Using the BioOdyssey™ Calligrapher™ MiniArrayer to Form Immobilized Protein Microarrays on Surface-Modified Glass Substrates

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Introduction

The development of adequate material platforms for high-throughput assays has become an important issue in the field of biotechnology, because such platforms can accelerate genomic, proteomic, and disease-diagnosis studies (Drickamer and Taylor 2002, Huang 2001). Furthermore, these platforms have significant potential to contribute to the advancement of drug discovery, tissue engineering, and stem cell technologies. Substrate materials that are commonly used in this area of research are glass, polystyrene, stainless steel, polypropylene, and gold. In order to incorporate suitable surface chemistries onto these substrates, various coating processes have been adopted, including dip or spin coating, surface grafting, plasma polymerization, chemical vapor deposition, self-assembled monolayers, and layer-by-layer deposition methods. Such functional substrate surfaces then allow the immobilization and study of specific individual biological factors — particularly those factors that can control the behavior of mammalian cells — in a microarray format (Anderson et al. 2004). The immobilization of combinations of such factors (i.e., cocktails) is also possible and enables true combinatorial studies.

We have designed cell microarray substrate coatings that provide a low cell-attachment background, functional groups for the covalent immobilization of biologically active signals, and excellent adhesion to the microarray substrate material (Kurkuri et al. in press, Thissen et al. in press). We used coatings made of random copolymers of glycidyl methacrylate (GMA) and polyethylene glycol methacrylate (PEGMA), which were deposited onto glass slides that had been previously coated with an allylamine plasma polymer (ALAPP) as a pinning layer. In the copolymer we used, GMA provided reactive oxirane groups for both biomolecule immobilization and attachment to the underlying ALAPP layer, while the other component, PEGMA, was thought to provide a low cell-attachment background. Different methods of attaching the copolymer to the underlying ALAPP layer (dip coating, spin coating, and grafting) were compared using surface analytical techniques.

In this study, a model protein (fluorescein isothiocyanate-labeled bovine serum albumin, BSA-FITC) was printed onto the copolymer coating using a BioOdyssey Calligrapher miniarrayer, and the conditions for protein printing — concentration, pH, temperature, and relative humidity (RH) — were optimized.

Methods

Surface Modification of the Glass Substrate

Microscope glass slides were cleaned by sonication in surfactant solution followed by soaking in NaOH solution and washing in ultrapure water. ALAPP coatings were deposited onto glass slides using a custom reactor. Copolymer of PEGMA/GMA prepared with different molar ratios was deposited onto glass slides using thermal initiator 2,2'-azobisisobutyronitrile (AIBN) by spin coating, dip coating, and in situ grafting. Bis (2-aminoethyl) polyethylene glycol 3400 was used as a crosslinker in the case of spin coating.

Microarray Printing

A BioOdyssey Calligrapher miniarrayer with temperature and humidity control was used for contact printing of BSA-FITC. An Arraylt round solid pin with a tip diameter of 375 µm (TeleChem International) was used for printing. The following printing conditions were used: Pin approach speeds to the source plate and glass slide were 15 and 5 mm/sec, respectively; dwell times in source plate and on glass slide were 1,000 and 35 ms, respectively. Protein concentration, pH, temperature, and RH were varied to determine the optimal conditions for microarraying.

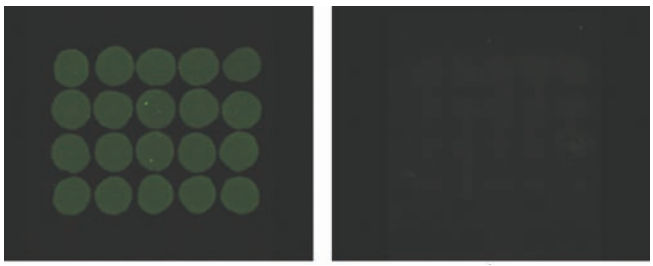
Glass slides were stored in the refrigerator for 10 hr after printing and subsequently washed and soaked in phosphate buffered saline (PBS), pH 7.4, at 37°C overnight. Slides were then washed with ultrapure water and dried by purging with dry nitrogen gas. Printed glass substrates were analyzed using a GenePix 4000A microarray scanner (Molecular Devices) at a resolution of 10 µm.

Results and Discussion

Proteins were first printed on the unmodified glass substrate to investigate whether the BSA-FITC bound to the bare glass surface. As shown in Figure 1A, the distribution of the protein was uniform within the spots. After overnight soaking of the glass slide in PBS, however, spots were completely washed off (Figure 1B), indicating that no chemical bonding occurred between the glass surface and the protein molecules.

When preparing protein-reactive glass surfaces, producing a uniform coating is highly desirable to reduce the influence of surface inhomogeneities on the spot-to-spot variation in the microarray. Previous surface analyses of PEGMA/GMA-coated slides have shown that spin-coated slides have the most uniform surfaces, followed by dip-coated slides and, lastly, grafted slides (Kurkuri et al. in press, Thissen et al. in press). Hence, in this study, spin- and dip-coated slides were further characterized in terms of their performance as microarray substrate surfaces. It was expected that proteins would be covalently immobilized on the coated substrate surface through the reaction of protein amino groups within the oxirane ring of GMA.

To investigate the optimal protein concentration and pH at which uniform circular spots can be reproducibly obtained, four protein concentrations (200, 100, 75, and 50 µg/ml) were prepared in buffers at four different pH values (pH 5, 7, 9, and 10) and printed at 10°C and 65% RH on glass slides spin-coated with PEGMA/GMA copolymer. As shown in Figure 2, protein spots can clearly be seen even after soaking in PBS



A B

Fig. 1. Binding of protein to unmodified glass substrate. Fluorescence images of BSA-FITC spots, before (A) and after (B) washing in PBS.

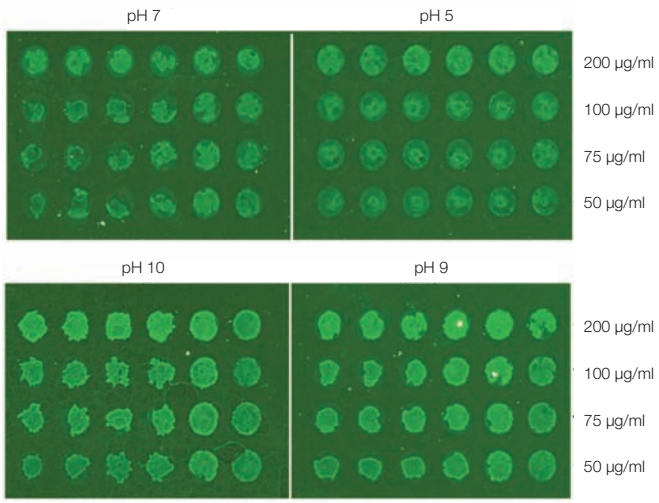


Fig. 2. Concentration and pH dependence of protein immobilization. Fluorescence images show arrays of BSA-FITC printed onto a glass slide that was spin-coated with PEGMA/GMA copolymer. Four different protein concentrations in buffer at four different pH values are shown.

overnight. All protein concentrations produced spots with detectable fluorescence intensity. The most circular spot geometry was observed when spots were printed using pH 5 buffer. Spots printed at pH 7 were also circular, but spots printed at pH 9 or 10 had irregular outlines. On the other hand, the spots printed at pH 9 or 10 showed greater fluorescence levels than those printed at pH 5 or 7. Similar results were obtained with dip-coated slides (data not shown).

Temperature and RH are known to play important roles in immobilization of proteins on microarray spots. The temperature at which the slide is held determines the kinetics of protein immobilization, while the RH influences the rate of evaporation of the nanoliter droplets. Therefore, experiments were performed to study the effects of temperature and RH on protein printing.

Images of dip-coated slides printed at different temperatures are shown in Figure 3. It is clear from the images that 10 and 15°C were ideal for printing biomolecules onto the reactive glass slides. In contrast, spots were incomplete when printing was performed at 5°C, presumably due to poor reaction kinetics. Printings obtained at temperatures of 10 and 15°C showed better protein attachment to the glass substrate. We selected 10°C for subsequent experiments in order to slow the evaporation of solvent from the protein solution, thus allowing the immobilization reaction to proceed from the liquid phase for a longer time. Binding to spin-coated slides showed similar temperature dependence (data not shown).

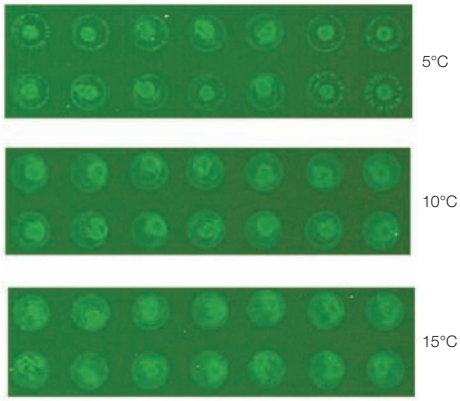


Fig. 3. Temperature dependence of protein immobilization. Fluorescence images show dip-coated glass slides printed with 75 µg/ml BSA-FITC in PBS, pH 5, at 65% RH. Three different temperatures are shown.

Figure 4 shows the effect of RH on protein attachment. Spots printed at 35, 45, or 55% RH were irregular in shape, and almost completely washed off. This may have been due to faster evaporation of solvent molecules from protein solution printed at lower RH. The spots printed at 65% RH remained attached even after soaking in PBS overnight, suggesting that higher RH is better for printing protein molecules onto reactive polymer coatings.

Conclusions

PEGMA/GMA copolymer coatings were prepared on glass slides using three different coating methods: spin coating, dip coating, and surface grafting. The BioOdyssey Calligrapher miniarrayer was successfully used to array proteins onto the modified glass substrates. Optimal protein concentration, pH, temperature, and humidity conditions for protein printing were identified. The surfaces presented in this study are currently used in cell microarray experiments in our laboratories, and initial cell culture results have confirmed the ability to immobilize biologically active compounds and have shown the intended low levels of cell attachment to these coated slides.

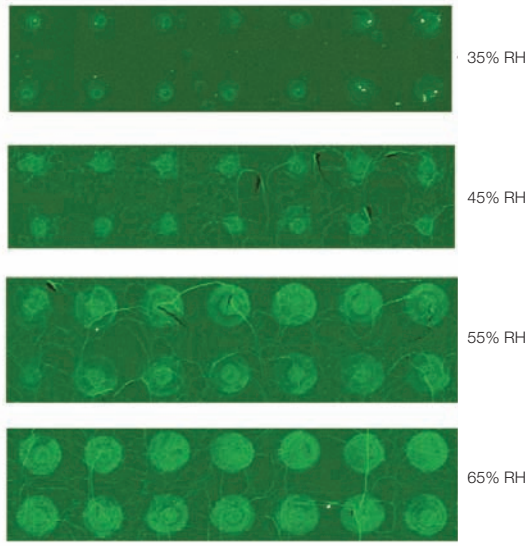


Fig. 4. The effect of RH on protein immobilization. Fluorescence images show 75 µg/ml BSA-FITC printed onto a dip-coated glass slide at 10°C and pH 5. Four different RH levels are shown.

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