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Micro-capillary sensor for imaging trypsin activity using confined nematic liquid crystals

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ABSTRACT

In this study, a simple and sensitive micro-capillary sensor for monitoring trypsin reactions using confined nematic liquid crystals (LCs) was developed. The confined LC droplet was formed through sequential injection of a LC and aqueous solution into an octyltrichlorosilane-pretreated capillary. When the confined LC droplet made contact with an aqueous mixture of phospholipids and poly-L-lysine (PLL) solutions, the electrostatic interaction between the phospholipid and PLL induced realignment of the phospholipid monolayer and produced an optical LC droplet texture of two bright lines. Trypsin can cleave peptides specifically on the carboxyl side of lysine, and in this case, trypsin activity is manifested as removal of this electrostatic interaction, leading to a change in the ordering of LC droplets confined in the capillary. Thus, the orientational transition of LC droplets results in a change in their optical response from two bright lines to a four-petal shape. Control experiments with deactivated trypsin or chymotrypsin confirmed the feasibility of this trypsin detection approach. Using the LC-based capillary sensing technique, trypsin levels as low as ~0.1 μg/mL could be detected. This research proposes that LC-based micro-capillary sensing systems can be utilized in the development of highly sensitive and simple sensors for detecting various enzymes.

1. Introduction

As one type of serine protease, trypsin is a digestive enzyme derived from the inactive protease trypsinogen and selectively cleaves peptide bonds on the carboxyl side of lysine and arginine amino acids [1]. Since it is related to the digestive enzyme cascade, trypsin is the most significant digestive enzyme. In the cascade of digestive enzyme activation, pancreatic proenzymes are transformed to their active forms in the intestine and they initiate autodigestion [2,3]. Hence, trypsin plays a major role in managing the pancreatic exocrine function.

Healthy people have trypsin concentrations of 0.25 ± 0.1 μg/mL in serum; however, pancreatitis patients have high trypsin concentrations of 1.4 ± 0.6 μg/mL in serum and patients with nutritional disorders have trypsin concentrations of <0.12 ng/mL [4]. In that way, some kinds of diseases are related to the trypsin level in serum. Therefore, many methods have been developed to detect trypsin concentrations, such as using optical nanoprobes [5], film techniques based on gelatin [6], surface-enhanced Raman scattering (SERS) [7], colorimetric assays [8], enzyme-linked immunosorbent assays (ELISA) [9], and fluorescence assays [10–13]. However, most of these methods are time-consuming or require an additional labeling process or specific instrumentation.

Over the past few years, liquid crystals (LCs) have drawn great interest for the important materials in the field of chemical and biological sensors [14–16]. Since the long-range orientational nature of LCs enables the amplification and transduction of bio-molecular interactions into optical signal visible under a polarized light microscope, LC-based sensing techniques have unique characteristics of simplicity, cost efficiency, rapid response, sensitivity, optical texture, and portability. Also, LC-based sensor does not require any sample labeling or specific instruments. Owing to these intrinsic LC features, LC-based sensing systems have been utilized for sensing in various fields, such as for DNA hybridization [17,18], interactions between ligands and receptors [19,20], enzyme reactions [21–23], and pH-driven phenomena [24].

LC-based detection technologies for monitoring trypsin reactions have also been developed and reported. Most of these techniques for detecting trypsin activity have used a LC film confined in a copper grid based on the bio-molecular interactions at the aqueous solution/LC interface [25,26]. Although LC film sensors are simple and sensitive, there are several drawbacks. The LC thin film sensing system is commonly fabricated on the glass slides with an eight well chamber (a volume of each well is approximately 3.5 cm^3). Thus, solutions are needed as hundreds of microliters for imaging, limiting the detection of small amount of substances. When preparing the LC films on the copper grids, excess LCs are extracted, wasting the materials. Moreover, the copper grids are easily oxidized and deformed, limiting their reusability. Because the eight well chamber is exposed to the external environment, aqueous solution/LC interfaces can be interrupted by heat, air flow, and certain vibrations. In another study, a different strategy for sensitive detection of trypsin using LC droplet patterns formed on a glass slide was

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developed [27]. This LC droplet pattern system has high sensitivity and easy fabrication. Since trace amounts of solution of interest are required for imaging, however, this system can stay stable for a few minutes at room temperature limiting its applications.

Here, we introduce a new type of LC-based trypsin sensor that is more stable, durable, rapid, sensitive and easily fabricated, and is reusable when compared with LC film sensing system. This new strategy uses confined LC droplets in a micro-capillary [28]. LC droplets immiscible with water were produced by sequential injection of LCs and an aqueous solution into an octyltrichlorosilane (OTS)-treated capillary (Fig. 1A). The orientational behavior of the confined LC droplets in the capillary was controlled by the properties of the aqueous solution, that is, whether or not it was a surfactant solution. When the aqueous solution is a non-surfactant solution, LC droplets maintain a homeotropic orientation. However, if the aqueous solution is a surfactant solution, surfactant molecules will be anchored to the LC/aqueous interface owing to their amphiphilic property, resulting in a change in the ordering of the LC droplets. The orientations of LC droplets in a non-surfactant solution and a surfactant solution are manifested as optical images of two bright lines and a four-petal shape, respectively. Based on this principle, a LC sensing technique for detection of trypsin was designed using the interaction between the positively charged poly-L-lysine (PLL) and the negatively charged phospholipid, 1,2-distearoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) sodium salt (DOPG). We considered that this electrostatic interaction prevented DOPG molecules from anchoring to LC/liquid interfaces, maintaining the homeotropic orientation of LC droplets. However, this interaction would be decreased by the presence of trypsin, which specifically cleaves peptides on the carboxyl side of lysine. An aqueous mixture of PLL and DOPG pre-incubated with trypsin led to a change in the ordering of LC droplets owing to the anchoring of DOPG molecules to LC/liquid interfaces (Fig. 1B). The orientational transition of a LC droplet was indicated by a change in its optical image from two bright lines to a four-petal texture (Fig. 1C). Through these results, we could confirm that the enzymatic reaction between trypsin and PLL was successful, decomposing PLL molecules substantially to induce the orientational transition of LCs.

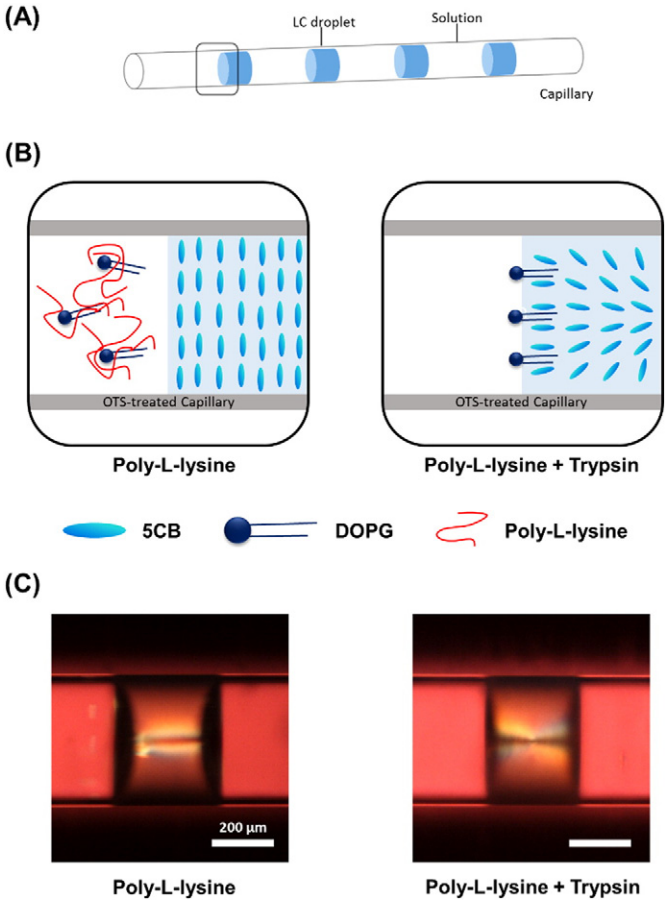

Fig. 1. The LC-based capillary sensing system. (A) 5CB confined in the micro-capillary, (B) schematic illustrations of the ordering change of 5CB droplets confined in an OTS-treated capillary as applied trypsin cleaves peptides on the carboxyl side of lysine, and (C) their optical images.

2. Materials and methods

2.1. Materials

LCs, 4-cyano-4′-pentylbiphenyl (5CB), were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). OTS was acquired from Alfa Aesar (Ward Hill, MA). *n*-Heptane (anhydrous), hydrogen peroxide (30% w/v), and sulfuric acid were purchased from Daejung Chemicals & Metals Co., Ltd. (South Korea). Methyl alcohol, ethyl alcohol, and methylene chloride were obtained from Duksan Pure Chemicals Co., Ltd. (South Korea). Micro-capillaries, polyethylene glycol sorbitan monolaurate (Tween-20), phosphate-buffered saline (PBS), DOPG, PLL (mol wt 150,000–300,000) (0.1% w/v), trypsin, α-chymotrypsin, trypsin inhibitor, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Deionized (DI) water (18.2 MΩ cm^{-1}) was collected from a Milli-Q water purification system (Millipore, Bedford, MA).

2.2. Octyltrichlorosilane (OTS) functionalization of micro-capillary

All micro-capillaries were immersed in piranha solution (H_2SO_4/H_2O_2, 7:3 v/v) at 80 °C for 1 h. Afterwards, the capillaries were rinsed with DI water, ethanol, and methanol, and then dried under gaseous N_2 and incubated in an oven at 120 °C for 8 h. Then, the cleaned capillaries were functionalized in a 10 mM OTS/*n*-heptane solution for 30 min and rinsed with methylene chloride, and then dried under gaseous N_2.

2.3. Preparation of DOPG solution

The DOPG solution was prepared using a procedure reported previously [26]. Simply, DOPG was dissolved in chloroform (50 mg/mL), and then dried using a stream of N_2 gas. The dried DOPG monolayer was then suspended in a PBS solution (pH = 5.8), with the DOPG concentration of interest.

2.4. Preparation of liquid crystals confined in OTS-treated capillary

Confined LC droplet patterns in OTS-treated capillary were fabricated with a method similar to the one reported previously [28]. Briefly, a small volume of 5CB (approximately 0.5 μL) was introduced into one end of an OTS-treated capillary. Next, the aqueous solution was then injected into the capillary using a syringe, and then 5CB was separated into several parts automatically. These LC droplets indicated the orientational states of 5CB in the capillary.

2.5. Optical examination of LC droplets using polarized light microscope

All LC droplets in micro-capillaries were observed using a polarization light microscope (Eclipse LV100POL, Nikon, Tokyo, Japan) and their optical images were taken using a digital camera (DS-2Mv, Nikon, Tokyo, Japan) attached to the microscope, using 5× objective lenses at a gain of 1.00×, resolution of 1600 × 1200 pixels, and shutter speed of 1/30 s. A retardation plate was added for determining the configuration of the detector as well as a crossed polarizer and an analyzer.