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Photonic crystals on copolymer film for label-free detection of DNA hybridization

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ABSTRACT

The presence of a single-nucleotide polymorphism in Apolipoprotein E4 gene is implicated with the increased risk of developing Alzheimer's disease (AD). In this study, detection of AD-related DNA oligonucleotide sequence associated with Apolipoprotein E4 gene sequence was achieved using localized-surface plasmon resonance (LSPR) on 2D-Photonic crystal (2D-PC) and Au-coated 2D-PC surfaces. 2D-PC surfaces were fabricated on a flexible copolymer film using nano-imprint lithography (NIL). The film surface was then coated with a dual-functionalized polymer to react with surface immobilized DNA probe. DNA hybridization was detected by monitoring the optical responses of either a Fresnel decrease in reflectance on 2D-PC surfaces or an increase in LSPR on Au-coated 2D-PC surfaces. The change in response due to DNA hybridization on the modified surfaces was also investigated using mismatched and non-complementary oligonucleotides sequences. The proof-of-concept results are promising towards the development of 2D-PC on copolymer film surfaces as miniaturized and wearable biosensors for various diagnostic and defense applications.

1. Introduction

DNA sensing studies traditionally involve solid-phase hybridization assays (Kang et al., 2010; Tel-Vered et al., 2012; Wang, 2005). These techniques typically employ an enzyme or nanoparticle-tagged secondary oligonucleotide binding to surface-immobilized DNA for either optical or electrochemical detection, thereby converting the hybridization event to a quantifiable signal. However, the detection of DNA hybridization by secondary probes is intrinsically complex, time-consuming and requires multiple layers of interacting components with high specificity. As a result, the detection of primary DNA hybridization would greatly simplify routine DNA assays. Label-free measurement of bioaffinity events is a potentially powerful tool, simpler and more efficient than secondary probe-based systems. In the past decade, our group and others have demonstrated the label-free detection of various clinically important biomolecules using electrochemical and optical biosensors (Aygun et al., 2017; Cheng et al., 2014; Ember et al., 2011; Endo et al., 2005a, 2005b; Huang et al., 2018; Kaatz et al., 2012; Kim et al., 2008; Ozkumur et al., 2010; Spuhler et al., 2010; Yurt et al., 2012).

In this report, a novel sensing surface comprising of photonic crystals (PCs) is introduced as a promising platform for optical detection of DNA hybridization. PCs are periodic optical nanostructures that possess a periodic dielectric structure with a photonic band gap, which does not allow light propagation of a specific wavelength range (John, 1987; Yablonovitch, 1993). Such control and manipulation of light allows optical communication applications to be studied experimentally according to Bragg's Law (Vlasov et al., 2005). The optical properties of PCs are related to their periodicity, size and average refractive index (Xu and Asher, 2004). Conveniently, these characteristics can be easily controlled using nano-imprint lithography (NIL). This printable photonics technology allows the fabrication of structural features down to the nanometer level using highly homogenous polymers (Endo et al., 2010, Aki et al., 2014; Guo, 2007).

In this study, DNA hybridization was detected by monitoring the Fresnel reflection change (Endo et al., 2010). Fresnel reflection is observed by the behavior of light travelling through media of varying refractive indices. Since the reflection peak wavelength is detected within the visible region, 2D-PC creates a promising and simple sensing platform. This emerging technology has been successfully applied towards the development of selective and sensitive biosensors (Alexeev et al., 2003; Aki et al., 2014; Cheng et al., 2014; Endo et al., 2008a, 2008b, 2010; Inan et al., 2017; Ma et al., 2009; Liu et al., 2017; Sharma et al., 2004). The multiplexed detection of cancer biomarkers was reported using quartz-based photonic crystal surfaces (Huang et al., 2012). We have also achieved the observation of a localized surface

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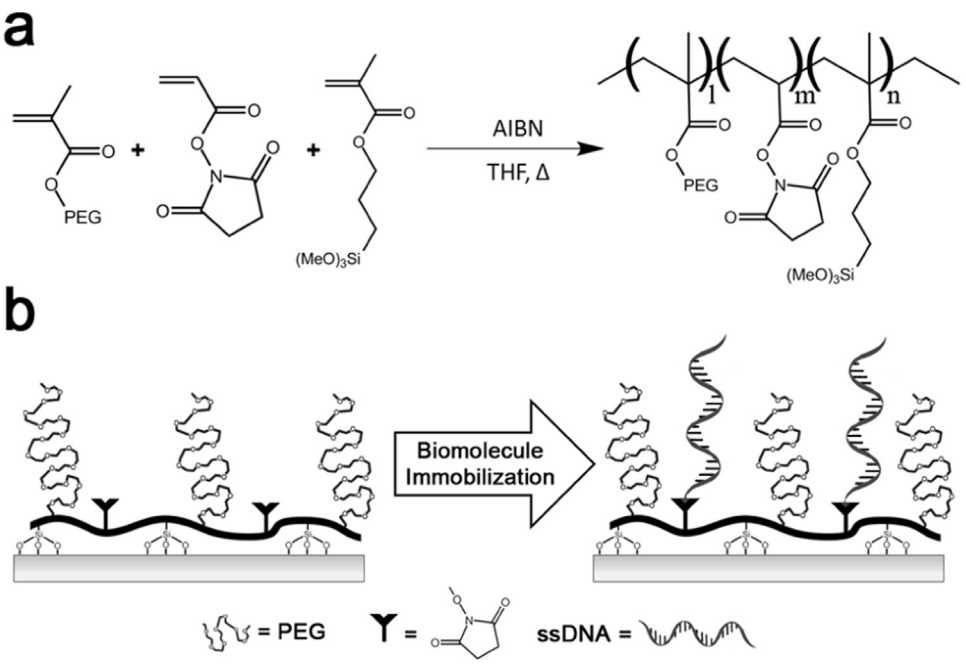

Fig. 1. (a) Synthetic scheme for the production of the multi-functional film, which contained l) protein-resistive, m) bio-reactive and n) anchor groups. (b) Schematic representation for specific immobilization of probe ssDNA on the multi-functional film.

plasmon resonance (LSPR) band by coating the surface of 2D-PC with a thin layer of Au. Noble metal nanoparticles exhibit rich LSPR properties from the collective oscillation of conduction electrons in response to optical excitation (Anker et al., 2008; Sagle et al., 2011). Stability and dispersion of deposition is difficult to control for nanoparticles significantly larger than 10 nm (McMahon and Emory, 2006; Park and Park, 2008). This problem was easily resolved, when Au sputtering was performed on the 2D-PC to form homogenously spaced Au nanostructures that produced an intense LSPR response for the first time in this report. As proof of principle, Apolipoprotein E gene (ApoE) was detected on these novel 2D-PC surfaces. Human ApoE is present in three isoforms (E2, E3 and E4) and related to two polymorphic codon sites (112 and 158) of the gene located on chromosome 19 (Bellis et al., 1997). These isoforms arise from 3 alleles: ε2, ε3 and ε4 (Siest et al., 1995). ApoE isoforms have been found in patients with type-III hyperlipidemia, atherosclerosis and Alzheimer's disease (AD) (Corder et al., 1993; Deming et al., 2015; Farrer et al., 1997; Morris et al., 2010). Inheritance of the ApoE single nucleotide polymorphisms is the only confirmed and consistently replicated risk factor for late-onset AD (Venter et al., 2001). Cramer et al. (2012) have recently reported that ApoE-directed therapeutics rapidly cleared amyloid-β deposits and reversed deficits in AD mouse models. Thus, reliable sensing platforms for ApoE gene is in high demand for the development of future AD therapeutics (Cramer et al., 2012; Landreth et al., 2013; Venter et al., 2001). Reports about the biosensor development for ApoE gene included electrochemical detection on disposable screen-printed electrodes (Ahmed et al., 2007; Marrazza et al., 2001), piezoelectrical detection on quartz crystals (Marrazza et al., 2000; Tombellis et al., 2000) and peptide nucleic acids in connection with electrochemical impedance spectroscopy (Guo et al., 2011). Compared to the previous methods of detection, the development of this novel ApoE sensor could potentially facilitate the development of cost-effective disposable biosensors in detection of clinically important biomolecules (Endo et al., 2016). Here, we report the fabrication and proof-of-concept results of a DNA hybridization sensor based on 2D-PC and Au-coated 2D-PC surfaces.

2. Materials and methods

2.1. Reagents

The oligonucleotides were purchased from BioBasic, Inc. (Markham, ON) as sodium salts. The probe was a 5′ disulfide-modified 23-bp long sequence from a fragment of the allele ε2 around codon 158. Fully complementary target DNA (FC), mismatch DNA (MM- single nucleotide polymorphism underlined) and non-complementary DNA (NC) were used in this study:

5′ to 3′:HS-S- ACC TGC AGA AGC GCC TGG CAG TG Probe
CAC TGC CAG GCG CTT CTG CAG GT FC
CAC TGC CAG GCA CTT CTG CAG GT MM
GAT TAG AGT CCC GCA ATT AAT CAT T NC

For the thiolated probes, the immobilization buffer was 1 M KH_2PO_4, (pH 3.8). Hybridization buffer contained 300 mM NaCl, 20 mM Na_2HPO_4, 0.1 mM EDTA (pH 7.4). All other reagents, unless stated otherwise were used as received from Sigma-Aldrich (Oakville, ON). EDTA and Tris-HCl were obtained from BioBasic Inc. (Markham, ON). In addition, ultra-pure water (18.3 MΩ cm) from Pall Cascada water purification systems was used in all preparations.

2.2. Fabrication and design of 2D-PC using nano-imprint lithography (NIL)

The mold for heat transfer was fabricated using computer aided design (CAD) system, electron-beam lithography, and nickel electrocasting. The film was prepared on ZeonorFilm ZF14 for NIL. After the fabrication of mold, heat transfer was performed using a semi-automatic nano-imprint apparatus (SCIVAX Corp. Ltd., Kanagawa, Japan, X-300). The co-polymer surface was pre-modified with dual functionality (Fig. 1a), which comprised of a protein resistant part (polyethylene glycol), anchor part (trimethoxysilane) and a bio-reactive part (N-acryloxysuccinimide). 2,2′-azobisisobutyronitrile (AIBN) and tetrahydrofuran (THF) were used as solvents (Ozkumur et al., 2010; Park et al., 2007). Then, 2D-PC film was immersed in a polymer solution that functionalized the surface with bio-inert (Siest et al., 1995) and bio-reactive properties. This provided anti-fouling characteristics on the film surface as well as sites that can bind to biomolecules. (Fig. 1b). For