A Liquid Crystal Biosensor for Liver Diseases

Sihui He[1], Jiyu Fang[2], and Shin-Tson Wu[1]

[1]CREOL, The College of Optics and Photonics, [2]Department of Materials Science and Engineering, University of Central Florida, Orlando, Florida 32816, USA

Abstract

The urinary concentration level of bile acids is a useful indicator for the diagnosis of liver diseases. Here we present a sensor platform based on the anchoring transition of nematic liquid crystals (LCs) at the surfactant-laden LC/aqueous interfaces for the detection of bile acids in urinary solution.

Keywords

Liquid crystal biosensor; Bile acids; Urinary test

1. Introduction

Bile acids are derivatives of cholesterol, which are synthesized in hepatocytes and then secreted into intestines. They are essential for the digestion and absorption of fat and fat-soluble vitamins through emulsification [1]. The concentration of bile acids is related to hepatic and intestinal functions [2, 3]. It has been shown that the normal concentration of urinary bile acid is less than 7 μM. In the case of liver diseases, the urinary bile acid concentration will increase to 10-100 μM [4]. Therefore, the concentration level of bile acids has long been used as a sensitive indicator for the early development of hepatic and intestinal diseases [5]. Chromatography-mass spectrometry methods are widely used for the detection of bile acids [6-8]. While the precision and selectivity of these methods are high, they require complex techniques and expensive equipment. For clinical applications, the most commonly used method for the detection of bile acids is the enzymatic colorimetry [9, 10]. These detection methods are mainly conducted in the blood. Another downside is that they have to use expensive enzymes and enzymatic reactions. The detection of bile acid concentration in urine has many advantages compared to the detection in blood. It does not require fasting, and it provides a time-average concentration. There's no need for venipuncture that many people have a strong aversion to. It is also less costly to perform. So the development of urinary bile acid test is highly desired. Our method features at its simplicity and low cost for self-test as a screen of liver diseases.

Liquid crystals (LCs) are anisotropic fluids, which have long-range orientational order. The orientation of LCs is extraordinarily sensitive to the change of the surface which they are in contact with. The surface-induced local order can be amplified over several tens of micrometers in LC phases due to the long-range orientational inherent of LCs. Thus, the optical amplification of LCs makes them a unique optical probe for sensing applications. Recently, LC based biosensors have been developed for the detection of bacterial, virus [11], enzymatic reactions [12], DNA hybridization [13], peptide–lipid membrane interactions [14], charged micromolecules [15, 16] and bile acids [17, 18].

In a previous publication [18, 19], we showed that the detection limit for cholic acid (one type of bile acids) depends on the nature of surfactants used for forming the surfactant-laden LC/aqueous interface. The influence of the LC structures including chain length, polar group, and core structure on the detection limit for cholic acid has been studied. By engineering the structure of LC, we can tune the detection limit from 1.5 μM to 96 μM. Higher detection limit can also be realized by using LC with alkyl groups at both end of the core structure and further increasing the chain length of the alkyl groups. In this paper, we demonstrate the detection of bile acids in synthetic urine and human urine. We also propose a device structure for this LC biosensor.

2. Results

The LC thin film-based biosensor is based on the anchoring transition of the LC at the sodium dodecyl sulfate (SDS)-laden LC/aqueous interface triggered by the interaction between SDS and bile acids. The SDS-laden LC/aqueous interface induces homeotropic anchoring of the LC. With the addition of bile acids, the competitive adsorption of bile acids at the SDS-laden LC/aqueous interface replaces the SDS from the interface, triggering the homeotropic-to-planar anchoring transition of the LC (Fig.1). The anchoring transition provides a simple optical sign for the rapid detection of bile acids.

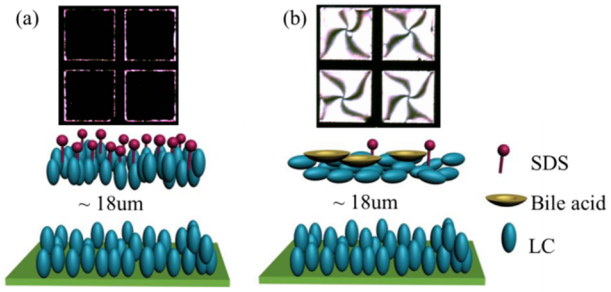

Fig. 1. Schematic illustrations of the optical appearance (top) and anchoring transition (bottom) of the LC at a SDS-laden LC/aqueous interface before (a) and after (b) being exposed to bile acids.

The major components in urine are urea, creatinine, and uric acid (Fig.2). They are potential interfering species for the detection of bile acids in urine. To understand the effect of these species on the detection of bile acids, we placed LC based biosensors into synthetic urine which contains urea, creatinine, and uric acid. The LC used in the biosensors is a mixture of 18.93 wt % of 5PCH and 81.07 wt % of 5CB. We have shown in our recent publications that the mixed LC-based biosensor gives a lower detection limit for screening bile acids in PBS solution [19]. We find that the LC based biosensors show no response to these potential species. We further test the sensitivity of the LC biosensor for bile acids in synthetic urine with and without creatinine and uric acid. The detection limit for bile acids is found to be unaffected by the presence of creatinine and uric acid.

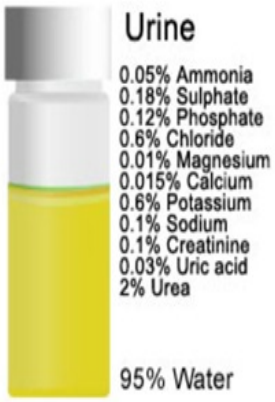

Fig. 2. Components of urine. (From http://nursingcrib.com/wp-content/uploads/urine.jpg)

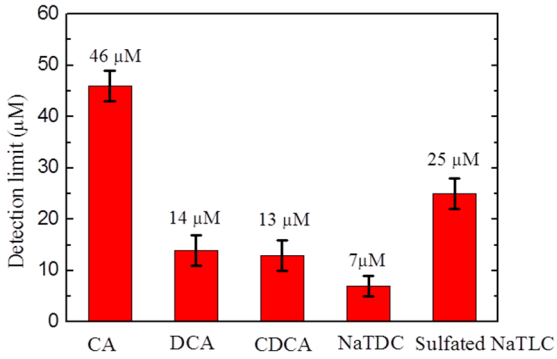

Fig. 3. Detection limit of the mixed LC-based biosensors for different bile acids in synthetic urine.

Fig. 3 shows the detection limit of the LC-based biosensors for different bile acids in synthetic urine. The detection limit here is determined by the transition of the optical appearance of LC biosensor in 2 hours. It varies for different bile acids. CA has three hydroxyl groups, while DCA and CDCA have two hydroxyl groups. CA is more hydrophilic than DCA and CDCA. This makes it less likely to penetrate into the SDS-laden LC surface, hence the detection limit increases. NaTDC is conjugated with taurine group. The taurine conjugation helps it stick out further into the aqueous phase once it adsorbs onto the SDS-laden LC surface, thus adopts flatter conformation [20]. This makes it more efficient in disrupting the SDS layer and consequently reduces the detection limit. For sulfated NaTLC which replaces the hydroxyl group at C-3 position by sulfate group, the hydrophobicity is greatly reduced, hence the detection limit increases. The detection limit of the LC-based biosensor for all bile acids we studied here is in the range of 10 μM - 100μM. Therefore, it is suitable for this LC biosensor to screen the possibility of liver diseases.

We find that the detection limit of the LC-based biosensors can be further tuned by the concentration of SDS laden at the LC-aqueous interface. Fig. 4 shows the detection limit of the LC-based biosensors fabricated with varied SDS concentrations. It linearly decreases with decreasing SDS concentrations. By reducing the SDS concentration to 10 μM, the detection limit for NaTLC can be reduced to 1μM.

Furthermore, we detect bile acids in human urine with the LC-based biosensors. Human urine was from a graduate student and then filtered with 0.22 μm pore size filters to remove some cells and large proteins. Compared to synthetic urine, human urine has more interfering species such as urobilinogen, phenol, and ascorbic acid. The concentration of urobilinogen in human urine was tested using a urine test strip and found to be 3.5 μM, which is within the normal range (< 17μM) [21]. The normal concentration of phenol is 100 ± 38.3 μM [22]. To test the interference of these species, we conduct the tests in synthetic urine with urobilinogen, phenol, and ascorbic acid, respectively. We find that the LC based biosensor is insensitive to 100 μM phenol and 240 μM ascorbic acid, respectively. However, the LC biosensor does response to 3.5 μM urobilinogen. In order to eliminate the influence of urobilinogen, we fabricated the LC-based biosensor by increasing the concentration of SDS to 230 μM. In this case, there is no anchoring transition of the LC at the SDS-laden LC-aqueous interface observed. When sulfated NaTLC with a concentration of 7 μM (in normal range) is added into human urine, the LC-based biosensor shows no response (dark) (Fig.5a). However, when the concentration of sulfated NaTLC in human urine is increased to 10 μM (in disease range), the LC-based biosensor responses by change its optical appearance (Fig. 5b). The responsive time is about 30 min. Our results clearly show the possibility of using the LC-based biosensors for screening liver disease by testing bile acids in urine of individuals.

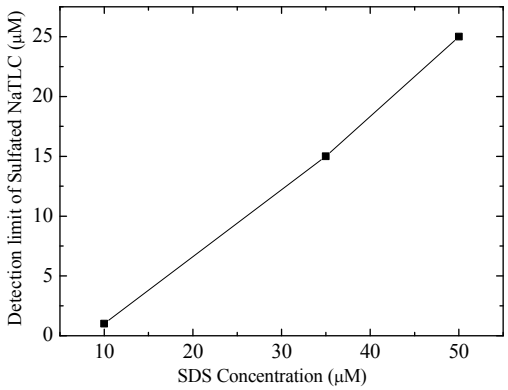

Fig. 4. Detection limit of the mixed LC-based biosensors for sulfated NaTLC as a function of SDS concentrations.

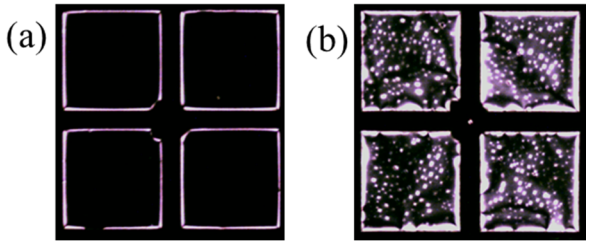

Fig. 5. Polarizing optical microscopy images of LC biosensors in human urine after addition of (a) 7 μM sulfated NaTLC and (b) 10 μM sulfated NaTLC for 30 min.

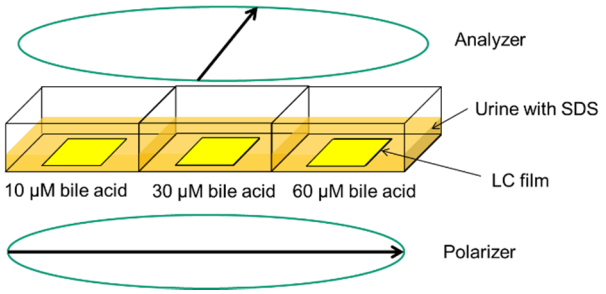

Fig. 6. Product design concept of LC biosensor to detect liver diseases.

Table 1. Possible liver diseases corresponding to different urinary bile acid concentrations. CIH is chronic inactive hepatitis, CAH is chronic active hepatitis, Comp. LC is compensated liver cirrhosis, Decomp. LC is decompensated liver cirrhosis, HCC is Hepatocellular carcinoma, AH is acute hepatitis, EHC is extrahepatic cholestasis, and IHC is intrahepatic cholestasis

UBA (μmol/L)	10-30	30-60	60- 100	100–230	>230
Most Possible Diseases	*CIH, CAH, Comp. LC, HCC*	*CAH, Comp. LC, Decomp. LC, HCC*	*Comp. LC, Decomp. LC, HCC, AH*	*Decomp. LC, AH, EHC, IHC*	*AH, EHC, IHC*

To make LC-based biosensors user friendly, we designed multi pixel LC biosensors as shown in Fig. 6. The pixel is made of a LC film with the LC-aqueous interface laden by SDS at different concentrations. A Polarizer and an analyzer are laminated on the bottom and top of the multipixel sensors, respectively. Thus, user just needs to fill each pixel with human urine for multi-tests by simply observing the color change of the LC film in each pixel with an optical microscope. This will allow user to detect the range of urinary bile acid concentration in human urine.

It has been shown that the range of urinary bile acid concentrations corresponds to different types of liver diseases (Table 1) [23]. The multipixel LC biosensors can provide user more information about the liver condition of individuals.

3. Conclusion

We have developed LC-based biosensors by molecular design to detect bile acids (biomarkers for liver diseases) in urine. The detection limit of the LC-based biosensors can be tuned by altering the nature of LCs and the nature and concentration of surfactants we used to modify the LC-aqueous interface. The LC-based biosensors can be used to selectively detect bile acids in human urine in the presence of a number of potential interfering species, which highlights their possibility for screening liver disease of individuals by testing bile acid concentrations in their urine. The LC-based biosensors are simple, rapid, and low cost without needing expensive and complex detection systems for signal transductions.

4. Acknowledgments

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5. References

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