

# Silicon-based microdialysis chip with integrated fraction collection

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**Abstract—** A silicon-based microdialysis chip that integrates sample acquisition and fractionation is demonstrated in this paper. A porous cellulose acetate film, created using the phase separation process, is suspended over silicon microchannels. Microfluidic fractionation is accomplished with passive microfluidic valves that are used to direct the dialysate into microfabricated collection chambers.

## I. INTRODUCTION

Real-time and continuous sampling of specific biomolecules provides an important and convenient means for monitoring individuals prone to high health risks. Point-of-care medical devices are preferred due to their fast diagnostic monitoring, which can improve medical decision-making. Microdialysis involves the use of a porous material to recover biomolecules from tissue. It is a sampling method for studying localized metabolic events by monitoring the chemistry of the extracellular space around an *in vivo* point of interest. The outflowing fluid from a microdialysis probe, called the dialysate, is usually collected in a fraction collector for analysis.

Conventional microdialysis probes are hand assembled, have limited useable lifetime, and can't provide the necessary temporal resolution required for external analytical tools due to large dead volume issues. Traditional fraction collectors use a series of tubes, which are sequentially filled with analyte based on a certain timing sequence. The drawbacks of timed fraction assay techniques primarily stem from the lack of integration between fraction collection and real-time sensing [1]. Current fraction collectors are bulky, measuring tens of centimeters or more in each dimension, and expensive, costing hundreds to thousands of dollars. These drawbacks lead to a lack of portability, in hospital settings this restricts patient freedom.

Microfluidic technology represents a revolution in laboratory experimentation, bringing the benefits of miniaturization, multiplicity, and automation to various industries such as genomics and proteomics research.

Miniaturized analytical instrumentation is attracting growing interest due to the potential for enhanced sensitivity and speed as well as the large reductions in overall size and weight [2]. Researchers have been exploring the possibility of using miniaturization in microdialysis systems to add integrated functionality (e.g. fluid pumping, analyte sensing, fraction collection), batch fabrication capabilities using semiconductor technology, as well as inherent advantages with using smaller devices [3][4].

The majority of microfabricated separation devices have been obtained by the integration of pre-fabricated polymeric membranes into the final device [5][6][7]. A monolithic microdialysis chip is presented that integrates fraction collection alongside a semi-permeable membrane. The microchannel fabrication technology allows porous cellulose acetate to be coated over silicon microchannels using a spin coat deposition. The design keeps the cellulose acetate from entering the channel and thus enables formation of a suspended membrane. Standard fabrication processes are used, preventing the need for adhesives, substrate bonding, or other complex assembly procedures.

By exploiting surface tension effects due to a hydrophobic coating, dialysate fills a set of collection chambers in a predetermined sequence using a passive valving technique. On-chip fraction collection is attained on the backside of the microdialysis chip using a set of three deep-etched silicon chambers (6  $\mu$ L in volume each), which are coated with a hydrophobic polymer. An eventual application involves integrating immunoassays directly onto the microfabricated collection chambers, allowing seamless integration of fraction collection and sensing.

## II. MICRODIALYSIS CHIP FABRICATION

### A. Cellulose Acetate Lacquer

Cellulose acetate (CA 394-60S & 398-3, 50%/50% mixture, Eastman Chemicals) was dissolved in the solvent n,n-dimethylacetamide (DMAC, Sigma Aldrich) at 10%

concentration. Additional components to the lacquer include 10% poly(ethylene glycol) (PEG 600, Sigma Aldrich), which is used as a pore former and hydrophilic agent, and 10% distilled water, which assists in the phase inversion technique. All concentration percentages are w/w.

The cellulose acetate powder was dried at 80°C for at least 24 hours in a convection oven to remove any traces of moisture. After all components were weighed and mixed, the solution was placed on rotary mill until the polymer was fully dissolved in the solvent, usually taking 2-3 hours. After adequate mixing, the homogeneous mixture was allowed to stand for at least 2 hours in a closed container to remove any air bubbles.

### B. Microchannel and Fraction Collector

The fabrication process is outlined in Fig. 1. Four inch diameter, double-polished, <100> oriented, n-type, 510 μm thick prime wafers with 2 μm of thermally grown silicon dioxide were used. The fabrication process begins with a lithography step that defines the location where a set of six 80 μm wide microchannels, arranged side-to-side, will be created. A backside aligner (Karl Suss MA6) was used to perform lithography on the backside, defining the area where the fraction collection chambers and fluidic inlet port are located. A 1.3 μm thick layer of photoresist (Shipley S1813, Microchem Corp.) was used for the patterning. The oxide layers were wet etched using a 6:1 buffered hydrofluoric acid (BHF) bath for 20 min. (etch rate ~100 Å/min) (step 1, Fig. 1).

Platinum microbridges of 5 μm width and spacing are patterned on the frontside using a lift-off process (step 2, Fig. 1). Using another contact aligner (Karl Suss MA56), the wafer was aligned to the oxide pattern. The same photoresist brand and coating thickness was used for both front and backside patterning. The wafer was then loaded into a vacuum chamber and DC magnetron sputtered with 50 Å of titanium (100 W, 5 mTorr, 30 sec.) and 0.1 μm of platinum (75 W, 17 mTorr, 2.5 min.). The titanium layer was used as an adhesion layer for the platinum. The wafer was then placed in an acetone bath for 1 hour and then ultrasonically agitated for 5 minutes to lift-off the photoresist layer.

Deep reactive ion etching (DRIE, Surface Technology Systems) was performed on the backside of the wafer until 30 μm of silicon was left (step 3, Fig. 1). The DRIE process parameters were set to a 12 sec. etch cycle time (130 sccm SF<sub>6</sub>, 13 sccm O<sub>2</sub>, 23 mTorr chamber pressure, 600 W coil power, 20 W platen power), and 8 sec. passivation cycle time (85 sccm C<sub>4</sub>F<sub>8</sub>, 12 mTorr chamber pressure, 600 W coil power, no platen power). The etch rate of the system varied between 2.0-2.3 μm/min. In the same process chamber, the wafer was then coated with a hydrophobic polymer coating by solely using the C<sub>4</sub>F<sub>8</sub> fluorocarbon gas (85 sccm C<sub>4</sub>F<sub>8</sub>, 12 mTorr chamber pressure, 600 W coil power, and no platen power). The wafer was mounted onto a carrier wafer using thermal release tape in order to provide better mechanical support during processing and also periodically inspected during the etching process to ensure the appropriate silicon etch depth. After the processing was finished, the wafer was placed on a hot plate to release it from the carrier.

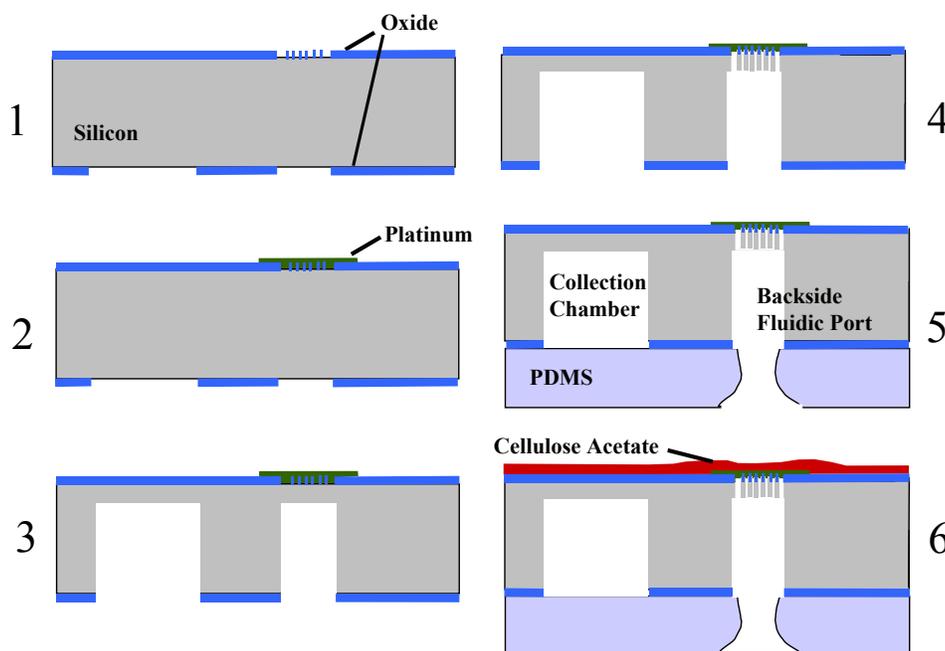


Figure 1. Schematic showing the fabrication of the microdialysis chip. (1) Patterning of front and backside oxide layer, (2) liftoff of platinum metal, (3) DRIE of backside, (4) DRIE and isotropic etch of frontside, (5) Bonding PDMS to backside, (6) Spin coat of cellulose acetate on frontside.

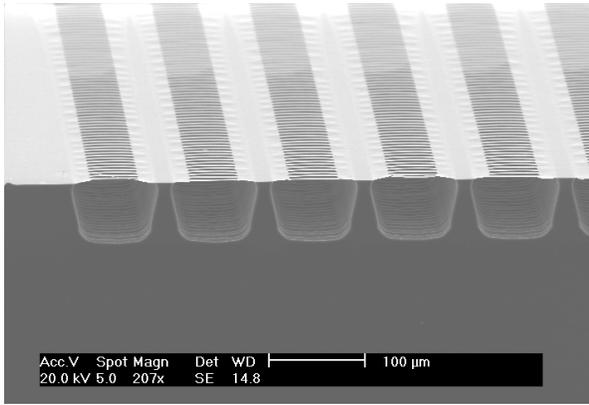


Figure 2. SEM micrograph showing a set of silicon trenches (each 100  $\mu\text{m}$  wide x 75  $\mu\text{m}$  deep, spaced 20  $\mu\text{m}$  apart) with platinum microbridges (5  $\mu\text{m}$  width and spacing) serving as the ceiling.

In step 4 of Fig. 1, formation of the frontside microchannels and completion of the through-wafer connection to the fluidic inlet port was made with a 25 min. DRIE, using the same parameters as on the backside etch, and an 8 min. isotropic etch (130 sccm SF<sub>6</sub>, 50 mTorr chamber pressure, 600 W coil power, 12 W platen power). This process releases and undercuts the platinum microbridges as shown in Fig. 2. These microbridges support the porous cellulose acetate film that gets coated over the microchannel region.

### C. Fluidic Interconnect and Polymer Coating

Substrates were dehydrated at 100°C in a convection oven to remove any moisture from the surface. The backside silicon surface and a PDMS sheet was cleaned and activated in an oxygen plasma chamber (PEIIA, Technics RIE) for 10 min. and subsequently bonded to each other (step 5, Fig. 1). The PDMS sheet has a hole punched into it to accommodate fluidic tubing. The frontside of the substrates were then exposed to an oxygen plasma for 10 min., removing organics

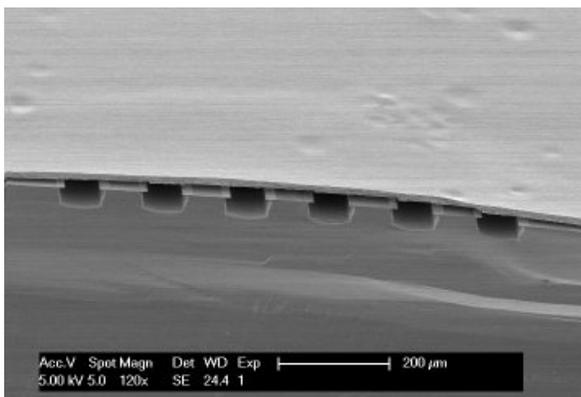


Figure 3. (Top) SEM micrographs showing the cross section of a set of six silicon microchannels trenches (each 80  $\mu\text{m}$  wide x 80  $\mu\text{m}$  deep, spaced 80  $\mu\text{m}$  apart) with suspended 10  $\mu\text{m}$  thick cellulose acetate membrane.

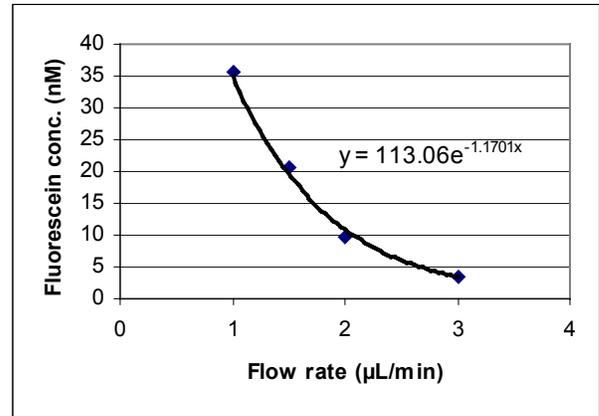


Figure 4. Plot showing the permeability of the cellulose acetate membrane to a 65 nM fluorescein solution for various flow rates. The governing equation of the trendline is shown on the plot.

from the surface and exposing hydroxyl groups. To assist in the adhesion of the polymer, a self-assembled monolayer was created on the surface by placing 2% (v/v) solution of octadecyltrichlorosilane (OTS) in anhydrous isopropanol on the substrate for approximately one hour. Substrates were then rinsed with acetone and isopropanol and dried at 100° C for 15 minutes.

The polymer lacquer is spin coated at 4 kRPM for 6 sec. at maximum acceleration (20 kRPM/s) over the frontside, coating the platinum microbridges yet not filling into the silicon trenches [8]. The substrate was then immediately placed in a room temperature, deionized water bath for at least 2 hours to remove the solvent in the lacquer, precipitate the polymer, and form a 10  $\mu\text{m}$  thick porous membrane. Spanning the cellulose acetate lacquer across the silicon microchannels is dictated by the physical properties of the lacquer and the substrate surface. An example set of parallel microchannels is shown in Fig. 3

## III. CHARACTERIZATION

### A. Permeability

A small well was made using silicon rubber above the microdialysis chip and then filled with 200  $\mu\text{L}$  of a 65 nM fluorescein solution. Fluorescein (molecular weight 332 Daltons) provides a method to quantify concentration based on measuring the fluorescence using a spectrofluorimeter. The chip was connected to a syringe pump through the inlet port and supplied a phosphate buffered saline (PBS) of pH 7.4 at varying flow rates. The dialysate was directed into a centrifuge tube and a fluid volume of 0.5 mL was collected to perform fluorimetric analysis. Having one side of the membrane with fluorescein and the other side with a buffered saline perfusate creates a concentration gradient. This drives the fluorescein molecules across the polymer membrane and into the chip. By controlling the flow rate, the fluid volume and concentration of the collected dialysate will vary as shown in Fig. 4.

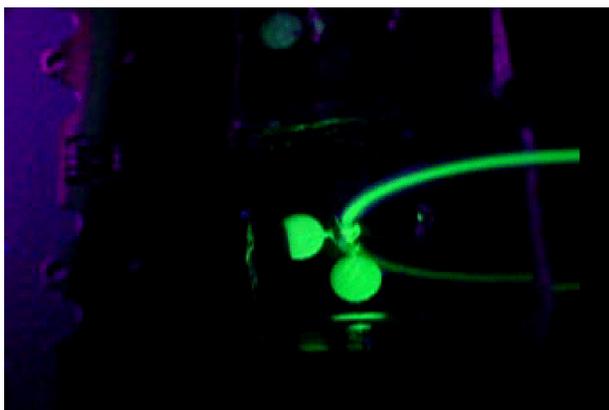
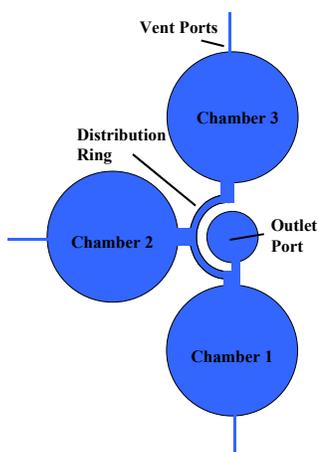


Figure 5. (Top) Schematic drawing of the fraction collector design. Each chamber is 4 mm wide x 480  $\mu\text{m}$  deep, creating a 6  $\mu\text{L}$  volume. (Bottom) Photograph showing collection chambers sequentially filled as fluorescein is pumped into the backside of the chip.

### B. Fraction Collection

A set of three collection chambers with a volume of 6  $\mu\text{L}$  each were designed in the pattern shown at the top of Fig. 5. As the dialysate exits through the outlet port and enters the beginning of the distribution ring, it encounters the entrance throat of chamber 1. Since the cross-sectional dimension of this throat region is larger than that of the distribution ring, the pressure barrier is smaller and the dialysate then enters the first collection chamber. Once a chamber fills, pressure build-up causes the dialysate to continue flowing around the distribution ring until the next entrance throat is approached. The bottom of Fig. 5 shows chamber 1 completely filled and chamber 2 being filled as a concentrated fluorescein solution is pumped into the microdialysis chip.

Small air vent ports are placed at the opposing end from where dialysate enters the collection chamber. As the dialysate fills the chamber, trapped air within the chamber is expelled through this port. Since these vent ports are 20  $\mu\text{m}$  wide, they have a small cross-sectional dimension and thus have the largest pressure barrier, not allowing fluid to leak during the filling process.

To observe the fraction collection capability of the chip, PBS buffer is pumped at a low flow rate of 0.2  $\mu\text{L}/\text{min}$ . After chamber 1 is filled (30 min. elapsed time), a 65 nM fluorescein solution is placed on the well above the cellulose acetate membrane and then removed immediately after chamber 2 is filled (1 hr. elapsed time). After all three collection chambers are filled, a sharp needle is used to puncture the PDMS and remove the dialysate. Table 1 shows the fluorescein concentration of each collection chamber. The highest concentration is in chamber 2 since that is when the fluorescein solution was added to the microdialysis chip.

TABLE I. FLUORESCENIN CONCENTRATION AT EACH CHAMBER

Collection Chamber	Fluorescein Concentration
Chamber 1	0 nM
Chamber 2	53 nM
Chamber 3	13 nM

### IV. CONCLUSION

A silicon-based microdialysis chip is presented that demonstrates acquisition and fractionation of a fluorescein sample solution. The permeability of a porous, phase-separated cellulose acetate membrane suspended over etched silicon microchannels is shown for various flow rates. Automated fraction collection is accomplished on-chip using passive microvalves that allow accurate temporal acquisition of analyte samples.

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