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ORIGINAL ARTICLES

Development of Novel Glucose Sensing Fluids with Potential Application to Microelectromechanical Systems-Based Continuous Glucose Monitoring

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Abstract

Background:

We have previously presented a microelectromechanical systems (MEMS) viscometric sensor for continuous glucose monitoring. The sensing fluid used therein was based on protein concanavalin A, which is known to have significant drawbacks, such as immunotoxicity and instability. To address this issue, a stable, biocompatible polymeric sensing fluid has been developed.

Methods:

In the polymeric sensing system, glucose reversibly formed strong ester bonds with the phenylboronic acid moiety on the poly(acrylamide-*ran*-3-acrylamidophenylboronic acid) (PAA-*ran*-PAAPBA) polymer backbone, resulting in cross-linking of the copolymers and an increase in the solution viscosity. The copolymers were synthesized via classic free radical copolymerization processes. The viscosity of the PAA-*ran*-PAAPBA, dissolved in phosphate-buffered saline buffer and in the presence of glucose at physiologically relevant concentrations, was measured by an Ubbelodhe viscometer and a prototype MEMS viscometric device.

Results:

Experimental results showed that the polymer molecular weight and composition depended on the solvent quantity, while the sensing fluid viscosity was determined by the polymer molecular weight and percentage composition of PAAPBA. The study of the temperature effect on viscosity showed that the polymer sensed glucose effectively under physiological conditions, although the high temperature lowered its sensitivity. Through proper adjustment of these parameters, a distinctive viscosity increase was observed when the glucose concentration increased from 0 to 450 mg/dl, which was detectable by our prototype MEMS device.

Conclusions:

We have successfully developed a stable, biocompatible polymeric system for the sensitive detection of glucose. MEMS experiments demonstrated that the sensing fluid was able to sense glucose at different concentrations. This sensing system can potentially enable highly reliable, continuous monitoring of glucose in interstitial fluid from subcutaneous tissue.

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Abbreviations: (AAPBA) *N*-3-acrylamidophenylboronic acid, (AIBN) 2,2′-azodiisobutyronitrile, (CGM) continuous glucose monitoring, (^{13}C NMR) carbon nuclear magnetic resonance, (Con A) concanavalin A, (DMSO) dimethyl sulfoxide, (^{1}H NMR) proton nuclear magnetic resonance, (ISF) interstitial fluid, (MEMS) microelectromechanical systems, (M_w) molecular weights, (NPAA) *N*-phenylacrylamide, (PAA-*ran*-PAAPBA) poly(acrylamide-*ran*-3-acrylamidophenylboronic acid), (PAA-*ran*-PNPAA) poly(acrylamide-*ran*-*N*-phenylacrylamide), (PBA) 3-aminophenylboronic acid, (PBS) phosphate-buffered saline

Keywords: affinity biosensors, boronic acid, copolymer, glucose sensing, MEMS

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Introduction

Diabetes is a metabolic disease characterized by persistent hyperglycemia (high blood sugar levels). Close monitoring of diabetes treatment with repetitive daily blood glucose measurements allows timely identification and correction of problematic blood sugar patterns. Continuous glucose monitoring (CGM) allows most timely detection of abnormal glucose levels and enables active intervention by taking carbohydrates or injecting insulin, which has been demonstrated to reduce the risk of diabetes-related complications.[1] Glucose sensors that are based on affinity-binding principles afford excellent stability and are less susceptible to biofouling when implanted subcutaneously. Thus, affinity CGM devices are attractive for long-term glucose monitoring applications. Currently, affinity glucose sensors are mostly based on the reversible binding of glucose to concanavalin A (Con A), a glucose-specific lectin. Such a 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] For example, Con A-based affinity glucose sensors have been fabricated using a solution of Con A and fluorescently labeled dextran. Glucose binding to Con A was detected via the resulting fluorescence change. This approach has been investigated further *in vitro* and *in vivo*, and there are active efforts in using this approach to optimized fluorescence-based sensors.[2,5–17]

We have previously developed a microelectromechanical systems (MEMS) viscometric sensor that was aimed at ultimately leading to a subcutaneously implantable device for CGM (**Figure 1a**).[18] The device used a vibrating microcantilever, which was situated in a microchamber and separated from the environment by a semipermeable membrane. Using a solution of dextran and Con A as the sensing fluid, the device measured the glucose concentration by detecting viscosity changes induced by the glucose–Con A binding through measurement of the cantilever's vibration parameters. While the device represents a first step toward a miniaturized, implantable MEMS sensor capable of long-term stable operation, there

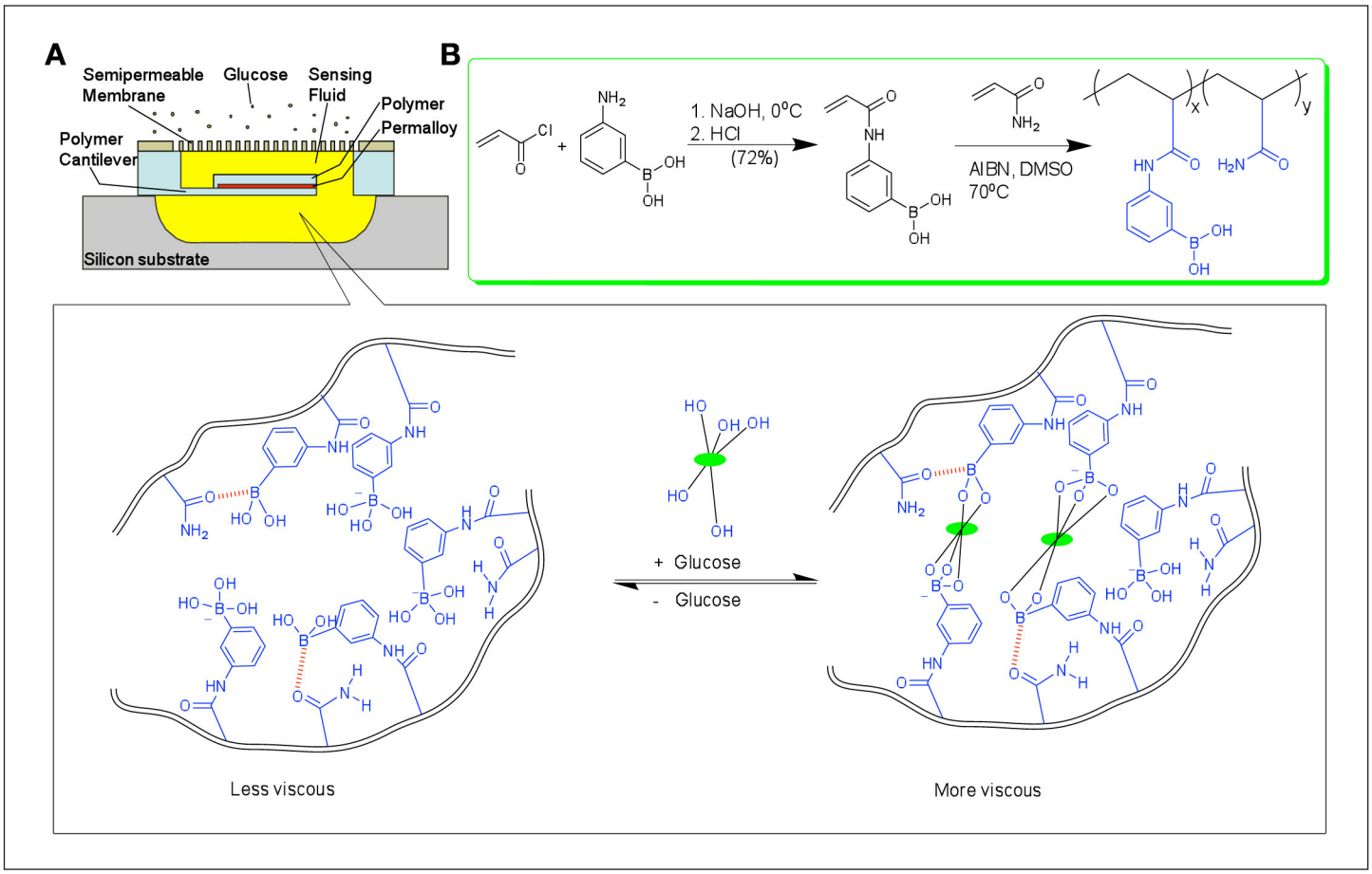

Figure 1. (a) Schematic illustration of the MEMS viscometric device design and its sensing mechanism. **(b)** Synthesis route of PAA-*ran*-PAAPBA.

are significant safety concerns with the toxicological properties of Con A. For instance, Con A is known to stimulate enhanced immunogenicity[19,20] and to induce antigen-specific cellular cytotoxicity.[21,22] The stability of Con A is also a concern. Although native dimeric Con A is fairly stable, removal of metal ions (Ca^{2+} and Mn^{2+}) will lower its stability considerably.[23] To address these concerns, new biocompatible sensing liquids for viscometric glucose sensing are highly desired.

It is well known that boronic acid binds reversibly to diols to form a cyclic boronate ester in aqueous media.[24] In general, the boronic acid is a biocompatible functional group with low cytotoxicity and low immunogenicity.[25] Thus, a variety of glucose sensors have been developed using boronic acid derivatives through different sensing mechanisms.[26–33] 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. This article reports on the development of a stable, biocompatible boronic acid-based polymer whose viscosity is specifically glucose dependent. In our design, the polymeric sensing fluid consists of poly(acrylamide-*ran*-3-acrylamidophenylboronic acid) (PAA-*ran*-PAAPBA) and physiological phosphate-buffered saline (PBS) at pH 7.4. The introduction of biocompatible hydrophilic PAA segments can improve the water solubility of the copolymer,[34] 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.[35,36]

While this article is focused on the sensing fluid development, it also presents preliminary measurement results using a prototype MEMS viscometric sensor.

Experimental Methods

Materials

3-Aminophenylboronic acid (PBA) was purchased from Oakwood Products, Inc. SnakeSkin™ pleated dialysis tubings (MWCO 3500) were purchased from Pierce Biotechnology, Inc. The Ubbelohde viscometer was obtained from Cannon® Instrument Company. Thermo Scientific HyClone Classical Powdered Hams F12 and fetal calf serum, D-(+)-fructose, and D-(+)-galactose were purchased from Thermo Fisher Scientific Inc. Dimethyl sulfoxide (DMSO)-d_6 and $CDCl_3$ were purchased from Cambridge Isotope Laboratories, Inc. All other reagents, including D-(+)-glucose, sodium azide, potassium phosphate monobasic, and potassium phosphate dibasic, were purchased from Sigma-Aldrich, Inc. Nanopure water was purified by the Milli-Q Ultrapure system from Millipore Corporation.

Preparation of Monomer N-3-Acrylamidophenylboronic acid (AAPBA)

Monomer AAPBA was synthesized adopting similar conditions as from Ivanov and colleagues.[37] PBA (5 grams, 36.5 mmol) was dissolved in a NaOH solution (2 *M*, 73 ml, 146 mmol) at 0°C. Cold acryloyl chloride (5.9 ml, 73 mmol) was added dropwisely to the vigorously stirred mixture over 15 minutes. A HCl solution (1 *M*) was added slowly to the reaction mixture until the pH reached 1.0. Many white solids precipitated, which were filtered, washed by cold water. The filtrate was extracted with ethyl acetate three times. The organic phase was washed with brine and evaporated to give off-white solids, which were combined with the aforementioned precipitates. Recrystallization in H_2O afforded 5.0 grams of off-white AAPBA crystals (yield: 72%). Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Mercury VX-300 spectrometer (Varian, USA) using DMSO-d_6 and $CDCl_3$ as solvents. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 10.06 (s, 1H, O = CNH), 8.01 (s, 2H, B-OH), 7.87 (s, 2H, Ar-H), 7.81 (d, J = 8.1 Hz, 1H; Ar-H), 7.49 (d, J = 7.2 Hz, 1H; Ar-H), 7.27 (t, J_1 = 7.5 Hz, J_2 = 7.8 Hz, 1H; Ar-H), 6.44 (dd, J_1 = 16.8 Hz, J_2 = 9.9 Hz, 1H; C = CHC = O), 6.23 (dd, J_1 = 17.1 Hz, J_2 = 2.1 Hz, 1H, C = CH_2), 5.72 (dd, J_1 = 9.9 Hz, J_2 = 2.1 Hz, 1H; C = CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 163.8, 138.8, 135.6, 132.7, 130.0, 128.4, 127.3, 126.0, 122.0. Control monomer *N*-phenylacrylamide (NPAA) was prepared as reported with a similar yield.[38] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, J_1 = 8.1 Hz, 2H; ArH), 7.51 (s, 1H, O = CNH), 7.37 (d, J = 1.8 Hz, 1H, Ar-H), 7.32 (t, J_1 = 6.6 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 7.13 (t, J_1 = 7.5 Hz, J_2 = 7.2 Hz, 1H; Ar-H), 6.44 (dd, J_1 = 16.8 Hz, J_2 = 1.5 Hz, 1H; C = CH_2), 6.24 (dd, J_1 = 16.8 Hz, J_2 = 10.2 Hz, 1H; C = CHC = O), 5.78 (dd, J_1 = 10.5 Hz, J_2 = 1.5 Hz, 1H; C = CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 164.4, 138.1, 131.6, 129.2, 127.9, 124.8, 120.6.

Preparation of PAA-ran-PAAPBA Copolymer

A typical free radical polymerization was conducted as follows: acrylamide (3.72 grams, 52.39 mmol), AAPBA (0.20 gram, 1.05 mmol), and 2,2′-azodiisobutyronitrile (AIBN) (21.5 mg, 0.13 mmol) were dissolved in DMSO. The mixture was bubbled by nitrogen for 30 minutes and subjected to a 70°C oil bath for 24 hours. After being cooled down to room temperature, the gel was subjected to dialysis against nanopure water for 24 hours.

The aqueous phase was precipitated by acetone and dried in a vacuum oven to yield 2.56 grams (65%) of white solids. A series of polymers with different percent compositions were prepared and characterized by ^{1}H NMR and viscometry.[39] ^{1}H NMR (300 MHz, D_2O): δ = 7.41 (bm, 4H; ArH), 2.06 (bm, 1H, O = CCH), 1.50 (bm, 2H, $-CH_2-$). Control polymer polyacrylamide-*ran*-*N*-phenylacrylamide (PAA-*ran*-PNPAA) was prepared and characterized similarly. ^{1}H NMR (300 MHz, D_2O): δ = 7.30 (bm, 5H; ArH), 2.07 (bm, 1H, O = CCH–), 1.50 (bm, 2H, $-CH_2-$). The viscosities of the copolymers were measured by the Ubbelohde viscometer in 0.12 *M* NaCl, pH 6.0, at 25°C.[39] According to the formula for polyacrylamide, the weight-averaged molecular weights (M_w) of PAA-*ran*-PAAPBA polymers were calculated from their intrinsic viscosities:

$$[\eta] = 5.31 \times 10^{-3} \times M_w^{0.79}$$

Experimental results are summarized in **Table 1**.

Microelectromechanical Systems Experiments

A prototype MEMS device was used to measure glucose-induced viscosity changes of the PAA-*ran*-PAAPBA sensing fluid. The device had a design similar to that shown in **Figure 1a** and is described in detail elsewhere.[18] The cantilever was a patterned Parylene thin film (250 × 250 × 7 μm^3 in dimension) anchored on a silicon substrate at one end and was suspended over a cavity, which, with an approximate average height of 250 μm, was etched isotropically into the silicon. A permalloy thin film (250 × 250 × 7 μm^3 in dimension) was electroplated on the cantilever at its free end. A polydimethylsiloxane microchamber 500 μm in height was formed above the cantilever. The chamber was filled with a PAA-*ran*-PAAPBA solution in which glucose was present at various concentrations. The cantilever, which was immersed in the solution, vibrated under the remote excitation from a home-made solenoid (2000 turns of a 250-μm-diameter copper wire on a 2-cm-diameter plastic core), which, under a driving voltage of 5 VRMS, produced a magnetic field strength of about 500 A/m perpendicular to the cantilever surface. The cantilever deflection was measured by an optical setup, in which a laser beam was directed onto the cantilever surface and the reflected light was measured by a photodetector connected to a lock-in amplifier.[18] The optical technique allowed proof-of-concept experimental data to be conveniently obtained; it will, however, be replaced in the future by transduction (e.g., piezoresistive or capacitive) methods that are more amenable to subcutaneously implantable applications.

Results and Discussions

A series of copolymers PAA-*ran*-PAAPBA (hereafter referred to as the polymer) were synthesized through classic free radical solution polymerization of acrylamide and AAPBA. Due to the possible binding between boronic acid and polar stationary phase-like silica of aqueous gel permeation chromatography, their weight-averaged molecular weights were characterized via common viscometry using Mark–Houwink parameters for polyacrylamide, because they were polyacrylamide analogs, by their intrinsic viscosity obtained under similar conditions used by Kuzimenkova and colleagues.[39] The final percent composition of the PAAPBA segment could be determined by ^{1}H NMR through the integration ratio of the aromatic protons to methylene and methine protons, which was fairly consistent with the initial loading molar ratio before polymerization (**Table 1**).

A variety of free radical polymerization conditions were tested, among which we discovered that the polymer

Table 1.
Characteristics of Polymers Prepared in DMSO at 70°C

Polymer[a]	Component molar ratio (AA/AAPBA/AIBN)	DMSO (ml)	Yield (%)	M_w[c]	PAAPBA% (theoretical)	PAAPBA%[d]
1	100/2/0.25	25	65	176,800	2.0%	2.3%
2	100/5/0.25	25	30	170,700	4.8%	5.0%
3	100/5/0.25[b]	25	44	214,200	0%	0%
4	100/2/0.25	35	82	129,200	2.0%	1.9%
5	100/5/0.25	35	64	171,100	4.8%	4.3%
6	100/8/0.25	35	33	71,700	7.4%	7.3%

[a] Polymers **1–6** are PAA-*ran*-PAAPBA, except **3**, which is the control polymer PAA-*ran*-PNPAA.
[b] Polymerization comonomer is AAPBA, except for polymer **3**, which is NPAA.
[c] Molecular weight was measured by viscometry.
[d] Percent composition was calculated by the integration ratio using ^{1}H NMR spectroscopy.

molecular weight was not under direct control by the molar ratio of initiator to monomers (**Table 1**). It was observed that using a 0.25/100 molar ratio of the initiator to acrylamide gave the best results with reproducible copolymer composition and higher molecular weight. Both solvent and AAPBA loading percentages seemed to play major roles in the polymerization. In general, less solvent (i.e., higher concentration) led to a higher polymerization rate and a larger polymer molecular weight. For example, 25 ml DMSO led to polymer **1** with a molecular weight of 176,800, whereas 35 ml DMSO led to polymer **4** with a molecular weight of 129,200. Other experiments showed that less DMSO (e.g., 15 ml) yielded insoluble polymer gels, which may have resulted from self-accelerated polymerization as a consequence of the high monomer concentrations, whereas 80 ml DMSO led to little product. However, polymer **5** was a result of an exception. However, only at a high AAPBA loading ratio did it seem to make polymerization difficult. A high AAPBA loading of an 8/100 molar ratio of AAPBA to acrylamide in the monomer mixture gave less product and a small polymer as polymer **6**. Above that loading ratio, polymerization only gave small oligomers, which were difficult to dissolve in PBS buffer after being lyophilized from a dialyzed aqueous solution, likely because of the low solubility of the high PAAPBA content.

All the viscosity experiments by capillary viscometer were conducted at room temperature around 25°C, except those for the temperature effect study in a water bath. After the polymer was dissolved in PBS (pH 7.4, 150 m*M* NaCl, 0.05% NaN_3), which mimics the physiological solution conditions, the polymer solution was loaded into the viscometer, followed by the addition of different amounts of glucose for varying glucose concentrations. The fluid kinematic viscosity obtained by a conventional Ubbelohde capillary viscometer was converted to viscosity because the polymer solution density was approximately the same as water. Because the main focus of our research was on the trend of viscosity change of the polymer in response to different glucose concentrations, the slight viscosity variations due to fluctuations of room temperature were neglected. In order to make sure of the accuracy of the fluid viscosity response, the sensing fluid was well mixed by bubbling through the middle size pore 10 times and then three measurements were taken for each data point. The viscosity values became steady within the short time of bubbling (less than 1 minute), which showed little variation even after hours, whose errors were all within the 2% range of the average value, suggesting that the system reached an equilibrium state quickly. It was reported that the time to reach equilibrium for most small boronic acid molecules could be as short as 30 seconds.[27] Because of the steric hindrance and mobility of the polymers, our sensing fluid could be a little slower, however still within 1 minute, to reach equilibrium. In the MEMS, the time response is dictated primarily by the time required for glucose to permeate throughout the sensor volume. Neither conventional nor MEMS viscometers were optimized to minimize this time. In future work, we plan to optimize the MEMS sensor design such that the characteristic diffusion distance in the MEMS device is optimized to achieve a dramatic speedup of the time response.

We investigated the glucose binding in response to the composition of the polymer. A series of polymers, **1–6**, were synthesized, where 2, 5, and 8 to 100 molar ratios of AAPBA to AA were initiated by the same amount of initiator in 25 and 35 ml DMSO, respectively. We found that around 45 mg/ml of polymer solution employed in all experiments measured by a conventional viscometer was able to give strong enough viscosity that has fallen

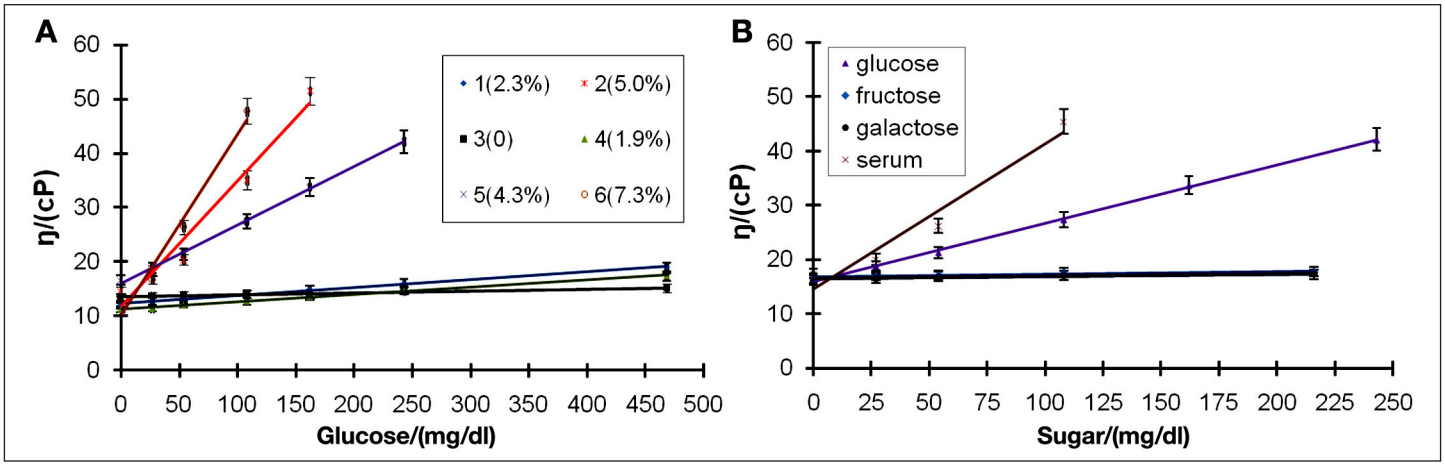

Figure 2. (a) Viscosity responses of polymers of different molecular weights and PAAPBA percentages (**1–3** with 176,800, 170,700, and 214,200; **4–6** with 129,200, 171,100, and 71,700). **(b)** Viscosity responses of polymer **5** to glucose, fructose, and galactose in PBS solution and glucose in dialyzed serum in PBS solution.

into the detecting range from 8.7 to 43.4 centipoises of our MEMS device,[18] beyond which we stopped viscosity measurements considering the accuracy of the viscometer. As shown in **Figure 2a**, polymers **1** and **4** of about 2% PAAPBA give similar responses to glucose from 0 to 468 mg/dl. Their viscosity change trend lines increase almost parallel to each other, whereas polymer **1** shows high viscosity because the PAAPBA percentage and the molecular weight of polymer **1** are higher than those of polymer **4**. Comparably, with about 5% PAAPBA moiety, polymers **2** and **5** show that their responses to glucose are dramatically stronger than those of polymers **1** and **4**. This suggests that the fluid viscosity is enhanced significantly when the PAAPBA percentage increases, as a higher PAAPBA percentage means more cross-linking spots along the polymer backbone. In the same glucose range, control polymer **3** without the phenylboronic acid group (using NPAA instead of AAPBA as the monomer) shows no obvious change, although its viscosity of the blank solution is high because of its high molecular weight. Polymer **6** shows lower viscosity than polymer **5** at glucose concentrations below 16 mg/dl because its molecular weight (71,700) is much lower than that of polymer **5** (171,100). When the glucose concentration of **6** is higher than 16 mg/dl, its viscosity outruns that of polymer **5** significantly. This result confirms that both molecular weight and PAAPBA percentage contribute to the sensitivity of the affinity sensing fluid to glucose.

Our future goal is to minimize and implant the MEMS affinity device under the skin and test the interstitial fluid (ISF) glucose concentration. Blood plasma is highly variable in the relative and absolute amounts of endogenous and exogenous solutes, many of which affect the strengths of the hydrogen bonds by competing with boronic acid. To overcome the most fundamental obstacle to the promised utility, the magnitude of this potentiated effect on glucose specificity was evaluated by sensing the possible residue sugars such as fructose and galactose in blood. This sensing fluid showed surprisingly high selectivity over them. As seen in **Figure 2b**, from 0 to 216 mg/dl, glucose caused a significant increase of viscosity by about 22.4 centipoises, whereas fructose and galactose increased by 1.3 and 1.1 centipoises, respectively. This dramatic difference indicated that although the chemical compositions were the same, the conformation difference of sugars played a key role in affinity binding. Due to the availability of sugar-free plasma, cell culture medium made of F12 with fetal calf serum for NIH 3T3 cell was dialyzed by a 3500-dalton MWCO dialysis tube against PBS to remove the sugars in order to mimic it. The addition of glucose caused a more acute response in serum than in PBS, indicating that some glucose-binding lectins or glycoproteins can enhance the fluid viscosity. In order to circumvent this problem, we designed a 3500-dalton MWCO dialysis membrane as the interface between the sensing fluid and blood in our MEMS device (**Figure 1a**), which only allowed molecules smaller than 3500 daltons, for example, glucose, fructose, and galactose, to diffuse through, while the copolymer cannot defuse out. More *in vitro* and *in vivo* studies will be conducted in the near future.

It is well known that temperature has a strong influence on viscosity. A higher temperature usually causes higher molecule mobility, thus lowering polymer solution viscosity. In order to make sure that the sensing fluid is applicable *in vivo*, the temperature effect study was proposed. Glucose was added to the solution of polymer **5** to make a glucose concentration of 54 mg/dl. In **Figure 3a**, the sensing fluid viscosity decreased almost linearly as temperature increased. As the body temperature

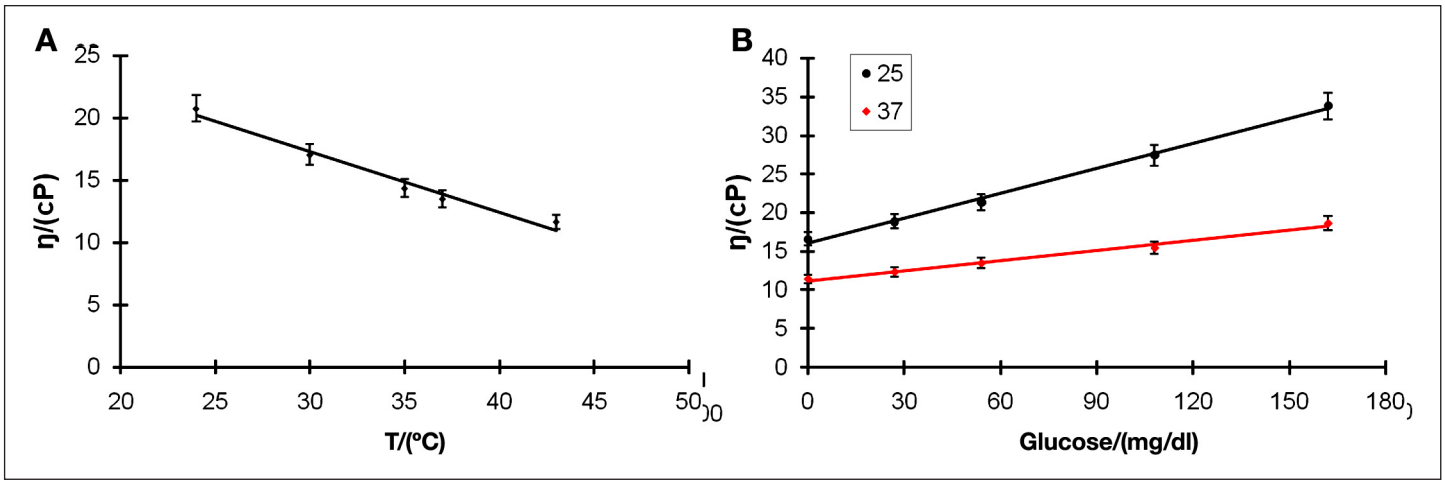

Figure 3. (a) Viscosity responses of polymer **5** at a 54-mg/dl glucose concentration to temperatures. **(b)** Comparison of viscosity responses of polymer **5** to glucose at 25 and 37°C.

fluctuates under some certain circumstances, for example, fever or surgery, the reading of the viscosity should be calibrated. At 43°C, its viscosity at 11.7 centipoises was even lower than 16.6 centipoises, which was the viscosity of blank fluid detected at 25°C. These phenomena may have resulted from dissociating of the polymer glucose cross-linked complex, high mobility of the cross-linked network, or both. In order to clarify this question, we tested polymer **5** viscosity responses to glucose at the physiological temperature of 37°C. As seen in **Figure 3b**, at 37°C, the sensing fluid can still respond to a glucose concentration change, although its slope is lower than that at 25°C. During normal physiologically relevant glucose levels (72–90 mg/dl), the viscosity increased from 14.4 to 15.2 centipoises that can be detected by our MEMS device whose resolution would reach a 0.45-mg/dl glucose concentration.[18] This result showed that the polymer was still able to sense glucose effectively, despite high mobility.

We also found with ~2% of PAAPBA segment in the polymer, the sensing fluid was able to give a strong signal that could be measured by our MEMS device. As shown in **Figure 1**, the MEMS affinity glucose sensor is based on a cantilever situated inside a microchamber. The cantilever is a polymer thin film anchored on the substrate at one end and is suspended over a cavity. Permalloy thin film strips are deposited on the cantilever at its free end and are covered with a thick reinforcement polymer layer to prevent curling of the cantilever because of the intrinsic stress mismatch between the polymer and metal thin films. Therefore, to demonstrate the applicability of the PAAPBA copolymer to MEMS-based affinity glucose sensing, the microchamber formed between the substrate and a 3500-dalton MWCO polymer membrane was filled with a solution of the PAAPBA copolymer. During operation, the cantilever was set in vibration by a remotely applied magnetic field, which acted on the permalloy strips. The viscosity change, caused by the interaction of glucose that permeated into and out of the chamber with the solution, altered the characteristics of the cantilever vibration, which can be measured to obtain the glucose concentration. In other words, the change of glucose concentration in the sensing fluid changed the viscosity of the sensing fluid and consequently altered the resistance of the sensing fluid applied to the cantilever vibration whose amplitude and frequency detected by the reflected light on the cantilever area was changed in the end.

The measured frequency response of the MEMS cantilever is shown in **Figure 4**, where the experimental data points are connected by straight line segments to guide the eye. It can be seen overall that the vibration amplitudes (**Figure 4a**) decreased with glucose concentrations. This agrees with the increased viscosity of the copolymer solution and viscous damping at higher glucose concentrations due to the increased media resistance caused by the cross-linking of boronic acid by glucose. A range of frequencies existed, approximately up to 60 Hz, over which the vibration amplitude changed most significantly with the glucose concentration. As the glucose concentration varied from 0 to 450 mg/dl, the resonance peak amplitude of the cantilever vibration decreased from 1.9 to 1 mV, with the resonance frequency shifting from 29.4 to 27.2 Hz. However, at all glucose concentrations, the phase of the cantilever vibration

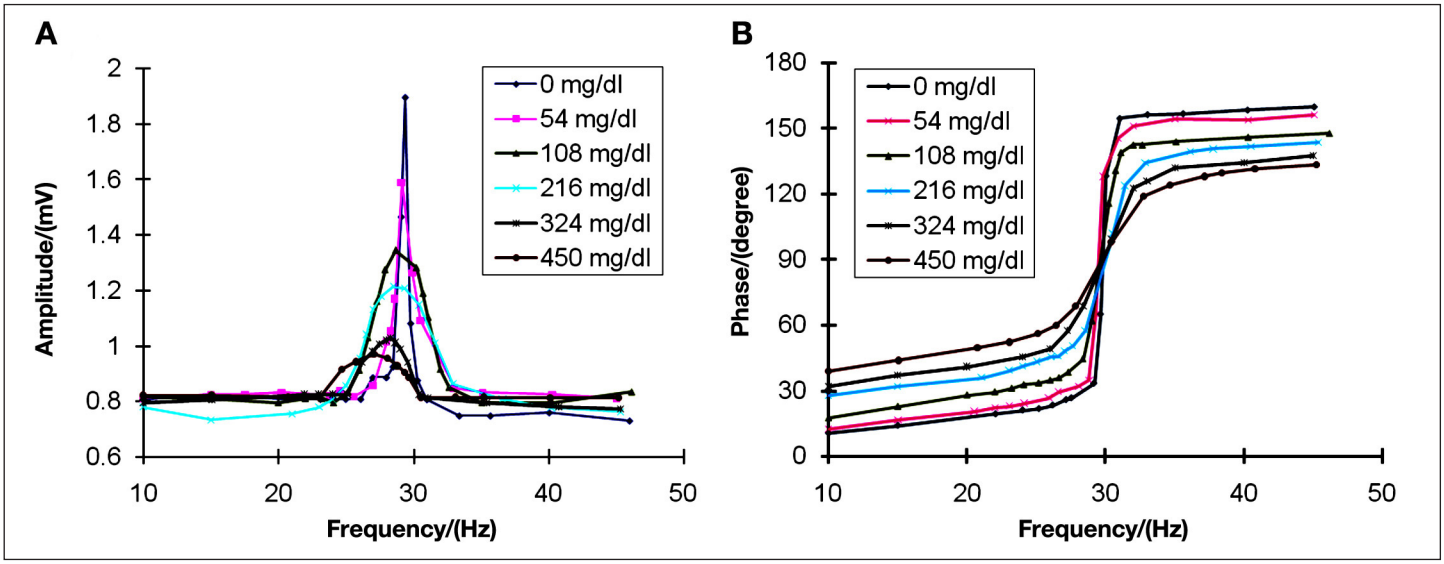

Figure 4. Vibration of a MEMS cantilever under sinusoidal magnetic excitation. The cantilever was immersed in a polymer solution of 1.9% PAAPBA with glucose present at various concentrations: **(a)** the amplitude and **(b)** phase of the cantilever tip deflection as a function of frequency.

(**Figure 4b**) transitioned from 0° at low frequencies to 180° at sufficiently high frequencies. This transition became increasingly less steep as the glucose concentration increased, which was consistent with the increased damping at higher glucose concentrations.

Conclusions

A novel glucose selective polymeric sensing fluid based on direct binding has been developed successfully. The polymers were easy to prepare through free radical polymerization, although their molecular weight and polydispersity were of little control. At the moment, they are not our major concern due to the dramatic viscosity change upon glucose binding. As a practical application, we hope to use an inexpensive, readily available polymer as exemplified. However, once we validate our sensing response from MEMS detection, if it requires better control over the polymer properties, controlled free radical polymerization of acrylamide in water such as reversible addition–fragmentation chain transfer[40] would be adopted to tailor our polymers, as the synthesis of a block copolymer of *N,N'*-dimetylacrylamide and AAPBA.[41,42]

This sensing fluid eliminated the usage of dextran, simplifying the sensing system and lowering the cost. The sensitivity of glucose was mainly dependent on the percent composition of the boronic acid monomer in copolymer, polymer molecular weight and temperature. Through proper adjustment of these parameters, the sensing fluid was able to detect and differentiate glucose from other blood residue sugars such as fructose and galactose. Unlike proteins, synthetic polymers are more stable for applications under physiological conditions and because they do not require any activation metal ions (unlike Con A), they can be used under different physiological environments.

Our MEMS prototype device was not equipped with temperature control for simplicity; however, an effort to integrate this capability is underway. Preliminary observations from this effort suggested that the device should be equally effective when operated at physiological temperatures. Temperature compensation is an important issue in practical applications and will be addressed in the future, possibly by incorporating differential measurements. Our final goal is to apply this fluid to MEMS affinity sensors and potentially enable highly reliable CGM in ISF.

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