Photo-Enhanced Luminescence in Bio-conjugated Quantum Dots for Ovarian Cancer Biomarkers

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ABSTRACT

Luminescent semiconductor quantum dots (QDs) can be readily conjugated with biomolecules and serve as sensitive probes for early detection of the cancer cells, specifically for ovarian cancer, which represents the most lethal gynecologic malignancy. We report on the luminescence characterization of the bio-conjugated QDs with CA125 antigen using linkage molecules. Kinetic curves of the bio-conjugated 655nm QD luminescence show both photo-enhancement and photo-degradation. Photo-enhancement is measured at various laser power densities, temperatures and laser wavelengths. The mechanism of the PL enhancement is discussed.

***Keywords*:** quantum dot, luminescence, ovarian cancer, kinetic, bio-conjugation

1 INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy. This largely reflects the fact that approximately 75% of cases are detected at advanced stages of disease, when cure is unlikely. In contrast, 5-year survival for patients with early stage ovarian cancer can exceed 90% [1]. It is possible therefore that detecting a greater number of patients with early stage disease by improving screening modalities could significantly improve overall survival. To date, detection of the secreted tumor marker CA 125 is the only biomarker available for screening and therapeutic monitoring, however it has limited sensitivity (70%). A novel approach to increase the sensitivity and specificity of early detection of cancer is through the application of nanotechnology, where luminescent semiconductor quantum dots (QDs) are conjugated with biomolecules [2]. Bioconjugation of QDs, i.e. the attachment of specific ligands to them, represents the convolution of biotechnology and nanotechnology yielding hybrid materials, processes and devices. In a case of early cancer detection this approach offers the potential to detect molecules in biological samples at levels below 10^{-7} [3]. We conjugated in this work core-shell CdSe/ZnS luminescence QDs with monoclonal mouse anti- CA 125 antibody (AB) as a potential serologic assay. Among different monoclonal antibodies potentially available for CA125 detection, we have selected OC-125 for QD-bioconjugation because it recognizes the defined peptide epitope of the target and can be compared with accepted clinical assays.

Tunable wavelength emission of the luminescence QDs was achieved from a variety of the inorganic semiconductors, predominantly of II-VI compounds such as CdSe, CdTe, CdS, etc. To obtain a noticeable quantum efficiency of the QD luminescence the core-shell structures can be effectively designed in a form of colloidal particles. A successful example represents CdSe/ZnS core/shell coupling, where large band-gap material (ZnS) serve as a surface passivating layer and as a barrier assisting the electron-hole confinement in the CdSe core [4]. A stability and efficiency of the QD luminescence is a critical aspect. Under intense laser or electron beam illumination it was observed a dramatic degradation of the luminescence intensity attributed to ionization of nano-crystals and subsequent trapping of the ejected electrons in the surrounding semiconductor matrix [5]. Therefore, even in the capped QDs with wide-gap semiconductor or embedded into ZnS matrix photo-degradation occurs. On the other hand, it was observed previously that the PL intensity is enhanced under light illumination in the bulk CdS and CdSe crystals [6, 7]. This was identified as light-induced defect reactions caused by donor-acceptor pair dissociation, assigned to a photo-chemical reaction. Similar effect of photo-induced PL enhancement was noticed in the glassy closed-packed film of QDs covered with ZnS film, however the process was not studied in details [5].

In this paper, we report on experimental study of the PL photo-enhancement effect in pure and bio-conjugated with OC125 antibody quantum dots.

2 EXPERIMENTAL

2.1 Samples

We sought to generate an assay prototype using the most widely accepted circulating biomarker for ovarian cancer – CA125. Development of the nano-assay was

based on immunometric methodology with the use of available QDs that were used as secondary antibody QD-F(ab) fragment conjugate, or were subjected to direct bioconjugation reaction with anti-CA125 primary antibody. For direct conjugation of QDs with OC125 mouse monoclonal antibody (DAKO, Carpinteria, CA), we used Qdot 655 Antibody Conjugation kit from Quantum Dot Corp. (Hayward, CA). The overall schema is: Si-wafer surface –anti-CA125 Ab (capture, clone M11) + Ag(CA 125) + anti-CA125 Ab (detector, clone OC125 –QD 655) ─ Read PL signal. PL spectrum from the Ag-Ab complex was measured utilizing described schema of solid phase sandwich format fluorometric assay and high-resolution PL spectroscopy, with confirmation of detected relative level of CA 125 in sample by reference ELISA. We utilized a capture (M11) and detector (OC125) antibody (Ab), designated as anti-epithelial ovarian carcinomas and as a reference standard the serial dilution of human CA 125 antigen (Ag) of high purity grade. Control wells/spots either lacked antigen or contained QDs 655-OC125 Abs only. The plasma samples from cancer patients were assayed using the reference ELISA kit for measurement of CA 125. The reportable (dynamic) range of CA125 detectable by the nano-assay is 0 to 500 U/ml, which reflects the physiological range of CA125 in blood. The samples for PL measurements represented ~3 mm spots deposited on a clean Si wafer surface as a substrate to minimize the luminescence background signal in the visible spectral range.

2.2 PL system

The PL spectroscopy was performed between 80K and room temperature, using a 50mW HeCd laser line at 325 nm or 200mW Ar^+ laser line at 488 nm as the excitation sources. Laser power density varied by use of a set of calibrated neutral density filters and could be focused down to 100 microns spot. At low intensity measurements the laser beam was un-focused with approximately 1.5 mm laser spot diameter at the sample surface. The PL signal was collected by optics, dispersed by a SPEX 500M spectrometer and recorded by a photo multiplier tube coupled with a lock-in amplifier. All system is computer controlled.

3 RESULTS

In Figure 1 we present luminescence kinetics of the bio-conjugated QDs measured at the maximum of the PL spectrum at 655nm. The kinetic data are collected at various power densities of the 325nm HeCd laser line which was used as the PL excitation source. At the highest laser power density of 500 W/cm^2 (focused laser spot down to 100 microns), we observe a strong photo-quenching, when the luminescence intensity degrades by a factor of three from its maximum value within 15 minutes (curve 1). At lower excitation power it is recognized that this photo-quenching follows an initial PL increase (hereafter, photo-enhancement) as shown on curve (2). When the laser power density is reduced more, both the enhancement and quenching kinetics are slowing down. At substantially reduced laser power of a few W/cm^2 it is possible to clearly separate the enhancement part of the kinetics as illustrated by the curve (3) in Figure 1. We concentrated in this study on the enhancement part of the PL kinetic curve only, which is strongly motivated as a potential mean to improve quantum efficiency of the bio-conjugated QDs.

The following observations were depicted based on this time-dependent PL study. (1) PL photo-enhancement can be quite substantial spanning the range from 10% up to 4-fold with respect to the initial luminescence intensity. (2) PL enhancement is observed on both pure QDs and bio-conjugated QDs. We noticed, however, that bio-conjugation affects noticeably a percentage of the enhancement and expand the process in time scale. In the bio-conjugated QDs the PL enhancement is typically stronger. (3) If the sample subjected to UV exposure was held in dark for definite time, the enhancement effect can be either fully recovered back (reversible effect) and the enhancement kinetics can be repeated again, or the effect can be non-reversible and show no recovery in the dark for at least 1 hour. Reversible behavior was found on QDs attached to F(ab) fragment used as a linkage molecule, while the non-reversible was observed on QDs without F(ab) bridge. (4) The enhancement effect is observed with both laser excitations at 325nm (HeCd) and 488nm (Ar^+).

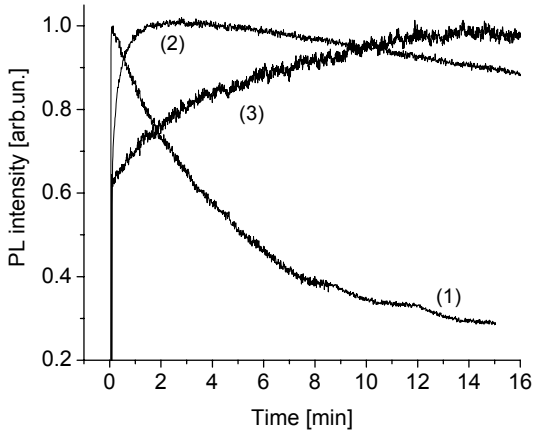

Figure 1: PL intensity variation of the 655nm luminescence band at room temperature and different laser power densities [W/cm^2]: (1) – 500; (2) – 20, (3) – 0.2.

In Figure 2 we show PL enhancement kinetic curves measured at different temperatures in the range from 80K to 300K. It is clear that PL enhancement effect is terminated at low temperatures and clearly observed at high temperatures. Specifically it is not found at temperatures in the range from 80K up to 210K and starts to be prominent in the range of 250K to 300K. It is interesting that the kinetic is not a single-exponential curve.

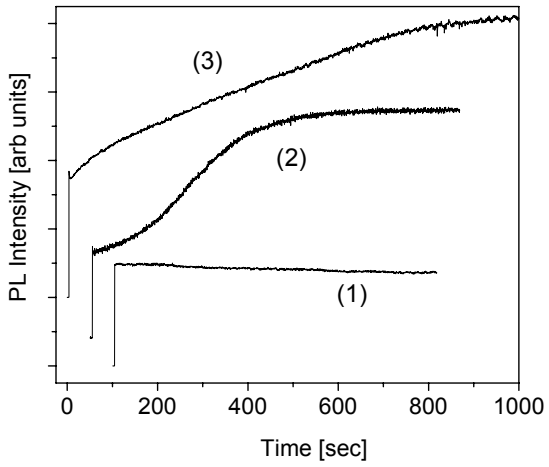

Figure 2: Kinetics of the PL enhancement measured at different temperatures:
(1) – 150K; (2) - 250K, (3) – 300K.

In Figure 3 we present temperature dependence of the PL intensity of bio-conjugated QDs. The sample was cooled down to 80K in dark, and was exposed to 325nm laser during heating cycle. The graph is plotted in the Arrhenius coordinates of the logarithmic PL intensity versus inverse temperature.

Following analytical dependence is typically describes the T-quenching of the PL intensity:

$$I(T) = I_0 \exp\left(\frac{E_a}{k_B T}\right) \qquad (1)$$

where E_a is the characteristic activation energy of the T-quenching process, k_B is the Boltzmann constant. In our case PL intensity shows exponential decline above 200K with E_a = 43meV (dotted line). We observe also that the curve shows substantial variation of the exponential quenching part, which can be attributed to the photo-enhancement process described above. This matches to the results in Figure 2. The temperature range of the PL enhancement according to the T-quenching curve is above 218K. In the insert we presented the temperature dependence of the PL band maximum position, which follows CdSe band edge temperature shift.

4 DISCUSSION

As we mentioned, the luminescence stability and quantum efficiency of the bio-conjugated quantum dots are two major concerns presenting both fundamental interest and practical importance. We can postulate based on the performed study that the process of the QD's luminescence photo-quenching, which deteriorate the PL efficiency, can be substituted with the opposite effect of the PL enhancement in a proper experimental conditions. It is obvious that the PL enhancement is strongly beneficial specifically for the early cancer detection where low PL output is one of limiting factors of the luminescence bio-marker methodology.

The effect of the QD luminescent photo-quenching was observed both in the photo- and cathodoluminescent experiments [5]. The model explaining qualitatively the photo-bleaching PL kinetics was attributed to multi-photon

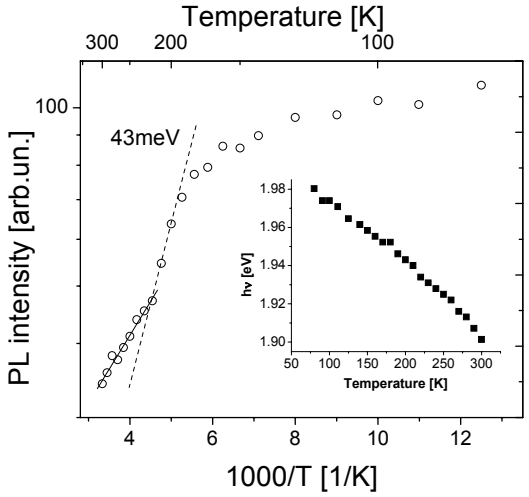

Figure 3: Temperature dependence of the QD PL intensity in the bio-conjugated QDs with partial PL enhancement at temperatures above 218K.

generation of the electron-hole pairs (excitons) within the same QD and subsequent Auger recombination. The energy released after non-radiative Auger recombination of one exciton is transferred to the second exciton, which inject the electron into a long-life trap states in the ZnS matrix. This process leads to a local charging of the QD after the light exposure and reduction of the luminescence efficiency as proposed by Efros and co-workers [8].

In our case we observe an opposite effect, when the PL output in increased after light exposure. We consider possible mechanisms. First, we emphasize that enhancement occur both under UV (325nm) and visible (488nm) laser exposure. The last has much smaller energy (2.54eV) than ZnS band-gap and polymer is also transparent at this wavelength. Therefore, the effect follows a direct light absorption and electron-hole pair generation in the CdSe core. These pairs either recombine radiatively yielding the characteristic PL band, or

alternatively can be captured by interface states which leads to their trapping or non-radiative recombination.

We summarize some specific features of the process. (1) It is observed only at high temperatures above 250K (Figure 2); (2) the enhancement rate is increased when laser intensity goes up (Figure 1); (3) it is observed in pure and bio-conjugated QDs; (4) rate, intensity and recovery depends on the origin of the conjugated bio-molecule and conjugation scheme. Keeping in mind (3) and (4) we may suggest that in the photo-enhancement process participate surface states (traps) at the CdSe/ZnS or ZnS/polymer interface. Capture of the photo-carriers by these states may lead to (a) compensation of the electric charge and altering the electrostatic field around the QD, or (b) passivation of non-radiative centers due to chemical bond reconstruction, which represents a photo-chemical reaction. Carrier capture rate by the surface states is increased with increasing of the electron-hole photo-generation rate (excitation light intensity). This is observed in the experiment. A recovery of the initial state (with reduced PL intensity) is obviously accounted for thermal release of the carriers from the surface trapping states and depends on the ionization energy of the traps. This trap energy is strongly affected and altered by a coupling of the QD with bio-molecules and may lead to reversible and non-reversible PL enhancement effect.

QD's surface charge can reduce the exciton PL intensity. This can be a result of the exciton ionization in the external electric field as observed in photo-conductivity study of close-packed glassy solids of colloidal CdSe QDs [9]. In the opposite case, compensation (neutralization) of the surface charge would lead to the PL increase due to stabilizing the exciton, increasing its binding energy, and reducing the PL thermal quenching. We emphasize that if only one type of photo-generated carriers is captured and the other left on the QD level, the Auger mechanism would reduce PL intensity as was predicted in [8] and observed experimentally in Ref. 5. Therefore, we postulate that both electron and hole must be captured by spatially separated and charged donor (D^+) and acceptor (A^-) surface defects, correspondingly. This leads to the neutralization of these defects according to the reactions

$$D^+ + e \Rightarrow D^0 \ ; \ A^- + h \Rightarrow A^0 \qquad (2)$$

and reduction of the surface charge (i.e. electric field), which reduces the exciton PL intensity. Alternatively, we may assume that non-radiative recombination defects are eliminated as a result bond restructuring at the QD surface due to a photo-chemical reaction. In this case, the recovery of the PL enhancement process is caused by a thermal reconstruction of chemical bonds. More experiments are required to identify a particular mechanism of the PL enhancement.

In conclusions, we studied a new effect of the luminescent enhancement in pure and bio-conjugated CdSe/ZnS core/shell quantum dots. The effect show a promise to enhance noticeably the QD quantum efficiency and to benefit the early cancer detection methodology based on QD bio-markers.

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