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Comparison of surface and hydrogel-based protein microchips

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Abstract

Protein microchips are designed for high-throughput evaluation of the concentrations and activities of various proteins. The rapid advance in microchip technology and a wide variety of existing techniques pose the problem of unified approach to the assessment and comparison of different platforms. Here we compare the characteristics of protein microchips developed for quantitative immunoassay with those of antibodies immobilized on glass surfaces and in hemispherical gel pads. Spotting concentrations of antibodies used for manufacturing of microchips of both types and concentrations of antigen in analyte solution were identical. We compared the efficiency of antibody immobilization, the intensity of fluorescence signals for both direct and sandwich-type immunoassays, and the reaction-diffusion kinetics of the formation of antibody–antigen complexes for surface and gel-based microchips. Our results demonstrate higher capacity and sensitivity for the hydrogel-based protein microchips, while fluorescence saturation kinetics for the two types of microarrays was comparable.

Keywords: Surface and hydrogel-based protein microarrays; Quantitative immunoassay; Immobilization efficiency; Fluorescence measurements; Time of signal saturation

Protein microchips with arrays of individual elements containing immobilized molecular probes specific to various target molecules in analyte solution present rapidly developing proteomic technology for different research and practical purposes (for review see, e.g., [1–4]). The most important applications include quantitative immunoassay of disease-associated markers, allergens, and biological toxins with sensitivity comparable to or higher than that of standard immunological methods. Other applications address protein–protein, protein–lipid, and protein–ligand interactions involved in enzymatic reactions. The rapid advance in microchip technology and a variety of techniques developed by different groups require systematic assessment and comparison of different platforms.

Current formats of protein microchips range from matrix microarrays in which the probes are fixed on glass, plastic, or another two-dimensional support, in the form of $M \times N$ matrix with M rows and N columns, to microwells, microspheres, and microfluidic systems. Probe molecules in the elements of matrix microarrays may be immobilized either on a substrate surface or in the gel pads. Here we study how the mode of immobilization affects the characteristics of protein microchips.

Specifically, we compare the efficiency of antibody immobilization, the intensity of fluorescence signals for direct and sandwich-type immunoassays, and the signal saturation kinetics for the protein microchips with antibodies immobilized on flat glass surfaces and within hemispherical gel pads.

The protocol for the manufacturing of surface microchips with the most favorable characteristics was chosen based on a recent review by Kusnezow et al. [4]. For the gel-based microchips, we applied the copolymerization technology described in our earlier publications [5–8]. To achieve maximal uniformity of the comparison, the two types of microchips were manufactured using similar equipment, the signals were measured with the same

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devices, and spotting concentrations of probes used for immobilization and concentrations of target molecules in analyte solution were identical. The uniformity of these parameters was thoroughly controlled in the course of the experiments.

For our comparative study we chose the antibody microchips developed for the detection of prostate-specific antigen (PSA)[1], a 240-amino-acid-long glycoprotein with molecular mass about 30 kDa. PSA is a tissue-specific tumor marker routinely used in clinical practice [9–11]. It has been approved by the FDA for early diagnostics of prostate cancer.

Our results demonstrate higher immobilization capacity and stronger fluorescence signals for the hydrogel-based protein microchips than for the microchips with surface-immobilized probes at the comparable kinetics of fluorescence saturation. Such differences are most likely related to the much longer interprobe distances in the gel pads than in the surface chips, the aqueous environment of immobilized compounds, and the absence of contacts with hydrophobic surfaces.

Materials and methods

Antibodies and analytes

PSA was obtained from Khema-Medica (Moscow). Monoclonal antibodies to PSA were purchased from CanAg (Sweden); antibodies PSA-30 (molecular mass 150 kDa) were used as immobilized antibodies, while PSA-66 were used as developing antibodies for sandwich immunoassay. Cy5 fluorescence dye, monosuccinimide ester, Sephadex G-25 coarse, and Bind Silane were purchased from Amersham Pharmacia Biosciences (Piscataway, NJ), Micro Bio-Spin chromatography columns were from Bio-Rad Laboratories (Hercules, CA), and glass slides for the fabrication of microarrays (Corning 2947 Micro Slides; 3 in. by 1 in.) were from Corning Glass Works (Corning, NY). Other chemical reagents were obtained from commercial suppliers and used without further purification.

Throughout this work we used the following buffer solutions: PBS (0.01 M phosphate buffer, pH 7.2, 0.15 M NaCl), PBST (PBS with 0.1% Tween 20), and blocking solution PBSP (PBS with 1% polyvinyl alcohol).

Fabrication of microchips

Gel-based microchips with immobilized antibodies covalently linked to the three-dimensional gel were fabricated by a polymerization-mediated immobilization method as described in [5,6,8]. Solutions containing gel-forming monomers and antibodies were transferred to the wells of a 384-well microtitration plate (Genetix, New Milton, UK) and spotted onto Bind-Silane-treated glass slides using a QArray pin robot (Genetix). Polymerization of the gel-forming composition was carried out under UV light with maximal wavelength of 350 nm, irradiation intensity 0.06 $\mu W/cm^2$ (GTE lamp F15T8/350 BL, Sylvania, Danvers, MA), for 40 min at 20 °C. Before carrying out on-chip immunoassays, biochips were washed in PBST and then with distilled water. After that, biochips were treated with PBSP for 40 min and washed with distilled water.

Surface antibody microchips were manufactured on glass slides treated with 3-(glycidyloxypropyl)triethoxisilane as described [4] with slight modifications. The slides were washed with 100% ethanol, etched overnight by immersion in 10% NaOH, cleaned by sonication in the same solution for 15 min, rinsed four times in water, washed twice in ethanol, and derivatized in a 3-(glycidyloxypropyl)triethoxisilane (Fluka, Germany) at room temperature for 3 h. After silanization, epoxysilanized slides were washed with toluene and dried with nitrogen. Phosphate buffer (0.015 M, pH 7.2) supplemented with 40% trehalose was used as spotting buffer. The antibodies were spotted using the QArray pin robot mentioned above. After spotting, the slides were incubated at 4 °C overnight, blocked for 40 min at room temperature in PBSP, and washed with distilled water.

For both gel-based and surface microchips the volume of spotting solution was about 1nl per spot.

For control purposes each set corresponding to a given antibody concentration in spotting solution $[Ab]$ consisted of four cells. We used graded twofold increases in concentrations for the neighboring four-cell sets. Specifically, the spotting concentrations for the analysis of immobilization efficiency ranged consecutively from $[Ab]_0 = 0.15$ mg/ml to $2^4 \times [Ab]_0$, while the concentrations of antibodies for the sandwich-type immunoassays and for the study of kinetic curves using direct immunoassays ranged from $[Ab]_0 = 0.1$ mg/ml to $2^3 \times [Ab]_0$. Additionally, the gel-based microchips also contained four-cell sets with gel pads without immobilized antibodies. An overview of the microchip scheme and the corresponding cells on gel-based and surface microchips is presented in Fig. 1.

The radii of the cells for both surface and gel-based microchips were 75 μm, while the distances between the centers of the cells were 250 μm. The reaction chamber was covered with a transparent plastic plate. The volume of the reaction chamber was about 40 μl (area $\sim 1 \times 1$ cm^2 and height 400 μm).

The study of microchips under microscope in transmitted light revealed distinct slightly oblate hemispherical gel pads on gel-based microchips (cf. [5,6]). There were no observable three-dimensional features on surface microchips.

Labeling with Cy5 fluorescence dye

To label PSA and antibodies at amino groups, 10 μl of Cy5 succinimide ester solution (protein:dye molar ratio 1:5) in freshly distilled dimethyl sulfoxide was added to

[1] *Abbreviations used:* PSA, Prostate-specific antigen; PBS, phosphate-buffered saline.

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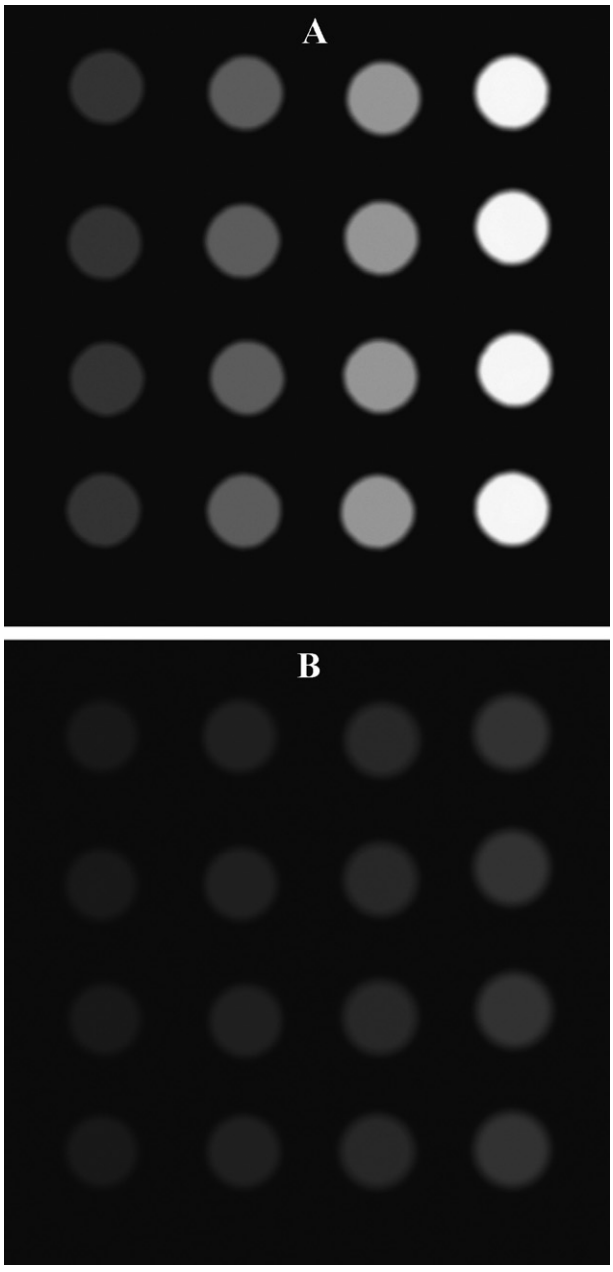

Fig. 1. Counterpart fragments of gel-based (A) and surface (B) microchips with the cells containing immobilized antibodies to PSA in different concentrations after binding with PSA and staining with Cy5-labeled antibodies (sandwich-type immunoassay). The concentrations of antibodies were 0.1, 0.2, 0.4, and 0.8 mg/ml of spotting solution (in columns from left to right, where each column of four elements corresponds to the same concentration of antibodies). Concentration of PSA in analyte solution was 60 ng/ml.

100 μl of PSA or antibody solution (2–5 mg/ml) in 0.01 bicarbonate buffer, pH 9.5, and the mixture was stirred for 1 h at 20 °C. Labeled PSA and antibodies were purified on a Micro Bio-Spin column with Sephadex G-25 coarse equilibrated with PBS. The protein:Cy5 molar ratio was determined by the absorbance at 280 and 650 nm and assessed to be about 1.0.

Microchip imaging and data analysis

Quantitative fluorescence measurements were carried out with a custom-built fluorescence microscope equipped with a cooled Charge-coupled device camera, Peltier thermotable, temperature controller, and a computer with data-acquisition board [12]. The signals from Cy5 dye were obtained using 649/670 nm excitation/emission filters. The exposure time was 1 s. Fluorescence signals from individual cells were processed using ImaGel Research program developed in our laboratory.

To assess the efficiency of immobilization and to measure the results of sandwich-type immunoassays, the chips were washed with water and the resulting fluorescence intensity was calculated using the formula

$$J = \frac{C - B_{d.c.}}{B_{r.g.} - B_{d.c.}}, \quad (1)$$

where C is the median fluorescence calculated for the image area occupied by a probe cell (or median value corresponding to the set of pixels covering image area). To take into account possible spatial inhomogeneity of the illumination source, the microchip slide was replaced by a slide of red glass of identical size, and the corresponding median fluorescence intensity within a position occupied by a gel pad, $B_{r.g.}$, was measured. This value was corrected by noise signal $B_{d.c.}$ produced by dark current at zero illumination intensity.

The corresponding fluorescence signals for direct antigen–antibody immunoassays during the study of kinetic curves were measured under buffer solution and were calculated as

$$J(t) = \frac{C(t) - C(0)}{B_{r.g.} - B_{d.c.}}, \quad (2)$$

where $C(t)$ is the integral fluorescence at moment t calculated for the image area occupied by a probe cell and $C(0)$ is the fluorescence of the same area at the initial moment $t = 0$.

Results

All experiments at a given concentration of antibodies in spotting solution (for the assessment of immobilization efficiency) and that of antigen in analyte solution (for both sandwich and direct immunoassays) were repeated twice. The lowest signal-to-noise ratio exceeded 2.0, while the relative scattering in experimental data was within 5–10%. The data presented below correspond to the median values for four-cell sets with identical concentration of immobilized antibodies (see Materials and methods and Fig. 1). All measurements were performed at 25 °C.

Antibody immobilization efficiency

The fraction of immobilized antibodies was assessed by comparing the fluorescence signals produced by Cy5-labeled antibodies after immobilization and washing. The chip was washed with PBST until the fluorescence signals remained constant. The degree of immobilization was

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calculated as the ratio of the fluorescence signals before and after washing.

Fig. 2 shows the dependence of immobilization efficiency for antibodies to PSA on the surface cells and in the gel pads on the concentration of antibodies in spotting solution. As is seen in Fig. 2, in a given range of concentrations the immobilization efficiency in the gel pads remains approximately constant ~30% with the enhancement of antibody concentration in spotting solution. At the same time, the corresponding efficiency for the surface cells drops rapidly beginning from concentrations $[Ab] \geqslant 2.5$ mg/ml. The possible explanation of such dependences will be presented below in the Discussion.

Sandwich immunoassay

As antibody microchips are used primarily for analytical purposes, we compared the sensitivity of gel-based and surface microchips in a sandwich-type immunoassay commonly used for the assessment of antigen concentration.

First, 40 µl of a solution containing PSA under study was applied on a chip, and biochips were kept at 25 °C for 17 h. PSA was diluted to desired concentration in PBSP with 0.15% polyvinylpyrrolidone. After incubation, biochips were washed for 20 min with PBST and then with distilled water. Second, to display the complexes formed between immobilized antibodies and PSA, a solution with Cy5-labeled antibodies against PSA (15 µg/ml) was incubated on a chip for 2 h. After subsequent washing with PBST for 20 min, fluorescent signals were recorded.

The resulting calibration curves at different concentrations of antibodies in spotting solution are shown in Fig. 3. Fluorescence signals obtained using the gel-based protein microchips turn out to be three- to fivefold higher than those obtained on the surface chips and cover a broader dynamical range than the corresponding signals on the surface microchips. The difference in the fluorescence intensities grows with the increase of antibody concentration in spotting solution.

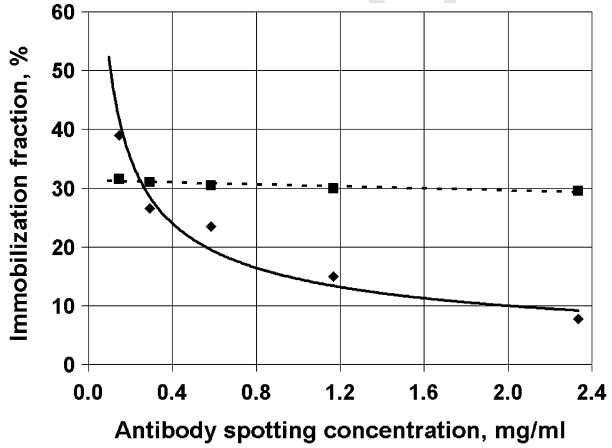

Fig. 2. Dependence of immobilization efficiency for antibodies to PSA on the surface cells (♦) and in the gel pads (■) on the concentration of antibodies in spotting solution. The antibodies were labeled with Cy-5.

Direct immunoassay and kinetics of antigen binding

Kinetic measurements were carried out using direct immunoassay for Cy5-labeled PSA applied on the microchips containing immobilized antibodies against PSA in different concentrations (cf. Fig. 1). Fluorescence signals from each cell were measured every 15 min for 50 h at the concentration of the antigen in buffer solution equal to $[Ag]_{sol} = 750$ ng/ml (or 2.5×10^{-8} M). Note that the concentrations of antigen used for direct immunoassay exceeded one-two orders of magnitude the concentrations typical for clinical measurements (and that used for sandwich type immunoassay above). These high concentrations of antigen were chosen to accelerate the kinetics of binding (cf. Eqs. (5) and (6) below) and to improve the visualization of cells under buffer solution. In additions, these concentrations cover the range $K_a[Ag]_{sol} \sim 1$, where K_a is the association constant for binding between immobilized antibodies and antigen, suitable for the experimental assessment of association constant (see Discussion).

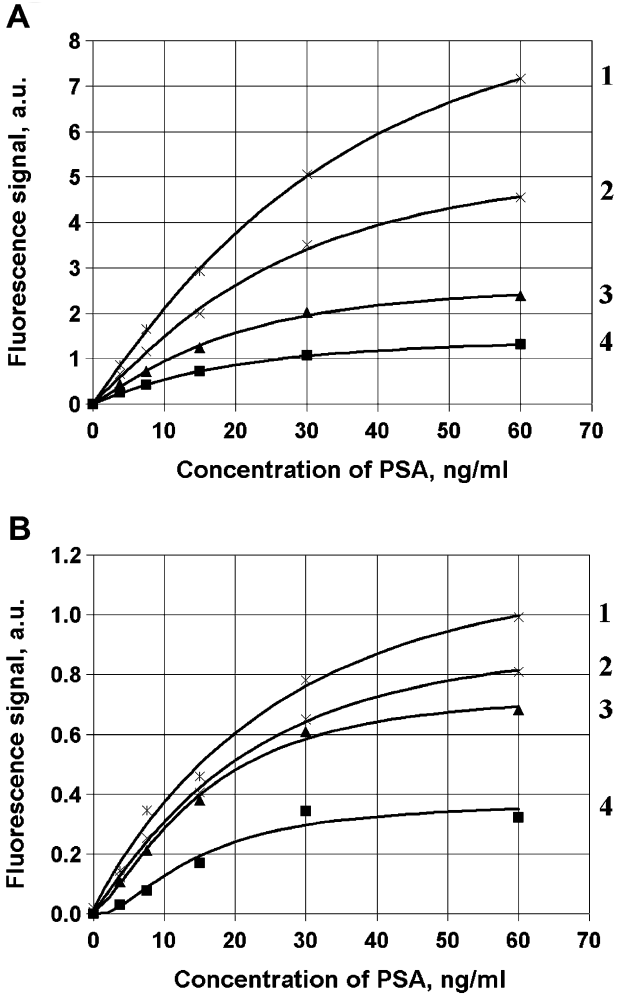

Fig. 3. Calibration curves for sandwich-type immunoassay of PSA obtained with gel-based (A) and surface (B) protein microchips at different concentrations of antibodies in spotting solution: 0.8 mg/ml (1); 0.4 mg/ml (2); 0.2 mg/ml (3); and 0.1 mg/ml (4).

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The corresponding kinetic curves are shown in Fig. 4. The saturation (or binding) time τ_B was determined from these curves as the time necessary for the fluorescence signal to reach 0.9 of the saturation level. Both the signals at saturation and those at binding times τ_B depend approximately linearly on the concentration of antibodies in spotting solution $[Ab]$, which are in this range approximately proportional to the number of immobilized antibodies in accordance with theoretical predictions (see Figs. 5 and 6 and Eqs. (3)–(6) below). The linear dependence of saturation time τ_B on $[Ab]$ shown in Fig. 6 indicates the diffusion-limited character of binding kinetics (cf. Eqs. (5)–(7) in Discussion). The intercepts of straight lines for the dependence of saturation time τ_B on $[Ab]$ with y axis corresponding to the limit of low concentration of immobilized antibodies $[Ab]$ provide the approximate estimates for the binding times at reaction-limited kinetics.

We observed about an order of magnitude higher intensity of signals at saturation for gel-based microchips compared to that for surface microchips, while the rates of binding were similar. Analogous results were also obtained at a lower concentration of antigen in buffer solution $[Ag]_{sol} = 375$ ng/ml (or 1.25×10^{-8} M).

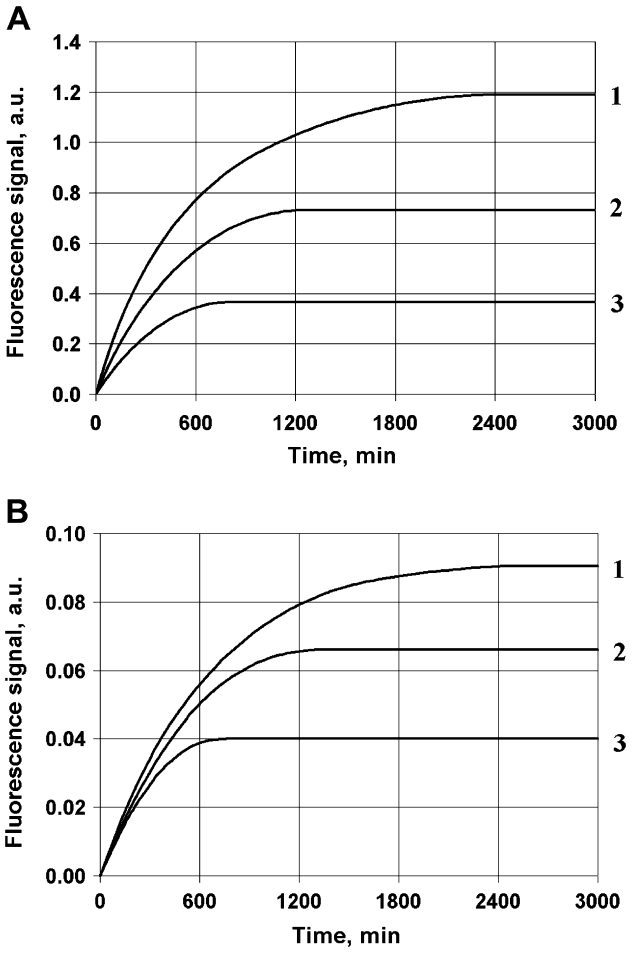

Fig. 4. Kinetic curves for the saturation of fluorescence signals on gel-based (A) and surface (B) protein microchips at different concentrations of antibodies in spotting solution: 0.8 mg/ml (1); 0.4 mg/ml (2); and 0.2 mg/ml (3). The data correspond to direct-type immunoassay with Cy5-labeled PSA.

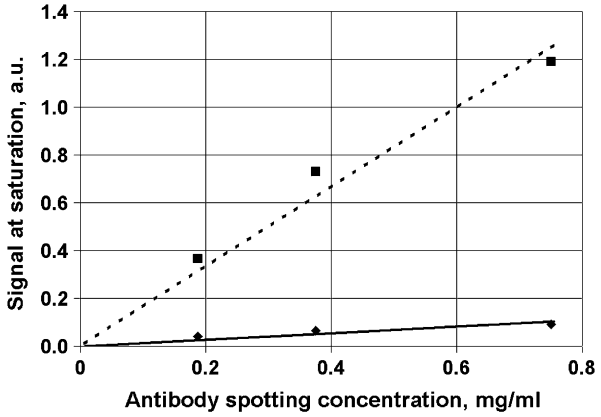

Fig. 5. Dependence of signals at saturation on the concentration of antibodies in spotting solution for the gel-based (■) and surface (♦) protein microchips. Concentration of PSA in analyte solution was 750 ng/ml. The lines (dashed line refers to gel-based microchip, while solid line refers to surface microchip) correspond to the best linear fit of experimental data.

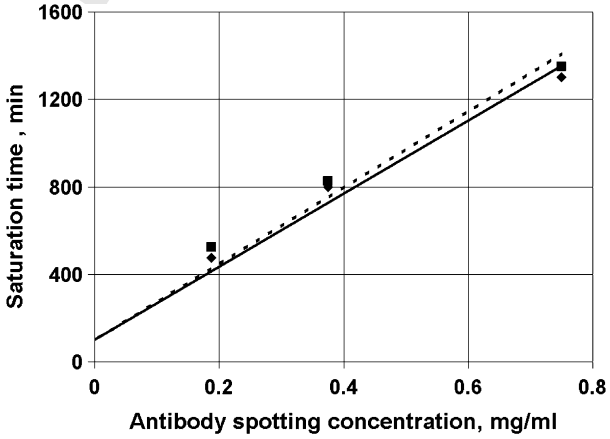

Fig. 6. Dependence of the saturation time on the concentration of antibodies in spotting solution for gel-based (■) and surface (♦) protein microchips. The concentration of PSA in analyte solution was 750 ng/ml. The lines correspond to the best linear fit of experimental data.

Discussion

Theoretical overview

To interpret the experimental results, we present below the relevant theoretical considerations.

In the case of direct immunoassay it is assumed that fluorescence signals at saturation J_{eq} detected from the cells of both gel-based and surface microchips are proportional to the number of complexes $[C]_{eq}$ formed between antigen and immobilized antibodies at the thermodynamic equilibrium

$$J_{eq}^{(gel)} = A[C]_{eq} = A[Ab_i]_{gel}\frac{K_a[Ag]_{sol}}{1+K_a[Ag]_{sol}} \quad \text{and} \tag{3}$$

$$J_{eq}^{(surf)} = \tilde{A}[\tilde{C}]_{eq} = \tilde{A}[\tilde{A}\tilde{b}_i]_{surf}\frac{K_a[Ag]_{sol}}{1+K_a[Ag]_{sol}}, \tag{4}$$

where A and $\tilde{A}$ are the apparatus constants for the gel pads and surface cells, respectively, K_a is thermodynamic association constant (which is equal to the ratio of association and dissociation rates $K_a = k_{ass}/k_{diss}$), $[Ag]_{sol}$ denotes the concentration of the antigen in solution, and $[Ab_i]_{gel}$ and $[\tilde{A}\tilde{b}_i]_{surf}$ correspond to the numbers of immobilized antibodies per unit volume of gel pad and per unit area of surface cell.

Assuming that the kinetics is diffusion limited, the corresponding characteristic times needed for signal saturation are defined by

$$\tau_{B,diff}^{(gel)} \cong \frac{R^2}{D_{gel}}[Ab_i]_{gel}\frac{K_a}{1+K_a[Ag]_{sol}} \quad \text{and} \tag{5}$$

$$\tau_{B,diff}^{(surf)} \cong \frac{R}{D_{sol}}[\tilde{A}\tilde{b}_i]_{surf}\frac{K_a}{1+K_a[Ag]_{sol}}, \tag{6}$$

where R is the radius of cells and D_{gel} and D_{sol} denote the diffusion coefficients corresponding to the diffusion of antigen in a gel without immobilized antibodies and in analyte solution. The derivation of Eq. (5) and its experimental justification can be found in [13]. It has to be noted that if the external transport is accelerated by mixing devices, the characteristic diffusion time R^2/D_{gel} should be replaced by an effective value dependent on specific conditions of mixing [14]. The related estimates for the surface kinetics [Eq. (6)] were obtained in Refs. [15,16].

In the case of reaction-limited kinetics the characteristic binding times are determined as

$$\tau_{B,react} \cong \frac{1}{k_{diss}+k_{ass}[Ag]_{sol}} \tag{7}$$

and do not depend on the concentration or density of immobilized antibodies $[Ab_i]_{gel}$ and $[\tilde{A}\tilde{b}_i]_{surf}$. As the parameters $[Ab_i]_{gel}$ and $[\tilde{A}\tilde{b}_i]_{surf}$ depend on the concentration of antibodies $[Ab]$ in spotting solution, the dependence of the observable binding times on $[Ab]$ may serve as the experimental test for the distinction between alternative regimes of kinetics: if the binding time depends on $[Ab]$, the kinetics is diffusion limited; if it does not, it is reaction limited.

Comparison of theoretical predictions with experimental results

Now we can use the theoretical predictions for the analysis of our experimental results.

It was implied above that the thermodynamic association constants K_a for the gel-based and surface microchips are similar. We checked this suggestion by studying the dependence of fluorescence signals on concentration of the antigen in solution $[Ag]_{sol}$ as described by Eqs. (3)–(6). In particular, we measured the signals at saturation and the saturation times for two concentrations of antigen $[Ag]_{sol} = 750$ ng/ml and 375 ng/ml (or 2.5×10^{-8} and 1.25×10^{-8} M). Based on these data, Eqs. (3)–(6) were used for the estimation of association constants. The resulting values were $K_a = (2.5\pm0.3)\times10^8$ M^{-1} for the gel-based microchips and $(2.6\pm0.2)\times10^8$ M^{-1} for the surface microchips. The close similarity of association constants in gel-based and surface protein microchips is in sharp contrast with those in oligonucleotide microchips, where steric restrictions imposed by the immobilization on a flat surface result in effective suppression of the association constant on surface chips compared with that on gel chips (see [15,17] and references therein).

Thus, the differences in fluorescence signals for both direct and sandwich immunoassays using gel-based and surface protein microchips observed in this work cannot be attributed to the differences in the association constants. In addition, such a suggestion would contradict the fact that saturation times $\tau_{B,diff}$ for chips of the two types are comparable, although the estimated values of the diffusion coefficient for the gel D_{gel} are four- to fivefold lower than the corresponding values measured in solution D_{sol} [18].

To further clarify the observable features, we assessed the mean distances between antibodies immobilized in the gel pads and on the surface cells. The numbers of immobilized antibodies per unit volume of gel pad $[Ab_i]_{gel}$ and per unit area of surface cell $[\tilde{A}\tilde{b}_i]_{surf}$ can be related to the concentration of antibodies in spotting solution as

$$[Ab_i]_{gel} = f_i^{(gel)}[Ab]_{spotsol} \quad \text{and} \tag{8}$$

$$\pi R^2[\tilde{A}\tilde{b}_i]_{surf} = \frac{2\pi}{3}R^3.f_i^{(surf)}[Ab]_{spotsol}. \tag{9}$$

Here $f_i^{(gel)}$ and $f_i^{(surf)}$ define the immobilization efficiencies (or the fractions of immobilized antibodies) for gel pads and surface cells respectively. Generally, these values depend on the concentration of antibodies in spotting solution $[Ab]_{spot\ sol}$ (see Fig. 1). The assessed range of concentrations of antibodies immobilized in the gel pads $[Ab_i]_{gel}$ was $2.9\times10^{-7} - 4.7\times10^{-6}$ M, while the relevant densities of antibodies immobilized on the surface cells $[\tilde{A}\tilde{b}_i]_{surf}$ amounted to $1.1\times10^{12} - 3.3\times10^{12}$ molecules/cm^2. To compare these values in comparable units, we calculated the effective surface density for antibodies immobilized in the gel pads

$$[\tilde{A}\tilde{b}_i]_{surf,eff} = R[Ab_i]_{gel} \tag{10}$$

obtained by vertical projection of volume concentrations $[Ab_i]_{gel}$ in hemispherical gel pads with the radius R onto their flat base. The relevant densities are reproduced in Fig. 7 and demonstrate that the effective surface density for antibodies immobilized in the gel pads can be an order of magnitude higher than the counterpart density for the surface antibody microchips.

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Using the data for the immobilization efficiencies f_i presented in Fig. 2 and Eqs. (8) and (9), the mean distances between immobilized antibodies were assessed as

$$d_{gel} = [Ab_i]_{gel}^{-1/3} \quad \text{and} \tag{11}$$

$$d_{surf} = [\tilde{A}\tilde{b}_i]_{surf}^{-1/2}. \tag{12}$$

The dependence of these values on the concentration of antibodies in spotting solution is shown in Fig. 8. The comparison of Figs. 2 (solid curve) and 8B reveals that the rapid growth in the immobilization efficiency for the surface protein microchips begins from the values of $[Ab]_{spot\ sol}$ corresponding to d_{surf} about ~10 nm, which is in a good accordance with the characteristic molecular sizes of antibodies determined by X-ray analysis [19] (see also other abundant X-ray data in PDB), light scattering [20], and atomic force microscopy [21]. The higher and nearly constant immobilization efficiency for the gel-based protein microchips appears to be concordant with sufficiently large mean distances d_{gel} between antibodies immobilized in the gel pads, ~100 nm, on the order of magnitude exceeding the characteristic molecular sizes of antibodies.

The proximity of antibody molecules immobilized on the surface cells may hamper their accessibility for the target antigen molecules and create steric restrictions for molecular antibody–antigen interactions. These effects may be approximated in Eqs. (4) and (6) by replacing the intensity of fluorescence at saturation and the binding time by modified expressions $J_{eq}^{(surf)} \to \alpha_{steric} J_{eq}^{(surf)}$ and $\tau_{B,diff}^{(surf)} \to \tau_{B,diff}^{(surf)}/\alpha_{steric}$, where the factor $0 < \alpha_{steric} < 1$ reflects steric restrictions produced by the close package of antibodies on the surface cells. This may explain the observable difference in the fluorescence signals at the comparable saturation kinetics in gel-based and surface protein microchips. These observable features should be partially attributed to the random orientation and partial denaturation for a fraction of antibodies immobilized on a substrate surface [16].

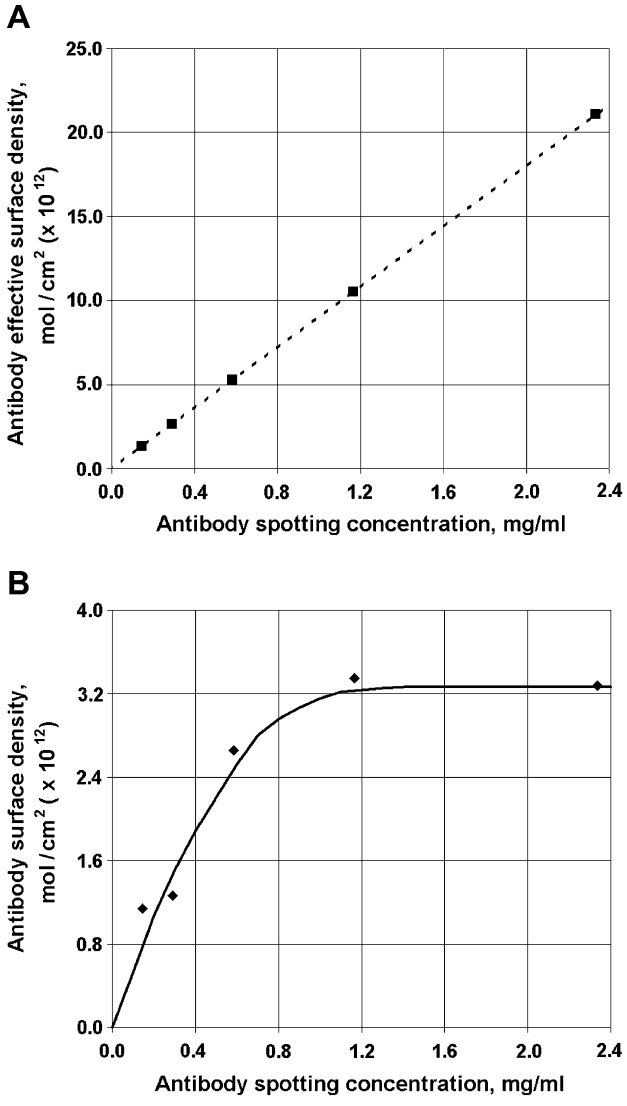

Fig. 7. Comparison of effective surface density for antibodies immobilized in the gel pads (A) [see Eq. (10)] with the corresponding density for antibodies immobilized on the surface cells (B) at different concentrations of antibodies in spotting solution.

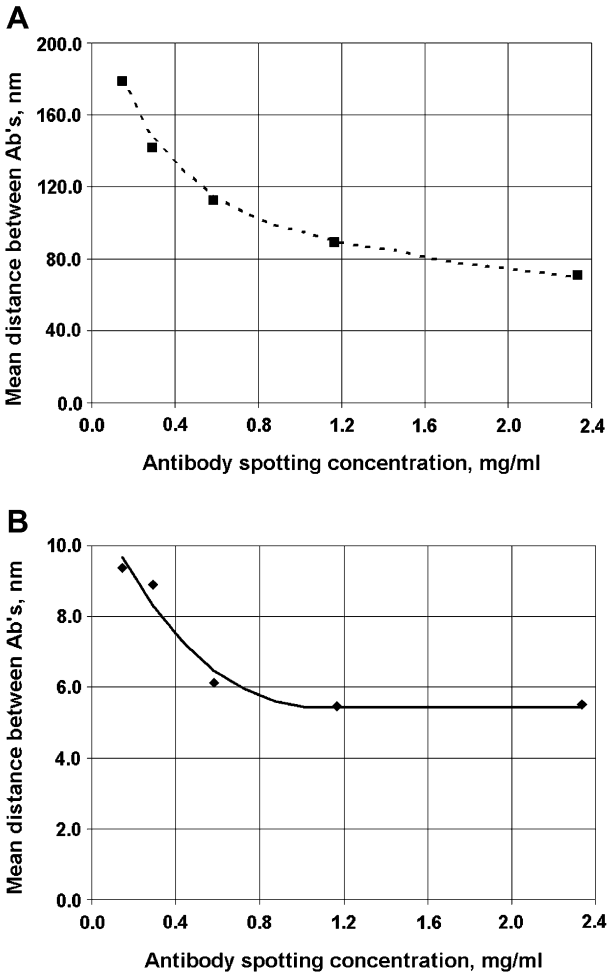

Fig. 8. Dependence of mean distance between immobilized antibodies on the concentration of antibodies in spotting solution for gel-based (A) and surface (B) protein microchips.

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Conclusion

Our results demonstrate that at the same concentration of antibodies in spotting solution gel-based microchips provide stronger fluorescence signals than the surface protein microchips. The relatively large molecular sizes of antibodies restrict the maximal density of the probes immobilized on the surface cells to $\sim(3–4)\times10^{12}$ molecules/cm^2, while the capacity of gel pads is much higher. Specifically, the concentration of antibodies immobilized in gel pads may attain 10^{-4}–10^{-3} M, which is two to three orders of magnitude higher than that used in our work. The vertical projection of concentrations $\sim 10^{-4}$–10^{-3} M in hemisphere with the radius 75 μm onto its flat base would provide the effective surface densities $\sim5\times10^{14}$–5×10^{15} molecules/cm^2, never attainable for the surface cells (cf. also Fig. 7). However, the limitations connected with the rates of binding kinetics impose the condition $[Ab_i]_{gel}K_a \leqslant 10^3 - 10^4$ and partially restrict the maximal capacity of gel pads.

Unlike the oligonucleotide microchips [15], the rates of signal saturation for the protein microchips of both kinds appear to be comparable. The observed saturation times for the surface antibody microchips in this work appear to be similar to those determined previously by Kusnezow et al. [16], while the corresponding times for the gel-based antibody microchips are close to values published earlier by our group [14].

It is worth noting that the diffusion fluxes for the hemispherical geometrical body (represented by the gel pad) turn out to be more efficient than the diffusion fluxes for the flat disk of the same radius and produce a more spatially homogeneous distribution of the antigen–antibody complexes (and, thus, the observable signals) than the hemispherical cells in the transient regime of binding [22]. If necessary, the binding kinetics may be accelerated about fivefold by the active mixing, which accelerates the transport of antigen in buffer solution [14] (see also [16]).

The choice of the optimal platform is one of the essential factors in the further development of protein microchip technology. The results of this work will be useful for the assessment of efficiency of different protein microchips.

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