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Evaluation of surface chemistries for antibody microarrays

Shannon L. Seurynck-Servoss [a], Amanda M. White [b], Cheryl L. Baird [a], Karin D. Rodland [a], Richard C. Zangar [a,*]

[a] *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99354, USA*
[b] *Statistical and Mathematical Sciences, Pacific Northwest National Laboratory, Richland, WA 99354, USA*

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Abstract

Antibody microarrays are an emerging technology that promises to be a powerful tool for the detection of disease biomarkers. The current technology for protein microarrays has been derived primarily from DNA microarrays and is not fully characterized for use with proteins. For example, there are a myriad of surface chemistries that are commercially available for antibody microarrays, but there are no rigorous studies that compare these different surfaces. Therefore, we have used a sandwich enzyme-linked immunosorbent assay (ELISA) microarray platform to analyze 17 different commercially available slide types. Full standard curves were generated for 23 different assays. We found that this approach provides a rigorous and quantitative system for comparing the different slide types based on spot size and morphology, slide noise, spot background, lower limit of detection, and reproducibility. These studies demonstrate that the properties of the slide surface affect the activity of immobilized antibodies and the quality of data produced. Although many slide types produce useful data, glass slides coated with aldehyde silane, poly-L-lysine, or aminosilane (with or without activation with a crosslinker) consistently produce superior results in the sandwich ELISA microarray analyses we performed.

Keywords: ELISA; Antibody; Microarray; Solid support

Sandwich enzyme-linked immunosorbent assay (ELISA)[1] microarrays have the potential to be a powerful tool for the screening and validation of biomarkers for cancer and other complex diseases, mainly due to the great promise of this technology for rapidly analyzing many antigens in very small sample volumes [1–6]. Even though the standard 96-well plate ELISA is routinely used for high-throughput detection of proteins, this platform has limited capacity to screen large numbers of proteins in an efficient manner, particularly when sample volumes are limited. In contrast, sandwich ELISA microarrays can simultaneously screen up to 50 proteins in small sample volumes (e.g., 15 μl of sample after dilution).

Originally, experimental protocols for ELISA microarray were based on a combination of the protocols for 96-well plate ELISA and DNA microarrays [6]. More recently, many studies have been directed toward optimizing conditions specifically for multiplexed protein assays, including determination of the necessary time for sample incubation [7–9], reduction of cross-reactivity between assays and nonspecific protein binding [10], and optimization of surface chemistries for immobilizing antibodies [11–14]. Despite these efforts, ELISA microarrays have yet to meet theoretical predictions for assay sensitivity [8], suggesting that assay conditions still have considerable room for improvement. That is, to achieve the full potential of the ELISA microarray technology, it will be necessary to develop an integrated platform that has been optimized at all processing steps.

* Corresponding author. Fax: +1 509 376 6767.
E-mail address: richard.zangar@pnl.gov (R.C. Zangar).

[1] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; DSS, disuccinimidyl suberate; BS^3, bis[sulfosuccinimidyl] suberate; PBS, phosphate-buffered saline; TSA, Tyramide Signal Amplification; Cy3, cyanine 3; RANTES, regulated on activation normal T cell expressed and secreted; HD, hydrophobic surface; ES, enhanced surface; NHS, *N*-hydroxysuccinimide.

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Slide surface chemistry is an important aspect of a robust ELISA microarray platform, a point that is highlighted by the large variety of surface modifications and chemistries that are commercially available. A number of characteristics that are associated with good performance of ELISA microarrays must be evaluated when choosing the ideal surface for antibody immobilization, including spot size and morphology, total antibody binding, background signal (both surrounding and within the printed spot), lower limit of detection, and reproducibility across chips and experiments. Therefore, a good surface chemistry for antibody immobilization must have (i) high binding capacity, (ii) an ability to retain antibody activity, (iii) low variability between slides, and (iv) high signal-to-noise ratios. We recently reviewed the literature on surface chemistries and found that there is a general failure to evaluate different surface chemistries in either a global or systematic manner [15]. As such, it has not been possible to critically determine which of the available slide chemistries are truly useful for antibody microarrays.

In this article, we evaluate 17 commercially available slide types based on the criteria listed above. The slides were evaluated by comparing standard curves for 23 different assays, all previously determined to have good assay sensitivity and specificity (R. Gonzalez and R.C. Zangar, unpublished data). We find that three-dimensional slide surfaces tend to have higher background than two-dimensional surfaces, likely due to an inability to efficiently wash and/or block the surface. We also observe that noncovalent chemistries for capturing the antibody can work nearly as well as covalent ones. Overall, these experiments identify superior slide chemistries for antibody capture and define some basic principles that contribute to these superior chemistries.

Materials and methods

Materials

Disuccinimidyl suberate (DSS) and bis[sulfosuccinimidyl] suberate (BS^3) were purchased from Pierce (Rockford, IL, USA). Purified antibodies and antigens were purchased from R&D Systems (Minneapolis, MN, USA), Lab Vision (Fremont, CA, USA), Fitzgerald (Concord, MA, USA), MBL International (Woburn, MA, USA), and BiosPacific (Emeryville, CA, USA). Blocking solution containing 10 mg/ml casein in phosphate-buffered saline (PBS) was purchased from Bio-Rad Laboratories (Hercules, CA, USA), the Tyramide Signal Amplification (TSA) system, including streptavidin-conjugated horseradish peroxidase, amplification diluent, and biotinyl tyramide, was purchased from Perkin-Elmer (Wellesley, MA, USA). Streptavidin-conjugated cyanine 3 (Cy3) was obtained from Amersham Biosciences (Piscataway, NJ, USA).

Erie Scientific (Portsmouth, NH, USA) kindly provided aminosilane-, epoxysilane-, poly-L-lysine-, and aldehyde silane-coated slides as well as hydrophobic and enhanced surface modifications for epoxysilane-coated slides. Full Moon protein slides were provided by Full Moon BioSystems (Sunnyvale, CA, USA); slides H, AL, and E were provided by Schott (Elmsford, NY); and dendron slides were provided by NSB Postech (Pohang, Korea).

Microarray preparation

A GeSiM NanoPlotter 2.1 (Quantum Analytics, Foster City, CA, USA) noncontact printer with humidity control was used to print the antibodies. All slides were used as provided by the manufacturer with the exception of aminosilane- and dendron-coated slides. In some cases, these slides were treated with a 0.2-mg/ml solution of the homobifunctional crosslinker DSS in ethanol to create a covalent binding site for amines. Alternatively, aminosilane-coated slides were treated with 0.3 mg/ml BS^3 in PBS to compare the two crosslinkers. After incubation with the crosslinker for 5 min, the slides were rinsed with 100% ethanol and dried under argon.

Capture antibodies were suspended in PBS to a concentration of 0.5–0.8 mg/ml. The spots were printed 0.5 mm apart, in quadruplicate, on each array. Each slide contained 16 arrays in an 8×2 pattern with 9 mm spacing between arrays. Following printing, the slides were incubated in the printer for 1 or 12 h, as suggested by the manufacturer (see Table 1 for details). The arrays were outlined with a Pap pen to create a hydrophobic barrier for processing arrays individually. The successful printing of the spots was confirmed by scanning the slides using the "red reflect" setting on a ScanArray Express HT microarray scanner (PerkinElmer). The slides were blocked at room temperature for 1 h in 10 mg/ml casein in PBS. Slides were then washed with PBS-T (0.05% Tween 20 in PBS) and processed immediately.

Slide processing

Slides were processed as described previously [16]. Briefly, all incubation steps were performed in a humid chamber at room temperature in the dark with gentle mixing. The slides were washed thoroughly between each processing step by soaking in PBS-T for 3–10 min. The slides were incubated with a mixture of antigens in 1 mg/ml casein in PBS for 4 h. Standard curves were created using a threefold dilution series of the antigen mix along with an antigen-free blank for eight total dilutions that were run in duplicate on each slide type. The slides were then incubated with biotinylated detection antibody at 25 ng/ml in 1 mg/ml casein in PBS for 1 h. The signal was amplified using the TSA system according to manufacturer's instructions, and then the slides were incubated with a solution of 1 μg/ml streptavidin-conjugated Cy3 in PBS-T for 30 min. A ScanArray Express HT laser scanner was used to image the Cy3 fluorescence signal on the slides.