Published in final edited form as:

Lab Chip. 2014 January 21; 14(2): 294–301. doi:10.1039/c3lc51026c.

A Differential Dielectric Affinity Glucose Sensor

Xian Huang^a, Charles Leduc^b, Yann Ravussin^b, Siqi Li^c, Erin Davis^c, Bing Song^a, Dachao Li^d, Kexin Xu^d, Domenico Accili^e, Qian Wang^c, Rudolph Leibel^b, and Qiao Lin^{a,*}

^aDepartment of Mechanical Engineering, Columbia University, New York, NY 10027, USA

^bDepartment of Pediatrics, Columbia University Medical Center, New York, NY 10032, USA

^cDepartment of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA

^dCollege of Precision Instrument and Opto-electronics Engineering, Tianjin University, Tianjin, 300072, China

^eDepartment of Medicine, Columbia University Medical Center, New York, NY 10032, USA

Abstract

A continuous glucose monitor with a differential dielectric sensor implanted within the subcutaneous tissue that determines the glucose in the interstitial fluid is presented. The device, created using microelectromechanical systems (MEMS) technology, consists of sensing and reference modules that are identical in design and placed in close proximity. Each module contains a microchamber housing a pair of capacitive electrodes residing on the device substrate and embedded in a suspended, perforated polymer diaphragm. The microchambers, enclosed in semi-permeable membranes, are filled with either a polymer solution that has specific affinity to glucose or a glucose-insensitive reference solution. To accurately determine the glucose concentration, changes in the permittivity of the sensing and the reference solutions induced by changes in glucose concentration are measured differentially. In vitro characterization demonstrated the sensor capable of measuring glucose concentrations from 0 to 500 mg/dL with resolution and accuracy of ~ 1.7 $\mu\text{g/dL}$ and ~ 1.74 mg/dL, respectively. In addition, device drift was reduced to 1.4% (uncontrolled environment) and 11% (5 °C of temperature variation) of that from non-differential measurements, indicating significant stability improvements. Preliminary animal testing demonstrated that the differential sensor accurately tracks glucose concentration in blood. This sensor can potentially be used clinically as a subcutaneously implanted continuous monitoring device in diabetic patients.

Introduction

Continuous glucose monitoring (CGM) for diabetes management can be effectively achieved by subcutaneously implanted sensors. Currently, such sensors are mostly based on enzymatic electrochemical glucose detection¹⁻³, even though irreversible glucose consumption, diffusion-dependent glucose reaction rate, and degradation of enzyme can significantly affect the device accuracy⁴, reliability and longevity². These issues can be addressed by use of alternative methods such as affinity sensing, involving equilibrium binding of glucose with specific receptors⁵⁻⁸. Affinity sensors utilize glucose-induced

changes in various properties of a receptor-functionalized material, such as fluorescence^{9, 10}, viscosity^{11, 12}, volume^{13, 14}, and electric conductivity¹⁵. However, these sensors contain either mechanically movable structures or require complex detector or actuator designs. These limitations pose miniaturization and integration challenges and give rise to reliability and robustness issues. In contrast, measurement of glucose-dependent dielectric properties is an attractive solution to fully realize the potential of affinity sensing.

Affinity dielectric sensors, which detect changes in dielectric properties caused by ligand-receptor binding, can be achieved by electrical impedance measurements. Such sensors have used DNA^{16, 17}, aptamers^{18, 19}, proteins^{20, 21}, and synthetic polymers^{22, 23} as receptors to specifically detect biomolecules in mostly *in-vitro* applications. An implanted dielectric affinity sensor capable of analyte detection has yet to be demonstrated. We have previously explored a dielectric sensor that detected glucose by measurement of permittivity changes of a synthetic polymer induced by its binding to glucose²⁴. That device demonstrated both sensitive and specific glucose detection, and exhibited improved reliability due to the elimination of mechanical moving parts that are commonly used in other MEMS affinity glucose sensors²⁵⁻²⁷. In addition, the device also suggested potential performance improvements by fine tuning the measurement frequency due to the frequency dependence of dielectric affinity glucose detection. However, as dielectric detection was strongly affected by fluctuations in environmental parameters (e.g., temperature), the dielectric sensor, which contained only one glucose-responsive sensing component, was susceptible to environmental interference, and, thus, not appropriate for implanted operation.

In this paper, we present a microelectromechanical systems (MEMS) differential dielectric sensor consisting of sensing and reference modules that are identical in design and placed in close proximity²⁸. The sensing module contains a glucose-sensing solution, while the reference module is filled with a reference solution that does not react with glucose. Differences in the measured signals from the two modules allow rejection of nonspecific disturbances and accurate determination of glucose concentration. Experimental results from *in-vitro* and *in-vivo* testing demonstrate that the sensor offers significant improvement in accuracy and stability in a compact format as compared with a single module dielectric sensor, and can potentially be used in clinical CGM applications.

Materials and Methods

Design and Fabrication

The differential dielectric sensor is based on a pair of capacitive detection modules (Fig. 1a to c). The modules are identical in design, each constructed from a microchamber that houses a pair of parallel electrodes. While the lower electrode rests on the substrate, the upper electrode is embedded in a suspended polymer diaphragm supported by an array of stiff posts, both preventing collapse of the diaphragm and stabilizing it from mechanical disturbance. The upper electrode and the embedding diaphragm are perforated, so that the gap between the electrodes forms a single connected volume with the remainder of the chamber. One microchamber contains a solution of a glucose binding polymer Poly(N-hydroxyethylacrylamide-*ran*-3-acrylamidophenylboronic acid) (PHEAA-*ran*-PAAPBA) (sensing polymer solution), while the other microchamber is filled with a solution of a polymer that does not respond to glucose (Poly(acrylamide) (PAA) (reference polymer solution). The chambers are each encased in a semi-permeable membrane, which prevents the polymers from escaping from the microchambers, while still allowing glucose to freely pass through. As a result, glucose molecules can diffuse through the semi-permeable membrane as well as the perforated diaphragm to reach the gap between the electrodes. The permittivity difference between the sensing and reference solutions can be determined from the differential capacitance, i.e., differences between the capacitances in the sensing and

reference chambers. This strategy allows accurate determination of the glucose concentrations by controlling for permittivity changes caused by nonspecific disturbances.

Details of the fabrication process²⁴ for the sensor are provided in Supplementary Information. Briefly, thin film deposition and patterning were used to produce perforated Parylene diaphragms (6×6 arrays of $50 \times 50 \mu\text{m}^2$ openings with $150 \mu\text{m}$ spacing) integrated with capacitive electrodes ($1 \text{ mm} \times 1 \text{ mm} \times 100 \text{ nm}$ for sensors used in *in-vitro* testing, and $0.5 \text{ mm} \times 0.5 \text{ mm} \times 100 \text{ nm}$ for sensors used in *in-vivo* testing) (Fig. S1 and Fig. 1d and f). The diaphragms are supported by posts that prevent the diaphragms from clapping onto the substrate. The resulting device with an overall dimension of $16 \times 8 \text{ mm}^2$ was placed in an acrylic flow cell (1 mL in volume and $5.3 \times 2 \text{ cm}^2$ in dimensions) outfitted with tubing for polymer and glucose solution handling during the *in-vitro* testing (Fig. 1a, b, and e). The device for *in-vivo* testing had reduced dimensions of $8 \times 8 \text{ mm}^2$ to accommodate the size of animals (Fig. 1f).

Experimental Setup and Characterization Methods

Two experimental configurations have been used to characterize the sensor. One of the configurations uses a lock-in amplifier (SR830, Stanford Research systems) that allows frequency-dependent dielectric measurement at a range of frequencies from 0.5 to 100 kHz; the other makes differential capacitance measurements via a more simplified scheme using a Σ - Δ capacitance digital convertor (CDC) (AD7746, Analog Devices) that supplies a excitation signal at a fixed frequency of 32 kHz (more details in Supplementary Information).

PHEAA-*ran*-PAAPBA, whose properties and ability in affinity glucose detection have been previously demonstrated²⁹, was used as the glucose sensing polymer (2840 mg/dL). PAA, a biocompatible polymer³⁰⁻³², which does not react with glucose^{33, 34}, was used as the reference polymer (1420 mg/dL). A series of glucose solutions with varying concentrations (50, 100, 200, 300, 400, and 500 mg/dL) were prepared by dissolving appropriate amount of glucose in 100 mL Ringer's solution (more details in Supplementary Information).

The *in-vitro* experiments were conducted in a typical laboratory setting. The presence of environmental disturbances (e.g. human activities, bench vibrations, temperature fluctuations, etc.) was compensated by the differential measurement approach, eliminating the need for tight environmental control. However, intentionally introduced changes in temperature were used to assess the temperature stability of the sensor, and were achieved via closed-loop control described elsewhere³⁵. Preliminary *in vivo* testing of the device was conducted at the Columbia University Medical Center in 10-week old C57BL/6J laboratory mice using a protocol approved by the Columbia Institutional and Animal Care Use Committee (Protocol Number: AAAD0381). Mice were sedated with inhaled 3-5% isoflurane and maintained throughout the experiments on isoflurane with sedation monitored by toe pinching every 10-15 minutes (more details in supplementary information). In addition, a local anesthetic, bupivacaine 2mg/kg, was injected subcutaneously at the incision site. Respiratory and heart rates were monitored. The sensor was implanted in the subcutaneous region of the interscapular space and allowed to stabilize for 30 minutes before measurements started. After completion of the experiments, mice were fully sedated (isoflurane increased to 5%) and euthanized. The implanted glucose sensors continuously measured the glucose concentrations in interstitial fluid (ISF), while the reference capillary blood glucose concentrations were monitored with a commercial glucose meter (Freestyle Lite, Abbott Diabetes Care) by tail nicking at specified frequencies.

The differential sensor was calibrated to predict ISF glucose concentration from the sensor output, which accounts for the time delay between ISF and blood glucose concentrations as

well as nonlinearity during the measurements³⁶. Modelling glucose exchange between blood and ISF in terms of diffusion between two compartments³⁷, the glucose concentrations in blood (G_1) and ISF (G_2) are related by³⁸

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

where k_{12} and k_{21} are forward and reverse flux rates of transcapillary glucose transportation, k_{02} is rate of glucose uptake by the subcutaneous tissue, and V_1 and V_2 are the compartment volumes of blood and ISF, respectively.

Due to the nonlinearity of affinity glucose sensing²⁸, the differential sensor capacitance (C_{out}) at a fixed frequency and ISF glucose concentrations (G_2) may be related by a quadratic equation:

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

where a , b , and c are constants. Combining Equations (1) and (2), the glucose meter reading (G_1) is then expressed using the following equation,

$$G_1 = a_1C_{out}^2 + a_2C_{out} + a_3C_{out}dC_{out}/dt + a_4dC_{out}/dt + a_5 \quad (3)$$

where a_1 , a_2 , a_3 , a_4 , and a_5 are constants that can be determined from least squares fitting using six reference blood glucose concentrations (i.e., measurement values of G_1) and the corresponding sensor output (C_{out}) obtained at the same time during the *in-vivo* experiments.

Results and Discussion

Steady-State Frequency Response at Different Glucose Concentrations

The frequency responses of the sensing and reference modules to the electric field (e-field) with a frequency from 0.5 to 100 kHz were obtained using a capacitance/voltage converter circuit. As shown in Fig. S2a, when the polymer solutions contained no glucose, the capacitance of the sensing and reference modules both decreased consistently from 0.5 to 20 kHz, and then increased slightly as the frequency further increased. The abnormal decreases of the module capacitances at low frequencies could be attributed to effects of electrode polarization and Maxwell-Wagner-Sillars polarization³⁹, which were caused by the formation of electrical double layers on the surface of electrodes and polymer molecules, respectively, leading to a rapid decrease of permittivity at low frequencies. In addition, the capacitance of reference module was smaller than the sensing electrodes, indicating that the PAA solution has lower permittivity as compared with the PHEAA-*ran*-PAAPBA polymer solution.

The capacitance changes in the sensing modules at glucose concentrations ranging from 50 to 200 mg/dL were also obtained *in vitro*. As shown in Fig. S2b, the single module capacitance decreased with increasing glucose concentration at frequencies higher than 10 kHz. In contrast, at frequencies between 0.5 and 10 kHz, the capacitance increased with glucose concentrations. This suggests that the sensitivity of differential capacitance detection can differ according to the selection of measurement frequencies.

The glucose-induced changes in the dielectric properties of PHEAA-*ran*-PAAPBA involve a complex interplay of several polarization mechanisms⁴⁰, such as dipole reorientation⁴¹, Maxwell-Wagner-Sillars polarization⁴², and electrode polarization⁴³. The binding between

PHEAA-*ran*-PAAPBA and glucose results in changes in polymer conformation, permanent dipole moments in the polymer solution, elastic resistance to the dipole motion in the *e*-field, and the distribution of electric double layers on the interface of the polymer and the solution⁴⁰. These effects combine to cause the solution permittivity, or capacitance of the sensing module, to vary with glucose concentration as seen in Fig. S2b. A crossover of the capacitance for different glucose concentrations at a frequency of approximately 10 kHz was observed, although its underlying cause is not clear and calls for further investigation. The nonlinearity of the sensor response in glucose concentration, which can be well represented by the relationship given in Eq. (3), was induced by the above-mentioned polarization mechanisms as well as the nonlinear dependence of affinity binding on glucose concentration^{44, 45}.

Device Response to Various Glucose Concentration Changes

The sensor was exposed to glucose solutions whose concentrations were alternated between two different values to characterize the device time responses. A typical sensor response measured at 32 kHz by CDC is shown in Fig. 2a, in which equilibrium glucose concentration in the test cell and microchambers was initially at 50 mg/dL and then changed to 100 mg/dL (at time 11 min) and then back to 50 mg/dL (at 28 min) by replacing the solution in the test cell. These concentrations of glucose are, from a clinical perspective, normoglycemic (100mg/dL) and hypoglycemic (50 mg/dL). Ignoring the time for glucose solution replacement (approximately 10 sec.), the time response, defined as the time required by the differential capacitance to reach 90% of the steady-state value⁴⁶, was determined to be 4.9 min and 7.8 min, respectively, when the glucose concentration changed from 50 to 100 mg/dL and then reversed back to 50 mg/dL. This response time was comparable to that for commercially available continuous glucose monitors⁴⁷, and could be reduced by decreasing the depth of the microchambers.

The sensor response to varying glucose concentrations in a physiologically relevant range was next investigated using the CDC. As the glucose concentration changed from 0 to 500 mg/dL (severe hyperglycemia), the differential capacitance of the sensor decreased steadily from 38.88 to 37.74 pF, indicating a decrease in the permittivity due to glucose binding with PHEAA-*ran*-PAAPBA (Fig. 2b). The sensitivity of the sensor was approximately 2.3 fF/(mg/dL) with a noise level of 0.057%. With the capacitance measurement resolution and accuracy of the CDC respectively given by 4 aF and 4 fF, this gives a glucose detection resolution and accuracy of 1.7 μ g/dL and 1.74 mg/dL, respectively. The sensor was capable of measuring glucose concentrations ranging from 0 to 500 mg/dL, although the differential capacitance gradually levelled off at the higher glucose concentrations. The change of 0.06 pF between the differential capacitance of the sensor at 400 and 500 mg/dL glucose concentration, while smaller than the capacitance changes at lower glucose concentrations, is sufficient for detection by the experimental setup. A larger glucose measurement range could potentially be achieved by optimizing the composition and concentration of the glucose-sensitive polymer. However, in practical terms, sensitivity at the lower end of glucose concentrations is more clinically relevant for initial use of this device in patients.

Reduction of Drift by Differential Measurements

The glucose-independent drift of the differential sensor, due primarily to temperature variations, was assessed at a fixed glucose concentration with the sensor temperature allowed to freely fluctuate over an extended measurement period³⁵. At a fixed glucose concentration of 50 mg/dL, the drift in the differential capacitance was significantly smaller than the drift in the single-module capacitance over a measurement period of 4 hours (Fig. 3a). The differential capacitance of the sensor was steady at 38.62 pF, while single-module capacitance decreased by 0.2 pF/hr (3467 ppm/hr), indicating that the differential sensor

offered effective compensation to environmental disturbances with excellent stability. The ability to reject common-mode disturbances was influenced by consistency between sensing and reference modules and the properties of sensing and reference polymer solution, and can be further optimized in future iterations of this device.

The device's ability to reject temperature fluctuation was characterized by varying the sensor temperature from 35 to 40 °C under closed-loop control at a fixed glucose concentration of 50 mg/dL. Both the differential capacitance and single-module capacitance were obtained for comparison (Fig. 3b). With a 6 °C temperature change, the differential and the single-module capacitances drifted by 0.15 and 1.3 pF, respectively, indicating that differential measurements effectively reduced the effect of temperature variation by 89% as compared with single module measurement. This result indicates that the differential sensor is a significant improvement over the single sensor device for use as an implantable device.

In-Vivo Sensor Characterization

Finally, we performed preliminary *in-vivo* characterization of the device in three laboratory mice. The glucose sensors that were implanted in the subcutaneous tissue of the sedated mice continuously measured the glucose concentrations in ISF (Supplementary Information, Fig. S3a), while a commercial glucose meter (Freestyle Lite, Abbott Diabetes Care) measured the glucose concentrations in capillary blood sampled from the mouse's tail tip every 5 minutes.

Immediately following the implantation of the differential sensors, there was an initialization period, during which glucose in ISF diffused into the initially glucose-free microchambers and the pressures within the microchamber and ISF equilibrated (Supplementary Information, Fig. S3b). This process ranged from 8 to 30 minutes and exhibited a typical sensor response as shown in Fig. S3b, in which the differential capacitance of a dielectric sensor was recorded over a period of approximately 8 minutes. The differential capacitance decreased steadily from 0.78 pF following the sensor implantation (time 0) to 0.68 pF at 2.5 minutes, indicating a decrease in the permittivity of the PHEAA-*ran*-PAAPBA polymer solution due to affinity binding between ISF glucose and the glucose-free sensing polymer. The differential capacitance stabilized at approximately 8 min, which was consistent with the *in-vitro* time response as shown in Fig. 2a.

The differential sensor output and the glucose meter readings were recorded following the completion of device initialization. The glucose meter readings and the changes of the differential capacitance calculated with respect to the value at the time of the first glucose meter reading are shown in Fig. 4a to 4c for all tested mice. Blood glucose concentrations were first allowed to vary physiologically. The glucose concentrations of mice were then reduced to hypoglycemic levels by intraperitoneal insulin administration at 68, 26, and 40 min, respectively (Fig. 4a to 4c). Subsequently, hyperglycemia was induced by intraperitoneal glucose administration at 98, 46, and 70 min, respectively. The device output closely tracked the commercial glucose meter, as blood glucose concentration varied over the measurement period ranging from 90 to 150 minutes. Time lag between the differential capacitance and the glucometer values were noted. These delays, ranging from 5 to 15 minutes, were considerably larger than the device-specific time delays as shown in Fig. 2a, and probably reflect, in part, physiological delay in equilibration of ISF and capillary blood.

The predicted glucose concentrations ($\hat{G}_1$) were obtained from calibrating measured differential capacitance changes with six reference glucose values. The reference values were selected through three measurement periods, in which the glucose concentrations remained approximately constant, were significantly increased, and significantly decreased,

respectively. The corresponding C_{out} at the reference points and the C_{out} obtained four or five minutes prior to the reference points were used to determine dC_{out}/dt in Eq.(3), leading to the determination of a_1, a_2, a_3, a_4 , and a_5 by solving a five-variant first-order equations. Therefore, $\hat{G}_1$ can be obtained from the corresponding C_{out} from Eq.(3).

The clinical accuracy of $\hat{G}_1$ as compared to G_1 measured by a glucose meter can be quantitatively assessed by constructing Clarke error grids⁴⁸, in which five zones (A, B, C, D, and E) are partitioned with different levels of clinical accuracy of $\hat{G}_1$ with respect to G_1 . Data points consisting of a G_1 and a $\hat{G}_1$, falling into Zone A (clinically accurate) and Zone B (clinically acceptable) are considered clinically useful.. However, a point falling in any of Zone C, D and E indicates that $\hat{G}_1$ may lead to significant deviations from the real values and erroneous clinical management.

The Clarke error grid constructed from our *in vivo* testing contains 61 measurement points (Fig. 4d). Overall, 83.6% of the measurement points were in Zone A and 16.4 % of points in Zone B, with none in other zones, indicating that all measurements from the sensors were clinically accurate or acceptable. In addition, the dielectric sensors exhibited a high correlation to the glucose meter readings (correlation coefficient = 0.962). The results from Clarke error grid analysis suggest that the sensors allow accurate measurement of ISF glucose concentrations when subcutaneously implanted for CGM.

Conclusions

A MEMS differential dielectric affinity sensor for accurate determination of glucose concentration in ISF is presented. *In vitro* characterization of the sensor demonstrates that the sensor can measure glucose concentrations ranging from 0 to 500 mg/dL at a fixed frequency of 32 KHz with a steady decrease of the differential capacitance from 38.88 to 37.74 pF and a resolution of $\sim 1.7 \mu\text{g/dL}$. The time response, defined as the time required for the differential capacitance to reach 90% of the steady-state value, was approximately 4.9 min in response to a glucose concentration change from 50 to 100 mg/dL. At a glucose concentration of 50 mg/dL, the drift of differential capacitance at 32 kHz of the device in an uncontrolled environment was negligible (47.5 ppm/hr) as compared with that in single module measurements (3467 ppm/hr). When ambient temperature of the device was increased from 35 to 40 °C, the variation in differential capacitance (at the same frequency) was more than eight times smaller than that in the single-module capacitance. In preliminary *in-vivo* testing of the device in laboratory mice the differential sensor output closely tracked blood glucose concentrations measured by a commercial glucose meter with 100% clinically acceptable accuracy. These results demonstrate that the differential dielectric sensor can potentially be used to enable reliable and accurate continuous glucose monitoring in patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge financial support from NSF (grant # ECCS-0702101) and the Columbia Diabetes and Endocrinology Research Center (NIH grant #DK63068-05 and NIH/NIDDK P30 DK63608-10).

References

1. Mastrototaro JJ. Diabetes Technol Ther. 2000; 2:13–18.
2. Heller A. Annu Rev Biomed Eng. 1999; 1:153–175. [PubMed: 11701486]

3. Gough DA, Kumosa LS, Routh TL, Lin JT, Lucisano JY. *Sci Transl Med*. 2010; 2:42–53.
4. Louchis K, O'Driscoll S. *Conf Proc IEEE Eng Med Biol Soc*. 2011;7670–7673. [PubMed: 22256115]
5. Shibata H, Heo YJ, Okitsu T, Matsunaga Y, Kawanishi T, Takeuchi S. *Proc Natl Acad Sci*. 2010; 107:17894–17898. [PubMed: 20921374]
6. Ballerstadt R, Evans C, Gowda A, McNichols R. *J Diabetes Sci Technol*. 2007; 1:384–393. [PubMed: 19885094]
7. Mansouri S, Schultz JS. *Nat Biotech*. 1984; 2:885–890.
8. Siegrist J, Kazarian T, Ensor C, Joel S, Madou M, Wang P, Daunert S. *Sensor Actuator B Chem*. 2010; 149:51–58.
9. Schultz JS, Mansouri S, Goldstein IJ. *Diabetes Care*. 1982; 5:245–253. [PubMed: 6184210]
10. Pickup JC, Hussain F, Evans ND, Rolinski OJ, Birch DJS. *Biosens Bioelectron*. 2005; 20:2555–2565. [PubMed: 15854825]
11. Ehwald R, Ballerstadt R, Dautzenberg H. *Anal Biochem*. 1996; 234:1–8. [PubMed: 8742073]
12. Zhao Y, Li S, Davidson A, Yang B, Wang Q, Lin Q. *J Micromech Microeng*. 2007; 17:2528–2537.
13. Barriet D, Siegel RA. *J Med Dev*. 2009; 3:027519.
14. Siegel RA, Gu Y, Lei M, Baldi A, Nuxoll EE, Ziaie B. *J Controlled Release*. 2010; 141:303–313.
15. Arnold FH, Zheng W, Michaels AS. *J Membr Sci*. 2000; 167:227–239.
16. Hleli S, Abdelghani A, Tlili A. *Sensors*. 2003; 3:472–479.
17. Ban C, Chung S, Park DS, Shim YB. *Nucleic Acids Res*. 2004; 32:e110. [PubMed: 15273279]
18. Li X, Shen L, Zhang D, Qi H, Gao Q, Ma F, Zhang C. *Biosens Bioelectron*. 2008; 23:1624–1630. [PubMed: 18339536]
19. Xu D, Xu D, Yu X, Liu Z, He W, Ma Z. *Anal Chem*. 2005; 77:5107–5113. [PubMed: 16097746]
20. Kharitonov AB, Alfonta L, Katz E, Willner I. *J Electroanal Chem*. 2000; 487:133–141.
21. Guan JG, Miao YQ, Zhang QJ. *J Biosci Bioeng*. 2004; 97:219–226. [PubMed: 16233619]
22. Daniels JS, Pourmand N. *Electroanal*. 2007; 19:1239–1257.
23. Zou Z, Kai J, Rust MJ, Han J, Ahn CH. *Sensor Actuat A-Phys*. 2007; 136:518–526.
24. Huang X, Li S, Davis E, Li D, Wang Q, Lin Q. *J Microelectromech Syst*. 2013 In Publication.
25. Huang X, Li S, Schultz JS, Wang Q, Lin Q. *J Microelectromech S*. 2009; 18:1246–1254.
26. Lei M, Baldi A, Nuxoll E, Siegel RA, Ziaie B. *Diabetes Technol Ther*. 2006; 8:112–122. [PubMed: 16472058]
27. Boss C, Meurville E, Sallese JM, Ryser P. *Biosensors Bioelectron*. 2011; 30:223–228.
28. Huang X, Oxsher J, LeDuc C, Ravussin Y, Wang Q, Accili D, Leibel R, Lin Q. 2011 16th International on Solid-State Sensors, Actuators and Microsystems Conference (TRANSDUCERS). 2011:970–973.
29. Li S, Davis EN, Huang X, Song B, Peltzman R, Sims DM, Lin Q, Wang Q. *J Diabetes Sci Technol*. 2011; 5:1060–1067. [PubMed: 22027298]
30. Risbud MV, Bhonde RR. *Drug Deliv*. 2000; 7:69–75. [PubMed: 10892406]
31. Gin H, Dupuy B, Bonnemaïson-Bourignon D, Bordenave L, Bareille R, Latapie MJ, Baquay C, Beziau JH, Ducassou D. *Biomater Artif Cells Artif Organs*. 1990; 18:25–42. [PubMed: 2334812]
32. Zarini E, Supino R, Pratesi G, Laccabue D, Tortoreto M, Scanziani E, Ghisleni G, Paltrinieri S, Tunesi G, Nava M. *Plastic and Reconstructive Surgery*. 2004; 114:934–942. 910.1097/1001.PRS.0000133425.0000122598.D0000133420. [PubMed: 15468401]
33. Himanshu AK, Bandyopadhyay SK, Bahuguna R, Ray DK, Sinha TP. *AIP Conf Proc*. 2011; 1349:204–205.
34. Shin S, Cho YI. *Int Commun Heat Mass*. 1993; 20:831–844.
35. Huang X, Li S, Davis E, Leduc C, Ravussin Y, Cai H, Song B, Li D, Accili D, Leibel R, Wang Q, Lin Q. *J Micromech Microeng*. 2013; 23:055020.
36. Huang X, Leduc C, Ravussin Y, Li S, Davis E, Song B, Wang Q, Accili D, Leibel R, Lin Q. *J Diabetes Sci Technol*. 2012; 6:1436–1444. [PubMed: 23294791]
37. Rebrin K, Steil GM. *Diabetes Technol Ther*. 2000; 2:461–472. [PubMed: 11467349]

38. Cengiz E, Tamborlane WV. *Diabetes Technol Ther.* 2009; 11:S-11–S-16. [PubMed: 19469670]
39. Kremer, F.; Schönhal, A. *Broadband dielectric spectroscopy.* Springer; 2003.
40. Huang X, Li S, Schultz JS, Wang Q, Lin Q. *Appl Phys Lett.* 2010; 96:033701–033703.
41. Block H, North AM. *Adv Mol Relax Proc.* 1970; 1:309–374.
42. Block H, North AM. *Adv Mol Relax Pr.* 1970; 1:309–374.
43. Feldman Y, Polygalov E, Ermolina I, Plevaya Y, Tsentsiper B. *Meas Sci Technol.* 2001; 12:1355–1364.
44. Zhang H, Williams PS, Zborowski M, Chalmers JJ. *Biotechnol Bioeng.* 2006; 95:812–829. [PubMed: 16937410]
45. Baker PF, McNaughton PA. *J Physiol.* 1976; 259:103–144. [PubMed: 182956]
46. McDonagh C, Bowe P, Mongey K, MacCraith BD. *J Non-Cryst Solids.* 2002; 306:138–148.
47. Keenan DB, Mastrototaro JJ, Voskanyan G, Steil GM. *J Diabetes Sci Technol.* 2009; 3:1207–1214. [PubMed: 20144438]
48. Clarke WL, Cox D, Gonder-Frederick LA, Carter W, Pohl SL. *Diabetes Care.* 1987; 10:622–628. [PubMed: 3677983]

Fig.1. Differential MEMS dielectric glucose sensors. (a) Schematic of the differential MEMS dielectric glucose sensor for *in-vitro* sensor characterization with (b) a differential sensor enclosed in the flow cell. (c) Side view of an unpackaged differential MEMS dielectric glucose sensor. Images of a differential MEMS affinity glucose sensor for *in-vitro* characterization: (d) before, and (e) after packaging. (f) A differential MEMS affinity sensor used in *in-vivo* testing.

Fig.2.

Sensor capacitance in response to glucose concentration changes. (a) Time course of the sensor capacitance at 32 kHz as the sensor responded to glucose concentration changes from 50 to 100 mg/dL, which was then reversed to 50 mg/dL. (b) Sensor capacitance under a sequence of glucose concentrations from 0 to 500 mg/dL.

Fig.3. Comparison of sensor capacitance output (a) over an extended time duration and (b) in changing temperature in single-channel and differential measurement as the glucose concentration was held constant at 50 mg/dL.

Fig. 4.

Experimental results of the sensor implanted in three tested mice (a) to (c). The results are presented as sensor capacitance changes as compared to readings from a commercial glucose meter. (d) Clarke error grid to assess the clinical accuracy of estimated glucose values obtained from calibrating differential capacitance with reference glucose values.