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Direct detection of genomic DNA by surface plasmon resonance imaging: An optimized approach

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ABSTRACT

The direct detection of specific sequences in genomic DNA samples is very challenging in the biosensor-based approach. In this work we developed an optimized strategy for the direct detection of DNA sequences in human genomic samples by a surface plasmon resonance imaging technology. As model study, the target analyte was identified in a DNA sequence mapping the human ABCB1 gene. The computed-assisted approach was here applied for probe design. After a preliminary evaluation of the probe functioning by the complementary synthetic target, the system was applied to the direct detection of the target sequence in human genomic DNA extracted from lymphocytes. To achieve this result, several steps aimed to improve the analytical performances of the biosensor were studied and optimized. The immobilization chemistry, based on thiolated probes, was adapted here to non-amplified sequence detection. DNA sample pre-treatments, i.e. genomic fragmentation by ultrasounds and dsDNA denaturation by thermal treatment were also investigated. A sandwich-like strategy, by using a secondary probe, was also applied to understand and confirm the selectivity of the developed biosensor in detecting ABCB1 gene in genomic samples. Finally, a reliable calibration curve of ABCB1 was obtained with an experimental detection limit of 140 aM. Furthermore, the biosensor was well regenerable, assuring up to thirty cycles of effective measurements.

1. Introduction

Direct detection of a genomic DNA sample, bypassing amplification steps, is a very attractive analytical challenge, especially in biosensor-based approaches with application to molecular clinical diagnostics. The possibility of shortening the pre-analytical steps, i.e. sample pre-treatments, may lead to reduced analysis times and costs with positive impact on the detection of clinically relevant targets. In addition to key applications to the detection of genetic disorders and polymorphisms or mutations, molecular diagnostics could have important reflections also in the emerging field of theranostics. In any case, the starting point in molecular genomics is the analysis of DNA samples. Most of the reported approaches deal with amplified DNA samples and very few of them reach sensitive detection of genomic DNA avoiding sample amplification, i.e. by PCR. Different transduction principles are applied to this aim: fiber optics, surface plasmon resonance (SPR) and SPR imaging (SPRi), piezoelectric detection (Almadidy et al., 2002; Bianchi et al., 1997; Kaewphinit et al., 2010; Minunni et al., 2005; Scarano et al., 2011). Concerning SPR-based biosensors, our group reported about human, animal and plant DNA detection directly in genomic samples using BIAcore family instrumentation, also applying restriction enzymes, reaching a detection limit of 10 mg L^{-1} (Minunni et al., 2007). D'Agata et al. used a secondary amplification by gold nanoparticles on the hybridized target, revealing detectable concentrations down to and attomolar (D'Agata et al., 2011) in case of mismatch analysis and 41 zM (D'Agata et al., 2010) for full complementary sequences.

DNA amplification by PCR or other approaches undoubtedly leads to a large enrichment of the target sequences, but it also may introduce errors and it can be an additional step in the pre-analytical phase, which eventually could represent a further risk of contamination of the sample. However, some inescapable pre-analytical steps are still applied to genomic DNA analysis, i.e. DNA extraction, fragmentation, and denaturation. DNA fragmentation can be achieved by ultrasounds (D'Agata et al., 2010) or enzymatic digestion with restriction nucleases (Minunni et al., 2005; Minunni, 2012).

In any case, the extracted DNA is a double helix, in which the target sequence, complementary to the immobilized probe, is hidden. Therefore, it is mandatory that, after the fragmentation, the double-stranded DNA (dsDNA) is opened to allow the target sequence contained in it to hybridize the probe. The denaturation process, both on PCR amplified or unamplified genomic samples, can be achieved by simple thermal treatment or by alternative strategies aiming to prolong the single-stranded DNA (ssDNA) life

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time in solution by preventing the re-annealing of double strands, i.e. using small hybridizing oligonucleotide sequences (Minunni et al., 2005).

We recently demonstrated how, behind dedicated pre-analytical key steps, in DNA-based sensing the rational design of suitable capturing sequences can be strategic for improving the analytical performances of the biosensor (Ermini et al., 2011).

In this paper we reported about a DNA biosensor development for sensitive human genomic DNA (hDNA) detection, using the SPR imaging-based technology. As model study, we selected a fragment of 511 mers belonging to the human ABCB1 gene, consisting in the genomic sequence to be targeted. The human ABCB1 gene, on chromosome 7, was considered of clinical interest due to the so-called rs1045642 (C3435T) polymorphism and its possible involvement in several diseases (Campa et al., 2012; Drain et al., 2011; Jaitner et al., 2012; Kesimci et al., 2012; Penna et al., 2011; Sharma et al., 2012; Teh et al., 2012).

First, a computed-assisted design of a capturing probe (CProbe) was here applied, following our previously reported approach (Ermini et al., 2011). The hybridization reaction with the selected CProbe was then monitored and optimized by using the synthetic complementary target. For the applicability to hDNA samples, several steps were considered, i.e. suitable biochip surface treatments, hDNA fragmentation by ultrasounds, and dsDNA denaturation by thermal treatment. Finally, the system was applied to the detection of ABCB1 gene directly into genomic hDNA extracted from lymphocytes and a reliable calibration of the system was successfully obtained in the range 0.14–2.8 fM. A sandwich-like strategy, by using a secondary detecting probe (DProbe), was also applied to understand and confirm the selectivity of the developed biosensor in detecting ABCB1 gene in genomic samples. The biosensor was well regenerable, assuring up to thirty cycles of effective measurements.

2. Materials and methods

2.1. Design of DNA probes

Probes design was performed by the free software OligoWiz 2.0 (Wernersson et al., 2007). This *in silico* evaluation can be applied to select highly performing probes for biosensing applications (Ermini et al., 2011). This can be achieved starting from any given DNA target sequence by the rational setting of five parameters, here imposed as follows: cross-hyb 44.8%, delta Tm 29.9%, folding 14.9%, position 0.0% and low-complexity 10.4%. In this study the 511 mers fragment of Homo sapiens ATP binding-cassette, subfamily B (MDR/TAP) member (ABCB1) on chromosome 7 (position 87138390–87138900 of the chromosome 7, position 3673–4183 of coding sequence of the gene) was the genomic target sequence. When the sequence of the target analyte was inserted in the program, a set of scored probes normalized to values between 0 (worst probe) and 1 (best probe) was obtained. Among the ranked probes, we selected two sequences: the so-called capturing and detecting probes (namely CProbe and DProbe). Finally a DNA sequence was chosen to act as negative control: Unspecific probe, i.e. a probe designed on the Enhanced green fluorescent protein gene, not present in human genome. Detailed information about designed probes is reported in Table 1.

2.2. Solutions and reagents

Thiolated DNA probes, 10 μm in immobilization solution (IS), were covalently bound on the biochip gold surface. IS consisted of 1 M KH_2PO_4 (Merck, Italy), pH 3.8. If not otherwise specified, a solution containing 1 μm 11-mercapto-1-undecanol (MU) and 1 μm 6-mercapto-1-hexanol (MCH) was used to passivate the gold surface of biochips, for 20 h in a dark moist chamber. A solution of 300 mM NaCl, 0.02 M Na_2HPO_4, 0.1 mM EDTA, pH 7.4, 0.05% (w/v) of TWEEN® 20 (Polyethylene glycol sorbitan monolaurate) was used as hybridization solution (HS) in SPRi measurements. A solution 100 mM HCl was used to regenerate the biochip surface after each measurement cycle. PDMS (Sylgard 184 Silicone Elastomer Kit, Dow Corning, UK), was used for the micro-welled mask preparation (Scarano et al. 2011). Unless otherwise stated, reagents were purchased from Sigma-Aldrich (Milan, Italy).

All solutions were prepared in MilliQ water (Millipore Corporation, Massachusetts, USA) and filtered using vacuum filter cups (Millipore E express plus, 0.22 μm) and syringe filters (Puradisc, cellulose acetate, 0.2 μm from Whatman GmbH, Dassel, Germany). All oligonucleotides were purchased from Eurofins MWG Operon (Ebersberg, Germany); the corresponding sequences are reported in Table 1. Human genomic DNA was purchased from Novagen (Piacenza, Italy).

2.3. SPRi instrumental asset

All measurements were performed by SPRi-Lab$^+$ instrument from Genoptics-Horiba Scientific (Orsay, France). Interactions between probe and analyte were followed in real time by an optical apparatus integrated with a homemade fluidic system. The whole instrumentation asset was reported previously (Scarano et al., 2010).

2.4. Probes immobilization

A micro-welled PDMS support was used for DNA probes immobilization in array format on SF-10 glass prisms coated with gold (50 nM-thick) (SPRi-Biochip™, Genoptics-Horiba Scientific, Orsay, France) as previously described by Scarano et al., 2010. Thiolated probe solutions (0.8 μL, 10 μm in IS) were spotted in each micro-well and let immobilize on the gold surface exploiting the affinity between sulphur and gold, forming a self-assembled monolayer (SAM). Two spots were used as reference surface (only IS).

Table 1
Probes and target sequences.

Gene fragment of ABCB1 (511 mer)	5' CTCACAAGGAGGGTCAGGTGATCAGGTAAAGGTAACAA CTAACCCAAACAGGAAGTGTGGCCAGATGCTTGTATACAGGTAAGGGTGTGATTTGGTTGCTAATTTCTCTT-CACTTCTGGGAAACCAGCCCCTTATAAATCAAACTATAGGCCAGAGAGGCTGCCACATGCTCCCAGGCTGTT-TATTTGAAGAGAGACTTACATTAGGCAGTGACTCGATGAAGGCATGTATGTTGGCCTCCTTTGCTGCCCTCA-CAATCTCTTCCTGTGACACCACCCGGCTGTTGTCTCCATAGGCAATGTTCTCAGCAATGCTGCAGTCAAACAG-GATGGGCTCCTGGGACACGATGCCCAGGTGTGCTCGGAGCCACTGAACATTCAGTCGCTTTATTTCTTTGC-CATCAAGCAGCTGAAAACAAGAGTTCACAGATCAACTTCAGGACCAGCACACTTTGAATGTAGCACAATTAA-CATCATTATTTCTTACACTGAAACTGCCAAGTTACTGTGAG 3'
CProbe	5' HS—$(CH_2)_6$—GTCACTGCCTAATGTAAGTCTC 3'
CTarget	5' GAGACTTACATTAGGCAGTGAC 3'
Unspecific probe	5' HS—$(CH_2)_6$—GAGGGCGATGCCACCTAC 3'
DProbe	5' GTGGTGTCACAGGAAGAGATT–BIO 3'

After probe spotting, the biochip was subjected to vacuum for 20 min and incubated in a moist chamber for 72 h. When required, biochip surface was finally passivated with an aqueous solution of two mercaptoalchols: 1 μm 11-mercapto-1-undecanol (MU) and 1 μm 6-mercapto-1-hexanol (MCH) (Sigma, Milan, Italy) for 24 h. Then surface was washed with water, dried and finally housed into the instrument.

2.5. SPRi measurements

Measurements with the synthetic target were performed under a constant HS flow of 6 μL min^{-1} (11 μL flow cell internal volume). The corresponding calibration was carried out by injecting 100 μL of the complementary target within the 5–250 nM range of concentration. Targets reached the flow cell about 5 min after injection, lasting for about 10 min in contact with the CProbe, and allowing to monitor the hybridization process in real-time. At the end of the injection, HS flow washed the surface, removing the analyte excess from the cell.

Injections of genomic hDNA samples were performed under a high HS flow of 6000 μL min^{-1}. After samples reached cell (1.0 s), the flow was lowered to 6 μL min^{-1} and the hybridization between the CProbe and the sample was monitored for 10 min. Finally running buffer washed the surface to remove the unbound analyte from the cell. The calibration was performed by injecting 100 μL of the pre-treated genomic hDNA in the concentration range 0.3–6.0 mg L^{-1} (0.14–2.8 fM).

During hybridization, the binding was visualized by sensorgrams showing the percentage of reflectivity variation ($\Delta R\%$) in real time (s). Moreover, digital images of the punctual variation of reflected light on the surface were collected. SPR signals were acquired as $\Delta R\%$ difference between before and after (5 min from washing) the analyte binding. The biosensor surface was regenerated by 24 μL min^{-1} for 1 min of 100 mM HCl, making probes available for consecutive measurements.

2.6. Genomic pre-treatment and electrophoresis

Genomic samples (100 μL, 2.8 fM, 6 mg L^{-1}) were fragmented by applying different protocols of ultrasonication (VC100, 100 Watt Model, Sonics, Newtown, CT, USA): ultrasonic bath and ultrasonic immersion probe. Furthermore, different amplitudes and times of sonication were tested (Table S1). Ultrasonic fragmentation of genomic hDNA was evaluated by agarose (Affymetrix, Santa Clara, CA, USA) gel electrophoresis (0.5% w/w of agarose in TAE buffer 1X) with ethidium bromide (Shelton Scientific, Romeoville, IL, USA) as intercalating agent (0.004% w/w) and 100 bp DNA ladder and λ/HindIII as molecular weight markers (Fermentas, Thermo Scientific, Milan, Italy). Electrophoretic bands and distributions were visualized by an UV transilluminator (TFX-20M, Vilber Lourmat, France) and images were captured by a digital camera. Digital images were transformed in grayscale and analyzed with the image processing software ImageJ (open source software). After ultrasonication, the dsDNA was denatured at 95 °C for 7 min in C1000 Thermal Cycler (Bio-Rad, Italy), then cooled in ice-bath for three seconds and directly injected in SPRi-Lab^{+} instrument (100 μL). The protocol for fragmentation was chosen on the basis of the magnitude and the specificity of the SPRi signal obtained experimentally.

3. Results and discussion

The *in silico* approach by OligoWiz 2.0 was here applied to define suitable probing sequences to be used for ABCB1 gene detection in non-amplified hDNA samples. The approach applied is displayed in Fig. 1.

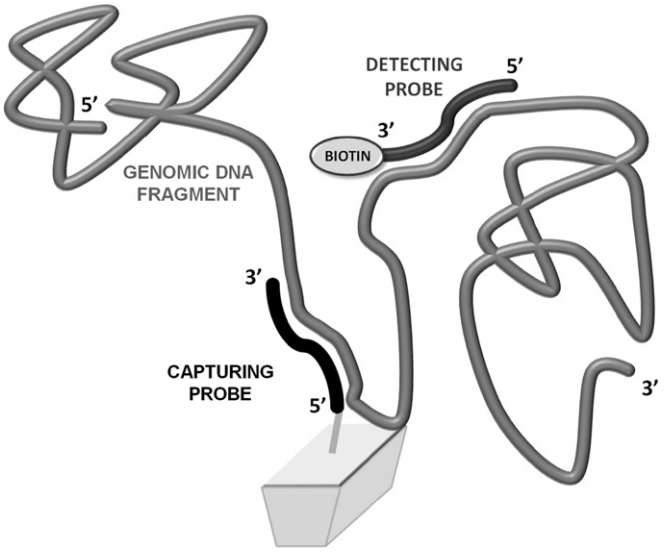

Fig. 1. Scheme of the strategy: thiolated CProbe, with estimated high performance in terms of selectivity, is immobilized on sensor surface, after incubating DNA thiolated probe 10 μM in IS on gold for 72 h in a moist chamber. This probe captures the complementary fragment in the genomic DNA sample. A secondary hybridization with DProbe on genomic DNA is performed, enhancing the signal and confirming the specificity of our strategy.

A 22 mers sequence, specific for ABCB1 gene, was selected as capturing probe (CProbe) and immobilized on the chip surface. This probe was conceived to selectively bind the target sequence on a higly conserved region of ABCB1 gene, with high specificity and selectivity. The target sequence was bound to the probe, and then exposed to the secondary hybridization with detecting probe (DProbe) to finally confirm the system selectivity in genomic DNA samples.

Key steps in sensor development were optimized to achieve sensitive and reliable genomic target conditions, i.e. sample pre-treatment (fragmentation and denaturation) and immobilization procedure.

3.1. In silico analysis

As previously reported by our group, improved analytical performances of a DNA biosensor can be achieved by the *in silico* rational design of probes (Ermini et al., 2011). Here the same computer-assisted approach was applied to design a selective strategy for the detection of a target sequence (511 mers, Table 1) on ABCB1 gene, used here as model system with potential application in theranostics. ABCB1 gene belongs to the ABC genes family, characterized by a high degree of homology. The CProbe was thus selected by OligoWiz 2.0 on the most conserved region of the 511 mers sequence, to assure a high degree of specificity, within the ABC genes. The selectivity of the CProbe was estimated by the Cross-hybridization (against the whole human genome DNA) score given by the program, resulting the highest value possible for this parameter, i.e. 1.0. Furthermore, an excellent score of 0.88 predicted high probing performances of CProbe.

To develop a sandwich-like assay, the DProbe was also selected by the program on a different region of the ABCB1 target sequence (see Fig. 1) with the aim to perform a secondary recognition, of the captured target gene. ABCB1 target sequence and nucleotidic probes are reported in Table 1.

3.2. Calibration of the biosensor with complementary target

Once selected, the capturing probe was immobilized on the gold surface of the biochip by thiol coupling, and its performance in binding the complementary synthetic target was experimentally tested by SPRi (Fig. 2).

The calibration was performed within the range of concentration 5–250 nM of synthetic complementary target, reaching signals up to $\Delta R\% = 1.38$. The sensor gave a linear response up to 25 nM, corresponding to a value of 0.45 of reflectivity variation percent. The average coefficient of variation percentage (CVav%) was here calculated as the percentage of standard deviations normalized for mean SPRi signals and averaged for all concentrations tested. This value resulted to be $< 5.8\%$ for each point of the calibration curve, demonstrating the reproducibility of the system. Unspecific probe was immobilized on the sensor surface as negative control and SPRi signals were monitored on it for all injected samples, for confirming the high selectivity and specificity of the interaction observed.

3.3. Genomic DNA detection

3.3.1. Pre-treatment optimization: ultrasonication and electrophoresis study

Genomic DNA fragmentation was achieved using two approaches. In particular, an immersion probe (dipped directly into the hDNA sample) or a sonication bath were used. The effect of amplitude frequency of ultrasounds and the sonication time applied were studied for both procedures (Table S1). Fragmentation efficiency was evaluated by agarose gel electrophoresis, obtaining a rough estimation of the fragmentation size (Fig. 3).

In particular, electrophoretic gel showed that the efficiency of fragmentation on genomic hDNA depended on the type of sonication applied. Fig. 3 shows the electrophoretical analysis, where the F1 sample was treated in an ultrasonic bath, whereas F2 and F3 samples were treated by immersing the ultrasonic probe directly in the genomic solution. The fragmented profile of each sample was compared to the untreated genomic DNA sample (lane G). F1 sample was characterized by a smear between 6557 and 23130 bp, meaning that a partial DNA fragmentation occurred respect to lane G. F2 and F3 samples showed an intermediate (100–1000 bp) and high (100–400 bp) degree of fragmentation, respectively. Therefore, only by the direct immersion of the ultrasonic probe (F2 and F3 lanes) a high DNA fragmentation was obtained. In this case, the highest amplitude applied was 20% (see Table S1), due to the rapid increase of temperature of the sample induced by the ultrasound probe. To evaluate the best protocol of sonication, fragmented hDNA samples were further thermally denatured and then tested at 2.8 fM in HS on biosensor. Among all sample tested, only F1 sample resulted to be detectable. Moreover, the F1 genomic direct hybridization on CProbe occurred only when the biochip surface was prepared with a suitable procedure (see next paragraph).

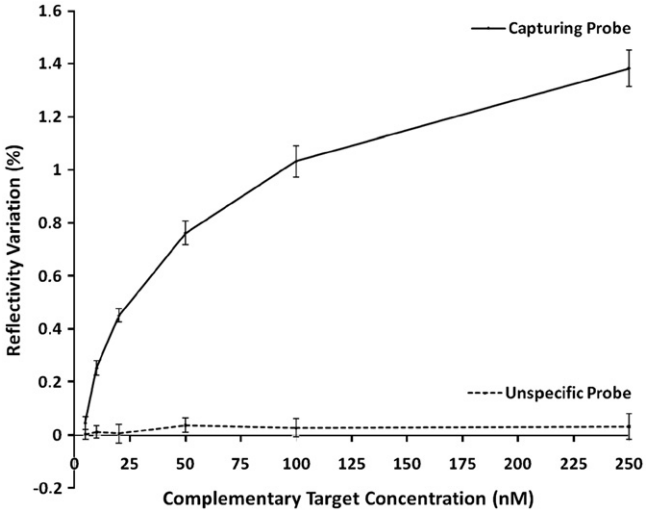

Fig. 2. SPRi signals recorded from CProbe and Unspecific probe immobilized on the biosensor surface by thiol coupling vs. different concentrations of the complementary synthetic target (from 250 nM to 5 nM). All measurement are performed under constant flux of HS (6 μl/min). Target are eluted in HS too and each injection volume is 100 μl (see Section 2). Contact time is 15 min and both dead time and washing time is 5 min.

3.3.2. Effect of alkane thiols on genomic DNA hybridization

The influence on the hybridization signal of alkane thiols used to passivate the biochip surface after probe immobilization was investigated. In particular, genomic samples were injected on biochipspassivated with thiols and on biochips not subjected to thiol passivation treatment. 2.8 fM of F1, F2 and F3 sonicated hDNA samples were injected separately on biochips modified by the two different procedures, and tested for their ability in hybridizing CProbe. No hybridization signal was obtained for the all three genomic samples (F1, F2 and F3) when the gold surface underwent thiol passivation (data not shown). Contrarily, on non-passivated biochips significant results were observed. In particular, among fragmented genomic samples, F1 gave a significant SPRi signal of 0.971 ($\Delta R\%$) on CProbe spots, and the hybridization in this condition was reproducible and specific, i.e. no interaction was recorded on bare gold, as displayed in the differential images of Fig. 4. Also the Unspecific probe (white-circled spots in Fig. 4) displayed the unexpected ability in binding the genomic samples (see next paragraph for discussion).

The different behaviors observed on passivated or not biochips could be explained by supposing that thiols may eventually modify the molecular packing of CProbe on receptor spots, leading to their reduced availability. For instance, possible disulfur superstructures of thiols could cause a reduced degree of freedom and/or a partial hindrance of the CProbe sequence.

3.3.3. Evaluation of the specificity by DProbe hybridization

In Fig. 4a, the differential image and the relative bar plot of SPRi signals obtained on F1 samples are reported. Biochip surface was modified by spotting the CProbe (6 spots), the Unspecific probe (2 spots), and two reference spots (2 spots, only IS). The Unspecific probe was chosen to be complementary to the EGFP gene, not present in the human genome. Therefore the SPRi signal obtained from the human genomic sample can be undoubtedly ascribed to unspecific interactions of the Unspecific probe. Data showed an intense signal on Unspecific probe ($\Delta R\% = 1.309$), higher than the signal on CProbe ($\Delta R\% = 0.971$). These results can be explained with the presence of fragmented genomic sequences able to hybridize Unspecific probe; contrarily, on CProbe spots, the lower signal could suggest the ability of the probe in catching selectively only fragments containing the corresponding ABCB1 target sequence.

To understand and confirm this assumption, after the hybridization on CProbe spots of F1 samples (Fig. 4a), the subsequent injection of DProbe (250 nM) was performed in a sandwich-like assay, as previously represented in Fig. 1. SPRi signals inferred from the secondary hybridization are reported in Fig. 4b. As expected, on Unspecific probe we obtained a signal enhancement of $\Delta R\% = 0.183$ whereas, on CProbe spots, a $\Delta R\% = 0.906$ was recorded. The relative intensity of the secondary hybridization

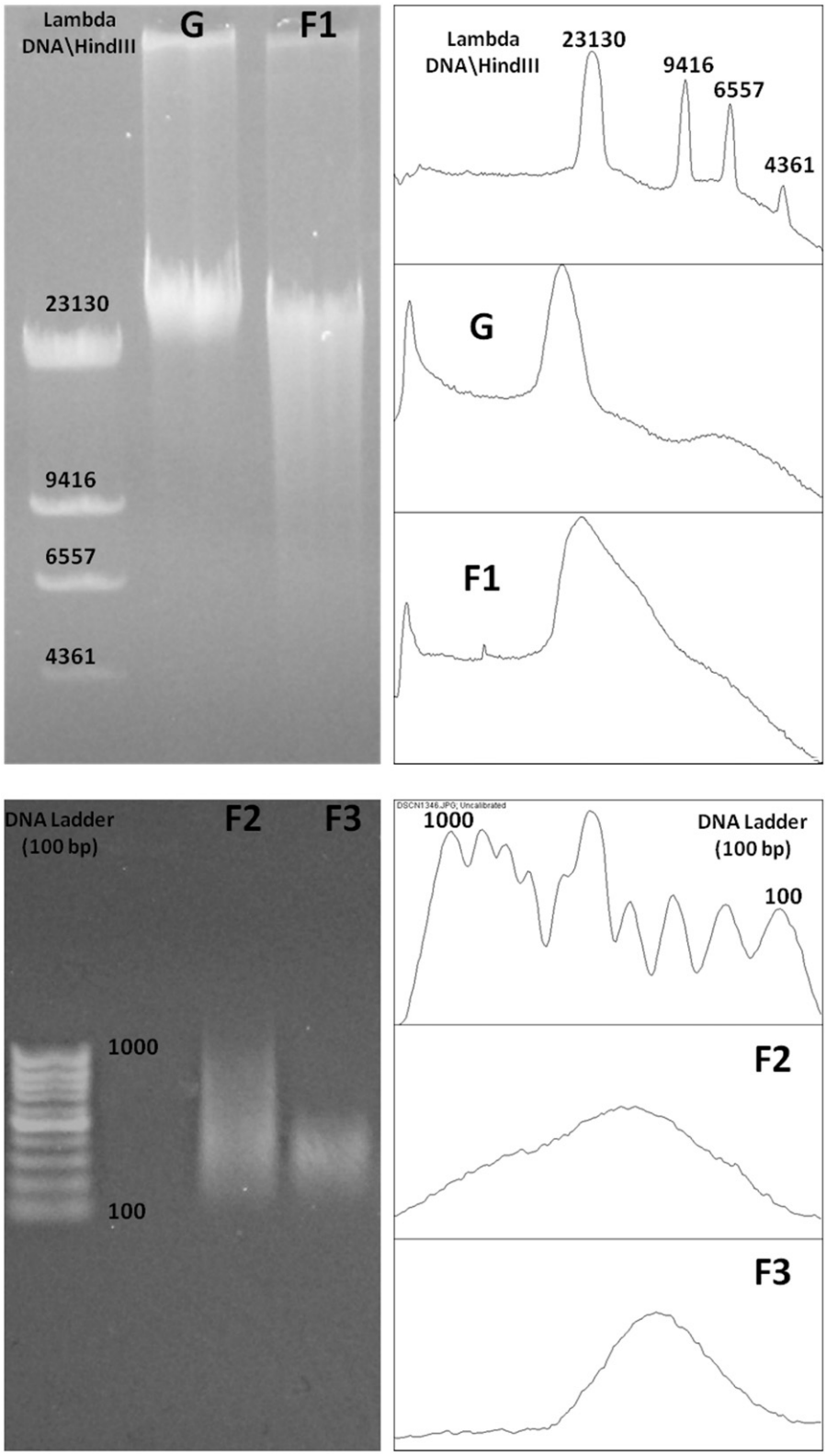

Fig. 3. Genomic DNA fragmentation for different ultrasonication procedures. Left: 0.5% agarose gel electrophoresis of genomic DNA samples. 100 bp DNA ladder and Lambda DNA/HindIII are used as markers. Right: distribution profiles of fragmentation visualized by ImageJ. Lane G is whole genomic DNA sample. Lane F1 is genomic DNA sample pre-treated in ultrasonication bath, and lane F2 and lane F3 are genomic samples pre-treated by immersing ultrasonic probe at different setups (Table S1).

indicated that the CProbe bound with high efficiency only the target sequence. In fact, DProbe was complementary to a different region of ABCB1 gene, confirming the specificity of the primary hybridization between the capturing probe and the genomic DNA fragment of interest. DProbe was previously tested in preliminary analyses on the biosensor to evaluate possible direct hybridization with immobilized probes (CProbe and Unspecific probe) even in absence of human genome. No crossing interaction was recorded among

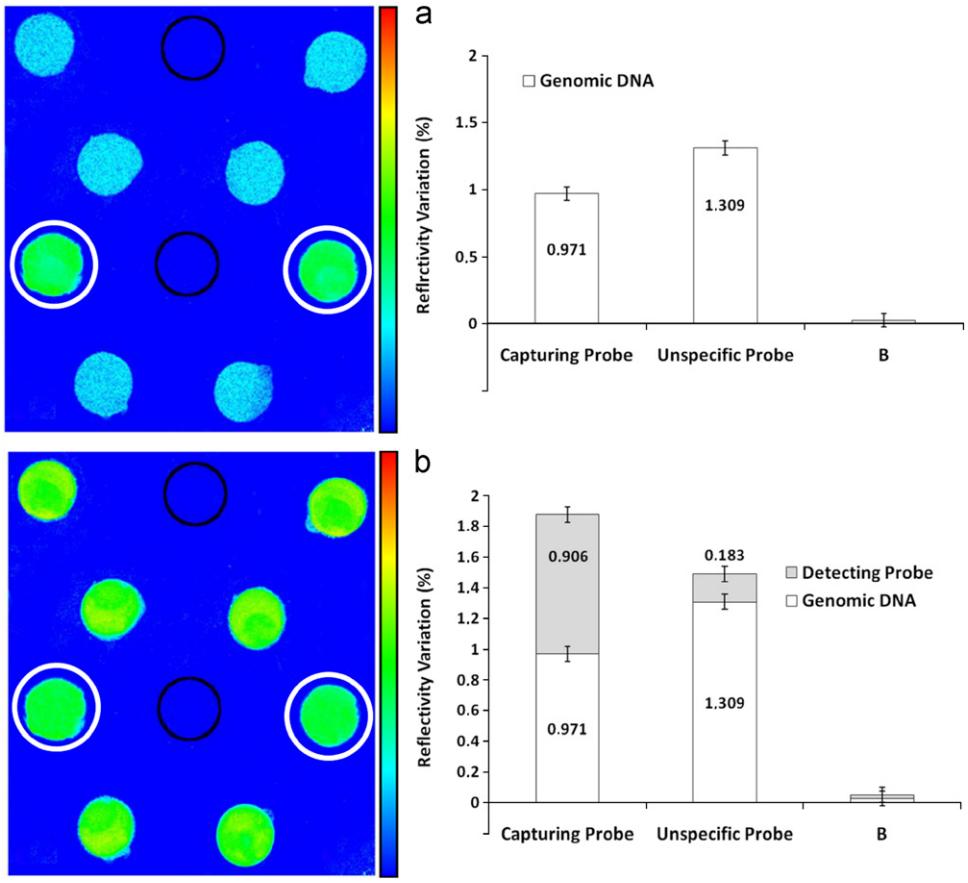

Fig. 4. Comparison between SPR signals obtained on CProbe and Unspecific probe, immobilized on the biosensor surface by thiol coupling, hybridized with pre-treated genomic F1 sample at 2.8 fM (100 μl in HS, the pre-treatment procedure is reported in Section 3.3.1) (a), and after the hybridization with DProbe (b), corresponding differential images of the spotted biochip surface are shown on the left. The bright spots represent areas where hybridization occurred between genomic DNA and capturing probe. Blank spots are circled in black and are reference spots where no probe is immobilized. Unspecific probe spots are circled in white.

probes, confirming that the sandwich-like assay carried out by using DProbe is a valid strategy to distinguish between specific and unspecific interactions possibly occurring on the sensor.

3.3.4. *Calibration of the biosensor with genomic DNA*

The biosensor was finally calibrated with genomic samples prepared by the same pre-treatment previously used to obtain sample F1. After thermal denaturation, genomic DNA was tested at the following concentrations: 0.14, 0.28, 0.70, 1.4, and 2.8 fM in HS. Points were fitted with a line, showing a good coefficient of determination (R^2=0.990), as reported in the upper part of Fig. 5a.

The experimental coefficient of variation %, averaged on all concentration points, resulted 17%, a very encouraging result considering the high complexity of the tested matrix and the absence of any signal amplification. The bottom part, Fig. 5b, reports the further application of the sandwich-like assay carried out with DProbe on each concentration of the genomic solutions tested. As shown in the bar plot, the SPRi signal enhancement (gray bar portion) due to the DProbe hybridization, resulted not directly correlated to the concentration of the tested DNA sample. This behavior could be due to the high variability of the lengths of fragmented DNA bound to the CProbe, and to possible intra- and inter-strand interactions among fragments. Single-stranded fragments hybridized on CProbe could actually expose their ends forming secondary structures impairing the secondary recognition with DProbe. The biosensor was successfully regenerable for up to thirty cycles of measurements (data not shown), opening encouraging perspectives in the direct detection of genomic sequences for clinical purposes by-passing PCR amplification.

The analysis time was of 30 min, considering sonication, denaturation and sample hybridization with both capturing and detecting probes.

4. Conclusions

Direct human genomic DNA detection is an attractive goal for diagnostics and theranostics, allowing faster analytical responses and cost reduction of assays, when compared with amplified DNA analysis. An optimized approach to directly detect very low concentrations (140 aM) of human genomic DNA in a multiarray SPRi biosensor was here described. As model system, the ABCB1 gene was selected and nucleic acid-based probes were selected by a computational-assisted approach. Effects of ultrasonic fragmentation on genomic DNA and of gold surface passivation after probe immobilization were studied. The best experimental conditions were found, ensuring the specific hybridization of fragmented DNA genomic sample on the specific probe. The biosensor was successfully regenerable for up to thirty cycles of measurements, foreseeing its potential application for routine analyses.

The developed biosensor allows the immobilization of a number of specific probes in a multi-array format, and could exploited for multi-analytes measurements and real time detection on whole

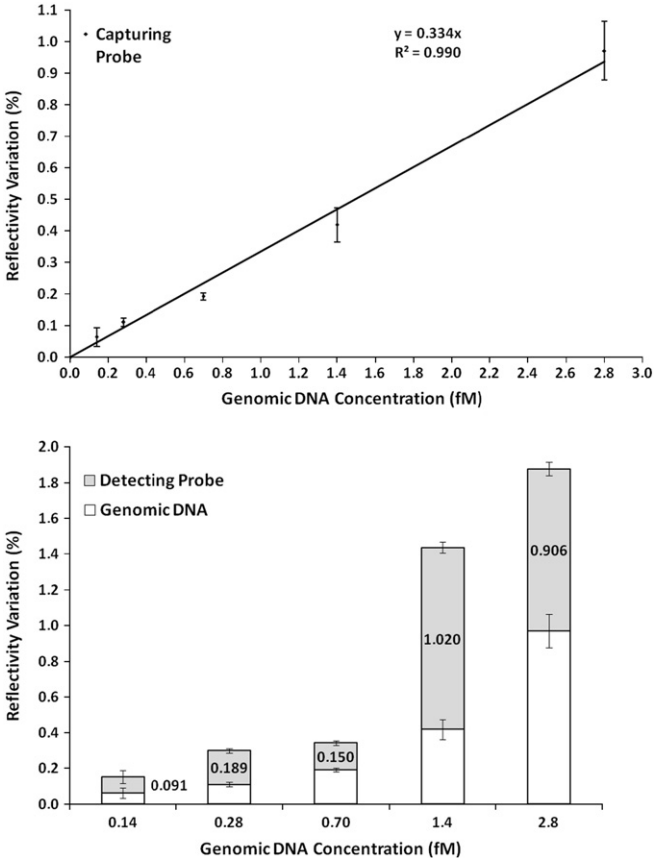

Fig. 5. Upper: SPRi signals recorded when different dilutions of the pre-treated genomic DNA flow on biosensor surface (HS flux of 6 µl/min, 100 µl injected, 15 min of contact time and both dead time and washing time is 5 min). Data are interpolated with a line with a very good correlation coefficient. Bottom: SPRi signals obtained after primary recognition of the genomic samples hybridizing with CProbe (immobilized on biochip surface by a thiol group) are coupled with signals enhancement obtained from subsequent injection of 250 nM DProbe in HS (see Section 2).

genomic samples. Moreover, the selectivity of this interaction was very well demonstrated by the use of a secondary detecting probe in a sandwich-like assay. This model strategy can be applied in the near future to the punctual detection of sequences of clinical relevance, i.e. point mutations, polymorphisms, and deletions.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2012.07.018.

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