DNA microarraying on compact disc surfaces. Application to the analysis of single nucleotide polymorphisms in Plum pox virus†

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The potential of using compact discs as high throughput screening platforms for DNA microarraying is discussed and applied to discriminate genetic variations of Plum pox virus.

Compact discs (CDs) are made from a 1.2 mm thick disc of polycarbonate (PC) plastic coated with a 100 Å reflective layer of aluminium, silver or gold protected by a transparent polymeric layer.[1,2] CD based technologies offer enormous possibilities as BioMEMS for molecular screening in biosensing. Similarly, the scope of microarrays has in recent years expanded impressively. As a rapidly maturing technology, microarrays pave the way for high-throughput proteomic and genomic exploration.[3] The high optical quality of the polymeric materials used for CD manufacturing makes them a promising platform for microarraying purposes.[4] Several attempts have already been made with optical detection systems. Disc-based immunoassay microarrays were developed by Kido *et al.*[5] who demonstrated the potential of this technology. Later, Remacle and coworkers described a method and device comprising capture molecules fixed on a disc surface for DNA and proteins detection.[6] An innovative approximation was also described by La Clair and Burkart[7] for molecular screening of proteins. The BioCD described by Nolte and Regnier[8] is an example of a spinning-disk, self-referencing interferometer that has a layer of antibodies. CD-Rs have been used as an inexpensive source of gold substrate for the fabrication of self-assembled monolayers.[9] All these above mentioned approaches unsuccessfully pursued the use of standard CD readers for detection and merely use the CD as a cheap medium support. Analyzing biological and chemical samples with a compact disc player has barely been attempted. On the research side, Barathur *et al.*[10] modified the normal CD drive for use with a laser-scanning microscope for analysis of a Biocompact disc assay. Other researchers[6] took advantage of the low cost, high resolution optical CD technology in analytical DNA array applications. The system comprised two optical detectors, one to detect the tracking information and the other for sample detection. On the other hand, Marc Madou's group have also focused on the fabrication of biosensing devices on disc-shaped polymer substrates similar to a CD, integrating a number of micro fluidic functions, such as fluid transfer control by spinning the disc and sample analysis.[11] Currently, Gyros AB (Uppsala, Sweden) and Eppendorf AG (Hamburg, Germany) commercialize products based on CD technology. These centrifuge-based fluidic platforms appeared as great technological innovations for clinical analysis; however, they are centrifugal analyzers with which fluid movement is achieved through rotation of the disc and employ optical detectors such as microscopes, phototubes, and CCD cameras.

Attaching probes on polymeric surfaces might be carried out by physical adsorption, covalently or through a general bridge system such as avidin–biotin. In this work, we propose the use of the bridge system due to its well-known good performance in a broad variety of applications, being also a simple way for the development of microarrays of nucleic acids and proteins. Therefore, the goal of this research[12] is two fold. First, to demonstrate the feasibility and performance of the polymers used for CD manufacturing as surfaces for DNA microarraying. Secondly, the application of the methodology to discriminate single nucleotide polymorphisms (SNPs) in plant viruses is also attempted.

The oligonucleotide sequences[13] used are shown in Table 1. The performance of the avidin coating process[14] was checked by spotting serial dilutions of probe A on coated and uncoated surfaces[15] followed by quantification of fluorescence.[16]

Different concentrations of avidin (10, 100 and 1000 mg/L) were used to test the coating efficiency on PC and polymethylmethacrylate (PMMA) surfaces. Contact angle and thickness measurements[17] were carried out in order to physically characterize the coated surfaces. PC and PMMA supports with 100 mg/L avidin showed contact angle measurements of 56.2° and 71.2°, respectively, which were lower than those of bare (81.6° and 75.8°) and 10 mg/L-coated surfaces (74.6° and 74.1°) of PC and PMMA, respectively. It is worth mentioning that in the case of PC a huge decrease was observed, indicating the successful immobilization of avidin. On the other hand, a slight decrease of contact angle was obtained for coated PMMA. Higher avidin coating concentration (1000 mg/L) did not significantly reduce contact angle measurements obtained with 100 mg/L. Physical measurements were also carried out to determine the homogeneity of the avidin film, and

Table 1 Nucleotide sequence of probes and target

Reference[a]	Sequence (5′–3′)
Probe A	$(T)_{15}$CCCGATTGACCAGCTAGCATT
Probe B	$(T)_{15}$CCCGATTGACCAGCTAGCATT
Probe C	$(T)_{15}$CCCGATTGACC**T**GCTAGCATT
Probe D	$(T)_{15}$CCCGATTGA**TT**AGCTAGCATT
Probe E	$(T)_{15}$CC**AT**ATTGACCAGCTA**T**CATT
Target	AATGCTAGCTGGTCAATCGGG

[a] Probes were modified with biotin at the 5′ end. Probe A and target were labeled with Cy5 at the 3′ end. Mismatched nucleotides are in bold.

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revealed a homogeneous immobilization of avidin on both supports (see the supporting information).[18]

A CD printer[19] was modified for spotting probes on PC and PMMA slides. Microarrays were patterned using the supplied software to print small, medium and large μm diameter spots and scanned for fluorescence to determine the areas of individual spots and their diameter distributions. In both supports, the most common diameters for small, medium and large spots were 150, 240 and 310 μm, respectively. It was clearly observed that as the spot diameter increased the range of diameter distribution became narrower.

Avidin coating (100 mg/L) and 0.01 μM probe A were used along with the experiments to optimize the protein adsorption.[20] The effect of pH on avidin adsorption clearly denoted that electrostatic forces are involved in the immobilization process.[18] The amount of biotinylated oligonucleotide immobilized was calculated by comparison of the relative fluorescence signals with those from a standard curve using the same target concentration. An immobilization yield of 52% and 9% for PC and PMMA was attained.

The sensitivity and efficiency of an immobilized capture probe to match its corresponding complementary target molecule were determined in hybridization model assays.[21] To adjust probe and target concentrations, a set of six coated surfaces were printed with probe B at concentrations ranging from 0.1 μM to 2 μM in PB, pH 9.0, and hybridized with a serial dilution of a complementary target molecule. Negative hybridization control experiments were also performed. As can be seen in Fig. 1, for any target concentration tested (2.5, 25, 50, 100 and 200 nM) probes developed maximum fluorescence in the range 0.5–1.0 μM on both supports, giving also the highest signal to noise ratio. Even though optimal probe concentration for both supports was of the same order of magnitude, fluorescence intensity was almost 10 fold higher on PC than in PMMA. Additionally, the influence of target concentration is depicted in the insets of Fig. 1. As is shown, for PC the optimal target concentration (25 nM) is two fold lower than in PMMA (50 nM). For hybridization yield determination, fluorescence data were interpolated on the reference curve, being 43% and 8% for PC and PMMA, respectively. Sensitivity in hybridization assays was estimated as the fluorescence background plus two times the standard deviation,[16] 5 nM and 10 nM being the minimum detectable target concentration on PC and PMMA, respectively. According to those results, PC showed better analytical performance than PMMA for DNA microarraying purposes.

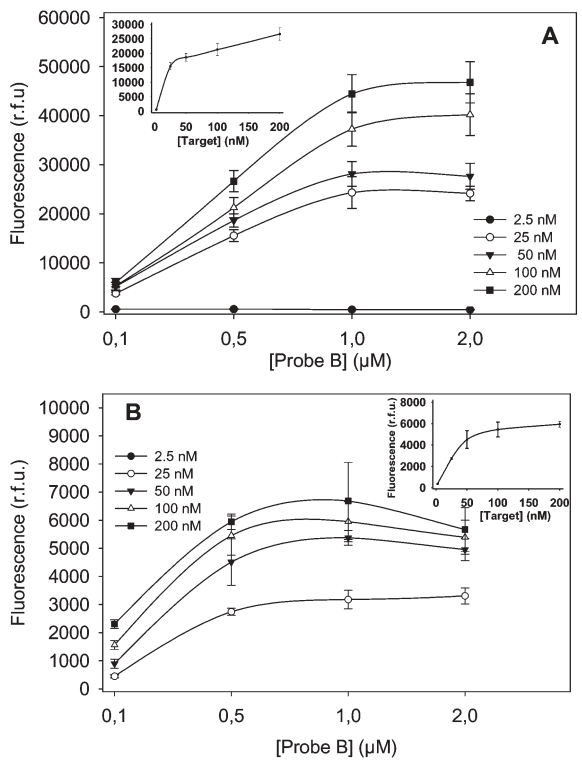

Fig. 1 Biotinylated probe saturation curves for (A) PC and (B) PMMA. Reported data are from replicates of nine spots for each oligonucleotide concentration.

Under the optimized conditions, the efficiency of the assay on PC was also evaluated by using full complementary (probe B) and mismatched probes (C, D and E) to target oligomer (see Table 1). As shown in Fig. 2, the fluorescence for the mismatched probes decreased as the concentration of formamide in hybridization buffer increased, being totally suppressed at 25% formamide. Indeed, even one single nucleotide mismatch was successfully discriminated with sufficient match/mismatch ratio.

The practical application of CDs for performing microarraying assays was assessed by the analysis of SNPs for the identification of Plum pox potyvirus (PPV).[22] Samples from different serogroups

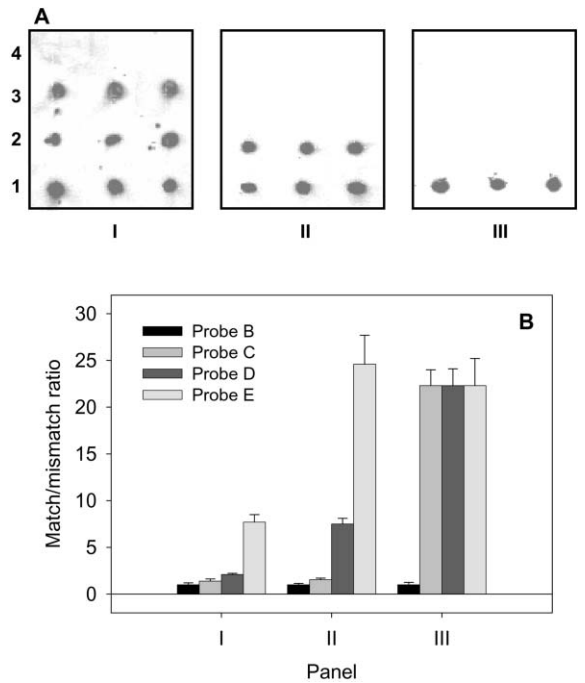

Fig. 2 Effect of formamide on hybridization assay efficiency. The arrays were printed in four rows of three identical spots. Row 1 – probe B (complementary). Row 2 – probe C (one mismatch). Row 3 – probe D (two mismatches). Row 4 – probe E (three mismatches). (A) Panel I – hybridization buffer without formamide, panels II and III – hybridization buffer with 10% and 25% formamide (v/v), respectively. (B) Match/mismatch ratio calculated as relative fluorescence between probe B and mismatched probe.

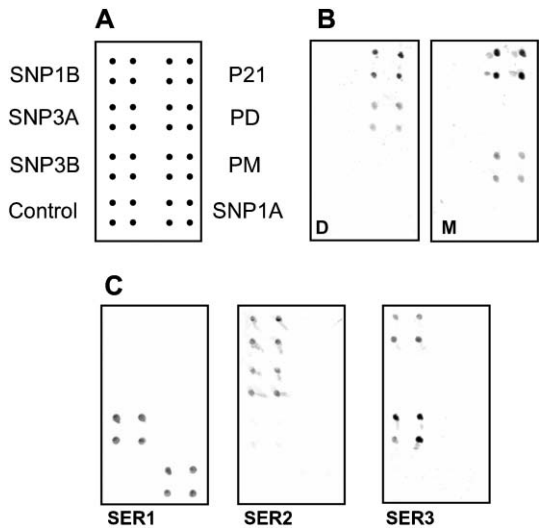

Fig. 3 Detection of Plum pox virus types and scoring SNP associated with serotypes. (A) Spot arrangement of the probes. (B) Results of the analysis of Plum pox virus samples. (C) Results of SNP analysis.

(SER1, SER2 and SER3) were used to approach SNP analysis. The identification of virus types and SNP tests were carried out on PC. As can be seen in Fig. 3, samples of virus type *Dideron* (D) specifically hybridized with both universal (P21) and D probes while type *Marcus* (M) did with both the universal and M probes, which implies a full score for discriminating different types of Plum pox virus.

The match/mismatch ratio reached was 17 and 18 for type M and D, respectively. These results confirm the CD array as an alternative method to detect viruses in a quick and reliable manner. Regarding SNP analysis, the results showed also a full match with the expected results (see Fig. 3C). Briefly, SER1 samples hybridized with probes SNP1A and SNP3B, SER2 samples with SNP1B and SNP3A while samples of SER3 specifically hybridized with SNP1B and SNP3B.[23] It is worth mentioning that the match/mismatch ratios were 3.7 and 4.5 for SER1 and SER3, respectively. On the other hand, SER2 was better scored with a match/mismatch ratio of 11.8. These values confirm the ability to analyze single nucleotide polymorphisms which are associated with different serotypes within PPV type D.

In conclusion, the preliminary evidence provided in this communication illustrates the potential of using compact disc surfaces as high throughput screening platforms to carry out a wide range of innovative experiments. Two of the main advantages of the CD platform are its very large area which can afford many different arrays or a few very large ones and using centrifugal force to move samples. The immobilization of biomolecules on CDs is a suitable strategy to perform genomic analysis. In terms of sensitivity and hybridization yield, good results were obtained using the polycarbonate face of CDs compared with PMMA.

It is remarkable to conclude that chemical derivatization of polycarbonate and polymethyl-methacrylate and the use of surface coating technologies will allow covalent and oriented immobilization of DNA or proteins for microarraying purposes. However, the incompatibility of polycarbonate with most of the common organic solvents will exclude its use as a microarray support for some chemical ligation steps. The more challenging research on which our group is currently working relates to the use of a modified CD player as detector, taking advantage of the simplicity of its reading technology to increase the sensitivity and portability of microarraying based assays. Therefore, herein demonstrated performances of compact disc surfaces as sensing platforms can be exploited for highly parallel analysis of general application in molecular recognition-based biochemical methods.

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12 This work was supported by the projects BIO2000-0243-P4-03 and BQU2003-02677 (CICYT, Spain).
13 DNA oligonucleotides were purchased from Tib Molbiol (Berlin, Germany).
14 Avidin (from egg white, extra-pure reagent) was from Sigma-Aldrich (Madrid, Spain). Buffers (PBS, 10 mM phosphate buffer, 150 mM NaCl, pH 7.4; PB, 150 mM phosphate buffer; CB, 50 mM carbonate–bicarbonate buffer, pH 9.6) were filtered through a 0.22 μm pore size disc before use. The polycarbonate reading faces of CD-Rs (PC) and PMMA slides (25 × 75 mm) were coated overnight at 4 °C with 100 μL avidin (100 mg/L in CB). Then, surfaces were extensively washed with water and dried by slight centrifugation.
15 PMMA slides, 1 mm thick, were kindly provided by Plexi S. A. (Valencia, Spain). CD-Rs were from U-Tech Media Corp., (Tau-Yuan Shien, Taiwan).
16 The fluorescence was measured adjusting the laser power and PMT settings at 100% and 600 V. The laser scanning system was from Axon Inst. (Union City, CA).
17 Contact angles were measured using an OCA 20 Video-Based Contact Angle Meter from DataPhysics Instruments, GmbH (Filderstadt, Germany). The thickness of the avidin film was measured using a Therma-Wave Opti-Probe 5220 (Fremont, CA).
18 The supporting information includes experimental aspects of the topology of coated surfaces and the influence of coating and printing buffer on fluorescence.
19 L. R. Allain, D. N. Stratis-Cullum and T. Vo-Dinh, *Anal. Chim. Acta*, 2004, **518**, 77. The Epson Photo Stylus 900 inkjet printer was from Epson Ibérica (Madrid, Spain).
20 For these experiments, CB buffer as coating buffer and 3XSSC (450 mM NaCl, 45 mM sodium citrate, pH 7.0) as printing buffer were used as controls.
21 Surfaces were incubated in hybridization chambers for 3 h at 42 °C. After the hybridization step, the supports were first washed with 0.3XSSC and then with 0.1XSSC and water for 1 min. Hybridization buffer was 5XSSC containing 250 μg/mL sheared salmon sperm DNA, 0.02% BSA (w/v), and 25% formamide (v/v).
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