Cytotoxic Effect of Silica Nanoparticle on some Tumor Cell Lines

Shatha Salah Asad[a] Khalid Mahdi Salih[b] Nahi Yousif Yassen[c]

[a]Al-kindy College of Medicine, Anatomy department/Histology, Baghdad University
[b]College of Science, Department of Biology, Al-Mustansiriya University, Baghdad, Iraq
[c]Iraqi Centre for Cancer & Medical Genetics Research, Al-Mustansiriya University

m12_moon@Yahoo.com

Abstract

Objective: Silica nanoparticles have been discovered to exert cytotoxicity in normal and cancerous human cells. Therefore, this study is aimed to evaluate the cytotoxic effect of silica nanoparticle on some tumor cell lines.

Materials and Methods: Six concentrations (100,200,400,600,800, and1000 µg/ml) of silicon oxide SiO_2nanoparticles (SONPs)with particle size 20-30 nm were prepared and their cytotoxic effect was tested on Hela, RD and ANM3 cancer cell lines as well as REF normal cell line as a control for 24 and 48 hr by using MTT assay to estimate the optical density (OD) of cell growth at wave length 500nm.

Results: All tumor cell lines (RD, HeLa, and AMN3) demonstrated significant inhibition in the cell growth index (GI%) after 24 and 48 hr exposure to all concentrations of SONPs, however the GI% of Ref cell showed non-significant change after both incubation periods of treatment. On the other hand, only RD cell line showed significant negative correlation (r=- 0.8178, P=0.047) between GI% and the concentration of SONPs after 24 hr exposure, while those HeLa, AMN3,and REF cell lines showed non-significant correlation whether after 24 hr or 48 hr of treatment.

Conclusion: The SONP *per se* at diameter 20-30 nm revealed significant cytotoxic effect based on MTT assay in different tumor cell lines, and the magnitude of their cytotoxic effect is dose-independent in the majority of cell lines which indicated that they are acting in cell type-dependent manner. Therefore, pathways by which SONPs induced their cytotoxic effect in tumor cells need further investigation.

Keywords: Silica nanoparticles, Cytotoxicity assay, MTT assay, Anti-tumor effect

Introduction

The terms silica refer to naturally occurring materials that composed of silicon dioxide (SiO_2), and can be classified into two types; the first one known as crystalline silica which is found in in the sand and rocks and responsible for many serious health problems such as pulmonary silicosis. However, the second type is known as amorphous silica, which is safer than crystalline silica, therefore, it is widely used in several kinds of material processing such as pharmaceutical products, paints, cosmetics, and food [1]. Moreover, with the development of nanoparticle field, it was found that amorphous silica at diameter size less than 100nm possess different physical and chemical properties with quietly different activities than native form, so the application of these nanoparticles is rapidly expanded in the field of nanomedicine [2]. These porous SONPs exhibited extraordinary qualities for application in biological field as a carrier for delivery several drugs into the body due to its biocompatibility and biodegradability, low toxicity and solubility. Although, injection of mesoporous silica subcutaneously in rats showed no toxicity effects, intraperitoneal and intravenous injections caused death which may be due to the formation of thrombus [3]. Another researcher

studied the cytotoxic effect of silica nanoparticle s*in vitro* and found that the smallest particles were revealed the most toxic effect, and the alteration in the chemical properties of the surface of these particles by special treatment act as the key factor regarding the toxicity aspect [4]. Although other metal oxide nanoparticles have antitumor activity *per se*, silica has been shown to be a good carrier for different anticancer drugs that can be loaded into SONPs and can replacing the need to use solvents that are often toxic for healthy tissues. Therefore, pore sizes together with surface treatments are the main features of silica nanoparticles that play a major role in cell-particle interactions and hence deter- mine toxicity of the material [5] . Several studies found that porous SONPs with pore size 2–50 nm has played a significant role as a carrier in pharmaceutical technology for its drug loading and controlled release drug in tumor sites particularly the delivery of doxorubicin that is a conventional anticancer drug against various types of cancers [6] [7] .

The application of silica nanoparticles is not restricted for drug delivery system but it is expanded to be involved in the immunotherapy approach for potential cancer treatment. This updated approach has been developed by conjugated an engineered silica particles with a selective antibody specific to HER-2 receptors which are overexpressed in the malignant cells of breast cancer and make it non responsive to the hormonal therapy [8]. Additionally, the using of SONPs as photosensitizers showed a cytotoxic effect to kill a considerable portion of the cancer cells *in vitro* higher than other photosensitizers that are previously used in this approach [9][10]. Furthermore, it was found that exposing cancer cells to ultrasonic field causes only inhibition in their proliferation rate, however, when these cells placed on SONPs with 10– 100 nm nanostructures resulted in complete destruction of these cells during additional ultrasonic exposure [11]. Recently, pancreatic malignant tumor cells can be destroyed by using near infrared laser which is another photo thermal approach used in the cancer therapy but using this approach in combination with colloid porous silica and dimethyl Sulphoxide solvent (DMSO-Psi)was found to be high enough to destroy the tumor and showed a sufficiently high cytotoxic effect *in vitro* [12].Thus this study is designed to investigate the ability of SONP s*per se* at certain particle size 20-30 nm *in vitro* to destroy or inhibit the proliferation of different cancer cells *in vitro* by using cytotoxicity assay based on MTT technique.

Materials and methods

Cell lines and culture

Four cell lines were obtained from Iraqi Center of Cancer and Medical Genetics Research, three of them represent tumor cell lines which include; murine mammary gland adenocarcinoma (AMN-3), pelvic rhabdomyosarcoma (RD**),** and cervical carcinoma (HeLa),and the fourth represent normal cell line which is transformed rat embryonic fibroblast (REF). The cells were cultured in RPMI 1640 medium containing 10% (v/v) fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified 5% CO2 incubator, and passaged once every 2–3 days [13].

Preparation of Silica nanoparticle (SONP)

Silicon Oxide SiO_2 nanoparticles(SONPs) were purchased from Nano Rahpouyan Mahan (NRM)/ Iran with a purity more than 99% and characterized by white color and their size range from 20-30 nm with bulk density less than 0.1g/cm^3. To prepare different concentrations of SONPs, 10 mg of powder was dissolved in 10 ml of RPMI 1640 medium free serum, to form a concentration of 1000 μg/ml, from which other six concentrations were prepared by using dilution method and RPMI 1640 free media which include 100,200,400,600, 800and 1000 μg/ml.

Cytotoxicity MTT Assay

The cytotoxic effect of different concentrations of SONPs on the proliferation of the adherent cells in 96-well microliter plate for 24 and 48 hr incubation periods has been tested according to [14] method by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay method [15] and the absorbance (OD) of wells was determined on a microplate reader at 500 nm. The average of OD for each concentration was determined and used for calculation of the growth index percentage (GI%) according to the following equation:

$GI\% = (B-A/A) \times 100$ (Where *GI%* is the percentage of growth index, A is the average of optical density of untreated wells (those treated with phosphate buffer saline), and B is the average of optical density of treated wells. Negative value of GI% means growth inhibition, while positive value means growth improvement [16].

Statistical analyses

Differences among independent samples were analyzed by ANOVA and HSD test, while associations between variables were assessed by using Pearson's correlation coefficient (r) test. Any P value < 0.05 was considered as statistically significant.

Results

All concentrations of SONPs caused significant reduction in the OD of all cancer cell lines whether after 24 hr or 48 hr of exposure, but non-significant reduction in those of REF cell line (Table 1 & 2).

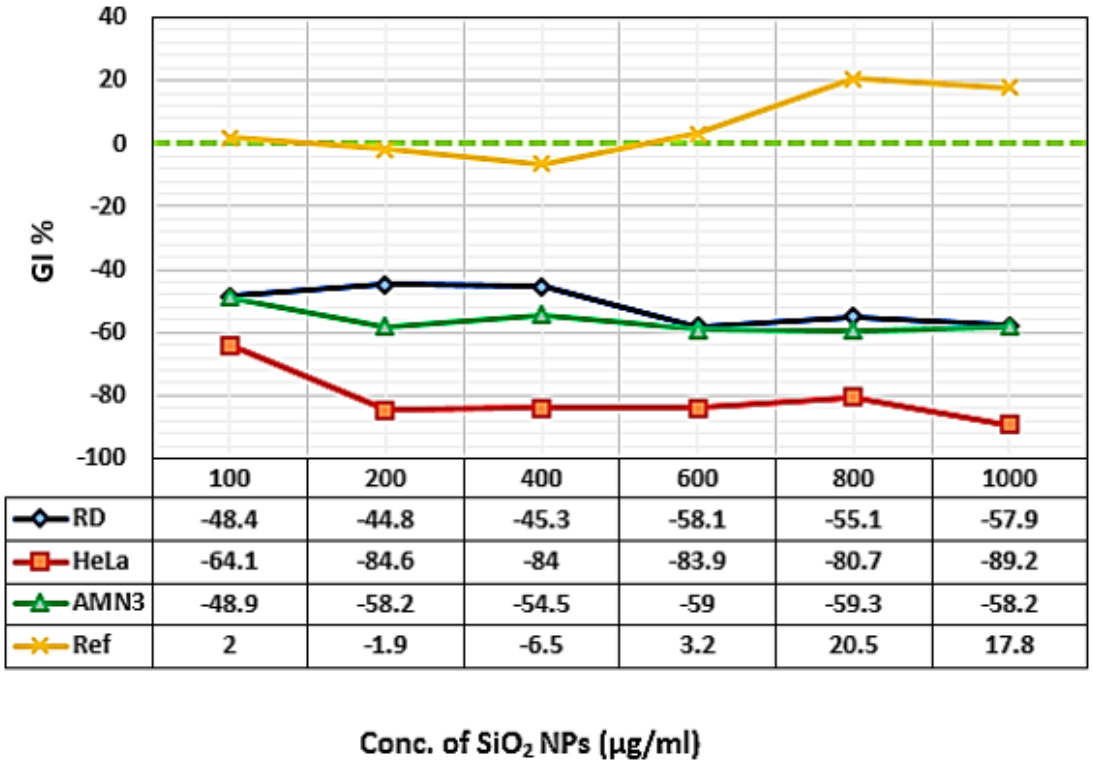
Table1. Optical density (OD) of different cell lines after 24 hr exposure to various concentrations of SONPs.

SONPs Conc. (µg/ml)	OD of Cell line (Mean ± SE)			
	RD	HeLa	AMN3	Ref
0 (control)	0.671 ± 0.082	1.473 ± 0.132	0.836 ± 0.116	1.305 ± 0.03
100	0.346 ± 0.049*	0.528 ± 0.045*	0.427 ± 0.024*	1.332 ± 0.085
200	0.370 ± 0.064*	0.226 ± 0.064*	0.349 ± 0.075*	1.280 ± 0.177
400	0.367 ± 0.077*	0.235 ± 0.063*	0.380 ± 0.074*	1.22 ± 0.091
600	0.281 ± 0.036*	0.237 ± 0.054*	0.342 ± 0.112*	1.347 ± 0.093
800	0.301 ± 0.020*	0.283 ± 0.043*	0.340 ± 0.092*	1.573 ± 0.240
1000	0.282 ± 0.021*	0.158 ± 0.054*	0.349 ± 0.025*	1.538 ± 0.132
(*) Significant difference at P < 0.05, Two-tail by one-way ANOVA test				

Table 2: Optical density (OD) of different cell lines after 48 hr. exposure to various concentrations of SONPs.

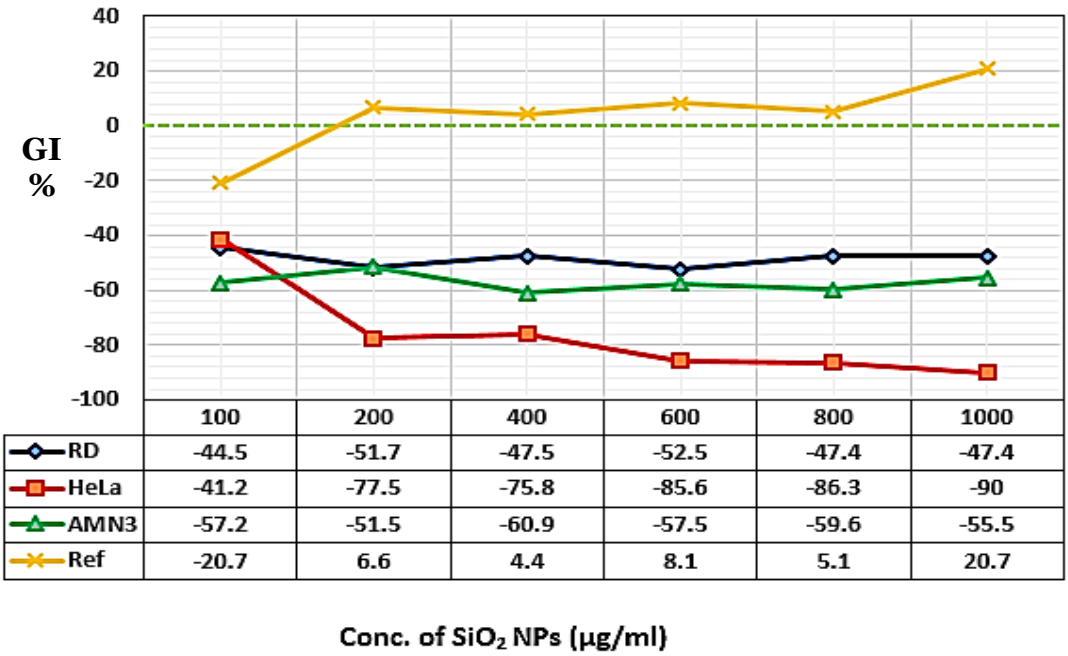
SONPs Conc. (µg/ml)	OD of Cell line (Mean ± SE)			
	RD	HeLa	AMN3	Ref
0 (control)	1.477 ± 0.025	1.410 ± 0.224	0.983 ± 0.059	0.135 ± 0.038
100	0.819 ± 0.053*	0.829±0.171*	0.42 ± 0.063*	0.107 ± 0.004
200	0.712 ± 0.055*	0.317±0.103*	0.467±0.062*	0.144 ± 0.020
400	0.774 ± 0.035*	0.340±0.106*	0.348±0.041*	0.141 ± 0.003
600	0.701 ± 0.063*	0.203±0.037*	0.417±0.034*	0.146 ± 0.008
800	0.776 ± 0.049*	0.193±0.032*	0.39 ±0.031*	0.142 ± 0.002
1000	0.776 ± 0.038*	0.141±0.023*	0.437±0.084*	0.163 ± 0.025
(*) Significant difference at P < 0.05, Two-tail by one-way ANOVA test				

When these values of OD were converted to growth index percentage according to previous equation, GI% of cancer cell lines after 24 hr is significantly reduced down to 57.9%, 89.2%, and 58.2% for RD, HeLa, and AMN3 respectively (Figure.1), while after 48 hr, GI% is also reduced down to 47.4%, 90%, and 55.5% respectively (Figure.2). In contrast, exposure of REF cell line to different concentrations of SONP for 24 hr and 48 hr showed non-significant change in GI % (Figure.1 & 2 respectively).



	100	200	400	600	800	1000
RD	-48.4	-44.8	-45.3	-58.1	-55.1	-57.9
HeLa	-64.1	-84.6	-84	-83.9	-80.7	-89.2
AMN3	-48.9	-58.2	-54.5	-59	-59.3	-58.2
Ref	2	-1.9	-6.5	3.2	20.5	17.8

Figure 1. The growth index percentage (GI %) of cell lines after 24 hr exposure to SiO_2 NPs.



	100	200	400	600	800	1000
RD	-44.5	-51.7	-47.5	-52.5	-47.4	-47.4
HeLa	-41.2	-77.5	-75.8	-85.6	-86.3	-90
AMN3	-57.2	-51.5	-60.9	-57.5	-59.6	-55.5
Ref	-20.7	6.6	4.4	8.1	5.1	20.7

Figure 2. The growth index percentage (GI %) of cell lines after 48 hr exposure to SiO_2 NPs.

According to the statistical analysis by Pearson correlation, the results showed that the GI % of all cell lines are dose-independent affected by SONPs treatment except for RD cell line which is significantly reduced as concentration of silicon oxide nanoparticles increases after 24 hr exposure as shown in Table 3.

Table 3. Correlation coefficient (r) of SONPs concentration with GI % of different cell lines.

Exposure period		SiO_2 NPs concentration vs GI % of			
		RD	HeLa	AMN3	Ref
4 hr	r	-0.8178	-0.6334	-0.6654	0.8013
	P	0.047	0.177	0.149	0.055
48 hr	r	-0.0493	-0.7845	-0.2177	0.7696
	P	0.926	0.064	0.679	0.073
Negative r value means reverse correlation, Positive r value means direct correlation					

Discussion

The cell viability of all cancer cell lines (RD, HeLa, and AMN3) was significantly reduced after 24 and 48 hr of exposure to different concentrations of SONPs based on MTT assay (Figure. 1&2) in dose-independent manner (Table.3). However this treatment didn't affect the viability of normal Ref cell line. Comparable to these results, [17] found that exposure of cultured human Broncho-alveolar carcinoma-derived cells (A549) to 15-nm or 46-nm silica nanoparticles for 24, 48, and 72 hr at concentration of (10 -100 μg/ml) causes reduction in the viability of these cells dependent on the dose and time of exposure. Similarly, little cytotoxic effects against normal cell lines were reported when treated with SONPs (14 nm)

at the concentration below 250 μg/ml within 48 hr, but treatment with SONPs at high concentrations (250-500 μg/ml) and long exposure time(72 h) caused oxidative stress in different cancer cell lines as well as membrane damage depending on their type A549>HOS > HeLa [18]. However, non-cytotoxic doses of 3 different sizes SONPs revealed that smallest particles showed an apparently higher-fold induction of genotoxic potential in lung cancer cells A549 [19], so the distinct physicochemical properties of nanoparticles can modulate their toxicity and modes of action because differences in such properties determine their interaction with the cell and even within the cell [20]. Furthermore, exposure of cultured Keratinocyte (HaCaT) from human epidermis to 15, 30 or 100 nm silica nanoparticles at serial concentrations up to 100 μg/ml caused damage in their DNA molecules due to their ability to induce oxidative stress that is increased with increasing concentration and decreasing size of nanoparticles [21]. Similar findings are reported when Murine macrophages (RAW264.7) and human epithelial lung (A549) cell lines are exposed to different sizes and different concentrations of SONPs for 4 and 24 hr [22] .

Comparable to the result of Ref cell line obtained in this study, [23] observed inverse dose-dependent relationships in human intestine cell line (HT-29) after a 24 hr exposure to Silica NPs with particle size of 100 nm, in which as the dose of 100 nm silica increases the higher NPs, the cytotoxic/genotoxic effect decreases. In recent study, it was found that exposure of two human cell lines; alveolar cells A549, and colorectal cells Caco2, as well as one murine fibroblast cell line Balb/c 3T3 for 24 hr to SONP suspensions at concentration of 3-100 μg/mL did not show toxic effects below 100 μg/mL based on neutral red uptake assay, although transmission electron microscopic (TEM) investigation after treatment revealed that NPs were internalized by these cells [24] . More recently, it was found that nanoparticles of amorphous silica prepared in three different process as pyrogenic, precipitated and colloidal around 20 nm particle size can produce significant cytotoxic and genotoxic effects in the fibroblasts (V79) cells cultured from the lung of Chinese hamster after 24 hr of exposure, but none of them induced intracellular reactive oxidative species, micronuclei or genomic mutations in V79 cells. In contrast, when these three different nanoparticles are prepared at diameter of 50 nm, negligible toxicity is yielded[25] .

It can be concluded that the SONP *per se* at diameter 20-30 nm revealed significant cytotoxic effect based on MTT assay in different cancer cell lines, and the magnitude of their cytotoxic effect is dose-independent in the majority of cell lines which indicated that they are acting in cell type-dependent manner. Therefore, pathways by which SONPs induced their cytotoxic effect in cancer cells needs further investigation particularly their ability to induce apoptosis, also their biocompatibility in vivo requires more attention.

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الخلاصة

الهدف: اكتشفت دقائق السليكا النانوية بإمتلاكها السمية الخلوية في الخلايا الطبيعية والسرطانية ، لذلك فأن هذه الدراسة تهدف الى تقييم التاثير السمي الخلوي لدقائق السيليكا النانوية على بعض خطوط الخلايا السرطانية.

المواد وطرق العمل: تم تحضير ستة تراكيز(١٠٠٠,٨٠٠,٦٠٠,٤٠٠,٢٠٠,١٠٠مايكروغم/مل)من الدقائق النانوية لثاني أوكسيد السليكون(السيليكا) وبحجم دقائق ٢٠–٣٠ نانوميتر وقد تم اختبار تأثيرها المسمم على خطوط الخلايا السرطانية (AMN3,Hela,RD)وكذلك على خط الخلايا الطبيعي REF كسيطرة ولفترتين تعريضيتين (٤٨,٢٤ ساعة) بإستخدام فحص MTT لقياس الكثافة الضوئية لنمو الخلايا عند طول موجي ٥٠٠ نانوميتر.

النتائج: اظهرت جميع خطوط الخلايا السرطانية تثبيط معنوي في نمو الخلايا السرطانية بعد ٢٤ و ٤٨ ساعة من التعريض لجميع تراكيز السيليكا النانوية, بينما كان التغير في نسبة تثبيط النمو غير معنوي في خلايا خط REFالطبيعية. من جهة أخرى تبين بأن خط خلايا RD كان الوحيد الذي أظهر علاقة سلبية معنوية (r= − 0.8178, P=0.047) بين معامل نسبة النمو وتركيز دقائق السليكا النانوية بعد ٢٤ ساعة من التعريض ، بينما بقية خطوط الخلايا (HeLa, AMN3,REF) أعطت علاقة غير معنوية سواء بعد ٢٤ ساعة أو ٤٨ ساعة من المعاملة.

الاستنتاج: إن دقائق السليكا النانوية بحد ذاتها وبحجم ٢٠–٣٠ نانوميتر أعطت وبشكل معنوي تأثيرا سميا خلويا في عدة خطوط من الخلايا الورمية المختلفة إستنادا لفحص الـ MTT وأن شدة تأثيرها التسممي غير معتمد على تركيزها مما يشير الى أن فعاليتها معتمدة على نوع الخلايا. لذلك فإن المسالك التي تحث من خلالها دقائق السليكا النانوية على التأثير السمي الخلوي في الخلايا الورمية تحتاج الى فحوصات أكثر.

الكلمات المفتاحية: دقائق السليكا النانوية، فحص MTT ، التأثير السمي الخلوي.