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Detection of the ovarian cancer biomarker CA-125 using chemiluminescence resonance energy transfer to graphene quantum dots†

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An immunoassay has been developed for the detection of the ovarian cancer biomarker CA-125 by utilizing the chemiluminescence resonance energy transfer to graphene quantum dots. This biosensor shows a wide linear range from 0.1 U mL^{-1} to 600 U mL^{-1} with a limit of detection of 0.05 U mL^{-1} for CA-125 in a buffer solution.

Ovarian cancer is among the fatal gynecological malignancies in women with a total 22 240 new cases and 14 030 deaths anticipated in U.S. in 2013.[1] It accounts for about 3% of all cancers among women and 5% of cancer deaths, which causes more deaths than any other cancer of the female reproductive system.[1] Nowadays, two clinical methods are normally used for the diagnosis of ovarian cancer, that is, the transvaginal ultrasonography and the blood measurements of the tumor marker CA-125. CA-125 is a biomolecule found in serum, which is associated with ovarian cancer. It has a threshold of 35 U mL^{-1} in normal human serum.[2] Elevated levels of CA-125 have been found in 80–90% of women with advanced-stage ovarian cancer; however, it is only 47% in patients with early-stage cancer.[3] Although a single protein biomarker such as CA-125 is not sufficient for early detection, the combination of CA-125 with other protein biomarkers is effective in the early detection of ovarian cancer.[4,5] Therefore, CA-125 test has been approved by the U.S. Food and Drug Administration (FDA) for monitoring the chemotherapy response, determining the prognosis and examining the recurrence, as well as the early-stage detection of ovarian cancer.[6]

Enzyme-linked immunosorbent assay (ELISA) is generally used for detection of biomarkers,[7,8] such as CA-125. Despite its high sensitivity, it requires several separation steps with strict washing processes, which is a time-consuming and tedious process. Additionally, various methods based on electrochemistry,[9–13] surface plasmon resonance,[13,14] fluorescence[15] and mass spectrometry[16] have been developed for the detection of CA-125. However, most of these methods still need thorough washing steps. Moreover, owing to the complexity of the processes involved in these methods, professional skills still are needed for operation and data interpretation, which makes it impractical for point-of-care (POC) diagnosis. Therefore, it is necessary to develop simple and effective portable sensors for CA-125 detection.

It is worth noting that optical biosensors have been constructed using the resonance energy transfer (RET) processes to gold nanoparticles[17–23] or to graphene oxide (GO).[24–28] Our previous studies have shown that fluorescence quenching by ultra-small gold nanoparticles or by GO follows a nanometal surface energy transfer (NSET) mechanism instead of a Förster resonance energy transfer (FRET) process.[17,18,24,28] Compared to FRET, NSET has a longer effective energy-transfer distance,[17,28] which provides the flexibility for construction of biosensors. However, for most of NSET- or FRET-based sensors, an external excitation source is required to excite the energy transfer processes from the donor (made of organic dyes or quantum dots) to the acceptor. To eliminate the external excitation source, the donor made up of organic dyes or quantum dots can be replaced with a chemiluminescence reagent, which enables the chemiluminescence resonance energy transfer (CRET) process.[28–34] Since an excitation laser is not needed for CRET-based sensors, the signal interferences from the background fluorescence that could be triggered by an external excitation source can be avoided, which can lower the signal-to-noise ratio and improve the sensitivity of sensors.[28,29,31]

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In this study, a simple and sensitive sensor has been developed for the detection of the ovarian cancer biomarker CA-125. In this sensor, graphene quantum dots (GQDs) are immobilized on a functionalized glass chip. Signal transduction is based on the CRET from the chemiluminescent reagent soluble in the aqueous solution to the GQDs on the solid chip. The use of GQDs as the energy acceptor avoids the photo-bleaching problem, which is usually associated with organic dyes. In addition, the use of GQDs enables the NSET mechanism,[28] which does not require the spectral overlap between the energy donor and the acceptor. This provides more flexibility for selection of the energy donors. Moreover, GQDs are typically hydrophilic, and are rich in the carboxylic acid moiety, which is convenient for bioconjugation. The capture antibody is linked to the GQDs on a transparent solid substrate, which has a potential for further development of high-throughput and automated sensor chips. Furthermore, fewer washing steps are needed during operation of the sensing system, which greatly simplifies the operation of the sensing assay.

The assembly of the chemiluminescent immuno-chip and the detection principle are shown in Fig. 1. The detailed characterization results obtained from each step can be found in the ESI.† The amino-modified glass chips were silanized with a 3-aminopropyl-trimethoxysilane (APTMS) layer (Fig. 1(a)). The GQDs were then immobilized *via* the electrostatic attraction to the positively charged amine group in the well array confined in the polydimethylsiloxane (PDMS) stencil (Fig. 1(b)). The capture antibody (cAb) that was specific to the CA-125 antigen was covalently linked to the GQDs through amide conjugation[35] (Fig. 1(c)). Bovine serum albumin (BSA) was then used to block the unreacted sites on the glass surface, forming the GQDs–cAb chip (Fig. 1(d)).

When the CA-125 antigen is absent in the immunoassay (Fig. 1(h)), the horseradish peroxidase (HRP) enzyme catalyzes the production of the reactive oxygen species (ROS) from H_2O_2, which oxidizes luminol to the singlet dianion, generating the excited electrons. When the electrons jump from the excited state to the ground state (that is, the singlet dianion becomes the ground-state dianion), chemiluminescence occurs[36] and the intensity of the emitted blue light is recorded using a fluorescence plate reader. In the absence of the CA-125 antigen, the HRP-labeled antibody (Ab–HRP) is far away from the GQDs due to the electrostatic repulsion between the Ab–HRP and the capture antibody (cAb) because they had the same charge polarity. The chemical reaction is catalyzed by the HRP and the reaction products are close to the HRP. Hence the dianion is also far away from the GQDs. As a result, there is no strong interaction between the dianion and the GQDs.

When the CA-125 antigen is present in the immunoassay (Fig. 1(e)), the antibody–antigen complex is generated, forming GQDs–cAb + CA-125. To enable the detection, this complex is exposed to Ab–HRP to form the sandwich structure, GQDs–cAb + CA-125 + Ab–HRP (Fig. 1(f)). Owing to the sandwich structure, HRP is in close proximity to the GQDs. The dianion catalyzed by the HRP enzyme is close to the GQDs, which enables the resonance energy transfer from the dianion to the GQDs, quenching the chemiluminescence (Fig. 1(g)). The higher the concentration of the antigen captured, the higher the concentration of

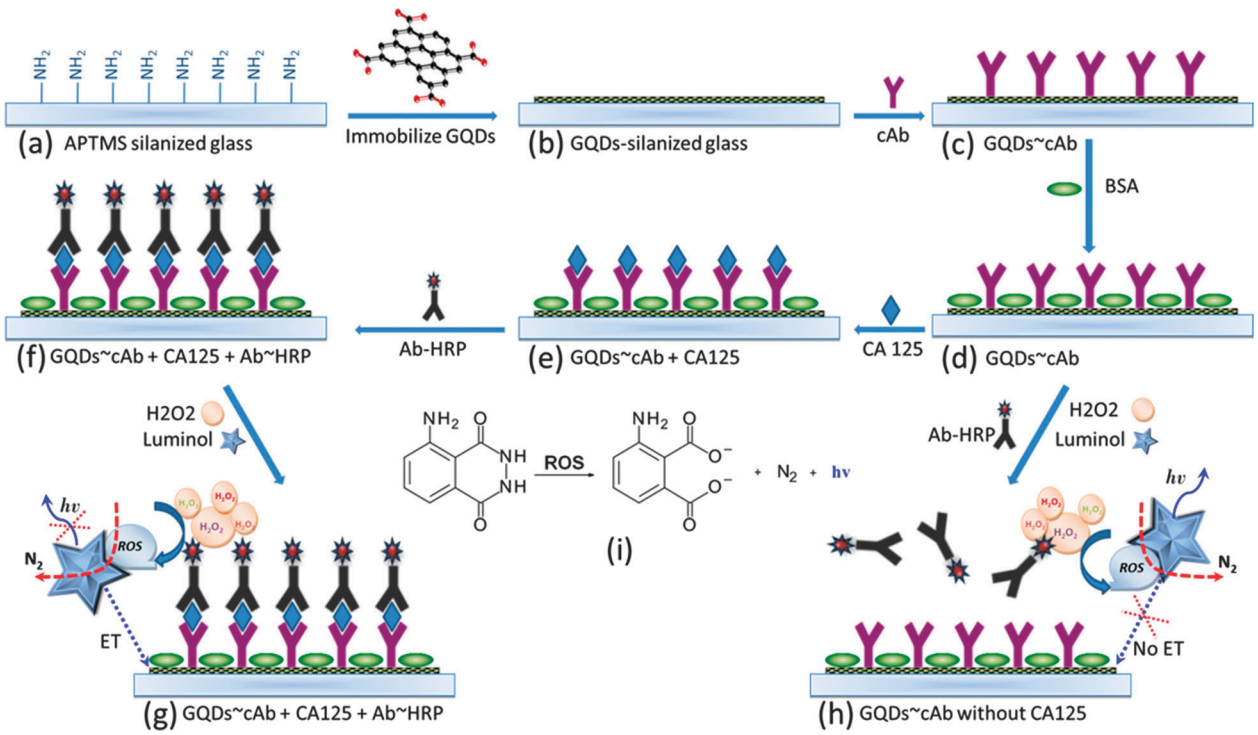

Fig. 1 Scheme of the assembly of the immunoassay and the detection principle.

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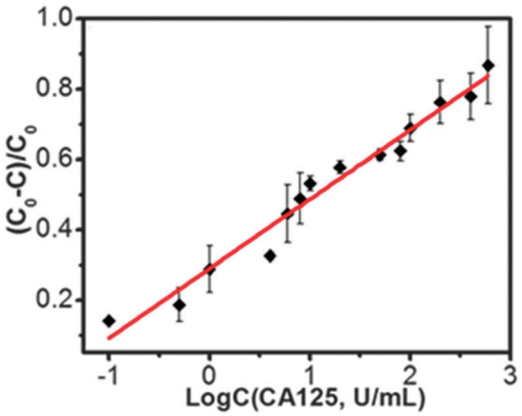

Fig. 2 Chemiluminescence measured from the immunoassay containing various concentrations of CA-125 antigen, showing the linear range.

Ab–HRP bound near the GQDs, which causes more energy transfer events that cause the chemiluminescence quenching. Hence, the chemiluminescence intensity is inversely proportional to the CA-125 concentration.

Based on the above sensing principle, a calibration curve (Fig. S5, ESI†) was obtained from the immunoassay containing various concentrations of CA-125 antigen as $y = 0.197x + 0.290$, (R^2 = 97.0%), where x is the logarithmic concentration of the antigen; and $y = (C_0 - C)/C_0$. Here C and C_0 are the chemiluminescence intensities of the assay in the presence and in the absence of CA-125, respectively. The normalized chemiluminescence intensity (y) exhibited a linear correlation over a wide range of CA-125 concentration from 0.1 U mL^{-1} to 600 U mL^{-1} (Fig. 2). The limit of detection (LOD) was estimated to be 0.05 U mL^{-1} at a signal-to-noise ratio of 3. The LOD value was far below the threshold of CA-125 (35 U mL^{-1}) in normal human serum.[2] It was comparable to the LOD values obtained from ELISA (0.05–0.3 U mL^{-1}, ref. 7) and better than those of the electrochemical sensors (1.8 U mL^{-1}, ref. 10, 1.29 U mL^{-1}, ref. 11, 0.5 U mL^{-1}, ref. 12) and the SPR senor (0.4 U mL^{-1}, ref. 14).

In order to evaluate the sensor performance in real-world samples, the chemiluminescence signal was recorded after capture of the antigen from the sample matrix containing 50% human blood plasma and 50% PBS buffer (Fig. S6, ESI†). The calibration curve showed a linear range from 0.1 U mL^{-1} to 600 U mL^{-1} even though the slope was a little lower than that obtained in the PBS buffer ($y = 0.183x + 0.245$, R^2 = 99.6%), giving a LOD of 0.08 U mL^{-1} at a signal-to-noise ratio of 3.

In summary, a rapid and sensitive immunoassay was developed for detection of CA-125 *via* chemiluminescence resonance energy transfer to the graphene quantum dots. The immunoassay exhibited a LOD of as low as 0.05 U mL^{-1} and a wide linear range from 0.1 U mL^{-1} to 600 U mL^{-1} for CA-125 in the buffer solution. It is an excellent substitute for a FRET-based assay because it eliminates the need for an external excitation source. This immunoassay exhibited a similar performance in the presence of 50% blood plasma with a LOD of 0.08 U mL^{-1}. The as-developed sensing platform can be further fabricated in an array chip-based device for high throughput multiplex detection.

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