The Effect of Silicon Nanowires on Hepatocellular Carcinoma: Cytotoxicity & Adhesion Effect

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ABSTRACT

Silicon nanowires are becoming increasingly important as a nanomaterial. In this paper, the cytotoxicity of silicon nanowires (SiNWs) on several cell lines, i.e., HepG2 (hepatocellular carcinoma), NCIH460 (large cell lung cancer), SF268 (glioblastoma), NIH3T3 (embryo fibroblast cell), RAW 264.7 (macrophage cell) and CRL1634 (skin fibroblast cell) were investigated. Cell proliferation profile and cell cycle phase were tested by flow cytometry tests. The effect of SiNWs on cell adhesion was assessed using HepG2 (hepatocellular carcinoma) cells. Viability results showed that SiNWs had certain cytotoxic effect on six cell lines. Flow cytometry results showed that cell proliferation profile and cell cycle phase did not change obviously between control cells and treatment cells. The results of the effect of SiNWs on HepG2 cell adhesion showed that SiNWs had inhibition effects on HepG2 cell adhesion, which may cause the disruption of cell-cell communication.

Keywords: silicon nanowires, hepatocellular carcinoma, cytotoxicity, adhesion

1 INTRODUCTION

Silicon nanowires are becoming increasingly important as a nanomaterial. Their outstanding properties such as quantum size effects, diameter-dependent thermal conductivity and large piezoresistance coefficient have attracted a lot of research interests, including applications as biological materials and devices [1, 2]. However, the bio-safety of nanomaterials is of great importance for further applications. Before SiNWs can be incorporated into new and existing biomedical devices, their potential adverse effects on biological systems should be thoroughly investigated. Herein, the cytotoxicity of SiNWs on several cell lines and the effect of SiNWs on HepG2 cell adhesion were reported.

2 EXPERIMENTAL SECTION

2.1 Sample Preparation

SiNWs were prepared using oxide assisted growth method, their typical diameters and lengths were about 20-30 nm and hundreds of nanometers to one micrometer, respectively [3]. (Figure 1)

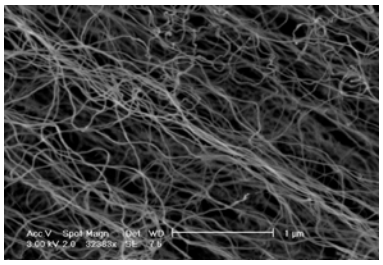

Figure 1 SEM photo of SiNWs

SiNWs stock suspension (1000 μg/ml) was prepared by dispersing SiNWs in sterile double-distilled water, followed by sonicating for 30 minutes. SiNWs suspensions which concentrations varying from 0.1 to 100 μg /ml were prepared by diluting the stock suspension with culture medium supplemented with 10% fetal bovine serum, 1% L-glutamine, penicillin, and streptomycin. Figure 2 shows the photo of SiNWs suspension.

Figure 2 Photo of SiNWs suspension

2.2 Cytotoxicity of SiNWs

HepG2, NCIH460, SF268, NIH3T3, RAW 264.7 and CRL1634 were obtained from American Type Culture

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Collection (ATCC, Manassas, VA). Cells were cultured in RPMI 1640 medium (HepG2, NCIH460, SF268, NIH3T3) or DMEM medium (RAW 264.7 and CRL1634) supplemented with 10% fetal bovine serum, 1% L-glutamine, penicillin, and streptomycin at 37°C in a humidified 5% CO_2 incubator.

Cytotoxicities of SiNWs suspensions on the above six cell lines were evaluated by Alamar Blue Assay [4]. Cells (6000 cells/well) were incubated in a 96-well cell culture dish for 8 hours at 37°C in a humidified 5% CO_2 incubator and then treated with SiNWs suspensions of different concentrations, i.e., 0.1, 1, 25, 50, 100 μg/ml for 48 hours at 37°C. Cells without SiNWs treatment were used as control and wells without cells were used as blank. After treatment, medium was removed and the cells were washed with PBS once, then 200 μL of Alamar Blue solution (2 mg/mL in cell culture medium) was added to each well and incubated for another 6 hours at 37°C. Fluorescence was read in a microplate reader (530 nm excitation, 584 nm emission). The relative cytotoxicity was expressed as percentage of $[OD_{sample}-OD_{blank}]$ / $[OD_{control}-OD_{blank}]$. Each experiment was performed in triplicate.

2.3 Flow Cytometry Tests

Flow cytometry tests were performed to study the differences between control cells and cells treated with 100 μg /ml for 24 hours in cell proliferation profile and cell cycle phase. Cells were washed three times with PBS and analyzed with FACS flow cytometer (Becton–Dickinson).

2.4 Effect of SiNWs on HepG2 Adhesion

To study the effect of SiNWs suspension on cell adhesion, HepG2 cells were incubated with 100 μg/ml of SiNWs suspension immediately after cell seeding (cell density was 10000 cells/ml) and further incubated for 48 hours. Cells without treatment were used as control. Morphologies of the cells were observed using optical microscope (Leica). The adherent cell number after treatment and none-treatment was counted.

Reverse transcription polymerase chain reaction (RT-PCR) was performed to examine the expression profiles of adhesion-specific genes, i.e. integrin, FAK, type I collagen, type III collagen and α-actin. HepG2 cells were treated with SiNWs suspension (100 μg/ml) for 18 and 48 hours, respectively (cells without treatment were used as control), and total RNA was extracted using TRI REAGENT (Molecular Research Center, Inc.) according to the manufacturer's instruction. Total RNA was quantified by biophotometer (Eppendorf, Hamburg, Germany), and the quality of total RNA was checked based on the 28S/18S rRNA ratio after agarose gel electrophoresis. Two micrograms of each total RNA sample were used for reverse transcription under standard conditions. The resulting cDNA was used as template in subsequent PCR. Sequences of interest were amplified using the following primer pairs: α-actin (5'-ATC TGG CAC CAC ACC TTC TA-3', 5'-AGC TCG TAG CTC TTC TCC AG), integrin (5'-GAC CTG CCT TGG TGT CTG TGC-3', 5'- AGC AAC CAC ACC AGC TAC AAT-3'), FAK (5'-GAA GTC TTC AGG GTC CGA TTG-3', 5'- CAT TCT CGT ACA CCT TAT CAT TCG-3'), type I collagen (5'-AAC ATG ACC AAA AAC CAA AAG TG-3', 5'-CAT TGT TTC CTG TGT CTT CTG G-3') , type III collagen (5'-CCC AGA ACA TCA CAT ATC AC-3', 5'- CAA GAG GAA CAC ATA TGG AG-3'). β-actin was used as endogenous reference housekeeping gene. PCR conditions were as follows: 30 s 94˚C, 30 s 58˚C, 30 s 72˚C, (5 min 94˚C, 5 min 72˚C) ×35 cycles. (α-actin, β-actin, integrin); 30 s 94˚C, 30 s 56˚C, 30 s 72˚C, (5 min 94˚C, 5 min 72˚C) ×45 cycles. (FAK, type I collagen, type III collagen).

Immunofluorescence staining analysis was performed to detect the location and relative abundance of protein actin. HepG2 cells with 100 μg/ml of SiNWs suspension were cultured on sterile glass cover slips at 37˚C in a humidified atmosphere of 5% CO_2 in air and cultured for 18 hours and 48 hours respectively. Cells without treatment were used as control. Protocols for performing immunofluorescence staining analysis can be found at the website of SANTA CRUZ BIOTECHNOLOGY, INC.

3 RESULTS & DISCUSSION

3.1 Cytotoxicity of SiNWs

The results from viability assay showed that the relative viability of six different cell lines compared to control responding to SiNWs suspensions in a dose-dependent manner. The results showed that SiNWs suspensions had certain inhibition effect on the six cell lines. A 10% to 30% decrease in cell viability was observed for SiNWs concentrations varying from 25 to100 μg/ml, respectively (Table 1).

Con / Viability (%)	0.1 (μg/ml)	1 (μg/ml)	25 (μg/ml)	50 (μg/ml)	100 (μg/ml)
HepG2	100.02	97.67	92.64	83.45	66.58
NCIH460	95.37	93.52	92.59	75.40	70.81
SF268	99.95	90.44	89.11	77.62	70.52
NIH3T3	97.88	95.26	92.41	85.12	69.42
RAW264.7	97.62	98.03	92.27	84.16	60.26
CRL1634	101.39	100.01	95.17	88.25	72.40

Table 1: Relative cell viability results of several cell lines

3.2 Flow Cytometry Tests

However, the results of flow cytometry tests showed the cell proliferation profile and cell cycle phase did not change obviously after 100 μg/ml SiNWs treatment (Figure 3).

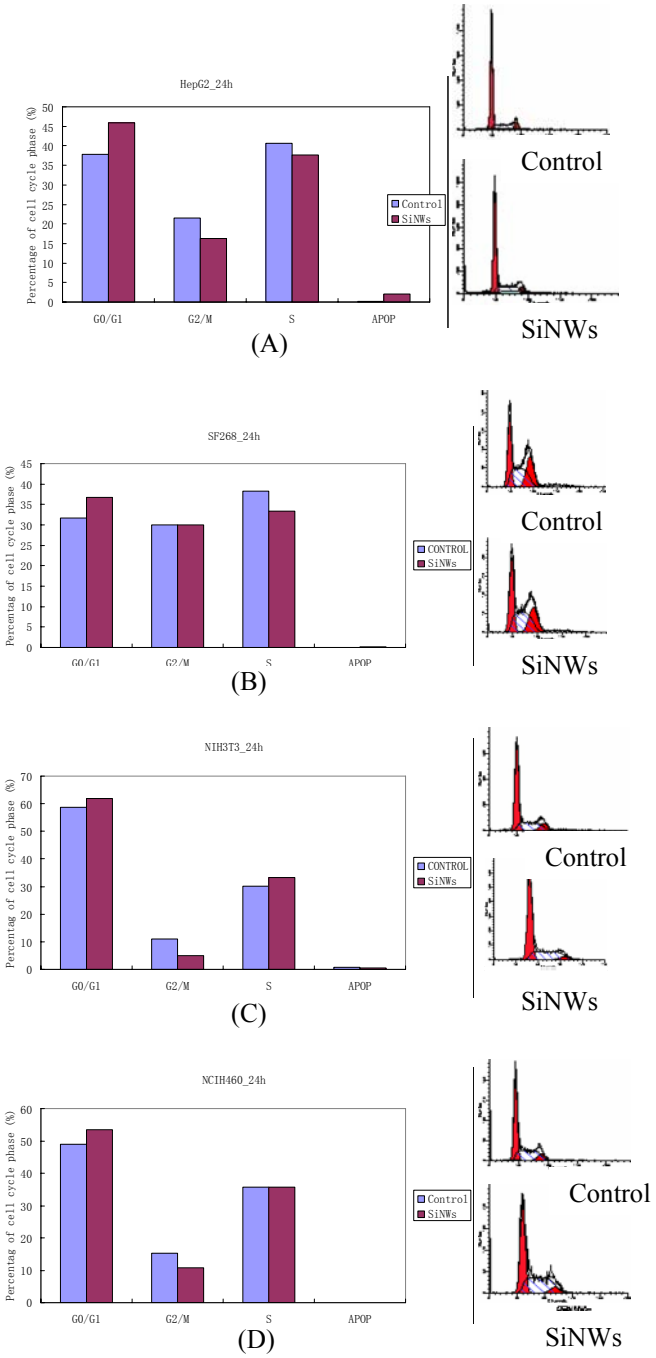

Figure 3: Flow cytometry results. (A) HepG2; (B) SF268; (C) NIH3T3; (D) NCIH460

3.3 Effect of SiNWs on HepG2 Adhesion

It is obviously that the morphologies of cells attaching and spreading after treated with 100 μg/ml of SiNWs suspension for 48 hours were different from the control cells. Most of the cells treated with SiNWs were tended to grow separately, detaching from the cell populations when compared to the control cells (Figrue 4).

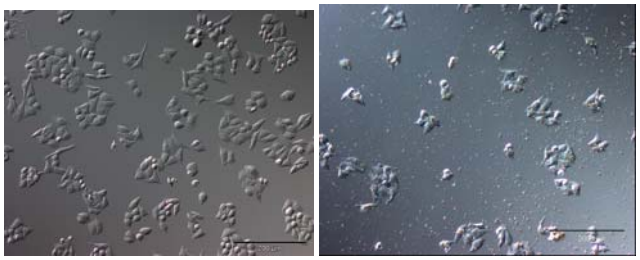

Figure 4: Morphologies of the HepG2 cells under optical microscope. HepG2 cells were exposed to 100 μg/ml of SiNWs suspension immediately after cell seeding (cell density was 10000 cells/ml) and incubated for 48 hours. (A) Control HepG2 cells, 48 hours; (B) Treated HepG2 cells, 48 hours

In order to get more evidence for the inhibition effects of SiNWs on HepG2 cell adhesion, the number of adhered cells with 5, 25, 100 μg/ml SiNWs suspensions treatment was counted. As a result, relative percentages of the adhered cells were 95.6%, 84.3%, 70.1%, respectively, when compared with 100% in the control.

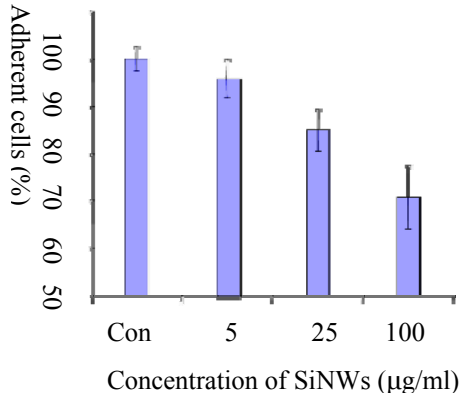

Figure5: Percentage of adherent cell number.

The RT-PCR results showed that after HepG2 cells were treated with SiNWs for 18 hours and 48 hours, the expression level of type III collagen and α-actin did not change significantly, while the expression of integrin, FAK and type I collagen were down-regulated, especially after 48 hours treatment (Figure 6).

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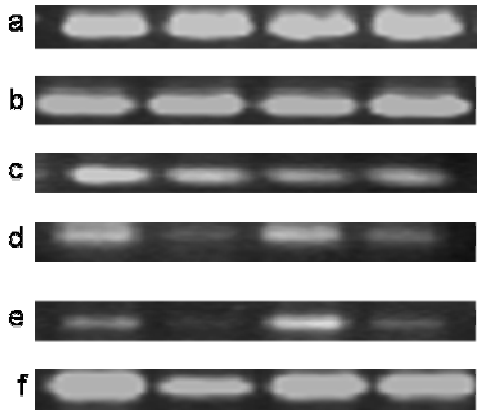

Figure 6: Images of 1.5% agarose gel electrophoresis for RT-PCR products to view the expression profile of adhesion-associated genes. Lane 1: Control, 48h; Lane 2: Treatment, 48h; Lane 3: Control, 18h; Lane 4: Treatment, 18h. (a) β-actin, endogenous reference housekeeping gene; (b) α-actin; (c) type III collagen; (d) FAK; (e) type I collagen; (f) integrin

Immunofluorescence results showed that the expression of protein actin did not change significantly, which was consistent with the gene expression profile of α-actin (Figure 7).

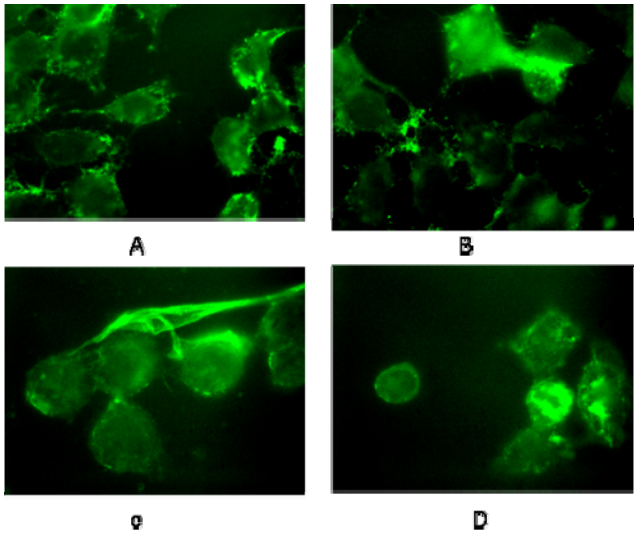

Figure 7: Immunofluorescence staining analysis of actin. (A) Control, 18h; (B) Treated, 18h; (C) Control, 48h; (D) Treated, 48h. Results showed that the expression level of actin did not change obviously between the control cells and treated cells both at 18h and 48h.

SiNWs suspension treatment resulted in the decrease in the adhered HepG2 cell number, the change of the adhesion and spreading morphologies of the cell, and the down-regulation of the adhesion-associated genes (type I collagen, integrin, FAK). Cell cytotoxicity test results showed the viability of the cells responded to SiNWs in a dose-dependent manner, with lower viability of the cells under higher concentration of SiNWs. From the results of HepG2, the decrease in viability is mainly due to the decreased adhesion abilities of the cells under SiNWs environment, rather than due to influence to cell cycle distribution.

The initial cell adhesion, including cell-cell adhesion and cell-ECM (extracellular matrix) adhesion plays a major role in cellular communication and regulation, and is of fundamental importance in the development and maintenance of tissues 5-7]. The present study suggested that SiNWs might affect HepG2 cell adhesion and spreading through the integrin- type I collagen pathway, rather than the actin-mediated pathway. SiNWs may have potential effect on cell proliferation and cellular communication and regulation based on our study. Further studies are needed to provide more detailed information on the bio-safety of SiNWs.

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