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Label-free electrical quantification of amplified nucleic acids through nanofluidic diodes

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ABSTRACT

A label-free method of quantifying nucleic acids in polymerase chain reaction (PCR) is described and could be the basis for miniaturized devices that can amplify and detect target nucleic acids in real time. The method takes advantage of ionic current rectification effect discovered in nanofluidic channels exhibiting a broken symmetry in electrochemical potential – nanofluidic diodes. Nanofluidic diodes are prototyped here on nanopipettes readily pulled from individual thin-walled glass capillaries for a proof of concept demonstration yet the basic concept would be applicable to ionic rectifiers constructed through other means. When a nanopipette modified in the tip region with cationic polyelectrolytes is presented with an unpurified PCR product, the tip surface electrostatically interacts with the amplicons and modulates its ionic rectification direction in response to the intrinsic charge of those adsorbed. Modulations are gradual and correlate well with the mass concentration of the amplicons above 2.5 ng/μL, rather than their sizes, with adequate discrimination against the background. Moreover, the tip surface, following a measurement, is regenerated through a layer-by-layer assembly of cationic polyelectrolytes and amplicons. The regenerated tips are capable of measuring distinct mass concentrations without signs of noticeable degradation in sensitivity. Further, the tips are shown capable of reproducing the amplification curve of real-time PCR through sequential steps of surface regeneration and simple electrical readout during the intermediate reaction stages. This suggests that nanopipettes as nanofluidic diodes are at a capacity to be employed for monitoring the PCR progress.

1. Introduction

Nanofluidics – analysis and exploitation of unique phenomena observed in fluids confined to nanoscale structures – has recently attracted burgeoning attention and could potentially lead to advanced devices for chemical and biomedical applications (Eijkel and van den Berg, 2005). For instance, fluidic channels, when scaled down to nanometer regime (typically 1–100 nm), physically confine and stretch nucleic acids (Tegenfeldt et al., 2004). Accordingly, facile integration of nanofluidic components into microfluidics has led to practical systems for entropic sieving of nucleic acids (Han and Craighead, 2000) and high-resolution analysis of stretched DNA molecules (Abad et al., 2011; Kim et al., 2011). Moreover, the unique ion transport behavior through artificial nanochannels has been well studied with a goal to better understand and emulate the function of transmembrane ion channels of biological origin (Cheng and Guo, 2010a; Plecis et al., 2005; Schoch et al., 2008). With a deep insight into its underlying mechanism, the utilization of this behavior has led to numerous innovative nanoelectrofluidic devices such as nanofluidic field effect transistors (Kalman et al., 2008; Nam et al., 2010, 2009) and ion current diodes for biosensing (Vlassiouk et al., 2009), molecule preconcentration (Hlushkou et al., 2012; Zangle et al., 2010), and molecule delivery (Karnik et al., 2006; Nguyen et al., 2010).

Nanofluidic diodes refer to the nanoporous structures that conduct ion current in one particular direction while suppressing it in the opposite direction (Cheng and Guo, 2010b). Such rectification effect occurs in a nanofluidic channel in which the critical dimension is comparable to the Debye length and ion concentration is governed by the wall surface charge rather than bulk concentration (Karnik et al., 2007; Kovarik et al., 2009; Vlassiouk et al., 2008). The electrical double layers of the walls overlap and the counter-ions dominate the transport. The rectification also requires a broken symmetry across the structure which could be as a result of asymmetric surface charge distribution (Karnik et al., 2007), or lopsided bath concentrations (Cheng and Guo, 2007), or simply asymmetric conical channel profile (Umehara et al., 2006). The effect is explained by the accumulation and depletion of cations and anions at the two entrances of the nanochannel in response to different bias polarities (Cheng and Guo, 2010b).

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A forward bias voltage applied across the nanochannel leads to enrichment of ions and an increased ion conductance whereas a reverse bias voltage depletes ions and results in a decreased ion conductance. Such devices offer new prospects for the controlled separation and sensing of diverse species in aqueous solutions (Ali et al., 2009).

A wealth of biosensing strategies has been reported using nanofluidic channels towards a rapid and cost-effective detection of analytes (Howorka and Siwy, 2009; Piruska et al., 2010). Many, however, apply a resistive-pulse sensing technique, the Coulter-counting principle, as elaborated in the detection of DNA labeled with a nanoparticle translocating through a nanopipette tip (Karhanek et al., 2005). With the recent studies geared towards ion current rectification and the dominant role of surface charge through such structures (Siwy et al., 2004; Siwy, 2006), new sensing strategies exploiting the rectification effect begin to emerge targeting, among various species, metal ions (Sa et al., 2010) and proteins (Ali et al., 2010b; Umehara et al., 2009; Vlassiouk et al., 2009). Moreover, a sequence-specific recognition of nucleic acids has been reported based on the hybridization of a target sequence of single-strand DNA to a complementary probe sequence immobilized on nanofluidic diodes (Ali et al., 2010a; Fu et al., 2009). In those approaches, however, recovery of the sensor surface for repeated measurements appears to be problematic and requires a careful dissociation of the capture probes from the hybridized targets, which poses a great challenge (Fan et al., 2003).

Nanofluidic diodes have not yet been fully explored for the quantitative DNA analysis. In molecular biology, routine DNA analysis often entails polymerase chain reaction (PCR), whereby a few copies of a specific sequence of DNA can be amplified to many copies so as to exceed the limit of detection in the subsequent steps (e.g. capillary or gel electrophoresis). Quantitative or real-time PCR (qPCR) measures the quantity of DNA as it gets amplified, through fluorescent intercalating dyes or sequence-specific reporter probes (Heid et al., 1996). However, the inhibitory effects of such fluorescent reagents and difficulty in miniaturizing and integrating optical components into a portable system for point-of-care diagnosis have elicited a growing interest in detecting PCR products through non-optical means. For instance, electronic field-effect (Fritz et al., 2002; Hou et al., 2006) and electrochemical (Luo and Hsing, 2009) methods have been successfully demonstrated for PCR quantification. Yet a simple, robust and cost-effective label-free strategy is still a remaining challenge. This challenge could potentially be overcome by nanofluidic diodes given their simplistic configuration and label-free real-time electrical readout capability.

In this study, we describe a simple and quantitative method of DNA detection using nanofluidic diodes and, for the first time, demonstrate their capability to quantify PCR products from an unpurified PCR mixture. Nanofluidic diodes used herein, glass nanopipettes, exhibit an asymmetric ion conductance owing to their conical channel (tip) structure and negatively charged surface (Wei et al., 1997). Prepared conveniently from a glass capillary on a commercial bench-top puller, nanopipettes offer a cost-effective route to nanofluidic diodes without the requirement of high-precision sophisticated semiconductor fabrication process. The surface functionalization adopted here draws its principle from electrostatic interactions between the charged polyelectrolyte layers and the device surface as previously implemented on the field-effect DNA sensors (Fritz et al., 2002; Hou et al., 2006). Thus, the tip region of a glass nanopipette gets deposited with a thin layer of an oppositely charged polyelectrolyte, poly-L-lysine (PLL). The charged polyelectrolytes have been recently explored to control the surface potential of quartz nanopipettes and the solid-state conical nanopores (Umehara et al., 2006). However, such nanofluidic diodes functionalized with cationic polyelectrolytes have never been applied before to detect DNA or PCR products but biotin–streptavidin and antibody–antigen interactions (Umehara et al., 2009). PCR products are double-stranded DNA and their electrostatic association to the tip surface through cationic polyelectrolytes is of particular interest for the convenience of evading the procedures to generate single-stranded sequences which would have been inevitable if hybridization capture probes were used.

The concept is briefly described in Fig. 1. First, a thin layer of PLL is electrostatically adsorbed onto the tip surface to overcompensate the negative surface charge of the native glass in preparation for the electrostatic adsorption of the intrinsically negatively charged DNA. The positive surface charge causes anions to predominantly fill the overlapped double layers and switches permselectivity from cations to anions, reversing the rectification direction of the current–voltage (I–V) relation (Fig. 1A). The rectification direction upon electrostatic adsorption of DNA to the PLL layer reverts back again with permselectivity in the overlapped double layer having switched back to cations (Fig. 1B). We show that not only does the reversal of the rectification direction signal the presence of DNA but also determine its concentration. This is because the reversal of the rectification direction occurs gradually and at a rate commensurate with DNA mass concentration in the range relevant to PCR conditions. We also show that nanopipettes can be reproducibly used without degradation to their sensitivity upon regenerating their surface with a PLL coating following each measurement. By applying the method to the amplification of a 600-bp segment of human ABO blood group gene, we further demonstrate that nanopipettes allow the PCR progress monitored through simple electrical measurements.

2. Material and methods

2.1. Glass nanopipette fabrication

Glass nanopipettes were fashioned from thin-walled borosilicate glass capillaries with an outer diameter of 1.0 mm and an

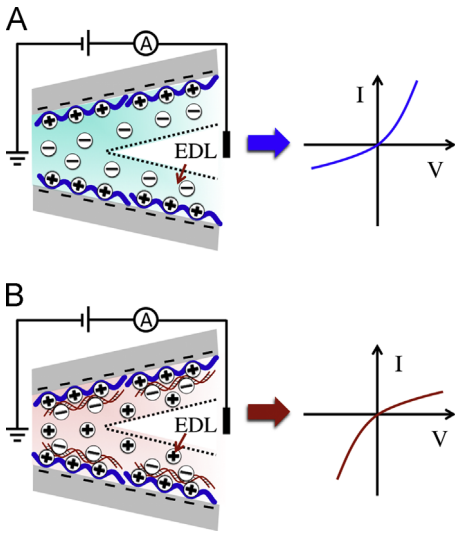

Fig. 1. Biosensor principle. Electrical detection of DNA based on its intrinsic charge modulating the ion current rectification of a nanofluidic diode. Cross-sectional schematics describe a conical nanofluidic diode profile, the nanopipette tip, with the electrical double layers (EDL) overlapped and filled predominantly with the counterions biased under the potentials applied to the electrodes. The current–voltage (I–V) plots are measured to evaluate the relevant ion current rectification. (A) The bare nanopipette tip exposed to cationic polyelectrolyte poly-L-lysine (PLL) adsorbs a thin layer of PLL (blue) on the negatively charged glass surface and thus switches permselectivity to anions; negative currents become suppressed. (B) Subsequently, the same tip exposed to DNA adsorbs a thin layer of DNA (red) on the PLL and accordingly switches permselectivity to cations; positive currents become suppressed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)