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Short Communication

A methacrylate-based polymeric imidazole ligand yields quantum dots with low cytotoxicity and low nonspecific binding

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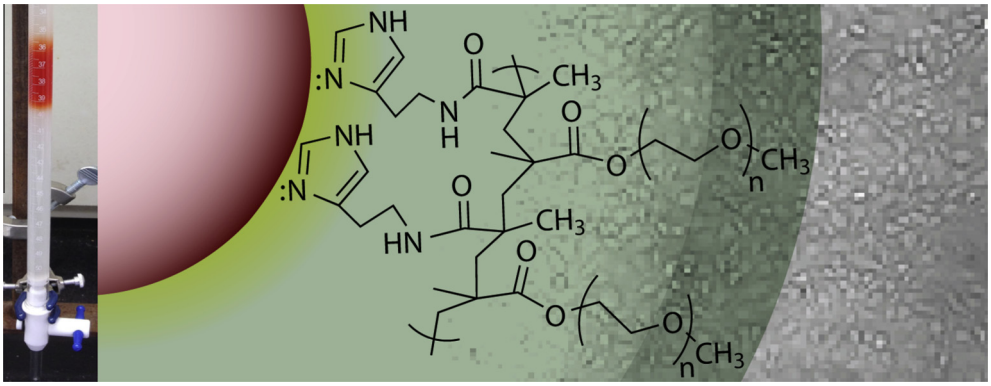
GRAPHICAL ABSTRACT



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ABSTRACT

This paper assesses the biocompatibility for fluorescence imaging of colloidal nanocrystal quantum dots (QDs) coated with a recently-developed multiply-binding methacrylate-based polymeric imidazole ligand. The QD samples were purified prior to ligand exchange via a highly repeatable gel permeation chromatography (GPC) method. A multi-well plate based protocol was used to characterize nonspecific binding and toxicity of the QDs toward human endothelial cells. Nonspecific binding in 1% fetal bovine serum was negligible compared to anionically-stabilized QD controls, and no significant toxicity was detected on 24 h exposure. The nonspecific binding results were confirmed by fluorescence microscopy. This study is the first evaluation of biocompatibility in QDs initially purified by GPC and represents a scalable approach to comparison among nanocrystal-based bioimaging scaffolds.

Abbreviations: QD, quantum dot; GPC, gel permeation chromatography; FBS, fetal bovine serum; HUVEC, human umbilical vein endothelial cell; MA-PIL, methacrylate-based polymeric imidazole ligand; PEG, poly(ethylene glycol); DHLA, dihydrolipoic acid.

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1. Introduction

Colloidal nanocrystal quantum dots (QDs) offer size-tunable absorption and emission spectra [1], large molar extinction coefficients [2,3] and 2-photon excitation cross-sections [4,5], and high photostability [6,7] compared to most molecular fluorophores. These properties have led to intense interest in QDs as light emitters in bioimaging [5,7–10]. Requirements for biocompatible nanocrystals include high colloidal stability, minimized nonspecific binding to cell surfaces and other biological substrates, and low toxicity on relevant timescales. Hydrophilic organic ligand coatings have been demonstrated as a route to biocompatible QDs with smaller hydrodynamic radii than are achieved by encapsulation strategies [11–14]. A key challenge for ligand exchange strategies is to install strongly-binding ligands in a highly repeatable and modular manner. Recently, several groups have developed co-polymer ligands in which multiple functional groups providing QD chelation, water solubility, and/or handles for derivatization are pendant on a polymer backbone [15–19]. Multiply-binding polymeric ligands are designed to provide biocompatible QDs with increased stability compared to those terminated with small molecules bearing the same chelating groups, as well as a high degree of tunability on the basis of polymer composition and length. We recently described the synthesis of a series of polymeric imidazole ligands with a methacrylate backbone (MA-PILs) and their use in preparing water-soluble QDs [20]. The methacrylate monomers exhibit increased stability compared to acrylate monomers, facilitating the control of polymer ligand properties through RAFT polymerization kinetics.

Here, we evaluated the biocompatibility of a series of representative MA-PIL multiply-binding ligand formulations through scalable nonspecific binding and cell viability assays. To ensure a consistent QD starting material, we employed gel permeation chromatography (GPC) purification of the QDs [21] to remove impurities and weakly bound species prior to ligand exchange to install the MA-PIL ligand. The result illustrates the potential of methacrylate-based polymeric ligands to form biocompatible nanocrystals for targeting and sensing applications. Furthermore, these techniques and the results can serve as the basis for fundamental study and practical optimization of multiply-binding polymeric ligands for biomedical applications of inorganic nanoparticles.

2. Materials and methods

2.1. Materials

Wurtzite CdSe/CdZnS core/shell quantum dots and MA-PIL ligands were synthesized as described previously [20], PEG side-chains were incorporated using poly(ethylene glycol) methyl ether methacrylate 500 from Sigma–Aldrich. Bio-Beads S-X1 GPC medium was obtained from Bio-Rad Laboratories. Toluene-d_8 (D, 99.5%) was obtained from Cambridge Isotope Laboratories. Decylamine (95%) was purchased from Sigma Aldrich. Oleylamine (80–90%) and Bis(trimethylsilyl) sulfide ($(TMS)_2S$; 95%) were purchased from Acros Organics. Rhodamine 590 chloride (R590, MW 464.98) was obtained from Exciton. Toluene (99.5%) and Tetrahydrofuran (THF, 99%) were purchased from Mallinckrodt Chemicals. Ethanol (200 proof) was obtained from Decon Laboratories. Acetone (99.9%) was purchased from VWR. Methanol (99.9%) was purchased from Fisher Scientific. Toluene was dried with activated 4A molecular sieves. THF was dried using the Puresolv system from Innovative Technologies. Synthetic or analytical procedures under inert conditions were carried out using Schlenk line techniques or in a glovebox under N_2 atmosphere. All media components were from Sigma Aldrich. 96 well plates with clear plastic bottoms and black walls were obtained from VWR. Calcein AM was obtained from Invitrogen.

2.2. Preparation of MA-PIL coated quantum dots

Prior to ligand exchange, QDs (5 nmol) were purified by one cycle of precipitation and redissolution followed by GPC in toluene solvent. Toluene was then removed under vacuum, and chloroform added (0.3 mL) to form a clear solution. This QD solution was mixed with a solution of the MA-PIL ligand (175:1 mol ratio, for example ~30 mg for 34K MA-PIL), also in chloroform. After 30 min stirring at room temperature, a small amount of methanol was introduced followed by 30 min additional stirring. The QDs were then diluted with ethanol and hexane was introduced, causing precipitation of the ligand-exchanged QDs. The solution was decanted and QDs were redissolved in aqueous phosphate buffer solution (pH 7.4), and filtered (0.2 μm pore size). Finally, samples were dialyzed into deionized water via repeated centrifugal filtration with a 50,000 molecular weight cutoff membrane prior to biocompatibility experiments.

2.3. Cell culture and preparation

Human umbilical vein endothelial cells (HUVECs) (American Type Culture Collection) were maintained in Ham's F12K medium supplemented with 10% fetal bovine serum (FBS), 0.1 mg/mL heparin, 30 μg/mL endothelial cell growth supplement, 100 units/mL penicillin, and 100 μg/mL streptomycin. Prior to experimentation, HUVECs were seeded at a density of 5×10^4 cells/well onto black-sided 96-well tissue culture plates and maintained for 24 h in supplemented Ham's F12K medium with 1% FBS to allow formation of confluent monolayers. All cultures were maintained at 37 °C in a humid atmosphere of 5% CO_2 and 95% air.

2.4. Calcein AM cell viability assay

To characterize the potential toxicity of MA-PIL QDs, Calcein AM was used to assess cell viability following exposure to QDs. Confluent HUVEC monolayers were incubated (37 °C, 5% CO_2) with QDs coated with MA-PILs exhibiting effective molecular weights of 10K, 22K, or 34K. QD solutions were prepared by adding small aliquots of MA-PIL QD solutions in DI water to supplemented medium containing 1% FBS to achieve an ultimate concentration of 100 nM QDs. Monolayers incubated with equivalent dilution of DI water or with 25 μM cadmium acetate served as the vehicle and positive controls, respectively. Following 24 h incubation, treatments were decanted and replaced with 1 μM Calcein AM diluted in phenol red-free, serum-free media. When taken up by living cells, the non-fluorescent Calcein AM probe is hydrolyzed by endogenous esterases to yield fluorescent acetoxymethyl ester, thereby allowing for a quantifiable measurement of cell viability. After 1 h incubation (37 °C, 5% CO_2), fluorescence was measured using a BioTek Synergy 2 multi-mode microplate reader equipped with excitation and emission filters of 485 ± 20 nm and 530 ± 25 nm, respectively, and using baseline (media containing Calcein AM) subtraction. Cell viability is reported as Calcein AM fluorescence normalized to the fluorescence observed for the vehicle. Each treatment was performed in triplicate, and results shown are the mean ± SEM of three independent experiments.

2.5. Nonspecific binding assay

To evaluate nonspecific interactions between MA-PIL QDs and cells, a static adhesion assay was implemented. Confluent HUVEC monolayers were incubated for 5 min with MA-PIL QDs exhibiting molecular weights of 10K, 22K, or 34K. QD solutions were prepared