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Personalized medicine and follow-up of therapeutic delivery through exploitation of quantum dot toxicity

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ABSTRACT

Tumor therapy using nanoparticles (NPs) is mainly aimed at using the NPs as carriers for therapeutic drugs or as mediators for external stimuli to generate heat. Recent studies have shown that the toxicity of NPs can also be specifically exploited to kill cancer cells. In the present work, we employ core-only CdTe quantum dots and study their cytotoxicity using a validated high-content screening approach. The data revealed a clear correlation between toxicity and quantum dot degradation, which could be monitored through loss of fluorescence intensity. Based on the *in vitro* data obtained, the *in vivo* dose was calculated relative to the estimated number of tumor cells based on luminescence measurements. The obtained results show a clear increase in reproducibility of the therapeutic effect compared to normal conditions, where a set dose of quantum dots was administered regardless of the tumor size. The therapeutic delivery could also be monitored *in vivo*, where the loss of fluorescence intensity correlated with the anticancer efficacy. The present work highlights the benefits of noninvasive imaging to monitor therapeutic delivery and to optimize treatment via personalized medicine.

1. Introduction

The field of nanomedicine is rapidly expanding, where the number of different nanoparticles (NPs) used and the precise applications are vastly increasing [1–3]. One of the major topics of interest is the use of NPs for tumor therapy [4–6]. Commonly, NPs are designed to deliver therapeutic cargo to the site of interest [7,8] or they can be exposed to external physical stimuli (*e.g.* alternating magnetic field or light) to locally generate heat that can kill the tumor cells [9,10]. The high level of interest is mainly driven by the substantial benefits associated to the use of NPs, which include the small size that enables the NPs to leave the blood stream through leaky (*i.e.* fenestrated) endothelium as typically observed for tumor-associated blood vessels and enter the tumor itself [10]. Owing to this ability, NPs have been found to increase the delivery of any therapeutic moiety to the tumor site compared to when the therapeutic moiety is administered by itself. A second benefit consist of the wide variety of different NPs possible, varying in size, shape, chemical composition and surface functionality. This plethora of different aspects enables one to generate a highly multifunctional NP that can simultaneously be targeted towards the tumor cells through surface attachment of specific ligands, be used for monitoring through multiple non-invasive imaging methods (*e.g.* optical imaging of fluorescently-labeled NPs, magnetic resonance imaging of magnetic NPs, optoacoustic imaging of gold NPs), trigger release of therapeutic cargo and generate therapeutic heat [11,12]. Though some of these applications have found clinical translation and others are undergoing clinical trials [13], the transition from the preclinical to the clinical stage is occurring very slowly [13]. The limited clinical translation is in part due to technical difficulties, where specialized equipment is needed to generate physical stimuli for triggered drug release of heat generation which is currently not commonly available. Another factor is the need for advanced chemistry, where the generation of targeted NPs bestowed with multiple functionalities are technically quite challenging, not easy to upscale and would be very costly.

Another issue with the use of NPs for therapeutic applications is their delivery to the region of interest. Various strategies have been tried to enhance the targeted delivery of the NPs, including conjugation with specific aptamers, ligands or antibodies. To date, no real substantial breakthroughs have yet been reported, where most studies result in only a minor portion (less than 10%) of the total amount of NPs to actually reach the region of interest [14]. One

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main issue is that for most cases of targeted delivery, only the material-related aspects are considered, such as size and shape, and the biological parameters are often overlooked. Different tumor types will have different levels of angiogenesis, which will determine the accessibility for NPs. Therefore, efficient NP delivery will depend on the individual nature of the tumor type, size of the tumor, and physicochemical properties of the NPs to be used [15].

In order to try and overcome these issues and to offer novel possibilities for the biomedical use of NPs, various research groups have suggested to try and exploit the toxicity of NPs as a novel means of therapy [16]. An interesting study by Sabella and colleagues [17] suggested that ion-releasing NPs in particular are prone to inducing high levels of cytotoxicity due to the so-called "lysosome-enhanced Trojan horse effect". In this effect, the natural acidic environment and degradative capacity of cellular endosomes will result in a biotransformation of the NPs and elicit metal ion release, resulting in a cascade of intracellular toxicity. Whereas some NPs have shown to possess cancer-specific toxicity profiles in a few preliminary studies, more work is needed to confirm these results and to evaluate whether this is still the case in a dynamic, multicellular environment and not simply an artefact caused by the artificial conditions used for cell culture. However, as most NPs have very distinct toxicity profiles, the local administration of the NPs enables a direct means to evaluate antitumor efficacy and to evaluate the potential of the NPs to be studied for tumor therapy [18].

To date, most studies involving NPs use a certain dosage of NPs that will be given to all animals of the same study group. However, tumor sizes between animals can vary widely and selection of animals with identical tumor sizes requires high numbers of animals to be removed from the study, which cannot be done out of ethical reasons. Alternatively, tumor sizes can be measured and treatments can be started upon reaching a predetermined tumor size, which is quite difficult from a practical point of view, as different animals will have to be studied at different time points, which can impede the interpretation and grouping of data and complicates the study design in terms of logistics. Together, these issues result in a high variability between different animals of the same study design, which hardens any statistical analysis. Here, we suggest that using personalized therapy offers a convenient solution to overcome these issues.

In the present work, we aim to evaluate whether personalized NP-mediated therapy can increase the repeatability of the applied therapy. To this end, we perform an in-depth screening of CdTe quantum dot cytotoxicity using a validated high content imaging approach [19–21], and try to establish a link between the therapeutic potential of the NPs due to ion leaching and their fluorescence intensity. Next, the IC_{50} value is determined which is then used to calculate the number of quantum dots required for the *in vivo* tumor therapy, where cell numbers are estimated based on their bioluminescence signal. The therapeutic efficacy of the quantum dots in this personalized approach is then compared to standard classical assays where one general dose of quantum dots is applied to evaluate the potential benefit of the personalized approach.

2. Materials and methods

2.1. Quantum dots

CdTe quantum dots were purchased from PlasmaChem GmbH (Germany), which provided the −COOH functionalized CdTe quantum dots as a dry powder. The particles had a core size of 5.01 nm as provided by the company and a maximum emission at 720 nm. Stock solutions of QDs for our following biological experiments were prepared by dissolving CdTe QDs in sterilized phosphate buffered saline (PBS, pH = 7.4) and filter sterilized through a 0.22 μm filter.

2.2. Cell culture

Human cervical cancer cells (HeLa), murine lung squamous tumor cells (KLN 205), and human lung carcinoma cells (A549) were used in the present study. HeLa and A549 cells were cultured in high glucose containing Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 2 mM L-glutamine, and 1% penicillin/streptomycin (Gibco, Invitrogen, Belgium). KLN 205 cells were cultured in high glucose RPMI, supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 1% non-essential amino acids and 1% penicillin/streptomycin. All cell types were maintained in a humidified atmosphere at 37 °C and 5% CO_2 and split 1/5 upon reaching 80% confluency.

2.3. Cell-nanoparticle interaction studies

For high-content imaging studies, all cell types were seeded at 3000 cells/well in a 96 well plate (Nunc, Belgium) after which the cells were allowed to attach overnight in a humidified atmosphere at 37 °C and 5% CO_2. Then, the cells were incubated with the CdTe quantum dots for 24 h in their full growth medium at concentrations of 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μg/ml. Every condition was performed in triplicate and results were analyzed based on the three repeats. The high-content imaging experiments were then performed based on previously validated methods. Full experimental details are described elsewhere [19–21].

2.4. Determination of pH effect on quantum dot stability

To assess possible endo- and lysosomal degradation of the CdTe quantum dots, an *in vitro* model system was used as described previously [22]. For these experiments, three different buffer systems were used which consist of PBS, supplemented with 40 mM sodium citrate to which an equal amount of 20% serum-containing, high glucose cell culture medium (DMEM) was added. Next, the buffer was divided in 3 parts and the pH of the respective buffer systems was adjusted to 7.4, 5.5 or 4.5 using HCl (2 N), equaling the pH of the cell cytoplasm, endosomes and lysosomes, respectively. As such, these buffer systems contain 10% serum (the physiologically relevant concentration as used for most cell culture conditions). These buffer systems were then filter sterilized using a 0.20 μm filter (Sartorius Minisart, Vivascience, Hannover, Germany) and were further used in the experiments described below.

To evaluate the effect of the pH on the fluorescence intensity of the CdTe quantum dots, the stock suspensions were diluted up to 2 ml using the three buffer systems, reaching a final concentration of 10 nM of quantum dots. These suspensions were then transferred to 10 wells per sample of a black 96 well plate (200 μl/well) and placed at 37 °C and 5% CO_2 in a humidified atmosphere to better simulate the intracellular environment. Fluorescence intensity levels were determined using a FluoStar Omega (BMG Labtech) plate reader instrument (λex: 410 nm; λem: 780 nm) after 1, 3, 5 and 7 days of further incubation. Data are expressed as mean ± SEM of three independent experiments and given relative to the values obtained for 10 nM quantum dots diluted in 10% serum-containing PBS at the initial time point.

The generation of free Cd^{2+} ions as a result of acid etching of the quantum dots under these conditions were measured by diluting the quantum dot stock suspensions up to 1 ml in the three buffer systems, reaching a final concentration of 10 nM of quantum dots.