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Assessment of cytotoxicity and artificial peroxidase activity of multi wall Carbon Nano tubes

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Abstract

Multi Wall Carbon Nano Tubes (MWCNTs) have enormous potential in biomedical researches. Structural and size-dependent properties such as length, diameter have notable effects on cytotoxicity which represent major challenge in this field. The aim of this research is evaluation of peroxidase mimic activity of MWCNTs as Nanozyme via Hydroquinone-H2O2 coupled colorimetric system cell viability which was tested on HU02 Human Fibroblast cells using different concentrations of MWCNTs by MTT assay. Results showed MWCNTs had weak catalytic efficiency in aquatic condition and Nanozyme activity of MWCNTs followed Michaelis–Menten curve as peroxidase mimic. Also experimental evidences demonstrated high potency of MWCNTs toxicity so that uptake of MWCNTs concentrations were critical factor in term of dose dependent inhibition of Human Fibroblast cells.

Keywords: Artificial Peroxidase, MWCNTs, Nanozyme, Hydroquinone

Introduction

Examining the toxicity of Nanostructures is very important in many purposes, especially biological applications such as drug delivery and biosafety. Generally, the toxic effects of Nanoparticles increase with their size shrinking which lead to increase in the surface-to-volume ratio. Also, due to the presence of various pores in the membrane of cells, that are larger in micrometers and Nanometers, it is possible to enter Nanomaterials into the cells and subsequently cellular destruction will be done. Researches have shown that the shape, dimensions and synthesis method of Nanoparticles are effective in allowing them to enter the cells and their ability to excrete them from the body and their toxicity. Because of large volumes of human body is content of water, the solubility of Nanoparticles and drugs in water affects cytotoxicity. (Jiang *et al.*, 2009) Moreover, the purity of Nanostructures and their lack of contamination are very important for the investigation of cytotoxic effects. For example, carbon Nanotubes produced by methods that use the iron as a catalyst are often contaminated with this. Studies have shown that iron removal from carbon Nanotubes reduces their toxicity (Kobayashi *et al.*, 2017; Podila and Brown, 2014) Therefore, it should be noted that the Nano toxicity is sometimes caused or aggravated by impurities.

The mechanism of cytotoxicity of some Nanoparticles on living cells is mainly osmosis and endocytosis in to the target cell which due to mitochondrial dysfunction, chromosomal abnormalities and inhibition of DNA replication and subsequently inhibition of cell proliferation. Nanozymes or Nano material enzyme mimics are part of the new Nano scale tools that are introduced as new diagnostic and therapeutic instruments and it seems to be necessary, to study of probable toxic effects of Nanozymes as Bio inspiration structures in term of their applications and sensitivities of living cells (Gao *et al.*, 2007; Wei, 2013). The aim of this research is evaluation of peroxidase mimic activity of Muti Wall Carbon Nano Tubes (MWCNTs) as carbon based Nanozyme and assessment of invivo toxicity

Material and Methods

Cell Culture and MTT assay

HU02 Human Fibroblast cells were seeded in 96-well plates at a density of 7 x 10^3 viable cells per well. Then, cells treated with (0, 0.005, 0.001, 0.0025, 0.005, and 0.0075) $\mu g.\mu L^{-1}$ of dispersed Carbon Nano tubes powders and the plate was incubated for 24h. Then, dispose of the supernatant to each well and the MTT added to the wells and placed it in CO_2 incubator for 4 hours at 37 ^{0}C. MTT assay was carried out using the ISO1099-3 protocol in dark since MTT reagent is light sensitive compound during incubation

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time, MTT was reduced by the succinate dehydrogenase mitochondria respiratory chain and absorbance of Formazan as artificial chromogenic (tetrazolium salt) were detected after 24 hour.

Artificial Peroxidase Activity Assay of MWCNTs Structures as Nanozyme

To evaluate peroxidase mimic behavior of MWCNTs, Coupled Chromogenic system was carried out based on HQ-H_2O_2 colorimetric assay. The kinetics Mode of Varian Cary 100 Bio Spectroscopy was used to measure v_{max}, K_m and K_{cat} parameters using 5µg/ml MWCNTs as Artificial Peroxidase Nanozyme and substrates concentrations were (0, 20, 40, 60, 80,100) mM in neutral aquatic condition.

Results and Discussion

MTT Assay

Results showed the enzymatically breakage of tetrazolium ring causes the formation of a formazan purple crystal through NADH/NADPH reduction. Basically, Formazan is artificial chromogenic reduced tetrazolium salt which is detectable by a microscope. Finally, the Purple color produced by the number of cells that were metabolically active is directly related. (Figure.1) Insoluble Formazan crystals were treated by DMSO before colorimetric assay for increase solubility. The absorbance of the solution was measured at 570 nm wavelength and the number of cells were calculated using the standard curve. Actually the greater activity of this enzyme, the greater the amount of color produced and consequently the absorption of light will be greater and therefore indirectly gives an estimate of the living cells (Figure.2a) (Figure.2b) Potency of Inhibitory of MWCNTs was measured by IC_{50} parameter which was calculated as 0.000206 µg.µL^{-1} and Inhibitory % curve and color change of micro plate wells can be seen in (Figure 3a) and (Figure 3b).

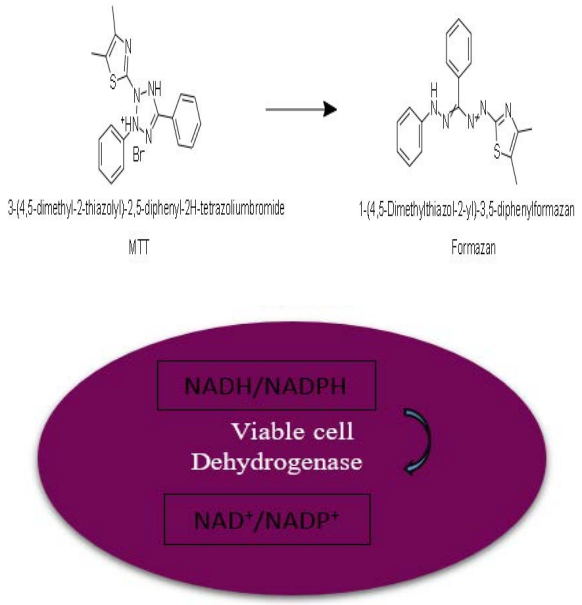
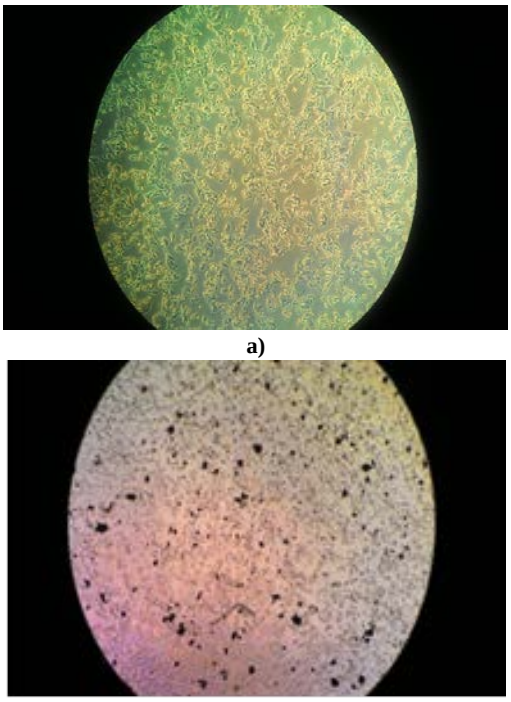


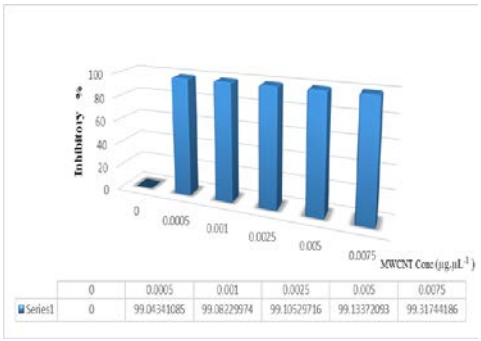
Fig. 1 MTT reaction and formation of Formazan



a)

b)

Fig 2. a) Human Fibroblast Cells (HU02) before MWCNTs Treatment. b) Human Fibroblast Cells (HU02) after Treated with selected Doses of MWCNTs



a)

b)

Fig. 3 a) Inhibitory Curve of MTT Assay b) MTT Test Micro plate

Artificial Activity of MWCNTs as Nanozyme

Peroxidase mimic activity of MWCNTs was measured by Michaelis -Menten equation so that the absorption changes was plotted as a function of time and double-reciprocal kinetic curve was drawn to demonstrate it. Velocity of Benzoquinone formation reaction as final product of catalytic reactions was distinctly measured under constant amounts of HQ as Chromogenic substrate and the change in the concentrations of the H2O2 and in second Michaelis-Menten curve, concentration of a H2O2 was constant and HQ concentration as second substrate was differed in distinct steady- state curve. Results showed Peroxidase mimic activity of MWCNTs as Nanozyme was occurred with hyperbolic curve (Figure 4) and steady- state kinetics parameters can be seen in (table 1).

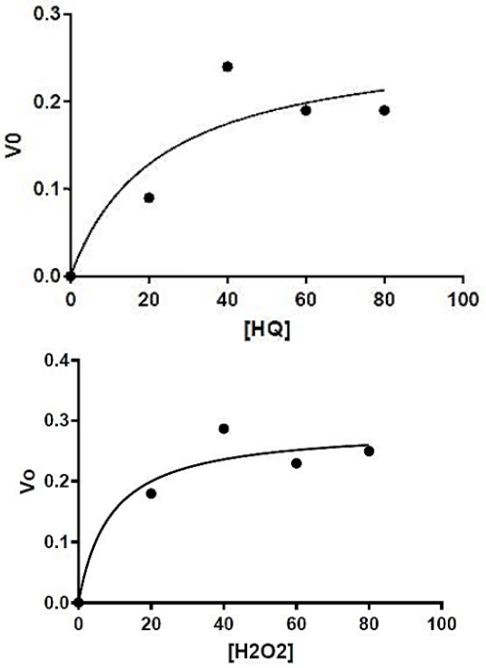


Fig. 4 Michaelis –Menten Curves of Peroxidase Mimic Activity of MWCNTs as Nanozyme

Table-1 Steady –State Kinetic Parameters of MWCNTs Peroxidase Mimic Activity in Aquatic Condition

Substrate	V_{max}	K_m (mM)	k_{cat} (s^{-1})
HQ	0.27	22.58	0.65
H2O2	0.289	8.92	0.69

Results showed Nanozyme kinetics parameters such as v_{max}, K_m and K_{cat} had lower values in comparison with natural peroxidase which indicates low catalytic efficiency. Researches have been demonstrated that pure MWCNTs are Carcinogenic agents and may be cause lung disorders and may attenuate if the fiber length is shorter. (Kobayashi *et al.*, 2017) also Khalid and colleagues showed toxicity of CNTs is dependent on geometry, composition and surface functionalization and it was suggested that wall-functionalized CNTs are safe to animal cells, (Khalid. P *et al.*, 2016). moreover, peroxidase-like catalytic activities of Carbon Nano Dots(C-Dots) was demonstrated by (Garg, Bisht, 2016) Carbon Nano Tubes have been defined as Carbon based Nanozymes are easy in rational design and their peroxidase like activity can be effectively tuned depending on physico-chemical factors such as pH, temperature, size and controlling the ratio of these parameters can make them more efficient Nano scale biocatalysts (Garg *et al.*, 2015; Wang *et al.*, 2016).

Conclusion

Cytotoxicity and Nanozyme peroxidase activity of MWCNTs have been investigated in this research. Inhibitory % curve and IC_{50} showed high toxicity potency of MWCNTs in Fibroblastic Cells (HU02). According to calculated Kinetics parameters, MWCNTs as Peroxidase mimic has limit catalytic efficiency in aquatic condition .The main reason for the restrictions seems to be the hydrophobicity of these carbon Nanostructures and the lack of complete recognition of the catalytic mechanism which lead to make challenges of understanding of their function .so using MWCNTs as diagnostic and therapeutic tools in intracellular conditions require specific types of functionalization which can greatly minimize their toxicity and inflammatory and MWCNTs surface layer engineering and chirality manipulations of surface atoms can provide conditions to create them more specific Nanozymes and kinetically perfect Nano catalysts with less damages and wider applications.

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