Proceedings of 2001 ASME International Mechanical Engineering Congress and Exposition
November 11-16, 2001, New York, NY

An Integrated Microfluidic Device for the Continuous Sampling and Analysis of Biological Fluids

Jeffrey D. Zahn[1,2], Ajay A. Deshmukh[1,3], Alexandros P. Papavasiliou[1,3], Albert P. Pisano[1,3] and Dorian Liepmann[1,2,3]
Affiliation: 1. Berkeley Sensor and Actuator Center 2. Department of Bioengineering 3. Department of Mechanical Engineering
497 Cory Hall University of California at Berkeley
Berkeley, CA 94720
(510) 642-9360, (510) 643-6637, liepmann@me.berkeley.edu

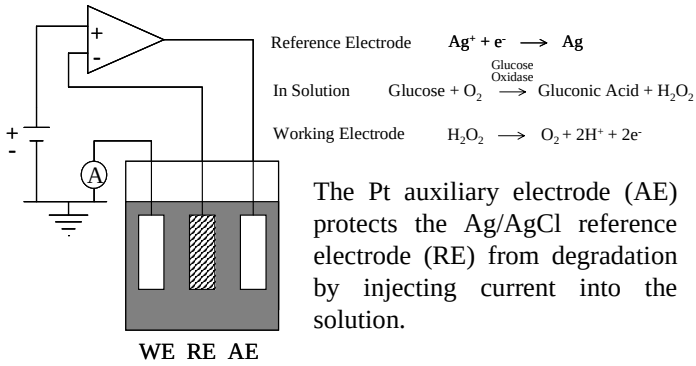
ABSTRACT

Enzyme based biosensors suffer from loss of activity and sensitivity. One major reason is due to large molecular weight proteins adsorbing on the surface of the sensor. These proteins affect sensor signal stability and disrupt enzyme function. Thus, one fruitful way to minimize the loss of sensor activity is to filter out large molecular weight compounds before they come in contact with the enzyme based biosensor. Further, the removal of free protein from biological solution helps to increase sensor accuracy and lifetime. Therefore, a microdialysis microneedle is introduced that is capable of excluding large MW compounds based on size. These microneedles have been integrated into a planar microfluidic system capable of sampling and analyzing biological solutions. The integrated microfluidic system includes the assembly of microneedles with on-chip flow channels and electronics together with previously designed positive displacement micropumps, microvalves and a planar electrochemical sensor for biological detection. Multichannel fluidic control for biological sampling, sensor cleansing and recalibration is demonstrated with integrated sensor operation.

INTRODUCTION

When microneedles are used to sample interstitial fluid, then biosensor stability and lifetime can be extended if metabolites are filtered through a microdialysis needle before fluid is moved onto the sensor. Protein adsorption onto a sensor seriously affects the stability and lifetime of a sensor. For this reason, many commercial enzyme based biosensors (e.g. glucose sensors) are single-use systems. In order to accurately monitor biochemical concentrations, sensors must be calibrated against a standard solution prior to use.

A glucose sensor uses the enzyme glucose oxidase to catalyze the reaction between glucose and oxygen to produce both gluconic acid and hydrogen peroxide (H_2O_2) (Lambrechts and Sansen, 1992). The H_2O_2 is subsequently oxidized at a platinum electrode to generate a current which is directly proportional to the glucose concentration (Fig. 1). If there is a loss of enzyme activity then H_2O_2 will not be produced and the current generated will be lower than it should be, leading to an inaccurate interpretation of the glucose concentration.



The Pt auxiliary electrode (AE) protects the Ag/AgCl reference electrode (RE) from degradation by injecting current into the solution.

Figure 1. (Left) Schematic of a three electrode electrochemical sensor. (Right) Summary of reactions which occur in a glucose sensor. Glucose oxidase is bound to the sensor and a current proportional to the glucose concentration is sensed at the Pt working electrode (WE).

A microfabricated dialysis microneedle (Fig. 2) has been developed which is permeable to small molecular weight (MW) molecules, yet excludes large MW compounds such as proteins. The needle is designed to penetrate just below the stratum corneum (the topmost layer of the skin), so analyte concentrations equilibrate between the interstitial fluid and the dialysis fluid. The concentration of many analytes in interstitial fluid, includ-

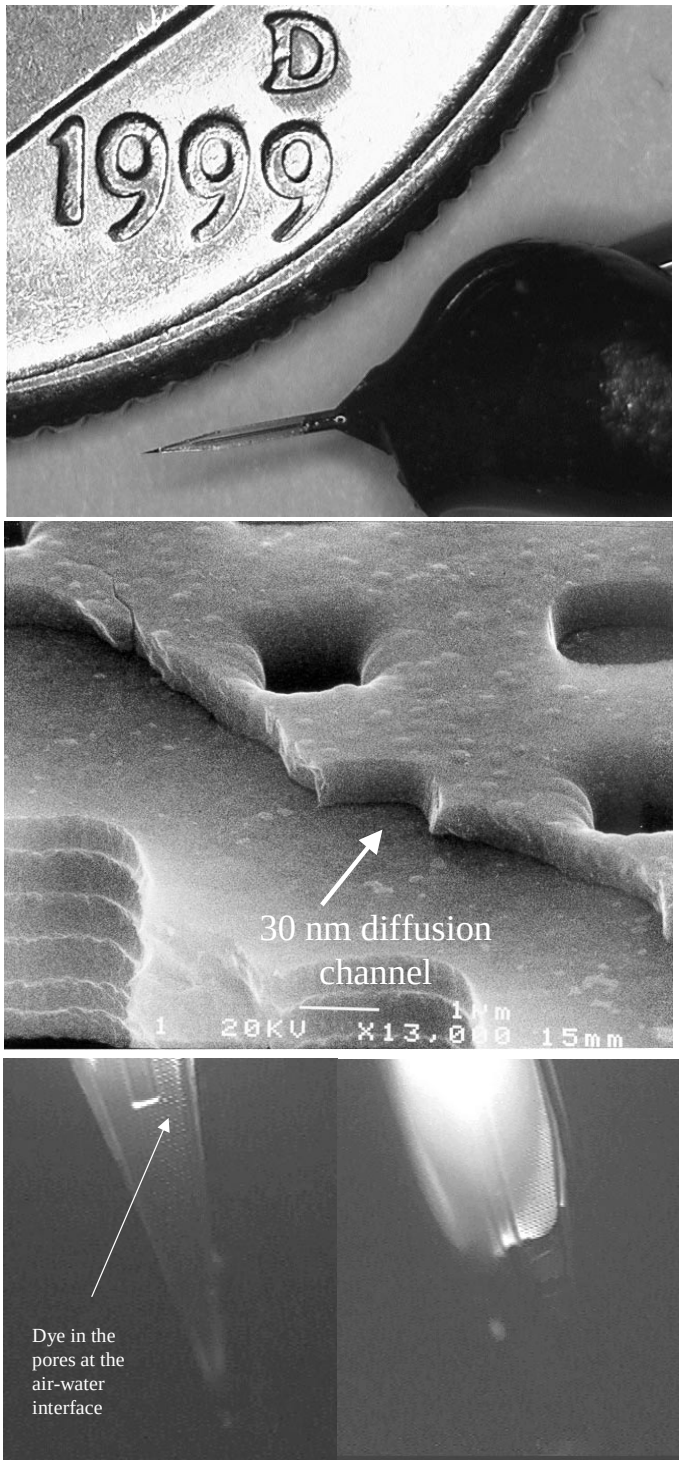


Figure 2. (Top) Packaged Microneedle next to a dime for size comparison. (Middle) Diffusion channel membrane. A thin (30 nm) opening can be seen between the polysilicon layers. The layers fractured at different points indicating they are separate. (Bottom) Fluorescent dye emerging from the microdialysis needle. Dye in equilibrium with a water droplet on the needle. Pores can be seen as small dots on the needle surface.

ing glucose, is strongly correlated with blood concentrations, while the superficial penetration of the needle under the skin minimizes patient discomfort.

Microdialysis needles are based upon the direct separation of large MW compounds from small analytes through the dialysis membrane. Dialysis fluid continuously flows so that the microdialysis membranes have a size exclusion property with convective filtration. If a smaller molecular weight protein permeates the membrane, its mobility will be retarded by the geometry of the membrane while fluid is continuously replenished. Therefore large MW proteins will never equilibrate across the membrane.

Design and Fabrication

Needles are fabricated as a polysilicon microshell on top of a silicon substrate using a silicon on insulator (SOI) device layer as a mechanical support (Fig. 3). The diffusion membrane is fabricated using a layered polysilicon sandwich with a thin thermally grown oxide (10-50 nm) between the layers, and appropriate etch access holes (Zahn, et. al. 2000).

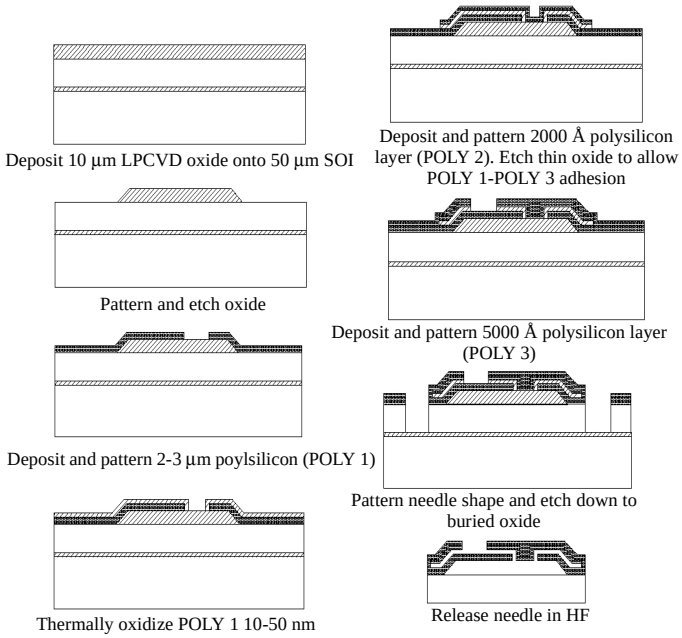


Figure 3. Fabrication details of the microdialysis membrane. The microshell is formed over a sacrificial oxide spacer layer. Several polysilicon layers are sandwiched around a thin sacrificial oxide which defines the diffusion channel. Appropriate access holes are etched into the polysilicon layers to allow small MW molecules to permeate across the membrane.

An integrated planar microfluidic system for the analysis of biological solutions has been fabricated (Fig. 4). It includes the integration of a microdialysis microneedle with a bioanalytical microchip. The device would be worn as a monitor to allow pre-

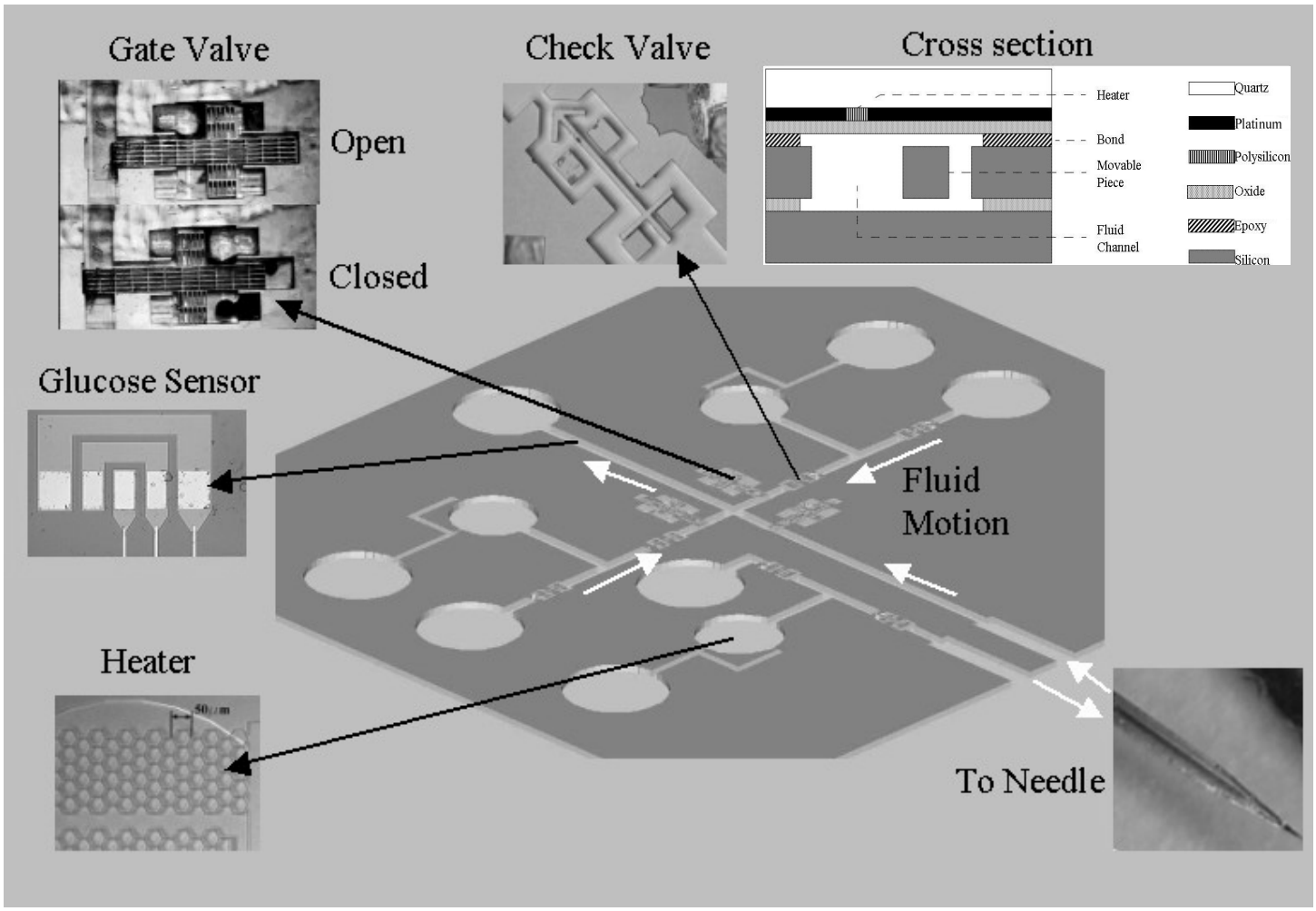


Figure 4. (Above) Schematic of a three channel sensing chip with integrated components including micropumps, microvalves, glucose sensor and a microdialysis needle. White arrows show the direction of fluid flow in each channel.

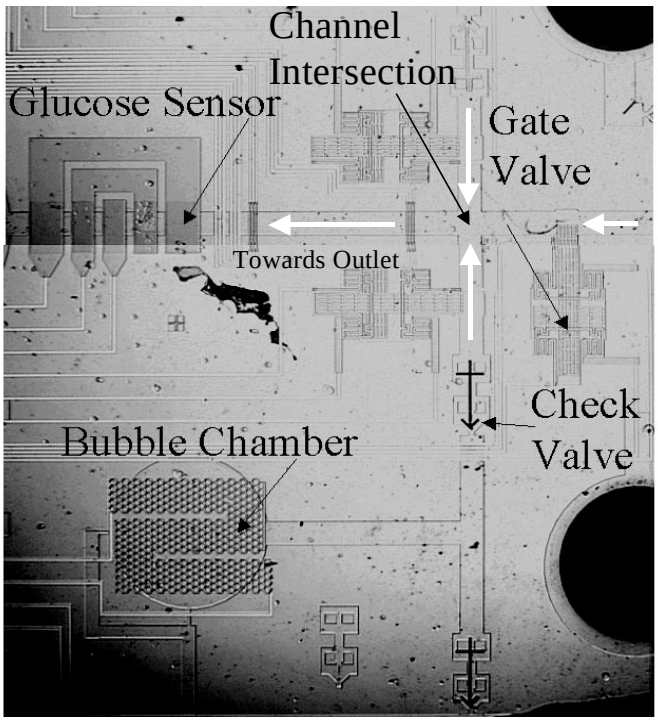


Figure 5. Composite photograph of the assembled device showing the salient features. The white arrows depict the direction of fluid flow in each of the three channels.

cise measurements of metabolite concentrations in interstitial fluid. Such a device could be used in a feedback control system with a drug delivery system. For example, the system could be used to sample interstitial fluid to determine glucose levels for diabetics. A second pump could then deliver insulin in a controlled manner as needed by the patient (Zahn, et. al., 2001).

The sensor module consists of a three channel design with planar positive displacement micropumps to move fluid, planar gate microvalves, and an integrated glucose sensor (Fig. 5). Each channel delivers a different fluid; interstitial fluid filtered through the microdialysis needle, phosphate buffered saline (PBS) to clean the sensor, and a stock concentration of glucose to recalibrate the sensor as the signal drifts.

Microvalves are necessary components to rectify and control fluid flow in any microfluidic device. Planar valves work upon a similar concept; a patterned, etched and released silicon piece may be used as the valve. The silicon piece can be moved within a fluidic microchannel to control fluid flow. The silicon piece can be moved in the channel to block fluid flow by increasing the resistance to flow. When the block is moved out of the chan-

nel the flow resistance is lowered. However, due to processing constraints there will always be a gap above and below the block because of a buried sacrificial layer of some thickness. A modest amount of leakage for many applications can be tolerated.

.A check valve (arrow shaped silicon piece Fig. 4 & 5) is moved by viscous drag on the valve body in the direction of fluid flow. As flow proceeds in the forward direction, the valve is dragged out the closed position and allows flow to proceed unhindered. When the flow reverses, the valve is pushed back into its seat and it blocks flow in the reverse direction. This is a directional check valve design since it allows flow preferentially in one direction over another but requires no energy input (Deshmukh, et. al., 2000).

The gate valve (Fig. 4 & 5) is a free floating silicon structure which may be moved into or out of a channel with bubble actuation (Papavasiliou, et. al., 1999, 2000). When bubbles are created to the right of the gate, they push the gate closed to the left. Then the gate can be opened by creating bubbles on the left side of the gate to push it open to the right. This valve is neutrally stable in the open and closed position, since once the valve is in one position no energy dissipation is required to hold the gate at the position, such as is the case with a spring tethered valve. In addition, fluid flow is normal to the direction of valve movement so the valve cannot be forced into an open position once it is closed. This movement of the gate valve body into the channel effectively closes off the channel allowing multichannel control over fluid delivery.

In order to move fluids along microfluidic channels a micropump must be developed. If bubble actuators are used in a channel, the fluid will be pushed on both sides of the bubble. Check valves may be placed in flow passages. A net pumping action may be obtained regardless of Reynolds numbers. Such a micropump consists of a bubble chamber and two directional check valves. Bubbles are created by polysilicon resistors on quartz which act as heaters (Fig. 4 & 5). Steam bubbles can be generated by heat dissipated when a current is run through the resistor. When the heat is removed, the steam will recondense and the bubble will collapse. When a bubble is created it acts as a piston and drives fluid out. The check valves allow fluid to flow only in one direction. When heat is no longer supplied, the bubble collapses and fluid is drawn in. By cycling bubble generation and collapse, a net pumping action occurs.

Finally, a glucose sensor has been integrated into the system. Fluid is always pumped towards the glucose sensor near the outlet. This ensures that fluid from each of the three channels can be sampled by the glucose sensor. Thus, a saline solution can be used to clean the sensor as needed, or a stock glucose solution can be used to recalibrate the sensor.

Due to the planar fluidic design, all fluidic components may be defined in a single lithography step, with a minimum of assembly. The fluidic components of an integrated system are made using a silicon on insulator (SOI) wafer and quartz dice. The SOI wafer has a 2 μm thick buried oxide layer and a 75 μm thick device layer. Two sequential deep reactive ion etches (DRIE) are performed; the first going all the way through the wafer to form through holes, while the second going only through the device layer to create the channels. A 1.3 μm thick wet oxide layer provides the mask for the second etch which defines all of the fluidic components, while a layer of 9.5 μm thick photoresist over the oxide serves as the mask for the first etch. After the DRIE processes, the valves are almost completely freed from the substrate in 5:1 buffered HF (BHF).

In order to fabricate electronics, a 3000 Å N^+ doped polysilicon, is first deposited onto the quartz wafer. The film is patterned into the polysilicon heaters, and conducting lines for the sensor. Then the wafer is patterned and 1000 Å platinum is sputtered at 5 mTorr, 100 W forward power and 150 sccm Argon. The pattern is lifted off in photoresist stripper (PRS 3000). This forms the conducting wires to the electronics and the working and auxiliary electrode of the glucose sensor. The layers are passivated with silicon oxide and/or silicon nitride, and windows for the electrical connections and electrochemical sensor are opened by RIE. The wafers are then patterned for silver liftoff to define the reference electrode. 1 μm silver is sputtered at 5 mTorr, 100 W forward power, and 150 sccm argon. The silver is then chlorinated in 1% $FeCl_3$ for one minute. The silver layer is then lifted off in PRS 3000. The quartz processing is summarized in Fig. 6.

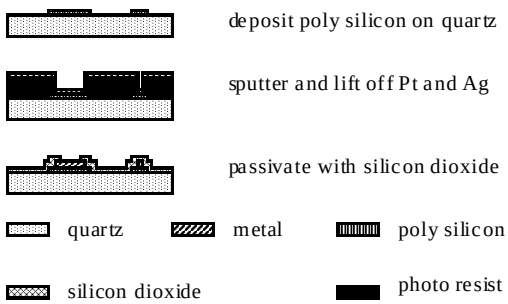


Figure 6. Simplified process flow for depositing electronics including polysilicon heaters and electrochemical cells onto quartz.

In order to bond the silicon and quartz die, a low viscosity epoxy (Epotek 301) is spun on at 10,000 rpm. The two dies are then bonded at a pressure of 100 kPa per die. The epoxy bonds and seals the dies but also traps the valve bodies.

An oxygen plasma (200 W in 600 mtorr of O_2) is used to remove the epoxy in the fluid channels. The plasma first removes the epoxy on the top of the channels, then ashes the epoxy in between the silicon and the quartz. The plasma etching is timed to free the valves. Once dies are assembled they are wirebonded onto a circuitboard package to allow application of electrical power to each of the microfluidic components and sensor independently.

A seat for a microneedle is defined using DRIE. To integrate the needle with the fluidics the microneedle is placed into the seat and sealed using a two-part epoxy. This ensures that the needle is fluidically coupled to the microfluidic chip.

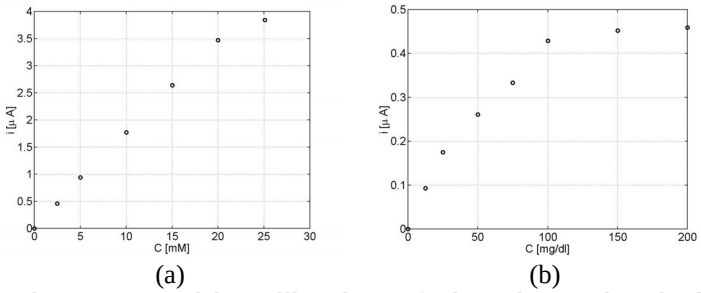
Glucose Sensor Operation

In order to verify the glucose sensor operation, the sensor was first calibrated using hydrogen peroxide (H_2O_2) and the signal recorded by cyclic voltammetry. A 10 µl drop of varying concentrations of H_2O_2 from 0 to 25 mM was placed onto the electrode pads. The signal was recorded using a HCH Instruments model 750A Electrochemical Workstation. The peak output at 0.7 V versus the reference electrode was recorded. The calibration plot is shown in Fig. 7a.

Once sensor operation was verified, the enzyme glucose oxidase was then bound on top of the sensor pad. First 3 mg of bovine serum albumin (BSA) was dissolved in 10 ml phosphate buffered saline (PBS). Then 1 mg (322 units) of glucose oxidase enzyme in 0.1 M sodium acetate was added to the solution. 2 µl of the BSA/glucose oxidase solution was placed onto the electrode surface. Then 2 µl of 2.5% glutaraldehyde in PBS was placed on top of the BSA to crosslink the protein and form a protein membrane. 10 µl of a glucose solution of varying concentrations was added to the sensor pad and the results recorded using amperometry. The sensor was biased at 0.7 V versus the reference electrode and the steady state current was recorded after 3 minutes of equilibration. The glucose calibration curve is shown in Fig. 7b. The steady state current is about half the signal of the H_2O_2 signal, and the signal seems to saturate after 100 mg/dl (~5 mM).

Flow and Filtration Through a Microdialysis needle

Fluid was infused through the needle using a syringe to verify that non-leaking interconnection was achieved. Afterwards, the needle was placed in a concentrated fluorescein solution for a half hour to allow material to equilibrate across the membrane. The fluid was then forced out of the needle with a syringe and the flow on the microchip was recorded to video. An upper and lower channel can be seen. The top channel exits the microchip into the microneedle. The bottom channel shows fluid which has exited the needle back into the chip. The fluid entering and exiting the microneedle after it was allowed to equilibrate in a fluorescein solution is shown in Fig. 8. The fluid exiting the needle is highly fluorescent showing the material flux across the microdialysis membrane. Since there was no dye in the solution which was infused into the needle from the flow channel, the dye had to diffuse into the needle from the fluorescein solution on the outside. This demonstrates the ability to use microdialysis needles to sample biological solutions by allowing compounds to equilibrate across the membrane into a dialysis fluid. The fluid can then be pumped downstream to a on-chip sensor for analysis of the solution.



(a) (b)

Figure 7. (a) Calibration of the electrochemical sensor with H_2O_2. (b) Output current versus glucose concentration.

Multichannel Pumping with Intermittent Valving

In order to increase the versatility of analytical devices there is a need for effective on-chip fluidic control. The ability to control independent microchannels by effective valving is necessary. Fluid in different channels should be moved independently to a desired location on the microchip. Each pump, valve and sensor was controlled as needed. The device was filled with water under vacuum. Filling under vacuum decreased the number of undesired bubbles in the device but did not completely eliminate the problem. Components were actuated independently in order to show effective fluid control of each channel. The movement of fluid in the device was recorded to video, and a series of frames were digitized to demonstrate fluid movement in the device. Since the actuation bubbles were generated thermally, the heat dissipated also produced bubbles in undesirable locations in the device. However, these undesired bubbles could also be used as tracer particles to follow fluid motion.

Two pumps were operated independently with intermittent valving. The heaters were actuated at 40 V to dissipate heat and grow a pumping bubble to direct fluid towards the device outlet. First, a pump at the top of the device (Pump 1) was actuated. The motion of fluid could be traced using tracer bubbles to show the fluid being pumped towards the channel intersection

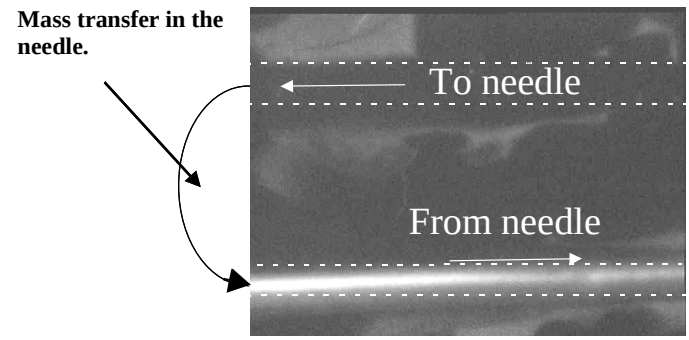


Figure 8. Two microchannels with flow entering and leaving the microdialysis needle after fluorescein dye has equilibrated across the dialysis membrane in the microneedle. The channels are delineated by the dashed white lines. The needle is not seen in the frame but forms a continuous fluid channel to the left of the frame.

and towards the device outlet (Fig. 9 Top).

Then a pump at the bottom of the device (Pump 2) was actuated. Now the fluid is pumped up and to the left towards the outlet (Fig. 9 Bottom). Each of these pumps were actuated separately and the flow was always towards the outlet of the device.

Next, one of the gate microvalves was closed and the downstream pump was activated to see how well the valve could rectify flow against an imposed pumping action. Even though the valve did rectify some flow, fluid was able to escape by flowing underneath the valve through the gap underneath the valve. Bubbles were seen slipping underneath the valve body (Data not shown).

Conclusion

This work presented a compact MEMS based integrated planar microfluidic system for the analysis of biological solutions. It represents one of the first steps required to realize a fully autonomous microfluidic system. A microdialysis microneedle was integrated with the analytical microchip. This system is a portable system capable of extracting, filtering and sensing glucose levels in interstitial fluid. This device could be using in a feedback control system with a drug delivery system. The system could be used to sample interstitial fluid to determine glucose levels for diabetics. A second pump could then deliver insulin through a microneedle in a controlled manner as needed by the patient.

Future research directions include defining the microdialysis needle as part of the microchip so no assembly is necessary. Better filing using degassed liquids to minimize undesired bubbles is also necessary to demonstrate controllable fluid flow with continuous sensor operation. In addition, as designs of planar microfluidic components (pumps and valves) mature they may be easily incorporated into a similar device.

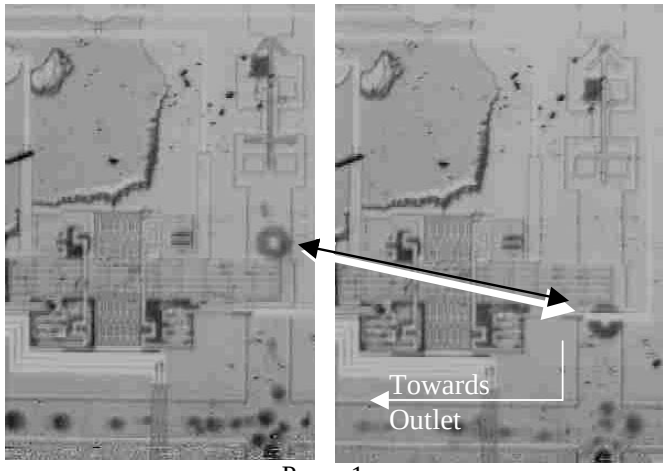
Acknowledgement

The authors would like to acknowledge the members of the Liepmann and Pisano Labs for their assistance. The authors are especially appreciative of Boris Stoeber for his help with the Electrochemical Workstation. All devices were fabricated at the U.C. Berkeley Microfabrication Laboratory. This work was funded by the DARPA Bioflips program.

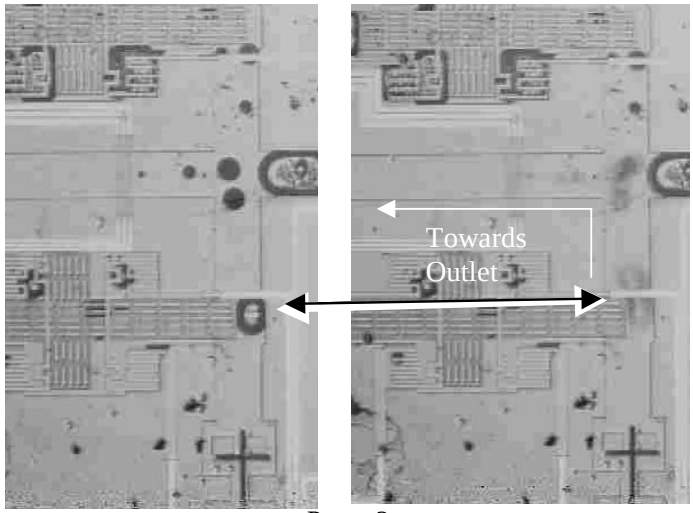
References

Deshmukh A.A., Liepmann D. and Pisano A.P., 2000 "Continuous Micromixer with Pusatile Micropumps," *Proceedings 2000 Solid State Sensor and Actuator Workshop* Hilton Head, S.C., pp. 73-76.

Lambrechts, M. and Sansen, W., 1992, "Biosensors: Microelectrochemical Devices," New York, NY, The Institute of Physics Publishing.



Pump 1



Pump 2

Figure 9. (Top) A series of frames showing net pumping towards the outlet when Pump 1 is activated. The tracer bubbles can be seen proceeding downwards and towards the outlet. (Bottom) Next Pump 2 is activated. Now the tracer bubbles proceed upwards and to the left towards the outlet.

Papavasiliou A.P., Liepmann D. and Pisano A.P., 1999, "Fabrication of Free Floating Silicon Gate Valve," *Proceedings of the ASME MEMS Division, IMECE, vol. 1* pp. 435-440.

Papavasiliou A.P., Liepmann D.and Pisano A.P., 2000 "Electrolysis Bubble Actuated Gate Valve," Proceedings 2000 *Solid State Sensor and Actuator Workshop* Hilton Head, S.C., pp. 48-51.

Zahn J.D., Deshmukh A.A., Pisano A.P. and Liepmann D., 2001, "Continuous On-Chip Micropumping Through a Microneedle," *14th Annual IEEE International MEMS-01 Conference*, January 21 - 25, Interlaken, Switzerland.

Zahn J.D., Trebotich, D.and Liepmann, D., 2000, "Microfabricated Microdialysis Microneedles for Continuous Medical Monitoring," *Proceedings of the First IEEE EMBS Joint Conference on Microtechnology in Biology and Medicine.*