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

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RATIONALE

BACKGROUND

Quantum dots (QDs) are known for their size-dependent optoelectronic properties and fluorescence. Applications of QDs include single molecule biophysics, optical barcoding, and *in vivo* imaging (Wagner, Knipe, Orive, 2019). QDs are described by an internal inorganic core surrounded by a shell of molecular ligands, commonly referred to as a bioactive coating (Bardajee & Hooshyar, 2011). 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 QD properties (solubility, functionalization, quantum yield, blinking properties) (Wagner, Knipe, Orive, 2019). Polymeric substances, including organic dendrons, linear or hyperbranched polymers and modified proteins have been proven to control the surface chemistry of QDs (Bardajee & Hooshyar, 2011).

Sodium alginate (Alg), a water-soluble biopolymer, consists of α-L-guluronate (G) and β-D-mannuronic acid (M) subunits. The renewable and biodegradable polysaccharide has been proven safe for biological applications. Alg contains the carboxylic acid functional group, making it an ideal candidate for QD linkage (Bardajee & Hooshyar, 2011).

IMPORTANCE

- CdSeTe (core) / ZnS (shell) QDs with coatings and functional amines (given peak emission wavelength (λ_{em}) = 820 nm) will be covalently linked with alginate biomaterial chains to form implantable alginate gels that can be non-invasively detected *in vivo* through infrared light

SOCIETAL IMPACT

- Water soluble QDs can act as a real-time *in vivo* deep tissue diagnostic agent in place of organic dyes
    - Optical properties of QDs are controlled by particle size, size distribution, surface chemistry, while the optical properties of organic dyes are dependent on electronic transition(s) (Resch-Genger, Grabolle, Cavaliere-Jaricot, Nitschke, Nann, 2008)

RESEARCH QUESTION

How do amine functionalized CdSeTe/ZnS QDs covalently linked with alginate compare to traditional non-invasive *in vivo* imaging techniques?

HYPOTHESIS

If amine functionalized CdSeTe/ZnS QDs are covalently linked to alginate biomaterial for the non-invasive detection of gel implants, then the amine-functionalized QDs will be stimulated and activated in the near-infrared region (NIR) of light.

REASONING

- In a similar study that aimed to investigate the applications of alginate-based biopolymers as multidentate ligands for CdS QDs, intensity levels for QDs were significantly higher than bioconjugated QDs and the size of CdS QDs remained relatively constant
    - Bioactive coatings are known to reduce QD signals
    - SEM and TEM images of bioconjugated QDs show that the morphology of Alg remained unchanged after linkage with CdS QDs
- Concluded that QDs allowed Alg to bind to the surface of QDs with relatively high stability, solubility and optical efficiency ratings (Bardajee & Hooshyar, 2011)

ENGINEERING GOAL

- To investigate the optical efficiency of amine functionalized CdSeTe/ZnS QDs when linked to alginate biomaterial

EXPECTED OUTCOME

- To successfully link amine functionalized CdSeTe/ZnS QDs with alginate biomaterial
- To test the stimulation and activation wavelengths of the bioconjugated CdSeTe/ZnS QDs
- It's expected that the CdSeTe/ZnS QDs will have comparable stimulation and activation wavelengths to amine functionalized CdSeTe/ZnS QDs covalently linked with alginate biomaterial

MATERIALS

- Amine functionalized CdSeTe (core) / ZnS (shell) QDs (cat# QD820-WS-yy: NanoOptical Materials, Carson, CA) (given peak emission wavelength (λ_{em}) = 820 nm)
- Sodium Alginate
- N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)
- Sulfo-NHS (N-hydroxysuflosuccinimide)
- Sodium dodecyl sulfate poly(acrylamide) gel electrophoresis (SDS-PAGE)
- Calcium Chloride
- Ethylenediaminetetraacetic acid (EDTA) chelator

PROCEDURE

Verification of the properties of CdSeTe/ZnS QD with fluorescence spectrophotometer

- Optical properties of QDs will be verified using fluorescence spectroscopy
    - Excitation wavelength (λ_{ex}) will be set to a constant value, while λ_{em} will be scanned over a range of values, and vice versa
- λ_{ex} and λ_{em} pairs will be obtained in correspondence to the near-infrared region (NIR)

Verification of alginate properties

- Gels will be made by fusing alginate particles from mixtures of 1% sodium alginate, QDs-alginate, and calcium chloride

- Gels (unfixed and formaldehyde-fixed) will be treated with ethylenediaminetetraacetic acid (EDTA) chelator to determine their stability

Covalent linkage of CdSeTe/ZnS QD with alginate

- QDs (with -NH_2) will be attached to alginate containing carboxylic acid groups (-COOH) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and sulfo-N-hydroxysulfosuccinimide (sulfo-NHS) reagents
    - Crosslinking reactions utilizes N-(3-dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDC) that has dermally acute toxicity
    - Chemical fume hoods will be used when handling chemicals
    - Waste chemicals will be collected in proper waste containers
        - Waste containers will be stored and collected by approved chemical disposal companies

Scanning electron microscopy (SEM)

- CdSeTe/ZnS QDs and CdSeTe/ZnS QDs linked with alginate will be imaged and their elemental composition will be evaluated using scanning electron microscopy (SEM) with (energy-dispersive X-ray spectroscopy) EDS detector

Sodium dodecyl sulfate poly(acrylamide) gel electrophoresis (SDS-PAGE)

- Linkages will be investigated through sodium dodecyl sulfate poly(acrylamide) gel electrophoresis (SDS-PAGE).

In vivo imaging

- QDs will be placed *in vivo* and optical efficiency will be measured using the Li-COR Pearl Trilogy

RISK AND SAFETY

- Laboratory safety requirements will be followed in accordance with the protocols listed in Safety Data Sheets, NIH Chemical Safety Guide, and NIH Biosafety in Microbiological and Biomedical Laboratories
- Online courses, NIH Laboratory Safety 101 and Working Safely with HIV and Other Bloodborne Pathogens, will be completed prior to laboratory work
- Personal protective equipment (PPEs) including laboratory coats, gloves, and eye goggles will be used

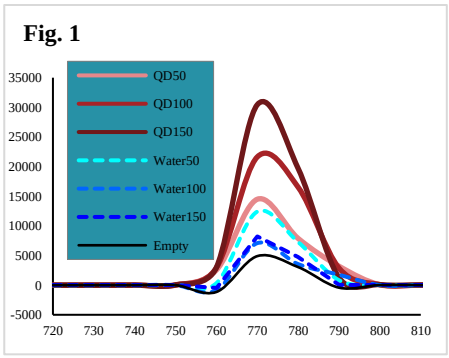
DATA ANALYSIS

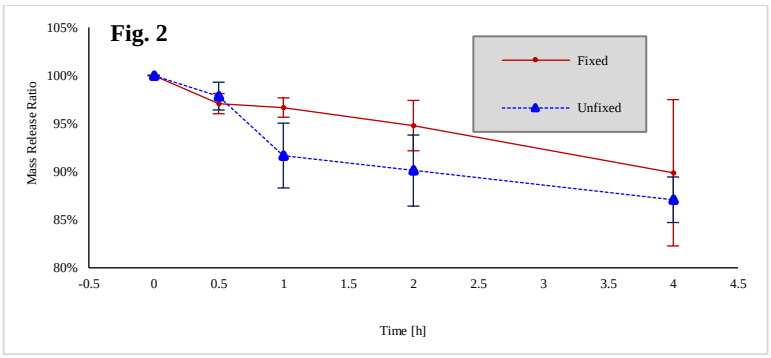
Fluorescence spectroscopy analysis (**Fig. 1**)

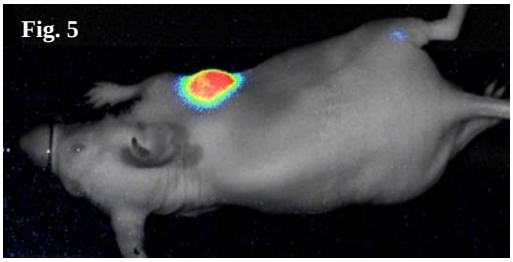
- Graphical analysis (x-axis: excitation/emission wavelength (nm); y-axis: emission/excitation intensity)
    - Volume of samples will be changed to ensure a dose-dependent relationship
    - QD (50 mL, 100 mL, 150 ml) will be compared against water (50 mL, 100 mL, 150 ml) to ensure that emission and excitation intensity is greater than that of water
        - Comparison between intensity of water and QDs ensures that the QD-alginate system can be applied to a biological system

Analysis of alginate properties (**Fig. 2**)

- Graphical analysis (x-axis: time (hr); y-axis: mass release ratio (%)
    - Degradation rate of alginate gels in EDTA chelator will be measured as a function of time







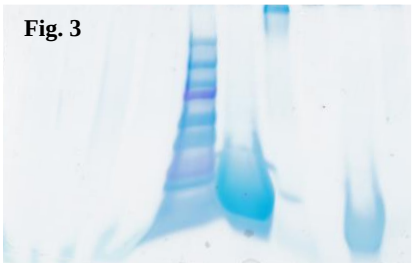
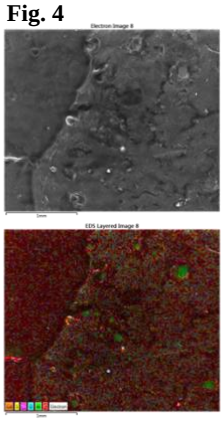


Fig. 4



Three tests will be used to ensure that CdSeTe/ZnS QDs are covalently linked to alginate biomaterial.

A. SDS-PAGE analysis (**Fig. 3)**
   - Image analysis: data will be compared against a marker
     - Lane samples: QDs (QD), alginate (A), QDs linked with alginate (QDA)
     - Larger molecular weight will signal that QDs linked with alginate biomaterial

B. SEM image analysis (**Fig. 4**)
   - Image analysis: EDS detector will be used to determine sample composition
     - Topographic surface analysis on the nanometer scale

C. *In vivo* imaging (**Fig. 5**)
   - Image analysis: efficiency of QDs will be measured *in vivo*
     - 800 nm channel allows for excitation wavelength of 785 nm and emission wavelength of 820 nm

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ADDENDUM
PROCEDURE

Dialysis of CdSeTe/ZnS QDs covalently linked with alginate post EDC/sulfo-NHS reaction

- CdSeTe/ZnS was dialyzed after EDC/sulfo-NHS reaction to remove excess salts from the samples prior to SEM imaging

Preparation of Carnoy's solution

- Prepared from 100 mL 40% formaldehyde, 900 mL, 95% ethanol, 0.5 g calcium acetate
- Alginate gels were soaked in 10 mL of Carnoy's solution for 8 hr

Verification of the properties of CdSeTe/ZnS QDs covalently linked with alginate using fluorescence spectroscopy

- Verify optical properties of QDs using fluorescence spectroscopy
    - Set excitation wavelength (λ_{ex}) to a constant value, while λ_{em} scans over a range of values, and vice versa
- λ_{ex} and λ_{em} pairs should be obtained in correspondence to the near-infrared region (NIR)