Carbon quantum dots-mediated direct fluorescence assay for the detection of cardiac marker myoglobin

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We report here fluorescence based assay using carbon quantum dots (CQDs) for the detection of cardiac marker myoglobin (Mb). CQDs with high fluorescence properties are synthesized and functionalized with generated anti-Mb-Aptamer to develop a sensitive assay platform with a detection limit of ~1 ng/ml and a wide detection range from 1 to 10^5 ng/ml.

Keywords: Carbon quantum dots, cardiovascular diseases, fluorescence assay, myoglobin.

CARBON quantum dots (CQDs) have tremendous potential because of their superiority in water solubility, chemical inertness, low toxicity, ease of functionalization and resistance to photobleaching[1]. They have been employed as novel, ideal fluorescent probes for bioimaging and smart sensing[2]. Sensitive and specific detection of protein biomarkers plays a crucial role in proteomics, clinical diagnostics, drug screening and biodefence applications[3]. Current methods for protein biomarkers are mostly based on the use of antibodies, which are not readily available and not well adapted to a rapid, sensitive strategy[4]. Recently, aptamers have been utilized as antibody alternatives functioning in a similar fashion with molecular recognition in a variety of diagnostic formats[5]. These specific recognition molecules are usually evolved from random oligonucleotide pool by a process known as 'systematic evolution of ligands by exponential enrichment' (SELEX)[6]. The synergies of nanotechnology in synthetic macromolecules and biomolecular recognition units are promising in developing novel diagnostic platforms for clinically important biomarkers[7].

Cardiovascular diseases are the leading cause of morbidity and mortality worldwide[8]. The increase in the incidence of cardiovascular disease will be particularly dramatic in India in near future and there is a growing demand for products that can provide rapid, quantitative and cost-effective diagnostic tests in virtually all patient-care settings. Several potential cardiac biomarkers have attracted attention because of their ability to predict future cardiovascular events[9]. Serum cardiac biomarkers such as myoglobin (Mb) have become the cornerstone for risk stratification and diagnosis of patients with an acute coronary syndrome[10]. The monitoring of cardiac markers requires high-precision assays acceptable to physicians for the proper diagnosis[11]. In the present study, we have generated specific aptamers screened from oligonucleotide library by SELEX method, against the most potential cardiac biomarker Mb[12]. We report here a fluorescence-based assay using specific aptamers immobilized on microtitre plate for direct detection of Mb, as shown in Figure 1 *a*.

CQDs were synthesized by thermal carbonization process, where 0.375 g of L-glutamic acid, 5 ml of 5 M HNO_3 and optimum concentration of glycerol (45%) were heated at 300°C in an oven for 6 h. Further, 10 ml of water was added into the solution followed by stirring for 30 min with cooling at room temperature. The resulting solution was ultracentrifuged at 10,000 *g* for 1 h. The supernatant was discarded and the final product was resuspended in water containing optimized concentration of nafion (5%) to form CQDs solution, which was further used for characterization and assay development. Standard solution of Mb (4 mg/ml) was prepared in PB (100 mM) and it was added to CQDs solution with an incubation at 37°C for 2 h followed by dialysis in PB buffer for 24 h. For morphological and structural characterization, transmission electron microscopy (TEM) was used (JEOL 2100 operating at 200 kV). The results show that the CQDs exhibit an average diameter of 3 ± 0.6 nm (Figure 1 *b*) and the crystalline lattices were consistent with graphitic carbons as shown in the SAED pattern (Figure 1 *c*). Further, the elemental composition of the selected area scan as determined by the energy-dispersive X-ray spectroscopy (EDX) (Figure 1 *d*), shows the presence of carbon and fluorine, which confirms the formation of nafion-coated CQDs. The excitation and emission of the synthesized CQDs were observed at 346 and 424 nm respectively (Figure 1 *e*). For assay development Mb solution was added to nafion-coated CQDs and incubated for 1 h followed by centrifugation at 17,000 rpm for 30 min to remove unbound CQDs. The absorption spectra of Mb-coated CQDs suggest an additional peak at 400 nm due to the Soret transition, which confirms the conjugation of Mb with nafion-coated CQDs. The microtitre

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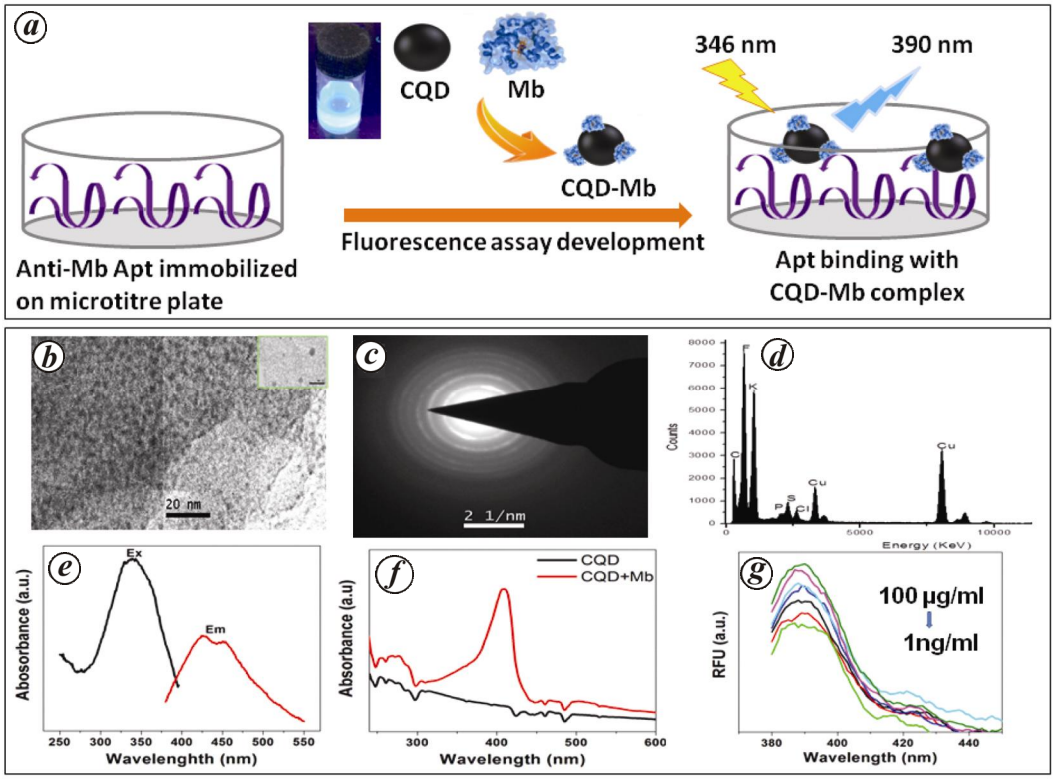

Figure 1. ***a***, Schematic showing carbon quantum dots (CQDs)-mediated direct fluorescence assay for the detection of myoglobin (Mb). ***b***, TEM photomicrograph showing CQDs. (Inset) Magnified view of a selected region. ***c***, SAED pattern of CQDs. ***d***, EDS spectra of CQDs. ***e***, Excitation and emission spectra of nafion-coated CQDs. ***f***, Absorption spectra of Mb-coated CQDs and CQDs. ***g***, Direct fluorescence assay for Mb using varying concentrations from 1 ng/ml to 100 µg/ml.

plates were coated with anti-Mb aptamer (2 µg/ml prepared in DNA-coating solution) by incubating overnight at 4°C. Varying concentrations of Mb-conjugated nafion CQDs were added and the fluorescence spectra were recorded in the range 380–450 nm. A subsequent increase in fluorescence intensity was observed with increase in Mb concentration, suggesting the specific interaction of Mb with anti-Mb aptamer immobilized on the microtitre plate.

Conclusion

A fluorescence-based detection method is presented for the selective detection of the cardiac marker Mb. This direct method provides a wide detection range using single bioreceptor, unlike the conventional sandwich ELISA tests. The developed biosensing platform will have great potential for the early diagnosis and management of cardiac diseases.

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