Talanta 98 (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Talanta

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

Influence of buffer composition on the distribution of inkjet printed protein molecules and the resulting spot morphology

Liyakat Hamid Mujawar [a,b,1], Aart van Amerongen [a,*], Willem Norde [b,c,2]

[a] Food and Biobased Research, Biomolecular Sensing and Diagnostics, Wageningen University and Research Centre, Bornse Weilanden 9, 6708 AA Wageningen, The Netherlands
[b] Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands
[c] University Medical Center Groningen, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 30 January 2012
Received in revised form
1 June 2012
Accepted 4 June 2012
Available online 18 June 2012

Keywords:
Microarray
Buffer
Spot morphology
BSA–biotin
Polystyrene
Inkjet printing

ABSTRACT

Producing high quality protein microarrays on inexpensive substrates like polystyrene is a big challenge in the field of diagnostics. Using a non-contact inkjet printer we have produced microarrays on polystyrene slides for two different biotinylated biomolecules, bovine serum albumin (BSA–biotin) and immunoglobulin-G (IgG–biotin), and studied the influence of buffer (composition and pH) on the spot morphology and signal intensity. Atomic force microscopy revealed the morphological pattern of the (biomolecule) spots printed from phosphate buffer (pH 7.4), phosphate buffered saline (pH 7.4) and carbonate buffer (pH 9.6). The spots showed an irregular crust-like appearance when printed in phosphate buffered saline (pH 7.4), mainly due to the high NaCl content, whereas spots of biomolecules printed in carbonate buffer (pH 9.6) showed a smooth morphology. In addition, the rinsing of these dried spots led to the loss of a considerable fraction of the biomolecules, leaving behind a small fraction that is compatible with the (mono)layer. It was confirmed by confocal laser microscopy that the quality of the spots with respect to the uniformity and distribution of the biomolecules therein was superior when printed in carbonate buffer (pH 9.6) as compared to other buffer systems. Particularly, spotting in PBS yielded spots having a very irregular distribution and morphology.

1. Introduction

Microarrays of various biomolecules (DNA, antibodies, serum albumin) have gained a lot of attention in the scientific world due to a wide range of potential applications [1,2]. Inexpensive sources of substrates like glass and plastics [3,4] are most commonly used for printing biomolecules. Printing with a non-contact microarrayer causes the biomolecules to physically adsorb onto these non-porous substrates, e.g., a polystyrene slide. Adsorption of a protein onto such substrate surface is influenced by various factors like temperature, humidity [5], pH [6] and composition. The charge of the biomolecule may also vary depending on the pH and the ionic strength of the medium, which may further influence the process of adsorption. In studying protein microarrays, two factors play an important role: firstly, the immobilization of the biomolecule onto the substrate, and secondly, the effect of immobilization on the functionality of the biomolecule. It has been reported that adsorption and functionality are interrelated such that the biomolecule which is more strongly adsorbed onto the surface has a higher activity [7]. However, by studying various conditions in the preparation of protein microarrays this assumption could not be confirmed [8–10]. The printing buffer used to print biomolecules could be modified with additives like triton-X-100 [11], glycerol [12,13] and trehalose [14]. End results showed that high density of protein immobilization and superior quality of the microspots were obtained when the printing buffer had an optimal concentration of glycerol (10%) and triton-X-100 (0.003%). Printing of biomolecules (antibodies) onto a polystyrene surface has been performed with phosphate buffered saline (pH 7.4) [15] and carbonate buffer (pH 9.6) [16] whereas printing of proteins like BSA has been previously done with carbonate buffer [17,18]. Yet, no clear evidence is available as to why a certain buffer system is to be preferred over others and this raises many unanswered questions.

In this research we addressed the first important factor in the preparation of protein microarrays, i.e., the immobilization of the biomolecule onto the substrate. The main objective was to analyze and compare the influence of buffers of varying

* Corresponding author at: Wageningen University and Research Centre, Food and Biobased Research, Biomolecular Sensing and Diagnostics, Bornse Weilanden 9, 6708 AA Wageningen, The Netherlands. Tel.: +31 317 480 164; fax: +31 317 483 011.
E-mail addresses: liyakat.mujawar@wur.nl (L.H. Mujawar), Aart.vanamerongen@wur.nl (A. van Amerongen), Willem.Norde@wur.nl (W. Norde).
[1] Tel.: +31 317 481 311; fax: +31 317 483 011.
[2] Tel.: +31 317 483540; fax: +31 317 483777.

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.06.006

compositions and pH on the distribution and spot morphology of different biomolecules on a polystyrene substrate. Atomic force microscopy was used to characterize the morphological pattern on the spot of printed biomolecules, and the distribution of the biomolecules over the spot was analyzed with confocal fluorescence microscopy studies. On these polystyrene slides we also compared the influence of printing buffers on a nucleic acid microarray immunoassay (NAMIA) and evaluated the compatibility of these buffers (based on spot characteristics and signal intensity) for biochip production and performance.

2. Materials and methods

2.1. Reagents

Bovine serum albumin biotin conjugate (BSA–biotin) was purchased from Thermo Scientific and biotin-SP-conjugated affinipure goat antimouse immunoglobulin (IgG–biotin) was from Jackson ImmunoResearch Laboratories. Anti-digoxygonin was purchased from Roche (Germany) whereas anti-Dinitrophenyl, anti-Fluroisothiocynate and anti-Texas red were from Invitrogen. DNA template for the *Corynebacterium bovis* and *Staphylococcus aureus* was provided by GD, Deventer (The Netherlands). The details of the PCR amplification are explained in *Electronic supplementary material* (ESM) 1. HTA polystyrene (PS) slides were purchased from Greiner BioOne. Carbon-alkaline phosphatase–neutravidin conjugate was prepared as described [19]. The substrate for the alkaline phosphatase was from Sigma (SigmaFAST BCIP/NBT). Streptavidin–Alexa-633 conjugate for labeling the biotinylated proteins was obtained from Invitrogen. 100 mM phosphate buffer saline (PBS) pH 7.4, phosphate buffer (PB) pH 7.4, and carbonate buffer (CB) pH 9.6 were prepared in Milli-Q water with resistivity of 18.2 MΩ cm^{-1}. Running buffer (100 mM borate buffer containing 1% BSA and 0.05% Tween20) was used as a diluent for the conjugates and also during the intermediate washing steps.

2.2. Apparatus

2.2.1. Microarrayer

Biomolecules were arrayed on the HTA-PS surface with a non-contact spotter, sciFLEXARRAYER S3 (Scienion AG, Berlin, Germany). The printer was placed in a hood to maintain constant temperature and humidity. Spotting of the biomolecules was performed at room temperature and 70% humidity. The voltage and pulse of the piezo-dispensing capillary (PDC) was optimized to print a droplet of 300 pL. The volume was kept constant throughout the entire process of spotting.

2.2.2. Reflectometry

Adsorption of BSA–biotin in various buffers was studied by reflectometry. The setup of the reflectometer has been explained in detail [20]. Si wafer strips (1 cm × 5 cm) were cleaned with ethanol followed by piranya treatment (1 part 30% H_2O_2 and 2 parts concentrated H_2SO_4) to remove any impurities from the surface. Finally, the wafers were rinsed with Milli-Q water and dried under N_2. A thin layer of PS was grafted onto the Si surface as described before [21]. The thickness of the PS layer (60–70 nm) was measured by ellipsometry.

2.2.3. AFM measurement

Spot morphology of printed biomolecules was characterized with atomic force microscopy, AFM (Veeco, NY, USA), in tapping mode (in air). For each spot 256 lines were scanned at a frequency of 0.4 Hz. Height data was compared to analyze morphological changes in the biomolecule printed using various buffers. The average thickness of the spot at various positions was obtained through section analysis (Nanoscope Analysis software).

2.2.4. CLSM imaging

Spots of biotinylated proteins were labeled with streptavidin–Alexa-633 conjugate and analyzed by confocal laser scanning microscopy (CLSM) to compare the distribution of the biomolecules in various buffers when printed on HTA-PS surface. Flurophore labeled biomolecules were analyzed using a confocal microscope (Carl Zeiss Axiovert 200 microscope, Zeiss, Jena, Germany) equipped with an LSM 5 Exciter. The configuration of the objective was LD Plan-Neofluar 40 × /0.6 Korr Ph2 M27. A He–Ne laser set at 633 nm was used. The size of the pinhole was 182 μm whereas the transmission was 11%. The dimension of the scanner was X: 160.40 μm and *Y*: 160.40 μm. Further analysis was carried out by the "Zen 2008" software.

2.3. Printing and labeling of BSA–biotin

The wells of the HTA-PS slide (*see Electronic supplementary material* (*ESM*) 2) were flushed with N_2 and mounted on the deck of the microarrayer. BSA–biotin (200 μg/mL) was prepared in PBS or CB and 20 μL of the diluted BSA–biotin was carefully placed into the wells of a Genetix 384 microtiter plate that was mounted on the deck as a reagent reservoir. Air bubbles, if present, were removed to prevent any interference during spotting. The microarrayer was programmed to print an array of one drop of BSA–biotin in different buffers on the HTA slide. After printing, the slide was incubated for 1 h in the sciPROCLIMATE unit at 37 °C and 70% humidity. Biotinylated BSA was incubated with 1% carbon-alkaline phosphatase–neutravidin conjugate (prepared in running buffer) for 1 h. The slide was rinsed 3 times with running buffer, followed by substrate incubation for 10 min. Finally, the slide was rinsed 3 times with Milli-Q water and air-dried.

2.4. Scanning and image analysis

Biomolecules labeled with carbon conjugates were scanned on an Epson 3200 Photo scanner (Seiko Epson, Nagano, Japan) with a resolution of 4800 dpi and 16 bit gray-scale and the images were saved as TIFF files. Spot intensity was measured by the TotalLab image analysis software, by placing a marker ring at the circumference of the spot. The total intensity or pixel gray volume of 16 replicate spots was calculated and compared with the spots printed in other buffers.

3. Results and discussion

3.1. Analysis of scanned images

Spots of carbon labeled biotinylated BSA printed in PBS and carbonate buffer showed differences in the appearance and signal intensity (Fig. 1). In PBS, spots of biotinylated BSA showed a tail-like appearance, commonly known as the spot-smearing effect [22], whereas in CB (pH 9.6) such tails were absent. The overall signal intensity of BSA–biotin in PBS was low as compared to those observed in carbonate buffer. These differences in spot appearance and intensity maybe due to weak binding of the BSA–biotin molecules onto the PS surface so that they were partly removed during the rinsing step or to a suboptimal conformation of the protein with respect to the binding of biotin by neutravidin in the subsequent step.

3.2. Reflectometry

To investigate the different spot formation of BSA–biotin in PBS and CB in more detail, reflectometry experiments were performed to study the adsorption of biotinylated BSA (2 μg/mL) onto PS in PBS (pH 7.4) and CB (pH 9.6). Experimental data showed that the adsorption of BSA–biotin in PBS (pH 7.4) reached 1.3 mg/m^2, which was much higher than that in CB (pH 9.6), 0.08 mg/m^2 (Fig. 2). A possible explanation for this observation could be the high saline content (137 mM NaCl) of PBS that may result in higher adsorption of BSA–biotin in this buffer. Therefore, the influence of NaCl was studied by comparing the adsorption in PBS with the adsorption in PB (PBS without NaCl; see Fig. 2). The results clearly demonstrate that in the absence of NaCl the adsorption of BSA–biotin was less. Still, the adsorption saturation in PB was much higher than in CB. The above results show that buffer pH along with the presence of saline plays an important role in the adsorption of protein onto the PS surface. The initial increase in the signal reflects the affinity of the protein for the substrate. Although the PS surfaces in the reflectometry experiment and the printing experiments are not identical, the results suggest that the signal intensity of the BSA–biotin in the printed spots (see Fig. 2) is not directly related to the adsorbed amount (see Fig. 2). The strong adsorption of these biotinylated BSA in PBS may interfere with the binding of streptavidin in the subsequent step due to steric hindrance.

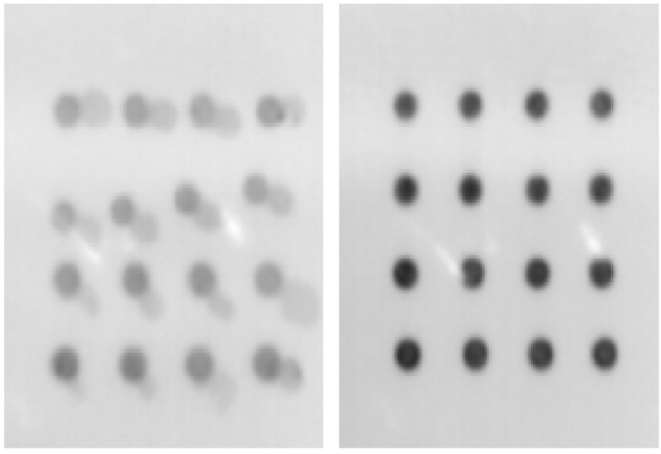

Fig. 1. Scanned image of HTA polystyrene slide: (*left*) BSA–biotin spotted in PBS (pH 7.4) and (*right*) BSA–biotin spotted in carbonate buffer (pH 9.6).

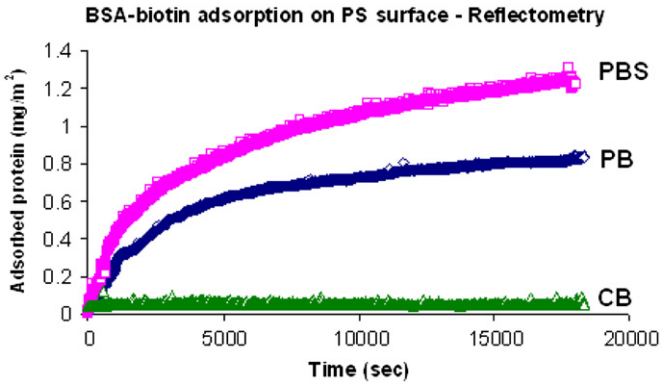

Fig. 2. Reflectometry data showing adsorbed amount of BSA–biotin in PBS (pH 7.4), PB (pH 7.4) and CB (pH 9.6) on polystyrene surface.

3.3. AFM analysis

To further study the discrepancy between signal intensity and the adsorbed amount of protein, spots of biotinylated BSA printed onto HTA-PS surfaces in PBS or CB were analyzed by AFM in tapping mode (in air). AFM images showed the differences in the spot morphology of BSA–biotin printed in PBS and CB (Fig. 3A). In carbonate buffer the dried spot had an even distribution with a thickness of 120 nm. In PBS, the distribution of biotinylated BSA showed an irregular pattern with clearly visible high crust-like regions. (Fig. 3A, 3-D image). The height of the BSA–biotin spot in PBS was 530 nm, which is 4.4 times higher than the observed thickness for the same protein in CB. It was hypothesized that the appearance of the high crust-like regions on the spot could be due to the presence of the high NaCl content in PBS buffer (137 mM NaCl). The salt could cause the biomolecules to aggregate [23] and could further interfere with an even distribution of the biomolecules on the surface of HTA-PS slide. The influence of the saline content in PBS was investigated by printed spots of BSA–biotin in PB (PBS without NaCl) on the same PS surface. The thickness of the dried spot in PB was 110 nm, which is almost 5 times less than in PBS. Spots of BSA–biotin in PB showed a different distribution pattern as compared to BSA–biotin in PBS. Both PB and CB do not have a high saline content and the spots of biotinylated BSA had similar dimensions with respect to the diameter and thickness for these two buffers, although a more even distribution was observed across the spot printed in CB.

AFM IMAGE TYPE	(A) BSA-BIOTIN			(B) IgG-BIOTIN		
	PBS (pH 7.4)	CB (pH 9.6)	PB (pH 7.4)	PBS (pH 7.4)	CB (pH 9.6)	PB (pH 7.4)
Height image						
3D image						
Height	530 nm	120 nm	110 nm	400 nm	100 nm	146 nm
Diameter	90 μm	100 μm	100 μm	100 μm	100 μm	120 μm

Fig. 3. AFM images for (A) BSA–biotin and (B) IgG–biotin in PB, PBS and CB printed on HTA polystyrene surface.

Interestingly, the buffer pH and salt composition hardly had any influence on the spreading of the drop on the PS surface. As shown in the figure, the diameter of the spot did not vary much. For PBS, CB and PB the diameter of the spot was 90 μm, 100 μm and 100 μm, respectively.

Antibodies are often used as ligands in immunoassays. Therefore, we also studied spots of a polyclonal goat IgG preparation by AFM. The printing conditions, the printing buffers and the substrate were the same as to those used for BSA–biotin. AFM images of the IgG–biotin spots also showed differences in the morphology and the thickness when printed in PB, PBS and CB. Here, also the pH and buffer composition did not influence the spreading of the drop as the diameter of IgG–biotin in PB, PBS and CB was between 100 μm and 120 μm (Fig. 3B). The thickness of the IgG–biotin spot in PBS was higher (400 nm) than in PB (146 nm) and CB (100 nm). Again, in PBS the appearance of the spot was rough with a crackled appearance, whereas in CB and PB the dried spot showed a much smoother and more uniform distribution on the surface of the PS slide.

3.4. Influence of rinsing on spots of BSA–biotin and IgG–biotin

The height data obtained from AFM analyses suggested that the spots of biotinylated biomolecules printed on the PS surface were not made up of just protein, especially if printed in PBS (see Fig. 3A). It was shown that the omission of salt substantially reduced the spot height, as shown in the spot printed in PB. Therefore, the structures appearing upon drying the spot most probably consisted of both protein and salt. We studied whether these structures were tightly or loosely bound by rinsing the HTA slide with Milli-Q water, followed by air-drying. Subsequently, the spots were again analyzed by AFM to observe any change in the thickness.

After rinsing the spots of biotinylated biomolecules, the height appeared to be reduced by more than 85%. The change in the thickness of the spots before and after rinsing for biotinylated proteins in PB, PBS and CB is shown in Table 1.The dimensions for BSA [24], IgG [25] and biotin [26] are shown in *ESM* 3.

The calculated thickness of the protein (*ESM* 3) and salt components present in 300 pL PBS printed on an area of $\sim 0.8 \times 10^{-8}$ m^2 would be approximately 206 nm after drying. However, as can be seen in Fig. 3A (BSA–biotin in PBS) 40% of the spot area was occupied by non-proteinaceous components whereas the remaining 60% was still empty (Evaluated by ImageJ software and MatLab). It implies that the average height of the salt deposits on 40% of the spot area amounts to 515 nm which is close to our experimental value of 530 nm.

Based on the concentration of protein (200 μg/mL) in the PBS, the volume of the drop to be spotted (300 pL) and the area of the spot after drying ($\sim 0.8 \times 10^{-8}$ m^2) the surface concentration of the protein deposited on the spot was calculated to be 7.5 mg/m^2. Assuming a packing density of 0.7 in the dried protein layer and taking a protein density of 1.37 g/cm^3, the thickness of a spot consisting of protein only would amount to 9.2 nm, assuming a protein spot coverage of only 60%. (The dimensions of various biomolecules and various components of the printing buffers are explained in the supplementary information, *ESM* 3–4).

Even after allowing for a large uncertainty in the assumptions underlying the calculation of the thickness of a spot solely consisting of protein and for a considerable experimental error in determining the thickness by AFM, it is obvious that the thicknesses of BSA–biotin and IgG–biotin spots before rinsing contain a large fraction of non-proteinaceous components. These components cannot be other than the salts from the buffer; indeed, the largest thickness before rinsing is observed for the spots in the buffer of high salinity, i.e. PBS. The influence of high saline content on the spot morphology has also been observed by Ressine et al., where they showed a salt crystallization effect [22] on the spot of the biomolecule when printed in PBS buffer. After rinsing the thickness of the spots were different but in the same range as the one calculated for pure protein layers on 60% of the spot area i.e. 9.2 nm. Moreover, these values are comparable with the dimensions of the protein molecules (see Table 1). These considerations strongly suggest that upon rinsing most of the salt is removed from the spots, leaving behind spots of which 60% of the spot area is covered by a (mono)layer mainly made up of protein.

Data in Table 1 shows the height of the dried spot (before rinsing) for BSA–biotin and IgG–biotin in three different buffers. The non-proteinaceous components present in the buffer along with the protein form structures with a final height of 400–500 nm (in PBS) and 100–150 nm in PB and CB. These structures are loosely bound onto the surface and tend to be washed away during the intermediate rinsing step, thus leaving behind a thin layer of protein on the PS surface. The average thickness of that layer was less than 20 nm which is compatible with a monolayer of albumin and immunoglobulin on the PS surface [27].

Based on the above observations, it is clear that the choice of printing buffer is very crucial for printing biomolecules onto a non-porous substrate like PS. The buffer composition (see Supplementary information) and pH play a vital role in packing of the biomolecules within the drying/dry spot. Use of a common buffer like PBS that has a high saline content (137 mM) results in high crust-like structures following drying of the printed droplet (Fig. 3) as compared to the same protein printed in the same buffer but without saline.

3.5. CLSM analysis

Following Alexa-633–streptavidin labeling of the immobilized biotinylated biomolecules the spots were scanned with a 40× objective at 633 nm wavelength. The PMT settings were kept the same for all the measurements. For the BSA–biotin spot in carbonate buffer, the fluorescence distribution was uniform throughout the spot as compared to BSA–biotin spots in PB and PBS, where the spots had an uneven distribution pattern (Fig. 4). CLSM images of BSA–biotin in PBS showed empty regions where no fluorescence was observed. Comparison with AFM data (Fig. 3A) showed the influence of the high saline content on the distribution of the BSA–biotin on the dried spot. The CLSM image for BSA–biotin in PBS was exactly the mirror image of the AFM data (Compare with Fig. 3A, BSA–biotin in PBS), which could be explained as follows: when a drop of BSA–biotin in PBS comes in contact with the PS surface, the surface area of the spot is not completely occupied with the BSA–biotin molecules, instead some portion of the surface is covered with buffer-salt moieties, especially NaCl which is in excess. Drying of the spot leads to the formation of a multilayer structure mainly composed of non-proteinaceous components which tend to be washed off during rinsing, leaving behind an empty space in the spot. Since Alexa–streptavidin molecules couple only to the biotinylated biomolecules,

Table 1
Change in the thickness of the spot of BSA–biotin and IgG–biotin in different buffers (before and after rinsing), based on AFM analyses (height data).

Buffer	Before rinse (nm)		After rinse (nm)		Loss in thickness (%)	
	BSA–bt	IgG–bt	BSA–bt	IgG–bt	BSA–bt	IgG–bt
PB	110	146	10	13	91	91
PBS	530	400	11	16	99	96
CB	120	100	18	13	85	87

the empty regions present within the spot of BSA–biotin in PBS showed no fluorescence.

BSA–biotin in PB showed a doughnut appearance with a strong fluorescence at the edges and a weak signal at the interior of the spot, whereas in CB the distribution pattern was more regular with an uniform fluorescence intensity throughout the spot (Fig. 4). IgG–biotin in all the three buffer systems showed less variation as compared to BSA–biotin. However, in carbonate buffer the distribution pattern was again more uniform than observed for the same biomolecule in PB and PBS (Fig. 4). The observations based on CLSM data reveal the distribution of biotinylated biomolecules on the PS surface and illustrated the influence of a high salt content on the final outcome. IgG–biotin printed in PB and PBS had a better distribution pattern throughout the spot as compared to BSA–biotin when printed in the same buffers.

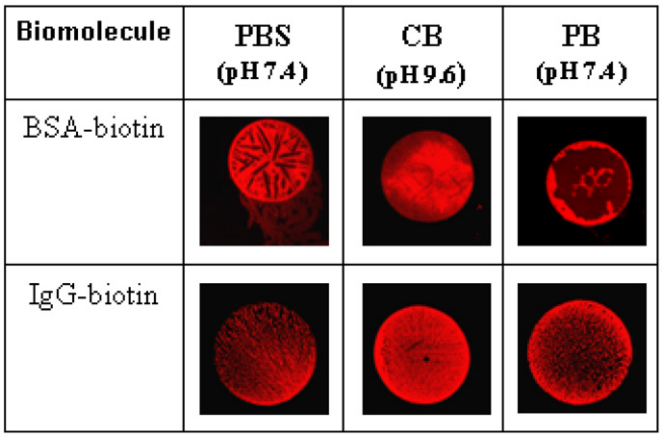

Fig. 4. CLSM images for (*upper lane*) BSA–biotin and (*lower lane*) IgG–biotin in PB, PBS and CB printed on HTA polystyrene surface.

3.6. *Influence of printing buffer on the assay performance on HTA-PS slide*

In order to analyze the influence of buffer on a real antibody assay, a one-step NAMIA [19] (nucleic acid microarray immunoassay) was performed on the HTA-PS slide (Fig. 5A). The layout of the microarray is shown in Fig. 5B. The antibodies specific for different tags (anti-DIG, anti- FITC, anti-DNP and anti-TR) were printed along with BSA–biotin (positive control) in PBS (pH 7.4) and CB (pH 9.6). A double labeled PCR amplicon (*C. bovis*) with FAM (Fluorescein amidite) at one end and biotin at the other end was incubated with a carbon-alkaline phosphatase–neutravidin conjugate. A similar incubation with another PCR amplicon for *S. aureus*, containing a DIG (digoxygonin) tag, was also used in a similar manner. The double-labeled amplicon was sandwiched in a one-step incubation for 1 h between these carbon nanoparticles conjugates and the immobilized antibodies resulting in black spots. On HTA-PS and when printed with CB (pH 9.6), the NAMIA spots had a more regular and defined shape without any spot-smearing effect (Fig. 5C, D). In PBS (pH 7.4), however, the NAMIA spots as well as the control spots showed a clear spot-smearing effect (tailing appearance), thus reducing the overall quality of the biochip.

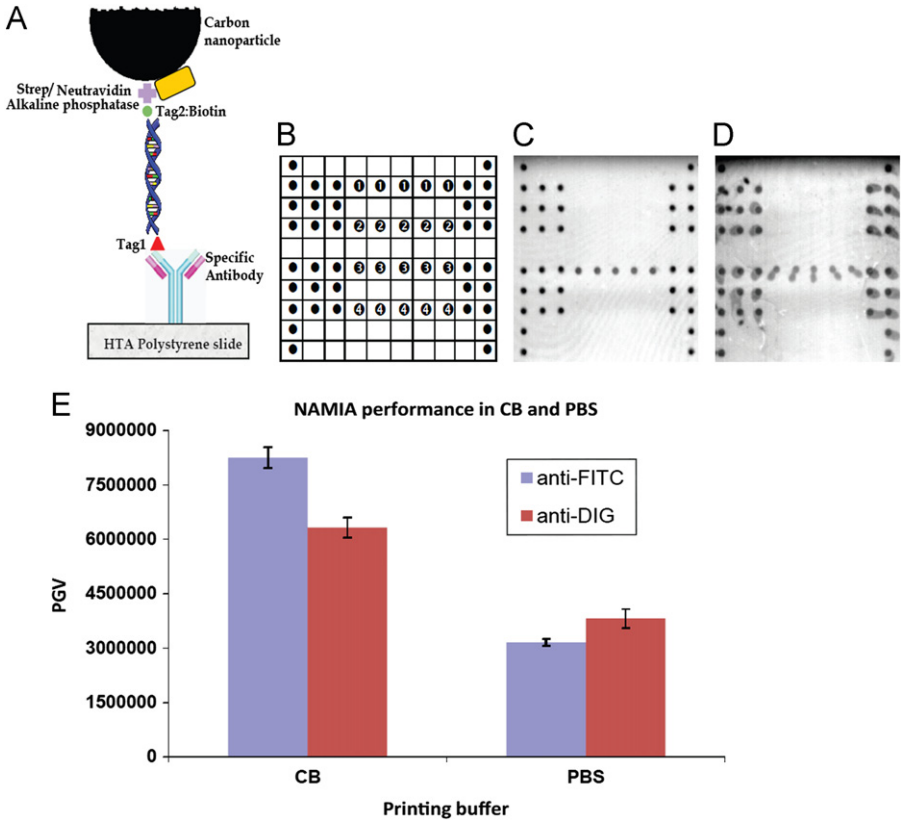

Fig. 5. (A) Schematic representation for the detection principle of NAMIA. (B) Layout of the NAMIA on HTA-PS slide: 1=anti-digoxigenin (*S. aureus*), 2=anti-DNP (*Streptococcus* spp.), 3=anti-FITC (*C. bovis*), 4=anti-TexasRed (*M. bovis*) 5=BSA–biotin control. (C) Scanned iamge of HTA slide showing a antibody assay (anti-FITC) performance in CB (pH 9.6) and (D) in PBS (pH 7.4). (E) NAMIA results for anti-FITC (against *C.bovis*) and anti-DIG (against *S.aureus*) when printed on HTA-PS slide in two different buffers.

The average pixel gray volume (PGV) was calculated for five NAMIA spots. It was found that when anti-FITC and anti-DIG were printed with CB (pH 9.6), the PGV (assay signal) was higher as compared to the same biomolecule when printed with PBS (pH 7.4). The PGV of the NAMIA spots for anti-FITC in PBS (pH 7.4) was ~38% less as compared to CB (pH 9.6; Fig. 5E). On the other hand the PGV of the NAMIA for anti-DIG in PBS was ~60% lower as compared to CB. As explained in Section 3.4, in the presence of PBS the spot coverage of the biomolecule was ~40%, leaving 60% of the spot area unoccupied. Therefore, the reduced spot coverage in PBS at least partly explains the reduced signals upon printing with PBS (pH 7.4) as compared to CB (pH 9.6).

4. Conclusion

In this study we have demonstrated the influence of printing buffers on distribution and spot morphology of the different biomolecules using a hydrophobic polystyrene slide as a substrate. Based on our experimental data we recommend that, upon printing onto a hydrophobic substrate, the printing buffer should be CB (pH 9.6). This will result in even spot morphology with a superior signal to noise ratio as compared to that of PBS, where our observations showed that the distribution of biomolecules and spot morphology were highly irregular. Due to the presence of a high NaCl content in PBS buffer, the spots show spot-smearing [22] and salt crystallization which is not acceptable for diagnostic purposes, as non-homogeneous signal intensity reduces the (data) output quality. On these hydrophobic polystyrene substrates, we also demonstrated and compared the performance of NAMIA for different buffers. Higher signal intensity and superior spot quality were achieved when CB (pH 9.6) was used instead of PBS (pH 7.4) as a printing buffer.

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2012.06.006.

References

[1] P. Angenendt, Drug Discov. Today 10 (2005) 503–511.
[2] M. Cretich, F. Damin, G. Pirri, M. Chiari, Biomol. Eng. 23 (2006) 77–88.
[3] M. Dufva, Biomol. Eng. 22 (2005) 173–184.
[4] M. Moschallski, J. Baader, O. Prucker, J. Rühe, Anal. Chim. Acta 671 (2010) 92–98.
[5] M.D. Kurkuri, C. Driever, G. Johnson, G. McFarland, H. Thissen, N.H. Voelcker, Biomacromolecules 10 (2009) 1163–1172.
[6] W. Kusnezow, A. Jacob, A. Walijew, F. Diehl, J.D. Hoheisel, Proteomics 3 (2003) 254–264.
[7] G. MacBeath, S.L. Schreiber, Science 289 (2000) 1760–1763.
[8] B.B. Haab, Curr. Opin. Biotechnol. 17 (2006) 415–421.
[9] G. Klenkar, B. Liedberg, Anal. Bioanal. Chem. 391 (2008) 1679–1688.
[10] B. Lu, M.R. Smyth, R. O'Kennedy, Analyst 121 (1996) 29R–32R.
[11] Y. Liu, C.M. Li, W. Hu, Z. Lu, Talanta 77 (2009) 1165–1171.
[12] N. Nath, R. Hurst, B. Hook, P. Meisenheimer, K.Q. Zhao, N. Nassif, R.F. Bulleit, D.R. Storts, J. Proteome Res. 7 (2008) 4475–4482.
[13] E.W. Olle, J. Messamore, M.P. Deogracias, S.D. McClintock, T.D. Anderson, K.J. Johnson, Exp. Mol. Pathol. 79 (2005) 206–209.
[14] A. Wolter, R. Niessner, M. Seidel, Anal. Chem. 79 (2007) 4529–4537.
[15] H. Sølling, B. Dinesen, Clin. Chim. Acta 130 (1983) 71–83.
[16] K. Sollier, C.A. Mandon, K.A. Heyries, L.J. Blum, C.A. Marquette, Lab Chip 9 (2009) 3489–3494.
[17] M. Mrksich, Chem. Soc. Rev. 29 (2000) 267–273.
[18] Z. Yong, Colloids Surf. B 48 (2006) 95–100.
[19] P.S. Noguera, G.A. Posthuma-Trumpie, M. van Tuil, F.J. van der Wal, A.d. Boer, A.P.H.A. Moers, A. van Amerongen, Anal. Chem. 83 (2011) 8531–8536.
[20] J.C. Dijt, M.A. Cohen Stuart, G.J. Fleer, Adv. Colloid Interface Sci. 50 (1994) 79–101.
[21] J.H. Maas, M.A. Cohen Stuart, A.B. Sieval, H. Zuilhof, E.J.R. Sudhölter, Thin Solid Films 426 (2003) 135–139.
[22] A. Ressine, G.r. Marko-Varga, T. Laurell, M.R. El-Gewely, Porous Silicon Protein Microarray Technology and Ultra-/Superhydrophobic States for Improved Bioanalytical Readout, in: Biotechnology Annual Review, Elsevier, 2007, pp. 149–200.
[23] V.B. Galazka, D. Smith, D.A. Ledward, E. Dickinson, Food Chem. 64 (1999) 303–310.
[24] A.K. Wright, M.R. Thompson, Biophys. J. 15 (1975) 137–141.
[25] K.B. Lee, S.J. Park, C.A. Mirkin, J.C. Smith, M. Mrksich, Science 295 (2002) 1702–1705.
[26] P. Hu, T. Tanii, G.-J. Zhang, T. Hosaka, I. Ohdomari, Sens. Actuators. B: Chem. 124 (2007) 161–166.
[27] R.J. Green, J. Davies, M.C. Davies, C.J. Roberts, S.J.B. Tendler, Biomaterials 18 (1997) 405–413.