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Xian Huang
Columbia University

Siqi Li
University of South Carolina - Columbia

Jerome S. Schultz
University of California, Riverside

Qian Wang
University of South Carolina - Columbia, wang@mail.chem.sc.edu

Qiao Lin
Columbia University, qlin@columbia.edu

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A dielectric affinity microbiosensor

Xian Huang,[1] Siqi Li,[2] Jerome S. Schultz,[3] Qian Wang,[2] and Qiao Lin[1,a)]
[1]*Department of Mechanical Engineering, Columbia University, New York, New York 10027, USA*
[2]*Department of Chemistry and Biochemistry and Nanocenter, University of South Carolina, Columbia, South Carolina 29208, USA*
[3]*Department of Bioengineering, University of California, Riverside, California 92521, USA*

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We present an affinity biosensing approach that exploits changes in dielectric properties of a polymer due to its specific, reversible binding with an analyte. The approach is demonstrated using a microsensor comprising a pair of thin-film capacitive electrodes sandwiching a solution of poly(acrylamide-*ran*-3-acrylamidophenylboronic acid), a synthetic polymer with specific affinity to glucose. Binding with glucose induces changes in the permittivity of the polymer, which can be measured capacitively for specific glucose detection, as confirmed by experimental results at physiologically relevant concentrations. The dielectric affinity biosensing approach holds the potential for practical applications such as long-term continuous glucose monitoring. © *2010 American Institute of Physics*. [doi:10.1063/1.3291669]

Affinity binding is a specific and reversible chemisorption process in which ligand and receptor molecules interact to form strong bonds.[1] Affinity biosensors achieve molecular recognition by affinity binding and have broad applications in clinical diagnostics,[2] genetic analysis,[3] drug discovery,[4] and environmental monitoring.[5] As a prominent example, affinity biosensors are applicable to continuous glucose monitoring (CGM), which most commonly involves a subcutaneously implanted sensor to measure glucose concentrations in an uninterrupted fashion throughout the day and night. Existing CGM devices typically use electrochemical detection of enzymatic reactions,[6] and are limited by large drift and poor stability.[7] In contrast, affinity glucose sensors are based on nonconsumptive equilibrium binding and can be highly stable and reproducible, holding the potential to allow long-term, reliable CGM applications. Affinity glucose sensors using protein[8,9] or synthetic polymer[10] receptors have been realized by a variety of transduction methods, such as glucose-induced changes in fluorescence intensity,[8] hydrogel volume,[11] or polymer solution viscosity.[7,9,12] Unfortunately, these methods are not well suited to fully implanted operation due to issues such as complications of optical access or mechanical moving parts.

This letter presents a distinctly different approach by exploiting permittivity changes of a polymer due to its specific, reversible affinity binding with an analyte. The approach is demonstrated using a proof-of-concept microsensor, which is fabricated using microelectromechanical systems (MEMS) technology and comprises a pair of parallel-plate capacitive electrodes sandwiching a solution of poly(acrylamide-*ran*-3-acrylamidophenylboronic acid) (PAA-*ran*-PAAPBA), a synthetic polymer with specific affinity to glucose. Experimental results demonstrate that the microsensor allows sensitive and specific detection of glucose at physiologically relevant concentrations. Thus, the dielectric affinity biosensing approach can be practically useful, for example, by potentially enabling a fully implantable sensor for long-term CGM.

In an electric field (E-field), a dielectric material undergoes polarization, i.e., short-range transport of positive and negative electric charges in opposite directions. The dependence of polarization on the frequency of an alternating-current (ac) E-field is represented by the complex permittivity, whose real and imaginary parts are, respectively, related to electric energy storage and dissipation within the material. Polarization is intimately influenced by the material's molecular structure. In the context of affinity biosensors, a liquid-phase dielectric material may undergo a molecular structure change as an embedded receptor binds to a target analyte. Thus, the permittivity changes can be measured to determine the analyte concentration.

To test this dielectric affinity biosensing principle, we have investigated the detection of glucose with a proof-of-concept microsensor equipped with the synthetic polymer PAA-*ran*-PAAPBA.[10] In this amphiphilic copolymer, the hydrophobic segment poly(3-acrylamidophenylboronic acid) (PAAPBA) contains boronic acid groups that can reversibly bind with glucose to form cyclic boronate esters in aqueous media, and the hydrophilic segment polyacrylamide (PAA) improves the overall water solubility.

The microsensor, fabricated using MEMS technology, consisted of a microchamber (volume: 7.8 μL) formed by two glass slides sandwiching a 3 μm thick photoresist spacer layer (Fig. 1). Gold thin films (100 nm in thickness) were deposited and patterned to form two electrodes (each 1×1 mm^2 in dimensions), respectively, on the top and bottom chamber walls, as well as a temperature sensor (a folded line 40 μm in width and approximately 2.3 mm in length, covering an area of 280×800 μm^2) on the bottom chamber wall. The electrodes and temperature sensors were passivated with a photoresist layer (500 nm in thickness). For testing, an aqueous solution of PAA-*ran*-PAAPBA, mixed with glucose or unspecific sugars, was introduced via an inlet and outlet into the microchamber. An ac E-field imposed on the electrodes caused the polymer polarization, which directly depended on glucose binding. Thus, the polymer permittivity could be obtained via the capacitance between the electrodes to determine the glucose concentration.

a)Electronic mail: qlin@columbia.edu.

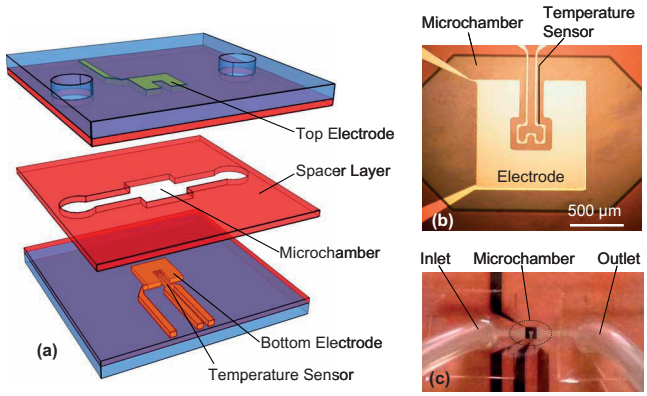

FIG. 1. (Color online) A dielectric affinity microbiosensor: (a) design schematic; and images of a fabricated device (b) before and (c) after packaging.

PAA-*ran*-PAAPBA polymers were synthesized in-house,[10] with three different molar ratios of acrylamide to 3-acrylamidophenylboronic acid (AAPBA) given by 50, 20, and 12.5, which corresponded to PAAPBA contents of 2%, 5%, and 7%, and molecular weights of 176800, 170700, and 71700, respectively. To prepare solutions of the polymers, 284 mg of each polymer was dissolved separately in 6 mL of phosphate buffered saline (PBS), which at pH 7.4 contained potassium phosphate (20 mM), sodium chloride (150 mM), and sodium azide (0.05%). D-(+)-glucose, D-(+)-galactose, or D-(−)-fructose (Sigma–Aldrich) was mixed with the polymer solutions, as appropriate, at varying concentrations to serve as the target analyte and unspecific interferents.

The microsensor was tested using the setup shown in Fig. 2(a). To eliminate thermally induced permittivity changes of the polymer, the temperature of the polymer solution was fixed at 37 °C using closed-loop control, in which the device was heated by a Peltier heater (Melcor, CP14) according to feedback from the integrated temperature sensor. The device was coupled to a capacitance/voltage transformation circuit [Fig. 2(b)] driven by a sinusoidal input of angular frequency $\omega=2\pi f$ from a function generator (Agilent, 33220A). All experiments were conducted at frequencies up to 100 kHz as allowed by a lock-in amplifier (Stanford Research Systems, SR844) used in output voltage measurements. When the microsensor was inserted into the circuit with the switch "T" in position "S" [Fig. 2(b)], an input voltage $U_{1S}e^{j\omega t}$ from the function generator yielded an output $U_{2S}e^{j\omega t}$. This was immediately followed by switching to a reference capacitor C_R with "T" in position "R" [Fig. 2(b)], with an input voltage $U_{1R}e^{j\omega t}$ yielding an output $U_{2R}e^{j\omega t}$. These allowed the device's complex admittance to be determined by $Y_S=G_S+j\omega C_S=j\omega C_R(U_{2S}^*U_{1R})/(U_{1S}^*U_{2R})$, where G_S is the equivalent conductance, and C_S the equivalent capacitance that is directly related to the polymer permittivity.

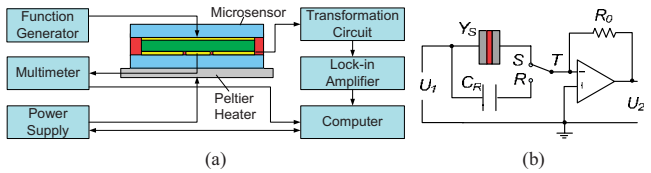

FIG. 2. (Color online) (a) Experimental setup. (b) A capacitance/voltage transformation circuit for sensor admittance measurements.

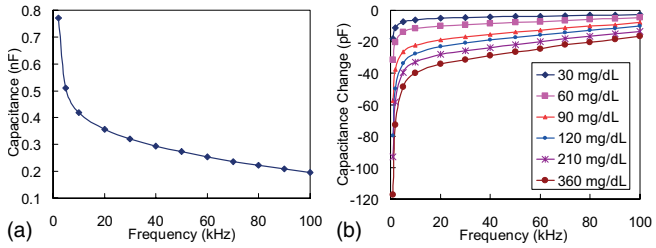

FIG. 3. (Color online) (a) Frequency-dependent equivalent capacitance of the microsensor in the absence of glucose (polymer PAAPBA content: 5%). (b) Differences between equivalent capacitances with and without the presence of glucose in the polymer.

With the microchamber filled with a glucose-containing polymer solution, we first investigated the microsensor response to varying glucose concentrations. The device's equivalent capacitance as a function of frequency is shown in Fig. 3 for the PAA-*ran*-PAAPBA polymer with 5% PAAPBA content. The sensor capacitance decreased consistently with the frequency [Fig. 3(a)] due to the dielectric relaxation of the polymer. At a given frequency, the capacitance decreased with increasing glucose concentration [Fig. 3(b)]. This suggests that the (real) permittivity of the polymer decreased due to glucose binding. Based on the resolution of our experimental setup (~70 μV), the device was estimated to be able to resolve glucose concentrations at about 0.5 mg/dL, which would be appropriate for CGM applications.

Next, using PAA-*ran*-PAAPBA polymers with different PAAPBA contents, we obtained the dependence of the equivalent capacitance on glucose concentration at a fixed frequency (100 kHz) (Fig. 4). Overall, the equivalent capacitance, and hence the polymer permittivity, exhibited a higher sensitivity to glucose concentration changes as the PAAPBA content increased. The responses of the polymers with 2% and 5% PAAPBA contents first varied approximately linearly, and then began to saturate as the glucose concentration increased. The polymer with 7% PAAPBA content was highly sensitive at the higher glucose concentrations. However, there was a significant decrease in sensitivity at the low end of the glucose concentration range, which was possibly due to a transition of the 7%-PAAPBA polymer from a liquid to a gel-like state at elevated glucose concentrations, a phenomenon not observed for the other polymer compositions.

Finally, the specificity of the microsensor was tested using the polymer with 5% PAAPBA content containing unspecific monosaccharides such as fructose and galactose (Fig. 5), which represented potential interferents with glucose

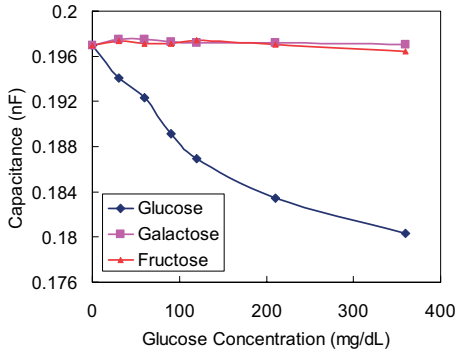

FIG. 4. (Color online) Equivalent capacitance of the microsensor with different polymer compositions at varying glucose concentrations (frequency: 100 kHz).

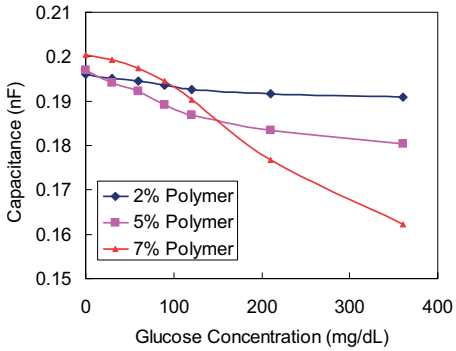

FIG. 5. (Color online) Equivalent capacitance of the microsensor with the polymer containing glucose monosaccharides galactose or fructose at varying concentrations (frequency: 100 kHz).

measurements. At all concentrations tested, the sensor responses to fructose and galactose were less than 0.4% and 5% of that to glucose. These unspecific responses can be considered negligible, given that physiological fructose and galactose concentrations are about 1000 times lower than those of glucose.[13,14] These results show that the dielectric affinity glucose sensing approach can be highly specific.

A number of polarization mechanisms, such as dipole reorientation, counterion diffusion, and interfacial polarization, contributed to the measured sensor capacitance. First, dipole reorientation[15] involves alignment with the applied E-field of permanent dipoles, which, for PAA-*ran*-PAAPBA, may include boronic acid and amide groups appended on the polyethylene backbone. Second, in counterion polarization,[16] appending groups of PAA-*ran*-PAAPBA are negatively charged, and cations (e.g., Na^+, K^+, and H_3O^+) are attracted to form a counterion cloud. Under the E-field, the counterions migrate unevenly within the cloud to contribute a net dipole moment. Finally, interfacial polarization involves dipole moments due to electrical double layers formed at the interfaces of the ionic buffer with polymer molecules (i.e., Maxwell–Wagner–Sillars polarization[15]) as well as the passivated electrode surfaces.[17] Crude estimate for the polymer suggests that the relaxation frequency of interfacial polarization is on the order of 1 GHz, while those of dipole reorientation and counterion polarization are on the order of a few kilohertz to a few tens of kilohertz. Thus, all of these polarization mechanisms may be significant for the polymer, and the relaxation behavior apparent from the rapid drop with frequency of the measured capacitance [Fig. 3(a)] could be mainly due to the relaxation of dipole reorientation and counterion polarization.

At a given frequency, the polarization behavior of PAA-*ran*-PAAPBA is influenced by glucose binding. AAPBA segments may bind with glucose at a two to one ratio to form cyclic esters of boronic acid, eliminating two hydroxyl groups. This may cause the net permanent dipole moments of AAPBA segments to decrease, thereby reducing dipole reorientation effects. Also, glucose binding may lead to variations in the net charge of polymer segments as well as in the polymer conformations, which would alter the electric double layer structure and result in changes in Maxwell–Wagner–Sillars and counterion polarization. Moreover, we conjecture that due to glucose binding, the polymer becomes partially cross-linked. This would increase the elastic resistance of the polymer to alignment of dipoles with the E-field, leading to a decrease in the polymer permittivity. These effects combine to explain that at a given frequency, the measured sensor capacitance decreased with glucose concentration [Fig. 3(b)].

In conclusion, we have presented an affinity biosensing approach that exploits changes in dielectric properties of a synthetic polymer due to its specific, reversible binding with an analyte. This approach has been demonstrated with a microsensor that uses the glucose sensitive polymer PAA-*ran*-PAAPBA. Glucose binding-induced permittivity changes of the polymer were measured capacitively at a controlled temperature to determine the glucose concentration. The measured device capacitance decreased consistently with frequency due to dielectric relaxation of the polymer. At a given frequency, the capacitance decreased sensitively and specifically with glucose concentration as the polymer becomes less polarizable due to glucose binding. These results demonstrate that the dielectric affinity biosensing approach is practically significant, and can potentially enable a fully implantable device for long-term CGM.

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