

Cytotoxicity of nanosize V₂O₅ particles to selected fibroblast and tumor cells

Siniša Ivanković^a, Svetozar Musić^b, Marijan Gotić^{b,*}, Nikola Ljubešić^c

^a Division of Molecular Medicine, Ruđer Bošković Institute, Bijenicka c 54, P.O. Box 180, HR-10002 Zagreb, Croatia

^b Division of Materials Chemistry, Ruđer Bošković Institute, Bijenicka c 54, P.O. Box 180, HR-10002 Zagreb, Croatia

^c Division of Molecular Biology, Ruđer Bošković Institute, Bijenicka c 54, P.O. Box 180, HR-10002 Zagreb, Croatia

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Abstract

Two kinds of nanosize V₂O₅ particles were synthesized in our own laboratory and concomitantly applied to V79 and L929 fibroblasts and SCCVII, B16F10 and FsaR tumor cells. The morphologies of the cells were monitored using an inverted inverse microscope equipped with digital camera, while quantitative determination of the cytotoxicity of nanosize V₂O₅ particles was measured using crystal violet bioassay. Twenty four hours after the addition of nanosize V₂O₅ particles (20 μM), noticeable changes in the morphology and density of fibroblast and cancer cells were observed. Reculturing in a freshly prepared medium for the next 24 h showed a high recovery effect on V79, SCCVII and B16F10 cells, while FsaR and L929 cells were seriously damaged and unable to recover. At a higher concentration of nanosize V₂O₅ particles (100 μM), the cytotoxicity of V₂O₅ prevailed against the recovery effect in all cell types. Quantitative measurements have shown that the resistance of investigated cell cultures to the cytotoxicity of nanosize V₂O₅ particles decreases in the order V79 > SCCVII > B16F10 > FsaR > L929. The high cytotoxic effect found on FsaR cells suggests that nanosize V₂O₅ particles could be regarded as poisoning material in the treatment of FsaR fibrosarcoma cells. Possible mechanisms involved in the cytotoxicity of nanosize V₂O₅ particles were discussed.

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1. Introduction

Nanoparticles have been extensively studied for their possible application in various fields in biomedicine, such as drug delivery, imaging, diagnostics, cell separation and purification, as well as in cancer treatment. Roughly, nanoparticles may be categorized into polymeric nanoparticles, polymeric micelles, dendrimers (macromolecular compounds that comprise a series of branches around the inner core), liposomes (small artificial vesicles of spherical shape that can be produced from natural non-toxic phospholipids and cholesterol), quantum dots (particles that are generally composed from group II–IV or III–V of the periodic table

with physical dimensions smaller than the excitation Bohr radius) and metal oxide nanoparticles that could be further categorized into ceramic, semiconducting or magnetic nanoparticles (Sahoo and Labhassetwar, 2003). Liposomes, polymeric nanoparticles and dendrimers are mainly used as drug carriers to overcome resistance phenomena (Vauthier et al., 2003) or to deliver and increase the efficacy of anti-cancer agents such as doxorubicin (Soma et al., 2000), mitoxantrone (Reszka et al., 1997), camptothecin (Miura et al., 2004) and others. On the other hand, metal oxide particles are active antitumor substances. In comparison with polymer and organic nanoparticles, metal oxide nanoparticles possess several advantages, such as compatibility with biological systems, possible tailoring of their size, shape and porosity or surface modification by different functional groups. These particles are also stable against

* Corresponding author. Tel.: +385 1 4561 123; fax: +385 1 4597 222.
E-mail address: gotic@irb.hr (M. Gotić).

the change in pH and/or temperature. In addition, in the metal oxide nanoparticles there are no swelling or porosity changes.

For example, magnetic metal oxide nanoparticles (magnetite and maghemite) have found application in cancer therapy (Jordan et al., 1999). These magnetic nanoparticles can be incorporated into malignant cells to generate heat under an alternating magnetic field by remagnetization losses. The heating so obtained at about 43 °C (hyperthermia) destroys cancer cells, because their oxygen supply via the blood vessels is not sufficient (Kawashita et al., 2005). The advantage of this technique is in its potential of treating embedded tumors in vital regions where surgical resection is not feasible. TiO₂ and Fe–TiO₂ nanoparticles have also been used in the treatment of tumor cells. Fujishima et al. (1986, 2000) and Cai et al. (1991, 1992) were the first to show that illuminated TiO₂ colloidal particles could be effective in killing tumor cells cultured in vitro and in vivo. In our previous work (Ivanković et al., 2003) it was shown that squamous carcinoma cells SCCVII cultured in vitro could be effectively killed by nanosize iron-doped TiO₂ particles in the presence of UV irradiation. Two different roles of iron were found: (a) the presence of iron dopant in nanosize TiO₂ particles stimulated the photokilling of cancer cells under UV irradiation and (b) iron provided the recovery of cell proliferation after reculturing in the dark. The advantage of the technique is its high selectivity, because the photocatalytic reactions only occur under illumination. The disadvantage of the photokilling effect is the difficulty of illuminating the tumor cell growth deep inside the body.

It is obvious that tumor cells could be treated with various metal oxide nanoparticles, and specifically V₂O₅ nanoparticles have an excellent potential due to the high cytotoxicity and antitumor effects of vanadium. However, while the cytotoxicity effects of various soluble vanadium salts (sodium metavanadate, vanadyl sulfate, ammonium vanadate, peroxovanadates, etc.) have been extensively investigated in the literature (Evangelou, 2002; Morinville et al., 1998; Mukherjee et al., 2004) the cytotoxicity effects of nanosize V₂O₅ particles were a new approach to this subject. The toxicity of vanadium salts differed among salt speciation and in the reference literature we did not find the standard vanadium salt to compare with the cytotoxicity of nanosize V₂O₅ particles. Generally, it is known that vanadium oxides could be more toxic than vanadium salts. In addition, Capella et al. (2002) showed that the same vanadium compounds could possess selective cytotoxicity to different cell lines; among two epithelial kidney cell lines, MDCK cells were resistant to vanadate whereas Ma104 cells, which expressed the multidrug-resistant phenotype, were highly sensitive to the same vanadium compound. These findings motivate us to apply nanosize V₂O₅ particles to five cell lines in order to determine (i) the cytotoxic effect of nanosize V₂O₅ particles (in vitro) and (ii) to determine if the nanosize V₂O₅ particle showed selective cytotoxicity among the selected cell lines.

Besides the cytotoxic effect of vanadium, there are numerous reports on the biological activities of specific vanadium compounds due to their insulin-mimetic effect (Morinville et al., 1998; Rehder, 2003; Tsiani and Fantus, 1997). These compounds encompass (a) the stimulation of glucose intake into living cells, thus lowering the blood glucose level, (b) the inhibition of glyconeogenesis and glycogenolysis, and (c) the inhibition of lipolysis. The therapeutic effects of vanadium were demonstrated in insulin tolerance, type II diabetic rodents which did not respond to exogenously administered insulin (Goldwaser et al., 2000). However, the possibility of explaining the cytotoxic effect of vanadium on the basis of its insulin-mimetic effect has never been used in the reference literature.

In an earlier paper (Ivanković et al., 2003) we showed that well-dispersed WO₃, TiO₂ and Fe–TiO₂ nanoparticles, synthesized and characterized in our own laboratory (Šijaković-Vujičić et al., 2004), were almost completely non-toxic to SCCVII cells. In contrast, some of these nanoparticles were highly cytotoxic to SCCVII cells, but only in the presence of UV irradiation. In the present work we report new results on the cytotoxicity of nanosize V₂O₅ particles, synthesized and characterized in our own laboratory, to selected fibroblast and cancer cell lines. Vanadium oxide nanoparticles applied to cancer cells showed quite a different cytotoxicity behavior in comparison with TiO₂ and Fe–TiO₂ nanoparticles. We have shown that nanosize V₂O₅ particles are cytotoxic to all cell lines in a dose-dependent manner per se and that there is no need for the UV irradiation assistance. Nanosize V₂O₅ particles killed most effectively the L929 and FsaR cells, whereas the fibroblast V79 cells and the squamous carcinoma cells SCCVII showed highest resistance to the treatment with nanosize V₂O₅ particles.

2. Materials and methods

2.1. Materials

Dowex[®] 50W-X (20–50 mesh) cation exchange resin with large effective pore size, (Bio-Rad Laboratories), vanadium(V)-triisopropoxide oxide (95–99%), VO[CHO-(CH₃)₂]₃, supplied by Alfa Aesar[®], isopropanol, (CH₃)₂-CHOH, by Aldrich, HCl (p.a.), NaVO₃ (p.a.) and NaOH (p.a.), by Kemika, were used as received. Water obtained from the Milli-Q purified system was used.

2.2. Synthesis of V₂O₅ nanoparticles

Nanosize V₂O₅ particles were synthesized using two different procedures. Sample V1 was synthesized by the sol-gel procedure using 187 ml of isopropanol, 25 g of vanadium(V)-triisopropoxide oxide and 25 ml of water. Synthesis was performed in an oil bath at 70 °C in a specially designed all-glass assembly with reflux and a bubbling extra dry N₂. Sample was dried in a Petri dish at 60 °C for 48 h. Calcination was performed at 240 °C.

Before being used in cytotoxicity experiments the V_2O_5 powder was weighed and resuspended in a certain volume of Millipore-Milli-Q purified water using an ultrasonic bath for 20 min (Gotić et al., 2003). This sample consisted of irregular particles of about 100 nm in size. TEM image, FT-IR and Raman spectra, XRD analysis and other microstructural characterization of this sample is given in Gotić et al. (2003).

Sample V2 was synthesized by a completely different procedure in comparison with sample V1. This sample was obtained by passing the 0.1 M $NaVO_3$ solution through Dowex cation exchange resin until the pH reaches a value slightly below 2.0. The clear-yellow solution thus obtained was aged in a closed flask at 90 °C for 5 days. The clear solution turned orange and red upon ageing, while its viscosity progressively increased. A dense colloid suspension with the color of rust (dark red) was formed. The dense colloid suspension thus obtained was poured into a very broad Petri dish and dried at 60 °C for 48 h under static-air conditions. Before being used in cytotoxicity experiments the isolated powder was weighed and resuspended in a certain volume of Millipore-Milli-Q purified water using an ultrasonic bath for 10 min. This synthesis resembles our synthesis of WO_3 particles (Gotić et al., 2000) and the synthesis of V_2O_5 according to Livage (Livage, 1998). Fig. 1 shows a TEM image of sample V2. This sample consists of quite uniform V_2O_5 nanoparticles of about 20 nm in size.

2.3. Suspensions

Suspensions of nanosize V_2O_5 particles were prepared by weighing an appropriate amount of V_2O_5 powders in a 50 ml volumetric flask and diluted with the Milli-Q purified water up to the mark. Suspensions were then ultrasonically dispersed for 15 min. Only the finely dispersed

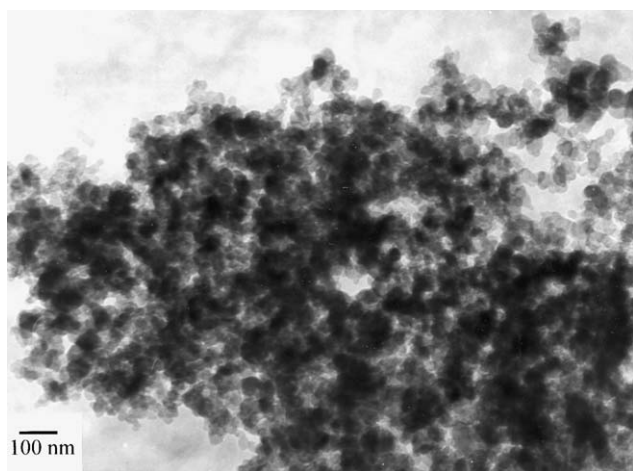


Fig. 1. TEM photograph of sample V2. This sample consists of quite uniform V_2O_5 nanoparticles of about 20 nm in size. TEM photograph of V1 sample that consists of V_2O_5 nanoparticles of about 100 nm was given in Gotić et al. (2003).

particles from the top of the suspensions were used in the cytotoxicity experiments. The proper concentration of nanosize V_2O_5 particles in the aqueous suspension was determined by a simple calculation of the weight/volume ratio, upon the evaporation of an aqueous phase up to dryness from the known volume of an aqueous suspension. The calculated concentrations of both V_2O_5 suspensions were adjusted to $1.0 \pm 0.1 \times 10^{-3}$ M. All weighing was performed on an analytical balance (5 decimals) produced by Sauter.

2.4. Cell culturing, incubation, survival and crystal violet bioassay

In the present work, model cell lines, V79 and L929 fibroblasts and SCCVII, B16F10 and FsaR tumor cells were used. L929, SCCVII and FsaR cells belongs to the same genetic background, i.e. they originated from mice strain C3H. SCCVII cells belong to the epithelial tumor of ectodermal origin, whereas FsaR cell line is of mesodermal origin, so that we can compare the tumors with the same genetic background and different hystogenesis. Mouse melanome B16F10 is originated from mice strain C57BL6. It is an experimental model cell line often used for the investigation of the sensitivity of various anticancer materials to melanoma. V79 Lung Hamster fibroblasts are a model cell line that has been frequently used in the investigation of vanadium toxicity. All cell lines were grown in a RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) in a humidified incubator with an atmosphere of 5% CO_2 in air at 37 °C.

For each experiment, SCCVII, FsaR, B16F10, V79 and L929 cells were harvested and plated on 24-well chambers (1 ml/well) at an initial concentration of approximately 5×10^4 cells/ml. When the cells reached confluence, the old cultured medium was replaced with a freshly prepared RPMI 1640 medium with FCS, then V_2O_5 suspensions (sample V1 or V2) were added. The final concentrations of V_2O_5 in 1 ml-well were adjusted to 20 or 100 μ M of V_2O_5 . Control cells were incubated in a vanadium-free medium. Ninety minutes after the addition of nanosize V_2O_5 particles, vanadium-treated and control cells were visualized (Fig. 2) using an inverted inverse microscope produced by Zeiss, model Axiovert 35, equipped with the digital microscopy camera processed with the Viewfinder program. After that the cells were incubated for 24 h, the old medium was replaced by a freshly prepared medium with FCS, and all cells were again visualized with an inverted microscope (Fig. 3). Then all cells were recultured in a freshly prepared medium for an additional 24 h and again visualized to determine the cell recovery effect (results not shown). The treated and untreated cells were fixed by the addition of 1% glutaraldehyde solution for 15 min, washed with deionized water, dried in air, allowed to stain with 0.1% crystal violet for 20 min, then extensively washed with deionized water and allowed to dry overnight. The cells thus fixed were again visualized (Fig. 4). To obtain

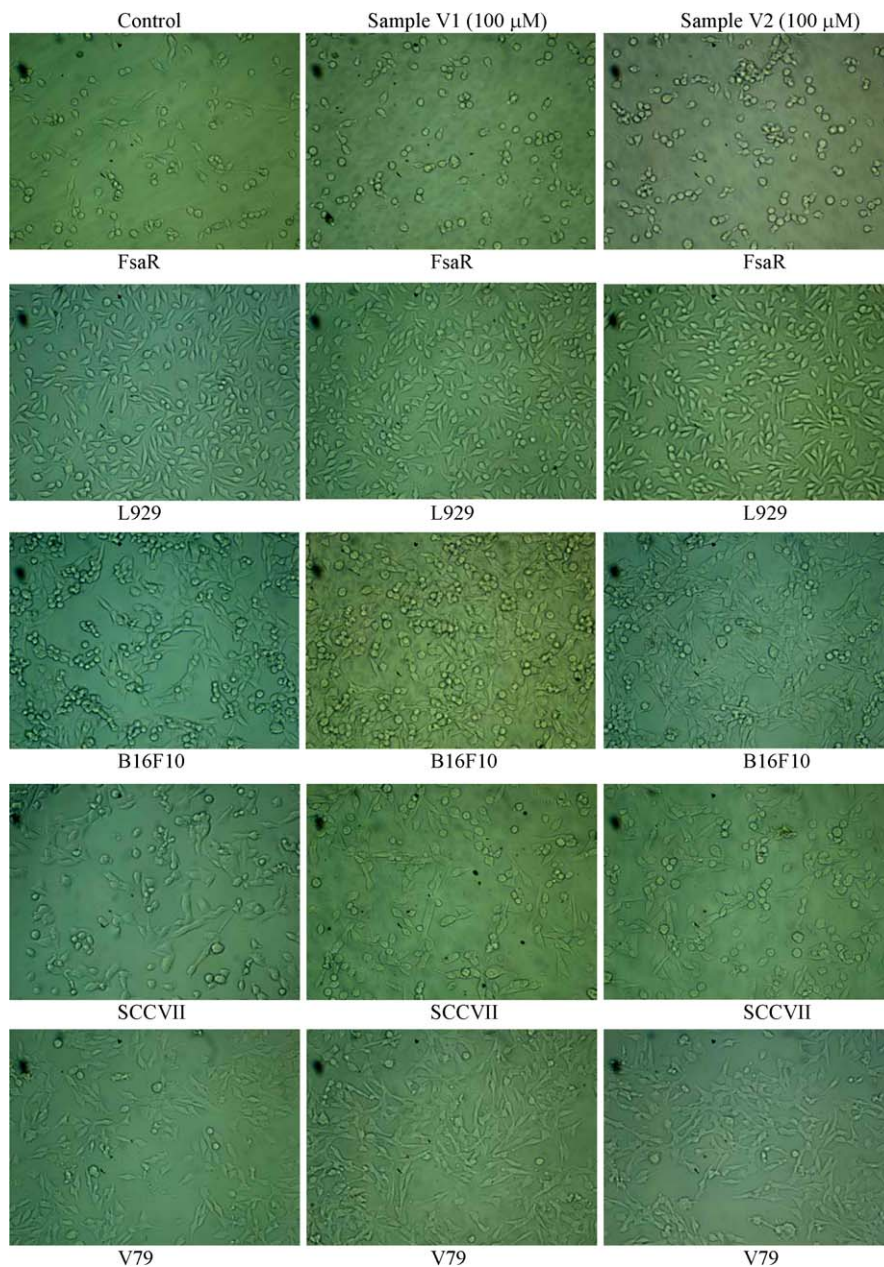


Fig. 2. Photomicrographs of control cells and concomitantly treated cells shortly (within 90 min) after the addition of 100 μM of V_2O_5 , as samples V1 and V2. All control cells are of high density with well-defined morphology characteristic of a certain line culture. Addition of nanosize V_2O_5 particles in the form of suspension did not destroy cell morphology, nor was there any influence on the density of cells.

quantitative data (Fig. 5), the dye was extracted from the cells using a 0.2% solution of Triton X-100, then absorbance was read at 590 nm using the UV–visible spectrophotometer, model Perkin Elmer (crystal violet bioassay).

3. Results

Fig. 2 shows photomicrographs of control cells and treated cells shortly (90 min) after the addition of 100 μM of V_2O_5 , as sample V1 or V2. All control cells are of high density, with well-defined morphology characteristic of a certain cell line culture. This figure shows that addition of both samples in the range of their highest concentration

of 100 μM did not produce extra material on the cells. Also, it is visible that addition of nanosize V_2O_5 particles in the form of suspension did not destroy cell morphology nor was there any influence on the density of cells (within 90 min). This is important, because even completely a non-toxic nanoparticle, such as TiO_2 nanoparticles, could be significantly toxic to the cells in the case that nanoparticles were not well-dispersed. In a previous work (Ivanković et al., 2003), we showed that the well-dispersed TiO_2 and Fe– TiO_2 nanoparticles were almost completely non-toxic to cells. The survival fraction of 100% for nanosize TiO_2 compared with control cells was reported in literature for HeLa cells (Cai et al., 1992). By contrast, the same but

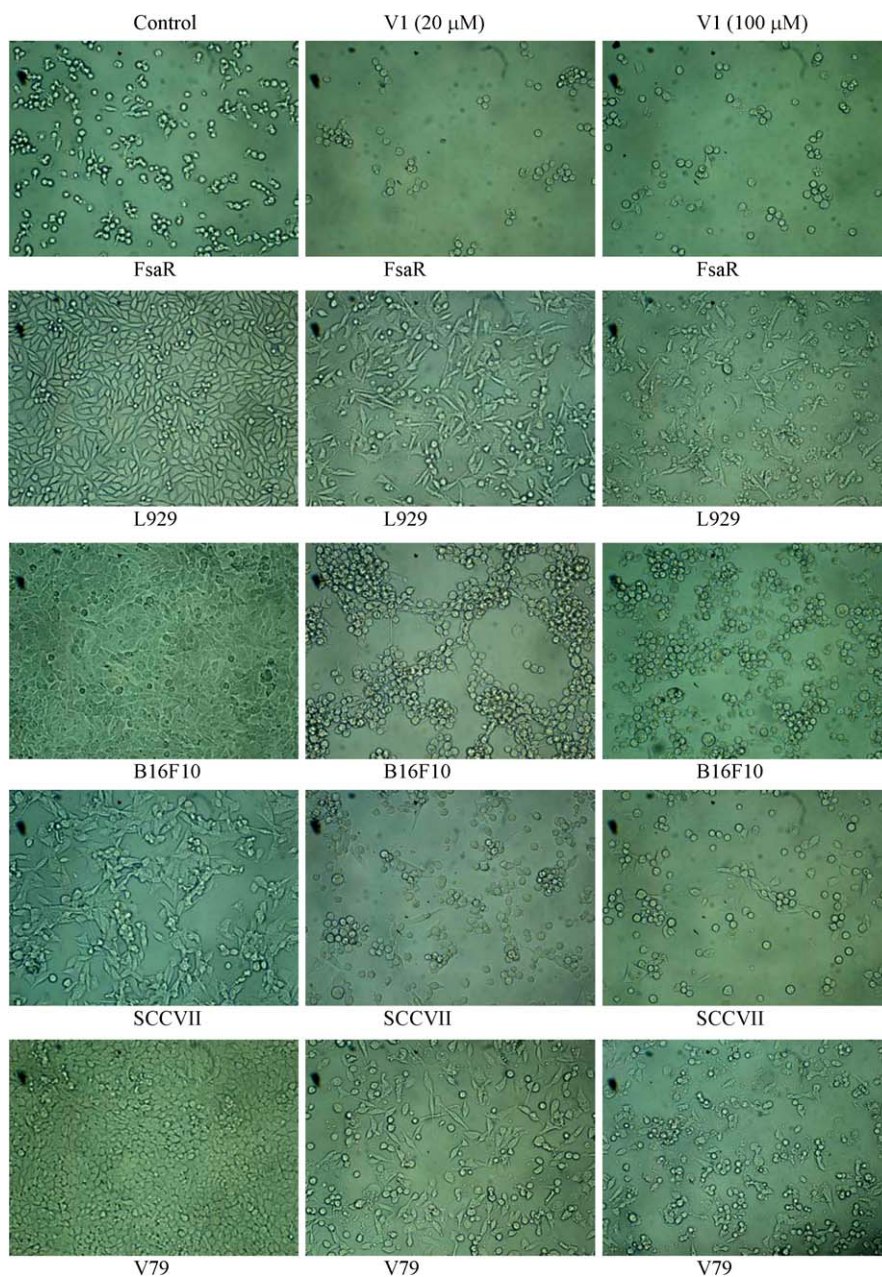


Fig. 3. Photomicrographs of control and treated cells 24 h after the addition of 20 and 100 μM of V_2O_5 , as sample V1. Both concentrations of V_2O_5 (20 and 100 μM) had an effect on the morphology and density of cells. V_2O_5 -treated cells became rounded and their number was reduced in comparison with control cells. At a higher concentration of V_2O_5 the effect was more pronounced. Fine yellow spots were seen inside the cells, the most clearly in B16F10 and SCCVII cells. These spots correspond to V_2O_5 nanoparticles that have enough time to accumulate and aggregate inside the cells.

not well-dispersed nanoparticles were cytotoxic to cells per se. These large particles produced a great deal of extra material deposited on the cells and thus “mechanically” damaged the cells (Ivanković et al., 2003). In addition, large particles are not able to penetrate into cells.

Fig. 3 shows photomicrographs of control cells and treated cells 24 h after the addition of 20 and 100 μM of V_2O_5 , as sample V1. This figure shows the effect of two different concentrations of V_2O_5 nanoparticles to cells. Sample V2 produced a similar concentration effect. Twenty four hours after the addition of 20 μM of V_2O_5 nanoparticles noticeable changes were visible in the morphology and density

of fibroblast and cancer cells. Generally, these cells became rounded and the number of cells was reduced in comparison with the control cell culture. Cell reduction and a change in the morphology of cells were more pronounced at a higher concentration of V_2O_5 . Also, fine yellow spots were seen inside all cells, the most clearly seen in B16F10 and SCCVII cells. These spots correspond to V_2O_5 nanoparticles that have enough time to accumulate and aggregate inside the cells.

In order to quantify the toxicity of nanosize V_2O_5 particles to fibroblast and cancer cell cultures, the treated and untreated live cells were fixed using the procedure given

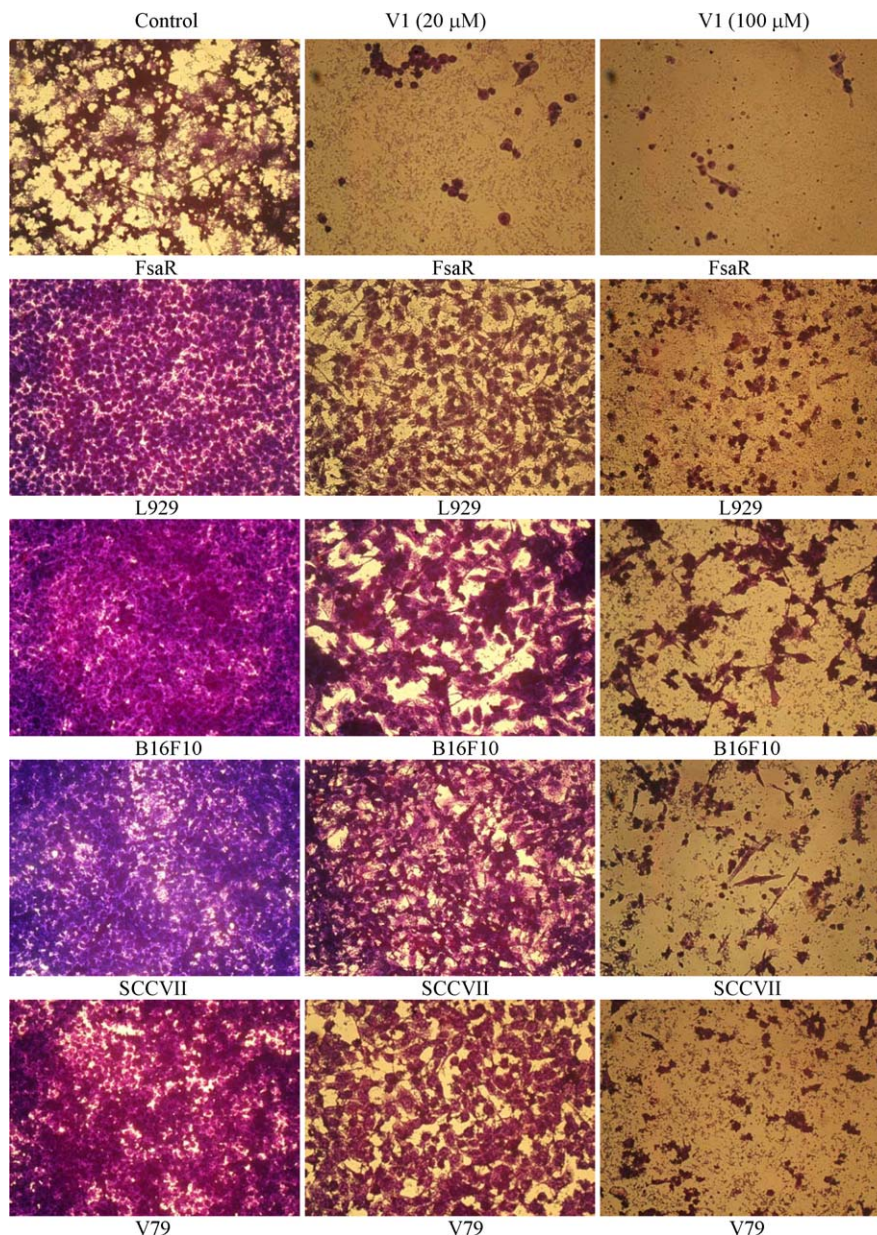


Fig. 4. Photomicrographs of fixed control and V_2O_5 treated cells, 24 + 24 h, after the addition of 20 and 100 μM of V_2O_5 , as sample V1. Fixed control cells contain an abundant number of cells with a well-defined morphology characteristic of the particular cell culture. In the presence of 20 μM of V_2O_5 added as sample V1, the number of FsaR and L929 cells was significantly reduced in comparison with control cells. Other cell cultures in the presence of 20 μM of V_2O_5 were not seriously damaged, the fibronectin filaments and cytoplasm were preserved, and there were many fixed cells with unaltered morphology. With the addition of 100 μM of V_2O_5 , all of the cell cultures were seriously damaged, the recovery effect is suppressed and an obvious reduction in the number of cells in all of the cell lines was observed.

in the experimental part of the present work. Fig. 4 shows photomicrographs of the fixed control and V_2O_5 treated cells 24 + 24 h after the addition of 20 and 100 μM V_2O_5 as sample V1. The blue color of crystal violet clearly showed the process that occurred in the cells took place in the presence of nanosize V_2O_5 particles. Fixed control cell cultures contain an abundant number of cells with well-defined morphology characteristic of the particular cell culture. In the presence of 20 μM of V_2O_5 added as sample V1, the number of FsaR and L929 cells was significantly reduced, the morphology of FsaR cells was com-

pletely altered and there were many dead and detached cells, whilst several colonies of rounded and dead cells were visible. Other cell cultures in the presence of 20 μM of V_2O_5 added as sample V1 were not seriously damaged, the fibronectin filaments and cytoplasm were preserved and there were many fixed cells with unaltered morphology. With the addition of 100 μM of V_2O_5 as sample V1 all cell cultures were seriously damaged and many detached cells were visible. Also, a highly altered cell morphology, a fragmentation of the nucleus, a roughly granulated cytoplasm and loosing of fibronectin filaments were noticed in cell cultures

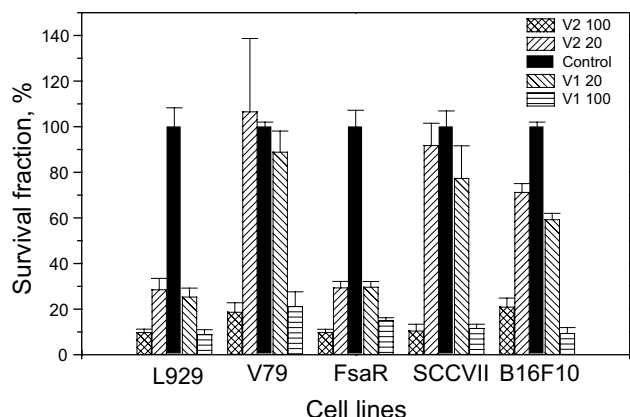


Fig. 5. Results of the surviving fraction (given in %) of V79 and L929 fibroblasts and FsaR, B16F10 and SCCVII cancer cells in dependence on the vanadium concentration added in the form of two different types of V_2O_5 particles, as samples V1 and V2. Larger V_2O_5 nanoparticles (sample V1) showed slightly higher cytotoxic effects; however, this was not statistically significant for any conclusion to be drawn. The resistance of investigated cell cultures to the cytotoxicity of nanosize V_2O_5 particles (20 μM) decreased in the order V79 > SCCVII > B16F10 > FsaR > L929. With the addition of 100 μM of V_2O_5 the survival fraction was below 22% in all the examined cell lines.

after the addition of 100 μM of nanosize V_2O_5 particles. It could be concluded that in comparison with the control cells, and also the 24-h old cell cultures (Fig. 3), the recovery effect approximately decreased in the order SCCVII > V79 > B16F10 > L929 > FsaR. The opposite effect is observed with a higher concentration of added V_2O_5 . With the addition of 100 μM of sample V1 the toxicity of V_2O_5 to fibroblast and cancer cells prevailed, the recovery effect is suppressed and an obvious reduction in the number of cells in all of the cell lines was shown.

Fixed cells were extracted using the 0.2% solution of Triton X-100, then the absorbance was read at 590 nm using the UV–visible spectrophotometer, (crystal violet bioassay). Fig. 5 shows the results of the surviving fraction (in %) of V79 and L929 fibroblasts and FsaR, B16F10 and SCCVII cancer cell cultures in dependence on the vanadium concentration added in the form of two different types of V_2O_5 nanoparticles, (samples V1 and V2). Larger V_2O_5 nanoparticles (sample V1) showed slightly higher cytotoxic effects; however, this was not statistically significant for any conclusion to be drawn. At a lower V_2O_5 concentration added (20 μM), V79 and SCCVII cell cultures showed a high resistance to the V_2O_5 cytotoxicity due to the high recovery effect, as mentioned above (Fig. 4). The B16F10 cell culture showed a relatively medium cytotoxicity to V_2O_5 , whereas the FsaR cell culture showed as low resistance as the L929 cell culture against the V_2O_5 cytotoxicity. With the addition of 100 μM of V_2O_5 the survival fractions were below 22% in all cell cultures. On the basis of the obtained quantitative data it can be concluded that the resistance of investigated cell cultures to the cytotoxicity of nanosize V_2O_5 particles decreased in the order V79 > SCCVII > B16F10 > FsaR > L929.

4. Discussion

In the present work the cytotoxic effect of laboratory-synthesized nanosize V_2O_5 particles on five cell lines was studied. Nanosize V_2O_5 particles were applied in the form of an aqueous suspension and it should be pointed here that there is no extra material settled on the cells, these particles were small enough to penetrate easily into the cells (Fig. 3).

The cytotoxicity effect of various soluble vanadium salts has been extensively investigated in literature (Capella et al., 2002; Cortizo et al., 2000; Ivancsits et al., 2002; Mannazzu et al., 2000; Mukherjee et al., 2004; Rehder, 2003; Zhang et al., 2003). Also, there are several in vitro studies about the cytotoxicity effect of commercially available V_2O_5 powders. For example, the cytokine gene expression and secretion in hepatocytes treated with toxic metals were studied (Dong et al., 1998). The metals selected as models for study were cadmium chloride, $CdCl_2$, (homogeneous solution) and commercial available vanadium pentoxide, V_2O_5 (suspension), both of which can readily produce oxidative damage. The genotoxicity of vanadium pentoxide in Chinese hamster V79 cells was also studied (Zhong et al., 1994). It was found that V_2O_5 was cytotoxic and aneuploidogenic to V79 cells. However, the cytotoxicity effects of nanosize V_2O_5 particles in order to obtain an anticancer agent are a new approach in the investigation of cytotoxicity of tumor cells. The great potential of V_2O_5 nanoparticles lies in the possibility of modifying their chemical composition (doping with other cation to obtain mixed metal oxides such as $V_2O_5-TiO_2$, $V_2O_5-Fe_3O_4$, etc.), morphology (plate like, fibrous, ribbon-like or tube-like nanoparticles) and surface (by adsorption of cytostatic to improve the cytotoxicity or by adsorption of polymer to improve the intracellular uptake and selectivity). Also, in comparison with the vanadium salts or other vanadium compounds applied in the form of a homogeneous solution, this heterogeneous system is stable against possible unwanted complexation with the various components present in the medium, and it could also be applied intravenously, if necessary. In a homogeneous solution it is very difficult to control the oxidation state of vanadium ions, whereas in solid matrix vanadium is stable against uncontrolled oxidation/reduction processes.

The investigated V79 Lung Hamster fibroblasts are known to be very sensitive against mutagenic agents. This type of cells easily forms micronuclei in the presence of mutagenic agents. However, in the present work a high resistance of V79 cells against the relatively small concentration of nanosize V_2O_5 particles (20 μM) was found. This could be explained by a functional adaptation of V79 cells to the environment permanently exposed to the oxidation/reduction processes prevailing in the lung. Because of these frequent oxidation/reduction processes in the lung, we could suggest that V79 cells possess a large amount of antioxidative enzymes able to scavenge the reactive oxygen species (ROS). It is generally accepted that one of the

mechanisms of cytotoxicity of V_2O_5 is the induction of ROS.

In comparison with the V79 cells that are of a mesodermal origin, squamous carcinoma cells SCCVII and mouse melanoma B16F10 cells belong to different cell types. These cells belong to the epithelial tumors of ectodermal origin, and they are also relatively resistance to the addition of nanosize V_2O_5 particles. Generally, tumor cells are basically organized to cope with the cytotoxicity agents that induce ROS. Higher levels of antioxidative enzymes in tumor cells were able to scavenge the ROS that could be generated by the addition of toxic agents, such as nanosize V_2O_5 particles. The mechanism explaining the resistance of tumor cells to ROS induced by cytotoxicity agents or heat by producing the endogenous tumor necrosis factor has already been discussed in the literature (Tsuji et al., 1992). By analogy with this mechanism, tyrosinase in B16F10 tumor cells could scavenge the ROS induced by the addition of toxic substances (Valverde et al., 1996).

On the addition of nanosize V_2O_5 particles, the L929 fibroblasts and FsaR fibrosarcoma cells showed the highest cytotoxicity effect. Both these cells are of mesodermal origin and in principle they are involved in similar anabolic and catabolic processes. In comparison with V79 cells, L929 and FsaR cells are not functionally adapted to the frequent oxidation/reduction processes, thus being very sensitive to ROS that could be generated by the addition of toxic agents such as nanosize V_2O_5 particles. Alternatively, besides the formation of ROS, another mechanism could participate in the cytotoxicity of V_2O_5 . It is well known that vanadium compounds inhibit various enzymes, in particular protein tyrosine phosphatases (PTP), thus allowing the stimulation of glucose intake into cells (insulin-mimetic effect). A high level of glucose inside the cells should affect the cellular metabolism. Moreover, if the supply of oxygen to cells is insufficient, in the sequence of reactions (glycolytic pathway) glucose will be converted into pyruvate and further into lactate as the end product. Accumulation of lactates decreases intracellular pH, which in turn has harmful effects on the activity of many enzymes, changes the net electric charge of membranes and other cellular metabolic processes. Generally, these two possible mechanisms, the generation of ROS and the decrease in cellular pH due to the accumulation of lactate could have an enhancing effect on the cytotoxicity of nanosize V_2O_5 to L929 and FsaR cells. Furthermore, the high cytotoxicity effect found on FsaR cells suggested that nanosize V_2O_5 particles could be regarded as poisoning material in the treatment of FsaR fibrosarcoma cells.

In the present work, a concentration-dependent cytotoxicity of nanosize V_2O_5 particles was observed. However, in contrast to a low concentration, a higher concentration of nanosize V_2O_5 particles (100 μ M) was very cytotoxic to all of the examined cell lines. At a higher concentration of V_2O_5 there was no recovery effect, neither there was any selective cytotoxicity. Since all of the cell lines were equally sensitive to the addition of a high concentration of V_2O_5 , a

mechanism other than the one discussed in the cytotoxicity of a low V_2O_5 concentration (20 μ M) should be considered. At a higher concentration of V_2O_5 due to the insulin-mimetic effect (“opening the door” (Rehder, 2003)), a huge quantity of glucose freely enters the cells (the concentration of D-glucose in the medium was 2 g/l). Glucose inside the cells has only one fate: it is phosphorylated, and further in the sequence of reactions converts into pyruvate (glycolytic pathway). As discussed above, in the oxygen-deficient environment (L929 and FsaR cells) pyruvate could be converted into lactate, which decreased intracellular pH and potentiated the cytotoxicity of V_2O_5 . However, the phosphorylation of D-glucose is a time-consuming and capacity-controlled reaction and as a consequence an increased level of non-phosphorylated glucose remained in cells. Thus, at a very high concentration of D-glucose inside the cells the glycolytic pathway is blocked. D-Glucose is osmotic active and could be converted into sorbitol in the polyol pathway that is an even more osmotic active molecule (Amano et al., 2002; Nakano et al., 2003). Since sorbitol with low-cell permeability cannot spread out from cells, it is accumulated in cells, leading to an elevated osmotic pressure and cell death. The proposed mechanism of V_2O_5 cytotoxicity due to its stimulation of glucose intake into cells (insulin-mimetic effect), which results in osmotic stress, fits really well the non-selective cytotoxicity of a high concentration (100 μ M) of nanosize V_2O_5 particles.

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