Attachment of CdSeTe/ZnS Quantum Dots to Alginate for Non-Invasive Detection of Gel Implants

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Abstract

Quantum dots (QDs), known for their size-dependent optoelectronic properties and fluorescence, have applications in *in vivo* imaging. Bioactive coatings, such as surfactants and polymers, limit cytotoxicity and allow for cell-specific targeting as well as alter the solubility, functionalization, quantum yield, and blinking properties of QDs. Due to high levels of endogenous absorbers, deep-tissue imaging requires excitation and emission wavelengths in the near infrared region (NIR), which mandate a wavenumber greater than 700 nm. CdSeTe/ZnS QDs with coatings and functional amines (-NH_2) were covalently linked with alginate biomaterial chains to form implantable alginate gels that can be non-invasively detected *in vivo* through infrared light. The amine functionalized CdSeTe/ZnS QDs, with the intent of developing a noninvasive imaging system, were confirmed to be stimulated and activated in the NIR. They were able to be covalently linked to alginate polymer chains and made into gel blocks. Future work will investigate the *in vivo* degradation of these QD-labeled alginate gels in animal models using a NIR fluorescence system for drug delivery.

1. Introduction

Semiconductor nanoparticles or quantum dots (QDs) have gained significant interest in biomedical imaging for their size-dependent optoelectronic properties and fluorescence **[7]**. QDs are described by an internal inorganic core surrounded by a shell of molecular ligands, commonly referred to as a bioactive coating (**Fig. 1**) **[1]**. Naked QDs are generally cytotoxic, reacting with oxygen species, and resulting in subsequent damage to cellular components. Bioactive coatings, such as surfactants and polymers, limit cytotoxicity and allow for cell-specific targeting as well as alter the solubility, functionalization, quantum yield, and blinking properties of QDs **[7]**. Polymeric substances, including organic dendrons, linear or hyperbranched polymers, and modified proteins have been used to control the surface chemistry of QDs **[1]**. Further, QDs have broad absorption spectra and narrow emission spectra, making them good candidates for *in vivo* imaging **[7,8]**. Due to high levels of endogenous absorbers,

theoretical modeling studies have indicated that two spectral windows, one at 700-900 nm and another at 1,200-1,600 nm, exist for *in vivo* QD imaging **[5]**.

Although the size, composition, and structure of QDs can predict their magnetic, optical, and electrical properties, the molecular structure of surface ligands controls the chemistry **[1,7]**. By covalently linking water-soluble ligands to the surface, bioconjugated QDs can be applied to biological systems where water is the primary solvent **[1]**.

Sodium alginate (Alg), a water-soluble polymer (**Fig. 2**), consists of α-L-guluronate (G) and β-D-mannuronic acid (M) subunits. The renewable and biodegradable polysaccharide has biological applications. Alg contains the carboxylic acid functional group, making it an ideal candidate for QD linkage **[1]**.

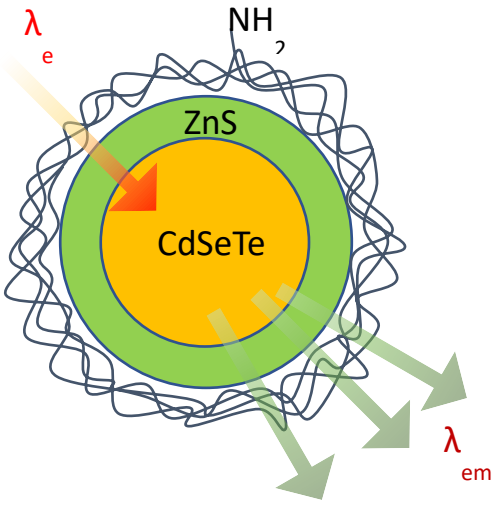

Fig. 1. Illustrative structure of CdSeTe/ZnS amine functionalized quantum dot.

Drug delivery systems (DDS) are vital to pharmaceutical and clinical sciences, controlling drug release through stimuli-responsive or smart materials **[6]**. By linking QDs with a water soluble polymer that acts as a DDS material, clinicians can non-invasively detect gel implants.

In this work, CdSeTe/ZnS QDs were

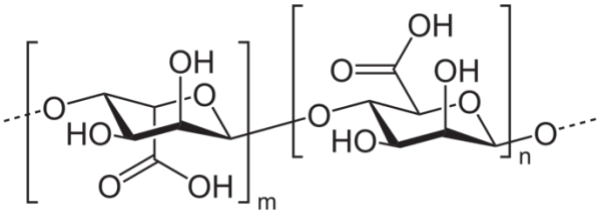

Fig. 2. Chemical structure of sodium alginate.

covalently linked with alginate biomaterial chains to form implantable alginate gels that can be non-invasively detected *in vivo* through infrared light. Our results showed that amine functionalized CdSeTe/ZnS QDs were responsive to excitation wavelengths (λ_{ex}) in the near-infrared region (NIR).

2. Materials and Methods

2.1. Materials

Amine functionalized CdSeTe/ZnS QDs with a given peak emission wavelength (λ_{em}) of 820nm were purchased from NanoOptical Materials (Carson, California). Sodium alginate, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (sulfo-NHS), calcium chloride ($CaCl_2$), ethylenediaminetetraacetic acid (EDTA) chelator, 2-ethanesulfonic acid (MES), sodium chloride (NaCl), sodium phosphate (Na_3PO_4), formaldehyde (CH_2O), ethanol (C_2H_5OH), calcium acetate ($Ca(C_2H_3O_2)_2$), potassium chloride (KCl), disodium phosphate (Na_2HPO_4), and

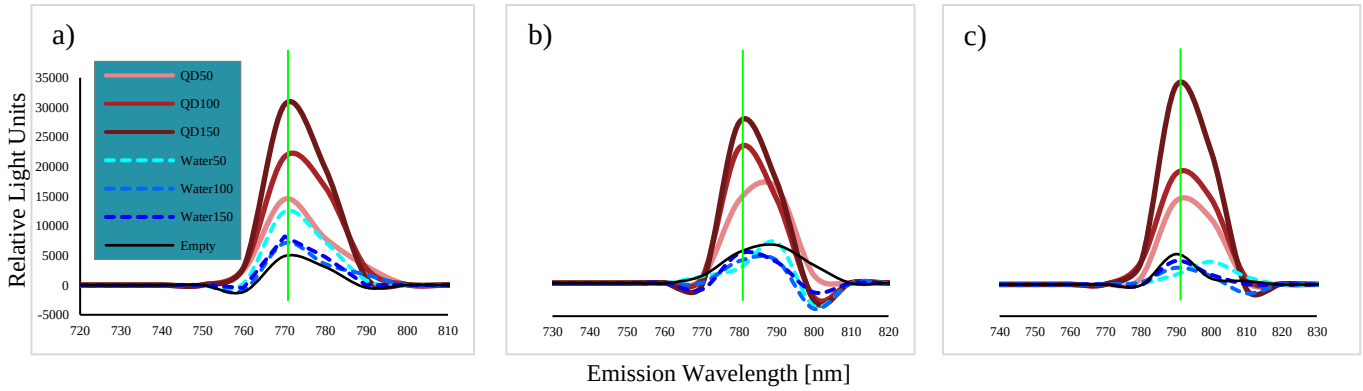

Fig. 3. Fluorescence spectra of CdSeTe/ZnS QDs at λ_{ex} of (a) 720, (b) 730, and (c) 740 nm before linkage with alginate.

monopotassium phosphate (KH_2PO_4) were purchased from Sigma Aldrich.

2.2. Absorption spectra of QDs

Fluorescence spectroscopy (Molecular Devices SpectraMax Plus 384 model, Downingtown, PA) was used to verify the optical properties of amine functionalized CdSeTe/ZnS QDs. An emission spectral scan of QDs was performed with emission wavelengths from 720-850 nm, 730-850 nm, and 740-850 nm and excitation wavelengths of 720 nm, 730 nm, and 740 nm, respectively. The spectra were obtained at 22.5°C, and the optical measurements were carried out with water as the reference.

2.3. QD-alginate conjugation

Phosphate buffered saline (PBS) was prepared from 0.8% sodium chloride (NaCl), 0.02% potassium chloride (KCl), 0.144% disodium phosphate (Na_2HPO_4), and 0.024% monopotassium phosphate (KH_2PO_4). Activation buffer was prepared from 1.0M MES and 0.5M sodium chloride (NaCl) at pH 6.0. Coupling buffer was prepared from phosphate-buffered saline (PBS), 100mM sodium phosphate (Na_3PO_4), and 150mM NaCl at pH 7.2. A two-step coupling of alginate and QDs using EDC and sulfo-NHS produced 1.0 mL QDs covalently linked with alginate (QDs-alginate). 200 µL sodium alginate in activation buffer was added to 200 µL EDC in coupling buffer and 200 µL sulfo-NHS in activation buffer. The solution reacted for 15 minutes at room temperature (≈23°C)

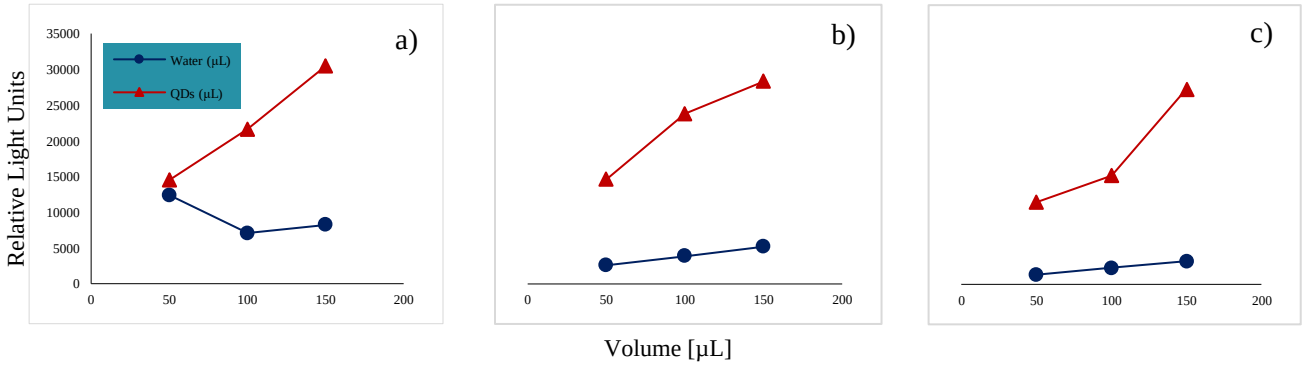

Fig. 4. Fluorescence spectra of QDs and water at (a) λ_{ex} of 720 nm and λ_{em} of 770 nm (b) λ_{ex} of 730 nm and λ_{em} of 780 nm, and (c) λ_{ex} of 740 nm and λ_{em} of 790 nm based on volume

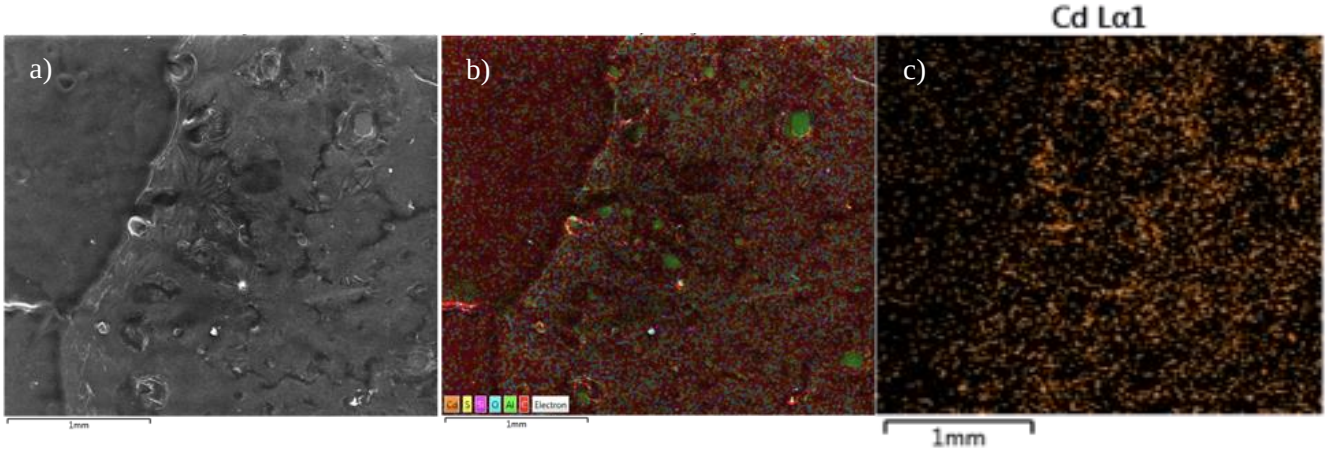

Fig. 5. SEM photographs of CdSeTe/ZnS amine functionalized QDs before linkage.

before 300 µL QD in 100 µL coupling buffer was added.

2.4. Scanning electron microscopy (SEM) analysis

QDs-alginate and QDs were dialyzed in distilled water for 12 h. SEM specimens, dialyzed QDs, were made by evaporating one drop of QDs on carbon coated grids. SEM images were taken by a scanning electron microscope (Quanta SEM, ThermoFisher Scientific, Waltham, MA) with energy-dispersive X-ray spectroscopy (EDS) detector operating at 30KV.

2.5. Characterization of alginate gels

Gels were made by fusing alginate particles from mixtures of 1% sodium alginate, QDs-alginate, and calcium chloride. Carnoy's solution was prepared from 100 mL 40% formaldehyde, 900 mL, 95% ethanol, and 0.5 g calcium acetate. QD-alginate gels were soaked in 10 mL solution for 8 h. Fixed and unfixed gels were treated with 1.0M ethylenediaminetetraacetic acid (EDTA) chelator, and their masses were measured during a 4 h period.

3. Results and Discussion

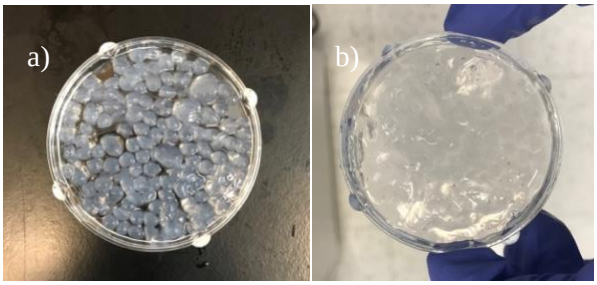

Fig. 6. QDs-alginate gelation.

3.1. Optical properties of QDs prior to linkage

To investigate the optical properties of amine functionalized CdSeTe/ZnS QDs, their fluorescence spectra was recorded prior to experimentation. The emission wavelengths were recorded from 720-850 nm, 730-850 nm, and 740-850 nm when the samples were exposed to excitation wavelengths of 720 nm, 730 nm, and 740 nm, respectively. **Fig. 3** shows the absorption spectra of different volumes (50, 100, and 150 µL) of amine functionalized CdSeTe/ZnS QDs (QD50, QD100, and QD150, respectively) while the concentrations of the QDs were constant. The samples emitted a dose dependent response with relatively low signals from water and the black opaque surface (empty well). When excited with λ_{ex} ranging from 720 to 740 nm, QD samples generated emission spectra

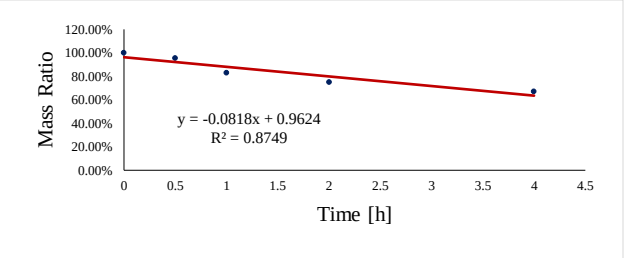

Fig. 7. Unfixed QDs-alginate gel degradation in EDTA.

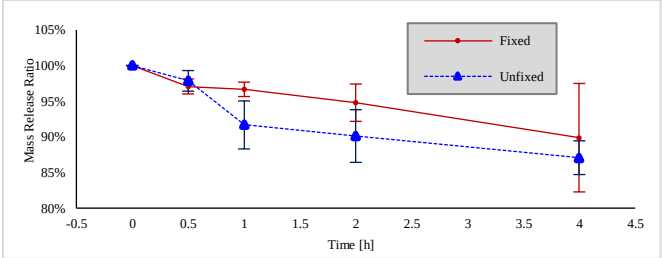

Fig. 8. Formaldehyde-fixed and unfixed QDs-alginate gel degradation in EDTA.

peaking at λ_{em} from 770 to 790 nm, which lie in the NIR region.

Fig. 4 shows the changes in absorbance between water and QD samples at 50, 100, and 150 µL during an emission spectral scan at 720, 730, and 740 nm. The greatest change in absorbance between water and QD at 50 and 150 µL was recorded during an emission spectral scan with λ_{ex} of 740 nm and λ_{em} of 790 nm. Under the stated conclusion, it was concluded that if CdSeTe/ZnS QDs were placed in a biological system, λ_{ex} at 740 nm and λ_{em} at 790 nm would provide for the most conclusive results.

3.2. SEM images

SEM did not distinguish individual QDs due to the resolution limitation. EDS detector images in **Fig. 5** confirmed the presence of Cd in dried samples of QDs.

3.2. Synthesis of QD-alginate particles

Alginate particles were synthesized and incubated with calcium ions with small quantities of EDTA to make fused gel blocks (**Fig. 6**). The covalently linked QD-alginate were also added to fabricate similar gels. Addition of 100mM EDTA to unfixed gels degraded the gel samples at a linear rate, represented by the equation: y=-0.0818x+0.9624 in **Fig. 7**, where y equals mass release ratio and x equals the time in hours. **Fig. 8** shows the degradation rate of formaldehyde-fixed and unfixed gels. Formaldehyde-fixed gels decreased the degradation rate, indicating that alginate chains were crosslinked. After four hours, the mass ratios of unfixed and fixed gels were found to be 87% and 90%, respectively.

4. Conclusion

In summary, CdSeTe/ZnS were covalently linked with alginate through EDC/sulfo-NHS chemistry. They were covalently linked to alginate polymer chains and made into gel blocks. Results showed that the amine functionalized CdSeTe/ZnS QDs were stimulated and activated in NIR light (**Fig. 3**). Alginate degradation experiments confirmed that formaldehyde-fixed gels decreased the degradation rate (**Fig. 8**). Future work will investigate the *in vivo* degradation of these QD-labeled alginate gels in animal models using a NIR fluorescence system for drug delivery.

Acknowledgements

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