Influence of silica particle internalization on adhesion and migration of human dermal fibroblasts

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1. Introduction

The applications of nanotechnology in biological field offer a great promise for biomedical sensing, diagnostics, and therapeutics [1,2]. As a type of non-metal oxides, the silicon dioxide (SiO2) particles have been extensively used as chemical mechanical polishing agents and additives to drugs, cosmetics, printer toners, varnishes and food [3]. In recent years, significant advantages have been shown when the SiO2 particles are used in the biomedical and biotechnological fields, such as DNA delivery [4,5], cancer therapy [6–8], enzyme immobilization [9] and biosensors for simultaneous assay of glucose, lactate, l-glutamate, and hypoxanthine levels in rat striatum [10]. In addition, the strategy of utilizing luminescent SiO2 nanoparticles bioconjugated with special ligands or antibodies for bioimaging has gained an increased interest [11,12]. Nevertheless, interaction of the colloidal materials with biological systems and environment could lead to unintended adverse effects, which are recently recognized and attracted broad attentions [13]. Accompanying with the well-established use of the SiO2 particles, their potential toxicity has been investigated [14], but the results are contradictory from different experiments. A wealth of information exists on the toxicity of silica, which includes the development of silicosis, lung cancer, and some autoimmune diseases such as systemic sclerosis, rheumatoid arthritis, lupus and chronic renal dysfunction [15–18]. Moreover, the SiO2 nanoparticles cause pro-inflammatory stimulation of endothelial cells [19]. It was reported that both the nanosized SiO2 and microsized SiO2 particles induce fibrogenesis in Wistar rats, although the former has milder adverse effect [20]. However, other studies showed that the silica nanoparticles are not toxic and can be used in vivo [21] or other biomedical fields [22].

Most of the previous studies focused on the acute cytotoxicity of nanoparticles, which may not be the only adverse effect caused by the nanoparticles. During the cellular uptake process, morphological changes and cytoskeleton disorganization may occur, which are indicative of abnormal cell development and certain cell functions impairment [23] and can not be efficiently and adequately detected by the simple cytotoxicity assay. In this regard, the biological effects of the SiO2 particles on cell functions such as the cell adhesion and migration need further investigation.

Herein the in vivo effects of SiO2 nanoparticles on cell functions such as the cell adhesion and migration need further investigation.
expression of adhesion related genes. The human dermal fibroblasts are the major type of cells in dermal layer of skin. Although the epidermal layer of skin provides a natural defense against contact penetration, both drug loaded particles and particles in environment can easily access to the dermal fibroblasts via injured or inflamed skin [24]. Moreover, dermal fibroblasts play a key role in the cell renewing system and wound healing process [23]. Study of the interaction between the SiO2 particles and dermal fibroblasts is therefore of great help to understand the influence of nanoparticles on the skin repair process.

2. Materials and methods

2.1. Materials

Rhodamine-B-isothiocyanate (RITC), amiloride-HCl, amantadine-HCl, sodium azide, genistein, 4,6-diamidino-2-phenylindole (DAPI), 2’7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma–Aldrich, LysoTracker® Green and rhodamine 123 were obtained from Invitrogen Corp. All other reagents were of analytical grade and used as received. The solutions were prepared using Millipore water purify by a Milli-Q water system.

2.2. Particles preparation and characterization

RITC doped SiO2 particles were synthesized according to literature [25] with minor modification. In brief, RITC conjugated triethoxysilane was prepared from 0.4 g 3-aminopropyl triethoxysilane (APS) and 11.8 mg RITC in 1 ml anhydrous ethanol under N2 atmosphere. A mixture of 0.3 ml tetraethyl orthosilicate (TEOS) and 1 ml RITC conjugated APS ethanol solution was injected into a mixture of 42.4 ml ethanol, 18.8 ml water and 0.8 ml NH3.H2O, or a mixture of 3.2 ml ethanol, 27 ml isopropanol, 11.3 ml water and 5.9 ml NH3.H2O to obtain particles with a smaller or larger size. Another 2.7 ml TEOS was added into the flask after 1 h. Five hours later, the reaction product was centrifuged at 10,000 g for 10 min and washed thoroughly with water and ethanol to obtain the silicon dioxide particles containing the fluorescent dye (RITC-SiO2). Solid products were obtained after centrifuged and dried at 50 °C overnight. These RITC-SiO2 particles were used throughout this work.

The particle size and morphology were characterized with a JEM-200 transmission electron microscope (TEM, JEMOL, Japan). Optical property of the RITC-SiO2 particles was detected at room temperature with a luminescence spectrometer (LS55, Perkin Elmer precisely) after they were dispersed in PBS under sonication. The ratio of the fluorescence intensity for these particles was obtained by comparing the integrated areas of the emission peaks.

2.3. In vitro experiments

2.3.1. Cell culture

Primary human dermal fibroblasts were obtained from spare skin tissues with agreement of patients and were used between passages 5 and 13 in this study. The cells were maintained with regular growth medium consisting of high-glucose DMEM (Gibco, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, and cultured at 37 °C in a 5% CO2 humidified environment.

2.3.2. Cellular uptake of RITC-SiO2 particles

Uptake of the SiO2 particles was determined by flow cytometry (FACS Calibur, Becton Dickinson BD). The cells were seeded on a 24-well plate at a density of 8 × 10⁵ cells per well and allowed to attach for 24 h. To determine the particle uptake rate and amount, the cells were incubated with 100 μg/ml of RITC-SiO2 particles for different times (0 h, 2 h, 4 h, 8 h, 12 h, 24 h). At determined time intervals, the cells were washed twice with PBS and harvested by trypsinization. The RITC incorporated in the SiO2 particles served as a marker to quantitatively determine their cellular uptake by flow cytometry and CellQuest Pro software. To study the uptake mechanism, the energy dependence of cell–particles interaction was assessed by treatment with sodium azide or low temperature at 4 °C. Different pharmacological inhibitors, including amiloride-HCl, amantadine-HCl and genistein, were used to treat the fibroblasts for 1 h before incubation with the SiO2 particles.

Fluorescence staining of lysosomes and cell nuclei and confocal microscopy were used to display the intracellular distribution of the SiO2 particles. Brieﬂy, after incubation with 100 μg/ml of RITC-SiO2 particles for desired time, the fibroblasts were carefully washed with PBS 3 times, then cultured with LysoTracker® Green and DAPI at 37 °C for another 30 min, and finally observed under confocal laser scanning microscopy (CLSM, LSM 510, Carl Zeiss).

2.3.3. Cell viability and proliferation assay

To determine cell viability, the cells were plated at a density of 1 × 10⁴ cells per well in a 96-well plate and cultured for 24 h. The medium was replaced with fresh medium containing the SiO2 particles of varying concentrations. After treatment for 10 h, 20 μl [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml)] was added to each well and the cells were further cultured at 37 °C for 3 h. For proliferation assay, the cells treated with the SiO2 particles for 10 h were allowed to grow in fresh regular culture medium for another 24 h followed by incubation with MTT reagent. The dark blue formazan crystals generated by the mitochondria dehydrogenase in live cells were dissolved with dimethyl sulfoxide to measure the absorbance at 570 nm by a microplate reader (MODEL 550, Bio Rad).

2.3.4. Measurement of mitochondrial membrane potential (MMP)

After the human dermal fibroblasts (5 × 10⁴ cells per well in a 24-well culture plate) were incubated with the SiO2 particles for 9.5 h, rhodamine 123 solution was added to a final concentration of 5 μg/ml [26]. The cells were then continually cultured for another 0.5 h, washed 3 times with PBS, and then observed under a fluorescence microscope (Axovert 200, Carl Zeiss). Subsequently, the cells were trypsinized and resuspended in PBS. The fluorescence intensity was determined via flow cytometry. For each analysis, at least 10,000 events were analyzed.

2.3.5. Measurement of intracellular reactive oxygen species (ROS)

The oxidation-sensitive probe DCFH-DA was employed for determination of the generation of ROS. DCFH-DA is a stable nonfluorescent molecule that readily crosses cell membranes, and can be oxidized to highly fluorescentDCF in the presence of intracellular ROS [27]. Briefly, the fibroblasts (5 × 10⁴ cells per well in a 24-well culture plate) were treated with the SiO2 particles for 10 h, washed and incubated with 10 μM of DCFH-DA for 30 min at 37 °C in dark. They were then washed, collected and analyzed by flow cytometry.

2.3.6. Cell adhesion assay

The cells were seeded on a 6-well plate at a density of 3 × 10⁴ cells per well and cultured for 24 h. After treated with 100 μg/ml SiO2 particles for 10 h, the cells were washed with PBS and detached with 0.25% trypsin, counted using a hemacytometer, and then placed into a 24-well plate with an equal number per well and allowed to adhere for 8 h or 24 h. Untreated cells were used as control. After the nonadherent cells were washed out with PBS, the adherent cells were counted at 12 different areas under an optical microscope. The quantification was repeated twice and each sample was assayed in triplicate. Data was averaged and normalized to that of the control.

2.3.7. Real-time RT-PCR analysis

Real-time quantitative reverse transcription polymerase chain reaction (Real-time RT-PCR) was performed to examine the expression profiles of adhesion specific genes for fibroblast, laminin and focal adhesion kinase (FAK) in the fibroblasts. Briefly, the cells were cultured with or without 50 μg/ml SiO2 particles for 24 h. Total RNA was extracted by using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions and quantified by using a biophotometer (Eppendorf, Germany). Total RNA samples (2 μg each) were used for reverse transcription under standard conditions using M-MuLV Reverse Transcriptase cDNA synthesis kit (Promega, USA). The resulting cDNA was used as template in subsequent PCR amplifications and the primer sequences used in this study are listed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous reference housekeeping gene. The Real-time PCR reactions were performed with the SYBR Premix ExTaq™ Kit (Takara, Japan) and iQ™ qPCR system (Bio Rad, USA). The relative gene expression values were calculated with the comparative ΔΔCt (threshold cycle) method, and normalized to the housekeeping gene.

2.3.8. Transwell assay

Cell migration assay was carried out according to literature with some modifications [28]. Brieﬂy, after cultured with 100 μg/ml SiO2 particles for 10 h, the fibroblasts were detached with trypsin. 1 × 10⁷ cells were suspended in DMEM containing 1% FBS and then seeded into a Millicell (8-µm pores; Greiner Bio-One, Germany) which was mounted into a well of a 24-well plate in prior. The DMEM culture medium containing 20% FBS was added to the culture well. The cells were allowed to migrate for 10 h at 37 °C and 5% CO2. After stained with 0.1% crystal violet, the migrated clones were photographed under an optical microscope. The cell number was counted at 12 different areas. Data were averaged from three parallel experiments, which were normalized to that of the control.

Table 1

<table>
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<tr>
<th>Gene</th>
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<th>Length</th>
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<tr>
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2.3.9. Wound healing assay

After 70–80% confluent cells were treated with 100 μg/ml SiO2 particles for 4 h, a linear wound was made by scratching with a pipette tip. The culture well was washed twice with PBS and incubated with fresh regular medium. Wound closure of the cells after 16 h or 24 h was observed and photographed using an optical microscope. The assays were repeated twice and each sample was assayed in triplicate.

2.4. Statistic analysis

Results are reported as mean ± standard deviation. Data were analyzed using t-test for differences. The significant level was set as p < 0.05.

3. Results and discussion

3.1. Characterization of SiO2 particles

The RITC doped SiO2 particles prepared according to the Stober sol–gel method had a spherical morphology with a uniform-size of 80 nm and 500 nm, respectively, as characterized by TEM (Fig. 1). The relative ratio of fluorescence intensity between the 80 nm and 500 nm silica particles was found to be 0.68, which was used to normalize the uptaken amount of the particles inside cells by FCM.

3.2. Cellular uptake

Since the RITC molecules were chemically bound to APS and doped inside the SiO2 particles, uptake of the particles could be conveniently monitored under a fluorescence microscope and quantified with FCM by determining the red fluorescence emitted from the SiO2 particles-treated cells. For the FCM study, the logarithmic fluorescence intensity of untreated cells was set between 10^6 and 10^7. Therefore, the cells emitting fluorescence with an intensity larger than 10^5 were considered as the RITC-SiO2 particles-labeled cells [29]. Fig. 2A presents the typical FCM plots of fibroblasts incubated without or with 100 μg/ml SiO2 particles with diameters of 80 nm and 500 nm for 24 h, respectively. It shows that most of the fibroblasts after treatment were labeled with RITC. To investigate the uptake process, the fibroblasts were incubated with 100 μg/ml SiO2 particles for different time intervals (Fig. 2B,C). The average fluorescence intensity per cell for both SiO2 particles increased along with the prolongation of the culture time before 12 h, and then leveled off for the 80 nm SiO2 particles but continually increased for the 500 nm SiO2 particles. In the prior 4 h, the fluorescence intensity ratio of the fibroblasts treated with the 500 nm and 80 nm SiO2 particles was 1:1, but increased to be about 2:1 and 3:1 after 8 h and 24 h, respectively. Fig. 2C shows that after incubation for only 2 h more than 70% of the fibroblasts were labeled with SiO2 particles. The percentage further reached to >90% after 10 h. This would mean that extension of the culture time has a more prominent effect on improvement of the uptaken amount in each cell than the number of labeled cells.

It is known that the size and surface properties of the nanoparticles play key roles in their adhesion and interaction with cells. Since both the SiO2 particles were synthesized using the same method and had the similar zeta potentials (−5.2 ± 0.2 mV vs −9.8 ± 1.2 mV for the 80 nm and 500 nm particles in culture medium with 10% FBS, respectively), the different uptaken amount should be attributed to the size difference. It has been reported that a diameter of 50 nm might be optimal for gold nanoparticles to be uptaken by Hela cells [30]. However, for Caco-2 cells treated with PLGA particles, the 50 nm particles showed much lower uptake amount than the 200 nm and 500 nm particles [31]. The difference in cellular uptake behavior of different size particles may imply diverse uptake mechanisms, which therefore should be further investigated.

It is known that the extracellular substance can be transported into cells through several different pathways such as nonspecific diffusion, phagocytosis and receptor-mediated or fluid phase endocytosis [32]. To determine the uptake mechanism of the SiO2 particles, the following experiments were carried out. First, the culture temperature was decreased to 4 °C or the cells were treated with 100 mM sodium azide. Fig. 3A shows that the uptake efficiency was significantly blocked (P < 0.01 at 4 °C and P < 0.01 by sodium azide treatment), suggesting that uptake of the SiO2 particles is an energy-dependent process but not through nonspecific diffusion. Second, the specific endocytic pathways were determined by addition of amiloride-HCl, amantadine-HCl and genistein into the culture medium. There are several possible endocytic pathways for internalization of exogenous particles, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis and clathrin-caveolae-independent endocytosis [33]. Amiloride-HCI can lock Na+ /H+ -channels and inhibit uptake the particles via macropinocytosis [34]; amantadine-HCl inhibits clathrin-mediated endocytosis by preventing budding of clathrin-coated pits [35]; and genistein usually blocks tyrosine kinases of the Src-family involved in caveolae-mediated uptake [36]. As shown in Fig. 3B, uptake of the both SiO2 particles was significantly blocked by either 2.5 mM amiloride-HCl or 1 mM amantadine-HCl. However, 100 μM genistein influenced apparently on the uptake of the 80 nm particles (about 20% block, P < 0.01) but not the 500 nm particles. Therefore, internalization of the SiO2 particles should be mediated by several cellular uptake mechanisms. For the 80 nm SiO2 particles, the macropinocytosis and clathrin-mediated endocytosis take the major role, and the caveolae-mediated uptake pathway contributes to some extent too. It is known that the particles with a diameter of
50–60 nm are generally internalized in caveolas. However, a recent research found that the caveolas could accommodate polymer nanoparticles up to 100 nm [37]. In this work, the caveolae-mediated uptake pathway for the 80 nm SiO₂ particles was also observed, suggesting compliant property of the caveolae. For the 500 nm SiO₂ particles, the internalization was mainly through the macropinocytosis and clathrin-mediated endocytosis pathway. The stronger blocking effect of amiloride-HCl to the 500 nm SiO₂...
particles (80% and 60% for the larger and smaller particles, respectively, $P < 0.05$) indicates that uptake of the particles of a larger size depends more intensively on the macropinocytosis pathway.

3.3. Intracellular distribution

In the cellular uptake process, the exogenous particles are enclosed into endosomes initially, which mature into late...
endosomes or multivesicular bodies and eventually fuse with lysosomes accompanying with a significant drop of pH. To track the SiO2 particles following their uptake, the lysosomal compartment of the cultured fibroblasts was stained with the LysoTracker Green probe, and the cell nucleus was stained with DAPI. Fig. 4 shows that after incubation with 80 nm or 500 nm SiO2 particles for 24 h, there were large amount of red particles dispersed in the cytosol, some of which overlapped with lysosomes judging from the yellow color in the merged images (the third column). The existence of SiO2 particles in the lysosomes confirms that the macropinocytosis and clathrin-mediated endocytosis are the major uptake pathways. No colocalization of the particles with the nuclei was observed, demonstrating that the SiO2 particles cannot penetrate the nucleus membrane during the 24 h culture (the cylinder-like nuclear pores are 25–30 nm wide [38]).

3.4. Cytotoxicity

MTT assay was used to assess the toxicity of the SiO2 particles to the fibroblasts. Fig. 5 shows that the particles with different size caused different extent of cytotoxicity. After the cells were exposed to 80 nm SiO2 particles at 5, 25, 50, 100, and 200 mg/ml for 10 h, the cell viability decreased monotonously. At 50 mg/ml and 100 mg/ml particle concentrations, the cell viability was decreased to 83.8% and 73.9% compared with the control group, respectively. However, the 500 nm SiO2 particles showed very weak cytotoxicity over the same particle concentration. For example, the viability was only decreased to 91.4% at 200 mg/ml, the highest dosage used herein. Therefore, the SiO2 particles with a smaller diameter caused more obvious adverse effects on the human fibroblasts in terms of cell viability. A continue culture of the fibroblasts in particles-free medium for 24 h showed that the 80 nm SiO2 particles also blocked the cell proliferation to a larger extent than the 500 nm SiO2 particles, which did not show significant difference to the control even at a pretreatment concentration of 200 mg/ml. One can thus conclude that the smaller SiO2 particles are more toxic to the human fibroblasts. This is consistent with a very recent study that amorphous spherical SiO2 particles of smaller size (15 nm) caused severely cytotoxic damage to endothelial cells. When the size of the SiO2 particles was larger than 60 nm and 104 or 335 nm, milder and very low cytotoxic response was recorded [39]. In our another experiment, however, the smaller SiO2 particles showed lower toxicity to HepG2 cells (data not shown). The discrepancies may arise from the differences of the fabrication conditions and the cell lines used or the culture conditions. It is understandable that the very small particles have a large ratio of surface area to weight, and thereby possess higher activity to bring side effects. To clarify this point, the following experiments were further conducted.

Mitochondria are the cellular organelles regulating cell metabolism and death pathways. MMP is a key indicator of mitochondrial health, which reflects the pumping of hydrogen ions across the inner membrane during the process of electron transport and oxidative phosphorylation, the driving force behind ATP production.
After the human dermal fibroblasts were cocultured with 80 nm and 500 nm SiO₂ particles and stained by rhodamine 123, assays of MMP in the cells were qualitatively performed by optical microscopy (Fig. 6A) and quantitatively by FCM (Fig. 6B). While the 500 nm SiO₂ particles did not bring significant influence, the 80 nm SiO₂ particles induced more severe loss of mitochondria membrane integrity and caused significant weakening of MMP (Fig. 6B). This result combination with the decrease of dehydrogenase activity in mitochondria (Fig. 5A) suggests that the mitochondria dysfunction of the dermal fibroblasts is one of the major reasons for the cytotoxicity caused by the SiO₂ particles.

Excess production of free radicals might be another important reason responsible for the cell damage. It is known that the DCF fluorescence intensity is a sensitive indicator of intracellular ROS. During the incubation DCFH-DA enters into cells and reacts with ROS to produce DCF, an oxidized fluorescent compound, which can be detected with excitation and emission wavelengths of 499 nm and 530 nm, respectively. As shown in Fig. 6C,D, compared with the control exposure of the cells to 100 μg/ml SiO₂ particles for 10 h did not significantly enhance the generation of intracellular ROS regardless of the particle size. This result is different from a previous report that the freshly fractured silica could generate ROS both on the particle surface and in phagocytic cells attempting to digest the silica particles, consequently induced protein and DNA damage [41]. The main reason for this discrepancy is believed the different sample statuses. In our experiment the particles were exposed to air for a relatively long time, and no particle fracture happened and thereby the active atoms catalyzing the ROS production were not exposed. One can thus conclude that the excess production of free radicals is not a reason for the cytotoxicity brought by the uptake of SiO₂ particles.

3.5. Cell adhesion

Cell adhesion is of crucial importance in governing a variety of cellular functions including cell growth, migration, differentiation, survival and tissue organization. To study the effect of SiO₂ particles uptake on cell adhesion, the fibroblasts were exposed to 100 μg/ml SiO₂ particles for 10 h, detached, and then allowed to adhere for another 8 h or 24 h. Fig. 7 shows that the morphologies, attachment, and spreading behavior of the particles-treated cells were different from the untreated control cells. The particles-treated cells became less elongated and were in a more round morphology (Fig. 7B–C), which is consistent with a previous report that the HepG2 cells incubated with silicon nanowires exhibited a weaker adherence [42]. Fig. 7D shows that compared with the control the relative percentages of the 24 h-adhered cells were significantly decreased to 80% and 57% after the cells were pre-incubated with the 80 nm and 500 nm SiO₂ particles, respectively. It is obvious that the particles with a larger size caused more impedance on the adhesion and spreading of the fibroblasts. Considering that the 500 nm SiO₂ particles had slighter influences on cell viability (Fig. 5) and mitochondrial membrane potential (Fig. 6), some other mechanisms, e.g., regulation of the related gene expression level (as shown in Fig. 8) or dysfunction of the cytoskeleton, should be involved in the impairment of cell adhesion.

It is known that the cell adhesion depends deeply on the interactions between cell membranes and extracellular matrix [43]. Fibronectin and laminin are two important extracellular matrix glycoproteins governing cell adhesion, spreading and migration [44,45], and FAK is an intracellular kinase localizing to focal adhesion and regulating both cellular adhesion and antiapoptotic survival signaling [46]. In order to explore the molecular mechanism

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**Fig. 7.** (A–C) Merge of bright field images and fluorescence images of fibroblasts adhered for 8 h. The cells were incubated without (A) or with 100 μg/ml of (B) 80 nm RITC-SiO₂ and (C) 500 nm RITC-SiO₂ particles for 10 h, respectively, detached, and then seeded to the culture plate with equal number and allowed to adhere for another 8 h or 24 h. Scale bar = 50 μm. (D) Quantitative analysis of the cell adhesion from the merge images as shown in (A–C). The data were normalized to that of the control. Asterisk indicates significant difference at p < 0.05.
of the inhibitory cell adhesion, real-time RT-PCR was performed to examine the expression level of fibronectin, laminin and FAK after the fibroblasts were cocultured with the SiO2 particles for 10 h (Namely, the cells used for the adhesion study in Fig. 7). Fig. 8 shows that the mRNA levels of fibronectin and laminin were significantly decreased after uptake of the 80 nm SiO2 particles, while all the mRNA expressions were reduced after uptake of the 500 nm SiO2 particles. Compared with those of the 80 nm SiO2 particles-treated fibroblasts, the fibronectin and FAK expressions were significantly lower for the cells treated with 500 nm SiO2 particles. This alteration tendency is consistent with the results shown in Fig. 7, confirming that the poorer adhesion after particles uptake is attributed to the lower expression of the adhesion related proteins and kinase. It also reveals that the larger particles cause more severe impedance of fibronectin and FAK expressions, while both particles induced significant influence on the laminin expression.

Compared with the results in Fig. 7A–C, the morphology alteration was not significant in the cellular uptake experiment (Fig. 4), in which the cells were firstly allowed to attach for 24 h and then treated with the SiO2 particles. It is known that the cells need to secrete extracellular matrix proteins while they attach to the culture plate. In the cellular uptake experiment, the extracellular matrix secreted by the fibroblasts in the first 24 h should take a role for the better adhesion and morphology maintenance. In Fig. 7, however, after treated with the SiO2 particles for 10 h the cells were washed with PBS, detached and then placed into a new 24-well plate and allowed to adhere for different time. Since exposure to the SiO2 particles strongly affected the expression of adhesion related genes (Fig. 8), the adhesion behavior of the control cells and the particle-treated cells were obviously different.

Fig. 8. Gene expression of fibronectin, laminin and FAK detected by real-time quantitative RT-PCR. The fibroblasts were cultured without or with RITC-SiO2 particles of different diameters for 10 h. The data were normalized to that of the control. Asterisk indicates significant difference at \( p < 0.05 \) vs. control sample.

Fig. 9. (A–C) Optical images of human dermal fibroblasts which crossed through the pores of transwell chamber and stained by crystal violet. Scale bar = 50 \( \mu \)m. The cells were incubated without (A) or with 100 \( \mu \)g/ml of 80 nm RITC-SiO2 (B) and 500 nm RITC-SiO2 particles (C) for 10 h, respectively, detached, and then seeded to the upper chamber with a cell number of \( 10^5 \) cells and incubated for another 10 h. (D) Quantitative analysis of the cell migration from the images as shown in (A–C). The data were normalized to that of the control. Asterisk indicates significant difference at \( p < 0.05 \).
3.6. Cell migration

The cell migration participates in a variety of physiological and pathological processes such as embryonic development, cancer metastasis, blood vessel formation and remodeling, tissue regeneration, immune surveillance and inflammation [47]. To investigate the effect of SiO2 particles uptake on cell migration, firstly, the Millicell separate culture plate inserts were used and the results are shown in Fig. 9. Fig. 9A shows that the control human dermal fibroblasts migrated efficiently through the filter of transwell chamber within a 10 h culture period. By contrast, a much smaller number of cells could be found on the filters after the cells were pre-treated with the SiO2 particles (Fig. 9B,C). The quantitative analysis confirmed that the relative migration efficacy of the fibroblasts was significantly decreased from 100%, the control, to 41% and 43% after the cells were pre-exposed to 100 μg/ml 80 nm and 500 nm SiO2 particles for 10 h (Fig. 9D), respectively. Unlike the cell adhesion, no significant difference on the cell migration was found between the 80 nm and 500 nm particles-treated cells.

As a consequence of change of cell migration ability, the wound healing process shall be greatly influenced. Therefore, in the next study, the “scratch” wound closure assay was further performed. The fibroblasts were cultured until ~80% confluence and then continually cultured for 4 h with or without the SiO2 particles, respectively. After the cell monolayers were scratched to form linear wounds, they were cultured for another 24 h in particle-free medium. Fig. 10 shows that the control cells migrated into the denuded area and recovered the exposed surface at a shorter time period than those treated with the particles. For instance, compared to the ~80% of the wound healing of the control at 24 h, only 15% and 40% percentages of the wounds were healed for the cells pre-treated with 80 nm and 500 nm SiO2 particles, respectively. The faster healing of the cells treated with the 500 nm SiO2 particles than those treated with 80 nm SiO2 particles is consistent with the cytotoxicity and cell proliferation results (Fig. 5), which is believed the responsible reason too since the wound healing process involves both cell proliferation and migration.

A previous study on the adverse effects of titanium dioxide particles also found significant inhibition of cell migration to the human dermal fibroblasts by using an agarose droplet assay [48]. However, another study demonstrated that no inhibitory effect was observed for A375 human melanoma cells after treatment with mesoporous silica nanoparticles. By contrast, the migration rate even increased by the stimulus from the sphere-shaped particles [49]. The inconsistent phenomena may arise from the different cell lines, particles and evaluation methods being used. Therefore, in order to elucidate the relevant cellular mechanisms more thoroughly, further investigations are needed to compare the effects of the same kind of particles on different cell lines, both from tumors and normal tissues.

4. Conclusions

Herein the monodispersed spherical SiO2 particles with diameters of 80 nm and 500 nm were used to study the cellular uptake by human dermal fibroblasts and the influence on cell functions thereof. Both particles accumulated into the fibroblasts in a short time. While the cell viability and mitochondrial membrane potential were more heavily affected by the smaller ones, the adhesion and migration ability of the fibroblasts were impaired by both particles regardless of their size. RT-PCR characterization demonstrated that the mRNA expression of adhesion relevant genes such as fibronectin, laminin and FAK was significantly decreased. The results indicate that internalization of the SiO2
particles can not only cause direct damage to cell viability, but also induce impairment of some essential cellular physiological functions. Our findings also imply that comprehensive assessment of the toxicity of nanomaterials is necessarily preformed before their applications in biological fields, in particular for in vivo applications.

Acknowledgments

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Appendix

Figures with essential color discrimination. Figs. 2, 4–7, 9 and 10 in this article are difficult to interpret in black and white. The full color images can be found in the on-line version, at doi:10.1016/j.biomaterials.2010.07.060.

References