

A new polymeric coating for protein microarrays

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Abstract

Despite the increasing interest in arraying proteins in a high-density format, several technical issues still impede the development of protein microarray technology. One of the major problems is the availability of substrates that are able to bind native proteins with high density. In this study, we investigated the suitability of a novel surface as a support for protein microarrays. A polymeric glass coating is obtained by physical adsorption of a *N,N*-dimethylacrylamide (DMA), *N,N*-acryloyloxysuccinimide (NAS), and [3-(methacryloyl-oxy)propyl]trimethoxysilyl (MAPS) copolymer. The coating procedure provides a fast and inexpensive method of producing hydrophilic functional surfaces. The slide performance was investigated in a protein–protein interaction experiment and in the assessment of rheumatoid factor (RF) in human serum samples. The results demonstrate that the ligands immobilized on the polymeric surface maintain an active conformation and are easily accessible, providing a detection limit of 54 amol/spot. Moreover, in the RF assay, after hybridization with the sera, the slides have a low background, leading to a detection limit of 900 amol/spot.

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Protein microarrays are becoming an important tool in proteomics, drug discovery programs, and diagnostics [1]. The amount of information obtained from small quantities of biological samples is significantly increased in the microarray format. This feature is extremely valuable in protein profiling, where samples are often limited in supply and unlike DNA, cannot be amplified [2]. Protein microarrays are more challenging to prepare than are DNA chips [3] because several technical hurdles hamper their application. The surfaces typically used with DNA are not easily adaptable to proteins, owing to the biophysical differences between the two classes of bioanalytes [4]. Arrayed proteins must be immobilized in a native conformation to maintain their biological function. Unfortunately, proteins tend to unfold when immobilized onto a support so as to allow internal hydrophobic side chains to form hydrophobic bonds with the solid surface [5]. The accessibility of the protein is also of crucial importance to

achieve proper recognition during hybridization; protein–substrate interactions reduce the accessibility of the target, leading to false negative results.

Another important requirement of the surface is to provide a low unspecific background because unwanted adsorption of proteins leads to false positive results. The presence of an aspecific background is one of the most severe problems in antibody microarrays [6]. The achievement of a low degree of unspecific binding is extremely difficult when the protein sample is a complex mixture of thousands of molecules [4].

Current microarray supporting materials can be divided into two major categories [7]: surfaces coated with gels, such as polyacrylamide and agarose, and surfaces derivatized with functional groups, such as aldehyde, epoxy, and amino groups (polylysine). Methods for on-chip protein analysis also include the ProteinChip array technology that is based on selective extraction and retention of proteins on chromatographic chip surfaces and analysis by laser desorption/ionization mass spectrometry [8].

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Recently, our group has introduced a new type of polymeric glass slide for DNA microarrays [9] obtained by adsorption of a copolymer of *N,N*-dimethylacrylamide (DMA),¹ *N,N*-acryloyloxysuccinimide (NAS), and [3-(methacryloyl-oxy)propyl]trimethoxysilyl (MAPS): copoly(DMA-NAS-MAPS). Each monomer confers to the copolymer a specific feature. NAS is the reactive group able to bind amino-modified DNA and primary amines of lysines and arginines in proteins. DMA, which forms the polymer backbone, facilitates polymer adsorption on the glass surface, whereas MAPS covalently reacts with free silanols and stabilizes the coating. The coating is innovative in that it adsorbs onto the glass surface very quickly (10–30 min) from a diluted aqueous solution. Therefore, the coating procedure is fast and robust, providing an inexpensive hydrophilic functional surface. The performance of glass slides coated with the copoly(DMA-NAS-MAPS) has been studied extensively in DNA microarray experiments [9].

In the current work, copoly(DMA-NAS-MAPS) slides were used as a microarray support for protein–protein interaction experiments and in the assessment of rheumatoid factor (RF) in human serum samples.

Materials and methods

Materials

DMA and MAPS were obtained from Sigma (St. Louis, MO, USA). NAS was obtained from Polysciences (Warrington, PA). Anti-rabbit immunoglobulin G (IgG) F(ab')₂ fragments specific, developed in goat (goat IgG specific for the Fab fragments) were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA).

Anti-human polyvalent immunoglobulins developed in goat (goat IgG), Tris, BSA, and Tween 20 were obtained from Sigma. Immunoglobulins from rabbit serum (rabbit IgG) were obtained from Life Line Lab (Pomezia, Italy). CodeLink Activated Slides were obtained from Amersham Biosciences (Piscataway, NJ, USA), and ArrayIt Super Aldehyde Substrates were obtained from TeleChem International (Sunnyvale, CA, USA).

Glass slide coating

Untreated microscope glass slides (Sigma) were pretreated with 1 M NaOH for 30 min and 1 M HCl for 1 h,

washed with water, and dried. After pretreatment, glass slides were immersed for 30 min in a copoly(DMA-NAS-MAPS) solution (1% w/v in water solution of ammonium sulfate at 20% saturation level). Slides were finally rinsed with water and dried under vacuum at 80 °C. Copoly(DMA-NAS-MAPS) was synthesized and characterized as described in [9].

Protein labeling

Solutions of goat IgG specific for the Fab fragments, rabbit IgG, and goat IgG (used as a “detection” secondary antibody) were labeled with the monofunctional *N*-hydroxysuccinimide (NHS) ester of the dye Cy3 (Amersham Biosciences) as recommended by the manufacturer. Briefly, Cy3-NHS and the protein (1 mg/ml) were dissolved in sodium carbonate–sodium bicarbonate buffer (0.1 M, pH 9.3). The solutions were mixed together and kept in the dark for 40 min. Labeled proteins were purified from the unreacted dye by gel permeation chromatography using a Hi Trap desalting column from Amersham Biosciences. The reactions were dialyzed against water in the dark at 4 °C. Molar concentrations for labeled immunoglobulins were calculated and corrected for the absorbance of the dye at 280 nm (approximately 8% of the absorbance at 552 nm) according to the following equation:

$$[\text{IgG}] = [A_{280} - (0.08A_{552})]/170,000, \quad (1)$$

where 170,000 M^{−1} cm^{−1} is assumed as the molar extinction coefficient for IgG. The dye-to-protein ratio (*D/P*) for the labeled IgG was calculated according to the following equation:

$$(D/P) = (1.13A_{552})/[A_{280} - (0.08A_{552})], \quad (2)$$

providing an estimated *D/P* of 4 for the goat IgG specific for the Fab fragments, 1 for the goat IgG, and 3 for the rabbit IgG. Labeled proteins were lyophilized and dissolved in the appropriate buffer.

Optimization of the spotting buffer

Cy3-labeled rabbit IgG at concentrations of 60, 30, 12, and 6 µg/ml was dissolved in the following spotting buffers: carbonate buffer (100 mM, pH 9.0), PBS buffer (100 mM PBS, 300 mM NaCl, pH 7.4), sodium acetate buffer (100 mM, pH 5.5), sodium citrate buffer (100 mM, pH 4.5), and sodium phosphate buffer (50 mM, pH 7.4). Protein solutions were spotted on copoly(DMA-NAS-MAPS) glass slides using an ArrayIt SpotBot Personal Microarrayer at a constant temperature of 20 °C with 70% humidity and were incubated in a humid chamber at room temperature. Protein arrays were scanned at different incubation times and checked for proper spotting to take out aberrant spots from the analysis and then were washed with PBS (20 min) and water (10 min)

¹ Abbreviations used: DMA, *N,N*-dimethylacrylamide; NAS, *N,N*-acryloyloxysuccinimide; MAPS, [3-(methacryloyl-oxy)propyl]trimethoxysilyl; RF, rheumatoid factor; IgG, immunoglobulin G; NHS, *N*-hydroxysuccinimide; *D/P*, dye-to-protein ratio; PMT, photomultiplier tube; *S/N*, signal-to-noise ratio; EIA, enzyme-linked immunoassay; ELISA, enzyme-linked immunosorbent assay; XRR, X-ray reflectivity.

and scanned again. Mean intensity values of 4×4 spot subarrays were calculated and plotted against spotted concentration.

Antibody Fab portion recognition on copoly(DMA-NAS-MAPS) slides

Rabbit IgG were dissolved in a PBS buffer at different concentrations and spotted on the copoly(DMA-NAS-MAPS) slides. After overnight binding in a humid chamber, printed slides were rinsed and blocked with BSA (2% w/v) in a phosphate buffer (50 mM, pH 7.2) for 1 h. The slides were incubated for 1 h with Cy3-labeled goat IgG specific for the Fab fragments, dissolved in the hybridization buffer (Tris-HCl, 0.1 M, pH 8; 0.1 M NaCl; 1% w/v BSA; 0.02% w/v Tween 20) at a concentration of 0.05 mg/ml. After washing with Tris-HCl (0.05 M, pH 9), 0.25 M NaCl, 0.05% Tween 20, PBS, and water, the slides were dried and scanned for fluorescence evaluation.

Sandwich immunoassay on microarray format

The capture antigen (rabbit IgG) was dissolved in a PBS buffer at different concentrations and spotted on the copoly(DMA-NAS-MAPS) slides, on CodeLink Activated Slides, and on ArrayIt Super Aldehyde Substrates using an ArrayIt SpotBot Personal Microarrayer at a constant temperature of 20 °C with 70% humidity. The slides were incubated overnight in a humid chamber at room temperature and subsequently washed for 20 min with PBS and for 10 min with water.

Printed slides were blocked with BSA (2% w/v) in a phosphate buffer (50 mM, pH 7.2) for 1 h and rinsed with water. The slides were then incubated with standardized human serum samples or control samples (a gift from Life Line Lab) containing different amounts of RF (0–485 IU/ml, determined by RF ELISA Auraflex, Alfa Biotech, Pomezia, Italy). The slides were incubated for 1 h with sera diluted 1:100 in the hybridization buffer (Tris-HCl, 0.1 M, pH 8; 0.1 M NaCl; 1% w/v BSA; 0.02% w/v Tween 20); washed with Tris-HCl (0.05 M, pH 9), 0.25 M NaCl, and 0.05% Tween 20; and rinsed with water before a 1-h incubation with the secondary antibody, a Cy3-labeled goat IgG dissolved in the hybridization buffer at a concentration of 0.04 mg/ml. After extensive washing with PBS and water, the slides were dried and scanned for fluorescence evaluation.

Scanning and evaluation

All fluorescence signals were detected with a ScanArray Express unit using the same photomultiplier tube (PMT) gain and laser power for all of the slides and were analyzed with the QuantArray software package (both from Packard Biosciences, Billerica, MA, USA). Data

were exported to Microsoft Excel software for further processing, and signal intensities were defined in fluorescence arbitrary units.

The signal-to-noise ratio (S/N) for fluorescence signals was calculated as the ratio between the mean spot signal intensity and the standard deviation of background intensity. The detection limit was defined as the lowest concentration of immobilized antigen at which the mean signal of intensities provided an S/N value of 3.

Results

Protein binding on copoly(DMA-NAS-MAPS) slides

Protein concentration, spotting buffer, and incubation time were optimized by arraying Cy3-labeled rabbit IgG, which is the target used in the antibody recognition experiments and in the sandwich immunoassay as the capture antigen. Fig. 1 shows the S/N values obtained on copoly(DMA-NAS-MAPS) slides with different concentrations of Cy3-labeled rabbit IgG dissolved in five spotting buffers.

Printed slides were incubated overnight in a dark humid chamber and were scanned before and after washing to determine the fluorescence intensity of immobilized proteins. An overnight incubation using PBS as the spotting buffer provided the highest S/N value. Among the different buffers, carbonate (pH 9.0) also provided a high S/N value. Its pH favors the reaction between protein amino groups and active esters on the surface. However, PBS was chosen as the spotting buffer for the following experiments because its pH is more suitable for maintaining the biological activity of the spotted proteins. The time required to achieve an optimal binding of the protein was also investigated.

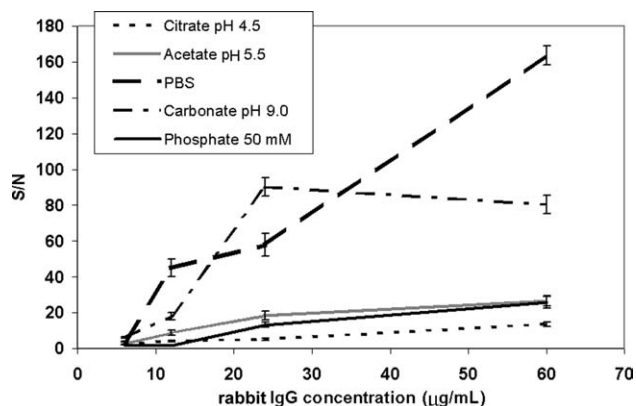


Fig. 1. Optimization of the spotting buffer. S/N values were obtained on copoly(DMA-NAS-MAPS) slides after overnight incubation of Cy3-labeled rabbit IgG dissolved at different concentrations in the following buffers: carbonate (100 mM, pH 9.0), PBS (pH 7.4), sodium acetate (100 mM, pH 5.5), sodium citrate (100 mM, pH 4.5), sodium phosphate (50 mM, pH 7.4). Experiments were carried out on nine replicates. Standard deviations are indicated by error bars.

Fig. 2 shows the binding kinetic of Cy3-labeled rabbit IgG dissolved in PBS at concentrations ranging from 6 to 60 $\mu\text{g}/\text{mL}$. Every slide was scanned before and after washing to detect the fluorescence intensity of the spots; background fluorescence did not change during experiments. The binding kinetic was independent from the

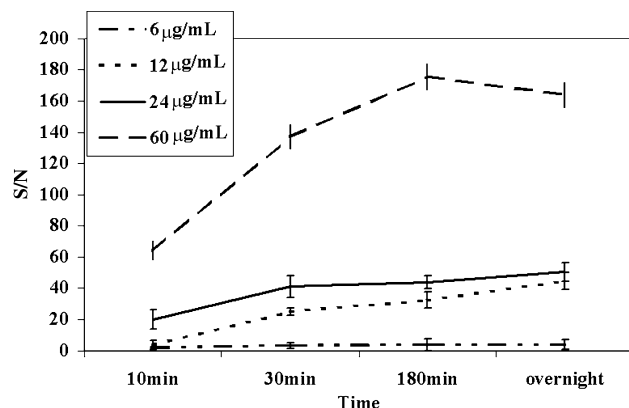


Fig. 2. Time course of protein binding. Cy3-labeled rabbit IgG, dissolved in PBS at concentrations ranging from 6 to 60 $\mu\text{g}/\text{mL}$, were spotted. Slides were incubated in a humid chamber in the dark for different lengths of time, scanned, washed extensively, and scanned again for the evaluation of fluorescence signals. Experiments were carried out on nine replicates. Standard deviations are indicated by error bars.

concentration of spotted proteins. The amount of immobilized protein reached a plateau for a reaction time longer than 3 h. A relationship was found between the concentration of spotted protein and the fluorescence intensity of the spots.

Antibody Fab portion recognition on copoly(DMA-NAS-MAPS) slides

A good support for antibody microarray assay should bind antibodies in an active conformation with the Fab regions freely exposed. To verify the suitability of the polymeric surface developed within this study, in antibody microarray assays, different concentrations of rabbit IgG and two negative controls (PBS and BSA, 1 mg/mL in PBS) were immobilized on the slide. After blocking with BSA, copoly(DMA-NAS-MAPS) slides were hybridized with a solution of Cy3-labeled immunoglobulins developed in goat, specific for the Fab fragments of rabbit IgG (Fig. 3A). The results are shown in Fig. 3B; no significant fluorescence signals were revealed for the negative controls, whereas a strong fluorescence was detected as a result of hybridization between the arrayed rabbit IgG and the Cy3-labeled goat anti-rabbit antibodies, specific for the Fab fragments. The limit of

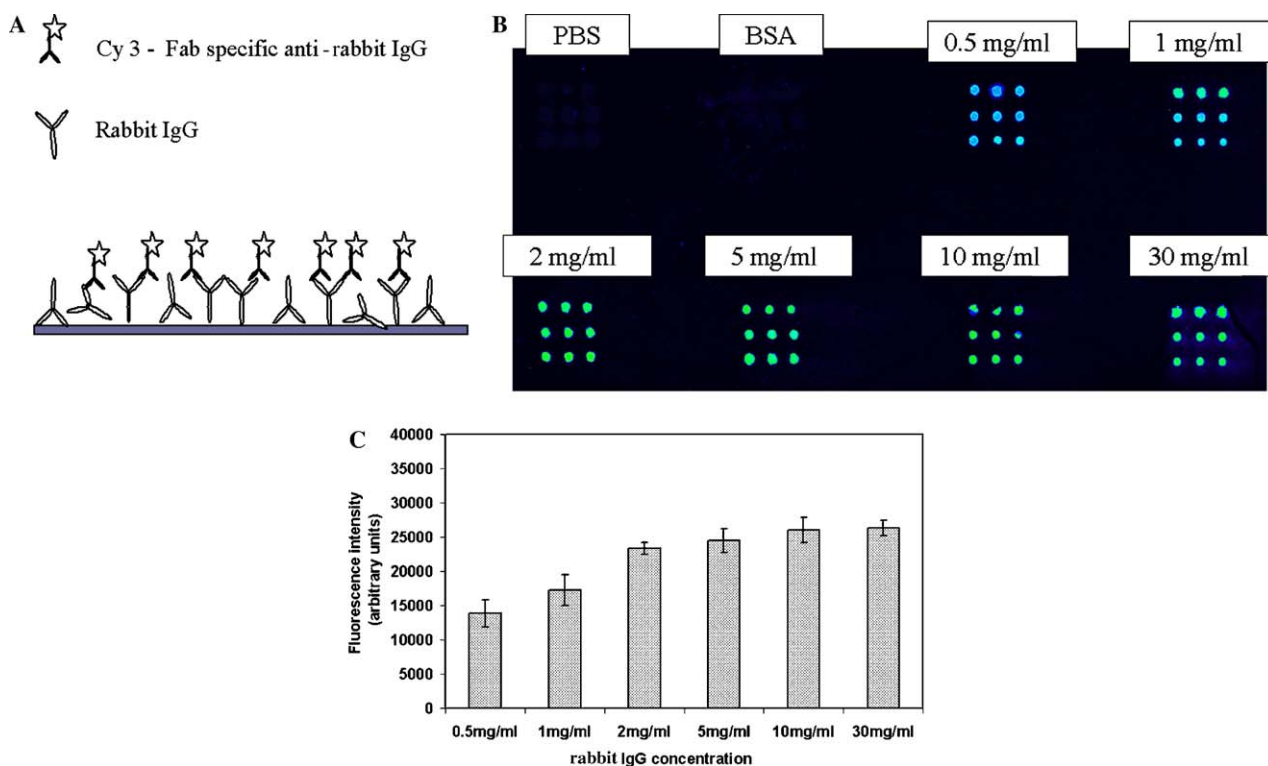


Fig. 3. (A) Schematic view of the antibody Fab portion recognition on copoly(DMA-NAS-MAPS) slides. Immunoglobulins from rabbit serum (rabbit IgG) were spotted on the slides. After overnight binding, slides were washed and hybridized with goat anti-rabbit IgG specific for the Fab fragments, labeled with Cy3. (B) Portion of the copoly(DMA-NAS-MAPS) slide after hybridization with Cy3 goat anti-IgG specific for the Fab fragments. The array is composed by eight subarrays of: PBS, BSA (two negative controls), and six concentrations of rabbit IgG ranging from 0.5 to 30 mg/mL. (C) Plot of fluorescence intensities versus rabbit IgG concentrations. Experiments were carried out on nine replicates. Standard deviations are indicated by error bars.

detection in these kinds of experiments resulted in 54 amol/spot. The detection limit was defined as the lowest concentration of immobilized antigen at which the mean signal of intensities provided (on hybridization) an S/N of 3 considering a spotting volume of 0.2 ml.

Sandwich immunoassay on microarray format

A fluorescent assay of the RF in human serum samples was carried out on copoly(DMA-NAS-MAPS) slides. The aim was to test the performance of the novel coating in real microarray experiments where the accessibility and conformation of the target are factors of crucial importance and where the high concentrations of background proteins (e.g., albumin, fibrinogen, antibodies) can complicate immunoassays by aspecific adsorption on surfaces. An identical assay was also carried out using two commercial slides: CodeLink Activated Slides and ArrayIt Super Aldehyde Substrates.

RFs are immunoglobulins of any isotype with activity directed against the antigenic sites on the Fc region of human or animal IgG. Seventy to eighty percent of patients with confirmed rheumatoid arthritis show an increase in the RF [10]; therefore, RF assay is a serological test widely used for the diagnosis of rheumatoid arthritis. Conventional serological tests for the measurement of RF rely on enzyme-linked immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA). In this study, we used a fluorescent sandwich assay, the schematic view of which is shown in Fig. 4. The “capture” antigens, rabbit IgG, were spotted on the slides. After overnight binding, the slides were incubated with serum samples containing the assayed antibody (RF), followed by incubation with Cy3-labeled anti-human polyvalent immunoglobulins, developed in goats (goat IgG).

In this type of test, it is important to reproducibly immobilize an appropriate amount of antigen on the slide and to maintain the antigen in an active conformation so

as to favor its specific interaction with the assayed antibody. The surface should not undergo nonspecific interactions with the serum components, which increase the background and hinder antigen–antibody recognition. In addition, the microarray surface should favor a highly specific interaction between the assayed analyte and the “detection” antibody to achieve high sensitivity. Fig. 5A shows a plot of fluorescence signals versus antigen

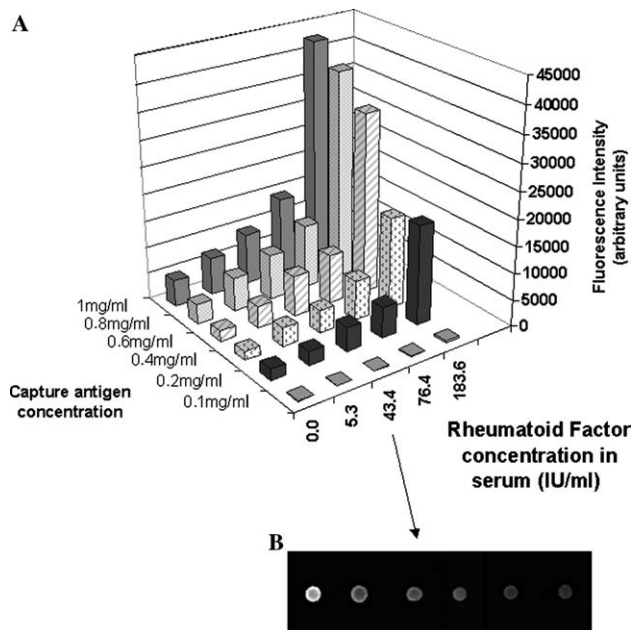


Fig. 5. (A) Plot of fluorescence signals obtained in the sandwich assay conducted on copoly(DMA-NAS-MAPS) slides. On every slide, six capture antigen concentrations, ranging from 0.1 to 1 mg/ml, were arrayed and then assayed by incubation with one of a set of five serum samples containing amounts of RF ranging from 0 to 183.6 IU/ml. Experiments were carried out on 16 replicates with an average standard deviation of 11.3%. (B) Example of an array (reconstructed image) composed of six concentrations of capture antigen (0.1–1 mg/ml) assayed with a serum containing 43.4 IU/ml of RF and finally revealed by Cy3-labeled goat IgG.

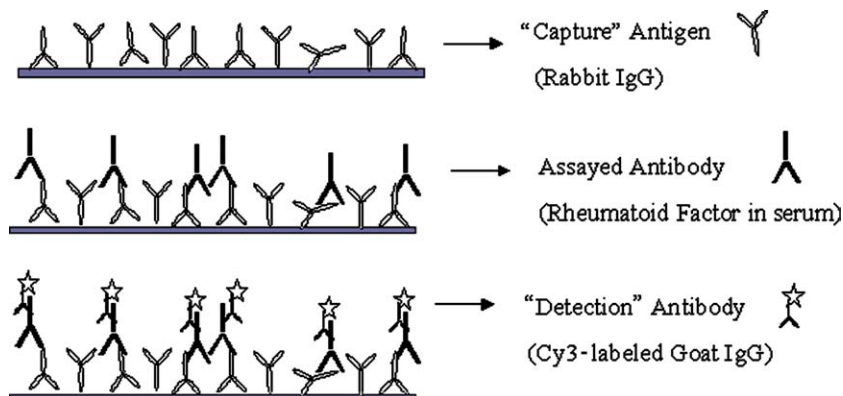


Fig. 4. Schematic view of the sandwich assay. The capture antigens, immunoglobulins from rabbit serum (rabbit IgG), were spotted on the slides. After overnight binding, slides were washed and hybridized with human serum samples containing different amounts of the assayed antibody (RF). After washing, RF was finally revealed by recognition with a secondary antibody, the anti-human goat immunoglobulins (goat IgG) labeled with Cy3.

concentration obtained in the sandwich assay, as illustrated in Fig. 4. On every slide, six concentrations of rabbit IgG (ranging from 0.1 to 1 mg/ml) and two negative controls (BSA (1 mg/ml) and PBS buffer) were arrayed. After an overnight incubation in a humid chamber, the slides were washed and hybridized with five different serum samples containing amounts of RF ranging from 0 to 183.6 IU/ml (as determined by the Auraflex RF ELISA test). Negative controls showed a negligible fluorescence, with an *S/N* value lower than 3, demonstrating the absence of a nonspecific interference in the assay. The average fluorescence signal of every subarray (each corresponding to one of the six capture antigen concentrations) was calculated, and the values are reported in Fig. 5A. The spots exhibit a reproducible shape with a diameter of 150 μ m, and their fluorescence intensity increases linearly with the concentration of the immobilized capture antigen. Fig. 5B shows a typical protein array obtained on copoly(DMA-NAS-MAPS) slides under these experimental conditions. The polymeric coating provided reproducible results and a linear dose–response relationship up to 76.4 IU/ml with a detection limit of 900 amol/spot.

Using the experimental conditions as described above, we compared copoly(DMA-NAS-MAPS) slides with two commercial microarray surfaces: CodeLink Activated Slides and ArrayIt Super Aldehyde Substrates. The linearity and reproducibility of the dose–response curves were comparable in the three cases, but we found a significant difference in the background intensities obtained in the assay with the different slides. Fig. 6 illustrates, for example, a portion of copoly(DMA-NAS-MAPS) (1), Code Link (2), and Super Aldehyde (3) slides used in the sandwich assay. *S/N* values obtained in this experiment are reported in

Table 1

S/N values of the portion of protein microarrays reported in Fig. 7, calculated from 16 replicates

Type of slide	<i>S/N</i> obtained with 0.2 mg/ml spotted rabbit IgG	<i>S/N</i> obtained with 0.8 mg/ml spotted rabbit IgG
copoly(DMA-NAS-MAPS)	6.2 $\pm$ 0.65	8.9 $\pm$ 0.71
CodeLink Activated Slides	2.6 $\pm$ 0.34	4.2 $\pm$ 0.45
ArrayIt Super Aldehyde	2.5 $\pm$ 0.27	5.2 $\pm$ 0.41

Table 1. The mean background signal intensities expressed in fluorescence arbitrary units were 300 for copoly(DMA-NAS-MAPS), 1400 for Code Link, and 1100 for Super Aldehyde. The differences in the mean background signal intensities strongly affect the sensitivity of the assay. The high sensitivity obtained with copoly(DMA-NAS-MAPS) slides results from the characteristics of the coating that prevent a nonspecific interaction between proteins and the surface during hybridization.

Sandwich assay with an internal calibration curve

The measurement of RF in three serum samples using an internal calibration curve was carried out on slides divided into eight portions by means of a hydrophobic pen. Eight subarrays (each containing 1 mg/ml of capture antigen) were printed on the surface. Six printed regions of the slides were used to generate a calibration curve; one region was for the unknown sample, and one region was for the negative control. The six subarrays of the calibration curve were hybridized with sera ranging in concentration from 0 to 485 IU/ml (one for every point of the calibration curve). The RF concentration in the samples was interpolated from the calibration curve of fluorescence intensities generated using calibration subarrays that were present on the same slide. The RF concentrations found in the three serum samples were 225–418, 16–30, less than 21 IU/ml, in full agreement with the results provided by the Auraflex RF ELISA test. The results demonstrate that the microarray assay is able to identify positive and negative sera with efficiency similar to that of the ELISA method. Moreover, the presence of an internal calibration curve offers a unique advantage over current immunoassay protocols in which calibration curves and samples are processed in separate tubes or wells. In these assays, matrix problems, which result from the differences between the calibrator and sample matrices, occur frequently and represent a known source of bias [11,12]. In contrast, in the microarray format, the sample signals refer to a calibration curve processed under the same physicochemical conditions, thereby reducing error sources.

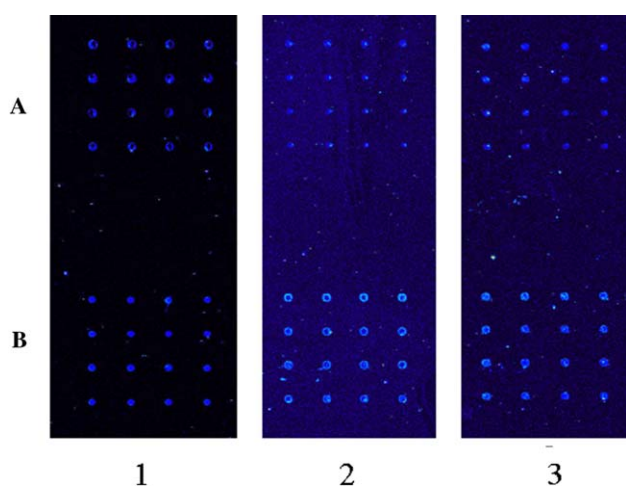


Fig. 6. Portion of protein microarrays obtained at the end of the sandwich assay illustrated in Fig. 3. The spotted capture antigen concentrations were 0.2 mg/ml (A) and 0.8 mg/ml (B). Incubation was with the serum sample containing 183.6 IU/ml on copoly(DMA-NAS-MAPS) slides (1), CodeLink Activated Slides (2), and ArrayIt Super Aldehyde (3).

Discussion

The aim of this study was to develop a simple and robust coating for microarray glass slides. A film bearing active esters was obtained by a combination of physical adsorption and covalent linking of a copolymer to a glass surface. The copolymer consists of three comonomers with different functions. DMA, the major constituent, interacts by hydrogen binding with the surface to which the polymer is irreversibly grafted on condensation of MAPS monomers, whereas the third ingredient, NAS, extends from the surface and binds the target molecules. The film was characterized by X-ray reflectivity (XRR) in a previous article. XRR measurements highlighted the presence of two polymeric layers of different thickness. The chains close to the wall, condensed with themselves and with the surface silanols, form a layer of 3.45 nm with a density of 1.8 g/cm³. The layer at the surface-air interface, made by less condensed chains, has a thickness of 1.8 nm and a density of 1.12 g/cm³ [9]. These are thickness values of the dry film. However, it is reasonable to assume that the film, on hydration, swells 10–15 times [13] due to the highly hydrophilic nature of the copolymer. In Fig. 7, a graphical representation of a copoly(DMA-NAS-MAPS) film with immobilized proteins is provided.

Two different experiments were carried out to illustrate the characteristics of this coating. In one experiment, the Fab portion of an antibody immobilized on the slide was efficiently recognized by the corresponding antibody. In the second experiment, the Fc domain of the capture antigen was specifically recognized. The results of the two assays demonstrate that both the Fab and Fc domains of the antibody are freely accessible. This is a considerable advantage because it overcomes one of the major problems of microarray assays, that is, the insufficient accessibility of the ligand. Copoly(DMA-NAS-MAPS)-coated slides immobilize antibodies in a random conformation, and no crosslinkers or spacers are required to separate the protein from the surface, as for most of the nonpolymeric slides

[14]. This is because the polymer chains act as a spacer between the protein and the surface. In addition, the active esters, which bind the protein, are spaced along the chains. Therefore, only a few groups of the protein are covalently bonded with the surface functional groups. Proteins are bonded to the matrix through one or two residues when the coupling is performed in non-polymeric coatings [15]. However, they entertain additional binding with the surface through hydrophobic, ionic, or hydrogen bonding interactions. Therefore, depending on the surface characteristics, different portions of the proteins are shielded, thereby reducing the recognition efficiency. The polymeric surface reported in this article constitutes an aqueous microenvironment in which the functional domains of the protein are freely exposed.

Concluding remarks

In this study, we investigated the suitability as a protein microarray substrate of a glass slide coated with a copolymer of DMA, NAS, and MAPS. The coating is formed by a fast, inexpensive, and robust procedure of simply immersing slides in a dilute aqueous polymer solution without the need for time-consuming glass pretreatments. This novel surface was used successfully in protein–protein interaction experiments and in the assay of RF in human sera. According to our data, copoly(DMA-NAS-MAPS) slides bind antibodies in an active conformation because the native conformation of the antigen-binding region of an antibody must be preserved to maintain its functional activity. Microarray surfaces that demonstrate good characteristics when used as substrates for antibodies can be used to study more complex protein–protein interactions in which the functionality of the immobilized partner is crucial [16]. Moreover, these slides allow one to identify positive and negative sera with efficiency comparable to that of an ELISA, with a detection limit of 900 amol/spot and a very low background signal.

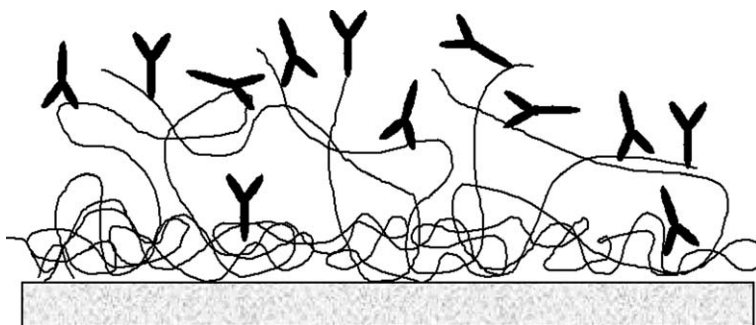


Fig. 7. Graphical representation of copoly(DMA-NAS-MAPS) polymer coating on glass support after hydration and antibody binding. The polymer chains close to the glass surface condense with themselves and with the silanols of the support. The chains at the surface-air interface are less dense due to the presence of loops and tails.

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