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Improved Temporal Resolution for in Vivo Microdialysis by Using Segmented Flow

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Abstract

Microdialysis sampling probes were interfaced to a segmented flow system to improve temporal resolution for monitoring concentration dynamics. Aqueous dialysate was segmented into nanoliter plugs by pumping sample stream into the base of a tee channel structure microfabricated on a PDMS chip that had an immiscible carrier phase (perfluorodecalin) pumped into the cross arm of the tee. Varying the oil flow rate from 0.22 to 6.3 $\mu\text{L}/\text{min}$ and sample flow rate from 42 to 328 nL/min allowed control of plug volume, interval between plugs, and frequency of plug generation between 6 to 28 nL , 0.6 to 10 s, and 0.1 to 1.7 Hz, respectively. Temporal resolution of the system, determined by measuring fluorescence in individual sample plugs following step changes of fluorescein concentration at the sampling probe surface, was as good as 15 s. Temporal resolution was independent of both sampling flow rate and distance that samples were pumped from the sampling probe. This effect is due to the prevention of Taylor dispersion of the sample as it was transported by segmented flow. In contrast, without flow segmentation temporal resolution was worsened from 25 to 160 s as the detection point was moved from the sampling probe to 40 cm downstream. Glucose was detected by modifying the chip to allow enzyme assay reagents to be mixed with dialysate as sample plugs formed. The resulting assay had a detection limit of 50 μM and a linear range of 0.2 to 2 mM. This system was used to measure glucose in the brain of anesthetized rats. Basal concentration was $1.5 \pm 0.1 \text{ mM}$ ($n = 3$) and was decreased 60% by infusion of high K^+ solution through the probe. These results demonstrate the potential of microdialysis with segmented flow to be used for in vivo monitoring experiments with high temporal resolution.

Keywords

Microdialysis; segmented flow; temporal resolution; glucose assay

INTRODUCTION

Microdialysis sampling is widely used for in vivo monitoring of chemicals in extracellular space of tissues such as heart, fat, liver, and brain.^{1–3} In chemical monitoring applications, temporal resolution is a key figure of merit because analyte concentrations can change rapidly.^{4–6} When using microdialysis sampling, temporal resolution is usually limited by mass sensitivity of the analytical method coupled to the probe i.e., sample must be collected long enough to obtain a detectable quantity. When techniques such as HPLC are used, the temporal resolution is often 10–30 min;⁷ however, coupling microdialysis to nanoscale analytical techniques such as capillary electrophoresis (CE), microbore liquid chromatography (LC), and electrochemical sensors have shortened sampling times to seconds.^{4, 8–18}

When using high sensitivity analytical methods, other factors can begin to limit temporal resolution achievable with microdialysis sampling. One inherent limitation is broadening of sample zones due to Taylor dispersion as they are transferred from sampling probe to analytical system.¹⁹ The effect of Taylor dispersion can be ameliorated by using high flow rate through the probe; however, this decreases relative recovery thus decreasing the concentration of analytes measured. Higher flow rates are also incompatible with smaller probes and alternative sampling methods such as low flow push-pull perfusion²⁰ or direct sampling²¹ that improve spatial resolution. Taylor dispersion can also be decreased by shortening the length of tubing connections; however, this approach is impractical for experiments involving freely moving animals. Thus, although temporal resolution of 3 s has been described for sampling from an anesthetized animal at high dialysis flow rates; temporal resolution is increased to 90 s for low flow rates or work with awake and freely moving animals.¹⁶ In this work, we describe coupling microdialysis probes to a microfluidic segmented flow system to avoid these limitations. Segmented flow can eliminate Taylor dispersion by localizing samples as aqueous droplets or plugs formed in a stream of water-immiscible carrier fluid.^{22–25}

A surge of recent research into segmented flows has shown the potential of this approach for chemical measurement. Droplets or plugs from femtoliter to microliter volume can be reproducibly created using a variety of microfluidic geometries including tee junctions,²⁶ Y-junctions,²³ and nozzles.²⁷ Furthermore, plugs can be manipulated for chemical analysis through reagent addition,^{28–30} rapid on-chip mixing,^{29, 30} and transfer to outside tubing.^{29–32} Recent applications of such systems include kinetic measurement,^{26, 33} synthesis,^{29, 34, 35} protein crystallization,^{32, 36} DNA analysis,³⁷ PCR,³⁸ cell sorting³⁹ and cell encapsulation.^{23, 40} Although avoiding dispersion or mixing of discrete samples is often cited as an advantage of segmented flow, this approach has not been described for chemical monitoring applications such as in vivo microdialysis.

The goal of this study was to combine in vivo microdialysis sampling with a segmented flow microfluidic device to conserve temporal resolution while sample plugs were transported from the probe to a downstream detection system. We determined conditions for obtaining dialysate flow segmentation on the scale needed for in vivo analysis, tested the effects of flow segmentation on temporal resolution, and demonstrated use of the system for analytical measurements by coupling it to an on-line enzyme assay for monitoring glucose in the brain of living rats. We demonstrate that temporal resolution of 15 s is possible and that this resolution is independent of both time to transport sample to the analytical system and dialysis flow rate.

EXPERIMENTAL SECTION

Chemicals

All chemicals were used as received. Perfluorodecalin, fluorescein, hexamethyldisilazane (HMDS), octadecyltrichlorosilane (OTCS), n-hexadecane, and methanol were purchased from Sigma-Aldrich (St. Louis, MO). Salts for artificial cerebral spinal fluid (aCSF) were purchased from Fisher Scientific (Chicago, IL). A glucose assay kit consisting of Amplex[®] red reagent, dimethylsulfoxide (DMSO), horseradish peroxidase (HRP), glucose oxidase (GOX), D-glucose, concentrated reaction buffer (0.05 M sodium phosphate, pH 7.4), and H₂O₂ was purchased from Invitrogen (Carlsbad, CA). All aqueous solutions were prepared with water purified and deionized to 18 M Ω resistivity using a Series 1090 E-pure system (Barnstead|Thermolyne Cooperation, Dubuque, IA).

Microdialysis Probes

Side-by-side microdialysis probes were constructed in-house as described elsewhere.^{41, 42} Briefly, two 40 μ m i.d. $\times$ 100 μ m o.d. fused silica capillaries (Polymicro Technologies,

Phoenix, AZ), held in place by a 250 μm i.d. capillary sleeve, were inserted into a 1 cm length of 200 μm diameter hollow fiber dialysis tubing that was sealed at one end with polyimide resin (Alltech Biotechnology, State College, PA). The dialysis tubing was made from regenerated cellulose and had 18 kDa molecular weight cut-off (Spectrum Laboratories, Rancho Dominguez, CA). Polyimide was used to coat the outside of the dialysis tubing except for the sampling region. A piece of 718 μm o.d. $\times$ 502 μm i.d. stainless steel tubing (Small Parts, Inc., Miramar, FL) was sleeved over the outside of the 250 μm capillary sheath to strengthen the probe. The probes had a 2 mm sampling tip unless stated otherwise.

Microchip Fabrication and Interface to Microdialysis

Sample plugs were formed by flowing dialysate and perfluorodecalin as an oil carrier phase into separate arms of a microchannel tee formed in PDMS as illustrated in Fig. 1A. Microchannels were formed by casting PDMS on silicon masters^{43, 44}. Silicon masters were fabricated using photolithography methods as described in detail elsewhere^{44–47}. Briefly, AZ 50XT photoresist (AZ Electronic Materials, Somerville, NJ) was spin-coated onto a 3-inch silicon wafer (HMDS vapor pre-treated, International Wafer Service, Colfax, CA) and exposed to UV radiation for 20 s (365 nm mercury line, 45 mJ/cm² power, Optical Associates, Inc. Milpitas, CA) through a mask to generate the desired pattern before development in AZ 400 K developer (AZ Electronic Materials, Somerville, NJ). The channels employed were 80 μm deep unless stated otherwise. Main channels (oil flow that carried plugs) were 250 μm wide and increased to 320 μm wide downstream in order to facilitate connection to silica capillaries. Inlet channels carrying aqueous flow were 125 μm wide for each branch. After casting PDMS over the molds, the layer containing channels was removed and treated with corona discharge (Model BD-20, Electro-Technic Products, Inc., Chicago, IL). The treated PDMS layer was sealed to an un-patterned PDMS layer. The sealed device was placed in a 120 °C oven for 10 min to ensure bonding and restore native hydrophobicity to the PDMS walls.

Chips had fused silica capillary connected for inlet flows of aqueous dialysate sample and oil carrier phase. Access ports for these connections were created using a blunt 18-gauge needle to punch holes through the PDMS over the appropriate channels (see Figure 1A).⁴⁸ Fused silica capillaries with 360 μm o.d. were inserted into the holes and used for connection to syringe pumps (Model 402, CMA Microdialysis, North Chelmsford, MA) via Valco unions (1/16" o.d. tubing, 0.15 mm bore, Houston, TX). Where microdialysis sampling was used, the 40 μm i.d. outlet capillary of the microdialysis probe was directly inserted into the port. The total length of this tube was 3.5 cm. Segmented flow was pumped out of the chip into a collection capillary consisting of a 40 or 70 cm long piece of 250 μm i.d. $\times$ 360 μm o.d. fused silica tubing. The inner surface of this capillary was made hydrophobic by pumping 1% (V/V) OTCS in n-hexadecane for 10 min at 6 $\mu\text{L}/\text{min}$. After derivatization the capillary was rinsed by pumping hexane, methanol and air through it in succession.⁴⁹ The modified capillary, with polyimide coating removed at one end, was inserted into the 320 μm wide channel between the two layers of the chip i.e., in the same plane as the main channel. After making all connections to fused silica capillaries, a sealant layer of PDMS was poured on top of the chips and cured on a hot plate at 85 °C for 3 h to seal capillaries to the chip.⁴⁸ Cyanoacrylate cement was applied to PDMS surface around inlet capillaries to prevent fluid leakage.

LIF Detection and Data Analysis

For visual inspection and monitoring of sample plugs, the chip or collection capillary was mounted on a Nikon inverted microscope (Eclipse TS100, Melville, NY). Photographs were taken through the microscope using a digital camera (FinePix F30, Fujifilm, Japan). When detecting fluorescence, the collection capillary or chip was mounted on an epi-illumination inverted microscope (Axiovert 100, Carl Zeiss Inc.). Fluorescence was excited using a 488 nm line of an Ar⁺ laser (Melles Griot, Carlsbad, CA) for fluorescein or the 543.5 nm line of a He-

Ne laser (Melles Griot, Carlsbad, CA) for Amplex[®] red. Fluorescence was collected through a 10x objective through appropriate filter sets and detected using a photometer (R3896, Hamamatsu Photonics, Bridgewater, NJ) mounted on the microscope. The photometer was set to low pass filter at 250 Hz. Fluorescence signals were collected via a data acquisition card (PCI-6036E, National Instruments, Austin, TX) at 1000 Hz using LabView program written in-house. Microsoft Excel 2007 (Microsoft, Redmond, WA), Igor Pro 6.01 (Wavemetrics, Inc., Lake Oswego, OR), and Cutter 7.0⁵⁰ were used for data analysis and graphing.

Testing of Temporal Resolution

The temporal response of the microdialysis/segmented flow system shown in Figure 1A was tested by equilibrating the dialysis probe in a solution of fluorescein dissolved in aCSF (145 mM NaCl, 2.68 mM KCl, 1.01 mM MgSO₄, 1.22 mM CaCl₂, 1.55 mM Na₂HPO₄, 0.45 Mm NaH₂PO₄, pH 7.4) and then rapidly altering the fluorescein concentration by using pipettes to quickly change the solution surrounding the probe while monitoring fluorescence of sample plugs either on the chip or downstream in the collection capillary. In some experiments, oil flowing through the main channel was replaced with aCSF to determine the effect of segmentation on temporal resolution.

Computational Modeling of Microdialysis Sampling

Microdialysis probe sampling dynamics were modeled using COMSOL Multiphysics[®] 3.3 (Comsol, Inc., Burlington, MA). 1 and 2 mm long probes were approximated as 200 μ m wide cylinders with a 40 μ m i.d. by 100 μ m o.d. capillary inserted to within 50 μ m of the bottom of the probe, a similar capillary outlet at the top of the probe and a 10 μ m thick membrane. The boundary layer (i.e., quiescent solution) around the probe was approximated to be 50 μ m thick for a well stirred solution (assuming Re $\sim$ 100).⁵¹ Diffusion was treated as uniform throughout the boundary layer, membrane and probe volume. All models were solved in three dimensions by modeling mass transport from the edge of the boundary layer into the perfusing flow into the probe as a function of time for 1 minute. The net concentration out of the probe was fit to a Hill equation by nonlinear regression using Origin[®] 6.0 (Microcal Software, Inc., Northhampton, MA) to generate data traces. The outlet capillary from the probe was modeled as a 3.5 cm long by 40 μ m i.d. capillary in two dimensions. Analyte concentration and response times were determined from the time-dependent concentration profile at the end of the capillary model. Constants used in the model were 4.25×10^{-6} cm²/s as diffusion coefficient for fluorescein,⁵² 993 kg/m³ for density of water, and 6.90×10^{-4} Pa s for viscosity of water (37 °C).

In Vitro Glucose Assay

Glucose assays were performed on-line by mixing reagents and dialysate within a chip to form sample plugs that were pumped to a detection zone in a collection capillary downstream of the mixing/plug formation point. Reagents were prepared from stock solutions of Amplex[®] red (10 mM in DMSO), GOX (100 U/mL in 0.05 M sodium phosphate reaction buffer, pH 7.4) and HRP (10 U/mL in reaction buffer, as described above) according to vendor instructions. Stock solution was stored frozen at -80 °C as single-use aliquots. For each day's experiment, stock solution aliquots were thawed on ice and then diluted with reaction buffer to produce separate enzyme (6 U/mL GOX and 0.6 U/mL HRP) and indicator (0.3 mM Amplex[®] red) solutions. These working solutions were kept on ice and shielded from light by aluminum foil at all times. To ensure activity of reagents, solutions were replaced every two hours.

For on-line glucose assay, the aqueous inlet channel shown in Figure 1A was modified to have 3 channels (one for HRP/GOX solution, one for Amplex[®] red solution, and one for dialysate) that merged to a single channel just before the tee intersection (details given in text). The flow rate for sample stream was 200 nL/min and for each reagent stream 50 nL/min. Oil flow rate

was 1000 nL/min. With these flow rates, each plug was expected to contain the concentrations recommended by the vendor, i.e. 0.1 U/mL HRP, 1 U/mL GOX and 50 μ M Amplex[®] red. To test and calibrate the on-line assay, glucose solutions with concentrations ranging from 0.1 mM to 5 mM were prepared in aCSF and sampled by microdialysis. Fluorescence of each resulting sample plug was detected 40 cm downstream using LIF as described above.

Surgery and in Vivo Glucose Monitoring

In vivo microdialysis experiments were performed on male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200–250 g. Rats were anesthetized with intraperitoneal (i.p.) injections of ketamine (65 mg/kg) and domitor (0.5 mg/kg) and mounted on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Rats were maintained under anesthesia for the entire experiment by giving i.p. injections of ketamine (32.5 mg/kg) and domitor (0.25 mg/kg) as needed. The probe was inserted into the nucleus accumbens (NAC) at coordinates +1.6 mm anterior, +1.2 mm lateral, and –8.1 mm ventral, measured from bregma, according to a rat brain atlas⁵³. After inserting the dialysis probe, the system was equilibrated by perfusing aCSF through the probe at 200 nL/min for 1 h before beginning measurements.

Glucose was measured in vivo similarly to the in vitro assay described above with some modifications. The length of the collection capillary from the chip to the detection window was increased to 70 cm to accommodate space requirements of the in vivo experiment. Flow rates were adjusted to 300 nL/min through sampling probe, 75 nL/min for each reagent stream and 1500 nL/min for oil to achieve a 17 min reaction time (i.e., time from the collection capillary to detection zone). A 6-port valve (Valco, Cheminert 04Y-0000H) was plumbed between the dialysate syringe pump and sampling probe to allow the dialysis perfusion solution to be switched. Solutions containing either aCSF or high K⁺ aCSF (100 mM KCl, 50 mM NaCl, 1.2 mM CaCl₂, 1.2 mM MgSO₄, 0.4 mM NaH₂PO₄, pH 7.40)⁵⁶ could be selected. In vitro calibration with glucose of known concentrations was performed prior to in vivo measurements.

RESULTS AND DISCUSSION

Effect of Flow Rates on Plug Volume and Time Interval

In a sampling system with on-line analysis that uses segmented flow, the sample plug volume, interval between plugs, and plug generation frequency have a large impact on both the temporal information that can be obtained and the analytical methods that should be adopted. The sample plugs must be large enough that the amount of analyte in them is higher than the mass detection limits of the analytical method. Sample plug generation frequency sets the upper limit to temporal resolution obtainable, e.g. a system that generates one sample plug every 10 s will have a temporal resolution no higher than 10 s. If on-line analysis is used, then the interval between plugs that are created must be longer than the minimum time required for each analysis (e.g., separation time in LC). In this work we used a microfluidic tee to segment flow from the dialysis probe. As demonstrated previously,^{54, 55} plugs generated in a tee can be controlled by changing relative flow rates of the sample and carrier fluid as well as dimensions of the channels. Although models have been developed to predict plug generation,⁵⁴ we found that experimentation was required to obtain the desired plug formation dynamics.

For this work, we sought to generate plugs from sample stream flow rates in the 0.1 to 1 μ L/min range at 1 to 10 s intervals to yield samples with low nanoliter volumes. This flow rate is typical for microdialysis and the plug formation frequency represents significant improvement in temporal resolution while generating plugs that are easily manipulated and analyzed. As shown in Figure 1, a tee with 125 $\times$ 80 μ m inlet channel and 250 $\times$ 80 μ m main channel allowed such plugs to be formed. For a given sample flow rate, increasing the oil flow rate decreased

plug volumes and intervals (Figure 1B and 1C). Decreasing the sample flow rate generated smaller plugs at longer intervals. The dynamic range of intervals was approximately 0.6 to 10 s. Plugs were reproducible with < 5% RSD in volume and < 8% RSD in interval time. Chip dimensions could also be varied to yield different ranges that might be appropriate for different applications. For example, with $50 \times 12 \mu\text{m}$ channels we generated plugs of 50 to 200 pL at < 1 s intervals. In some cases, it may be desirable to independently control sample size and interval between plugs; however, this is not possible with the tee junction. An active system where plugs are formed by a trigger would allow such independent control but would also necessitate a more complex instrument.

Conservation of Temporal Resolution with Segmented Flow System

After demonstrating controlled sample plug formation, we tested the potential to preserve temporal resolution in comparison to a continuous flow system during microdialysis sampling. For these experiments, step changes in fluorescein concentration were made at the probe surface while recording response curves by LIF detection. A dialysis flow rate of 200 nL/min was used representing a relatively low flow rate that generates high relative recovery (approximately 53% for glucose) but is usually not associated with good temporal resolution. For the continuous flow system the oil flow was replaced by aCSF. Recordings were made both near the tee junction and 40 cm downstream for both continuous flow and segmented flow (see Figure 2A and 2B). A comparison between upstream and downstream response curves for both systems demonstrates the advantage of segmented flow over continuous flow for conserving temporal resolution. (Temporal resolution and response time in this discussion refer to the time from the initial increase in signal to the steady state signal and does not include the delay time associated with flowing from the probe to the detection window.) With segmented flow, on-chip and downstream detection produced response curves that exactly overlapped (Figure 2C), verifying prevention of axial dispersion between plugs and conservation of temporal resolution after sampling. In contrast, severe deterioration in temporal resolution, represented as a broadened transition zone, was observed with continuous flow (Figure 2D), due to axial dispersion of sample zones during transport at low flow rate through a capillary.

We next explored the upper limits of temporal resolution with this system. To do this, we repeated the step change experiment but with stirred solutions and probes equilibrated to 37 °C. Stirring is expected to decrease the distance required for analyte to diffuse to the probe while the elevated temperature increases diffusion coefficients. Using this approach we observed response times of ~ 30 s (time from 10–90% of maximal signal) at 200 nL/min dialysis flow rates (see Figure 3A). Increasing the dialysis flow rate to 1 $\mu\text{L}/\text{min}$ did not improve the temporal resolution (Figure 3B). Decreasing the dialysis probe length by half to 1 mm resulted in an approximately 2-fold improvement in response time to 15 s at both flow rates (Figure 3C and 3D). Thus, response time scaled with membrane length rather than flow rate. These results suggest that mass transport across the membrane, and not Taylor dispersion, limits temporal resolution under these conditions.

To further explore this effect, we modeled the response of a dialysis probe to step changes in concentration in a stirred solution at 37 °C. The model was based on the geometry of the probe (see Experimental section and Figure 4A). The system was considered to have a 50 μm boundary layer and diffusion coefficient within the membrane was considered to be the same as solution. These conservative estimates have the effect of making observed responses limited primarily by flow and diffusion within the probe and tubing and not transport across the membrane. As shown in Figure 4, this model predicts response times of 11.9 s for a 2 mm probe at 0.2 $\mu\text{L}/\text{min}$. Increasing the flow rate to 1 $\mu\text{L}/\text{min}$ decreased the response time to 3.5 s. Cutting the probe length in half yielded a small decrease in response time to 8.0 and 2.1 s with 0.2 and 1 $\mu\text{L}/\text{min}$ flow rates, respectively. Thus, when the transport across the membrane

is not limiting, the response times are 2 to 8-fold faster than those measured experimentally. Furthermore, in contrast to experimental results, the dialysis flow rate has a bigger effect on response time than membrane length. This result further supports the conclusion that transport across the membrane is a limiting factor in response time in this system. Lower effective diffusion coefficients within the membrane,^{19, 56} adsorption to the membrane, and flow leakage through the membrane are all factors that can slow transport and therefore alter response time. Accurate knowledge of these processes would be required to correctly predict response times. This work suggests that improved membranes or sampling without membranes, such as direct²¹ or push-pull sampling,²⁰ would be required to further improve the temporal resolution.

On-Chip Glucose Assay with Segmented Flow

To demonstrate the potential of the microdialysis to segmented flow system for chemical analysis, we integrated an on-line glucose enzyme assay. The sampling chip was modified with a triple-branch inlet to enable addition of assay reagents to the sample stream 1 mm upstream of the plug formation point. As illustrated in Figure 5A, sample stream from the microdialysis probe flowed in the middle branch while enzymes (GOX, HRP) and dye (Amplex[®] red) were infused from the two side branches. Sample stream flow rate was 200 nL/min while the reagent stream flows were 50 nL/min each. This net flow rate resulted in sample plugs forming at 4 s intervals. Mixing and enzymatic reaction took place to form a fluorescent product (see Figure 5B for reaction scheme) within plugs as they were transported in a capillary from the microfluidic chip to detection window. Reaction time could be adjusted by varying the length of the capillary as well as oil flow rate. Although the assay kit suggested a 30 min incubation time before detection, we found that 17 min was enough to distinguish different glucose concentrations. This reaction time could be achieved by a 40 cm capillary at 1 μ L/min oil flow rate. An illustration of the raw fluorescence signal from the plug enzyme assay at the detection point during a step change from 0.2 to 1 mM glucose is shown in Figure 5C. This trace illustrates the uniformity of signal intensities across different plugs for a given glucose concentration. Indeed, the assay yielded 2.3% RSD ($n = 56$) at 1 mM glucose. The assay also had a linear response up to 2 mM glucose and a detection limit of 50 μ M glucose (see Figure 5D). The trace in 5C also illustrates the preservation of temporal resolution for this experiment even though the sample plugs required 17 min for transport from the sampling to detection points. This effect represents a substantial advantage of the segmented flow system for assays that require long reaction times. This result further illustrates that the segmented flow system can maintain temporal resolution regardless of downstream processes.

In Vivo Monitoring of Extracellular Glucose in Rat Brain

We next investigated if the system with glucose assay was robust enough for in vivo measurements. Glucose plays a critical role for providing energy to the brain. Regional cerebral activity, for example during cognition, has been associated with local glucose utilization.^{57–59} Brain glucose concentrations also change with infarct⁶⁰ and spontaneous depolarization after head injury¹⁸. Therefore, glucose dynamics is of interest for research and clinical applications.

In this preliminary trial to couple the segmented flow system to an animal, probes were inserted into the NAC of anesthetized rats and perfused at 300 nL/min with aCSF. Basal concentrations of glucose measured by the method were 1.5 ± 0.1 mM ($n = 3$), in agreement with previous work.⁶¹ To evoke a change in glucose concentration, 100 mM K⁺ was perfused through the probe. As illustrated in Figure 6, after a 24 minute delay (7 min for perfusion solution to reach rat brain and 17 min for samples to reach the detection point), the concentration of glucose began to decrease reaching a minimum of $43 \pm 9\%$ ($n = 4$) of basal concentration after 30 min of exposure. Re-perfusion with regular aCSF brought glucose concentration back to basal in

less than 10 min. The results of this experiment coincide well with previous reports which showed 70% decrease of glucose in NAC over 60 min and 25% decrease in striatum over 30 min.^{62, 63} Differences in conditions such as use of anesthetized animals in this experiment versus awake animals in other reports likely accounts for the small quantitative differences observed.

The purpose of these in vivo experiments was to illustrate that the system could be used in a practical application to measure chemical dynamics. In view of pre-existing glucose assays that can be coupled on-line to microdialysis,^{18, 60} and the observation that extremely rapid changes were not observed in this experiment, it is not likely that this method will have primary application for in vivo glucose monitoring. The flow-segmented approach will have more impact as assays are developed for neurotransmitters and other compounds that are expected to have faster concentration changes. Indeed, a particular advantage of the approach described here compared to sensors is the ease with which the assay can be changed to target other analytes. We also believe that the general concept of using sampling with segmented flow to preserve temporal resolution will have utility beyond brain chemistry to monitoring other environments, such as chemical reactions or other biological systems.

CONCLUSION

Segmented flows have received significant attention in chemical analysis in recent years primarily for their potential in high-throughput analysis. In this paper, we reduce to practice the possibility that microfluidic segmented flows can be used to prevent temporal distortion in a sampling and monitoring experiment. Temporal resolution for microdialysis was maintained at 15 s regardless of downstream processes thus allowing good temporal resolution to be maintained for experiments requiring long connection capillaries (such as freely moving animals) or assays that involve long reaction times. The use of segmented flow with sampling represents a significant advance for sampling approaches because it decouples analysis time from the temporal resolution that is possible. It also provides a convenient approach for manipulating nanoliter volume fractions that are generated with performing high temporal resolution measurements. Our experiments suggest that future research aimed at improving temporal resolution should be directed towards improving sampling processes. Furthermore, coupling to other assay systems will be important in allowing this approach to extend beyond enzyme assays. Extension of the method to other assays and coupled to different sampling probes will likely yield systems with temporal resolution that approaches that of many sensors.

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Figure 1.

(A) Illustration of microdialysis/segmented flow system used in this work. Arrows indicate direction of flow. Micrograph in inset illustrates plug generation at a tee junction. (Aqueous stream contains food dye for visualization.) Aqueous channels had a width of 125 μm and main channel with segmented flow had a width of 250 μm . (B) Dependence of plug volume on oil flow rate at different aqueous flow rates (Q_w) for structure shown in Figure 1A. (C) Dependence of interval time between plugs on oil flow rate at Q_w for structure shown in Figure 1A.

Figure 2.

Illustration of on chip and on capillary detection points for comparing temporal resolution of segmented flow (A) and continuous flow (B) systems. Step change of fluorescein concentration from 50 nM to 100 nM was made at the probe surface and response curves at the two detection points were recorded for segmented flow (C) and continuous flow (D). For (C), the data points represent the maximal fluorescence recorded from each sample plug as it passed through the detector. The top time axis is for the downstream (capillary) detection point and the bottom for the on-chip detection point in both graphs. Sampling flow rate was 200 nL/min and cross-sectional flow rate (perfluorodecalin or aCSF) was 1 μ L/min. Microfluidic chip conditions were the same as described in Figure 1.

Figure 3.

Response obtained at both low and high sampling flow rate with microdialysis probes with different membrane lengths. (A) 2 mm probe at 200 nL/min; (B) 2 mm probe at 1 μ L/min; (C) 1 mm probe at 200 nL/min; (D) 1 mm probe at 1 μ L/min. Fluorescein concentration was changed from 2 μ M to 5 μ M at the probe surface. Cross-sectional flow rates were 1 μ L/min for 200 nL/min sampling rate and were 4 μ L/min for 1 μ L/min sampling rate. Data traces are raw output from LIF detector and show detection of individual sample plugs. Temporal resolution, defined as the time during which signals increased from 10% to 90% of the maximum intensity, is marked on each graph. Chip conditions were the same as described in Figure 1.

Figure 4.

Simulation of response to step change in fluorescein concentration at a microdialysis probe using COMSOL. (A) The geometry of the dialysis probe was the same as those used experimentally i.e., 200 μm inner diameter, 220 μm outer diameter, side-by-side inlet and outlet capillaries and a 40 μm i.d. by 3.5 cm long exit capillary. Curved arrow indicates direction of dialysis flow. The steady state concentration gradient for unit concentration outside the probe is shown over the geometry in this illustration. (B) The response shown is the concentration change at the outlet of the exit capillary following a step change from zero to unit concentration at time zero. The probe lengths and dialysis flow rates are given in the legend.

Figure 5.

Glucose assay with segmented flow system. (A) Micrograph of microchannel network used for enzymatic assay within plugs. Food dye has been added to the Amplex® red and GOX/HRP streams for visualization. (B) Reaction scheme for the enzymatic assay. (C) LIF response when glucose concentration was changed at the probe from 0.2 to 1 mM. The inset shows an amplified view of the trace. Data are raw traces showing detection of individual plugs. (D) Calibration curve for glucose sampled by microdialysis and assayed using this system. Glucose concentrations are sampled concentrations.

Figure 6.

(A) Overview of system for in vivo glucose assay. (B) Time course of extracellular glucose concentration in the NAC of rats infused high K^+ (100 mM) aCSF through the probe. Time on axis is time since switch was made to high K^+ . Black bar indicates application of K^+ corrected dead volume of system. Axis on the left is relative fluorescence unit (RFU) for the maximal signal in each plug while right axis expresses data as the percentage of basal glucose concentration.