# Zeolite micropattern for biological applications

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**A facile method was established using composition-gradient pattern on zeolite surface to guide the deposition and formation of chemical and biomolecular patterns with features as small as five microns.**

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 increasing the probe density per sites and optimizing the spatial presentation of probe molecules.[3] Spotting is one of the earliest and most established techniques for making DNA microarrays.[1] It delivers liquid solution by bringing a pin or needle into contact with the surface or by projecting a liquid droplet through a nozzle under pressure. Liquid volumes of 50 pL to 100 nL can be routinely delivered resulting in a spot size of 75 to 500 μm. Despite the initial concern for the large thermal and shear stresses in ink-jet printing, it proved to be a convenient and cost-effective method for depositing complex patterns of biomolecules on surfaces. The method is highly reproducible and can print DNA, proteins and cells with volume and spot size as small as 24 pL and 30 μm, 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.[6] Manipulation of surface chemistry through judicious use of self-assembly monolayer achieves optimum surface linkage and presentations of biomolecules.[7] Mirkin and coworkers[8] used AFM stylus to write molecular patterns on surfaces with linewidth as small as 15 nm. Oligonucleotide has been patterned on surfaces using this method.[9]

Surfaces coated with molecular linkers that bear photo-removable protective groups can be patterned by photolithographic process. Irradiation creates deprotected regions that are available for linkage with the biomolecules.[10] This method has been successfully employed for microarray production. Using e-beam lithography, oligonucleotides features smaller than ten microns were obtained.[11] Soft lithographic processes using microfluidics can direct the deposition of biomolecules and cells in prefabricated channels on biochips.[12] Microstamping transfer biomaterials onto a surface by using a stamp containing the desired pattern[13] 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.

The zeolite micropattern shown in Fig. 1a 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. 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 $SiO_2$: 8 $Al_2O_3$: 10 $Na_2O$: 1 TPAOH: 40 000 $H_2O$ at 423 K for 48 h. 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. 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 at%. The zeolite possessed a preferred (101) film orientation according to the X-ray diffraction (XRD, Philips PW1825).

Methyl groups were grafted onto the ZSM-5 surface by refluxing the sample in a dry toluene (99.5%, LabScan) solution

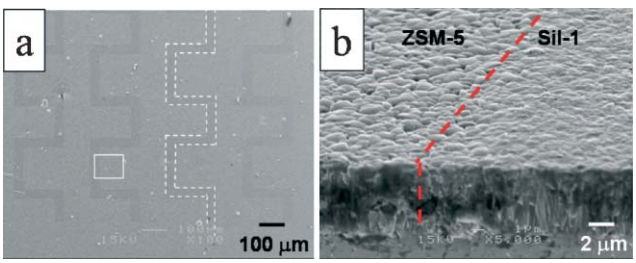


**Fig. 1** (a) SEM micrograph of aluminium-rich square-wave patterns of ZSM-5 against a Sil-1 background. (b) A high magnification picture of the sample cross-section taken near the boxed area shown in (a).

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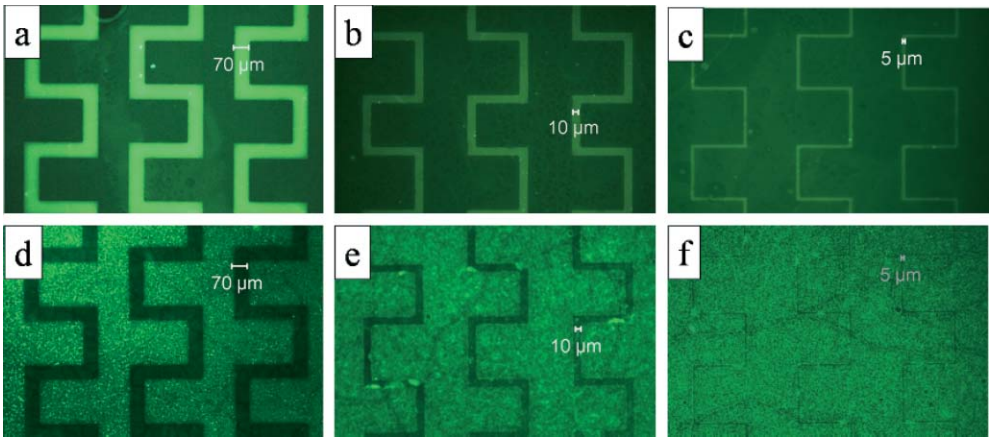


**Fig. 2** BSA-FITC (a–c) and FITC (d–f) depositions on 70 μm, 10 μm and 5 μm ZSM-5 square-wave pattern.

containing 4.4 × $10^{-7}$ 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 $NH_4F$ at 298 K. The unprotected zeolites outside the square-wave patterns were etched to a depth of 2 μm at a rate of 0.33 μm $min^{-1}$. 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 $SiO_2$: 5 TPAOH: 20 000 $H_2O$. 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. Ten hours of synthesis at 403 K is sufficient to fully regrow Sil-1 on the etched portion of the sample as shown in Fig. 1b. The SEM picture shows that the ZSM-5 and Sil-1 layers have the same height. Without elemental contrast, it is difficult to differentiate the two zeolites from the figure. The ZSM-5 and regrown Sil-1 display identical crystal size and morphology. XRD analysis indicated that the regrown Sil-1 also exhibit a preferred (101) film orientation similar to the ZSM-5. The surface roughness of the patterned surface is about ±0.2 μm.

Three samples with pattern linewidths of 70, 10 and 5 microns were prepared using the above procedure. 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 $min^{-1}$ to avoid crack formation in the zeolite film layer. Fluorescein isothiocyanate conjugate bovine serum albumin (BSA-FITC, Sigma) was deposited by simply immersing the patterned zeolite samples in 1 mg $ml^{-1}$ aqueous BSA-FITC 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 is 450–490 nm. Figs. 2a–2c display the fluorescent pictures of BSA-FITC incubated samples with pattern linewidths of 70, 10 and 5 microns. It is clear from the pictures that the protein preferentially adsorbed on ZSM-5 and not on Sil-1. The numbers of BSA-FITC molecules deposited on 70, 10 and 5 microns patterns were determined to be roughly 2.5 ± 1.2 × $10^{14}$ molecules per $cm^2$, which is close to a monolayer coverage. The protein was electrostatically adsorbed on the charged ZSM-5 surface and can be removed by a simple acid wash.

The adsorbed BSA-FITCs can also be removed by air calcination at 673 K for 24 h. Inspection by fluorescent microscope showed that fluorescent signal is absent in the calcined samples. Fluorescein isothiocyanate (FITC, Sigma) was deposited from a 1 mg $ml^{-1}$ solution and the excess FITC was washed away with phosphate buffered saline solution. Fig. 2d–2f show that the FITC molecules preferentially adsorbed on Sil-1 and not on ZSM-5 creating an exact negative image of that of Fig. 2a–2c. The results demonstrate that the use of composition-gradient pattern in zeolite is an inexpensive and convenient way to direct the deposition of chemical and biological molecules into organized surface features. 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.

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