Biologically Functionalized Nanochannels on Ferroelectric Lead Zirconium Titanate Surfaces

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ABSTRACT

We have demonstrated the feasibility of selective functionalization of a ferroelectric thin film, allowing it to be used for the manipulation of charged biomolecules. This hybrid inorganic-biomolecular platform may find use in electrically actuated valves capable of controlling flow in nanochannels. The nanochannels are patterned on a ferroelectric lead zirconium titanate (PZT) thin film grown on a platinum electrode substrate. The channels are fabricated using a new bilayer resist method in order to create addressable encapsulated channels in one process step. A bacterial virus (bacteriophage) is used to identify ligands that selectively bind to the PZT surface. After attaching the biomolecules in specific locations along the channels, a voltage is applied to the PZT layer resulting in polarization of the surface, thus creating charges that cause the biomolecules to tether. Such tethering can be used like a valve in a nanofluidic channel. The biological valve discussed in this paper would enable many applications, including "lab-on-a-chip" and controlled medical drug delivery.

Keywords: nanofluidics, nanochannels, heptapeptides, ferroelectrics, piezoelectrics, nanovalves.

1 INTRODUCTION

1.1 Opportunities in Nanofluidics

In the last several years, fluid control on the micron scale (i.e. microfluidics) has been an area of significant interest. Many of these devices have been based on micro-electromechanical systems (MEMs) and alternative architectures such as organic valves [1]. Microfluidic devices have been fabricated using a variety of methods, ranging from thermal decomposition of buried patterned channels, to fabrication of trenches via plasma etch or hot embossing followed by trench capping. Our work focuses on an alternative method by using a bilayer resist in an inverted configuration normally used for *T*- and *Γ*– gate fabrication. This method is capable of yielding sub-100 nm nanochannels with high aspect ratios and sub-500nm alignment [2].

As fabrication processes improve, the new frontier is in the nanometer regime. It is difficult to scale to nanometer dimensions the same designs that work well in the micron regime, some being still in the theoretical stage [3].

1.2 PZT as a material for nanofluidics

The surface of polarizable ferroelectric materials can be used to manipulate electrically charged biological molecules through the electrical charges appearing on it. A ferroelectric crystal is a material capable of being polarized in the reverse direction when an electric field is applied. Lead zirconium titanate (PZT) is a material that exhibits one of the highest polarization and piezoelectric effect (i.e. lattice deformation upon application of an electric filed), and both phenomena potentially can be used in a wide range of devices such as non-volatile ferroelectric memories and piezoelectrically actuated MEMS/NEMS devices [4]. PZT thin films with controlled composition and microstructure and excellent properties have been synthesized by sputter and laser ablation deposition, sol-gel methods, and metalorganic chemical vapor deposition (MOCVD) [5]. For the work presented in this paper, we have elected to use the sol-gel method as the means to prepare the PZT thin films. Future work will focus on extending these studies on MOCVD deposited PZT, since it enables better crystallographic growth control and therefore better control on the induced polarization in the device.

1.3 Biologically functionalized PZT

Recently, it has been shown that peptides can possess a high affinity for a variety of inorganic substrates and that they can be isolated from combinatorial libraries expressed on filamentous bacteriophage [6,7]. These peptide ligands offer numerous advantages over organic ligands since they are nontoxic, compatible with aqueous chemistry, and can be readily produced using standard biological techniques. Most importantly, by isolating these peptides from a combinatorial library, ligands can be identified that can specifically derivatize any desired material, allowing the functionalization of specific components of fabricated MEMS devices. In prior work [8] we reported the successful use of this approach to identify a hepapeptide sequence that selectively binds to PZT. Here we evaluate the applicability of this sequence to the fabricated nanochannels that will form the basis of a nanofluidic

device. A final design for the nanochannel device is being finalized, and a prototype is on schedule for fabrication. This valve would prove useful in many applications, including the lab on a chip and in controlling medical drug delivery.

2 EXPERIMENTAL

2.1 Preparation of phage functionalized PZT

For these experiments, PZT films were prepared using a sol gel technique. A 10% excess lead acetate [$Pb(OC_2H_3)_2$], titanium isopropoxide [$Ti(OC_3H_7)_4$] and zirconium isopropoxide [$Zr(OC_3H_7)_4$] were mixed with acetic acid and n-propanol. Additional n-propanol was added to optimize the viscosity of the final precursor solution. Spin coating was used to deposit PZT layers with $PbZr_{0.47}Ti_{0.53}O_3$ composition onto $Pt/Ti/SiO_2/Si$ substrates at 3,000 rpm for 30 s. The films were dried at 450 °C for 10 min; followed by annealing at 650 °C for 30 min in a tube furnace to ensure full crystallization.

Following annealing, the PZT film-based samples were cut into 0.5 x 0.5 cm sections and immersed in 2 ml of a phosphate buffer (10 mM potassium phosphate, 100 mM NaCl, pH = 7.5). Subsequently, 100 µl of phage expressing the PZT-specific peptide as a pIII fusion peptide was added (1×10^9 pfu/ml). The mixture was shaken at 75 rpm for one hour. The PZT sample was then rinsed with phosphate buffer. It was placed in a phosphate buffer containing 0.5% BSA and shaken for 15 min. It was rinsed with more buffer, and immersed in 2 ml of buffer. 10 µl of biotinylated anti-fd antibody was added (3.5 mg/ml), and the mixture was shaken for 1 hr. The PZT sample was rinsed with buffer and placed in fresh buffer. 25 µl of FITC-tagged avidin was added (1 mg/ml), and the mixture was shaken an additional hour. The sample was removed and rinsed a final time with buffer. After functionalization, all samples were stored in phosphate buffer at 4 °C.

2.2 Nanochannel Fabrication

The resist materials used to form the nanochannels are UV113 (Shipley Inc.) and ZEP520 (Zeon Corporation). The UV113 resist is based on a polar polymer (polyhydroxystyrene), while the ZEP520 resist is based on a non-polar polymer (methylstyrene/chloromethylacrylate). Because of the difference in polarity, these polymers do not mix. In addition, the developer for UV113 is aqueous based TMAH solution, while for ZEP520 it is Xylenes and both leave the other resist intact. The UV113 resist is about 5 times more sensitive than ZEP520 and is used to form the walls of the channels, while the ZEP520 resist is used to form the top and ceiling. An example of buried micron and sub-micron channels on silicon substrates is shown in Figure 1 [2]. Our plan is to integrate this type of channel onto functionalized PZT substrates to fabricate biologically controlled nanofluidic devices.

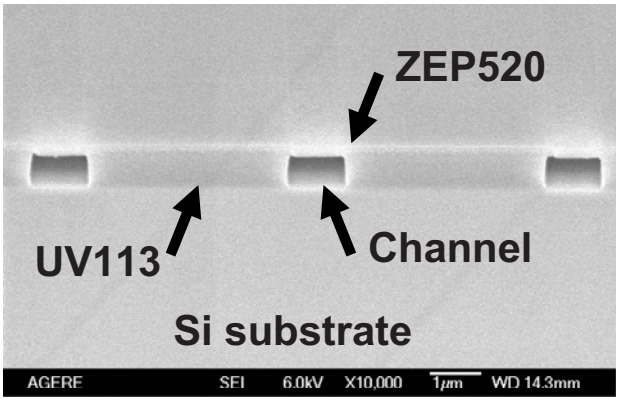

Figure **1**. Cross-section SEM micrograph taken at 45 degrees of buried 1 micron polymer channels fabricated on a silicon substrate. (Ref. 2)

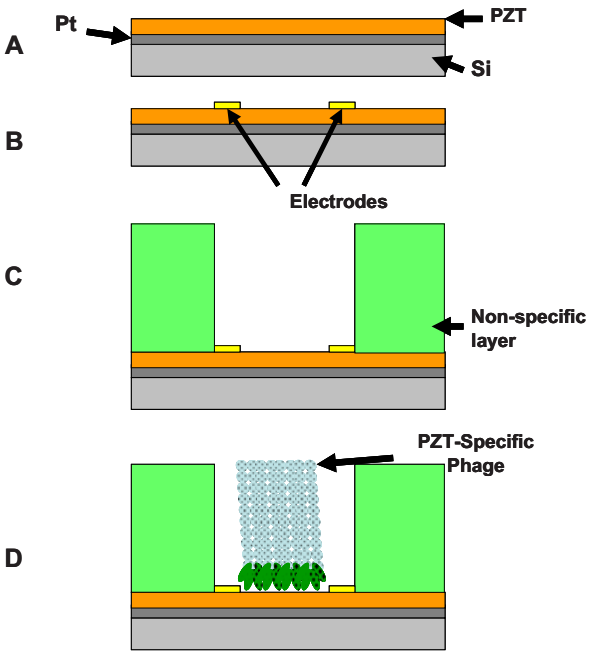

Figure **2.** Fabrication sequence of nanochannels to study PZT functionalization for attachment of specific peptide. (A) PZT is deposited over a Pt electrode; (B) Electrodes are fabricated using liftoff on the PZT substrate; (C) A non-specific layer is patterned to expose only the PZT between the electrodes deposited on the surface of the PZT-sensitive specific phage; (D) sample is functionalized with PZT-sensitive specific phage.

A key aspect of the integration of the nanochannels fabrication process onto functionalized PZT is that the PZT-sensitive specific peptide does not bind to the other components of the device. For this purpose, we fabricated electrodes on the surface of the PZT and channel trenches that overlap the electrodes. In this case the process flow is as follows: a) Electrode process: spin coat PZT with 200 nm of ZEP520:Anisol solution 1:1 at 2000 rpm; bake at 150

C for 3 min; expose electrode patterns using a Raith 150 e-beam tool at 30 KV, with a dose of 80 μC/cm^2; develop in Xylenes for 60s; N_2 dry; sputter 40 nm of Au on 1 nm of Cr adhesion layer; do liftoff of the metal thin film using N-methyl 2-pyrrolidone (Microposit remover 1165, Shipley) heated at 60 ˚C for 3 min and ultrasonic agitation for 2 min; rinse with acetone and isopropanol; N_2 dry; b) Trenches over electrodes process: spin coat PZT with 200 nm of ZEP520:Anisol solution 1:1 at 2000 rpm; bake at 150 ˚C for 3 min; expose electrode patterns using a Raith 150 ebeam tool at 30 KV, with a dose of 80 μC/cm^2; develop in Xylenes for 60s; N_2 dry; dip in TMAH 0.26N solution for 2 minutes; N_2 dry. A summary of the process steps is illustrated in Figure 2.

3 RESULTS AND DISCUSSION

3.1 Phage compatibility with nanofluidic structure

The vendors do not provide the compositions of UV113 and ZEP520 photoresists. Therefore, their surface chemistry after the fabrication process is also unknown. It is important then to experimentally determine the binding specificity of the peptide to these surfaces. For these reasons, Si wafers were coated with the desired photoresists and subjected to a similar functionalization procedure as the PZT to determine if the biological ligands possessed any affinity for the photoresists. Every possible development condition of the desired photoresist was tested to determine the conditions that would minimize nonspecific binding of phage particles to the resist. Figure 3 displays such experimental data for three photoresists, HSQ, UV113, and ZEP. The data in Figure 3 indicates that the phage has great affinity for HSQ, but little affinity for ZEP and UV113. For this reason, ZEP and UV113 were chosen for future experiments.

To functionalize these nanofluidic devices, it is also important to determine if the phage can selectively bind PZT in the presence of other materials. For this reason, Au electrodes were deposited onto sol gel PZT films, and these structures were modified with bacteriophage expressing the TAR-1 peptide and labeled with antibody and a fluorescent tag (Control experiments have previously shown that the fluorescent tag used in these experiments can bind to Au. To prevent this, the sample was first treated with 1 mM dodecanethiol). Figure 4A shows an optical micrograph of this system. In Figure 4B it can be clearly seen that the PZT is fluorescent while the Au is nonfluorescent, indicating the phage is preferentially binding the PZT.

3.2 Integration of Biofunctionalization and Nanofluidic structures

The next step is to test the selectivity of the PZT specific peptide in a system where the resist channel is fabricated over the gold electrodes. Figures 5 and 6 show details of the fabricated structures. They illustrate how the electrode is buried under the ZEP520 resist and that only the areas where there are trenches in the resist are the gold electrodes and the PZT surface exposed to the buffer with the PZT specific peptide. Once the structure was fabricated, the sample was immersed in the buffer solution for more than 24 hrs. While in the liquid, a fluorescence image at 520 nm wavelength was obtained, Figure 7, of a structure with 500 nm wide trenches, the same one shown in Figures 5 and 6. We observed the same intensity in the narrower trenches down to 250 nm.

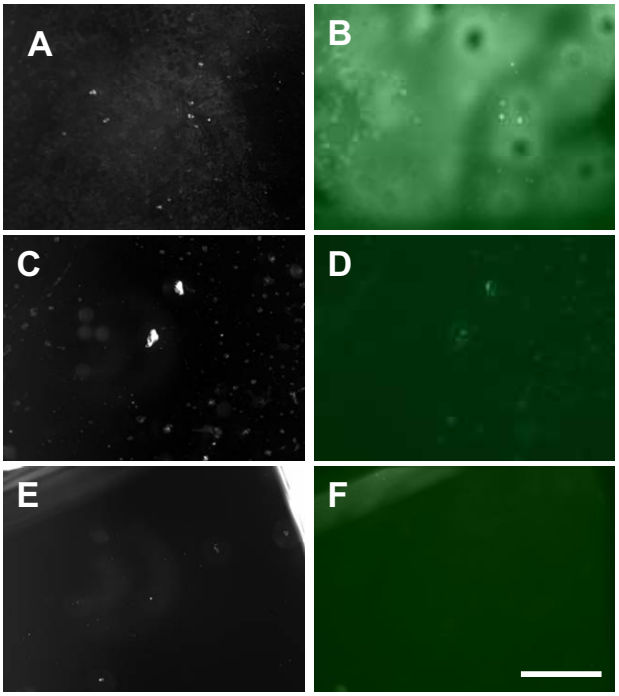

Figure **3**: Optical micrograph images of photoresist samples treated with bacteriophage expressing the PZT-sensitive specific peptide TAR-1, including a bright field image (A, C, E) and fluorescence image (B, D, F) for the photoresists HSQ (A & B), UV113 (C & D), and ZEP (E &F). The scale bar in F is 10 mm, and all images are taken at the same magnification.

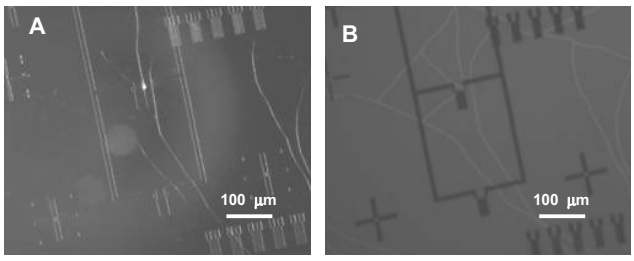

Figure **4**: Optical micrographs of sol gel prepared PZT films modified with Au electrodes and functionalized with bacteriophage expressing the TAR-1 peptide, including bright field (**A**) and fluorescence (**B**) images.

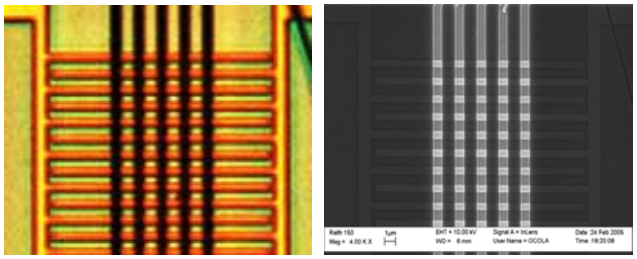

Figure **5**. Interdigitated electrodes with trenches in ZEP520 patterned over. (Left) optical micrograph showing both buried electrodes under the ZEP520 resist and the trenches. ZEP520 is transparent to light. (Right) SEM micrograph of the same electrodes. ZEP 520 is not transparent to electrons so only trenches are visible.

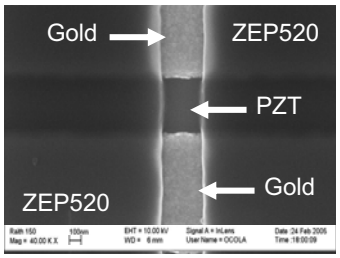

Figure **6**. Details of SEM micrograph shown in Figure 5. Micrograph shows two gold electrodes, one on the top and one on the bottom of the image. The ZEP 520 resist layer is covering the electrodes and the PZT layer with exception of the open trench in the middle.

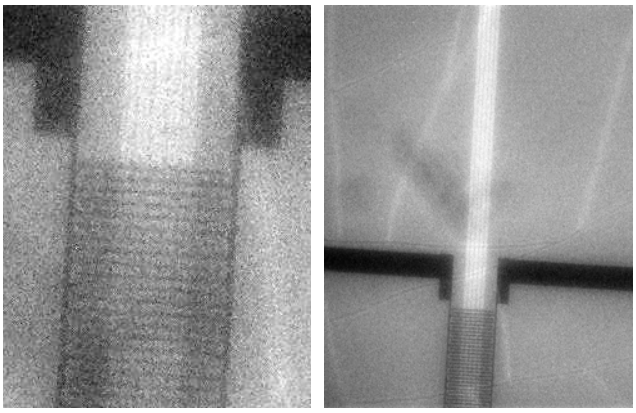

Figure **7**. Fluorescence images of functionalized sol-gel PZT surfaces. Functionalization is restricted to patterned trenches in a ZEP520 top layer, 500 nm wide, that overlap gold interdigitated electrodes.

4 SUMMARY AND CONCLUSIONS

In summary, this paper shows results of an ongoing research project to integrate a heptapeptide into a nanofluidic system. In order to accomplish this, a multidisciplinary team has been assembled to address multiple aspects of material science, biology, nanofabrication and surface science. The results obtained so far are encouraging and indicate that the device is feasible. Further work is in progress to improve the attachment of the phage onto the PZT surface and to achieve a better understanding of this interaction.

In conclusion, we have demonstrated that PZT is a material that can be used as part of a device that requires high polarizability that is compatible with an aqueous environment. We have also demonstrated that a heptapeptide sequence can be synthesized via phage display techniques to bind specifically to the PZT surface. Finally we have shown initial results of integration of these two aspects into a nanofluidic device with interdigitated electrodes.

ACKNOWLEDGEMENTS

Work supported by the U.S. Department of Energy under contracts W-31-109-Eng-38.

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