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Research on the interaction mechanism between quantum dots and radionuclides for the improvement of Cerenkov luminescence imaging

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Cerenkov luminescence imaging (CLI) is a relatively new optical molecular imaging technique. The nature of Stokes shift in quantum dots (QD) can be used to improve the quality of CLI. However, the interaction mechanism of QD with Cerenkov light remains unclear. In this work, the interaction mechanism between QD and radionuclides emitting β rays, γ rays, and Cerenkov light was investigated. The 96-well plates were used to test the different levels of radioactivity of radionuclides with different QD concentrations. Transparent vials were used to determine the relationship between QD fluorescence intensity and the distance from QD to the radionuclide. In addition, black paper was used to block the transmission of Cerenkov light through the QD vials. A linear relationship was found between the number of photons and the radioactivity of radionuclides when the QD concentration was kept constant. Similarly, the number of photons was linearly related to the QD concentration when the radioactivity of radionuclides was kept constant. Furthermore, with the increases in the distance between radionuclides and quantum dots, the number of photons was exponentially decreased. Meanwhile, the number of photons emitted from QD excited by Cerenkov light accounted for 20% the total number of photons excited by ^{131}I radionuclide. The result proved that QD was not only excited by Cerenkov light but also by other rays.

Cerenkov luminescence imaging, quantum dots, radionuclide

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1 Introduction

In recent years, Cerenkov luminescence imaging (CLI), as a novel optical molecular imaging technique, has opened up a new application field [1–4]. CLI is based on Cerenkov light signals emitted by radionuclides used in nuclear imaging [5]. In 2009, Robertson et al. [1] verified the feasibility of *in vivo* optical imaging with Cerenkov light via a charge-coupled device (CCD). In 2010, Li et al. [6] studied Cerenkov luminescence tomography (CLT) to reconstruct the model with homogeneous and heterogeneous phantoms for the first time. However, the application of CLT was inevitably limited by several factors in two aspects. Firstly, photon energy of Cerenkov light is so low that it is difficult to collect imaging information. Secondly, reflection, refraction, scattering, and absorption in tissues lead to low penetration of Cerenkov light.

Dothager, Goiffon, Gammon, and other research groups used Cerenkov radiation energy transfer (CRET) to solve the shortcomings of CLI [7–10]. Gammon et al. combined

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quantum dots (QD) with radionuclides because charged particles decayed by radionuclides could emit Cerenkov light and found that the Stokes shift in QD formed highly red-shifted emission spectra, intensified the penetrability of Cerenkov light in tissues, and greatly improved the quality of CLI. Herein, this kind of imaging method can be called Cerenkov-light-excited QD imaging (CLEQI). Recently, QD excited by Cerenkov light was experimentally studied. The new red-shifted light from QD excited by Cerenkov light makes the greatest contribution to imaging quality improvement.

At present, the interaction mechanism for Cerenkov light-excited QD imaging is obscure. The theory that "the new red-shifted light makes the greatest contribution of imaging quality improvement, and the light comes from QD excited by Cerenkov light" is still doubtable and confusing [11–14]. This paper aims to investigate the interaction mechanism between QD and radionuclides emitting β particles, γ-rays, and Cerenkov photons.

2 Methods

To investigate the mechanism, the ^{131}I isotope, which emits β rays (99%) and γ rays (1%), was used. The maximum energy of β rays was 0.6065 MeV, and the main energy of γ rays was 0.364 MeV. The QD used was water-soluble CdTe with a size of 50 nm and could be detected in aqueous suspensions through the dynamic optical detection method. Fluorescence emission peak focus was at 660 nm and full width at half maximum (FWHM) was 54 nm. Imaging was carried out using a small-animal IVIS (In *Vivo* Imaging System) equipped with Lumina II optical imaging system (Perkin Elmer).

The experiment was divided into four parts. The first two parts were completed with six 96-well plates (Figure 1(a)). Isotopes of different levels of radioactivity were placed in the 96-well plates so that the number of photons could be measured by the IVIS while QD concentration was kept constant. Similarly, QDs of different concentrations were placed in the 96-well plates so that the number of photons could be measured while radioactivity of isotope was kept constant. As shown in Figure 1(b), the isotope and QD were placed in two transparent vials separately. The number of photons in the region of the QD vial was measured after the distance "*d*" was changed. The relationship between the distance and QD fluorescence intensity was then analyzed. Finally, to investigate the quotient from QD excited by Cerenkov light, Cerenkov light emitted by the isotope should not hit the QD vial. For this purpose, black paper was placed between the isotope and the QD vial. After the Cerenkov light was fully blocked by black paper, the number of photons in the region of the QD vial was measured.

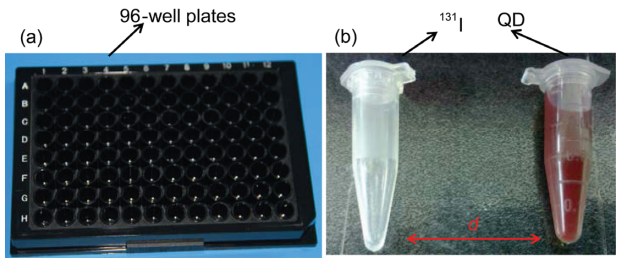

Figure 1 Schematic diagram of the experiment. (a) Isotope and QD were mixed in a 96-well plate; (b) the isotope and QD were placed in two vials separately.

3 Results

3.1 Effects of different levels of radioactivity on QD excited by isotope

The relationship between QD fluorescence intensity and isotopes with different levels of radioactivity was discussed here. Figure 2 shows the optical image of 96-well plates with isotopes having different levels of radioactivity. Here, the QD concentration in the 96-well plates was 0.5 mg/mL, whereas the radioactivity levels of isotopes were 5, 10, 15, 20, 30, and 50 μCi, respectively. The number of photons increased as radioactivity increased. As shown in Figure 3, the data from the number of photons and radioactivity can be fitted to obtain a linear curve ($R^2 = 0.993$).

Meanwhile, we took the 6th well as a sample (Figure 2) and measured the number of photons at different wavelengths (500 to 700 nm). At a filter bandwidth of 20 nm, a distinct fluorescence emission peak at 660 nm was detected (Figure 4). This result is in accordance with the characteristic of QD. In Figure 4, the dashed line represents the spectrum detected by pure ^{131}I without QD and the solid line represents the spectrum of Cerenkov light. Some fluctuations were observed in the spectrum of QD before 620 nm. These fluctuations may be caused by background interference and the photons produced by Cerenkov light.

3.2 Effect of different concentrations on QD excited by isotope

In addition to the effect of isotopes with different levels of radioactivity on QD, the change in the QD concentration also affects QD fluorescence intensity. Isotopes were placed in the 96-well plates. The concentrations of QDs were 0.1,

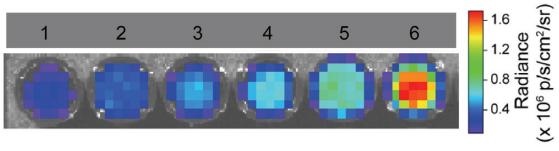

Figure 2 IVIS image of the 96-well plate. QD concentration in the 96-well plate was 0.5 mg/mL; the radioactivity levels of isotopes in the 96-well plate 1–6 were 5, 10, 15, 20, 30, and 50 μCi, respectively.

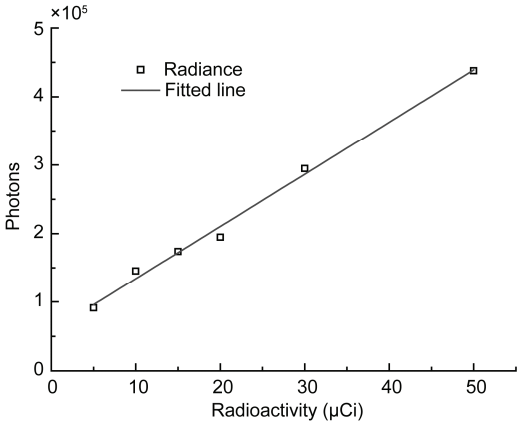

Figure 3 The number of photons increased as radioactivity increased. Data from the number of photons and radioactivity can be fitted to obtain a linear curve ($R^2 = 0.993$).

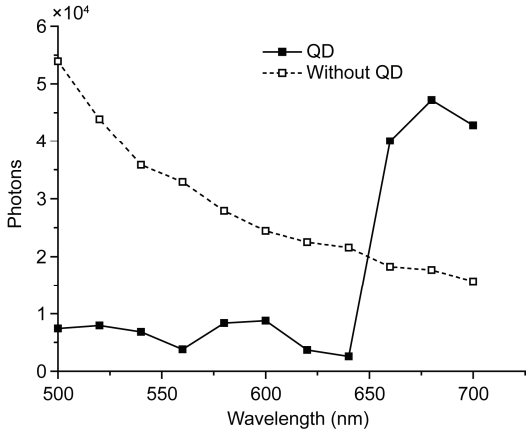

Figure 4 The dashed line represents the spectrum detected using pure ^{131}I without QD and the solid line represents the spectrum of Cerenkov light. The spectrum of QD excited by the isotope is in accordance with the characteristic of fluorescence of QD.

0.2, 0.3, 0.4, and 0.5 mg/mL; the radioactivity of isotope was constantly 20 μCi. The number of photons increased as QD concentration increased (Figure 5). Similar to Figure 3, Figure 6 shows that a linear relationship exists between the number of photons and QD concentration ($R^2 = 0.935$).

Similar to the first part of the experiment, in order to analyze the excitation of QD quantitatively, we took the 5th well as a sample and measured the number of photons at different wavelengths (500 to 700 nm). Distinct fluorescence emission peaks were detected at 660 nm at a filter bandwidth of 20 nm (Figure 7). Similar to the results shown in Figure 4, some fluctuations were found in the spectrum of QD before 600 nm.

3.3 Effects of different distances on QD excited by isotope

We also investigated the relationship between QD fluorescence intensity and the distance from QD to the radionuclide. In the experiment, isotope and QD were placed in two vials separately. The number of photons from the QD vial was measured after the distance "*d*" between the isotope and QD vials was changed. The QD concentration was 0.5 mg/mL, whereas the radioactivity level of isotope was 50 μCi. As shown in Figure 8, the number of photons decreased gradually as "*d*" increased. More accurately, when the data were fitted using an index function plus a constant term, a good agreement between the index function and the experimental data was obtained (fitting function: Photons/$10^5 = 10e^{-d/2}+2$, $R^2 = 0.985$).

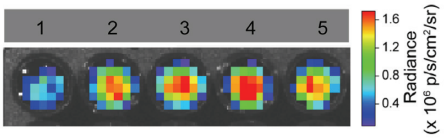

Figure 5 IVIS image of the 96-well plate. Concentrations of QD 1–5 were 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively; radioactivity of isotope was constantly 20 μCi.

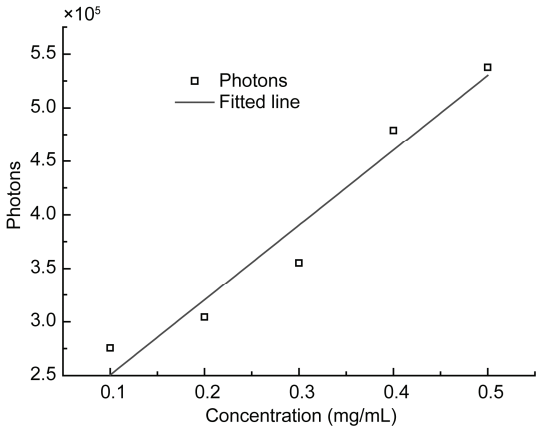

Figure 6 The number of photons increased as QD concentration increased. Data from the number of photons and concentrations can be fitted to obtain a linear curve ($R^2 = 0.935$).

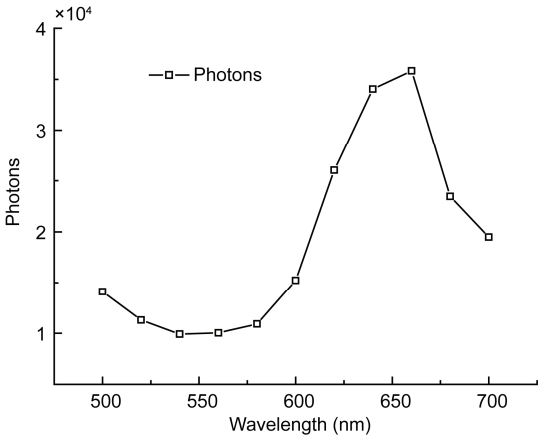

Figure 7 The spectrum of QD excited by isotope is in accordance with the characteristic of fluorescence of QD.

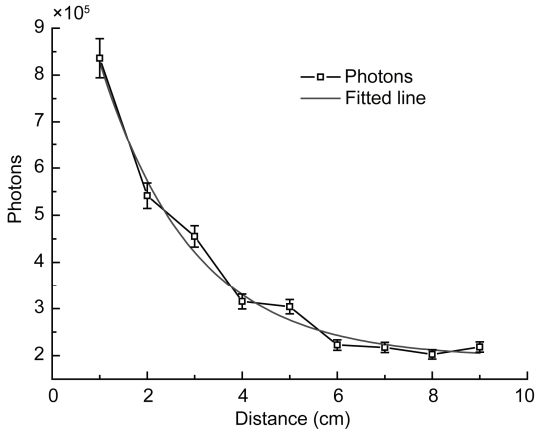

Figure 8 The number of photons decreased gradually as "*d*" increased. The data were fitted with an index function plus a constant term (fitting function Photons/ $10^5 = 10e^{-d/2}+2$, $R^2 = 0.985$).

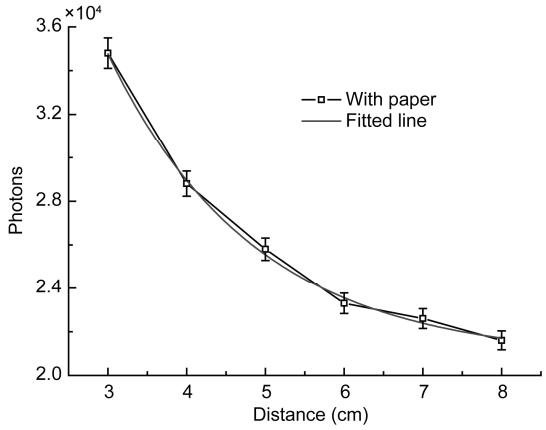

Figure 10 The number of photons decreased gradually as "*d*" increased. The result was similar to that obtained when black paper was not used. The data were fitted with an index function plus a constant term (fitting function: Photons/$10^4 = 7e^{-d/1.9}+2$, $R^2 = 0.997$).

3.4 Contribution of Cerenkov light to the excitation of QD

^{131}I is a kind of β– decay nuclide that emits β rays (99%) and γ rays (1%). Therefore, three kinds of rays from the ^{131}I isotope can reach the QD area, including β rays, γ rays, and Cerenkov light. This experiment aims to find the relationship between Cerenkov light and QD.

As shown in Figure 9, to separate Cerenkov light from other rays, a piece of thin black paper was placed between the isotope vial and QD vial. As a result, the Cerenkov light emitted by ^{131}I was stopped by the black paper completely and only the β rays and γ rays reached the QD vial.

Shielded by the black paper, the number of photons decreased gradually as "*d*" increased. When the data were fitted using an index function plus a constant term (Figure 10), a good agreement between the index function and the experimental data was obtained (fitting function: Photons/ $10^4 = 7e^{-d/1.9}+2$, $R^2 = 0.997$).

To analyze the relationship between Cerenkov light and QD fluorescence intensity, the proportional relationship in the area of QD (with and without black paper) was detected in a setup where the distance "*d*" varied from 3 to 8 cm. The ratio was computed by dividing the number of photons (with paper) by the number of photons (without paper). As shown in Figure 11, the ratio remains at about 0.8 when the distance is increased. This finding demonstrates that the quotient from QD excited by Cerenkov light is 20%, whereas the quotient from other rays is 80%. The comparison can be performed by considering the shaded areas in the histogram, as shown in Figure 11.

When black paper was used, QD was excited only by β rays and γ rays because black paper prevented QD excitation by Cerenkov light. According to the results, when QD was excited by radionuclides, the contribution of Cerenkov light to QD was only about 20%. Through the process of CLEQI, QD is only excited by β rays and γ rays and Cerenkov light has no substantial contribution. Therefore, it is proved that the theory that "the new red-shifted light makes the greatest contribution of imaging quality improvement, and the light comes from QD excited by Cerenkov light" is inaccurate.

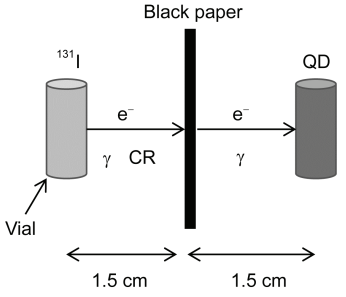

Figure 9 Contribution of Cerenkov light to the excitation of QD. To separate Cerenkov light from the other rays, a piece of thin black paper was placed between the isotope vial and QD vial. The Cerenkov light emitted by ^{131}I was stopped by the black paper completely and only β rays and γ rays reached the QD vial.

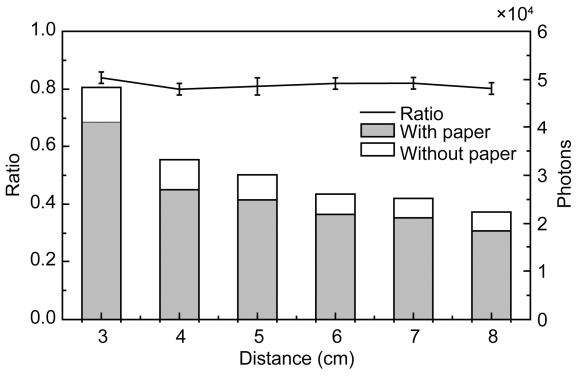

Figure 11 The ratio of the number of photons emitted by QD excited by Cerenkov light is 20%.

4 Discussion and conclusions

We investigated the excitation mechanism of QD by isotope. When QD was excited by isotopes of different levels of radioactivity, the resulting data from the number of photons and radioactivity could be fitted with a linear curve. When QD of different concentrations was excited by isotope, the number of photons increased as QD concentration increased. The data from the number of photons and QD concentration could be fitted with a linear curve. The number of photons decreased gradually as "*d*" increased, and a good agreement between the index function and the experimental data was obtained. For ^{131}I radionuclide, the quotient of the number of photons emitted from QD excited by Cerenkov light was around 20%, whereas the quotient excited by the β rays and γ rays was around 80%. These results proved the inaccuracy of the previous research theory. QD was not only excited by Cerenkov light but also by other rays.

This paper focused on the QD excited by Cerenkov light. Further studies should focus on the excitation laws of QD by β rays, γ rays, and other rays.

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