Fabrication of a Flexible Amperometric Glucose Sensor Using Additive Processes

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Advances in accurate sensing of blood glucose concentrations have contributed to improved control of blood sugar levels for patients with type 1 diabetes.1,2 Type 1 diabetes is a pancreatic and endocrine disease in which blood glucose is poorly controlled because the patient’s beta cells cannot generate sufficient insulin. Long-term exposure to elevated glucose levels can lead to complications such as retinopathy, nephropathy, and cardiac disease. There is no known cure for type 1 diabetes and it has traditionally been controlled by the use of frequent glucose measurement with a blood glucose meter and insulin injections in order to maintain blood glucose levels within an acceptable range. The recent development of continuous glucose sensors now allows real-time monitoring of blood sugar levels.3 Portable infusion pumps allow continuous subcutaneous infusion of insulin and deliver basal and bolus doses of insulin to assist in maintaining glycemic control.4,5

A glucose sensor is a critical component of an artificial pancreas and research in this area has been extensive.1,2 An artificial pancreas integrates continuous glucose sensing with automated delivery of the hormone insulin with or without glucagon to appropriately control glucose levels.6,7 The concept of a closed loop artificial pancreas has been around for many years, beginning with the work of Albisser et al.8,9 The number of research studies on artificial pancreas controllers has increased in recent years including those using model predictive controllers,10–13 those using proportional integrative derivative (PID) or PID-like controllers,14–16 and those using fuzzy logic.17,18

A sensor within an artificial pancreas needs to accurately measure glucose concentrations over the wide range typically found in patients with diabetes (i.e. 2–30 mM in the interstitial fluid) to minimize the risk of over/under delivery of insulin and glucagon.1,2 Other desirable characteristics of a sensor for an artificial pancreas are long working lifetimes to minimize the inconvenience of replacing the device, and low cost to facilitate widespread usage. Amperometric glucose sensors are specified to work for 7 days, and typically have a mean absolute relative difference (MARD, percent error) in the 12–18% range. Castle et al. found that redundancy of sensing elements can somewhat reduce the MARD and can markedly reduce the frequency of very large egregious errors.19

Continuous glucose sensors have been made using a wide variety of substrates and electrode materials. Harrison et al.20 fabricated a glucose sensor based on a platinum wire, which was dip-coated with an active enzyme layer. Yu et al.21 designed a coiled platinum wire with an integrated Ag/AgCl reference electrode wire, which was successfully tested in rats for a period of up to 56 days. Endo et al.22 developed another sensor with integrated Ag/AgCl reference electrode and a platinum wire which provided continuous glucose monitoring in anesthetized animals. Other glucose sensor devices have been fabricated on flexible polymer substrates. Kudo et al.23 constructed a sensor on a polydimethylsiloxane (PDMS) substrate, with platinum working electrode and Ag/AgCl counter/reference electrode. Sensors with the same design were also fixed to PDMS contact lenses and successfully tested in rabbits.24 Li et al.25 used a polyimide substrate to fabricate a spirally rolled flow-through catheter for implantation in blood vessels, where the sensor had gold working and counter electrodes with a Ag/AgCl reference electrode.

Additive manufacturing is of increasing interest for the fabrication of high value components, which can be customizable with reduced environmental impact.26 Key benefits of additive manufacturing are (i) the reduction of processing steps compared to standard microfabrication based approaches, which require multiple deposition, pattern, and etch steps for each functional layer; (ii) the potential reductions in cost that result from reduced steps, and (iii) unique functionality including integration on flexible substrates.27 Recently additive manufacturing has become of much more interest for medical applications, where unique two- and three-dimensional structures can be effectively constructed for individual patients.28,29 Printing is one of the most appealing additive manufacturing approaches since it is inherently low cost, and can simultaneously deposit and pattern the films. Examples of inkjet printed materials include glucose oxidase,30,31 metal conductors,32 and adhesives.33

In this paper, we demonstrate the advantage of printing and additive processes to fabricate amperometric glucose sensors using a thin, metallized, and flexible polyimide substrate as a starting material. High-resolution electrohydrodynamic (e-jet) printing was used to pattern functional glucose oxidase (GOx) enzyme inks on plated platinum working electrodes. Amperometric measurements indicate

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that the enzyme is still active after e-jet printing. The thickness of the permselective layer was optimized to selectively limit the glucose diffusion rate through the membrane compared to oxygen diffusion, which allows for a linear response to the glucose concentration over the range of interest. Our results illustrate that low cost additive manufacturing approaches can be used to fabricate amperometric glucose sensors, and these can potentially be applied to other enzymatic sensing approaches.

Experimental

Materials.— Bovine serum albumin (BSA), 1H,1H,2H,2H-perfluorodecanethiol, ferric nitrate, acetaminophen and octadecanethiol were purchased from Sigma Aldrich (St. Louis, MO). Thiourea and glucose was obtained from Alfa Aesar (Ward Hill, MA). Glutaraldehyde was acquired from Electron Microscopy Sciences (Hatfield, PA). Glucose oxidase and FeCl₃ were obtained from Amresco (Solon, OH). NaCl, KCl, NaH₂PO₄, Na₂HPO₄ were acquired from Avantor Performance Materials (Center Valley, PA). The e-jet printing needle (glass micropipette) was purchased from World Precision Instruments (Sarasota, FL). Perfluorosulfonic acid (PFSA) in 1-propanol and water was purchased from Ion Power (New Castle, DE). The photoresist SU-8 was acquired from Microchem (Westboro, MA). Sygldard 184 PDMS was obtained from Dow Corning (Midland, MI). The Pt and Ag plating solution were purchased from Technic (Cranston, RI). Au (150 nm thick) on 12.7 μm polyimide film was acquired from Sheldahl (Northfield, MN). Milli-Q water (18.2 MΩ cm) was used in all sample preparation.

Amperometric sensor electrode fabrication.— A glucose sensing device comprised of six Pt working electrodes and one common Ag/AgCl reference/counter electrode, was patterned on a Au coated PI substrate by micro-contact printing as has been described previously. 36–38 In brief, a PDMS stamp was fabricated from an SU-8 master that was patterned on a silicon substrate using photolithography (MJBi, Suss Micro Tec Group, Garching, Germany). A self-assembled monolayer (SAM), octadecanethiol, was applied to the PDMS stamp by immersion in 2 mM octadecanethiol (ethanol as solvent) for 5 min. The SAM was transferred from the PDMS stamp to the Au film on the PI substrate after ~15 s contact time. After the SAM was transferred the unprotected Au was etched at a rate of 10 nm/min in 20 mM Fe(NO₃)₃ and 30 mM thiourea solution at pH ~2. 39 The octadecanethiol SAM was removed from the Au surface by UV-Ozone treatment followed by an ethanol rinse. The surface was subsequently cleaned by an oxygen plasma (PE-100, Plasma Etch, Inc. Carson City, NV).

Selective electroplating was performed by making electrical contact to the working or reference electrode contact pads. Pt (~12 nm) was electroplated on the working electrodes at a plating bath temperature of 65 °C and a current density ~54 A/m² for 48s. Ag (~200 nm) was electroplated on the counter/reference electrode at a plating bath temperature of 30 °C and a current density ~107 A/m² for 25s. Chloridization of the Ag was performed by reacting the Ag electrode with a 50 mM FeCl₃ solution for 2 min at room temperature. 40 The chloridization was performed soon after electroplating to minimize the formation of silver oxide and silver sulfide. Electroplated Ag showed homogeneous coverage on Au coated PI substrates, where a uniform layer of sub-micron AgCl particles was observed after chloridization.

A custom-built e-jet printer was used to deposit and pattern GOx ink onto the Pt working electrodes. The e-jet printer features a high-voltage amplifier (Trek 677B, Trek, Inc. Medina, NY), which applies a voltage between the printing needle and the substrate. The substrate was placed on a carrier that has a computer controlled motorized stage using x and y-axis linear steppers (Parker MX80L T03MP, Parker Hannifin Corp. Cleveland, OH) to move the substrate with respect to the printing needle, and a two-axis tilting stage (Edmund Optics 70 mm Micrometer Tilt Stage. Edmund Optics Ltd. York, UK) to level the substrate. The printing needle can be moved up and down using a computer controlled z-axis linear stepper motor (Parker MX80ST 02MSJ, Parker Hannifin Corp. Cleveland, OH). The printing process was observed through a microscope camera (Edmund Optics Infinity 2, Edmund Optics Ltd. York, UK) that was illuminated with fiber optic lamps. Custom control software was written in LabView to control the stage x-y motion and velocity, and the pulse voltage, duration, and frequency. The printing needle was coated with a film of 20 nm sputtered 60/40 Au/Pd alloy to provide electrical conductivity, using a Cressington Sputter Coater 108auto. Needles with an inner diameter of 5 and 30 μm were used for these studies. Prior to printing, the needle tip was dipped in liquid 1H,1H,2H,2H-perfluorodecanethiol for one minute, which forms a hydrophobic SAM on the outer metal surface of the needle. This monolayer prevents excessive wetting of the needle by the GOx ink. Printing backpressure was controlled by a pressure transducer (ControlAir 500-AF, ControlAir Inc. Ambler, NH) that was connected to a syringe and forced the ink to the tip of the needle. Normal operating pressures were between 0.5 and 3 psi. The needle bias was set using a National Instruments controller (SCB-80, National Instruments Inc. Austin, TX) and a voltage amplifier, which was connected to the printing needle, while the substrate was grounded. Printing was achieved by pulsing the voltage in a square wave, where the frequency and amplitude of the wave were controlled by the software. To print in the controlled cone-jet mode, the lower voltage of the pulse was sufficient to hold the fluid meniscus in a Taylor cone whereas the higher voltage of the pulse caused fluid to eject from the tip of the cone. 41–43 To print specific patterns, the e-jet printing software reads a text file which contains commands to move the x- and y- stages, and to modulate the voltage. Adjusting the stage velocity and voltage pulsing frequency allows the control of droplet size and spacing between droplets. Prior to printing the substrate was treated with UV/ozone for 15 min to ensure good surface wetting. The printed patterns were characterized using atomic force microscopy (AFM, Bruker Innova, Santa Barbara, CA), optical microscopy (Zeiss Axiotom, Pleasonton, CA), and optical profilometry (ZeScope, Zemetrics, Zygo Corp. Middlefield, CT).

Printing parameters on the e-jet printer (voltage, pulse frequency, stage velocity, pressure, and working distance) were adjusted to give repeatable patterning. Printing was accomplished by biasing the needle at a constant 600 V, which was pulsed with a peak of up to 800 V for frequencies between 10–30 Hz. The distance between the printing needle and the substrate was typically 100 microns. The printed GOx patterns were characterized by optical microscopy and AFM to help optimize the printing parameters for uniform coverage of the electrode surfaces. To improve wetting of the GOx ink we performed a UV/ozone treatment of the Pt/Au/PI substrates prior to printing.

Glucose oxidase ink.— An aqueous GOx and BSA ink was developed for e-jet printing of the enzyme films. The dissolved GOx was stabilized with the addition of 5% v/v of glycerol to the aqueous base. The glycerol helps to keep the enzyme from crystallizing prematurely, and reduces the surface tension of the GOx ink, which aids in printing. It was determined that a mixture of 18 mg Gox and 2 mg BSA per mL of water gave good mechanical stability of the printed enzyme layer, and provided good sensitivity to glucose. In order to crosslink the GOx film onto the sensor, the GOx and BSA were premixed with 0.4 mg/mL glutaraldehyde just prior to printing on the Pt working electrode surface. The total GOx and BSA concentration was 20 mg/mL, which gave a thick cross-linked enzyme layer, while still allowing uniform printing for over 40 minutes (i.e., the time required to print several devices). The functional ink conductivity, viscosity, and surface tension were determined to be 3.2 × 10⁻² μS/m, 1.1 × 10⁻³ Pa·s, and 60 mN·m⁻¹, respectively.

Permselective membrane.— Solutions between 1% and 30% by weight of PFSA in 1-propanol and water were used to fabricate thin polymer films onto the surfaces by spin-coating (Laurel WS-400BZ-6NPP/LITE, Laurell Technologies Corp. North Wales, PA). Polymer films were formed using rotation speeds of 1000–3000 RPM for
60–90 seconds, and the film thicknesses were measured by profilometry (Alpha Step 500, KLA-Tencor Inc. Milpitas, CA).

**Electrochemical glucose sensing.**— Characterization of glucose sensor devices was performed by placing the sensor in an electrochemical cell containing phosphate buffered saline (PBS) solution composed of 137 mM NaCl, 2.5 mM KCl, 4 mM NaH₂PO₄, and 16 mM Na₂HPO₄, with a pH of 7.4. The electrochemical cell solution was purged with Ar during experiments, in order to stir the solution and reduce oxygen levels in the solution to that of mammalian interstitial fluid (45 torr or 0.08 mM). An oxygen sensor (NeuLog Inc. Rishon-Lezion, Israel). During amperometric measurements the Pt working electrodes were polarized at +600 mV relative to the integrated Ag/AgCl reference electrode, and the current was monitored while the concentration of glucose was increased by pipetting in concentrated glucose solutions. The glucose concentration was varied between 0–32 mM for these measurements, which corresponds to the relevant clinical interstitial fluid glucose levels of diabetic patients. All the experiments were performed on 3 separate devices.

**Results and Discussion**

**Sensor design.**— In Figure 1a we show the wrapping of the sensors around catheters with high radius of curvature ~250 μm. A cross sectional schematic of the sensor with each of the layers labeled (not to scale) has been shown in Figure 1b. The three key factors that allow accurate glucose sensing are formation of platinum electrodes with well-defined areas and good catalytic activity, deposition of glucose oxidase layers with uniform thickness, and use of permselective layers with good control over the relative effective diffusion rates of glucose and oxygen. Each of these processes will be discussed below. In Figure 1c we show the operating principle of an amperometric glucose sensor. In brief, the oxidation of glucose by molecular oxygen is catalyzed by immobilized GOx, which results in the formation of H₂O₂. The H₂O₂ is then oxidized at the Pt working electrode that is biased at +600 mV with respect to the Ag/AgCl reference electrode, resulting in an electrochemical current. This electrochemical current can be used to quantify the amount of glucose in the interstitial fluid.

In Figure 2a we show optical microscope images of Au films on PI substrates. The Au films were patterned using microcontact printing and etching. These patterns replicated the stamp without visible defects or loss of resolution. The yield of these Au microelectrodes exceeded 90% on a typical batch of devices, as determined by optical microscopy and two-point electrical measurements. For electroplating, we used a patterned photoresist to define the regions where the Pt and Ag were electroplated on the Au microelectrodes. Electroplating of either Pt or Ag layers was conducted by placing the substrate in the appropriate plating solution and applying a potential to achieve the desired current density. The thickness of plated Ag (200 nm) and Pt (12 nm) film was determined by profilometry. The plated Ag counter/reference electrode and Pt working electrodes are shown in Figure 2b. Each working electrode has a planar surface area of approximately 0.32 mm², whereas the total reference electrode area was approximately 4.74 mm². A 2 μm thick SU-8 layer was used as a passivation layer on the sensor and to provide wells for the working or counter/reference electrodes.

**Electrohydrodynamic printing.**— In Figure 3 we show uniformly spaced e-jet printed droplets of very small GOx features (~5 μm in diameter) which were obtained using a needle with an inner tip diameter of 5 μm. These results indicate that very fine and uniform GOx features can be obtained using e-jet printing compared to ~15–40 μm diameter dots produced by typical inkjet printing. Further optimization of the printing parameters of e-jet printed GOx inks was necessary to pattern uniform films. For these studies different drop spacings were obtained by adjusting the stage velocity (v) and voltage square wave pulse frequency (f), while using a needle with an inner tip diameter of 30 μm. Using a stage velocity of 1200 μm/s and a frequency of 10 Hz (Figure 4a), we found that the ratio of drop generation and the voltage frequency was ~ 1. This ratio can be maintained up to f = 20 Hz (Figure 4b), however above f = 30 Hz the printed

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**Figure 1.** (a) The wrapping of the sensors around catheters with high radius of curvature ~250 μm, (b) schematic of a cross section of the amperometric glucose sensor on polyimide substrate and (c) schematic of the sensors function and chemical reactions in the glucose oxidase (GOx) layer and on Pt working electrodes.

**Figure 2.** Optical microscopy images of (a) patterned Au on PI substrate prepared via microcontact printing, and (b) after Pt and Ag electrodes deposition by electroplating.
drops (Figure 4c) showed irregular size and/or spacing. This may be
due to Taylor cone distortion, with poor recovery at higher frequen-
cies resulting in a reduction in the ratio of drop generation and voltage
to below 1.41 Therefore, the optimized pulse frequency to print our GOx ink was determined to be 20 Hz, which allowed us
to generate the most uniform drop patterns for high-speed printing.
The stage velocity was also optimized to go from isolated droplets to continuous lines of GOx ink, by slightly overlapping each droplet
with its previous neighbor. At a frequency of 20 Hz and velocity of
1200 μm/s (Figure 4d), multiple small droplets can be distinguished,
which results from the stage velocity being too high. At a frequency
of 20 Hz with the stage velocity reduced to 700 μm/s (Figure 4e),
the profile of individual droplets could still be recognized within the
coalescing line. When the stage velocity was reduced further to 500 μm/s (Figure 4f), we were able to produce a smooth, continuous
line with no evidence of individual droplets. We show AFM measure-
ments in Figure 4g to 4h, for the same printing conditions as Figure 4d to 4f, respectively. These AFM images confirm that individual drops, elongated drops, and continuous lines were observed, respectively. The line width was determined to be approximately 30 μm wide for the printing conditions optimized for continuous lines.

Once continuous lines were obtained, a printing pattern with line spacing of 30 μm was used so that adjacent lines could coalesce and create a continuous film of GOx on the Pt working electrodes. In Figure 5a we show images of the e-jet printer depositing the GOx ink on two working electrodes of the sensor. It takes approximately 1 min to print two layers of GOx on a single working electrode, which corre-
responds to a total printing time of 6 min for the entire amperometric
current sensor. An optical profilometry image of a single working electrode with two layers of e-jet printed GOx is shown in Figure 5b.

Electrochemical test.— Spin-coating was used to provide uniform
film thicknesses for the permselective membrane (PSM), which al-
lowed us to evaluate the effect of PSM film thickness on sensor performance.46,47 It has been suggested that exposing the cross-linked GOx layer to concentrated acidic polymer solution may denature some of the enzyme.22 Therefore, a thin "base coat" using 1% PFSA solution, about 60 nm thick, was applied before subsequent applica-
tions of thicker membranes with more concentrated PFSAs solutions. Chronoamperometric data was obtained after application of the PSM on the flexible glucose sensors. The experimental data is shown in Figure 6a where the amperometric current is monitored for three different PSM thicknesses. In all cases the current increases in a step-
wise fashion as the glucose concentration is increased. The increase in amperometric current is plotted versus glucose concentration in Figure 6b. In the upper portion of Figures 6a and 6b we show data from a two-coat membrane, with a total PFSA thickness of 300 nm. A highly nonlinear current response to glucose concentration was obtained, which suggests that the film does not effectively limit diffusion of glucose to the enzyme layer, and the oxidation rate of glucose is limited by low oxygen concentrations in the glucose solution (to approximate the O2 concentration in the interstitial fluid). We found that increasing the PFSA membrane thickness to 3 μm significantly reduced the amperometric current (compared to the 300 nm PFSA membrane), but did not significantly extend the linear working range of the glucose sensor. The 3 μm PFSA coated sensor still only gave a linear response up to 15 mM of glucose. A nonlinear current response was observed above this concentration since glucose oxidation was again limited by oxygen concentrations through the membrane, which suggests that the 3 μm thick PSM still does not effectively limit diffusion of glucose to the enzyme layer. An even thicker 7 μm coating of PFSA further reduced the amperometric current, however it also improved the sensor function such that a linear sensor response up to 32 mM glucose (slope of current density vs. glucose concentration ~ 4.8 × 10−5 A·cm−2·mM−1) could be obtained. The extended linear region of this sensor is a result of an optimized membrane thickness that limits the flux of glucose with respect to oxygen through the membrane layer to the GOx enzyme layer, such that the enzyme re-
action is limited by glucose rather than oxygen concentration.

Since PFSA is negatively charged, it has been shown to effectively limit the diffusion of negatively charged interfering compounds (e.g., ascobic and uric acids).48 However it is necessary that the PFSA
membrane also limits the diffusion of neutral interfering compounds such as acetaminophen, which can be oxidized directly on the Pt indicating electrodes. Prior studies have shown that acetaminophen interference can be eliminated when PFSA is used as an inner membrane. For our glucose sensor, PFSA was used as an outer membrane and it was necessary to determine if this structure can also through the thin membrane. For the 3 μm thick membrane also limits the diffusion of neutral interfering compounds such as acetaminophen, which can be oxidized directly on the Pt indicating electrodes. Prior studies have shown that acetaminophen interference can be eliminated when PFSA is used as an inner membrane.

For our glucose sensor, PFSA was used as an outer membrane and it was necessary to determine if this structure can also block interference molecules. The arrows in Figure 6a indicate when 0.13 mM of acetaminophen was added to the electrolyte at the end of the experiment. A rapid increase in current was observed for the 300 nm PFSA membrane, indicating that acetaminophen readily diffuses through the thin membrane. For the 3 μm thick PFSA membrane there was only a slight current increase due to acetaminophen interference, indicating that acetaminophen still diffuses through this thicker membrane. However, we found that interference of acetaminophen was totally suppressed when using the 7 μm thick PFSA membrane, which suggests that thicker PFSA membranes are effective in minimizing interference from acetaminophen. The glucose sensor response was also tested six months after fabrication and showed negligible signal loss (see Table 1).

Conclusions

This study demonstrates that a continuous glucose sensor can be fabricated on a flexible polyimide substrate using additive technologies. We were able to use electrohydrodynamic printing to deposit and pattern a biological ink (containing glucose oxidase, bovine serum albumin, and glutaraldehyde as a cross-linking agent) with high resolution on the sensors working electrodes. The GOX enzyme was found to be active after e-jet printing. PFSA was used as a perselective membrane and its thickness was optimized to obtain a linear response for glucose concentrations up to 32 mM, as well as to minimize the detection of interfering molecules (e.g., acetaminophen). The flexible sensor design developed in these studies can ultimately be used within a closed loop artificial pancreas control system thereby reducing the number of components in such a system.

Acknowledgments

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References


Table 1. Chronoampereometry of glucose sensor response to 32 mM glucose concentration with printed GOX on Pt working electrodes and a 300 nm, 3 μm, and 7 μm outer PFSA membrane. All the experiments were conducted 3 times.

<table>
<thead>
<tr>
<th>PFSA coating</th>
<th>300 nm</th>
<th>3 μm</th>
<th>7 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>As prepared</td>
<td>860 ± 40 (nA)</td>
<td>450 ± 40 (nA)</td>
<td>300 ± 30 (nA)</td>
</tr>
<tr>
<td>6 months storage</td>
<td>790 ± 40 (nA)</td>
<td>420 ± 30 (nA)</td>
<td>290 ± 30 (nA)</td>
</tr>
</tbody>
</table>

Figure 6. (a) Chronoamperometry of glucose sensor response to glucose concentration with printed GOX on Pt working electrodes and 300 nm, 3 μm and 7 μm thick outer PFSA membrane. Arrows indicate addition of 0.13 mM acetaminophen. (b) Current density vs. glucose concentration for these devices.