

# Porous Enzymatic Membrane for Nanotextured Glucose Sweat Sensors with High Stability toward Reliable Noninvasive Health Monitoring

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Development of reliable glucose sensors for noninvasive monitoring without interruption or limiting users' mobility is highly desirable, especially for diabetes diagnostics, which requires routine/long-term monitoring. However, their applications are largely limited by the relatively poor stability. Herein, a porous membrane is synthesized for effective enzyme immobilization and it is robustly anchored to the modified nanotextured electrode solid contacts, so as to realize glucose sensors with significantly enhanced sensing stability and mechanical robustness. To the best of our knowledge, this is the first report of utilizing such nanoporous membranes for electrochemical sensor applications, which eliminates enzyme escape and provides a sufficient surface area for molecular/ion diffusion and interactions, thus ensuring the sustainable catalytic activities of the sensors and generating reliable measurable signals during noninvasive monitoring. The as-assembled nanostructured glucose sensors demonstrate reliable long-term stable monitoring with a minimal response drift for up to 20 h, which delivers a remarkable enhancement. Moreover, they can be integrated into a microfluidic sensing patch for noninvasive sweat glucose monitoring. The as-synthesized nanostructured glucose sensors with remarkable stability can inspire developments of various enzymatic biosensors for reliable noninvasive composition analysis and their ultimate applications in predictive clinical diagnostics, personalized health-care monitoring, and chronic diseases management.

## 1. Introduction

The increasing demand for predictive clinical diagnosis and personalized health care has been attracting research efforts on developing reliable sensors on various sensing platforms (e.g., wearable bands, tattoos, and textile) in the past decades to tackle the challenges with point-of-care measurements and analysis.<sup>[1–5]</sup> Due to the unique properties of desirable selectivity, high sensitivity, and ease of device miniaturization, electrochemical sensors that convert the detected concentrations of analytes through chemical redox reactions into measurable electrical signals have been explored for a wide variety of biomarkers including metabolites, proteins, ions, and heavy metal.<sup>[6–8]</sup> Among them, glucose in human fluid is a significant biomarker for diabetes diagnostics, which is one of the most prevalent diseases around the world.<sup>[9,10]</sup> However, the commercially available glucose monitors are still operated in an invasive manner and mostly require drawing blood from patients, which introduce inconvenience, pain, and potential microbial infection to

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diabetic patients who need to have glucose level monitored routinely.<sup>[9,11]</sup> In this regard, noninvasive glucose sensors provide a painless option for continuous and long-term monitoring without compromising the patients' compliance, which is highly desirable.<sup>[4,6]</sup> Body fluids, such as interstitial fluid, sweat, saliva, and tears, also contain a wealth of biomarkers, and provide distinct capability to be extracted and analyzed in a non-invasive/minimal invasive manner, thus become alternatives to blood to achieve insightful physiological information for personalized health care.<sup>[12,13]</sup> Among a variety of body fluids, sweat contains abundant analytes and provides the distinct advantage of continuous sampling on various sites, which make it a feasible and ideal extracted fluid for noninvasive monitoring.<sup>[12,14]</sup> However, due to the limitation on the sensor stability and reliability, there is still a lack of conclusive/verified results to reveal the correlations between glucose level in blood and sweat, while there are a few studies showing the possible correlations.<sup>[15–18]</sup> For instance, the most well-developed glucose sensors are enzyme-based electrochemical sensors with much superior selectivity and sensitivity compared with enzyme-free sensors, which also suffer from surface poisoning by intermediates/chloride ions that leads to unsatisfying sensing reliability.<sup>[6,9,19]</sup> However, the determination of glucose level by enzyme-based sensors can be affected with the immobility of enzymes and their catalytic activities, especially for noninvasive measurements that involve interferences from surrounding factors (e.g., temperature, pH values, and mechanical frictions).<sup>[20,21]</sup> While progressive attempts have also been made to develop a variety of nanomaterials (e.g., metal oxides and carbon nanotubes) for fabrication of glucose sensors, it is still challenging to achieve reliable sensing without loss of sensitivity and selectivity for noninvasive sensors.<sup>[6,20]</sup> Therefore, developing glucose sensors with superior reproducibility and sensing stability for reliable noninvasive monitoring is imperatively required especially in the field of predictive diagnostic and routine monitoring of diabetes.<sup>[9,22]</sup>

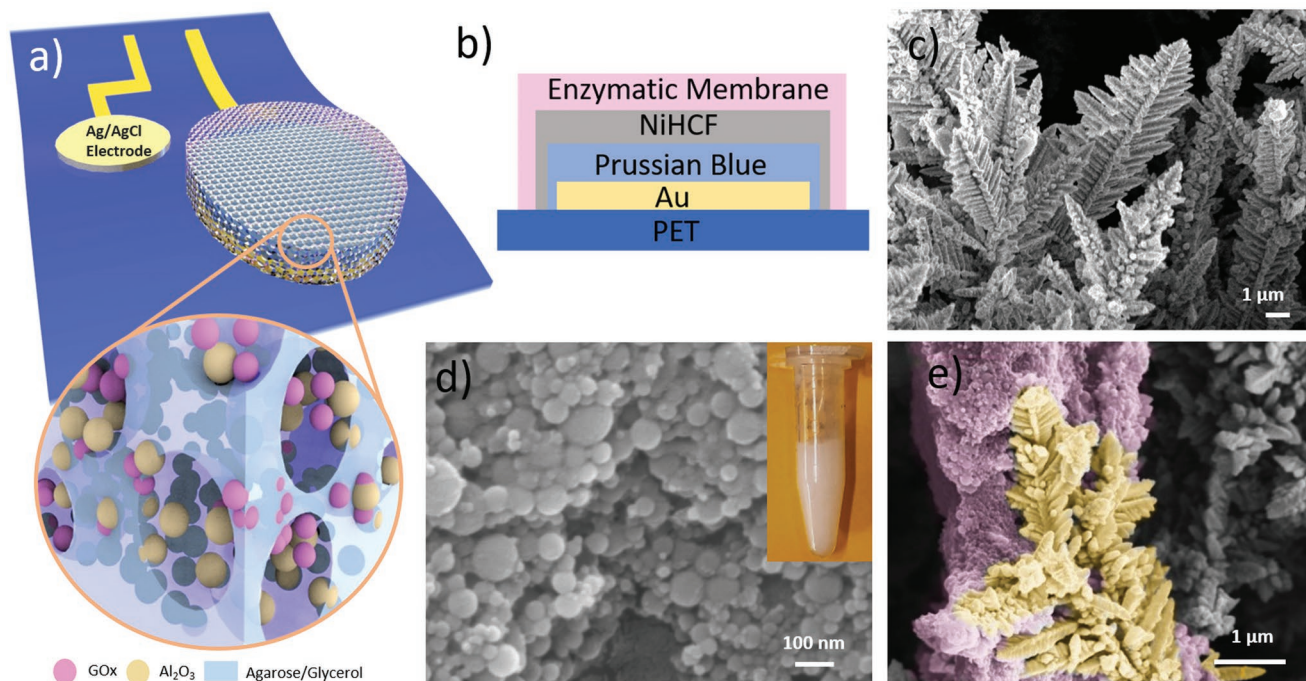
Herein, a porous membrane for effective enzyme immobilization is utilized in conjunction with modified nanotextured electrode solid contacts to achieve glucose sensors with largely enhanced sensing stability for noninvasive monitoring. The nanoporous structured enzymatic membranes provide scaffolds that eliminate enzymes escaping directly into measured fluids and greatly increase surface area for molecular/ion interactions.<sup>[23,24]</sup> Thus they ensure the sustainable catalytic activities of the sensors and generate reliable measurable signals. To the best of our knowledge, it is the first demonstration to adopt such nanoporous membranes for electrochemical sensor applications. Furthermore, the solid contacts of sensor electrodes are texturized with dendritic nanostructures that serve as anchors to the porous enzymatic membranes and achieve mechanical robustness of the sensor devices. Compared with planar electrodes, these nanotextured electrodes with large surface area also reduce potential drifts on redox peaks due to electrolyte concentration variations.<sup>[25,26]</sup> The as-assembled nanostructured glucose sensors demonstrate reliable cyclic sensitivity and long-term stable monitoring with minimal response drift for up to 20 h, which deliver a significant enhancement compared with most of the reported sensor operational time in a few hours. The sensing stability of the as-fabricated noninvasive

sweat glucose sensors is also demonstrated based on a microfluidic sensing patch with sweat extracted via an iontophoresis approach. The strategy of integrating nanostructures into electrochemical sensor designs, especially the nanoporous enzymatic membranes developed in this work, provides an innovative methodology to realize a variety of stable and reliable electrochemical sensors geared toward reliable noninvasive physiological monitoring.

## 2. Results

The device architecture of the as-fabricated glucose sensor for amperometric noninvasive monitoring is schematically shown in **Figure 1a,b**. Briefly, the enzymatic glucose sensing electrode is coupled with a Ag/AgCl electrode that serves as the reference/counter electrode to form the sensor device, and such a two-electrode system is a common strategy for low-current electrochemical sensing.<sup>[1]</sup> Typically, in the presence of glucose oxidase (GOx) as enzyme and oxygen, glucose in the fluid can be selectively oxidized into gluconolactone and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>[22]</sup> Though H<sub>2</sub>O<sub>2</sub> as an oxidizer can release free electrons to go through conductors and generate electrical responses, it could also introduce polarization on the conductors and leads to significant drift on response signals.<sup>[26]</sup> Therefore, a layer of ferric ferrocyanide, which is also known as Prussian blue (PB), is generally adopted as ion-to-electron transducer.<sup>[6,18]</sup> The PB layer involves in the redox reaction with H<sub>2</sub>O<sub>2</sub>, and the glucose level in the fluid can then be identified with the amplitude of the generated currents. As the reduction potentials of PB in glucose solution is close to 0 V, external power sources for the glucose sensors can be eliminated and interference from potential bias can be reduced as well.<sup>[27]</sup> The fabrication of the glucose sensor electrode starts from texturizing the electron conductors (e.g., thermal evaporated gold (Au) film and printed conductive carbon) with Au dendritic nanostructures obtained from an overpotential electrochemical deposition method.<sup>[28]</sup> Afterward, PB as redox active material and nickel hexacyanoferrate (NiHCF) as stabilizing layer to PB are conformably deposited on the nanotextured conductor, respectively, as shown in **Figure 1c** and **Figure S1a** (Supporting Information). To realize effective immobilization and trapping of enzyme, an emulsion that contains agarose, glycerol, and aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) nanoparticles to create porous scaffold is mixed with GOx (inset photo of **Figure 1d**) and nanoporous enzymatic membranes can form on the nanotextured electrodes by direct drop casting (**Figure 1d**). Note that Al<sub>2</sub>O<sub>3</sub> provides high isoelectric point that is favorable for GOx immobilization.<sup>[6]</sup> As shown in the colored scanning electron microscope (SEM) images in **Figure 1e** and **Figure S1b–f** (Supporting Information), the nanotextured electrodes serve as anchors to the porous membranes, which contributes to the remarkable enhancement on sensor mechanical robustness and sensing stability. For comparison, the SEM image of bulk agarose enzymatic membrane on nanotextured electrode is shown in **Figure S2** (Supporting Information).

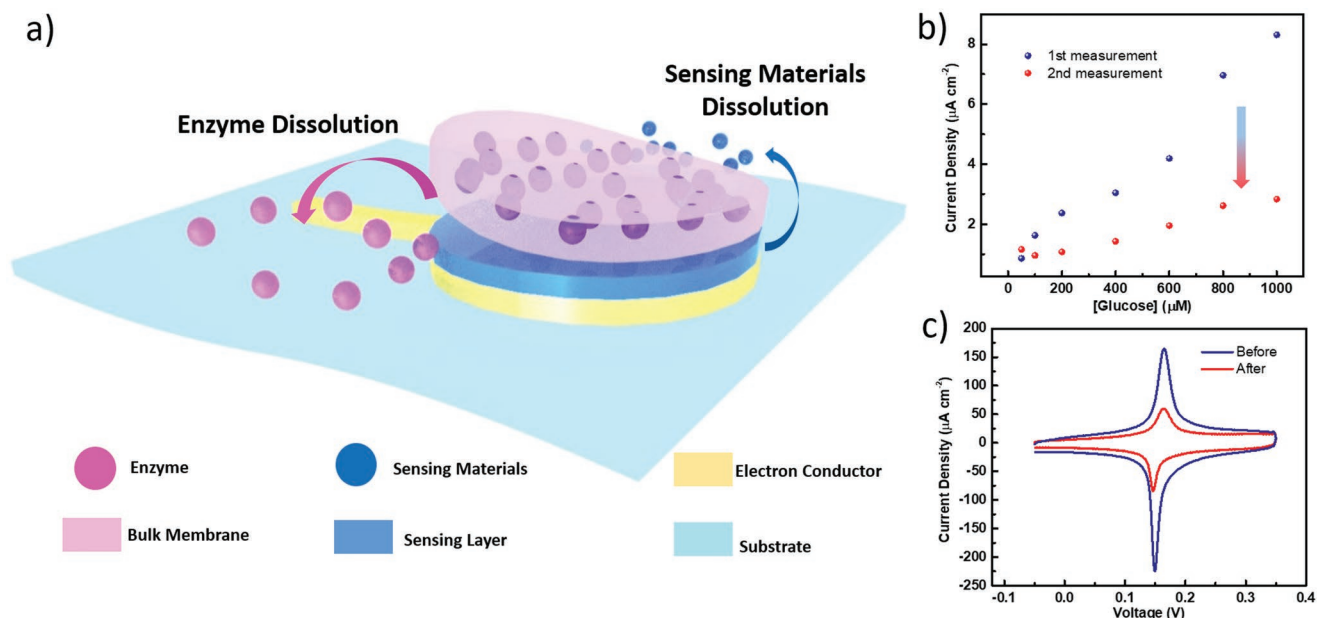
The as-developed strategy to realize stable sensing aims at tackling the challenge on sensing stability for conventional glucose sensors. As shown in **Figure 2a**, the most commonly



**Figure 1.** a) Schematic illustration of nanotextured glucose sensors with porous enzymatic membrane. b) Cross-section schematics of the working electrode. SEM images of c) nanotextured NiHCF/PB/Au electrode. d) Porous membranes (inset: photo of the membrane emulsion). e) Porous membrane anchored to the nanotextured electrode.

reported enzyme-based glucose sensors are mainly based on planar electrodes and GOx immobilized within permeable films, such as chitosan and agarose bulk membranes.<sup>[1,29]</sup> However, it has been discovered that such glucose sensors suffer from degraded amperometric responses and observable

drift within several hours of operation.<sup>[18,21]</sup> Specifically, with refreshed glucose electrolytes, significant decrease in the sensitivities and response current densities is observed on sensors with different electron conductors (i.e., Au and printed carbon), as shown in Figure 2b and Figure S3a–c (Supporting



**Figure 2.** Challenges for stable and reliable noninvasive glucose monitoring based on conventional sensors with planar electrodes and bulk enzymatic membranes. a) Schematic illustration of possible factors attribute to the instability of conventional glucose sensors based on planar electrodes and bulk enzymatic membranes. b) Significantly decreased sensitivity within two measurement cycles. c) CV curves of PB electrode before and after emerging in DI water for 1 min.

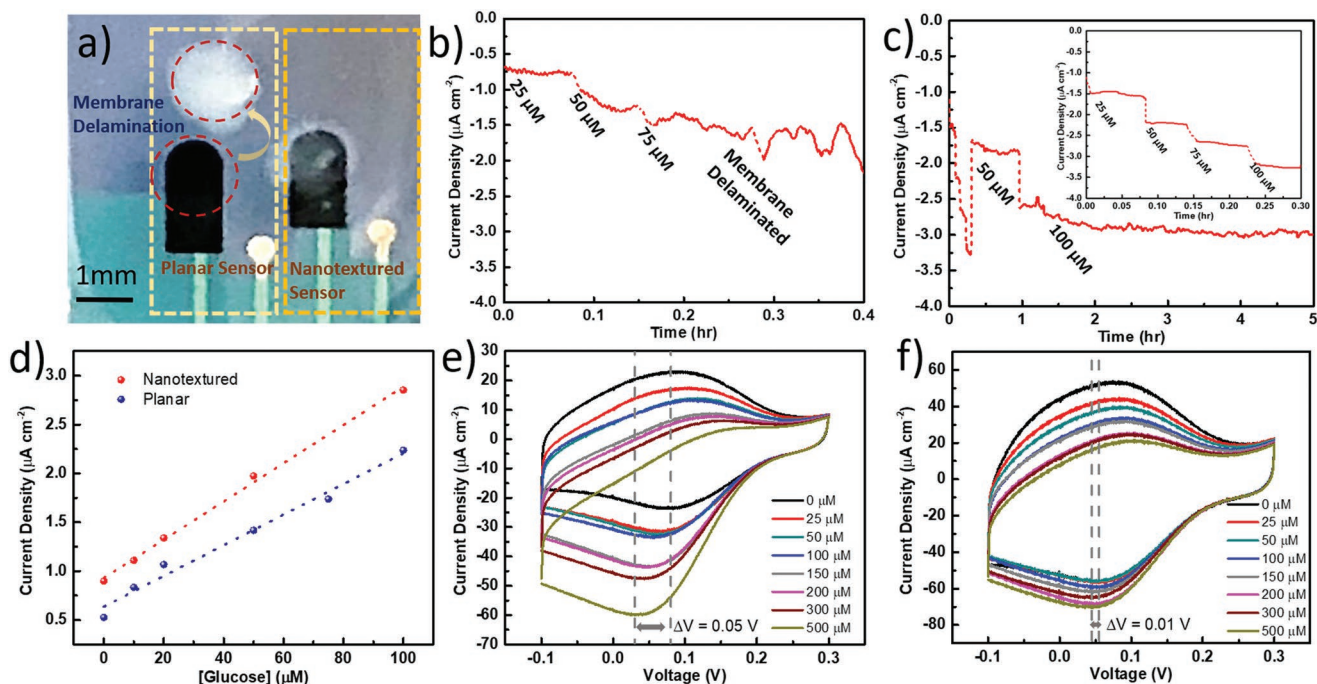


Information). The sensitivities remain mostly stable when the concentrations of glucose electrolytes are manually tuned by adding concentrated glucose or phosphate-buffered saline (PBS) buffer solution into the original electrolyte solutions. However, large variations of sensitivity can be observed after replacing the original electrolytes with refreshed ones. Therefore, it indicates that the dissolution of active materials, including enzymes and PB, into the flowing or refreshed fluids bears a significant responsibility for the degradation on sensing performances, as illustrated in Figure 2a. On the one hand, it is highly possible that the GOx immobilized on the surface of bulk membranes can be washed away while those inside the bulk membranes are less accessible to the glucose electrolytes, together with the decreased catalytic activities of enzymes in an ambient environment, which results in the decreased oxidation of glucose and less  $\text{H}_2\text{O}_2$  generation.<sup>[19]</sup> Thus, the redox reaction will be less active and generates decreased amperometric response. Therefore, effective trapping of enzymes within the membranes and sufficient electrolyte ion contact are essential for reliable glucose sensing. As indicated from the amperometric responses of glucose sensors with porous membrane on planar thin film electrodes (Figure S3d, Supporting Information), the sensitivity remains stable during cyclic variation of glucose concentrations with refreshed electrolytes, which confirms the enhancement on sensing stability with the as-fabricated nanoporous enzymatic membranes, and a desirable sensor reproducibility can also be achieved (Figure S3e, Supporting Information). On the other hand, while PB delivers superior catalytic and selective redox capability with  $\text{H}_2\text{O}_2$  (both of which are three orders of magnitude higher than platinum, which is also commonly adopted for clinical applications),<sup>[27,30]</sup> it suffers from inherent instability in aqueous solutions and the decreased amount of PB on electrodes can be indicated by its largely reduced redox peak heights in the cyclic voltammograms (CVs) after immersion in deionized (DI) water for 1 min (Figure 2c). The decreased amount of PB as ion-to-electron transducer on electrode will significantly affect the redox reaction rate and generates unstable/decreased response currents. It has been reported that NiHCF, which is isostructural to PB while chemically stable and catalytically inactive, can be a promising candidate to serve as stabilizing layer without interfering the redox reactions.<sup>[27,30,31]</sup> The  $\text{H}_2\text{O}_2$  sensors based on the NiHCF modified PB electrodes maintain the sensitivity with refreshed testing solutions (Figure S4a, Supporting Information), which is rarely achieved on sensors with bare PB. The effectiveness of stabilization of PB with NiHCF layer is also demonstrated with the CV curves of glucose sensors with/without NiHCF layers in a supporting electrolyte containing 0.1 M KCl and 0.1 M HCl after 20 h long-term measurements (Figure S4b, Supporting Information). The sensor with NiHCF delivers the typical PB redox peaks, while the sensors without such stabilizing layer showed significant decrease trend of PB. With such a NiHCF stabilizing layer, the deterioration from the instability of PB layer can be eliminated and the as-fabricated glucose sensors demonstrate stable amperometric response for over 9 h long-term measurements in constant glucose solutions, compared to the fluctuated and decreased currents from the ones without NiHCF, as shown in Figure S4c (Supporting Information).

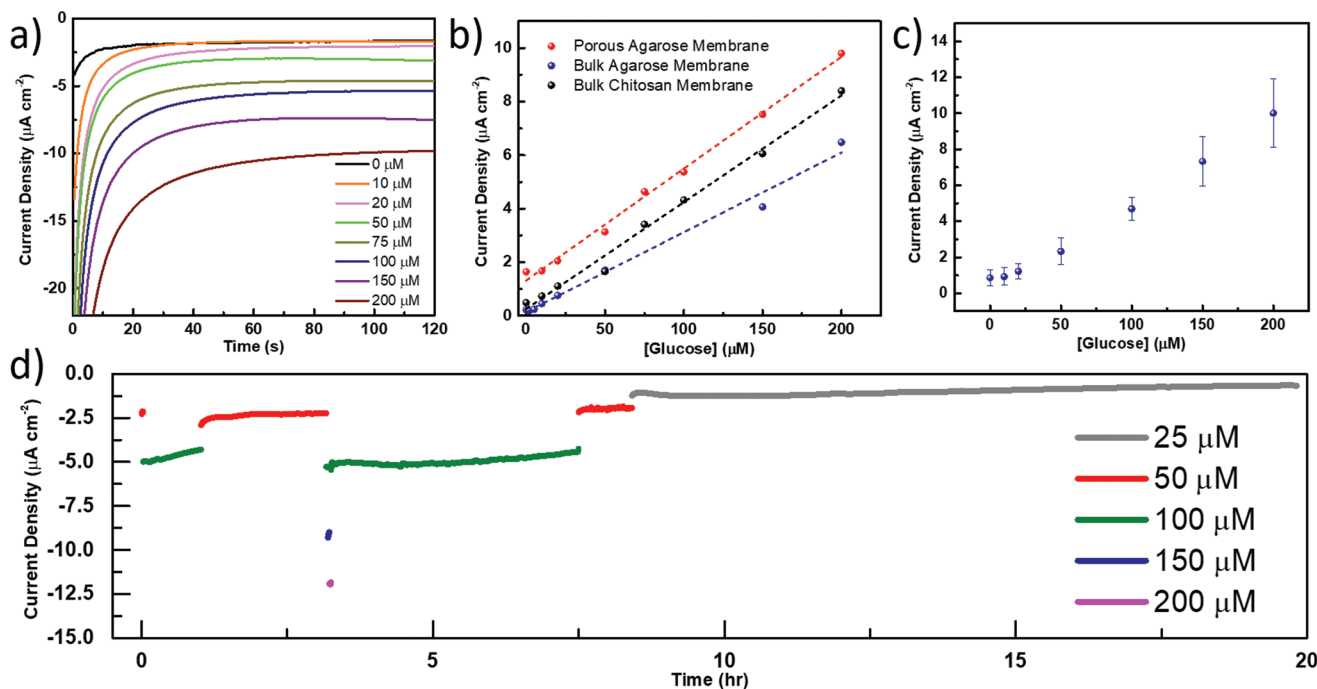
While the nanoporous enzymatic membranes introduce improvement on sensing stability, it is observed that such porous enzymatic membranes consisting of nanoparticles have less adhesion to electrodes compared with the conventional chitosan/agarose bulk membranes. As shown in Figure 3a, membranes are prone to delaminate from the planar electrodes with disturbance in the testing electrolytes and result in obvious response fluctuation (Figure 3b), which can be ascribed to the interfered catalytic activity and ion transfer. Therefore, enhanced adhesion between the porous enzymatic membranes and electrode solid contacts is crucial to achieve stable sensing performance. As shown in Figure 3a, the adhesion of the porous enzymatic membrane onto the electrodes is effectively improved with the nanotextured electrodes and the as-fabricated sensors delivered stable amperometric responses in glucose solutions with constant or dynamically tuned concentrations for several hours, as shown in Figure 3c.

Apart from serving as the anchors to the porous membranes to achieve mechanical robustness of the sensors, the nanostructured electrodes with conformal active materials deposition also deliver enhanced electrochemical properties. As shown in Figure S5a (Supporting Information), the largely increased area enclosed by CV curves indicates the enlarged electrochemically active surface area compared with the planar ones. Moreover, reduced intrinsic impedance and facilitated ion diffusion can also be indicated with the smaller intercept and higher slope in the electrochemical impedance spectrum (EIS) measurements (Figure S5b, Supporting Information). Therefore, the ion-to-electron transduction is promoted and contributes to a larger response current density as shown in Figure 3e. Besides, owing to the nature of capacitive currents during the redox reaction, especially with large current density in high concentration electrolytes, charge accumulation on the limited interface areas hinders effective ion-to-electron transduction and results in a built-in potential at the electrode interfaces and response drift.<sup>[26]</sup> As shown in Figure 3f, a PB reduction peak shift of 0.05 V is observed on the planar sensors with glucose concentration increased from 0 to  $500 \times 10^{-6}$  M, while it is suppressed to less than 0.01 V with the dendritic nanostructures, as shown in Figure 3g. Meanwhile, even larger peak shifts are observed on sensor electrodes with intentionally separated enzymatic membranes (Figure S5c,d, Supporting Information), which also confirms the deterioration on sensing performance resulted from the delamination of membranes. In this regard, the nanostructures contribute to both the mechanical and electrochemical robustness of the glucose sensors, and coherently enhance the sensing stability with the nanoporous enzymatic membranes.

The performance of the as-fabricated nanostructured glucose sensors, with nanoporous enzymatic membranes firmly anchored onto nanotextured electrodes, is systematically characterized in vitro. The sensing responses are measured chronoamperometrically in PBS-based  $0\text{--}200 \times 10^{-6}$  M glucose solutions with remarkable sensing limit down to  $10 \times 10^{-6}$  M, as shown in Figure 4a. A linear relationship is extracted between current density and analytic concentrations delivering a sensitivity of  $41.8 \text{ nA } \mu\text{M}^{-1} \text{ cm}^{-2}$ , which is in the same range of the sensors with bulk agarose ( $39.9 \text{ nA } \mu\text{M}^{-1} \text{ cm}^{-2}$ ) and chitosan ( $40.9 \text{ nA } \mu\text{M}^{-1} \text{ cm}^{-2}$ ) membranes (Figure S6 of the Supporting



**Figure 3.** Performance enhancement of nanostructured glucose sensors with porous enzymatic membranes. a) Photos showing delamination of porous enzymatic membrane from planar electrode (left) after 1 h measurement and enhanced adhesion to nanotextured electrode (right). Amperometric response of b) planar glucose sensors with membrane delamination occurs during measurements and c) nanotextured glucose sensors without membrane delamination (inset: focused amperometric response in the first 20 min). d) Sensitivities of planar and nanotextured glucose sensors. CV curves of e) planar and f) nanotextured glucose sensors in different concentrations of glucose/PBS buffer solutions at the scan rate of  $100 \text{ mV s}^{-1}$ .

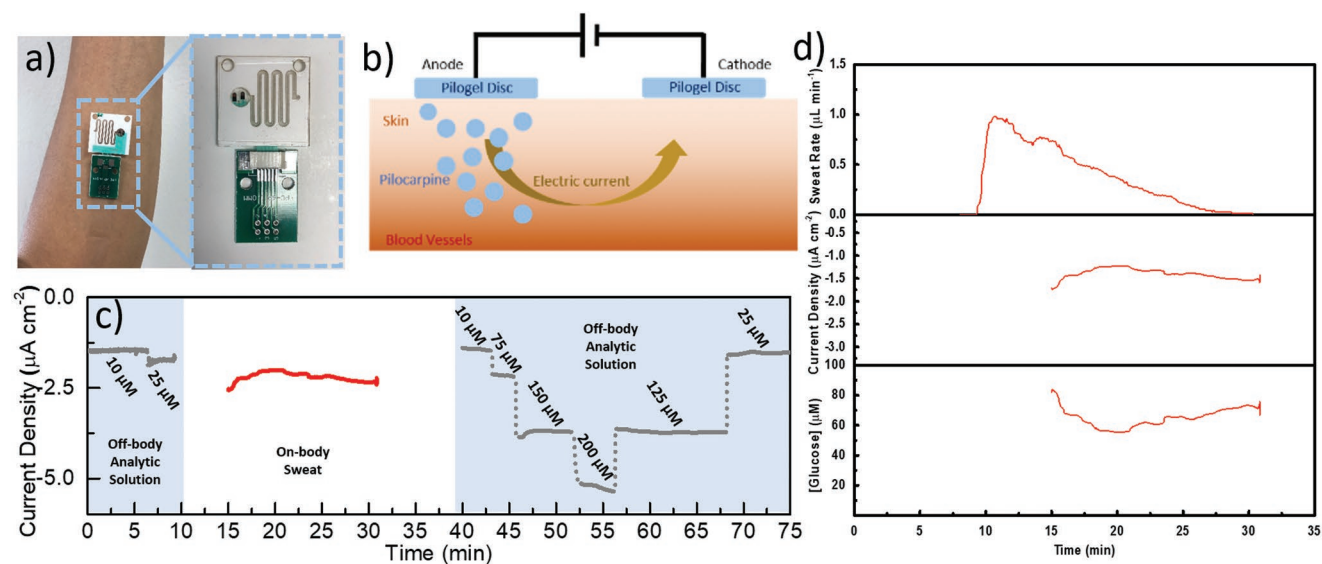


**Figure 4.** Performance evaluation of as-fabricated nanostructured glucose sensors. a) Amperometric responses of glucose sensor based on printed carbon/nanotextured Au/PB/NiHCF/porous enzymatic membrane. b) Sensitivity of glucose sensors with different membranes. c) Reproducibility of the as-fabricated nanostructured glucose sensors (six samples). d) 20 h long-term measurement of the as-fabricated nanostructured glucose sensors.

Information, and Figure 4b). Such results indicate that introducing the  $\text{Al}_2\text{O}_3$  nanoparticles and glycerol as additives in agarose to create porosity in enzymatic membranes introduces negligible interference to the sensor sensitivities.<sup>[32,33]</sup> Moreover, the remarkable reproducibility of the developed fabrication process for nanostructured sensors with nanoporous enzymatic membranes is demonstrated as shown in Figure 4c. Notably, the as-fabricated nanostructured glucose sensors demonstrate reliable amperometric response in the glucose solutions with dynamically tuned or constant concentrations and showed negligible current drift (Figure S7a–c, Supporting Information). More significantly, a 20 h long-term sensing stability with reversible sensitivity in refreshed glucose sensors is recorded and such result is also repeatable as shown in Figure 4d and Figure S7d (Supporting Information). Due to the fact that long-term stability has been a critical challenge for electrochemical glucose sensors, especially for noninvasive sensors, such continuous measurements of up to 20 h have rarely been seen before. Note that current drift can still be observed, which is a natural outcome of possible decrease in local concentration of glucose, loss of enzyme activity during long-term measurement in ambient. However, it is verified that the sensors are capable to generate currents in similar level with refreshed standard glucose solutions, which proves the sensor sensitivity is well retained. And it indicates the effectiveness of stabilization of active materials, including enzyme and PB, as well as the enhanced mechanical robustness without membrane delamination, based on the rationally designed nanostructures.

The feasibility of using the as-fabricated nanostructured glucose sensors for wearable applications is demonstrated by integrating them onto a printable sensing patch. Such a sensing patch can be conveniently and conformally attached on various body parts, including the wrists, arms (Figure 5a), and forehead. Sweat sampling is realized with assistance of iontophoresis approach as illustrated in Figure 5b, in which

pilocarpine is delivered across skin by applying a small current, and stimulates the sweat glands to induce sweat.<sup>[2,18]</sup> The as-extracted sweat can fill into the reservoir embedded with the as-fabricated glucose sensors and generates amperometric responses. Driven by the pressure from sweat perspiration and capillary force, the sweat further transports along the microfluidic channels embedded with a pair of Ag lines as electrical impedance sweat rate sensor, enabling continuous and dynamic assessment on freshly extracted sweat.<sup>[34]</sup> The generated signals can be wirelessly transmitted to a Bluetooth-enabled mobile handset with combination of a transceiver circuit board, which demonstrated its potential applications for point-of-care monitoring. As shown in Figure 5c, the as-fabricated sensors allow simultaneous measurements in analytic glucose solutions as precalibration/postcalibration, and in situ analysis of sweat glucose for around 20–30 min with effective sweat collection. The reliability of such sweat glucose monitoring is indicated with no obvious degradation on sensitivity in the postcalibration process compared with the precalibration values. Note that a detection error in the range of around  $20\text{--}25 \times 10^{-6} \text{ M}$  could be observed, which might come from both operational disturbance and detection variations. As displayed in Figure 5d, the measured sweat glucose level is first observed to experience a slight decrease, which could be attributed to the dilution effect caused by the increased sweat amount in the reservoir.<sup>[1]</sup> To achieve a comprehensive physiological information via such noninvasive sweat sensing, research efforts to reveal the correlation between sweat glucose and blood glucose level are still required. For instance, there can be significant variations on the glucose levels and sweat rates among different subjects (Figure S8, Supporting Information).<sup>[35]</sup> Besides, the effect of sweat rate variations and interferences during monitoring, including stress/pressure from physical contacts, has not been verified.<sup>[11]</sup> Future work can couple the as-fabricated nanostructured glucose



**Figure 5.** Demonstration of on-body sweat glucose monitoring. a) Nanostructured glucose sensors integrated on a sensing patch with microfluidic channels and Bluetooth module. b) Schematic illustration of iontophoresis process. c) On-body sweat glucose measurement with pre/postcalibration in analytic glucose solutions. d) Measured sweat rate pattern and sweat glucose concentrations.



sensors with pH and temperature sensors into integrated sensing patch for more accurate calibration and population clinical investigation.

### 3. Conclusions

Despite the significant advances on perspiration biosensors, intensive research efforts are still required to explore sensors that are suitable for reliable long-term and real-time assessment of the human body physiological state and to realize point-of-care monitoring.<sup>[12,15]</sup> It holds significance to tackle the challenge on growing aging population that poses heavy stress on the limited clinical resources.<sup>[10]</sup> Among a variety of biosensors, the development of reliable glucose sensors has been the subject of concerns due to its crucial applications in clinical investigation, especially for diabetes diagnostic. Currently, glucose level is mainly determined within blood or urine, which is complicated and inconvenient for routine measurements as it is essential to determine the treatment effectiveness and prevent diabetic emergencies for patients. While glucose monitoring without interruption or limiting the user's mobility is highly desirable, commercially available noninvasive glucose sensors can be rarely found, mainly due to the challenge of instability issues. In this work, we successfully developed nanostructured glucose sensors with highly enhanced performance stability for noninvasive monitoring. The immobilization and trapping of enzymes onto the sensors are effectively improved with dedicated fabricated nanoporous membranes and the large porosity of the enzymatic membranes provides highly increased surface area for interactions with molecular/ion in fluids. Meanwhile, the sensor electrodes are texturized with dendritic nanostructures to prevent delamination of the enzymatic membranes and reduce potential drifts on redox potential by charge accumulation effects. The as-assembled glucose sensors demonstrate the capability for long-term stable monitoring with minimal response signal drift for up to 20 h, which is rarely reported for noninvasive enzyme-based glucose sensors. The integration of the as-fabricated nanostructured glucose sensors into a printable and wearable sensing patch incorporated with wireless transmission module further demonstrates its potency in practical applications for point-of-care monitoring. And it provides a highly attractive tool for future population study so as to obtain insight understanding of correlations between sweat glucose concentrations/sweat rate patterns and physiological state of human body. The approach to create nanostructured sensors with stability enhancement on noninvasive glucose monitoring demonstrated in this work can also be exploited for a variety of biosensors to realize reliable noninvasive composition analysis, which is imperatively demanded especially for predictive clinical diagnostics, personalized health-care monitoring, and chronic diseases management.

### 4. Experimental Section

*Chemicals and Commercial Electronics:* All the chemicals were reagent grade and used as received.

*Fabrication of Nanostructured Glucose Sensors:* The sensors can be fabricated on both Au and carbon-based electrodes. The fabrication process of Au electrodes on polyethylene terephthalate (PET) substrates was the same as in our previously reported work.<sup>[2]</sup> The carbon electrodes were fabricated by roll-to-roll printing and integrated into sensing patches.<sup>[29]</sup> The functionalization of the electrodes was realized via electrochemical depositions, conducted in a three-electrode configuration using a potentiostat (PCI4G300, Gamry Instruments). The electrolyte for Au dendritic nanostructures growth was a mixture of  $50 \times 10^{-3}$  M H<sub>2</sub>AuCl<sub>4</sub> and  $50 \times 10^{-3}$  M HCl. The deposition was conducted by applying a periodic voltage wave with amplitude of  $-2$  V, frequency of 50 Hz, and duty cycle of 50% in gold plating solution for 60 cycles, leading to formation of around 20  $\mu$ m Au dendritic nanostructures. For comparison, the planar Au film was also fabricated via a similar method under a periodic voltage wave with amplitude of  $-0.8$  V. PB deposition was realized under a periodic voltage wave with amplitude of 0.5 V, frequency of 1 Hz, and duty cycle of 10% for 60 cycles in a fresh electrolyte containing  $2.5 \times 10^{-3}$  M FeCl<sub>3</sub> and  $2.5 \times 10^{-3}$  M K<sub>3</sub>[Fe(CN)<sub>6</sub>] in the supporting solution. The supporting solution is a mixture of 0.1 M KCl and 0.1 M HCl.<sup>[30,36]</sup> NiHCF layer was then deposited via CV scanning deposition with a potential window of 0–0.8 V at a scan rate of 100 mV s<sup>-1</sup> for ten cycles in a fresh electrolyte containing  $1 \times 10^{-3}$  M Ni(NO<sub>3</sub>)<sub>2</sub> and  $0.5 \times 10^{-3}$  M K<sub>3</sub>[Fe(CN)<sub>6</sub>] in 1 M KCl. To prepare porous enzymatic membranes, 1 mg Al<sub>2</sub>O<sub>3</sub> nanoparticles were dissolve in 100 mg mixture of agarose/glycerol/DI water (1.5:95 w/w/w) and kept in 80 °C for 6 h and using vortex mixer to improve the uniformity every 1 h. Emulsions for bulk agarose membranes contain 1 wt% agarose, and 1 wt% chitosan solution with 2% acetic acid was prepared for bulk chitosan membranes. 1 mg GOx powder was first dissolved in 100  $\mu$ L PBS (pH 7.2), and the as-prepared enzymatic solution was mixed with membrane emulsion in a volume ratio of 1:2. 1  $\mu$ L of the emulsion was then drop-cast onto the electrode and dried in the ambient environment. The solutions/emulsions were stored at 4 °C when not in use.

*Fabrication of Biosensing Patch:* Three-electrode configuration sensor was manufactured by roll-to-roll rotary silk screen printing. PET substrate (Melinex ST506 125  $\mu$ m) was run through 4 m long, heated (140 °C) ovens with speed of 2 m min<sup>-1</sup> to prevent substrate shrinkage during the process. Four layers were printed including (I) lower silver wiring, (II) carbon electrodes, (III) dielectric layer, and (IV) upper silver wiring. The alignment between these layers (I–IV) was achieved with a camera-assisted module on the printing line. Printed silver and carbon inks were cured in ovens while dielectric material was UV-cured. The microfluidic channels were fabricated by cutting through a double-sided adhesive tape (3M 9965) with 355 nm UV-laser (Master OEM laser system DPL 015\_3, Elas Ltd). Laser processing was also used for cutting through holes on the cover lid (hydrophilic coated polyester film 3M 9984), which was then manually bonded with the microfluidic layer as well as with the electrode layer.

*Characterization and Measurements:* Various analytical techniques were utilized to characterize the as-synthesized nanoparticles and as-fabricated printable supercapacitors and gas sensor. Morphologies were characterized using field-emission SEM (JSM-7100F, Japan). Chemical compositions distributions were studied by energy dispersive spectrometer (EDS) (JSM-7100F, Japan). Amperometric response and CV measurements on glucose sensor electrodes based on a three-electrode configuration were performed on an electrochemical workstation (CHI 660E, USA). EIS measurements based on a three-electrode configuration were performed on an electrochemical workstation (Gamry Instruments, USA) with the frequency ranges from 100 kHz to 0.01 Hz with a potential amplitude of 10 mV. The performance of the glucose sensors was tested chronoamperometrically under PBS buffer solution containing varying glucose concentrations and based on a two-electrode configuration. All measurements were paused while refreshing solutions and continued after a waiting period of 120 s. On-body evaluation of the sweat glucose was performed in compliance with the protocols that were approved by the Institutional Review Board at University of California, Berkeley (2015-05-7578). Three

healthy subjects, aged 20–30, were recruited from the University of California, Berkeley campus. All subjects gave written, informed consent before participation in the study. Data were directly recorded in a mobile phone via a customized application. The quantification of sweat rate was interpreted from the impedimetric response of sweat rate sensors following our previous reported methods.<sup>[34]</sup>

## Supporting Information

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

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Y.L. and A.J. developed the device design. Y.L. developed the fabrication process, carried out the experiments, characterization, and data analysis, and drafted the manuscript. M.B. and H.Y.Y.N. helped with sensor characterization and manuscript revision. L.K., S.U., E.J., T.H., C.L., and J.H. designed and fabricated the biosensing patch. W.J. and Z.Y. provided support on device fabrication. Y.L., Z.F., and A.J. completed the manuscript. All authors read and approved the final manuscript. The authors thank Y. Zhang and Alex H. K. WONG from Materials Characterization and Preparation Facility (MCPF), the Hong Kong University of Science and Technology for their help on SEM analysis. The authors also acknowledge J. Zhao, Dr. X. He, B. Maskey, G. B. Zhang, and Dr. Y. Zhao for their support in this work. This work was supported by the National Science Foundation (NSF), Nanomanufacturing Systems for Mobile Computing and Mobile Energy Technologies (NASCENT), Berkeley Sensor and Actuator Center (BSAC), National Natural Science Foundation of China (Project 51672231), and Hong Kong Innovation Technology Commission (ITS/115/18).

## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

glucose sensor, nanotextured electrode, porous enzymatic membrane, stable and reliable noninvasive monitoring

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