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A molecular beacon biosensor based on the nanostructured aluminum oxide surface

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ABSTRACT

A new class of molecular beacon biosensors based on the nanostructured aluminum oxide or anodic aluminum oxide (AAO) surface is reported. In this type of sensor, the AAO surface is used to enhance the fluorescent signals of the fluorophore-labeled hairpin DNA. When a target DNA with a complementary sequence to that of the hairpin DNA is applied on the sensor, the fluorophores are forced to move away from the AAO surface due to the hybridization between the hairpin DNA and the target DNA, resulting in the significant decrease of the fluorescent signals. The observed signal reduction is sufficient to achieve a demonstrated detection limit of 10 nM, which could be further improved by optimizing the AAO surface. The control experiments have also demonstrated that the bioassay used in the experiments has excellent specificity and selectivity, indicating the great promise of this type of sensor for diagnostic applications. Since the arrayed AAO micropatterns can be fabricated on a single chip in a cost-effective manner, the arrayed sensors could provide an ideal technical platform for studying fundamental biological process and monitoring disease biomarkers.

1. Background

Deoxyribonucleic acid (DNA) is a molecule that contains the genetic information for all known living organisms and many viruses (Jones and Baylin, 2002; Lockhart and Winzeler, 2000) Hence, DNA sensor is a critical platform for understanding fundamental biological process and unrevealing the causes of diseases. DNA sensors based on different operational principles have been developed for the past decades. Examples include optical, electrochemical, magnetic, and quartz crystal microbalance (QCM) based DNA sensors (Nelson et al., 2001; Drummond et al., 2003; Kouassi and Irudayaraj, 2006; Wang et al., 2012). For optical DNA sensors, a variety of optical methods such as the evanescent optical field, resonant rings and optical grating coupler have been used for detecting DNA (Nelson et al., 2001; Sun and Fan, 2011; Liu and Tan, 1999). For instance, surface Plasmon resonance (SPR) technique has been proved to be a viable technical platform for DNA detection (Nelson et al., 2001). Electrochemical method offers another approach for DNA sensing. Electrochemical DNA sensors provide a cost-effective technical platform for the detection of DNA sequences or genes associated with human disease with high sensitivity and selectivity. There are a variety of approaches for electrochemical detection such as direct electrochemistry of DNA, electrochemistry at polymer-modified electrodes, just to name a few (Drummond et al., 2003). Some efforts have also been devoted to using magnetic nanoparticles for DNA detection. Single strands of oligonucleotides can be effectively immobilized onto amino- and carboxylate-functionalized magnetic nanoparticles or gold-coated magnetic nanoparticles using the streptavidin-biotin interaction. Experiments have demonstrated that the gold-coated magnetic nanoparticles are ideal DNA sensors (Kouassi and Irudayaraj, 2006; Fan et al., 2005). For the QCM-based DNA sensor, when the target DNA binds to the probe DNA on the QCM, the mass of the biomolecules changes, resulting in a shift of the resonant frequency of the QCM (Wang et al., 2012).

One of the promising optical DNA sensors is to use molecular beacons (MBs) for sensing due to their high sensitivity, specificity and reliability (Du et al., 2003; Xiao et al., 2006; Sun and Fan, 2012; Fang et al., 1999; Peng et al., 2009). Usually the MB is a hairpin DNA functionalized at one end with a fluorophore and at the other end with a quenching agent or using a metal-coated substrate to quench the fluorescent signals. Without the target DNA, the quencher is in close proximity to the fluorophore, no fluorescent signal is generated. By adding a complementary target DNA, the loop of hairpin DNA is unfolded, the fluorophore moves away from the quencher, resulting in fluorescent signal generation. To our knowledge, most of the reported MB sensors employ a quenching agent or the metal-coated substrate as fluorescent quencher. Herein, we investigate if a nanostructured aluminum oxide or anodic aluminum oxide (AAO) surface itself could be used

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as a fluorescent enhancer, thereby developing a new type of MB biosensor.

For the past, using the unique optical inference fringes of the AAO thin film, AAO thin film-based interferometry sensors have been developed and mainly used for label-free biodetection (Zhang et al., 2010, 2011; 2012; He et al., 2014). Recently it has been found that the AAO surface can also significantly enhance the fluorescent signals when the fluorophores are in close proximity to its surface (Li et al., 2012, 2013; Li and Que, 2014). It has been found that the enhancement factor is in the range of two to three orders of magnitude compared to the glass surface when the fluorophores are directly attached to the AAO surface (Li et al., 2012, 2013; Li and Que, 2014; Yin et al., 2014). Different from metal-enhanced fluorescence (MEF), which requires a spacer between the metal surface and fluorophores to avoid quenching effect, the AAO surface offers the maximum fluorescence enhancement when the fluorophores directly contact with its surface, but the enhancement reduces dramatically when the gap between the AAO surface and the fluorophores increases. The enhancement mechanism of the AAO surface is not totally understood and thus requires further studies. However, based on the previous research, this enhancement probably results from the following main reasons. First, optical scattering of the AAO surface may play a very important role in the fluorescence enhancement. The surface scattering effects of the AAO surface cause the redistribution of the electromagnetic fields with high surface intensities, resulting in the enhanced fluorescence (Fujii et al., 2004; Zhang et al., 2012; Ganesh et al., 2007). Second, the fluorescence enhancement might also result from the evanescent electrical field from the surface of the nanoscale AAO grains (Fig. 1(a)), similar to other reported metal oxide nanoscale materials (Dorfman et al., 2006; Zhao et al., 2008; Gu et al., 2008). This assumption has been validated by evaluating the effect on the fluorescent signals from the gap between the AAO surface and the fluorophores by coating a layer of poly (methyl methacrylate) (PMMA) between the AAO surface and the fluorophores (Li, 2014). It has been found that the significant enhancement of the fluorescence occurs only in the range of one hundred nanometers from the AAO surface. At and beyond 100 nm from the AAO surface, the fluorescence enhancement becomes significantly weak. This property can be exploited for a new type of MB biosensor.

The principle of the new MB biosensor is illustrated in Fig. 1(b-c). In this case, a hairpin DNA with one end attached with a fluorophore emits strong fluorescent signal when it is immobilized on the AAO surface and its loop is closed (Fig. 1(b)). The fluorescent signal reduces dramatically when the loop is open if it is hybridized with a complementary target DNA (Fig. 1(c)). The sequence of "on" and "off" of fluorescent signals of this type of MB biosensor is just opposite from that of other previously reported MB sensors (Du et al., 2003; Xiao et al., 2006; Sun and Fan, 2012; Fang et al., 1999; Peng et al., 2009), which might open a new avenue for fluorescent-based bioassay. Compared to the noble metal (i.e., Au or Ag) nanoparticles or nanostructures based MB biosensors (Du et al., 2003; Xiao et al., 2006; Sun and Fan, 2012; Fang et al., 1999; Peng et al., 2009), the AAO based MB biosensor offers at least the following advantages. (1) It is simple and inexpensive to prepare AAO surface using the anodization process with high uniformity and repeatability; (2) the material (aluminum) for fabricating the MB sensors is much cheaper than Au or Ag based MB sensors; and (3) using the lithography-based fabrication process, hundreds or thousands of AAO based microscale MB biosensors can be fabricated in an efficient manner for high throughput application.

2. Methods and materials

2.1. *Fabrication of the anodic aluminum oxide micropatterns on glass substrate*

An ITO glass substrate is first cleaned by DI water, acetone, ethanol, and then DI water for 20 min with each solution. Then 2 μm aluminum is deposited on the substrate with 10 nm Ti as an adhesion layer by E-beam evaporation. The measured surface roughness of the Al thin film is in the range of 6–12 nm, which is smooth enough for carrying out the anodization process. Then, one-step anodization process (in 0.3 M oxalic acid) is performed to form anodic aluminum oxide. After this step, the AAO micropatterns are fabricated using the process developed in Yin et al. (2004) using standard optical lithography and wet etching process.

2.2. *Chemicals and materials*

The reagents (DNA samples) are custom-synthesized at Integrated DNA Technologies, Inc. Their sequences are summarized in Table 1. The reagents include three types of DNA powers with different sequences. Hairpin DNA (H1 DNA) (with 5′ amino-terminated) has a fluorophore at its 3′ end. Target DNA (T1 DNA) has

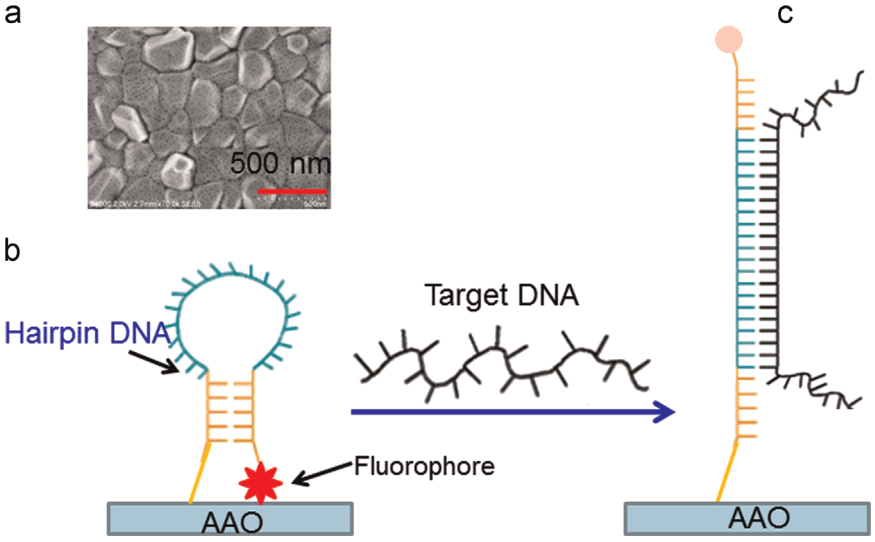

Fig. 1. (a) SEM image of an AAO surface showing nanoscale domains; (b-c) operational principle of the AAO surface based molecular beacon biosensor.