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Cytotoxic effects of nickel nanowires in human fibroblasts

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ABSTRACT

The increasing interest in the use of magnetic nanostructures for biomedical applications necessitates rigorous studies to be carried out in order to determine their potential toxicity. This work attempts to elucidate the cytotoxic effects of nickel nanowires (NWs) in human fibroblasts WI-38 by a colorimetric assay (MTT) under two different parameters: NW concentration and exposure time. This was complemented with TEM and confocal images to assess the NWs internalization and to identify any changes in the cell morphology. Ni NWs were fabricated by electrodeposition using porous alumina templates. Energy dispersive X-ray analysis, scanning electron microscopy and transmission electron microscopy imaging were used for NW characterization. The results showed decreased cell metabolic activity for incubation times longer than 24 h and no negative effects for exposure times shorter than that. The cytotoxicity effects for human fibroblasts were then compared with those reported for HCT 116 cells, and the findings point out that it is relevant to consider the cellular size. In addition, the present study compares the toxic effects of equivalent amounts of nickel in the form of its salt to those of NWs and shows that the NWs are more toxic than the salts. Internalized NWs were found in vesicles inside of the cells where their presence induced inflammation of the endoplasmic reticulum.

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1. Introduction

Advancements in fabrication and characterization techniques, as well as the development of new functionalization methods, have increased the interest in nanostructures to provide treatments for certain diseases, to improve our understanding of molecular biology and as tools for a wide range of medical applications. Nanoparticles (NPs) of spherical shape have been the most widely studied nanostructures for their use in drug delivery [14,43], diagnostic tools [15,25,42] or as contrast agents in medical imaging [41]. Cylindrical nanostructures such as NWs have been shown to provide improved performance for some biomedical applications. By adjusting the radius and the length, high aspect ratios are achievable. The composition along the wire can be precisely modulated to have multiple domains with different properties [38], allowing single or multiple functionalization using ligands. In addition, magnetic NWs have higher magnetic moment per unit of volume than NPs, allowing them to exert large forces and torques [16,34]. This last statement has been supported by two comprehensive studies that compared the performance of nickel (Ni) NWs and NPs for cell separation, with the results showing improved performance of NWs over NPs [12,17]. Additionally, the saturation magnetization of NWs is over an order of magnitude higher than that of NPs [12,13].

Recent studies have elucidated the potential application of silicon NWs and polycaprolactone NW surfaces in tissue engineering and bone regeneration by showing their capability to support adhesion and proliferation of cells with elongated morphologies [24,37]. Other studies [10] have demonstrated the advantages of NWs in magnetic biosensing, where biomolecules of interest are labeled for their detection and identification. By using NWs, detection can be achieved in the absence of an external magnetic field due to the large remanent magnetization. Moreover, Ni NWs can be internalized by cells such as immortalized fibroblasts [34], HeLa and human colorectal carcinoma cells (HCT116) [26], rat marrow stroma cells, osteoblast cells (MC3T3-E1) and osteosarcoma cells (UMR-106)

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[30]; a capability that can be exploited for diverse disease treatments such as cancer therapy [5].

Cytotoxicity studies of NWs have shown a dependence on factors like material and size. A high concentration of NWs might produce very low toxicity and good biocompatibility in some cell lines such as HeLa cells [34], 3T3 fibroblasts (CRL-1658) [11] and L929 mouse fibroblast [20], while being highly toxic for other cell lines including mesenchymal stem cells [35] and human colorectal carcinoma cells (HCT 116) [28]. These results together with other contributions have made Ni NWs very interesting for biomedical applications.

Information about the adverse effects of nanostructures on cells and tissues would enable the systematic design of suitable nanostructures for biomedical applications. Cytotoxicity studies provide vital information about the cellular mechanisms involved in nanostructure internalization and toxicity. NWs interact with the cell membrane, triggering their internalization mainly through endocytosis by enclosing them in membrane vesicles (e.g., late endosomes or lysosomes) to be degraded, recycled back to the extracellular environment, transported across cells, or to reach other organelles such as mitochondria [8,39]. According to some studies, the endocytosis effectiveness can be influenced by the length of the nanostructures, resulting in the activation of membrane receptors specific for a cellular uptake pathway [22,23]. Smaller nanostructures are internalized more efficiently than longer ones with similar surface characteristics [36]. Additionally, the surface charge seems to influence the amount of nanostructures taken up by cells. Non-phagocytic cells have shown a preference for cationic NPs while phagocytic cells take up more efficiently anionic NPs [1]. The toxic effects of nanostructures inside cells can be driven by their physicochemical properties, such as retention time inside the cell, surface properties, and toxic metabolites. The adverse effects include morphological and structural changes, genotoxicity, and biochemical alterations that trigger different cellular responses such as cell-cycle and proliferation irregularities, diminution of mitochondrial function, activation of cell signaling pathways and cell death [8]. It has been revealed that cells are able to break up iron NWs aggregates into smaller ones that were later degraded [32]; the NWs and the remains of the degradation were found either in vesicular compartments or directly dispersed in the cytosol. The resulting ionic forms due to NW degradation are able to interact with and alter the intracellular environment [11].

A review article about Ni carcinogenesis [22] concluded that Ni^{2+} ions released from NPs reached the nucleus in greater amounts than Ni^{2+} ions from water-soluble Ni(II) sulfate, resulting in a higher cytotoxicity. According to the authors, this was due to the relatively inefficient uptake mechanisms of water-soluble Ni(II) compounds (i.e. diffusion, iron/calcium channels). Some other studies have shown that the toxicity of a material in a certain form (e.g. NW) cannot be inferred from the toxicity of the same material in a different shape (e.g. NP) [34]. A good example is asbestos, a "benign" silicate that is highly toxic in its fibrous form [34]. Therefore, cytotoxicity studies must be carried out for specific materials in specific geometries and for specific cell lines.

This work aims to study the viability of human fibroblasts when grown in the presence of Ni NWs at different concentrations and for incubation times of 24, 48 and 72 h. The results are compared to those obtained for Ni salt to get further insight into the cytotoxic mechanisms. In addition, the NW internalization assessment is approached using TEM and confocal images that as well elucidate any changes in the fibroblasts. To our knowledge, this is the first systematic cytotoxicity study done in human fibroblasts WI-38 using ferromagnetic NWs, where the toxic effects of equivalent amounts of Ni in its ionic form and in its NW form are compared.

2. Materials and methods

2.1. Fabrication and characterization of Ni NWs

Ni NWs were fabricated by electrodeposition in highly ordered porous aluminum oxide (PAO) templates. High purity Aluminum (Al) disks (99.999% purity, Goodfellow) of 2.5 cm in diameter and 0.5 mm in thickness were subjected to cleaning and electropolishing to remove contaminants from the substrate surface. After that, a two-step anodization process using 0.3 M oxalic acid was used to obtain a highly ordered PAO template. The first anodization was carried out by applying a constant voltage of 40 V to the Al disk for 24 h, maintaining the temperature within 2–4 °C and keeping the solution under constant stirring (~200 rpm). The result was an aluminum oxide (alumina) layer on the Al surface with disordered pores and ordered domains on the Al substrate. The alumina layer was chemically removed by using a chromium-based solution consisting of 0.2 M CrO_3 and 0.4 M H_3PO_4 in DI water at 30 °C for 12 h. The second anodization was carried out using the same setup and under the same conditions of the first anodization process, with the exception of the anodization time being 20 h. The result was a membrane with highly ordered nanopores and a narrow diameter distribution. To prepare the sample for the electrodeposition process, the aluminum on the backside of the Al disk was removed using a copper solution 1.67% (w/v) of $CuCl_2{\cdot}2H_2O$ and 49.16% (v/v) of HCl. Afterwards, the pores were opened using 5% (v/v) phosphoric acid solution and a gold layer (~200 nm) was sputtered on the backside of the membrane. These three steps were done in order to establish a good electrical contact between the pore and the base of the substrate. Finally, the pores were filled with Ni by direct current electrodeposition using a Ni solution consisting of 46 g/L of Ni(II) chloride hexahydrate ($NiCl_2{\cdot}6H_2O$), 40 g/L of boric acid (H_3BO_4) and 300 g/L of Ni(II) sulfate hexahydrate ($NiSO_4{\cdot}6H_2O$). For the electrodeposition process, a reference voltage of −1 V (vs. Ag/AgCl) was used and the time was adjusted to obtain Ni NWs of 1 μm in length. After electrodeposition, the gold layer on the backside of the sample was removed using reactive ion etching. The template pieces were then put in an Eppendorf tube containing 1 ml of 1 M sodium hydroxide (NaOH) solution and left for 24 h to selectively dissolve the alumina. The NaOH solution was replaced with 1 ml of chrome solution and left for 24 h in a Thermomixer® comfort (Eppendorf) at 40 °C and 300 rpm. The Eppendorf tube was put in a magnetic holder (Dynamag™-2) to collect the NWs, and then the chrome solution was discarded and replaced with ethanol. The NWs were suspended and shaken for cleaning and disposal of traces of NaOH, chromium-based solution and aluminum membrane. This step was repeated several times to ensure complete removal of the chromium-based solution.

Energy dispersive X-ray analysis (EDX) was used to obtain the elemental composition of the Ni NWs. Scanning electron microscopy (SEM) was used to visualize the filled pores, the alumina template and released Ni NWs. Transmission electron microscopy (TEM) was used to characterize their morphology and to study the oxide layer formed on their surface. To estimate the number of NWs corresponding to each sample, the number of pores was calculated for a defined area of the alumina template from SEM images using ImageJ software. This value was multiplied by the total area and divided by the area of the alumina template from the SEM image.

2.2. Cell culture

Human fibroblasts WI-38 (ATCC® CCL-75™) cells were cultured in Eagle's minimum essential medium (EMEM Quality Biological Inc.) supplemented with Sodium Pyruvate (Gibco®), non-essential aminoacids solution (MEM NEAA Gibco®), 10% fetal bovine serum

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