Effects of Nanostructurized Silicon on Proliferation of Stem and Cancer Cell

L. A. Osminkina[1], E. N. Luckyanova[1], M. B. Gongalsky[1], A. A. Kudryavtsev[2], A. Kh. Gaydarova[3,4], R. A. Poltavtseva[4], P. K. Kashkarov[1,5], V. Yu. Timoshenko[1], and G. T. Sukhikh[4]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 1, pp. 91-95, January, 2011
Original article submitted January 25, 2010

In vitro experiments showed that stem and cancer cells retained their viability on the surface of porous silicon with 10-100 nm nanostructures, but their proliferation was inhibited. Silicon nanoparticles of 100 nm in size obtained by mechanical grinding of porous silicon films or crystal silicon plates in a concentration below 1 mg/ml in solution did not modify viability and proliferation of mouse fibroblast and human laryngeal cancer cells. Additional ultrasonic exposure of cancer cells in the presence of 1 mg/ml silicon nanoparticles added to nutrient medium led to complete destruction of cells or to the appearance of membrane defects blocking their proliferation and initiating their apoptotic death.

Key Words: *silicon nanoparticles; stem cells; cancer cells; ultrasound; proliferation*

Silicon nanoparticles are now intensely studied as the basis for the creation of new diagnostic agents and drugs [3,4,7,8,10]. This interest is explained by biocompatibility and biodegradation of silicon nanocrystals (nc-Si) [3,4,8,10] and by recently discovered possibility to obtain these nanoparticles in great amounts for use in food industry [1]. The possibility of adding nc-Si to toothpastes and to foodstuffs to prolong their shelf life is now studied [3,4,5,7]. Recent studies revealed no appreciable cytogenetic and teratogenic effects of nc-Si *in vivo*. Their slight genotoxic activity in mouse bone marrow and brain cells was demonstrated [1]. On the other hand, porous silicon nanoparticles demonstrated their useful properties in brachiotherapy [13] and photodynamic therapy [2].

We studied *in vitro* effects of nanostructurized silicon surfaces and nanoparticles on proliferation of stem and cancer cells, including combined effects of silicon nanoparticles and ultrasonic exposure for stimulation of the effect of nanoparticles.

MATERIALS AND METHODS

Nanostructurized silicon surfaces were obtained by standard electrochemical pickling of crystal silicon (c-Si) plates of p-type conduction with surface orientation (100) and specific resistance of 25 mΩ×cm in HF(50%):C_2H_5OH solution at pickling current density of 60 mA/cm^2. Layers of the so-called mesoporous (pSi) silicon with porosity of about 50% were thus formed on a c-Si sublayer [6]. The thickness of pSi layer was 15 μ. Atomic force microscopy showed nanostructures (protrusions and depressions of 10-100 nm) on pSi layer. Silicon nanoparticles were prepared by pulverization of several types of silicon powders in a FRITSCH Pulverisette 7 planetary mill for 30 min by a previously described method [5]. Nanoparticles based on crystal silicon (nc-cSi) and porous silicon (nc-pSi) were prepared. nc-cSi nanoparticles were prepared from powder made from fragmented c-Si

[1]Physical Faculty, M. V. Lomonosov Moscow University; [2]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region; [3]I. M. Setchenov Moscow Medical Academy; [4]V. I. Kulakov Center of Obstetrics, Gynecology, and Perinatology; [5]Kurchatov Institute, Moscow, Russia. ***Address for correspondence:*** osminkina@vega.phys.msu.ru. L. A. Osminkina

plates. nc-pSi nanoparticles were prepared from detached films of porous silicon formed by electrochemical pickling as described above. Transmission electron microscopy showed that the nc-cSi specimens were crystal granules of 100 nm, while nc-pSi consisted of porous medium-sized (20-100 nm) particles with 2-5-nm pores.

In order to study cell proliferation processes, presterilized plates with pSi layers were plunged for 24 h into 30-mm Petri dishes with DME, after which the medium was replaced with complete nutrient medium (DMEM+glutamin+10% fetal calf serum) and the cells were inoculated. Stem cells were isolated from human fetal spinal cord and cultured throughout up to 10 passages before the use in the experiments. After 5, 10, and 16 days of cell culturing on nanostructurized plates, the plates were removed and stained with fluorescent stains (Acridine Orange and Propidium Iodide mixture) for further analysis. After 5-min staining the plate with cells was plunged in fresh phosphate buffer and analyzed under a fluorescent microscope. The cells on pSi surfaces were additionally examined under a LEO 1455 VP scanning electron microscope.

The effects of silicon nanoparticles were studied as exemplified by Hep 2 human laryngeal cancer cells and 3T3 NIH mouse fibroblast cultures. The cells were cultured in DME with 10% fetal serum, 5% CO_2, and antibiotics in Carrel's vessel (25 cm^2, 5 cm^3). After 24 h of culturing, silicon nanoparticles (suspension in culture medium) were added. The cells cultured under the same conditions without nanoparticle powder served as the control. In addition, effects of ultrasound (US) on cell proliferation were studied. Culture medium was completely replaced after US exposure in all cultures, including the control (without nanoparticles and US). After 24 h, the cells were removed with trypsin, counted, and their phase composition was analyzed on a PAS III flow cytofluorometer. Ultrasonic exposure was carried out in an UZV6-0.063/37 ultrasonic tank at working frequency of 37 kHz and maximum power of 62 W. The mean power density was no more than 0.5 W/cm^2, but the intensity varied in different places of the tank, which was taken into consideration [9]. Culture vessels were placed into zones with the maximum US power (center of the tank, 2 W/cm^2) and in zones of the minimum intensity (tank edges, 0.2 W/cm^2). It is noteworthy that the maximum intensity was similar to that of therapeutic US [11] and could lead to warming of the biological systems. In order to minimize this thermal effect, constant temperature (37°C) in the US tank was maintained with a thermostat. The results of cell count estimation were statistically processed and expressed as the mean arithmetic±error of the mean (M±m). The significance of differences was evaluated using Student's t test (p<0.05).

RESULTS

After 5 days of culturing, the count of stem cells on pSi surface was 0.4±0.1 of cell count in control cultures; 60±10% cells on pSi surface were flat, while the rest were globular (Fig. 1, *a*). In the control, the relative content of flat cells was 95±5%. After 10 days of culturing, the cell count on pSi surface decreased to 0.3±0.1 of control, while the relative content of flat cells on pSi surface decreased to 20±5% (Fig. 1, *b*). Detailed analysis of cells by scanning electron microscopy (Fig. 1, *e*, *f*) showed similar results. After 16 days of culturing, the cell content on pSi surface was 0.35±0.15 of control, *i.e.* virtually the same as after 10 days of incubation. This indicated certain adaptation of cells to existence on pSi surface. The relative number of cells flattened on nanostructurized surface increased to 80±10%, they looked similar as control cells. These results indicated that stem cells were viable, but their proliferation on nanostructurized silicon surface was inhibited during that period. Similar results (inhibition of cell proliferation) were observed in experiments with Hep 2. This indicated similarity of the processes determining the behavior of different cell types on the surface of this material. The mechanism of this effect could be determined by local electrical fields on the surface and by the purely chemical effects of orthosilicic acid forming during partial dissolution of pSi in water [3].

The presence of silicon nanoparticles in concentrations of 1 mg/ml and lower virtually did not appreciably change (p>0.05) proliferation of Hep 2 and 3T3 NIH cells. A slight increase in the counts of 3T3 NIH cells was observed at silicon nanoparticles concentrations of 0.5-2.5 mg/ml. Increasing nanoparticles concentration above 3 mg/ml led to a significant reduction

TABLE 1. Relationship between Percentage of 3T3 NIH and Hep 2 Cells and nc-pSi Silicon Nanoparticles Concentration in Nutrient Solution (% of Control)

Nanoparticles concentration, mg/ml	3T3 NIH	Hep 2
0.15	100±10	100±10
0.3	100±10	100±10
0.5	105±10	96±9
1.0	106±11	94±8
1.5	117±12*	84±8*
3.0	80±8*	70±7*
4.0	74±7*	68±5*
5.0	56±6*	65±5*

Note. Here and in Table 2: *p≤0.05 compared to the control.

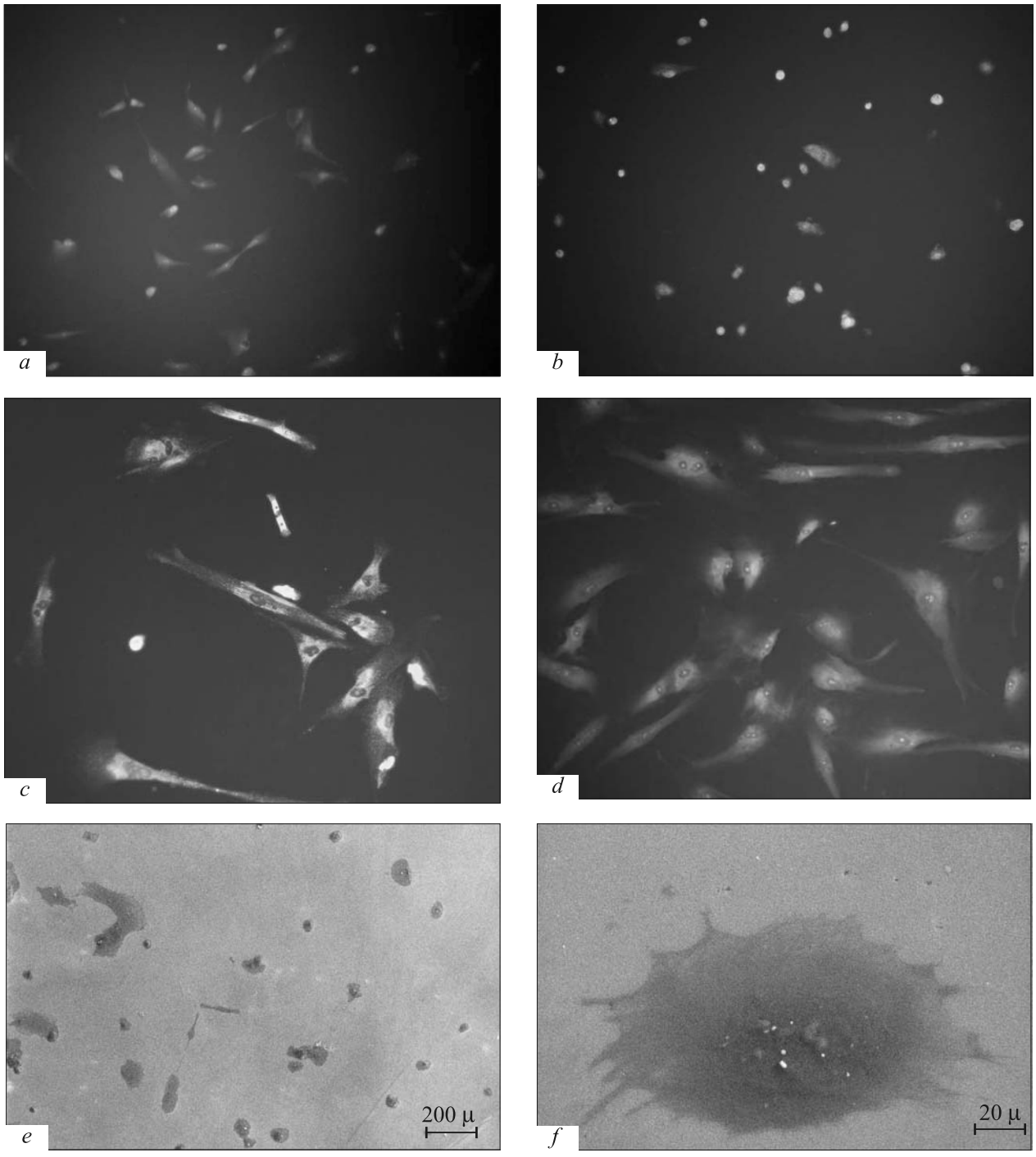

Fig. 1. Stem cells under a fluorescent microscope after 5 (*a*), 10 (*b*), 16 (*c*) days of culturing on pSi surface and after 16 days (*d*) on Petri dish surface (control), and under a scanning electron microscope (*e*, *f*) after 10 days of culturing on pSi surface.

of cell count in comparison with the control (Table 1).

Study of combined effects of silicon nanoparticles and US exposure on Hep 2 cell proliferation showed that US exposure of the maximum intensity (2 W/cm^2) led to removal of 30±5% of control (without nanoparticles) cells from the surface of culture vessel. The cells remaining on the surface and detached cells, inoculated in Petri dishes, were viable and actively proliferated. Ultrasonic exposure in the presence of nanoparticles led to a significant reduction of cell count. The cells were completely detached from culture vessel surface at the maximum US intensity. In experiments with nc-cSi nanoparticles the cell content reduced to 0.25±0.05% (4-fold) of control after 30-min

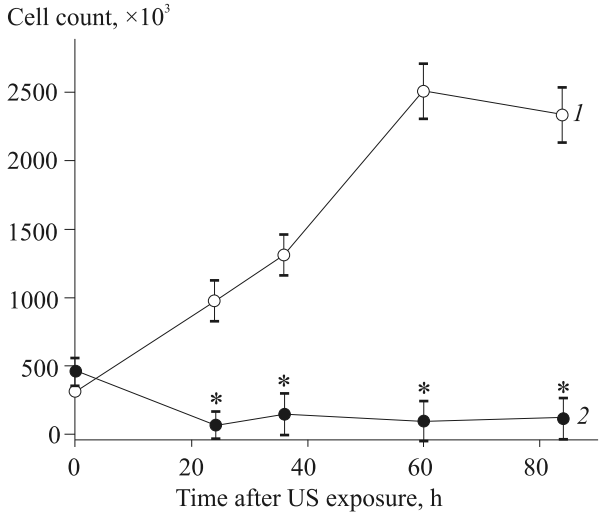

Fig. 2. Changes in the count of Hep 2 cells after US exposure (30 min, minimum intensity) in the absents (*1*) and presents (*2*) of 1 mg/ml nc-pSi nanoparticles. *$p<0.05$ compared to the control.

US exposure of the maximum intensity (Table 2). In the presence of smaller nanoparticles (nc-pSi), the cell count after US exposure was below the detectable level. Microscopic studies of vessel surface and nutrient medium volume after their cleansing from nanoparticles and replacement of nutrient solution for a fresh portion showed few small groups of 1-10 cells with extremely low proliferative activity.

These results indicated that silicon nanoparticles caused cell damage under conditions of US exposure. Presumably, nanoparticles served as centers of the appearance of cavitation bubbles leading to cell destruction. The highest effect in experiments with nc-pSi can be explained by hydrophobic nature of the surface of these nanoparticles [6], presumably stimulating local heat release near the nanoparticles as a result of US

TABLE 2. Count of Hep 2 Cells (×10³) after US Exposure (30 min, Maximum Intensity) in the presence of nc-pSi and nc-cSi Nanoparticles in a Concentration of 1 mg/ml

Cells	Control	US exposure
With nc-cSi	240±20	50±5*
With nc-pSi	200±20	<0.1*

exposure. Mechanical injury of cells by the nanoparticles swinging in the US wave ("nanoscalpel" effect) is also possible.

The counts of control cells and cells with nc-pSi nanoparticles directly after US exposure of low intensity (0.2 W/cm²) virtually did not differ, being within the range of statistical error ($p>0.05$). However, the count of control cells continued to increase with time, while that of cells incubated with nanoparticles decreased until complete cessation of proliferation during 80 h (Fig. 2).

The phase composition of cells after US exposure of low intensity is presented (Fig. 3). The distribution curves for control cells (Fig. 3, *a*) corresponded to the phase distribution in a normally proliferating culture (without US exposure or nanoparticles). The distribution curves for cells with nanoparticles exposed to US showed significant distortions (Fig. 3, *b*): SG1 phase of the cell cycle appeared, which attested to apoptosis [12].

Hence, the data indicate the possibility of using silicon nanostructures for regulation of stem and cancer cells proliferation. For example, the surface of nanostructurized silicon films inhibits stem cell proliferation, which can be used for their preservation until further use. The results of combined exposure

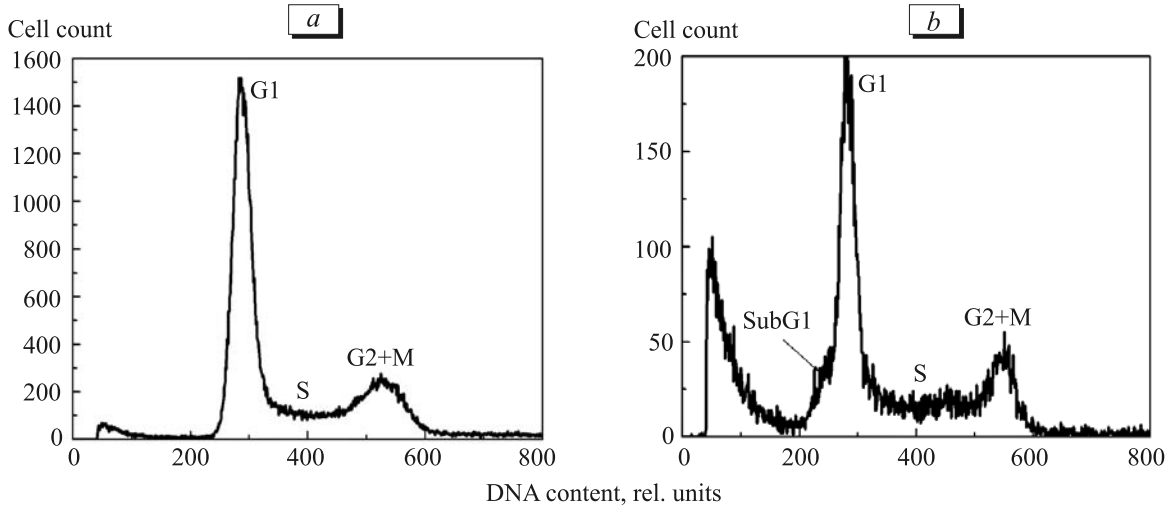

Fig. 3. Distribution of cultured Hep 2 cells by cell cycle phases after 30-min exposure to US of minimum intensity in the absents (*a*) and presents (*b*) of 1 mg/ml nc-pSi nanoparticles.

of cancer cells to silicon nanoparticles and ultrasound depended on US intensity: the cells were completely destroyed or developed defects leading to loss of proliferative activity and subsequent apoptotic death. These data can be used in medical practice, presumably for the treatment of cancer. Further experiments, including *in vivo* tests, should be carried out for more ample evaluation of the potentialities of these bioactive characteristics of silicon nanostructures and nanoparticles.

The authors are grateful to S. S. Abramchuk (Moscow State University) for measurements by transmission electron microscopy, to R. A. Sennov (Moscow State University) for measurements by scanning electron microscopy, and to A. V. Revishchev (Institute Gene Biology, Russian Academy of Sciences) for assistance in measurements by the fluorescent microscopy.

The study was partially supported by the Ministry of Education and Science of the Russian Federation (State Contract No. 02-513.12.3058). Specimens of nanostructures were made using the equipment at M. V. Lomonosov Moscow State University.

REFERENCES

1. A. D. Durnev, A. S. Solomina, N. O. Daugel-Dauge, *et al.*, *Byull. Exp. Biol. Med.*, **149**, No. 4, 429-433 (2010).
2. V. Yu. Timoshenko, A. A. Kudryavtsev, L. A. Osminkina, *et al.*, *Pis'ma Zh. E. T. F.*, **83**, No. 9, 492-495 (2006).
3. S. Anderson, H. Elliott, D. J. Wallis, *et al.*, *Phys. Status Solidi a*, **197**, 331-335 (2003).
4. L. T. Canham, *Adv. Mater.*, **7**, No. 12, 1033-1037 (1995).
5. L. T. Canham, *Nanotechnology*, **18**, No. 185704, 1-6 (2007).
6. A. G. Cullis, L. T. Canham, and P. D. J. Calcott, *J. Appl. Phys.*, **82**, No. 3, 909-965 (1997).
7. R. Jugdaohsingh, S. H. Anderson, K. L. Tucker, *et al.*, *Am. J. Clin. Nutr.*, **75**, No. 5, 887-893 (2002).
8. S. P. Low, N. H. Voelcker, L. T. Canham, and K. A. Williams, *Biomaterials*, **30**, No. 15, 2873-2880 (2009).
9. I. P. Marangopoulos, C. J. Martin, and J. M. Hutchison, *Phys. Med. Biol.*, **40**, No. 11, 1897-1908 (1995).
10. J. H. Park, L. Gu, G. von Maltzahn, *et al.*, *Nat. Mater.*, **8**, No. 4, 331-336 (2009).
11. C. A. Speed, *Rheumatology* (Oxford), **40**, No. 12, 1331-1336 (2001).
12. H. Steller, *Science*, **267**, 1445-1449 (1995).
13. K. Zhang, S. L. Loong, S. Connor, *et al.*, *Clin. Cancer Res.*, **11**, No. 20, 7532-7537 (2005).