Vacuum 146 (2017) 606–613

Contents lists available at ScienceDirect

Vacuum

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

Quantum dots and carbon dots based fluorescent sensors for TB biomarkers detection

Dhiman Bhattacharyya, Prashant K. Sarswat*, Michael L. Free

Department of Metallurgical Engineering, University of Utah, Salt Lake City, UT 84112, USA

ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form
31 January 2017
Accepted 2 February 2017
Available online 3 February 2017

Keywords:
Quantum dots
Biomarkers
Sensors
Tuberculosis
Colorimetry

ABSTRACT

The clinical use of volatile organic compounds (VOCs) detection for improved and rapid diagnoses of various diseases possesses a significant potential for the correct and timely screening of such diseases. Tuberculosis (TB) is a major pulmonary disease of concern, and can potentially be diagnosed using breath analysis techniques. Various sensing methods have been demonstrated and utilized to detect TB-VOCs. Some of the methods are based on electrical signal or change in resistance of device due to VOCs while some methods utilize mass spectroscopy. A low cost and quick method is very essential at this stage. Hence, in this paper, we report the synthesis of stable colloidal suspensions of CdSe QDs as well as carbon dots as a viable photoluminescent platform for detection of TB biomarkers. Such novel CdSe/carbon dots based sensing solution with tunable excitation and emission properties acts as a fluorescent probe for selective detection of TB biomarkers. The development of a smart, tunable fluorescent sensor system can provide an economically feasible solution and pave the way for rapid disease diagnostics.

1. Introduction

Rapid, point-of-care (POC) disease diagnostics presents several challenges including cost, detection time, equipment portability, and performance. Detection of volatile organic compounds (VOCs) based disease biomarkers from the breath provides a potential solution to the aforementioned problems [1]. The clinical use of VOCs identification for improved and an accurate diagnoses of pulmonary [2,3] and non-pulmonary [4–7] diseases possesses a significant potential for the rapid screening of such diseases. Tuberculosis (TB) is a major pulmonary disease of concern, which can potentially be diagnosed via breath analysis. In a recent report, world health organization (WHO) states that almost 9.6 million people worldwide are infected with TB each year, while ~3 million people do not get the basic care they need and ~1.5 million people die of the disease, causing TB to be the leading cause of death worldwide alongside HIV [8]. A VOCs detection based approach that falls under the "moderate complexity assays" group for TB diagnostics was presented [9]. In addition to the capability of giant African pouch rats (apopo) for sniffing TB, the technologies enlisted for VOC based approach include TB Breathalyzer (Rapid Biosensor Systems), Breathlink (Menssana), Aeonose (The eNose Company), Breath analysis instrument (Metabolomx), and Prototype Breathalyzer (Next Dimensions Tech). The TB Breathalyzer utilizes the evanescent wave technology leading to reduction in fluorescent signal when the TB antigen displaces fluorescently coated analogues and bonds to the antibody in the device [10]. Breathlink identifies the markers of oxidative stress and the disease using proprietary algorithms where alveolar breath VOC samples are separated by gas chromatography and detected with flame ionization (GC-FID) or surface acoustic wave detection (GC-SAW) with high sensitivity [11]. The Aeonose utilizes the basic concept of an electronic nose, or machine olfaction, where a measurement generates complex multi-dimensional data for each measurement combined with a pattern recognition technique that interprets the complex data and relates it to a target value or class [12]. Metabolomx uses a colorimetric sensor array and breath analysis instrument. The colorimetric sensors help to capture the chemical signature pattern of the complex mixture of VOC's present in breath thereby helping in identifying tuberculosis, colon cancer, and lung cancer detection [13]. It is evident that although the aforementioned technologies fall under the VOC based detection category, none of them effectively detects the specific VOCs found in the exhaled breath of TB patients.

* Corresponding author. 135 S, 1460 E, Room 412, Salt Lake city, UT 84112, USA.
E-mail address: saraswatp@gmail.com (P.K. Sarswat).

http://dx.doi.org/10.1016/j.vacuum.2017.02.003
0042-207X/

1.1. General methods for VOB detection and colorimetry approach

The prominent volatile organic biomarkers (VOBs) in the breath of smear positive tuberculosis patients have been detected using solid phase microextraction fibers with GC-MS analysis [14,15]. Recently, the capability of electrochemical detection of these predominate TB VOBs using metal functionalized titania nanotubes array based sensing platform has been demonstrated [1,16]. Although such sensors provide an excellent platform for rapid and non-invasive detection, sensitivity might be an issue in the case of extremely low biomarker concentrations, which is critical for early detection or latent TB subjects. Colorimetric sensors for VOCs and odor detection and quantification have been a topic of great interest, due to their increased sensitivity correlated to change in color or fluorescence even at low concentrations of the VOBs [17–22].

Colorimetric assays [23] warrant inevitable mention of a critical component, quantum dots. Quantum dot nanocomposites have been utilized for the delivery of both small and large molecular weight therapeutics [24], as well as for sensing applications. Fluorescence assisted quantum dot (QD) based protein detection has been utilized for sensing of the multiple cancer biomarkers [25,26]. CdS quantum dots can bind effectively to methyl viologen molecules [27]. In addition, EDA/EDTA modified graphene quantum dots based fluorescent sensor has demonstrated promising capabilities for the detection of dipicolinic acid, an important anthrax biomarker [28]. In other research, Cu-Zn-Sn based chalcogenide-quantum dots tethered to a rhodamine-fluorene derivative have shown immense potential for selective detection of VOBs specific to lung cancer [29]. Amine terminated gold nanodendrites sensitized quantum dots (AuND-QDs) assembly has been successfully employed for sensing of highly explosive and environmentally detrimental trinitrotoluene (TNT) [30]. Therefore, it is evident that the development of a quantum dot based fluorescence platform is particularly attractive for application in biological labeling, disease detection, drug delivery, and explosive compound monitoring. Also, it is important to note that for most sensing applications, the quantum dots are coupled with a ligand which can selectively bind with the biomarker of interest and render fluorescence at the same time. The predominate TB biomarkers can be classified as methyl esters. Zhang et al had demonstrated that modified metalloporphyrins can catalytically oxidize fatty acid methyl esters [31]. Also, protoporphyrin IX dimethyl ester has been demonstrated to be a potent photosensitizer of human nasopharyngeal carcinoma. In addition, metalloporphyrins have been declared as nearly ideal for detection of metal ligating vapors facilitated by their open coordination sites for axial ligation [19]. Further, large spectral shifts upon ligand binding, intense coloration, together with their ability to provide differentiation based on metal selective coordination, renders metalloporphyrins indispensable for VOCs detection [19]. Although the QD - metalloporphyrin nanohybrid photo-label seems viable, its specificity towards the methyl ester based TB biomarkers might be low. Therefore, we suggest an alternative QD based photo-label with higher selectivity and specificity towards the biomarkers.

For biological applications, QDs typically utilize ZnS as the outermost shell because of its role as a physical barrier to prevent potentially toxic core materials such as CdSe from leaching out to the surrounding environments [32]. In addition, the higher band gap of ZnS (~3.7 eV) confines the excitons to the cores and prevents them from interacting with the surrounding environment, thereby maintaining high fluorescence quantum yields (QYs) [32]. These QDs exhibit a tendency to aggregate in the absence of any ligands. The ligands essentially provide enhanced functional stability in a cooperative binded ensemble. In this regard, polymeric ligands appended with functional pendant groups render significantly enriched functionality to the QDs. Among the more common chemical groups able to coordinate with Zn at the QD surface, thiols form reasonably stable bonds, leading to their ubiquitous use as the surface anchoring group on many discrete QD ligands. Polymeric ligands appended with multiple thiols as pendant groups yield robust hydrophilic QDs [32]. Thiolated ligands, especially the monothiolated variety, are prone to oxidation in air and under light with the presence of QDs potentially contributing to this as a photocatalyst. On the other hand multidentate thiolated ligands are extremely susceptible to disulfide formation and cross-linking, thereby reducing their functionality. Researchers have shown that pyridines demonstrate excellent surface binding capabilities and can be utilized as ligand anchors with QDs in place of thiols. Pyridine is well-known for use in replacing the original hydrophobic ligands on the QD surfaces and has been extensively utilized to fabricate QD-based thin film devices such as solar cells [33]. Also, Pyridine is a good electron donor due to the presence of a pair of isolated nitrogen electrons that are delocalized over its entire aromatic ring [34]. CdSe quantum dots show a good electron affinity, meaning that the nitrogen readily coordinates with them to form a stable complex. In addition Guo et al. [35] showed pyridine coatings of the QDs was essential for obtaining densely packed monolayers of QDs with minimum clumps over large areas of graphene.

Here, we report the synthesis of stable colloidal suspensions of CdSe QDs as well as carbon dots as a viable photoluminescent platform for detection of TB biomarkers. Such novel CdSe/Carbon quantum dots based sensing solution with tunable excitation and emission properties acts as a fluorescent probe for selective detection of TB biomarkers. The development of a smart, tunable fluorescent sensor system can provide an economically feasible solution and pave the way for rapid disease diagnostics. We have presented preliminary results including emission, absorbance, and binding behavior of selected TB VOBs with QDs and CDs.

2. Experiments

2.1. Synthesis of quantum dots

For synthesis of CdSe quantum dots, 15 mg of cadmium oxide was first dissolved in 0.5 ml of oleic acid and 10–12 ml of 1-octadecene. This mixture was gently heated in order to completely dissolve the cadmium oxide and to form cadmium oleate. Stirring was continued until reaching a temperature of ~225 °C. Prior to preparation of cadmium based precursor, synthesis of selenium precursor is essential. For such synthesis 30 mg of selenium powder was mixed with a solution containing 5 ml of 1-octadecene and 0.5 ml of trioctylphosphine. This solution was heated and stirred for dissolution of selenium. The full dissolution of selenium resulted in a transparent solution. This precursor was stored for CdSe dots preparation. The selenium precursor (~1 ml) was injected on a bath containing cadmium oleate precursor that was kept at ~ 225 °C. Note that growth of CdSe QDs started soon after mixing. A small amount of QDs mixture was quickly withdrawn and the process was repeated at predermined intervals. In this way, QDs of different size can be prepared and named as S1, S2, S3, S4, and S5. The size of QDs was as follows: S1 < S2 < S3 < S4 < S5.

2.2. Synthesis of carbon dots

Carbon dots were synthesized using a carbonaceous source. For CDs synthesis, 15 ml of a drink containing sugar, was mixed with an appropriate amount of ethanol and oleic acid. Such mixture was gently heated and monitored for ~1.5 h at a temperature of ~225° C.