Journal of Fluorescence (2022) 32:1733–1741
https://doi.org/10.1007/s10895-022-02954-x

ORIGINAL ARTICLE

A Turn Off Fluorescence Probe Based on Carbon Dots for Highly Sensitive Detection of BRCA1 Gene in Real Samples and Cellular Imaging

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Received: 21 October 2021 / Accepted: 11 April 2022 / Published online: 8 June 2022

Abstract

In this research, DNA-modified carbon dots (CDs) were exploited to construct a fluorescence assay for breast cancer genes (BRCA1, a potential marker for cancer diagnosis) detection. For this purpose, water-soluble synthesized CDs were functionalized with 19 mer-modified oligonucleotides (capture probe). By adding the DNA target, the specific binding between the DNA probe and DNA target causes fluorescence quenching. The assay displayed a fine capability of sensing the BRCA1 gene with a linear range ($R^2 = 0.9918$) of 36 attomolar (aM) to 532 femtomolar (fM) and a detection limit of 2 attomolar. This homogeneous process does not need additional separation and washing steps of un-hybridized DNA. To assess the selectivity, the prepared biosensor responses were evaluated in solutions containing single-base mismatched DNA sequences, three-base mismatched DNA sequences, or non-complementary DNA sequences, separately. To demonstrate the practical application of the designed biosensor, the extracted DNA from blood samples of breast cancer patients was utilized as real samples. When the CDs-DNA bioassay was exploited in the imaging of MCF-7 cancer cells, strong fluorescence emission was observed. After incubation times, both the cells' size and shape remained unchanged. The results validated that the CDs are an extremely great bioimaging candidate in disease diagnosis, biomedicine investigation, and managing cancer diseases.

Keywords Turn off fluorescence · Breast cancer 1 gene · Carbon dots · MCF-7 imaging

Introduction

The study of nucleic acids sequences has a critical potential for the routine application of efficient management and diagnosis of initial medicine. With technical progress, total genome sequencing is becoming a clinical device, causing a motion toward personalized medicine based on a person's genetics. However, for numerous uses that need quick outcomes, novel analytical procedures will be required to make the routine testing for DNA and RNA biomarkers affordable and applied for mainstream medicine. Nanomaterials have a multitude number of benefits as biomolecular detectors. Great surface-to-volume ratios combined with nanoscale dimensions permit target binding events to result in great signal alterations [1]. Developments in the utilization of nanomaterials for DNA biosensing have prompted the progress of effective sensors with higher signal processing. Nanomaterial-based biosensors have established numerous uses in food, environment, and health management for the determination of several targets [2]. Recently, a remarkable number of DNA biosensors have been appeared using distinctive optical or electrochemical features of various nanomaterials such as metal nanoparticles [3, 4], quantum dots (QDs) [5, 6], carbon nanotubes (CNTs) [7], graphene and its derivatives [8, 9] and other carbon-based nanoparticles

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[10]. DNA nanoassays are significant devices in the field of genetics, criminology, pharmacogenetics, pathology, and the medical/food industry.

Recently, fluorescent carbon dots (CDs), as a novel kind of the carbon nanomaterial family, are fascinating QDs for many applications owing to their stable photoluminescence, high aqueous solubility, low toxicity, robust chemical inertness, and easy functionalization [11, 12]. Researchers have been working hard to produce CDs with excellent properties using different methods or precursors. Compared with conventional chemicals, natural sources are becoming more and more attractive because they are non-toxic, inexpensive, and renewable [13]. Owing to environmental concerns, the consumption of hazardous chemicals used in nanomaterials synthesis limits their biocompatibility and applications. Thus, the green preparation of nanomaterials is a highly attractive research topic [14]. Fluorescence assays offer a great number of advantages, like relatively higher sensitivity, simpler design approaches, and being cost-effective [15]. Fluorometric methods have been effectively exploited for detecting biomolecules.

In previous researches [16, 17], CDs were applied as donor fluorescence to construct FRET assays that possess attractive features, including the feasibility of developing a biosensor without the necessity of using multi-step synthesis procedures. In this study, *Ferulago angulata* (*Chavil* in Persian) was used as a carbon precursor for CDs synthesis via the pyrolysis method. *Ferulago angulata* is from the family *Apiaceae* that grows in the western and central of Iran. Phytochemical examinations have verified the presence of phenolic and flavonoid compounds, anthocyanin, steroid, and tannin. The anticancer effect of *F. angulata* extract on HT-29, HEP-G2, T47D, MCF-7, HeLa-60, HUVEC, Raji (non- Hodgkin's B-cell lymphoma), U937, KG-1 A, VEGF-A, VEGFR-2, AGS, and PBL cell lines have been investigated [18]. Moreover, the produced multicolor fluorescent (blue, green, and yellow) CDs have been investigated as a fluorescent probe for fast, selective, and extremely sensitive quantification of BRCA1 gene by measuring the decreasing the fluorescence signal and also were successfully utilized in multicolor imaging of MCF-7 cancer cells.

Experimental

Apparatus

All of the fluorescence analyses were recorded on a Varian Cary Eclipse spectrofluorometric, utilizing a micro quartz cell (1 cm × 1 cm, 300 µL) in fast scanning mode. Both excitation and emission slits were set at 10 nm. Ultraviolet-visible (UV–vis) absorption spectra were acquired by UV–vis spectroscopy (Varian Cary 5000 UV–vis absorption spectrometer, Agilent Technologies, USA), a range of 220–800 nm. All optical analyses were done in triplicate under ambient conditions. The CDs' morphology was recorded by an H600 transmission electron microscopy (TEM, Hitachi, Japan) under a quickening voltage of 160 kV. The Fourier transform infrared (FT-IR) Bruker-Vector 22 spectrometer (Germany) was utilized for functional groups characterization of CDs. The optical density (OD) was recorded on a nanodrop (Thermo fisher 2000, American). A laser scanning confocal microscope (Nikon, ECLIPSE, Ti, Japan) was used for relative cell imaging.

Materials and reagents

1-Ethyl – 3- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS), were purchased from Abcam Company (Cambridge, UK, http://www.Abcam.com). Dialysis bags (molecular weight cut off = 1000 Da) ordered from Sigma-Aldrich (https://www.sigmaaldrich.com).

The 19-mer synthetic oligonucleotides were synthesized by BIONEER (Bioneer, South Korea), and purified by HPLC. The related sequences were as follows:

Capture probe (C1): 5′NH_2-GAT TTT CTT CCT TTT GTT C 3′.

Target DNA (Complementary): 5′GAA CAA AAG GAA GAAAAT C 3′.

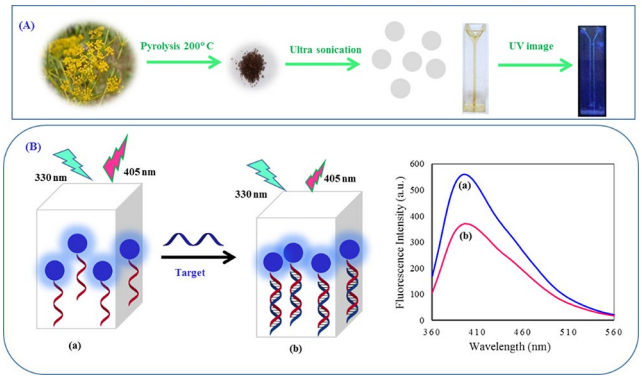
One-base mismatch: 5′CAA CAA AAG GAA GAA AAT C 3′.

Three-base mismatch: 5′CAA CAA AAG CAA CAA AAT C 3′.

Non-complementary: 5′CCT TGT TGG ACT CCC TTC T 3′.

Synthesis of CDs

CDs were synthesized by pyrolysis of *Ferulago angulata*. In a common process, the *Ferulago angulata* was first thoroughly washed with deionized water. After drying in an oven at 80 °C for 5 h, the pieces were mechanically crushed into fine powder. Then, 0.5 g powder was put into a typical ceramic crucible and carbonized at the temperature of 200 °C for 2 h with a heating rate of 5 °C/min, respectively. The carbonization was also performed at three different temperatures (200 °C, 300 °C, and 400 °C). After cooling down to room temperature, 0.2 g of resultant brown product was ultrasonically dispersed in 35 mL deionized water to form a homogeneous yellow solution (Scheme 1 A). The large dots were removed through centrifugation (4000 rpm, 10 min) and then resultant CDs were dialyzed against ultra-pure water through a dialysis tubing (molecular weight cut-off



Scheme 1 The schematic illustration of the synthesis of CDs (A) the fluorescence method for BRCA1 gene detection (B)

= 1000) for 48 h. The obtained CDs solution was kept in a dark place in the refrigerator for further characterization and use.

Attachment of DNA onto CDs

The terminal carboxylic groups on the CDs surface can be engineered for further coupling with numerous amino-modified biomolecules through covalent amide bond formation [19]. For this purpose, EDC and NHS solution (100 mmol L^{-1}, 100 µL) were added to 1000 µL of CDs solution (1.15 mg mL^{-1}) under stirring. After incubation at 4 °C for 2 h, 40 µL of DNA probe solution (100 nmol L^{-1} in 0.01 M PBS) and 750 µL of PBS (0.01 M, pH 7.4) were added to this mixture under vigorous stirring at room temperature. Excessive EDC and NHS were removed by dialysis [20]. The CDs-DNA probe was preserved at 4 °C for the following applications.

Extracting DNA from whole blood samples and quality of the extracted DNA

Blood from patients with adjuvant breast cancer was utilized for genome DNA extraction. Human blood samples were collected in vacutainer tubes containing EDTA. DNA extraction was performed using the salting-out method that is a solution-based protocol and avoids the usage of organic solvents [21]. The extracted DNA has been stored at -20 °C for future use [22]. For measuring the DNA quality, the 260/280 absorbance ratio of each sample was also evaluated using a spectrophotometer (Thermo – Nanodrop 2000) [23]. The 260/280 adsorption ratio was obtained as standard (1.7–1.8) for all extracted DNA samples.

Preparation of the sensing method

Examinations were accomplished in a reaction volume of 300 µL that comprised CDs (38.3 µg mL^{-1}), phosphate buffer solution, 10 mM, pH = 7.4. The as-prepared CDs solution has been utilized to investigate the selectivity of the probe against some DNA sequences. Various DNA sequences, including one base mismatch, three base mismatch, and non-complementary DNA target were mixed with CDs dispersions by gentle shaking.

Cellular cytotoxicity test

The cytotoxicity of CDs against MCF-7 cell lines was evaluated using standard MTT assay (3- (4,5-dimethylthiazol-2-yl) 5,2-diphenyltetrazolium bromide). MCF-7 cancer cells were first seeded in 96-well plates at a density of 2500 cells $well^{-1}$ and located in an incubator at 37 ° C in under 5% CO_2 for 2 days. The culture medium was then separated and DMEM containing numerous concentrations of CDs (0.0-0.5 mg mL^{-1}) was added and incubated at 37 ° C for 24 h. After that, the culture medium was separated and 200 µL of fresh DMEM containing 10% MTT (20 µL) was added to each well. After 3 h, the supernatant solution was substituted with 100 µL of DMSO to dissolve MTT. Finally, the optical density of each sample was recorded at 545/630 nm.

Cell imaging

The capability of the synthesized CDs can be demonstrated by studying the MCF-7 cell imaging. MCF-7 cells were cultured in 12-well plates with a density of 7000 cells per well and placed in an incubator at 37 ° C under 5% CO_2 for 48 h. The cells were then treated with a fresh culture medium containing several concentrations of CDs (0.0-0.5 mg mL^{-1}) for 24 h at 37 ° C. Finally, the cells were washed with PBS three times to eliminate the remaining CDs. For optical imaging, the MCF-7 cell images were performed (at reaction times 20 h) by a Nikon ECLIPSE microscope.

Results and Discussion

Characterization of CDs

The presence of CD surface functional groups was studied using the FT-IR spectra (Fig. 1 A). Broadband in the range of 3200–3550 cm^{-1} is ascribed to O-H and N-H stretching vibrations. And the band in the 2900 – 2800 cm^{-1} region is attributed to C-H stretching vibrations. The bending vibrations of the C-N groups can appear at 1423 cm^{-1}, and the characteristic band at 1640 cm^{-1} is caused by C = O stretching vibration. A peak centered at 1571 cm^{-1} is assigned to the bending vibration of C=C. A absorption band of about 1115 cm^{-1} is related to the stretching vibrations of C-O.

These data exhibit that the synthesized CDs possess COOH, OH, and amine functional groups, resulting in their excellent solubility in water without the need for further chemical modification [24, 25]. The formation of CDs with a size of 1.9 ± 0.6 nm was confirmed using a TEM image as shown in Fig. 1B. The TEM image exhibits that the CDs are spherical / quasi-spherical and have a narrow size distribution between 1.0 and 2.7 nm. The optical feature is one of the most significant properties of CDs for biological applications. CDs generally have robust absorption in the ultraviolet region with decreasing intensity of sequence absorption in the visible region (Fig. 1 C). The absorption band below 300 nm is generally related to the π-π* transition of the C=C bond of the carbon core, while the tail band of 300 to 500 nm can be attributed to the inherent absorption of n → π* transition of the C = O bond in carbon cores [26]. This can be regarded as the feature of the CDs synthesized by the carbonization of carbon-based materials. As the inset photographs display the CDs solution is yellow colored under daylight, while it emits bright blue fluorescence under UV excitation. One of the interesting features of CDs is the dependence of their emission spectra on the excitation wavelength, which is commonly observed in most CDs (Fig. 1D). The fluorescence emission spectra of CDs were acquired by enhancing the excitation wavelength from 290 to 360 nm at 10 nm intervals. The maximum emission wavelength of CDs was 405 nm as it is excited at 330 nm. As the excitation wavelength enhances, the red-shift of emission wavelength and decrease of fluorescence emission intensity can be observed. The excitation-dependent feature is common in carbonous fluorescent nanomaterials which can attribute to various particle sizes or the presence of different emissive traps on the surface of CDs. Therefore, it leads to the fact that the smaller size of particles are excited at lower wavelengths, while particles with larger sizes get excited at higher wavelengths [27].

Optimization of Test Conditions

To investigate the application of CDs as a fluorescence probe in biological conditions, the fluorescence stability of the aqueous solution of CDs was evaluated. Figure 2 A shows that the fluorescence intensity of CDs is approximately constant in the pH range of 6–8, the physiological environment is usually pH 7.4, which is useful for imaging applications. As shown in Fig. 2B, NaCl solution of 0.03 to 0.8 M was added to the aqueous solution of CDs and their fluorescence intensity was almost constant up to about 0.167 M. The stability of the aqueous solution of CDs-DNA probe and upon addition of BRCA1 target to CDs-DNA probe system was investigated (Fig. 2 C-F). The fluorescence intensities were calculated throughout 60 min that the results revealed the

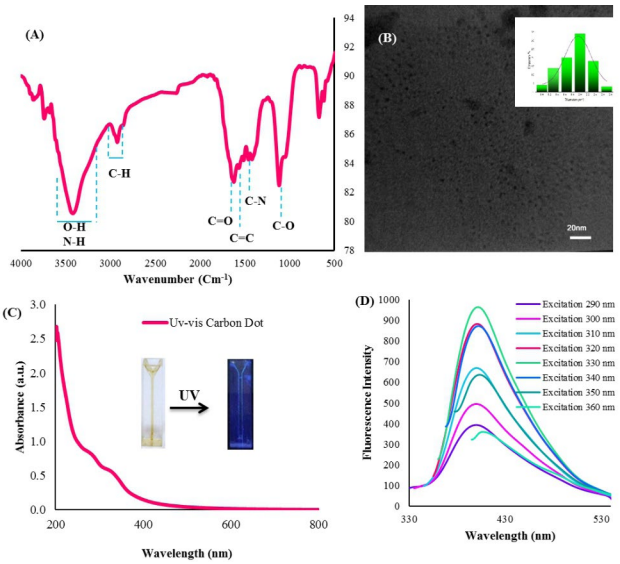


Fig. 1 FTIR spectrum of CDs (A) TEM image of CDs and inset: CDs particle size distribution diagram (B) Uv-vis spectrum of CDs, inset: solution of CDs under visible light (left) and under ultraviolet light (right) (C) Fluorescence spectrum of CDs at different excitation wavelengths from 280 to 350 nm (D)

signal remained almost unchanged. To guarantee a good interaction between probe and target, the fluorescence response was measured within a reaction time of 20 min. NH_2 modified DNA probe can be conjugated to the carboxyl group of CDs by covalent bonding [17]. The stabilization of the DNA probe on the surface of the CDs was confirmed using fluorescence spectra (Fig. 3 A). Covalent bonding between CDs and DNA probes using the activating agent leads to enhancing the rigidity of the fluorophore structure and increasing the fluorescence intensity [28]. Moreover, the successful immobilization of the DNA probe on CD was proved by electrochemical impedance spectroscopy (EIS). As revealed in Fig. 3B, the bare GCE exhibited a small semicircle, while the resistance enhanced for the electrode modified with CD. For the CD-DNA probe immobilized on the electrode, a further increase in resistance was detected, which confirmed the CD modification with the DNA probe. As shown in Fig. 3 C, different concentrations of DNA probes (0.5, 2.0, 5.0, 10.0, and 20 nM) were investigated and the best fluorescence response was obtained for a concentration of 2.0 nM of DNA probe. Therefore, 2.0 nM was selected as the optimal amount of the DNA probe.

Analytical Performance of the Designed Biosensor

The performance of the present biosensor was evaluated by measuring the dependence of the fluorescence response on the concentration of the BRCA1 target sequence under optimal conditions. As shown in Fig. 3D, the fluorescence intensity decreases with increasing BRCA1 concentration,

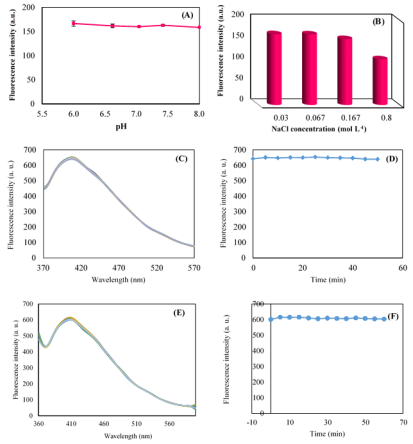


Fig. 2 Graph related to the fluorescence stability of CDs at pH 6–8 (A); in the presence of NaCl concentration of 0.8 – 0.3 M (B); Fluorescence spectra of CDs-DNA probe (C); CDs-DNA probe upon the addition of o.15 fM BRCA1 target (E) in 60 min interval; and graph related to the fluorescence stability of CDs-DNA probe (D); CDs-DNA probe upon the addition of o.15 fM BRCA1 target (F) in 60 min interval

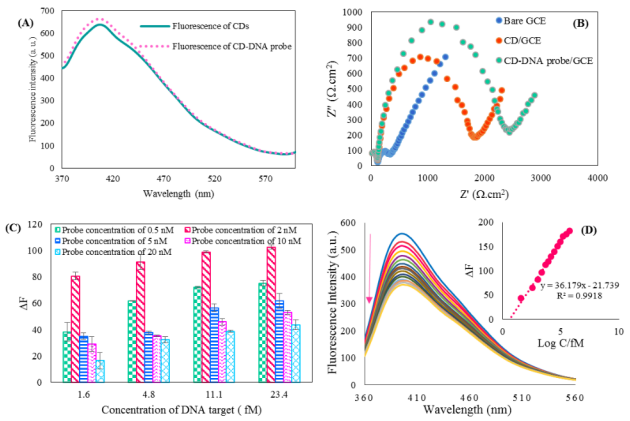


Fig. 3 Fluorescence spectra of CD and DNA probe functionalized CD (A); Nyquist plots of GC electrode, GCE/nafion/CD and GCE/nafion/CD-DNA probe in phosphate buffer (pH 7.4) containing 2.5 mM $[Fe(CN)6]^{3-/4-}$ and 0.1 M KCl, applied potential was 0.2 V at the frequency range of 0.1 Hz-10 kHz (B);
The effect of DNA probe concentration to modification of CDs surface (C); Fluorescence spectra of CDs modified with DNA probes in the presence of different concentrations of target sequence and inset: linear correlation of fluorescence intensity and logarithm of DNA target concentrations from 36 aM to 532 fM (D)

while a good linear correlation ($R^2 = 0.9918$) between the logarithm of the target sequence concentration from 36 aM to 532 fM and the fluorescence intensity was obtained with a detection limit of 2 aM. As can be seen in Table 1, the performance of the constructed assay was compared to earlier BRCA1 gene biosensors reported in the literature. From Table 1, it can be realized that the detection range was wider and the sensitivity was higher than the previous reports. This biosensor has reached a high sensitivity only with a very simple method and without a complex signal amplification procedure. Moreover, the fluorescence assay was easy to operate and had a fast response time. According to the above experimental outcomes and previous studies DNA probes can bind with the target, a promising detection mechanism is suggested (Scheme 1B).

The fluorescence intensity of CDs gradually decreased when BRCA1 gene solution was gradually added to the CDs-DNA probe solution. Quenching mechanisms of CDs can be due to static quenching, dynamic quenching, inner filter effect (IFE), energy transfer, and photoinduced electron transfer (PET). The mechanism of the reaction was investigated by detecting the excitation, emission spectra of CDs, the absorption spectrum of the target, and the average fluorescence lifetime of the CDs-DNA probe before and after the addition of the BRCA1 gene. From Fig. 4 A, the overlap of the absorption spectrum of a target ($\lambda_{max} = 260$ nm) with the emission spectrum of the CDs was negligible. So, the possible fluorescence quenching mechanism can be probably due to static quenching or dynamic quenching. To prove the static quenching or dynamic quenching mechanism, the average fluorescence lifetime of the CD-DNA probe was measured in the absence and presence of a quencher (Fig. 4B). The values of an average lifetime were calculated to be 1.61 and 1.56 ns for fluorophore without and with a quencher. Because the average lifetime amount remains almost constant, the sensing mechanism can be static quenching. Moreover, UV–vis absorption spectra of CDs, CDs-DNA probe, DNA target, and CDs-DNA probe-target system were measured. The absorbance band of the DNA target was at 260 nm. The CDs-DNA probe-target system also exhibited the absorbance band at around 260 nm, and it signified the creation of the CDs-DNA probe-target complex (Fig. 4C). To further investigate the static mechanism, we tested the effect of temperature increase (Fig. 4D). An increase in temperature can lead to reducing in the stability of the ground-state complex, so decreases the influence of static quenching [29]. Therefore, the quenching mechanism of present method was static quenching. The quenching efficiency was examined with the Stern-Volmer equation. This equation;

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

Where K_{SV} is the Stern–Volmer quenching constant, [Q] is the concentration of DNA target, and F_0 and F are the fluorescence intensities at 405 nm before and after adding DNA target, respectively [30]. Fig. 4D displayed a good linearity in the range of 0.16 fM-6.8 fM ($R^2 = 0.9964$). K_{SV} was calculated as 14.7×10^6 μM^{-1} from the slope of Fig. 4D.

Table 1 Nanomaterial-based DNA sensors for determination of BRCA1gene

Detection technique	Nanomaterials	Linear range	Detection limit	Ref.
Electrochemical	Gold nanoparticle cluster	100 aM to 1 nM	50 aM	[3]
Electrochemical	Single walled carbon nanotube (SWCNT)	-	378.52 nM	[7]
Electrochemical	Graphene	1 fM to 1 nM	100 aM	[8]
Electrochemical	Quantum dots	5 aM to 5 fM	1.2 aM	[34]
Fluorescence	Quantum dots and silver nanoclusters	0.5 pM to 1.0 nM	0.12 pM	[6]
Fluorescence	Carbon nano dots	up to 200 nM	270 pM	[10]
Fluorescence	Gold nanoparticle	0.29 pM to 29 nM	0.29 pM	[35]
Fluorescence	Carbon dots	36 aM to 532 fM	2 aM	This work

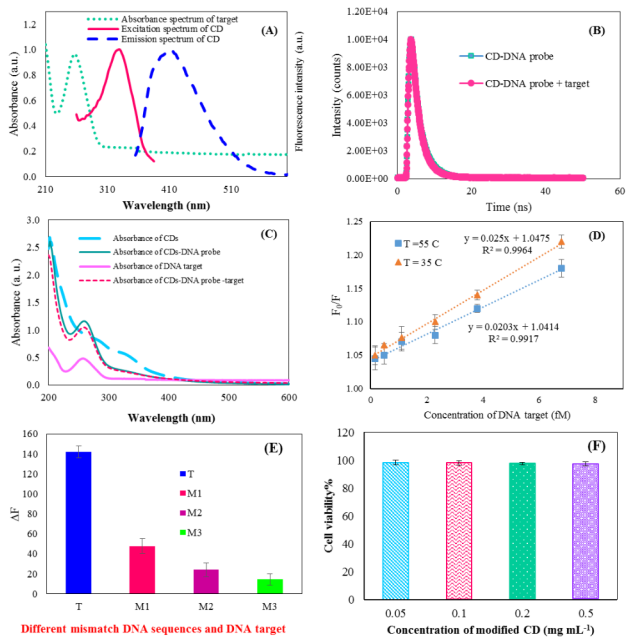


Fig. 4 Absorbance spectra of BRCA1 gene (target) (green), excitation (pink) and emission (dark blue) fluorescence spectra of CD (A); Fluorescent lifetime of CD-DNA probe before and after adding target (B); Absorbance spectra of target, CD, CD-DNA probe before and after adding target (C); The fluorescence responses (plot of F_0/F) of the CDs-DNA probe after the addition of different concentration of DNA target at 35 and 55 °C (D); Fluorescence response exhibiting the selectivity of the biosensor with 3 fM concentration of DNA target (E); Evaluation of CDs toxicity for MCF-7 cancer cells (F)

In general, direct, sensitive, and selective quantification of biomarkers is very challenging. To improve the selectivity of the DNA-based sensors, the DNA probe should be conjugated on the surface of the nanomaterials via a covalent bond to prevent the non-specific binding as well as other side reactions. Amidization process using EDC/NHS was extensively utilized for the covalent functionalization of different ligands on the surface of the nanostructures, which is based on reaction among carboxyl or amino groups on the surface of the nanostructures with amino or carboxyl ended ligands. Therefore, the high selectivity of designed biosensors could be because of the covalent conjugation of DNA probe on CDs surface as well as the high affinity of DNA target to DNA probe [19, 31]. To assess the selectivity of the biosensor, the sensor was assessed in different solutions containing one base mismatch DNA sequence (M1), three bases mismatch DNA sequence (M2), and non-complementary DNA sequence (M3), and the target sequence (T). As shown in Fig. 4E, the designed biosensor shows excellent selectivity towards the target molecule (T), and the other three sequences show little response. Hence, the biosensor of the BRCA1 gene has a good selectivity to T. CDs-DNA probe possess high affinity towards DNA target to form a non-fluorescent complex, leading to obvious fluorescence quenching. Hence, the CDs-DNA probe revealed high sensitivity and selectivity for DNA target, which is ascribed to the static quenching and the strong complex interaction among CDs-DNA probes and DNA targets.

Quantification of BRCA1 Gene in Extracted DNA Samples

To demonstrate the practical application of the designed biosensor, extracted DNA from breast cancer patients was utilized as a real sample. The amount of extracted DNA samples was determined using a nanodrop and diluted twice. 1 µL of diluted DNA sample was added to the biosensor. Using the calibration equation, the amount of the extracted DNA sample was calculated. Then 33 aM of the target sample is added to the above solutions, and the recovery is calculated, the results are shown in Table 2.

Evaluation of CDs Toxicity and Cellular Imaging

CDs are well regarded for their outstanding biocompatibility and low toxic nature, which is also an essential requirement for bio applications. The cytotoxicity of CDs has been widely studied both in vitro and in vivo. In vitro imaging application is achieved to afford comprehensive data on the imaging capability, distribution, and toxicity of probes in cells. The toxicity investigation of CDs is worth examining due to their nanoscale dimensions and their potential for bioimaging. The cytotoxicity of the red, green, blue photoluminescence CDs was examined by using a standard MTT

Table 2 Results from nanobioensor and nanodrop spectrophotometry for detection of DNA extracted from breast cancer patients

Sample	Spiked BRCA1 (aM)	Detected BRCA1 (aM) by our method	Recovery%	RSD%	Detected DNA by nanodrop
1	0	11.75		2.2	10.5
	33	41.58	90.4	3.6	
2	0	14.82		4.3	12.6
	33	47.42	98.8	3.2	
3	0	17.24		2.2	12.45
	33	48.19	93.8	0.61	

assay against MCF-7 cancer cells [32, 33]. To evaluate the efficiency of CDs in cell imaging, the cytotoxicity of CDs was evaluated by adding different concentrations of CDs (0.05, 0.1, 0.2, 0.5 mg mL^{-1}) to MCF-7 cancer cells. The relative viability of MCF-7 cancer cells exposed to CDs was determined by MTT assay at 545/630 nm. As shown in Fig. 4F, CDs showed negligible toxicity to MCF-7 cancer cells up to 0.5 mg mL^{-1} within 24 h of preparation. These results demonstrate that functionalized CDs can be utilized as non-toxic labels for cellular imaging applications. MCF-7 cancer cell imaging was adjusted during the 24 h reaction time. MCF-7 cancer cells treated with CDs did not show significant changes in shape. As shown in Fig. 5 (A-L), a strong blue, green, and yellow photoluminescence on MCF-7 cell membranes exhibited that CDs had entered the cells and that fluorescence properties had been maintained in the cellular medium, while no radiant light in the core is not visible. The images displayed that MCF-7 cells are well labeled with CDs.

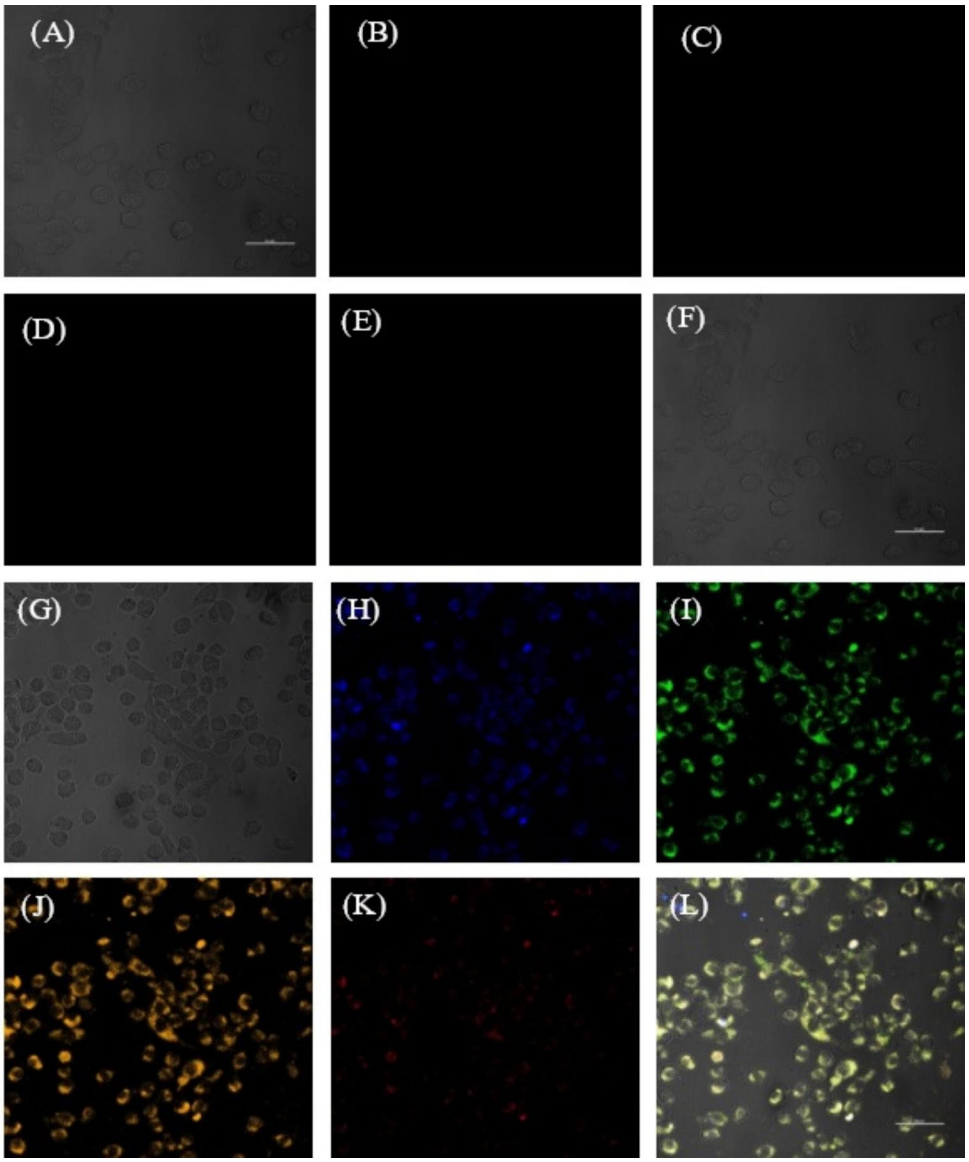


Fig. 5 Confocal laser scanning microscopic images of MCF-7: control (A-F) cells and cells treated with 0.5 mg mL^{-1} of CDs-DNA probe (G-L) excitation by bright field (A & G), Blue (B & H), Green (C & I), Yellow (D & J), Red (E & K) and Merge (F & L)

Conclusions

The construction of a sensitive biosensor for the detection of the BRCA1 gene was reported using CDs synthesized from the local plant. These CDs were identified and described using spectroscopic and microscopic techniques. The low cytotoxicity of the CDs ensured the activity of the cells and sustained the sensing efficiency and MCF-7 cells is successfully imaged with a fluorescence microscope. Compared to prior researches, our consequences also demonstrate a promising analysis of the BRCA1 gene with a large detection range with a comparable detection limit was attained. Most significantly, selective imaging of cancer cells using CD-DNA suggests its potential in biological applications in bioassay as well as in cancer diagnosis and would possess the capability to be widened for the quantification of other biomarkers with a large linear range and low detection limit.

Acknowledgements The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 97041) for the financial support.

Authors' Contributions MP helped through supervision, project administration, funding acquisition, SM performed investigation, data curation, conceptualization, writing - original draft and editing, RKh contributed to supervision, Editing. ZH performed cell culture experiments and a cellular cytotoxicity test. KM helped by supervision. MP Supplied the blood samples from breast cancer patients.

Funding The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 97041) for the financial support.

Data Availability All data generated or analyzed during this study are included in this published article.

Code Availability Not Applicable.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest in the publication of this article.

Ethics approval/declarations Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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