Bioassays

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

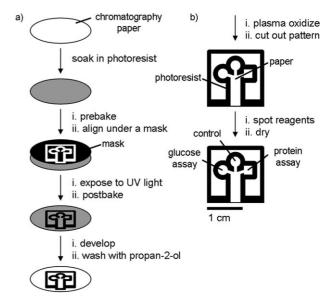
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Herein we describe a simple method for patterning paper to create well-defined, millimeter-sized channels, comprising hydrophilic paper bounded by hydrophobic polymer. We believe that this type of patterned paper will become the basis for low-cost, portable, and technically simple multiplexed bioassays. We demonstrate this capability by the simultaneous detection of glucose and protein in 5 μL of urine. The assay system is small, disposable, easy to use (and carry), and requires no external equipment, reagents, or power sources. We believe this kind of system is attractive for use in lessindustrialized countries, in the field, or as an inexpensive alternative to more-advanced technologies already used in clinical settings. $^{[1-4]}$

The analysis of biological fluids is necessary for monitoring the health of populations, [2] but these measurements are difficult to implement in remote regions such as those found in less-industrialized countries, in emergency situations, or in home health-care settings. [3] Conventional laboratory instruments provide quantitative measurements of biological samples, but they are unsuitable for these situations as they are large, expensive, and require trained personnel and considerable volumes of biological samples. [2] Other platforms for bioassays provide alternatives to more-expensive instruments, [5-7] but the need remains for a platform that uses small volumes of sample and that is sufficiently inexpensive to be widely used, particularly in less-industrialized countries.

We believe that patterned paper may be one of the least expensive platforms available for developing assays. We made assay devices based on paper by patterning photoresist onto chromatography paper to form defined areas of hydrophilic paper separated by hydrophobic lines or "walls"; these patterns provide spatial control of biological fluids and enable fluid transport, without pumping, owing to capillary action in the millimeter-sized channels produced. Patterns in paper make it feasible to run multiple diagnostic assays on one strip of paper while still using only small volumes of a single sample. In a fully developed technology, patterned photoresist would be replaced by an appropriate printing technology, but patterning paper with photoresist is 1) convenient for prototyping these devices and 2) a useful new micropatterning technology in its own right.

We patterned chromatography paper with SU-8 2010 photoresist as shown in Scheme 1a and as described below:



Scheme 1. Diagram depicting the method for patterning paper into millimeter-sized channels: a) Photolithography was used to pattern SU-8 photoresist embedded into paper; b) the patterned paper was modified for bioassays.

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we soaked a 7.5-cm-diameter piece of chromatography paper in 2 mL of SU-8 2010 photoresist for 30 s, spun it at 2000 rpm for 30 s, and then baked it at 95 °C for 5 min to remove the cyclopentanone in the SU-8 formula. We then exposed the photoresist and paper to 405-nm UV light (50 mW cm⁻²) for 10 s through a photomask (CAD/Art Services, Inc.) that was aligned by using a mask aligner (OL-2 Mask Aligner, AB-M, Inc). After exposure, we baked the paper a second time at 95 °C for 5 min to cross-link the exposed portions of the resist. The unpolymerized photoresist was removed by soaking the paper in propylene glycol monomethyl ether acetate (PGMEA) for 5 min and by washing the pattern with propan-2-ol (3×10 mL). The paper was more hydrophobic



after it was patterned, presumably owing to residual resist bound to the paper, so we exposed the entire surface to an oxygen plasma for 10 s at 600 millitorr (SPI Plasma-Prep II, Structure Probe, Inc) to increase the hydrophilicity of the paper (Scheme 1b).

The patterned paper can be derivatized for biological assays by adding appropriate reagents to the test areas (Scheme 1b and Figure 1b). We demonstrate this concept by detecting glucose and protein, [8] but the surface should also be suitable for measuring many other analytes as well.^[7] The glucose assay is based on the enzymatic oxidation of iodide to iodine,[9] in which a color change from clear to brown is associated with the presence of glucose. [10] The protein assay is based on the color change of tetrabromophenol blue (TBPB)

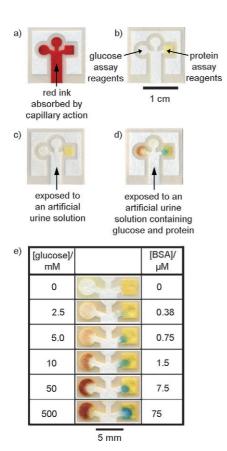


Figure 1. Chromatography paper patterned with photoresist. The darker lines are cured photoresist, whereas the lighter areas are unexposed paper. a) Patterned paper after absorbing Waterman red ink (5 μ L) by capillary action. The central channel absorbs the sample by capillary action and the pattern directs the sample into three separate test areas. b) Complete assay after spotting the reagents. The square region on the right is the protein test and the circular region on the left is the glucose test. The circular region on the top was used as a control well. c) Negative control for glucose (left) and protein (right) by using an artificial urine solution (5 μ L). [18] d) Positive assay for glucose (left) and protein (right) by using a solution that contained 550 mm glucose and 75 μ m BSA in an artificial urine solution (5 μ L). The control well was spotted with the potassium iodide solution, but not with the enzyme solution. A similar control containing the enzyme solution, but not the iodide, gave identical results (data not shown). e) Glucose and protein detection assays by using varying concentrations of glucose and BSA.

when it ionizes and binds to proteins; [11] a positive result in this case is indicated by a color change from yellow to blue.

For the glucose assay, we spotted a 0.6 m solution of potassium iodide (0.3 µL), followed by a 1:5 horseradish peroxidase/glucose oxidase solution (0.3 μL; 15 units of protein per mL of solution). For the protein assay, we spotted 0.3 µL of a 250 mm citrate buffer solution (pH 1.8) in a well separate from the glucose assay and then layered a 3.3 mm solution (0.3 μL) of tetrabromophenol blue (TBPB) in 95 % ethanol over the citrate buffer solution. The spotted reagents were allowed to air dry at room temperature. This preloaded paper gave consistent results for the protein assay regardless of storage temperature and time (when stored for 15 days both at 0°C and at 23°C and wrapped in aluminum foil). The glucose assay was sensitive to storage conditions and showed a decreased signal for assays run 24 h after spotting the reagents (when stored at 23 °C); when stored at 0 °C, however, the glucose assay was as sensitive after day 15 as it was on day

We measured artificial samples of glucose and protein in clinically relevant ranges (2.5-50 mm for glucose and 0.38-7.5 µM for bovine serum albumin (BSA))[12,13] by dipping the bottom of each test strip in a premade test solution (5 µL; Figure 1 d). The fluid filled the entire pattern within approximately 1 min, but the assays required 10-11 min for the paper to dry and for the color to fully develop. [14] In all cases, we observed color changes corresponding roughly in intensity to the amount of glucose and protein in the test samples, in which the lowest concentrations define the lower limits to which these assays can be used (Figure 1e). For comparison, commercially available dipsticks detect glucose at concentrations as low as 5 mm $^{[7,9]}$ and protein as low as 0.75 μm ; $^{[6,15]}$ these limits indicate that these paper-based assays are comparable in sensitivity with commercial dipstick assays. Patterned paper-based assay formats also allow for the measurement of multiple analytes.

This paper-based assay is suitable for measuring multiple samples in parallel and in a relatively short period of time. For example, in one trial, one researcher was able to run 20 different samples (all with 550 mm glucose and 75 µm BSA) within 7.5 min (followed by another 10.5 min for the color to fully develop). An 18 min assay of this type-capable of measuring two analytes in 20 different samples—may be efficient enough to use in high-throughput screens of larger sample pools.

In the field, samples will not be measured under sterile conditions, and dust and dirt may contaminate the assays. The combination of paper and capillary action provides a mechanism for separating particulates from a biological fluid. As a demonstration, we purposely contaminated the artificial urine samples with quantities of dirt, plant pollen, and graphite powder at levels much higher than we might expect to encounter in the field. These particulates do not move up the channels with the sample and therefore do not interfere with the assay (Figure 2).

Paper strips have been used in biomedical assays for decades because they offer an inexpensive platform for colorimetric chemical testing.^[1] Patterned paper has characteristics that lead to miniaturized assays that run by capillary

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Communications

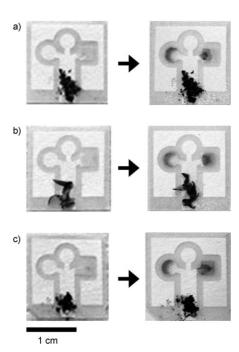


Figure 2. Assays contaminated with a) dirt, b) plant pollen, and c) graphite powder. The pictures were taken before and after running an artificial urine solution that contained 550 mm glucose and 75 μm BSA. The particulates do not move up the channels during the assay.

action (e.g., without external pumping) with small volumes of fluids. These methods suggest a path for the development of simple, inexpensive, and portable diagnostic assays that may be useful in remote settings, and in particular, in less-industrialized countries where simple assays are becoming increasingly important for detecting disease and monitoring health.^[16,17]

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