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Physico-Chemical Characterization of Nickel Nanowires and Analysis of Its Anti-Cancer Properties Against Panc-1, Pancreatic Cancer Cells

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Abstract

We have investigated the cellular toxicity and anti-cancer effects of Ni NWs in one of the most aggressive human pancreatic ductal cancer (Panc-1) cell lines with the objective of development of a potential cell selective pancreatic cancer treatment strategy. Ni NWs were fabricated in a custom-made setup utilizing the electrodeposition method. Elemental analysis, crystallographic structure, and morphological properties of the synthesized Ni NWs were investigated using, X-Ray Diffraction (X-RD) and Scanning Electron Microscopy (SEM), respectively. Two commercially available and straight forward distinct cytotoxicity procedures including 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and trypan blue (TB) Assay were utilized to determine the qualitative and quantitative cytotoxicity and anti-cancer effects of Ni NWs. The elemental composition study and XRD demonstrated that the Ni NWs prepared in this study are pure Ni with face-centered cubic (fcc) lattice rather than Ni compounds. Both the MTT and TB assays, qualitatively and quantitatively confirmed the cytotoxic and anti-cancer effects of Ni NWs treated Panc-1 cells in vitro in both concentration and exposure-time dependent manners. We explored the molecular mechanisms associated with the biological pathway involved in Ni NWs induced toxicity against Panc-1 cells. Our results evidence that Ni NWs show strong candidacy for targeting cell selective pancreatic cancer therapy.

Keywords

Nickel Nanowires, Anti-Cancer Effects, Panc-1, Pancreatic Cancer

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1. Introduction

Cancer has been defined as a condition of abnormal, uncoordinated, and excessive cell division under which tissue is formed and growth persists [1]. If left untreated, cancer ultimately leads to death of the organism. Human cancer is actually comprised of more than 200 different diseases [1] that show great complexity [2]. When the pancreas cells become malignant, the resulting cancer is termed pancreatic adenocarcinoma [3]. The term “apoptosis” refers to the

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process of programmed cell death (PCD) in which a particular cell is an active participant in its own demise [4-5]. In cancerous cells, apoptosis induction is considered very helpful in management, therapy, and prevention of cancer [6-7]. Current therapeutics does not reflect the full heterogeneity of pancreatic cancers due to the resistance to apoptosis and does not suffice for a successful treatment. Therefore, synthesis of novel anticancer drugs continues to be a potential topic for pancreatic cancer research.

Cancer nanotechnology is an interdisciplinary area of research in applied biosciences, engineering, and medicine. Nickel (Ni), a basic element that is part of metalloproteins, is vital for living beings [8]. In small quantities, there is no report supporting that Ni has substantial carcinogenic effects on the human body. Furthermore, it has been reported that Ni has apoptogenic capabilities [9]. However, at high concentrations, Ni is carcinogenic with low mutational activity and does not induce apoptosis [9-10]. Nanostructural metallic wires such as Ni NWs are objects of high interest due to their physico-chemical characteristics. More specifically, the electrical, physical, chemical, magnetic, and catalytic properties of these nanoscale materials are different from their macro scale metallic counterparts, making them useful for various biomedical applications [11]. Cytotoxicity study of magnetic nanomaterials is a key consideration for biomedical applications. Very little is known about the cytotoxic and anti-cancer effects of nickel nanowires (Ni NWs) on mammalian cells and their interaction with proliferating cancer cells. Therefore, we studied the cytotoxic and anti-cancer effects of Ni NWs on Panc-1 cells using novel integrated bionanotechnological approaches to understand the corresponding biological pathways with the objective of developing a novel pancreatic cancer treatment therapy.

2. Experimental

2.1. Synthesis and Physicochemical Characterization of Ni NWs

An electrodeposition syringe method was used to synthesize Ni NWs [9]. The Ni NWs were characterized structurally and morphologically using XRD, and SEM. The surface morphology of Ni NWs was investigated using SEM analysis (FESEM-6330F, JEOL, Ltd, Tokyo, Japan). Crystallographic structure of the Ni NWs was determined by x-ray diffraction (XRD, Brucker D8 discover) with Cu-Kα radiation (λ=0.1542 nm) in θ-2θ mode. For these analyses, Ni NWs were dissolved in deionized water, then a few drops of the suspension were placed on native oxide p-Si (100) (resistivity 12-25 Ω-cm) substrates. Finally, the prepared samples were left to dry overnight.

2.2. Cell Culture

The Panc-1 cell cultures were maintained according to American Type Culture Collection (ATCC) protocol [5].

2.3. Phase Contrast Microscopy (PCM)

Morphological apoptogenic characteristics assessment of the Ni NWs induced Panc-1 cell was accomplished using phase contrast microscopy (PCM). The Zeiss inverted phase contrast 40 CFL microscope with Spot basic camera software was used. As a negative control, Panc-1 cells without Ni NWs treatment were used in all experiments. PCM also was used to confirm the Ni NWs internalization by Panc-1 cells.

2.4. Working Suspensions of Ni NWs, Administrations to the Cancer Cells, Internalizations, and Resulting Metabolic Activity

Deionized water was used to wash fabricated Ni NWs several times until a pH of 7.00 was attained. Then, a magnet was used to collect the Ni NWs and water was discarded. The Ni NWs were then resuspended in fresh DMEM cell culture medium to the final desired concentration of 3 Ni NWs for each cell [5]. This concentration was verified using a haemacytometer and a Zeiss inverted phase contrast 40 CFL microscope with Spot basic camera software. Before the experiments, the Ni NWs were sonicated for 5-10 minutes for well dispersion. In order to ensure a low cytotoxicity, the Ni NWs to cell ratio was kept low. Ni NWs internalization, qualitative metabolic activity, the internalization of Ni NWs through endocytosis, and the qualitative metabolic activity of Panc-1 cells were investigated.

2.5. Cell Culturing and Cytotoxicity Assay with MTT for Proliferation Study

A slightly modified ATCC protocol was followed to culture the Panc-1 cells [5]. Cytotoxicity was determined using MTT cell viability assay Kit. Absorbance was measured with a Biotek µQuant microplate reader at 570 nm with a reference OD at 630 nm.

2.6. Cell Viability by TB Exclusion Assay

Cell viability was determined utilizing a modified TB exclusion method. Cells were grown on 6-wells plates up to 70% confluency ($1X10^4$ cells /well) in a 2 ml medium. The cells were then washed with 1X PBS (10mM, pH 7.4) and treated with different concentration of Ni NWs for 24 hours. They were then washed with 1X PBS, trypsinized, and transferred to 1.5 ml micro-centrifuge tubes where they were spun down and resuspended in 0.5 ml 1X PBS to an approximate concentration of $1X10^5$ cells/ml. Following this, 0.1 ml of 0.4% TB stain was added to the cell suspension and was mixed thoroughly. Cells

were allowed to stand for 5 minutes at room temperature. The viable cells excluded dye due to maintaining an intact cell membrane, whereas non-viable cells stained blue as a result of their damaged membrane. Cells were immediately counted using a haemacytometer, under PCM. The experiments were performed at least three times in triplicates.

2.7. Statistical Analysis

Results of all experiments were depicted as mean ± SE. One way analysis of variance (ANOVA) was used to evaluate the significance of difference with a probability level of $p < 0.05$ being considered statistically different from the control.

3. Results and Discussions

3.1. Structural and Morphological Characterization of Synthesized Ni NWs

The Figure 1 shows the SEM image of Ni NWs which replicate the channels of the template used for fabrication. The diameter and length of these Ni NWs are found to be the same as the pore diameter and thickness of the template anodisc membrane. SEM measurements confirm that an individual Ni NW has an average diameter of 215 nm and 6.5 µm length.

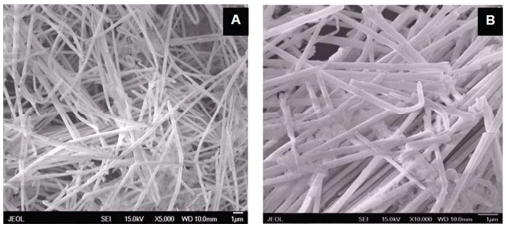

Figure 1. SEM of Ni NWs at magnification of A) 5,000X and B) 10,000X.

The corresponding XRD profile for the resultant Ni NWs is shown in Figure 2A. Two characteristic peaks for Ni NWs (2θ = 44.5 and 51.8 degrees) marked by their indices [(111) and (200)] were observed. It should be noted that the Ni (111) plane is dominant over Ni (200) plane, indicating that the Ni NWs are growing with a favored orientation of the (111) plane in the direction of growth. X-ray peaks correspond to face-centered cubic (fcc).

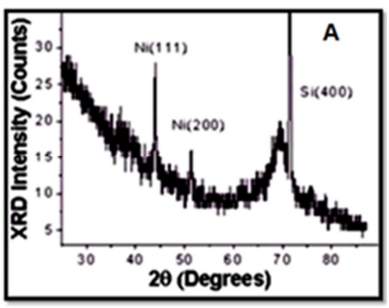

Figure 2. A) XRD profile of Ni NWs.

It should be noted that the presence of carbon, aluminum, and silicon is due to the pre-existing contamination from carbon and aluminum in the SEM chamber and using the silicon wafer as a substrate for Ni NWs. A small percent of oxygen has also been observed as a result of exposure of the Ni NWs to the environmental air. This reveals that the resultant Ni NWs are pure Ni metal.

3.2. Internalization of Ni NWs by Panc-1 Cells

The Figures 3A-B portray the internalization of Ni NWs by Panc-1 cells. The PCM micrograph in figure A shows Panc-1 cells incubate with Ni NWs for 24h. Figure 3B depicts the same micrograph shown in Figure 3A, but with focus on the Ni NWs.

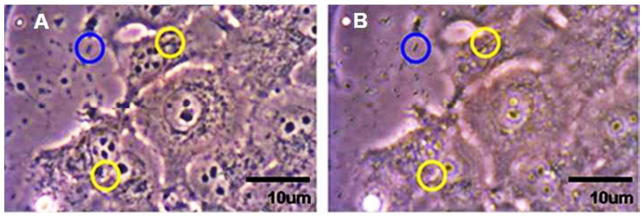

Figure 3. Ni NWs internalization by Panc-1 cell line through endocytosis.

These PCM images support that Ni NWs are easily internalized by Panc-1 cells due to their nanoscale size, thus validating their biocompatibility. The yellow circles indicate the Ni NWs position inside cells and blue circles identify the uninternalized Ni NWs on the empty flask surface.

3.3. Ni NWs Cytotoxicity in Panc-1 MTT Assay

The Figures 4A-F depict the MTT assay results that show the effect of Ni NWs as concentration dependent reduction of viability in Panc-1 cells. The quantitative MTT assay portrayed that the dose and exposure-time are two main factors in Ni NWs cytotoxicity in Panc-1 [9].

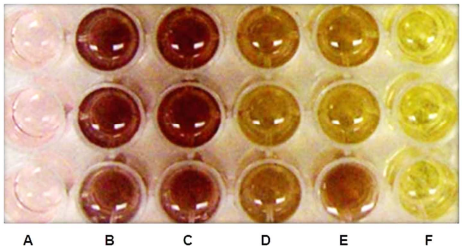

Figure 4. Qualitative cytotoxic MTT assay of Panc-1cells after 24 h of Ni NWs exposure. A) DMEM media only, B) cells and DMEM media without treatment (control), C, D, E, and F) with Ni NWs treatment of 5, 50,100 and 200 µl respectively for 24 hours.

3.4. TBE Assay Showing Ni NWs Induced Panc-1 Cytotoxicity

The Panc-1 cell viability was determined by TB exclusion

assay. Figure 5 shows the apoptogenic action of Ni NWs on viability of Panc-1 cells. The viability of the control cells was set to 100%. Viability relative to the control is presented in the subsequent image B. Panc-1 cells were treated with Ni NWs for 24 hours. Control cells were maintained in Dulbecco's Modifies Eagle Medium (DMEM) for the appropriate experimental time periods. Cell viability assay shows the role of Ni NWs in reduction of viability of Panc-1 cells. Results indicate that apoptotic cells with compromised membrane permeability detach from each other and thus stain blue with TB dye.

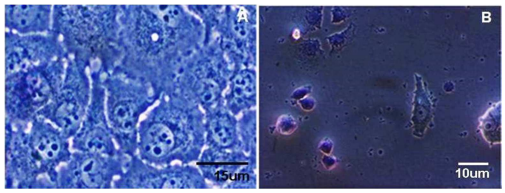

Figure 5. TB assay showing Panc-1 cytotoxicity by Ni NWs. A) Untreated cells (control), B) internalized Ni NWs showing initiation of membrane blebbing, cell shrinkage, and detachment from flask surface and each other. In Image B, TB stained dead cells blue as a result of their permeabilized membrane, while live and healthy cells did not stain by TB dye.

3.5. Characteristics of Apoptosis Depicting Anti-Cancer Effects

Morphologically detectable apoptosis characteristics can include changes in cell size, shape, plasma membrane permeability, and formation of apoptotic bodies [4-5, and 12-13]. Figures 6A-F show optical microscopic images verifying characteristics of apoptosis on Panc-1 cells treated with Ni NWs.

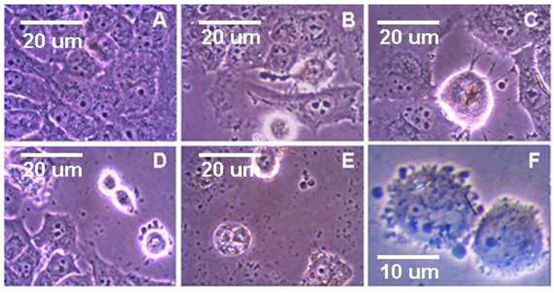

Figure 6. Morphological characteristics of apoptosis. A) Control, B) cell detachment from surface, shrinkage C) rounded cells and spike formation, D) membrane blebbing increases, chromatin condensation, E) finally apoptotic body formation in vitro, and F) magnified version showing Ni NWs induced rounding and membrane blebbing in Panc-1 cells.

4. Conclusion

From the experimental results, it is clearly evident that Ni NWs are capable of inducing dose and exposure-time dependent cytotoxicity in cancerous Panc-1 cell lines. This cytotoxicity appears in Panc-1 cells by way of a PCD mechanism referred to as apoptosis, which is a hallmark of cancer prevention [9]. This study validated the apoptogenic effects of Ni NWs on Panc-1 cells. Therefore, Ni NWs show excellent potential as novel anti-pancreatic cancer agents.

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