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Luminescent properties of ZrO_2:Tb nanoparticles for applications in neuroscience

A. Słońska [a,b,1], J. Kaszewski [a,b,c,1,*], E. Wolska-Kornio [c], B. Witkowski [c], Ł. Wachnicki [c], E. Mijowska [d], V. Karakitsou [a], Z. Gajewski [e], M. Godlewski [c], M.M. Godlewski [a,b]

[a] Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland
[b] Veterinary Research Centre, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Nowoursynowska 100, 02-797 Warsaw, Poland
[c] Institute of Physics, Polish Academy of Sciences, Al. Lotników 32/46, 02-668 Warsaw, Poland
[d] Institute of Chemical and Environment Engineering, West Pomeranian University of Technology, Pułaskiego 10, 70-322 Szczecin, Poland
[e] Department of Large Animal Diseases with Clinic, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Nowoursynowska 100, 02-797 Warsaw, Poland

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ABSTRACT

In this paper a new generation of non-toxic nanoparticles based on the zirconium oxide doped with 0.5% Tb and co-doped by the range of 0–70% with Y was evaluated for the use as a fluorescent biomarker of neuronal trafficking. The ZrO_2:Tb nanoparticles were created by microwave driven hydrothermal method. Influence of the yttrium content and thermal processing on the Tb^{3+} related luminescence emission was discussed. The higher intensities were achieved, when host was cubic and for the nanoparticles with 33 nm. Presence of yttrium was associated with the energy coupling of the host and dopant, wide excitation band is present at 309 and 322 nm before and after calcination respectively.

For the experiment on living primary neurons, nanoparticles doped with 0.5%Tb and 7%Y were chosen based on their luminescence emission intensity. Recently transfer of the nanoparticles through the barriers in the organism including blood–brain barrier following their alimentary absorption was confirmed (Godlewski and Godlewski, 2012). This raised the possibility of the nanoparticle application as a tool in the neuroscience, and the question of potential mechanisms of nanoparticle turnover in neurons. Concentration of 0.001 mg/ml of ZrO_2:0.5%Tb 7%Y in growth medium was added to the primary murine culture medium, and the intracellular trafficking of nanoparticles was observed following 15 min pre-incubation period. ZrO_2:0.5%Tb 7%Y nanoparticles were dynamically absorbed by the neurons and the dynamic passage of transport vesicles containing ZrO_2:0.5%Tb 7%Y nanoparticles was observed along the neuronal processes and in between two neighbouring neurons. Reassuming, the ZrO_2:0.5%Tb 7%Y nanoparticles proved to be biocompatible and a valid tool to assess intracellular trafficking dynamics in the neurobiology.

1. Introduction

Nanotechnology is one emerging scientific field of significant potential in various commercial and medical applications, including food prevention, medicines, cosmetics, chemicals, biomedicine, and light sources [2–4]. First nanoparticles (NPs) for biological applications have been initially developed around highly fluorescent CdTe, CdS and CdSe core usually encapsulated in the ZnO or ZnS shell [5,6], drawing a lot of attention due to their photostability and their unique physicochemical properties that promoted them as potential candidates for biomedical applications [7]. These remarkable features led to the use of this first generation of NPs in many biological applications, including cancer therapy, cellular imaging and delivery of various molecules into cells [8]. However, the release of heavy-metal ions when applied to a living organism could not be entirely prevented [9]. Consequently, the major concern about using first generation NPs in vivo regarding their potential toxicity continued [10,11], as in many cases, NPs consist of a core cell-structure typically involving toxic heavy metals, such as Cd or Hg. Second significant limitation of the first generations of NPs regards to their excitation wavelength range localized within UV bands, of limited penetration and high phototoxicity in living organisms. As a result, focus was placed on the NPs core, taking into account that

* Corresponding author at: Institute of Physics, Polish Academy of Sciences, Al. Lotników 32/46, 02-668 Warsaw, Poland.
E-mail addresses: jaroslavuss@wp.pl (J. Kaszewski), mickgodl@hotmail.com (M.M. Godlewski).
[1] Both authors equally contributed to the paper.

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an increased biocompatibility of such NPs can be achieved by substituting the toxic core-forming metals with biocompatible substances, such as Si or encapsulating the NPs in a lipid [12]. The promising applications of nanoparticles in the medical field, especially in the treatment of cancer, autoimmune diseases and nerve injury [13–15] led us to formulate the new generation of NPs based on the transition metal oxides and rare-earth doping. The relatively high yields of fluorescence of ZrO_2:Tb following excitation by the commercially-available laser wavelengths form the visible spectrum showcased these nanoparticles as a viable tool for above-mentioned applications.

Zirconium dioxide (ZrO_2) is wide band-gap semiconductor with three main polymorphic forms: monoclinic (m) ($P2_1/c$) [16], tetragonal (t) ($P4_2/nmc$) [17] and cubic (c) ($Fm3\,m$) [18]. At the room temperature thermodynamically stable is m form, at 1170 °C shifts to the tetragonal, which transforms to the cubic one at 2715 °C. However, both t and m phases may exist at the room temperature as the metastable. It was observed, that given crystalline phase can be stable at the temperature much lower than the temperature of phase shift, but only if the critical size is exceeded [19]. Garvie has given the values of particle sizes for ZrO_2 [20]. This phenomenon is called stabilization and can be also achieved by doping with foreign ions. Usually, the ions with lower valency are used, and Y^{3+} is the most common stabilization agent [21]. The compensation of charges is accomplished by introduction of the oxygen vacancies into the host lattice. Doping with different ions results in the change of zirconia properties e.g. mechanical, electrical or optical. Lanthanide ions are interesting as guests, since they modify optical and magnetic properties of the host lattice [22]. There are known reports on the ZrO_2 doping with Eu [23], Er [24], Tm [25] and others. Interesting properties shows Tb^{3+} ion with electronic configuration $4f^8$. It belongs to the non-Kramers family and exhibit luminescence emission in the visible range, mainly in the green region. This emission is due to the intrashell 4f–4f transitions $^5D_4 \rightarrow {}^7F_J$ within Tb^{3+} ions. Hydrothermal technique of oxide materials crystallization employs properties of critical or subcritical water. Such a conditions may be achieved by increasing the temperature and pressure of water, however by definition hydrothermal conditions require the water to perform $P > 100$ kPa, $T > 25$ °C [26]. The technique exhibits number of advantages in materials synthesis: high reaction rate, narrow distribution of resulting product grain sizes, control over crystal shapes, low temperature of process and possibility of new phase crystallization. Further development of hydrothermal method resulted in application of microwave radiation as the heating agent. It increased the purity class of synthesized materials and additionally enhanced reaction rates [27]. Microwave-hydrothermally zirconias luminescent properties were studied in the cases of Pr^{3+} [28] and Eu^{3+} [29] doping, to conclude that structural properties of the host lattice strongly influence luminescence efficiency.

Recently, it was suggested that NPs are able to permeate into the brain tissue [1,6,30] and this translocation can happen both directly, via the axonal transport from olfactory epithelium, and indirectly, by passing to the bloodstream and crossing the blood brain barrier. In accordance, similarly to other metal oxide NPs, it has been documented that ZnO NPs are able to reach the brain of experimental animals after oral administration [31] and inhalation [32]. Following the lack of sufficient knowledge about the uptake, trafficking and toxicology issues of the NPs in the nervous system, especially for human neuronal cells, we aimed at creation and characterization of the NPs for the neurophysiological studies based on transition metals. To verify our experimental system we evaluated the performance of ZrO_2:Tb nanoparticles on the primary murine neuron culture. Luminescence efficiency of ZrO_2:Tb nanoparticles was studied in the light of structural properties of the zirconia host lattice.

2. Material and methods

If not mentioned otherwise all of the chemicals were purchased from Sigma–Aldrich (Poland).

2.1. Ethics statement

All animals were handled and cared for according to Polish and European Animal Care and Use guidelines and regulations. All procedures involving live animals were approved by a local ethics committee and confirmed to applicable international standards agreement No. 44/2012.

2.2. ZrO_2:Tb nanoparticles preparation

The series of ZrO_2:Tb samples were prepared. Concentration of terbium was fixed to 0.5 mol% in all the samples. Content of yttrium varied from 0 to 70 mol%. Typical sample was prepared as follows: in 200 ml of distilled water $ZrO(NO_3)_2{\cdot}xH_2O$, $Tb(NO_3)_3{\cdot}5H_2O$ and $Y(NO_3)_3{\cdot}5H_2O$ were dissolved to form clear solution. The solution was constantly stirred and 25% water ammonia solution was rapidly added to the pH value of 10. The resulting residue was triply washed in distilled water and placed in the Teflon vessel. Then the vessel with the reaction mixture was closed in the microwave hydrothermal reactor (Ertec, Poland) to perform the reaction. Process was conducted at 6 MPa by 20 min. After cooling down the reactor, the product was dried overnight at 40 °C. The powder was divided into 6 parts, each was calcined at 200, 400, 600, 800, 1000 °C and one was left uncalcined.

2.3. Nanoparticles characterization

X-ray diffraction (XRD) measurements were performed with Phillips X'Pert powder diffractometer working with Bragg–Brentano geometry. The measurements were conducted in the 2Θ range from 20° to 90° with a step of 0.05° and at counting time of 3 s. The Cu Kα radiation (1.54 Å) was used in all the experiments. The morphology of the samples was examined with transmission electron microscopy (TEM, FEI Tecnai F20). The scanning electron microscopy (SEM) measurements were conducted with high resolution (1 nm) Hitachi SU-70 microscope. The photoluminescence (PL) emission and excitation spectra were taken using CM2203 spectrofluorimeter (Solar). The source of excitation was 150 W xenon lamp. The instrument was equipped with R-928 Hamamatsu photomultiplier.

2.4. Primary culture of murine neurons

Balb/c (H-2d) mice were used to establish primary culture of murine neurons, as described before [33]. Neuronal cells were plated onto poly-D-lysine with laminin-coated culture slides (5×10^4 cells per well) and cultured in B-27 Neuron Plating Medium consisting of neurobasal medium, B27 supplement, glutamine (200 mM), glutamate (10 mM), antibiotics (penicillin and streptomycin) with 10% supplement of foetal and equine serum (Gibco, Life Technologies Polska Sp. z o. o., Warsaw, Poland) and maintained at 37 °C with 5% CO_2. Four days after plating the medium was removed and replaced with Neuron Feeding Medium (B-27 Neuron Plating Medium without glutamate). In this medium murine neurons were maintained for the next 4 days, prior to treatment.