Synthetic Metals 244 (2018) 92–98

Contents lists available at ScienceDirect

Synthetic Metals

journal homepage: www.elsevier.com/locate/synmet

Fluorescence biosensor based on gold-carbon dot probe for efficient detection of cholesterol

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ARTICLE INFO

Keywords:
Cholesterol
Fluorescence
Biosensor
Carbon dot
Gold nanoparticles

ABSTRACT

Cardiovascular diseases have raised serious concerns in recent times with a huge number of mortality all over the world. Herein, we report a fluorescence probe, comprising of carbon quantum dots (CD) and gold nanoparticles (AuNP) for efficient sensing of cholesterol, the most important cardio-risk-marker. A simple hydrothermal method has been employed to synthesize the gold-carbon-dot nanohybrid (AuCD). Cholesterol oxidase immobilized AuCD (Ch-AuCD) showed prominent fluorescence emission at the excitation wavelength of 360 nm. Förster resonance energy transfer (FRET) has been observed between Ch-AuCD and graphene oxide with visible fluorescence intensity *turn off*. This has been recovered by the addition of a range of concentrations of cholesterol which *turn on* the fluorescence intensity. This 'on-off' mechanism could efficiently detect cholesterol within the concentration range of 10–100 nM (lower detection limit ~10 nM). Moreover, selectivity of the probe towards cholesterol has been ascertained by analyzing a range of interfering analytes, *viz.* ascorbic acid, urea, glucose, galactose and L-cysteine. Thus, we forward this FRET based 'nano-switch' with high selectivity and sensitivity for efficient sensing of cholesterol. Further, ease of detection endorses this fluorescence-based method for real sample analysis, after scrutinizing the allied factors.

1. Introduction

Observing the worldwide rate of heart diseases, the periodic monitoring of cardiovascular risk markers is inevitable. Plasma cholesterol, being one of the most prominent heart disease risk marker has raised serious concern [1]. Low density lipoprotein (LDL), the so-called *bad cholesterol*, beyond the normal range initiates pulmonary or coronary blockage and subsequent cardiac strokes [2–5]. So, a rapid, accurate and selective sensor for cholesterol is highly desired. Recent advances in biosensors confront issues like selectivity, sensitivity and ease of handling [6]. Thus, development of advanced biosensors demand the incorporation of these attributes. Besides the conventional spectrophotometric techniques, extensive research has been carried out for electrochemical detection of cholesterol [7–10]. However, low selectivity, tedious and expensive sensing processes restrict their commercial viability [11]. Saxena and Das recently reviewed the potentiality of nanomaterials in the fabrication of cholesterol biosensors. In this review, they have shown the variation of selectivity, sensitivity and detection limit of the biosensors, based on the electronic, optical and catalytic properties of the nanomaterials [12].

Fluorescence based biosensors have been delved into recently for efficient sensing of a range of bio-analytes [13–15]. Förster resonance energy transfer (FRET) mechanism has been employed in the domain of biosensors for micro-molar (μM) or nano-molar (nM) level detection of analytes [16,17]. Owing to the excellent fluorescence properties, nanoparticles and quantum dots have been explored for fabricating fluorimetric-biosensors [18,19]. Carbon-quantum dots (CD) are of great interest due to their cheap precursors and easy synthetic protocols [20]. Excitation wavelength dependent fluorescence behaviour of CD aids distinctive advantage in fluorescence based biosensing [21]. It is witnessed that on interaction with bio-analytes, semiconductor quantum dots and nanoparticles show fluorescence quenching, which can be used for ascertaining the biosensing ability of a nanomaterial.15] Gold nanoparticles (AuNP) have also been used in FRET based studies either as a fluorophore or as a quencher [14]. Again, two dimensional (2D) nanostructures like graphene, graphene oxide (GO), reduced graphene oxide (RGO), MoS_2 *etc.* have also been used as quenchers in FRET based fluorescence biosensing [13,22–25]. Herein, we demonstrate the development of a fluorescent probe by the combination of CD and AuNP (AuCD). Cholesterol oxidase (ChOx) has been immobilized onto the

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https://doi.org/10.1016/j.synthmet.2018.07.010
Received 8 February 2018; Received in revised form 27 June 2018; Accepted 16 July 2018

probe for selective sensing of cholesterol [26]. GO, being a strong fluorophore, has been utilized for turning-off the fluorescence intensity of cholesterol oxidase immobilized AuCD (Ch-AuCD), as FRET is very much probable between Ch-AuCD and GO. In a recent report, Gu et al. witnessed FRET between MoS_2 and hyaluronic acid immobilized AuNP, which was recovered by micromolar addition of hyaluronidase [13].

Though some recent reports overrule the use of specific enzymes for cholesterol detection, still they could not address selectivity at very high sensitivity. Priyadarshini et al. reported a gold-carbon dot based nanoconjugate for enzyme free sensing of cholesterol with high selectivity. However, they could achieve sensitivity only up to milli molar level [15]. So, herein we have described the efficiency of a fluorescent probe based on Ch-AuCD for highly sensitive detection of cholesterol. Addition of cholesterol has been used to *turn-on* the fluorescence intensity, which ascertains the specific and selective sensing. Thus, we have put forward a '*nano-switch*' that can be operated by *on and off* mechanism, based on FRET.

2. Experimental Section

2.1. Materials

D-(+)-glucose (~99.5% pure), gold(III) chloride ($HAuCl_4$), sodium citrate tribasic dehydrate (SCT ~99.0% pure), graphene oxide (GO, 10 mg/mL, dispersion in water), cholesterol (~92.5% pure) and cholesterol oxidase (ChOx, 100 U/mL from *Streptomyces* sp.) were procured from Sigma Aldrich (USA). Allied chemicals and reagents were purchased from, Merck, India and used without further purification.

2.2. Synthesis of AuCD

AuCD was synthesized by a single step hydrothermal method. D-(+)-glucose (30 mg) was taken in 50 mL of water in a 100 mL conical flask. A solution of $HAuCl_4$ (0.01 M) was prepared in water and added to glucose solution. The mixture was allowed to stir at room temperature for 30 min. Then, SCT (0.01 M) was added drop wise to the reaction vessel [27]. After the addition, the reaction mixture was poured into a Teflon lined hydrothermal reactor and heated at 180 °C for 6 h in a hot air oven. After the completion of the reaction, the vessel was cooled to room temperature. The resultant product was AuCD with ruby-purple colour. Solid content of the solution was determined by drying 1 mL of the solution in a glass petri-dish.

2.3. Preparation of Ch-AuCD

ChOx (100 U/mL) was diluted 100 times in Milli-Q water and immobilized onto AuCD by physical adsorption technique [28]. Briefly, diluted ChOx solution (1 U/mL) was added to 1 mL of AuCD, taken in a 15 mL glass bottle and stirred for 5 h at 4 °C, followed by ultrasonication for 15 min. The immobilized nanohybrid system was encoded as Ch-AuCD.

2.4. Characterization

UV-visible spectra were recorded in a SPECORD-200 spectrophotometer (SPECORD, Germany). X-ray diffraction patterns were analyzed by a Rigaku (Rigaku, Japan) X-ray diffractometer at the scanning speed of 2.0° min^{-1} (scanning range $2\theta = 10°$-60°). Morphology of the nanomaterials was studied by a field emission scanning electron microscope (Zeiss Sigma VP FE-SEM, Germany). Distribution pattern, morphological parameters and shape-size accords of AuCD were studied by a High-Resolution Transmission Electron Microscope (HRTEM, JEOL, JEMCXII, Japan) at an operating voltage of 200 kV.

The excitation dependent fluorescence emission of Ch-AuCD was studied in a Horiba, Fluorolog-3, USA, spectrofluorometer. Emission spectra were recorded in the excitation wavelengths range of 300–400 nm. Quantum yield was also determined by using an integrating sphere method. The same spectrofluorometer was used in various fluorescence based measurements for the detection of cholesterol.

2.5. Ch-AuCD as fluorescence probe for cholesterol sensing

A solution of Ch-AuCD (3 mg/mL) was taken in a 3 mL quartz cuvette, into which different concentrations of the quencher (GO) was added. Decrease in the fluorescent intensity of Ch-AuCD was recorded within the emission range of 380–700 nm, at the excitation wavelength of 360 nm. Amount of the quencher was optimized by recording the quenching pattern in the concentration range of 5–55 μg/mL (see results and discussion 0–55). A linearity calibration curve was plotted to elucidate the relationship between the fluorescence intensity *turn-off* and concentration of GO. The optimum concentration was calculated and was added to the working Ch-AuCD solution. A varying concentration (10–100 nM) of cholesterol was added to Ch-AuCD/GO to record the fluorescence intensity *turn-on*. Each reading was taken after incubating Ch-AuCD/GO with a particular concentration of cholesterol for 20 min at room temperature. Recovery of the quenched fluorescence intensity was measured within the same emission range as stated above. The relation between the *turned-on* fluorescence intensity and the concentration of cholesterol was determined by linearity-fit curve plotted in Origin 8.5 Pro software.

Selectivity of the sensor towards cholesterol was investigated in presence of interfering bio-analytes *viz.* ascorbic acid, urea, glucose, galactose and L-Cysteine [15]. Static fluorescence spectra of Ch-AuCD were recorded in presence of these biomolecules under similar experimental conditions. Same excitation wavelength and interfering analyte concentrations (50 nM) were maintained as that of cholesterol. If an analyte possesses interfering affect, it would enhance the fluorescence intensity. On the other hand, the inert analytes would leave the intensity unaltered. Thus, comparison of fluorescence intensities of the probe in presence interring elements and cholesterol provides the extent of selectivity of the sensing platform.

3. Results and discussion

3.1. Structural and morphological analysis of Ch-AuCD

Synthesis of quantum dots has attained great attention in recent decades owing to their immense potential in opto-electronic devices, biosensors, solar concentrators, single-photon devices and *in vivo* medical imaging [29]. Variation in the synthetic-protocol has a direct impact on the shape and size accords as well as the properties of the quantum dots [30]. Chemical and laser ablation, electrochemical carbonization, microwave irradiation, hydrothermal and solvothermal methods are the commonly employed techniques for synthesizing quantum dots [31]. However, hydrothermal method has been widely adopted for synthesising carbon based quantum dots [20]. De and Kark in 2013, proposed a mechanism for the formation of CD from a carbohydrate rich source, banana extract [32]. Accordingly in the present work, high temperature and pressure initiated the dehydration and decomposition of glucose, followed by condensation, aromatization and carbonization. Finally, nuclear burst of the resulted clusters yielded highly water soluble CD.

Again, synthesis of AuNP is primly carried out by the reduction of a gold salt, most commonly $HAuCl_4$ by a variety of reducing agents of natural or synthetic origin. [27]33 AuNP are generated *via* a sequence of reactions. Initially small nuclei are formed, followed by their coalescence to generate bigger particles. Subsequent slow growth along with the reduction of $HAuCl_4$ resulted in the formation of colloidal AuNP [34]. Shape and size is greatly dependent on the concentration of the precursor and the reaction conditions. The present method provides