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DNA immobilisation procedures for surface plasmon resonance imaging (SPRI) based microarray systems

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Abstract

Two different surface chemistries have been studied for the development of surface plasmon resonance imaging (SPRI) based DNA microarray affinity sensors: (1) 11-mercaptoundecanoic acid–poly(ethylenimine) (MUA–PEI) and (2) dextran procedures. The MUA–PEI method consists of assembling a multilayer on the basis of electrostatic interactions formed with: 11-mercaptoundecanoic acid (MUA), poly(ethylenimine) (PEI) and extravidin layers. The dextran procedure involves assembling a multilayer formed with 11-mercaptoundecanol, dextran and streptavidin layers, which are linked by covalent bonds. The oligonucleotide probes are immobilised onto the sensor surface as spots forming a matrix 14×14, which is spotted by a robot, while the target sequences are free in solution. The system allows the interaction (hybridisation) monitoring, in real-time and in parallel, of unlabeled oligonucleotide solution targets to oligonucleotide probes immobilised on a 196 spots matrix. Using oligonucleotides as probes and targets, both functionalised surfaces have been evaluated in view of their application to the diagnosis of gene mutations involved in human diseases. In particular, we demonstrate the ability to detect, in parallel, several mutations causing human cystic fibrosis (CF), which lie within exon 10 of the human cystic fibrosis transmembrane conductance regulator (CFTR) gene. The immobilised probes were complementary to sequences corresponding the mutant or wild type alleles. Two deletions of three bases (ΔF508 and ΔI507) and four single nucleotide polymorphisms (M470V, Q493X, V520F and 1716 G > A) were investigated. In both functionalised surfaces, the system showed the capacity to discriminate normal and mutant sequences differing by a single base.
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Keywords: DNA immobilisation chemistry; Surface plasmon resonance imaging; DNA microarray; Real-time monitoring; Gene mutation

1. Introduction

The completion of the Genome project opens a new era for medical research and health care. Among the new challenges, particular attention is drawn on the role that human genome variation plays in diseases, and on how mutations or prognosis markers can be detected. In this context, a major focus is the development of new, effective, reliable, user friendly and high-throughput systems for the detection of gene mutations. To this aim, the development of efficient sensor systems allowing simultaneous detection of a large number of mutations is needed. Indeed, most genetic disorders are heterogeneous at the molecular level, and are caused by a large number of various mutations. Among these disorders, cystic fibrosis (CF), the most frequent autosomal human genetic disease in Caucasians, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Van Biervliet et al., 2005). The main mutation (accounting for 70% of CF chromosomes) corresponds to a single phenylalanine deletion at position 508 of the protein (ΔF508), but more than 1000 of other, less frequent mutations, have also been identified.

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The cystic fibrosis molecular tests currently performed in clinical laboratory hospitals are able to identify 30–35 mutations, including the most frequently found in the Caucasian population. These diagnostic kits allow detection of 85% of the defects. This coverage level is insufficient when non-Caucasian or mixed populations are studied, as their spectrum of mutations is more diverse. To reach 90–95% coverage, a genetic test able to screen for at least 100 mutations is necessary. In addition to the increased complexity of populations at risk due to migrations and admixing, the numbers of genetic tests to carry out also augment with the progress in disease knowledge and the need to analyse individuals with CF-related disorders such as chronic bronchiectasis or congenital absence of the vas deferent (CABVD). In addition, the ability to analyse in parallel a set of single nucleotide polymorphisms (SNPs) markers located in relevant genome loci (such as regulatory regions), would greatly improve these disease diagnostic and prognosis tools. Such novel DNA chips, in addition to mutation detection, could allow a one-shot evaluation of the associated patient's genetic background, providing as an added-value predictive information as to the possible evolution of their disease.

In the last few years, DNA microarray technology has become a valuable tool for genomic screening (Campàs and Katakis, 2004), since it allows the study of many DNA sequences in the same experiment rather than one sequence after the other (Bowden and Brennan, 2004). DNA microarray technology therefore provides larger amounts of data faster than previously possible, and holds great promises in genetic diagnosis.

Currently, in the most commonly employed microarray systems, the detection and monitoring of DNA hybridisation relies on the use of radiolabeled and fluorescent probes (Fouqué et al., 2005; Kohlhammer et al., 2004; Deng et al., 2004). Fluorescence, due to low background noise, has a high sensitivity, but requires the use of costly reagents and time-consuming labeling procedures.

The surface plasmon resonance imaging (SPRI) technology is based on the traditional surface plasmon resonance (SPR) principle (Kretschmann and Raether, 1968) with the exception that the organically functionalised metal surface is imaged on a CCD camera by an imaging lens (Zizlsperger et al., 1998; Guedon et al., 2000).

The SPR imaging is a label-free method that allows simultaneous monitoring of hybridisation across an array of immobilised DNA probes. In a SPRI experiment, local changes in the reflectivity from a thin metal film are exploited to detect in real-time hybridisation onto the molecules tethered to the surface.

In the present paper, we used the SPR imaging to monitor the hybridisation reaction of oligonucleotide targets with six different CF oligonucleotide probes immobilised on a 196 spots array. We also assessed the specificity and the selectivity of the system in the discrimination between perfectly matched and mismatched hybrids.

The interface between the sensor surface and the biological systems to be studied is a vital component of all surface-sensitive sensor systems. Receptors attached to the solid support must retain their native conformation and binding activity. This attachment must be stable over the course of a binding assay and, in addition, sufficient binding sites must be accessible to the solution phase to interact with the analyte. Most importantly, the support must minimize non-specific binding of the sample (Kasemo, 2002; Tombelli and Mascini, 2000). Various chemical modification procedures permit such coupling by the use of specific molecular tags, containing functional moieties with high affinity for the chosen substrate (Ferretti et al., 2000).

In this paper we compare the performance of two microarray biosensors developed using two different immobilisation chemistries. The first strategy adopted for probe immobilisation consists of modifying the gold surface with a polyelectrolyte film of branched poly(ethylenimine) (PEI), linked to gold via a first layer of 11-mercaptoundecanoic acid. A last high-density extravidin layer binds biotinylated probes due to the strong affinity of the extravidin–biotin complex (Bassil et al., 2003). In the second immobilisation procedure, the gold surface is modified with 11-mercaptoundecanol and carboxylated dextran to immobilise streptavidin and then the biotinylated probes (Tombelli et al., 2002; Löfås and Johnsson, 1990).

2. Experimental

2.1. Reagents

11-Mercaptoundecanoic acid (MUA), 11-mercaptoundecanol, poly(ethylenimine) (number-average molecular mass: 10 kDa, refractive index $n = 1.53$ according to Sigma–Aldrich), extravidin ($n = 1.45$ (Piscevic et al., 1995)) and streptavidin ($n = 1.45$ (Piscevic et al., 1995)), as well as 1× PBS (25 mM phosphate-buffer with 0.137 M NaCl, pH 7.4), epichlorohydrin, 2-methoxyethyl ether (diglyme), bromoacetic acid, *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) were purchased from Sigma–Aldrich (France). Dextran was from Amersham Biosciences (France). All reagents used to prepare buffers were purchased from Sigma–Aldrich.

2.2. Experimental set-up

The SPR imaging monitoring of the biochemical interactions was performed by coupling a chemically modified gold covered glass slide to a prism (SF 10) (Edmund Optics, England) via index matching oil. Conventional BK7 ($n = 1.515$) glass slide substrates were covered with an extra-thin (2.5 nm) layer of chromium on which a 47 nm gold film was vapor deposited. Index matching oil ($n = 1.720$) was purchased from Cargille.

The DNA:DNA hybridisation reaction monitoring was carried out with the set-up described in Bassil et al. (2003) and Maillart et al. (2004).

2.3. DNA samples

Synthetic biotinylated oligonucleotide probes were ordered from Proligo (France). Synthetic oligonucleotide targets were purchased from Sigma–Genosys (Cambridge, England). The probe (P) sequences used are: 1, ΔF508 wild type (P-ΔF508-WT) = 5′ biotin $(T)_{17}$ ATA TC**A TCT TT**G GTG 3′; 2, ΔF508

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