Sensors and Actuators B 230 (2016) 260–271

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

# Heat-transfer based characterization of DNA on synthetic sapphire chips

M.S. Murib[a,*], W.S. Yeap[a], Y. Eurlings[a], B. van Grinsven[a], H.-G. Boyen[a,c], B. Conings[a], L. Michiels[b], M. Ameloot[b], R. Carleer[a], J. Warmer[d], P. Kaul[d], K. Haenen[a,c], M.J. Schöning[e], W. De Ceuninck[a,c], P. Wagner[a,f]

[a] Hasselt University, IMO, Wetenschapspark 1, B-3590 Diepenbeek, Belgium
[b] Hasselt University, BIOMED, Agoralaan, B-3590 Diepenbeek, Belgium
[c] IMEC vzw, IMOMEC, Wetenschapspark 1, B-3590 Diepenbeek, Belgium
[d] Bonn-Rhein-Sieg University of Applied Sciences, von Liebig Str. 20, D-53359 Rheinbach, Germany
[e] Aachen University of Applied Sciences, INB, Heinrich-Mussmann-Str. 1, D-52428 Jülich, Germany
[f] KU Leuven, Soft Matter and Biophysics Section, Celestijnenlaan 200 D, B-3001 Leuven, Belgium

ARTICLE INFO

*Article history:*
Received 23 December 2015
Accepted 7 February 2016
Available online 10 February 2016

*Keywords:*
Synthetic sapphire
Nanocrystalline diamond
Heat-transfer method
Fourier-transform infrared spectroscopy
Thermogravimetric analysis
X-ray photoelectron spectroscopy
Confocal fluorescence microscopy
Single-nucleotide polymorphisms in DNA

ABSTRACT

In this study, we show that synthetic sapphire ($Al_2O_3$), an established implant material, can also serve as a platform material for biosensors comparable to nanocrystalline diamond. Sapphire chips, beads, and powder were first modified with (3-aminopropyl) triethoxysilane (APTES), followed by succinic anhydride (SA), and finally single-stranded probe DNA was EDC coupled to the functionalized layer. The presence of the APTES-SA layer on sapphire powders was confirmed by thermogravimetric analyis and Fourier-transform infrared spectroscopy. Using planar sapphire chips as substrates and X-ray photoelectron spectroscopy (XPS) as surface-sensitive tool, the sequence of individual layers was analyzed with respect to their chemical state, enabling the quantification of areal densities of the involved molecular units. Fluorescence microscopy was used to demonstrate the hybridization of fluorescently tagged target DNA to the probe DNA, including denaturation- and re-hybridization experiments. Due to its high thermal conductivity, synthetic sapphire is especially suitable as a chip material for the heat-transfer method, which was employed to distinguish complementary- and non-complementary DNA duplexes containing single-nucleotide polymorphisms. These results indicate that it is possible to detect mutations electronically with a chemically resilient and electrically insulating chip material.



## 1. Introduction

Molecular electronics and bio-analytical sensing have in common that both require an in-depth understanding the electrical properties of molecules and molecular layers on surfaces [1]. In recent years, biological detection has been revolutionized by surface-based methods for the detection of biological targets such as cells [2], DNA [3] and proteins [3]. In biomedical sciences, the detection of DNA hybridization or protein binding to surfaces is accomplished through a variety of "biochip" devices, lending particular importance to biologically modified surfaces [4]. Regarding the substrate materials for biosensors, extensive work has been carried out on gold [5–9], silicon [10–12], glass [13], and carbon-based materials [14–18]. Gold seems ideal for electronic sensing principles but is not the most economic choice. The degradation of silicon and glass interfaces in aqueous solutions limits their use to that of disposable biosensors [19,20]. Carbon-based materials such as diamond or graphene are often used in electrochemical biosensors due to their inertness, low electrical resistivity and beneficial mechanical properties [14–18]. Different graphene-based materials were used as an electrochemical platform for the detection of a variety of chemicals and biomolecules [21]. However, the surface modification of graphene is rather difficult to accomplish because of its surface inertness due to the $\pi$ orbitals [22]. Chemical-vapor deposited (CVD) diamond has proven to be an excellent sensor-chip material due to its chemical inertness, stability at elevated temperatures, biocompatibility, and excellent thermal conductivity [23]. Moreover, diamond can be doped with boron up to metallic conductivity values [24]. So far, CVD-diamond chips have been

* Corresponding author. Ghent University, Department B-9000 Gent, Belgium of Information Technology INTEC, Sint-Pietersnieuwstraat 41, B-9000 Gent, Belgium.
*E-mail address:* mohammed.murib@aum.edu.kw (M.S. Murib).

http://dx.doi.org/10.1016/j.snb.2016.02.027
0925-4005/

employed for electronic sensor experiments on chemical- and thermal DNA denaturation [14–17], and in combination with aptamers [25], enzymes [26], and glucose [27]. Also, conducting diamond has been shaped into microelectrode arrays for eye-retina implants [28].

Recently, Deng and Toh functionalized a free-standing nanoporous alumina membrane with a specific DNA sequence of the dengue virus [29]. Ultra-thin platinum layers were deposited on both sides of the alumina membrane to be used as working- and counter electrodes for differential pulse voltammetry and impedance measurements. Couniot et al. employed aluminum-made microelectrodes, passivated with a thin aluminum-oxide layer, for the selective detection of bacteria [30]. In the present contribution, we will show that synthetic sapphire on its own can serve as a platform material for biosensors with properties comparable to those of nanocrystalline diamond. Sapphire shows outstanding chemical inertness, wear resistance, and biocompatibility [31]. Hence, it is widely used as an implant material in crystalline- or ceramic form such as in hip implants [31], dental implants [32], and endosseous implants [33]. Moreover, its wide optical transmission band from ultra-violet (UV) to near-infrared (near-IR) [34] suggests application potential in optical biosensors [35]. Due to the fact that sapphire has a high thermal conductivity [34] and a low thermal expansion [36], it appears also favorable for use in optical sensors operating in a broader temperature regime. Table 1 summarizes the physical properties of several materials, *i.e.*, sapphire, diamond, silicon, quartz, and copper regarding their thermal expansion, thermal conductivity, and hardness. A previous theoretical study based on elastic light scattering showed that sapphire and diamond have a comparable optical detection capability for the binding of proteins [37]. So far, sapphire has not been used as a platform material for biosensors based on electronic readout principles due to its electrically insulating behaviour [38]. However, thanks to the biosensoric heat-transfer method HTM, the immobilization platform does not necessarily need to be electrically conducting [16]. Moreover, synthetic sapphire is relatively cheap with a price of *ca.* 10 € for 1 $cm^2$ chip surface in single-crystalline, highly oriented quality. For comparison, nanocrystalline diamond surfaces come at about 25 €/$cm^2$. Hence, we report here on the surface modification of several sapphire-based materials including amorphous powders, polycrystalline microbeads, and single-crystalline chips with carboxylic groups and double-stranded DNA. We also show that the heat-transfer method, applied to the sapphire-liquid interface, allows to detect single-nucleotide polymorphisms in DNA.

## 2. Experimental

### 2.1. Surface functionalization

#### 2.1.1. Hydroxylation

As a starting point, the sapphire powder and beads were used for optimizing the DNA coupling protocol. The surface composition after each functionalization step was hereby followed by TGA (thermogravimetric analysis) and FT-IR (Fourier-transform infrared spectroscopy). The sapphire beads (ALODUR) were bought from Treibacher Industrie AG (Althofen, Germany) and the powder was purchased from Nabaltec AG (Schwandorf, Germany) under the commercial name Granalox NM 9622. Details on the synthesis and physical characteristics of these materials can be found on the company websites. Prior to any functionalization, the powders and the beads were annealed in air at 500 °C for 5 h to remove any surface-bound organic impurities. Single-side polished single-crystalline $\alpha$-$Al_2O_3$ chips, orientation (0,1,$\bar{1}$,2), were purchased from CrysTec GmbH (Berlin, Germany).

The chip dimensions were $10 \times 10 \times 1$ $mm^3$ and the root-mean-square roughness of the polished surfaces, measured by atomic force microscopy, was 0.44 nm over an area of $1 \times 1$ $\mu m^2$. All sapphire samples were ultrasonically cleaned in acetone, ultrapure water (Sartorius Stedim Biotech, Ultra Pure Water System Type 1), and isopropanol for 20 min in each bath. Next, the sapphire samples were immersed in 10 mM $HNO_3$ solution at room temperature for 30 min to create OH groups on the surface [42,43]. Finally, the sapphire samples were rinsed again with ultrapure water and dried with nitrogen gas. The sequence of all surface-functionalization steps is illustrated in Fig. 1, starting with the $HNO_3$ treatment in Fig. 1a.

#### 2.1.2. Silanisation

Silanisation of the sapphire samples was carried out by liquid-phase deposition of silanes in an organic solvent [44]: the samples were placed in a 600 mM solution of (3-amino-propyl) triethoxysilane (APTES, 99%; Sigma–Aldrich, Steinheim, Germany) in toluene (≥99.9%, Sigma–Aldrich) for 15 h in a nitrogen-filled glovebox. To wash off unbound APTES molecules, the samples were rinsed with toluene, followed by tetrahydrofuran (THF, ≥99.9%, Sigma–Aldrich). After drying the samples under nitrogen flow, they were cured for 2 h at 150 °C, resulting in an especially strong adhesion of the APTES layer to the surface [44]. This way, the surface becomes modified with amines, allowing for the subsequent reaction with succinic anhydride (SA) in order to yield a carboxyl-terminated surface [45].

Succinic anhydride (SA, ≥99.9%, Sigma–Aldrich) was used as a cross-linker molecule to ensure the binding of the amino group of APTES to the 5′-$NH_2$ terminus of the oligonucleotides by providing carboxylic groups at the surface. For this purpose, the samples were placed overnight in a solution of 800 mM of SA in acetonitrile (ACN) (anhydrous, 99.8%, Sigma–Aldrich), after which they were rinsed with pure acetonitrile and dried under nitrogen gas. The amine-terminated surface reacts with one of the two carbonyl groups of the SA molecules, causing the anhydride ring to open and to form a covalent bond with this carbonyl group. The other carbonyl group is released to form a free carboxylic acid-terminated surface [46]. In principle, the outlined protocol can be further simplified by using COOH-terminated DNA from the beginning, meaning that the crosslinker SA will not be necessary anymore.

#### 2.1.3. DNA grafting

Table 2 shows the base sequences of the probe DNA (36 bases), the corresponding full-match target DNA (29 bases), target DNA with a resulting CC mismatch at base pair 7, and target DNA with a CC mismatch at base pair 20. The position of the mismatches with respect to the probe DNA is underlined and indicated in bold letters. The first 7 adenine bases at the 5′ terminus in the probe DNA served as a spacer to minimize steric hindering during hybridization due to the proximity of the solid surface. The amine ($NH_2$) modification at the 5′ terminus in the probe DNA is used as a linker group of the DNA to the carboxyl-terminated surface. The target-DNA fragments carry a fluorescent Alexa-488 label at the 5′ end to allow for an optical verification of the presence of target DNA (and probe DNA) on the chip surface by confocal fluorescence microscopy.

The ‘zero-length’ crosslinker EDC 1-ethyl-3-3-dimethylaminopropyl-carbodiimide (EDC, Thermo Scientific, Erembodemgem, Belgium) was used for the covalent coupling of the 5′ terminus of amino-modified ssDNA fragments (36 bases) to the carboxylic acid-terminated surface in 2-[*N*-morpholino]-ethanesulphonic acid (MES) buffer at 4 °C. For this, the carboxylic acid-terminated sapphire samples (powders, beads, and chips) were incubated for 2 h in a solution made of 30 μL of probe DNA (10 pmol/μL) and 20 μL EDC (0.26 M) in MES buffer (25 mM, pH