Nanofluidic BioMEMS

RLE Group
Micro / Nanofluidic BioMEMS Group

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Overview of group

Nanofluidic BioMEMS group in RLE (Han group) is exploring various ways that the micro/nanofabrication techniques can be used for advanced biomolecule manipulation and separation applications. It is now possible to reliably fabricate nanofluidic gaps and filters that have regular, controllable structures, with near-molecular dimensions (10 -100nm). One can take advantage of these nanofluidic structures for advanced separation and manipulation of various biomolecules and bioparticles, including cell, cellular organelles, DNA, protein, and carbohydrates. The research of Nanofluidic BioMEMS group is currently actively designing, fabricating and testing the new kinds of molecular sieves and filters that can be essential for the next-generation biomolecule assays in the new era of genomics, proteomics and glycomics. At the same time, the subject of molecular stochastic motion and molecular interaction with nanostructure is actively studied, in order to provide firm theoretical and scientific ground for the development of novel nanofluidic molecular filters.

1. Biomolecule Separation in Nanofluidic Filters

Sponsors
National Science Foundation (CTS-0347348)
National Institute of Health (EB005743)
SMA-II FRP-1

Project Staff
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We have recently published a paper on Anisotropic Nanofilter Array (ANA) design for continuous flow protein size separation[1]. In this paper, we demonstrated separation of various molecules (native proteins, SDS-coated proteins, long and short DNA) by utilizing different sieving mechanisms. Structural anisotropy is the key to the high-throughput, continuous flow separation, and also ideally suited for preventing system clogging, since larger molecules are prevented from entering many nanofilters[2]. In this paper, we also demonstrated electrostatic sieving, which separates proteins based on their charge characteristics (pI values).

We are fabricating denser nanofilter array for enhancing separation resolution. This device was fabricated by e-beam lithography, and has ~200nm structural period. E-beam lithography was assisted by visiting graduate students from the collaborating group (Prof. Kim) in Korea University, Seoul, Korea. The fabrication process was fully characterized and finished, and separation experiments are under way. Figure 1 shows the SEM image of fabricated devices. Based on previous estimation, we expect to have 5~10 fold increase in separation speed (with same obtained resolution)[3], which would be better than the current state-of-the-art separation technique (capillary gel electrophoresis).

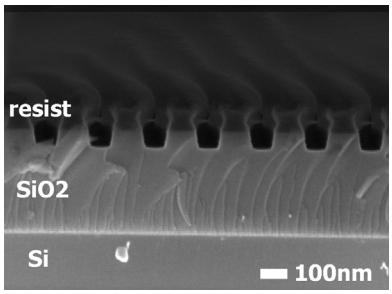

Figure 1: 200nm period nanofilter array (cross section)

Since theoretical model for separation in nanofilter array is essential for future engineering, we have invested significant resources in this direction. We have recently characterized both separation selectivity[4] and dispersion[5] for 1D nanofilter array[3], based on Ogston sieving and other established theories. Developing models for ANA device[1] would be more important, and will likely be possible by numerical (computational) approaches. We expect to achieve these goals in a separate program (SMA-II), while in this program we will mainly focus on device engineering.

2. Nanofluidic Biomolecule Preconcentration for Enhancing Biomolecule Detection

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Project Staff
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Leon Li
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Using the dual-nanochannel design of preconcentration device, we recently realized integration between nanofluidic preconcentrator and bead-based immunoassay[6]. The design of this device (Figure 2) allows one to increase the local biomolecule concentration at the vicinity of primary antibodies, giving the immuno-sensor better sensitivity and faster binding kinetics. With 30 min preconcentration, we were able to enhance the immunoassay sensitivity (with molecular background) by more than 500 fold from 50 pM to the sub 100 fM range. Moreover, by adjusting the preconcentration time, we can tune the dynamic range of the detection of the immunoassay arbitrary covering 6 logs concentration variation (from 100-10,000 ng/ml to 0.1-10 ng/ml), which is critical for multiplexed detection of many target molecules. This was achieved with the existence of simulated 'background' molecules (another fluorescent molecules) at much higher (~1000x) concentration. Binding signal obtained from these experiments was not affected by the existence of background molecules, which suggests that direct detection from complex mixture (such as serum) could be possible.

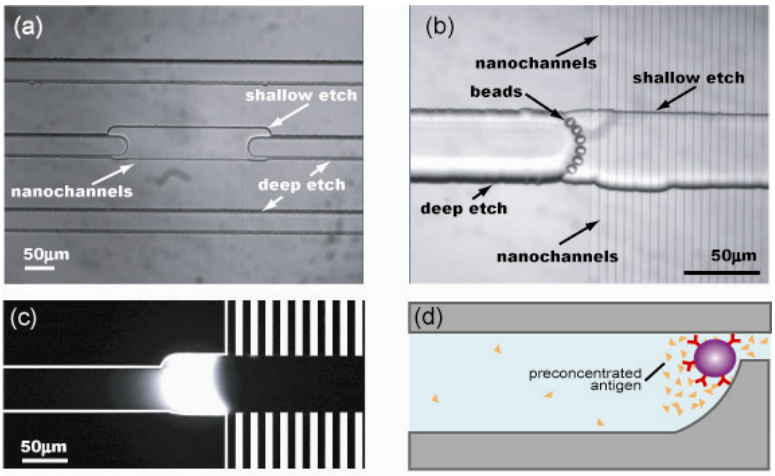

Figure 2: Enhancing bead-based immunoassay with nanofluidic preconcentrator. (a~b) Micrograph of channel and beads used for experiments (c) Fluorescent signal from concentrated protein plugs (d) schematic diagram of integrated bead-based immunosensor (e) Binding reaction progress at different target concentration. Binding reaction kinetics are very slow at concentrations much lower than K_d of antibody-antigen reaction. (f) Binding curve measured with/without preconcentration steps in the system. Not only the sensitivity of detection is improved, but the binding kinetics are improved yielding much more robust, faster binding reaction. Adapted from ref [6].

We recently developed a novel method to create preconcentrator in PDMS microfluidics platform[7, 8]. By incorporating soft membrane materials (Nafion), we increased the plug volume of the concentrator by several hundred times. This will pave the way for integration with mass

spectrometry and other biosensors, which will be sought in the following years. The key to the increased plug volume is enhancing the nanochannel (membrane) throughput. We are currently studying novel nanochannel fabrication strategy for more efficient preconcentration system. Flow / current / concentration profile measurements in and around of nanochannel yielded many insights about this nonlinear electrokinetic systems, which was (and will be) presented in various venues[9, 10].

3. Fabrication of Nanofluidic Membrane for Biomolecule Separation

Sponsors
KIST (Korean Institute of Science and Technology) - IMC (Intelligent Microsystems Center)

Project Staff
Pan Mao

Nanofluidics has gained tremendous successes in the last few years because they provide unique capability in biomolecular manipulation and control. For nanofluidic applications, one critical issue is the availability of reliable, reproducible fabrication strategies for nanometer-sized structures. A simple technique, without nanolithography or special tools, has been developed to generate planar nanochannels with precise control of depths to the nanometer scale for many applications including separation and preconcentration. However, two big issues with these planar nanofluidic channels are the limited fluidic conductance that results and low throughput.

We have been able to fabricate 25μm deep trenches with a uniform gap size (lateral trench width) as narrow as 55nm throughout the depth of the trench (Fig 1). The aspect ratio (depth-to-width ratio) of vertical nanochannels can be as high as 400. With this technique, we could make much deeper trenches by simply increasing KOH etch time. The ultimate limitation of the maximum depth achievable by KOH etching is yet to be investigated. We expect that such a limitation would not be a serious issue for attaining our target thickness of ~50μm. Hence, we should be able to etch the narrow trenches to a depth of hundreds of micrometers by KOH etching, which will be investigated in the future.

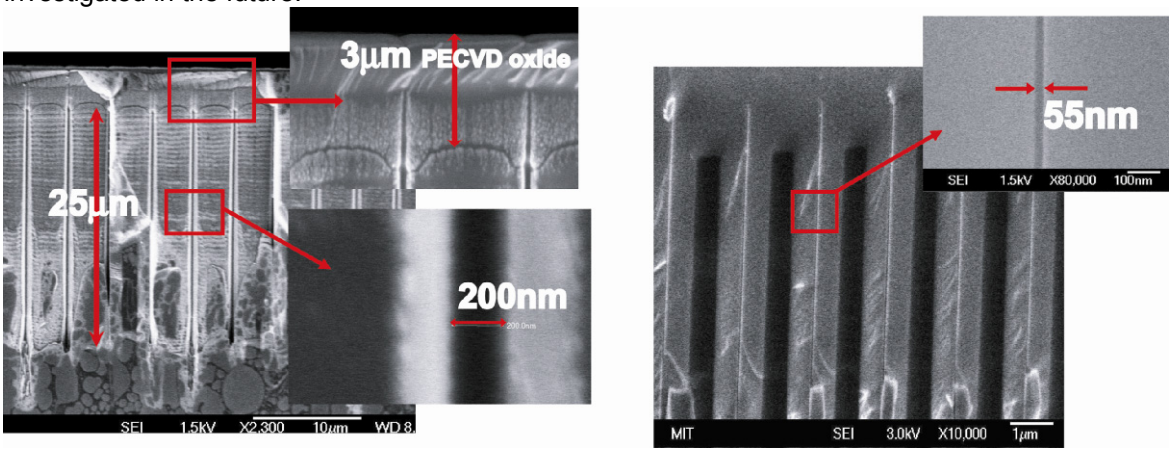

Figure 1 Cross-sectional SEM micrograph of slot-like vertical nanochannels with a gap size of 200nm (left) and 55nm (right). The depths are 25 μm by 15 min KOH etching. The close-up shows complete trench sealing by 3 μm PECVD oxide.

We have also characterized the DRIE process for generating high-aspect-ratio trenches since it has the capability of etching various channel patterns, including squares, circles, and even

irregular geometries. In this project, we use regular pillar array structures for molecular sieving, which can not be done only with KOH etching. By tuning the DRIE parameters, we have been able to obtain 35μm deep channels with an undercut less than 1μm and sidewall angle less than 1° deviation from the vertical direction, as shown in Figure 3. The scallops are less than 20nm in the entrance region and become negligible in the other regions a few micrometer away from the channel entrance. Also, SEM shows rather smooth sidewalls when looking from the side. There is no clear limitation of the maximum depth we can push to with DRIE.

4. Continuous-flow pI-based separation of biomolecules using diffusion potential

Sponsors
KIST (Korean Institute of Science and Technology)-IMC (Intelligent Microsystems Center)
National Science Foundation (CTS-0347348)
Merck-MIT postdoctoral fellowship

Project Staff
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In this work, we have developed a simple microfluidic chip that can sort biomolecules based on their isoelectric point (pI) values in a simple buffer system. The new method differs from previous approaches such as transverse isoelectric focusing or free-flow electrophoresis in that this process involves no external power supply and no special ampholyte. Instead, we utilize the diffusion potential generated by the diffusion of different buffer ionic species in-situ at the laminar flow junction. The use of diffusion potential in microfluidics was previously demonstrated with the mass transport of dye molecules between the two streams. However, they did not explicitly demonstrate a separation of two species. In our device, we establish a laminar flow junction between two buffers with different pH and concentrations. A potential gradient is developed across the liquid junction, generating a high-enough electric field to mobilize and to collect biomolecules at the boundary when their pI values fall between the two buffer pH values. In Figure 1, the MALDI-MS result of the two-step-sorted sample is shown. In this two-step sorting experiment, we tried to collect 1 pH unit fractionation of the sample containing three peptides. Corresponding to the result of the ingle-step sorting, shown in Figure 7, we achieved a decrease of the relative intensity of pI 9.7 after the first sorting step at pH 7.0. From an initial concentration ratio of 5:1:3 for $C_{pI\ 9.7}$:$C_{pI\ 6.3}$:$C_{pI\ 5.1}$, it changed to to $C_{pI\ 9.7}$:$C_{pI\ 6.3}$:$C_{pI\ 5.1}$ = 0.9:1:3.2., which means that the amount of the positive peptide pI 9.7 was reduced by 5.5 times through the first sorting step. The other two negative peptides maintained their initial concentrations. After the second sorting at a lower pH value of 6.0, the ratio was changed to $C_{pI\ 9.7}$:$C_{pI\ 6.3}$:$C_{pI\ 5.1}$ = 0.9:1:0.7. This result demonstrates that the amount of the negatively charged peptide pI 5.5 was reduced by 4.5 times through the second sorting using NaCl as sheath buffer. After these two sequential sorting steps, the relative intensity of the peptide 6.3 has significantly increased from 20% to 100% and therefore it is well visible (or detectable) in the diagram. This exactly corresponds to what we intend to achieve with the two-step sorting of the sample mixture as sample preparation, namely to remove the highly-abundant molecules from the proteomic sample mixture and thus to make the low-abundant molecules detectable for subsequent sensing using mass spectrometry.

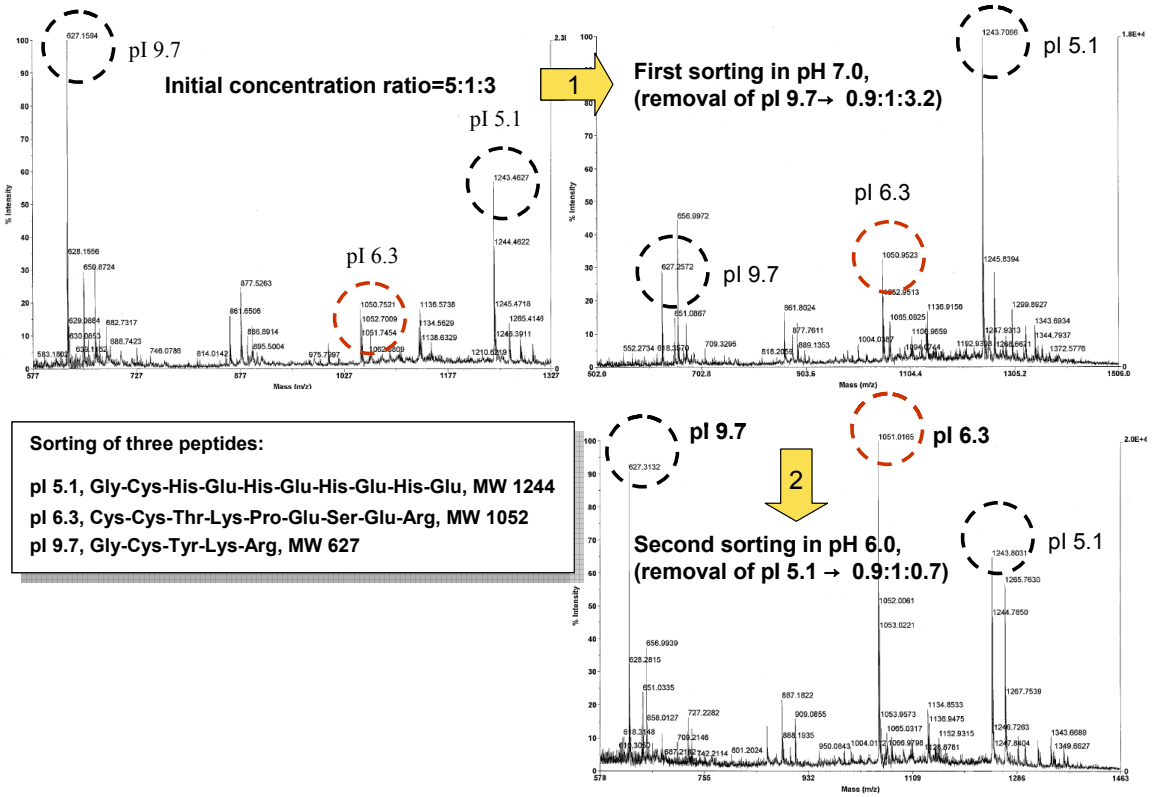

Figure 1: MALDI-MS result for two-step sorting of three peptides (pI 9.7, 6.3 and 5.1). After two sorting steps, the highly abundant peptides have been removed and the low-abundant peptide became visible in the MS diagram.

5. Integrated Microfluidic Device for preconcentration and detection of multiple biomarkers

Sponsors
NIH-NCI (CA119402)

Project Staff
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Phillip Dextras
Kris Payer
Ying-Chih Wang

In this project, we aim to integrate nanofluidic protein preconcentrator with suspended microchannel resonator (SMR) label-free biosensors, as a portable, label-free biomarker detection platform. We are currently developing an integrated nano- and microfluidic system for the parallel detection of multiple cancer biomarkers. The system will be based on the nanofluidic concentrator developed by the Han lab and is expected to achieve a detection limit for biomarker concentration that is significantly lower than what is possible with existing immunoassays such as ELISA and radioimmunoassays. A key attribute of the nanofluidic concentrator design is that the region where biomolecules are concentrated must not be larger than about 10-100 picoliters. In order to measure the concentration of specific biomarkers within this region, we propose to integrate the concentrator with a detector of similar volume in order to avoid dilution. The detection method is conceptually similar to ELISA; however, the readout of the

biomarker/antibody binding is based on the direct detection of mass that absorbs to the surface of a sensor known as the suspended microchannel resonator (SMR). The SMR detector has a volume of 10 picoliters and a mass resolution of near 100 femtograms. Thus, we anticipate that the combined concentrator and SMR detection system will allow a specific biomarker to be detected at a resolution near 1 pg/mL. Since the SMR provides a real-time readout and the concentrator is easily controlled by an applied voltage, the integrated system can operate in a closed-loop mode in which the sample is amplified until the biomarker concentration reaches the vicinity of the disassociation constant of the biomarker/antibody complex. Thus, the linear range of the detector can be dynamically tuned over a wide range of biomarker concentrations.

Since one of the challenges in the project is the complexity of biomarker samples (e. g. serum). Using the recently-developed bead-based preconcentration-immunoassay microdevices[6], we have tested both preconcentration and immunobinding affinity at different background conditions. While immunobinding signal itself was not significantly affected by the serum background, preconcentration of complex, serum sample forced aggregation of majority proteins (albumin) in the preconcentration zone, therefore interfered with enhanced detection. It was determined that removal of most abundant protein species using commercially available affinity columns (albumin removal kits) was effective way to deal with this issue. These kits are known to decrease the concentrations of a few (6-12) of most abundant proteins by ~100 fold. Target molecules in depleted serum were able to bind effectively to the primary antibody, when the abundant proteins were removed from the sample. Continuous, linear increase in binding signal even in depleted serum conditions demonstrates that direct detection might be possible in the integrated device in the future, with only minmum sample fractionation.

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Jianping Fu (ME), "Microfabricated Nanofilter Array Based Devices for Advanced Biomolecule Separation," Ph.D. degree, June 2007.