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Liquid crystal as sensing platforms for determining the effect of graphene oxide-based materials on phospholipid membranes and monitoring antibacterial activity

Yibin Wei, Chang-Hyun Jang*

Department of Chemistry, Gachon University, San 65, Bokjeong-Dong, Sujeong-Gu, Seongnam-City, Gyeonggi-Do, 461-701, Republic of Korea

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ABSTRACT

Understanding the mechanism of the cellular interaction of graphene oxide (GO)-based materials is vital for their biomedical and environmental applications. In this study, we developed a promising method for imaging the interaction between chitosan (CS)-reinforced GO and the phospholipid membrane based on a nematic liquid crystal (LC) sensing platform. Direct observation of the CS-GO-induced rupture of the pre-adsorbed phospholipid monolayer supported by an LC thin film was achieved using a polarizing optical microscope (POM). The disruption process was clearly monitored in real-time using this method. Moreover, because lipids at the Gram-negative bacterial cell membrane could be transferred onto the interfaces of micro-meter-sized sessile LC droplets, we found the LC droplets could be used to distinguish live *Escherichia coli* (*E. coli*) cells from dead cells. CS-GO efficiently reduced the cellular integrity of *E. coli* which was detectable by LC droplets; thus, the LC-based approach could be used for monitoring the antibacterial activities of GO-related materials. These experimental LC sensing platforms offer simple and direct observation methods for evaluating the effect of GO-based materials on the cell membrane and may be useful in corresponding antibacterial applications.

1. Introduction

Since Geim and Novoselov shared the Nobel Prize in Physics 2010 for their pivotal contributions to the isolation of graphene from bulk graphite using adhesive tape, graphene-based materials have gained increasing attention in various applications [1–4]. As one of the most important derivatives of graphene, graphene oxide (GO) has been extensively investigated because of its high mechanical strength and good water solubility. In addition, GO is rich in oxygen-containing functional groups (carboxyl, hydroxyl, carbonyl, and epoxy) in the carbon lattice. The presence of these functional sites enables facile surface modification with biocompatible polymers such as polyethylene glycol (PEG) [5], polyvinyl alcohol (PVA) [6,7], poly-L-Lysine (PLL) [8,9], and chitosan (CS) [10–12]. Recently, a number of studies have used those biopolymer grafted GO for biomedical applications in fields such as drug delivery, biosensing, antimicrobial therapy, and cancer therapy [12–18]. Thus, the most fundamental mechanisms involved in cell membrane interactions with GO-based materials should be thoroughly understood. However, although several possible pathways have been suggested and evaluated by both indirect experimental observation [19] and theoretical-based molecular dynamics (MD) simulations [20,21], the investigation of the exact mechanism remain challenging.

Liquid crystals (LCs) are a type of soft matter that serves as an indicator responding to external signals and have attracted great interest for their utility in sensing applications [22,23]. The transitions in orientation of LC molecules for surface anchoring have been used as an optical amplification medium because LCs are highly sensitive to small changes in external conditions, making them attractive for studying interfacial interactions. LCs have been proposed for detecting defects in graphene as a characterization tool. Son et al. [24] reported a "top-down" method in which they aligned liquid crystal molecules on graphene film to directly observe the domain size and defect distribution, relying on the distinct birefringence properties of LCs between crossed polarizers.

In the past decade, LCs have been shown to be promising in the biosensing field and numerous studies have employed the ordering transitions of LC materials for sensing a range of biochemical events and biomolecules [25]. The technology for monitoring the interaction between the phospholipid membrane and intrusive drugs with LCs has rapidly developed in recent years. Abbott

* Corresponding author.
E-mail address: chjang4u@gachon.ac.kr (C.-H. Jang).

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et al. [26] first confined LC molecules into an electron microscopy used grid to form an LC thin film and then phospholipids were decorated onto the LC film surface. The introduction of specific binding events with phospholipids may shift the orientation of LCs from a homeotropic state to a planar state, which can be observed using a polarizing optical microscope (POM). This method offers label-free and in situ monitoring of the interaction between phospholipid membranes and external chemical signals without complex instrumentation. We also successfully imitated a series of biomimetic phospholipid-disruption processes using this method [27,28]. Another LC biosensing platform based on LC droplet patterns [29,30] has been considered as the new generation of LC sensing techniques and has shown high sensitivity because of its three-dimensional structure and sensitive layer at the aqueous/LC interface. Transitions in the LC droplet configuration can be induced by variety of interfacial adsorbates for examining molecular interactions. Although numerous methods are available for fabricating LC droplets, several possible configurations of LC droplets have defined, including bipolar, uniaxial, axial, escaped radial, pre-radial, and radial [31]. In 2009, the use of LC emulsion droplets for the experimental detection of bacteria was reported by Sivakumar et al. [32], who found the Gram-negative bacteria with lips on the cell membrane could induce LC emulsion droplets to form a radial configuration. However, few studies [33–35] have evaluated the potential mechanism of this process. It is believed that bacteria can be regarded as 'active' particles for disrupting the orientation of LCs. All of those previous studies demonstrated the potential of using LCs to study the interactions between phospholipid membranes and GO-related materials. Moreover, given the feasibility of using LC droplets monitoring bacteria, LC-based sensing platforms may be useful for imaging of practical antimicrobial activities of GO-related materials.

CS is a typical biopolymer with unique properties such as good biocompatibility and biodegradability, allowing its widespread bioapplication (e.g. in drug/gene delivery and as an antimicrobial agent) [36]. In this study, we report a new perspective of LC sensing platform for examining the interaction between the phospholipid membrane and CS-grafted GO (CS-GO) as well as monitoring the antibacterial activities of CS-GO material. GO nanosheets were synthesized and then modified with CS chains via amide linkage. Changes in the LC thin film were observed by POM, which confirmed that the GO nanosheet could puncture the phospholipid membranes and the reinforced CS-GO showed better efficiency for destructing the phospholipid membranes. Exploiting POM to distinguish the orientation ordering of LC droplet textures, the fabricated sessile LC droplets indicated the state of Gram-negative *Escherichia coli (E. coli)* cells. Because lipids on the *E. coli* cell membrane may be transferred onto LC droplets, the ordering of LC molecules at the aqueous/LC interface aligned homeotropically; however, dead bacteria without intact cellular structures caused the sensitive LC layer to become planar. Incubation of *E. coli* with CS-GO efficiently reduced the cellular integrity of the bacterial cells, and sessile LC micro-droplets were successfully used for determining CS-GO-killed bacteria.

2. Materials and methods

2.1. Materials

Nematic LC, 4-cyano-4′-pentylbiphenyl (5CB) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Premium glass microscope slides were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Copper electron microscope grids were purchased from Gilder Grids Co., Ltd. (Grantham, UK). Graphite powders were obtained from Bay Carbon (Bay City, MI, USA). Hydrogen peroxide (30% w/v) and octyltrichlorosilane (OTS) were purchased from Alfa Aesar (Haverhill, MA, USA). Acetone, ethanol (anhydrous), 1 M hydrochloric acid, *n*-heptane, and concentrated sulfuric acid were purchased from Daejung Chemicals & Metals Co., Ltd. (Gyeonggi-do, Korea). Chitosan (CS, low-molecular weight), acetic acid, 85 wt.% phosphoric acid, potassium permanganate, 10 mM phosphate-buffered saline (pH 7.4), *N*-hydroxysuccinimide (NHS), 1-ethyl-2-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used as received without further purification. Deionized (DI) water (18.2 MΩ cm^{-1}) was generated by a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Fabrication of LC sensing platforms

Two types of LC sensing platforms were used in this study, including the LC thin film confined by electron microscope-used grids and sessile LC micro-droplets. Glass slides were cleaned in piranha solution (70% H_2SO_4/30% H_2O_2) for 30 min at 85 °C under a stream of gaseous N_2 (0.2 MPa). The slides were then sequentially rinsed with DI water, ethanol, and methanol and dried under a stream of gaseous N_2, followed by heating to 120 °C overnight prior to OTS deposition. The cleaned slides were immersed in 0.2% OTS/*n*-heptane solution for 30 min at room temperature. The slides were rinsed with methylene chloride and dried under a stream of N_2.

For the LC thin film system, OTS-treated glass slides were attached to the bottom of an eight-well chamber. Copper grids were then placed onto the chamber supported by the glass slides, and approximately 2 μL of 5CB was dispensed onto the grid. Excess LC was carefully removed by a capillary tube. Finally, the LC thin film surface was decorated with self-assembled phospholipid monolayer at room temperature.

Sessile LC micro-droplets were prepared as we previously described [37]. Briefly, 2% (v/v) nematic 5CB was freshly prepared in anhydrous *n*-heptane. After dropping 1 μL of the prepared mixture, the micrometer-scale LC droplet matrix was immobilized on OTS-pretreated glass slides as the organic solvent evaporated. The transition of the sessile LC droplet configuration was recorded when the droplets were in contact with solutions of interest.

2.3. Preparation of self-assembled phospholipid membrane

Self-assembled phospholipid monolayers were prepared as previously described [27]. DOPG dissolved in chloroform was gently dried with N_2 gas and was placed under vacuum for at least 3 h. The dried phospholipid was then re-suspended in PBS (pH 7.4) to a final concentration of 1 mM. Next, the phospholipid suspension was sonicated three times, each for 5 min, to obtain a clear solution. Finally, the solution was filtered twice through a 0.22-μm filter and was used within 1 day of preparation. The phospholipid monolayer was formed by contacting the copper grid impregnated with 5CB to the aqueous solution of phospholipids in the optical cell.

2.4. Preparation of E. coli and CS-GO antibacterial activity

Escherichia coli cells were incubated in Luria-Bertani medium at 37 °C and harvested during the mid-exponential growth phase after 15 h. The obtained cultures were then centrifuged at 5000 rpm for 2 min to pellet cells, which were washed three times with isotonic saline solution to remove residual macromolecules and other growth medium constituents. Bacterial cell suspensions contained 10^7–10^8 CFU/mL in isotonic saline solution prior to use. To evaluate the antibacterial activity of CS-GO, the prepared *E. coli* cells