Zeolite Micropattern on Biological Application

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ABSTRACT

Zeolite micropattern created from structurally similar hydrophobic Sil-1 and hydrophilic ZSM-5 zeolites, and tested for the immobilization of fluorescein isothiocyanate conjugate bovine serum albumin (BSA-FITC), biotin conjugate bovine serum albumin (Biotin-BSA), Hochest 33342 and rhodamine conjugate α- bungatoxin, were preferentially deposited onto hydrophobic ZSM-5 patterns regardless of the pattern geometry. Being inorganic, the zeolite micropattern does not deteriorate with time and can be readily reused by simply burning away the deposited chemicals and biomolecules. This suggests that compositional micropattern of zeolite could be used to create high resolution array of biomolecules on flat substrate.

Keywords: zeolite, micropattern, BSA-FITC, biotin-BSA, Hochest 33342, rhodamine conjugate α- bungatoxin, molecules.

1 INTRODUCTION

The deposition and immobilization of biomolecules are important for the creation of DNA, protein and cell arrays that are essential for rapid drug discovery, new diagnostic assays and emerging bio-MEMS and cellomic technologies. The goal is to achieve well defined spatial patterns of bioactive molecules of precise quantity in a fast and reliable manner. [1,2] The high cost of biomolecules and recent advances in detection technology motivate the creation of smaller and finer pattern features. High signal fidelity is obtained by either increasing the probe density per sites or optimizing the spatial presentation of probe molecules [3]. Spotting is one of the earliest and most established techniques for making DNA microarrays. A pin or needle was stored with desired solution and then contact with the surface or the desire liquid was delivered to surface through a nozzle under pressure. Using this method, a spot size of 75 to 500 mm can be created. Ink-jet printing is a convenient and cost-effective method for depositing complex patterns of biomolecules on surfaces even it posses a large thermal and shear stresses in the initial stage . This method is highly reproducible and can print DNA, proteins and cells with volume and spot size as small as 24 pL and 30 mm, respectively [4,5]. Surface pretreatment, proper design of pin and nozzle and appropriate solution chemistry and viscosity are a must to achieve good control on the spot size. Manipulation of surface chemistry through judicious use of self-assembly monolayer achieves optimum surface linkage and presentations of biomolecules. Mirkin and coworkers used AFM stylus to write oligonucleotide patterns on surfaces with linewidth as small as 15 nm [6]. Microarray was created by coating the surface with molecular linkers that bear photo sensitive groups can be patterned by photolithographic process. Irradiation creates regions that are available for linkage with the biomolecules [7]. Using e-beam lithography, oligonucleotides features smaller than ten microns were obtained [8]. Soft lithographic processes using microfluidics can direct the deposition of biomolecules and cells in prefabricated channels on biochips [9]. Microstamping transfer biomaterials onto a surface by using a stamp containing the desired pattern13 and proved to be a convenient technique for creating features as small as 20 microns. This work describes the use of composition-gradient pattern created on a zeolite-coated surface to guide the deposition of biomolecules.

2 METHODOLOGY

2.1 Construction of zeolite micropattern

The sample was prepared by growing a thin layer of ZSM-5 film on silicon wafer. The wafer was seeded with a uniform layer of zeolite nanocrystals. The 100 nm TPA-silicalite-1 seeds were prepared by hydrothermal synthesis from a solution containing fumed silica and tetrapropylammonium hydroxide (TPAOH) at 403 K for 8 h. The seeds were purified by a series of centrifugation and washing steps and suspended in dry ethanol to give a 1 wt% seeding solution. The seeds were spin-coated at 4000 rpm (P6000 Speciality Coating System) on silicon and calcined at 823 K for 8 h. A five micron thick ZSM-5 film was grown from a clear synthesis solution containing 80 SiO2: 8 Al2O3: 10 Na2O: 1 TPAOH: 40 000 H2O at 423 K for 48 h. Methyl groups were grafted onto the ZSM-5 surface by refluxing the sample in a dry toluene (99.5%, LabScan) solution containing 4.4 6 X 10^{-7} M methyltriethoxysilane (99%, Aldrich Chemicals) at 393 K for 18 h. Three micron thick photoresist layer (HPR-207) was spin-coated on the surface of ZSM-5 film and the square-wave patterns were transferred by photolithographic process. The sample was etched by a buffer oxide etchant (BOE, Olin) containing 1 HF : 6 NH4F at 298 K. The unprotected zeolites outside the square-wave patterns were etched to a depth of 2 mm at a rate of 0.33 mm min21. The remaining photoresist was stripped using acetone solvent (99.5%, LabScan) and the etched sample was placed in an aluminium-free, Sil-1 synthesis solution with a composition of 40 SiO2: 5 TPAOH: 20 000H2O. The Sil-1 zeolite preferentially grew on the etched area of the sample. After ten hours of synthesis at 403 K, sil-1 was re-growing on the etched portion of the sample. The samples were calcined in a furnace at 673 K for 24 h to remove the methyl groups from the ZSM-5 surface and free the zeolite pores of the TPA (tetrapropylammonium) template molecules. The heating rate was kept at 0.5 K per min to avoid crack formation in the zeolite film layer.

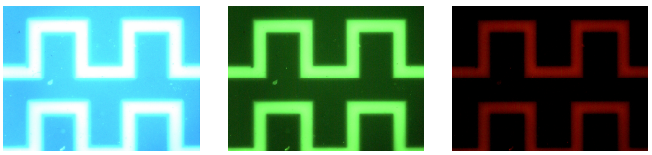
2.2 Use of zeolite micropattern to guide the deposition of biomolecules

Fluorescein isothiocyanate conjugate bovine serum albumin (BSA-FITC, sigma) in 1 mg/ml, rhodamine conjugate α- bungatoxin (sigma) in 1ng/ml and hochest 33342 in 1ng/ml, were deposited by simply immersing the patterned zeolite samples in each aqueous suspension for an hour. The incubated samples were washed six times with phosphate buffered saline (PBS) solution before rinsing with deionized, distilled water. The sample was then examined under fluorescent microscope (BX41, Olympus). The light shutter is fully open during the operation and the excitation wavelength of 330-385 nm for hochest 33342, 450–490 nm for fluorescein isothiocyanate and 510-550nm for rhodamine. Biotin conjugate bovine serum albumin (Biotin-BSA, sigma) was deposited by simply immersing the patterned zeolite sample in biotin-BSA suspension for 30 minutes. The incubated samples were washed six times with phosphate buffered saline (PBS) solution in every five minutes before rinsing with deionized, distilled water. The sample was then incubated with PBS solution overnight to ensure the excess Biotin-BSA was washed away, the sample was then immersing in 1μg/ml Flour594-Streptavidin for 30 minutes and followed by six times of PBS washing, the sample then examined under fluorescent microscope.

3 RESULTS AND DISCUSSIONS

In the synthesis solution, TEOS serves as the silica precursor whereas TPAOH molecules serve as the zeolite growth directing agents, and as the source of hydroxide ions for maintaining the alkalinity of the solution. Increasing the amount of TPAOH in the synthesis solution produced larger grains that were less elongated and more square. The crystal length remains unchanged with dilution, but the width decrease resulting in elongated shape. Precautions were taken to prevent deposition of unwanted zeolite powder on the growing film by placing the sample horizontally in the synthesis solution with the seeded surface facing downward. The Sil-1 zeolite preferentially grew on the etched area of the sample, since the methyl groups attached to the ZSM-5 surface inhibited the zeolite growth on the square-wave patterns. X-Ray fluorescent spectroscopy (XRF, JEOL JSX-3201Z) and X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI5600) indicated that the ZSM-5 film has a uniform aluminium content of 6 perecntage. The zeolite possessed a preferred (101) film orientation according to the X-ray diffraction (XRD, Philips PW1825).

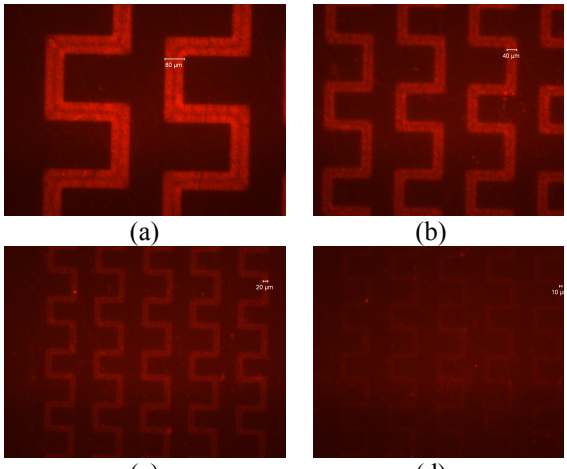
The zeolite micropattern consists of aluminium-rich, square-waves made of ZSM-5 zeolite on a background of aluminium-free Sil-1 zeolite. The ZSM-5 and Sil-1 are medium pore MFI-type zeolites with similar crystal structure and differ mainly in their composition. Sil-1 zeolite containing only silicon and oxygen atoms is hydrophobic, while ZSM-5 with isomorphous substituted aluminium atoms is hydrophilic. Figure 1 show that BSA-FITC, rhodamine conjugate α- bungatoxin and hochest 33342 are preferentially absorbed on ZSM-5 and not on Sil-1. Initially, all of them adsorbed on both ZSM-5 and Sil-1 by electrostatic interaction and hydrophobic force respectively. After washing with P.B.S solution, the biomolecules that electrostatically adsorbed on the charged ZSM-5 surface is remain attached while the biomolecules attached on Sil-1 by hydrophobic force is detached. This results 2c. The results demonstrate that the use of composition-gradient pattern in zeolite is an inexpensive and convenient way to direct the deposition of biomolecules into organized surface features.



(a) (b) (c)

Figure 1. Fluorescent microscope picture of (a) hochest 33342, (b) FITC-conjugated BSA and rhodamine conjugate α- bungatoxin molecules deposited onto the zeolite micropattern.

For Biotin-BSA and flour594-Streptavidin essay, it was clear from the fluorescent picture in Figure 2 that the deposited Biotin-BSA was able to conjugate with Flour594-Streptavidin and they were deposited on ZSM-5 only. This suggested that deposited Biotin-BSA is still active to conjugate with streptavidin. The results demonstrate that the use of composition-gradient pattern in zeolite is able to direct the deposition of chemical and biological molecules into organized surface features in an inexpensive and convenient way. The prefabricated zeolite micropattern is easy to make and is durable. Months of storage did not affect its use. Being chemical and heat resistant, the zeolite micropattern can be chemically cleaned, sterilized and even heat treated at high temperature (up to 823 K). It is also CMOS compatible and can be easily integrated onboard bioMEMs



(a) (b) (c) (d)

Figure 2. Fluorescent microscope pictures of Biotin-BSA conjugated with Flour594-Streptavidin on zeolite micropatterns with linewidth of (a) 80, (b) 40, (c) 20 and (d) 10 microns.

4 CONCLUSIONS

A facile method was established using composition-gradient pattern on zeolite surface. The zeolite micropattern consists of aluminum-rich, square-waves made of ZSM-5 zeolite on a background of aluminum-free Sil-1 zeolite. Studies show that hochest 33342, FITC-conjugated BSA and rhodamine conjugate α- bungatoxin were preferentially deposited onto the ZSM-5 pattern.

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