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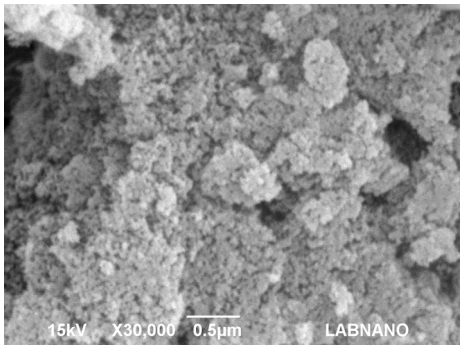
Hybrid suspension of nanodiamonds-nanosilica/titania in cytotoxicity tests on cancer cell lines

Michael Nazarkovsky[a], Heber L. de Mello[b,c], Rodrigo C. Bisaggio[b,c], Luiz A. Alves[b], Volodymyr Zaitsev[a,*]

[a] Chemistry Department, Pontifical Catholic University of Rio de Janeiro, 225 Marquês de São Vicente Str., Gávea, 22435-900 Rio de Janeiro, RJ, Brazil
[b] Laboratory of Cellular Communication, Oswaldo Cruz Institute, Fiocruz, 4365 Brasil Av., Manguinhos, 21040-360 Rio de Janeiro, RJ, Brazil
[c] Biotechnology Departament, Federal Institute of Rio de Janeiro, 121 Senador Furtado Str., Maracanã, 20270-021 Rio de Janeiro, RJ, Brazil

GRAPHICAL ABSTRACT

Skeletal structure of hybrid nanodiamond-nanosilica/titania xerogel after drying the suspension in normal saline solution (0.9 wt% NaCl).



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ABSTRACT

The present communication reports the results of application of stable hybrid solid dispersed phase-based (nanodiamonds-nanosilica/titania) suspensions in cytotoxicity tests, using two different cell lines – the mouse sarcoma (J774.G8) and the ovarian carcinoma (OVCAR 8). It is also demonstrated that binary (1 wt% of nanodiamonds and 5 wt% of nanosilica/titania) suspension is more stable than individually prepared (nanodiamonds alone) in normal saline solution (0.9 wt% NaCl). With the help of scanning electron microscopy the structure and the morphology of hybrid aggregate particles in dried suspensions (xerogels) were examined. In normal saline solution the subject suspensions do not experience the sedimentation by dilution within 24 h after addition of identical dispersion medium, while the negative zeta-potential (−28.8 mV) decreases to zero (4-times dilution) and the solid phase is characterized by aggregates size at ≤160 nm from the DLS measurements. The tests of biocompatibility (cytotoxicity) did not demonstrate any statistically significant toxicity at the suspension in the concentrations range of 0.01–0.155% (in respect to nanodiamonds in the suspensions diluted by cell culture, wt./vol) of the suspensions.

* Corresponding author.
E-mail address: vnzaitsev@puc-rio.br (V. Zaitsev).

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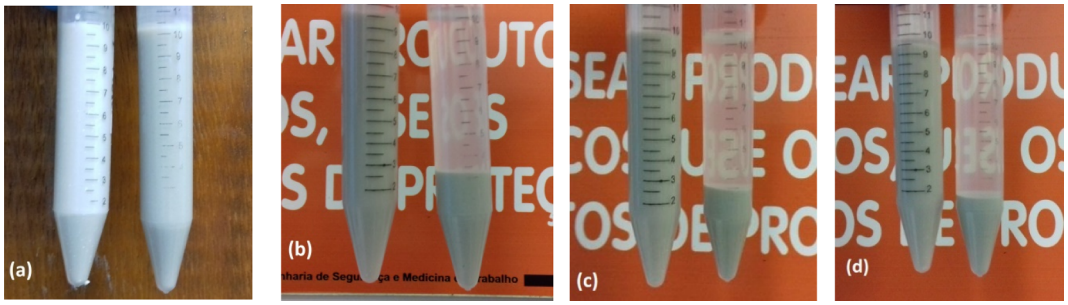

Fig. 1. Hybrid suspensions of nanodiamonds-nanosilica/titania (in total 6 wt%) (left) and single nanodiamonds (1 wt%) (right): 0 h *(a)*, 3 h *(b)*, 4 h *(c)* and 24 h *(d)*.

In terms of biomedical application, the nanodiamonds (NDs) hold a unique position among biocompatible drug supports and have shown themselves to good advantage in a flexibility in tuning the surface active sites and in the applied approaches [1,2]. Titania or silica/titania nanoparticles have been proven to exhibit anticancer effect in variously designed experiments. Particularly, the necessity of crystalline titania phase was required by its ability to generate reactive oxygen sites (ROS) under X-ray, near infrared (NIR), and/or ultrasound treatment – this is a key property of TiO_2 intended for fight against cancer [3–9]. Chemical and colloidal stability of the nanoparticle suspensions under physiological conditions (saline buffers and/or cell cultures) is crucial for their biomedical application since it allows to maintain physicochemical properties, biocompatibility and cure efficiency. Earlier it was demonstrated that NDs are chemically stable in biological environments, but they tend to precipitate at physiological ionic strength [10]. It seems that combination of NDs with silica can improve bioactivity of the resulted suspension. Therefore, a certain attention was dedicated to application of silica and NDs both in a form of a simple mixture [11] and as a core-shell nanocomposite [12–14]. At the same time, the information about composition and colloidal stability of such binary suspensions with nanodiamonds is very limited. For example, the stability of nanosilica-nanodiamond suspension was studied by Goncharuk et al. [15] with dispersion in water only. The present communication is dedicated to elaboration and investigation of colloidal stability of hybrid solid phase consisting of nanodiamonds and nanosilica/titania mixed oxide (15 wt% of TiO_2) dispersed in normal saline solution (dispersion medium). The suspension labeled as hybrid owing to combination of two different by the class inorganic materials.

In this research the suspension was developed and adapted for anticancer composition, whereas the principal difference consists in alteration from water to normal saline solution to serve as a dispersion medium for TiO_2-modified fumed nanosilica.

Tumor cells have metabolic characteristics different from normal ones [16]. With the perspective of using the subject suspension in a therapy for cancer treatment, the cells involved in the present study are originated from the mouse sarcoma (J774.G8) and the ovarian carcinoma (OVCAR 8), which would better approximate the results to a possible cancer treatment [17]. As components to prepare suspensions, the detonation nanodiamonds (RT-DND, Ray Technologies Ltd, Israel) and fine silica/titania nanocomposites (at the specific surface area of 300 m^2/g and the titania crystallites size of 15 nm) were used. The latter had been synthesized, analyzed and described elsewhere [18]. As a dispersion medium, normal saline solution, NSS (0.9 wt% of NaCl, from ADV Farma) was used. Such a medium served to substitute water in order to prevent cellular osmotic shock and dilution of the suspensions in the cytotoxicity investigation.

To study cytotoxicity, the cell lines (J774.G8 and OVCAR 8) were cultured in 96 wells plates in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (FCS). For the cytotoxic assays, cells were incubated for 24 h varying concentrations of the dispersed nanodiamonds-nanosilica/titania phase by dilution of the suspension. After incubation in the presence of nanodiamonds, each well was washed three times with phosphate buffer solution (PBS) and then incubated in a fresh medium for another 24 h. After the incubation, the culture medium was removed and a fresh medium containing 12.5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) was added. After 3 h of incubation at 37 °C, the medium was removed and 100 μL of DMSO (Sigma) were added and the absorbance was analyzed at $\lambda = 570$ nm, using a Spectramax 190 spectrophotometer (Molecular Devices).

The suspension was prepared by dispersing nanodiamonds together with nanosilica/titania powder in normal saline solution by exploit of a UP200S (Hielscher) sonicator at 70% of the nominal power 200 W for 2 min resulting in the following concentrations of the solid components: nanodiamonds – 1 wt%, nanosilica/titania – 5 wt%. As a reference, individual suspension of nanodiamonds was prepared at the identical concentration in NSS.

The study of the morphology of dry suspensions (xerogels) was carried out using a scanning electron microscopy, SEM (JSM 6490 LV, JEOL) at Brazilian Center for Research in Physics (CBPF). The particle size distribution and ζ-potential were measured by instrumentality of a SZ-100 (Horiba).

Replacing water with NSS and incorporation of titania into nanosilica must have faced the risk of destabilization and coagulation. Individual suspensions of nanodiamonds undergo the loss of stability (right), while hybrid suspensions (left) are viewed to be stable for 24 h of the examination in NSS (Fig. 1). The suspension is characterized by a negative interface charge of $\zeta = -28.8$ mV. Such an absolute value is less than 30 mV though, the stabilization of the suspension can be affected by structural factor even modifying the acidity of the nanosilica surface, where surface Ti-O-Si sites (more acidic then Si-O-Si) occur.

Diluting the suspension with NSS leads the ζ-potential to decrease

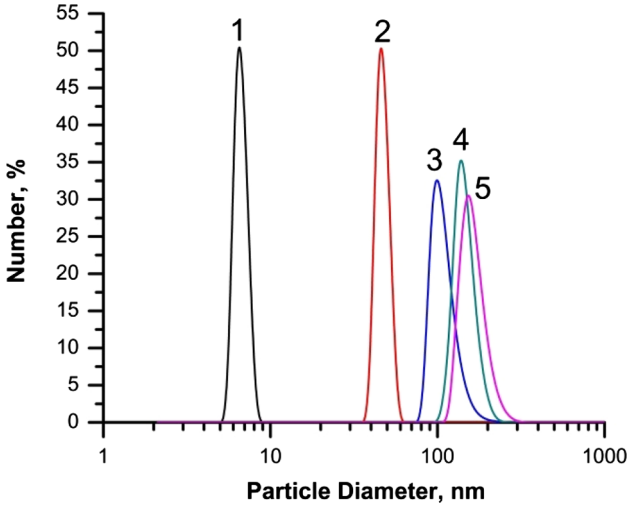

Fig. 2. The semilogarithmical particle size distribution in nanodiamonds-nanosilica/titania suspensions – before dilution *(1)*, at ×1.25 *(2)*, ×1.5 *(3)*, ×2 *(4)* and ×4 *(5)* times of dilution with normal saline solution.

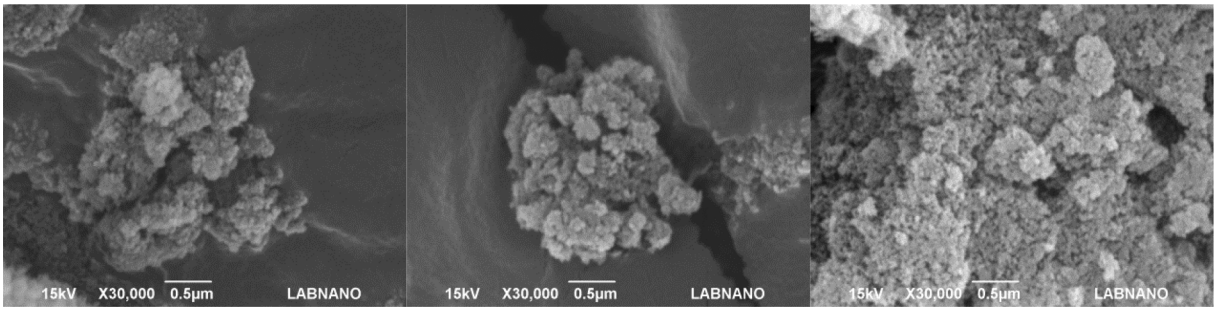

Fig. 3. The SEM images of the solid phase after drying hybrid nanodiamonds-nanosilica/titania suspensions in normal saline solution.

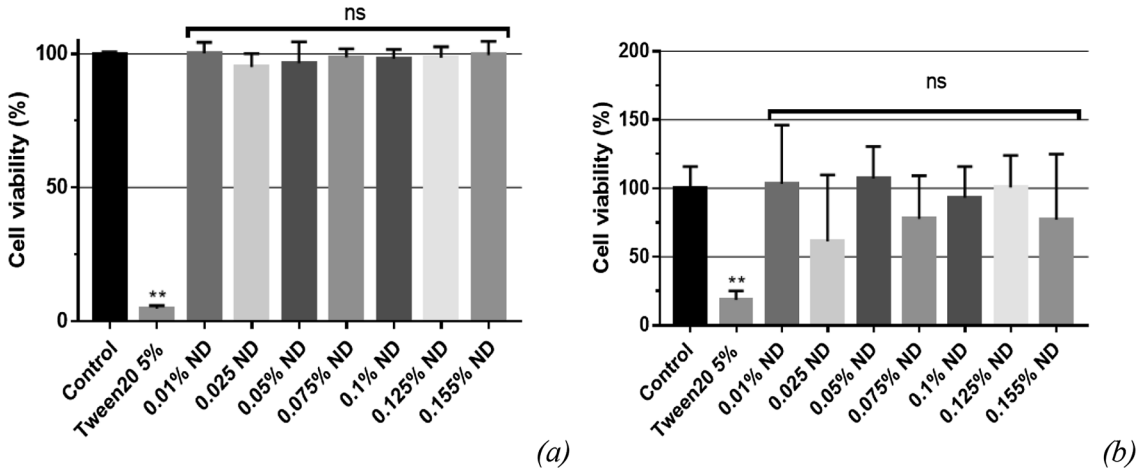

Fig. 4. The bar-plotted cytotoxicity results on J774.G8 *(a)* and OVCAR8 *(b)* cell lines incubated for 24 h with different concentrations of nanodiamonds-nanosilica/titania suspensions. Asterisk indicates a significant difference in respect to the control and *ns* is "not significant".

down to zero with a loss of aggregative stability owing to formation of agglomerates with a monomodal particles size distribution (Fig. 2). In particular, respective values of ζ-potential at ×2 and ×4 times dilutions of the initial suspension are −0.1 and 0.0 mV

The mechanism of the suspension stability was suggested by Goncharuk et al., as was mentioned above. Accordingly, it was assumed that the interaction between hydrophobic and hydrophilic sites of NDs with respective hydrophilic sites of nanosilica takes place, whereas NDs' hydrophobic groups may be blocked, since during the ultrasonification, primary particles of both nanooxides and nanodiamonds are exposed to an inconstant relative orientation. The hybrid aggregates may contain an adsorbed layer of water molecules with a consequent thermodynamic prevention of the total destabilization of the suspension and precipitation of the dispersed phase [19].

Dried suspension sample demonstrates a coagulation structure typical for colloidal xerogels (Fig. 3). It provides a proof to the fact that liquid phase is confined inside the spaces (voids) among the agglomerates. Moreover, this observation favours the view that the mixed "spongiform" agglomerates are bond strongly.

Viable cells with active metabolism convert MTT into a purple colored formazan product due to mitochondrial activation likely involving reaction with NADH or similar reducing molecules that transfer electrons to MTT. Dying, the cells lose their capacity to convert MTT into formazan [20]. Culturing the cell lines J774.G8 and OVCAR8 for 24 h at varied concentrations of the suspensions diluted by cell culture media (0.01–0.155 wt/vol%) and analyzing their viability with MTT, no statistically significant toxicity was detected (Fig. 4).

With this communication we emphasize an innovative step in the nanodiamonds suspension stabilization in the normal saline solution for potential use in biological systems. The suspension containing nanodiamonds is instantiated by the stability due to the combination with silica/titania nanocomposite which is a carrier of active component (titania) to produce reactive oxygen sites. Numerically, the cytotoxicity tests have shown a low toxicity of the hybrid suspension for the mouse sarcoma cell line J774.G8 and the ovarian carcinoma cell line (OVCAR 8). Thus, our results show that the hybrid nanomaterials we developed are suitable for biomedical application and, particularly, for sonodynamic therapy for cancer treatment.

Declaration of Competing Interest

The authors state that there are no conflicts of interests to declare during the research and the Manuscript's preparation.

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