Sub-micron Patterning on Polymer Films for Protein Arrays

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ABSTRACT

Patterning proteins at the sub-micron and nanoscale has many uses, including fabrication of protein arrays for diagnostic and sensor applications. Many groups have reported micron scale protein patterns using photolithography; however, smaller scales have not been realized. In this study, we demonstrate sub-micron protein patterns using photolithography. Patterning is achieved by chemical transformation of pH-reactive polymer films. Site-specific immobilization of streptavidin within a protein resistant background is demonstrated. This methodology could be utilized for the development of high density proteins arrays for biotechnology applications.

INTRODUCTION

The emerging technology of protein micro and nanoarrays offers exciting possibilities for biosensor applications [1, 2]. The resolution of commercially available arrays is limited due to the robotic printing techniques used in fabrication [3]. As an alternative approach, we have recently developed a methodology for protein patterning using pH-responsive polymer films and photolithography. This technique uses poly(3,3'-diethoxypropyl methacrylate) (PDEPMA), which contains reactive acetal groups that hydrolyze to aldehydes in the presence of acid (Figure 1). Aldehydes readily react with aminooxy-functionalized compounds [4] without the addition of any other reagent and can be reacted with amines via reductive amination [5]. We first demonstrated micron scale protein patterning (18 x 18 micron features) using PDEPMA, the photoacid generator (PAG) triphenylsulfonium triflate, and deep ultraviolet light (λ_{max}=248 nm). In the current study, we examine the utility of PDEPMA for protein patterning at the sub-micron scale.

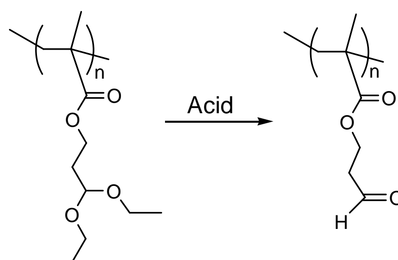

Figure 1. Poly(3,3'-diethoxypropyl methacrylate). Upon exposure to acid, acetal side chains are converted to aldehydes.

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EXPERIMENTAL DETAILS

3,3’-diethoxypropyl methacrylate was synthesized and polymerized according to previously described procedures [6, 7]. Silicon wafers were coated using C_4F_8 plasma deposition (C_4F_8 flow rate: 100 sccm; argon flow rate: 10 sccm; pressure: 15 mT; inductively coupled plasma power source: 300 W at 13.56 MHz; process time: 10 sec; wafer temp: 30 ºC). PDEPMA was then spin-coated onto the substrates using a 2 % w/w solution in chloroform containing diphenyliodonium-9,10-dimethoxyanthracene-2-sulfonate (DIAS; 5 wt % PAG/polymer; Aldrich). Films were exposed to i-line light through a mask, which contained arrays of features ranging from the micron to sub-micron scale, for 4 sec with a GCA 6300 I-Line Wafer Stepper. Films were then incubated with aldehyde reactive probe (ARP; *N*-(aminooxyacetyl)-*N'*-(D-biotinoyl) hydrazine; 2 mg/ml; Molecular Probes) for 45 min, followed by Alexa Fluor 568 labeled streptavidin (5 µg/ml; Molecular Probes) for 45 min. *O*-(Methoxypoly(ethylene glycol))-hydroxylamine (M_n= 5,000) was synthesized according to a previously described procedure [8]. Additional samples were incubated with ARP as described above, then flood exposed to i-line light for 4 sec, and stained with the aminooxy-terminated poly(ethylene glycol) (PEG) (1 mg/ml; 45 min). Films were finally incubated with Alexa Fluor 568 labeled streptavidin (5 µg/ml) for 45 min. All reagents were diluted in Milli-Q H_2O. Samples were rinsed with Milli-Q H_2O (3 x 5 min) after each incubation step. Fluorescence was visualized with a Zeiss Axiovert 200 fluorescent microscope equipped with an AxioCam MRm monochrome camera. Pictures were acquired and processed using AxioVision LE 4.1. Films were also examined with a MultiMode™ Atomic Force Microscope (AFM; Digital Instruments) in tapping mode.

RESULTS

To site-specifically hydrolyze acetal side chains on PDEPMA to aldehydes, polymer films were exposed to 365 nm light through a mask, which contained both micron and sub-micron features. In order to pattern proteins at locations exposed to i-line light, samples were first incubated with a biotinylated hydroxylamine (ARP), which binds to aldehydes via an oxime linkage. Films were then incubated with red fluorescent Alexa Fluor 568 streptavidin. Streptavidin bound specifically to locations of light exposure, with minimal background staining (Figure 2A).

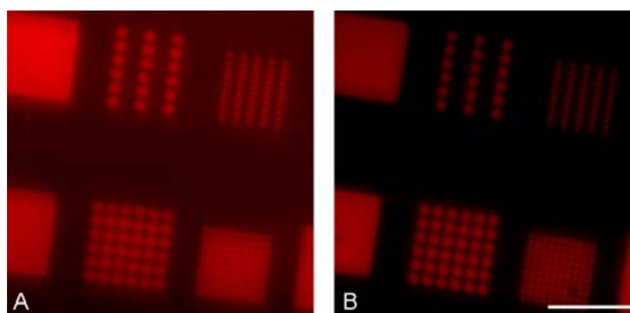

Figure 2. Streptavidin patterns. A: A PDEPMA film was exposed to 365 nm light through a mask. The film was then incubated with a biotinylated aldehyde reactive probe (ARP), followed by red fluorescent Alexa Fluor 568 streptavidin. B: A PDEPMA film was first exposed with i-

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line light through a mask and then incubated with ARP. The sample was then flood exposed to i-line light, incubated with an aminooxy terminated PEG, and finally incubated with Alexa Fluor 568 streptavidin. Note the reduction in non-specific binding. Scale bar = 40 μm.

Despite the minimal background binding with streptavidin, other proteins may result in greater fouling. To eliminate this possibility, the background area was passivated with poly(ethylene glycol) (PEG) because this polymer is known to be generally resistant to proteins. Therefore, an aminooxy-terminated PEG was synthesized. To immobilize this PEG, PDEPMA plus PAG films were exposed to i-line light and patterned with biotin as described above. The films were then flood exposed to hydrolyze the remaining acetals to aldehydes. Following incubation with aminooxy terminated PEG, which reacted with the newly converted aldehydes, streptavidin was immobilized to the original biotin patterns (Figure 3). Red fluorescent streptavidin bound specifically to the biotin, with even less non-specific binding than films without PEG (Figure 2B).

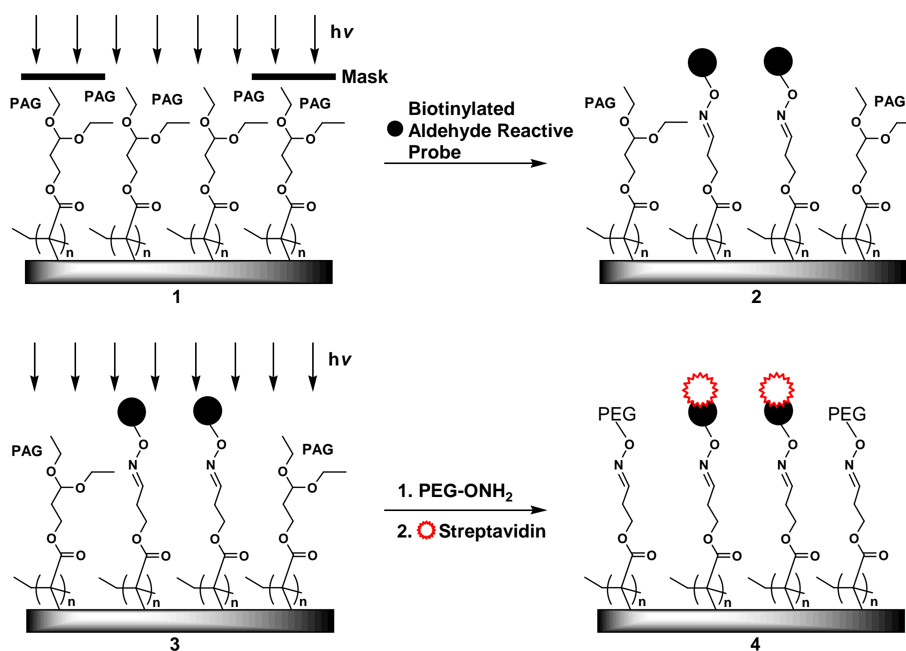

Figure 3. Streptavidin patterning. Following exposure to i-line light through a mask (1), acetal groups were hydrolyzed to aldehydes, which allowed for conjugation to a biotinylated aldehyde reactive probe (2). Films were then flood exposed to i-line light (3), which hydrolyzed the background acetals to aldehydes. Aminooxy-terminated poly(ethylene glycol) then bound to the background aldehydes, while streptavidin immobilized to the biotin patterns (4).

Films were also examined by AFM in tapping mode. Conversion to aldehydes resulted in a decrease in the height of the polymer film, which was likely due to the release of small molecules and subsequent collapse of the film. AFM images revealed patterned features down to 500 nm (Figure 4). It was difficult to visualize the bound streptavidin due its immobilization within wells and dry conditions of the AFM; however, fluorescent imaging verified the patterned

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streptavidin within larger features (Figure 2). Attachment of biotinylated proteins has also now been demonstrated [9], indicating that the attached streptavidin retains its bioactivity.

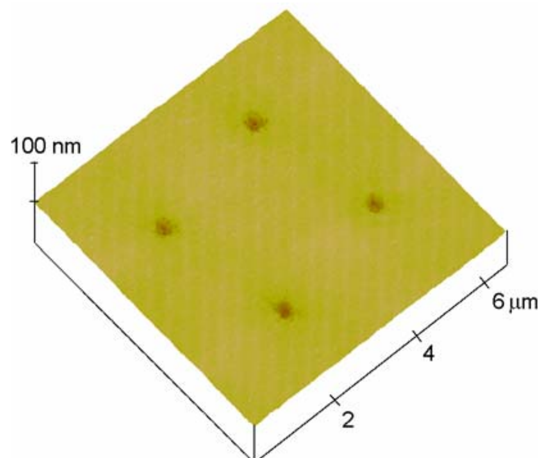

Figure 4. Sub-micron patterns. Upon exposure to i-line light, films collapsed upon PAG activation and aldehyde conversion. Streptavidin was immobilized to the patterned aldehydes with a non-fouling PEG background. Image was acquired using AFM in tapping mode.

CONCLUSIONS

We have prepared micron and sub-micron pattern sizes for immobilization of streptavidin in a protein-resistant PEG background. Due to the multiple binding sites on streptavidin for biotin, biotinylated ligands can be attached to the arrays for use in enzyme linked immunosorbent assays. Alternatively antibodies could be functionalized with an aminooxy and directly conjugated to aldehydes on the polymer film. The I-line Stepper allows for alignment, thus multiple proteins could be patterned using this methodology for the development of biosensors and protein arrays with feature sizes on the sub-micron scale.

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REFERENCES

1. J. W. Silzel, B. Cercek, C. Dodson, T. Tsay and R. J. Obremski, *Clin. Chem.* **44**, 2036-2043 (1998).
2. D. S. Wilson and S. Nock, *Angew Chem Int Ed Engl* **42**, 494-500 (2003).
3. S. Choudhuri, *J Biochem Mol Toxicol* **18**, 171-179 (2004).

https://doi.org/10.1557/PROC-0900-O09-35 Published online by Cambridge University Press

4. L. A. Marcaurelle, Y. S. Shin, S. Goon and C. R. Bertozzi, *Organic Letters* **3**, 3691-3694 (2001).
5. R. C. Horton, T. M. Herne and D. C. Myles, *J Am Chem Soc* **119**, 12980-12981 (1997).
6. K. L. Christman and H. D. Maynard, *Polymer Preprints* **46**, 1286 (2005).
7. K. L. Christman and H. D. Maynard, *Langmuir* **21**, 8389-8393 (2005).
8. T. L. Schlick, Z. Ding, E. W. Kovacs and M. B. Francis, *J. Am. Chem. Soc.* **127**, 3718-3723 (2005).
9. K. L. Christman, M. V. Requa, V. E. Enriquez-Rios, S. C. Ward, K. A. Bradley, K. L. Turner and H. D. Maynard, *Nano Let* **submitted**, (2005).

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