A Liquid Crystal Biosensor for Liver Diseases

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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.

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.
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.
To make LC-based biosensors user friendly, we designed multipixel 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.

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5. References


