Liquid Crystal Interactions with Nanostructure and Applications to Diagnostics and Medical Research

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ABSTRACT

Liquid crystals are rigid organic molecules that exhibit orientational behavior on surfaces. The orientation of liquid crystals can be controlled by the design of surfaces with regular nanoscale features and/or defined chemistries. The orientation of liquid crystals on such surfaces can be exploited to sensitively report the binding of proteins to the surface [1-6]. Liquid crystal based detection of proteins can be the basis for a new generation of diagnostics and research tools. The use of liquid crystals to detect proteins enables the development of label free, rapid, sensitive and multiplexed assays.

Keywords: liquid crystals, nanotechnology,antibodies, diagnostics

INTRODUCTION

The control of the orientational behavior of liquid crystal molecules is the basis for their use in the display of information on surfaces, such as in laptop computer monitors. In the past decade, however, a body of knowledge has accumulated that describes the interactions of liquid crystals with proteins [7-9] viruses [10] and cells [11]. Understanding of these interactions now permits the design of new research tools for protein detection and new diagnostics.

Liquid crystals assume a preferred orientation on a surface within seconds. Changes in the orientation of a film of liquid crystals results in changes in the optical properties of that film and thus in changes of the appearance of the surface of that film when viewed through cross polars.
Previous studies have shown that the nanometer scale of the surface topography influences the response of liquid crystals to proteins bound on a surface [6]. In addition, studies have shown that the optical properties of liquid crystals on surfaces can provide quantitative information on proteins bound to those surfaces [1,7].

A variety of surfaces, such as glass, polymers and gold films can be fabricated or chemically functionalized to align liquid crystals [12-16]. New methods for detection of proteins that are label free, inexpensive, and adaptable to robotic processing and high throughput screening are in demand. We present data to demonstrate the use of liquid crystals to detect and report the presence of specific target proteins, such as antibodies.

Affinity contact printing has been used to selectively capture target proteins from solution and print them in a patterned fashion on a surface [17] Recently, Tingey et al.,[8] reported the use of liquid crystals to image affinity microcontact printed proteins using liquid crystals. We describe the use of liquid crystals to report and quantify the presence of antibodies deposited on surfaces using affinity contact printing.

Common methods for antibody detection include enzyme linked immunoassays (ELISA), Western blots, and serum neutralizations. Microbead-based methods are under development and a variety of agar gel diffusion and hemagglutination based tests are commonly used for disease diagnosis. Liquid crystal based detection of antibodies offers significant advantages over existing methods for detection of antibodies. In a liquid crystal based diagnostic assay, the liquid crystals perform three critical steps: detection, amplification and signal transduction. The use of a single reagent to perform all three steps eliminates the need for secondary antibodies, labels, and amplification systems.

The sensitivity of liquid crystal to proteins bound to surfaces, is not dependent on amino acid sequence. In the case of antibodies, this means that antibodies of any isotype or from any species can be detected with equal sensitivity and without the need for species or isotype specific secondary antibodies. This is a major advance in diagnostic technology suitable for monitoring outbreaks of zoonotic diseases such as West Nile virus or avian influenza.

Materials and Methods

To capture antibodies from serum samples, polydimethylsufoxide (PDMS) was prepared using a Sylgard 184 elastomeric kit (Dow Corning), and was placed into an O_2 plasma to create silanol groups at the PDMS surface. The PDMS was functionalized with a recombinant envelope (E) protein (L2 Diagnostics, New Haven CT) of the West Nile virus. A monolayer of aminopropyltriethoxylsilane (APES) was formed on the PDMS and a crosslinker of disuccinimidyl substrate was used to covalently bind the E protein to the surface. To capture specific antibodies from serum, the E protein functionalized PDMS was incubated with serum from an immunized rabbit (L2 Diagnostics, New Haven CT). After an incubation period, the surface was washed with phosphate buffered saline and dried with a stream of nitrogen.

To detect captured antibodies, the PDMS surface was brought into brief contact with a printing surface to permit transfer of the antibodies from the PDMS. To prepare the printing surface, glass microscope slides were cleaned by exposure to an oxygen plasma and then were coated with a 10 nm thick layer of gold deposited by electron beam evaporation over a 1 nm thick layer of titanium. Amine self assembled monolayers (SAMs) were formed on the surfaces of the gold coated slides by immersion of the slides in aminothiophenol (ATP).

To form an optical cell for detection of the antibodies, a top surface was applied to the printing surface. The two surfaces were separated by 25 μm thick mylar strips. A glass slide was coated with Tridecafluoro-1,1,2,2-tetrahydrooctyl)triethoxysilane (OTS) to form the upper surface of the cell. The liquid crystal 4-cyano-4'pentylbiphenyl (5CB),obtained from EM Industries, Hawthorne NY, was introduced into the optical cell by capillary action. To detect the presence of antibody by a change in the orientation of the liquid crystals, the optical cell was viewed under a polarizing light microscope. Digital images were captured, converted to an 8 bit grey scale and analyzed using the Image/J NIH freeware. The amount of light passing through the liquid crystal film as imaged between cross polars was determined.

Results

We confirmed that the recombinant E protein was successfully immobilized on the surface by the chosen immobilization chemistry. We performed the immobilization on a silicon wafer and measured the increase in thicknesses of the surface following each immobilization step. We observed the large increase in thickness, approximately ten fold, following the incubation of the functionalized surface with rabbit antiserum to protein E. In contrast, incubation of the surface with a control rabbit sera resulted in a small increase in thickness, indicating little binding of non-specific serum proteins to the E protein.

The PDMS surfaces functionalized with E protein were exposed to rabbit antisera, rinsed, dried and brought into contact with a printing surface that presented amine groups. The antibodies transfer to the printing surface, leaving the E protein covalently bound to the PDMS. The printing surface is designed to orient a film of liquid crystals in a homeotropic fashion (that is perpendicular to the plane of the surface). When thus oriented, the plane of polarized light is retained as it passes through the optical cell and the surface of the cell appears dark when viewed through cross polars. In contrast, proteins deposited on the printing surface prevent the ordering of the liquid crystal film. The plane of polarization is disrupted in regions of bound protein and the light that emerges from the cross polarizing filters is proportional to the amount of protein present on the surface. Figure 1 illustrates the use of affinity contact printing coupled with liquid crystal detection to detect antibodies in rabbit serum to the E protein.

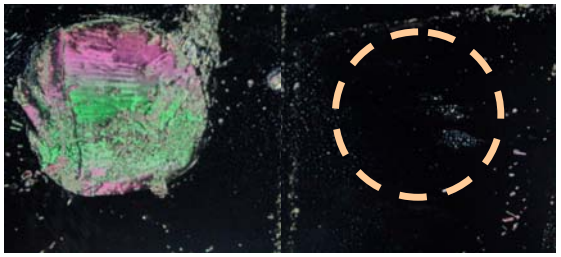

Figure 1. Photomicrographs of optical cells between crossed polars reporting capture of WNV antibody present in rabbit serum (1:5 dilution). The bright appearance on the left is caused by the presence of WNV antibody on the functionalized gold surface and a planar alignment of liquid crystal. The dark appearance on the right is due to homeotropic (perpendicular to surface) alignment on the amine-terminated monolayers in the absence of WNV antibody in the negative serum. The dashed lines on the right represent the area where the E protein functionalized stamp was applied after incubation in rabbit serum negative for anti-WNV antibody.

To investigate the reproducibility of this method for antibody detection, we assayed a single positive rabbit sera and a single negative rabbit sera 10 times each. The ten replicates of the positive sample were all positive

(appeared bright) by visual appearance and the ten negative replicates were all negative (appeared dark) by visual appearance. The average light intensity for all positive and all negative replicate assays are presented in Figure 2. The data indicate good reproducibility and a clear distinction between positive and negative samples. Using this approach to the detection of antibodies, we have shown that the response of liquid crystals is proportional to the concentration of antibodies in the rabbit serum.

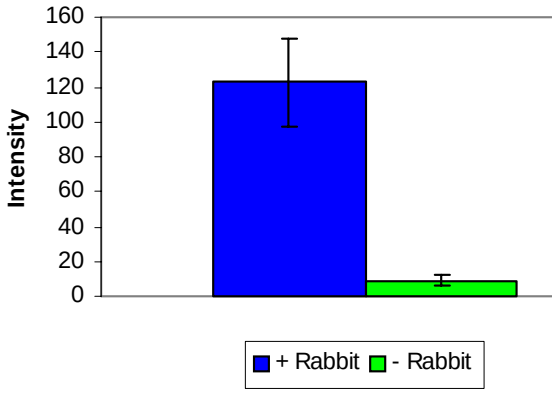

Figure 2. Reproducibility of liquid crystal detection of antibodies. Ten independent assays were conducted on a single WNV antibody positive or WNV antibody negative rabbit sera (1:5 dilutions). Standard deviations are shown by the corresponding error bars.

Studies are ongoing to translate liquid crystal based antibody detection into a technology that will advance serodiagnostic testing. Our goal is to develop a rapid, inexpensive laboratory assay for detection of specific antibodies that will be readily adaptable to robotic systems and that will permit a high degree of multiplexed testing.

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