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PMMA Isocyanate-Modified Digital Discs as a Support for Oligonucleotide-Based Assays

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A mild chemical procedure for the derivatization of PMMA as the isocyanate on rigid supports is described. The proposal is based on spin-coating the support with a hydroxy-modified PMMA polymer, followed by treatment with (3-isocyanatopropyl)triethoxysilane, developing an isocyanate-ended PMMA. Oligonucleotide hybridization assays performed on this surface demonstrate that the process is simple and highly effective and agree with the results of other modified materials, including aminated PMMA, gold, and glass. As a demonstration, it has been applied to covalently attached aminated oligonucleotides on a modified audio−video compact disc (CD) surface to perform DNA probe hybridization assays. Measurements were carried out with a CD player, by detecting the solid microspots precipitated from an enzymatic reaction (HRP-TMB). This new approach, being a competitive technique, opens a broad horizon of applications for point of care or *in situ* needs.

INTRODUCTION

The concept of hybrid systems has become an active area of research. In them, a molecular event is detected by its ability to alter the transmission of a digital sequence; a true interaction between digits and molecules occurs (*1*).

Compact disc (CD)-based technology offer enormous possibilities as BioMEMS (Bio-micro-electromechanical systems) for molecular screening in biosensing (*2*). In 2000, Kido et al. demonstrated the potential of this technology, developing disc-based immunoassay microarrays (*3*). Other authors (*4*−*6*) have been working on polycarbonate (PC) surface modification of discs to attach proteins and nucleic acids. Also, Yu (*7*) has developed strategies for the preparation of high-quality SAMs for electrochemical analysis on gold-metalized CDs. Regarding the use of CD reader technology, Barathur et al. modified the standard CD drive to use a laser-scanning microscope for analysis of a biocompact disc assay (*8*). Potyrailo et al. (*9*) used conventional optical disc drives for quantitative detection of Ca^{2+} and other ionic species by sensitive films deposited on conventional CD and DVD optical discs.

Preliminary evidence displayed by our research team (*10*) illustrated the potential of using compact disc surfaces as high throughput screening platforms. The immobilization of biomolecules on the PC face of CDs was a suitable strategy to perform genomic analysis, providing good results in sensitivity and hybridization. These studies were performed employing avidin−biotin methodology and using fluorescence microarray scanning for detection. The challenge now was the use of the PMMA-modified compact discs as support and the CD player as detector.

The chemical derivatization of PMMA, a material employed as a protective layer for the metallic film of the CDs,[1] would allow covalent and oriented immobilization of DNA or protein for microarraying. Lacquering the metallic layer of the compact disc with a chemically modified polymer is an interesting alternative for developing surfaces with specific chemical moieties and physical properties to covalently attach nucleic acid probes; advantages arise from the possibility to derivatize the polymer in solution after application on metal and the facility to apply it by spin-coating, sputtering, etc., achieving well finished surfaces. The problem is that most of the methods employed for the chemical modification of PMMA surfaces (*11*, *12*) require use in fluidic structures, affecting the optical and mechanical properties of the polymer. So, the described approaches are not applicable for our purposes.

PMMA microspheres have been successfully used for the immobilization of enzymes by aldehyde oxidation of the hydroxyl groups introduced in the PMMA using poly(vinyl alcohol) (*13*), design of microfluidic devices, metal deposition (*14*), and immobilization of proteins and DNA. Henry et al. (*15*) showed that aminated PMMA sheets can be used for the immobilization of enzymes in the restriction digestion of dsDNA. Waddell et al. (*16*) have developed a method using *N*-lithiodiaminepropane-modified PMMA slides as substrate to test the immobilization and hybridization of oligonucleotides in a microarray format. Christensen et al. (*17*) also proposed a chemical procedure to functionalize PMMA in an easier and faster manner than that previously described, by using hexamethylenediamine and glutaraldehyde or sulfo-EMCS ([*N*-ϵ-maleimidocaproyloxy]sulfosuccinimide ester). The density of immobilized and hybridized DNA was similar to those obtained on other PMMA-modified surfaces and silanized glass.

Although isocyanates potentially represent an ideal covalent DNA or protein immobilization chemistry (*18*), none of the previously published modifications of PMMA employ the isocyanate moiety for the covalent attachment of biomolecules. Isocyanates can react with many functional groups including amines, hydroxyls, and carboxyls, using soft conditions and aqueous media. Their reactivity renders them highly versatile, since these groups are present in nucleic acids, proteins, and other biomolecules. All these reasons make isocyanate highly attractive to develop derivatized supports for the covalent immobilization of probes.

In this work, a new DNA covalent immobilization approach based on the isocyanate modification of PMMA and its application by spin-coating on the metallic layer of the CDs is proposed. The goal of this research also involves a demonstra-

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[1] Standard compact discs are made from a 1.2 mm thick disc of polycarbonate coated with a 100 Å reflective layer of aluminium, silver, or gold protected by a transparent polymeric layer, usually poly(methyl methacrylate) (PMMA).

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tion of the potential of our proposal by the development of microarray-based assays, reading the hybridization results with a CD player as detector.

EXPERIMENTAL PROCEDURES

Reagents. PMMA (ref 99530) sheets (1 mm thickness) were from Röhm (Darmstad, Germany). Ethanolamine and (3-isocyanatopropyl)triethoxysilane (ICPTS) were purchased from Acros (Barcelona, Spain) and Fluka (Madrid, Spain), respectively. Dowanol, Tween 20, streptavidin−horseradish peroxidase (strept-HRP) from *Streptomyces avidinii*, and 3,3′,5,5′-tetramethylbenzidine (TMB) substrate were purchased from Sigma-Aldrich (Madrid, Spain). Oligonucleotides (see Table 1) were from Sigma-Genosys (Suffolk, UK). All commercially available products were used without further purification. Oligonucleotide solutions were printed onto the desired surface by tip contact using a stamper, 6 × 4 pins, from V&P Scientific, Inc. (San Diego, CA).

Buffers employed were as follows: Saline sodium citrate (10 × SSC, 0.9 M sodium chloride, 0.09 M sodium citrate, pH 7); carbonate buffer (10 × CB, 0.5 M sodium carbonate, pH 11 and pH 9.6); phosphate buffer saline (10 × PBS, 0.08 M sodium phosphate dibasic, 0.02 M sodium phosphate monobasic, 1.37 M sodium chloride, 27 M potassium chloride pH 7.5); PBS-T (PBS containing 0.05% Tween 20); MES buffer (0.1 M 2-morpholinoethanesulfonic acid monohydrate, pH 3.5).

Apparatus. The Energy Dispersion Spectra (EDS) were obtained with a scattering electron microscope Jasco-SEM, resolution 59 eV (Easton, MD). Fluorescence scanner Genepix 4000B, from Axon Instruments (Union City, CA), was used to read fluorescent probes immobilized on chip.

Compact Discs. Golden CD-Rs, partially transparent (30% reflectivity), provided by U-Tech Media Corp. (Tau-Yuan Shien, China) were directly treated, keeping its original shape, or cut in chips of 55 × 25 mm size when necessary. These low reflectivity recordable compact discs (L-CD, 1.2 mm thick polycarbonate discs, with a gold reflective layer of 30 nm, 12 cm diameter) allow the laser (780 nm) to have two functions: (a) reflected light (30%) lets the laser follow the track, maintaining the rotation of the disc (autotracking), and (b) transmitted light (70%) reaches the microarray spots located on the upper side of the CD, detecting the hybridization intensities by the amount of precipitate produced by an enzymatic marker (HRP) and a substrate (TMB).

Disc Reader. A standard CD player from Plextor LLC (Fremont, CA) was used as detector. The drive uses the servo control system to center and focus the beam on the spiral data track across the whole disc surface. The CD reader uses the original design of the CD players, taking advantage of the CD driver optical system to accurately illuminate the polycarbonate face of the L-CD (partially reflecting the laser and transmitting the rest through the PMMA surface to the photodiode). The controlled parameters of the optical disc drive include positioning of the laser toward the disc, scanning the whole disc surface while controlling the spatial resolution, and the linear rotation velocity of the disc. A planar photodiode (SLSD-71N6, Silonex, Montreal, Canada), 25.4 mm long, with a spectral sensitivity of 0.55 A/W at 940 nm, and a spectral range between 400 and 1100 nm, transmitted laser light and converted it into an analog electrical signal.

A reflective photosensor (EE-SY125, Omron, Scahumburg) used to detect the analytical areas includes an infrared LED of 950 nm and a phototransistor with a sensing distance ranging from 0.5 mm to 2 mm. The operational principle of the photosensor is based on the detection of the different reflectivities between the sensing object and the disc. For that, the analytical areas are marked in the outer rim of the disc by low reflectivity trigger footprints of 3.5 cm size. Because the unmarked perimeter presents higher reflectivity, the photosensor detects the marked areas, providing a trigger signal to the data acquisition board in order to start capturing data exclusively from those zones.

A custom-built electronic board (DAB) incorporates the planar photodiode and the photosensor. The function of the board is 2-fold. First, the detection of analytical areas, and second the amplification of the analog signal. Both functions are carried out at the same time the CD drive performs its original function of reading and writing data. In this way, during the data acquisition process only the signals from the detection areas are digitized by the data acquisition board (DAQ), stored in the computer and deconvoluted into an image for further quantification. A scheme of the developed detection system is shown in Figure 1. The detector system is controlled by software running on a Windows-based computer connected to the PC through a universal serial bus interface (USB2.0) to become portable.

Custom software was written in Visual C++. The software provides the control for the CD/DVD driver to scan the surface of the disc, to modulate the disc linear rotation velocity to a specified spatial resolution, and to write data on the CD or DVD. Also, the software provides the control to the data acquisition board to configure the sampling frequency according to the disc rotation speed and desired angular resolution. To completely scan the surface of the L-CD, the software simulates the writing process of a 700 MB size file to a controlled disc rotation speed. The scan begins from the inner tracks of the disc, following the continuous spiral toward the outer tracks. The captured data of each detection area are represented within a sector that is formed by a set of arcs centered over a radial direction, starting from the inner toward the outer radial. The software collects the data of each area and stores them in independent files in uncompressed binary format and displays them into a graphical gray scale code image.

Because of the spatial difference between samples taken horizontally (each 13 μm) and vertically (each 1.6 μm), a graphical horizontal adjustment is done to display a proportional $x-y$ image. This software allows the image to be exported in a gray scale code to a compressed tif format or bitmap. Then, the images were processed with Photoshop 7.0 (Adobe Systems Inc., San Jose, CA), to map the lightest and darkest pixels into black and white before quantifying with GenePix software (Axon Inst., Union City, CA). Signal intensities of each spot were calculated by background subtraction.

Disc Surface Modification. *(a) PMMA Transamination.* PMMA (310 mg) was dissolved in THF (3 mL). Ethanolamine (1 mL) was added, and the mixture was refluxed for 5 h. After that, THF was removed under vacuum and the solid was dissolved in CH_2Cl_2. The organic phase was washed with MES buffer until the total removal of ethanolamine. The organic layer was dried with $MgSO_4$ and the solvent evaporated, providing the transaminated polymer as a colorless solid. The ^{1}H NMR spectrum showed 50% transaminated compound. ^{1}H NMR ($CDCl_3$): δ (ppm) 3.79 (t, CH_2OH), 3.57 (s+m, CH_3O and CH_2-NHCO), 1.87−1.78 (m, CH_2), 0.091 (s, CH_3), 0.806 (s, CH_3).

(b) Coating. The transaminated polymer was dissolved in Dowanol (5%). One milliliter of this solution was pipetted onto the metalized (gold) side of a standard L-CD for spin-coating at a centrifugal force of 47.33*g* (840 rpm). After that, the solvent was removed at 52 °C for 30 min.

(c) Isocyanate Treatment of Coated L-CD. Coated L-CDs were immersed into a solution of (3-isocyanatopropyl)triethoxysilane in 2-propanol (1%) for 10 min and cured at 62 °C for 15 min. Finally, they were washed with 2-propanol and dried at 62 °C.

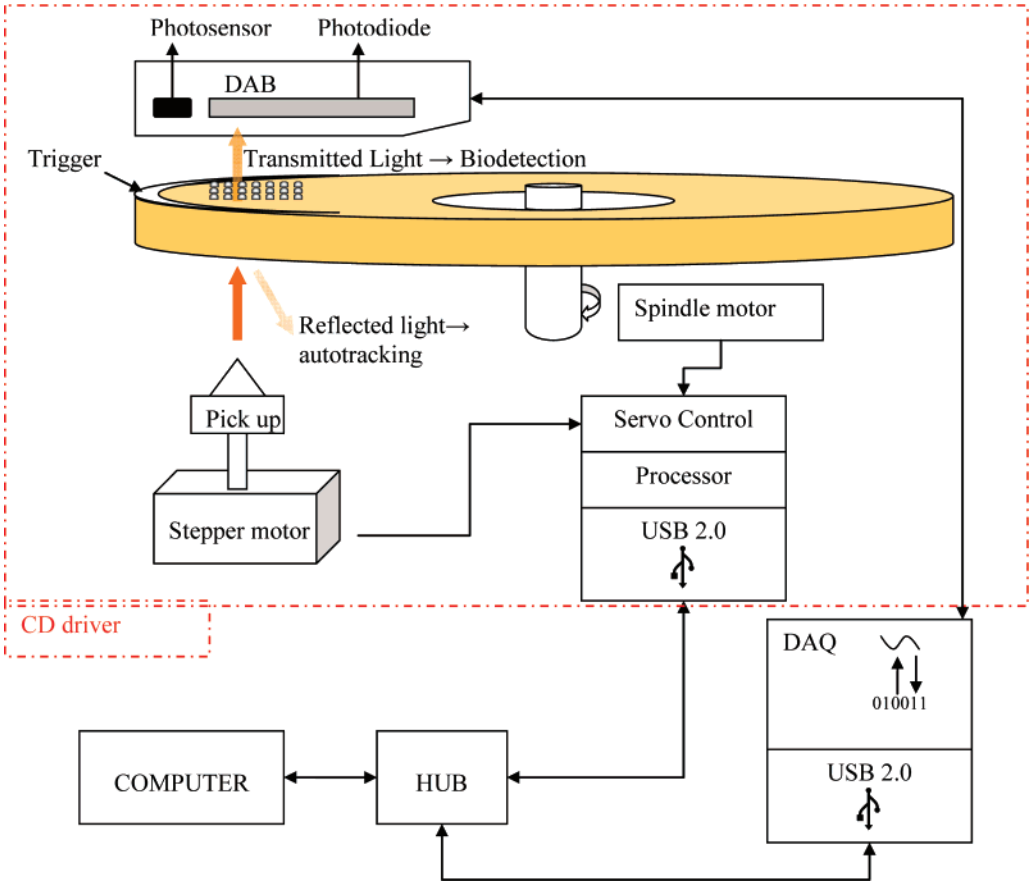

Figure 1. Schematic representation of the detection system. The set of servo systems (spindle and stepper motors) of the CD drive allows disc rotation and laser scanning. The transmitted light through the disc is transformed by the photodiode into an analog electrical signal (RF signal). At the same time, the photosensor detects the trigger footprints, starting the data collection on disc. The amplification/ detection board (DAB) is integrated into the CD drive unit and contains the planar photodiode and the photosensor. The data acquisition board (DAQ) digitizes the analog signals and transfers them to the computer for processing. On the disc, one detection area is represented.

Surface Behavior: DNA Immobilization and Hybridization Assays. Different 5′ Cy5-labeled and/or 3′ NH_2-ended oligonucleotides were employed for DNA immobilization and hybridization assays (Table 1). Amino-labeled DNA capture probes (SYM12 and SYM23) were serial diluted in carbonate buffer pH 11 ranging from 0.01 to 5 μM; 18 replicates (2 matrix of 3 × 3 spots) of each dilution were spotted on PMMA-modified chips. The fluorescence intensity of the spots was registered by the laser scanning detector to obtain a standard curve for the probes. Similar calibration curves for the Cy5-labeled target DNA were made. After immobilization and hybridization, the fluorescence signal of the spots was quantified and the amount of immobilized and hybridized DNA was calculated from the respective calibration curves.

Six 5′ Cy5-labeled, 3′ NH_2-ended oligonucleotide solutions (1 and 10 μM) were prepared employing different solvents: H_2O, 1 × PBS pH 7.5, and 1 × CB pH 11. Microarrays (3 × 3) of each solution were tip-contact-printed onto isocyanate-modified surfaces. L-CDs were then incubated for 2 h in a humid, dark chamber at room temperature. Then, the printed surfaces were washed with PBS-T and water and dried.

For the hybridization assays, the aminated probe SYM22 was covalently attached to the isocyanate surface as described above. Cy5-labeled target-DNA SYM8 was serial diluted between 0.1 and 1 μM in a mixture containing 6 × SSC, 0.6% SDS, 0.1% salmon sperm DNA, and 2% BSA. A 100 μL amount of the complementary oligonucleotide solution was dispensed on the microarrays and spread under a coverslip (22 × 22 mm). The temperature of hybridization was 37 °C, performing the incubation in a dark and humidified chamber for 1 h. After that, the slides were washed with 0.1 × SSC, 0.5% SDS for 15 min, and with 0.1 × SSC (5 min). Finally, the chips were rinsed with water and air-dried.

Table 1. Nucleotide Sequence of Probes and Targets Used

name	sequence (5′ to 3′)	5′ end	3′ end
SYM12	ACTGGTCGATCG	Cy5	C_6-NH_2
SYM23	TTACGATCGATTAGTTAGCCC-$(T)_{15}$	Cy5	C_7-NH_2
SYM8	AATGCTAGCTAATCAATCGGG	Cy5	none
SYM25	AATGCTAGCTAATCAATCGGG	biotin	none
SYM22	$(T)_{15}$-CCCGATTGATTAGCTAGCATT	none	C_7-NH_2

For hybridization assays on standard L-CDs, each disc was divided into eight printing zones and SYM22 was covalently attached as described above. Afterward, 35 μL of OVA in 1 × CB (pH 9.6) was dispensed on each printed area, spread under a coverslip, and incubated for 10 min at room temperature in a moist chamber. Then, L-CDs were washed 5 min with PBS-T and 5 min with water and air-dried. Biotin-ended target DNA (SYM25) was serial diluted between 0.001 and 0.2 μM in a mixture containing 6 × SSC, 0.6% SDS, 0.1% salmon sperm DNA, and 2% BSA. A 35 μL amount of the complementary oligonucleotide solution was dispensed on the microarrays and spread under a coverslip, performing the hybridization at 37 °C in a dark and humidified chamber, for 1 h. The discs were washed with PBS-T (15 min) and water (5 min) and air-dried. Following a similar procedure described by Cao et al. (*19*), 35 μL of 10 μg/mL streptavidin−HRP in 1 × PBS were deposited on each zone of the L-CD and incubated again for 20 min in a moist chamber at 37 °C. After rinsing with PBS-T (15 min) and water (5 min) and drying, 35 μL of TMB substrate solution was dispensed, causing a blue precipitate to appear on

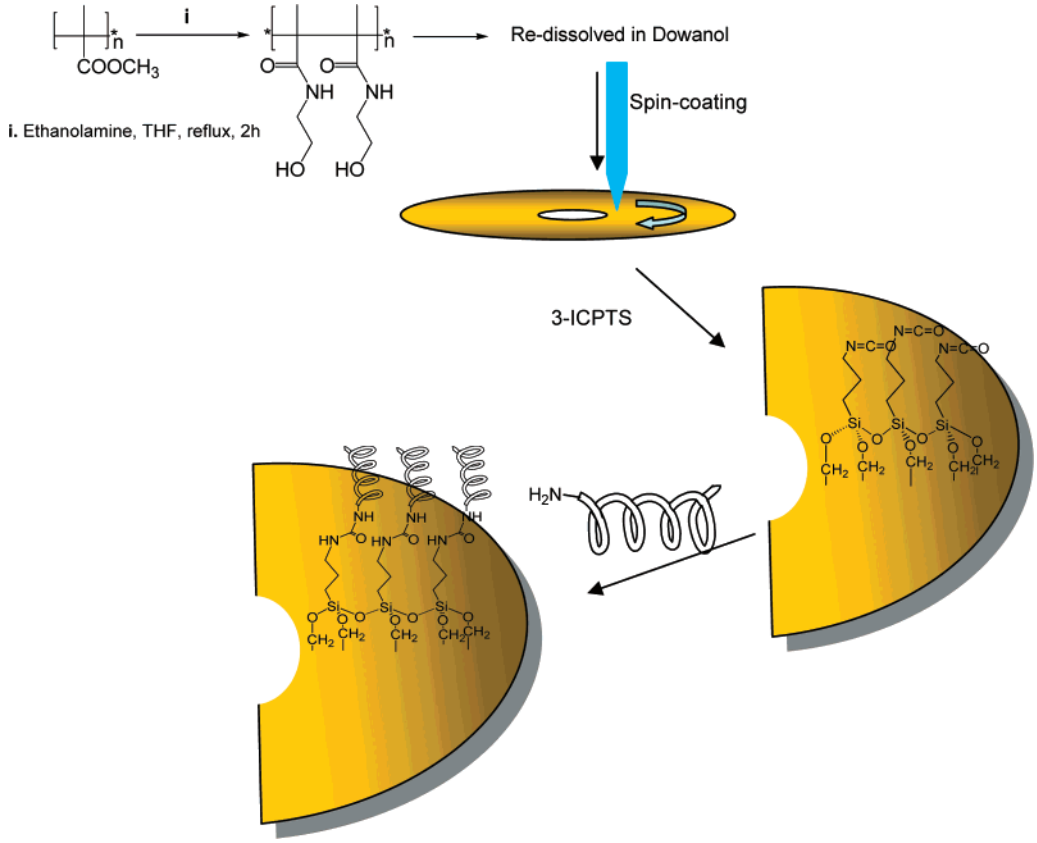

Figure 2. Scheme of the method for the covalent attachment of amino-ended oligonucleotides on L-CD surface.

the microarrays after 3 min. Results were read with the CD player as described above.

RESULTS AND DISCUSSION

L-CD Surface Modification. To achieve the covalent attachment of oligonucleotides onto the isocyanate-modified L-CD surface, the following three-step approach was investigated: (i) methyl ester transamination of PMMA employing ethanolamine in THF; (ii) spin-coating of the hydroxylated PMMA dissolved in Dowanol; (iii) introduction of the isocyanate moiety by the ether formation between the hydroxyl groups on PMMA and 3-isocyanatopropyltriethoxysilane (Figure 2).

The employment of organosilane allowed us to introduce a thin and homogeneous layer when working in a controlled manner. On the other hand, as isocyanate was chosen to attach the aminated oligomer, a urea bond was obtained. This bond is highly stable and easy to perform. The high reactivity of the isocyanate group results in a rapid coupling reaction and is particularly useful for covalent attachment of biomolecules such as nucleic acids, peptides, carbohydrates, lipooligosaccharides, etc.

The reaction of PMMA dissolved in THF with ethanolamine produced the aminolysis of the methyl ester, providing hydroxylated PMMA with a yield of around 50%, determined by NMR.

The modified polymer was dissolved in Dowanol for application of a thin layer onto the L-CD by spin coating and drying as described in the Experimental Section. The film thickness was 60 nm (measured by AFM and ellipsometry) with a high degree of homogeneity and reproducibility (tested by AFM and SEM).

For the organosilane treatment, selecting the proper solvent was a crucial point; thus, the mechanical and optical properties of the L-CDs must be unaltered in order to read the signals resulting from the hybridization assays. Most of the tested solvents (diethyl ether, tetrahydrofuran, dichloromethane, hexane) were promptly discarded; thus, they partially dissolved the base polymer (PC) and caused drastic alterations on the L-CD support. Finally, the selected solvent was 2-propanol. Also, different reaction concentrations, ranging from 0.1% to 5%, and times, between 10 min and 2 h, were studied.

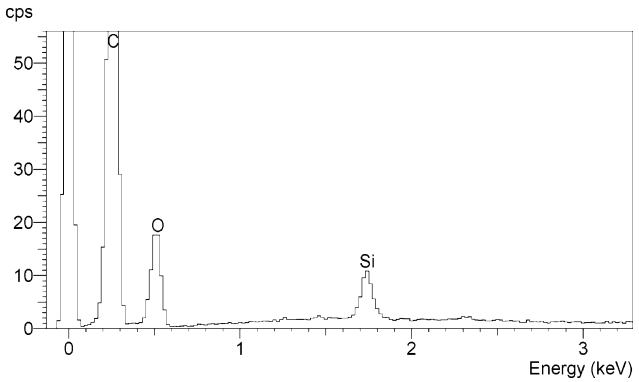

Figure 3. ED spectrum of the isocyanate-modified PMMA-L-CD surface.

To detect the effectiveness of ICPTS reaction onto hydroxylated PMMA-coated L-CDs, ED spectra were recorded; the presence of Si indicated that the organosilane reaction worked in a proper manner (Figure 3). Also, to examine the success of the reaction, the treated L-CDs were cut in chips (55 × 25 mm) and immersed into 4.5 mM fluoresceinamine dissolved in 2-propanol for 20 min. After washing, fluorescence was measured (λ_{exc} 485 nm; λ_{em} 580 nm), and the results were compared with different control chips to eliminate the solvent effect. This control is necessary, as the consecutive treatments produced an alteration of the surface and the fluorescein could swell the chip, resulting in high fluorescence signals. For that, treatment with fluoresceinamine was carried out for every step of the approach (raw L-CD, L-CD coated with hydroxylated PMMA, and isocyanate-treated L-CD) and compared.

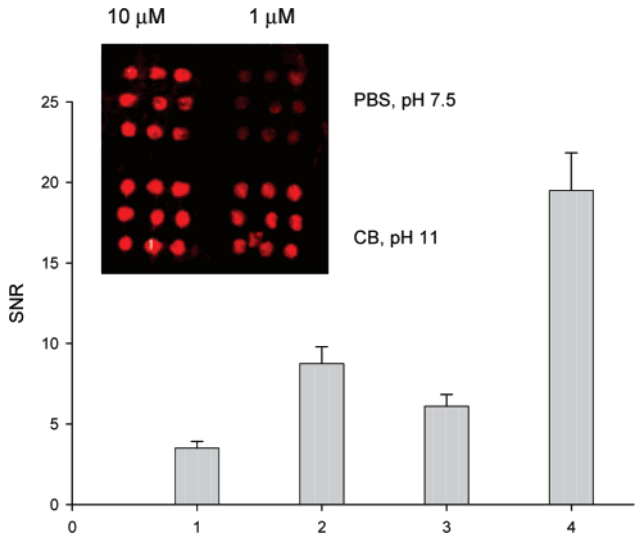

Figure 4. Results for oligomer immobilization on the isocyanate-modified L-CD surface using different buffers: (1) PBS, pH 7.5, 1 μM of probe; (2) PBS, pH 7.5, 10 μM of probe; (3) CB, pH 11, 1 μM of probe; (4) CB, pH 11, 10 μM of probe.

During the optimization process it was observed that the longer the treatment with organosilane the lower difference in fluorescence values with respect to the controls (similar conditions as for silanization but without the organosilane). This is explained considering that the solvent employed for the organosilane treatment did not alter the disc surface when short silanization times were applied, but prolonged exposure of PMMA to 2-propanol damaged the chip surface, lowering its transparency and producing a gummy texture.

Final conditions were chosen, limiting the time exposure to the organosilane solution (10 min, 1%) and curing the plastic after the treatment for 15 min at 62 °C. Oligomer immobilization was performed after the surface preparation.

DNA Immobilization. Under the chosen reaction conditions, L-CDs were prepared to assay the covalent attachment of oligonucleotides. For that, 1 μM and 10 μM 5′ Cy5-labeled, 3′ aminated oligonucleotide (SYM23) solutions were prepared in water, PBS buffer (pH 7.5), and carbonate buffer (pH 11). The oligonucleotide solutions were deposited onto the modified PMMA surfaces by tip-contact-printing. After immobilization, as explained in the Experimental Section, discs were cut in chips and the fluorescence of Cy5-labeled probes read using microarray laser scanning.

The best results were those employing CB, pH 11 (Figure 4), while results for water were far from that expected; the fluorescence at 1 μM and 10 μM was almost the same. This could be due to the unspecific adsorption and electrostatic forces. When working at pH values higher than 9, most of the amine groups in the oligomer exist as free amines and the nucleophilic attack of nitrogen to the isocyanate group is possible, providing a urea bond. However, at pH lower than 7, many of the amine groups exist as their ammonium salt and the covalent attachment is not possible, although it must be taken into account that this charged structure can adhere to the surface by electrostatic interactions as well as by hydrogen bonds.

In parallel, and in order to check the oligomer probe covalent attachment quality, several controls were performed employing raw L-CD, and hydroxyl-PMMA-coated L-CDs, raw and treated with 2-propanol. In all cases, the fluorescence signal was less than 10% of the fluorescence observed in isocyanate-modified L-CDs. Besides, to assess that all the attached oligomers were covalently bound to the disc surface, controls employing Cy5-labeled oligonucleotide without amine functionalization (SYM8) were printed on activated PMMA discs (blank assay), working in a way similar to that with amine-ended oligonucleotides. Again, the fluorescence signal was at the background level.

When the disc was inserted in the standard CD player and a recording simulation was done, the L-CD was read without presenting internal errors. It was concluded that the treatment proposed for oligomer immobilization on discs leave the L-CD available for further common use such as data recording, and the L-CD surface remained, to the naked eye, unaltered after the chemical treatment and oligomer attachment. This opens up an interesting application field of the developed methodology for performing chemical assays on the top of treated PMMA-L-CDs, using the bottom side (PC) to read it with the CD player laser, and for the registration of the analytical data and other management information. Another advantage of this methodology is the application of widely studied organosilane chemistry on CDs. By making use of these reagents, different ways of biomolecule covalent attachment are possible; for example, by employing a mercaptoorganosilane, thiol-ended biomolecules could be attached by formation of disulfide bridges.

To estimate the density of DNA single strands covalently attached to the surface, calibration assays were performed on treated L-CDs, employing SYM23 (analogous to SYM22, but Cy5-labeled). Considering the tip-contact dispensed volume (3 nL) and the averaged spot diameter, through the calibration curve, an immobilization density about 2 pmol/cm^2 for a 5 μM concentration of the spotted oligonucleotide was estimated. This value was in agreement with data found in the literature for glass (*20*) (2 pmol/cm^2), PMMA (*17*) (4 pmol/cm^2), and gold (*21*) (3 pmol/cm^2).

Hybridization on L-CD. One of the main concerns when preparing DNA microarrays is to achieve a good yield in hybridization assays. To check the performance of the developed approach, several experiments were conducted employing different concentrations of both DNA probe (1, 5, 10, and 20 μM) and Cy5-labeled complementary DNA strand (0.1, 0.2, 0.5, and 1 μM). The hybridization density (pmol/cm^2) was calculated from interpolation of the registered fluorescence value in the corresponding calibration curve.

Figure 5 shows the hybridization density reached at different sDNA probe concentrations, indicating that the best results correspond to 5 μM sDNA. Besides, it was observed that when probe concentration as high as 20 μM was assayed, the hybridization did not give good results; the spots were surrounded by a halo of fluorescence and presented a "black hole" at the center of the spot. This could be due to the high surface coverage with DNA probes that may cause a crowding effect, lowering the accessibility of the probes (*21*). Probably, repulsive electrostatic and steric interactions also increased with probe density. Likewise, by increasing the concentration of the complementary oligonucleotide solution, no significant improvement of hybridization efficiency was detected. Consequently, it was assumed that 0.1−0.2 μM hybridization solution and 5 μM DNA probe were the optimal for this assay. Related to immobilization density, the maximum hybridization efficiency was achieved between 2 and 4 pmol/cm^2. These results were in agreement with studies (*17*) performed on chemically modified PMMA, which provide oligonucleotide densities around 0.5 pmol/cm^2, employing similar hybridization conditions.

Finally, in order to demonstrate that the developed method is fully applicable to standard CD technology, discs were treated according to the described procedure, and different concentrations of SYM 22 probe (1, 5, 10, and 20 μM) were immobilized on the L-CD surface. After hybridization with serial dilutions of SYM 25 (0.2 to 0.001 μM), the L-CD was treated with HRP-conjugated streptavidin for 20 min, washed, and developed with

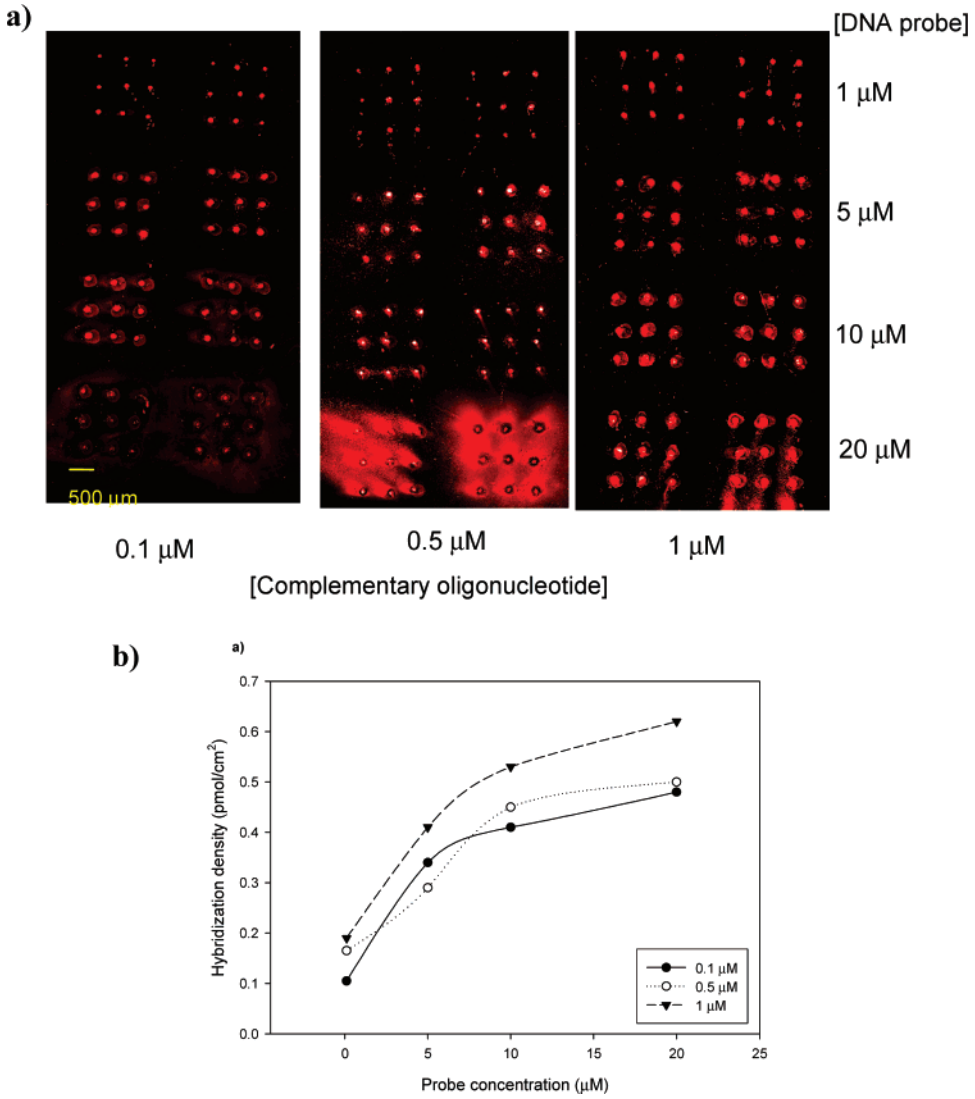

Figure 5. (a) Microarray picture of the hybridization assays performed on PMMA-modified L-CD employing Cy5-labeled complementary oligonucleotide and reading by microarray laser scanning. (b) Curves of hybridization density using different probe and target concentrations.

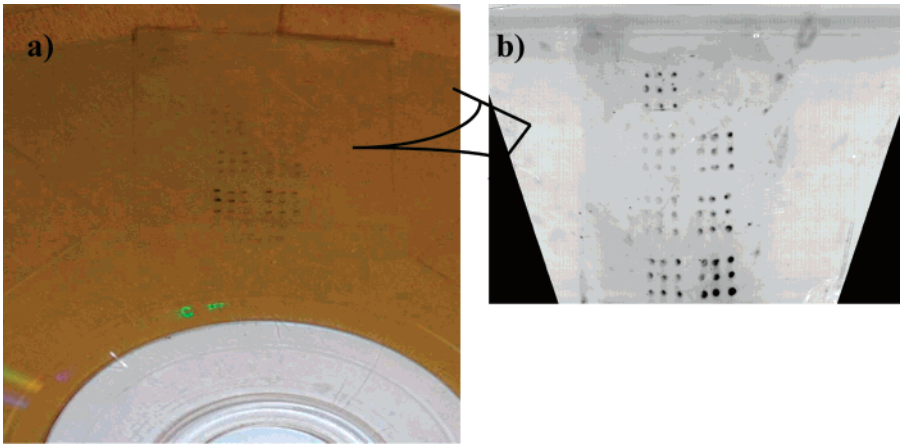

Figure 6. (a) Image of the microarray (blue spots) developed on modified L-CD after hybridization assay. (b) Magnification of the same image obtained with the CD player.

TMB for 3 min; the blue precipitate appeared in the matrixes where the hybridization happened (Figure 6) as read with the CD player.

Several controls were performed, avoiding the different working protocol steps: isocyanate treatment, covalent attachment of probe, OVA blocking of remaining isocyanate groups, hybridization solution and streptavidin−HRP incubation; none of them displayed blue points except when the OVA treatment was suppressed, as was expected.

Related to the detection system, similar trends where achieved employing conventional readers (Axon detector) or CD player. Thus, the best results were obtained for 5 μM of probe and 0.2 μM of hybridization solution although it was possible to detect concentrations of 0.005 μM hybridization solution (even by the

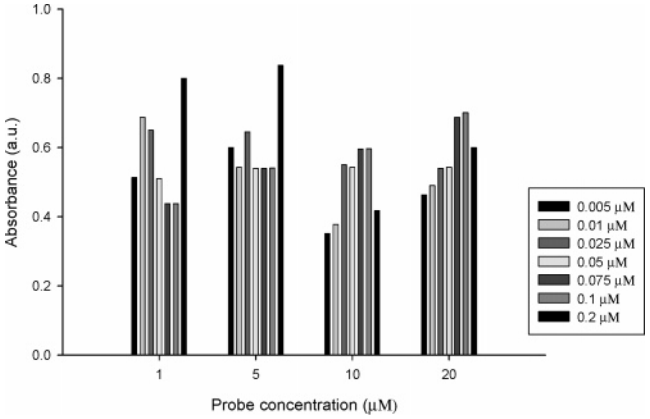

Figure 7. Signal intensity values read with the CD player for different probe and target concentrations.

naked eye). This is because of the higher sensitivity of the HRP labeling; thus, the developing procedure is based on an enzymatic reaction. The absorbance intensities of different concentrations of probe and target are shown in Figure 7. The most remarkable was that the highest signal is obtained for 5 μM probe and 0.2 μM target, as that which occurred in the assays with fluorescence detection. Also the high signal obtained for the lowest concentrations of hybridization solution provides an idea of the good sensitivity achieved from the disc isocyanate modification and CD player detection approach. These assays demonstrate that the proposed system works for qualitative detection; no quantification was intended, as the quantification protocol will depend on the application, but in general, intensity values can be converted into biologically relevant outputs by comparing the experimental and control elements present in a given microarray (*22*).

CONCLUSION

In this work a PMMA isocyanate modification is proposed for the covalent attachment of biomolecules, employing well established organosilane chemistry. The method retains the physical properties of the support, and its application is versatile and clean. The procedure is easily carried out and is compatible with aqueous media chemistry, which is crucial for its bioutility. Immobilization densities about 2 pmol/cm^2 were reached with oligonucleotides. The values were in agreement with other described methods for microarrays on modified PMMA, glass, and other surfaces.

The application on standard recordable compact discs opens an interesting field because of its potential advantages such as low fluorescent background, high quality materials at very low cost, high available surface (90 cm^2), and further use of laser technology found in the CD players as detectors, to be applied at point of need to health care or *in situ* environmental analysis.

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