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A MEMS differential viscometric sensor for affinity glucose detection in continuous glucose monitoring

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Abstract

Micromachined viscometric affinity glucose sensors have been previously demonstrated using vibrational cantilever and diaphragm. These devices featured a single glucose detection module that determines glucose concentrations through viscosity changes of glucose-sensitive polymer solutions. However, fluctuations in temperature and other environmental parameters might potentially affect the stability and reliability of these devices, creating complexity in their applications in subcutaneously implanted continuous glucose monitoring (CGM). To address these issues, we present a MEMS differential sensor that can effectively reject environmental disturbances while allowing accurate glucose detection. The sensor consists of two magnetically driven vibrating diaphragms situated inside microchambers filled with a boronic-acid based glucose-sensing solution and a reference solution insensitive to glucose. Glucose concentrations can be accurately determined by characteristics of the diaphragm vibration through differential capacitive detection. Our *in-vitro* and preliminary *in-vivo* experimental data demonstrate the potential of this sensor for highly stable subcutaneous CGM applications.

Keywords

Viscometric glucose sensor; differential measurement; affinity detection

Introduction

Subcutaneously implanted sensors can effectively achieve continuous glucose monitoring (CGM) for diabetes management. Existing CGM sensors [1–3] and representative commercial available systems [4–6] predominantly use electrochemical detection techniques [7–12], in which irreversible consumption of glucose, diffusion-limited glucose reaction rates, and interferences from electrochemically active compounds may potentially cause the degradation of functional enzyme and affect the device accuracy [13], reliability and longevity [10]. In contrast, glucose affinity sensing [14–20], which uses the equilibrium binding of glucose with receptors to realize specific glucose detection, has shown great promise as an alternative for glucose monitoring. These methods prevent the local glucose concentration from changing and do not generate unfavorable reaction products. Most importantly, affinity sensing is considerably more tolerant of biofouling because of the use

of equilibrium binding [21, 22], as the accumulation of biological material on the implanted sensor surface does not affect the measurement accuracy.

Affinity glucose sensors based on microelectromechanical systems (MEMS) technology hold the potential for glucose sensing, enabling miniaturization that would lead to improved measurement time response and minimized invasiveness. MEMS sensors have been used to measure glucose-induced changes in fluorescence intensity [23, 24], viscosity [25–27], hydrogen swelling [28], conductivity [29], and permittivity [30]. In addition, MEMS technology has also demonstrated interstitial fluid (ISF) sampling devices, which, with potential application to affinity glucose detection, employ microdialysis [31, 32], microneedles [33], sonophoresis [34], and microablation [35]. We have previously reported affinity glucose sensors based on a vibrational cantilever [25] or diaphragm [26]. These devices each contains a single glucose-detection module that measures the glucose-induced viscosity change in a solution of a glucose-sensitive polymer, and have demonstrated the feasibility of MEMS-based affinity sensors for CGM. However, these devices are affected by fluctuations in temperature and other environmental parameters, and hence cannot be subcutaneously implanted for accurate and reliable CGM applications.

To address these issues, we present a MEMS differential sensor that can effectively reject environmental disturbances and allow accurate glucose detection. The sensor consists of two magnetically driven vibrating diaphragms each situated inside a microchamber [36, 37]. One of the microchambers is filled with a glucose-sensing solution, while the other microchamber contains a reference solution that does not react with glucose. As glucose permeates through a semi-permeable membrane into each chamber, the viscosity of the sensing polymer solution increases due to glucose binding, while the viscosity of the reference solution only changes with environmental disturbances [38, 39]. Thus, measurement of the viscosity difference between the two chambers through differential capacitive detection of the vibration damping allows determination of the glucose concentrations while rejecting common mode disturbances. This paper reports systematic study of this differential viscometric sensor, including both *in-vitro* and preliminary *in-vivo* experiments conducted to quantify the performance of the sensor. Results from these experiments demonstrate that due to the differential design and MEMS fabrication techniques, the sensor offers significantly improved accuracy and stability with compacted footprint, and reveals the potential for use in fully implantable continuous glucose monitoring applications.

Materials and Methods

Principle and Design

The MEMS differential affinity glucose sensor is based on a pair of surface-machined freestanding diaphragms that vibrate under an external AC magnetic field [26] and are each situated inside a microchamber (Fig. 1). One chamber (the sensing chamber) is filled with a solution of a glucose-affinity polymer (the sensing solution), while the other chamber (the reference chamber) contains a solution of a polymer that does not bind with glucose (the reference solution). These chambers are sealed with a semi-permeable membrane that allows glucose to permeate freely while preventing the polymers from escaping. A moving electrode is embedded in each of the diaphragms and is separated from a fixed electrode on a substrate below by an air gap, forming a diaphragm-based position-sensing capacitor. Permalloy thin-film strips are also integrated on each of the diaphragms and are passivated along with the moving electrodes to avoid direct contact with the polymer solutions.

Poly(N-hydroxyethylacrylamide-*ran*-3-acrylamidophenylboronic acid) (PHEAA-*ran*-PAAPBA), a synthetic polymer that recognizes glucose by specific affinity binding [40], is

used as the glucose sensitive polymer in the device. PHEAA-*ran*-PAAPBA is an amphiphilic copolymer containing two components: a hydrophobic glucose-sensitive component poly(acrylamidophenylboronic acid) (PAAPBA) and a hydrophilic, nonionic component poly(N-hydroxyethyl acrylamide) (PHEAA) (Fig. 2a). PHEAA primarily serves to improve the overall water solubility of the copolymer, and also possibly provides additional neighbor coordinating effects to enhance the binding of boronic acid to glucose [40]. When added to an aqueous solution of PHEAA-*ran*-PAAPBA, glucose binds reversibly to the phenylboronic acid moieties in the PAAPBA segments to form strong cyclic boronate ester bonds, resulting in an increase in the viscosity of the solution (Fig. 2b), while having almost no response to other potential interferents, such as fructose, galactose, and sucrose. In contrast, poly(acrylamide) (PAA), which does not bind to glucose and has a glucose-independent solution viscosity, is used as a reference polymer [38, 39].

As free glucose molecules permeate through the semi-permeable membrane into the sensing chamber, they bind with PHEAA-*ran*-PAAPBA, likely causing the polymer to become cross-linked [40]. This increases the viscosity of the polymer solution and hence the viscous damping to the vibrational diaphragm. In the reference chamber, the viscosity and the vibration damping of the PAA solution are glucose-independent and only vary with environmental parameters (e.g., temperature). The differential capacitive sensors that are formed by the fixed electrodes on the device substrate and the moving electrodes embedded within the diaphragms measure the differences in the vibration amplitudes of the diaphragms, allowing accurate determination of the glucose concentration in the face of environmental fluctuations.

Fabrication Process

The fabrication of the device began with thermal evaporation and patterning of chrome (Cr) (5 nm) and gold (Au) (100 nm) to form two adjacent fixed electrodes ($500\text{ }\mu\text{m} \times 500\text{ }\mu\text{m}$) on a SiO_2 -coated silicon wafer (Fig. 3a). A Parylene (diX C Parylene dimer, Kisco Conformal Coating, LLC) passivation layer (1 μm in thickness) was then deposited through chemical vapor deposition, followed by spin-coating and patterning a sacrificial photoresist layer (AZ 4620, AZ Electronic Materials) to define a pair of electrode air gaps ($500 \times 500 \times 5\text{ }\mu\text{m}$ each) as well as channels for photoresist removal (Fig. 3b). An additional Parylene layer (1 μm in thickness) was then deposited to seal the sacrificial photoresist, followed by thermally deposited Cr/Au (5/100 nm) to form moving electrodes and permalloy seed layer (Fig. 3c). Subsequently, defined by photoresist (AZ4620) patterns (5 μm in thickness), strips of Permalloy ($220 \times 35 \times 3\text{ }\mu\text{m}$) were electroplated using an established recipe [41] (Fig. 3d). This was followed by removal of the photoresist with photoresist stripper (AZ 400T, AZ Electronic Materials), patterning of the moving electrodes ($500 \times 500\text{ }\mu\text{m}$), and deposition of a final Parylene passivating layer (3 μm) (Fig. 3e, and Fig. 4b and 4c). Aluminum (100 nm in thickness) was then thermally deposited and patterned as a mask layer for opening etching holes ($250 \times 250\text{ }\mu\text{m}$) to expose the sacrificial photoresist and bonding pads for electrical contacts through the Parylene layers by reactive ion etching (Fig. 3f). The aluminum was thereafter removed by an aluminum etchant (Aluminum Etchant Type A, Transene company, Inc). After wafer dicing (Figure 4d), a SU-8 layer (SU-8 2050, MicroChem corp.) 80 μm in thickness was spin-coated and patterned on the device, forming circular microchambers (1 mm in diameter and 0.06 μL in volume) as well as the inlets and outlets for polymer solution handling (Fig. 4e). The diaphragms ($520 \times 520\text{ }\mu\text{m}$) were finally released by removing the sacrificial photoresist in SU-8 developer [42] (80 °C) for 20 minutes (Fig. 3g). The etching holes were then sealed by epoxy (HP 250, Devcon Inc.), resulting in a pair of free standing diaphragms separated from the underneath substrate by sealed air gaps. The SU-8 microchambers were in turn bonded using epoxy to a regenerated cellulose semi-permeable membrane (Membrane Filtration Products, Inc.) with a thickness

of 20 μm and a molecular weight cutoff of 6 kD (Fig. 3h). Dimensional information of the glucose sensor is given in Fig. 4a.

Experimental Method

Chemicals and reagents used in the experiments include PHEAA-*ran*-PAAPBA and PAA, which were synthesized in house by free radical polymerization [40]. D-(+)-glucose were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS), pH 7.4, was prepared by diluting a Ringer's stock solution (Nasco Inc.) with sterile water (Fisher Scientific) at a ratio of 1:9. To prepare the sensing polymer solution, 284 mg of PHEAA-*ran*-PAAPBA with an hydroxyethylacrylamide (HEAA) to AAPBA molar ratio of 20 (or approximately 5% of PAAPBA content in the polymer) and a molecule weight of 188,600, was dissolved in 6 mL of PBS, while the reference polymer solution was prepared by dissolving 142 mg of PAA (molecular weight: 0.6×10^6 to 9×10^6) [43] in PBS (6 mL) to achieve roughly compatible viscosity as the sensing polymer solution. Glucose stock solution (1 M) was prepared by dissolving glucose (180 mg) in PBS to 10 mL. A series of glucose concentrations (60, 90, 180, 360, and 500 mg/dL) was prepared by further diluting the stock solution with PBS.

During testing, the microchambers were filled with solutions of PHEAA-*ran*-PAAPBA and PAA, respectively. To facilitate *in-vitro* device characterization, a test cell (volume: 300 μL) was constructed from an acrylic sheet directly above the MEMS device [26]. A glucose solution at a given concentration was introduced into the test cell, where it was allowed to permeate through the device's semi-permeable membrane to interact with PHEAA-*ran*-PAAPBA in the sensing chamber. Because the test cell was about 5,000 times larger in volume than the microchambers, it was assumed that the glucose concentration inside the microchambers equalized to the given glucose concentration in the test cell when the glucose permeation reached an equilibrium.

The diaphragm vibrations were excited and measured using an instrumentation setup in both the *in-vitro* and *in-vivo* experiments (Fig. 5). A cylindrical magnet attached perpendicularly to the shaft of a brushless DC motor (Anaheim Automation) spun with a maximum rated speed of 4000 RPM. The motor shaft was in parallel to the chip plane and perpendicular to the Permalloy strips, which were hence subjected to an AC magnetic field, inducing the vibration of the diaphragms. The average diaphragm vibration amplitudes were measured by an Σ - Δ ** capacitance digital converter (CDC) (Analog Devices, AD7746), which provided a square wave excitation with an amplitude of 3.3 V to the sensor electrodes and converted the amount of charges on the sensor electrodes to a capacitance value with a acquisition frequency at 90 Hz. This CDC is capable of measuring a capacitance change of $\pm 4\text{pF}$ with a measurement resolution and accuracy at 4 aF and 4 fF, respectively. The measurement range of the CDC can be adjusted by changing the excitation voltage of 3.3 V to a smaller value. To measure the differential capacitance, the CDC applied an AC excitation voltage to the fixed electrodes, while the moving electrodes in the diaphragms were connected to pins for differential capacitance measurement of the CDC, respectively. The capacitances of the sensing and reference electrodes at rest were determined to be 81.5 and 63.5 pF, which were obtained by calibrating the measured capacitance values with the measured value of a commercial capacitor with known capacitance. The CDC and computer software can be programmed to either obtain the maximum capacitance difference of the sensing and the reference electrodes (Differential capacitance) or the single-module capacitance that is solely from sensing electrodes.

The *in-vitro* experiments were conducted in a laboratory, where common environmental disturbances, such as human activities, modest bench vibrations, and temperature fluctuations were present. Such disturbances were compensated by the differential measurement approach, which eliminates the need for tight environmental control and is

appropriate for practical glucose monitoring applications. However, intentional temperature variations were introduced through closed-loop temperature control during the characterization of the effects of temperature on the device stability. In this closed-loop control system, a multimeter (Agilent 34410A) measured the device temperature through a k-type thermocouple on the device. The measured temperature was then used as feedback by the control system to impact appropriate power from a DC power supply (Agilent E3631A) to a thin film heater passivated by kapton and placed directly below the device. In *in-vivo* testing, the sensor was subcutaneously implanted in the back of a sedated mouse, whose glucose concentration was controlled by glucose and insulin administrations. The device temperature was uncontrolled and was determined solely by the body temperature of the tested mouse. The implanted glucose sensor continuously measured the glucose level in ISF, while a commercial glucometer (Abbott Diabetes Care, Freestyle Lite) sampled blood sugar levels in the mouse's tail at specified frequencies.

Sensor Calibration

In-vivo experimental results from the differential glucose sensor can be calibrated using a method that refers to glucose meter readings and resolves the time lags between ISF and blood glucose concentration as well as nonlinearity during the measurements, resulting in predicted glucose concentrations. In this method, blood and ISF are considered as two separate glucose-containing compartments that exchange glucose through capillary walls [44]. As a result, ISF glucose values can be determined by the diffusion rate of glucose between blood to ISF and the rate of glucose uptake by subcutaneous tissues [45]. The concentration gradient in the ISF glucose can be expressed as

$$dG_2/dt = -(k_{02} + k_{12})G_2 + k_{21}V_1/V_2G_1 \quad (1)$$

where G_1 and G_2 are blood and ISF glucose concentrations respectively. k_{12} and k_{21} are the flux rates of forward or reverse glucose transport across the capillary. k_{02} is the glucose uptake into the subcutaneous tissue, and V_1 and V_2 are the volumes of blood and ISF respectively. This equation accounts the time lag between the differential capacitance and the glucose meter readings due to exogenous mass transfer [46]. However, the same equation is also applicable to account for the time lag caused by the endogenous glucose diffusion within the glucose sensors.

Existing electrochemical CGM sensors predominately use a simple linear equation $y = ax + b$ as a representation between CGM sensor outputs and blood glucose values [45, 47]. In this equation, a and b are constant, while x stands for a blood glucose value in corresponds to a sensor output y in a form such as electric current and voltage. Assuming zero current or voltage output from glucose sensors in a glucose-free environment ($b = 0$), a reference glucose value from the glucose meter and a corresponding sensor output (a glucose concentration pair) can be used to determine a . If $b \neq 0$, two glucose concentration pairs can be used to obtain both a and b [48].

However, affinity glucose sensors in general exhibit nonlinearity in response to glucose concentration changes as *in-vitro* demonstration below. Thus we may use a quadratic equation as

$$G_2 = aC_{out}^2 + bC_{out} + c \quad (2)$$

to represent the relation between the differential sensor capacitance (C_{out}) and ISF glucose concentrations (G_2) with constant indexes a , b , and c . Combining Eq. (1) and (2) yields

$$G_1 = a_1 C_{\text{out}}^2 + a_2 C_{\text{out}} + a_3 C_{\text{out}} dC_{\text{out}}/dt + a_4 dC_{\text{out}}/dt + a_5 \quad (3)$$

where a_1 , a_2 , a_3 , a_4 , and a_5 are constants that can be determined from least squares fitting using six glucose meter readings (G_1) and the corresponding sensor output (C_{out}). Thus, for a given C_{out} , the predicted glucose value, denoted by $\hat{G}_1$, can be obtained from Eq. (3).

Results and Discussion

This section presents *in-vitro* and *in-vivo* experimental results from the MEMS differential viscometric glucose sensor. We first evaluated the device response to various glucose concentrations. The temporal course of the diaphragm vibration due to glucose concentration changes was then obtained to determine the device's time response and examine its reversibility. In addition, the ability of the device in rejecting disturbances, such as temperature fluctuations and osmotic pressure was characterizations in terms of its temperature stability and signal drift. Finally, a preliminary experiment with a sedated lab mouse was performed.

Sensor Response to Glucose at Physiologically Relevant Concentrations

Sensor response to varying glucose concentrations in a physiologically relevant range was first investigated to characterize the device resolution in glucose detection. During the experiments, the magnet was spun at a fixed frequency of 13 Hz. As the glucose concentration changed from 0 to 500 mg/dL, the differential capacitance of the device decreased steadily from 28.0 to 27.4 pF, indicating a decrease in the difference of the diaphragm vibration amplitudes and an increase in the viscous damping due to glucose binding with PHEAA-*ran*-PAAPBA (Fig. 6). The sensitivity of the sensor in this glucose concentration range was hence determined to be approximately 1.2 fF/(mg/dL). With the capacitance measurement resolution and accuracy of the CDC respectively given by 4 aF and 4 fF, this allowed for the determination of the resolution and accuracy of the device for glucose concentration measurements to be 0.3 µg/dL and 0.3 mg/dL, respectively.

Sensor Time Response to Glucose Concentration Changes and Reversibility

The sensor was then exposed to glucose solutions whose concentrations were changed between two different values to characterize the device time responses. For example, glucose concentration in the test cell and microchambers was initially equilibrated at 60 mg/dL. Next, glucose solution in the test cell was replaced with another glucose solution at a concentration of 90 mg/dL at time 10 min. After achievement of equilibrium throughout the test cell and microchambers, the test cell was finally refilled with a glucose solution at 60 mg/dL at time 28 min. During the equilibration processes, the differential sensor capacitance, at a fixed frequency of 13 Hz, was measured as a function of time. As shown in Fig. 7, it can be seen that as the glucose concentration varied from 60 to 90 mg/dL, the differential sensor capacitance decreased with time, corresponding to a decrease in the sensing diaphragm vibration amplitude as well as an increase in the viscous damping to the diaphragm vibration. The capacitance finally saturated to a constant level, reflecting that the process of glucose permeation and binding had reached equilibrium. In the reverse process as the glucose concentration in the test cell was decreased from 90 to 60 mg/dL, the sensor capacitance increased with time, indicating an increase in the vibration amplitude of the sensing diaphragm due to the reduced viscous damping.

To quantitatively estimate the device response time, each glucose concentration change was approximated to be a step function, i.e., it was completed instantaneously. This was appropriate because the process of each glucose solution or buffer replacement in the test

cell was completed within 10 seconds, which could be reasonably neglected when compared to the time constants to be obtained. The time dependence of the equilibration processes in response to all glucose concentration changes could be adequately represented by a first-order exponential function with the time constant obtained via least-squares fitting. The time response, defined as the time required by the differential capacitance to reach 90% of the steady-state value [49], was determined to be 2.5 min as the glucose concentration increased from 60 to 90 mg/dL, and 4.3 min as the glucose concentration changed reversely from 90 to 60 mg/dL. The longer reverse time constant could be due to the smaller diffusivity of glucose molecules in the initially more viscous polymer solution. These time constants are consistent with those for previously reported viscometric sensors containing a single sensing module [26], and is smaller than response times of commercially available continuous glucose monitors, which range from 5 to 15 minutes [50–53]

These experiments also allowed us to quantitatively assess the reversibility of the device response by examining in sensor output as the glucose concentration undergoes a change that was later reversed. For example, consider the glucose concentration change from 60 to 90 mg/dL, and then back to 60 mg/dL (Fig. 7). The average sensor output during two time intervals at 60 mg/dL, first from 0 to 7 min and then from 32 to 36 min, was computed to be 27.642 and 27.636 pF. Thus, the difference in the average sensor output was only 6 fF, or 0.02%. Note that this reversibility is achieved by rejection of common-mode temperature fluctuations without temperature control (below), and is hence appropriate for implantable applications.

Temperature Stability in Differential and Single-Module Measurements

The ability of the device to reduce the effects of temperature fluctuations was characterized. The temperature of the device was varied from 34 to 40 °C under closed-loop control at a fixed glucose concentration of 60 mg/dL. Both the differential capacitance and single-module capacitance (i.e., the capacitance between the sensing electrodes, with that between the reference electrodes ignored) were obtained (Fig. 8). Corresponding to the 6 °C temperature change, the differential and the single-module capacitances changed by 0.8 and 2.7 pF, respectively. Thus, differential measurements reduced the effects of the temperature variations on glucose concentration measurements by 70%. This would lead to a significant improvement in the stability and accuracy of the sensor when used as an implantable device. The compensation of temperature variations could be further optimized by reducing the mismatch between the glucose-free capacitances associated with the sensing and reference diaphragms due to device fabrication variations as well as viscosity differences between the sensing and reference solutions.

Drift in Sensor Output in Differential and Single-module Measurements

The glucose-independent drift in differential and single-module sensor capacitance was assessed at a fixed glucose concentration without controlling the temperature. The sensor was exposed to a 60 mg/dL glucose solution over an extended period about 5 hours (Fig. 9). During this period, the CDC was programmed to alternate in recording the differential and single-module capacitances under the same environmental conditions (e.g., temperature variations and lighting). We observed that the drift in the differential sensor capacitance was significantly smaller than the drift in the single-module capacitance. The differential capacitance of the sensor changed from 27.24 at 0 min to 27.23 pF at 320 min, corresponding to a drift rate of approximately 1.8 fF/hr or 60 ppm/hr, while this value was 94 fF/hr (1100 ppm/hr) in the single-module measurements. These results demonstrate that the differential device is capable of compensating for environmental disturbances and providing excellent stability. The ability of rejection common-mode disturbances is influenced by factors such as the diaphragm fabrication variations, compositions of sensing

and reference polymer solutions, and parasitic capacitances, and can hence be optimized for accurate and reliable glucose concentration measurements.

A Preliminary Animal Experiment

Finally, we have performed preliminary *in-vivo* characterization of a MEMS differential viscometric sensor with a laboratory mouse. The glucose sensor was implanted in the subcutaneously tissue of the sedated mouse to measure the glucose concentrations in ISF continuously (Fig. 10a), while a commercial glucose meter (Abbott Diabetes Care, Freestyle Lite) measured the glucose levels in the capillary blood sampled from the mouse's tail tip every 10 minutes after a glucose administration and every 5 minutes after an insulin administration. ISF glucose concentrations obtained from the calibrated MEMS differential sensor was compared with the corresponding blood glucose concentrations as well as changes in the measured differential capacitance, which is calculated with respect to the differential capacitance value at the time of the first glucose meter reading (Fig. 10b). Six blood glucose concentration values were used for sensor calibration, consisting of three pairs of values at times when the glucose concentration stayed roughly at a constant ($t = 20$ and 30 min), experienced a significant increase ($t = 61$ and 97 min) and exhibited a significant decrease ($t = 135$ and 143 min), respectively. The corresponding device output (C_{out}) values at each of these times and at time 4 min prior were used to approximately calculate the derivative dC_{out}/dt in Eq. (3). It can be seen that the ISF glucose concentrations predicted by the MEMS sensor closely followed blood glucose concentrations from the glucose meter over the entire measurement period of 180 minutes. For example, from the time of glucose administration ($t = 0$) to the time of insulin administration ($t = 125$ min), the blood glucose concentration increased from 193 to 500 mg/dL, corresponding to a rise in ISF glucose concentration from 210 to 440 mg/dL. Following insulin administration at $t = 125$ min, the mouse's blood glucose concentration rapidly dropped from 500 to 322 mg/dL at $t = 130$ min, which was accompanied by a decrease of the MEMS sensor-measured ISF glucose concentration from 410 to 320 mg/dL.

Due to limitations in the early animal testing protocol in which insulin administration was not sufficient to reduce large glucose concentration decreases, all of the measurements occurred in the hyperglycemic range. In addition, the calibrated glucose concentrations exhibit larger fluctuations as compared with changes in differential capacitance and readings from the glucose meter. This might have been caused by a combination of factors. First, the non-optimal selection of time intervals for numerical differentiation in Eq. (3) could have resulted in larger apparent variations in glucose concentrations in the presence of rapid changes in differential capacitance. Second, the differential sensor minimized but did not completely eliminate the effects of external disturbances. Finally, the discrete reference readings from the glucose meter might have missed fluctuating blood glucose concentrations in between readings, which were detected by the MEMS sensor. These apparent fluctuations will be investigated in future work, which could involve improvement of numerical differentiation methods, better match in geometry and operation of sensing and reference chambers and diaphragms, and comparison of results from the MEMS sensor and from other continuous glucose sensors [54]. Nonetheless, these early preliminary data suggest that the MEMS differential viscometric sensor holds the promise for measurement of ISF glucose concentrations in an *in-vivo* setting.

Conclusions

A differential MEMS affinity glucose sensor that uses a biocompatible glucose-responsive polymer has been presented. The sensor consists of a pair of parylene diaphragms, which are situated inside two microchambers (sensing and reference chambers) and are driven magnetically. The sensing chamber is filled with a PHEAA-*ran*-PAAPBA polymer solution,

which specifically recognizes glucose via affinity binding, while the reference chamber is filled with PAA, a reference polymer that does not respond to glucose. A semi-permeable membrane seals the chambers, preventing the polymers from escaping while allowing glucose to permeate into the device. As glucose binds with PHEAA-*ran*-PAAPBA, the polymer becomes cross-linked. This changes the viscosity of the polymer solution and the damping to the diaphragm vibration. On the other hand, the viscosity in the reference PAA polymer solution is glucose-independent and varies only with environmental parameters. Thus, the glucose concentration can be determined by capacitively measuring the difference in the vibration between the sensing and the reference diaphragm.

In-vitro experimental results have shown that the sensor experienced a decrease in the differential capacitance of approximately 0.6 pF when the glucose concentration increased from 0 to 500 mg/dL. The time constant of the sensor was approximately 1.48 minutes during a glucose concentration change from 60 to 90 mg/dL. In addition, the sensor also exhibited excellent reversibility; the differential capacitance of the sensor measured at two separated measurements at 60 mg/dL glucose concentration agreed within 99.97%. In addition, by varying the device temperature from 34 to 40 °C at a glucose concentration of 60 mg/dL, the differential capacitance only changed by 0.8 pF, which is at least three times smaller than the change in the single-module capacitance, indicating the sensor's ability in resisting temperature variations. By exposing the sensor to a 60 mg/dL glucose concentration for an extended measurement period of 5 hours, the differential sensor output exhibited low drift (1.8 fF/h), which is appropriate for long-term, stable CGM. Moreover, preliminary *in-vivo* experimental results have indicated that the MEMS sensor-measured ISF concentration closely follows blood glucose concentrations in laboratory mice as measured by a commercial glucose meter. These *in-vitro* and *in-vivo* characterization results indicate that our sensor has its potential applications in long term CGM with improved stability in facing of environmental disturbances. Future animal experiments will be pursued with more extended measurement periods as well as improved experimental procedures that allow the assessment of differential sensors in the entire physiological glucose concentration range.

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Fig. 1. Schematic of the MEMS differential glucose sensor: (a) Side view and (b) top view of the capacitive glucose sensor. The unit in the figure is in μm .

Fig. 2. Poly(N-hydroxyethylacrylamide-*ran*-3-acrylamidophenylboronic acid) (PHEAA-*ran*-PAAPBA), a glucose-specific polymer. (a) Polymer composition. (b) Specificity of the polymer in the presence of other sugars.

Fig. 3. Fabrication processes. (a) Bottom electrode deposition and patterning. (b) Parylene deposition and sacrificial layer patterning. (c) Parylene and moving electrode deposition. (d) Permalloy electroplating. (e) Moving electrode patterning and additional Parylene layer deposition. (f) Aluminum mask deposition and RIE patterning of underneath Parylene layers. (g) SU-8 microchamber patterning and sacrificial layer removal to release diaphragms. (h) Semi-permeable membrane attachment and epoxy seal of photoresist etching holes.

Fig. 4. Micrographs of (a) 3-dimensional structure of a single vibrational diaphragm taken by an optical profiler, (b) top view of a single vibrational diaphragm and (c) its enlarged view, (d) the MEMS differential glucose sensor (d) before and (e) after integrating with SU-8 microchambers.

Fig. 5. Experimental setup for in-vitro and in-vivo characterization of the MEMS differential glucose sensor.

Fig. 6. Sensor response to glucose variations. The diaphragm vibration amplitude (indicated by the differential sensor capacitance) decreases due to the higher viscous damping from glucose binding. (The glucose concentration changes occurred at time 0, 22, 40, and 55 min, respectively.)

Fig. 7. Time course of the diaphragm vibration amplitude (indicated by the differential sensor capacitance) as the sensor responded to glucose concentration changes from 60 to 90 mg/dL (occurring at time 10 min), which was then reversed to 60 mg/dL (occurring at time 28 min).

Fig. 8. Comparison of sensor capacitance output as a function of temperature in single-module and differential measurements.

Fig. 9. Drift measurement. The device was exposed to 90 mg/dL glucose solution for approximately 5 hours.

Fig. 10.

Preliminary in-vivo testing. (a) Sensor implantation in a laboratory mouse. (b) Differential capacitance changes and predicted glucose concentration from the differential sensor as compared to readings from a commercial glucometer. Glucose and insulin solutions were injected at the time of 0 min and 125 min to change the glucose concentration of the mouse.