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A planar microfabricated fluid filter

James P. Brody [a,*], Thor D. Osborn [a], Fred K. Forster [b], Paul Yager [a]

[a] *Center for Bioengineering, Box 352141, University of Washington, Seattle, WA 98195-2141, USA*

[b] *Department of Mechanical Engineering, Box 352600, University of Washington, Seattle, WA 98195-2600, USA*

Abstract

Many blood tests must be performed on plasma without cellular matter present. In the standard laboratory protocol, pure plasma is obtained through centrifugation. In order to produce a miniaturized blood sensor, a method to separate plasma other than centrifugation is needed. We describe the design, fabrication, and testing of a fluid filter that fulfils this need, and also has some features of general interest. Results from a particular device show that we can easily remove 16 μm diameter spheres from the fluid. This filter can be reusable, can potentially remove particles as small as 0.1 μm, and is easily fabricated.

Keywords: Fluid filters; Microfluidic; Surface tension

1. Introduction

Chemical analysis of biological samples is constrained by sample size. Withdrawing a few milliliters of blood from an adult may have little effect, but repeating this procedure every hour or even withdrawing this amount once from an infant can significantly alter the health of the subject. For these reasons, a miniaturized blood analysis system would be useful. Furthermore, while many sophisticated tests that have great importance for critical care can be performed in major hospital laboratories, a substantial impact could be made on the practice of emergency medicine if some key tests could be performed on the patient at the site of injury.

As part of a program for the development of a miniaturized portable system for monitoring the chemistry of blood, we are using silicon microfabrication to produce minimally sized optical cells for monitoring fluids. For some assays it is vital to make measurements in the absence of red blood cells, so some form of separation of cells from plasma is required. This paper describes a microfabricated filter that provides a plasma sample of a few nanoliters starting from a microliter of whole blood. This filter can be an element in an integrated system of microfluidics and optics for tests of medical interest on blood, but also has applications in many other areas of analytical chemistry.

At least two other microfabricated fluid filters exist in the literature [1,2]. Neither of these is appropriate for our needs. We require, first, that there is a method for carrying away blood cells from the filter, allowing reuse, and second, that the device is easily integrated into our system.

Stemme and Kittilsand [1,3] used a technique to filter out particles as small as 50 nm, but the design cannot be fabricated on the surface of a wafer as it requires flowing through the wafer. For gases, their filter seems to perform well, but surface tension caused problems when filtering liquids [4]. We avoid this problem by using the selectivity of the etch along the {100} planes of silicon to provide a gradual transition from a sub-micrometer opening to a larger one.

Wilding et al. [2] designed and tested a filter as a small part of their pioneering work demonstrating the manipulation and flow of blood in a microfabricated device. They use a more conventional approach to filtration. Their minimum dimension is determined by a photolithographic process, and so they have a limit of about 1 μm. This is more sensitive to defects and requires tighter constraints on manufacturing than a process that relies on the etching time to define the size.

Recently we have constructed and tested a microfabricated device that provides a separation mechanism based on diffusion. This device brings two flow streams, one containing particles and the other a dilutant, into contact and then separates them. At low Reynolds numbers, this can be done without any gross mixing of the fluid. However, mixing by diffusion does take place. Since the diffusion constant is a function of the linear size of a particle, separation based on size is achieved. This device can easily separate 0.5 μm particles from a fluid and should also work on protein-sized

* Corresponding author. Phone: +1 206 616 1928. Fax: +1 206 616 1984. E-mail: jpbrody@u.washington.edu.

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molecules. This can be achieved with a minimum device dimension of greater than 10 μm.

2. Fluid dynamics

New microfluidic elements need to be designed in order to develop a chemical analysis system at the micrometer scale. A short list of the functions of these elements might be: moving fluid around, separating particles from fluid, mixing two fluids, and heating and cooling fluids.

Two things need to be kept in mind when designing microfluidic elements. First, for a practical device, manufacturing needs to be considered. The number of mask steps and wafer/wafer or wafer/glass bonding steps needs to be minimized. Secondly, size scaling must be considered. Fluid-dynamic behavior is directly related to the Reynolds number of the flow. In microdevices, if the velocity decreases as the linear dimension (the device works in a fixed time at all scales), then the Reynolds number varies in proportion to the square of the length scale. As devices are miniaturized further, the Reynolds number will inevitably be reduced.

The Reynolds number is the ratio of inertial forces to viscous forces. As the Reynolds number is reduced, flow patterns depend more on viscous effects and less on inertial effects. Below a certain Reynolds number, say 0.1 (based on lumen size for a system of channels with bends and lumen-size changes), inertial effects can essentially be ignored [5]. Ideally, a microfluidic device will not require inertial effects to perform its tasks, and will therefore have no inherent limit on its miniaturization due to Reynolds number effects. Our filter design, while significantly different from previous reported designs, operates in this range.

2.1. Design constraints

The system constraints are that the device needs to provide a few hundred picoliters of plasma within a few seconds. It also needs to be reusable, so that clogging should be minimized and reversible. The sizes and velocities (100 μm wide and 100 μm s^{-1}) indicate a Reynolds number ($Re=\rho lv/\eta$) of about 10^{-2} so that the fluid is in a regime where viscosity dominates over inertia.

2.2. Poiseuille's law

The magnitude of the pressure drop needed to obtain an average velocity, v, of a fluid with absolute viscosity, η, and density, ρ, through a circular channel (length, l, diameter, d) can be calculated from Poiseuille's law [6],

$$\frac{P}{l}=\frac{32\eta v}{d^2}$$

Using $v=100$ μm s^{-1} and $d=100$ μm, we get a pressure drop equivalent to about 0.3 mm H_2O per cm of channel length. Since Poiseuille's equation is only strictly valid for circular flow channels and our channels are V-shaped grooves, it can only be considered as an approximate relation between the variables represented.

2.3. Surface tension

When a liquid is introduced into a device there is at first an effective pressure, $P_{\text{eff}}=P_0+P_{\text{st}}$, equal to the sum of the applied pressure, P_0, and a force with an equivalent pressure due to the surface tension [6],

$$P_{\text{st}}=\frac{\gamma\cos\theta}{r}$$

P_{st} is a function of the surface tension of the fluid, γ, the contact angle of the fluid with the surface, θ, and the radius of curvature of the fluid surface, r.

For hydrophilic surfaces, cos θ is close to 1, and for small channels no applied pressure is needed to wet the device. This is referred to as 'wetting by capillary action'. However, once the device is completely wet, one has to worry about the exit area. In our case, the radius of curvature of the fluid in the exit area was several millimeters, so that the pressure due to the surface tension was negligible.

With a channel width of 100 μm, P_{st} is about 1 cm H_2O, so surface tension on the exit channel is significant. However, since the EPW (ethylenediamine: pyrocatechol: water) etch attacks the {100} planes of silicon, the corners as etched are not as sharp as shown in Fig. 1. This results in a gradual widening of the channel to 1 mm, which reduces the effect of the surface tension.

This effect also occurs in the barrier region. Using an etch that gave a vertical (90°) profile, instead of the 55° characteristic of the {100} planes of silicon, would require a pressure as large as one atmosphere to overcome the surface tension in a 0.1 μm gap.

Since this filter design is self priming, it can be operated in two modes. In one-shot mode, a drop (1 μl) of blood contacts the entrance port. The blood would be drawn down

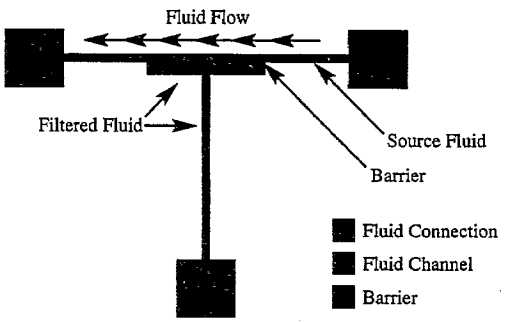

Fig. 1. A schematic drawing of the filter design (not to scale). The fluid enters the device from the top right port and flows to the exit port at left. Some particle-free fluid is pushed across the barrier and down to the filtered fluid exit port. By controlling the pressure drop between the top right and bottom ports, we can control how quickly and how much filtered fluid is drawn out. Shear forces inside the horizontal channel act to prevent clogging of the barrier by particles.

the channel and plasma would be drawn through the filter without any applied pressure. This would provide several nanoliters of plasma within a few seconds. Once fluid filled the device, flow would stop. There would be no way to flush this sample out of the device, and it would have one use.

The second mode, continuous-flow mode, requires an applied pressure head. The relative pressure between the three ports (entrance, fluid exit, and filter exit) can be controlled to provide a continuous stream of filtrate or to induce some reverse flow through the filter, which is useful for removing particles stuck on it.

3. Fabrication

A three-mask-level process was needed to fabricate the device. The first level defined connection ports, which were etched completely through the wafer to the rear side of the silicon. The second level defined the fluid-transport channels, and the third level defined the maximum size of particles that could flow through the filter.

We started with 4″ chrome masks made to our specifications by Photo Sciences, Inc. (Torrance, CA) and 3″ wafers ({100}, n-type) with 500 nm of SiO_2 grown on them.

Wafers were cleaned in a Piranha bath (H_2SO_4:H_2O_2, 2:1) before processing. A primer (HMDS spun on at 3000 rpm) was used to enhance photoresist adhesion. About 1 μm of AZ-1370-SF (Hoechst) photoresist was deposited by spin coating (3000 rpm), and this was followed by a soft bake (30 min at 90°C).

A contact aligner was used to align and expose the wafers. Exposure time was varied to yield best results. No post-exposure bake was done. Wafers were developed in AZ-351 (Hoechst) (diluted 4:1) for 1 min, and rinsed in DI water. Blue tack tape (Semiconductor Equipment Corporation, Moorpark, CA) was applied to the backsides of the wafers to protect the oxide from the oxide etch.

The wafers were immersed in a buffered oxide etch (BOE, 10:1 HF (49%) and NH_4F (10%)) for 11 min to etch away completely the unprotected oxide. The blue tack tape was removed by hand, and the photoresist was removed in an acetone rinse.

Silicon etching was done in an EDP mixture (EPW F-etch) [7] set up in a reflux boiling flask. This etch attacks the {100} planes of silicon at a rate of about 100 μm per hour. Fluid-attachment ports were etched in the first step. Flow channels between fluid ports and the filter region were etched in the second step. The barrier was etched approximately 1 μm in the final step.

After final processing the wafers were once again cleaned in a Piranha bath and rinsed in DI water. They were then diced into individual devices.

We used anodic bonding [8] to attach Pyrex glass to the silicon devices (see Fig. 2). We obtained 1″ square pieces of Pyrex glass (100 μm thickness) from Esco Products Inc (Oak Ridge, NJ). First, the silicon and Pyrex glass were immersed in a solution of H_2O_2, NH_4OH, and H_2O (1:4:6) heated to 50°C. This process removes any organic matter on the surfaces and also makes them hydrophilic. After 20 min in this solution, the silicon and Pyrex were rinsed with DI water and dried. Anodic bonding was done at 400°C with 400 V applied between the glass and the silicon.

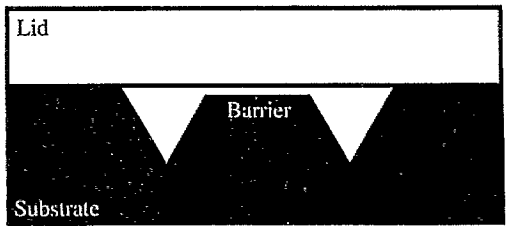

Fig. 2. A cross section of the filter shown in Fig. 1 (not to scale). The channels in the silicon substrate are etched using EPW, an anisotropic etchant. This gives the characteristic V-shaped grooves. This profile is critical to preventing surface tension lock. The barrier is etched in a different step from the channels and can be anywhere from 0.1 μm to a few micrometers from the lid. The lid is Pyrex glass and is attached to the substrate by anodic bonding.

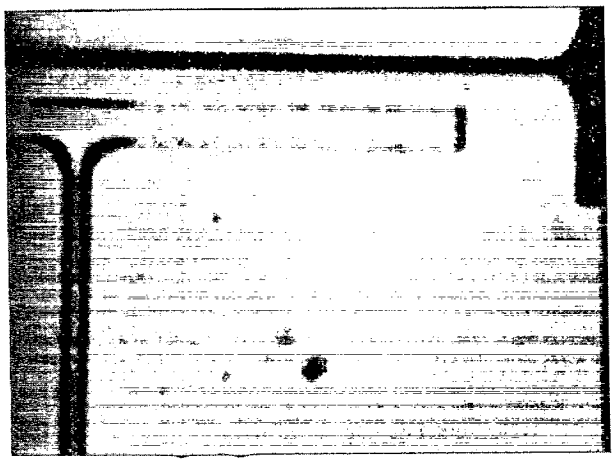

Fig. 3. A reflected light image of one of our devices. Whole blood enters at the top right and flows across the dark channel to the left. (The dark channel is 100 μm wide.) Some plasma crosses the barrier and fills the region at the bottom. A connecting channel carries the plasma down. Compare this with Fig. 1.

A reflected light image of one of the filter devices is shown in Fig. 3.

4. Experiment

Testing was done by flowing dilute suspensions of fluorescent microspheres through the device. We added one drop of 16 μm diameter fluorescing microspheres (1% solids, from Duke Scientific, Palo Alto, CA) to 5 ml of DI water and prepared a similar mixture of 2.6 μm diameter spheres. The 16 μm spheres fluoresce in the green and the 2.6 μm diameter spheres fluoresce in the red, making them easily discernible by eye.

This mixture was introduced into the device, and observations were made using a Zeiss ICM-405 inverted micro-

scope. We observed the fluorescence of the spheres using a silicon-intensified target camera (SIT-66x, Dage-MTI) and recorded the observations on video tape. Some images were also digitized using a frame grabber (Data Translation) and NIH Image software.

The pressures at the inlet port and at the exit port of the filtered liquid were controlled by filling a tube to the appropriate height with liquid. The exit port of the unfiltered liquid was held constant to within a few mm H_2O.

The experiment proceeded by first inserting a mixture of fluid and particles at the inlet port. Once this wet the entire device (one-shot mode), we observed as some particles built up on the edge of the barrier. Typically, some 16 μm spheres would build up along the barrier, but not to any significant depth.

We then added the solution containing 2.6 μm spheres to the inlet and the flow was driven with a pressure head of about 1 cm H_2O. These spheres freely flowed through the channel and some crossed the barrier.

After observing this for some time, we pressurized the filter exit port to about half the pressure being put on the entrance port. This resulted in a backflow across the barrier, quickly forcing the 16 μm spheres back into the flowstream, where they were carried to the exit port.

5. Results

Results are shown in the following digitized images. In Fig. 4, 16 μm fluorescent spheres have just been introduced into the channel. A few flowing from right to left are visible, along with some pressed against the barrier. Note that none has passed through the barrier. In Fig. 5, taken after the introduction of the 2.6 μm spheres, these smaller spheres are easily seen. Most are flowing from right to left, but note that some easily pass through the barrier that is trapping the 16 μm spheres.

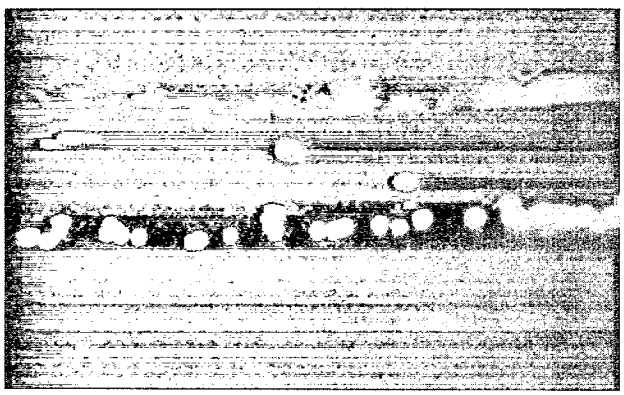

Fig. 4. A fluorescent image of microspheres (16 μm in diameter) flowing from right to left. The channel in this image is about 50 μm deep and 200 μm wide, wider than the device shown in Fig. 3. Note that some of the fluorescent spheres adhere to the barrier across the middle of the image, but none is below this line.

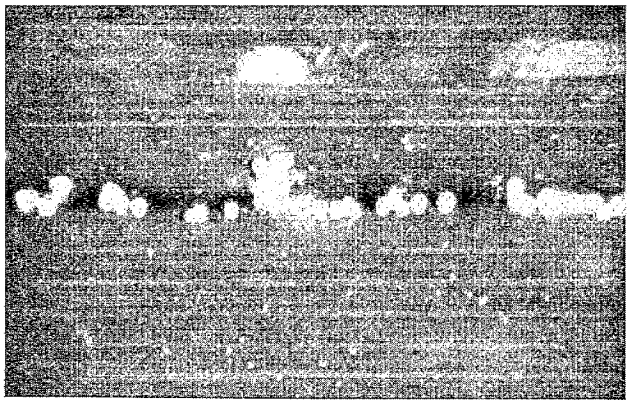

Fig. 5. Fluorescent image after 2.6 μm diameter spheres have been added. These smaller spheres can be seen flowing through the channel from right to left. Note that here, some 2.6 μm spheres go past the barrier into the filtrate area. This filter stops 16 μm spheres and allows 2.6 μm spheres to pass.

6. Conclusions

We have designed, fabricated, and tested a planar microfabricated filter suitable for separating plasma from whole blood.

This filter has several unique features. It uses a sloped profile to provide a gradual transition from the narrow filter region to the wide flow channel. This reduces the pressure needed to overcome the surface tension. It uses a tangential flow to reduce clogging. If particles build up on the filter beyond a certain level, they are carried downstream. Particles can be dislodged from the filter by a slight backpressure.

We estimate that, with slight modifications to support the glass above the barrier, this design could be used for removing particles as small as 0.1 μm from a liquid.

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Biographies

James P. Brody is a research assistant professor at the Center for Bioengineering, University of Washington. He received a Ph.D. in physics from Princeton University in 1994. His research interests lie in developing microfluid systems for studying biological problems.

Thor D. Osborn received a Ph.D. in bioengineering from the University of Washington in 1994. From 1994 to 1995 he was a senior fellow at the Center for Bioengineering. He is now employed at Allied Signal in Seattle, Washington.

Fred K. Forster is an associate professor of mechanical engineering. He received a Ph.D. in aeronautical engineering from Stanford University in 1972. He has been at the University of Washington since 1974, where his research has been in the application of mechanical engineering to medicine with particular emphasis on fluid dynamics and acoustics.

Paul Yager is a professor at the Center for Bioengineering, University of Washington and director of the Microbiosensor Technology Laboratory at the Washington Technology Center. He received a Ph.D. in chemistry from the University of Oregon in 1980. From 1980 to 1987 he was at the Naval Research Laboratory in Washington, DC. In 1987 he moved to the University of Washington, where his research has been in lipid chemistry. He has discovered a number of ways to sense chemically induced shifts in lipid systems and developed these into biosensors. More recently, he has become interested in using silicon microfabrication techniques to develop miniaturized laboratory instrumentation.