Acta Biomaterialia 48 (2017) 195–205

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

# Coating of Quantum Dots strongly defines their effect on lysosomal health and autophagy

Karen Peynshaert [a,b], Stefaan J. Soenen [c], Bella B. Manshian [c], Shareen H. Doak [e], Kevin Braeckmans [a,d], Stefaan C. De Smedt [a,b,*], Katrien Remaut [a,b]

[a] Lab of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B9000 Ghent, Belgium
[b] Ghent Research Group on Nanomedicines, Ghent University, Ottergemsesteenweg 460, B9000 Ghent, Belgium
[c] Biomedical MRI Unit/MoSAIC, Department of Imaging and Pathology, Catholic University of Leuven, Faculty of Medicine, UZ Herestraat 49 Box 7003, B3000 Leuven, Belgium
[d] Centre for Nano- and Biophotonics, Ghent University, B9000 Ghent, Belgium
[e] Institute of Life Science, College of Medicine, Swansea University, Singleton Park, Swansea SA2 8PP, Wales, United Kingdom

ARTICLE INFO

*Article history:*
Received 14 June 2016
Received in revised form 24 September 2016
Accepted 15 October 2016
Available online 17 October 2016

*Keywords:*
Quantum Dot
Biocompatibility
Lysosome
Autophagy

ABSTRACT

In the last decade the interest in autophagy got an incredible boost and the phenomenon quickly turned into an extensive research field. Interestingly, dysfunction of this cytoplasmic clearance system has been proposed to lie at the root of multiple diseases including cancer. We therefore consider it crucial from a toxicological point of view to investigate if nanomaterials that are developed for biomedical applications interfere with this cellular process. Here, we study the highly promising 'gradient alloyed' Quantum Dots (QDs) that differ from conventional ones by their gradient core composition which allows for better fluorescent properties. We carefully examined the toxicity of two identical gradient alloyed QDs, differing only in their surface coatings, namely 3-mercaptopropionic (MPA) acid and polyethylene glycol (PEG). Next to more conventional toxicological endpoints like cytotoxicity and oxidative stress, we examined the influence of these QDs on the autophagy pathway. Our study shows that the cellular effects induced by QDs on HeLa cells were strongly dictated by the surface coat of the otherwise identical particles. MPA-coated QDs proved to be highly biocompatible as a result of lysosomal activation and ROS reduction, two cellular responses that help the cell to cope with nanomaterial-induced stress. In contrast, PEGylated QDs were significantly more toxic due to increased ROS production and lysosomal impairment. This impairment next results in autophagy dysfunction which likely adds to their toxic effects. Taken together, our study shows that coating QDs with MPA is a better strategy than PEGylation for long term cell tracking with minimal cytotoxicity.

**Statement of Significance**

Gradient alloyed Quantum Dots (GA-QDs) are highly promising nanomaterials for biomedical imaging seeing they exhibit supremely fluorescent properties over conventional QDs. The translation of these novel QDs to the clinic requires a detailed toxicological examination, though the data on this is very limited. We therefore applied a systematic approach to examine the toxicity of GA-QDs coated with two commonly applied surface ligands, this while focusing on the autophagy pathway. The impact of QDs on this pathway is of importance since it has been connected with various diseases, including cancer. Our data accentuates that the coating defines the impact on autophagy and therefore the toxicity induced by QDs on cells: while MPA coated QDs were highly biocompatible, PEGylated QDs were toxic.



## 1. Introduction

Nanotechnology is a rapidly evolving field with a growing potential for a wide range of applications. These applications naturally require the design of highly functional though biocompatible nanomaterials (NMs). Among the most extensively investigated

* Corresponding author at: Ghent Research Group on Nanomedicines, Ghent University, Ottergemsesteenweg 460, B9000 Ghent, Belgium.
*E-mail address:* Stefaan.Desmedt@ugent.be (S.C. De Smedt).

http://dx.doi.org/10.1016/j.actbio.2016.10.022
1742-7061/

NMs for biomedical imaging applications are Quantum Dots (QDs), which are semiconductor nanocrystals with a size ranging from 2 to 100 nm [1]. They possess supremely advantageous optical properties including a very high and stable fluorescence intensity that is strongly resistant to photobleaching [2]. In addition, they are known for their broad excitation spectrum and narrow emission profile enabling efficient multiplexing [3]. Based on these features QDs have been promoted as eminent materials for *in vivo* and *in vitro* biomedical applications such as tumor visualization and intracellular trafficking [1,4–6].

With the aim to enhance the biocompatibility and optical properties of the conventional core-shell QDs, many QD designs and compositions have been investigated. Recently, gradient alloyed (GA) QDs were developed (Fig. 1) [7]. The gradient structure ensures that instead of the usual size-tunable emission of conventional QDs, the emission spectra of GA-QDs can be subtly altered by adjusting their chemical composition [8,9]. This solves issues related to size limitation sometimes occurring in biological labeling and allows multiplexing of QDs without size-related changes in sensitivity [8,10].

Despite their excellent properties, the translation of QDs in general toward biomedical applications is limited, mainly due to concerns about their toxicity. It is widely established that this toxicity, at least *in vitro*, is mainly attributed to the leaching of toxic cadmium ions and the formation of reactive oxygen species (ROS) that can induce secondary toxic effects such as DNA damage and apoptosis [11–15]. To avoid these toxic pathways, several groups are attempting to develop a more biocompatible QD core by synthesizing e.g. cadmium-free QDs [16,17]. However, next to core composition the surface chemistry of the QD can greatly influence toxicity by affecting its cellular interactions.

Several research groups have recently reported that various types of NMs can modulate (macro)autophagy [18,19]. Autophagy is a highly conserved catabolic process essential for maintaining cellular homeostasis (Fig. 2). It is usually present at a basal level in every cell where it functions as a cytoplasmic housekeeper for organelle and protein quality control. In addition, autophagy serves as a cytoprotective process that is induced to support the cell in stressful conditions such as starvation or oxidative stress [20]. Autophagy perturbations have been associated with the pathogenesis of multiple diseases including cancer, neurodegeneration and liver disease [21–23]. To date, the exact influence of cellular NM exposure on the autophagic process remains unclear. Various studies have reported clear induction of autophagy, resulting in cell death [18], whereas others have described an inhibition on autophagosome clearance, which can also result in cell death [24]. The direct induction of cell death through the autophagy process however remains a topic of debate, as autophagy is mainly a self-preservation process and any alterations observed during cell death could simply be the result of secondary unrelated bystander effects of the cell trying to recover [25]. In line with the latter view, it has been suggested that the induction of autophagy could be beneficial, as the overall toxicity of NMs could be reduced by the protective effects of autophagy [26,27].

Considering the impact of autophagy induction and inhibition as described above, we believe it is critical to characterize the influence of NMs on autophagy from a nanotoxicological point of view [18]. However, despite abundant reports on NM-modulated autophagy, only a few studies exist related to QDs. Furthermore, very little attention has yet been paid to the potential harm induced by the highly promising GA-QDs [28]. In addition, many of those studies are limited to one type of coating or lack data on intracellular uptake [18].

In this paper we look into the influence of QD surface chemistry on the toxicity and its underlying causes by comparing two types of QDs that only differ in their coating. We opted for polyethylene glycol (PEG) as a coating, since this is the most commonly applied coating strategy in biomedical applications to reduce unspecific protein binding and prevent aggregation. In addition, PEGylation is known to increase the blood circulation time of particles by preventing NP uptake by the reticuloendothelial system [29–31]. As a second coating strategy we selected the short ligand 3-mercaptopropionic acid (MPA) based on previous reports that observed limited toxicity with this coating.[29–31] We therefore wanted to analyze this promising observation further with a special focus on autophagy since the influence of MPA coating on this pathway remains unexplored so far. To this end, we examined the aggregation profile, cellular uptake, cytotoxicity and associated ROS levels of QDs, and studied their effect on lysosomes and autophagosomes - the two most essential organelles of the autophagy pathway.

## 2. Methods

### 2.1. Materials

Two types of spherically shaped Gradient Alloy Quantum Dots were purchased from Mesolight LLC (Little Rock, Arkansas, USA). Both types have a gradient $CdSe_xS_{1-x}$ core surrounded by a ZnS shell (Fig. 1). The GA-QDs only differ in their surface coating: one particle is coated with polyethylene glycol (PEG) with terminating carboxyl groups while the other one is coated with 3-mercaptopropionic acid (MPA). Both QDs have an emission maximum at 580 nm (Supplementary Fig. S1), and exhibit very similar quantum yields, i.e. 60% for PEG-QDs and 65% for MPA-QDs. All QD dispersions were diluted from the original colloidal suspen-

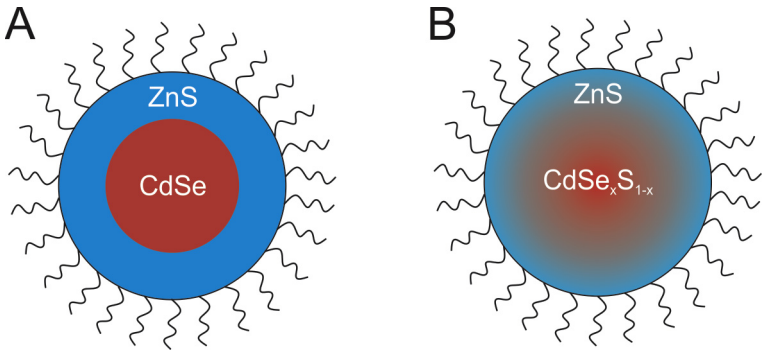


**Fig. 1.** Schematic design of a core-shell QD (A) and a gradient alloyed QD (B). A core-shell QD commonly exists of a metal core enveloped by an inorganic shell and a coating that renders them water-soluble and allows for further conjugation [76]. In case of gradient alloyed QDs, the defined core-shell interface within the QD is replaced by a gradient composition.