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## A hydrogel-based glucose affinity microsensor

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### Abstract

We present a hydrogel-based affinity microsensor for continuous glucose measurements. The microsensor is based on microelectromechanical systems (MEMS) technology, and incorporates a synthetic hydrogel that is attached to the device surface via *in situ* polymerization. Glucose molecules that diffuses into and out of the device binds reversibly with boronic acid groups in the hydrogel via affinity binding, and causes changes in the dielectric properties of the hydrogel, which can be measured using a MEMS capacitive transducer to determine the glucose concentration. The use of the *in situ* polymerized hydrogel eliminates mechanical moving parts found in other types of affinity microsensors, as well as mechanical barriers such as semipermeable membranes that are otherwise required to hold the glucose-sensitive material. This facilitates the miniaturization and robust operation of the microsensor, and can potentially improve the tolerance of the device, when implanted subcutaneously, to biofouling. Experimental results demonstrate that in a glucose concentration range of 0–500 mg/dL and with a resolution of 0.35 mg/dL or better, the microsensor exhibits a repeatable and reversible response, and can potentially be useful for continuous glucose monitoring in diabetes care.

### Keywords

affinity sensor; continuous glucose monitoring; hydrogel

## 1. Introduction

Continuous glucose monitoring (CGM), which involves highly frequent and repetitive measurements of glucose, can detect abnormal glucose concentrations in diabetes patients in a timely manner. Existing CGM devices often rely on electrochemical detection of enzymatic reactions<sup>1–3</sup>. While commonly used for glucose sensing, these devices are typically hindered by large drift and insufficient accuracy because of the irreversible,

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consumptive nature of electrochemical reactions. Affinity sensors, which are based on non-reactive equilibrium binding of glucose with a specific receptor<sup>4, 5</sup>, can potentially overcome these limitations. Affinity glucose sensing can be implemented in microsensors, which have used measurements of affinity binding-induced changes in physical properties such as volume<sup>6, 7</sup>, viscosity<sup>8, 9</sup>, fluorescence<sup>10, 11</sup> and electric conductivity<sup>12</sup>. However, these efforts have required the use of a semi-permeable membrane as a physical barrier or mechanically movable structures, which can increase the complexity and limit the reliability of the devices. In contrast, affinity dielectric sensors that detect the glucose-dependent dielectric properties can effectively address these limitations.

Affinity sensors that are based on dielectric measurements have been used in applications such as detecting or quantifying biochemical targets under excitations at various frequencies. Example of these applications include determination of protein concentration<sup>13, 14</sup>, detection of DNA<sup>15, 16</sup>, and monitoring of bacteria<sup>17, 18</sup>. Affinity glucose microsensors utilizing dielectric measurements have however not been widely explored. We have previously reported measurements of the permittivity of a polymer solution as the polymer binds to glucose microsensors<sup>19–21</sup>. While demonstrating the potential in sensitive and selective detection of the glucose through dielectric measurements both *in vitro* and *in vivo*, the polymer solution required sealing using a semi-permeable membrane that significantly increased the complexity, limited the level of miniaturization, and affected the reliability of the microsensor.

This paper presents an affinity microsensor that measures glucose concentrations *via* the dielectric response of a hydrogel embedded in a capacitive transducer. The microsensor is fabricated using microelectromechanical systems (MEMS) technology, and the hydrogel is synthetically prepared, non-toxic and polymerized *in situ* in the device. Reversible affinity binding of glucose with boronic acid groups in the hydrogel changes the dielectric properties of the hydrogel, which can be measured using a MEMS capacitive transducer to determine the glucose concentration. The design of the microsensor eliminates the use of mechanical moving parts found in other types of affinity microsensors that are not amenable to miniaturization<sup>7, 22</sup>. The hydrogel is directly immobilized onto the surface of the transducer and will be stable over time, allowing the device to eliminate the use of a semipermeable membrane that are otherwise required to hold the glucose-sensitive material<sup>20</sup>, and potentially offer improved tolerance to biofouling during implanted operation. Experimental results demonstrate that in a glucose concentration range of 0–500 mg/dL and with a resolution of 0.35 mg/dL or better, the hydrogel-based microsensor is capable of measuring glucose in a repeatable and reversible manner, and holds promise to enable CGM in a stable, accurate and rapid manner.

## 2. Method

### 2.1. Principle and design

The affinity glucose microsensor utilizes a synthetic glucose-sensitive hydrogel, which consists of N-3-acrylamidophenylboronic acid (AAPBA) as the glucose-sensing component, and acryl N-Hydroxyethyl acrylamide (HEAA) as the hydrophilic component. The hydrogel uses tetraethyleneglycol diacrylate (TEGDA) as the cross-linker and 2,2'-Azobis (2-

methylpropionamidine) dihydrochloride (AAPH) as the polymerization initiator. When glucose binds reversibly to the phenylboronic acid moieties in the AAPBA segments to form strong cyclic boronate ester bonds, a change in the dielectric properties of the hydrogel occurs and can be measured to determine the glucose concentration.

The dielectric properties of the hydrogel can be represented by the complex permittivity:  $\epsilon^* = \epsilon' - \epsilon''$ , where the real permittivity  $\epsilon'$  represents the ability of the hydrogel to store electric energy, while the imaginary permittivity  $\epsilon''$  is related to dissipation of energy. When the gap between the electrodes of a parallel-plate transducer is filled with the hydrogel (Figure 1), the transducer can be represented by a capacitor (effective capacitance:  $C_x$ ) and resistor (effective resistance:  $R_x$ ) connected in series. Correspondingly, the real and imaginary parts of the complex permittivity are related to these parameters by  $\epsilon' = C_x/C_0$  and;  $\epsilon'' = 1/(\omega R_x C_0)$ , where  $C_0$  is the capacitance when the electrode gap is in vacuum. The interactions of the hydrogel with glucose in general cause changes in its composition and conformation, and hence changes in its dielectric properties  $\epsilon'$  and  $\epsilon''$ . Thus, the transducer's effective capacitance and resistance will hence change and can be measured to determine the glucose concentration.

The transducer is enabled by MEMS technology and uses a pair of parallel electrodes sandwiching the hydrogel (Figure 2). The upper electrode is perforated to allow passage of glucose molecules, and is passivated within a perforated diaphragm to avoid direct contact with the hydrogel. The perforated electrode and diaphragm are supported by microposts so that they do not collapse onto the lower electrode on the substrate. Glucose molecules reversibly bind with the hydrogel, thereby changing the hydrogel's complex permittivity. While changes in the real and imaginary parts of the complex permittivity can be used to determine the glucose concentration, we in the present work focus on the real permittivity, which can be interrogated via measurement of the capacitance between the electrodes, to determine the glucose concentration.

## 2.2. Fabrication

To fabricate the MEMS capacitive transducer, a chrome (Cr)/gold (Au) film (5/100 nm) was deposited by thermal evaporation and patterned to form the lower electrode (500  $\mu\text{m} \times 500 \mu\text{m}$ ) on a  $\text{SiO}_2$ -coated wafer. The patterned gold electrode was then passivated with Parylene (1  $\mu\text{m}$ ). This was, followed by deposition of an S1818 sacrificial layer (5  $\mu\text{m}$ ) and an additional Parylene layer (1.5  $\mu\text{m}$ ). Another Cr/Au (5/100 nm) film was patterned to form the upper electrode and passivated by another Parylene layer. An SU-8 layer was then patterned to form a channel and anti-collapse microposts between the electrodes. The Parylene diaphragm was patterned with reactive ion etching (RIE) to form perforation holes that allow glucose permeation. The sacrificial photoresist layer was removed with acetone to release the diaphragm. The device fabrication process is shown in Figure 3(a).

The hydrogel was prepared *in situ* in the capacitive transducer. First, a mixture of the hydrogel components (AAPBA, HEAA, TEGDA, and AAPH) in solution was deoxygenated by nitrogen gas for 30 minutes, and was then injected into the device, filling the gap between the parallel electrodes. The device was placed in a nitrogen environment and heated for 4 hours at 70°C. The hydrogel was formed between the parallel electrodes, as shown in Figure

3(d). The hydrogel-integrated device was rinsed with water and ethanol to remove unreacted monomer and reagents.

### 2.3. Materials

The hydrogel was synthesized in house via free radical polymerization with AAPBA and HEAA monomers. An HEAA to AAPBA molar ratio of 9 (or approximately 10% AAPBA content among all monomers) was adopted. Then a solution consisting of AAPBA (1.1% w/v), HEAA (5.5% v/v), TEGDA (0.08% v/v), and AAPH (0.16% w/v) in distilled water was prepared for polymerization. A stock solution (0.1 M) of glucose was prepared by dissolving D-(+)-glucose (0.9 g) in distilled water to 50 mL. Glucose solution at varying concentrations (40, 70, 90, 180, 300, and 500 mg/dL) was prepared by diluting the stock solution.

### 2.4. Experimental setup

During testing, we placed the device in an acrylic test cell (2 mL in volume) filled with glucose solution (Figure 4). The device was connected to a capacitance/voltage transformation circuit driven by a sinusoidal input from a function generator (Agilent, 33220A), which imposes an AC electric field on the electrodes of the device to induce a glucose concentration-dependent change in the permittivity of the hydrogel. The resulting changes in the effective capacitance  $C_x$  of the capacitance/voltage transformation circuit are determined by measuring the output voltage ( $U_{out}$ ) from a given input AC voltage ( $U_{in}$ ). All experiments were conducted at frequencies in a range of 1 to 100 kHz as allowed by a lock-in amplifier (Stanford Research Systems, SR844) used in output voltage measurements.

## 3. Results and Discussion

We first investigated the microsensor response to different glucose concentrations under bias voltages of different frequencies (Figure 5). First, we observed that, at each of a series of physiologically relevant glucose concentrations (0–500 mg/dL), the effective capacitance of the device, and hence the permittivity of the hydrogel, decreased with increasing frequency over the entire frequency range tested (1–100 kHz) (Figure 5a). This is consistent with the dielectric relaxation of the hydrogel, in which the dielectric properties of the hydrogel have a momentary delay with respect to a changing electric field<sup>23</sup>. The dielectric properties of the hydrogel in an electric field are in general influenced by a number of mechanisms of polarization (i.e., shift of electric charges from their equilibrium positions under the influence of an electric field<sup>24</sup>), such as electronic polarization, ionic polarization, dipolar polarization, counterion polarization, and interfacial polarization. Electronic polarization and ionic polarization involve the distortion of electron clouds with nucleus and the stretching of atomic bonds, while counterion polarization and dipolar polarization reflect redistribution of ions and reorientation of electrical dipoles<sup>25, 26</sup>.

At a given frequency, the effective capacitance of the hydrogel increased consistently with glucose concentration in the entire range tested (0–500 mg/dL). This is clear from the device's frequency response (Figure 5a), and can be more conveniently examined when the device's response is plotted versus the glucose concentration (Figure 5b). For example, at 30

kHz, the effective capacitance increased from 16.2 pF to 24.8 pF as the glucose concentration increased from 0 mg/dL to 500 mg/dL. This reflected that the binding between the hydrogel and glucose significantly influences the polarization of the hydrogel, which may include changes in the hydrogel's structural conformations, permanent dipole moments, elastic resistance to the dipole rearrangement in the electric field, and electric double layer characteristics. These effects, which are highly complex and require elucidation through further in-depth studies, combine to result in the glucose concentration dependence of the hydrogel's dielectric properties, explaining the observed variation of the device's effective capacitance with glucose concentration.

It can be seen from Figure 5b that the dependence of the effective capacitance on glucose concentration is in general nonlinear over the full glucose concentration range tested (0–500 mg/dL). Thus, in practical applications, a calibration curve represented by a lookup chart or nonlinear equation<sup>27</sup> can be used to determine the glucose concentration from a measured effective capacitance value. Meanwhile, it is interesting to note that this dependence became considerably more linear in glucose concentration ranges that are moderately smaller but of strongest relevance to continuous glucose monitoring. For example, in a range of 40–300 mg/dL, the effective capacitance at a given frequency was approximately linear with glucose concentration as indicated by the linear fits. In such a range, a linear calibration equation may hence be adequate for the determination of glucose concentration from measurement results.

We conducted the above-mentioned experiments in triplicates to examine the ability of the microsensor to measure glucose concentrations in a repeatable manner and with adequate sensitivity (Figure 5b). At all glucose concentrations, the standard error in the effective capacitance was less than 0.91 pF (2.3%), indicating excellent repeatability. In addition, at all of the measurement frequencies used, the resolution and range of glucose measurement resolution were found to be appropriate for continuous glucose monitoring. Considering 30 kHz for example, the sensitivity of the microsensor was approximately  $15 \text{ fF}(\text{mg/dL})^{-1}$  in the glucose concentration range of 0–40 mg/dL. With a capacitance measurement resolution of 3 fF as allowed by our measurement setup, the device's resolution for glucose concentration measurement was correspondingly estimated to be 0.2 mg/dL. At a signal-to-noise ratio of 3, this yielded a detection limit of 0.6 mg/dL, well below the physiologically relevant glucose concentration range (typically greater than 40 mg/dL<sup>28</sup>). For glucose concentrations within 40–300 mg/dL, the sensitivity was approximately  $23 \text{ fF}(\text{mg/dL})^{-1}$ , corresponding to an estimated resolution of 0.12 mg/dL. At higher glucose concentrations (300–500 mg/dL), the nonlinear sensor response experienced a gradual declination in sensitivity and resolution (respectively to  $8.4 \text{ fF}(\text{mg/dL})^{-1}$  and 0.35 mg/dL at 500 mg/dL) as an increasingly small number of binding sites remained available in the hydrogel. These sensor characteristics, appropriate for practical applications, are comparable to those of commercially available electrochemical sensors (e.g., 1 mg/dL over a glucose concentration range from 0 to 400 mg/dL<sup>29, 30</sup> or 500 mg/dL<sup>31</sup>) as well as other research-stage boronic acid-based affinity sensors (e.g., 0.3 mg/dL<sup>32</sup> over a range from 0 to 300 mg/dL<sup>33</sup> or 540 mg/dL<sup>34</sup>) for continuous glucose monitoring.

We investigated the response of the hydrogel-based microsensor to glucose as compared to its response to potential interferents. Nonspecific molecules exist in interstitial fluid and can interact with boronic acid, which is the glucose sensitive component of our hydrogel. These molecules include fructose (~1.8 mg/dL), galactose (~1.8 mg/dL), lactate (~9 mg/dL), and ascorbic acid (~1.32 mg/dL). We tested the hydrogel-based microsensor on these molecules and found that the resulting response was substantially lower than that to glucose. For example, at the same concentration of 90 mg/dL, the effective capacitance change (measured at 30 kHz) due to fructose, galactose, lactate and ascorbic acid was found to be 17%, 38%, 32% and 28% of that due to glucose, respectively (Figure 6a). Here, the effective capacitance change is calculated according to  $C = C - C_0$  where  $C$  is the effective capacitance at a given glucose (or interferent) concentration, and  $C_0$  is the effective capacitance in the absence of glucose and interferents. Considering that the physiological concentrations of the potential interferents were about one order of magnitude lower than that of glucose, the microsensor was determined to be sufficiently selective for measurements of glucose in interstitial fluid for CGM applications.

While boronic acid binds to all diol-containing molecules, the selective response of the microsensor to glucose over the potential interferents could be attributed to the unique binding behavior between boronic acid and glucose. At a 1:1 ratio, boronic acid in fact binds more strongly to fructose than glucose. However, with a high concentration of boronic acid moieties (which was the case for our hydrogel), boronic acid can bind with glucose at a 2:1 ratio<sup>35–37</sup>. We exploited this property in previous work and developed solution-phase, viscometrically based affinity microsensors<sup>37, 38</sup>. In this work, we postulate that the existence of 2:1 binding between glucose and boronic acid moieties played a major role in the microsensor response, by causing additional crosslinking of the hydrogel that could lead to the augmentation of elastic resistance to electric field-induced dipole reorientation. The rather insignificant device response to the potential interferents (fructose, galactose, ascorbic acid and lactate) could, on the other hand, be attributed to a lack of this 2:1 binding mode.

We also studied the role of boronic acid in glucose recognition to gain further insight into the principle and operation of the microsensor. Using hydrogels containing with and without AAPBA content, we obtained the dependence of the effective capacitance on glucose concentration at a fixed measurement frequency (e.g., 30 kHz, Figure 6b). It also can be seen that when using an AAPBA-free hydrogel, the microsensor exhibited negligible changes in the effective capacitance in response to glucose concentration changes. This is in contrast to the strong glucose-induced response of the microsensor when it was equipped with the 10%-AAPBA hydrogel (shown in Figure 5b and reproduced in Figure 6b), indicating that boronic acid moieties in the hydrogel were critically responsible for the desired recognition of glucose by the microsensor.

Finally, we tested the device with time-resolved glucose concentration measurements to assess its ability to track glucose concentration changes in a consistent and reversible manner. The measured microsensor output at 30 kHz varied from 16.5 pF at 0 mg/dL to 24.8 pF at 500 mg/dL (Figure 7). In particular, when the device was exposed to a glucose concentration after experiencing another sample that was either higher or lower in concentration, virtually the same effective capacitance value was consistently obtained. For



example, the effective capacitance at 40 mg/dL over the two periods (from 20 to 38 minutes, and from 321 to 341 minutes) were respectively 16.87 pF and 16.73 pF, agreeing within 0.8%. Similarly, the reversibility was within 3.4% and 1.3% for the measurement data at glucose concentrations of 180 and 300 mg/dL, respectively. This indicates that because the binding between glucose and boronic acid moieties in the hydrogel, the microsensor possesses excellent reversibility in response to glucose concentration changes. The time constant of the response (i.e., the time for the sensor to reach 63% of the steady state response) was approximately 16 min. This time constant was attributable to the relatively large thickness of the hydrogel in the proof-of-concept device (~200  $\mu\text{m}$ ), through which glucose molecules must diffuse to interact with the capacitive transducer. As the glucose diffusion time decreases with the square of the hydrogel thickness, it is expected that thinner hydrogels can be used to effectively obtain more rapid time responses.

## 4. Conclusions

We have presented a hydrogel-based affinity glucose microsensor that measures glucose concentration through dielectric transduction. The device consists of a pair of thin-film parallel capacitive electrodes sandwiching a synthetic hydrogel. Glucose molecules permeate into the hydrogel through electrode perforations, and bind reversibly to boronic acid moieties of the hydrogel. This induces changes in the dielectric polarization behavior, and hence the complex permittivity, of the hydrogel. Thus, the effective capacitance between the electrodes, which is directly related to the real part of the complex permittivity, can be measured to determine the glucose concentration. The use of an *in situ* polymerized hydrogel simplifies the design of the microsensor, facilitates its miniaturization and robust operation, and can potentially improve the tolerance of the device, when implanted subcutaneously, to biofouling. Testing results showed that the effective capacitance of the device, 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 effective capacitance increased consistently with glucose concentration, suggesting that the affinity binding between glucose and boronic acid moieties caused the real permittivity of the hydrogel to increase. At 30 kHz, the measurement resolution of the microsensor was estimated to be 0.2, 0.12 and 0.35 mg/dL in the glucose concentration ranges of 0–40, 40–300, and 300–500 mg/dL, respectively. When subjected to time varying glucose concentration changes in the full 0–500 mg/dL range, the microsensor response was consistent and reversible. The time constant of this response was approximately 16 min, which can be readily improved by using thinner hydrogels for reduced glucose diffusion distances. These results have demonstrated that the microsensor can potentially allow accurate and consistent measurement of glucose concentration in interstitial fluid for continuous glucose monitoring.

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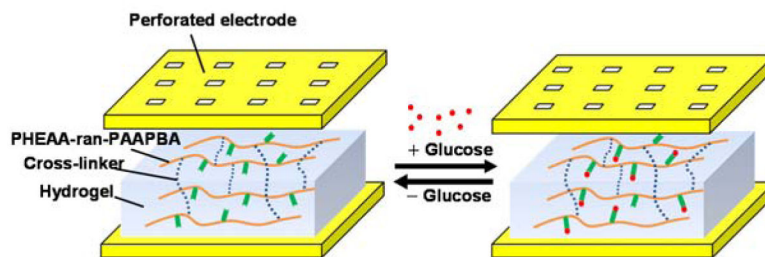
**Kexin Xu** received the Ph.D. degree in precision instrument engineering from Tianjin University, Tianjin, China, in 1988. From 1992 to 1999, he was a senior research fellow and team leader for non-invasive glucose measurement techniques in the Laboratory of Basic Technologies, KDK Corporation, Kyoto, Japan. He obtained his professorship from Tianjin University in 2000. Since 2002, he has been the Dean of the College of Precision Instrument and Opto-Electronic Engineering, Tianjin University. His research interest includes the design theory of acousto-optic tunable filter and spectroscopic technology, rapid detection of milk ingredients, monitoring of air and flue gas composition, non-invasive glucose monitoring, etc.

**Qian Wang** received the B.S. and Ph.D. degrees in chemistry from Tsinghua University, Beijing, China, in 1992 and 1997, respectively. After postdoctoral research with Prof. Manfred Schlosser at the University of Lausanne, Switzerland, and with Prof. M. G. Finn at the Scripps Research Institute, he started as an Assistant Professor at the University of South Carolina in 2003, where he has been a Full Professor since 2011 and currently is the Robert L. Sumwalt Chair of Chemistry and Carolina Distinguished Professor. He has published over 170 publications in peer reviewed journals and maintained an active research program which focuses on using chemical biology tools to probe intracellular activities and the development of hierarchically structured nanomaterials to study the cooperative response of cells to extracellular matrixes.

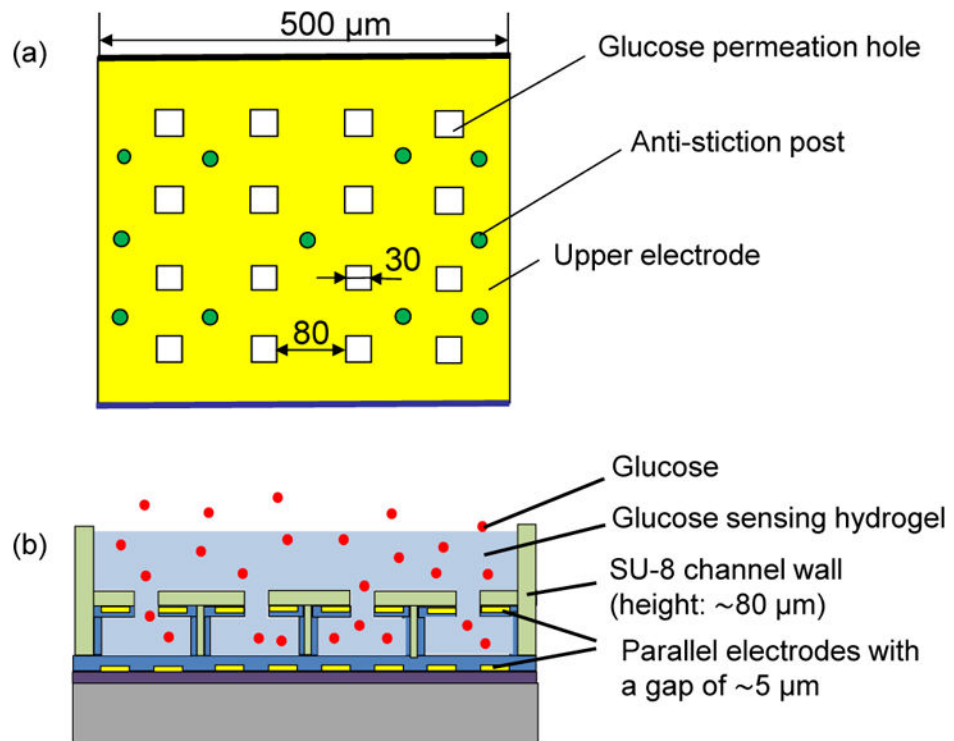
**Qiao Lin** received his Ph.D. in Mechanical Engineering from the California Institute of Technology in 1998 with thesis research in robotics. He conducted postdoctoral research in microelectromechanical systems (MEMS) at the Caltech Micromachining Laboratory from 1998 to 2000, and was an assistant professor of Mechanical Engineering at Carnegie Mellon University from 2000 to 2005. He has been an associate professor of Mechanical Engineering at Columbia University since 2005. His research interests are in designing and creating integrated micro/nano systems, in particular MEMS and microfluidic systems, for biomedical applications.

### Highlights

- An affinity microsensor is presented for continuous glucose monitoring.
- The device surfaces are functionalized with a hydrogel via *in situ* polymerization
- Glucose is detected by its affinity binding to the hydrogel's boronic acid moieties.
- Glucose detection has been made in 0–500 mg/dL range at 0.35 mg/dL resolution.

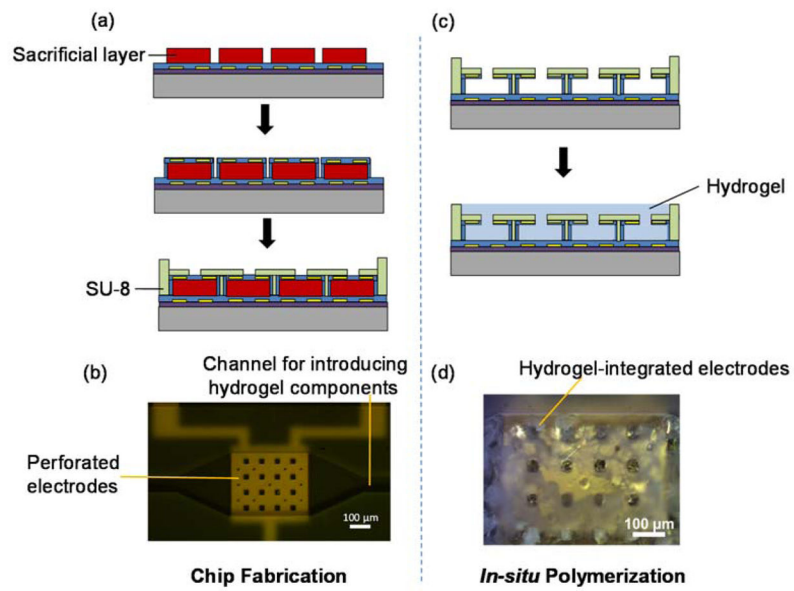


**Figure 1.**  
Principle of hydrogel-based microsensor.

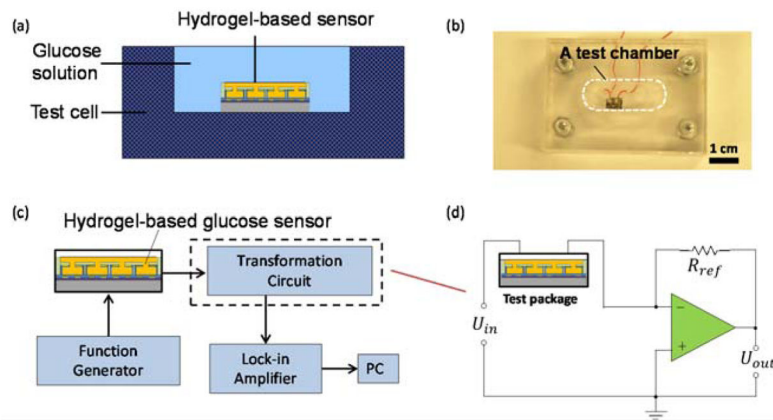


**Figure 2.** Schematics of the affinity microsensor: (a) top view and (b) side view.

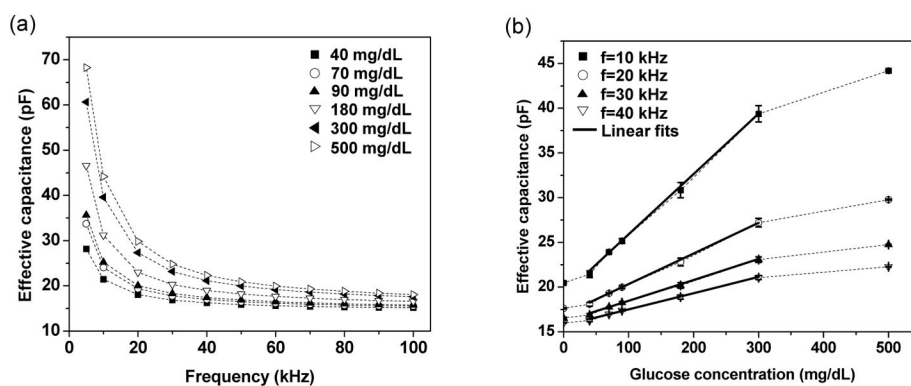




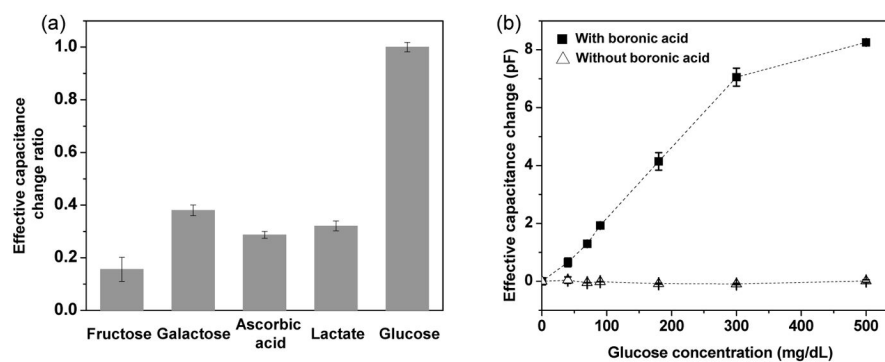
**Figure 3.** Chip fabrication: (a) standard fabrication procedures and (b) image of a fabricated capacitive transducer. *In situ* polymerization: (c) hydrogel integration in the transducer and (d) image of the hydrogel-integrated device.



**Figure 4.** Experimental setup: (a) Schematics of a testing setup. (b) Image of the testing setup. (c) Experimental setup. (d) A capacitance/voltage transformation circuit.



**Figure 5.** Effective capacitance of the microsensor averaged from triplicate measurements. (a) Dependence of effective capacitance on measurement frequency. (b) Dependence of effective capacitance on glucose concentration, where error bars reflect standard errors and linear fits (solid lines) have a coefficient of determination  $R^2$  ranging from 0.993 to 0.995. (Data points are connected by dashed lines to guide the eye.)



**Figure 6.** Selectivity and role of boronic acid for the microsensor: change of the effective capacitance calculated with reference to measurement in the absence of glucose or interferents (frequency: 30 kHz). (a) Ratio of the interferent-induced effective capacitance change to the glucose-induced effective capacitance change (concentration: 90 mg/dL for glucose and each of the interferents including fructose, galactose, ascorbic acid and lactate). (b) Effective capacitance change to glucose when boronic acid moieties were present (10% AAPBA content) or absent in the hydrogel (data points are connected by dashed lines to guide the eye).

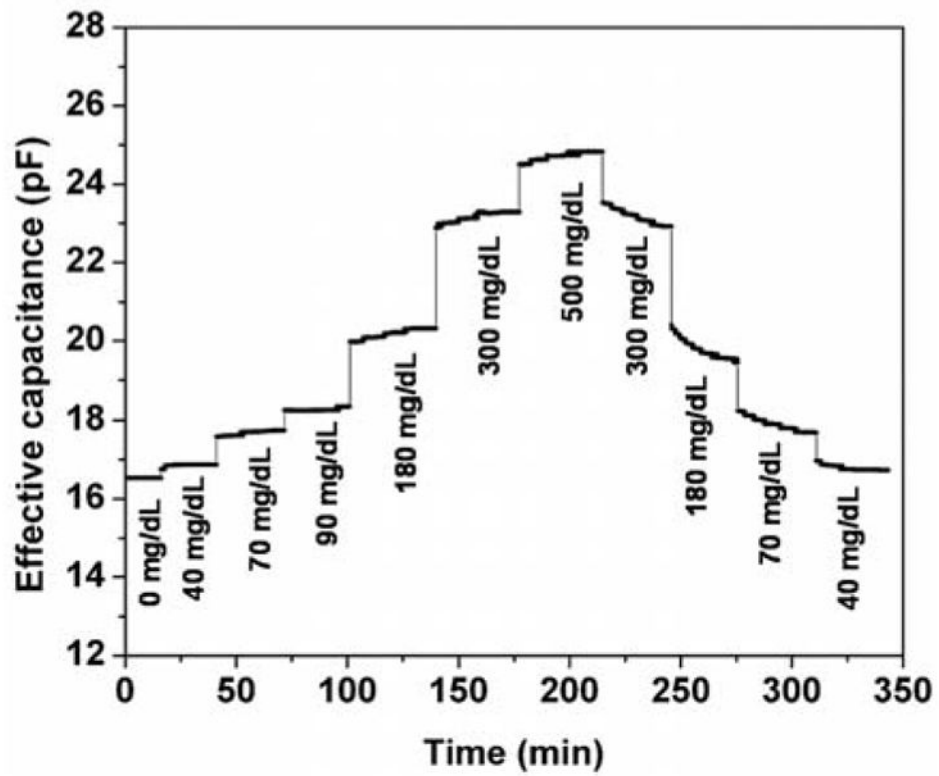


Figure 7. Time-resolved device response to time-varying glucose concentration. (Bias voltage frequency: 30 kHz.)