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Direct and label-free monitoring oligonucleotide immobilization, non-specific binding and DNA biorecognition

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ABSTRACT

DNA binding chemistry on silicon surface has been investigated. Aminated oligonucleotide probes were immobilized on the chip surface by chemical silanization and further covalent attachment. The chemistries employed were the classical 3-aminopropyltriethoxysilane/glutaraldehyde and, for comparison purposes, the novel 3-isocyanatepropyltriethoxysilane, that allows the direct attachment of the aminated probe. Alternatively, a thiolated oligonucleotide was also photochemically immobilized by means of a thioether linkage. The experiments were followed label-free by Dual Polarization Interferometry. All chemical and photochemical methods gave rise to a probe density immobilization in the order of 1.0–2.5 $\times$ 10^{10} molecules/mm^2, similar to the values reported for other chemistries. The obtained data suggest that DNA strands are anchored in a different conformation depending on the immobilization method employed. In order to avoid non-specific binding of target molecules, ethanolamine and inert proteins were assayed, and successful results were obtained when using small size proteins such as gelatine. Hybridization efficiency was around 20% for aminosilane-based immobilized probes, and more than 4-fold this value when the other immobilization methods were employed. The ability for recognition complementary DNA strands discriminating non-complementary ones was applied for species identification in mixtures.

1. Introduction

The studies and uses of nucleic acids have been growing up for decades, and the trend is to keep on in first line. Thus, basic research on nucleic acids provides fundamental knowledge for in vivo and in vitro applications. DNA-based methods have also been employed in many areas, examples being forensic science [1], environmental control [2] and biosensing [3]. Additionally, new issues addressed to both array-like platforms [4] and lab-on-a-chip arrangements [5] are currently under development.

Nearly all nucleic acid-based methods employ the recognition element immobilized on a surface, so DNA immobilization has been extensively studied in the last years, and many attachment chemistries have been described [6]. Among all the supports used in biosensing, silicon is one of the most popular because this is a highly versatile solid, readily functionalized, biocompatible, and its derivatization for attaching biomolecules has been deeply studied [7].

Many strategies have been developed for detecting DNA hybridization processes. The employment of labels such as fluorophores is well-known, facilitated by the PCR amplification of the target and recommendable to routine high-load analysis. However label-free detection retrieves better information and the processes to be monitored are more similar to what is happening in life or biological systems.

There are several label-free technologies for biosensing and related applications, e.g. Quartz Crystal Microbalance (QCM) [8], Surface Plasmon Resonance (SPR) [9] and interferometry [10]. Among the latter ones, Dual Polarization Interferometry (DPI) is a very interesting option.

DPI is considered a reliable technique for monitoring binding events happening on a silicon oxynitride surface in real time. Basically [11], light from a laser is passed through a sandwiched waveguide structure, and an interference pattern is detected on the opposing side by a CCD camera. The phase shift of transverse magnetic (TM) and transverse electric (TE) modes is recorded in real time, and data are resolved; so that only one value of thickness and absolute refractive index at a given timepoint t will satisfy Maxwell's equations of electromagnetism for both

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TM and TE polarizations. Differences in the waveguide mode dispersion between the TE and TM modes allow unique solutions for adlayer thickness and refractive index, and these data can be converted to surface mass and density. Thus, this technique provides label-free precise measurements, in real time, enabling details of the structure and function of biomolecules to be elucidated. Hence, high-quality information on the orientation, distortion, and efficiency of immobilization procedures as well as the interaction events can be obtained. It is remarkable that a DPI instrument is a quantitative analytical tool, not a sensor. DPI routinely and reproducibly provides simultaneous quantitative data on real-time changes in dimension (resolution < 0.1 Å) and density (resolution < 0.1 pg/mm^2) of biomolecules immobilized on the chip surface, without recourse to tagging, but it is not intended as a sensor system for the highly sensitive determination of the agents that provoke these small changes [12].

DPI is frequently compared to SPR [13] because both are based on similar principles. However, SPR utilizes only the TM mode, while DPI takes advantage of measuring both the TM and the TE polarizations [14], which allows the simultaneous determination of both effective refractive index and thickness values. That is clearly a great advantage over SPR and other optical biosensing techniques, which can only report relative changes of refractive index [15]. Combining the thickness, mass and density data allows not only to monitor the binding but also to retrieve structural information at molecular level, and this is the main benefit of DPI.

Another difference between DPI and SPR or QCM relies on the different solid substrate employed. QCM and SPR use gold as platform, while DPI employs silicon-based chips, this material having the advantage of its low cost, versatility and applicability [7]. Nevertheless, both DPI silicon [16] and SPR gold [17] can be coated with a polymer in order to expand and improve the attaching chemistry.

Applications of DPI can be found in any area where microscopic surface properties need to be monitored, provided that silicon-based chips act as a supports. Interesting reports are the monitoring of lipids [18], polyelectrolytes [19] and other multilayers [20], events happening in liposomes [21], as well as the study of induced conformational changes in proteins [22], molecular aggregations [23], basic protein–protein interactions [24–26] and protein crystallization [27].

Nucleic acids chemistry and their interactions have also been monitored by DPI, in terms of DNA multilayer films formation [28], interactions of DNA aptamers with small molecules [29,30] and even with metal ions [31]. However the studies of DNA hybridization are scarce. Two interesting pioneer papers describe a basic study about monitoring DNA immobilization and hybridization. In the first one [32], single stranded DNA immobilization by passive adsorption, covalent linkage and avidin–biotin interaction is studied, and hybridization is monitored. In the other [33], authors covalently immobilize aminated oligonucleotides on commercial amine-derivatized chips, then block with BSA the remaining active sites, and finally add a complementary oligonucleotide for hybridization, all the three processes being monitored. Both papers are focused on monitoring DNA immobilization and hybridization, with useful discussions, but other issues such as unspecific binding or practical applications are scarcely addressed, there being little or none good contribution papers on this topic.

The aim of the present paper is to describe a basic study of DNA immobilization and hybridization by means of DPI including variables such as chip derivatization (silanization) as well as different modes of DNA anchoring including photochemical activation [34]. Attention is especially paid to the use of different blocking agents to avoid unspecific binding but maintaining specific hybridization. The applicability of the DPI for DNA screening and other practical issues is also discussed. The paper pretends to show that DPI can be a very useful tool for understanding and helping in the designing of reliable biosensors, due to the conformational information provided.

2. Materials and methods

2.1. Materials

Synthetic oligonucleotides for immobilization, hybridization and control are shown in Table 1, and were supplied by Sigma–Aldrich (Madrid, Spain) and TibMolbiol (Berlin, Germany). Silanizing agents: 3-aminopropyltriethoxysilane (APTES), 3-(triethoxysilyl)propyl isocyanate (ICPTS) and 3-glycidoxypropyltrimethoxysilane (GOPTS), as well as blocking proteins: bovine serum albumin (BSA), gelatine, lysozyme and casein, were supplied by Sigma–Aldrich. Ethanolamine, 2-mercaptoethanol and 4,7,10-trioxatridecane-1,13-diamine supplied by Sigma–Aldrich were also used as a blocking agents. All other common chemicals were analytical grade and Milli-Q purified water was employed in all stages.

Phosphate buffered saline (PBS, 10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4), sodium saline citrate (SSC, 150 mM NaCl, 15 mM sodium citrate, pH 7) and carbonate buffer (CB, 100 mM sodium carbonate, pH 9.12) solutions were used as a carrier or medium in all assays. They were previously filtered through a 0.45 μm PVDF filter and degassed by sonication under vacuum prior to be employed.

2.2. Instrumentation

All experiments were performed at 20 °C with an Analight Bio200 Dual Polarization Interferometer (Farfield Scientific Ltd., Crewe, UK). The apparatus consisted of a helium neon laser (λ = 632.8 nm), a sensor chip (24 mm × 5.8 mm) clamped inside a thermostated block (temperature control within 1 mK) and a 1024 × 1024 element-imaging device.

Silicon oxynitride chips (FB 100, Farfield, UK) were used for measurements. The sensor surface has two fluidic interfaces, 2 μL dead volume each, named channel 1 and channel 3, with an additional waveguide reference area, channel 2, having a dielectric cover with constant refractive index.

Carrier and other solutions were flowed using a double-channel precision syringe pump (Harvard Apparatus PHD 2000 Infusion, Kent, UK) and injections were carried out by means of a dual injection valve. The state of polarization of the input beam was switched (50 Hz) between TE and TM using a ferroelectric crystal before passing through the sandwiched waveguide structure, the interference pattern being detected on the opposing side by the element-imaging device. Analight DAQ and Analight Explorer (Farfield) software packages were used for system managing/data acquisition and data treatment, respectively.

2.3. Chip silanization and oligonucleotide immobilization

For silanization using APTES, the chemical vapor deposition or dry method was employed. It consisted of exposing the chip surface to silane vapor at room temperature in a dessicator [35,36], followed by baking at 160 °C for 30 min. Silanized chip was placed in the DPI device, starting the data collecting, and the surface was activated by injecting glutaraldehyde (5%, v/v in PBS, 250 μL at 50 μL/min). Then, on-line oligonucleotide immobilization was carried out by injecting the aminated probes (0.1 μM in PBS, 150 μL at 10 μL/min).

For ICPTS chip derivatization, silanization was accomplished as described for APTES, but changing the silanizing agent. Once the chip was placed in the DPI, glutaraldehyde was not necessary and