Journal of Diabetes Science and Technology
Volume 5, Issue 5, September 2011

SYMPOSIUM

Synthesis and Development of Poly(*N*-Hydroxyethyl Acrylamide)-*Ran*-3-Acrylamidophenylboronic Acid Polymer Fluid for Potential Application in Affinity Sensing of Glucose

Siqi Li, Ph.D.,[1] Erin N. Davis,[1] Xian Huang, M.S.,[2] Bing Song, M.S.,[2] Rebecca Peltzman,[1] David M. Sims,[1] Qiao Lin, Ph.D.,[2] and Qian Wang, Ph.D.[1]

Abstract

Background:

In previous work, we described viscosity and permittivity microelectromechanical systems (MEMS) sensors for continuous glucose monitoring (CGM) using poly[acrylamide-*ran*-3-acrylamidophenylboronic acid (PAA-*ran*-PAAPBA). In order to enhance our MEMS device antifouling properties, a novel, more hydrophilic polymer-sensing fluid was developed.

Method:

To optimize sensing performance, we synthesized biocompatible copolymers poly(*N*-hydroxyethyl acrylamide)-*ran*-3-acrylamidophenylboronic acid (PHEAA-*ran*-PAAPBA) and developed its sensing fluid for viscosity-based glucose sensing. Key factors such as polymer composition and molecular weight were investigated in order to optimize viscometric responses.

Results:

Compared with PAA-*ran*-PAAPBA fluid of a similar binding moiety percentage, PHEAA-*ran*-PAAPBA showed comparable high binding specificity to glucose in a reversible manner and even better performance in glucose sensing in terms of glucose sensing range (27–468 mg/ml) and sensitivity (within 3% standard error of estimate). Preliminary experiment on a MEMS viscometer demonstrated that the polymer fluid was able to sense the glucose concentration.

Conclusions:

Our MEMS systems using PHEAA-*ran*-PAAPBA will possess enhanced implantable traits necessary to enable CGM in subcutaneous tissues.

J Diabetes Sci Technol 2011;5(5):1060-1067

Author Affiliations: [1]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina; and [2]Department of Mechanical Engineering, Columbia University, New York, New York

Abbreviations: ([1]H NMR) proton nuclear magnetic resonance, (AAPBA) *N*-3-acrylamidophenylboronic acid, (AIBN) 2,2′-azodiisobutyronitrile, (CGM) continuous glucose monitoring, (Con A) concanavalin A, (DMSO) dimethyl sulfoxide, (HEAA) *N*-hydroxyethyl acrylamide, (MEMS) microelectromechanical systems, (MW) molecular weight, (PAA) polyacrylamide, (PAA-*ran*-PAAPBA) poly(acrylamide-*ran*-3-acrylamidophenylboronic acid), (PAAPBA) 3-acrylamidophenylboronic acid, (PHEAA) poly(*N*-hydroxyethyl acrylamide), (PHEAA-*ran*-PAAPBA) poly(*N*-hydroxyethyl acrylamide-*ran*-3-acrylamidophenylboronic acid), (THF) tetrahydrofuran

Keywords: antifouling, boronic acid, continuous glucose monitoring, microelectromechanical systems, poly(acrylamide-*ran*-3-acrylamidophenylboronic acid), poly(*N*-hydroxyethyl acrylamide)-*ran*-3-acrylamidophenylboronic acid)

Corresponding Author: Qian Wang, Ph.D., Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter St., Columbia, SC 29208; email address wang@chem.sc.edu

Approximately 21 million people in the United States and 171 million worldwide suffer from diabetes mellitus. A continuous glucose monitoring (CGM) device allows timely detection of abnormal glucose levels and enables active intervention by taking carbohydrates or injecting insulin, which has been shown to reduce the risk of diabetes-related complications.[1] Currently, affinity glucose sensing is most commonly based on reversible binding of glucose to concanavalin A (Con A), a glucose-specific lectin. The system has excellent specificity, as there are no other sugars at significant concentration levels in blood serum that might interfere with Con A binding.[2–4] This approach has been extensively investigated *in vitro* and *in vivo*, and there are active efforts to develop optimized fluorescence-based sensors.[2,5–12] To enhance the reliability of affinity-based sensors, sensors with fully electronic readout via viscosity measurement have been reported.[13–17] However, there were significant safety concerns with the toxicological properties of Con A. For instance, Con A is known to stimulate enhanced immunogenicity[18,19] and to induce antigen-specific cellular cytotoxicity.[20,21] Stability of Con A is another problem. Metal ions were found to strongly influence its stability. Although native dimeric Con A was fairly stable, removal of metal ions (Ca^{2+} and Mn^{2+}) dramatically lowered its stability.[22]

To address these concerns, new biocompatible sensing liquids for viscometric glucose sensing are highly desired. In general, boronic acid is a biocompatible functional group with low cytotoxicity and low immunogenicity.[23] Therefore, considerable research interests have been attracted to develop a variety of glucose sensors through different sensing mechanisms in polymers. For example, fluorescent changes due to photoelectron transfer, fluorescence resonance energy transfer, or internal charge change have been used to build fluorescent sensors.[24] Asher and colleagues[25] introduced the use of polymerized crystalline colloidal array for colorimetric detection of glucose. Lei and associates[26] reported a thin-film wireless pressure sensor using a phenylborate-based hydrogel that could bind with glucose, resulting in swelling. Arnold and coworkers[27] reported preliminary data from a conductometric sensor with a boronic acid immobilized in a hydrogel, whose binding to permeated glucose changed the ionic concentration conductivity, resulting in change of conductivity. Zhang and colleagues[28] and Hoare and Pelton[29] developed a poly(*N*-isopropyl-acrylamide) copolymer microgel sensor for glucose that is based on the volume change caused by repulsion of the boronate groups. Huh and associates[30] showed improved potentiometric sensing capability using enzymatic polymerized self-doped copolymer of poly(aniline-*co*-3-aminobenzeneboronic acid). However, there has been no report on boronic-acid-based glucose sensing systems that exploit viscosity changes, even though such systems have the potential to allow fully integrated biocompatible CGM devices.

We developed microelectromechanical systems (MEMS) viscosity[31–34] and permittivity[35] sensors that were aimed for the continuous monitoring of glucose levels in diabetes patients. For example, a typical viscometric device[32,33,36] is shown in **Figure 1**. Glucose sensing was based on affinity binding principles using a solution of poly(acrylamide-*ran*-3-acrylamidophenylboronic acid) (PAA-*ran*-PAAPBA) as the sensing fluid. Glucose concentration was determined by detecting viscosity changes induced by the binding of glucose to PAA-*ran*-PAAPBA through measurement of the cantilever's vibration parameters.

In order to make our MEMS implantable for subcutaneous CGM, antifouling polymer coating would be convenient and cost-effective. Many antifouling polymers have some common properties, such as being electrically neutral, hydrophilic, and hydrogen bond receptors but not hydrogen bond donors.[37] Poly(*N*-hydroxyethyl acrylamide) (PHEAA) is a hydrophilic, nonionic polymer with hydroxyl groups on the side chain covering a hydrophobic carbon-carbon backbone. It has been found to be less adhesive to proteins than polyacrylamide (PAA). While introduction of hydrophilic PAA segments can improve the water solubility of the copolymer,[38] as well as possibly provide the additional neighbor coordinating effect via carbonyl oxygen and boron chelating to enhance the binding of boronic acid to carbohydrates,[39,40] incorporation of an additional hydroxyl group to the

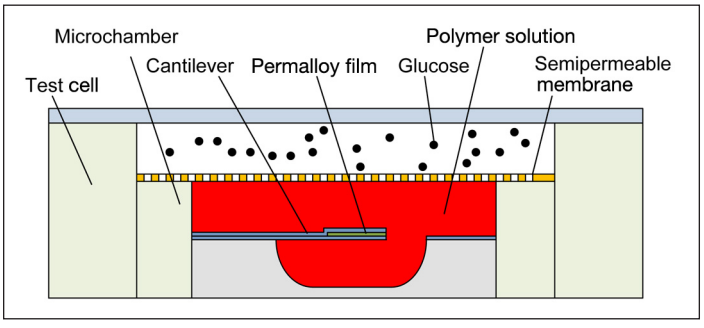

Figure 1. Schematic illustration of the MEMS viscometric device and its sensing mechanism design.

side chain may further enhance this additional neighbor coordinating effect. Therefore, we report the first synthesis of novel biocompatible boronic-acid-based polymers and development of another polymeric-sensing fluid that can be used in MEMS affinity glucose sensors. In our present work, a nonionic hydrophilic monomer *N*-hydroxyethyl acrylamide (HEAA) replaces acrylamide as the comonomer to prepare boronic acid containing random copolymers to investigate boronic-acid-based interaction with sugars. While this article is focused on sensing fluid development, preliminary measurement results using a cantilever-based MEMS viscometric sensor are also presented.

Methods

Materials

Compound 3-aminobenzene boronic acid monohydrate was purchased from Frontier Scientific, Inc. SnakeSkin Pleated Dialysis Tubing (MWCO 3500) was purchased from Pierce Biotechnology, Inc. Ubbelohde viscometer was obtained from Cannon Instrument Company. Acryloyl chloride, D-(-)-fructose, D-(+)-glucose, D-(+)-lactose, D-(+)-galactose, D-(+)-sucrose, D-(+)-cellobiose, D-(+)-mannose, hydroxyethyl acrylamide, sodium carbonate, and sodium chloride were purchased from Sigma-Aldrich, Inc. Nanopure water was purified by Milli-Q Ultrapure system purchased from Millipore Corporation.

Synthesis of N-*3-Acrylamidophenylboronic Acid*

The monomer *N*-3-acrylamidophenylboronic acid (AAPBA) was prepared by following a similar procedure mentioned in the literature.[41] Starting material 3-aminobenzene boronic acid monohydrate (3.4 g, 21.9 mmol) was dissolved in 80 ml of tetrahydrofuran (THF)/H_2O (1:1 ratio) and chilled to 4 °C by ice-water bath. $NaHCO_3$ (3.7 g, 44.0 mmol) and acryloyl chloride (3.57 ml, 44.1 mmol) were added slowly to the mixture. The reaction was stirred and allowed to warm up naturally to ambient temperature overnight. THF/H_2O was then evaporated to dry under vacuum to give an off-white solid. This solid was dissolved in ethyl acetate (50 ml), filtered, and washed with water (50 ml), saturated sodium bicarbonate (50 ml), water (50 ml), and brine (50 ml). It was dried by anhydrous Na_2SO_4 (15 g), and the solvent ethyl acetate was evaporated. The solid was then recrystallized in water to give 2.74 g of off-white AAPBA crystals (yield 65%). Proton nuclear magnetic resonance (^{1}H NMR) using dimethyl sulfoxide (DMSO) as the solvent was used to confirm the structure. ^{1}H (300 MHz, DMSO-*d6*): δ 10.06 (s, 1H, NH), 8.01 [s, 2H, B-$(OH)_2$], 7.86, 7.81–7.78, 7.49–7.47, 7.29–7.24 (s, d, d, t, 1H each, ArH), 6.48–6.39 (2d, 1H, vinyl CH_2), 6.26–6.19 (dd, 1H, vinyl CH_2), 5.73–5.69 (dd, 1H, vinyl CH).

*Synthesis of Poly(*N*-Hydroxyethyl Acrylamide-*Ran*-3-Acrylamidophenylboronic Acid) (PHEAA-*Ran*-PAAPBA)*

A typical free radical polymerization was used following the same method as in our previous paper.[32,33] *N*-hydroxyethyl acrylamide (5.46 ml, 52.7 mmol), AAPBA (0.5 g, 2.63 mmol), and 2,2′-azodiisobutyronitrile (AIBN; 0.022 g, 0.13 mmol) were dissolved in 35 ml of DMSO. Then the solution was bubbled with nitrogen for 1 h and put into an oil bath at 70 °C for 24 h under a nitrogen atmosphere. The resultant gel was dissolved by Nanopure water and subjected to dialysis by SnakeSkin Pleated Dialysis Tubing (MWCO 3500) against Nanopure water for 24 h. The solution was dropwisely added to acetone, and precipitate was stirred in acetone for at least 10 min before filtered and dried in a vacuum oven. Resultant white polymer was then characterized using viscometry and ^{1}H NMR as in the reference.[32,33] A typical ^{1}H NMR is as follows: ^{1}H (300MHz, DMSO-d_6): δ 8.01 [br, 2H, B-$(OH)_2$], 7.64–7.31 (m, 4H, ArH), 4.65 (m, H, $HOCH_2/H_2O$), 3.51 (m, 2H, CH_2NH), 3.18 (m, 2H, CH_2OH), 1.85 (m, 1H, CHC=O), 1.52 (m, 2H, CH_2CH).

Polymer Molecular Weight Measurement

Weight-average molecular weights (MWs) of the polymers were determined by dissolving 40 mg of polymer in 2 ml of 0.12 M NaCl (pH 6) buffer.[32,33] Using an Ubbelohde viscometer, viscosity was measured at 25 °C and MW was calculated from the intrinsic viscosity according to the Mark–Houwink equation for PAA:

$$[\eta]=5.31 \times 10^{-3} \times M_w^{0.79}. \quad (1)$$

Viscosity-Sensing Studies

Upon addition of a saccharide, viscometric response of the polymer was measured by dissolving 90 mg of polymer in 2 ml of sodium phosphate buffer (0.1M, pH 7.4). This solution was then added to an Ubbelohde viscometer and the kinematic viscosity was recorded at 25 °C. These measurements were taken in triplicate in order to assure accuracy. Kinematic viscosity was then converted to viscosity.

Glucose Binding Reversibility Study

Polymer 1 (114.2 mg) was dissolved in 4 ml of 0.1 M sodium phosphate buffer (blank). After measuring the

viscosity of the blank, 200 mg/dl of glucose was added to the solution and viscosity was measured again. The solution was then dialyzed against 0.1 M sodium phosphate buffer (400 ml) with polyethylene glycol 8000 (18.0 g) through the dialysis membrane (MWCO 3500) in order to see if the cross linking between boronic acid and glucose was reversible.

Microelectromechanical Systems Experiments

A prototype MEMS device (**Figure 1**) was used to measure glucose-induced viscosity changes of the PHEAA-*ran*-PAAPBA-sensing fluid in a range from 0 to 480 mg/dl. The schematic design of the device is shown in **Figure 1** and is described in detail elsewhere.[31] Briefly, a parylene microcantilever (400 × 250 × 7 μm^3 in dimension) embedded with Permalloy thin film was immersed into the polymer solution and vibrated under external AC magnetic field. Environmental glucose can permeate through a semipermeable cellulose acetate membrane and bind with the polymer solution, changing the viscosity of the polymer solution as well as the damping to the cantilever vibration. As a result, glucose concentration can be determined from the cantilever vibration characteristics that were measured by an optical lever, in which a converged laser beam was directed onto the cantilever surface and reflected light from the Permalloy film was measured by a position-sensitive detector that was connected to a lock-in amplifier. This optical setup provided a rapid assessment of the polymer functionality and could be replaced by electrical (e.g., piezo-resistive or capacitive) transduction methods for *in vivo* animal studies.

Results and Discussions

The PHEAA coating on a capillary was discovered to interact least with acidic and basic proteins because of its hydrophilic nature.[42] Thus, grafting PHEAA from our MEMS device surfaces could potentially improve the antifouling properties. In order to take advantage of the antifouling property of PHEAA and to investigate the effect of hydroxyethyl group on boronic acid binding to glucose, a series of PHEAA-*ran*-PAAPBA copolymers with differing monomer ratios were synthesized by classic free radical polymerization (**Figure 2**). The percentage yield for these polymers were relatively high, ranging from 77% to 90%, with varying MWs (**Table 1**). Because they were PAA derivatives with only a slight difference in polymer structures, their viscosity profiles were presumed similar to that of PAA. Thus, their MWs were measured and calculated approximately using the method shown previously for PAA-*ran*-PAAPBA.[32,33] The percentage of

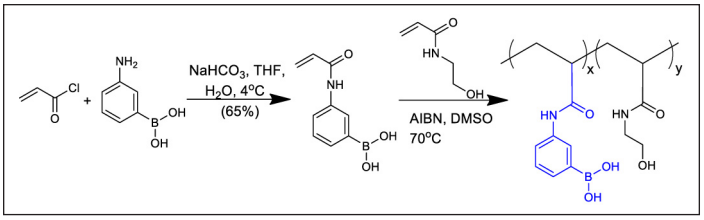

Figure 2. Synthesis of AAPBA monomer and its glucose-sensitive polymer derivative PHEAA-*ran*-PAAPBA.

Table 1.
Characteristics of Polymers Synthesized in 35 ml Dimethyl Sulfoxide at 70 °C

Polymer	HEAA/AAPBA/AIBN	MW[a]	Yield (%)	PAAPBA %[b]
1	20:1:0.05	188,600	77	4.7
2	20:1.6:0.05	260,600	90	4.2
3	20:0:0.50	346,800	77	0
4	20:1:0.05[c]	130,000	43	4.7

[a] MW was determined using viscometry.
[b] The percentage composition of PAAPBA was calculated by ^{1}H NMR spectroscopy.
[c] The reaction mixture for polymer 4 (positive control) was Acrylamide/AAPBA/AIBN.

3-acrylamidophenylboronic acid (PAAPBA) in the polymer was calculated by the ratio of proton integration of aromatic protons to its sum with that of ethylene protons on hydroxyethyl group.

The AAPBA ratio in the reaction mixture (HEAA/AAPBA/AIBN) was an important factor in preparing the polymers. With a high AAPBA ratio (e.g., HEAA/AAPBA/AIBN was 20:5:0.05 or 20:7:0.05), it was difficult to dissolve resultant polymers completely in water or buffer for MW and component characterization (not listed in **Table 1**), presumably resulting from the incorporation of higher content of hydrophobic PAAPBA segments. However, when the AAPBA ratio was low, a polymer with high MW (505,800 Da) was obtained, which resulted in a high initial fluid viscosity that was beyond Ubbelohde viscometer accurate measurement range (from 7 to 35 cP). When it was set to 20:0:0.5 (no AAPBA in the polymerization), the MW (346,800 Da) of the control polymer 3 PHEAA was much higher than those of polymers 1 and 2 (188,600 and 260,600 Da). These results suggested radical polymerization was slightly hampered by the presence of boronic acid moiety, which agreed with our previous synthesis of PAA-*ran*-PAAPBA.[32,33] Therefore, a monomer ratio around 20:1:0.05 was adopted in order to ensure successful preparation of feasible polymer-sensing fluids.

Following the same viscosity measurement procedure used by our previous PAA-*ran*-PAAPBA fluids, all viscosity experiments were done in triplicate, and the results were all within 1% error range, which assured the repeatability of our measurements. Similar to the response speed of PAA-*ran*-PAAPBA-sensing fluids to glucose, PHEAA-*ran*-PAAPBA fluids quickly reached an equilibrium state within a time frame of a few seconds of bubbling to mix them, whose viscosity also showed no obvious variation even after hours. Though the time to reach equilibrium for most small boronic acid molecules could be as short as 30 s, PHEAA-*ran*-PAAPBA showed comparable response speed to PAA-*ran*-PAAPBA.[32,33] As the polymer-sensing fluid will be segregated in a nanoliter chamber of MEMS by a semipermeable membrane that only allowed small molecules such as water and glucose to pass (**Figure 1**), sheer thinning of the fluids caused by direct contact with blood flow was not considered as an influential factor for time response at the present study by Ubbelohde viscometer, though PHEAA was reported to be more resistant to sheer thinning than PAA.[42] Instead, time response is primarily dictated by the time required for glucose to permeate throughout the sensor volume. In the future, we plan to speed up the time response of the MEMS sensor via optimization of the characteristics diffusion distance in the MEMS device.

We also investigated PHEAA-*ran*-PAAPBA concentration impact on viscosity response to glucose inspired by previous experiments with PAA-*ran*-PAAPBA. When the polymer 1 concentration was 20 mg/ml, the fluid barely responded to the addition of glucose (data not shown). However, at 45 mg/ml, its response was highly evident as shown in **Figure 3**. So in the following sugar sensing experiments, all polymer concentrations were set to 45 mg/ml. Responses of PHEAA-*ran*-PAAPBA of different MWs and control polymers to glucose were inspected. As shown in **Figure 3**, polymer 4 sensed glucose from below hypoglycemia region 27 to 162 mg/dl, a normal glycemia concentration, with a standard error of estimate of 9%. Polymer 2 covered a little further to 243 mg/ml, the threshold of hyperglycemia range, with a better standard error of estimate of 4%. Polymer 1 demonstrated the best coverage from 27 to 468 mg/ml, with lowest standard error of estimate 3%, suggesting it would be an ideal fluid to sense hypoglycemia, normal glycemia, and hyperglycemia to a far end. As our MEMS device was able to measure viscosity range from 8.7 to 43.4 cP,[36] polymer 1 was tested in our prototype MEMS device (**Figure 1**) and discussed later. Compared with the 2006-launched commercial Medtronic's Paradigm® REAL-Time system whose sensitivity was 80.3% at hypo-

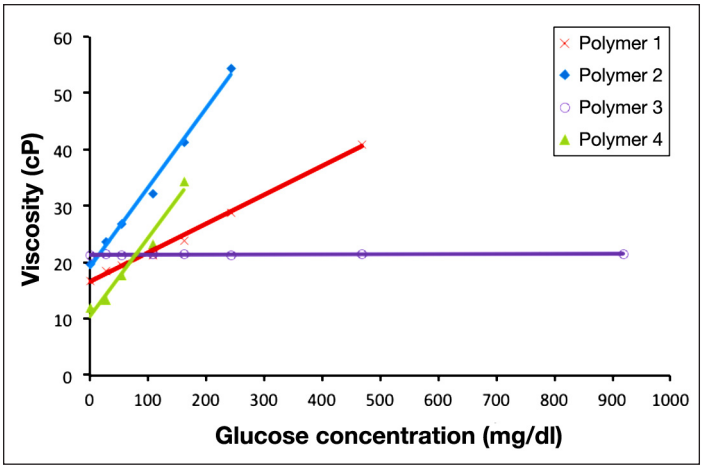

Figure 3. Viscosity response of polymer 1 to 4 to the addition of glucose.

glycemia and 80.6% at hyperglycemia,[43] our optimized MEMS device using polymer 1 as sensing fluid could be more sensitive.

Negative control polymer 3, which possessed the same PHEAA component but no boronic acid moiety, did not show any obvious change upon addition of glucose up to 918 mg/dl, confirming the presence of PAAPBA was the determining factor to glucose response. Thus these polymer fluids should follow similar glucose sensing mechanism where two boronic acid moieties were bound to one glucose in furanose form as we discuss with PAA-*ran*-PAAPBA.[32,33] Polymer 2 had sharper response to the addition of glucose than polymer 1, though polymers 1 and 2 were of similar PAAPBA percentage (4.7% versus 4.2%). The MW of polymer 1 was 188,600 Da, much lower than that of polymer 2 (260,600 Da), indicating longer polymer chains were easier to cross link and increase resulting complex viscosity because there are more boronic acid moieties on the longer polymer chain available to bind glucose. These results implied that both PAAPBA percentage and MW are important factors for viscosity-based glucose sensing, which agreed with our previous conclusions for polymers PAA-*ran*-PAAPBA results.

Positive control polymer 4 showed that its initial viscosity 12 cP was slightly over half of that of polymer 2 (20 cP), which was consistent with the fact that its MW 130,000 Da was almost half of that of polymer 2 according to the Mark–Houwink equation. However, it gave a similar response trend as polymer 2 did to glucose addition. Compared with polymer 1, polymer 4 of the same PAAPBA percentage and smaller MW showed sharper response to glucose. These results suggested that, instead of bringing in additional neighbor-coordinating

effect on boron and thus enhancing glucose sensing, the introduction of the hydroxyethyl group on amide lowers the sensitivity of polymer to glucose, presumably due to the introduction of more steric hindrance caused by the hydroxyethyl group. On the other hand, the hydroxyethyl group is more hydrophilic, which enabled polymers 1 and 2 to detect glucose in a larger range than polymer 4 did.

In order to observe the saccharide binding capability of PHEAA-*ran*-PAAPBA, polymer 1 was chosen as the sensing polymer for sugars such as fructose, galactose, mannose, maltose, and glucose. **Figure 4** showed that the viscosity of polymer 1 solution increased dramatically upon increase of glucose concentration from 0 to 468 mg/dl. On the contrary, viscosity remained unchanged upon addition of other sugars, indicating polymer 1 solution responded selectively to glucose.

Reversibility of the interaction between glucose and PHEAA-*ran*-PAAPBA was investigated by dissolving 28.5 mg/ml of polymer 1 following the same conditions outlined in a previous paper.[32] Our results showed that the polymer continued to respond to the addition of glucose even after 1 week (seven cycles). However, after each cycle, the response magnitude decreased from 17.7 to 0.9 cP as shown in **Figure 5**, which could be attributed to the loss of polymer to the contact surface of dialysis. Because the polymer fluid without glucose was already very viscous, it tended to stick to the interior surface of Ubbelohde viscometer and the dialysis membrane. Therefore, the more contact of the polymer to these surfaces, the more polymers were stuck on them. Though the viscosities of fluid without glucose were still similar, due to the decrease of available amount of polymers to bind glucose, viscosity also decreased as a result.[31] Such situations can be circumvented if the dialysis setup is designed properly. For example, in a MEMS device where polymer loss was minimized, reversibility was reproducible and no decay was observed.[31]

We also measured the frequency response of glucose concentration changes using polymer 1 as the sensing fluid in the MEMS device (**Figure 1**). While PAA was so viscous that it was hard for injection, PHEAA behaved approximately like a Newtonian fluid at low shear rate.[44] Thus, we did not investigate shear rate impacts on glucose sensing experiment. Because our polymer fluid will be confined in the sensing chamber by a 3500 MWCO membrane, blood flow and pressure fluctuation over the membrane may apply high enough shear rate on the sensing fluid to cause fluid shear thinning and

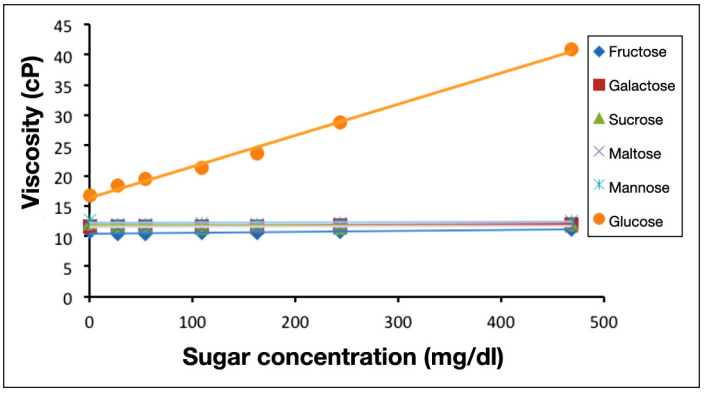

Figure 4. Viscosity response of polymer 1 to the addition of different carbohydrates.

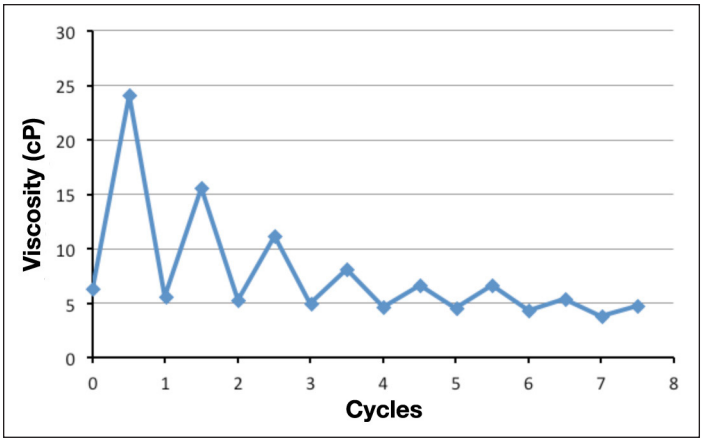

Figure 5. Reversibility study using 28.5 mg/ml of polymer 1. The low points being just the polymer with no glucose (after dialysis for 12 h) and the high points being the viscosity responses to the addition of 200 mg/dl of glucose.

influence viscosity sensing. Further *in vivo* experiments are necessary to illustrate such impacts on CGM in the future.

Measured frequency response of vibrational amplitude and phase of the MEMS cantilever is shown in **Figure 6**. It can be seen that vibration amplitudes decreased with glucose concentration in the measured frequency range from 10 to 1500 Hz (**Figure 6A**). This is consistent with an increase of the polymer solution's viscosity and viscous damping at higher glucose concentrations. The cantilever phase shift curves cross at approximately 500 Hz (**Figure 6B**), which can be considered the natural frequency of the cantilever. In addition, as glucose concentration varied from 0 to 480 mg/dl, the resonance peak amplitude of the cantilever vibration decreased from 5.53 to 1.23 mV, with resonance frequency shifting from 350 to 200 Hz, indicating a damping coefficient from 0.71 to 0.92. This was again consistent with increases of polymer solution viscosity and viscous damping at higher glucose concentrations.

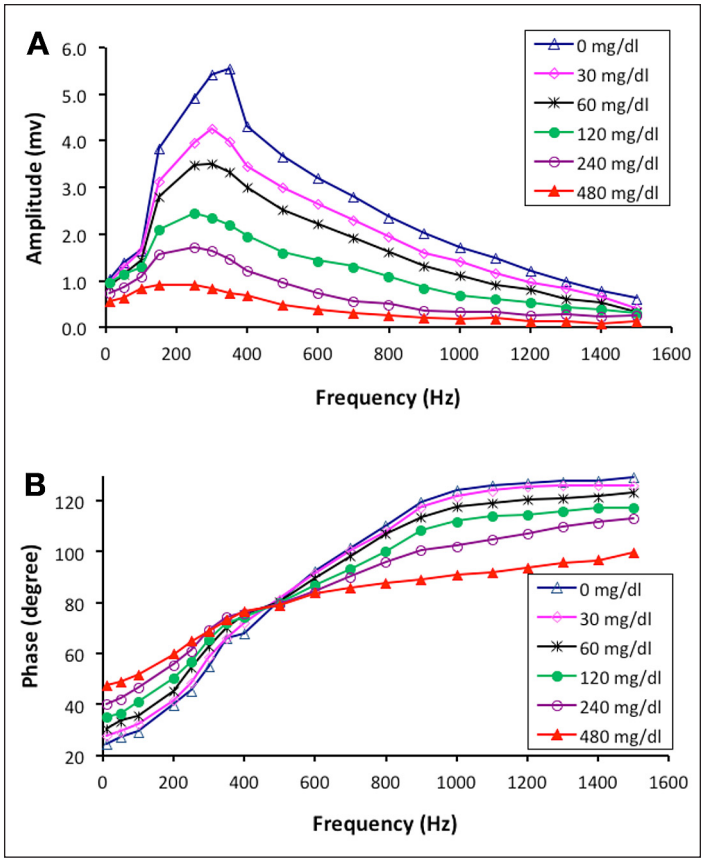

Figure 6. Vibration of a MEMS cantilever under sinusoidal magnetic excitation. The cantilever was immersed in a polymer 1 solution with glucose present at various concentrations: **(A)** the amplitude and **(B)** phase of the cantilever tip deflection as a function of frequency.

Conclusions

We report here the first synthesis of novel boronic-acid-containing polymer PHEAA-*ran*-PAAPBA and its application in glucose sensing. Syntheses of monomers and polymers were straightforward. Compared with PAA-*ran*-PAAPBA fluid of a similar binding moiety percentage, PHEAA-*ran*-PAAPBA showed comparable high binding specificity to glucose in a reversible manner and even better performance in glucose sensing in terms of glucose sensing range and sensitivity. Preliminary experiment on a MEMS viscometer demonstrated that PHEAA-*ran*-PAAPBA fluid was able to measure physiological glucose concentrations. This new polymer can potentially improve the antifouling properties of boronic-acid-based sensing liquid. We expect that our MEMS systems using PHEAA-*ran*-PAAPBA could possess enhanced implantable traits necessary to enable CGM in subcutaneous tissues. We plan to perform *in vivo* tests using our MEMS device and to test the biocompatibility of our device in the future study.

As polymer fluid concentration, polymer composition, and MW are important factors that determined sensing fluid viscometric responses, upon proper adjustment of polymer fluid concentration, their sensitivity can also be adjusted to fit the desired glucose concentration range. If precise control of polymer MW is required for future precise control of polymer fluid response to glucose, living polymerization technique and reversible addition–fragmentation chain transfer polymerization would be a great option to adopt.[40,41] Such polymer MWs would also be characterized by an absolute technique such as small angle light scattering,[45] compared with current, less accurate relative viscometry technique.

Funding:

This work was supported by the National Science Foundation (NSF-ECCS-0702101) and the Magellan Scholarship through the University of South Carolina.

References:

1. Department of Health and Human Services; Centers for Disease Control and Prevention. National diabetes fact sheet, 2007. www.cdc.gov/diabetes/pubs/pdf/ndfs_2007.pdf.
2. Ballerstadt R, Schultz JS. Competitive-binding assay method based on fluorescence quenching of ligands held in close proximity by a multivalent receptor. Anal Chim Acta. 1997;345(1-3):203–12.
3. Schultz JS, Sims G. Affinity sensors for individual metabolites. Biotechnol Bioeng Symp. 1979;(9):65–71.
4. Schultz JS, Mansouri S, Goldstein IJ. Affinity sensor: a new technique for developing implantable sensors for glucose and other metabolites. Diabetes Care. 1982;5(3):245–53.
5. Mansouri S, Schultz JS. A miniature glucose sensor based on affinity binding. Biotechnol.1984;2(10):885–90.
6. Meadows DL, Schultz JS. Design, manufacture and characterization of an optical fiber glucose affinity sensor based on a homogeneous fluorescence energy transfer assay system. Anal Chim Acta. 1993;280(1):21–30.
7. Russell RJ, Pishko MV, Gefrides CC, McShane MJ, Coté GL. A fluorescence-based glucose biosensor using concanavalin A and dextran encapsulated in a poly(ethylene glycol) hydrogel. Anal Chem. 1999;71(15):3126–32.
8. Ballerstadt R, Schultz JS. A fluorescence affinity hollow fiber sensor for continuous transdermal glucose monitoring. Anal Chem. 2000;72(17):4185–92.
9. Liang F, Pan T, Sevick-Muraca EM. Measurements of FRET in a glucose-sensitive affinity system with frequency-domain lifetime spectroscopy. Photochem Photobiol. 2005;81(6):1386–94.
10. Ballerstadt R, Gowda A, McNichols R. Fluorescence resonance energy transfer-based near-infrared fluorescence sensor for glucose monitoring. Diabetes Technol Ther. 2004;6(2):191–200.
11. Ballerstadt R, Polak A, Beuhler A, Frye J. *In vitro* long-term performance study of a near-infrared fluorescence affinity sensor for glucose monitoring. Biosens Bioelectron. 2004;19(8):905–14.

12. Friedl KE. Novel biosensors for long-term *in vivo* physiological monitoring. Diabetes Technol Ther. 2004;6(2):201–2.
13. Ballerstadt R, Ehwald R. Suitability of aqueous dispersions of dextran and concanavalin A for glucose sensing in different variants of the affinity sensor. Biosens Bioelectron.1994;9(8):557–67.
14. Ehwald R, Ballerstädt R, Dautzenberg H. Viscosimetric affinity assay. Anal Biochem. 1996;234(1):1–8.
15. Beyer U, Ehwald R, Fleischer LG. Post-stress thickening of dextran/concanavalin a solutions used as sensitive fluids in a viscosimetric affinity assay for glucose. Biotechnol Prog. 1997;13(6):722–6.
16. Beyer U, Ehwald R. Compensation of temperature and concanavalin A concentration effects for glucose determination by the viscometric affinity assay. Biotechnol Prog. 2000;16(6):1119–23.
17. Beyer U, Schäfer D, Thomas A, Aulich H, Haueter U, Reihl B, Ehwald R. Recording of subcutaneous glucose dynamics by a viscometric affinity sensor. Diabetologia. 2001;44(4):416–23.
18. Kataoka T, Oh-Hashi F, Sakurai Y. Immunogenicity and amplifier cell production by tumor vaccines enhanced by concanavalin A. Gann. 1982;73(2):193–205.
19. Enker WE, Craft K, Wissler RW. Augmentation of tumor-specific immunogenicity by concanavalin A in the Morris hepatoma 5123. J Surg Res. 1974;16(1):66–8.
20. Möeller E. Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens. Science. 1965;147:873–9.
21. Phillips JH, Lanier LL. Lectin-dependent and anti-CD3 induced cytotoxicity are preferentially mediated by peripheral blood cytotoxic T lymphocytes expressing Leu-7 antigen. J Immunol. 1986;136(5):1579–85.
22. Zahnley JC. Effects of manganese and calcium on conformational stability of concanavalin a: a differential scanning calorimetric study. J Inorg Biochem. 1981;15(1):67–78.
23. Yang W, Gao X, Wang B. Boronic acid compounds as potential pharmaceutical agents. Med Res Rev. 2003;23(3):346–68.
24. Fang H, Kaur G, Wang B. Progress in boronic acid-based fluorescent glucose sensors. J Fluoresc. 2004;14(5):481–9.
25. Asher SA, Alexeev VL, Goponenko AV, Sharma AC, Lednev IK, Wilcox CS, Finegold DN. Photonic crystal carbohydrate sensors: low ionic strength sugar sensing. J Am Chem Soc. 2003;125(11):3322–9.
26. Lei M, Baldi A, Nuxoll E, Siegel RA, Ziaie B. A hydrogel-based implantable micromachined transponder for wireless glucose measurement. Diabetes Technol Ther. 2006;8(1):112–22.
27. Arnold FH, Zheng WG, Michaels AS. A membrane-moderated, conductimetric sensor for the detection and measurement of specific organic solutes in aqueous solutions. J Membrane Sci. 2000;167(2):227–39.
28. Zhang Y, Guan Y, Zhou S. Synthesis and volume phase transitions of glucose-sensitive microgels. Biomacromolecules. 2006;7(11):3196–201.
29. Hoare T, Pelton R. Engineering glucose swelling responses in poly(*N*-isopropylacrylamide)-based microgels. Macromolecules. 2007;40(3):670–8.
30. Huh P, Kim SC, Kim Y, Wang Y, Singh J, Kumar J, Samuelson LA, Kim BS, Jo NJ, Lee JO. Optical and electrochemical detection of saccharides with poly(aniline-co-3-aminobenzeneboronic acid) prepared from enzymatic polymerization. Biomacromolecules. 2007;8(11):3602–7.
31. Huang X, Li S, Schultz JS, Wang Q, Lin Q. A MEMS affinity glucose sensor using a biocompatible glucose-responsive polymer. Sens Act B. 2009;140(2):603–9.
32. Li S, Davis EN, Anderson J, Lin Q, Wang Q. Development of boronic acid grafted random copolymer sensing fluid for continuous glucose monitoring. Biomacromolecules. 2009;10(1):113–8.
33. Li S, Huang X, Davis EN, Lin Q, Wang Q. Development of novel glucose sensing fluids with potential application to micro-electromechanical systems-based continuous glucose monitoring. J Diabetes Sci Technol. 2008;2(6):1066–74.
34. Huang X, Li S, Schultz JS, Wang Q, Lin Q. A capacitive MEMS viscometric sensor for affinity detection of glucose. J Microelectromechanical Sys. 2009;18:1246.
35. Huang X, Li S, Schultz JS, Wang Q, Lin Q. A dielectric affinity microbiosensor. Appl Phys Lett. 2010;96(3):033701.
36. Zhao Y, Li S, Davidson A, Yang B, Wang Q, Lin Q. A MEMS viscometric sensor for continuous glucose monitoring. J Micromech Microeng. 2007;17(12):2528–37.
37. Dalsin JL, Messersmith PB. Bioinspired antifouling polymers. Mater Today. 2005;8(9):38–46.
38. Kitano H, Kuwayama M, Kanayama N, Ohno K. Interfacial recognition of sugars by novel boronic acid-carrying amphiphiles prepared with a lipophilic radical initiator. Langmuir. 1998;14(1):165–70.
39. Hall DG, ed. Boronic acids: preparation and applications in organic synthesis and medicine. Weinheim: Wiley-VCH; 2005.
40. Yang X, Lee MC, Sartain F, Pan X, Lowe CR. Designed boronate ligands for glucose-selective holographic sensors. Chemistry. 2006;12(33):8491–7.
41. Roy D, Cambre JN, Sumerlin BS. Triply-responsive boronic acid block copolymers: solution self-assembly induced by changes in temperature, pH, or sugar concentration. Chem Commun (Camb). 2009;(16):2106–8.
42. Albarghouthi MN, Stein TM, Barron AE. Poly-*N*-hydroxyethyl-acrylamide as a novel, adsorbed coating for protein separation by capillary electrophoresis. Electrophoresis. 2003;24(7-8):1166–75.
43. Keenan DB, Cartaya R, Mastrototaro JJ. Accuracy of a new real-time continuous glucose monitoring algorithm. J Diabetes Sci Technol. 2010;4(1):111–8.
44. Albarghouthi MN, Buchholz BA, Huiberts PJ, Stein TM, Barron AE. Poly-N-hydroxyethylacrylamide (polyDuramide): a novel, hydrophilic, self-coating polymer matrix for DNA sequencing by capillary electrophoresis. Electrophoresis. 2002;23(10):1429–40.
45. Levine HI, Mark EH, Fiel RJ. Small-angle light scattering characteristics of pyran copolymer-divalent cation coacervates. J Colloid Interface Sci. 1978;63(2):242–50.