Using the BioOdyssey™ Calligrapher™ MiniArrayer to Form a Polymer Array for Studying Cell-Surface Interactions

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Introduction

Microarray analysis has revolutionized genomic studies and has given insight into various biological systems by enabling high-throughput, cost-effective analysis. DNA and protein microarrays have been useful for wide-scale screening of protein-protein interactions and for gene profiling. In addition to these analyses, microarray technology can be used to scan for the interaction of biomolecules with other synthetic materials that are of interest as biomaterials. This is useful because a large number of synthetic materials with diverse properties are available, and in order to find out whether these materials are biocompatible and suitable for various applications, such as DNA immobilization, protein entrapment, or cell growth, a format that can effectively screen for biomolecular interactions with a material of interest must be developed. The obvious solution is the development of material arrays that are subsequently able to interact with biomolecules of interest.

The formation of material arrays, particularly polymer arrays, has been investigated, and the interaction of biomolecules and cells with these arrays has been studied (Anderson et al. 2005). Understanding cell-surface interaction is particularly important for biomaterial applications. It is well known that variations in the surface chemistry, topography, wettability, and roughness can greatly alter the behavior of cells at a surface. Understanding how cells behave at surfaces enables one to manipulate cell behavior and can lead to the development of advanced biodevices. One could envision, for example, the development of smart materials for coating implants that would enable the implants to interact with cells involved in the immune response in a way that would minimize harmful scarring. Such a material could improve the integration of implants into the body. Finding suitable materials for such applications demands an extensive and detailed understanding of cell-surface behavior. Libraries of various polymers can be screened to identify disparate properties that might be used to evoke the cellular behavior of interest.

One application of interest is the development of transfected-cell microarrays. These arrays are used to study the behavior of cell clusters, each transfected with a specific gene of interest (Hook et al. 2006). Creating transfected-cell microarrays involves, in brief, first spotting a library of plasmids of interest in an array format, then seeding cells onto the array. Cells that attach over the DNA spots take up the plasmid and express the encoded protein. This technique demands the spatial control not only of a DNA microarray, but also of the cells grown on the DNA spots. Furthermore, the DNA itself must be controlled so that it does not migrate across the surface, but remains available for uptake by cells.

To this end, we sought to develop a polymer array that would be suitable for interactions with both DNA and cells. We initially chose to investigate polyethylene imine (PEI), because it is a polycation that interacts with the negatively charged phosphate groups on the DNA backbone (Pei et al. 2001). In this article, we describe a method for the formation of a PEI array that could subsequently be used in living cell microarray studies.

Methods

A 1 mg/ml solution of PEI (MW 70,000, Fluka) was arrayed onto a polyethylene glycol (PEG) surface using a BioOdyssey Calligrapher miniarrayer equipped with an ArrayIt 375 µm diameter solid pin (TeleChem International, Inc.) that delivered approximately 4.0 nl/spot. Unless otherwise specified, printing was conducted at a temperature of 16°C, a humidity of 65%, an approach speed of 20 mm/sec, and a dwell time of 10 ms. The PEG surface was formed as previously described (Hook et al. 2005). In brief, bare silicon was modified with an allylamine plasma polymer (ALAPP). The amine functionality of this polymer was used for grafting aldehyde-functionalized PEG to the surface using reductive amination. Initially, the formation of the PEI array on the PEG surface was optimized by altering the humidity in the range of 57–64% and the temperature in the range of 3–37°C. The approach speed of the pin and the dwell time of the pin in contact with the surface were adjusted to optimize spot formation. PEI arrays were scanned using a GenePix 4000A microarray scanner (Molecular Devices Corporation). PEI spots were analyzed using ImageQuant v. 5.2 software (GE Healthcare).

Once PEI array formation had been optimized, the array was analyzed for its ability to permit cell attachment. Formation of the array on a low-fouling surface (PEG) meant that the attachment of cells seeded to the surface was limited to the polymer spots. Human embryonic kidney cells (HEK 293) and neuroblastoma cells (SK-N-SH) were seeded onto the PEI array. After incubation for 24 hr, cells were stained with fluorescein diacetate, a common cell vitality stain. Cells were then analyzed by fluorescence microscopy.

Results and Discussion

Initially, the optimized formation of PEI arrays was of interest. Thus, PEI printing was conducted at various temperatures, humidities, approach speeds of the pin to the surface, and dwell times of the pin in contact with the surface. Fluorescent images of PEI spots formed under these various conditions are shown in Figure 1. The diameter and variability of the pixel height, calculated as the standard deviation of the height of the total number of pixels for each spot, was calculated and these data are also shown in Figure 1. It should be noted that the data presented in Figure 1A and 1B were obtained on separate days, so the pixel intensity values are not quantitatively comparable.

A number of key conclusions can be drawn from the data in Figure 1. First, printed PEI spots always have a rim, as a result of capillary flow as the spot dries (Deegan et al. 1997). Formation of this rim results in increased variability across the spots. It can be seen from Figure 1A that decreasing both temperature and humidity increases the variability as a result of increasing the thickness and height of the rim. Altering humidity has little effect on the spot diameter. On the other hand, decreasing temperature increases the spot size. In order to minimize variability, a humidity of 65% and a temperature of 30°C were selected for further PEI array formation.

To further optimize the formation of PEI spots, the approach speed and dwell time of the pin were altered. The PEI spots formed as a result of altering these parameters are shown in Figure 1B. Interestingly, altering dwell time appeared to have little effect on the size or morphology of spots. In contrast, increasing the approach speed from 5 to 20 mm/s resulted in a decrease of spot diameter from 580 µm to 490 µm and decreased the spot variability, presumably by minimizing the formation of the polymer rim. Thus, an approach speed of 20 mm/s and a dwell time of 10 ms were selected for subsequent PEI printing.

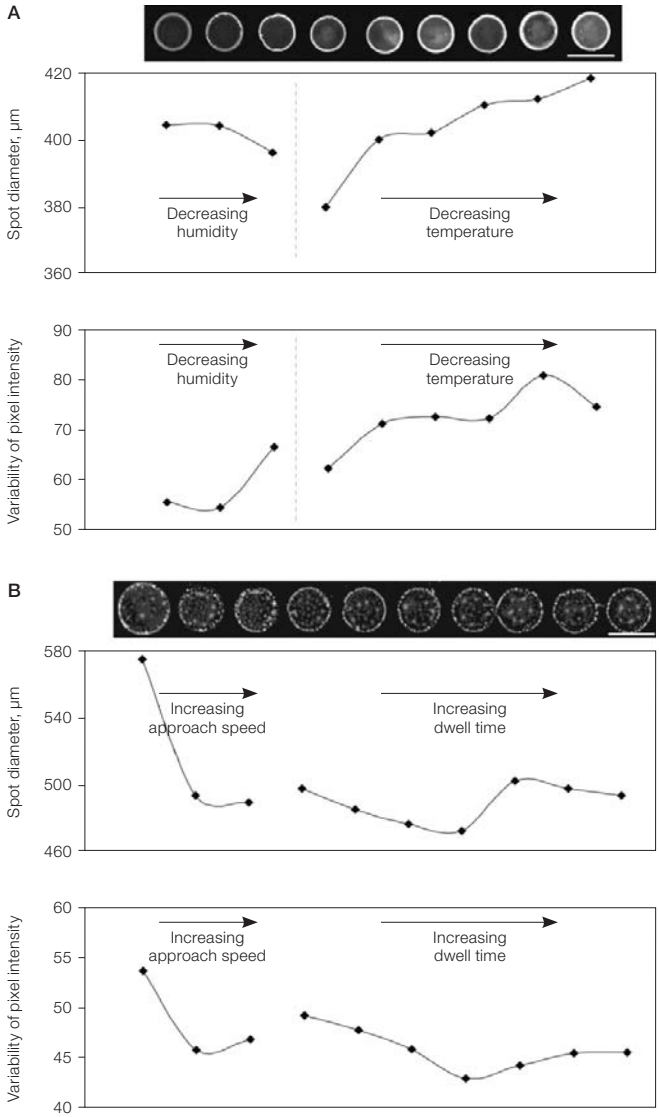

Fig. 1. Characterization of the spotting of PEI onto an ALAPP-PEG surface. A, 1 mg/ml PEI spots spotted at varied humidity and temperature. From left to right, the first three spots correspond to humidity of 64%, 61%, and 57%. The next six spots correspond to temperatures of 37°C, 30°C, 20°C, 10°C, 5°C, and 3°C. **B**, 1 mg/ml PEI spots spotted at varied approach speed and dwell time. From left to right, the first three spots correspond to approach speeds of 5 mm/s, 10 mm/s, and 20 mm/s. The next seven spots correspond to dwell times of 0 ms, 5 ms, 20 ms, 50 ms, 100 ms, 250 ms, and 500 ms. The spot diameter and the variability of pixel intensity for each spot are graphed underneath each spot. Scale bars, 500 µm.

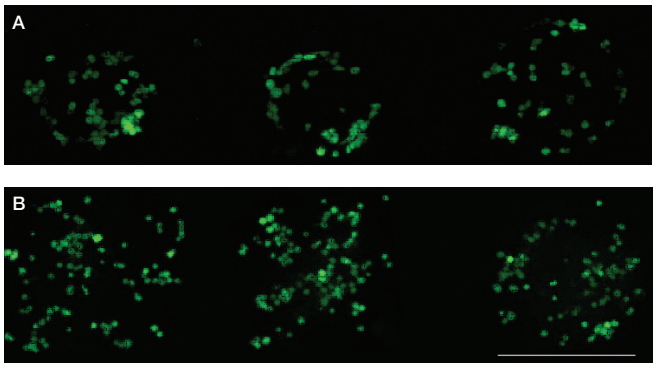

Fig. 2. Fluorescence scan of spatially controlled cell attachment to PEI spots on a PEG background. Cells were stained with fluorescein after 24 hr incubation on surface: **A**, SK-N-SH cells; **B**, HEK cells. Scale bar, 400 µm.

Finally, to investigate cell attachment to PEI microarrays, PEI was spotted onto ALAPP-PEG surfaces, and HEK 293 or SK-N-SH cells were seeded onto the surface. After incubation for 24 hr to allow cell attachment, cells were stained with fluorescein to allow observation and to assess the viability of the cells. The resulting fluorescent images are shown in Figure 2. Importantly, spatially directed attachment of both cell lines was observed, such that cells only attached to the PEI spots. Moreover, PEI at this spotting concentration appeared to be nontoxic over a 24 hr period. Interestingly, the SK-N-SH cells appeared to show a greater affinity for the rim of the spots (Figure 2A), where the PEI surface concentration was higher. This result suggests that either PEI actively promotes SK-N-SH cell attachment or the interior of the spot does not contain a complete PEI coating and that in some regions the underlying PEG layer is exposed, preventing cell attachment. The former explanation is supported by the observation that HEK 293 cells appeared to cover the PEI spots more evenly (Figure 2B).

This study demonstrated the ability of the BioOdyssey Calligrapher miniarrayer to successfully form a polymer microarray that can subsequently be used for cell attachment studies. Variation of various printing parameters altered the properties of the spots formed, and thus demonstrated the need for stringent control of these parameters to ensure reproducible spot formation. Tuning of these parameters allows one to optimize the parameters of the printed spots. After optimizing PEI spot formation, the ability to spatially control cell attachment using a PEI array on ALAPP-PEG films was demonstrated. These techniques will enable the further investigation of other polymeric materials and their interaction with various biomolecules in a high-throughput fashion.

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