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Biosensors by means of the laser induced forward transfer technique

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ABSTRACT

This work presents the direct laser printing of thiol-modified oligonucleotides onto the surface of 3-glycidoxypropyltrimethoxysilane (GOPTS)-functionalized ultra-thin Low temperature oxide (LTO)/Si membranes for the fabrication of a label-free capacitive biosensor. During the hybridization of the target oligonucleotides with their immobilized complementary strands, various interaction forces develop, which contribute toward the deflection of the ultrathin LTO membranes, resulting in a capacitance shift. A number of different parameters were investigated for the laser printing conditions of the oligonucleotides that in turn affect the maximum deflection of the membranes and therefore the capacitance variation.

1. Introduction

Laser induced forward transfer (LIFT) is a direct write technique that allows the deposition of liquid and solid materials with high spatial resolution. The LIFT technique was first employed by Bohandy et al. [1] for the deposition of metals and since then, has been used for the printing of many different types of materials such as superconductors [2] and polymers [3], but most importantly for the printing of biomolecules such as cells [4], proteins [5] and DNA [6].

The technique relies on the displacement of the material to be deposited from a donor substrate to a receiver substrate. The donor substrate in most liquid phase LIFT experiments consists of three layers [7,8]. The first layer must not demonstrate spectral absorption to the laser beam (quartz), the second layer is an absorbing titanium layer (Ti) and the third layer corresponds to the material to be transferred. Irradiation of the donor substrate results in the rapid heating of the metal film and the explosive boiling of the adjacent liquid, thus producing a high vapor cavity, which expands and drives through the remaining film [9]. The receiving substrate is placed parallel to the donor substrate, so that, following irradiation, the biomaterial is printed onto the former.

The LIFT technique has significant advantages over several other deposition techniques such as spotting [10], inkjet [11] and photolithography [12] since it is a contactless direct write technique, which does not require the use of masks or nozzles. Both LIFT and droplet-on-demand inkjet printing techniques have the ability to deposit micrometer-sized droplets of ink into user-defined patterns. However, since LIFT technique is nozzle less, it can handle a wider range of ink properties and does not suffer from clogging and material compatibility issues associated with the more prevalent nozzle-based techniques [13].

In this paper we present the direct laser printing of three different oligonucleotide probe sequences onto the surface of capacitive sensors' membranes [14]. The capacitive sensor consists of 60 ultrathin circular LTO/Si membranes onto which the oligonucleotide probe solutions are deposited for the fabrication of the biosensor and 4 aluminum (Al) sensors which are used as a reference for the electronic readout circuit.

The first oligonucleotide was fully complementary to the target analyte in solution, while the second probe formed one internal base pair mismatch with the same target sequence. The third oligonucleotide was non-complementary to the target sequence and was used as a reference sequence to quantify non-specific binding (Fig. 1). The interaction of the DNA probes with their targets can be detected by the stress that develops across the surface of the capacitive membranes. As it has already been reported [15,16], a number of forces contribute toward the phenomena observed across the surfaces of the membranes such as electrostatic repulsions and steric hindrance between the DNA strands, all of which, however, can be shielded off effectively with the use of a buffer of high ionic strength. Recently published work suggests that the major contributing factor toward the surface stress recorded upon DNA immoblization and hybridization is due to hydration forces [17]. As it has been previously shown by our research group, maximizing probe grafting density enhances the

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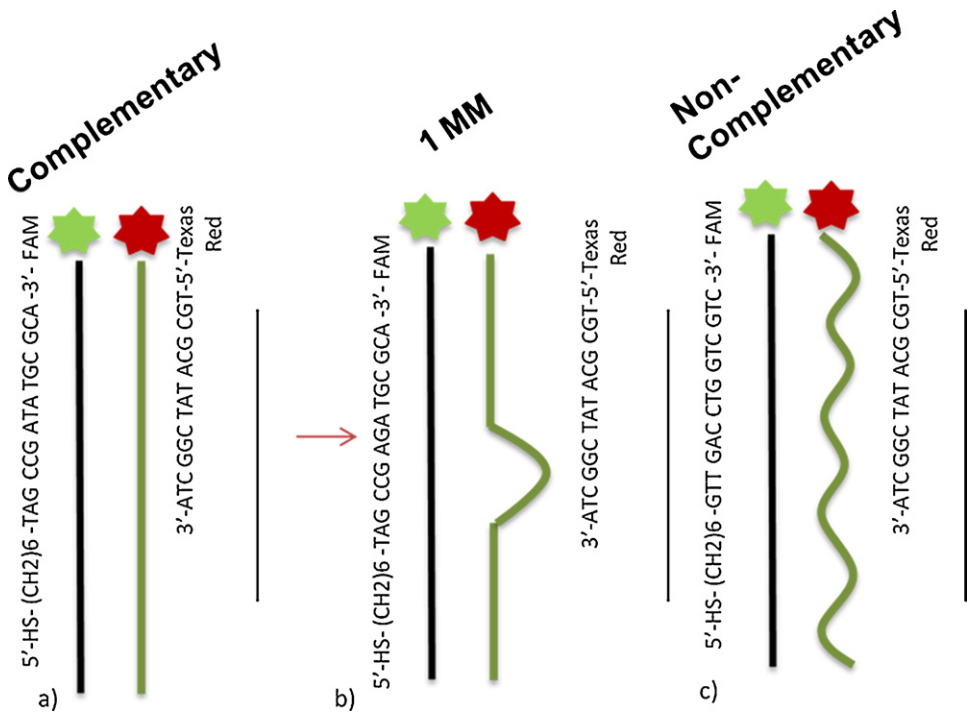

Fig. 1. Schematic representation of the hybridized (a) fully complementary probes, (b) one mismatch probes and (c) non complementary probes, to the target analyte in solution.

hydration forces that develop following target recognition by the deposited oligonucleotides [18]. Detection of single base pair mismatches is achieved by the different surface stress that develops from that recorded when fully complementary strands hybridize. As far as the non-complementary probes are concerned, the surface stress that develops due to non-specific binding is negligible.

2. Materials and methods

2.1. Materials

All reagents were obtained from Aldrich Chemical. All solutions were made with deionized water (18 MΩ cm resistivity) from a Millipore MilliQ system. DNA oligonucleotides were purchased from Eurofins MWG Operon (Ebersberg, Germany). The 15 nucleotide bases-long probe oligomers contained a 5′-thiol C5 linker and were labeled with FAM (6-carboxyfluorescein) at their 3′ end. The sequences of the complementary, the one base pair mismatch and the non-complementary to the target analyte probes were 5′-HS-(CH2)6-TAG CCG ATA TGC GCA-3′-FAM, 5′-HS-(CH2)6-TAG CCG AGA TGC GCA-3′-FAM and 5′-HS-(CH2)6-GTT GAC CTG GTC GTC-3′-FAM respectively. The sequence of the target oligonucleotide was 5′-TGC GCA TAT CGG CTA-3′ and was tagged with Texas Red at the 5′ end. 6-Mercapto-1-hexanol (MCH) was used in a 1.0 mM solution in deionized water. A 2% 3-glycidoxypropyltrimethoxysilane (GOPTS) in 95% ethanol was used for the functionalization of the surfaces. DNA deposition and hybridization were performed from 1.0 M potassium phosphate buffer (0.5 M KH_2PO_4, and 0.5 M K_2HPO_4, pH 8). A washing solution (10 mMNaCl, 5 mM tris(hydroxymethyl)aminomethane-Tris, buffer, pH 7.4) was used to remove unbound probes or target DNA.

2.2. Sensor description

Each capacitive biosensor element consists of a silicon membrane, 1 μm in thickness and 200 μm in diameter. The membrane is formed using a self-alignment process [19], where boron implantation is carried out through a SiO_2 window to create a highly boron-doped region (>7×10^{19} cm^{-3}), which, following selective wet etching of undoped Si, results in the fabrication of the sensing element, a thin flexible membrane. The membrane stands on the rim of the circular SiO_2 cavity as shown in Fig. 2.

In the final steps of the biosensor fabrication process, the surface of the membrane is passivated with a 0.5 μm thick low temperature silicon oxide (LTO) layer and functionalized with GOPTS, onto which the oligonucleotide probes are immobilized. The array also includes four Al capacitors, which are structures where the flexible membrane electrode has been replaced by a fixed electrode of aluminum over the SiO_2. These elements can be used as reference.

Hybridization events between the immobilized probe and the target analyte in solution result in the development of surface stress on the membrane, which increases or decreases the gap between the flexible and the fixed electrodes resulting in a capacitance shift.

2.3. Laser printing of the biomaterial

The LIFT experiments were carried out according to the set-up previously published [18], using a pulsed Nd:YAG laser (266 nm wavelength) with a pulse duration of 10 ns and a high power imaging micromachining system by which the whole printing process was monitored through a customized microscope system, equipped with a camera, which enabled accurate alignment of the target and substrate materials.

A target holder was fixed onto a computer-controlled $x-y$ translation stage, allowing a maximum 25 mm × 25 mm movement and having a 1 μm positioning resolution. A LabVIEW program was used in order to synchronize the x–y motion with the laser. Following the exit of the laser beam from the laser it passes through an attenuator to a rotating base controlled by another LabVIEW program and then it is expanded by a telescopic system of lenses as it finally goes through a circular mask.

The microarrays were printed using a 15× objective to focus the laser beam onto the donor substrate. The donor substrate consisted of a Ti (40 nm) film on quartz plate, onto which the thin liquid film of