A biosensor of high-density lipoprotein of human serum on a liquid crystal and polymer composite film

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ABSTRACT

A biosensor for the concentration of high-density lipoprotein (HDL) in human serum on a liquid crystal and polymer composite film (LCPCF) is demonstrated. The sensing mechanism is based on a polar-polar interaction between orientation of LC directors and HDL in human serum. The concentration of polar HDL in human serum affects the orientations of LC directors at the interface between LCPCF and the human serum. In addition, the surface free energy of LCPCF changes with the applied voltage due to the electrically tunable orientations of LC directors anchored among the polymer grains of LCPCF. As a result, the droplet motion of human serum on LCPCF under applied voltages can sense the concentration of HDL in human serum.

Keywords: Liquid-crystal devices; liquid crystals, biosensor, high-density lipoprotein; human serum.

1. INTRODUCTION

High-density lipoprotein (HDL) is one of the major carriers of cholesterol in the human blood. In general medical examination, the measured concentration of high-density lipoprotein cholesterol (HDL-C), which is the cholesterol easters inside HDL and also proportional to a HDL concentration, are used to indicate the risk of cardiovascular diseases. Many literatures are also reported that HDL-C concentrations in human serum are related to breast and lung cancers, non-Hodgkin's lymphoma, and reducing the risk of Alzheimer's disease and dementia.[1] Many methods are proposed and demonstrated to measure HDL-C or HDL concentrations. [2] The developed methods are usually expensive and not easy to be used at one's home. Recently, we have developed an electrically switchable surface, liquid crystal and polymer composite film (LCPCF), and also developed many applications based on LCPCF, such as electrically tunable

Liquid Crystals XVII, edited by Iam Choon Khoo, Proc. of SPIE Vol. 8828, 88280I

focusing lenses, polarizer-free electro-optical switches, and sperm testing devices.[3-10] The switchable surface free energy or switchable surface tension of LCPCF mainly results from orientations of liquid crystal molecules anchored among the polymer grains under applied pulsed voltages.[11] In this paper, we demonstrate a biosensor for high-density lipoproteins of human serum on LCPCF. The sensing mechanism is based on the balance of surface free energy between HDL and anisotropic LC molecules resulting from the polar-polar interaction between HDL and anisotropic LC molecules. The HDL samples with controlled parameters of human serum show the collapse distance of the sample drops increases with the HDL concentration. This study can help us to develop biosensors using switchable surfaces for sensing polar biosamples, such as HDL of human serum.

2. STRUCTURE AND SAMPLE PREPARATION

To fabricate LCPCF on the indium tin oxide (ITO) glass substrate, we mixed a nematic LC mixture E7 (Merck) and a liquid crystalline monomer (4-(3-Acryloyloxypropyloxy)-benzoic acid 2- methyl-1, 4-phenylene ester) at 70:30 wt % ratios and then filled the mixture into an empty cell with a gap of ~7 μm which consists of a glass top substrate and a patterned ITO bottom glass substrate. The top substrate of the cell was overcoated with a thin polyimide (PI) layer and then rubbed along x-axis as shown in Fig. 1(b). After filling, the cell was exposed to a UV light with intensity I = 10 mW/cm2 for ~60 min at 70 ^{0}C. After the phase separation and photo-polymerization, the top glass substrate was peeled off by a thermal-releasing process. A solidified LCPCF was then obtained with 7 μm thickness and 15 nm root-mean-squared roughnesses. As to the electrodes, the ITO electrodes on the glass substrate were etched with interdigitated chevron patterns, shown as the zigzag electrodes in the Fig. 1(b). The zigzag ITO strips have corner angles of 150o. The width and gap of the electrode strips are 4 μm and 14 μm, respectively. In order to manipulate a HDL drop on LCPCF, the two regions of the interdigitated chevron electrodes were patterned identically, as shown in Fig. 1(b). The distance between two regions is 20 μm and the width of each region is 2 mm.

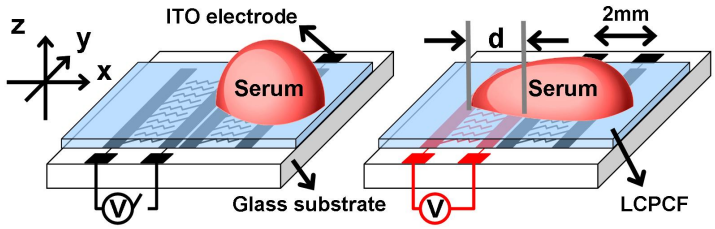

Figure 1 : Left: The serum drop is placed on LCPCF without voltage. Right: The voltage is applied at the left region of the electrodes. The serum drop moves toward left and the collapse distance is d.

3. EXPERIMENTAL RESULTS AND DISCUSSION

After the human bloods were collected from the human beings in Chimei Medical Center, the anticoagulant was added into the tubes with human blood. The sample tubes were placed for 30 min and then were centrifuged at 2000 rpm by a centrifuge (Table Top Centrifuge 4000, Kubota Corporation) for 10 minutes. After the red blood cells were deposited on the bottom of the tube, we removed the liquid or so-called blood serum above deposition of the red blood cells to other clean tubes. The blood serum or so-called human serum in the tube was the serum sample for testing. Such human serum consists of water, proteins and lipoproteins. The lipoproteins also consists of many particles, such as chylomicron (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein(HDL). The parameters related to the serum were measured by an automatic biochemical analyzer (Hitachi automatic analyzer 7600-110), such as total protein, albumin, total cholesterol, triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), the ratio of albumin to globin (A/G). The concentration of HDL is proportional to the concentration of high density lipoprotein-cholesterol (HDL-C). From collecting samples from many people, we specially selected samples of human serums with different HDL-C ranging from 18 mg/dL to 92 mg/dL, and other parameters are controlled in the similar range of concentrations: LDL-C ranging from 103mg/dL to 153mg/dL, triglyceride (TG) ranging from 102mg/dL to 165mg/dL, total protein (i.e. Albumin + Globulin) ranging from 6.1 mg/dL to 7.6 mg/dL, albumin ranging from 3.7 mg/dL to 4.9 mg/dL, and A/G (i.e. the ratio of albumin to globin) ranging from 1 to1.9.

A HDL drop of 3 μl was dropped on LCPCF and then applied 200 V_{rms} square pulses (f = 1 kHz) to the left electrodes shown in right figure in Fig. 1(b) for a time duration of 500 ms. The region of the left electrode is more hydrophilic under inhomogeneous distribution of LC reorientations induced by fringing electric fields. We then recorded the motion of the HDL drop on LCPCF by a CCD camera (JAI CV-M30) with a frame rate of 360 frames/sec and measured the contact angles of the HDL drops by a contact angle measurement (FTA 1000 Analyzer System). When we recorded the dynamics of the HDL drop, the position difference of the left of the HDL drops at voltage-on and at voltage-off was defined as the collapse distance.

In order to validate the relation between the contact angle and the HDL-C concentration, and the relation between the collapse distance and the HDL-C concentration, we prepared more samples with different HDL-C concentrations. Then we measured the contact angles at V=0 and at V= 200 V_{rms}, and also measured the collapse distance, as shown in Fig. 2. In Fig. 2, the contact angle decreases from 76.2 degree to 73.8 degree at V=0 and the contact angle decreases from 63.2 degree to 43.3 degree at 200 V_{rms}. We also plotted the collapse distance as a function of HDL-C concentration, as shown in Fig. 3. The collapse distance also increases from 0.37 mm to 0.98 mm as the HDL concentration increases. As we can see that larger the contact angle difference, longer the collapse distance. Moreover, contact angle difference and the collapse distance increase as the HDL-C concentration increases. According to the HDL-C guideline suggested by the American Heart Association and National Institutes of Health (NIH), people suffer from high risk of heart dieases

when the HDL-C is lower than 40 mg/dL. That means the collapse distance is less than 0.4 mm. People suffer from medium risk of heart dieases when the HDL-C is between 40 mg/dL and 60 mg/dL. This indicates the collapse distance is around 0.5-0.8 mm. In addition, people are in optimal condition considered protective against heart diseases when the HDL-C is larger than 60 mg/dL or the collapse distance is larger than 0.8 mm. We can define the sensitivity as the ratio of collapse distance to HDL-C concentration. The sensitivity is ~8.6×10^{-3} mm×dL/mg. In order to improve the sensitivity of LCPCF, LC materials with high polarity and high dielectric anisotropy should be adopted. The electrode patterns can be improved. The surface morphologies of LCPCF can be adjusted in order to lower the hysteresis of LCPCF(i.e. the difference between advancing angle and receding angle).

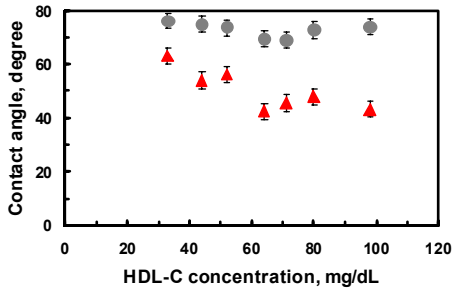

Figure 2: The contact angle as a function of the HDL-C concentration at V=0 (grey dots), and at V=200 Vrms (red triangles).

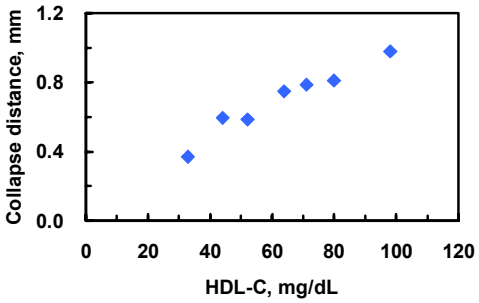

Figure 3: The collapse distance as a function of the HDL-C concentration.

The wetting properties of the surface on LCPCF we developed are switchable due to the electrically tunable orientations of LC molecules anchored among the polymer grains on LCPCF. We have reported the physical mechanism of tunable wettability of LCPCF in previous literatures.[11] The LC molecules of LCPCF consist of three part: cyano(CN)-group (terminal group), alkyl-group (terminal group), and biphenyl/terphenyl part (core part). The surface free energy or surface tenstion of LCPCF is switched from 36×10^{-3} J/m^2 to 51×10^{-3} J/m^2 when the CN-group of LC molecules tilts up 32 degree with applied pulsed voltages. When a HDL drop is placed on LCPCF at a null voltage (i.e.V=0), LC molecules are aligned along x-direction. The biphenyl/terphenyl of LC molecules contacts with the

HDL/LC interface and LC molecules have a relative weak interaction with HDL compared to the case of the titling CN-group. When the applied voltage V exceeds threshold voltage (V_{th}), the CN-group (or one of the terminal groups) of LC molecules tilt up toward the HDL because of two reasons. The first one is LC molecules are reoriented by the electric fields. The applied electric field helps to overcome the anchoring force from the boundaries of the polymer grains and the elastic force from LC materials. The second one is HDL is a polar fluid. As a result, the polar-polar interaction between HDL and LC molecules results in CN group of LC molecules toward the interface in order to balance of surface free energy at the interface. In addition, the polarity of HDL concentration increases as the HDL concentration increases. According to the interaction between the orientations of LC molecules and HDL, the biosensor is designed based on HDL droplet manipulation on LCPCF, as illustrated in Fig. 2. A HDL drop is placed on LCPCF and the two regions of patterned ITO elecrodes on a glass substrate beneath the LCPCF. At V=0, HDL drop is placed on the right region of electrodes. The HDL drop collapses to the left as we apply pulsed voltage on the left region of electrodes. This is because the left region of LCPCF is more hydrophilic to HDL due to the tilting CN-groups of LC molecules in the left region of LCPCFand the biphenyl/terphenyl of LC molecules in the right region of LCPCF. As a result, the HDL drop experience a net Young's force to move the HDL drop. In addition, the viscosity of HDL drop is high and then the HDL drop collapses instead of doing the translation motion. When HDL concentration is higher, the polar-polar interaction at HDL/LC interface is larger and then the region applied electric fields on LCPCF is more hydrophilic. Thus, the collapse distance d is larger as HDL concentration is higher. By droplet manipulation on LCPCF, a biosensor for HDL can be realized. Actually, LCPCF senses HDL concentration, instead of HDL-C concentration. In the experiments, we compared the paramenters provided by the Chimei Medical Center who meaused HDL-C instead of HDL.

4. CONCLUSION

We demonstrate a biosensor for HDL-C adopted a droplet manipulation on LCPCF. The sensing mechansim mainly based on the polar-polar interaction between HDL and anisotropic LC molecules. The HDL samples and the human serum samples with controlled parameters show the collapse distance of the drops is related to the HDL-C concentration. The proposed method is not only useful to sense HDL, but also can apply to other biosamples as long as the bisamples exhibit the polarity and the polarity increases with the concentration without changing the surface tensions. For practical applications, we can design pixelated electrodes for LCPCF in order to guide the human blood drop and anticoagulant on LCPCF and then combine them together. After the acceleration of the combined drop on LCPCF, the blood cell and human serum are separated on LCPCF. Then the human serum is guided to the sensing area for testing the collapse distance. This study opens a window to develop a portable devices sense HDL using droplet manipulation on an electrically switchable surface.

The authors are indebted to Mr. Kuei-Hung Chuang (NCTU) for technical assistance, and Chimei Medical Center for providing human serum samples. This research was supported partially by Chimei Medical Center and partially by the

National Science Council (NSC) in Taiwan under the contract no. NSC 101-2112-M-009 -011 -MY3.

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