Analytical Biochemistry 450 (2014) 37–45

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Evaluation of homo- and hetero-functionally activated glass surfaces for optimized antibody arrays

María González-González [a], Raquel Bartolome [a], Ricardo Jara-Acevedo [b], Juan Casado-Vela [c], Noelia Dasilva [a], Sergio Matarraz [a], Jacinto García [d], J.A. Alcazar [d], J.M. Sayagues [a], Alberto Orfao [a,*], Manuel Fuentes [a,*]

[a] Centro de Investigación del Cáncer/IBMCC (USAL/CSIC)–IBSAL, Departamento de Medicina and Servicio General de Citometría, University of Salamanca, 37007 Salamanca, Spain
[b] ImmunoStep, 37007 Salamanca, Spain
[c] Spanish National Research Council (CSIC)–Spanish National Biotechnology Centre (CNB), 28049 Madrid, Spain
[d] Servicio de Cirugía, Hospital Clínico Universitario de Salamanca–IBSAL, 37007 Salamanca, Spain

ARTICLE INFO

Article history:
Received 29 November 2013
Accepted 7 January 2014
Available online 15 January 2014

Keywords:
Antibody arrays
Surface functionalization
Antibody immobilization
Protein microarrays
Functionalized surfaces

ABSTRACT

Antibody arrays hold great promise for biomedical applications, but they are typically manufactured using chemically functionalized surfaces that still require optimization. Here, we describe novel hetero-functionally activated glass surfaces favoring oriented antibody binding for improved performance in protein microarray applications. Antibody arrays manufactured in our facility using the functionalization chemistries described here proved to be reproducible and stable and also showed good signal intensities. As a proof-of-principle of the glass surface functionalization protocols described in this article, we built antibody-based arrays functionalized with different chemistries that enabled the simultaneous detection of 71 human leukocyte membrane differentiation antigens commonly found in peripheral blood mononuclear cells. Such detection is specific and semi-quantitative and can be performed in a single assay under native conditions. In summary, the protocol described here, based on the use of antibody array technology, enabled the concurrent detection of a set of membrane proteins under native conditions in a specific, selective, and semi-quantitative manner and in a single assay.

The study of molecule interactions and the assessment of multiprotein profiling in biological samples are becoming increasingly relevant [1–6]. One of the current favorite tools for such aims is the use of miniaturized array-based technology, enabling the attachment of multiple molecules on solid surfaces for screening purposes. In this sense, protein arrays can be defined as collections of chemically defined miniaturized areas (spots, <250 μm^2 each) arranged on a solid surface (typically glass) able to attach proteins of interest through chemical moieties that act as capture agents [1,2,4–9]. Such protein arrays enable the screening, identification, characterization, and quantification of target proteins and other biomolecules using small sample volumes [1–5,7–10]. Protein arrays represent a promising tool in biomedical research and may be used for biomarker discovery, for drug discovery, and (eventually) for clinical diagnostic purposes [2,11–20]. In particular, the diagnostic impact of these approaches at the clinic resides in the possibility of performing massive comparisons of expression patterns from different types of biological samples allowing the detection of altered expression profiles. As an example, protein arrays proved to be valid for early detection of minimal amounts of infiltrating cells in tissues [13,15].

However, protein arrays still present several drawbacks undermining their applicability that need to be overcome [2,6,8,9,17]. Reviewing the literature, it is widely acknowledged that many different variables contribute to the usability of this technology, including improved protein immobilization, function stabilization, structure preservation, optimal orientation of the binding molecules, and array surface functionalization with chemical moieties [2,6,8,9,13,14,17,21,22]. One issue frequently neglected is that arrayed proteins are often unstable or unable to keep their native conformation [2,6,8,9,16]. In this regard, the optimization of surface chemistries binding proteins may be crucial because different chemistries may significantly influence the binding properties of the arrays.

The ideal surface activation chemistry for protein array applications should accomplish the following characteristics: (i) should have high binding capacities, (ii) should not alter antibody specificity and binding capabilities, (iii) should have low inter- and intraarray variability, (iv) should provide high signal-to-noise ra-

* Corresponding authors. Fax: +34 923294743.
E-mail addresses: orfao@usal.es (A. Orfao), mfuentes@usal.es (M. Fuentes).

0003-2697/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2014.01.002

tios, and (v) should be stable at least for several months [1,4,22–26]. In the case of antibody arrays (where the proteins attached on the surface of arrays are composed exclusively of panels of antibodies), other characteristics should also be considered, including (vi) the size and morphology of the spots, (vii) antibody binding properties, (viii) background signaling, and (ix) required low detection limits and good reproducibility [23].

Several authors previously compared different surface chemistries (mainly using commercially available glass arrays), described their characteristics, and tested their ability to detect and identify biomolecules attached on them [7,26]. Briefly, one of the main conclusion s reached in previous reports is that antibody immobilization, through either noncovalent or covalent chemistries, seems to work similarly. However, that assumption considered only frequently commercially available chemistries, based exclusively on mono-functional chemistries (e.g., amines, epoxy or aldehyde groups) in combination with cross-linkers such as bis-(sulfosuccinimidyl) suberate (BS3),[1] *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), and succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC).

In this article, we show that hetero-functional chemistries may also be used for manufacturing protein arrays and provide data supporting that such chemistries are highly advantageous, compared with homo-functionalized chemistries, favoring the correct functional orientation of antibodies on the surface of arrays and, thus, improving their binding capabilities. We also propose a two-step antibody binding strategy comprising antibody immobilization by ionic/affinity adsorption followed by covalent attachment. This two-step immobilization approach significantly improves antibody immobilization yield.

A wide range of hetero-functional chemistries are commercially available. Therefore, although only a small range of hetero-functional chemistries were included here, we provide the basis for novel array designs with improved binding capabilities. Finally, we successfully address the identification of human leukocyte antigens (CD) isolated from peripheral blood cells using arrays functionalized in-house with optimized chemistries. Our preliminary results strongly support the suitability of the antibody immobilization strategies proposed and their application to address biological questions.

Materials and methods

Chemicals

The procedures described in this article required the use of the following chemicals: acetone (Panreac, Spain), 3-(2-amine ethylene) propyl-methyl dimethoxysilane (MANAE), 3-glycidoxy-propyl-trimethoxysilane (GPTS), amino-phenyl-boronic (AMPB), Tween 20, sodium periodate, iminodiacetic acid (IDA) (Sigma, USA), BS3, SPDP, SuperBlock casein solution (Pierce, USA), and Tyramide Signal Amplification (TSA) reagent (PerkinElmer, USA). Purified mouse anti-human CD proteins (see Table 1) were supplied by ImmunoStep (Spain). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG secondary antibodies were obtained from GE Healthcare (UK). High-purity ($\geqslant$98%) chemicals and distilled water were used. A source of compressed air (2–3

[1] *Abbreviations used:* BS3, bis-(sulfosuccinimidyl) suberate; SPDP, *N*-succinimidyl 3-(2-pyridyldithio) propionate; SMCC, succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate; CD, leukocyte antigens; MANAE, 3-(2-amine ethylene) propyl-methyl dimethoxysilane; GPTS, 3-glycidoxypropyl-trimethoxysilane; AMPB, amino-phenyl-boronic; IDA, iminodiacetic acid; TSA, Tyramide Signal Amplification; HRP, horseradish peroxidase; PDMS, poly-dimethylsiloxane; PBS, phosphate-buffered saline; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; CV, coefficient of variation.

Table 1
Anti-human monoclonal antibody reagents used to spot the antibody arrays.

Number	Antibody	Clone	Isotype
1	CD61	C17	IgG1
2	CD45	D3/9	IgG1
3	CD22	HIB22	IgG1
4	CD56	B-A19	IgG1
5	CD56	MEM188	IgG2a
6	CD58	HI58a	IgG1
7	CD21	HI21a	IgG2a
8	CD235a	HI264	IgG2a
9	CD52	HI186	IgG2b
10	CD40	HI40a	IgG1
11	CD1a	HI149	IgG1
12	CD42b	HIP1	IgG1
13	Isotypic control IgG2b	IgG2b	IgG2b
14	CD53	HI29	IgG1
15	CD11c	BU-15	IgG1
16	CD33	HIM3-4	IgG1
17	CD62L	HI62L	IgG2a
18	CD7	HIT7	IgG1
19	Anti-kappa	HIgk	IgG1
20	CD40	HI40a	IgG1
21	CD62e	TEA2/1	IgG2b
22	CD20	LT-20	IgG2a
23	CD20	BC1	IgM
24	CD41	HIP8	IgG1
25	Anti-Lambda	HIgl	IgG3
26	CD11a	TP1/40	IgG1
27	CD11b	DCIS1/18	IgG2a
28	CD15	MCS-1	IgG3
29	CD19	A3-B1	IgG2a
30	CD34	HI273	IgG2b
31	CD38	GR7A4	IgG1
32	CD42a	GR-P	IgG2a
33	Isotypic control IgG2a	IgG2a	IgG2a
34	CD26	TP1/19	IgG2b
35	CD57	HI57a	IgM
36	CD59	VJ1/12.2	IgG2a
37	CD71	FG2/12	IgG1
38	CD8	143-44	IgG1
39	CD98	FG1/8	IgG1
40	CD99	HI156	IgG2a
41	CD105	2H6F11	IgG1
42	CD16	GRM1	IgG2a
43	Isotypic control IgG1	IgG1	IgG1
44	Anti-HLA DQ	HI118	IgG1
45	CD2	HIP11	IgG1
46	CD25	TP1/6	IgG2b
47	CD28	204-12	IgG2a
48	CD3	33-2A3	IgG2a
49	CD14	47-3D6	IgG1
50	CD18	GRF1	IgG1
51	CD31	TP1/15	IgG2a
52	CD49b	TEA1/41	IgG1
53	CD49c	VJ1/6	IgG1
54	CD49d	ALC1/1	IgG1
55	Anti-HLA DP	HI43	IgG1
56	CD29	VJ1/14	IgG2b
57	CD5	33-1C3	IgG2a
58	Isotypic control IgM	IgM	IgM
59	CD4	HP2/6	IgG2a
60	CD43	TP1/36	IgG1
61	CD45	D3/9	IgG1
62	CD6	MAE-1C10	IgG1
63	CD62P	P.selKO2.22	IgG2b
64	IREM2 (CD300e)	UP-H2	IgG2a
65	CD45RA	GRT22	IgG1
66	CD45RB	MC5/2	IgG1
67	CD45RC	RP1/12	IgG1
68	CD9	VJ1/20	IgG2a
69	CD10	HI10a	IgG1
70	Isotypic control IgG3	IgG3	IgG3
71	Anti-HLA DR	GRB1	IgG2a

Note. All reagents were obtained from ImmunoStep (Spain).