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Single Cell Chromatography, LDRD Feasibility Study

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Single cell chromatography

LDRD Feasibility Study Final Report

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Introduction

A limitation in the mass spectrometry of biological materials is the reduced ion formation caused by sample complexity. We proposed to develop an enabling technology, single cell planar chromatography, which will greatly increase the amount of chemical information that can be obtained from single biological cells when using imaging mass spectrometry or other surface analysis methods.

The sample preparation methods were developed for the time-of-flight secondary mass spectrometer (ToF-SIMS) at LLNL. This instrument has a measured zeptomole (10^{-21} mole, 600 atoms) limit-of-detection for a molecule with a mass to charge ratio of 225[1]. Our goal was to use planar chromatographic separation to approach similar low limits of detection even with the chemically complex contents of a single cell. The process was proposed to reduce ion suppression and at the same time expose more of the cell contents to the ion beam.

The method of work was to deposit biological cells on a silicon chip with suitable chromatographic and electrical properties, dissolve the cell with a droplet of solvent, allow the solvent to evaporate, and then allow the movement of cell contents laterally by immersing an edge of the chip in to a chromatographic solvent, that then moves through the chromatographic matrix allowing the components to interact with, and be separated by, the chromatographic substrate. This process is a miniaturized version of thin layer chromatography with detection by surface mass spectrometry

Materials and Methods

The proposed work required a microscope platform to deposit nanoliter droplets to test the chromatographic process and for later dissolution of cells. Figure 1 shows the system consisting of a microscope, micromanipulator, and 500 nanoliter syringe with homemade fused silica capillary needle.

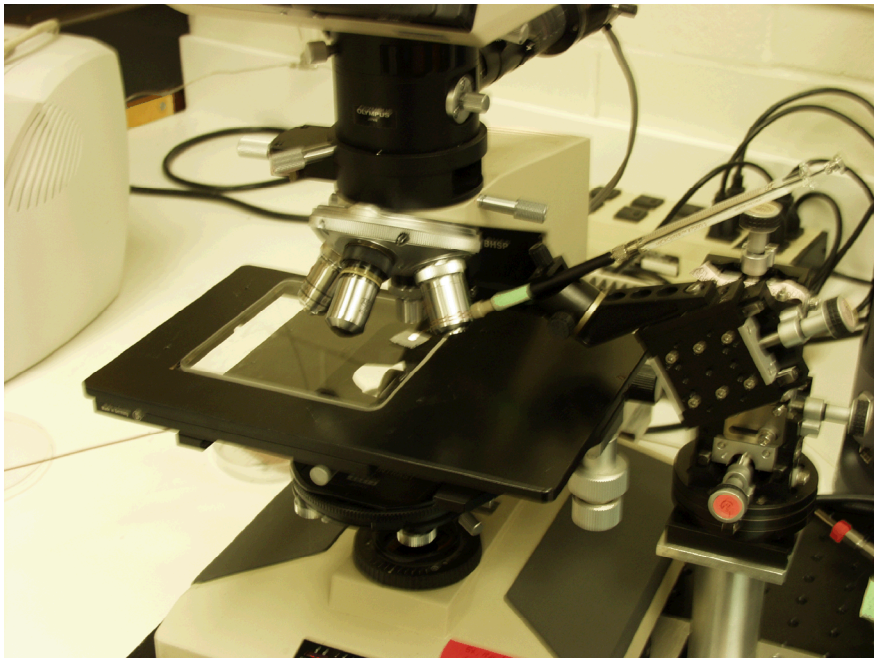

Figure 1. Microscope, micromanipulator, and syringe.

Substrate synthesis

Most of the effort was spent on the development of chips for the chromatographic process. Suitable chips need to have a thin (2-4 micron) macroporous structure on top of a conductive silicon chip. Biological cells have a diameter of 10 to 40 μm, and are about 6 μm thick.

Coating of chips was done by the sol-gel process, using many variations. In this process a solution containing metal-organic compounds grows by polycondensation to form a porous glass. Spin coating onto glass or silicon chips was done, varying the concentration of precursors and the spin rate. Many coated chips were produced, but variations in coating thickness, and nucleation sites resulting in imbedded islands, led to the search for improved methods. Figure 2 shows results from profilometry measurements of a monolithic silica nanocomposite coating, made by the method of Constantin and Freitag, a mixture of tetraethoxysilane and octyltriethosysilane [2]. These results show thickness in the desired range, 5μm, but thickness variation was excessive for good results in the ToF-SIMS instrument with biological cells.

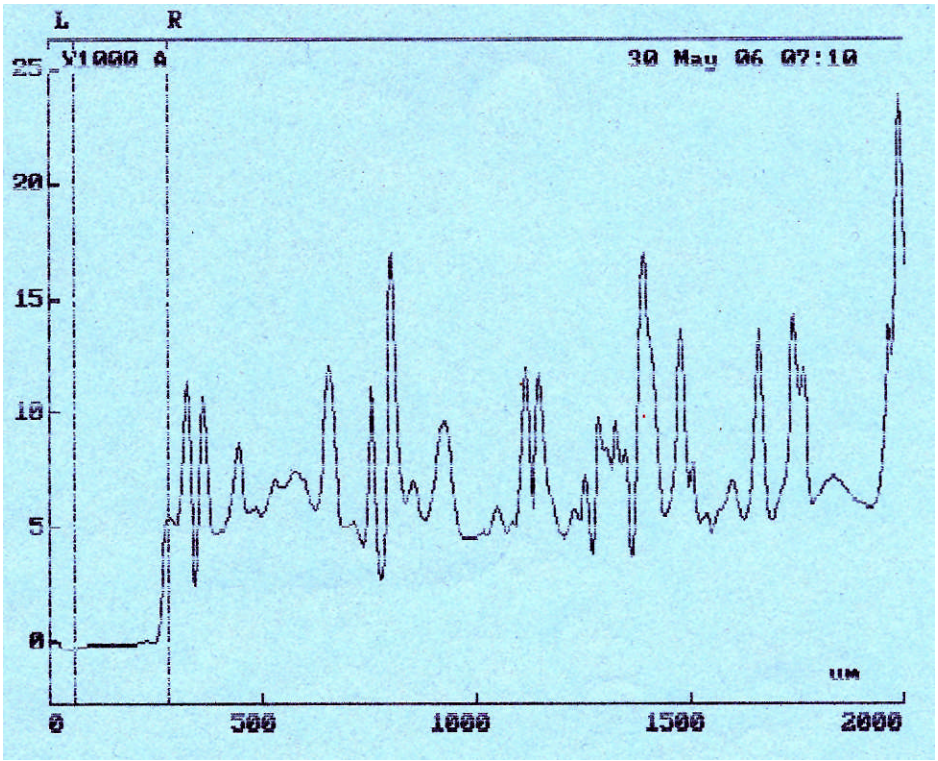

Figure 3. Profilometry plot of silicon chip surface, showing average thickness of 5 microns, but a rough surface with imbedded particles, some over 15 microns in height.

A new method, dip coating of a methyltrimethoxysilane-bromocresol sol-gel [3] was finally successful in producing defect-free areas in the center of chips. Figure 3 shows some non-homogenous chips in the rear, but some thin and suitable chips for testing in the foreground.

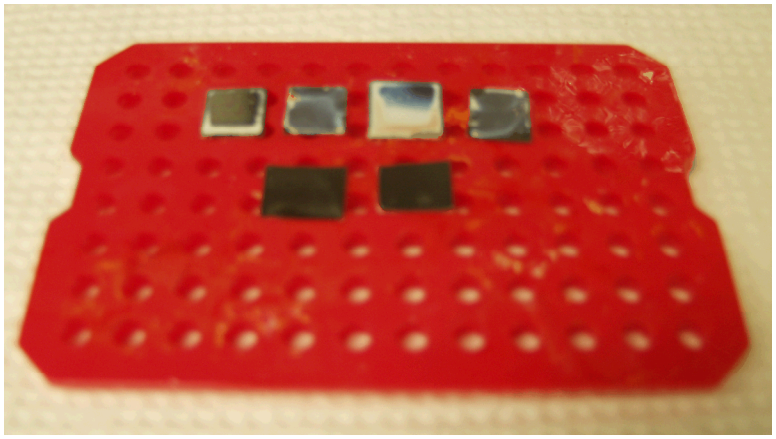

Figure 3. Chips coated by dipping.

We also investigated two other controlled chip types, etched micropillars produced at LLNL, and carbon nanotube coated chips, also produced at LLNL. The micropillar chips were fragile in handling, and did not have the desired solvent transport properties for chromatography, and were not investigated further.

Silicon chips coated with carbon nanotubes were more successful. The surfaces were, very uniform in thickness, and deposition of polar solvents showed the porosity desired for chromatographic behavior.

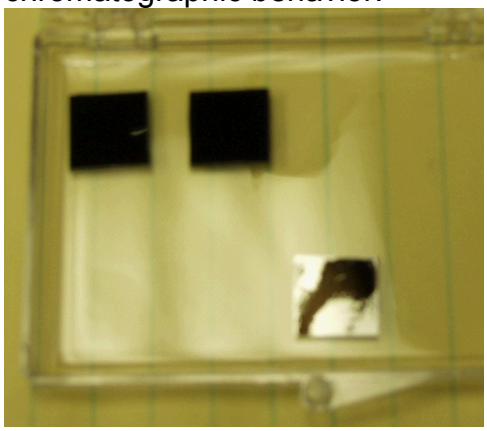

Figure 4. Silicon chips coated with carbon nanotubes. Chip in lower right shows most of the nanotube matrix detached as a result of immersion in water.

The adhesion of the nanotubes to the silicon is a problem, making them fragile to handling and making it difficult to add the aqueous cell suspensions necessary for the ultimate goal of depositing and growing cells on them.

Results

Chromatographic behavior of manufactured chips

Each chip type was investigated for the movement of solvents used for the chromatography of the major biological materials like lipids and peptides. Typical solvents were mixtures of ether, methanol, chloroform. Solvent movement was observed by dipping an edge in a glass chromagography jar with a saturated atmosphere as well as by observing the behavior of liquids applied with the syringe under a microscope. It was determined the micropillar chips did not have useful porous characteristics to move solvents, but the dip cast coated chips and those with carbon nanotubes were suitable.

ToF-SIMS of chemical mixture applied to a carbon nanotube chip

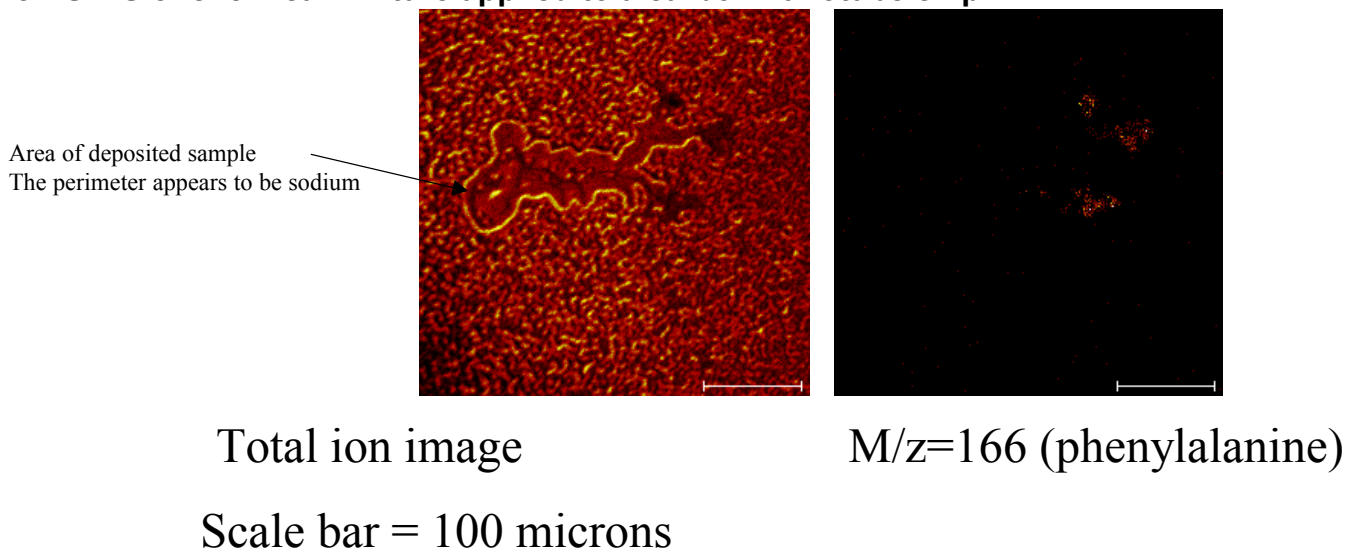

Figure 5. Left: Total ion image of carbon nanotube surface with solution of phenylalanine added. Droplet perimeter can be seen as sodium ion signal, left. Right: plot of mass to charge 166, the observed ion from phenylalanine. Ions moved to the right by dipping the left edge in ether/chloroform/methanol 20:70:10.

Growing cells on the coated chips

Common laboratory-cultured MCF-7 cells were deposited upon coated chips and grown in standard laboratory culture-ware with no indication of reduced viability. Cells attached and looked normal in just a few hours. Before use, chips with attached cells were rinsed in phosphate-buffered saline (PBS) and then in briefly immersed in water and immediately blown dry with a stream of argon gas. This procedue produced chips with numerous attached cells free of the salts associated with the cell culture medium and the PBS.

Dissolution of cells

Another needed step for single cell analysis is the dissolution of the cell and cell contents to allow the chemical constituents to be deposited within the porous chromatographic material. A goal here is to dissolve the cell using as little volume as possible to keep the cell contents concentrated. Solvents and solvent mixtures were deposited via microsyringe on the surface of cells grown on the chip coating. Mixtures of dimethyl sulfoxide and water (50:50) dissolved the cells and had minimal spreading. The deposition of droplets on the scale of mammalian cells (about 20 microns diameter) was difficult with our apparatus, droplets were typically 2-3 times

the cell diameter. Figure 5, left panel demonstrates the movement of solvent within the nanotube mesh area of the original sample, but not further, with this solvent system.

Conclusion

We demonstrated the synthesis of thin macroporous chips suitable for chromoatography, a platform the dissolution of mammalian cells placed on these. Experiments in the ToF-SIMS instruments showed poor ion formation from the primary ion beam on a porous surface, reducing the gains expected from dissolving and concentrating the cell contents.

Future work in this area could overcome the porous surface problem by transferring cell contents to a new chip by flash sublimation, a method shown to efficiently transfer biological molecules with minimal degradation [4-6].

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