Video Article

Dry Film Photoresist-based Electrochemical Microfluidic Biosensor Platform: Device Fabrication, On-chip Assay Preparation, and System Operation

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Abstract

In recent years, biomarker diagnostics became an indispensable tool for the diagnosis of human disease, especially for the point-of-care diagnostics. An easy-to-use and low-cost sensor platform is highly desired to measure various types of analytes (*e.g.,* biomarkers, hormones, and drugs) quantitatively and specifically. For this reason, dry film photoresist technology - enabling cheap, facile, and high-throughput fabrication - was used to manufacture the microfluidic biosensor presented here. Depending on the bioassay used afterwards, the versatile platform is capable of detecting various types of biomolecules. For the fabrication of the device, platinum electrodes are structured on a flexible polyimide (PI) foil in the only clean-room process step. The PI foil serves as a substrate for the electrodes, which are insulated with an epoxy-based photoresist. The microfluidic channel is subsequently generated by the development and lamination of dry film photoresist (DFR) foils onto the PI wafer. By using a hydrophobic stopping barrier in the channel, the channel is separated into two specific areas: an immobilization section for the enzyme-linked assay and an electrochemical measurement cell for the amperometric signal readout.

The on-chip bioassay immobilization is performed by the adsorption of the biomolecules to the channel surface. The glucose oxidase enzyme is used as a transducer for electrochemical signal generation. In the presence of the substrate, glucose, hydrogen peroxide is produced, which is detected at the platinum working electrode. The stop-flow technique is applied to obtain signal amplification along with rapid detection. Different biomolecules can quantitatively be measured by means of the introduced microfluidic system, giving an indication of different types of diseases, or, in regard to therapeutic drug monitoring, facilitating a personalized therapy.

Video Link

The video component of this article can be found at https://www.jove.com/video/56105/

Introduction

Over the past two decades, diagnostic applications have become elementary for in-depth studies on the development of global public health. Traditionally, laboratory diagnostic tools are used for the detection of diseases. Even though they still play a key role in the diagnosis of diseases, point-of-care testing (POCT) performed near the patient or by the patient himself has become more and more commonplace in recent years. Especially in such cases that require immediate treatment, such as acute myocardial infarction or diabetes monitoring, the rapid confirmation of a clinical finding is essential. Hence, there is a growing need for POCT devices that can be operated by non-experts and that are concurrently capable of performing precise *in vitro* diagnostic tests in a short time[1,2,3,4].

Remarkable improvements have already been achieved in the field of POCT. However, there are still many challenges to overcome[5,6,7,8]. For a POCT platform to be successfully launched to the market and to be competitive with laboratory diagnostics, the device must strictly fulfill the following requirements: (i) provide precise and quantitative test results that are consistent with laboratory findings; (ii) have short sample-to-result times, enabling the immediate treatment of the patient; (iii) feature uncomplicated and easy handling, even when operated by untrained individuals, and require minimized user intervention; and (iv) comprise of a low-cost sensor unit designed for single-use applications. Furthermore, equipment-free diagnostics are favorable, mainly in resource-poor environments[3,4,6].

Due to these severe requirements, only two POCT systems based on electrochemical detection (*e.g.,* blood glucose test strips) and on lateral flow immunoassays (*e.g.,* home pregnancy tests) have been successfully launched to the market so far. However, both systems suffer from disadvantages such as poor performance (*i.e.,* blood glucose monitoring has inaccurate test results and lateral flow assays only provide qualitative (positive or negative) measurement results)[4,6]. These drawbacks of conventional POCT systems have led to an increasing demand on exploring new technologies that offer fast, low-cost, and quantitative detection at the point of care[4,5].

To meet these challenges facing POCT devices, DFR technology has been recently employed for the fabrication of disposable and low-cost biosensors[9,10,11,12,13,14]. Compared to soft and liquid lithographic materials, such as PDMS or SU-8, DFRs present many benefits: they (i) are available in a variety of compositions and thicknesses (from a few microns to several millimeters); (ii) have a very rough surface area, which facilitates adhesion to various materials; (iii) feature excellent thickness uniformity; (iv) offer cheap, facile, and high-throughput fabrication for mass production; (v) are easy to cut with various low-cost tools, like a simple pair of scissors; and (vi) allow for the creation of three-dimensional structures, such as microfluidic channels, by stacking multiple DFR layers on top of each other.

On the other hand, DFRs in general have a relatively poor resolution compared to liquid photoresists, which is mainly caused by the film thickness and by the increased distance between the mask and the DFR due to the protective foil, which additionally enables light scattering. Still, for the manufacturing of integrated microfluidic biosensors, DFRs are highly suitable for low-cost mass production.

Therefore, we present in this work the fabrication and application of a DFR-based electrochemical microfluidic biosensor. The detailed protocol describes each production step of the biosensor platform, the on-chip immobilization of a DNA-based model assay, and its electrochemical readout using the stop-flow technique. This universal platform enables the detection of numerous kinds of biomolecules, using different assay technologies (*e.g.,* genomics, cellomics, and proteomics) or assay formats (*e.g.,* competitive, sandwich, or direct). Based on such a DFR platform, our group previously successfully demonstrated the rapid and sensitive quantification of various analytes, including antibiotics[13,15,16] (tetracycline, pristinamycin, and ß-lactam antibiotics), troponin I[17], and substance P[18].

Protocol

1. Fabrication of the Microfluidic Biosensor Using DFR Technology

1. **Preparation of the PI wafers.**
    1. Cut a PI substrate into 6-in round wafers. Put the PI wafer in an oven at 120 °C for roughly 1 h for a dehydration bake.

2. **First photolithography step for the lift-off process.**
    1. Program the spin-coater to a 30-s spinning time at 3,000 rpm, with an acceleration of 2,000 rpm/s. Place the PI wafer on the spin-coater and fix it, applying a vacuum. Dispense 2 mL of a resist, enabling the lift-off process, and start the spin-coater program. Remove the wafer from the spin-coater and soft-bake the PI wafer on a hot plate for 2 min at 100 °C.
    2. Adjust the desired mask for patterning the electrodes on the PI wafer with the naked eye and expose it to 400 mJ/cm^2 UV light (365 nm). Place the wafer in a bowl filled with a resist-matching developer on an orbital shaker and let it shake slightly for 1 min.
    3. After the excess resist is removed, use a shower to rinse the PI wafer with deionized water (DI-water) on a wet bench and dry it afterwards using compressed air.

3. **Deposition of platinum for the formation of the electrodes.**
    1. Use a standard physical vapor deposition process to deposit 200 nm of platinum onto the wafer[19].
    2. Place the wafer in a bowl. Add the matching remover to the bowl and remove the lift-off resist while slightly shaking the wafer on an orbital shaker until all excess platinum is removed. Rinse and dry the PI wafer as described in step 1.2.3.

4. **Second photolithography step: Forming the insulation layer.**
    1. Put the PI wafer in an oven preheated to 120 °C for 5 min to dehydrate it.
    2. Program the first step of a spin-coater to 5 s of spinning time at 500 rpm. Program the second step for 30 s at 4,000 rpm, with an acceleration of 700 rpm/s.
    3. Place the PI wafer on the spin-coater and fix it, applying a vacuum. Dispense 4 mL of an appropriate epoxy-based photoresist before starting the spin-coater program.
    4. Soft-bake the coated wafer for 3 min at 95 °C on a hot plate.
    5. Adjust the mask for the insulation layer on the wafer using the respective alignment marks and an ocular lens. Expose the wafer to 100 mJ/cm^2 UV light (365 nm).
    6. For a post-exposure bake, place the wafer on a preheated hot plate for 2 min at 95 °C.
    7. Place the wafer into a bowl and develop the resist with a suitable developer (*e.g.,* 1-methoxy-2-propyl-acetat for 2 min, in the case of SU-8) on an orbital shaker.
    8. Rinse the PI wafer first with isopropanol and then rinse and dry it as described in step 1.2.3.
    9. Hard-bake the photoresist in an oven for 3 h at 150 °C.

5. **Plasma-assisted cleaning of the electrodes.**
    1. To remove the photoresist residues, use oxygen plasma with a standard plasma unit. Start the cleaning program, using 200-W low frequency power with 100% O_2 flow, a rate of 250 sccm, and a total time of 3 min.
    2. Place the PI wafer into the plasma chamber, fix the wafer on the grounded plate using glass lids, and start the plasma process.

6. **Silver and silver chloride deposition: Creating the on-chip reference electrode.**
    1. Passivate the platinum contact pads of the wafer with a UV-sensitive adhesive tape. Cut the foil in to 5 mm-wide and 11 cm-long stripes and attach them to the wafer, protecting the parts not to be deposited with silver.
    2. For the silver deposition, put a sonic bath (35 kHz) into a fume cupboard and insert a container of silver electrolyte solution (100 g/L Ag, pH 12.5) into the bath. Set the sonic bath to room temperature and the power to 10%.
    Caution: Silver electrolyte solutions are highly toxic and should be handled with extreme care. The fumes should never be inhaled.
    3. Connect the bulk contact of the reference electrodes to a constant current source. Connect the counter electrode, a silver wire that is immersed in the silver electrolyte solution, to the current source using standard connecting cables.
    4. Set the current source to DC and to a current density of -4.5 mA/cm^2, resulting in a silver deposition rate of approximately 0.3 µm/min. Start the sonic bath and let the current source run for 10 min. Rinse the wafer with DI-water

5. For the chlorination of the reference electrode, insert a vessel of 0.1 M potassium chloride (KCl) solution into the sonic bath. Connect the bulk contact of the wafer and a platinum electrode that is immersed in the KCl solution with the constant current source.
6. Set the current source to DC and to a current density of +0.6 mA/cm^2, resulting in a deposition rate of approximately 0.075 µm/min. Start the sonic bath and let the current source run for 7.5 min.
7. Rinse and dry the PI wafer, as described in step 1.2.3.
8. Remove the UV-sensitive tape after a UV (365 nm) exposure of 300 mJ/cm^2 and rinse and dry the PI wafer, again as described in step 1.2.3.

7. **Third photolithography step: Using DFRs to create the microfluidic channels.**
   1. Cut the needed DFR layers to a size similar to that of the wafer (20 x 20 cm^2). Fix the desired mask onto the exposure unit and align the DFR layer onto the mask using the naked eye. Illuminate the resist with 250 mJ/cm^2 UV light (365 nm).
   2. Remove the protective foil of the photoresist and develop the resist for roughly 2 min (depending on the structures) in a 1% sodium carbonate (Na_2CO_3) solution using a standard sonic bath (35 kHz) preheated to 42 °C and a sonic power of 100%. To stop the reaction immediately, shake the resist for 1 min in a 1% HCl bath on an orbital shaker. Rinse and dry each DFR layer, as described in step 1.2.3.
      NOTE: In total, four DFR layers need to be processed as mentioned in step 1.7: one channel layer, one cover layer, and two backside layers (preventing the biosensor from bending at the end).

8. **Lamination of the DFR layers on the PI substrate.**
   1. Place the PI wafer onto a transparency overhead foil and fix it using standard adhesive tape. Adjust the channel DFR layer on the PI wafer under a microscope, using the respective alignment structures.
   2. For the lamination, use a standard hot roll laminator and preheat the top roll to 100 °C and the bottom roll to 60 °C. Set the pressure to 3 bar, with a forward speed of 0.3 m/min. Place the wafer with the fixed DFR layer in the middle of the laminator and start it; the DFR is pushed through the laminator. Repeat the step after rotating the wafer by 180°.
   3. To prevent the bending of the biosensor, laminate the two developed backside layers onto the wafer following steps 1.8.1-1.8.2.

9. **Employing a hydrophobic stopping barrier and sealing the chip.**
   1. Remove the protective layer from the front side of the DFR channel layer by placing standard adhesive tape at one end of the DFR and pulling it upwards. Use a hand dispenser with 0.004-in tubes to dispense small droplets of dissolved polytetrafluoroethylene into the wells of the insulation layer. To seal the microfluidic chip, laminate the cover DFR layer onto the channel layer, as described in step 1.8.

10. **Hard-baking the microfluidic biosensor.**
    1. Remove all protective foils on the cover and backside DFR layers. Cut the biosensors into strips using an ordinary pair of scissors. Cure the chips in an oven at 160 °C for 3 h.

2. On-chip Assay Immobilization Procedure

1. **Adsorption of avidin in the immobilization area of the channel.**
   1. Prepare a 100 µg/mL avidin solution in 10 mM phosphate-buffered saline (PBS) at a pH of 7.4.
   2. Dispense 2 µL of the avidin solution onto the inlet of the biosensor.
      NOTE: The channel is filled by capillary forces until the fluid reaches the hydrophobic stopping barrier.
   3. To ensure that the fluidic keeps stopping at the barrier during the whole incubation time, dispense 2 µL of DI-water on the outlet of the chip. Incubate the chip at 25 °C for 1 h in a closed container.
   4. Remove excess reagents through the inlet of the chip by applying a vacuum. Wash the channel with 50 µL of wash buffer (PBS with 0.05% Tween 20), dispensed on the outlet while the vacuum is being applied. Dry the channel for 30 s while the vacuum is being applied.

2. **Blocking the channel surface to inhibit unspecific binding.**
   1. Prepare a 1% bovine serum albumin (BSA) solution in 10 mM PBS at a pH of 7.4.
   2. Pipette 2 µL of the 1% BSA solution on the inlet and 2 µL of DI-water on the outlet of the channel. Incubate the chip at 25 °C for 1 h in a closed container. Remove excess reagents and dry the channel as described in step 2.1.4.

3. **Incubation of DNA oligos labeled with biotin and 6-FAM.**
   1. Prepare different concentrations (0.001-5 µM) of the DNA solution in 10 mM PBS at a pH of 7.4.
   2. Dispense 2 µL of one concentration of the DNA solution on the inlet and 2 µL of DI-water on the outlet. Incubate the chip at 25 °C for 15 min in a closed container.
   3. Repeat step 2.3.2 for the other DNA concentrations using additional biosensor chips.
   4. Remove excess reagents and dry the channel as described in step 2.1.4.

4. **Immobilization of GOx-labeled 6-FAM antibodies.**
   1. Formulate a 5 µg/mL concentration of 6-FAM antibodies in 10 mM PBS at a pH of 7.4.
   2. Introduce 2 µL of the antibody solution to the inlet and 2 µL of DI-water to the outlet ofthe channel. Incubate the labeled antibodies at 25 °C for 15 min in a closed container. Remove the excess reagents, as described in step 2.1.4.

3. Amperometric Signal Detection Using the Stop-flow Technique

1. **Preparation of the substrate solution for electrochemical detection.**
   1. Formulate a 40-mM glucose solution in 0.1 M PBS at a pH of 7.4 in ultra-pure water.

2. For the preliminary treatment, prepare a 0.1 M PBS solution at a pH of 7.4 in ultra-pure water.

2. **Preliminary treatment of the working electrodes.**
   1. Use the custom-made chip holder to allow for the easy placement of the chip and a fluidic and electric connection.
   2. Electrically connect the biosensor to the potentiostat. Ensure the fluidic contact of the microfluidic channel to a syringe pump and to the reagent reservoir using the custom-made adapter and tubes. Start the laptop that is connected to the potentiostat and start the syringe pump controller, controlling the fluidic flow through the chip.
   3. Start the syringe pump manually, with 0.1 M PBS at a flow rate of 20 µL/min. At the working electrode versus the on-chip reference electrode, apply an alternating voltage of 0.8 and -0.05 V for 5 s each for 30 cycles. Following this, oxidize the working electrode by applying a voltage of 0.8 V for 60 s.

3. **Assay readout using the stop-flow technique.**
   1. To start the assay signal readout, wait until the measured current signal stabilizes. Stop the syringe pump and switch the reagent from 0.1 M PBS to the 40-mM glucose solution and start the software at the syringe pump controller; it will automatically stop the flow for 1, 2, or 5 min and will then restart the flow again. Observe the resulting current peak.
   NOTE: For the analysis of the data, either the current peak or the charge of the peak can be considered, as described in the model of an electrochemical signal readout (**Figure 2**).

Representative Results

Design and Fabrication of the Microfluidic Biosensor Platform:

The fabrication of the microfluidic biosensor chips is realized on the wafer-level by standard photolithographic techniques employing multiple DFR layers. This fabrication strategy relies on the lamination of developed layers of DFRs on a platinum-patterned PI substrate, forming the microfluidic channels. A short summary depicting the different fabrication steps is given in **Figure 1**. A single 6-in wafer comprises 130 microfluidic biosensors, each with a dimension of 8 × 10 mm^2.

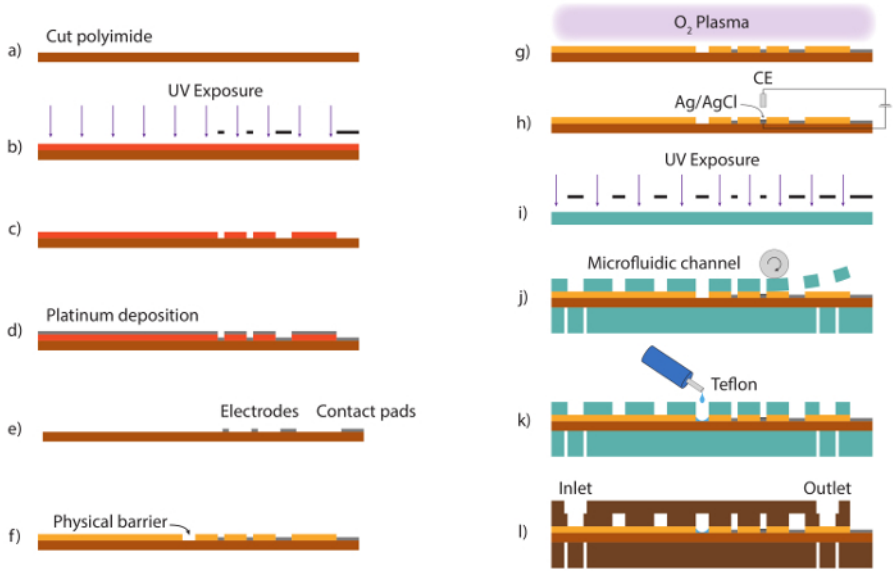

Figure 1. Graphical illustration of the different fabrication steps of the microfluidic biosensor platform. a) Cut the PI substrate into 6-in round wafers. b) Exposure of a possible spin-coated lift-off resist using the respective mask for platinum patterning. c) Substrate after exposure, post-exposure back, and developing the photoresist. d) Physical vapor deposition of platinum on the substrate. e) Lift-off process to remove the excess photoresist. f) Spin-coating of SU-8, forming an insulation layer. g) O_2 plasma process to remove SU-8 residues on the Pt electrodes. h) Galvanic deposition of the Ag/AgCl reference electrode. i) UV exposure and development of different DFR layers. j) Lamination of the DFR layers onto the PI wafer. k) Dispensing solved polytetrafluoroethylene, forming a hydrophobic stopping barrier between the immobilization capillary and the electrochemical cell. l) Final electrochemical microfluidic biosensor. Please click here to view a larger version of this figure.

Each biosensor consists of one microfluidic channel, separated into two distinct areas by a hydrophobic stopping barrier: an immobilization section and an electrochemical cell, marked in red and blue, respectively, in **Figure 2**. The immobilization part of the microchannel has a surface volume of 10.34 mm^2 and a volume of 580 nL, resulting in a high surface-to volume ratio of 155 cm^{-1}. The electrochemical cell includes an on-chip silver/silver chloride reference electrode and a counter and working platinum electrode. This separation of the immobilization area and the electrochemical readout of the assay prevents any contamination of the electrodes with biomolecules and therefore inhibits electrode fouling. Furthermore, it enables the precise metering of the immobilization reagents by capillary filling.

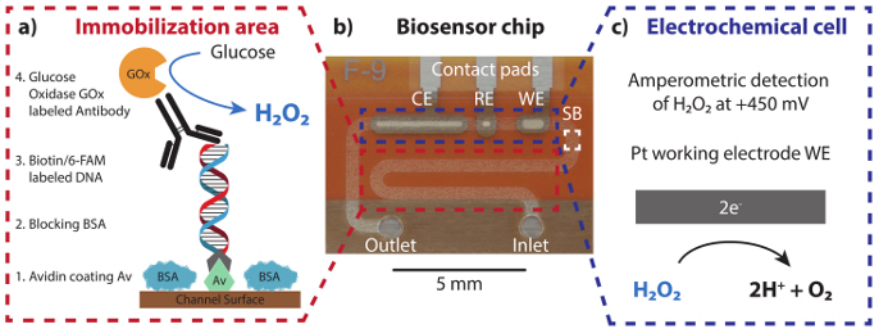

Figure 2. Illustration of the operating principle of the electrochemical microfluidic platform. a) Schematics of a model assay based on avidin. b) Photograph of the microfluidic biosensor showing its main elements, including the counter electrode (CE), the reference electrode (RE), the working electrode (WE), and the stopping barrier (SB). The immobilization area is highlighted in red, and the electrochemical cell is marked in blue. c) Schematic reaction of the oxidation of the produced hydrogen peroxide at the Pt working electrode for amperometric detection inside the electrochemical cell. Please click here to view a larger version of this figure.

By employing DFRs for the fabrication of the sensor, the manufacturing process allows for high-throughput on the wafer-level. Therefore, the costs can be kept down to a minimum. The development of all DFR layers is done using simple and low-cost foil masks and a vacuum exposure unit, rather than costly chrome masks and a mask aligner. In addition, the reduction of the necessary clean-room process steps to a minimum cuts the costs for the fabrication even more. In total, the fabrication procedure takes a workload of roughly 10 h, excluding the baking times and the physical vapor deposition of the platinum.

On-chip Assay Incubation:

The on-chip assay incubation is done only by capillary forces. By pipetting drops of different reagents onto the inlet of the microfluidic channel, the fluids are driven through the channel by capillarity action until they reach the hydrophobic stopping barrier. Under steady-state conditions, the reagents are then incubated for a distinct time. This passive system enables the capillary filling of the channel, without the need of any external instrumentation like a syringe pump, and allows for the precise metering of the reagents, as the fluid automatically stops at the stopping barrier. Also, the workflow is consistent with that of a conventional ELISA and it works with high-viscosity liquids like serum or blood.

For the purpose of this experiment, the chip operation is demonstrated by a simple test assay employing the avidin-biotin interaction, as shown in **Figure 2**. The on-chip assay incubation starts with the adsorption of the avidin to the capillary surface for 1 h, followed by a blocking step with BSA for another 1 h. Subsequently, 6-FAM/biotin-labeled DNA is incubated in the immobilization capillary for 15 min, where it binds to the avidin molecules. Between each incubation step, any unbound biomolecules are removed by a washing step. The wash buffer is applied via the channel outlet using a custom-made vacuum adapter. In the last step, glucose oxidase-labeled antifluorescein antibodies are introduced to the channel for 15 min. After that, the biosensor is ready for the electrochemical readout, using a 40-mM glucose solution as the substrate.

Amperometric Signal Readout Using the Stop-flow Technique:

For the signal readout of the immobilized assay, the enzymes product (here, H_2O_2), produced in the presence of its substrate, glucose, can be electrochemically detected at the working electrode in the electrochemical cell. To achieve fast detection along with signal amplification, the so-called stop-flow technique is used[13]. In this method, the flow of the substrate is stopped, which leads to an accumulation of the product inside the capillary. The amount of produced H_2O_2 therefore depends on the amount of bound glucose oxidase and is dependent on the amount of bound biotinylated DNA. By restarting the flow, the enhanced H_2O_2 concentration is subsequently flushed through the electrochemical measurement cell, resulting in a current peak signal, as illustrated in **Figure 3**.

For data analysis, the stop-flow technique offers two different parameters: the maximum height and the charge (*i.e.,* the integral) of the peak signal. Both parameters are directly proportional to the stopping time and can therefore be used for analyzing the data. Considering the data evaluation, when using the peak height, the signal height depends on the maximum H_2O_2 concentration and thus on its diffusion coefficient and the applied stop time. The longer the stopping time, the higher the measured signal response. For this reason, the gauged peak height remains constant, down to a minimum length of the immobilization capillary. This special feature of the stop-flow technique allows for a drastic decrease in chip dimensions, particularly in the capillary length, while preserving the sensitivity of the sensor.

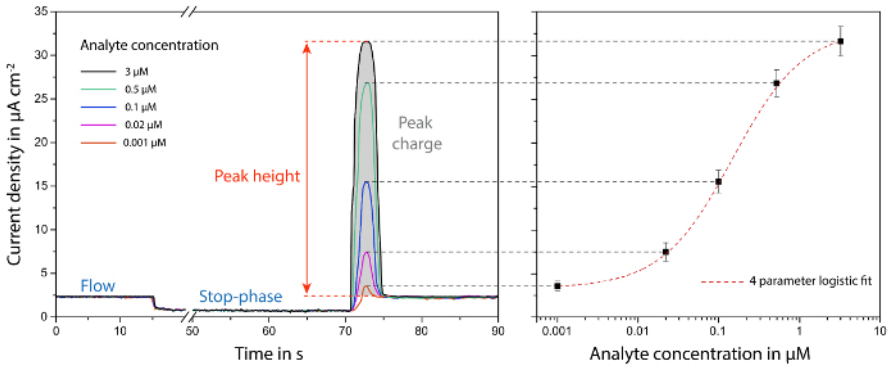

Figure 3. Model of a typically obtained electrochemical signal readout of an enzyme-linked assay using the stop-flow technique. a) A flow of 40-mM glucose solution at a rate of 20 µL/min was applied. During the stop phase (1, 2, or 5 min), the enzyme production of H_2O_2 continues. By restarting the flow, the accumulated H_2O_2 is flushed through the electrochemical cell, where the hydrogen peroxide is electrochemically detected. By repeating the measurement several times (error bars; SEM), an on-chip calibration curve b) is obtained. Please click here to view a larger version of this figure.

Discussion

The protocol presented here for the fabrication of a microfluidic electrochemical biosensor enables the development of a low-cost, compact, and easy-to-use platform for the detection of biomolecules. Depending on the assay used afterwards on the biosensor, several different biomarkers can be detected. This makes the platform very versatile and provides broad access to various fields of applications, from standard diagnostic tests (*e.g.,* determining the presence of specific diseases at the doctor's office) to point-of-care applications (*e.g.,* the therapeutic drug monitoring of a patient for individualized drug therapy). Especially in point-of-care diagnostics, miniaturized biosensors have many advantages over conventional methods, which usually require extensive laboratory equipment, specialized employees, and large reagent and sample volumes and have long turnaround times.

The fabrication of this biosensor requires strictly following the protocol; otherwise, manufacturing problems can occur. One of the most often observed issues is the misalignment of the different layers. This can be easily solved by using a more advanced apparatus for the fabrication process, which allows for the easier and more precise alignment of the different layers.

The DFR technology offers the possibility of fast and low-cost fabrication. On the other hand, the technology is limited in terms of resolution. For example, microfluidic channels of very small structures (less than 100 µm) cannot be realized using the protocol presented here. In such a case, the photolithography must be performed by a high-precision mask aligner with glass photomasks. In addition, a too-wide channel can also be problematic, since the channel could bend down, reducing the height of the channel and influencing the microfluidic behavior of the chip.

We believe that DFR technology will be more present in future applications because it can easily be scaled up for commercial use. The mass-production of DFR-based microfluidic sensors will be no hindrance when it hits the market because the technique is well-established in other fields of application (*e.g.,* flexible electronics).

In terms of reducing the complexity of the whole system, our future work will focus on the design and implementation of a disposable microfluidic cartridge that includes the biosensor chip; a waste reservoir; and pre-loaded reagents, such as wash buffer and other assay components. For the amperometric measurement, the delivery of the sample and other reagents can then be provided by pneumatic actuation. This will be performed by the handheld reader and will move through the microfluidic biosensor chip to a waste reservoir.

Disclosures

The authors have nothing to disclose.

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References

1. Spindel, S., Sapsford, K. E. Evaluation of optical detection platforms for multiplexed detection of proteins and the need for point-of-care biosensors for clinical use. *Sensors (Basel).* **14** (12), 22313-22341 (2014).
2. Luppa, P. B., Bietenbeck, A., Beaudoin, C., Giannetti, A. Clinically relevant analytical techniques, organizational concepts for application and future perspectives of point-of-care testing. *Biotechnol Adv.* **34** (3), 139-160 (2016).
3. Gauglitz, G. Point-of-Care Platforms. *Annu Rev Anal Chem.* **7** (1), 297-315 (2014).
4. Jung, W., Han, J., Choi, J. W., Ahn, C. H. Point-of-care testing (POCT) diagnostic systems using microfluidic lab-on-a-chip technologies. *Microelectron Eng.* **132**, 46-57 (2014).

5. Yager, P. *et al.* Microfluidic diagnostic technologies for global public health. *Nature.* **442** (7101), 412-418 (2006).
6. Fu, E., Yager, P., Floriano, P. N., Christodoulides, N., McDevitt, J. T. Perspective on diagnostics for global health. *IEEE Pulse.* **2** (6), 40-50 (2011).
7. Yager, P., Domingo, G. J., Gerdes, J. Point-of-care diagnostics for global health. *Ann Rev Biomed Eng.* **10**, 107-44 (2008).
8. Dincer, C., Bruch, R., Kling, A., Dittrich, P. S., Urban, G. A. Multiplexed point-of-care testing - xPOCT. *Trends Biotechnol.* **35** (2017).
9. Horak, J., Dincer, C., Bakirci, H., Urban, G. A disposable dry film photoresist-based microcapillary immunosensor chip for rapid detection of Epstein-Barr virus infection. *Sens Actuators B Chem.* **191**, 813-820 (2014).
10. Jobst, G., Gamp, T. *Method for the fabrication of a "lab on chip" from photoresist material for medical diagnostic applications.* **2**, 1-8 (2010).
11. Kling, A., Dincer, C., Armbrecht, L., Horak, J., Kieninger, J., Urban, G. Electrochemical microfluidic platform for simultaneous multi-analyte detection. *Procedia Eng.* **120**, 916-919 (2015).
12. Armbrecht, L., Dincer, C., Kling, A., Horak, J., Kieninger, J., Urban, G. Self-assembled magnetic bead chains for sensitivity enhancement of microfluidic electrochemical biosensor platforms. *Lab Chip.* **15**, 4314-4321 (2015).
13. Dincer, C. *et al.* Designed miniaturization of microfluidic biosensor platforms using the stop-flow technique. *Analyst.* **141**, 6073-6079 (2016).
14. Weltin, A., Kieninger, J., Enderle, B., Gellner, A. K., Fritsch, B., Urban, G. A. Polymer-based, flexible glutamate and lactate microsensors for in vivo applications. *Biosens Bioelectron.* **61**, 192-199 (2014).
15. Kling, A. *et al.* Multianalyte Antibiotic Detection on an Electrochemical Microfluidic Platform. *Anal Chem.* **88** (20), 10036-10043 (2016).
16. Bruch, R. *et al.* Clinical on-site monitoring of ß-lactam antibiotics for a personalized antibiotherapy. *Sci Rep.* (2017).
17. Horak, J., Dincer, C., Qelibari, E., Bakirci, H., Urban, G. Polymer-modified microfluidic immunochip for enhanced electrochemical detection of troponin i. *Sens Actuators B Chem.* **209**, 478-485 (2015).
18. Horak, J., Dincer, C., Bakirci, H., Urban, G. Sensitive, rapid and quantitative detection of substance P in serum samples using an integrated microfluidic immunochip. *Biosens Bioelectron.* **58**, 186-192 (2014).
19. Mattox, D. M. *Handbook of Physical Vapor Deposition (PVD) Processing.* Elsevier Inc. (2010).

