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Microdialysis Coupled with an Embedded Systems Controller and CMOS Image Sensor

Alan John Rosenbloom, Heer Robin Gandhi, George Lopez Subrebost

Abstract—**Continuous monitoring of specific metabolite and drug levels within a patient's blood can contribute to shorter hospital stays and more successful treatment of both chronic and acute diseases. Intravenous microdialysis is an attractive technology for continuous venous blood sampling that can be used to manage tight glucose control and to sample a large variety of molecules from human blood. In combination with lab-on-a-chip architectures, microdialysis could provide continuous monitoring of important diagnostic and therapeutic substances. Unfortunately, microdialysis is inherently variable and non-transparent, i.e., errors in sampling cannot be detected and corrected in real-time. A portable microdialysis system is presented that gauges membrane diffusive capacity by using a fluorescent tracer, providing a method to track the intrinsic variability. An embedded systems controller and CMOS image sensor is used to measure and wirelessly communicate fluorescent tracer levels. The controller has the capability to generate alarms when probe performance deteriorates, making microdialysis both more accurate and robust for clinical use. The potential to integrate a microparticle-based, turbidimetric vancomycin immunoassay with microdialysis is also demonstrated by using a CMOS image sensor to detect changes in turbidity.**

I. INTRODUCTION

Tight blood glucose control (TGC) in patients in the intensive care unit (ICU) has been shown to reduce mortality and lessen complications [1,2]. TGC requires frequent determination of blood glucose that is accurate and rapidly available [3]. The present technology for obtaining these readings is by the use of point-of-care (POC) glucose meters and fingerstick blood sampling. This method has been shown to be unreliable in critically ill patients and has been suspected of causing inaccurate insulin doses that lead to harmful glucose levels for patients [4,5]. It is also expensive and time consuming for the nursing staff and uncomfortable for the patient. Technology that could achieve improved accuracy with frequent and rapid blood glucose readings and little or no nursing attention would improve safety and reduce costs of TGC.

Intravenous microdialysis (MDL) is a versatile and highly effective method to sample blood and provides a platform to implement TGC. MDL has been cited in nearly 11,000 research studies as a method to obtain and accurately measure a multitude of molecular targets from tissue and the bloodstream. MDL is performed by circulating buffer through a small catheter (0.5 mm diameter probe) that has microscopic holes as shown in Figure 1. Molecules (such as glucose) that are present in the blood and surrounding the probe diffuse through the holes and into the circulating buffer. This dialysate is then collected and analyzed.

Continuous blood glucose monitoring via microdialysis would eliminate repeated testing and allow adjustment of glucose level more smoothly, quickly, and with increased safety. However, there are factors that create variability with estimates of blood glucose obtained by microdialysis [6]. Fouling of the probe, i.e., clogging of its pores by blood proteins or clotting, can decrease the recovery of glucose. By placing a fluorescent tracer in the circulating microdialysis buffer, it is possible to continuously monitor the diffusive capacity of the membrane and compensate for variable analyte recovery. Diagnostic systems involved in clinical decision making must have alarms to prevent the acquisition of inaccurate data. An embedded systems controller that manages self-diagnostic routines, such as diffusive capacity monitoring during microdialysis, can fulfill this function as well as improve accuracy.

Previous work by our research group has shown that a microdialysis catheter can be combined with an embedded systems controller to continuously monitor membrane diffusive capacity [7]. The system was limited due to the low signal-to-noise ratio inherent with the photodiode detector and limited volume throughput with a 500 micron ID tubing. With a single photodiode, the complete surface is subjected to the emitted fluorescence signal along with noise sources (excitation light signal). The signal-to-noise ratio of this setup is about 24 dB.

For faster throughput and lower dead volumes, small diameter tubing is necessary. Using 150 micron ID tubing will decrease sample volume and fluorescence by a factor of 11x. To compensate for the smaller signal, a color CMOS image sensor is shown in this paper to provide the greatest sensitivity for measuring fluorescence in the dialysate tubing. With a CMOS image sensor the signal-to-noise ratio can be improved to 48 dB using windowing, which is a factor of 16x improvement. Based on the intrinsic specifications of the

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A. J. Rosenbloom is with the Institute for Complex Engineered Systems, Carnegie Mellon University, Pittsburgh, PA. 15213 USA; Phone: 412-268-8638; fax: 412-268-6571; e-mail: alan2@andrew.cmu.edu.

H.R. Gandhi is with the Electrical and Computer Engineering Dept., Carnegie Mellon University, Pittsburgh, PA. 15213 USA; Phone: 412-268-5212; e-mail: hgandhi@andrew.cmu.edu.

G.L. Subrebost is with the Institute for Complex Engineered Systems, Carnegie Mellon University, Pittsburgh, PA. 15213, Carnegie Mellon University, Pittsburgh, PA 15213 USA; Phone: 412-268-5212; e-mail: georgel@andrew.cmu.edu.

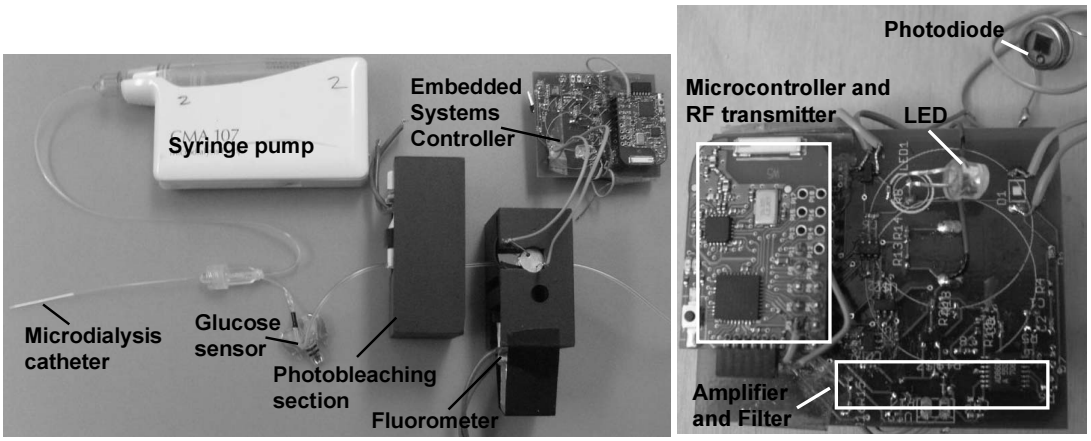

Fig. 1: (Left) Continuous glucose microdialysis setup that uses an intravenous microdialysis catheter, in-line glucose sensor, and embedded systems controller to monitor the inaccuracies in the glucose recovery. (Right) Embedded systems controller includes a Texas Instruments MSP430 controller and a custom printed circuit board for signal amplification and filtering. are shown.

CMOS image sensor and windowing, the light sensitivity will increase by a factor of 480x over a standard photodiode.

The potential to integrate a microparticle-based, turbidimetric drug immunoassay with microdialysis is also demonstrated by using the CMOS image sensor to detect changes in turbidity. Various manufacturers provide turbidimetric assays that allow the measurement of more than thirty different drugs. In addition, researchers have shown the capability to detect static light scattering of latex microsphere agglutination in a microfluidic immunoassay device [8]. By combining intravenous microdialysis with a glucose sensor or a turbidimetric assay, glucose and a variety of drug levels can be continuously measured in the blood of patients to guide various therapies.

II. Methods and Materials

A. Microdialysis catheter, glucose sensor, and fluorometric measurements with a CMOS image sensor

A portable syringe pump was used to provide different flow rates while in vitro glucose sampling was performed with a microdialysis catheter (see Figure 1, left). Downstream and in-line with the microdialysis catheter, the self-diagnostic monitoring techniques and glucose sensor were assembled. A miniature, fluorometer setup (8 cm x 4 cm x 2.5 cm) was fabricated to measure fluorescence of the dialysate. Numerous researchers have demonstrated miniature fluorescence detection systems with various dyes [9,10]. The microdialysis probe was placed in a 15 mL test tube that contained glucose at a concentration of 180 mg/dL in a 1X CMF-PBS buffer. An electrochemical glucose sensor, Medtronic CGMS® iPro™, was used to sense dialysate glucose and produce an average value at 5 minute intervals. An embedded systems controller (MSP430, Texas Instruments, Dallas, TX), shown on the right side of Fig. 1, is used to control the self-diagnostic events and transmit measured fluorescence value to a nearby laptop.

B. Embedded systems controller

A custom printed circuit board, attached to the embedded system controller, is used to efficiently amplify the photodiode signal and determine the fluorescence level in the dialysate. Its secondary tasks are to sustain itself in terms of power usage as well as retain or transmit photodiode data. To perform these tasks the setup is governed by a central controller that controls the functioning of the complete system.

The fluorescein dye fluoresces at a 521 nm wavelength when blue light (488 nm wavelength) is projected on to the dialysate tubing. The photodiode is subjected to this emission radiation as well as other external noise sources (60 Hz noise from light sources such as fluorescent light tubes). To suppress this noise, the source 480nm light emitting diode (LED) light is modulated to provide an identifier for subsequent filtering and amplification. The fundamental signal frequency chosen for this operation is 965 Hz and an active band-pass analog filter (2^{nd} order, signal gain 40 dB) is implemented using discrete off-the-shelf components. The signal gain is programmable using a digital potentiometer so that the central controller can calibrate the gain of the system based on the input signal level. This prevents the signal from saturating if the optofluidic setup is altered. The amplified and filtered analog signal is sent over to the on-chip analog to digital converter (ADC) on the central controller.

To measure the fluorescence level digitally the LED is focused on the dialysate tubing for approximately 500 ms. Within this time frame, the ADC is programmed to sample the amplified signal for 2,560 samples. The average DC level and AC RMS level of the signal are measured using digital computations on the controller. The analog gain can be calibrated using this information and all the data henceforth is measured relative to the calibrated signal. The AC RMS signal level is what denotes the fluorescence level.

C. Turbidimetric measurements with a CMOS image sensor

Turbidimetric measurements were performed using a CMOS image sensor (Omnivision OV7690) and a 592 nm

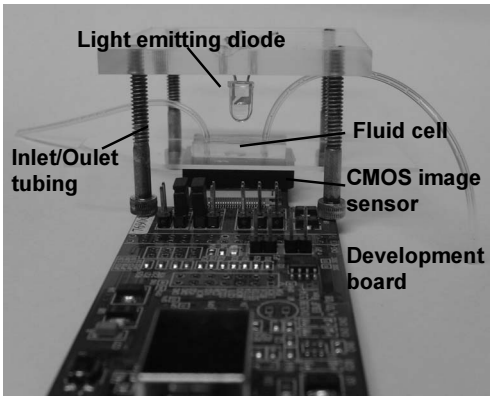

Fig. 2: Experimental setup to measure the turbidity of a fluid volume. The fluid cell is placed between the CMOS image sensor (bottom section) and the light emitting diode (top section).

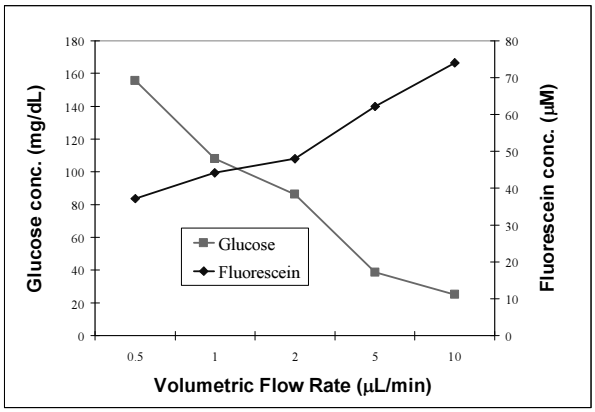

Fig. 3: *In vitro* glucose absolute recovery and fluorescein absolute loss at varying volumetric flow rate. The microdialysis probe was placed in a test tube containing 180 mg/dL glucose while the microdialysis buffer contained 100 μM fluorescein.

amber-colored LED as shown in Figure 2. A microparticle-based immunoassay for the antibiotic drug called vancomycin (QMS® Immunoassay, Thermo Fisher Scientific) was used during the experimental testing. A 20 μL fluid cell was placed between the LED and CMOS imager. The fluid cell is made from 3 mm-thick polydimethylsiloxane (PDMS). This enclosed cell is made by bonding this PDMS sheet to a 1 mm-thick glass side. The bond was formed by placing the PDMS sheet in an oxygen plasma (100 W for 15 s) to render its surface hydrophilic and form permanent bonds with the glass surface. In addition, the PDMS sheet was hole-punched to provide ports for inlet and outlet fluidic tubing.

Images were captured every 20 seconds (total capture time of 20 minutes) as the drug coated microparticle reagent is rapidly agglutinated in the presence of the antibody reagent and in the absence of any competing drug in the sample. The binary image data was then processed through MATLAB software (Mathworks, Natick, MA).

III. Experimental Results

A. In vitro glucose microdialysis with self-diagnostics

Combined tracer out-diffusion, glucose in-diffusion

A decrease in tracer diffusion rate quantifies the loss of diffusion capacity by fouling. The glucose sensor is combined with this modified retrodialysis method in order to evaluate the accuracy of using fluorescein as a metric for glucose trans-membrane diffusion. The microdialysis catheter was placed into a 15 mL test tube that contained a 180 mg/dL glucose solution. A 100 μM fluorescein solution was used as the microdialysis buffer while the volumetric flow rate on the syringe pump was varied to five different levels (0.5, 1, 2, 5, and 10 μL/min). As the flow rate increases, less time is allowed for diffusion to occur (more glucose remains in the test tube, more fluorescein remains in the probe buffer). Glucose absolute recovery and fluorescein absolute loss are shown in Figure 3. Since the fluorescein molecule is approximately twice the size of glucose (376 g/mol vs. 180 g/mol), it should provide an early warning if fouling begins to occur on the probe membrane.

B. Fluorometric and turbidimetric imaging with a CMOS image sensor

Fig. 4 compares the linearity of the CMOS image sensor by plotting the actual fluorescein concentration versus the CMOS imager measured fluorescein concentration. The 150 micron ID capillary was filled with six different concentrations of fluorescein (0, 5, 25, 50, 75, and 100 μM). As the fluorescein concentration increased, the dye fluorescence (green color, 520nm emission) became more visible.

Turbidimetric imaging was also performed with the CMOS image sensor. Captured images from the CMOS image sensor of a microparticle-based vancomycin immunoassay are shown in Fig. 5. A 0 μg/mL vancomycin solution was mixed with the two reagents in the immunoassay and then the solution was photometrically measured using the CMOS image sensor. The vancomycin concentration corresponds to the highest rate of agglutination and should provide the greatest visual discrepancy between images taken at different times. Images

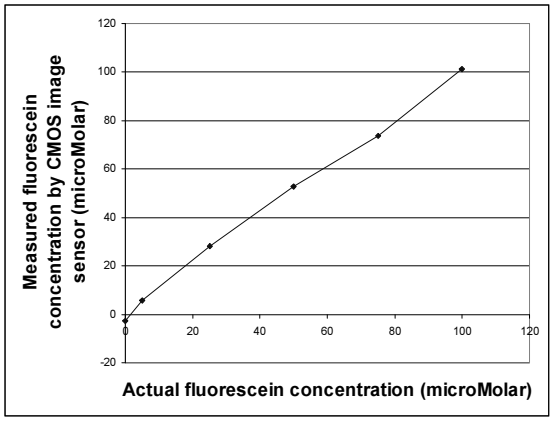

Fig. 4: Plot that compares the actual and CMOS imager measured fluorescein concentrations. Six different concentrations were used (0, 5, 25, 50, 75, and 100 μM).

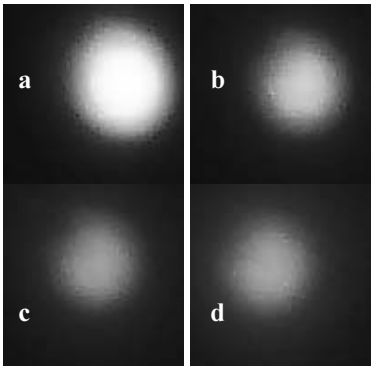

Fig. 5: Series of images taken by a CMOS image sensor showing a decrease in pixel intensity caused by increased turbidity (a, b, c, d correspond to time t=0, 5, 10, 15 minutes, respectively). A microparticle-based, vancomycin immunoassay was used at 0 μg/mL vancomycin concentration (the greatest rate of agglutination of the microparticles).

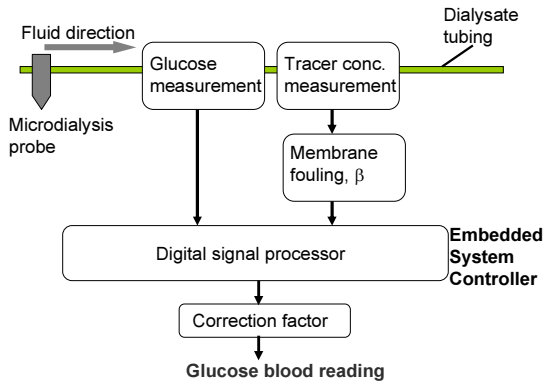

Fig. 6. System design showing the data from the glucose sensor and continuous monitoring technique enter the embedded system controller and provide a correction factor to glucose blood level readings.

at four different times (0, 5, 10, and 15 min.) demonstrate the capability to visualize differences in turbidity. The center bright spot is the visual representation of the LED light passing through the turbid fluid and imaged by the pinhole CMOS imager. Before acquiring images it was necessary to calibrate the setup to begin with unsaturated images. The binary image data was processed using MATLAB® software by windowing the analysis to a 40,000 pixel region that contains the spot region. A simple filter was used to remove any saturated light regions. As time progressed, the pixel light intensity decreased significantly. In addition, the amount of light scatter increased as noticed by the black image surrounding the spot region becoming lighter in color.

IV. Discussion

Microdialysis has the potential to continuously sample the bloodstream with minimal invasiveness. Since the technique recovers a dialysate sample, hundreds of molecules are obtained simultaneously. Drugs, metabolites, cytokines, chemokines, growth factors and many other targets can be repeatedly measured in association with disease processes and treatments. This data-rich fluid can be assayed on chip with the growing capability of lab-on-a-chip sensor technologies.

A fundamental requirement for microdialysis to become a clinical tool is that collected analytes truly reflect the composition in the blood. In this paper, a modified retrodialysis method that uses a fluorescent tracer allows continuous measurements of the probe membrane diffusive capacity. A CMOS image sensor with 300,000 pixels was able to deliver the required sensitivity to measure fluorescence levels in 150 micron ID tubing. In addition, the CMOS image sensor has the capability to measure light intensity changes within a turbidimetric drug assay.

Future work will involve the development of an empirical model that accounts for microdialysis inaccuracies (membrane fouling, ultrafiltration, and osmosis) and their interactions. This model will be uploaded to the embedded system controller and provide a correction factor to glucose recovery values as shown in Figure 6. Together these results and their implications take bring microdialysis closer to being a robust clinical tool.

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