A MULTICHANNEL MICROFABRICATED DEVICE FOR CONVECTION ENHANCED DRUG DELIVERY

K.B. Neeves*, W.M. Saltzman** and W.L. Olbricht*

* School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA
** Department of Biomedical Engineering, Yale University, New Haven, CT, USA

kbn4@cornell.edu

Abstract:
Convection enhanced delivery uses pressure gradients to distribute a therapeutically relevant concentration of drug throughout a targeted region of tissue. A limitation of this technique is the lack of control over the ultimate distribution of infused drugs because flow is influenced locally by the physiology of targeted regions. This paper describes a microfabricated device comprising an array of fluidic channels that can infuse or withdraw fluid from tissue and, therefore, act as fluid sources and sinks to better control the distribution and penetration of infused drugs. The device is a silicon/polymer hybrid that is rigid enough to penetrate tissue and capable of delivering flow rates of 0.1 to 2 μL/min. Devices were tested in agarose gels to determine the feasibility of infusing and withdrawing fluid from a poroelastic medium. Positive pressure infusions of up to 20 kPa (2 μL/min) were tolerated without evidence of backflow. Negative pressure withdrawals lead to compression of the gel at the channel opening which impeded flow. Our results suggest that fluid sinks should be passive and have low hydraulic resistance.

Introduction

Convection enhanced drug delivery (CED) is a promising new therapeutic method for treating diseases of the brain [1]. In contrast to most localized drug delivery methods that rely on diffusion mediated transport, CED uses pressure gradients to distribute drugs into tissue. Small molecules [1], proteins [2], nucleotides [3], and viral vectors [4] infused using CED can penetrate over large regions of the brain. The conventional CED protocol involves advancing into a targeted region a catheter that is attached to a syringe pump. Constant volumetric flow rates on the order of 0.1 to 1 μL/min are delivered for hours to days depending on the size of the targeted region and the physical properties of the drug molecule. However, it is difficult to control the distribution of infused solutions because of heterogeneous features in normal [5] and disease afflicted [6] tissues. For example, in the normal brain the hydraulic permeability of white matter is much larger than that of gray matter, which can significantly affect flow patterns. Similar behavior is observed in the necrotic regions of tumors. The elevated pressure found in tumors further complicates the issue by pushing molecules towards the periphery of a tumor.

Elimination mechanisms of infused drugs must be considered. Therapeutic proteins often have slow binding kinetics, and they can bind non-specifically to the extracellular matrix and to non-targeted cells. To achieve a therapeutically relevant exposure over a sustained period requires long infusion times. Drugs can penetrate beyond the targeted region because of long infusion times and ultimately find their way into healthy tissue.

One strategy to control the distribution of infused compounds is to control the magnitude and direction of the local pressure gradient that dictates the direction fluid flows. This strategy is commonly used on a much large length scale in secondary and tertiary extraction of petroleum. Petroleum extraction involves arranging a number of source (infusion) and sink (withdrawal) wells to optimize recovery of displaced oil[7]. A particularly common configuration is the five spot well, where a single source is surrounded by four sinks equidistant from the infusion source. Streamlines emanating from the source lead directly to a sink. These types of multipole flows could be a helpful in controlling the penetration and distribution of infused drugs.

In this study we report the fabrication of a microfluidic device for multipole flows in brain tissue. The channel outlets were arranged in a five-spot configuration. The probes were characterized in agarose gels that mimic some of the poroelastic behavior of brain tissue. The physiological, material, and fabrication limitations are discussed including suggestions for improved multipole performance.

Materials and Methods

Devices were fabricated using standard micromachining techniques on a silicon substrate. A detailed explanation of the fabrication process is given in Neeves et al. [8]. Briefly, double-sided polished 300 μm thick silicon wafers were deep reactive ion etched (DRIE) on the backside using a silicon dioxide etch mask to define the thickness of the shanks and protrusions (Figure 1a). Next, a layer of polyimide was patterned to act as a base for the channels (Figure 1b). The polyimide acts as an adhesive layer for a parylene structural layer that defines the channels. The channel height (10-15μm) and width (25-50 μm) were defined by a sacrificial photoresist layer (Fig-

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ure 1c) and coated with a thin layer (5 μm) of parylene (Figure 1d). The silicon probe geometry was defined by a second DRIE step (Figure 1e). Finally, the channels were cleared of photoresist in an acetone bath (Figure 1f).

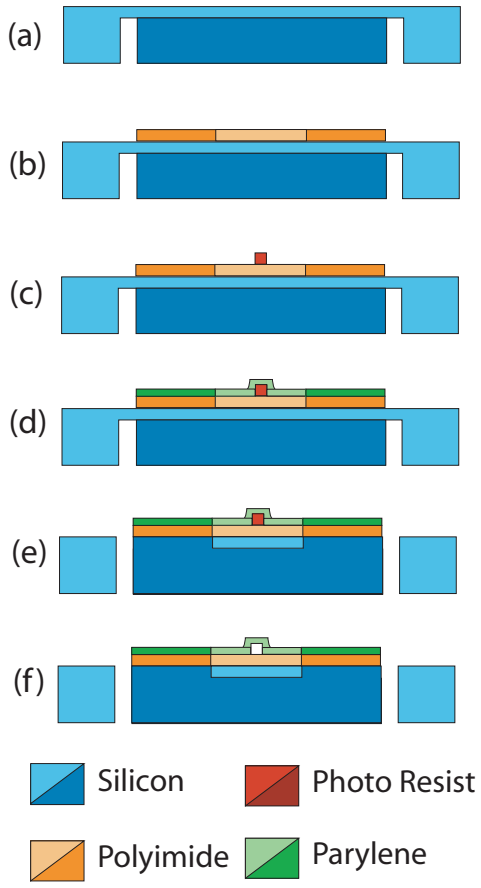

Figure 1: Summary of fabrication process; a) Deep reactive ion etching (DRIE) of backside silicon, b) Patterning of polyimide adhesion layer, c) Patterning of sacrificial photoresist layer, d) Deposition and patterning of parylene channel top, e) DRIE of frontside silicon, f) Dissolution of sacrificial photoresist in acetone.

Devices were attached to micropipettes (OD=1 mm, ID=0.58 mm) and polyimide tubing (OD=0.36 mm, ID=0.32 mm) using two part epoxy. The micropipette was attached to the channel on the middle shank and polyimide tubing was attached to the other four channels. The micropipette was then backfilled with 1% (w/v) Evans Blue labeled albumin (69 kDa) and inserted into a micropipette holder on a micromanipulator. The micropipette holder was connected to a programmable pressure injector (World Precision Instruments PM8000, Sarasota, FL, USA) with low compliance polyethylene tubing. The pressure injector can be programmed to both infuse (positive pressure) and withdraw (negative pressure) fluid at pressures of up to 500 kPa. Compressed high purity nitrogen was used as a pressure source. The flow rate through the devices was determined by measuring the speed of the advancing liquid front in the graduated micropipette.

Agarose gels (0.6% w/v) were used as a brain tissue analog to characterize the delivery of dyes from the devices. Flow in agarose at this concentration has been shown to mimic some characteristics of pressure driven flow in brain tissue [9]. Agarose powder was added to Tris-Borate-EDTA (TBE) buffer and heated in a 90 °C water bath for one hour in a capped vial. The hot solution was poured into 100 mm tissue culture plates and allowed to gel for two hours at room temperature. The devices were inserted 5 mm into the gels at a rate of 1 mm/sec using a piezoelectric motor attached to a micromanipulator.

The hydraulic permeability was calculated as a function of infusion or withdrawal pressure to characterize the gel mechanics. A constant hydraulic permeability suggests that the local flow resembles flow through a rigid pore. A flow rate-dependent hydraulic permeability suggests poroelastic behavior in which the agarose gel is dilated or compressed by the applied pressure.

In a rigid pore model the hydraulic permeability is defined as a constant that represents the conductivity of flow under a pressure gradient as described by Darcy's Law.

$$\mathbf{v} = -\kappa \nabla P \tag{1}$$

where v is the fluid velocity, κ is the hydraulic permeability, and P is the pressure. When the microfabricated channel is inserted into the gel, a small spherical void of radius a forms at the tip of the channel. Fluid exiting the channel fills this spherical void before it flow into the gel. The pressure in the void is constant. The velocity at the spherical void-gel interface is related to the volumetric flow rate through the channel according to:

$$Q(a) = v(a) 4\pi a^2 \tag{2}$$

where $Q(a)$ is the volumetric flow rate and $v(a)$ is the fluid velocity at the void-gel interface, $r = a$. Solving eq. (1) in spherical coordinates with boundary conditions that on the void surface the pressure is $P(a)$ and that the pressure goes to zero as the radius approaches infinity, and substituting into eq. (2) gives

$$Q(a) = 4\pi a \kappa P(a) \tag{3}$$

Thus, the hydraulic permeability can be determined by measuring the flow rate, $Q(a)$, for various values of the pressure, $P(a)$. Eq. (3) suggests that a plot of $Q(a)/4\pi a$ as a function of $P(a)$ should give a straight line whose slope is an apparent permeability of the rigid pore medium. The result is an "apparent permeability" because it is measured only at one point. To determine the true permeability the pressure would need to be measured at several radial positions.

Results

Each probe consisted of five channels on three insertable shanks (Figure 2). The cross-sectional areas of

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the shanks were 75 X 100 μm and they were six and seven millimeters in length. There were two channels on each outside shank and one channel on the middle shank. The cross-sectional area for each channel was 25 X 10 μm. Each channel had its own protrusion for connecting tubing. The dimensions of the device were designed for insertion into the caudate putamen of rats, which largest volume of homogeneous gray matter in the rat brain.

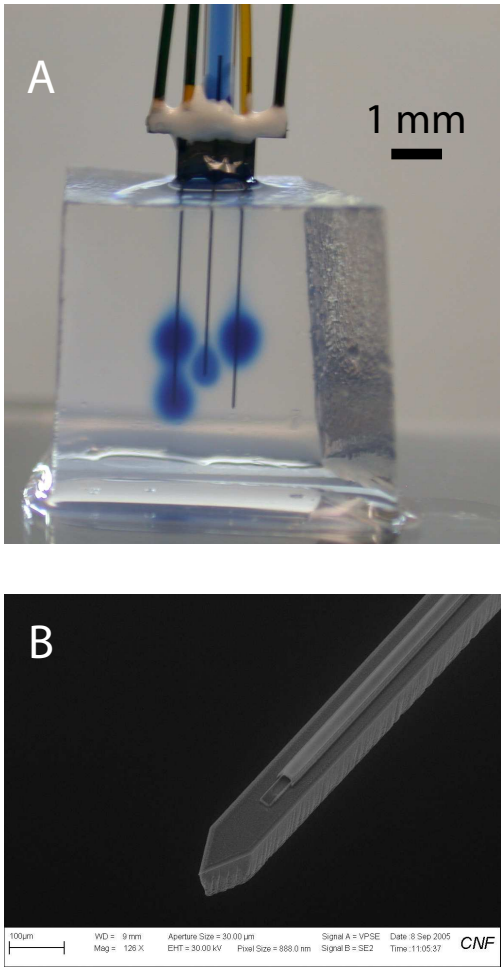

Figure 2: (A) Infusion of Evans Blue labeled albumin through four of the channels into 0.6% agarose. B) Scanning electron micrograph of the tip of the middle silicon shank with parylene channel on top.

Constant pressure infusions were controlled by a pressure injector, which sets the pressure upstream of the channel to a desired value. However, there is a significant pressure loss over the length of the channel, and this must be taken into account to find the infusion pressure, $P(a)$, at the channel exit. To determine this pressure loss as a function of flow rate, flow rates through the channel were measured for several positive and negative pressures with the exit of the channel outlet immersed in a water reservoir (zero gage pressure). The pressure drop ΔP between the injector and the channel exit under positive and negative pressure was found to be ΔP (in kPa) = $120Q$ (in μL/min). Therefore, during an experiment, Q was measured and this relationship was used to find ΔP, which was then subtracted from the injector pressure to determine $P(a)$.

Positive pressure infusions at 10, 35, 70, and 140 kPa were performed in triplicate into 0.6% agarose. A linear fit (R^2=0.88) to eq. (3) yielded a hydraulic permeability of 6.2 X $10^{-}12$ m^2/Pa s, which suggests that the gel can be described by a rigid pore model in this pressure range. Positive pressure higher than 140 kPa resulted in backflow due to separation of the gel and shank.

Negative pressure withdrawals of 10, 35, and 70 kPa produced unexpectedly low flow rates. The relationship between withdrawal pressure and flow rate was nonlinear and therefore eq. (3) could not be used to calculate a permeability. However, the trend showed a decrease in permeability with increasing withdrawal pressure. Clearly, under negative pressure the rigid pore model fails and the poroelastic properties of the gel must be considered.

Discussion

Heterogeneous or anisotropic tissue structures in the brain can limit the effectiveness of CED [10] by making it difficult to predict the distribution of infused drugs. Even in homogeneous tissue regions, the extent of penetration is difficult to control during long infusions. We have fabricated a microfluidic device for testing the feasibility of multipole flows to better control the distribution of infused drugs. The small dimensions of the microfabricated device make it possible to have in close proximity several independent channels for infusing or withdrawing fluid.

However, the results of this study show that mechanical properties of poroelastic media like agarose and brain tissue may limit the range of positive and negative pressures that than can be be achieved *in vivo*. Backflow of infused solutions along the shaft of the delivery vehicle sets an upper bound on infusion rates. In the devices presented here, the maximum positive pressure at the channel outlet for infusion into 0.6% agarose was approximately 20 kPa (corresponding to an injector pressure of 140 kPa).

In the case of negative pressure applied for withdrawing fluids, we hypothesize that the adjacent poroelastic media is compressed by the net force on the gel due to the pressure gradient at the channel outlet. Compression of the gel leads to a decrease in the local hydraulic permeability that impedes flow into the channel. Results showed that increasing the magnitude of the negative pressure in the withdrawal channel did not increase the fluid withdrawal rate, presumably because the gel permeability at the channel tip decreases with increasing magnitude of the applied negative pressure. Therefore, a better strategy may be to apply no negative pressure in the withdrawal channels. However, a passive sink would need to provide a low resistance path for fluid relative to the poroelastic media. The high pressure drop in our microfluidic withdrawal channels, owing to their

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small dimensions, indicates that many channels would be needed to withdraw a significant amount of fluid. In a hypothetical closed system, if fluid were infused through one channel at a rate of 1 μL/min, 40 channels (cross-sectional area 25 X 10 μm, length 10 mm) would be required to withdraw all of the fluid at a pressure drop of 3 kPa per channel (the normal interstitial pressure of the brain). However, the number of required withdrawal channels would decrease significantly if each were made larger. The largest channels we have fabricated using sacrificial photoresist and a parylene structural are 75 X 20 μm. Only two sinks would be required for a channel this size.

Conclusions

A microfabricated device was fabricated on a silicon substrate with microfluidic channels capable of infusing fluid at flow rates that are comparable to conventional CED protocols (0.1 to 1 μL/min). The device was tested in an agarose brain analog to determine the feasibility of using multipole flows in a poroelastic media. We identified material and operational restrictions that limit the range of infusion and withdrawal rates in the device. Infusion pressures must remain less that 20 kPa to prevent significant backflow up the shaft of the device. Active withdrawal of fluid by the using negative pressure results in compression of the adjacent gel and a decrease in the local hydraulic permeability. Our results suggest that to achieve multipole flow, passive sinks with minimal hydraulic resistance are necessary for the withdrawal of fluid from poroelastic media.

Acknowledgements

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References

[1] BOBO, R. H., LASKE, D. W., AKBASAK, A., MORRISON, P. F., DEDRICK, R. L., and OLDFIELD, E. H. Convection-enhanced delivery of macromolecules in the brain. *Proc. Natl. Acad. Sci. U.S.A.*, 91:2076–2080, 1994.

[2] LONSER, R. R., WALBRIDGE, S., GARMESTANI, K., BUTMAN, J. A., WALTERS, H. A., VORTMEYER, A. O., MORRISON, P. F., BRECHIEL, M. W., and OLDFIELD, E. H. Successful and safe perfusion of the primate brainstem: in vivo magnetic resonance imaging of macromolecular distribution during infusion. *J. Neurosurg.*, 97:905–913, 2002.

[3] GROOTHUIS, D. R., BENALCAZAR, H., ALLEN, C. V., WISE, R. M., DILLS, C., DOBRESCU, C., ROTHHOLTZ, V., and LEVY, R. M. Comparison of cytosine arabinoside delivery to rat brain by intravenous, intrathecal, intraventricular and intraparenchymal routes of administration. *Brain Res.*, 2000.

[4] REN, H., BOULIKAS, T., SÖLING, A., WARNKE, P.C, and RAINOV, N. G. Immunogene therapy of recurrent glioblastoma multiforme with a liposomally encapsulated replication-incompetent semliki forest virus vector carrying the human interleukin-12 gene a phase i/ii clinical protocol. *J. of Neuro-Oncol.*, 64:147–154, 2003.

[5] NGUYEN, T. T., PANNU, Y. S., SUNG, C., DEDRICK, R. L., WALBRIDGE, S., BRECHBIEL, M.W., GARMESTANI, K., BEITZEL, M., YORDANOV, A. T., and OLDFIELD, E. H. Convective distribution of macromolecules in the primate brain demonstrated using computerized tomography and magnetic resonance imaging. *J. Neurosurg.*, 98:584–590, 2003.

[6] BOUCHER, Y., BREKKEN, C., NETTI, P. A., BAXTER, L. T., and JAIN, R. K. Intratumoral infusion of fluid: estimation of hydraulic conductivity and implications for the delivery of therapeutic agents. *Br. J. Cancer*, 78(11):1442–1448, 1998.

[7] BEAR, J. *Dynamics of Fluids in Porous Media*. Elsevier, Amsterdam, 1972.

[8] NEEVES, K. N., FOLEY, C. P., LO, C. T., SALTZMAN, W. M., and OLBRICHT, W. L. Fabrication and characterization of microfluidic probes for convection enhanced drug delivery. *Under Review*.

[9] CHEN, S., GILLIES, G. T., BROADDUS, W. C., PRABHU, S. S., FILLMORE, H., MITCHELL, R. M., CORWIN, F. D., and FATOUROS, P. P. A realistic brain tissue phantom for intraparenchymal infusion studies. *J. Neurosurg.*, 101:314–322, 2004.

[10] VOGELBAUM, M. A. Convection enhanced delivery for the treatment of malignant gliomas: symposium review. *J. of Neuro-Oncol.*, 73:57–69, 2005.

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