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The effect of nanoparticle degradation on poly(methacrylic acid)-coated quantum dot toxicity: The importance of particle functionality assessment in toxicology

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ABSTRACT

Colloidal semiconductor nanoparticles (quantum dots) have attracted a lot of interest in technological and biomedical research, given their potent fluorescent properties. However, the use of heavy-metal-containing nanoparticles remains an issue of debate. The possible toxic effects of quantum dots remain a hot research topic and several questions such as possible intracellular degradation of quantum dots and the effect thereof on both cell viability and particle functionality remain unresolved. In the present work, poly(methacrylic acid)-coated CdSe/ZnS quantum dots were synthesized and characterized, after which their effects on cultured cells were evaluated using a multiparametric setup. The data reveal that the quantum dots are taken up through endocytosis and when exposed to the low pH of the endosomal structures, they partially degrade and release cadmium ions, which lowers their fluorescence intensity and augments particle toxicity. Using the multiparametric method, the quantum dots were evaluated at non-toxic doses in terms of their ability to visualize labeled cells for longer time periods. The data revealed that comparing different particles in terms of their applied dose is challenging, likely due to difficulties in obtaining accurate nanoparticle concentrations, but evaluating particle toxicity in terms of their biological functionality enables an easy and straightforward comparison.

1. Introduction

The use of colloidal nanoparticles (NPs) in technological and biomedical applications is vastly increasing [1,2]. There are currently over 800 consumer products containing NPs, including many foods, beverages and cosmetics. The nanotechnology industry is growing very rapidly, and is predicted to have a total value of US$3.1 trillion by the year 2015 [3]. Currently, NPs are omnipresent in many different consumer products, but the number of biomedical applications is still limited due to several questions remaining on the possible induction of toxic side-effects by NPs [4–6]. Uncertainties regarding the safety of NPs are being fed by the continuous discovery of new pathways and mechanisms by which NPs may interfere with cellular wellbeing, which can either be beneficial for biomedical purposes or pose serious threats to human health [7]. One example is the recent finding that NPs can induce DNA damage and chromosome mutations, as shown for carbon nanotubes that were found to selectively stabilize human telomeric i-motif DNA and inhibit telomerase activity [8]. To progress towards use of NPs without risks, more data are required on the (toxic) effects of NPs on cells, tissues and whole organisms [9]. In order to fulfill the current needs in nanotoxicity research, NPs should be screened rapidly on a variety of cells under standardized conditions, enabling a comparison of data obtained for different materials and between different research groups [10]. In this view, we recently established a multiparametric methodology that looks at the interactions between cultured cells and NPs in order to get a profound knowledge of the possible effects of these materials on cultured cells [11]. Using a variety of cell types that have shown great potential for nanotoxicity research [12–14], being primary human umbilical vein endothelial cells (HUVECs), murine C17.2 neural progenitor cells and rat PC12 pheochromocytoma cells, the obtained results are representative for a wide variety of cell types. This methodology therefore allows for determining the non-cytotoxic levels of NPs (i.e. the concentration of NPs

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appropriate for cell labeling applications) and additionally helps in unraveling the mechanisms that are involved in the cytotoxic profile of the NPs under investigation. Furthermore, by comparing the data obtained against data for other NPs that have been tested under identical conditions, physicochemical features of the NPs that contribute to their cytotoxicity can be defined, paving the way for a more rational and safer NP design.

One important aspect with respect to rigorous NP toxicity testing is the design, purification and characterization of the NPs under investigation [15]. If any cytotoxic effects are to be ascribed to specific physicochemical features of the NPs, it is of utmost importance to test well-characterized NPs free of contaminants or impurities [16]. In this direction, poly(methacrylic acid) (PMA)-coated CdSe/ZnS core–shell quantum dots (QDots) are a useful system, as these NPs are well defined and have been extensively characterized. QDots are small semiconductor NPs that possess several exciting features such as high photostability, narrow and tunable emission spectra and high brightness [17]. Owing to these properties, QDots have shown great potential for many biomedical applications, including cell labeling applications [18–21], long-term tracking of (single) molecules [22], in vivo imaging [23,24] and photodynamic therapy [25,26]. However, despite their excellent photophysical properties, their toxicity, in particular due to the release of Cd^{2+} ions [27,28], remains an issue of debate [29,30].

2. Materials and methods

2.1. Nanoparticles

CdSe/ZnS colloidal nanoparticles were synthesized and made water-soluble as described in the Supplementary information. The nanoparticles were carefully characterized as also described in full detail in the accompanying Supplementary information.

2.2. Cell culture

C17.2 neural progenitor cells and PC12 cells are maintained in high glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 5% horse serum, 2 mM L-glutamine and 1% penicillin/streptomycin (Gibco, Invitrogen, Merelbeke, Belgium). Cells were maintained in a humidified atmosphere at 5% CO_2 and fresh medium was given every other day. C17.2 cells were passaged (1/10) when reaching 90% confluency. PC12 cells were grown in 25 cm^2 cell culture flasks (Corning, Amsterdam, The Netherlands) that were coated with collagen (rat tail collagen type I, Invitrogen, Belgium) and passaged (1/5) when growing in small clumps (~5 cells per clump and reaching 70–80% confluency). Fresh medium was given every other day.

For some long-term experiments, such as the effect of intracellular pH on Cd^{2+} in time, and the toxicity derived from this free Cd^{2+}, non-proliferating cell cultures are required as dilution of the number of particles per cell due to cell division abolishes any attempt to analyze these parameters. Therefore, in the current study, non-proliferating cell populations were established to enable the investigation the time-dependent effects of the intracellular environment on QDot functionality and toxicity. Next to analyzing these effects without the problem of exponential QDot dilution, these conditions also better mimic the in vivo conditions where autologous cells or stem cells after transplantation show minimal proliferation. To establish non-proliferating cell populations, cells were exposed with 60 μM Apigenin (Sigma–Aldrich, Bornem, Belgium) together with the Sil NP exposure. After removal of the medium, fresh media containing 60 μM Apigenin were used, where media were replaced for 50% every other day with fresh Apigenin-containing medium for the duration of the experiments. Under these conditions, cell death was found to be minimal and cell proliferation was reduced to ~9% of the normal value. Furthermore, removal of the medium with normal cell culture medium not containing any Apigenin resulted in a recovery of cell proliferation to near-control levels after ~3 days.

Primary human umbilical vein endothelial cells (HUVECs) were kindly provided by Dr. Aldo Ferrari (ETH Zurich, Switzerland). For cultivation, cells were kept in 75 cm^2 cell culture flasks (Corning, Amsterdam, The Netherlands) coated with collagen (rat tail collagen type I, Invitrogen, Belgium) prior to cell seeding. The cells were maintained in endothelial cell basal growth medium and growth supplement (Cell Applications, Tebu-Bio, Le Perray en Yvelines, France) and passaged (1/5) when reaching 80–90% confluency. Every other day, fresh medium was given. For HUVEC cells, the Apigenin treatment resulted in slight toxic effects and proliferation could be impeded better using serum-free conditions. To establish non-proliferating HUVEC cultures, cells were given endothelial cell serum-free defined medium (Cell Applications, Tebu-Bio, Le Perray en Yvelines, France) when reaching high levels of confluency. Confluent HUVEC monolayers could then be maintained for at least 1 week without any observable signs of cell death or reactive oxygen species (ROS) induction.

2.3. Cell–nanoparticle interaction studies

The following cell–NP interactions were studied; a full methodology can be found in the Supplementary information that accompanies this paper.

2.3.1. Intracellular QDot localization

To evaluate possible endosomal localization of the QDots, C17.2, HUVEC or PC12 cells were seeded in collagen-coated 35 mm diameter glass bottom MatTek dishes (MatTek Corporation, Ashland, MA, USA) at 4×10^4 cells per dish in 1.5 ml of full culture medium. Cells were allowed to settle overnight prior to being incubated with the lipophilic membrane tracer dye 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO; Molecular Probes, Invitrogen, Belgium) for 30 min at 2.5 μg ml^{-1}. Next, a mixture of the QDot at 15 nM and the lipophilic dye DiO (2.5 μg ml^{-1}) in full cell medium was prepared and added to the cells for 30 min at 37 °C at a humidified atmosphere. Subsequently, the media were removed, cells washed three times with phosphate buffered saline (PBS) and fixated with 4% paraformaldehyde (PFA) for 15 min at ambient temperature prior to visualization using a Nikon Cs1 confocal laser scanning microscope (Nikon Belux, Brussels, Belgium).

Cellular uptake of the PMA-coated QDots was also evaluated using transmission electron microscopy (TEM), following 24 h exposure of the various cell types to 10 nM of QDots, as described in the Supplementary information.

2.3.2. Quantitative determination of cellular QDot levels

The number of QDots per cell was determined by measuring the cellular Cd^{2+} levels using the Measure-iT kit (Molecular Probes, Invitrogen, Merelbeke, Belgium) as described in the Supplementary information.

2.3.3. Effect of pH on QDot stability

The effect of pH on QDot fluorescence intensity and release of Cd^{2+} ions was determined by preparing three buffer solutions of pH 7.4, 5.5 and 4.5, after which the QDots (5 nM) were exposed to these buffers for a period up to 5 days. Every 24 h, fluorescence intensity or Cd^{2+} release was measured, as described in the Supplementary information.

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