ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

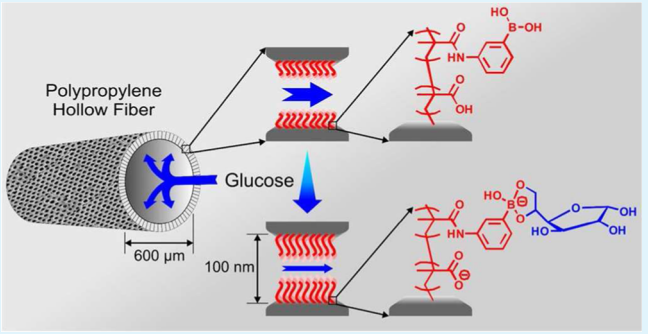
Glucose Monitoring Using a Polymer Brush Modified Polypropylene Hollow Fiber-based Hydraulic Flow Sensor

Nicolas Fortin and Harm-Anton Klok*

Institut des Matériaux and Institut des Sciences et Ingénierie Chimiques, Laboratoire des Polymères, École Polytechnique Fédérale de Lausanne (EPFL), Bâtiment MXD, Station 12, CH-1015 Lausanne, Switzerland

Supporting Information

ABSTRACT: Tight regulation of blood glucose levels of diabetic patients requires durable and robust continuous glucose sensing schemes. This manuscript reports the fabrication of ultrathin, phenylboronic acid (PBA) functionalized polymer brushes that swell upon glucose binding and which were integrated as the sensing interface in a new polypropylene hollow fiber (PPHF)-based hydraulic flow glucose sensor prototype. The polymer brushes were prepared via surface-initiated atom transfer radical polymerization of sodium methacrylate followed by postpolymerization modification with 3-aminophenyl boronic acid. In a first series of experiments, the glucose-response of PBA-functionalized poly(methacrylic acid) (PMAA) brushes grafted from planar silicon surfaces was investigated by quartz crystal microbalance with dissipation (QCM-D) and atomic force microscopy (AFM) experiments. The QCM-D experiments revealed a more or less linear change of the frequency shift for glucose concentrations up to ∼10 mM and demonstrated that glucose binding was completely reversible for up to seven switching cycles. The AFM experiments indicated that glucose binding was accompanied by an increase in the film thickness of the PBA functionalized PMAA brushes. The PBA functionalized PMAA brushes were subsequently grafted from the surface of PPHF membranes. The hydraulic permeability of these porous fibers depends on the thickness and swelling of the PMAA brush coating. PBA functionalized brush-coated PPHFs showed a decrease in flux upon exposure to glucose, which is consistent with swelling of the brush coating. Because they avoid the use of enzymes and do not rely on an electrochemical transduction scheme, these PPHF-based hydraulic flow sensors could represent an interesting alternative class of continuous glucose sensors.



KEYWORDS: *polymer brushes, surface-initiated atom transfer radical polymerization, glucose sensor, phenylboronic acid, hydraulic permeability*

INTRODUCTION

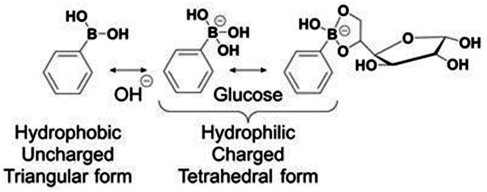
Diabetes mellitus is a chronic metabolic disease in which patients suffer from high blood glucose levels because either insulin production is inadequate or the body's cells do not respond properly to insulin, or both. Diabetes represents one of the largest health concerns of the 21st century, and the World Health Organization (WHO) has predicted a total of ∼300 million diabetes patients worldwide in 2025.[1] Everyday life of diabetic patients involves frequent blood glucose monitoring and multiple daily insulin injections. However, this treatment does not provide the tight glycemic regulation afforded by a healthy pancreas. As a consequence, there is an interest in devices that allow continuous monitoring of blood glucose levels.

The development of a glucose sensor requires strategies that enable to selectively bind this target molecule and transduce this molecular recognition event in a macroscopic and measurable output. The molecular strategies that have been developed for the recognition and binding of glucose can be divided in three main categories.[2,3] The first group is based on the enzyme glucose oxidase (GOx), which converts glucose to gluconic acid.[4−6] A second important class of glucose sensors use lectins, and in particular concanavalin A (Con A), to selectively bind glucose.[7,8] Although they have been widely investigated, these protein-based glucose detection schemes also have some drawbacks, which are primarily due to the limited stability of the proteins and the associated limitations with regards to sterilization, durability, and lifetime.[9−12] Synthetic ligands that are able to selectively bind glucose represent an attractive alternative and could provide the enhanced stability that is needed for long-term operation and sterilization. One such class of synthetic, glucose-binding ligands are phenylboronic acid (PBA) derivatives, which have been extensively investigated because of their stability, resistance to moist heat and their ability to be sterilized.[13,14] Scheme 1 shows the structure of PBA and illustrates the

Received: November 12, 2014
Accepted: February 13, 2015
Published: February 13, 2015

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

Scheme 1. Schematic Illustration of Glucose Binding by PBA



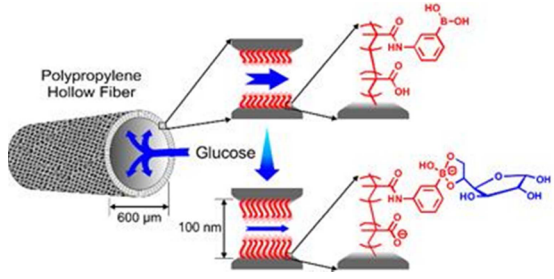
glucose binding process. In aqueous solution, PBA is present in two coexisting forms; an uncharged, hydrophobic form and a charged, more hydrophilic form.[15−17] Binding of glucose shifts the equilibrium toward the charged form, which can be used to trigger a variety of signal transduction schemes as will be discussed below.

A wide variety of PBA incorporating synthetic polymer-based systems has been developed that are able to selectively bind glucose and transduce this event, for example, into a colorimetric response or a change in conductivity or swelling properties. Kataoka et al. have prepared glucose-responsive hydrogels containing phenylboronic acid derivatives, which showed changes in their equilibrium swelling degree upon glucose binding.[18−22] Conceptually related PBA containing microgels that swell or shrink upon exposure to glucose have been reported by several other groups as well.[23−26] Asher et al. introduced the use of polymerized crystalline colloidal arrays for the colorimetric detection of glucose.[27,28] Arnold et al. developed a conductometric sensor based on a phenylboronic acid modified hydrogel, in which glucose binding resulted in a change in the ionic conductivity.[29] Liu et al. have developed multifunctional ratiometric probes based on poly(*N*-isopropylacrylamide-*co*-(*N*-acryloyl-3-aminophenylboronic acid)) microgels, which were modified with FRET dyes that allowed to monitor both glucose concentration and temperature.[30] In addition, several research groups have developed micellar systems based on PBA-functionalized polymers, which assemble or dissociate upon exposure to glucose.[31−35] Highly selective holographic glucose sensors with a very low lactate dependence that operate at pH 7.4 have been developed by Lowe et al.[14,15] Other reports have used surface-initiated polymerization techniques to modify surfaces with ultrathin glucose-responsive layers ("polymer brushes"). Surface-initiated polymerization results in chain-end tethered assemblies of polymer chains that are colloquially referred to as polymer brushes.[36] Ivanov et al., for example, have reported the synthesis of phenylboronic acid containing polymer brushes by surface-initiated free radical copolymerization from glass plates and capillary tubes.[37,38] These polymer brush films were investigated as substrates for carbohydrate-mediated cell adhesion. Zauscher et al. have reported the postpolymerization modification of poly(*N*-isopropylacrylamide)-*co*-poly(acrylic acid) brushes, which had been grafted from Si_3N_4 cantilevers and gold substrates using surface-initiated atom transfer radical polymerization (SI-ATRP), with 3-aminophenylboronic acid (APBA).[39] Glucose binding resulted in cantilever deflection, which was used as a read-out for glucose detection. PBA containing polymer brushes have also been grown from silicon surfaces using reversible addition−fragmentation chain transfer (RAFT) polymerization to produce QCM-D-based glucose sensors.[40]

Surface-initiated polymerization techniques possess a number of characteristics that make them attractive tools for the fabrication of glucose-sensitive thin polymer films. First of all, being an example of a bottom-up fabrication strategy, the use of surface-initiated polymerization methods is not limited to planar two-dimensional substrates, but also provides a means to uniformly and conformally coat complex three-dimensional and/or porous substrates, which can be challenging using conventional film forming techniques (e.g., spin coating) or grafting-onto protocols. Grafting onto approaches, which also result in polymer brush-type films, in contrast to surface-initiated polymerization strategies, however, are more restricted in the range of film thicknesses and the maximum film thicknesses that can be generated. Surface-initiated polymerization can generate densely grafted assemblies of chain-end attached polymer chains that are tethered at distances that are shorter than the radius of gyration of the corresponding polymer in solution and thus enforce a stretched chain conformation. Upon exposure to an appropriate external stimulus, these surface-grafted polymer chains can transition from an extended to a more collapsed state. These stimulus-induced conformational transitions of surface-tethered polymer chains provide attractive opportunities for the development of innovative thin polymer sensory interfaces.

Glucose binding induced conformational transitions in PBA-containing polymer brush films have been previously explored for the development of cantilever[39] and QCM-D-based glucose sensor formats.[40] When films are grafted from a suitable porous substrate, the changes in film thickness that accompany the swelling/collapse of a PBA-functionalized polymer brush film may also lead to changes in hydraulic permeability of the substrate, which potentially provides another strategy to capitalize upon the unique conformational properties of surface-grafted polymers for the development of novel glucose detection schemes. This study reports a first set of experiments that explores the feasibility of PBA-functionalized polymer brush films as the sensory interface for a prototypic hydraulic flow based glucose sensor. The prototype that is investigated in his study is based on a microporous polypropylene hollow fiber (PPHF) membrane that is modified with an ultrathin PBA containing polymer coating (Scheme 2). Glucose binding

Scheme 2. Schematic Illustration of the PBA Functionalized Polymer Brush Coated PPHF Based Glucose Sensor

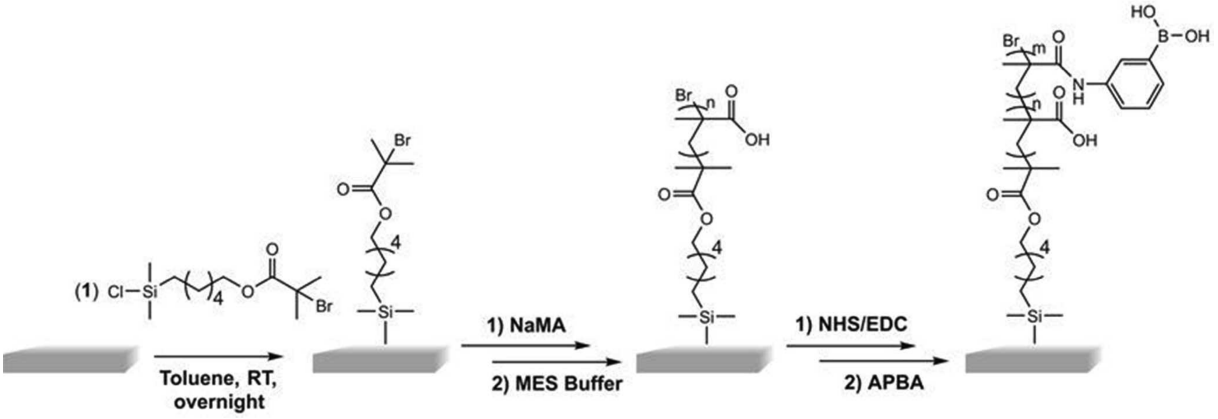


results in swelling of the PBA modified polymer brush coating, which induces a change in the hydraulic permeability of the fiber that can be used as a readout to monitor variations in glucose concentration. This new detection scheme could be potentially interesting for the development of continuous in vivo glucose detection systems and an alternative to, for example, electrochemical glucose sensors as it avoids the use of enzymes and does not rely on an electrochemical transduction pathway.

This report consists of two parts. The first part will discuss the preparation and glucose-responsiveness of PBA containing

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

Scheme 3. Synthesis of PBA Functionalized PMAA Brushes on SiO_x Substrates



polymer brushes grown from planar silicon oxide substrates. These brushes were prepared via SI-ATRP and subsequent postpolymerization modification with PBA. Producing these brushes first on planar silicon oxide substrates allowed to use quartz crystal microbalance with dissipation (QCM-D) monitoring and atomic force microscopy (AFM) experiments to study the glucose-responsiveness of these polymer coatings. In the second part, the synthetic strategy developed for the modification of the planar substrates is used to modify microporous PPHF membranes. The glucose responsiveness of the resulting fibers is subsequently investigated by hydraulic permeability measurements.

EXPERIMENTAL SECTION

Materials. PPHF membranes MICRODYN (MD 070 FP 1L; inner diameter, ~ 600 μm; pore size, 100 nm) were kindly provided by Microdyn Nadir. To remove additives, samples were Soxhlet extracted with acetone for 24 h. Thereafter, the specimens were dried under vacuum at room temperature for 24 h and subsequently stored under nitrogen until use. Silicon wafers were cut in 80 × 50 mm pieces, and silicon coated quartz crystal (QCM) chips were purchased from ICM (Oklahoma City, OK). Sodium methacrylate (99%) (NaMA), Cu(I)bromide (99.999%), Cu(II)bromide (99.999%), bromine (99.5%), 1,1,4,7,10,10hexamethyltriethylenetetramine (HMTETA, 97%), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC, 98%), *N*-hydroxysuccinimide (NHS, 98%) and 3-aminophenylboronic acid (APBA, 98%) were purchased from Aldrich and used as received. The atom transfer radical polymerization (ATRP) initiator, (6-(2-bromo-2-methyl)propionyloxy)-hexyldimethylchlorosilane ((**1**), Scheme 3), was synthesized as described previously.[41] All solvents were purified and dried using a PureSolv automated solvent purification system. Ultrahigh quality water with a resistance of 18.2 MΩ·cm (at 25 °C) was obtained from a Millipore Milli-Q gradient machine fitted with a 0.22 μm filter.

Methods. Polymer brushes were grown from silicon wafers and silicon coated QCM chips, which were cleaned using a microwave-induced oxygen plasma system (Diener electronic GmbH, Germany). Fourier transform infrared (FTIR) spectrophotometry analysis of modified PPHFs was carried out on a nitrogen purged Nicolet Magna-IR 560 spectrometer equipped with a Specac Golden Gate single reflection diamond ATR system. Planar brush modified substrates were analyzed with grazing angle attenuated total reflectance (ATR) FTIR spectroscopy using a nitrogen purged Nicolet 6700 FT-IR spectrometer equipped with a VariGATR grazing angle (ATR) accessory (Harrick Scientific Products, Inc., New York) fixing the incident angle at 65°. Each spectrum was acquired by collecting 64 scans at a resolution of 4 cm^{-1}. X-ray photoelectron spectroscopy (XPS) measurements were performed on an Axis Ultra system from Kratos Analytical with a conventional hemispheric analyzer and a monochromatic Al Kα (1486.6 eV) source. The X-ray source was run at 150 W and 10^{-9} mbar. The area analyzed was 700 × 350 μm at an angle of 90° relative to the substrate surface. The software CasaXPS was used to fit the XPS spectra peaks. Atomic force microscopy (AFM) was performed in tapping mode on a Veeco Multimode Nanoscope IIIa SPM controller (Digital Instruments, Santa Barbara, CA) using NSC36/AIBS cantilevers with a spring constant of 0.6 N/m. QCM measurements were performed at 25 °C with a Q-Sense E4 system (Q-Sense, Sweden) recording the third overtone. The setup used to characterize the hydraulic permeability of the PPHF membranes is illustrated in Figure S1 (Supporting Information). A piece of PPHF membrane (20 mm length) was glued to a 20 gauge needle at one end, while the other end was blocked. A feed tank containing the testing solution was connected to a compressed air line, which was used as a pressure source. The pressure can be adjusted between 1.010 and 1.500 bar and measured using a pressure sensor interfaced with a computer. An electronic balance, which was linked to a computer, was used to continuously weigh the permeate solution. The PPHF modified needle was mounted in the luer lock support and equilibrated for 30 min in the corresponding testing solution prior to any measurements. Mass flux measurements were performed at room temperature using a pressure of 1.300 bar. The applied pressure inside the closed system induces an increase in the liquid weight (the permeate solution) collected in the beaker. The mass flux is determined from the linear regression (slope) of the signal recorded. The hydraulic permeability of the PBA-modified PPHF membranes was evaluated using a 100 mM phosphate buffer at pH 9 with and without 100 mM glucose.

Procedures. *Immobilization of the ATRP Initiator on Planar Silicon Substrates.* First, the silicon wafers were sonicated for 5 min in acetone and then dried. The silicon substrates were then exposed to an oxygen plasma (180 W, 10 min) and kept overnight in the dark in a 10 mM solution of **1** in anhydrous toluene. Afterward, the slides were extensively rinsed with chloroform, dried under nitrogen and transferred to the appropriate reactors for the polymerizations. Silicon oxide coated QCM chips were modified in the same way but were not sonicated.

Photobromination of the PPHF Membranes. A piece of PPHF membrane (60 mm length) was introduced in a 5 × 150 mm (diameter × length) Pyrex glass tube, which was subsequently placed in a 250 mL Schlenk flask. The Schlenk flask with the PPHF membranes was left overnight under vacuum at room temperature. Thereafter, the Pyrex glass tube was sealed with a septum and purged with nitrogen for 5 min, and a droplet (~20 μL) of bromine was introduced with a syringe. After 5 min, when the bromine had vaporized, the tube was placed in front of a Hamamatsu LC6 high-pressure vapor mercury lamp, which was equipped with a condenser lens in order to obtain a uniform illumination of the fiber. The lamp was operating at 100% intensity and placed at a distance of 33 cm from the Pyrex tube to generate a spot with a diameter of 12 cm and light intensity of 67 mW·cm^{-2} (±5%) between 230 and 400 nm (33 mW·

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

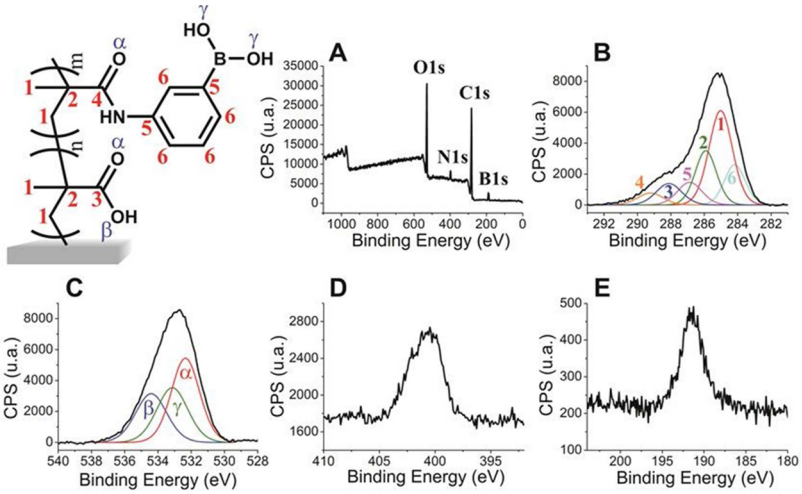


Figure 1. (A) XPS survey and (B) high-resolution C 1s, (C) O 1s, (D) N 1s, and (E) B 1s scans of a 105 nm thick PMAA brush postmodified with phenylboronic acid (the numbers and Greek letters refer to the different C and O atoms in the chemical structure of the PBA-functionalized PMAA brush that is included as an insert).

cm^{-2} between 320 and 400 nm). During irradiation, the Pyrex tube was rotated through one-third of a turn each 7 min to allow a uniform bromination of the substrate. After an irradiation time of 21 min, the light source was switched off, and the tube was purged with nitrogen for 60 min. After that, the samples were kept under vacuum for 24 h at room temperature to remove residual bromine.

Surface-Initiated Atom Transfer Radical Polymerization. SI-ATRP of NaMA was carried out following a procedure adapted from a protocol that was published earlier.[42] A reaction system consisting of NaMA, CuBr, $CuBr_2$, and HMTETA in the following molar ratios: 148:1:0.6:3.5 was used. First, two solutions containing the catalyst, respectively, monomer solution were prepared in two different Schlenk tubes. To prepare the catalyst solution, 141.45 mg (0.63 mmol) of $CuBr_2$, 1035 μL (3.8 mmol) of HMTETA, and 15.5 mL of Milli-Q water were added in a Schlenk tube, and the mixture was subsequently subjected to three freeze−pump−thaw cycles. Once the solution was degassed, 155.25 mg (1.1 mmol) of CuBr was added under nitrogen and allowed to dissolve. In a separate Schlenk tube, a solution of 16.05 g (162 mmol) NaMA and 34.5 mL of Milli-Q water was prepared. The mixtures were stirred until a homogeneous solution was obtained and then degassed by two freeze−pump−thaw cycles. After that, the catalyst solution was cannula transferred into the Schlenk tube containing the monomer solution. After 10 min, when the system had become homogeneous, the polymerization mixture was transferred under nitrogen, using a cannula, into a polymerization reactor containing the appropriate ATRP initiator modified substrates, and the reaction was allowed to proceed for the desired time at room temperature under nitrogen atmosphere. After polymerization, the substrates were thoroughly rinsed with deionized water and ethanol and then left for 1 h in a 10 mmol MES buffer solution at pH 5. Micropatterned polymer brushes for AFM studies were prepared from patterned ATRP initiator modified silicon wafers, which were obtained as previously described.[43]

Postpolymerization Modification Reactions. First, the carboxylic acid groups of the poly(methacrylic acid) (PMAA) brush modified substrates were activated by placing the samples in a solution of EDC (0.1 M) and NHS (0.2 M) in deionized water for 4 h and rinsing with ethanol. To introduce APBA, the NHS-activated PMAA brushes were immersed overnight in a 16.0 mg/mL solution of APBA in 10 mM phosphate buffer (pH 7.2). After that, the substrates were rinsed three times with water for 20 min each and finally dried under nitrogen.

RESULTS AND DISCUSSION

Synthesis and Characterization of PMAA−PBA Brushes on Planar Silicon Substrates. Prior to embarking on the modification of the PPHFs with the proposed glucose-sensitive brushes, the synthetic strategy toward these PBA modified PMAA brushes was first elaborated on planar silicon substrates as outlined in Scheme 3. PBA functionalized PMAA brushes were prepared both on planar silicon wafers and on silicon oxide coated QCM-D crystals. The former samples were used to study the kinetics (more precisely, the growth profiles) of the SI-ATRP process and for the chemical analysis of the postpolymerization modification reaction, whereas the QCM-D substrates were used to investigate the glucose responsiveness of the PBA functionalized polymer brushes.

The synthesis of the PMAA brushes starts with the immobilization of the ATRP initiator functionalized chlorosilane derivative (**1**) on the SiO_x substrate, followed by SI-ATRP of sodium methacrylate (NaMA) in water at room temperature and a final acidification step. Figure S2 (Supporting Information) illustrates the evolution of the dry film thickness of the PMAA brushes as a function of polymerization time as determined by AFM analysis on micropatterned substrates. The $CuBr/CuBr_2$/HMTETA catalytic system allows for a fast brush growth and PMAA brushes with thicknesses >200 nm are obtained in less than 30 min.[42] Postpolymerization modification of the PMAA brushes with 3-aminophenylboronic acid (APBA) was performed in aqueous solution at pH 7.2 and started with EDC/NHS activation of the carboxylic groups.[44] After that, the resulting NHS-activated polymer brushes were reacted with APBA. The introduction of the PBA groups was monitored with XPS. Figure 1 presents survey XPS scans as well as high resolution C 1s, N 1s and B 1s spectra of a 105 nm thick PBA postmodified PMAA brush. The corresponding spectra of a 100 nm thick, unmodified PMAA brush are shown in Figure S3 (Supporting Information). The elemental compositions of the different brush samples that can be obtained from the XPS analyses are summarized in Table S1 in the Supporting Information. The survey spectra reveal the presence of C 1s and O 1s signals for the PMAA brush whereas the survey spectrum of the PBA modified brush reveals C 1s, O 1s, N 1s and B 1s signals. For the PMAA brush, the high-resolution C 1s signal can be fitted with the expected peak area ratios using three model Gaussian/Lorentzian curves, which correspond to the aliphatic carbon atoms of the polymer backbone (C−C/C−H, 285.0 eV), the secondary carbons of the carboxylic groups (C−C═O, 285.9 eV) and the carbon

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

atoms of the carboxylic groups (O−C=O, 289.2 eV). The C 1s high resolution scan of the PBA modified PMAA brush is more complicated (Figure 1B). This is partly due to the presence of additional signals resulting from the PBA moiety and partly due to the fact that not every PMAA repeat unit in the brush is modified with a PBA group. The survey XPS scans of the PBA modified PMAA brushes, however, reveals two additional signals at ∼400 and ∼191 eV, which correspond to the N 1s and B 1s signals and reflect the successful introduction of the PBA groups. From the [B 1s]/[C 1s] XPS ratio it was estimated that 36% of the PMAA repeat units in the polymer brush were modified with a PBA moiety. The PBA modified PMAA grafted QCM-D crystals that were used for the experiments discussed in the next section were prepared using identical reaction conditions and thus are assumed to present the same amount of PBA moieties.

Glucose-Responsiveness of PBA Functionalized Brushes Grafted from Planar Silicon Substrates. The glucose-responsiveness of the PBA functionalized brushes was studied using the quartz crystal microbalance with dissipation monitoring (QCM-D) technique. As an example, Figure 2 compares changes in the third overtone of the resonance frequency (Δf) and the dissipation (ΔD) of a QCM-D crystal coated with an unmodified PMAA brush (d = 100 nm) with that of a QCM-D crystal coated with a PBA modified PMAA brush (d = 105 nm) upon exposure to glucose concentrations varying between 0.1 and 25 mmol/L at pH 9. The experiments were carried out by exposing polymer brush coated samples to pH 9 buffer and injecting glucose solutions of defined concentrations at specific times. The experiments were performed at pH 9, since the pK_a of PBA is 8.8 and the boronate form of PBA (Scheme 1) is favored to facilitate glucose binding.[16] To investigate the reversibility of glucose binding, after a certain time, the glucose solution was replaced again by pH 9 buffer. As illustrated in Figure 2A, the PMAA brush coated crystal only revealed a very minor drift in the resonance frequency shift (∼40 Hz) and the dissipation (∼ −6 × 10^{-6}) upon exposure to aqueous glucose solutions. Similar drifts have been observed in other QCM-D experiments with PMAA brushes[45] upon exposure to aqueous buffer solutions and are tentatively attributed to the slow stabilization of these polyelectrolyte polymer films in the different media. The PBA modified PMAA brush coated crystal, in contrast, shows a decrease in frequency and an increase in dissipation upon exposure to glucose. The intensities of the frequency and dissipation shifts decrease with deceasing glucose concentration to be almost undetectable at a glucose concentration of 0.1 mM. Note that the small drift that was observed for the unmodified PMAA brush is (almost) not visible for the PBA-functionalized brush as the putative polyelectrolyte effect is dominated by the PBA-mediated glucose binding. The changes in both the resonance frequency and dissipation indicate that the observed response is a combination of a mass loading effect on the polymer brush and a change in viscoelastic properties. The observed frequency shifts reflect glucose binding to the PBA functionalized brushes, whereas the increase in dissipation indicates a change from a rigid-collapsed polymer film to a more soft-swollen structure.

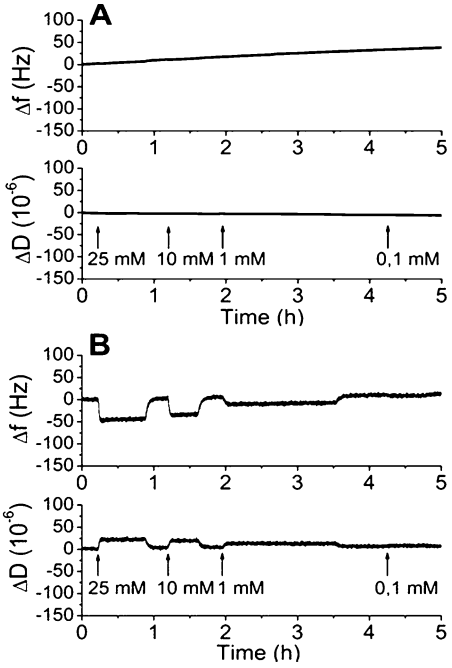


Figure 2. (Top) Changes in Δf and (bottom) ΔD upon exposure of a QCM-D chip coated with (A) a nonmodified PMAA brush (d = 100 nm) and (B) a PBA postmodified PMAA brush (d = 105 nm) to different glucose concentrations.

The QCM-D experiments summarized in Figure 2 also provide a first insight into the response kinetics of the PBA-functionalized polymer brush films. From the results in Figure 2, Figure S4 (Supporting Information) can be obtained, which plots the evolution of the normalized frequency shift (defined as $(F - F_0)/(F_{eq} - F_0)$) as a function of time for a 105 nm thick PBA-functionalized PMAA brush film upon exposure to 1−50 mM glucose solutions. Taking the time it takes for the QCM-D sensor to reach equilibrium as a measure for the response time, Figure S4 (Supporting Information) indicates response times that vary from ∼3 min for 50 mM to ∼6 min for 1 mM glucose solutions. It is interesting to compare the response times of these thin PBA-modified thin polymer films with those of macroscopic PBA-functionalized hydrogels. The response times of the thin PBA-containing films reported here compare very favorably with those of macroscopic hydrogels, which typically require more than 1 h to reach swelling equilibrium upon changing glucose concentration.[20−22] The much faster response kinetics of the polymer brush films is attributed to the shorter diffusion pathway for the glucose molecules through these films, as compared to bulk hydrogels. Along the same lines, Figure 2 also provides information on the response kinetics upon exposing a glucose-loaded PBA-functionalized PMAA brush to a pH 9 buffer solution that does not contains glucose (Supporting Information, Figure S5). Interestingly, the release kinetics are much slower (∼9 min) and independent of the glucose concentration that was used during the glucose binding cycle.

To study the effect of glucose binding on the swelling properties of the brushes, AFM experiments were carried out on micropatterned PBA-modified PMAA brushes that were grafted from planar silicon wafer substrates. Figure 3A plots the thicknesses determined from cross-sectional analysis of micro-patterned brushes, both in the "dry" state and in contact with pH 9 buffer with and without 25 mM glucose. These results were compared with the variation of frequency and dissipation observed under similar conditions on a PBA modified QCM chip (Figure 3B). The QCM-D results shown in Figure 3B differ from those in Figure 2 in that Figure 3B shows the

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

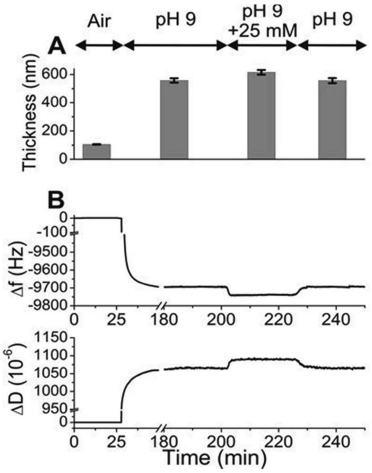


Figure 3. Variation of (A) film thickness and (B) Δf and ΔD of a 105 nm thick PBA postmodified PMAA brush upon exposure to pH 9 solutions with and without 25 mM glucose.

changes in Δf and ΔD starting with a dry brush. After 30 min, the dry brush is exposed to pH 9 PBS buffer and left to equilibrate for 30 min before starting the experiment. The dry PBA modified PMAA brush has a thickness of about 105 ± 1.7 nm. When placed in pH 9 buffer, in the absence of glucose, the polymer brush swells significantly to a thickness of 560 ± 16 nm. At these high pH values, the PBA modified PMAA chains transition from a collapsed and protonated state to a hydrated and deprotonated one, which results in swelling of the polymer film. Swelling of the PMAA-based brush film is accompanied by a large decrease of Δf of ~9.7 kHz, which reflects adsorption of water in the polymer brush, as well as an increase in ΔD of $\sim 1075 \times 10^{-6}$, which is indicative for a change from a more rigid to a softer (swollen) polymer film. Introduction of 25 mM glucose at pH 9 inside the QCM chamber results in a decrease of frequency of about 44 Hz and an increase of the dissipation of 26×10^{-6}. AFM cross-sectional analysis reveals that exposure to glucose results in a ~ 10% (~58 nm) increase in brush thickness. This observation is consistent with results by Zauscher et al., who recorded a 25% increase of thickness for a 140 nm thick PBA-functionalized poly((*N*-isopropylacrylamide)-*co*-(acrylic acid)) brush upon exposure to a 50 mM glucose solution.[39] The AFM thickness analysis in Figure 3A indicates that glucose binding by the PBA modified PMAA brushes is not just a mass-loading effect, but also results in swelling of the brush film, which is consistent with the observed changes in ΔD.

From the QCM-D results illustrated in Figure 2 and Figure 3, a response curve, which plots the change in Δf as a function of glucose concentration, can be obtained. Figure 4 shows that Δf increases in a relatively linear fashion up to a glucose concentration of ~10 mM and then gradually levels off at a glucose concentration of ~20 mM. This is a practically useful response range for a glucose sensor considering the fact that blood glucose levels can vary from ~5 mM for a healthy adult up to 11.1 mM for diabetic patients in case of hyperglycemia.[46] To assess the reversibility of glucose binding to the PBA functionalized PMAA brush coatings, we exposed a QCM chip modified with a 105 nm thick PBA functionalized PMAA brush alternatingly to a pH 9 buffer solution without or with 25 mM glucose at 25 °C. As illustrated by the data in Figure 5, the

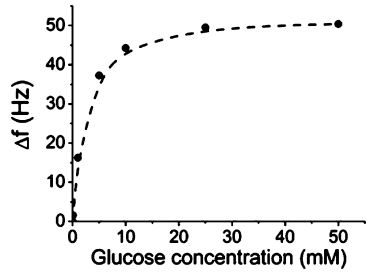


Figure 4. Shift in Δf for a 105 nm thick PBA-modified PMAA brush as a function of glucose concentration (dotted line serves as a guide to the eye).

response of the PBA functionalized brush was completely reversible for up to seven switching cycles.

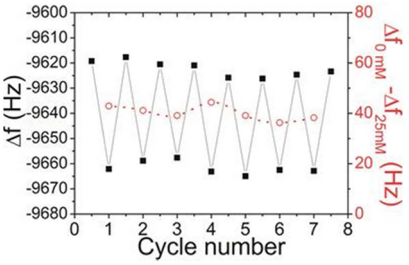


Figure 5. (Δf; ■) Frequency shift and frequency shift difference ($\Delta f_{0\ \mathrm{mM,glucose}} - \Delta f_{25\ \mathrm{mM,glucose}}$; ○) observed upon exposing a QCM chip modified with a PBA functionalized PMAA brush (d = 105 nm) alternatingly to pH 9 solutions without or with 25 mM glucose over seven switching cycles.

All the experiments discussed so far were carried out with pH 9 glucose solutions. This pH was selected because the pK_a of PBA is 8.8, and the boronate form of PBA (Scheme 1) is favored to facilitate glucose binding.[16] Ideally, the PBA functionalized polymer brushes should allow glucose monitoring at pH 7.4 so that they could be operated, for example, with fresh blood samples without the need for further sample preparation. The glucose response of the PBA functionalized PMAA brushes at pH 7.4 and pH 8 was evaluated in additional QCM-D experiments. Figure 6 compares the shift in Δf for a 105 nm thick PBA functionalized PMAA brush upon exposure to 25 mM glucose at pH 9, 8, and 7.4. As illustrated by the data in Figure 6, glucose binding at pH 8 and pH 7.4 still results in a shift in Δf, albeit almost 4 times smaller as compared to pH 9. This reflects the equilibrium between the neutral, trigonal form of PBA at low pH and the tetrahedral, ionized boronate form at

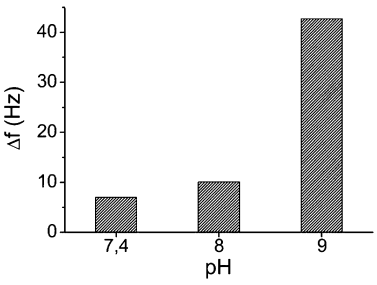
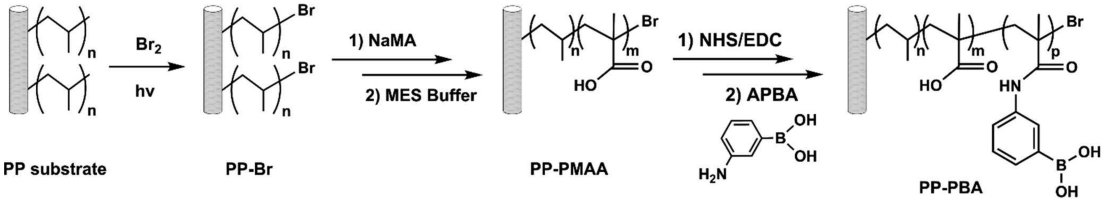


Figure 6. Frequency shift (Δf) measured for a 105 nm thick PBA-modified PMAA brush upon exposure to 25 mM glucose at three different pH values.

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

Scheme 4. Synthesis of PBA Functionalized PMAA Brushes on PPHF Substrates



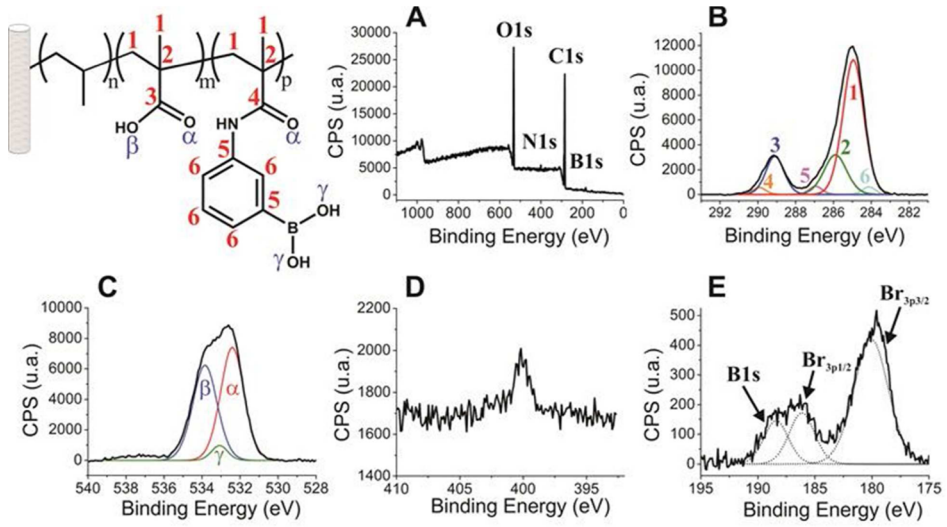


Figure 7. (A) XPS survey and (B) high-resolution C 1s, (C) O 1s, (D) N 1s, and (E) B 1s scans of a PBA functionalized PMAA brush modified PPHF. (Inset) Chemical structure of the PBA-functionalized PMAA brush; numbers and Greek letters refer to the different C and O atoms.

pH values higher than the pK_a. At pH 9, glucose has a high affinity with the tetrahedral boronate species to generate a 1:1 PBA−glucose complex. With decreasing pH, however, this equilibrium progressively shifts toward the hydrophobic, uncharged form with a concomitant decrease of the potential active sites available for the binding with the glucose.[16] While the results in Figure 6 indicate that the PBA-functionalized PMAA brushes in principle can be used to detect glucose at physiological pH, it is also obvious that (the pK_a of) PBA is not the ideal choice to analyze blood glucose because the observed frequency shifts at pH 7.4 are significantly smaller as compared to those at pH 9. The optimal pH operation window of the polymer brush films, however, may be shifted to pH 7.4 by incorporation of tertiary amine moieties in the brush or by using substituted PBA derivatives.[47−50]

The experiments discussed above were carried out with solutions that contained exclusively glucose. An important aspect that has not been mentioned so far is the potential interference of other component present in blood, since boronic acids are well-known to bind a variety of diols. While glucose dominates, blood also contains various other sugars, including e.g. fructose. Fructose has a much higher binding constant toward PBA as compared to glucose (560 M^{-1} versus 11 M^{-1} at pH 8.5),[16] yet is present at much lower concentrations in blood (typically around 10 μM).[51] In previous QCM-D experiments with PBA-containing polymer brush films that were prepared via surface RAFT polymerization, we demonstrated that the frequency shifts observed for these polymer brush films upon exposure to 5 mM glucose were not affected by the presence of 10 μM fructose as a competing diol.[40]

PBA Functionalized PMAA Brush Coated PPHF Based Glucose Sensors. Having developed a protocol for the preparation of PBA modified PMAA brushes and assessed the feasibility of these coatings to reversibly bind (and respond to) physiologically relevant glucose concentrations, the second part of this manuscript discusses the integration of this polymer brush platform into PPHFs with the aim to develop a prototype of the glucose sensor that was outlined in Scheme 2. The modification of the PPHF with the PBA functionalized PMAA brush coating is outlined in Scheme 4. The synthetic protocols are identical to those used for the modification of the planar silicon substrates, except for the first reaction step. Instead of using chlorosilane chemistry, we subjected the PPHFs to a photobromination treatment to introduce ATRP active groups.[52]

Photobromination of the PPHFs was monitored using XPS. Survey, C 1s, and Br 3d high-resolution scans are included in Figure S6 in the Supporting Information. Analysis of the elemental composition revealed a bromine content of 12.1%. The subsequent growth of the PMAA brushes from the PPHF substrate via SI-ATRP can be monitored by ATR-FTIR spectroscopy (Figure S7, Supporting Information). The gradual increase in intensity of the carboxylic acid carbonyl band at 1710 cm^{-1} reflects the increase in brush thickness as a function of polymerization time. The relatively linear increase in intensity of this FTIR band (after normalization with respect to the polypropylene C−C/C−H vibrations at 2917 cm^{-1}) reflects the ability of the SI-ATRP process to tune the film thickness of the PMAA brushes.

NHS activation of the PMAA brush modified PPHFs and the subsequent postpolymerization modification with PBA was performed using the same protocols that were discussed earlier

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

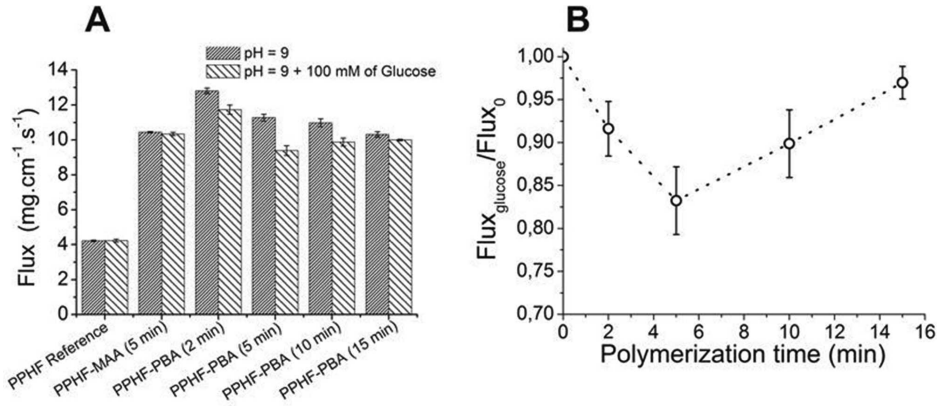


Figure 8. (A) Hydraulic permeability of an unmodified PPHF membrane, a PMAA brush modified PPHF, and various PBA functionalized PMAA brush modified PPHFs upon exposure to pH 9 aqueous solutions with and without 100 mM glucose; (B) relative flux (defined as the ratio between the flux measured with and without glucose in pH 9 PBS buffer solution, i.e., $flux_{pH\ 9\ buffer+100\ mM\ glucose}/flux_{pH\ 9\ buffer}$) as a function of the polymerization time that was used to grow the PMAA brush coating.

for the preparation of the planar silicon oxide supported brushes. Each of the reaction steps on the PPHF was followed using FTIR and XPS analysis. Figure S8 (Supporting Information) compares the ATR-FTIR spectra of an unmodified PPHF, a PMAA brush modified PPHF, an NHS activated PMAA brush modified PPHF, and a PBA functionalized PMAA brush coated PPHF (all obtained after a polymerization time of 10 min). Upon reaction of the PMAA brushes with NHS, the broad band at ~3100−3500 cm^{-1}, as well as the bands at ~1699 and ~1189 cm^{-1} in the FTIR spectrum of the unmodified PMAA brush, which correspond, respectively, to the O−H stretching, the C═O vibration, and the C−OH stretching, vanish (Figure S8, Supporting Information). At the same time, two characteristic bands at ~1801 and ~1757 cm^{-1} appear, which can be assigned, respectively, to the ester carbonyl stretching and the vibration of the succinimidyl carbonyls.[44,53] After the NHS activated PMAA brushes react with 3-aminophenylboronic acid for 24 h, the broad FTIR band above ~3300 cm^{-1} corresponding to the O−H stretch appears again, the two characteristic bands of the NHS groups (at ~1806 and ~1756 cm^{-1}) disappear, and various new bands between ~1629−1702 cm^{-1}, which are due to the monosubstituted amide groups appear, all indicating the successful introduction of the PBA moieties.

Figure 7 presents the XPS survey spectra as well as C 1s, O 1s, N 1s, and B 1s high-resolution scans of a PMAA brush prepared with a polymerization time of 10 min after NHS activation and postpolymerization modification with PBA. In agreement with the elemental composition of the PBA modified PMAA brushes, the survey spectrum shows the appearance of C 1s, O 1s, N 1s, and B 1s, as well as Br 3p and Br 3d signals. As illustrated in the high-resolution scan in Figure 7E, the B 1s signal, which reflects the PBA that is introduced in the PMAA brush film, partly overlaps with the Br 3p1/2 signal, which is due to residual bromine that has not been used to initiate the ATRP process. Due to the partial overlap of the Br 3p1/2 signal, the XPS [N 1s]/[C 1s] ratio was used to estimate the PBA content in the PMAA brush coatings. From the spectra that are shown in Figure 7 this resulted in a degree of modification of 12.5%. A series of experiments was performed in which the SI-ATRP reaction time was varied from 2 to 15 min, while the conditions of the PBA postpolymerization modification reaction were unchanged. These variations in the thickness of the PMAA brush, however, were not found to lead to significant changes in the PBA content of the brushes (Table S1, Supporting Information).

In a final series of experiments, we investigated the feasibility of the PBA functionalized PMAA brushes to transduce variations in glucose concentration in changes of hydraulic permeability across the coated PPHFs, as illustrated in Scheme 2. The setup that was used in these experiments is illustrated in Figure S1 in the Supporting Information. Figure 8A shows the results of these hydraulic permeability measurements, which were carried out using pH 9 buffer solutions with and without 100 mM glucose for an unmodified PPHF, a PPHF modified with a PMAA brush obtained after a polymerization time of 5 min and four PBA functionalized PMAA brush coated PPHFs that differ in the polymerization time (i.e., thickness) that was used to graft the PMAA brush (2, 5, 10, and 15 min). Figure S9 in the Supporting Information summarizes the results of similar experiments that were carried out using 25 mM glucose solutions. The results in Figure 8A illustrate that the water permeability across the unmodified PPHF is relatively small compared to the brush modified fibers, which reflects the hydrophobic character of the PPHF. Whereas the flux through PPHFs coated with the unmodified PMAA brush is not influenced by the addition of glucose, the PPHFs that are coated with the PBA functionalized PMAA brush layers show a decrease in flux upon addition of glucose. This decrease in flux is consistent with the observed swelling of the brush upon binding of glucose (vide supra), which leads to a decrease in the effective pore diameter of the PPHFs. As illustrated in Figure 8B, the extent to which the flux decreases upon binding of glucose depends on the polymerization time that was used to grow the brush (i.e., the brush thickness). This effect of brush thickness on the permeability may be due to the fact that the swelling of thin brushes only causes a minor reduction in effective pore diameter, which becomes more pronounced as the thickness of the brush increases. As some point, however, when the brush thickness is roughly equal to the pore diameter of the PPHF, the pores are effectively filled, and glucose-induced swelling has little effect on the flux across the membrane. This is illustrated in Figure 8B, which shows that F/F_0 first decreases with increasing polymerization time (i.e., brush thickness), but that for thicker brushes (at polymerization times of ~15 min) glucose binding only results in a very

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

minor reduction of the flux compared to an unmodified PMAA coated PPHF. The gradual "filling" of the nanosized pores of the PPHF substrate is also illustrated by the gradual decrease in flux with increasing polymerization time that is observed for pH 9 solutions that do not contain glucose. Similar results were obtained when a 25 mM instead of a 100 mM glucose solution was used, albeit with a smaller decrease in F/F_0, which reflects the smaller glucose concentration (Figure S9, Supporting Information) and indicates that the proposed sensor configuration in principle allows to correlate variations in hydraulic permeability with changes in glucose concentration.

CONCLUSIONS

Glucose-sensitive polymer brushes have been prepared via surface-initiated atom transfer radical polymerization (SI-ATRP) of sodium methacrylate followed by postpolymerization modification with 3-aminophenylboronic acid. These polymer brushes have been grown both from planar silicon substrates modified with an organosilane-functionalized ATRP initiator and from PPHF membranes that were subjected to photobromination prior to SI-ATRP. QCM-D and AFM experiments on samples grown from planar silicon substrates indicated that the PBA functionalized brushes are able to bind glucose over a range of physiologically relevant concentrations in a reversible manner and revealed that glucose binding was accompanied by an increase in film thickness (swelling) of the brush. Application of the PBA-functionalized PMAA films on the surface of a hollow PPHF enabled the preparation of a first prototype of a hydraulic flow sensor. Binding of glucose results in the swelling of the PBA-functionalized brush coating and concomitantly a decrease in the hydraulic permeability of the PPHF. Because the detection scheme presented here avoids the use of enzymes and does not rely on an electrochemical transduction pathway, it could be potentially interesting for the development of continuous in vivo glucose detection systems.

ASSOCIATED CONTENT

Supporting Information

Illustration of the hydraulic permeability setup, PMAA film thickness versus polymerization time, additional XPS and FTIR data as well as results from hydraulic permeability experiments carried out using 25 mM glucose solution. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: harm-anton.klok@epfl.ch. Fax: + 41 21 693 5650. Tel.: + 41 21 693 4866.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the European Commission under the IST framework program 7 for Information and Communication Technology, ICT (FP7-ICT-2009-4, grant 247138, www.apathome.eu). The authors are grateful to N. Xanthopoulos for his support with the XPS experiments and Dr. C. Sugnaux for valuable discussions.

REFERENCES

(1) King, H.; Aubert, R. E.; Herman, W. H. Global Burden of Diabetes, 1995−2025: Prevalence, Numerical Estimates, And Projections. *Diabetes Care* **1998**, 1414−1431.
(2) Wang, J. Electrochemical Glucose Biosensors. *Chem. Rev.* **2008**, *108*, 814−825.
(3) Wu, Q.; Wang, L.; Yu, H.; Wang, J.; Chen, Z. Organization of Glucose-Responsive Systems and Their Properties. *Chem. Rev.* **2011**, *111*, 7855−7875.
(4) Alonso, B.; Armada, P. G.; Losada, J.; Cuadrado, I.; González, B.; Casado, C. M. Amperometric Enzyme Electrodes for Aerobic and Anaerobic Glucose Monitoring Prepared by Glucose Oxidase Immobilized in Mixed Ferrocene−Cobaltocenium Dendrimers. *Biosens. Bioelectron.* **2004**, *19*, 1617−1625.
(5) Jing-Juan, X.; Hong-Yuan, C. Amperometric Glucose Sensor Based on Coimmobilization of Glucose Oxidase and Poly(*p*-phenylenediamine) at a Platinum Microdisk Electrode. *Anal. Biochem.* **2000**, *280*, 221−226.
(6) Clark, L. C.; Lyons, C. Electrode Systems for Continuous Monitoring in Cardiovascular Surgery. *Ann. N.Y. Acad. Sci.* **1962**, *102*, 29−45.
(7) Cella, L. N.; Chen, W.; Myung, N. V.; Mulchandani, A. Single-Walled Carbon Nanotube-Based Chemiresistive Affinity Biosensors for Small Molecules: Ultrasensitive Glucose Detection. *J. Am. Chem. Soc.* **2010**, *132*, 5024−5026.
(8) Cheung, K. Y.; Mak, W. C.; Trau, D. Reusable Optical Bioassay Platform with Permeability-Controlled Hydrogel Pads for Selective Saccharide Detection. *Anal. Chim. Acta* **2008**, *607*, 204−210.
(9) Caves, M. S.; Derham, B. K.; Jezek, J.; Freedman, R. B. The Mechanism of Inactivation of Glucose Oxidase from Penicillium Amagasakiense under Ambient Storage Conditions. *Enzyme Microb. Technol.* **2011**, *49*, 79−87.
(10) Katano, H.; Sugimoto, Y.; Uematsu, K.; Hibi, T. Kinetic Sudy of the Thermal Inactivation of Glucose Oxidase in the Presence of Denaturant and Stabilizer by Means of Bioelectrocatalysis Method. *Anal. Sci.* **2011**, *27*, 979−983.
(11) Koschwanez, H. E.; Reichert, W. M. In Vitro, In Vivo, and Post Explantation Testing of Glucose-Detecting Biosensors: Current Methods and Recommendations. *Biomaterials* **2007**, *28*, 3687−3703.
(12) von Woedtke, T.; Jülich, W. D.; Hartmann, V.; Stieber, M.; Abel, P. U. Sterilization of Enzyme Glucose Sensors: Problems and Concepts. *Biosens. Bioelectron.* **2002**, *17*, 373−382.
(13) Ivanov, A. E.; Thammakhet, C.; Kuzimenkova, M. V.; Thavarungkul, P.; Kanatharana, P.; Mikhalovska, L. I.; Mikhalovsky, S. V.; Galaev, I. Y.; Mattiasson, B. Thin Semitransparent Gels Containing Phenylboronic Acid: Porosity, Optical Response, and Permeability for Sugars. *J. Mol. Recognit.* **2008**, *21*, 89−95.
(14) Domschke, A.; Kabilan, S.; Anand, R.; Caines, M.; Fetter, D.; Griffith, P.; James, K.; Karangu, N.; Smith, D.; Vargas, M.; Zeng, J.; Hussain, A.; Xiaoping, Y.; Blyth, J.; Mueller, A.; Herbrechtsmeier, P.; Lowe, C. R. Holographic Sensors in Contact Lenses for Minimally-Invasive Glucose Measurements. *Sensors* **2004**, *3*, 1320−1323.
(15) Pan, X.; Yang, X.; Lowe, C. R. Evidence for a Cross-Linking Mechanism Underlying Glucose-Induced Contraction of Phenylboronate Hydrogel. *J. Mol. Recognit.* **2008**, *21*, 205−209.
(16) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. The Relationship Among pK_a, pH, and Binding Constants in the Interactions between Boronic Acids and Diols—It Is Not as Simple as it Appears. *Tetrahedron* **2004**, *60*, 11205−11209.
(17) Springsteen, G.; Wang, B. A Detailed Examination of Boronic Acid−Diol Complexation. *Tetrahedron* **2002**, *58*, 5291−5300.
(18) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. Totally Synthetic Polymer Gels Responding to External Glucose Concentration: Their Preparation and Application to On−Off Regulation of Insulin Release. *J. Am. Chem. Soc.* **1998**, *120*, 12694−12695.
(19) Kikuchi, A.; Suzuki, K.; Okabayashi, O.; Hoshino, H.; Kataoka, K.; Sakurai, Y.; Okano, T. Glucose-Sensing Electrode Coated with

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640

Polymer Complex Gel Containing Phenylboronic Acid. *Anal. Chem.* **1996**, *68*, 823−828.
(20) Matsumoto, A.; Kurata, T.; Shiino, D.; Kataoka, K. Swelling and Shrinking Kinetics of Totally Synthetic, Glucose-Responsive Polymer Gel Bearing Phenylborate Derivative as a Glucose-Sensing Moiety. *Macromolecules* **2004**, *37*, 1502−1510.
(21) Matsumoto, A.; Yamamoto, K.; Yoshida, R.; Kataoka, K.; Aoyagi, T.; Miyahara, Y. A Totally Synthetic Glucose Responsive Gel Operating in Physiological Aqueous Conditions. *Chem. Commun.* **2010**, *46*, 2203−2205.
(22) Matsumoto, A.; Yoshida, R.; Kataoka, K. Glucose-Responsive Polymer Gel Bearing Phenylborate Derivative as a Glucose-Sensing Moiety Operating at the Physiological pH. *Biomacromolecules* **2004**, *5*, 1038−1045.
(23) Ancla, C.; Lapeyre, V.; Gosse, I.; Catargi, B.; Ravaine, V. Designed Glucose-Responsive Microgels with Selective Shrinking Behavior. *Langmuir* **2011**, *27*, 12693−12701.
(24) Farooqi, Z. H.; Khan, A.; Siddiq, M. Temperature-Induced Volume Change and Glucose Sensitivity of Poly[(*N*-isopropylacrylamide)-co-acrylamide-co-(phenylboronic acid)] Microgels. *Polym. Int.* **2011**, *60*, 1481−1486.
(25) Hoare, T.; Pelton, R. Engineering Glucose Swelling Responses in Poly(*N*-isopropylacrylamide)-based Microgels. *Macromolecules* **2007**, *40*, 670−678.
(26) Zhang, Y.; Guan, Y.; Zhou, S. Synthesis and Volume Phase Transitions of Glucose-Sensitive Microgels. *Biomacromolecules* **2006**, *7*, 3196−3201.
(27) Alexeev, V. L.; Sharma, A. C.; Goponenko, A. V.; Das, S.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N.; Asher, S. A. High Ionic Strength Glucose-Sensing Photonic Crystal. *Anal. Chem.* **2003**, *75*, 2316−2323.
(28) Asher, S. A.; Alexeev, V. L.; Goponenko, A. V.; Sharma, A. C.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N. Photonic Crystal Carbohydrate Sensors: Low Ionic Strength Sugar Sensing. *J. Am. Chem. Soc.* **2003**, *125*, 3322−3329.
(29) Arnold, F. H.; Zheng, W.; Michaels, A. S. A Membrane-Moderated, Conductimetric Sensor for the Detection and Measurement of Specific Organic Solutes in Aqueous Solutions. *J. Membr. Sci.* **2000**, *167*, 227−239.
(30) Wang, D.; Liu, T.; Yin, J.; Liu, S. Stimuli-Responsive Fluorescent Poly(*N*-isopropylacrylamide) Microgels Labeled with Phenylboronic Acid Moieties as Multifunctional Ratiometric Probes for Glucose and Temperatures. *Macromolecules* **2011**, *44*, 2282−2290.
(31) Du, P.; Mu, B.; Wang, Y.; Liu, P. Glucose and Temperature Dual-Responsive Monodispersed Hollow Nanospheres via Facile One-Pot Two-Step Process. *Mater. Lett.* **2012**, *75*, 77−79.
(32) Jin, Q.; Lv, L.-P.; Liu, G.-Y.; Xu, J.-P.; Ji, J. Phenylboronic Acid as a Sugar- and pH-Responsive Trigger to Tune the Multiple Micellization of Thermo-Responsive Block Copolymer. *Polymer* **2010**, *51*, 3068−3074.
(33) Roy, D.; Cambre, J. N.; Sumerlin, B. S. Sugar-Responsive Block Copolymers by Direct RAFT Polymerization of Unprotected Boronic Acid Monomers. *Chem. Commun.* **2008**, 2477−2479.
(34) Wang, B.; Ma, R.; Liu, G.; Liu, X.; Gao, Y.; Shen, J.; An, Y.; Shi, L. Effect of Coordination on the Glucose-Responsiveness of PEG-*b*-(PAA-co-PAAPBA) Micelles. *Macromol. Rapid Commun.* **2010**, *31*, 1628−1634.
(35) Zhao, L.; Ding, J.; Xiao, C.; He, P.; Tang, Z.; Pang, X.; Zhuang, X.; Chen, X. Glucose-Sensitive Polypeptide Micelles for Self-Regulated Insulin Release at Physiological pH. *J. Mater. Chem.* **2012**, *22*, 12319−12328.
(36) Barbey, R.; Lavanant, L.; Paripovic, D.; Schüwer, N.; Sugnaux, C.; Tugulu, S.; Klok, H.-A. Polymer Brushes via Surface-Initiated Controlled Radical Polymerization: Synthesis, Characterization, Properties, and Applications. *Chem. Rev.* **2009**, *109*, 5437−5527.
(37) Ivanov, A. E.; Eccles, J.; Panahi, H. A.; Kumar, A.; Kuzimenkova, M. V.; Nilsson, L.; Bergenståhl, B.; Long, N.; Phillips, G. J.; Mikhalovsky, S. V.; Galaev, I. Y.; Mattiasson, B. Boronate-Containing Polymer Brushes: Characterization, Interaction with Saccharides and Mammalian Cancer Cells. *J. Biomed. Mater. Res. Part A* **2009**, *88A*, 213−225.
(38) Ivanov, A. E.; Kumar, A.; Nilsang, S.; Aguilar, M.-R.; Mikhalovska, L. I.; Savina, I. N.; Nilsson, L.; Scheblykin, I. G.; Kuzimenkova, M. V.; Galaev, I. Y. Evaluation of Boronate-Containing Polymer Brushes and Gels as Substrates for Carbohydrate-Mediated Adhesion and Cultivation of Animal Cells. *Colloid Surf., B* **2010**, *75*, 510−519.
(39) Chen, T.; Chang, D. P.; Liu, T.; Desikan, R.; Datar, R.; Thundat, T.; Berger, R.; Zauscher, S. Glucose-Responsive Polymer Brushes for Microcantilever Sensing. *J. Mater. Chem.* **2010**, *20*, 3391−3395.
(40) Sugnaux, C.; Klok, H.-A. Glucose-Sensitive QCM-Sensors via Direct Surface RAFT Polymerization. *Macromol. Rapid Commun.* **2014**, *35*, 1402−1407.
(41) Schüwer, N.; Klok, H.-A. A Potassium-Selective Quartz Crystal Microbalance Sensor Based on Crown-Ether Functionalized Polymer Brushes. *Adv. Mater.* **2010**, *22*, 3251−3255.
(42) Tugulu, S.; Barbey, R.; Harms, M.; Fricke, M.; Volkmer, D.; Rossi, A.; Klok, H.-A. Synthesis of Poly(methacrylic acid) Brushes via Surface-Initiated Atom Transfer Radical Polymerization of Sodium Methacrylate and their Use as Substrates for the Mineralization of Calcium Carbonate. *Macromolecules* **2007**, *40*, 168−177.
(43) Tugulu, S.; Harms, M.; Fricke, M.; Volkmer, D.; Klok, H.-A. Polymer Brushes as Ionotropic Matrices for the Directed Fabrication of Microstructured Calcite Thin Films. *Angew. Chem., Int. Ed.* **2006**, *45*, 7458−7461.
(44) Wang, C.; Yan, Q.; Liu, H.-B.; Zhou, X.-H.; Xiao, S.-J. Different EDC/NHS Activation Mechanisms between PAA and PMAA Brushes and the Following Amidation Reactions. *Langmuir* **2011**, *27*, 12058−12068.
(45) Schüwer, N.; Klok, H.-A. Tuning the pH Sensitivity of Poly(methacrylic acid) Brushes. *Langmuir* **2011**, *27*, 4789−4796.
(46) American Diabetes Association. Standards of Medical Care in Diabetes-2012. *Diabetes Care* **2012**, *35* (Supplement 1), S11−S63.
(47) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. Fluorescence Sensors for Monosaccharides based on the 6-Methylquinolinium Nucleus and Boronic Acid Moiety: Potential Application to Ophthalmic Diagnostics. *Talanta* **2005**, *65*, 762−768.
(48) DiCesare, N.; Lakowicz, J. R. Spectral Properties of Fluorophores Combining the Boronic Acid Group with Electron Donor or Withdrawing Groups. Implication in the Development of Fluorescence Probes for Saccharides. *J. Phys. Chem. A* **2001**, *105*, 6834−6840.
(49) Horgan, A. M.; Marshall, A. J.; Kew, S. J.; Dean, K. E. S.; Creasey, C. D.; Kabilan, S. Crosslinking of Phenylboronic Acid Receptors as a Means of Glucose Selective Holographic Detection. *Biosens. Bioelectron.* **2006**, *21*, 1838−1845.
(50) Katoka, K.; Miyazaki, H.; Okano, T.; Sakurai, Y. Sensitive Glucose-Induced Change of the Lower Critical Solution Temperature of Poly[*N*,*N*-dimethylacrylamide-co-3-(acrylamido)phenylboronic acid] in Physiological Saline. *Macromolecules* **1994**, *27*, 1061−1062.
(51) Kawasaki, T.; Akanuma, H.; Yamanouchi, T. Increased Fructose Concentrations in Blood and Urine in Patients With Diabetes. *Diabetes Care* **2002**, *25*, 353.
(52) Lavanant, L.; Pullin, B.; Hubbell, J. A.; Klok, H.-A. A Facile Strategy for the Modification of Polyethylene Substrates with Non-Fouling, Bioactive Poly(poly(ethylene glycol) methacrylate) Brushes. *Macromol. Biosci.* **2010**, *10*, 101−108.
(53) Dordi, B.; Schönherr, H.; Vancso, G. J. Reactivity in the Confinement of Self-Assembled Monolayers: Chain Length Effects on the Hydrolysis of *N*-Hydroxysuccinimide Ester Disulfides on Gold. *Langmuir* **2003**, *19*, 5780−5786.

DOI: 10.1021/am507927w
ACS Appl. Mater. Interfaces 2015, 7, 4631−4640