and long pristine SWCNT on the concentration of DNA in both cell lines. The effect of different functionalization periods on DNA concentration was also observed.

4. Discussion

Both SWCNT-COOH and SWCNT-COOH conjugated to OctaAmmonium-POSS demonstrate a peak of 270 nm upon FTIR imaging. However, the peak for SWCNT-COOH conjugated with OctaAmmonium-POSS appears to be lower. The presence of OctaAmmonium-POSS is illustrated as a peak at SWCNT-COOH conjugated with OctaAmmonium POSS in its lower level in comparison to SWCNT-COOH alone. The presence of OctaAmmonium-POSS decreases the peak absorbance level. When OctaAmmonium-POSS is absent, a peak in the same area as with SWCNT-COOH is observed.

TEM images likewise demonstrate successful functionalization. The TEM image of pristine SWCNT appear as clustered tubes. The SWCNT ultrasonication was used for breaking up the clusturs and prevent the aggregation of the SWCNTs. The SWCNT were then treatedwith acid. the image clearly shows less clustered tubes with a black layer formed on the tube surface. It is assumed this layer is due to the presence of carboxylic acid. Further tests were conducted to prove this hypothesis.

The next test used to further prove the presence of the carboxylic acid on the SWCNT was confocal microscopy. Following the treatment of both short and long SWCNTs with acid, the materials were reacted with HPA lectin-FITC and viewed under the confocal microscope. Lectins are oligomeric proteins with carbohydrates binding sites.Due to the presence of protein, lectin consists of NH2 group. The COOH group on the surface of the SWCNT has affinity to form an amide bond with the NH2 group of the lectin. In this experiment, the lectin was attached to the FITC. As a result, in any area that fluorescence colour was observed under the confocal microscopy the presence of lectin can be confirmed.

After adding OctaAmmonium-POSS to the SWCNT-COOH, TEM images illustrate the presence of black dots and a less clustered SWCNT. The black dots are due to the presence of OctaAmmonium-POSS. Following characterization of SWCNT, the HT29 and MCF7 cell lines were exposed to differently functionalized SWCNTs.The one-way ANOVA test was employed to evaluate the significanceof the difference between groups.With respect to both HT29 and MCF7 cell lines, results have illustrated both an increase in cell metabolism and DNA concentration over a 72 hr period. It was seen that this increase is greater in the case of HT29 in comparison to MCF7, which clearly demonstrates greater metabolic activity and cell proliferation of the HT29 cell line in comparison to MCF7. In both cases, during the 48 hr-72 hr interval, the DNA concentration and metabolic activity increase more than it does between 24 hr- 48 hr, indicating that major cell proliferation occurs after the second day.

This study also aimed to investigate the effect of OctaAmmonium-POSS as a functionalization agent to reduce the toxicity of SWCNT. Initially, the effect of various concentrations of OctaAmmonium-POSS on cellular metabolism and any change in DNA concentration was observed. The effect of exposure of OctaAmmonium-POSS on cellular metabolism and DNA concentration were investigated in the next stage of the experiment. Results showed that no significant alteration occurred at a cellular metabolic level or DNA concentration after OctaAmmonium-POSS concentration was increased. This indicates thatincreasing the dose of POSS has no effect on the cell lines. This is because the POSS is biologically safe and has no cytotoxic characteristics. For this reason, it was concluded that OctaAmmonium-POSS can be used as a functionalizing agent, conjugated to SWCNTs so as to

increase its dispersability and reduce toxicity. Functionalization of SWCNT molecules with OctaAmmonium-POSS can substantially enhance the solubility and processability of the nanocomposite. Evidence of biocompatibility and the amphiphilic properties of the OctaAmmonium-POSS nanocomposite have already prompted researchers to patent this molecule for use at the vascular interface in devices such as stents [20,21].

POSS has been used extensively as filler in manufacturing the OctaAmmonium-POSS nanocomposite polymer for surgical application, as well as for drug delivery [22,23,24]. By functionalisation of SWCNT with OctaAmmonium-POSS, nanoparticle toxicity is reduced [25]. The OctaAmmonium-POSS nanocomposite has likewise been used to coat quantum dots (QDs) to reduce their toxicity [26,27]. Moreover, POSS is known as a material resistant to degradation and has anti-calcification effects, superior biocompatibility,and is currently being assessed for use in medical implants [28].

An aim of the study was to conjugate the carboxy (-COOH) functional group through the covalent bond on the surface of the SWCNT rather than using a weak bond such as non-covalent or Van der Waals bond (Figure 11) It has been indicated by different researchers that the attachment of the non-covalent bond would not be suitable as the functional group will likely dissociate from the SWNCT's surface[28,29,30].

The OctaAmmonium-POSS is another type of POSS molecule, which consists of eight NH2 groups [31]. The presence of eight –NH2 group allows the OctaAmmonium-POSS to be conjugated through the covalent bonds to the –COOH group on the surface of the SWCNT [19]. For this reason, the OctaAmmonium-POSS was used for further conjugation to SWCNT-COOH, increasing SWCNT dispersion in biological systems, due to its amphiphilic nature.

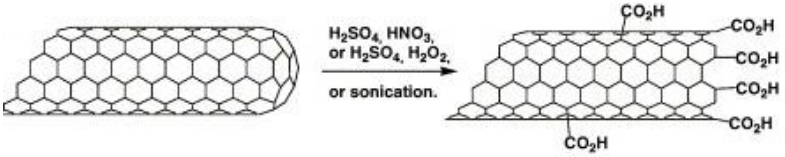

Figure 11: Schematic diagram of SWCNT functionalization process [32].

Prior to considering the effect of functionalization on the toxicity of SWCNT, the effect of adding pristine SWCNT on cell metabolism and DNA concentration was assessed in line with whether adjusting the length would affect the toxicity of the SWCNT. It was determined that addition of all four concentrations of pristine SWCNT significantly reduced the metabolism of both cell lines. Results showed that regardless of the size of the SWCNT, by increasing the pristine SWCNT's concentration from 0.125 mg/ml to 1.0 mg/ml, cell metabolic activity and DNA concentrations significantly decrease. The dose dependent effect of the SWCNT means by increasing theconcentrationthere will be more SWCNT that can reach the nucleus following exposure to the cancer cell, further interrupting cell division. This trend was observed with the MCF7 cell line. However, SWCNTs decreased MCF7 cellular metabolism to a greater extent than observed in HT29 cells. This was the case with both short and long SWCNT, a phenomenon which might be accounted for due to less cell division taking place in MCF7 compared to HT29 cells.

Increasing the size of the SWCNT is one factor which has been shown to increase its toxicity [33]. It is known that once a particle hits the cell membrane, it will be phagocytosed However, round nanoparticles are engulfed and internalised more efficiently than those that are rod shaped, such as SWCNT [34,35]. Various research groups have shown that smaller nanoparticles are engulfed more thoroughly by the cell membrane than larger ones Large rod shaped nanoparticles such as SWCNT could not be processed via phagocytosis as easily as small SWCNT [36,37] Also demonstrated by various researchers was that large pristine nanoparticles could generally result in the rupture of the cell membrane once engulfing begins. A short functionalization period will result in coating of only a portion of the SWCNT and those not coated would normally rupture the cell membrane with their sharp ends. The chance of rupture with the short SWCNT is lower than with long SWCNTs.

In HT29 and MCF7 cells, following the addition of various concentrations of long SWCNT, the cell's metabolic activity and DNA concentration significantly decrease in comparison to the addition of short SWCNT. This trend was observed at all three time intervals. For example, with respect to cell line MCF7 and long SWCNT, DNA concentration results show that at concentrations 0.250 mg/ml, 0.5 mg/ml and 1 mg/ml, DNA concentration is decreased significantly more than with 0.125 mg/ml. However, in the case of short SWCNT, concentrations 0.5 mg/ml and 1.00 mg/ml of SWCNT appeared more toxic than concentrations 0.125 mg and 0.25 mg/ml.

Future experimental aims include further investigation of the influence of functionalization on the reduction of SWCNT toxicity. Generally, pristine SWCNTs are not biocompatible [38]. The pristine SWCNTs are clustered and networked materials. Functionalization is a process in which the SWCNT is treated with different chemical materials, including combinations of HNO3 and H2SO4, OctaAmmonium-POSS and PEG, resulting in dissociation of Van der Waals forces between the nanotube [39]. As a result, the tubes will be less clustered and less toxic Moreover, coating the surface of the SWCNT will increase its hydrophilicity [33]. During this process, the sharp end of the

SWCNT, which normally results in cellular rupture, will disappear. In our experiment, it was demonstrated that longer duration of SWCNT exposure to HNO3:H2SO4 correlated to higher cellular DNA concentration and metabolic activity. This is because the functionalization process will result in dissociation of the Van der Waals forces between the tubes and as a result, the SWCNT will appear less clustered. A longer functionalization period is linked to more SWCNT dissociation and release from clustered arrangements. This breakdown of cluster arrangement would increase the probability of better SWCNT engulfment. A longer functionalization period of SWCNT could also result in the loss of any sharp ends present on the SWCNT, which may normally result in the rupture of the cell membrane. Many impurities normally used as catalytic agents during SWCNT synthesis can also be removed following a successful functionalization process. These impurities are known to cause oxidative stress to the cells by generating reactive oxygen species. The impurities can be removed following a successful functionalization process [40].

Other factors permitted through the functionalization process include SWCNT diameter expansion [41]. Once the SWCNT is functionalized, it becomes thicker, leading to a lower chance of SWCNT entering the nucleus and interrupting normal DNA function. Functionalization will result in opening the networked and clustered arrangement of the SWCNT. It changes their properties, making them hydrophilic, which allows them to better interact with the cell membrane, and to be potentially used as tools for other applications such as drug delivery and thermal treatment of cancer [42,43]. It has been reported that the functionalization of long SWCNT has also resulted in reduction of SWCNT toxicity, but with a lower magnitude than noted with short SWCNT. This is because longer SWCNTs result in further interruption of engulfment.

In the case of the HT29 cell line, it was shown that shorter SWCNTs need a lower treatment period with acid (minimum 2 hrs) than the longer SWCNT (minimum 5 hrs) in order to increase HT29 metabolic activity. Long SWCNT requires a longer treatment time with acid in order to become less clustered (there is more interaction between the tubes) than the short SWCNTs. Considering smaller SWCNTs require less treatment time with acid compared to longer SWCNTs for their toxicity to be reduced, it is understood they are less toxic.

Comparing the two cell lines, MCF7 demonstrated that in the case of the short SWCNT, following a 5 hr functionalization period, cellular metabolic activity significantly improved. In the case of HT29, functionalization of short SWCNT for as little as 2 hr provided a significant increase in the metabolic activity of the HT29 cell line. This could be due to various reasons including that MCF7 cell division is slower than HT29 cell division and so there are less cells available. This means that in the case of MCF7, as there is a higher ratio of SWCNT to cell, even a small number of non-functionalized SWCNTs will result in a massive reduction in normal cellular activity. The other reason could relate to the uptake mechanism and the rate of uptake of the different cell lines. A 2 hr functionalization process of SWCNT results in a decreased SWCNT cluster arrangement and formation of a thick layer of carboxylic acid on the SWCNT surface. In the case of MCF7, the cell's metabolic activity decreases to the same level as pristine SWCNT following the addition of SWCNT functionalized for as long as 2 hr. This demonstrates, that with respect to MCF7, the functionalized SWCNT can still diffuse through the cell membrane and reach the nucleus. However, in the case of HT29, following a 2 hr functionalization of SWCNT, the metabolic activity significantly increases in comparison to pristine SWCNT, meaning less SWCNT can diffuse into the HT29 cell line following a 2 hr functionalization. The smaller pores of HT29 cell membrane or its slower uptake mechanism in comparison to the MCF7 cell line may account for this phenomenon. As mentioned earlier, this experiment was designed to investigate the best functionalization technique to reduce toxicity of SWCNT. Results from treating the SWCNT with acid clearly illustrate that with short and long SWCNT, the longer the SWCNT stays in contact with the acid, the greater the metabolic activity and DNA concentration of the cell observed in the case of both short and long SWCNT.

Future investigations may aim to study the effect of OctaAmmonium-POSS as a material for reducing SWCNT toxicity. Initially, the aim of the experiment was to investigate the effect of OctaAmmonium-POSS non-covalently conjugated to the surface of the SWCNT. Doing so, a pattern cannot be observed with respect to the increase of cell's metabolic activity and the DNA concentrations; however, in most cases, the conjugation of OctaAmmonium-POSS has demonstrated increased cellular metabolic activity and DNA concentration in both cell lines. The main reasons that the conjugation of OctaAmmonium-POSS is not as effective as treating the SWCNT with acid, which results in conjugation of SWCNT with carboxylic acid group, could be due to different factors. One factor could be the weak non-covalent bonding formed between the SWCNT's surface and the OctaAmmonium-POSS, which could result in easy dissociation of the OctaAmmonium-POSS from the SWCNT surface. A second factor includes non-uniform distribution of OctaAmmonium-POSS on the SWCNT's surface. With respect to conjugating the OctaAmmonium-POSS onto the SWCNT already functionalized with acid, at almost all OctaAmmonium-POSS concentrations, it was seen that following its conjugation to the SWCNT-COOH, cellular metabolism and DNA concentration significantly improve. This could be due to the uniform distribution of OctaAmmonium-POSS. The presence of carboxylic acid on the SWCNT's surface could provide for a wider area for the OctaAmmonium-POSS to attach. Additionally, the covalent bond formed between the OctaAmmonium-POSS and the SWCNT-COOH surface is much stronger than the non-covalent attachment of the OctaAmmonium-POSS. As a result, it is less likely for the OctaAmmonium-POSS to disassociate from the SWCNT's surface.

5. Conclusion

Application of different functional groups have been investigated by

diverse research groups. This experiment has indicated significant reduction of SWCNT's toxicity following its functionalization with carboxylic acid and OctaAmmonium-POSS. The first part of the experiment has been investigated on characterisation of SWCNT. Different characterisation techniques such as TEM, Confocal microscopy, FTIR and UV-VIS spectroscopy have proved the successful attachment of the carboxylic acid and Octa-Ammonium-POSS to the surface of the SWCNT. From this it can be concluded that carboxylic acid can act as as a strong platform for further attachment of Oca-Ammonium-POSS to the surface of the SWCNT. Subsequently, the DNA assay and Alamar blue tests have been used to investigate the effect of functionalization agent and duration of functionalization exposure on different lengths of the SWCNT.

This study analysed toxicity levels of 2 sizes of SWCNTs on 2 different cell types by altering various SWCNT properties. From the study it can be concluded that Octa-ammonium-Poss is a good candidate for functionliazation of SWCNT. However the use of carboxylic acid as a platform for the attachment of the Octa-ammonium poss to the surface of the SWCNT would siginifacntly improve it's action. It can aslo be concluded that the long SWCNT is more toxic than the short SWCNT.short SWCNTs that were functionalized for a longer duration, which resulted in coating of the most surface area of the SWCNT, were associated with less toxicity when compared to shorter functionalization times. Extent of toxicity was best represented on the MCF7 cell line, likely because of its increased proliferative potential in comparison to the HT29 cell line. By conjugating OctaAmmonium-POSS to the carboxylated SWCNT, cell metabolic activity was comparatively improved, indicating that such a conjugating agent is likely to be beneficial if it were to be used at the level of a clinical trial. By devising new methods to minimise CNT toxicity, SWCNTs can have widespread translational potential. To achieve such success, future studies should aim to better understand the intricacies of carbon nanotubes and how to manipulate them to be clinically advantageous.

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