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Enhancing detection sensitivity in quantitative protein detection based on dye-doped liquid crystals

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ABSTRACT

A quantitative protein assay and label-free immunodetection based on dye-doped liquid crystal (DDLC) was developed in this study. The black dye incorporated as the dopant in the DDLC mixture features a high dichroic ratio and wide visible-light absorption band so that the level of disturbance in DDLC orientation in the presence of biomolecules can be readily observed with the naked eye. Due to the absorption and anisotropic features of DDLC, transmission spectrometry was exploited to establish a quantitative method for DDLC-based biosensing. Our results indicate that the transmittance of DDLC decreased with increasing concentration of bovine serum albumin (BSA), a common protein standard, and the detection sensitivity can be improved by using linearly polarized light during spectral measurement. A linear correlation between the spectral parameters of DDLC and BSA concentration was thus derived to facilitate protein quantitation. As a demonstration of clinical relevance, a label-free DDLC-based immunoassay for the cancer biomarker CA125 was manifested by reacting CA125 with immobilized anti-CA125 antibody. The transmittance of DDLC decreased with increasing concentration of CA125, but when a nonspecific protein such as BSA was reacted with the anti-CA125 antibody, the transmission spectrum was similar to that in the absence of CA125, suggesting that immunodetection via the DDLC-based biosensing platform was specific. It is evident from this study that with its unique dichroic and optical properties, DDLC is a potential biosensing material with capabilities of both qualitative detection as required in rapid screening as well as quantitative bioassay as in cancer screening.

1. Introduction

A wide variety of liquid-crystal (LC)-based biosensors have been designed and extensively studied since the first biological application of LC was published in 1998 [1]. The basic principle of LC-based biosensing originates from the sensitive response of LC orientation to external stimuli as a result of its optical anisotropy (i.e., birefringence). In a typical LC-based biosensing platform, the order of LC molecules is usually induced by an aligning reagent, such as dimethyloctadecyl [3-(trimethoxysilyl)propyl] ammonium chloride (DMOAP) to orient LC homeotropically (i.e., vertically) at a LC–glass interface [1–5]. The disturbance in the homeotropic alignment of LC due to the presence of biomolecules at the interface is reflected through the change in optical texture, which can be readily observed under a polarizing optical microscope [3–5]. Numerous other LC-based biosensors rely on textural transition at the LC–water interface to detect biomolecules [6–10]. Nevertheless, establishing quantitative measures for a technology that has long been counting on qualitative textural observation is a persisting technical hurdle in LC-based biosensors.

Our previous studies have shown that the birefringence of LC plays a pivotal role in determining the detection limit of biosensing. The alignment of HDN, a nematic LC of larger birefringence than that of 5CB, which is commonly used in most LC-based biosensing studies, is more sensitive to the disturbance caused by biomolecules, thus giving rise to a lower detection limit [11–13]. This led us to investigating the potential of various types of LC phases other than the nematogen 5CB in biosensing and the correlations of the optical anisotropy and electro-optical properties of LC to protein concentration in an attempt to develop quantitative approaches [14–18]. We demonstrated that, in an externally applied electric field, the electric capacitance of the nematic HDN and the electro-optical properties of dye liquid crystal (DLC) can be utilized in protein quantitation [14,15]. Moreover, transmission spectrometry was proved to be a valuable tool for protein quantitation based on LCs with unique absorption in the visible wavelength, such as cholesteric LC, blue-phase LC, and DLC [15–18].

In this study dye-doped LC (DDLC) was applied as the sensing

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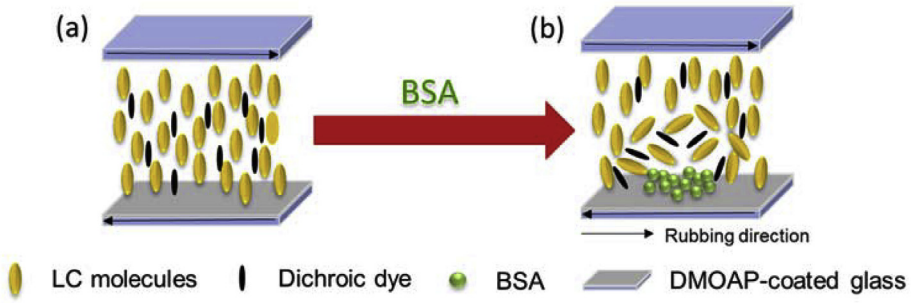

Fig. 1. Schematic of the DDLC-based biosensor. (a) Both the dichroic dye and LC molecules are directed by DMOAP to align vertically in the absence of BSA. (b) When sufficient amount of BSA is immobilized on one of the DMOAP-coated glass substrates, the molecular orientation of both the dye and LC is disturbed and, in turn, deviated from homeotropic alignment.

element in protein assay and immunodetection. DDLC is one of the LC components regularly employed in the LC display (LCD) technology, but its potential in biosensing has not been explored. Compared with DLC, in which the chromophore is covalently conjugated to LC molecules [15], the dye in DDLC was blended into a LC mixture as a dopant whose relative amount to the LC host can be easily adjusted. The black dye incorporated in the DDLC of this study is one of the commercial dyes with the highest dichroic ratio and order parameter, with a wide absorption band within the visible spectrum and a preference for aligning parallel to the long axes of LC molecules. When the initial homeotropic alignment of DDLC was disturbed by biomolecules such as the protein standard bovine serum albumin (BSA) at the LC–glass interface, the orientation as well as optical absorption of the black dye changed accordingly. A correlation between the absorption of DDLC and the amount of immobilized protein can therefore be derived by transmission spectrometric analysis for the purpose of quantitation. To improve the sensitivity of DDLC-based biosensing, the advantage of using polarized incident light in transmission spectrometry was discussed for the first time, to the best of our knowledge, in this study. Finally, the clinical relevance of the DDLC-based biosensor was demonstrated in the label-free immunodetection of the cancer biomarker CA125.

2. Experimental

The surfactant DMOAP as the aligning agent and the standard protein BSA were provided by Sigma–Aldrich. The optical glass substrates NEG AT35 (22.0 mm × 11.0 mm × 1.1 mm) were obtained from Ruilong Glass, Taiwan. The DDLC mixture was prepared by mixing the nematic LC host E7 (Merck, birefringence $\Delta n = 0.2255$ at the wavelength of 589 nm and temperature of 20 °C) [19] uniformly with the display-grade black dichroic dye S428 (Mitsui, Japan) at a concentration of 2 wt%. Recombinant human CA125 (MUC16) and monoclonal mouse anti-human CA125 antibody were manufactured by R&D Systems and Santa Cruz Biotechnology, respectively.

To fabricate the DDLC-based biosensor, clean optical glass substrates were immersed in an aqueous solution containing 1% (v/v) DMOAP and sonicated for 15 min at room temperature, followed by rinsing twice with DI water for 15 min to remove excess DMOAP. The glass slides were then dried under a stream of nitrogen and baked at 85 °C for 15 min. In order to immobilize BSA, 40 μl of an aqueous solution of BSA at a designated concentration was dispensed at the center of a DMOAP-coated glass substrate and allowed to react in an unperturbed environment in an oven at 30 °C for 2 h. After rinsing with DI water to remove excess BSA, the glass substrate was dried at 30 °C in an oven for 10 min. Prior to the assembly of a DDLC cell for biosensing purpose, glass substrates coated with DMOAP and immobilized with BSA, as well as those with DMOAP coatings only, were rubbed twice unidirectionally using a rayon-cloth rubbing machine at a rotation speed of 1500 rpm, a plate speed of 22 mm/s and a pile impression of 0.6 mm. Each BSA-immobilized glass substrate was paired with a DMOAP-coated one with 8-μm rod spacers in between to create the desired cell gap. Finally, DDLC was injected into the cell and distributed through capillary action to create a thin layer of DDLC sandwiched between two glass substrates. The optical texture of the DDLC cell was acquired at room temperature with an Olympus BX51 polarizing optical microscope. All transmission spectra were measured at room temperature by an Ocean Optics HR2000+ fiber-optic spectrometer equipped with an Ocean Optics HL2000 halogen light source. In some measurements, a polarizer was set between the light source and the DDLC cell to produce linearly polarized light and to control its polarization state.

To perform the CA125 immunoassay, 7 μl of an aqueous solution of 0.1 μg/ml anti-CA125 antibody was dispensed on the DMOAP-coated glass substrate and reacted at 30 °C for 2 h to immobilize the antibody. After rinsing with DI water, CA125 was reacted with the immobilized anti-CA125 antibody by dispensing 30 μl of an aqueous solution of CA125 at the designated concentration and covered with a clean cover glass. After reacting for 30 min at room temperature, the cover glass was removed and the glass substrate was rinsed with DI water to remove unbound CA125.

3. Results and discussion

Protein detection and quantitation in DDLC-based biosensing rely on the level of disturbance in the homeotropic alignment of both the dye and LC molecules caused by various concentrations of immobilized BSA (Fig. 1). In the absence of BSA, the long axes of the dye and LC molecules were oriented perpendicularly to the plane of the glass substrate due to the vertical anchoring effect induced by DMOAP, as shown in Fig. 1(a). In contrast, the alignment of both the dye and LC molecules was disturbed when sufficient BSA was immobilized on the DMOAP-coated substrate, suggesting that the anchoring effect of the aligning reagent was blocked or weakened by BSA (Fig. 1(b)).

The change in molecular orientation of the birefringent LC molecules affects their interaction with light, which in turn alters the optical texture of DDLC when observed under a polarizing optical microscope. At a BSA concentration of 10^{-7} g/ml or lower, the optical texture of DDLC was completely dark, similar to that in the absence of biomolecules (Fig. 2). This implies that DDLC molecules remained homeotropically aligned and the amount of BSA was insufficient to disturb DDLC alignment. As BSA concentration increased, the optical texture of DDLC went through a transition from dark to bright, which is indicative of the gradual deviation of DDLC alignment from the homeotropic anchoring directed by DMOAP.

The high contrast ratio of the black dye in the anisotropic DDLC also enables the change in DDLC orientation with BSA concentration to be observed with the naked eye. As shown in Fig. 3, the grey level of the circled area in the DDLC cell, where BSA was immobilized, was enhanced with increasing concentrations of BSA, while the grey level remained unchanged in the area outside the circled area without BSA immobilization. Biosensing based on DDLC therefore offers the advantage of colorimetric detection, which can be of use in developing rapid screening tests that require fast response and simplicity for qualitative analysis. The amount of protein present can be estimated simply by comparing the grey level of a DDLC cell with those of a series of "standard" DDLC cells containing known concentrations of BSA.