SECM Investigation on the Effect of ZnO Nanoparticles on Nasopharyngeal Cancer Cell Respiration

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ABSTRACT

The cell respiration through glycolytic pathway in cancer cells leads to the accumulation of a high level of NADH, which is normally channeled to the electron transport chain as the energy fuel in respiration-competent cells. The increase in NADH leads to drug resistance and survival advantage of cancer cells. For developing new nanodrugs for cancer therapy the effect on respiration need to be investigated in a quantitative way. Herein, the cell respiration process in human nasopharyngeal cancer cells is investigated with the help of Scanning Electrochemical Microscope (SECM). At the presence of ZnO nanoparticles the respiration process is substantially diminished and the rate of this "respiration blockade" increases with increasing particle concentration. This offers new approaches for treating cancer.

Keywords: cell respiration control, ZnO nanoparticles, Scanning Electrochemical Microscopy, nasopharyngeal cancer cells

1 INTRODUCTION

In cancer cells and other rapidly proliferating cells, energy is produced predominantly by glycolysis (Warburg effect) [1] suggesting that a shift away from that mechanism might suppress tumor growth. The metabolic alteration in energy production has been observed in many cancer types, including solid tumors and leukemia. It is now recognized that the Warburg effect represents a prominent metabolic characteristic of malignant cells [2,3,4].The exact mechanisms for these metabolic alterations need to be elucidated, malfunction of mitochondrial respiration or "respiration injury" due, in part; to mitochondrial DNA (mtDNA) mutations/deletions is thought to be an important contributing factor. Certain mutations to these mtDNA may cause defect in respiratory chain, forcing the cells to increase glycolysis to maintain their ATP supply. Cancerous cells require constant sufficient supply of ATP for their active metabolism environmental factors, especially hypoxic conditions in the tumor tissue microenvironment, may also force cancer cells to use glycolytic pathways to generate ATP to meet their energy supply [5]. Under hypoxic conditions, cancer cells have to use the "energy-inefficient" glycolytic pathway to generate ATP, leading to accumulation of a high level of NADH, which is normally channeled to the electron transport chain as the energy fuel in respiration-competent cells [2-5]. Drugs that can produce shift in these energy production pathways and the NADH transport could offer new approaches for treating cancer. The reaction involving is

$$\text{Glucose} + NAD^{+} + 2P + 2ADP \longrightarrow 2 \text{ pyruvate} + 2NADH + 2ATP + 2H^{+} + 2H_2O.$$

The electron transport chain accomplishes harnessing the energy released by this transfer to the pumping of protons ($\mathbf{H^{+}}$) from the matrix to the intermembrane space.

It has been proved that the integration of nanotechnology and biology provides new opportunity for the development of novel materials in the nanometer size range that may be applied to many potential applications in biological science and clinical medicine [6-8]. When reduced to the nanoscale realm, unique size-dependent properties of nanomaterials, including nanoparticles (NP), are manifested [9]. The major properties of nanomaterials that make them differ from their bulk counterparts include an increase in relative surface area and quantum effects, which can affect chemical reactivity and other physical and chemical properties [7-9]. NP are of a few of nm and the cells are of the size of few microns, so NP can enter inside the cells and can readily interact with biomolecules on both the cell surface and within the cell and potentially affect cellular responses in a dynamic and selective manner. Materials that exploit these characteristics become attractive for use in novel biomedical applications [10-13]. Various types of nanomaterials including quantum dots and metal oxide nanoparticles have been shown to induce the generation of reactive oxygen species resulting in modification and damage of cellular components [13]. However, to the best of our knowledge, no respiration studies reported so far, to understand the effect of reactive oxygen species on the glycolytic pathway of cell respiration in cancer cells. Herein, this report; we investigate the possibility of controlling the cancer cell respiration with ZnO nanoparticles. The ZnO nanoparticles used in this study can induce oxidative stress and hence influence the production of NADH. This paper describes the measurement of the cell respiration in human nasopharyngeal cancer cells with the help of a commercially available SECM [14-22]. The real time investigation of the cell respiration is carried out in the presence of ZnO nanoparticle.

2.1 EXPERIMENTAL

ZnO nanoparticles are synthesized in aqueous solution at near-neutral pH and low temperature. Dynamic Light

Scattering studies shows diameter of the ZnO nanoparticles in the range of 50-120 nm (Figure 1).

The crystalline structure of ZnO nanoparticles was measured with X-ray diffraction pattern. The XRD pattern of the nanoparticle is shown in the support figure 2. It was clear that all the ZnO NPs gave the similar crystal diffraction pattern. The peaks could be indexed to the hexagonal wurtzite ZnO with lattice constants in agreement with the values in the standard database.

The absorption spectrum of the ZnO nanoparticles prepared was shown in support figure 3. UV scanning was performed from 250 to 400 nm. The peak maximum obtained at 370nm**,** which corresponds to the maximum value of molar absorptivity

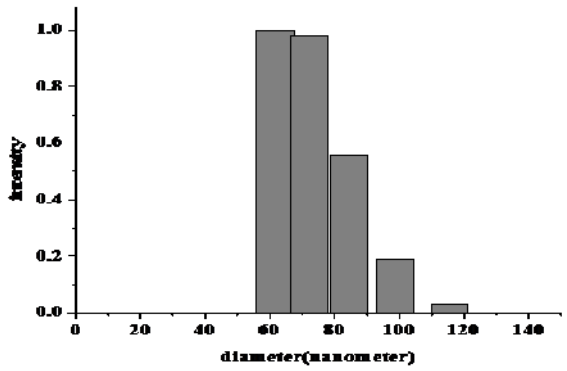

Figure1: Dynamic Light Scattering spectra of ZnO nanoparticles

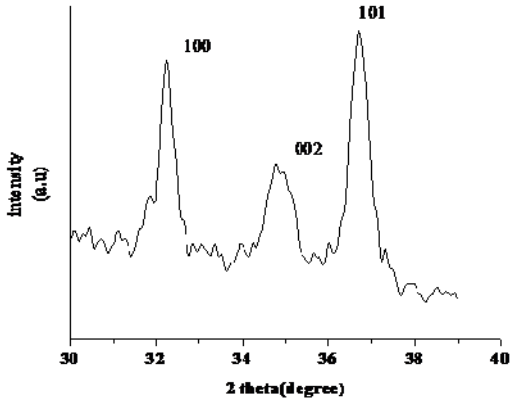

Figure 2: XRD pattern of ZnO Nanoparticle

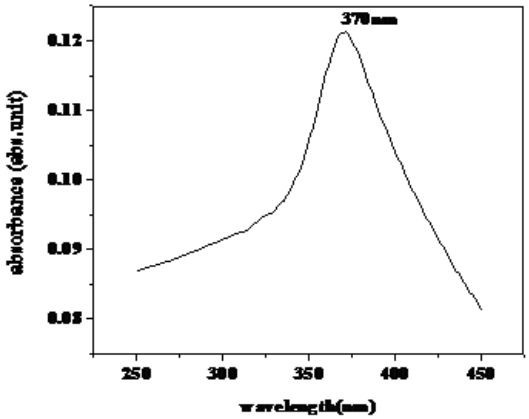

Figure 3: absorption spectra of ZnO nanoparticle

Cytotoxicity of biofriendly ZnO nanoparticles synthesized was determined by 3-(4, 5- dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) assay. It is a colorimetric test based on the selective ability of viable cells to reduce the tetrazolium component of MTT in to purple colored formazan crystals. The assay was done on three different concentrations 2mM, 6mM and 10mM of ZnO nanoparticles at varying time intervals. Triton X-100 was taken as the positive control for cytotoxicity and normally cultured cells without nanoparticles as negative control. The following figures represents plot of concentration versus optical density (at 570nm) and the experiments were done at time intervals of 4, 6, 8,10 and 12 hours.

The cytotoxicity effects of biofriendly ZnO nanoparticles to human nasopharyngeal cancer cells were studied using MTT method (figure 5). The results showed that ZnO nanoparticles kill the cancerous cells depending on time as well as concentration basis. Even 4-hour incubation with the ZnO nanoparticles showed nearly half the populations diminish. More cell death was observed as time increases. Several types of nanomaterials including quantum dots and metal oxide nanoparticles have been shown to induce the generation of reactive oxygen species resulting in modification and damage of cellular proteins, DNA and lipids which can lead to cell death. Here a concentration and time dependent increase in ROS production in the oral cancer cells following ZnO nanoparticle exposure that finally leading to cell death were observed.

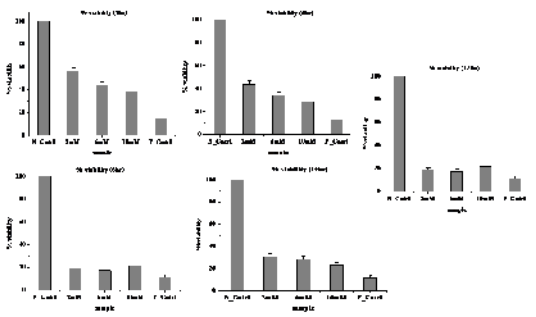

Figure 5: Cytotoxicity of ZnO nanoparticle

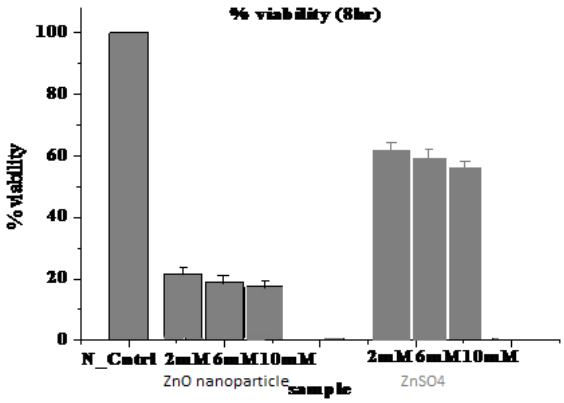

Figure 6: comparitive cytotoxicity study of ZnO Nanopaticle with $ZnSO_4$

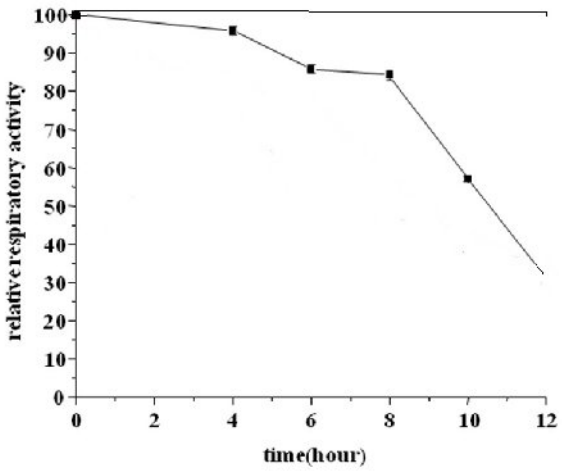

Figure 7: Respirative activity of cancer cell with respect to time

A comparative study has been carried out with the ZnO nano particle and ZnSO4 is depicted in figure 6

The cell respiration study is carried out in a commercial SCEM. From the graph (Figure 7) it was evident that the respiratory activity diminishes on time basis. As the incubation time increases oxygen reduction current decreases. The respiratory activity rate diminishes because oxygen concentration in the close environment of the cell may vary during oxidative stress processes due to its consumption.

2.2Conclusion

In Conclusion, insitu monitoring of the local respiratory activity of Human nasopharyngeal epithelial cancer (KB) cell during ZnO nanoparticles exposure is carried out with the help of SECM. ZnO nanoparticle can substantially influence the respiration process of nasopharyngeal epithelial cancer cells. The finding of cells toxicity, towards the potential disease causing cells, indicates a potential utility of ZnO NPs in the treatment of cancer and/or autoimmunity

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