Supporting Information for

Patterned Paper as a Platform for Inexpensive, Low Volume, Portable Bioassays

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Patterning paper with SU-8 photoresist. A photo-mask was printed using a laser-photoplotter (CAD/Art Services, Inc). A 7.5 cm chromatography paper disk (1 Chr, Whatman International Ltd.) was soaked in 2 mL of 100% SU-8 2010 photoresist (MicroChem, Inc) for 30 s at room temperature. Excess photoresist was removed from the paper by spinning it on a Headway spin coater at 2000 rpm for 30 s. To pre-bake the photoresist, we heated the paper on a plate warmer at 65 °C for 1 min and at 95 °C for 5 min, and then cooled it under ambient conditions. The paper disk was exposed to 405 nm UV light (50 mW/cm^2) for 10 s through the photo-mask (CAD/Art Services, Inc) using a mask aligner (AB-M, Inc). To post-bake the photoresist, we heated the disk to 65 °C for 1 min and to 95 °C for 5 min, and then cooled it under ambient conditions. Unpolymerized photoresist was removed by immersing the disk in room temperature propylene glycol monomethyl ether acetate (PGMEA) for 5 min. The disk then was rinsed with 2-propanol (3×10 mL) to remove any remaining unpolymerized photoresist and was air dried for 1 h. The paper disk was exposed to oxygen plasma (SPI Plasma-Prep II, Structure Probe, Inc) for 10 s at 600 millitorr to increase the hydrophilicity of the paper.

Glucose assay. A 0.6 M potassium iodide solution (0.3 μL) (Sigma) in Millipore-purified water was spotted on the paper using a micropipettor and allowed to dry in ambient conditions. After it dried, 0.3 μL of a 5:1 glucose oxidase/horseradish peroxidase reagent (15 units of protein per mL of solution) (Sigma) was spotted on top of the potassium iodide, and allowed to dry.[1] The glucose assay reagents were obtained in the form of a Starch Assay Kit from Sigma.

Protein assay. A priming solution (0.3 μL) (92% water, 8% ethanol by volume, 2.5 g/L polyvinyl alcohol and 250-mM citrate buffer at pH 1.8) was spotted on the paper with a micropipettor and allowed to dry in ambient conditions.[2] A reagent solution (0.3 μL) (95% ethanol, 5% water by volume, 3.3 mM tetrabromophenol blue) was spotted on top of the priming solution and dried at room temperature for 3 min.

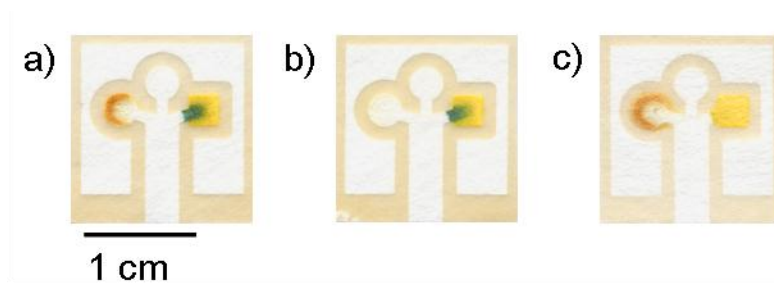
Preparation of standard test solutions. An artificial urine solution was prepared according to the recipe provided by Brooks and Keevil.[3] The artificial urine solution contained 1.1 mM lactic acid, 2.0 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2.0 mM magnesium sulfate, 10 mM sodium sulfate, 7.0 mM potassium dihydrogen phosphate, 7.0 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride all mixed in Millipore-purified water. The pH of the solution was adjusted to 6.0 by addition of 1.0 M hydrochloric acid. All reagents were obtained from Sigma-Aldrich. A stock solution of 550 mM glucose and 75 μM bovine serum albumin (BSA) was prepared in the artificial urine solution. The stock solution was diluted to the desired standard concentrations of 2.5, 5.0, 10, 50, 75 and 500 mM of glucose and 0.38, 0.75, 1.5, 7.5, and 50 μM of BSA.

Running the assay. A 5 μL sample solution was transferred to a Petri dish with a micropipettor. The bottom of the paper was dipped into the solution, and the solution was absorbed into the paper by capillary action. After 10.5 min, the test areas were evaluated for color changes and the device was scanned using an Epson Perfection 1640SU scanner on default settings.

Control assays. To demonstrate that glucose does not interfere with the protein assay and that BSA does not interfere with the glucose assay, we ran standard solutions of each analyte individually as well as a standard solution of both analytes combined (Figure S1). The intensity of the color does not change for either assay.

Figure S1. (a) Device run with an artificial urine solution containing 75 mM glucose and 50 μM BSA, (b) device run with an artificial urine solution containing 50 μM BSA, (c) device run with an artificial urine solution containing 75 mM glucose.

Figure S1



References

[1] Cypress Diagnostics, Urine-9 product insert, http://www.diagnostics.be/products/clinical/quicktest/Urine/urine-9.htm

[2] M. J. Pugia, J. A. Lott, J. A. Profitt, T. K. Cast, *J. Clin. Lab. Anal.* **1999**, *13*, 180-187.

[3] T. Brooks, C. W. Keevil, *Lett. Appl. Microbiol.* **1997**, *24*, 203-206.