Electronic Supplementary Material (ESI) for Analyst.

Printing silicone-based hydrophobic barriers on paper for microfluidic assays using low-cost ink jet printers

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Supporting Information

Experimental

Materials

Tetraethyl orthosilicate (TEOS, Aldrich), tetrakis(dimethylsiloxy)silane (QM^H_4, Gelest), tris(pentafluorophenyl)borane [$B(C_6F_5)_3$, 95%, Aldrich], 1,3-dimethyltetramethoxysilane (DMTMDS) (Gelest), cetyltrimethylammonium bromide (CTAB, Sigma-Aldrich), Triton X-100 (poly(ethylene glycol) *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether, Sigma-Aldrich), sodium dodecylsulfate (SDS, BioShop), B-PER® direct bacterial protein extraction reagent (Product #1861465, Thermo Scientific), chlorophenol red (CPR, Sigma-Aldrich), isopropyl-β-D-thiogalactopyranoside (IPTG, Bioshop), Whatman™ qualitative paper grade 1 and quantitative ashless paper grade 41 paper (Whatman) were obtained from the indicated suppliers and used as received. Tryptic soy broth (TSB) media was composed of bacto tryptone (BD), potassium phosphate (Caledon), dextrose (D-glucose, Caledon), sodium chloride (Bioshop) and peptone (BD). All alcohols used were reagent grade and used as received.

Siloxane formulations

Printable formulations were produced by first preparing catalyst stock solutions. For P1, $B(C_6F_5)_3$ was added to TEOS (0.125 g, 0.6 mmol) or DMTMDS (0.136 g, 0.6 mmol) to give stock solutions of 0.016 M (2 mg, 0.004 mmol) and 0.063 M (4 mg, 0.008 mmol), respectively. For the P2A formulation (Table 1S), 40 mg (0.078 mmol) of catalyst was dissolved in 1 mL of a 1:1 MeOH and IPA mixture. Stock solutions could be kept for several hours or days if stored under relatively dry conditions. The P2B ink would be freshly prepared for a series of experiments. The printable P1 formulation was prepared in a very specific order by first combining the stock solutions with the appropriate alcohols and finally with QM^H_4. *Note that this reaction will generate ethane or methane, respectively, as a byproduct: mixtures containing catalyst should not be stored in a closed container* (Figure 1S).

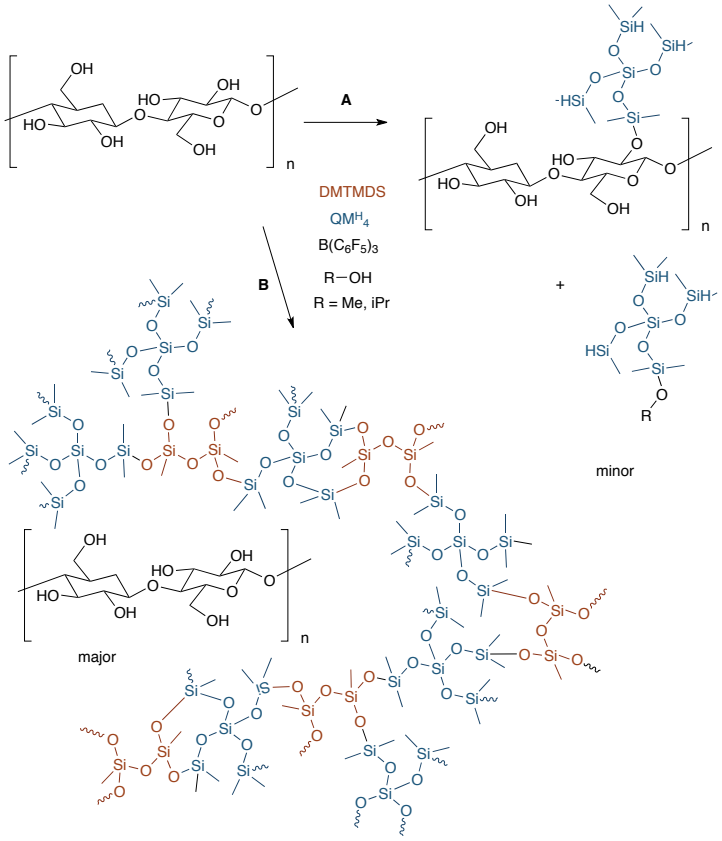


Figure 1S: General schemes of the PR reaction to form new siloxanes from TEOS or DMTMDS, respectively.

Table 1S: All siloxane formulations to form silicone barriers on paper. Siloxanes and catalyst amounts are reported in mmol and alcohols are reported in mL.

	Recipe					
Reagents	M1	M2	M3	P1	P2A	P2B
DMTMDS				0.6	-	0.6
TEOS	0.6	0.6	0.6			
QM^H_4	0.6	0.6	0.6	0.6	-	0.6
$B(C_6F_5)_3$	0.008	0.008	0.008	0.004	0.08	-
MeOH	1.0	0.1	0.05	0.05	0.5	0.05
IPA	-	-	0.05	0.05	0.5	0.05
Printer Method [a]	-	1P	1P	1P	2P	
App [b]	-	4	4	4	2[c]	2[c]

[a] Printer Method = Number of printers used to form silicone barriers (e.g. P2A and P2B used together utilizing the 2 printer methodology (2P)).

[b] App = number of applications or printing passes.

[c] P2A and P2B were printed once on each side of the paper, thus a total of 2 applications/printing passes.

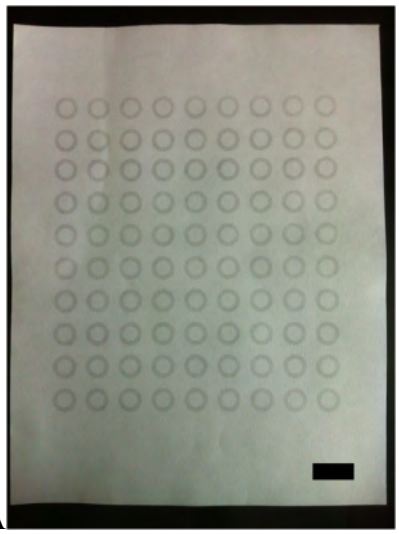
Printing protocols

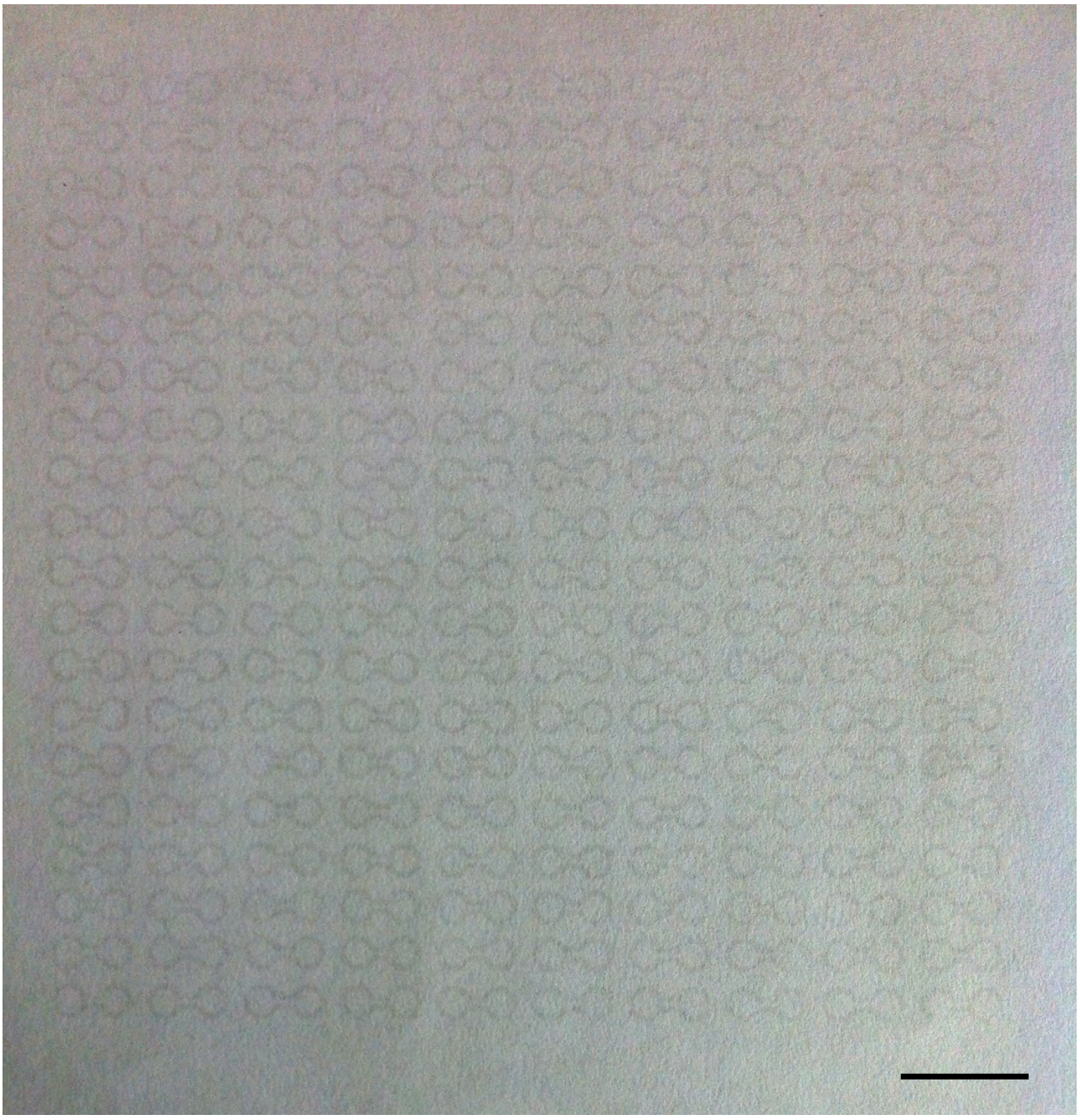
Ink-jet printing siloxane barriers from a single printer

A Canon Pixma MP280 was used to ink-jet print the siloxane containing solutions (M1-M3, P1, P2A/P2B) onto Whatman™ filter paper grade 1, which was cut into 8.5 x 11 inch pieces. PG-210 black ink cartridges that can dispense ink droplets of 25 pL (picoliters), were reconstructed in the following manner. The sponges inside the cartridge and the tank cover slip were removed. The black ink was removed and the cartridge was thoroughly cleaned with water and air dried to reduce the amount of residual water. Once dry, the siloxane-containing solutions were loaded into the cartridge. The printer was controlled by a personal computer and hollowed out circles (like the letter "O") of 1.1 cm diameter were constructed using Microsoft Word and were printed onto the paper. Up to 4 printing passes (Table 1) were performed with the printing formulations to optimize the amount of siloxane deposited on paper, and then rapidly cured with a heat gun for 10 s.

Ink-jet printing siloxane barriers from two printers

Two Canon Pixma MP280 printers were used to keep the catalyst separate from the other ingredients. The first printer was used to print only the catalyst mixture P2A, while the second printer was used to lay the siloxane mixture P2B on top of the dried catalyst solution: cure began once the two solutions mixed leading to a siloxane-based barrier. It was not possible to use a single printer with two separate ink cartridges, as the automatic cartridge cleaning protocol performed by the printer (which cannot be shut down) leads first to cross contamination followed by clogging. To print two solutions on the same page location (with proper alignment) from two different printers, 4 document templates in Microsoft Word were devised: 1 template was used to print the front side and 1 for the back side of the paper for each printer. Once the front and back side templates were properly aligned, formulations P2A and P2B, respectively, were loaded into their respective printers. P2A was first printed on both the front and the back side of the paper with 1 pass each. Then P2B was printed on the front and back sides with 2 passes each and the treated surface. Although cure was relatively rapid at room temperature, greater reproducibility in barrier formation was observed when the samples were heated with a heat gun for 10 s (total of 2-3 mins for fabrication). Typically a series of circles were printed onto the paper, but 200 objects (2 hollowed circles connected by a channel) were also readily printed onto full sheets of paper (Figure 2S).

A  B



C 

Figure 2S: 90 hollowed circles (A) and hollowed circles (400/page) with channels connecting adjacent circles (B). Objects were inkjet printed using inks P2A/P2B with the 2 printer methodology. C is a magnified image of B. Scale bars = 2 cm (A, B) and 5 mm (C).

Wax printed hydrophobic barriers

For comparative studies with the most commonly used hydrophobic barrier, wax, identical hollowed circles were printed on Whatman filter paper grade 1 as previously described[1, 2] with one pass through a Xerox Phaser 8560 wax printer and then heated at 120°C in an oven for approximately 2 min and allowed to cool. In one case, the process was repeated 4 times to load extra wax onto the paper.

Surface tension measurements

The surface tension of the tested surfactant solutions were measured with Optical Contact Angle OCA20 (Future Digital Scientific Corp) using the pendant drop method with a 500 μL Hamilton syringe and a 1.65 mm needle.

Testing the robustness of the hydrophobic barriers

The ability of a given ink to constrain aqueous flow through paper was affected by the ink, number of printing passes and the type of paper. Summaries of the robustness of each of the inks are provided below.

Distilled water (DI) was utilized on its own and in solutions of 1% w/v SDS, 1% v/v Triton X-100 and 1% w/v CTAB in 75 mM phosphate buffer at pH 6.8. The concentrations of all surfactant solutions were chosen to be well above their critical micelle concentrations.[3-5] A commonly used cell lysing solution, 10% v/v B-PER was also prepared. The B-PER concentration was chosen to correspond to standard concentrations used for cell lysing experiments. Chlorophenol red (CPR) was then added to each solution as a colorimetric and fluorescent dye for both optical and fluorescent imaging. Each surfactant solution (15 µL) was added into the printed hollowed circles of either siloxane- or wax-based barriers for comparative studies. Solvents were used as received and were tested against the hydrophobic barriers by placing 15 µL of the given solvent into the printed hollowed circles.

M1

Manually pipetting the dilute ink M1 (Table 1S) onto Whatman filter paper grade 41, and briefly heating, led to a hydrophobic barrier that could resist water penetration (*note that small amounts of gas may be observed during heating* – this is evidence of alkane release during the PR reaction). By contrast, under the same conditions on Whatman filter paper grade 1 a barrier was not achieved. The most notable difference between the two Whatman papers is the pore size of the cellulosic network: 11 µm and 20 µm for Whatman #1 and Whatman #41, respectively. The larger surface area on Whatman #1 paper required more silicone delivered from a more concentrated ink

M2

After concentrating the siloxane content in the ink (M2, Table 1S), consistent hydrophobic barriers could be produced when manually pipetted onto Whatman #1 paper. These barriers were also capable of withstanding water penetration. However the ink's surface tension was too low, and the M2 loaded black ink cartridges had dripping issues when printing leading to the clogging of the cartridge itself.

M3

To solve the low surface tension issue seen with M2, a wide range of solvent formulations were screened and 50:50 methanol:isopropanol ratio was found to allow for M3 (Table 1S) to be printed without dripping while retaining its reactivity on paper. When testing the robustness of the barriers with various surfactant solutions (10% B-PER, 1% SDS, 1% Triton X-100, 1% CTAB), all of them were capable of penetrating the silicone barriers, even if the barriers were applied by hand.

P1

Forming more robust hydrophobic barriers required the siloxane ink formulation to be modified with more hydrophobic siloxanes. Thus, several hydrophobic alkoxysilanes were tested with QM^{H}_{4} to determine a suitable substitute for TEOS, which doesn't contain any hydrophobic moieties. DMTMDS was found to be a suitable replacement, as it was capable of consistently forming hydrophobic barriers on paper and could also be inkjet printed. However, clogging of

the cartridges was still observed. As shown in Figure 3S and Figure 7S, the siloxane ink after cure did not penetrate into and hydrophobize the paper evenly,

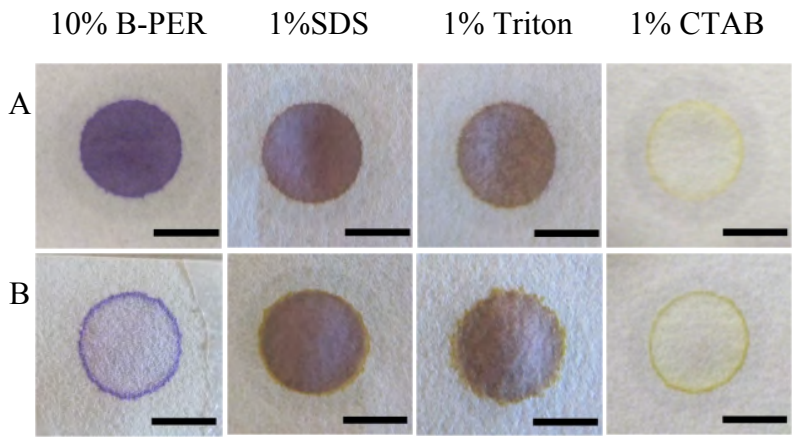
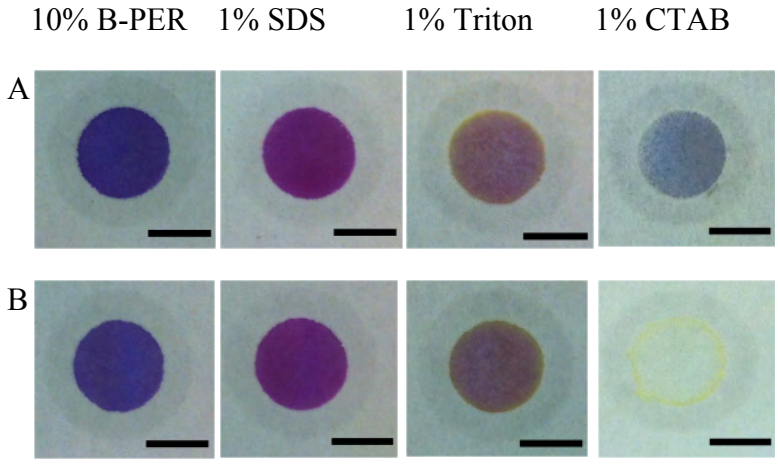


Figure 3S: P1 printed hollowed circles with CPR surfactant solutions on the printed side (A) and the backside (B). Scale bars = 5mm.

P2A and P2B

The minimum thickness required to contain surfactant solutions was tested with the preferred 2 printer method and as Figure 4S C and D demonstrates, a barrier thickness of 0.7 mm is the limit of containing the B-PER surfactant solution. Due to the larger unmodified internal surface area of the two hollowed circles, an additional 15 µL of CPR surfactant solution was added to each. Figure 4S D showed that a barrier thickness of 0.6 mm was just below the limit for surfactant solutions, however water could be contained within these. Note that unconstrained solutions (15 µL) expanded to approximately 7.4 mm.



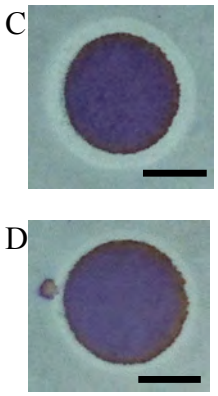


Figure 4S: Images of both printed sides (B, C) of the silicone hollowed circles (2 printer method) with CPR surfactant solutions of 10% B-PER, 1% SDS, 1% Triton X-100 and 1% CTAB added to them. Barriers with thicknesses of 1 mm (D) and 0.6 mm (E) demonstrated the minimum thickness required to contain surfactant solutions. Scale bars = 5 mm (A – D).

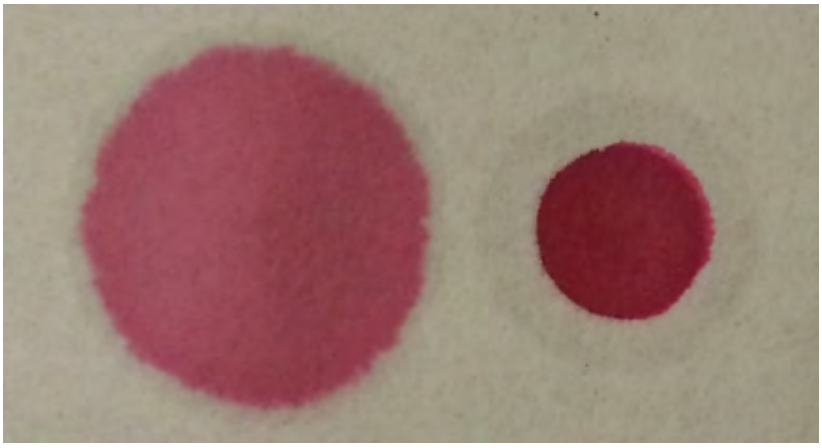


Figure 5S: Difference in spreading efficiency of a 15 µL aqueous solution unconstrained (left) and constrained (right) by a silicone barrier.

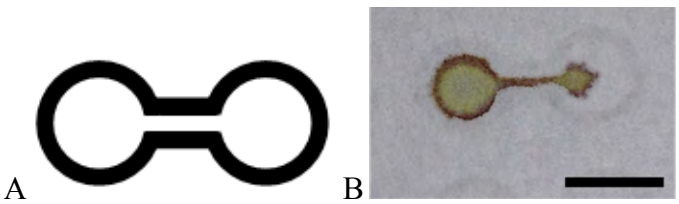


Figure 6S: Structure of hollowed circles with channels printed onto paper (A) using the 2 printer method and then tested for their ability to contain a 10% B-PER/CPR solution (B). Scale bar = 5 mm.

The ability to print a large number of hollowed circles with the smallest printable unmodified channels to contain and transport a surfactant solution was tested. 400 silicone

hollowed circles with channels (Figure 2S A; 200 objects of the image shown in Figure 6S A) were printed with barriers 1 mm thick. In addition, Figure 6S B show the result after a drop of a 10% B-PER/CPR solution (~50 µL) was added to a hollowed circle. The fluid was capable of travelling across an unmodified cellulose channel approximately 0.4 mm width. This demonstrated that large screening tests for biological assays with microfluidic capabilities appear possible with surfactant based solutions using silicone barriers.

Imaging of hydrophobic barriers

Fluorescent Images

Optical and fluorescent images were obtained with an Olympus BX51 microscope fitted with a Q-imaging Retiga EXi camera and with the Image Pro-Plus software. By utilizing a rhodamine fluorescent filter with an excitation wavelength from 530-550 nm and a long pass emission filter at 590 nm, fluorescence images of CPR were obtained.

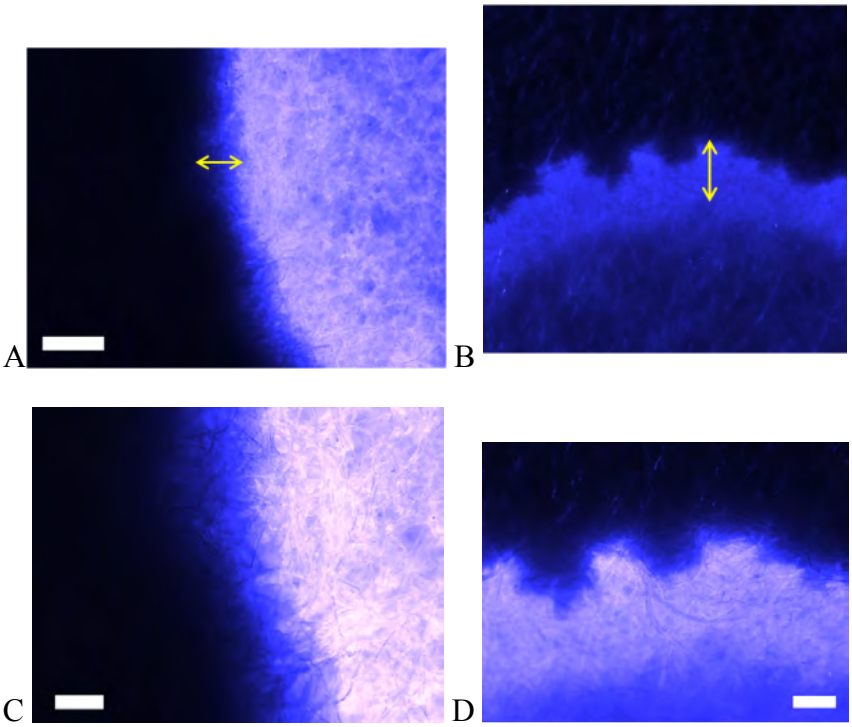


Figure 7S: Fluorescent images of a 10% B-PER solution with a CPR dye added to the P1 barriers on the printed side (A) and the backside (B). Yellow arrows show the discrepancy in hydrophobization between the two faces of the paper. Scale bars = 500 µm. (C,D) Blow up of A,B: Scale bar = 200 µm.

SEM

Scanning electron microscopy (SEM) images were taken with a JEOL 7000f FE-SEM after coating samples with 15 nm of gold. Electron dispersive spectroscopy (EDS) was performed with the same instrument on cross-sectional slices of the silicone barriers on paper. First, the silicone modified paper was infiltrated with Spurr's epoxy resin in a vacuum oven for 24 hours and then transferred to flat embedding molds where they polymerized overnight at 60 °C. 500 nm slices were cut on a Leica UCT Ultramicrotome and finally adhered to a polished SEM stub. Line mapping of the fibers was done using the Oxford Instruments Inca software.

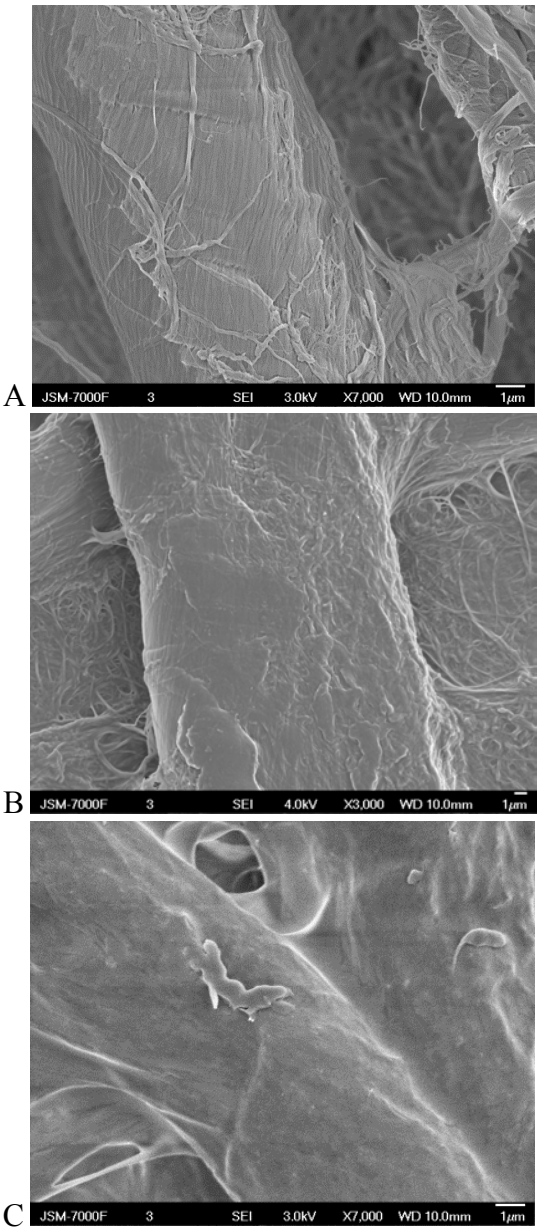


Figure 8S: SEM images of Whatman #1 paper that is unmodified (A), wax modified (B) and P1 siloxane-modified (C). SEM images at various magnifications of Whatman #1 paper that is unmodified (A), wax modified (B) and P1 siloxane-modified (C). 300 x magnification.

The resulting printed barrier from P2A and P2B was quite resilient: crumpling of the modified paper appeared to have no discernible difference in the ability of the hydrophobic siloxane barriers to withstand water, even after several washings of hexanes. SEM images of the crumpled barriers depict only minor cracking of the cured silicone around the cellulose fibers (Figure 9S). Separating the inks meant that clogging of the cartridges did not occur, and the barriers formed were capable of containing the surfactant solutions.

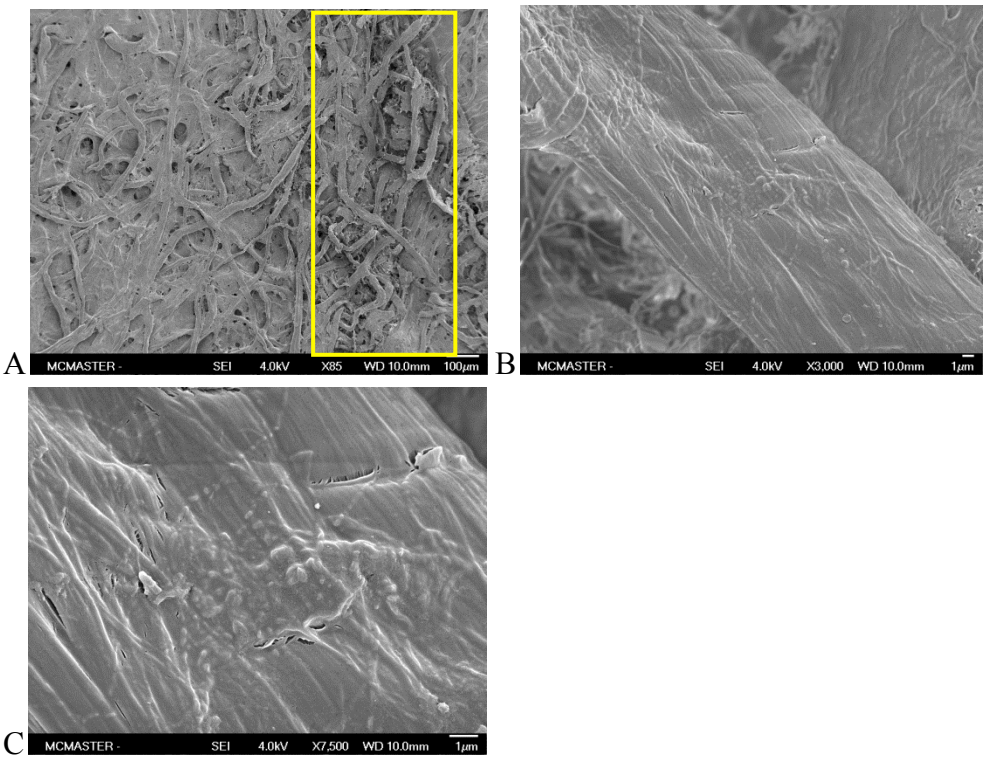
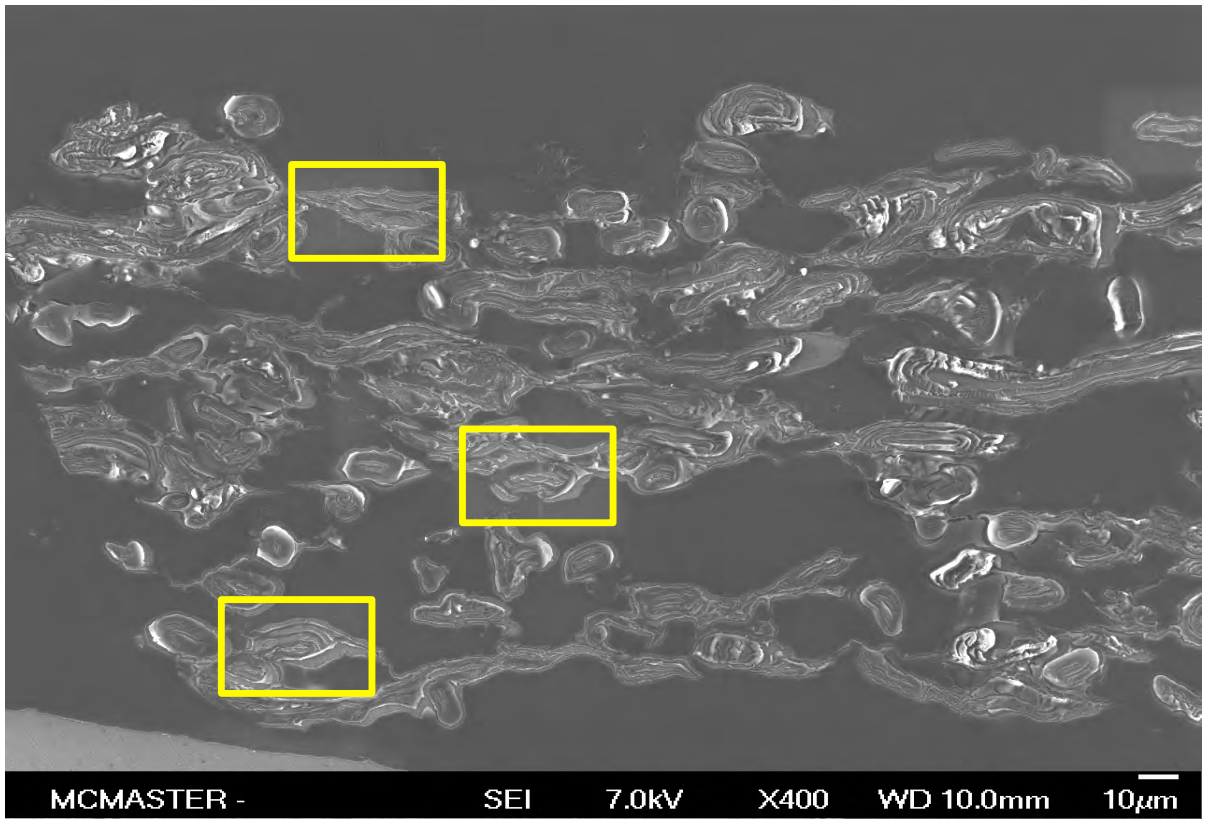


Figure 9S: SEM images of folded and crumpled paper that was soaked overnight in hexanes at various magnifications. Yellow box depicts the fold in the paper and the region where B was obtained. C is a magnification of B.

Examining the extent of hydrophobization by EDS

Cross-sections of the hydrophobized paper were analyzed by EDS to determine the depth and location of the silicone barriers. Figure 10S A shows a cross-sectional SEM image of the Whatman paper and the locations of the EDS line mappings. Figure 10S B – D shows that the majority of the cross-sections are unsurprisingly composed of carbon (blue line). However, they also depict the presence of silicone throughout the paper cross-section by the strong silicon and oxygen signals (green and red lines respectively) compared to a weaker carbon signal. More interestingly, the silicones seem to locate themselves around the cellulose fibers, between fibers and even within pockets of the fibers themselves. Figure 10SB shows cellulose fibers that run horizontally to one another with a strong silicone signal at the top of the image depicting the surface of a fiber, and another smaller signal in the middle of the image that appears between fibers. A strong silicone signal is found within the cellulose fiber as shown in the middle of the image of Figure 10SD. This indicates that the siloxane ink can penetrate cellulose fibers with pores or openings quite quickly before curing.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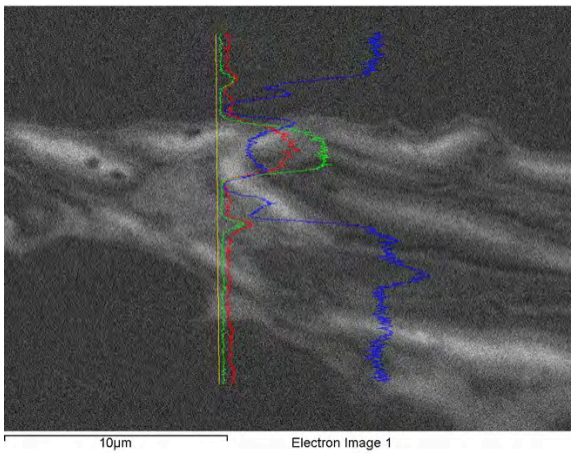
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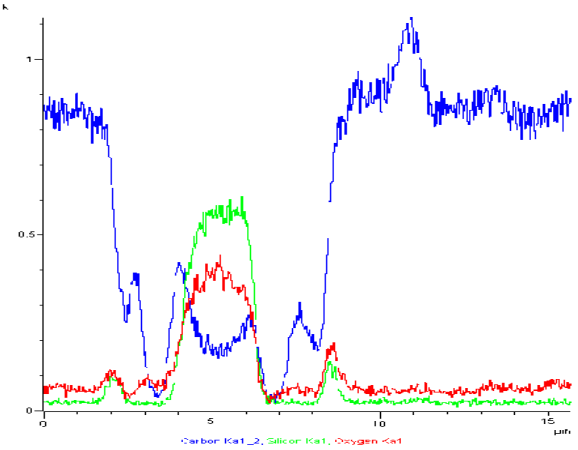
Electron Image 1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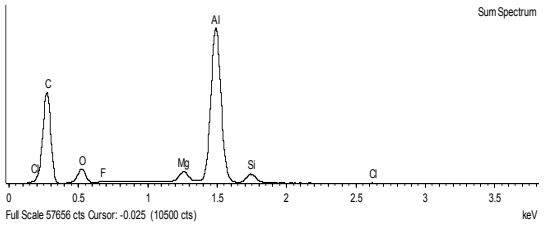
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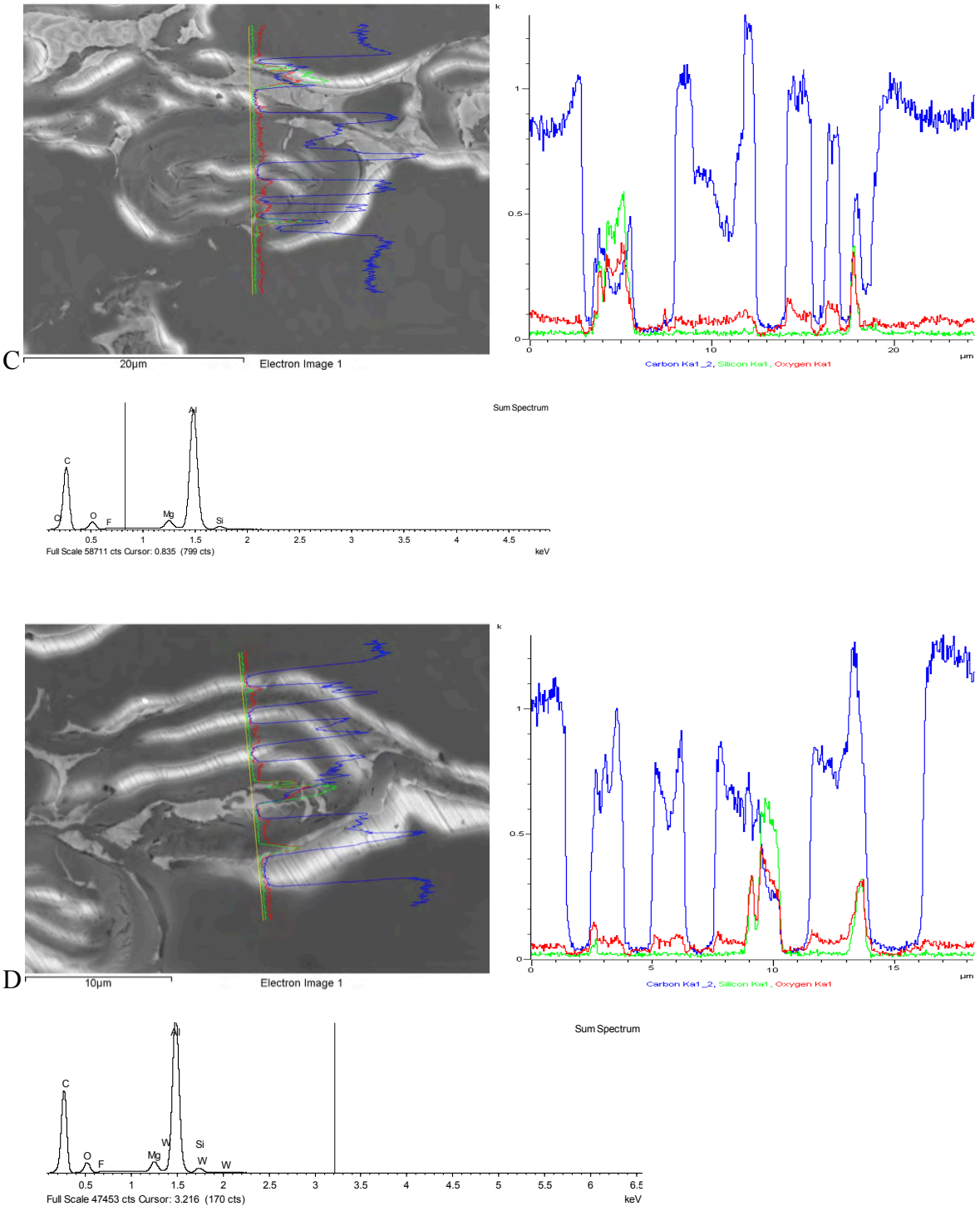

Figure 10S: Cross-sectional view of the silicone modified paper and the locations of the EDS line mappings in yellow boxes (A) of both printed sides (B, D) and middle section of the paper (C). Green line = silicon, red line = oxygen and the blue line = carbon.

Biological Assay

Detecting β-galactosidase from lysed E. coli cells within the hydrophobic barriers

Total coliform test strips were adapted from the previously reported tests strips for total coliform detection.[6] Circles of 1.1 cm in diameter were delimitated by a printed wax-based hydrophobic barrier or a siloxane-based hydrophobic barrier, using the 2 printer method explained above. A sensing zone was printed inside the circle with a Canon Pixma MP280 printer. A layer of poly-L-arginine hydrochloride (P-Arg, 2% w/v in Milli-Q water) was first printed, followed by one layer of chlorophenol red β-galactopyranoside (9 mM in MilliQ water). In the original article by Hossain et al.,[6] poly(vinylamine) (PVA) instead of P-Arg was utilized as a capturing agent to concentrate the formed dye. However, due to challenges with printability with poly(vinylamine), P-Arg was used instead. *E. coli* ATCC 25922 cells were grown overnight at 37 ºC in TSB media. IPTG (2 µL/mL) was added to the media to induce the β-galactosidase enzymatic production. Cells were lysed with B-PER® direct bacterial protein extraction reagent following the manufacturer protocol (9:1 ratio of cells in suspension to B-PER). 15 µL of cell lysate was dropped onto the circle test strips.

References

1. E. Carrilho, A. W. Martinez and G. M. Whitesides, *Anal. Chem.*, 2009, **81**, 7091-7095.
2. E. Carrilho, A. W. Martinez and G. M. Whitesides, *WO2010102294A1*, 2010, **President and Fellows of Harvard College**.
3. E. J. Regalado, J. Selb and F. Candau, *Macromolecules*, 1999, **32**, 8580-8588.
4. C. Cuypers, T. Pancras, T. Grotenhuis and W. Rulkens, *Chemosphere*, 2002, **46**, 1235-1245.
5. N. Li, S. Liu and H. Luo, *Anal. Lett.*, 2002, **35**, 1229-1238.
6. S. M. Z. Hossain, C. Ozimok, C. Sicard, S. Aguirre, M. M. Ali, Y. Li and J. Brennan, *Anal. Bioanal. Chem.*, 2012, **403**, 1567-1576.