Biosensors and Bioelectronics 80 (2016) 315–322

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

# Functional surface engineering of quantum dot hydrogels for selective fluorescence imaging of extracellular lactate release

Xiaomeng Zhang, Shushu Ding, Sumei Cao, Anwei Zhu *, Guoyue Shi

*School of Chemistry and Molecular Engineering, East China Normal University, 500 Dongchuan Road, Shanghai 200241, PR China*

ARTICLE INFO

*Article history:*
Received 12 October 2015
Received in revised form
17 January 2016
Accepted 29 January 2016
Available online 30 January 2016

*Keywords:*
Lactate
Quantum dot hydrogel
Fluorescence resonance energy transfer
Cell
Fluorescence imaging

ABSTRACT

Selective and sensitive detection of extracellular lactate is of fundamental significance for studying the metabolic alterations in tumor progression. Here we report the rational design and synthesis of a quantum-dot-hydrogel-based fluorescent probe for biosensing and bioimaging the extracellular lactate. By surface engineering the destabilized quantum dot sol with Nile Blue, the destabilized Nile-Blue-functionalized quantum dot sol cannot only self-assemble forming quantum dot hydrogel but also monitor lactate in the presence of nicotinamide adenine dinucleotide cofactor and lactate dehydrogenase through fluorescence resonance energy transfer. Notably, the surface engineered quantum dot hydrogel show high selectivity toward lactate over common metal ions, amino acids and other small molecules that widely coexist in biological system. Moreover, the destabilized Nile-Blue-functionalized quantum dots can encapsulate isolated cancer cells when self-assembled into a hydrogel and thus specifically detect and image the extracellular lactate metabolism. By virtue of these properties, the functionalized quantum dot hydrogel was further successfully applied to monitor the effect of metabolic agents.



## 1. Introduction

Semiconductor nanocrystals, socalled quantum dots (QDs), have generated great research in the fluorescent bioimaging and biosensing (Freeman and Willner, 2012; Medintz et al., 2005; Somers et al., 2007; Wu and Yan, 2013). For biological applications, most fluorescent probes and applications were based on the monodisperse water-soluable QDs that were synthesized by the strategies such as surface functionalization with water-soluble ligands, silanization and encapsulation within block-copolymer micelles (Zhu et al., 2012; Zrazhevskiy et al., 2010). Very recently, enormous efforts have been made toward the self-assembly of colloidal QDs into hydrogels and aerogels (Arachchige and Brock, 2007a; Gaponik et al., 2008; Jeong et al., 2011). The specially designed QD gels kept the quantum confinement effect and retained the luminescence of the colloidal QDs (Arachchige and Brock, 2007b). Moreover, the pore sizes of the nanocrystal gels ranging from meso to macro size is exceptionally advantageous for the encapsulation of a variety of biomaterials from proteins to whole cells in a functional state (Yuan et al., 2013), which opens opportunities to fabricate biocompatible sensors. Obviously, the QD gel has great potential to act as a multifunctional platform for fluorescent bioanalysis. However, up to now, most of the attention was paid to extend the material range of the nanocrystal gels (Gaponik et al., 2012). Very fewer attempts were reported to demonstrate bioimaging and biosensing application although some pioneering papers explicitly stressed on the necessity of further processing materials to improve their properties relevant to applications in optical sensing (Mohanan et al., 2005). Recently, a mercaptosuccnic acid (MSA)-capped CdTe QD hydrogel was developed for fluorescent sensing of dopamine based on the simple encapsulation of tyrosinase in a QD hydrogel (Yuan et al., 2013). However, it is likely that the luminescence of the MSA-capped CdTe QD hydrogel is sensitive to many ions as well as dopamine due to the ionic interaction with the surface of QDs, showing no selectivity in biological applications (Chen and Rosenzweig, 2002). Thus, it remains challenging work to create a QD hydrogel-based probe that possesses an appropriate combination of selectivity and controlled self-assembly for biosensing and bioimaging. In addition, it is of great interest to exploit QD hydrogel as a multifunctional platform for probing multiple dynamic bioprocesses. Since various redox enzymes comprise the common nicotinamide adenine dinucleotide ($NAD^+$) cofactor (Bardea et al., 1997), it is reasonable to expect that the reduced form of nicotinamide adenine dinucleotide (NADH)-sensitive QD hydrogel could provide a versatile gel probe to analyse $NAD^+$-dependent enzymes, as well as to detect their substrates.

In this paper, we report on the self-assembly of destabilized Nile-Blue-functionalized CdTe QDs into a QD hydrogel that fluorescent probe NADH with high specificity and their use to follow

* Corresponding author.
*E-mail address:* awzhu@chem.ecnu.edu.cn (A. Zhu).

http://dx.doi.org/10.1016/j.bios.2016.01.083
0956-5663/

$NAD^+$-dependent biocatalytic transformation. Furthermore, we incorporated the QD hydrogel into HeLa cells and monitored the extracellular lactate release and the effect of metabolic agent.

## 2. Experimental section

### 2.1. Reagents and materials

Cadmium chloride ($CdCl_2$), sodium borohydride ($NaBH_4$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) were purchased from the Sinopharm Chemical Reagent Co., Ltd. Sodium tellurite ($Na_2TeO_3$) was obtained from Alfa Aesar. Mercaptosuccinic acid (MSA), suberic acid bis(3-sulfo-N-hydroxysuccinimide ester) sodium salt ($BS^3$), Nile Blue (NB), bovine serum albumin (BSA), reduced and oxidized forms of nicotinamide adenine dinucleotide (NADH and $NAD^+$), L-lactic dehydrogenase (LDH, from porcine heart), propidium iodide (PI), sodium L-lactate, sodium pyruvate and α-cyano-4-hydroxycinnamic acid (α-CHC) were supplied by Sigma-Aldrich. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), citrate and sodium tetraborate ($Na_2B_4O_7$) were purchased from Aladdin. HEPES was obtained from J&K Scientific. Deionized water (18 MΩ cm) was used from a Hitech science tool laboratory water purification system. The culture medium was obtained from Gibco.

### 2.2. Instrumentation and apparatus

Transmission electron microscopy (TEM) images were obtained with a JEOL JEM-2100 microscope. Infrared (IR) spectrum was taken on a Thermo Scientific Nicolet iS50 spectrometer. Fluorescence emission spectra was measured on a Hitachi F-7000 Fluorescence spectrophotometer. UV–vis absorption spectrum was recorded by a Shimadzu UV-1800 spectrophotometer. The cell images were taken by an Olympus Fluoview 1000 confocal laser scanning microscope. Fluorescence lifetime measurements were performed on a compact fluorescence lifetime spectrometer C11367 Quantaurus-Tau from Hamamatsu.

### 2.3. Synthesis of MSA-coated CdTe QDs

MSA-coated CdTe QDs were synthesized according to a modified procedure (Ying et al., 2008; Yuan et al., 2013). First, $CdCl_2$ (58.7 mg), MSA (0.1 g) and $Na_2TeO_3$ (17.6 mg) were dissolved in 100 mL of boric–citrate buffer (15 mM $Na_2B_4O_7$ and 15 mM citrate, pH=7.0) at room temperature. Then, 0.1 g of $NaBH_4$ powder was added rapidly to the above precursor solution with stirring. Finally, the mixture was refluxed at 100 °C under open-air conditions for about 12 h to get 99 mL of 3 μM MSA-coated CdTe QDs.

### 2.4. Preparation of MSA-capped CdTe QD hydrogel

The CdTe QD hydrogel was prepared according to the previously reported gelation method (Yuan et al., 2013). In brief, 2 mL of ethanol was added into 2 mL of 3 μM MSA-coated CdTe QD solution. The resulting precipitate was collected by centrifugation (6000 rpm) and dried under air flow. After dispersing the precipitated QDs in 1 mL of 50 mM phosphate buffer (pH 7.4) and standing at room temperature (25 ± 2 °C) for 4 days, CdTe QD hydrogel was prepared.

### 2.5. Fabrication of Nile-Blue-functionalized CdTe QD hydrogel

Functional surface engineering of CdTe QD hydrogel was performed as follows: First, CdTe QD sol from sonicating the above CdTe QD hydrogel was mixed with 1 mM BSA. Then, EDC (10 mM) was added and shaken at room temperature for ~2 h for the conjugation. Next, unreacted EDC and BSA were removed by centrifugation and the QDs were redispersed in 1 mL of 10 mM phosphate buffer (pH 7.4). To this solution was added 50 μL of 1 mg $mL^{-1}$ $BS^3$ stock solution, and the mixture was shaken for ~30 min. After purifying the QDs by centrifugation and redispersing them in 1 mL of 10 mM phosphate buffer (pH 7.4), 5 μL of 1 mg $mL^{-1}$ Nile Blue (NB) stock solution (3:2 ethanol/water) was added and the mixture was shaken for 16 h. To eliminate excess NB, precipitation by NaCl and methanol followed by centrifugation were performed. Finally, the QD precipitates were redispersed in 1 mL of 10 mM phosphate buffer (pH 7.4) and left at room temperature for several hours to form the NB-functionalized CdTe QD hydrogel. It is obvious that the hydrogel regeneration is much faster than that of the first gelation, which is associated with the completion of partial removal of the MSA molecules and/or the incomplete destruction of the QD hydrogel by sonication (Jeong et al., 2011).

### 2.6. Cell culture and in Vitro fluorescence imaging

HeLa cells were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 units $mL^{-1}$ penicillin and 100 μg $mL^{-1}$ streptomycin. When in the proliferative period, HeLa cells were passaged and dispersed in a 35-mm Petri dish containing 2 mL of phosphate buffer (pH 7.4). To this cell suspension was added 500 μL of ~6 μM NB-functionalized CdTe QD sol. Then, the cell suspension was incubated in a 5% $CO_2$/95% air incubator at 310 K for 3 h. To validate whether the obtained QD hydrogel encapsulating HeLa cells can fluorescence image the lactate release, $NAD^+$ (2 mM) and LDH (50 U $mL^{-1}$) were added gently, and then confocal fluorescence images were collected after 15 min. The excitation wavelength was 488 nm, and the fluorescence emission between 550 nm and 650 nm was collected for cell imaging. The confocal microscope settings, such as transmission density, brightness, contrast and scan speed, were held constant to compare the relative fluorescence intensity.

## 3. Results and discussion

### 3.1. Synthesis and surface engineering of QD hydrogel

The process for the synthesis and surface engineering of CdTe QD hydrogel illustrated in Fig. 1 was described above in the experimental section. As a starting point of our study, a homogenous and transparent colloidal solution of mercaptosuccinic acid (MSA)-

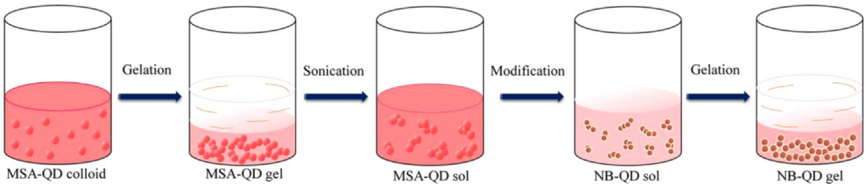


**Fig. 1.** Schematic representation of the fabrication process of Nile-Blue-functionalized CdTe QD Hydrogel.