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Photo-click chemistry to create nucleic acids dextran-based microarrays.

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Abstract

Different hydrogels are reported in the literature to create generic platforms for protein microarray applications. Here, a novel strategy was developed to obtain high performance microarrays. It uses a dextran hydrogel to covalently immobilize oligonucleotides and proteins. This method employs aqueous solutions of dextran methacrylate (Dx-MA), which is a biocompatible photopolymerizable monomer. The approach promotes the covalent attachment of the capture probes inside the hydrogel by a thiol-acrylate coupling reaction by light while the dextran polymer is formed. The hydrogel microarrays were prepared on different surfaces, such as Blu-ray disk, polycarbonate or alkene functionalized glass slides, and showed high probe loading capability and good biorecognition yields. This methodology presents certain advantages, like low cost, short analysis times, low limit of detection, and multiplex capability, among others. The confocal fluorescence microscopy analysis demonstrates that the receptor immobilization and the biorecognition event take place inside the hydrogel and not merely on the surface.

Introduction

Microarray technology has received growing interest in the recent years because of its potential advantages such as miniaturization, multiplexing and high throughput screening capabilities.[1, 2] Microarrays, frequently printed chips, are considered a collection of probes arrayed in an ordered manner onto a solid support that allow many analytes to be detected simultaneously, and they find application in different fields including clinical diagnosis, genomics, forensic and food and environmental control. [3, 4]

Despite their high degree of development, there are still some critical issues to improve before achieving optimal the microarray technology performance. One of those key points is the way the probe is anchored to the surface, which considerably determines the system's reproducibility and sensitivity. Many works are found in the literature in which nucleic acids and proteins probes are immobilized onto the support by adsorption, covalent or by electrostatic forces.[2, 5, 6] Among them, covalent attachment is normally preferred for its robustness and directionality. However, problems associated with low immobilization densities or the denaturalization of the probe anchored to the surface often appears.

Much work has been done on the covalent immobilization of biomolecules for microarrays or biosensing [7]. Approaches for effective immobilization include the use of polymer brushes [8], the optimization of linkers and horizontal spacers [9], especially for gold surfaces functionalized with thiol-gold SAMs [10], dendrimers [11, 12], or tuning surface properties [13]. A wide range of chemical-bonding strategies are used, and range from standard carbodiimide coupling or imine formation to designs based on more sophisticated reactions, such as click-chemistry [14–16][17][18, 19]. However, plenty of interest is still shown in developing new immobilization approaches. Special emphasis is placed on providing easy high-efficiency methods for probe immobilization and biorecognition events that can be broadly applied.

One way of dealing with these difficulties is to employ a hydrogel for immobilization.[20] Hydrogels offer the advantages of changing from a 2D to a 3D space to immobilize the probes by increasing the surface mass loading capacity. The hydrogel also provides a hydrophilic environment that mimics the biological environment and reduces the non-specific adsorption of proteins. Thus, hydrogels applied to microarrays provide high signal-to-noise ratios given the combination of probes immobilization at high density and low nonspecific binding.[8, 9] Currently, hydrogel based biochips are commercially available but simplification in manufacturing and broadening the range of available immobilized probes is still demanded.

Cross-linked dextran-hydrogels (CDH) are known to provide favorable extracellular matrix conditions with high water content and biocompatibility, so they have been modified and studied in different bioapplications such as SPR biosensing,[10, 11] scaffolds for tissue engineering,[12, 13] antibacterial films,[14, 15] or controlled delivery systems.[29]

As for using CDH for the covalent immobilization of probes for microarray technology, most approaches perform probe immobilization well either after creating the CDH layer on the support [17-19] or prior to dextran layer formation.[31] In both cases classic coupling chemistries are employed for probe anchoring. Thus, very few approaches use alternative methods like those which employ reactions based on click-chemistry[21, 22] to immobilize probes after CDH layer deposition.

Recently, our group developed a pool of photo-click reactions that employ UV light to activate thiol-bearing probes to promote the covalent link to alkene, alkyne or epoxy groups present on the surface.[34–37] All those reactions met the advantages associated with click-chemistry such as proceeding under mild conditions in the presence of oxygen, being regioselective, tolerating many functional groups, performing in neat or benign solvents like water, and providing quantitative or near-quantitative yields.[38] The microarrays developed by these approaches show improved performance of classic coupling methods by providing better sensitivity and making the process quicker and cleaner.

In the search for further improvements, here we propose a method by which the CDH are formed by the photo-crosslinking of acrylate derivatized dextran, and the covalent attachment of thiol-bearing probes takes place simultaneously to CDH layer formation. During the polymerization process, CHD are also covalently attached to surfaces that have epoxy, alkene, acrylate or alkyne moieties. Here, CHD microarrays are created on acrylate functionalized glass and BluRay Disc (BD) which have an epoxy or vinyl based protective layer. Microarrays can then be prepared in very short times, following a clean rapid procedure that employs only water as a solvent. Light plays a double role by catalyzing both the polymerization of acrylate-dextran monomers and the covalent attachment of the probe by thiol-acrylate coupling.

Materials and Methods

Chemicals

Glass microscope slides were obtained from Labbox (Barcelona, Spain). 25 Gbyte Bluray Dics (BD) were ordered from MediaRange GmbH (Bad Soden, Germany). Commercial 2D-Epoxy and 3D-Epoxy glass slides were purchased from PolyAn GmbH (Berlin, Germany) Albumin from bovine serum ≥98 % lyophilized powder, anti-Bovine Albumin antibody produced in rabbit, Dextran from *Leuconostoc spp*. (Mr 450,000-650,000), Glycidyl methacrylate (GMA), 4-dimethylaminopyridine (DMAP), dimethylsulfoxide (DMSO), D_2O and ethanolamine were purchased from Sigma-Aldrich Quimica (Madrid, Spain). 3-(Mercaptopropyl)trimethoxysilane and 3-(Trimethoxysilyl)propylmethacrylate were from Acros (Spain). The employed oligonucleotides were supplied by Sigma-Genosis (UK). The used sequences are shown in Table 2. Alexa Fluor (Alx) 647 NHS Ester was from by ThermoFisher (Spain). The labeled bioreagents were prepared in the laboratory following the supplier's instructions.

Table 1. Name and sequence of the different employed oligonucleotides

Name	Sequence (5'- 3')
Probe 1[a]	SH-$(T)_{15}$-CCCGATTGACCAGCTAGCATT
Probe 1*[a]	SH-$(T)_{15}$-CCCGATTGACCAGCTAGCATT-Cy5
Probe 2	H_2N-C_7-$(T)_{15}$CCCGATTGATTAGCTAGCATT
Probe 3	SH-ATTCACTCAACATCAGTCTGGTAAGCTAGTGAAT
Probe 4	SH-ATCGACAAGTGTGAGTTCTACCATTGCCAAAAGTCGAT
Target 1*	Cy5-AATGCTAGCTGGTCAATCGGG
miR-182*	Cy5-UUUGGCAAUGGUAGAACUCACACU

[a] Comparing to the commercial 2D-Epoxy slides, Probe 1 had an amine group instead of a thiol group, which we referred to as an "aminated Probe 1".

Instrumental techniques

Microarray printing was carried out with a low volume non-contact dispensing system Biodot AD1500 (Irvine, CA, USA). Contact angle measurements were taken with Attension Theta Lite and images with OneAttension both from Biolin Sci (Spain). Irradiation was carried out with UV-Ozone Cleaning System FHR (Ottendorf, Okrilla, Germany). Absorption measurements were taken with NanoDrop 2000 from Thermo Scientific (Spain). Microarray fluorescence measurements were carried out with a homemade surface fluorescence reader (SFR) equipped with a CCD camera, as described elsewhere [39] and with a GenePix™ 4000 B Array Scanner. Image data processing was performed with the GenePix Pro 4.0 software (Axon, CA, USA). The fluorescence confocal microscopy measurements were taken by a Leica TCS-SP2-AOBS at 633 nm excitation and 670 nm emission laser lines. XPS spectra were acquired with SEGE 150 equipment from SPECA GMBH-Surface Analysis (Germany).

Experimental Procedures

Synthesis of acrylate-dextran (Dx-Ma)

Methacrylate groups were incorporated into Dextran by modifying the procedure described by van Dijk-Wolthuis.[40] Briefly, Dextran (Mr 450,000 - 650,000, 1.7 g) was dissolved in DMSO (15 mL). Once the polymer was dissolved, DMAP (0.34 g) and GMA (1 mL) were added. The reaction was carried out at room temperature, in the dark, with stirring for 72 h. The product was precipitated in cold EtOH (200 mL) and washed several times with EtOH. After freeze-drying, the polymer modification was assessed by NMR (in D_2O), and ATR-FTIR.

Surface preparation

To prepare BD microarrays, BDs were used in raw after washings with isopropanol and deionized water (DIW) and drying. They were cut into slides of 5×2.5 cm whenever necessary.

For the acrylate-modified surfaces, glass slides were washed with isopropanol and water and dried. Then they were activated with ozone surface cleaner for 10 min before being immersed in a toluene solution containing 10% (w/v) of the corresponding organosilane. After 1 h of stirring at room temperature, substrates were cleaned with toluene, isopropanol and cured at 120 ºC for 30 min. Successful functionalization was assessed by measuring WCA on surfaces before and after silanization.

Oligonucleotide immobilization and hybridization assays by Dx-Ma

An aqueous solution of Dx-Ma was prepared by dissolving 1 g of the modified dextran in 10 mL of deionized water (DIW). This solution was employed as a solvent to prepare several concentrations (0.5, 0.1 and 0.05 μM) of Probe 1, Probe 2, Probe 3, and Probe 4, which were spotted onto the solid support by creating matrices of 4 spots per row (30 nL/spot). Generally, an extra row having Probe 1* at 0.5 μM was also included as a positive immobilization control. The microarrays were irradiated for 40 s at 254 nm (50 $mW \cdot cm^{-2}$), washed with water, and dried. After that, hybridization with Target 1* in SSC5×, from 0.25 to 0.0001 μM, was carried out for 1 h at 37 ºC. Then the chips were washed with SSC1× and dried. Fluorescence was registered with the homemade surface fluorescence reader (SFR) and quantified by GenePix 4.0 software.

Oligonucleotide immobilization and hybridization assays onto 2D-Epoxy and 3D-epoxy slides

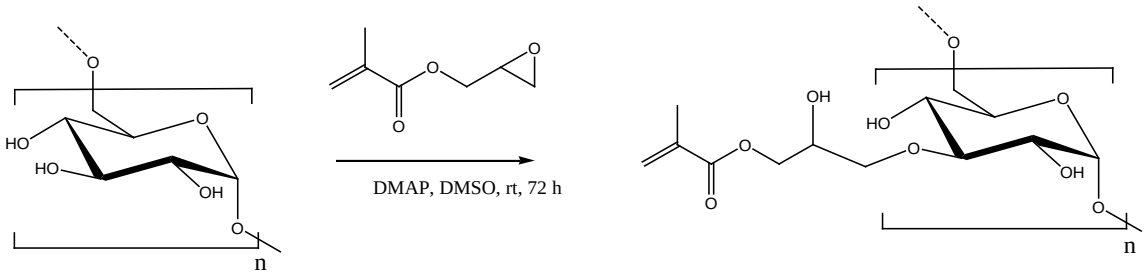
Microarrays were created following the supplier's instruction. To do so, aminated Probe 1 was dissolved in carbonate buffer (pH 11) at 1 μM. The 4x4 microarrays were spotted (30 nL/spot) at 24 ºC and 70% relative humidity. One of the rows contained the aminated Probe 1 labeled with a fluorophore, while the others were of the unlabeled aminated Probe 1. Then, slides were incubated overnight in a humidity chamber filled with 50 mL SSC1x. Later, the slides were dried at 80 ºC and blocked with 50 mM ethanolamine in 0.1 μM Tris buffer (pH 9) for 60 minutes. The surface was sequentially washed with SSC 4×, SSC 2× + 0.1% (w/v) SDS, SSC 1×, SSC 0.2× and SSC 0.1×, and finally with deionized water.

Microarrays were hybridized with Target 1* at 0.5, 0.25, 0.12 and 0.06 μM in SSC 5× for 60 minutes at 37 ºC as above. Fluorescence was read with the SFR and quantified as before.

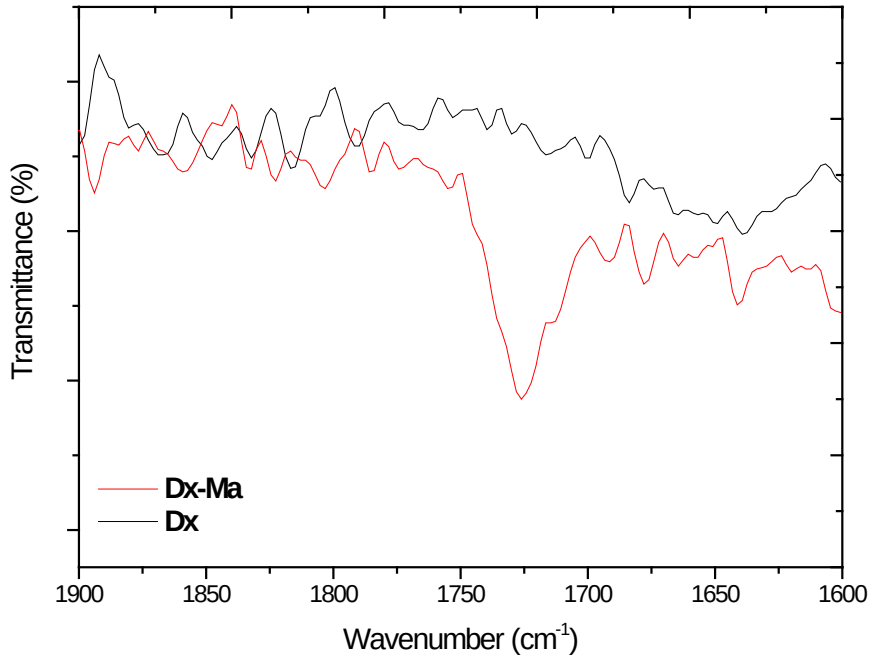
Discussion and Results

First, dextran polymer was modified with the methacrylate groups to have a photocrosslinkable polymer (Dx-Ma) as indicated in **Chart 1**. For this purpose, a modification of a protocol previously described was used.[40] The incorporation of acrylate into dextran was confirmed by ^{1}H-NMR, with the presence of signals at 6.15 and 5.7 ppm, corresponding to vinyl H, and at 1.91 ppm, corresponding to methyl group (Fig 1). The ATR-FTIR spectrum showed the band at 1728 cm^{-1}, which corresponded to the carbonyl stretching of methacrylate (Fig 1).

Chart 1 Dx-Ma synthesis



a)



b)

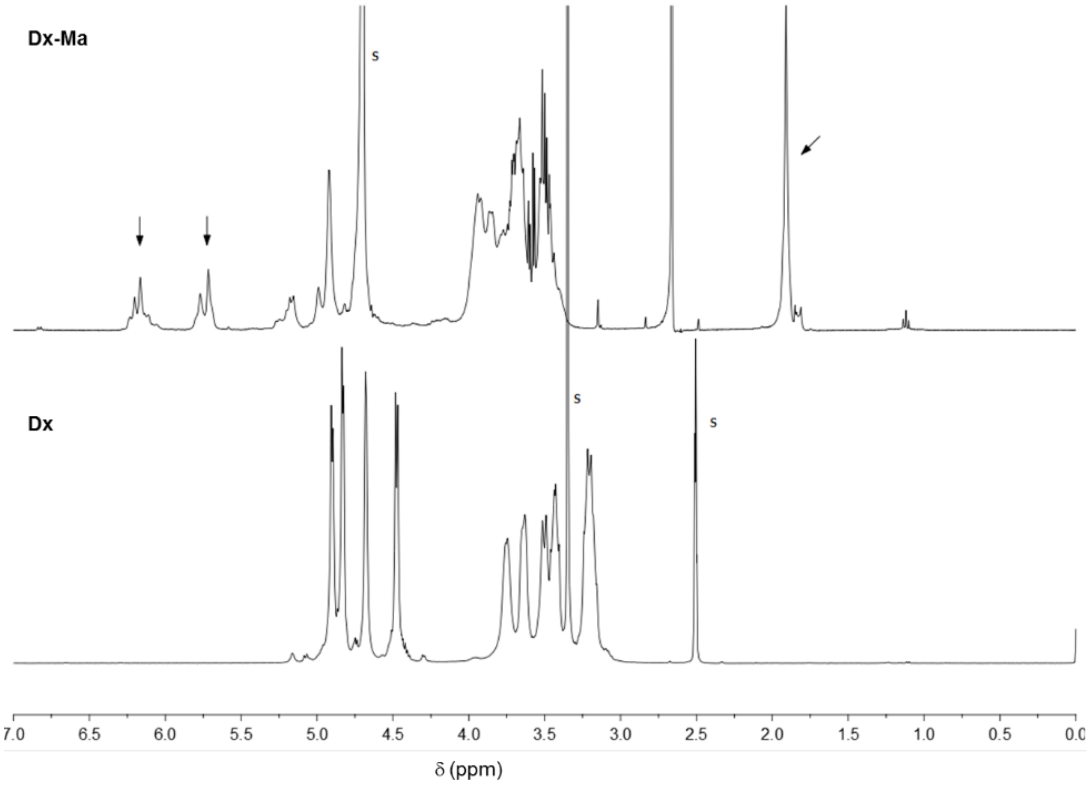


Fig 1a) ATR-FTIR spectra of Dx and Dx-Ma, the presence of a band at 1728 cm^{-1} reveals the successful incorporation of methacrylate moieties. **b)** ^{1}H-NMR spectra comparison between dextran (below) and acrylate modified dextran (above). The presence of acrylate moieties is verified by the signals at 6.15, 5.7 and 1.91 ppm (black arrows) attributed to vinyl H and methyl H, respectively.

To optimize the hydrogel formation for microarray purposes, BD were used as a solid support. BDs are easily available chip platforms on the mass scale. This type of digital discs has an external protective polymeric layer based on vinyl or epoxy resins (composition varies according to the manufacturer). Thus the remaining vinyl/epoxy groups on the surface will allow the hydrogel to be anchored to the surface during its photocrosslinking. Moreover, given the chemical composition of the anti-dust and anti-scratch protective layer, a low fluorescence background was observed when the fluorophore-labeled proteins or oligonucleotides were incubated on the surface. This indicates that low non-specific adsorption takes place on the surface.

Dx-Ma was dissolved in water at different w/v ratios (from 1:10 to $1:10^{8}$), spotted onto the BD surface and irradiated for 30 s at 254 nm. After washings the quality of spots was inspected by the naked eye. Only the microarrays created with the 1:10 ratio were visible and offered acceptable uniformity. So, this ratio was set for further experiments. Other aqueous buffers were also assayed instead of water (SSC1×, PBS1×) and provided similar results.

The water contact angle changed from 80º for the raw BDs to approximately 13º after Dx-Ma deposition (ESI, Fig S1).

After ensuring the right and reproducible formation of the Dx-Ma hydrogel, the co-immobilization of nucleic acids while the hydrogel was forming was studied. It was hypothesized that thiol-bearing oligonucleotide probes would be covalently attached to the hydrogel by thiol-acrylate photocoupling. To demonstrate this, a microarray was prepared where increasing

concentrations of 5'-thiolated, 3'-Cy5 labeled Probe 1* (from 0.1 to 0.0125 µM) in Dx-Ma 1:10 in water were spotted onto BDs and irradiated several times (40s, 1 min, 3.5 min and 7 min). In the microarray several rows with non thiolated 3'-Cy5 labeled Target 1* at the same concentrations as Probe 1* were also added. After extensively washing with water, fluorescence was recorded and quantified. For 40 s of irradiation, fluorescence was only detected for Probe 1* which proves the key role of thiol moiety in probe immobilization. For longer irradiation times, Target 1* also remained entrapped inside the hydrogel, probably due to the high degree of crosslinking in the polymer which prevents oligonucleotide from leaving. (Figure 2)

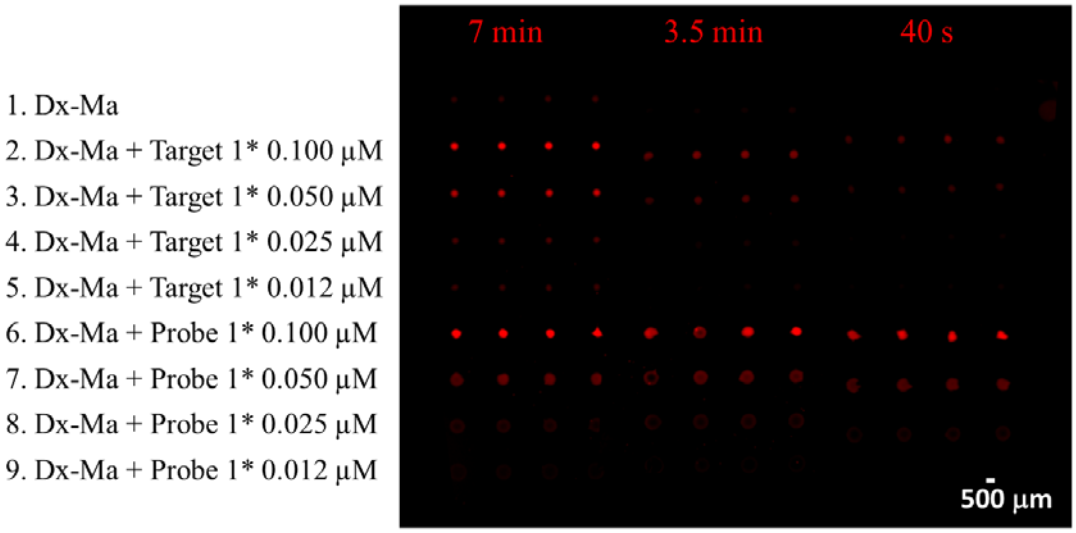


Fig 2 Fluorescence images for the Dx-Ma microarray irradiated for 7 min, 3.5 min and 40 s in the presence of Probe 1* or Target 1* at different concentrations. For the 40 s irradiation, only thiol bearing Probe 1* are immobilized in the microarray.

Buffers like TC and PBS1x were also assayed instead of water for immobilization but they did not result in any significant improvement, and water was set as the solvent for microarrays construction. Besides, irradiation times below 40 s were assayed (from 5 s to 30 s). The hydrogel remained attached to the surface from the 20 s irradiation time, but fluorescence spot quality was optimal for the times exceeding 30 s.

The XPS analysis demonstrated the successful immobilization of Probe 1*. On the one hand, the atomic percentage on the surface with Dx-Ma showed the presence of S and N atoms, which did not appear for Dx-Ma alone (Table 2). On the other hand, C1s peak deconvolution indicated the presence of bonds C=C, and of bonds C-N and C-S at 284 and 284.6 eV, respectively, which were attributable to the oligonucleotide structure. (Fig 3)

Table 2. Atomic percentage provided by XPS for BD+Dx-Ma and BD+Dx-Ma+Probe 1. nd denotes non detected.

Quantification (Atomic %)	**O 1s**	**C 1s**	**Si 2p**	**N 1s**	**S 2p**
CHD	35.6	63.7	0.7	nd	nd
CHD+Probe 1*	38.6	60.5	nd	0.6	0.3

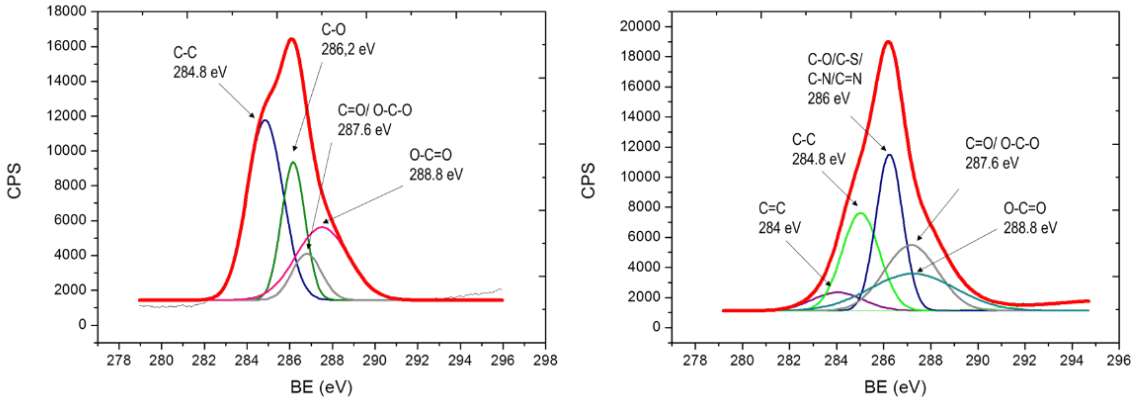


Fig 3 C 1s peak deconvolution for the XPS spectra of BD+Dx-Ma (left) and BD+Dx-Ma+Probe 1. The contribution of bonds C-S, C-N and C=N are attributed to the presence of oligonucleotide.

By bearing in mind that the protective layer on top of BDs has vinyl or epoxy groups, the thiolated probes can be directly photoattached by thiol-ene and thiol-epoxy coupling chemistries.[35, 37] A comparison between the direct attachment and the immobilization through the developed hydrogel was made. A microarray was prepared with Probe 1* and Probe 1 at 0.5 µM both in Dx-Ma 1:10 and directly spotted onto the BD surface. The spotted probes were irradiated for 40 s. After water washing and hybridization with Target 1* (1 µM in SSC5×), fluorescence was quantified and compared. The same experiment was also run by irradiating for 4 min to facilitate probe attachment directly to the surface without Dx-Ma. As seen in Fig 4, the Dx-Ma hydrogel increased the amount of immobilized probe and, thus, hybridization. Longer irradiations made spot quality worse with a coffee ring effect, and also the photobleaching of Probe 1* when spotted without Dx-Ma.

The hybridization signal was 3-fold higher for the Probe 1 immobilized via Dx-Ma than the direct immobilization, and it resulted in slightly higher signals for the 30 s irradiations than for 4 min ones.

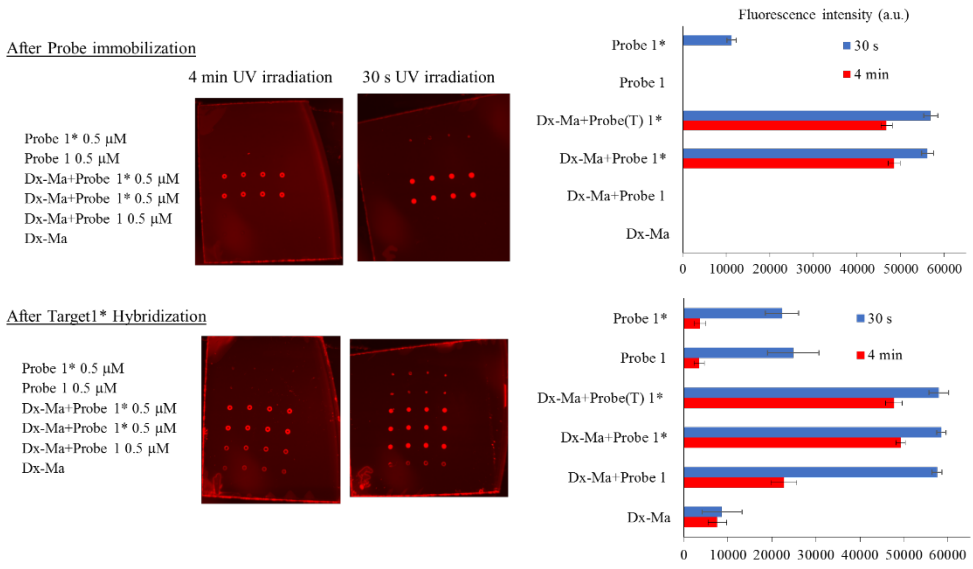


Fig 4 Fluorescence intensity obtained for microarrays when comparing the hybridization with Target 1* by Probe 1 immobilized via Dx-Ma or directly onto the surface. Both the immobilization of Probe 1 and the hybridization with Target 1* provides much higher signal intensity when immobilizing via Dx-Ma.

To demonstrate selectivity in both covalent attachment and hybridization, and in order to assess sensitivity, a microarray was prepared by printing Probe 1 and Probe 2 at several concentrations (0.5, 0.1 and 0.05 μM). Both probes had the complementary sequence to Target 1*, but Probe 2 bore an amine group at the 5' end instead of a thiol group. A 5'-thiolated probe with a non-complementary sequence, Probe 3, and the positive immobilization control, Probe 1*, were also included in the array. After immobilization, the hybridization with Target 1* in SSC5× at lowering concentrations (0.25, 0.1 and 0.05 μM) was done, and fluorescence was recorded and compared. As seen in Fig 5, only those rows containing Probe 1 provided significant fluorescence, which correlated with the concentration of Target 1* used in the hybridization steps. So, this experiment demonstrated that, on the one hand, a thiol group is needed for efficient probe immobilization, and, on the other hand, the specificity of hybridization.

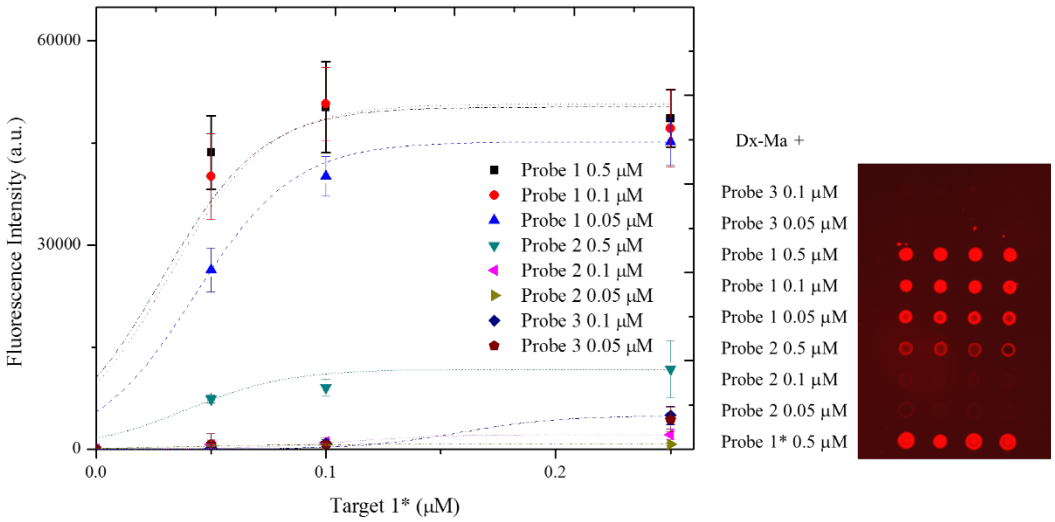


Fig 5 Comparison of the fluorescence intensity obtained for the increasing Target 1* concentrations in microarrays which immobilized the following probes via Dx-Ma: a thiolated complementary strand (Probe 1), an aminated complementary strand (Probe 2) and a thiolated non complementary strand (Probe 3).

To determine the sensitivity achieved by the developed hydrogel, new microarrays were created where Probe 1 was immobilized at different concentrations (0.1, 0.2 and 0.4 μM) and hybridization with Target 1*, from 0.0001 to 0.1 μM in SSC5×, was carried out as before.

The obtained fluorescence intensities were similar for the different Probe 1 concentrations, with very good performance found even for the lowest probe concentration. In Fig S2, ESI the hybridization curve is shown for 0.1 μM of Probe 1. The linear range went from 0.0001 to 0.01 μM, which is a wide interval, and was also accomplished for the higher Probe 1concentrations. A comparison of the fluorescence intensities achieved for the different concentrations of Probe 1 is found in the Supp Info (ESI, Fig S3 and S4). The limit of detection was calculated as the concentration that provides the blank signal plus 3-fold its standard deviation, which was 85 pM, and compared very well with previous reported sensitivities using analogous probes and different ways to anchor.[35–37]·[41, 42] The reached value is limited by the sensitivity of the SFR device, but a lower LOD could be reached using a more sensitive fluorescence detector.

Microarray reproducibility was assessed hybridizing 10 slides with 4x4 spots of Probe 1 0.1 μM with Target 1* 0.25 μM. Intra-chip RSD was 10% while inter-chip RSD was 14%.

Probe immobilization performance and hybridization capacity were compared to other standard microarray surfaces. To do so, 2D-Epoxy and 3D-Epoxy glass slides were purchased from a commercial supplier. They can immobilize probes through their amino groups. Thus, microarrays of aminated Probe 1 were created following the supplier's protocols. Then microarrays were hybridized with Target 1* and compared to our Dx-Ma microarray by checking several parameters. The whole comparison can be found in Table 3.

Table 3: Comparison of commercial 2D- and 3D-Epoxy microarrays to the DX-Ma microarrays.

Surface	[Probe 1] (μM)	Immobilization time (h)	Blocking	Immobilization Fluorescence signal (Probe 1*)	Hybridization fluorescence signal (Target 1* 0.25 μM)	SNR	Fabrication + Assay time
2D-Epoxy	10	Overnight	50 mM ethanolamine 60 min	10939±680	8582±1387	30.5	26 h
3D-Epoxy	10	Overnight	50 mM ethanolamine 60 min	19687,0 ±1789,3	10875±965	52	26 h
BD Dx-Ma	0.5	40 s	none	25518 ±3810	19009 ± 3040	397	3 h
BD Dx-Ma	0.1	40 s	none	10925,7±400	17215±1658	180	3 h

Our approach offers greater immobilization and hybridization than commercial slides, and the time to prepare and run the assay is significantly shorter. Besides, no blocking is needed and a 100-fold less amount of probe is needed to achieve similar fluorescence signals. On the contrary, the deviation in the data is worse than in commercial slides, which could be an issue to be improved in further developments. Nevertheless, the deviation in the fluorescence values for Dx-Ma still falls within the range of acceptable values. In ESI, Figure S5 the fluorescence image for the three microarrays can be compared.

The approach herein proposed can be employed onto any surface containing acrylate, alkene or thiol groups that can react with Dx-Ma to be tethered to the surface. As glass is a common support for microarrays, we tested the versatility of the developed hydrogel by assaying its performance on acrylate derivatized glass slides. The chemical functionalization of glass surfaces was carried out by the widely reported organosilane chemistry as described in the Materials and Methods section. The success of the chemical surface functionalization was assessed by measuring the change in the water contact angle, which varied from 20º raw glass to 73º for a modified surface. The WCA for the acrylated glass modified with Dx-Ma was 12º (ESI, Fig S1). Probe 3, Probe 1 and Probe 1* were spotted at 0.2 μM in Dx-Ma 1:10 and in buffer without Dx-Ma, and the microarray was irradiated under the above-mentioned conditions, and after washing steps, hybridization was done with lowering Target 1* concentrations (from 0.1 to 0.0001 μM) (Fig 6). The best spot quality and fluorescence intensity were achieved when immobilizing probes with the hydrogel, although specific hybridization with the probes directly tethered to the surface was also achieved. The limit of detection calculated for this surface was 92 pM, which is similar to

that observed for the BD surfaces. A comparison between the hybridization curves obtained for Probe 1 immobilized by Dx-Ma and directly on the surface is shown in Supp Info (ESI, Fig S6).

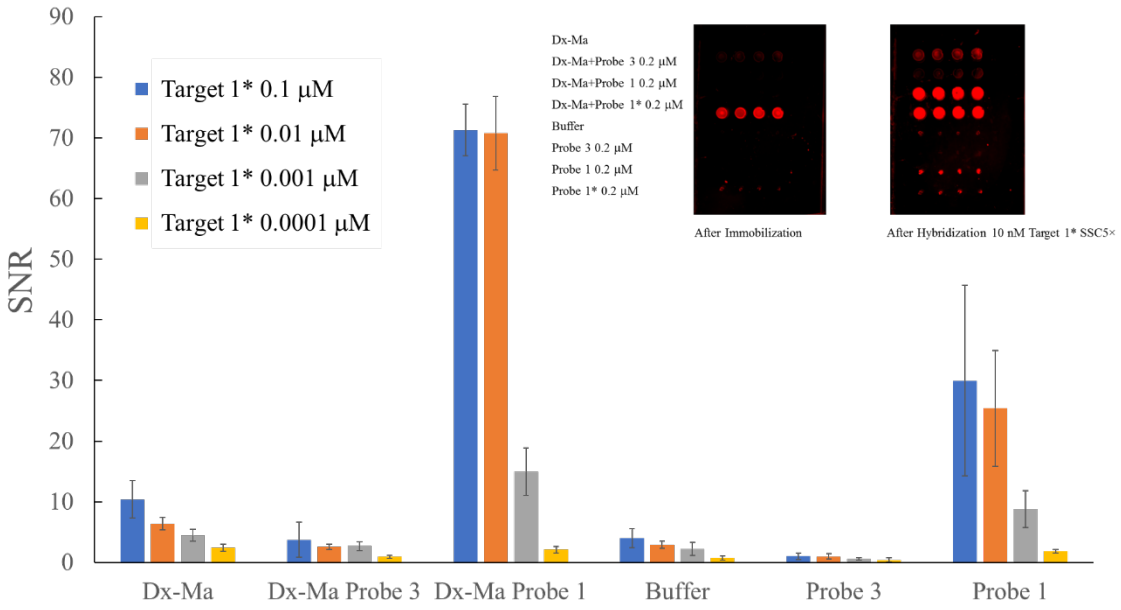


Fig 6 Hybridization performance for Dx-Ma microarrays created on acrylate-glass. Effective hybridization is only detected when immobilizing the thiolated complementary strand to Target 1*. Fluorescence SNR was 2-fold for Probe 1* immobilized via Dx-Ma than for Probe 1* directly photoattached to the acrylate surface. The signal-to-noise (SNR) ratio is plotted. Probe 1*, used as a positive immobilization control, is not included in the bars graph. Fluorescence was measured by SFR at the same gain and acquisition time as before.

The Dx-Ma hydrogel was also tested with miRNA-182, which is overexpressed in prostate cancer patients and provides prognosis information. [43] A glass microscope slide was modified with acrylate organosilane and Dx-Ma microarrays were created to contain the specific thiolated probe for miR-182 (Probe 3) at 0.1 and 0.5 μM. Hybridization was performed with lowering concentrations of fluorescence labeled miR-182 and signal was quantified. In this case, a commercial fluorescence confocal scanner was employed instead of the SFR. As seen in Fig 7, a good correlation was observed with the amount of target. The limit of detection was 2.92 pg/μL, which is below the regular amounts of circulating miRNA that fall within the ng/μL range. Further information is found in Fig S9, ESI.

Thus, the developed approach can be of broad application, and could be used to effectively immobilize different thiolated probes onto any alkene, thiol, or acrylate ended surface.

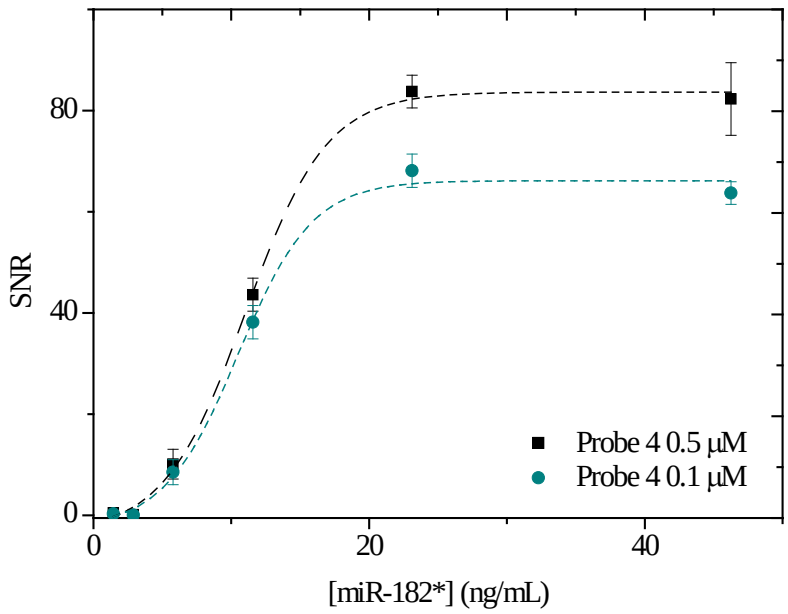


Fig 7 Hybridization plot for a Dx-Ma microarray created onto acrylate derivatized glass slide with Probe 4, specific towards miR-182

Conclusions

Here we report the modification of a dextran polymer with acrylate units to be employed to prepare hydrogel-based microarrays of nucleic acids. Oligonucleotide probes are covalently linked to the dextran polymer by thiol-acrylate coupling click chemistry. Dextran hydrogel formation, its fixation to the support surface, and probe covalent attachment are carried out in one step which takes only a few seconds. The process is activated by light and is carried out in water.

Thanks to its simplicity, quickness and mild conditions, the approach is an interesting possibility for developing microarrays with thiolated probes that are covalently attached. Probe loading is high, and the hybridization done with the complementary target is specific.

The method goes one step forward in the use of thiol-ene click-chemistry for microarray applications and combines the speed and orthogonality of click chemistry reactions with the high loading capacity and increased sensitivity of 3D microarrays.

The microarrays created in this way were demonstrated on BDs and acrylate derivatized glass but can be potentially applicable to any surface with acrylate, alkene, or epoxy groups. Hence, this is a simple way to prepare high-performance microarrays.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest. All the authors contributed equally to the paper.

Electronic Supporting Information

WCA for the BD and the Dx-Ma modified BD, the hybridization curves for Probe 1 and Target 1, and the hybridization curves for miRNA-182.

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