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Size and shape influence of luminescent orthovanadate nanoparticles on their accumulation in nuclear compartments of rat hepatocytes

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ABSTRACT

In this paper the process of nonfunctionalized negatively charged orthovanadate nanoparticle accumulation and redistribution in cells dependent on their shape and size was investigated. Aqueous colloidal solutions of $nReVO_4:Eu^{3+}$ (Re = Gd, Y, La) luminescent nanocrystals of different sizes and shapes have been synthesized. The average sizes of spherical particles were 2, 20, and 300 nm, of spindle-like particles – 22 × 6.3 nm, and of rod-like particles – 57 × 4.4 nm. Luminescence of $nReVO_4:Eu^{3+}$ nanocrystals was effectively excited by UV and visible irradiation. By means of luminescence microscopy and luminescence microspectroscopy, it has been revealed that spherical nanocrystals with an average diameter of 2 nm tend to accumulate mainly in the rat hepatocyte nuclei *in situ* and also in the isolated nuclei of these cells. An additional experiment has shown that nanoparticles reveal tropism to nuclear structural components. The penetration into nuclei does not require any modifications of the surface of nanoparticle and is governed by the shape and size of nanoparticle and also is determined by the cellular type.

1. Introduction

In the last decade there has been a substantial increase in the application of nano-sized materials in biomedical research. New opportunities appearing from the development of nanotechnologies are believed to be the key for solving a whole number of problems concerning molecular diagnostics and disease therapy. Nano-scaled materials have become also increasingly important for the development of new fluorescent probes for *in vitro* and *in vivo* imaging [1–4] both experimentally and clinically. The family of such nano-sized fluorescent materials includes semiconductor quantum dots [5–8], gold nanoparticles, nanoshells and lanthanide-doped inorganic nanocrystals [9–12]. Moreover, sufficient progress in biomedical research is associated with engineering nanoparticles that are capable of targeted delivery of both imaging agents and active compounds into the site of action in living body. In comparison with other small molecule contrast agents, the advantage of nanoparticles (NPs) is their large surface area and the possibility of surface modification for further conjugation or encapsulation of therapeutic agents in large quantities [13].

Size, shape and surrounding of NPs are the key factors determining peculiarities of their interaction with biological structures [14–16]. However, the correlation of particle parameters with their ability to penetrate into cells remains unclear. It depends to a large extent on the methodological approach of NP's effect identification and registration *in vitro* and *in vivo*. The methods based on direct visualization of NPs in biological systems are the most effective. Luminescent nanomaterials allow direct observing of bioobject — NP interaction [17,18].

Recently some authors have reported the application of various rare-earth-doped inorganic NPs as a fluorescent probes *in vitro* and *in vivo* experiments. It was shown that such materials possess a number of attractive features (high photostability, absence of typical for quantum dots "photoblinking effect", low toxicity) and are very prospective for biomedical application [19–21]. One of the main advantages of doped inorganic NPs is also a narrow spectral lines governed by the type of doped ions and matrix composition. Stokes shift in inorganic nanoluminophores activated with rare-earth (Re) ions is more than 200 nm that allows avoiding the autoluminescence from biomolecules and observing just the luminescence of inorganic probes [13,18].

The main disadvantage of Re-doped NPs, which restricts their biomedical application, is insufficient brightness of their luminescence that requires laser sources and highly sensitive CCD cameras to excite and collect luminescence.

Recently we have reported the method for the wet-chemical synthesis of brightly luminescent $ReVO_4:Eu^{3+}$ nanoluminophores [22,23]. The possibility of NP's registration in hepatocytes was investigated by means of luminescence microscopy and luminescence microspectroscopy [24]. One of the attractive features of orthovanadate nanoparticles is a possibility to control their size and shape during the synthesis procedure keeping almost unchangeable such parameters as surrounding, composition, charge, mass concentration, *etc.* It allows tracing the influence of NP's size and shape on efficiency and pattern of their interaction with biological objects.

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In the present paper we have visualized the penetration of vanadate NPs activated by europium ions into hepatocytes and isolated nuclei of rat hepatocytes and fibroblast-like cells L929 for comparison. For this purpose, aqueous colloidal solutions of $ReVO_4:Eu^{3+}$ (Re = Gd, Y, La) nanocrystalline luminophores of different sizes and shapes, but similar composition and surrounding have been used.

2. Materials and methods

2.1. Synthesis of the $nReVO_4:Eu^{3+}$ NPs

The synthesis of $nReVO_4:Eu^{3+}$ (Re = Gd, Y, La) aqueous colloidal solutions has been carried out according to the method reported earlier [23]. Briefly, 10 mL of aqueous solution of rare-earth chlorides (0.01 mol/L) was mixed with 8 mL of sodium ethylenediaminetetraacetate (EDTA 2Na) solution (0.01 mol/L). Then, the obtained solution 8 mL Na_3VO_4 (0.01 mol/L) was flowed drop by drop (pH = 13). The mixture was intensively stirred by using a magnetic stirrer until yellowish transparent solution is formed. The solution was heated on a water bath for 30 min till 24 h at 90 or 100 °C.

Synthesis duration, temperature and stoichiometric composition were chosen empirically until reproducible geometric parameters of solid phase are achieved. Obtained colorless transparent solution scatters light under the side illumination (Tyndall cone). Then, the solution was cooled and dialyzed against water for 24 h to remove the excess of ions. A dialysis membrane with a molecular weight cutoff of 12 KDa was used. The composition of spherical particles is $Gd_{(0.6–0.8)}Y_{(0.1–0.3)}Eu_{(0.1)}VO_4$, spindle-like – $Gd_{(0.9)}Eu_{(0.1)}VO_4$, rod-like – $La_{(0.9)}Eu_{(0.1)}VO_4$.

2.2. Biological material preparation

Isolated rat hepatocytes from male Wistar rats were obtained by the method described by Wang after dissociation of the liver with 2 mM EDTA [25]. Cell viability was assessed *via* trypan blue exclusion test. The viability 95% and yield 1.5×10^7 cells/g liver compared well with those of previously described. Isolated hepatocyte nuclei were obtained as described by Kaufmann [26]. For obtaining nuclear a liver was homogenized using Downs's homogenizer in ice-cold 0.25 M sucrose solution containing 5 mM $MgSO_4$, 50 mM Tris, and pH 7.4. The homogenate was filtered through Muslin cloth and spun at low speed 800 g for 5 min. The pellet was rinsed and spun twice as it was described above. Vanadate NPs (1 g/l) were loaded into hepatocytes in suspension (10^6 cell/mL) and isolated cell nuclei *via* their short-term incubation in 5% glucose isotonic solution for 1.5–3.0 h at RT. Such condition of mild hypothermia is optimal for maintenance of integrity and function of this cell preparation [27]. L929 cells (ATCC CCL 1) are a fibroblast-like cell line cloned from strain L. The parent strain was derived from normal subartaneous areolar and adipose tissue of a male C3H/An mouse. The loading NPs into the cells were carried out in the condition described for isolated hepatocytes.

2.3. Instruments

Cell visualization was carried out by luminescent microscope Olympus IX 71 with magnification of 1000; 1500 times in conditions of oil immersion. Luminescence was excited by a xenon lamp 75 W using a band-pass 460–490 nm excitation filter. Luminescence was collected using an emission long-pass 510 nm filter.

Luminescence spectra were recorded with a USB4000 (Ocean Optics, USA) spectrometer-based optoelectronic system connected to the optical outputs of luminescent microscope Olympus IX 71. Such system allows recording luminescence spectra from local sites within observable objects, including local sites of single cells.

Transmission electron micrographs of the particles were taken using TEM-125K ("SELMI") electron microscope. Samples were prepared by evaporating dilute solution droplets onto carbon coated copper discs.

Analysis of DNA melting curves was carried out by means of SPECORD 200 spectrometer with temperature controls.

3. Results

To study the orthovanadate NP accumulation in rat hepatocytes, aqueous colloidal solutions with solid phase consisted of NPs with different sizes and shapes (standard deviation does not exceed 15% of an average diameter), have been synthesized (Fig. 1). Vanadate NPs stabilized by EDTA are negatively charged, stable in solutions more than 2 months and possess bright luminescence [21,23]. It is known that inorganic electrolytes provoke solid phase coagulation and precipitation [28]. Therefore, for incubation of vanadate NPs with cells, salt-free buffer 5% glucose solution was used.

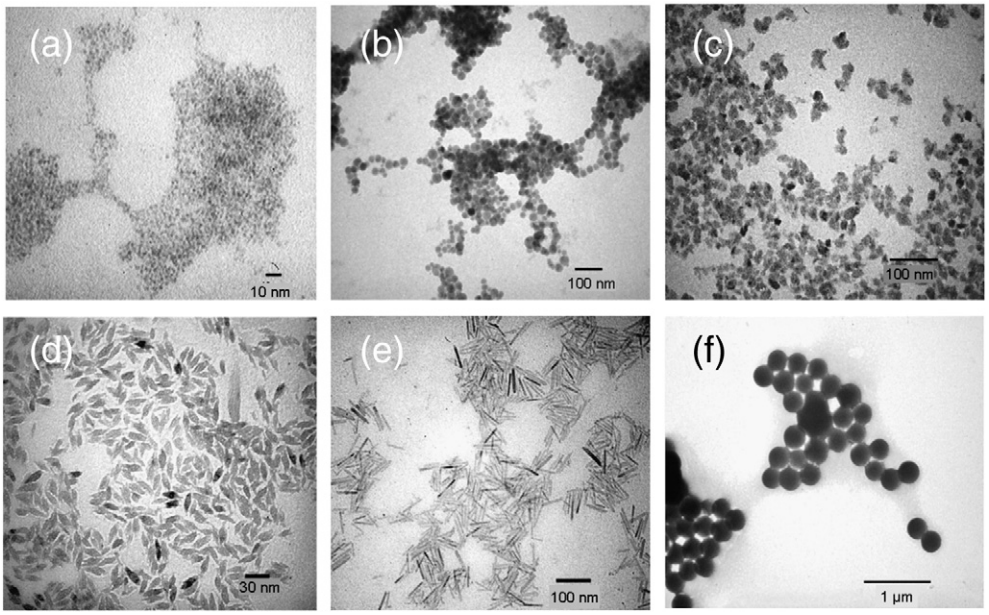

Fig. 1. TEM images of $ReVO_4:Eu^{3+}$ nanocrystals in colloidal solutions. Spherical particles with an average size of 2 nm — a, 20 nm — b, 300 nm — f; asymmetric sphere-like shaped — c; spindle-like — d and rod-like particles — e.