Full Paper**Enzyme-free Detection of Glucose with a Hybrid Conductive Gel Electrode***Shofarul Wustoni, Achilleas Savva, Ruofan Sun, Eloise Bihar, and Sahika Inal**

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Current assays for glucose monitoring rely predominantly on glucose oxidation-catalyzing enzymes because of the high specificity of enzyme-substrate interactions. Enzymes are however expensive, suffer from instability during fabrication, operation and storage, and necessitate complex procedures for integration with transducer materials. These challenges, rendering the enzyme-based sensors disadvantageous for routine glucose monitoring, can be overcome by non-enzymatic sensors. Here, for the enzyme-free detection of glucose, an electroactive gel is developed via one-pot polymerization. The functional material is a hybrid of the conducting polymer poly(3,4-ethylenedioxythiophene):polystyrenesulfonate and a polyacrylamide gel functionalized with phenylboronic acid. As an electrode, the gel exhibits a specific current response to glucose within the standard concentration range measured in the complex blood-like medium. When integrated as the lateral, micrometer-scale gate electrode of an organic electrochemical transistor (OECT), the channel current is proven to be sensitive to the presence of glucose in the measurement solution. The advantage of the OECT based sensor compared to the amperometric electrode is its miniaturized form, amplified input signal as well the elimination of a reference electrode. Adaptable to different geometries, this conducting gel exhibits multifunctionality within its soft, gel-like architecture, that is, mixed ionic and electronic conductivity and glucose specific electrical response.

Introduction

Glucose is the primary nutrient of living systems to perform a variety of cellular metabolic processes including cellular respiration and biosynthesis.^[1,2] In case of malfunction, cells may end up not taking up glucose sufficiently, which leads to abnormal levels of glucose build up in the bloodstream. The high concentration of glucose in blood is typically associated with the disease, diabetes. Diabetes is a pandemic disorder which was one of the top ten leading causes of death worldwide in 2015 and it is predicted to become the tenth most burdensome disease by 2030.^[3-5] A good part of diabetes treatment as well as its early diagnosis involve self-monitoring of the amount of glucose in biological fluids. Thus, powerful, low-cost and easy-to-use glucose sensors are on high demand.

The inherent specificity and electrochemical reversibility of enzymes poise them as the biorecognition element of choice for a wide range of metabolites. The majority of current glucose sensors are thus electronic and based on the redox enzyme, glucose oxidase that undergoes redox reactions with the analyte, glucose. In these platforms, the enzyme transforms glucose into gluconolactone and hydrogen peroxide (H_2O_2). H_2O_2 reacts with a metal electrode in its vicinity, resulting in a change in the flow of electrical current which is directly proportional to concentration of glucose that has reacted with the enzyme. The electrical potential required to oxidize H_2O_2 however matches that for the oxidation of several other species, such as ascorbic acid, dopamine and uric acid, naturally present in blood. These species generate interference, in other words, false read-outs which may lead to the decision of excess intake of insulin.^[6] Strategies to improve the selectivity for glucose involve enhancing the catalytic activity of electrode materials,^[7] using anti-interference coatings,^[8] and integrating electron mediators that lower the operation potential of the sensors.^[9] Despite their success, these approaches rapidly increase fabrication costs and device complexity. Moreover, enzymes are inherently sensitive to small changes in their environment (such as temperature and humidity)

and tend to lose their activity over time. Another shortcoming involves the high cost of enzyme production and purification.^[10] Continuous monitoring of glucose levels would thus benefit from a technology that does not rely on enzymes and omits tedious procedures for the fabrication of the functional sensor component.

A variety of micro and nanostructured electrode materials with high geometrical surface area such as those made of noble metals of Ag, Pt, Au or composites of CuO, NiO, MnO or ZnO can directly catalyze glucose.^[11-13] The limited abundance of these materials, however, decreases the chances of translation to practice. Furthermore, as they can electrochemically interact with other redox active molecules,^[14,15] selectivity remains an issue. Another alternative route for enzyme-free glucose sensing is the use of synthetic boronic acid-based receptors. A phenylboronic acid (PBA) unit reversibly binds to diols of glucose to form five- or six-membered cyclic boronic esters.^[16,17] Boronic acid derivatives have thus been utilized as the biorecognition element in various sensor platforms involving optical,^[18,19] mechanical,^[20,21] or electromagnetic^[22,23] transducers, as an alternative to glucose oxidase. A breakthrough work with PBAs is the glucose sensing contact lens made of acrylamide-based hydrogel involving a fluorescent dye conjugated PBA.^[24] PBA derivatives have also been integrated with electronic transducers for electrochemical sensing of glucose.^[25,26] Miyahara and co-workers functionalized the gate dielectric of a field effect transistor with PBA to detect the change in the surface potential of gate electrode upon glucose complexation with the dielectric layer.^[27,28] In these studies, two major routes were followed to functionalize the devices with PBA: PBA was attached to the transducer surface either as a part of a self-assembled monolayer (SAM) or it was immobilized within a polymeric network that was deposited as a layer. Such fabrication processes are not monolithic, thus hindering for adaptation into different geometries and form factors. Another major drawback of these platforms is the disintegration of the functional layer

over time, limiting the stability of the sensors. These challenges point to the need of an enzyme-free platform with structural simplicity while accommodating selectivity and sensitivity.

In this work, we demonstrate an enzyme-free, conductive gel electrode that is responsive to glucose. The complete sensor is electropolymerized at a single step on gold coated flexible or rigid substrates. The conductive gel (CG) is composed of the conducting polymer poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) and a poly(acrylamide) gel functionalized with phenylboronic acid (PAAm:PBA). The PAAm gel acts as a scaffold accommodating the PBA units that complex with glucose. PEDOT:PSS is, on the other hand, the electronic transducer of the platform, which translates the volumetric changes that take place upon glucose binding into measurable electronic signals. The CG electrode changes its electrical properties in response to glucose within a physiologically relevant concentration range and without significant interference from other electroactive species present in complex media. The response is reversible within a modest concentration range and stable over four days of operation involving numerous measurements each day. Due to the simplicity of fabrication, the material can be integrated into devices with different geometries, allowing for miniaturization. When used as the micrometer-scale gate electrode of an organic electrochemical transistor (OECT), we detect specific changes in the channel (output) current as a function of the glucose concentration in the electrolyte. The OECT circuitry amplifies the glucose response of the gate electrode at the channel, showing better resolved output currents compared with the conventional amperometric sensors of the same geometry. This conductive gel prepared via a straightforward synthetic route combines soft, gel-like properties with glucose sensitive electrical conductivity.

2. Results and Discussion

2.1. Compositional and Electrochemical Analysis of the CG

The first distinguishing characteristics of the CG is associated with its fabrication. We perform a single step electropolymerization procedure to assemble the three components, i.e., the receptor (PBA), the reporting component (PEDOT:PSS) and the PAAm gel (Figure 1a). Compared with other techniques for polymer deposition, electropolymerization gives the flexibility to form hybrid structures compatible with a variety of conducting substrates.^[29] This hybrid architecture ensures structural integrity as well as strong adherence to the substrate, promising for operational stability. To verify successful incorporation of all the components on the Au coated, flexible polyimide substrate, we, firstly, perform elemental analysis using X-ray photoelectron spectroscopy (XPS). Figure 1b and c show high resolution scans from N 1s and B 1s regions that reveal the presence of nitrogen and boron atoms on the gel surface (max. resolution depth ca. 10 nm), respectively. The XPS spectrum of N 1s comprises of two peaks associated with amide/amine moieties (401 eV) and protonated amino groups (403 eV), originating from PAAm and/or PBA.^[30] XPS B 1s spectrum displays a distinct peak at 193 eV confirming the presence of PBA.^[31] On the contrary, for PEDOT:PSS film that is electropolymerized in the absence of the PBA and PAA in the reaction mixture, XPS spectra are featureless in these regions (Figure S1). Further insight into the gel composition comes from FTIR studies. The CG spectrum exhibits distinct bands typical for C=O and N-H vibrations, attributed to PBA units and the acrylamide, in addition to those of PEDOT:PSS (Figure S2). The morphology of CG surface is also significantly different compared to that of PEDOT:PSS (Figure 1d-e). The hybrid gel displays a rough but homogeneous, microstructured surface whereas PEDOT:PSS exhibits a relatively smooth film similar to what has previously been reported.^[32,33] From a cross-sectional image, we estimate the thickness of the CG to be ca. 100 nm (Figure 1f). Together, we conclude that the recognition and reporter units of the sensor are

successfully incorporated into a gel-like architecture via a simple, low cost electropolymerization technique.

Figure 1. Compositional and surface characterization of the conducting gel (CG) electrode electropolymerized on an Au coated, flexible substrate. **a)** Schematic illustration of the CG electropolymerization. **b)** High resolution N 1s XPS spectra of the CG. The peak at 403 eV (red lines) is assigned to the characteristic bond of protonated amino groups of PAAm, while the one at 401 eV (blue lines) is attributed to amine and amide groups of PBA and PAAm. **c)** High resolution B 1s XPS spectrum of the CG demonstrates a characteristic peak for $\text{B}-\text{O}$ bond (193 eV). In b) and c), green lines represent the background signal while the black lines are the raw spectra. SEM images showing **d)** microstructured CG surface and **e)** rigid and smooth surface of electropolymerized PEDOT:PSS. **f)** Cross sectional image of the CG sandwiched between the Au contact (100 nm) and the Pt layer that is used as protective coating during FIB-SEM.

PAAm matrix is known to undergo volumetric changes upon interactions of the immobilized PBA groups with diols.^[34] Our CG has a relatively rough surface (RMS roughness of 21.2 nm) when it is dry (Figure 2a). Once incubated in PBS solution, it swells with water and therefore the roughness decreases to 15.2 nm (Figure 2b). Upon introduction of 10 mM of glucose into the solution, the surface topography changes significantly with roughness diminishing down to 7.4 nm (Figure 2c and see Figure S3 for height profiles). As glucose molecules complex with PBA units, the soft, swollen gel undergoes further swelling and transforms into a more “expanded” architecture. These distinct changes occur because glucose binding leads to the charged form of boronic acid as depicted in Figure 2d. These charged groups are then responsible for the osmotic expansion of the network.^[34] For the case of the CG, the presence of PEDOT:PSS does not hinder glucose-triggered swelling of the hybrid gel as the conducting polymer itself swells to nearly double its size due to the diffusion of water molecules and ions into the film.^[35] A microporous network such as the one of the CG should allow for glucose diffusion. Quartz crystal microbalance with dissipation monitoring (QCM-D) studies can evidence these interactions and glucose uptake. QCM-D is a molecular balance to quantify the adsorption/desorption processes occurring on top of a film coated on a quartz crystal sensor.^[36] We treat the raw QCM-D data (change in frequency and dissipation signals, Figure S4) with the viscoelastic model to estimate the amount of glucose that is coupled to the CG (Figure 2e). As glucose diffuses into the CG and binds to the PBA units, the complex formed transforms the gel into a polyanion while releasing H_3O^+ as byproduct (Figure 2d).^[37] We consider that the increase in CG mass is solely due to the glucose taken up as H_3O^+ molecules are extracted out to the electrolyte. From the mass calculated, we deduce that 1.31×10^{14} glucose molecules are complexing with PBA groups which have a density of *ca.* 1.67×10^{14} units cm^{-3} . When we assume that the byproduct is instead trapped inside the gel along with glucose, we derive 1.19×10^{14} molecules and 1.51×10^{14} units cm^{-3} for the incorporated amount of glucose and the

site density of PBA, respectively. In both cases, the glucose taken up by the gel accounts much less than the amount that is present in the solution flowing through the chamber.

Figure 2. Atomic force microscopy (AFM) images (1 μm x 1 μm) of the CG obtained in **a)** air, **b)** PBS solution and **c)** the presence of 10 mM of glucose in PBS. **d)** Illustration of the binding event between glucose and the PBA units on the PAAm chain. **e)** The mass added on the CG that is coated on a QCM-D sensor as the glucose concentration increases in the solution. The glucose additions are marked by the arrows. The last step involves rinsing the sensor with PBS.

Since the hybrid gel undergoes morphological and volumetric changes as a result of interactions with glucose, we turn our attention to its electrochemical behavior. The CG coating on the gold electrode lowers the impedance of the gold dramatically due to the bulk interactions of the electrolyte ions with the conducting polymer,^[38] advantageous when transducing ionic signals (Figure S5). The cyclic voltammetry (CV) curve of the CG electrode measured in PBS shows that the gel preserves the capacitive behavior of PEDOT:PSS (Figure 3a, red lines, see Figure S6 for the CV of bare PEDOT:PSS). As glucose concentration increases, we observe a gradual

increase in both oxidation and reduction currents (Figure 3a). Electrochemical capacitance is 2.93 mC and 3.85 mC in the absence and presence of 10 mM glucose, respectively. With an increase in glucose concentration, a new reduction peak at ca. 0.55 V appears (inset, Figure 3a). The shape of this CV curve resembles that of the conductive gel that was electropolymerized in the absence of PBA (Figure 3b). Importantly, neither the PBA-free gel nor the pristine PEDOT:PSS film exhibits an electrical response to glucose, shown in Figure 3b and Figure S6a, respectively, evidencing that PBA units are required for interactions with glucose and their complexation effects the electrochemical properties of the hybrid material. In agreement with the binding induced increase in capacitance, we observe a decrease in the magnitude of the electrochemical impedance of the CG electrode, most pronounced at high frequencies ($>1\text{Hz}$) (Figure 3c). Glucose dependent impedance profile is specific to the CG: we observe no change for the PBA-free gel or the PEDOT:PSS itself (Figure 3d and Figure S6b). We attribute these changes to the mobility of ions within the CG which becomes higher as the gel expands. These results are in agreement with the findings of Daikuzono *et al* who reported similar glucose-triggered changes in the electrochemical impedance spectra of interdigitated carbon electrodes coated with a thin layer of PBA functionalized hydrogel.^[39] Swelling of the network induced by binding of sugar molecules to boronate species improves the diffusion of electrolyte ions inside the hybrid gel. The net result is an increase in current flow.

Figure 3. The effect of glucose on the electrochemical characteristics of the CG recorded in PBS. The CV curve of the CG electropolymerized in the **a)** presence and **b)** absence of PBA. CV measurements were performed at a scan rate of 0.1 mV s^{-1} over a potential window of -0.2 to 1 V vs Ag/AgCl . Electrochemical impedance spectra of the CG fabricated **c)** with and **d)** without PBA, prior to and after addition of glucose into PBS. These measurements were performed at the open circuit potential (V_{oc}). Corresponding Nyquist plots of the two gels are shown in **e)** and **f)**.

To further understand the effect of glucose uptake on the electrochemical properties of CG, we examine the Nyquist plots shown in Figure 3e. The CG electrode has lower electrochemical capacitance and conductivity than the gel that does not contain PBA groups as well as bare PEDOT:PSS (Figure 3f and Figure S6c, respectively). This is an expected outcome as the CG involves non-conducting PAAm and PBA in its structure. The Nyquist plot of the CG includes a semicircle at high frequencies followed by a linear part characteristic of lower frequencies attributed to diffusion-controlled charge transfer.^[40] With addition of glucose, the diameter of

this semicircle becomes smaller, i.e., reduced charge-transfer resistance (Figure 3e). The increase in glucose concentration also causes a distinct shift in the linear part of the curve. The linear part of the curve with an angle of 45° at low frequencies is typically attributed to diffusion of ionic species from the electrolyte into the electrode.^[41] As the CG swells, PEDOT:PSS becomes more permeable to electrolyte ions which readily penetrate inside the gel and reach electroactive sites — similar to the findings of Willner et al.^[42] Thanks to the volumetric interactions of solution ions with PEDOT:PSS and the bulk coupling between ionic and electronic charges therein, the gel is able to operate as a glucose sensor. These changes are again specific to PBA groups (Figure 3f and Figure S6c for the Nyquist plots of PBA-free gel and PEDOT:PSS, respectively). These results show that we can transduce glucose binding induced variations in the volume of the CG into a well-detectable electrochemical output.

2.2. Sensor Characteristics

2.2.1. The CG electrode on a flexible substrate

Swelling of the hybrid gel upon glucose uptake affects the electrochemical properties of PEDOT:PSS. PEDOT:PSS is an organic mixed conductor which accommodates simultaneous hole and cation transport. In its neutral, conducting (oxidized) state, holes of PEDOT are charge-compensated by the sulfonate groups of PSS. The polymer film undergoes changes in its doping state upon exchange of cations with the electrolyte:^[43]

Injection of cations (C^+) and simultaneous accumulation of electrons (e^-) provided by the Au electrode underneath the film switch the polymer to a semiconducting (reduced) state (right hand side of equation 1). Glucose uptake transforms the CG to a relatively more hydrated and expanded state, an environment ideal for drifting cations to access PEDOT:PSS sites to compensate the polyanions. This, in turn, leads to *enhanced reduction currents*. The modulation of ion permeability into the gel as a result of glucose binding interactions is the transduction

mechanism of our sensor, as depicted in Figure 4a. We evaluate the sensor performance by measuring its current at a pre-set voltage (-0.2 V) applied for 60 s upon addition of increasing concentrations of glucose into the measurement solution (PBS) Figure 4b shows that the current increases gradually with an increase in glucose concentration from 0.05 mM to 20 mM, i.e., a clinically relevant range for healthy and diabetic blood glucose levels (see Table S1 for the target glucose level in blood).^[44-46] While this range corresponds to a large spectrum of blood glucose concentrations,^[47,48] it particularly covers the regions of hypoglycemia (low blood glucose, <3 mM) and hyperglycemia (high blood glucose, >8 mM) for the samples obtained during fasting.^[44-48] Above 20 mM, the sensor is no longer responsive to glucose (Figure S7). The platform yields excellent analytical characteristics as evidenced from the calibration curve, i.e. the current response as a function of sugar content (Figure 4c). The device exhibits a wide dynamic range of detection from 0 mM to 20 mM with a sensitivity of ca. $0.23 \mu\text{A mM}^{-1}$ above 2 mM. The limit of detection is ca. 0.061 mM of glucose. Notably, we found that the sensitivity to glucose can be optimized by controlling the electropolymerization time. While the most sensitive electrode is the one that has been polymerized only for 5 minutes, longer deposition times resulted in thicker but less conducting gels (Figure S8). The sensor is, however, also responsive to D-fructose since the PBA possesses suitable structural framework for other saccharides containing 1,2- and 1,3-diols units to bind. The current shows linear dependence on both sugars in the range of 2 – 20 mM, while the sensitivity for fructose is much lower (ca. $0.13 \mu\text{A mM}^{-1}$) (Figure 4c). Nevertheless, glucose concentration in blood, as well as other biological fluids, is typically several orders of magnitude higher than that of other saccharides.^[49] We also examined the potentiometric response of the sensor by recording the changes in the open circuit potential (V_{oc}) as a function of sugar (glucose and fructose) concentrations (Figure S9). The V_{oc} decreases to more negative values with an increase in the concentration of these analytes. The attachment of glucose/fructose to PBA units generates a negative charge on the tetragonal complex of PBA as shown in Figure 2d. We postulate that the accumulation of these negative

charges leads to the observed trend in V_{oc} . Potentiometric response seems, however, not to be the ideal sensor output to distinguish between the two sugars, we therefore selected amperometric monitoring for the following experiments.

Figure 4. Glucose detection mechanism and the sensing performance of the CG electrode. **a)** Illustration of the sensing scheme: Swelling of the CG upon binding of glucose changes its electrical response. Under de-doping potentials, the gel becomes more permeable to electrolyte cations; the reduction current increases and its color changes to darker blue. **b)** The change in the electrode current upon successive additions of glucose (from bottom to top, addition of 0, 0.5, 1, 2, 4, 6, 8, 10, 15, 20 mM of glucose). The current was monitored at a potential of -0.2 V

vs V_{oc} applied for 60 s. **c)** Calibration curve of the sensor for glucose (squares) and fructose (circles) ($N=3$). **d)** The current output of CG electrodes measured in PBS (A), in blood-like medium comprising of hemoglobin (3.5 g/dL) and in human serum albumin (13 g/dL) in PBS (B), and in a mixture of B with non-specific redox active molecules (uric acid, ascorbic acid and dopamine present at concentrations relevant to the physiological levels in blood) (C). The current was monitored in the absence and presence of 1 mM of glucose in each medium ($N=3$). **e)** Calibration curve of the sensor measured in PBS and in mixture C. The inset represents the response of the electrode magnified for the specific concentration range (5.5-to-8.0 mM) beneficial to screen blood samples for the risk of diabetes.

Next, we evaluated the sensor performance in complex media. In order to mimic human blood environment, the sensor was operated in the presence of the most abundant proteins in blood, hemoglobin and human serum albumin.^[50] These proteins increase the magnitude of the background current (Figure 4d, red bars, B). When potentially interfering species present in blood (i.e. ascorbic acid, uric acid and dopamine) are added into this solution, the current of the electrode increases further (red bars, C). Despite the higher initial current in the absence of glucose in complex media, the sensor gives a similar current output in response to glucose (black bars, compare A and C). Although PEDOT:PSS can oxidize redox active components of biological fluids, the interference is avoided thanks to the operation at low negative potentials. Thus, the presence of proteins or redox active components does not hinder the sensitivity of the CG to glucose. Nevertheless, for practical use, normalizing the read-outs with a reference, PBA-free electrode measuring alongside the CG would improve the accuracy of recordings. When operated in the complex, blood like-media, the current has a linear dependence on glucose concentrations within the range of 2 - 10 mM, with a sensitivity of ca. $0.42 \mu\text{A mM}^{-1}$ and a dynamic range comparable to that in PBS (Figure 4e). Healthy patients are known to exhibit a blood glucose level of around 4-5.4 mM (pre-meal), whereas for patients with diabetes, the

target value varies between 4 and 7 mM (Table S1).^[44-46] We highlight the sensitivity of the sensor developed in this work at the specific range of 5.5 to 8 mM, beneficial to screen blood samples collected pre-meal (fasting) for the risk of diabetes (inset of Figure 4e). We also investigated the effect of pH on sensing performance. The CG electrodes measured in acidic conditions (e.g. pH 5 and 6) exhibit lower current values in response to a given amount of glucose (Figure S10a-d) and the dynamic range is narrower (i.e., saturation at ca. 10 mM) (Figure S10e). This is attributed to the weaker binding affinity of PBA to glucose in acidic media, leading to a smaller change in the ion exchange capacity of the electrode.^[51,52] Our sensor operates best at physiological pH, possibly because the pKa of this glucose-PBA complex is close to 7.2.^[48,53] Nevertheless, blood has typically a pH of ca. 7.4 and this environment promotes the interactions between PBA and glucose.

As a recognition unit, one obvious advantage of the PBA over enzymes (oxidoreductases) which are prone to denaturation should be its stability. When the measurement solution containing 4 or 8 mM glucose is replaced with PBS, the current reverts to its initial, glucose-free value (Figure S11a-b). For 15 mM of glucose, however, the sensor response is no longer reversible (Figure S11c-d). We attribute this to glucose that remains bound in the gel. In fact, when the QCM-D sensor is rinsed with PBS after being exposed to 15 mM glucose, we observe that it keeps most of the gained mass, as shown in Figure 2e. When the device is kept in ambient conditions without any encapsulation and subject to at least 4 measurements each day over a period of 7 days, it is still sensitive to glucose. The electrode exhibits a stable current response to 1 mM of glucose over 4 days of operation (Figure S11e). At the 4th day, however, the current in the presence of glucose is higher than what is measured at day 1. The background signal (measured in PBS at the beginning of each day) starts deviating from its initial value after 5 days of operation (Figure S11e). We postulate that the increase in the background current is related to cations trapped within the CG network upon continuous doping/de-doping cycles

during operation as evidenced by the changes in Raman spectra (Figure S12). Upon multiple measurements, the gel architecture becomes more permeable to electrolyte ions, which leads to the observed instability.

2.2.2. The CG electrode as the planar gate of an OECT

Due to the simplicity of fabrication, CG can be integrated into different device formats. A device type that has shown record-high transconductance (g_m) values beneficial for biochemical sensing applications is the OECT.^[54] OECTs comprise of a conjugated polymer film in the channel (predominantly PEDOT:PSS based formulations) and a variety of electrode materials used as the gate, the most common being the top gate Ag/AgCl. As the electrolyte ions interact with the bulk of the channel, the transistor transduces ionic fluctuations of the electrolyte into electronic signals with particularly high amplification. This feature renders OECTs ideal for bio-transduction. For instance, when the gate electrode of an OECT is functionalized with glucose oxidase, the reaction at this electrode is translated into large changes in the channel current.^[55,56] The output current is then correlated to glucose concentration in the solution that the gate is exposed to.

Figure 5. The CG gated OECT operates as a glucose sensor (a) Schematic of the OECT comprising CG at the gate electrode and PEDOT:PSS in the channel [gate dimensions: $500 \mu\text{m}^2$, channel dimensions: $10 \mu\text{m}$ (length) x $100 \mu\text{m}$ (width)]. (b) Transfer curves (the source-drain current (I_D) vs gate voltage (V_G)) and transconductance (g_m) characteristics of OECTs gated by the CG electrode (filled symbols) as well as the PBA-free gel (hollow symbols) using PBS as the electrolyte. The source-drain voltage (V_D) is -0.6 V . (c) NR of OECT sensors gated with the CG electrode (filled symbols) as well as the PBA-free gel (hollow symbols). NR is calculated from the chronoamperograms shown in Figure S14b and c ($V_D = -0.4 \text{ V}$, $V_G = -0.2 \text{ V}$). (d) Comparison of the sensor output signals generated by the OECT and when the micrometer-scale CG is operated as a conventional amperometric electrode ($V = -0.2 \text{ V}$ vs Ag/AgCl). The current of the latter sensor is order of magnitudes lower than the OECT.

Electropolymerization offers spatially resolved functionalization and stable interconnections of the material on the surface, a significant advantage over other solution-based deposition methods commonly used to coat electrode surfaces with enzymes/bio-functional layers. To

showcase the robustness of our CG, we employ it as the micrometer-scale gate electrode of a microfabricated OECT (Figure 5a). The “functional” CG modified electrode gates the PEDOT:PSS channel efficiently, albeit with lower transconductance (g_m) than a PBA-free PEDOT:PSS based gel (Figure 5b, see Figure S13 for output characteristics). This is expected as CG contains a larger content of non-conducting components. For this device, we also observe a shift in the gate voltage (V_G) that yields the maximum transconductance, e.g., for the PBA-free gel this voltage is at 0.1 V and it shifts to -0.05 V for the CG. The g_m is relatively flat over a broad range of V_G , accounting to ca. 2 mS. Next, we monitor real-time changes in the channel current (I_{DS} vs. time) as successive amounts of glucose added to the measurement solution (Figure S14a-c). A gate voltage that maximizes the g_m was applied and after stabilization of the current, glucose concentration in the electrolyte was gradually increased. Only the gate that was modified with CG generates a gradual change (either increase or decrease depending on the polarity of the V_G) in the source-drain current (I_{DS}) with respect to the increase in glucose concentration (Figure 5c, see Figure S14c for I_{DS} vs. time for the PBA-free gel electrode). Notably, for all the devices, the gate current is in the range of several nAs, order of magnitudes lower than I_{DS} (Figure S14d-f). When the device is operated at $V_G = -0.2$ V, PEDOT:PSS in the channel is oxidized with anions injected from the electrolyte while the CG gate is reduced by the cations. We know that upon complexing with glucose, CG becomes more permeable to cations, which causes an increase in its capacitance (see Figure 3). Increasing glucose concentration in the solution, therefore, acts as an additional voltage at the gate and its net effect is further electrochemical doping of the channel, i.e., higher I_{DS} (Figure S14b). On the other hand, when the device is operated at the opposite polarity ($V_G = +0.2$ V), glucose induces further de-doping of the channel (cation/anion injection into the channel/gate), however its effect is not well pronounced (Figure S14a). Indeed, the CV curves in Figure 3a show that glucose uptake impacts more on the reduction current rather than the oxidation current because CG is inherently

in the oxidized state and the anions drifting in the film cause only a little change in its capacitance.

While eliminating the use of a reference electrode on one hand, the OECT provides an amplified response compared to an amperometric sensor built using the same material. The current that is generated by the micrometer scale electrode in response to glucose, measured using a three-electrode system, is order of magnitude lower than the output signal of the OECT bearing the CG as its gate electrode (Figure 5d). One obvious advantage of the CG is therefore its lateral (on chip) adaptation into a high gain, miniaturized device type such as the OECT. We are able to pattern the functional gel on a micrometer scale area with high precision and at a single step without any chemical modifications. This enables the development of portable and integrated sensors and, as such, expands the application scope of our PBA based enzyme-free functional material.

3. Conclusion

We presented a one-pot fabrication of a conductive gel comprising PEDOT:PSS and PBA functionalized PAAm. Each component has a particular purpose in the sensing scheme: PBA interacts specifically with glucose, PAAm along with its electroactive environment expands volumetrically which allows for more electrolyte ions to reach PEDOT:PSS. The mixed conductor PEDOT:PSS then converts these changes in ionic fluxes into well measurable electrical signals. Successful incorporation of the components into the hybrid gel was evidenced using XPS, SEM and FTIR studies. AFM and QCM-D studies showed and quantified the glucose uptake and subsequent swelling of the CG. The CG was applied as a sensor in two configurations: as an amperometric electrode and integrated in an OECT. As an electrode, CG demonstrates quantitative detection of glucose in a clinically relevant concentration range (0.5-20 mM) and selective sensing performance in blood-like complex media. When employed as

the gate electrode of an OECT, CG rendered the device sensitive to glucose. This work is the first demonstration of an all-polymer based, non-enzymatic glucose sensitive electroractive material and its application in an OECT. With a reliable performance in sensing glucose and easy operation, future studies will focus on maturing this technology to surpass enzymatic sensors.

4. Experimental Section

Materials: 3,4-ethylenedioxythiophene (EDOT), Poly(4-styrenesulfonic acid) (PSS), Acrylamide (AAm), 3-(Acrylamido)phenylboronic acid 98% (PBA), N,N'-Methylenebis(acrylamide) (MBIS), (3-Glycidyloxypropyl)trimethoxysilane (GOPS), sodium dodecylbenzenesulfonate (DBSA), ethylene glycol (EG), D-(+)-glucose, D-(+)-fructose, hemoglobin (Hb), human serum albumin (HSA), uric acid, ascorbic acid, and phosphate buffer saline (PBS, pH 7.4), were all purchased from Sigma-Aldrich and used as received. All aqueous solutions were prepared with ultrapure water (Millipore Milli-Q). Glucose solutions with different concentrations were prepared in PBS solution. The channel of OECTs is composed of PEDOT:PSS (PH1000, Heraeus).

Fabrication of the gel electrode: A 175 μm thick Kapton (polyimide) film was used as the substrate and cut with laser into a specific circular geometry (0.7 mm in diameter) as shown in Figure 1a. A 100 nm thick Au layer was then sputtered on the top of the substrates and further deposition of the CG was carried out by electropolymerization. To remove any contaminants, the electrodes were cleaned in acetone and sonicated for 30 min, followed by a rinsing and soaking in DI water under sonication for 30 min. We started the fabrication of CG by mixing the pre-gel solution with 10 mM of EDOT in 0.08 wt% aqueous PSS. The pre-gel mixture contained 70 wt% AAm (monomer), 20 wt% PBA (recognition unit) and 10 wt% MBIS (cross-linker). This mixture was then placed inside the electrochemical cell with three-electrode

configuration including an Ag/AgCl reference electrode and a Pt counter electrode while the Au coated Kapton was used as the working electrode. The electropolymerization was performed in an aqueous solution using a potentiostat (Autolab PGstat128N, MetroOhm). The deposition lasted for 5 minutes (potentiostatic mode at 1 V), optimized to develop the best performing electrode (Figure S8). The CG coated electrodes were finally immersed in DI water to remove excess unreacted small molecules and then dried with N₂ gas.

Compositional and surface characterization: X-ray photoelectron spectroscopy (XPS) measurements were performed using AMICUS/ESCA 3400 KRATOS instrument equipped with achromatic Al K α X-ray source (1468.6 eV). The source was operated at voltage of 10 kV with 10 mA current generating a power of 100 Watts. The elemental narrow scan region was acquired using a step of 0.1 eV. The pressure in the analysis chamber was in the range of 10⁻⁷ Pa during the course of the measurement. The obtained spectra were calibrated to reference of C 1s at 284.8 eV. The spectra were deconvoluted using Gaussian and Lorentzian methods and background subtraction was carried out by Tougaard method. Fourier transform infrared (FTIR) spectra were recorded in the range 500-4000 cm⁻¹ using Thermo Scientific Nicolet iS10. We used a scanning electron microscope (SEM) (NovaNano) for morphological analysis of the films. Atomic force microscopy (AFM) measurements were conducted with an Agilent 5500 instrument equipped with a liquid cell. The films were prepared on sputtered glass/Ti/Au substrates and mounted on the liquid cell. All the measurements performed in tapping mode with Veeco MULTI40A at k=0.9Nm⁻¹ using f₀ 33-50 kHz AFM probes. The thickness of the gels was measured using a mechanical profilometer (Dektak 8 Advanced Development Profiler, Veeco) with a scan step of 33.33 μ m s⁻¹.

Quartz crystal microbalance with dissipation monitoring (QCM-D): We performed QCM-D measurements using a Q-sense analyzer (QE401, Biolin Scientific). CG was electropolymerized

on QCM-D crystals using Autolab PGstat128N potentiostat coupled with Q-sense electrochemistry module as described above. The three-electrode setup was comprised of Ag/AgCl reference, Pt counter and the QCM-D sensor as the working electrode. The measurements were carried out at 24°C with a flow rate of 50 μLmin^{-1} controlled by a peristaltic pump. After the stabilization of QMC-D signals in PBS, the change in frequency (Δf) and dissipation (ΔD), we injected aliquots of glucose solutions into the chamber and monitored the glucose uptake, i.e., changes in Δf and ΔD signals. To quantify the mass added on the sensor (Δm) during glucose uptake, we used the Sauerbrey equation:

$$\Delta m = \frac{-17.7}{n} \Delta f_n \quad (2)$$

where n is the number of the overtone selected for the calculations and -17.7 is a constant calculated based on the resonant frequency, active area, density and shear modulus of the piezoelectrically active quartz crystal.

Electrochemical measurements: Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and chronoamperometry measurements were performed in a standard electrochemical cell using Ag/AgCl reference electrode and Pt counter electrode and a potentiostat (Autolab PGstat128N, MetroOhm). All the electrochemical measurements took place in cell inside a grounded Faraday cage. The electrolyte was PBS and we added different concentrations glucose into this solution (1, 4 and 10 mM) while maintaining its volume. CV curves were recorded by sweeping the potential between -0.3 to 1 V vs. Ag/AgCl using a scan rate of 100 mVs^{-1} . Impedance spectra were recorded at a DC voltage of 0 V vs. open circuit potential (V_{oc}) and an AC modulation of 10mV over a frequency range of 0.1-10000 Hz. For chronoamperometry measurements, we applied a voltage pulse of -0.2 V vs V_{oc} for 60 s and measured current in the absence and presence of glucose. Each measurement was conducted by subsequent additions of increasing concentrations of glucose solutions after a steady current in

PBS was recorded. We determined the read-out signal in the absence of glucose (I_0) by the steady, background current obtained in PBS solution. The device response was measured at different time points upon addition of different concentrations of glucose (I_t) and subtracted from the baseline current (I_0). The device performance was then normalized according to the following equation:

$$NR = \frac{I_t - I_0}{I_0} \quad (3)$$

The limit of detection (LOD) was calculated using a formula of $LOD = (3 \cdot SB)/m$,^[57] where the SB is standard deviation of the blank sample of PBS with 0 mM glucose and m is the slope from calibration curve.

Fabrication and operation of the OECT as glucose sensor: OECTs were fabricated photolithographically using a parylene-C peel-off method, as described previously.^[58,59] The resulting device structure is shown in Figure 5a, with the defined channel width (W) and length (L), while the lateral gate electrode has an area of $500 \mu\text{m} \times 500 \mu\text{m}$. PEDOT:PSS (PH 1000) was mixed with EG (5 vol%), DBSA (0.002 vol%) and GOPS (1 wt%) and sonicated for 30 min. The dispersion was then filtered through $1 \mu\text{m}$ glass fiber filters and spin coated on the channel at 3000 rpm for 30 sec. After spin-coating, the sacrificial Parylene-C layer was peeled off and the films were annealed at 140°C for 1 hour in ambient conditions. The Au gate electrode was then modified with the CG prepared identically as described above. All measurements were performed using the lateral gel coated electrode as the gate electrode and PBS solution (with and without glucose) as the electrolyte. The steady-state measurements of the OECT (output and transfer curves) and the pulsed gate voltage experiments were performed using a Keithley 2612A with customized LabVIEW software. The normalized response of OECT channel current was calculated using equation 3. For the chronoamperometric measurements and

obtaining calibration curves, the transistors were operated at a constant V_D and V_G and current outputs were simultaneously recorded during additions of glucose.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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This work demonstrates, for the first time, an all-polymer based, non-enzymatic, glucose sensitive electroactive material. The conducting gel is fabricated via a single-step electropolymerization and employed either as an amperometric sensor or as a micrometer-scale gate electrode of an organic electrochemical transistor. At both configurations, this hybrid material offers selective and sensitive electrical sensing performance at low operation voltages.

Organic electrochemical transistors, conductive gels, PEDOT:PSS, phenylboronic acid, glucose sensors

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Enzyme-free Detection of Glucose with a Hybrid Conductive Gel Electrode