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Implementing heat transfer resistivity as a key element in a nanocrystalline diamond based single nucleotide polymorphism detection array

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ABSTRACT

In this article, we report on the label-free real-time thermal monitoring of the denaturation of specific DNA fragments and its potential to detect and quantify single nucleotide polymorphisms (SNPs). Probe DNA, consisting of a 36-mer fragment was covalently immobilized on nanocrystalline chemical vapour deposition (CVD) diamond platforms and hybridized with a 29-mer target DNA fragment (full matching and/or with a point mutation). It was observed that the change in heat transfer resistance upon denaturation is dependent on the amount of DNA hybridized to the nanocrystalline diamond (NCD) surface. Furthermore the possibility to distinguish between a full matching sequence and its singularly mutated counterpart, when bound to the same NCD surface, was investigated. NCD surfaces were selectively hybridized with both full matching and mutated DNA fragments at different ratios (3:1, 2:2 and 1:3). A clear bipartite response in heat transfer resistivity was observed upon simultaneous denaturation of these DNA fragments. Denaturation temperature could be used to identify the DNA fragment to which each partial response could be attributed. Moreover, the partial increases in heat transfer resistivity related to the hybridized amount of non-mutated or mutated DNA, respectively. These results imply that heat transfer resistivity is a technique which can be used to (i) quantify DNA fragments of interest, (ii) detect and (iii) quantify SNPs in a mixture of mutated and non-mutated DNA fragments. Moreover, it illustrates the potential of this technique to detect SNPs without the necessity to design complex microarrays.

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1. Introduction

Diamond has proven to be an excellent platform for biomedical research due to its outstanding material properties such as chemical inertness, high thermal conductivity, and electronic properties [1]. In addition, intrinsic diamond displays a high chemical and electrochemical stability and has a wide band gap (5.5 eV) [2–4]. In recent years there has been a major development of DNA electrochemical biosensors such as field-effect sensors [5–7] and sensors monitoring electrical surface properties such as conductance, resistance [8] and capacitance [9]. In addition, diamond proved to be a good transducer for protein sensing as well [10–12]. In previous work it was established that the monitoring of changes in heat transfer resistance of double stranded DNA (dsDNA), which was single stranded covalently bound to a nanocrystalline diamond (NCD) surface, upon denaturation is an interesting approach to measure duplex stability and as such discriminate between different DNA sequence fragments [13]. The change in heat transfer resistance upon denaturation of the dsDNA to ssDNA was hypothesized to be due to the difference in geometrical configuration between dsDNA and ssDNA, which is, respectively, collapsed or erected on the surface. In the future this technique can allow for the development of a fast and low effort, label-free biosensor to detect gene sequence polymorphism based hereditary diseases such as Alzheimer's disease [14], phenylketonuria [15] and specific types of cancer [16]. Alternatively, a biosensor to distinguish between closely related target and non-target organisms, based on 16S rRNA gene sequence polymorphisms, can be developed with this technique. Such a sensor would be widely applicable to detect human pathogens in patients [17,18], food industry [19] and

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drinking water production, to identify plant pathogens in agriculture [20] or to detect bacterial contaminants during food production [21]. Elaborating on previous results [13] this study focuses on the effect size of heat transfer resistance changes as a function of the attached amount of target DNA. For this purpose, hybridization was restricted to a range of specific areas of the NCD surface. To do so, a PDMS flow cell was designed to divide the NCD surface in four identical and fully isolated areas, in which hybridization could take place. The change in heat transfer resistance in response to denaturation was analysed in function of hybridization surface coverage (25%, 50%, 75% or 100%). Secondly, it is investigated if one can distinguish between a full matching sequence and a point mutated sequence when bound to the same NCD surface. The same PDMS flow cell was used to selectively hybridize NCD surfaces with full matching DNA fragments and singularly mutated sequences at different ratios (3:1, 2:2 and 1:3). It was investigated whether the theoretical difference in stability between the two types of DNA fragments would also translate into a two-step response in heat transfer resistance upon denaturation. The aim of this article is to gain insight in the surface coverage to effect size ratio as an analogue to a dose response curve and to show an early stage DNA micro-array where one can identify two different sequences with different melting temperatures by means of only two thermocouples and an adjustable heat source.

2. Experimental

2.1. Synthesis of nanocrystalline diamond

10×10 mm^2 doped (10–20 kΩcm) p-type crystalline silicon wafers (100) were seeded with a water-based colloid of ultra-dispersed detonation (nano)diamond. NCD films with thicknesses of ~300 nm and grain sizes of 100 nm were grown on this silicon substrate, using microwave plasma-enhanced chemical vapour deposition (MPECVD) in ASTEX reactor equipped with a 2.45 GHz microwave generator. This is achieved by using a standard mixture of 15 sccm methane gas (CH_4) and 485 sccm hydrogen gas (H_2) to deposit the NCD thin films on the silicon wafer. The growth was performed under a pressure of 45 Torr, and temperature of 750 °C, the microwave power was set to 4000 W. The growth rate was ~ 390 nm/h.

2.2. Sample preparation

Probe DNA, consisting of a 36-mer single stranded DNA (ssDNA) fragment (3′-CCA AGC CCC CAT ATG TAC CCG ACG TCC CC - A AAA AAA C_6H_{12}-NH_2-5') was covalently immobilized on NCD electrodes. First, the diamond surface is hydrogenated. The hydrogenation is done at 700 °C during 30 s at 3500 W, 12 kPa and 1000 sccm hydrogen gas (H_2) [22]. After hydrogenation the samples are placed inside a glovebox under nitrogen atmosphere. The hydrogenated NCD is then covered with a thin film of unsaturated fatty acid (10-undecenoic acid) and exposed to UV radiation (254 nm, 265 mW/cm^2) for 20 h under nitrogen atmosphere. The double bonds of the unsaturated fatty acid chains will break and a covalent bond with the hydrogen-terminated diamond is established. This process is mediated by photoemission from the surface as proposed for the photochemical grafting of alkenes to silicon surfaces [3,4]. The fatty acid layer is about 2 nm thick [23]. The unbound fatty acid chains are washed off using acetic acid and milliQ water at 120 °C. In this way a carboxyl (COOH) terminated NCD surface is obtained. Zero-length 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide (EDC) is used for the covalent coupling of the 5′ side of an amino-modified 36-mer ssDNA fragment to the carboxyl-terminated surface in 2-[N-morpholino]-ethannesulphonic acid (MES) buffer at 4 °C. These conditions were previously reported to result in a probe areal density of 8×10^{12}/cm^2, as evaluated by X-ray photoemission spectroscopy [13].

2.3. PDMS flow cell

A mold is fabricated in Teflon (PTFE) to serve as the master template, through numerical control milling. Four identical triangles, which occupy an area of 10 mm^2 each, with a height of 1 mm are cut from a PTFE block, as can be seen in Fig. 1. The master mold is then encapsulated in uncured polydimethylsiloxane (PDMS) polymer (Sylgard 184, Dow Corning), which is mixed in a 10:1 ratio with the curing agent. The mixture is degassed for 30 min at an absolute pressure of 50 kPa, to remove trapped gas bubbles. Curing of the PDMS in an oven at 120 °C takes approximately 20 min. The cured daughter-mold can be gently peeled from the master template and cut to the required dimensions with a lancet. A biopsy punch (Miltex) with a 1 mm inner diameter is used to core both an inlet and outlet as connection ports for each triangular cavity. PTFE tubing (1.2 mm outer diameter) is press-fitted in the connection ports [24], to form a reliable connection without additional bonding or molding. Finally, the PDMS mold is pressed onto the diamond covered silicon substrate. As such it provides four completely separated identical areas for hybridization on the NCD sample.

2.4. Selective hybridization

In a following step, 6 μl Alexa-488-modified ssDNA (100 pmol/μl) is mixed with 14 μl 1× PCR buffer and per mm^2 4 μl of this mixture was selectively added to specific areas of the ssDNA-modified NCD sample by means of the PDMS flow cell. As such, about 10^5 times more DNA is provided for hybridization than probe DNA is available on the NCD surface. The sample is then incubated at 35 °C for 2 h for hybridization to take place. Non-specifically bound DNA is removed using a double washing step. In a first step, the sample is washed with 2× saline sodium citrate (SSC) + 0.5% sodium dodecyl sulphate (SDS) for 30 min. Secondly, the sample is washed twice with 0.2× SSC at 30 °C for 5 min. Finally, the sample is rinsed with phosphate buffered saline (PBS) of pH 7.2 and stored in PBS at 4 °C [2]. The use of low salt concentrations in comparison to the hybridization buffer and by washing at temperatures lower than hybridization temperature, premature denaturation is avoided. Both full match ssDNA fragments (5′-GGT TCG GGG GTA TAC ATG GGC TGC AGG GG-3′) and ssDNA fragments with a point mutation at position 20 (5′-GGT TCG GGG **C**TA TAC ATG GGC TGC AGG GG-3′) were used for hybridization. Because of the abundant amount of DNA provided for hybridization, all probes available on the NCD surface can be assumed hybridized.

2.5. Fluorescence imaging

Fluorescence images were taken on a Zeiss LSM 510 META Axiovert 200 M laser scanning confocal fluorescence microscope. To excite the FAM-488 fluorescence dye, a 488 nm argon-ion laser was used with a maximum intensity at the sample surface of 30 μW to avoid bleaching during the image acquisition. The peak emission has a longer wavelength of 518 nm due to vibrational relaxation of the FAM molecule after photon absorption. All images were collected with a 10 × 0.3 Plan Neofluar air objective with a working distance of 5.6 mm. The pinhole size was 150 μm and the laser intensity was set at 10%. The detector gain, being a measure for the photomultiplier

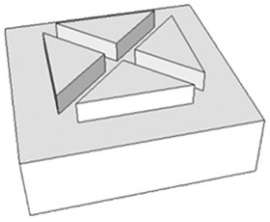

Fig. 1. CAD drawing of the flow cell master mold.

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