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Label-free detection of viruses on a polymeric surface using liquid crystals

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ABSTRACT

In this study, we demonstrated a label-free detection of viruses using liquid crystals (LCs) on a polymeric surface with periodic nanostructures. The polymeric nanostructures, which hold sinusoidal anisotropic patterns, were created by a sequential process of poly-(dimethylsiloxane) buckling and replication of the patterns on a poly-(urethane acrylate) surface containing a film of gold. After immobilization of human cytomegalovirus- and adenovirus-antibodies onto the polymeric surface treated with a mixed self-assembled monolayer, a uniform appearance reflecting the uniform orientation of 4-cyano-4′-pentylbiphenyl (5CB) was observed. Conversely, binding of viruses to their antibody decorated surface induced a random appearance of 5CB from the random orientation of 5CB. The uniform to random orientational transition of 5CB indicates that the anisotropic topography of the polymeric surface was masked after specific binding of viruses to the antibody decorated surface. We evaluated the specificity of the binding events by confirming topographical changes and optical thickness using atomic force microscopy and ellipsometry, respectively. These results demonstrate that polymeric surfaces with continuous anisotropic patterns can be used to amplify the presence of nanoscopic virions into an optical response of LC, as well as expand the scope of LC-based biological detection on polymeric solid surfaces.

1. Introduction

For the last few decades, thermotropic liquid crystalline materials (e.g., 4-cyano-4′-pentylbiphenyl (5CB)) have been coupled with chemical and biological detections due to their high sensitivity to molecular level interactions on nanostructured solid surfaces [1–3]. The orientational properties of liquid crystals (LCs), which have a long-range ordering nature [4], have facilitated the transduction and amplification of molecular interactions into optical changes visible under a polarized microscope through the use of simple instrumentation [5–7]. Previously, various strategies such as oblique deposition of gold [8], micro-contact printing [9], mechanical shearing [10], enzymatic metal deposition [11], and introduction of DNA-protein complexes [12] were exploited to construct original technologies for LC-based biological detections on solid surfaces. However, the applications of these individual systems have been confirmed on a case-by-case basis based on biological phenomena that are not integrated into a single system. Although large-scale biological detections have been carried out on obliquely deposited gold surfaces based on topography masking strategy [13,14], the limitations of small-scale anisotropic topography of the surface (amplitude, <3 nm) [15] should be overcome because a high areal density of primarily immobilized biomolecules, not a target molecule, is essential for enhancing detection sensitivity, but can bring unwanted and premature topography masking at the same time.

Therefore, our group recently established a generalized method for detection of a variety of biological events that occur on a solid surface using the optical response of 5CB. By developing a novel method that enables quantitative control of anisotropic surface topography [16], we established an integrated LC-based detection system on a solid surface. For example, DNA hybridization [17] and protein–protein binding events [18] were detected based on distinct changes in the optical response (from uniform to random) of 5CB on the polymeric substrates. The change in optical response was caused by the orientational transition of 5CB that originated from the specific binding between biomolecules that mask the anisotropic nanostructures of the polymeric substrate.

Here, we report a specific detection of viruses using 5CB based on the detection mechanism on a polymeric solid substrate. Several previous studies have exploited the ordering transition of 5CB upon contact of viruses with a solid surface. Tercero Espinoza et al. observed planar to homeotropic anchoring of 5CB on positively charged poly-L-lysine (PLL) treated gold surfaces incubated with vesicular stomatitis virus solutions [19]. Similarly, Jang et al. investigated the orientational behavior of 5CB on a PLL

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treated gold surface presenting electrostatically bound viral particles that possess different enveloped structures (enveloped- and non-enveloped viruses) [20]. Although these studies exploited the orientational behavior of 5CB on the virus decorated surface, the lack of specificity was not resolved for substantive specific virus detection.

In this study, we demonstrated a LC-based label-free specific detection of viruses on polymeric substrate containing periodic nanostructures. This study focused on the detection of human cytomegalovirus (HCMV) and adenovirus (AdV) because they are important contributors to severe disease outbreaks in immuno-compromised individuals, such as cancer patients who receive anticancer treatment, AIDS patients, organ or bone-marrow transplantation recipients, newborn infants, etc. [21,22]. Uniform orientation of LCs was observed after immobilization of the HCMV and AdV antibodies onto gold-deposited polymeric surfaces treated with a mixed self-assembled monolayer (SAM). However, specific binding of HCMV and AdV to their antibodies induced random orientation of LCs attributed to the masking of the surface topography of the polymeric surface (Fig. 1). The results of this study suggest that a LC-based detection method could specifically detect viruses on a solid surface and demonstrate that polymeric substrates with sinusoidal wave patterns could be universally applicable for LC-based biological detection.

2. Experimental details

2.1. Materials

Titanium (99.999%) and gold (99.999%) were obtained from Unlimited Enhanced Technology (South Korea). Glass microscope slides were obtained from Matunami (S-1215, Japan). Nematic liquid crystal 4-cyano-4′-pentylbiphenyl (5CB) was purchased from Tokyo Chemical Industry Co., Ltd. (C1555, Japan). UV-curable polymer was acquired from Minuta Technology (MINS-311RM, South Korea). Polished silicon (1 0 0) wafers were obtained from Silicon Sense (Nashua, NH). Octyltrichlorosilane (OTS), 3-aminopropyltriethoxysilane (APTES), succinic anhydride (SA), 16-mercaptohexadecanoic acid (MHA), triethylene glycol mono-11-mercaptoundecyl ether, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), and phosphate buffered saline (PBS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Antibodies (sc-52400 and sc-80671) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All protein solutions were made in 0.01 M PBS (pH 7.4 at 25 °C). Dialysis kits (66,494 and 66,383) for virus solutions were purchased from Thermo Scientific (Rockford, IL, USA). All aqueous solutions were prepared with high purity deionized water (18 MΩ cm) generated using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Cleaning of substrates

Glass microscope slides and silicon wafers were cleaned using a piranha solution (70% H_2SO_4/30% H_2O_2. Caution: piranha solution reacts violently with organic materials and should be handled with extreme caution; do not store the solution in closed containers) for 1 h at 80 °C. After removal from the cleaning solution, the substrates were rinsed with copious amounts of deionized water, ethanol, and methanol and dried under a stream of gaseous N_2. The cleaned substrates were then stored overnight in an oven at 120 °C.

2.3. Virus culture and preparation

Maintenance and propagation of primary human Foreskin Fibroblasts, human embryonic kidney cells, HCMV Towne strain and adenoviruses have been described previously [23]. To minimize physical adsorption to the antibody decorated surface, serum was removed from the virus solutions using Slide-A-Lyzer Dialysis Cassettes immediately before the experiment according to the manufacturer's protocols (Thermo Scientific, Rockford, IL, USA).

2.4. Preparation of octyltrichlorosilane (OTS)-functionalized silica substrate

The piranha-cleaned glass slides and silicon wafers were immersed into an OTS/n-heptane solution for 30 min. The substrates were then rinsed with methylene chloride and dried under a stream of N_2. Next, the OTS-treated glass slides were tested for homeotropic alignment by observing the orientation of 5CB sandwiched between two OTS slides. Any slide that did not display homeotropic alignment was discarded.

2.5. Procedure for the buckling of polymer thin films

Flat PDMS (Sylgard 184; Dow Corning) substrates were prepared by mixing siloxane base resin and crosslinking agent (10:1 weight ratio) and then casting against a polystyrene Petri dish (10 cm in diameter). The films were then left under low pressure to allow trapped air bubbles to be removed from the mixture, after which they were cured overnight at 60 °C. Next, the PDMS was cut into 2.9 cm × 5.8 cm slabs and exposed to oxygen plasma (Covance, Femto Science) at 150 W with a flow rate of 20 sccm to generate a thin, silica-like film on the surface of the substrate. The plasma-treated PDMS was subsequently wrapped around a cylindrical surface after 3 h, after which the sinusoidal wave patterns were generated within the brittle layer of PDMS upon relaxation of the strain.

2.6. Replication of polymeric substrates

Polystyrene (PS, Mn = 44,000), 3 wt% in toluene, was spin-coated onto piranha cleaned glass substrates and baked in an oven for 24 h at 60 °C. The layer of PS (thickness = 500 nm) was used to promote adhesion between the glass microscope slide and the film of poly-(urethane acrylate) (PUA). The PUA solution was then spin-coated onto the PS-coated glass substrates at a rate of 2000 rpm for 2 min. The PUA-coated substrates were imprinted with the PDMS mold and then polymerized by UV light exposure (365–435 nm, 15 mW/cm^2) for 2 min.

2.7. Deposition of gold films

For use in combination with liquid crystals, semi-transparent films of gold with thicknesses of 200 Å were deposited onto patterned PUA substrates mounted on rotating planetary using an electron beam evaporator. The rotation of the substrates on the planetary mount ensured that the gold was deposited without a preferred direction of incidence. A layer of titanium (thickness = 80 Å) was used to promote adhesion between the PUA substrate and the gold film. The rate of deposition of gold and titanium was 0.2 Å/s, and the pressure in the evaporator was <5 Torr during deposition.

2.8. Preparation of mixed self-assembled monolayers (SAMs)

The gold coated polymer substrates were functionalized with mixed SAM by immersing the surface into ethanolic solutions of 0.8 mM 16-mercaptohexadecanoic acid and 0.2 mM of triethylene glycol mono-11-mercaptoundecyl ether at room temperature.