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Lab-on-a-chip thermoelectric DNA biosensor for label-free detection of nucleic acid sequences

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ABSTRACT

This study describes a thermoelectric method for detection of nucleic acid sequences that is based on measurement of the heat that is released when two DNA sequences hybridize. Microfluidic biosensor with an integrated thin-film antimony/bismuth thermopile was fabricated and successfully used to detect the heat released during DNA hybridization reaction. The thermopile was attached to the external surface of the lower channel wall and measured the dynamic change in temperature caused by the reaction. The intrinsic rejection of common-mode thermal signals by the thermopile in combination with hydrodynamic focused flow allows measurement of temperature changes on the order of 10^{-4} K without control of the ambient temperature. The microfluidic calorimeter contains a single flow channel, 100 μm high and 12 mm wide, two inlets and single outlet. An oligonucleotide probe, immobilized on the inner surface of the lower channel of the biosensor hybridized to its complementary target that is introduced through one of the inlets. The total sum of the area under the curve of the thermoelectric response was calculated as 30 μVs. The accuracy of the method to discriminate between complimentary and non-complimentary targets sequences was investigated by measuring the thermoelectric response when a mismatched sequence was introduced in the device. The described thermoelectric lab-on-a-chip device accurately detected complimentary and non-complimentary sequences. The applications of the DNA biosensor include detection of specific nucleic acid sequences for gene identification and pathogen detection.

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1. Introduction

The field of DNA biosensor technology has rapidly grown over the last several decades. DNA biosensors are used to obtain DNA sequence specific information in a fast and simple manner and have wide range of application including DNA based diagnosis and pathogen detection [1]. Biosensors couple a biological recognition element that interacts with the target analyte and a physical transducer that translates the biorecognition event into a signal. Common transducing elements, including optical, electrochemical or mass-sensitive devices, generate light, current or frequency signals, respectively [2]. DNA biosensors are bio affinity sensors based on a selective binding of nucleic acids to a surface immobilized probe [3].

Microfluidic technology has been successfully used in the fields of gene identification and detection of nucleic acid hybridization. Lab-on-a-chip technology allows sensitive detection of DNA, provides enhanced mass transport of the analytes, and offers the possibility for parallel processing and integration in complex systems [4]. The rate of nucleic acid hybridization within a microfluidic channel is affected by several factors such as flow velocity, DNA concentration and channel height. Reduction of the channel height combined with higher flow rate enhances the mass transport of the target DNA to the surface immobilized probes and increases the hybridization signal. Compared to passive hybridization, the hybridization of DNA in a microfluidic channel generates higher signal intensities at lower concentration of the target DNA [5].

Sensitive detection of nucleic acid sequences requires amplification of specific genomic targets. While fluorescent detection is the most widely used technology for detection of amplified nucleic acid sequences, it suffers from a number of limitations. It is difficult to consistently incorporate fluorophore labeled nucleotides into DNA strand during the amplification process. As a result, it is hard to achieve the consistent and accurate generation of labeled DNA samples [6]. Fluorescence detection requires sensitive equipment such as charge-coupled devices (CCD) cameras or microarray scanners [7,8]. Such technological requirements limit the access of many labs to the technology. Intercalating dye binds to all the double-stranded nucleic acid sequence, including non-specific products

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that provide a false estimation of the target concentration [9]. The use of a molecular beacon requires sophisticated design and optimization of the dye/quencher pair, and this limits its use in biosensors applications [10].

Contrary to fluorescent detection that requires labeling with various chemical reagents in order to detect target nucleic acid, the label-free thermoelectric method, described in this work, provides direct information about the nucleic acid sequence by measuring the heat generated during the DNA hybridization reaction. The probe nucleic acid sequence is immobilized to the surface of the device while the target sequence is introduced in the system. The detection of the heat is performed in a microfluidic biosensor with an integrated thin-film antimony–bismuth (Sb/Bi) thermopile. The thermopile sensor is composed of 60 thermocouples connected in series that converts the thermal energy into electrical energy. The output voltage is proportional to local temperature difference between the measuring and the reference junctions. The sensitivity of the thermopile is determined by the Seebeck coefficient that represents the magnitude of an induced thermoelectric voltage in response to a temperature difference. The Seebeck coefficient for the Sb/Bi thermopile used in the experiments is 7 $\mu V\,mK^{-1}$.

Thermopiles are used as sensors in microfluidic calorimetry for detection of biochemical reactions because they have a high-common mode thermal noise rejection ratio and are well suited for miniaturization [11]. Microfluidic calorimeter with an integrated thermopile offers several advantages, including label-free detection, low sample volume and, most importantly, accuracy and cost-effectiveness [12,13]. The output of a calorimeter with an integrated thermopile depends on the flow rate, the concentration of the reactants as well as where the location of the reaction zone relative to the measuring junction of the thermopile [14].

The described thermoelectric DNA biosensor provides rapid, easy to use and sensitive technology for detecting nucleic acid sequences that have applications in the areas of DNA based diagnosis and pathogen detection. The rationale behind the thermoelectric method is based on the fact that the heat that is released during DNA hybridization depends on the length and the structure of the nucleic acid sequence. Introduction of single or several mismatched base pairs in the target strand will affect the amount of heat released during the hybridization reaction. In this work, we measured the heat released during the hybridization of 23 bases long probe with its complimentary sequence. The calculated amount of heat released during the reaction is $-182.2\,kcal\,mol^{-1}$. Introduction of five mismatches in the target sequence reduces the enthalpy of the reaction to $-141.9\,kcal\,mol^{-1}$ [15,16]. During the hybridization reaction, hydrogen bonds are formed between the two complementary strands of nucleic acids that lead to the formation of the double stranded nucleic acid molecule. Two hydrogen bonds are formed between adenine and thymine and three hydrogen bonds are formed between cytosine and guanine [17]. The identity of the adjacent bases also affects the enthalpy of the hybridization reaction. As a result the amount of heat released during a DNA hybridization event is a function the length and the structure of the single strands [18]. Since the amount of heat that is released during a hybridization event is sequence specific, target nucleic acid can be identified by measuring the amount of heat that is released during its hybridization to a surface immobilized sequence with known structure.

Detection and discrimination of specific nucleic acid sequences is required for gene expression analysis, detection of genetic mutations, pathogen detection and genotyping. The hybridization reaction occurs in two steps: initial nucleation formation that is followed by a zippering reaction. Nucleation occurs when a few base pairs form a short hybrid. The nucleation rate depends on the temperature, DNA concentration, solvent viscosity, and ion concentration. In a typical hybridization reaction, numerous nucleation events take place until the correct base pair is formed. The rate-limiting step of the DNA hybridization reaction is the actual nucleation reaction that is the formation of a few correct base pairs. Once the nucleation is completed, it is followed by a quick hybridization of the rest of the molecule [19]. When the hybridization reaction occurs at the surface of the device, the rate of access of the probe to the target is decreased. Reduced access to the nucleation sites leads to lower rate of hybridization reaction. Mathematical simulations of hybridization kinetics at the interface of solid and liquid phases reveal that the hybridization process occurs via two mechanisms: direct hybridization from the bulk phase or hybridization after an initial nonspecific adsorption step to the surface followed by surface diffusion to the probe [20].

We report a lab-on-a-chip method for direct detection of nucleic acid sequences that is based on measurement of the heat released during DNA hybridization reaction using thin-film thermopile senor. Additionally, we investigated the specificity of the method to discriminate between complimentary and non-complimentary sequences. The effect of surface passivation on non-specific adsorption of DNA to the inner surface of the channel wall and its effect on the thermoelectric response is evaluated.

2. Materials and methods

2.1. Thermopile fabrication

Antimony–bismuth thermopiles with 60 thermocouple junction pairs were manufactured using custom designed metal shadow masks containing the patterns for creating the thermopile's thin metal lines (Town Technologies, Inc., Somerville, NJ). Two different shadow masks were designed for the deposition of bismuth and antimony metals. A rectangular piece of 75 mm × 25 mm polyimide film was placed behind the shadow mask, designed to create the bismuth line pattern. The thin-film was suspended above the evaporator heat source in the metal evaporation chamber. Bismuth shots (Sigma–Aldrich, St. Louis, MO) were heated and the vapors condensed on the polyimide film to form the bismuth metal line pattern. The polyimide film was removed from the evaporation chamber, and the shadow mask for antimony deposition was aligned to overlap with the bismuth lines at the thermocouple junctions. The metal evaporation process was repeated using antimony shots (Sigma–Aldrich, St. Louis, MO). Following the deposition of the antimony, the polyimide support containing the thermopiles was removed from the metal evaporation chamber, tested for electrical continuity and protected from physical damage using thin polyimide tape. The thermopile sensor is shown in Fig. 1.

2.2. Nucleic acid probe immobilization

Biotinylated probe (5′ GGA CTA TAA AGA TAC CAG GCG TT 3′), the complimentary oligonucleotide (5′ TTA ACC GGT ACG AAC GCC TGG TAT CTT TAT AGT CCA TC 3′) and the non-complimentary sequence (5′ AAA ATC GGC ACC GGT GGA GCG ATC 3′) which have been designed for pyrosequencing system quality control assessment, were synthesized and HPLC purified by IDT (Coralville, IA) [21]. The probe and the target sequences were suspended in phosphate buffer, pH 7.2 (BDH®, VWR International, West Chester, PA) to final concentration of 100 μM. A rectangular area that measures 8 mm × 3 mm was marked on the streptavidin coverslip (Arrayit, Sunnyvale, CA) and located directly atop the measuring junctions of the thermopile. Twenty μL of the biotinylated probes were pipetted within the marked area. The coverslip was incubated in a humidity chamber (37 °C, 90% humidity) for 15 min and washed using 1 × SSC buffer (VWR International, West Chester, PA). A schematic of the probe immobilization on the coverslip is shown in Fig. 2.

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