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A dielectric affinity glucose microsensor using hydrogel-functionalized coplanar electrodes

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Abstract

This paper presents a dielectric affinity microsensor that consists of an in situ prepared hydrogel attached to a pair of coplanar electrodes for dielectrically based affinity detection of glucose in subcutaneous tissue in continuous glucose monitoring applications. The hydrogel, incorporating *N*-3-acrylamidophenylboronic acid that recognizes glucose via affinity binding, is synthetically prepared on the electrodes via in situ gelation. When implanted in subcutaneous tissue, glucose molecules in interstitial fluid diffuse rapidly through the hydrogel and bind to the phenylboronic acid moieties. This induces a change in the hydrogel's permittivity and hence in the impedance between the electrodes, which can be measured to determine the glucose concentration. The in situ hydrogel preparation allows for a reduced hydrogel thickness ($\sim 10\ \mu\text{m}$) to enable the device to respond rapidly to glucose concentration changes in tissue, as well as covalent electrode attachment of the hydrogel to eliminate the need for semipermeable membranes that would otherwise be required to restrain the sensing material within the device. Meanwhile, the use of coplanar electrodes is amenable to the in situ preparation and facilitates glucose accessibility of the hydrogel, and combined with dielectrically based transduction, also eliminates mechanical moving parts often found in existing affinity glucose microsensors that can be fragile and complicated to fabricate. Testing of the device in phosphate-buffered saline at pH 7.4 and 37 °C has shown that at glucose concentrations ranging from 0 to 500 mg/dL, the hydrogel-based microsensor exhibits a rapid, repeatable, and reversible response. In particular, in the glucose concentration range of 40–100 mg/dL, which is of great clinical interest to monitoring normal and low blood sugar levels, the device response is approximately linear with a resolution of 0.32 mg/dL based on effective capacitance and 0.27 mg/dL based on effective resistance, respectively. Thus, the device holds the potential to enable reliable and accurate continuous monitoring of glucose in subcutaneous tissue.

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Keywords

Affinity sensing; Coplanar electrodes; Synthetic hydrogel; Continuous glucose monitoring

1 Introduction

Diabetes mellitus is a metabolic disease involving high blood sugar levels in patients. Subcutaneously implanted sensors can effectively achieve continuous glucose monitoring (CGM) to reduce the risk of complications caused by hypoglycemia or hyperglycemia, thereby enabling effective management of diabetes mellitus (Keenan et al. 2009). Existing CGM sensors are predominantly based on the electrochemical detection of enzymatic reactions (Ricci et al. 2005; Luo et al. 2013; Heo et al. 2011). While electrochemical CGM sensors are well established, the irreversible consumption of glucose during the electrochemical reactions can potentially induce changes in the local equilibrium glucose concentration, causing significant inaccuracies (Heller 1999). In addition, since electrochemical reaction rates are diffusion-dependent, any changes in the diffusive properties of the reagents on the sensor's surface due to biofouling (e.g., by cell deposition and capsule formation) tend to further decrease the accuracy of the device (Liu et al. 1998). Also, functional enzymes used in electrochemical sensors are often found to degrade over time, while interference from electrode-active chemicals can compromise the device accuracy, reliability, and longevity.

As an alternative to electrochemical detection, affinity glucose sensing relies on the equilibrium binding of glucose with receptors to achieve specific glucose detection and has been an area of active research on both conventional and micro/nanoscale sensor platforms (Huang et al. 2014; Ballerstädt and Ehwald 1994; Schultz et al. 1982). The primary advantage to such sensors lies in the non-consumptive nature of affinity binding, which avoids sensor-induced change of the local glucose concentration and generation of unfavorable reaction by-products. Furthermore, affinity sensing is considerably more tolerant of biofouling as the change of the diffusion rate results in an increase in the equilibration time without affecting the accuracy of the readings. Affinity glucose sensors based on microelectromechanical systems (MEMS) technology are promising in achieving miniaturized subcutaneous implantable CGM devices. MEMS has been applied to affinity glucose sensors using proteins (Kuenzi et al. 2010), synthetic polymers (Li et al. 2008), and hydrogel receptors (Shibata et al. 2010; Shang et al. 2016; Lei et al. 2006; Guenther et al. 2014), based on measurements of glucose-induced changes in physical properties of the sensing material such as the fluorescence intensity (Shibata et al. 2010), viscosity (Zhao et al. 2007), or volume (Lei et al. 2006; Guenther et al. 2014). Unfortunately, these methods are not well suited to implanted operation due to issues such as requirements of optical access or mechanical moving parts. In contrast, dielectric affinity sensors that detect changes in the glucose-dependent permittivity of the sensing material can alleviate these limitations and are hence highly attractive. Our group previously reported our preliminary work exploring hydrogel-based affinity glucose sensors (Shang et al. 2016). That work demonstrated the potential of hydrogels for affinity glucose sensing, but was limited by a very slow time response (~16 min) due to the use of a thick hydrogel layer (~250 μm), which

required long diffusion times between the sensor surface and surroundings. The hydrogel layer, manually dispensed by a pipette to the sensor surface, also posed challenges in secure adhesion to and uniform coverage of the surface.

The present work was aimed at developing a hydrogel-based MEMS affinity glucose sensor that is robustly constructed and rapidly responsive. The device consists of a coplanar capacitive transducer coated with a thin ($\sim 10\ \mu\text{m}$) hydrogel layer. Binding between the hydrogel and glucose causes a change in the permittivity of the hydrogel, which is measured from the impedance of the transducer to determine the glucose concentration. Key to the rapid responsiveness and robust construction of the device is the adoption of a substantially thinner hydrogel ($\sim 10\ \mu\text{m}$), achieved by the innovative use of in situ gelation, to speed up the time response (potentially down to 1.2 s). Additionally, the device adopts a coplanar capacitive transducer, which, compared with the parallel-plate based configuration used in our preliminary work (Shang et al. 2016), is considerably more robust and easier to fabricate, is amenable to in situ hydrogel preparation, and fully avails the hydrogel layer to glucose exchange. The device has been tested in phosphate-buffered saline (PBS) solution at pH 7.4 and 37 °C. Testing results showed that in a glucose concentration range of 0–500 mg/dL the microsensor exhibited a repeatable and reversible response to glucose with a resolution down to 0.32 mg/dL based on effective capacitance and 0.27 mg/dL based on effective resistance, thereby demonstrating the potential of the device for continuous glucose monitoring.

2 Materials and methods

2.1 Principle and design

The hydrogel-based affinity sensor is integrated with a glucose-responsive hydrogel formed by in situ radical polymerization by successive addition of *N*-3-acrylamidophenylboronic acid (AAPBA) as free radical glucose-sensitive monomer, acryl *N*-hydroxyethyl acrylamide (HEAA) as the hydrophilic monomer, tetraethyleneglycol diacrylate (TEGDA) as the cross-linker and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) as the polymerization initiator. When diffusing into the affinity hydrogel, glucose binds reversibly to phenylboronic acid moieties in the AAPBA segments to form strong cyclic boronate ester bonds, inducing partial cross-linking of the hydrogel matrix and causing a molecular structure change. As electric polarization is strongly influenced by the material's molecular structure changes, the binding process of the glucose to the immobilized affinity hydrogel induces changes in its dielectric properties, which can be detected using an inter-digitated sensor (Fig. 1).

The complex permittivity of hydrogel is represented by $\epsilon^* = \epsilon' - j\epsilon''$, where the real permittivity ϵ' represents the ability of the hydrogel to store electric energy, while the imaginary permittivity ϵ'' is related to dissipation of energy (Kremer and Schönhal's 2012). When the hydrogel is integrated on the IDEs, the microsensor can be represented by a capacitor and a resistor connected in series. The complex interaction of hydrogel with glucose can cause changes in charge distribution, double-layer structure, composition as well as conformation of the hydrogel, which will in general induce changes in both the real part and imaginary part of dielectric properties of hydrogel. Thus, the microsensor's

effective capacitance and resistance can be affected by the interaction of glucose to hydrogel and are measured to determine the glucose concentration.

The MEMS microsensor adopts a coplanar capacitive transducer using IDEs. The IDEs situated on the silicon dioxide substrate consist of 40 pairs of gold electrodes with a thickness of 50 nm, 1.5 mm length, 20 μm width, and interspacing (Fig. 2a, b). A thin layer of hydrogel ($\sim 10 \mu\text{m}$) is covalently attached to the sensor surface via in situ radical polymerization to substantially speed up the time response. When immersing the sensor to glucose solution, glucose molecules can rapidly diffuse through the thin layer of hydrogel and reversibly bind with the hydrogel, changing its complex permittivity, which can be interrogated via measurement of the impedance between the IDEs to determine the glucose concentration. An on-chip resistive temperature sensor is integrated for monitoring the surface temperature and providing feedback to a temperature control system (Fig. 2c).

2.2 Fabrication

The hydrogel-based microsensor chip was fabricated using the standard MEMS fabrication process. A thin layer of metal (Cr/Au 5/45 nm) was deposited on a SiO_2 substrate by thermal evaporation and patterned to form the inter-digitated electrodes and on-chip resistive temperature sensor. The hydrogel thin film was integrated on the electrode using in situ radical polymerization. Specifically, a mixture of the deoxygenated hydrogel components was first spin coated on the microsensor surface and then heated to 70 $^{\circ}\text{C}$ in nitrogen to initiate in situ gelation via radical polymerization from a mixture of monomers, cross-linker, and initiator dissolved in water.

2.3 Materials

The hydrogel was synthesized in house via free radical polymerization of the pre-mixed solution of AAPBA (1.1% w/v), HEAA (5.5% v/v), TEGDA (0.08% v/v), and AAPH (0.16% w/v). D-(+)-glucose and phosphate-buffered saline (PBS, 0.15 M, pH 7.4) were bought from Sigma-Aldrich. A glucose stock solution (500 mg/dL) was prepared by dissolving D-(+)-glucose (2.5 g) in 500 mL PBS. Then glucose solutions (40, 80, 100, 200, and 300 mg/dL) were prepared by further diluting the stock solution with PBS.

2.4 Experimental procedures

During testing, the microsensor was placed in an acrylic flow cell (1.2 mL in volume) filled with glucose solution to be tested (Fig. 3a). To minimize thermally induced dielectric property change of the hydrogel and solution, during all experiments the temperature of the test cell was controlled at 37 $^{\circ}\text{C}$ by closed-loop control of a Peltier heater (Melcor, CP14) according to feedback from the integrated resistive temperature sensor. The microsensor was coupled to an impedance/voltage transformation circuit driven by a small sinusoidal input from a function generator (Agilent, 33220A) (Fig. 3b), which imposes an AC field between the IDEs of the microsensor to induce a glucose concentration-dependent change in the permittivity of the hydrogel. The impedance of the microsensor can be determined by measurements of the amplitude and phase of the system output voltage V_{out} , detected using a lock-in amplifier (Stanford Research Systems, SR830) from a known input voltage V_{in} , via the impedance/voltage transformation circuit. We calculated the effective capacitance and

effective resistance of the microsensor based on an equivalent circuit that consists of a serial connection of a capacitor C_x and resistor R_x representing the microsensor. The microsensor's complex impedance can be determined by $Z_x = R_x + 1/(j\omega C_x) = -V_{out}/(R_f V_{int})$, where R_f is the reference resistance.

3 Results and discussion

We first investigated frequency dependence of the microsensor response to various glucose concentrations from 0 to 500 mg/dL over selected frequencies in the range of 1–100 kHz. Then we characterized the time-resolved response of the microsensor to a sequence of glucose variations under a specific frequency, to assess its potential applicability to glucose monitoring. Finally, we tested the response of the microsensor to other potential interferents including fructose, galactose, and lactate at their physiological concentrations to compare the sensor's response to a normal glucose concentration of 100 mg/dL.

3.1 Frequency dependence of device response

We first characterized the frequency dependence of the sensor response to various glucose concentrations. In the experiment, a series of glucose concentrations (40, 80, 100, 200, 300, and 500 mg/dL) in PBS buffer were tested sequentially. For instance, PBS buffer was first introduced to the test cell and an AC power source was applied to the microsensor. The microsensor was then allowed to reach equilibrium followed by a frequency sweep from 1 to 100 kHz. The tested frequency range was chosen between 1 and 100 kHz since the unwanted parasitic impedance induced by electrical double layer becomes more dominant at frequency less than 1 kHz (Oh et al. 2003) and the maximum frequency can be detected using lock-in amplifier is 100 kHz. The test was conducted in triplicate to examine the ability of the microsensor to measure glucose concentrations in a repeatable manner. The same experiments were repeated for all other concentrations, from which the steady-state effective capacitance and effective resistance of the microsensor, as a function of the excitation frequency, were computed from the output voltage of the measurement circuit and plotted as a function of frequency (Fig. 4a, b). The microsensor's effective resistance and effective capacitance were both decreased consistently with the increasing frequency due to the dielectric relaxation of the hydrogel. A number of polarization mechanisms (i.e., the shift of electric charges from their equilibrium positions under the influence of an electric field) (Kremer and Schönhal's 2012), such as dipole reorientation, counterion diffusion, and interfacial polarization, contributed to the measured response of the microsensor.

At the same time, the effective capacitance and the effective resistance were recorded as a function of glucose concentration at three chosen frequencies (1, 2, 5 kHz) (Fig. 5a, b). At a specific frequency, the microsensor's effective capacitance decreased monotonically with glucose concentration in the entire range tested (0–500 mg/dL), while the effective resistance of the microsensor increased monotonically with the entire tested glucose concentration. Specifically, at 1 kHz, the effective capacitance decreased from 297.2 to 246.6 pF and the effective resistance increased from 11.58 to 14.57 kΩ as the glucose concentration increased from 0 to 500 mg/dL. This was reflected in the binding between the hydrogel and glucose, which influenced the polarization of the hydrogel. The AAPBA units

appended on the hydrogel backbone could form cyclic boronate esters with glucose molecules at a 1:1 or 2:1 ratio, leading to a change in the net permanent dipole moments of AAPBA units (or their dipole reorientation). In addition, based on our previous observation (Li et al. 2009), the binding with glucose can induce partial cross-linking of the hydrogel, which in turn will increase the elastic resistance of the dipole rearrangement in the electric field, leading to a change in the hydrogel complex permittivity. The combination of these effects can contribute to the concentration-dependent sensor response to solution glucose levels.

Next, we characterized the microsensor's sensitivity, i.e., change in effective capacitance or resistance due to glucose concentration change (C/c_{glucose} , R/c_{glucose}). The device characteristics indeed generally varied with the glucose concentration due to the nonlinearity of the sensor response. With the capacitive transducer operating at 1 kHz for glucose concentration ranging from 0 to 500 mg/dL tested, the sensitivity, calculated at a given concentration value, varied from 410 fF(mg/dL)⁻¹ to 15.8 fF(mg/dL)⁻¹ based on effective capacitance and from 18.9 to 2.8 Ω(mg/dL)⁻¹ based on effective resistance, respectively. Using an estimated noise of 90 μV and 0.085° in the magnitude and phase of the output AC voltage of the measurement circuit and instrumentation, the corresponding resolution in glucose concentration in this range was estimated to be from 0.27 to 3.2 mg/dL based on effective capacitance and from 0.20 mg/dL to mg/dL based on effective resistance, respectively. It is interesting to note that the sensor response, while nonlinear in the full range, was quite linear from 40 to 100 mg/dL, with $R^2 = 0.99$. In this range, the sensitivity was 270 fF(mg/dL)⁻¹ based on effective capacitance and 11.09 Ω(mg/dL)⁻¹ based on effective resistance, and the resolution was 0.32 mg/dL based on effective capacitance and 0.27 mg/dL based on effective resistance, respectively.

3.2 Device response to time-dependent glucose concentration changes

We then measured the time-resolved response of the sensor to a sequence of glucose variations to assess its potential applicability to glucose monitoring. Noting that as the measurement frequency increased, the sensor response became more diminished because of dielectric relaxation (Woo et al. 2012), we performed the measurements at a fixed frequency of 1 kHz, which offered a pronounced sensor response while minimizing the effect of the electrical double layer on the electrode surface on the device's impedance. The test cell was initially filled with PBS buffer and then replaced with PBS solution of glucose, first sequentially at concentrations of 40, 80, and 300 mg/dL, and then sequentially in the reverse order at the same set of concentrations. From the measurement results (Fig. 6a, b), it was seen that both the effective capacitance and resistance were consistent at a given glucose concentration setpoint, regardless of whether that setpoint resulted from an increase or decrease from the glucose concentration used in the preceding test. For example, the effective resistance at a glucose concentration of 80 mg/dL over the two periods, approximately defined by the internals from 30 to 40 min (when the glucose concentration increased from 40 to 80 mg/dL) defined by the internals from 56 to 66 min (when the glucose concentration decreased from 300 to 80 mg/dL), was, respectively, 12.845 and 12.895 kΩ, which agree within 0.4%. Similarly, the reversibility was found to be within 0.6 and 0.8% for the measurement data at 40 and 0 mg/dL glucose concentrations, respectively.

This indicates that the device was highly reversible in response to glucose concentration variations.

The time dependence of the measured sensor output could be well represented by an exponential function, and based on a least squares fit, exhibited a time constant of min for the effective capacitance and 1.8 min for the effective resistance. These time constants are a substantial reduction from that (16 min) calculated for our previously reported device (Shang et al. 2016) and are comparable to those (0.65–1.30 min) estimated for the commercially available electrochemical continuous glucose sensors (Keenan et al. 2009). To understand the physical significance of these time constants, we consider the transport of glucose molecules to the sensor surface. When there was a change of glucose concentration in the solution fed into the test flow cell, glucose molecules were first transported through the flow cell to the liquid–hydrogel interface and then diffused through the hydrogel layer and bound to boronic acid moieties (Fig. 3a). The time constant for glucose transport through the flow cell, estimated via simulation of convective–diffusive mass transfer (under conditions used in the tests and with a glucose diffusivity of $4.5 \times 10^{-11} \text{ m}^2/\text{s}$), was approximately 1.5 min and comparable to the time constants of the measurement data. On the other hand, the time constant for glucose diffusion through the hydrogel, estimated via one-dimensional diffusion (under conditions used in the tests and with a glucose diffusivity of $4.5 \times 10^{-11} \text{ m}^2/\text{s}$), was approximately 1.2 s and the glucose–boronic acid binding was expected to be as fast as milliseconds (Ni et al. 2012). These two latter time scales were both substantially smaller than the time constants of the measurement data. Thus, it was concluded that the measured time constants were dominated by those of convective–diffusive transport of glucose in the test flow cell and did not reflect the true response time (which could be as low as several seconds) of the hydrogel-based sensor. Note that the flow cell was not part of the glucose microsensor and was only used as an in vitro testing tool. Measurement of the microsensor's true response time would require a new test setup, to be constructed in future work, capable of generating glucose concentration changes at the sensor surface within a much shorter time duration.

3.3 Device response to potential interferents

Boronic acid is known to bind, in addition to glucose, with oligosaccharides through boronic acid–diol interactions (Wu et al. 2013). Oligosaccharides that exist in blood mainly include fructose and galactose. Lactate, which is present in blood at a typical concentration of 11.2 mg/dL (Krogstad et al. 1996), could be the primary interferent in glucose detection in a physiological sample. Fructose (~1.8 mg/dL) and galactose (~1.8 mg/dL) are present in blood at much lower concentrations. We tested the device on these potential interferents at their physiological concentrations. At 1 kHz, the effective capacitance change of the device due to lactate (12 mg/dL), fructose (2 mg/dL) and galactose (2 mg/dL) was determined to be, respectively, 4.3, 4.7, and 2.1% of that induced by glucose (100 mg/dL), while the effective resistance change to the interferents at the physiological concentrations above was, respectively, 4.5 and 2.2% of that due to glucose (100 mg/dL). The device was hence considered appropriately selective for measurements of physiological glucose concentrations.

4 Conclusions

We have presented a dielectric affinity microsensor that consists of an in situ prepared hydrogel attached to a pair of coplanar electrodes for dielectrically based affinity detection of glucose in subcutaneous tissue. The hydrogel, incorporating *N*-3-acrylamidophenylboronic acid (AAPBA) that recognizes glucose via affinity binding, is synthetically prepared on the electrodes via in situ polymerization. When implanted in subcutaneous tissue, glucose molecules in interstitial fluid diffuse rapidly through the hydrogel and bind to the phenylboronic acid moieties. This induces a change in the hydrogel's complex permittivity and hence in the impedance between the electrodes, which can be measured to determine the glucose concentration.

The microsensor offers several distinct advantages. The in situ hydrogel preparation allows for a reduced hydrogel thickness ($\sim 10\ \mu\text{m}$) to enable the device to respond rapidly to glucose concentration changes in tissue, as well as covalent electrode attachment of the hydrogel to eliminate the need for semipermeable membranes that would otherwise be required to restrain the sensing material within the device. Meanwhile, the use of coplanar electrodes is amenable to the in situ preparation and facilitates glucose accessibility of the hydrogel, and combined with dielectrically based transduction, also eliminates mechanical moving parts often found in existing affinity glucose microsensor that can be fragile and complicated to fabricate.

Experimental results showed that the effective capacitance and effective resistance of the microsensor, in a measurement frequency range of 1–100 kHz, responded consistently to glucose concentration changes ranging from 0 to 500 mg/dL. At a given frequency, the device's effective capacitance decreased, while the effective resistance increased with glucose concentration as the hydrogel likely became less polarizable due to the glucose-hydrogel binding. At a frequency of 1 kHz, the measurement resolution of the microsensor in the glucose range (40–100 mg/dL) was estimated to be 0.32 mg/dL based on effective capacitance measurements and 0.27 mg/dL based on effective resistance measurements. In response to time-varying glucose concentration changes, the device exhibited a highly reversible response. Additionally, the measurement data showed a time constant of 1.6 min based on effective capacitance and 1.8 min based on effective resistance. These time constants were determined to be due to convective–diffusive transport of glucose in the experimental setup, with the actual time response of the device expected to be considerably faster (possibly down to several seconds). These results demonstrate that the device holds the potential to enable reliable and accurate continuous measurement of glucose in subcutaneous tissue for continuous glucose monitoring applications.

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Fig. 1.
Principle of the hydrogel-based MEMS affinity sensor

Fig. 2. Schematics of the MEMS hydrogel-based affinity CGM sensor: **a** top view, **b** side view, **c** image of a fabricated microsensor. The unit in the figure is in μm

Fig. 3.
a Side view of the test flow cell. **b** Experimental setup. **c** Impedance/voltage transformation circuit

Fig. 4. Effective capacitance and resistance of the microsensor averaged from multiple measurements. **a** Dependence of effective capacitance on measurement frequency. **b** Dependence of effective resistance on measurement frequency, where *error bars* reflect standard errors

Fig. 5. Effective capacitance and resistance of the microsensor from triplicate measurements. **a** Dependence of effective capacitance on glucose concentration. **b** Dependence of effective resistance on glucose concentration, where *error bars* reflect standard errors

Fig. 6. Time course of the sensor response at 1 kHz as the microsensor responded to time-varying glucose concentrations. **a** Effective capacitance. **b** Effective resistance