Fast and reliable protein microarray production by a new drop-in-drop technique

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In contrast to DNA microarrays, production of protein microarrays is an immense technological challenge due to high complexity and diversity of proteins. In this paper we investigate three essential aspects of protein microarray fabrication based on the highly parallel and non-contact TopSpot[1] technology: evaporation of probes during long lasting production times, optimization of protein immobilization and improvement of protein microarray reproducibility. Evaporation out of the printhead reservoirs was reduced to a minimum by sealing the reservoirs with gas permeable foils or PDMS frames. This led to dramatically lowered setup times through the possibility of long-term, ready-to-print storage of filled printheads. To optimize immobilization efficiency 128 printing buffers were tested by printing two different proteins onto seven different microarray slide types. This way we were able to reduce the CV of spot diameter on the microarray slide below 1.14%. To remarkably increase protein immobilization efficiency on microarray slides the commonly used EDC-NHS system (a laboratory method for immobilization of proteins) was miniaturized by using a new drop-in-drop printing technique. Additionally the very fast UV cross-linking was used to immobilize antibodies. The optimized system was used to produce antibody microarrays and with it microarray ELISA experiments were performed successfully.

Introduction

In the last few years microarray technology has become a powerful tool for highly parallel analysis of biological molecules. Miniaturization, automation and parallelization the decrease in costs of often expensive materials and lead to faster analyzing times.[2] Over the years DNA microarray technology especially has been established in many fields of applications.[3] But despite the success of DNA microarrays in gene expression profiling or mutation mapping, it is the activity of encoded proteins that directly manifests the gene function.[4] Protein microarrays have been applied in basic research, diagnostic as well as pharmaceutical research. Especially, antibody microarrays have the potential to revolutionize protein expression profiling.[5]

But as the biochemistry of proteins is orders of magnitude more complex than DNA biochemistry the production of protein microarray is much more difficult.[6] Simply using the most commonly used contact based pin printing technology to produce protein microarrays would led to problems arising from the varying adhesion forces between probes, needles and the substrate surface leading to inhomogeneous amounts of proteins on the microarray slide. Additionally the influence of mostly used metallic pins on protein structure remains unclear. So due to high complexity and diversity of proteins, production of protein microarrays is a big technological challenge and requires very flexible printer systems.

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Different microarray applications need different buffer systems, depending on used proteins, coupling chemistry of used microarray slides or surface properties. We expect that only non-contact technologies meet the requirements. But, like pin printers, most of the non-contact based technologies are assembled from single (dispensing) units. So they have limitations in speed and the risk of carry over of the arrayed spots. The TopSpot technology overcomes these problems and enables high throughput printing of protein microarrays.

Principle of operation

The TopSpot technology is based on a micromachined printhead; the fabrication of TopSpot printheads is described in ref.1 The printhead formats allow the simultaneous application of 24 and 96 different probes in one step, respectively. The TopSpot printhead consists of three layers, pyrex glass, silicon and another pyrex layer. The printing probes are contained in reservoirs drilled in the upper pyrex glass wafer and can be filled automatically by standard liquid handling robots. The glass wafer is bonded to the intermediary silicon wafer by anodic bonding. The reverse side of the silicon wafer is bonded to a thin pyrex glass wafer with a square opening for the outlet nozzles (Fig. 1a). A recess in the upper Pyrex (actuation chamber) opens the nozzles to the upper side of the printhead. Every reservoir is connected with one nozzle in the central area of the print head (nozzle array) *via* an etched microchannel system in the silicon wafer. Liquid is drawn to the nozzles simply by capillary forces. The actuation of the printhead

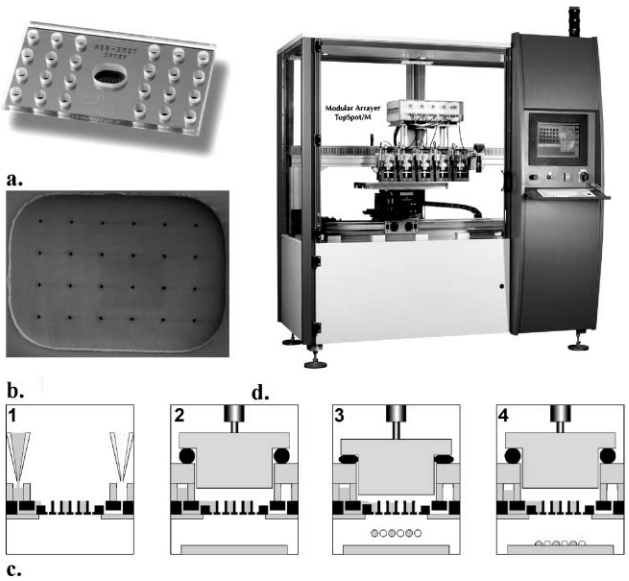

Fig. 1 (a) Picture of a printhead, (b) SEM picture of the 24 nozzles on the bottom side of the printhead, (c) working principle of TopSpot. 1, Filling of the printhead reservoirs. 2, Placing a piston into the printhead. 3, Actuation by moving the piston. 4, Retraction of the piston. (d) Picture of the used TopSpot® Modular Arrayer (TopSpot®/M). The instrument can handle up to 5 printheads simultaneously. A camera system and dedicated software make it possible to have an integrated quality assurance.

is done by a piezostack actuator and depicted in Fig. 1c. The actuator drives a piston into the actuation chamber of the micromachined printhead. This generates a pressure pulse that affects all nozzles simultaneously. If the pressure pulse amplitude is large enough, it overcomes the capillary forces of the channels and the surface tension of the fluids in the nozzles and droplets are accelerated out of them. To achieve a homogenous ejection of the droplets, the nozzle array is coated with a hydrophobic silane.[7]

One of the major advantages of the TopSpot technology over other nanoliter dispensing systems (*e.g.* inkjet printer) is that every nozzle can be supplied with another printing medium at the same time. So in contrast to the present procedures (*e.g.* pin printer, piezo tips) a contact-free high throughput production is possible.

In this work the TopSpot® Modular Arrayer'' (TopSpot®/M) was used for the production of microarrays.[8] The instrument is able to use up to 5 printheads simultaneously. The mobile axis system of TopSpot®/M can handle up to 40 standard substrates. An integrated camera system and dedicated software enables an integrated quality assurance (Fig. 1d.).

In earlier publications we discussed the use of the TopSpot technology for the printing of oligonucleotides.[1] This publication reports on three main aspects of protein microarray production: evaporation of probes out of the printhead reservoirs during sometimes long lasting production times, optimization of protein immobilization and improvement of the protein microarray reproducibility due to high spot homogeneity.

Chemicals and materials

a. Buffers

10 × PBS buffer [1.54 M phosphate buffered saline], Gibco BRL; 20 × SSC buffer [3.3 M sodium chloride sodium citrate], Gibco BRL; Sodium phosphate buffer [1 M = 141.96 g Na_2HPO_4, 137.99 g $NaH_2PO_4 \cdot H_2O$ in 2000 ml H_2O (deion.). Carbonate buffer [1 M: 10.6 g sodiumcarbonate, 8.4 g sodiumbicarbonate in 200 ml H_2O (deion.)]; Borate buffer [1 M: 12.37 g boric acid, 4 g NaOH in 200 ml H_2O (deion.)]; Boric acid, Sigma-Aldrich; Sodium carbonate, Sigma-Aldrich; Sodium bicarbonate, Sigma-Aldrich; DMSO [dimethylsulfoxide] [$(CH_3)_2SO$], Merck; Glycerol 99% [$C_3H_8O_8$], ACROS ORGANICS; Betaine monohydrate [$C_5H_{11}NO_2 \times H_2O$], Fluka; Washing solution: 4 × SSC/0.2% Tween20/0.1% SDS.

b. Surfactants

Nonidet P40 Substitute, ionic surfactant, Fluka; RBS N, non-ionic surfactant pH 7, Roth; SDS (sodium n-dodecyl sulfate), Roth; Tween20, Sigma-Aldrich.

c. Proteins and immobilization chemicals

BSA (Bovine Serum Albumin), fraction V, VWR; Donkey anti Rabbit IgG-Cy5 (H+L), Dianova; Donkey anti Mouse IgG-Cy5 (H+L), Dianova; Donkey anti Goat IgG-Cy5 (H+L), Dianova; Goat anti Human Protein C, American Diagnostics; Mouse anti BSA, Sigma Aldrich; Sheep anti Rabbit IgG-Cy3, Sigma Aldrich; Rabbit anti Sheep IgG, Sigma Aldrich; Ethyldimethyl-aminopropylcarbodiimide, EDC, Sigma Aldrich; N-hydroxy-succinimide, NHS, Sigma Aldrich.

d. Microarray slides

ArrayLink hyphob, epoxy-modified glass slides, Genescan Europe AG; CMT-GAPS2, amino-modified glass slides, Corning; QMT Epoxy, epoxy-modified glass slides, Quantifoil; PMMA, plastic slides, Genescan Europe AG; PamChip, porous microarray matrix, PamGene International B.V.; Nexterion Slide Aminosilane, amino-modified glass slides, Schott Nexterion AG; ez-rays aminosilane, amino-modified glass slides, Apogent Discoveries.

e. Sealing foils

PTFE membrane, 0.2 μm pores, GoreTex; PTFE membrane with PES supporting tissue, 0.2 μm pores, Schleicher & Schuell GmbH; Silicone membrane, 50 shore, Helmuth Socke GmbH; Silicone membrane, 60–70 shore, Helmuth Socke GmbH; Gas permeable adhesive seal, AB-0718 clear, microtiterplate sealing, Abgene House; PDMS, Sylgard 184, Dow Corning.

f. Devices and software

UV StrataLinker 2400, Stratagene, USA; Bioanalyzer 4F/4S Fluorescencereader, La Vision Biotech, Germany; Highspeed-Videostroboscope MOCON-RT, VISIT GmbH & Co. KG, Germany; NeuroCheck®, NeuroCheck, Germany.

Results and discussion

Three main aspects of protein microarray production were examined: optimization of protein immobilization, evaporation of probes during long lasting fabrication times and improvement of protein microarray reproducibility.

Carry-over test

In our publication we investigated printing with 24 channel printheads. Parameters for diameter and pitch of the nozzles were 50 μm and 500 μm. In this highly parallel system it is of prime interest to evaluate that no cross-talk between the 24 nozzles occurred. Since the printhead is re-used it is very important to evaluate the washing procedure of the printhead to guarantee no carry over of former fillings to the next usage of the printhead. This was already shown for oligonucleotides applications.[9] To develop and test the washing procedure for protein applications every second reservoir was filled with Cy5-labeled BSA or antibodies (each 200 μg ml^{-1}) and the other reservoirs with pure printing buffer. The array was printed onto microarray slides and detected *via* fluorescence reader (La Vision Biotech, Germany, sensitivity up to <0.8 Cy3 molecules μm^{-2}). Without cross-talk between the 24 nozzles of the printhead they should look like a checker board. Afterwards the checkerboard-like filled printhead was cleaned according to the following washing procedure: First a rinsing step with 5% v/v RBS N, followed by an ultrasonic bath, a second rinsing in deionized water and a second ultrasonic bath with deionized water. Then the checkerboard-like filling was inversed, so every nozzle, which was filled with Cy5-labeled BSA or antibodies (each 200 μg ml^{-1}) before is now filled with buffer solution. In the fluorescence reader, no remnants of former filling should be detectable, not even at very high exposure times.

Extensive printing of checkerboard like arrays showed on the one hand no cross talk of the different microchannels, nozzles and dispensed droplets even after thousands of printed arrays. On the other hand the re-usage of printheads showed that after washing and re-using of printheads no carry-over of formerly printed media was detectable (Fig. 2).

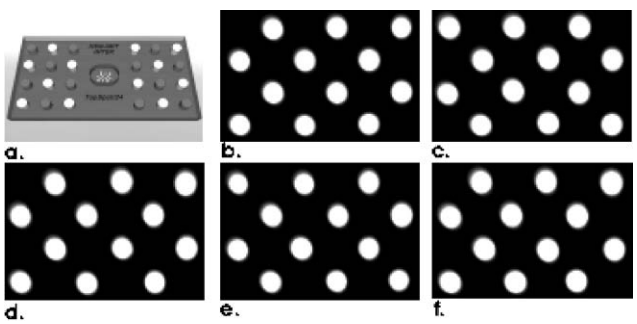

Fig. 2 Carry-over test: The printhead was filled with Cy5-labeled BSA and buffer like a checker board. a, The printed microarrays were evaluated in a fluorescence reader b. Afterwards the printheads were washed, refilled with the inversed checkerboard filling and printed. c, The whole procedure was repeated 5 times, b to f. In the quantification of the 4 × 6 arrays only the spots with Cy5-BSA are visible, that means both no cross-talk between nozzles and no carry-over occurs.

So the experiments ascertain carry-over and cross-talk free printing of protein microarrays. With it a prime critical point of microarray production is solved enabling high quality and high throughput protein microarray fabrication.

Avoiding evaporation of probes

Microarray production runs sometimes take a long time, depending on the amount of features on the microarray and batch size. Evaporation of probes out of the printhead reservoirs could lead to a gradient of concentration during the print run or in the worst case to blackout of printhead nozzles due to salt out effects. To achieve a highly reproducible microarray quality it is of prime interest to reduce this evaporation to a negligible minimum. To avoid evaporation out of the printhead reservoirs six reservoir sealings were tested to cover directly the printhead reservoirs during printing. Additionally a peltier cooling unit is implemented on the TopSpot printer device to cool the printhead near to the dew point during usage. Deionized water was filled into the printhead reservoirs and printed until one of the reservoirs was empty. The maximum number of prints was counted and compared to the theoretically possible number of prints without any evaporation.

Experiments showed that the cooling effect of the integrated peltier cooling unit is insufficient. Almost no effect of the cooling was detectable. Beside this, cooling generally increases the viscosity of printing solution. High viscosity is a critical parameter for dispensing fluids, so it is favorable not to increase the viscosity of printing solution by cooling down to low temperatures.

As an alternative solution six sealing foils were tested to cover directly the printhead reservoirs during printing. In the experiments evaporation was reduced to a minimum by sealing the reservoirs with commercially available gas permeable foils or selfcasted PDMS frames. With it, nearly the theoretical maximum number of dispensing cycles with a printhead filling was achieved (Fig. 3). So even long lasting microarray

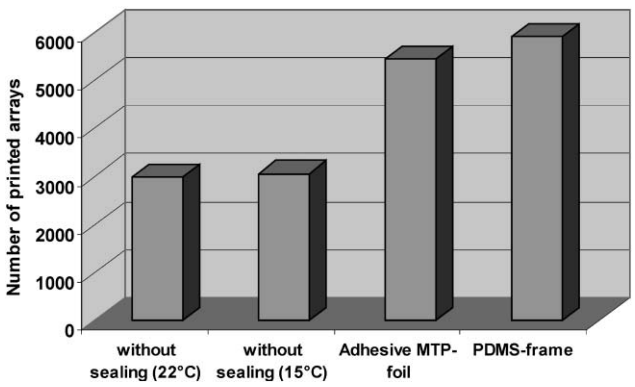

Fig. 3 Maximum achievable dispensing cycles with and without reservoir sealing during printing. Theoretically, with a droplet volume of 0.83 nl and a reservoir filling of 5 μl deionized water per reservoir 6024 dispensing cycles should be possible. Without sealing the cooling showed almost no effect (both approx. 3000 prints). Using the adhesive MTP-foil (5437 prints) and the PDMS frame (5913 prints) increased the number of maximum prints close to the theoretical value; proof of low evaporation out of the reservoirs.

production runs result in high reproducible microarray quality with no gradient of concentration of probe molecules and blackout of printhead nozzles due to salt out effects.

Storage of filled printheads

A dew-point controlled stage was developed as external cooling station for storage of filled printheads. The filled printhead was stored on the stage 0.3 °C from the dew-point and was re-used in several printing runs during one week. In the fluorescence reader the change of Cy3 signal of printed spots was measured. The small changes in the measured Cy3 signal of printed spots showed the very low evaporation out of the printhead reservoirs. The printheads remained ready-to-print. So setup times for protein microarray production could be lowered dramatically and with it reagent consumption of expensive protein solutions is reduced.

Reproducibility of protein dispensing

During printing protein microarrays the reproducibility of the spot diameter and horizontal deflection of dispensed spots was evaluated. Therefore a stroboscopic camera was used to evaluate droplet tear off, flight and impact on microarray slides of different printing media in a time resolved measurement. Due to the arrangement of the nozzles on the printhead in several lines it is only possible to evaluate 6 but not all 24 nozzles at the same time by this stroboscopic method. Therefore we used a fixed camera system, mounted on the TopSpot printer device. Each printed array on a slide was photographed automatically right after the print. By geometric outline analysis (NeuroCheck®) the spot diameter and horizontal deflection of dispensed spots from their correct 500 μm spacing was determined.

With it we were able to improve the reproducibility of droplet dispensing depending on used printing buffer (Table 1). The optimized printing buffers (highest protein immobilization) showed CVs of spot diameter below 1.14% on Corning GAPS2, the slides with highest spot reproducibility. The evaluation of spot deflection showed an average deflection lower than ±15 μm from their correct 500 μm spacing. This represents less than 7.5% of the spot diameter and represents no problem for the spot finding of fluorescence scanners.

Both the high spot position and spot diameter reproducibility of the optimized system enabled an increase of spot density by a factor of 4 by printing between spots of already printed arrays at 500 μm pitch, resulting in a 250 μm spacing of spots (Fig. 4). An online quality control of the microarray printing run is possible based on the mounted camera system.

Table 1 Scheme of printing buffer optimisation: 128 different protein printing buffers in different concentrations and pH values were used to solve two different proteins, which were printed onto seven microarray slide types. After immobilization a fluorescence reader was used to evaluate the efficacy of immobilization

Printing buffer (each in 0.5, 0.4, 0.3, 0.2, 0.1, 0.05 M)	pH range			
Sodium phosphate	6.0	6.8	7.4	8.0
PBS	6.0	6.8	7.4	7.8
SSC	6.0	6.5	7.0	7.6
Borate (not in 0.5 M, 0.4 M)	7.7	8.0	8.5	9.0
Borate (not in 0.5 M, 0.4 M)	9.2	9.6	10.0	10.4
Sodium carbonate	9.2	9.6	10.0	10.4

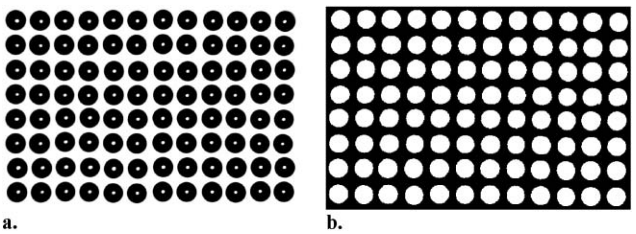

Fig. 4 Higher spot densities were achieved by printing between spots of already printed arrays (500 μm pitch), resulting in a 250 μm spacing of spots. a. Image of spots using the mounted camera system (online quality control). The light dots in the middle of the spots are from reflection of light of the wet droplets. b. Fluorescence reader image of spots after immobilization.

Protein immobilization

Protein microarray usage depends on good and reproducible immobilization of proteins to the slide surface. To optimize protein immobilization we investigated over 128 different protein printing buffers by printing two different proteins (BSA-Cy5 and Donkey anti Goat IgG-Cy5 antibody) onto seven microarray slide types (Table 2). The proteins were immobilized to the slide surface over 2 h in a humid box at 37 °C. Afterwards unbound material was removed by washing the slides 3 times for 10 min in washing solution, rinsed quickly in deionized water and dried with nitrogen. For the evaluation of protein immobilization the fluorescence intensity was measured in the fluorescence reader. Both protocols were used as standard protocols.

The results showed that immobilization is highly dependent on the interaction of used slide surface modification and appropriate printing buffer. The results of the printing buffer optimization are summarized in Table 2. Best immobilization results were obtained on ArrayLink hyphob (Genescan), CMT-GAPS2 (Corning) and QMT Epoxy (Quantifoil) microarray slides, independent of protein used.

Table 2 Summary of the printing buffer optimisation. Best immobilization was achieved at low salt concentrations and higher pH values, independent of buffer used. Only very small variations were observed between the different printed proteins. The buffers showed CV of the spot diameter on Corning slides under 1.14%. Comparing the buffers, absolute best results were achieved with sodium phosphate and sodium carbonate buffer, independent of used microarray slide

Printing buffer	Highest immobilization of DaM IgG-Cy5	Highest immobilization of BSA-Cy5	CV of spot diameter on Corning slide
PBS	0.05 M pH 7.8	0.05 M pH 7.4	0.53
Sodium phosphate	0.1 M pH 8.0	0.1 M pH 8.0	0.51
Borate	0.1 M pH 9.6	0.05 M pH 7.7	0.47
SSC	0.05 M pH 7.6	0.1 M pH 7.0	1.14
Sodium carbonate	0.1 M pH 9.6	0.1 M pH 9.6	0.49

Increasing protein immobilization by a new drop-in-drop printing technique

To increase protein immobilization on microarray slides a new drop-in-drop printing technique was established to use the well known EDC-NHS affinity ligand coupling chemistry.[10] NHS-esters react with amines to form amide bonds. EDC is a zero-length cross-linker and effects direct coupling between carboxylates (–COOH) and primary amines ($–NH_2$). The problem of using the EDC-NHS system for microarray printing is that the reaction starts immediately after the two partners are mixed. So if the printhead reservoirs are filled with a mixture of both reaction partners and protein the immobilization reaction starts already in the printhead. In the following printing sequence this led to protein arrays on the first printed slides which are more weakly immobilized than arrays on the last slides. Another problem is the risk of protein clogging, leading to blackouts of printhead nozzles.

To circumvent these problems the drop-in-drop printing technique was implemented. Two printheads were used in parallel. The first printhead was filled with 200 μg ml^{-1} Donkey anti Mouse IgG-Cy5 with 10 vol% EDC in the optimized printing buffer. The second printhead was filled with optimized printing buffer and 10 vol% NHS. This printhead was used to print immediately into the humid spots of the formerly printed array of the first printhead. With it the EDC–NHS reaction starts for every array at a defined starting point independently of position in the printing sequence.

Essential for the drop-in-drop technique is to understand what happens if a droplet hits a printed spot on the slide. The experiments with a stroboscopic camera showed that neither the printed spot nor the flying droplet burst when hitting one another. Also it could be shown that it is possible to hit precisely a formerly printed spot. Compared to the ''single spot'' the diameter of the ''double spot'' increased about 20% depending on the microarray slide properties used, ranging from hydrophobic to hydrophilic (Fig. 5a and b). But very important for the technique was the fact that it was highly reproducible.

While using the EDC-NHS system for protein immobilization we were able to increase the fluorescence signal depending on microarray slide used. Maximum increase was detectable on the PMMA slides (30.5×) followed by CMT GAPS2 (6.6×) and ArrayLink hyphob (2.4×) compared to the standard immobilization protocol (Fig. 5c). On the other hand the immobilization time could be reduced to 30 min (instead of 2 h) without less immobilization. The good results on PMMA are affected by two facts: Very low immobilization while using the standard protocol and the fact that PMMA offers carboxyl groups on the surface, which are used by EDC and NHS to form an aminoreactive NHS-ester.

The system showed successfully its potential to increase the immobilization. The drop-in-drop technique is ideal for the EDC-NHS system, but also applicable with other two component coupling systems. Above all it enables a defined starting point of the coupling reaction independently of position in printing sequence and therefore it circumvents the risk of protein clogging and leads to no blackouts of printhead nozzles.

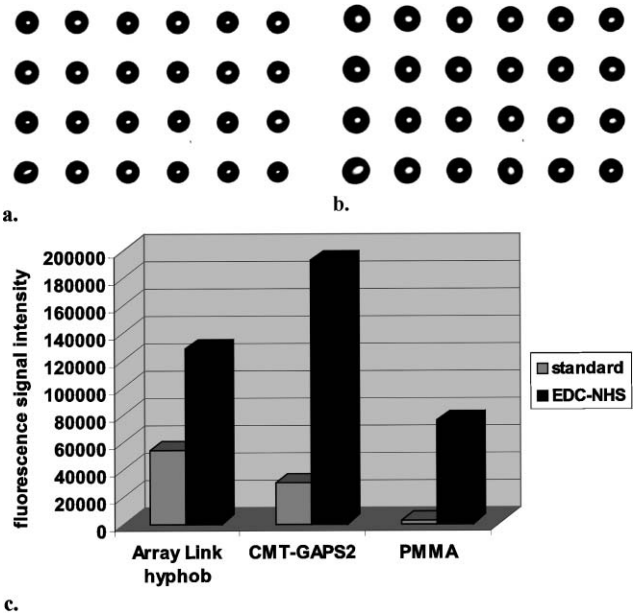

Fig. 5 Drop-in-drop printing. a, First array of a printhead: Donkey anti mouse IgG 200 μg ml^{-1} in 0.1 M sodium phosphate pH 8 with 10 vol% EDC on ArrayLink hyphob. b, Second array printed onto the provided array, one second later, with another printhead: 10 vol% NHS in printing buffer. In evidence the precise hitting of both arrays resulting in increased spot diameters. c, The standard immobilization (printed on doubled spot size) compared to the drop-in-drop EDC-NHS immobilization. The fluorescence signal of printed 200 μg ml^{-1} Donkey anti mouse IgG-Cy5 was increased up to 30 fold.

An inherent problem of all non-oriented protein immobilization, like for example with all chemical surface modification of common microarray slides as well as the EDC-NHS method, is the potential of inadvertent masking of epitopes of antibodies, active sites of enzymes or binding sites of receptors. So a validation of the performance of each immobilized antibody has to be performed. This is required for all chemical immobilization strategies (common microarray slides as well as EDC-NHS method). We have performed a complete microarray-ELISA to evaluate the amount of immobilization and accessibility of epitope (*see following section*). Another strategy would be the specific orientation of capture agents that their binding sites are oriented toward the solution phase as shown by Peluso *et al.*[5] A disadvantage of this techniques is the need of antibodies that are site-specifically modified on the carbohydrate domain of the Fc region of the antibody.

Fast protein immobilization by UV cross-linking

UV cross-linking is a very fast way to immobilize DNA molecules to microarray slides. We used UV cross-linking to immobilize antibodies on ArrayLink hyphob slides. But it is very important that the UV radiation does not destroy the epitope accessibility of the immobilized proteins.

An array of Goat anti human antibodies was printed and immobilized by UV energy. A different amount of UV energy was used after printing, before printing and both before and

after printing the arrays on the slide. As a control the standard protocol was performed. Afterwards the amount of immobilization and accessibility of epitope was evaluated by performing a microarray-ELISA.

In the experiments the concerns about destroying epitope did not come true. In contrast, the results showed almost doubled signal intensity of arrays on the UV cross-linked microarrays compared to the standard protocol (Fig. 6). But very importantly the UV cross-linking takes only about 2 min in contrast to 120 min immobilization time of the standard protocol. Particularly interesting are the high immobilization results of slides which were only pre-treated with UV radiation (before printing the array). We suppose the formation of ozone on the slide, which is very reactive and therefore enhancing the immobilization of antibodies. In consequence it could be shown that very fast UV cross-linking is applicable, at least for antibody microarrays.

Microarray ELISA

Finally, the previously optimized printing buffer system (0.1 M sodium phosphate pH 8.0) was used to produce and perform a microarray ELISA and cross-check the data of the optimization experiments in an applied approach. A dilution series of BSA (100–12.5 μg ml^{-1}) was printed and immobilized on four different slides. As a negative control Donkey anti goat and Goat anti human Protein C antibodies (100 μg ml^{-1}) were used. For the printing the previously optimized printing buffer system was used. The arrays were immobilized and washed according to the standard protocol. Then the arrays were incubated with Mouse anti BSA (7.5 μg ml^{-1}) for 1 h at room temperature in a humid box. Afterwards the slides were washed once again according to the standard protocol. For detection the array was incubated with Donkey anti Mouse IgG-Cy5 (7 5 μg ml^{-1}) for 1 h at room temperature in a light-protected humid box and washed with the standard protocol.

The result of the sandwich ELISA on different microarray slide types showed clearly a printed dilution series of BSA (Fig. 7), whereas the negative controls Goat anti human Protein C and Donkey anti Goat IgG spots showed only very low signal. With the experiment the application of the TopSpot printing system for functional protein microarray production is shown.

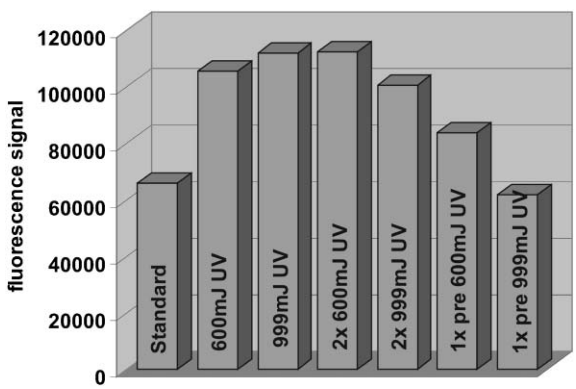

Fig. 6 Arrays of Goat anti human antibodies were printed on ArrayLink hyphob slides. A different amount of UV energy was used for immobilization: after printing (600 mJ, 900 mJ), before and after printing (2 × 600 mJ, 2 × 999 mJ) and only before printing the arrays (1 × pre 600 mJ, 1 × pre 999 mJ). Immobilization and accessibility of epitope was evaluated by a microarray-ELISA. Almost doubled signal intensity of UV cross-linked microarrays was observed compared to the standard protocol, but requiring only 2 min immobilization time. UV pre-treatment of slides resulted also in increased immobilization, requiring no post-treatment. No antibody epitope damage was detectable.

Conclusion

We have optimized the highly parallel TopSpot printing system for protein microarray production using 24 channel printheads with a nozzle spacing of 500 μm. The experiments ascertained carry-over and cross-talk free printing of protein microarrays. While using gas permeable foils or PDMS frames around the printhead reservoirs we reduced evaporation out of the printhead to a minimum during microarray printing. The printing buffer system was optimized biochemically and microfluidically, leading to CV of spot diameter on the microarray slide below 1% and therefore to highly reproducible protein microarray production. An integrated camera system was used for online quality control of the printing run. Two improved immobilization strategies were tested successfully. The drop-in-drop printing technique increased the signal up to 30 fold compared to the standard protocol. The very fast UV cross-linking was used to immobilize antibodies without a detectable effect on the performed microarray ELISA.

Outlook

A new printhead washing station will be evaluated in further experiments. In contrast to the single washing station washing

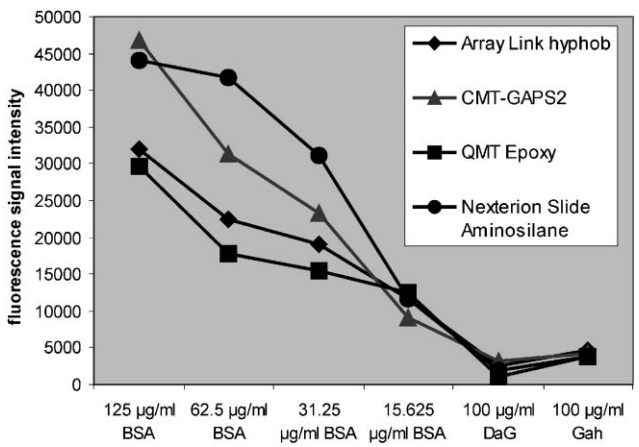

Fig. 7 Microarray ELISA with a TopSpot printed antibody microarray: The arrays were incubated with Mouse anti BSA (7.5 μg ml^{-1}). For detection the array was incubated with Donkey anti Mouse IgG-Cy5 (7.5 μg ml^{-1}). The evaluation of fluorescence image showed clearly the printed dilution series of BSA, whereas the negative controls Goat anti human Protein C and Donkey anti Goat IgG spots showed only very low signal.

of multiple printheads in one step will be possible. The promising results of the drop-in-drop technique will be tested with other two component coupling systems. Also the influence of UV cross-linking on other proteins should be studied.

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