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

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Paper-based microfluidic devices exhibit many advantages for biological assays. Normally, the assays are restricted to certain areas of the paper by hydrophobic barriers comprised of wax or alkyl ketene dimers (AKD). Neither hydrophobic barrier is able to constrain aqueous solutions of surfactants, which are frequently used in biological assays. We demonstrate that rapidly curing silicone resins can be inkjet printed onto pure cellulose paper using inexpensive thermal ink-jet printers. The Piers-Rubinsztajn (PR) reaction dominates the cure chemistry leading to cellulose fibers that are surface coated with a silicone resin. The resulting barriers are able to resist penetration by surfactant solutions and even by the lower surface energy solvents DMF and DMSO. The utility of the barrier was demonstrated using a coliform assay based on detection of β-galactosidase.

Introduction

Microfluidic, paper-based analytical devices (μPAD) offer a variety of advantages over traditional laboratory-based bioassays as a platform for the detection of various analytes in qualitative or quantitative assays.[1-6] Such devices typically use hydrophobic barriers to control liquid movement, and move liquids by wicking rather than external pumping,[7] making them suitable for use in countries with limited resources.

Several methodologies for forming hydrophobic barriers have been reported, including wax printing/patterning,[2, 8, 9] ink-jet printing,[10, 11], spraying techniques,[12] laser ablation,[13] plasma treatment[14], etc. Wax and AKD are perhaps the most widely used materials to create hydrophobic barriers, as these are cost-effective and can be used with a number of printing techniques.[15] However, many reagents that are commonly found in bioassays (enzyme activity,[16, 17] toxicity studies,[18, 19] etc.), including low surface energy surfactants, alcohols and solvents like DMSO or DMF will breach these barriers.

Sol-gel derived methylsilsequioxanes (MSQ) have been described as a barrier-forming material that can be ink-jet printed and which resists breaching by surfactants and some low surface tension liquids.[20] However, this material requires an expensive, research-grade piezoelectric ink-jet printer for barrier fabrication, making it unsuitable for direct printing of μPADs in resource-limited settings. In addition, the barriers required ~6 h to cure, making high-speed μPAD production difficult. More recently, Deiss et al. produced robust Teflon based barriers on paper, which also utilized a research-grade dispenser for automation.[21]

A goal of the current study was to develop robust barrier materials that could be easily printed using inexpensive and widely available thermal ink-jet printers, to facilitate access for researchers to μPADs. We evaluated a Canon thermal inkjet printer, which is inexpensive (ca. CDN$40), has a built in sheet feeder to allow rapid printing of multiple pages, and can use aqueous inks without the need to modify the viscosity or surface tension. A major hurdle to overcome was condensation of the ink in the nozzle due to the heating of the ink associated with the thermal inkjet process, which can lead to rapid clogging of cartridges. In addition, the method of hydrophobization and characterization of printed barriers are important.

We have been exploring the utility of the Piers-Rubinsztajn (PR)[22, 23] reaction as a novel method for silicone elastomer formation, The reaction utilizes a Lewis acidic boron catalyst $B(C_6F_5)_3$ (~0.3 mol %) to facilitate the condensation of hydrosilanes with alkoxysilanes to form new siloxane bonds ($R_3Si\text{-}H + R'OSiR''_3 \rightarrow R_3Si\text{-}OSiR''_3 + R'H$) and an alkane byproduct (Figure 1).[23, 24] This reaction is extremely rapid (seconds to minutes at 25 °C) and is capable of forming various silicone elastomers,[25, 26] resins,[27] and other materials.[28]

Silicones have a lower surface energy than hydrocarbons and it was therefore hypothesized that they may be better able to contain aqueous solutions on paper, particularly those containing surfactants. We describe the thermal ink-jet printing of silicone precursors (siloxanes) onto porous filter papers that are rapidly converted into hydrophobic silicone resin barriers. This is the first description of printing rapidly curing, low surface tension siloxane inks from a standard ink-jet printer. The beneficial characteristics of the printing process, including the ability to localize surfactant-containing aqueous solutions, are demonstrated using a paper-based sensor for coliform detection developed by Hossain et al.[29]

Materials and methods

A Canon Pixma MP280 was used to ink-jet print the siloxane-containing solutions onto Whatman™ #1 filter paper, which was cut into 8.5 x 11 inch pieces. PG-210 black ink cartridges, that

can dispense ink droplets of 25 pL, were cleaned before use (ESI †). Tetrakis(dimethylsiloxy)silane (QM^H_4) and 1,3-dimethyl-tetramethoxysilane (DMTMDS, Gelest) and tris(pentafluoro-phenyl)-borane ($B(C_6F_5)_3$, 95%, Aldrich) were used as received.

Inks (ESI † Table 1S) were prepared by diluting the starting materials in methanol/isopropanol mixtures, placed into the ink cartridge and then printed onto the Whatman paper. After printing, the paper was optionally heated for a few seconds using hot air from a heat gun to cure the silicone prior to testing. Full details about printing and barrier testing are found in the ESI †.

Results

Thermal ink-jet printers commonly use inks with both low surface tension (30-40 mN/m) and viscosities (1-5 cP).[30, 31] Formulation of the uncured silicone ink required consideration of "printability", the rate of curing before and after printing, and performance of the resulting cured silicone barrier. A wide variety of commercially available precursor siloxanes permit one to tune the physical properties of the oil, foam,[28] elastomer[32] or resin produced by the PR reaction. Extensive optimization experiments based on hydrosilanes/hydrosilanes plus alkoxysilanes/alkoxy-modified silicones (data not shown) demonstrated that a resin formed from the reaction of QM^H_4 + DMTMDS balanced these printability requirements most effectively (Figure 1).

Two main issues needed to be addressed when formulating inks for the Canon printer: (i) achieving a suitable surface tension and (ii) avoiding clogging of the ink cartridge due to reaction of the ink in the cartridge. The PR reaction requires complexation of the boron catalyst $B(C_6F_5)_3$ with the SiH group of the hydrosilane.[23] However, due to the strong Lewis acidity of the catalyst, Lewis basic reagents such as alcohols also coordinate to the boron catalyst and inhibit the PR reaction. Thus, an alcohol was chosen as diluent for the siloxane precursors both to control surface tension and to mitigate premature cure. Note that silyl ether formation between hydrosilanes and alcohols (R_3SiH + $HOR' \rightarrow R_3SiOR'$ + H_2) can occur under the same reaction conditions, but at a much slower rate, over days rather than seconds. While several inks were examined, the best performing were formulated in a combination of methanol/isopropanol – which had ideal properties in terms of both viscosity and surface tension – as a complete ink P1 used on a single printer, or separated into two inks used on two printers P2A/P2B (ESI † Table 1S).

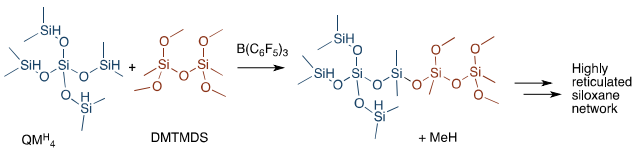

Figure 1: The PR reaction leading to a silicone resin.

After formulations of the siloxane "ink" were loaded into clean and dry 'black ink' cartridges, hollowed circles (ID 7 mm, OD 11 mm) were printed on Whatman #1 paper.[#] When P1 was printed onto cellulose, a hydrophobic domain rapidly formed that was tightly anchored to the substrate. Greater reproducibility in preparing hydrophobic barriers was observed when a short heating step (~ 10s hot air) was used after printing to help evaporate the solvent. Attempts to use single or multiple passes of ink demonstrated that 4 passes were required to create effective silicone barriers that were not 'breached by water (15 µL) (ESI †). Unfortunately, this ink exhibited a relatively short 'pot life' as, even in alcoholic solvents, the PR reaction occurred slowly, leading to polymer formation and clogging of cartridges after about 1 hour of use.

For inks P2A and P2B, two printers were utilized to create the silicone barriers. P2A (catalyst mixture) was first printed once on each side of the Whatman paper, with a subsequent printing of P2B (siloxane mixture) once on each side of the paper using a separate printer. In this case, the catalyst never contacts the siloxane ink components except on the paper and clogging of the nozzles was not observed.[▽] Since formulations P2A and P2B do not independently react over time, they can be stored for much longer time periods than the reactive mixture P1. Although the initial setup is slightly more complicated, the two-printer method is preferred: both mixing and chemistry occur directly on paper.

A comparative study between wax printed barriers[2] and the inkjet printed silicone barriers was performed using surfactant-containing solutions. Standard solutions representing different surfactant classes were prepared from anionic (SDS), non-ionic (Triton X-100) or cationic (CTAB) surfactants, with surface tensions of 36.6 ± 0.2, 31.4 ± 0.2 and 35.5 ± 0.2 mN/m respectively. In addition, B-PER (31.3 ± 0.2 mN/m), which is commonly used for cell lysis[29] (the exact composition is a trade secret of Thermo Scientific), was investigated to demonstrate the ability to retain cell lysates in a defined area of the paper.

Wax-based barriers (1.5 mm thick) were breached by all of the surfactant solutions over time, even if the wax circle was created using 4 passes of the wax ink (2.7 mm, Figure 2A). By contrast, robust barriers could be prepared using 4 printing passes with ink P1 (1.7 mm wide on the top face and 1.5 mm on the back side, ESI † Figure 3S): that were not breached when surfactant solutions (15 µL) were added into the circles. However, the surfactant solutions were observed to penetrate further into the silicone barrier on the bottom side, which appeared as a lightly colored corona on the printed side of the paper.

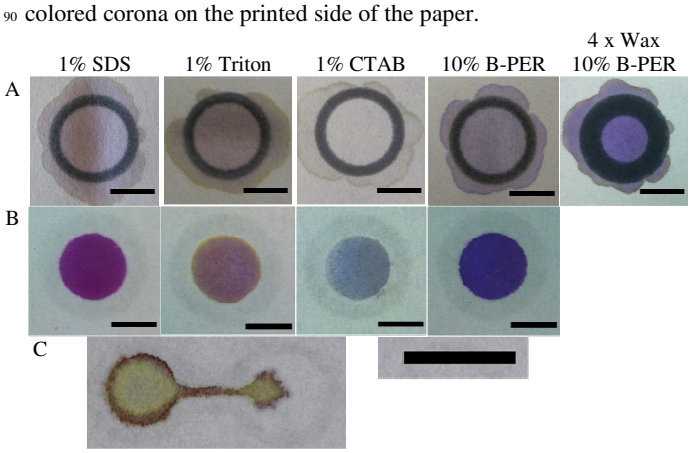

Figure 2: A: Wax-based hollow circles (internal diameter = 3.7 mm) stained with CPR surfactant solutions 1 or 4 passes of wax. B: P2A/P2B printed hollowed circles on Whatman #1 paper with CPR surfactant solutions. C: Silicone barrier width 2 pt, 0.7 mm. Scale bar = 5 mm.

Analogous silicone barriers were printed using 2 printers: one with a cartridge containing the catalyst P2A and the other a

mixture of the hydro- and alkoxy-silanes P2B (ESI † Table 1S). Using this protocol, both sides of the paper were printed (once on each side for each ink formulation) as full pages of hollowed out circles (90 in total) or smaller hollowed out circles connected by channels (200 objects) can be fabricated within a relatively short time frame (~2-3 min, ESI † Figure 2S, 5S). The robustness of the barriers derived from P2A and P2B was demonstrated with the different surfactant solutions (Figure 2B). In no case was the barrier (1.9 mm wide on both sides) breached, but unlike those described with P1 using the one printer method, the barriers appeared to be uniform on both sides of the paper: no corona was observed. Surfactant solutions could travel through untreated cellulose channels as narrow as 0.4 mm wide constrained by silicone barriers as narrow as 1 mm (Figure 2C). This means the barrier could be printed in a standard 384 well array (or slightly higher density), with each 3.1 mm diameter well surrounded by 0. 7 mm silicone. Note that an unrestrained 15 µL droplet forms a 7.4 mm diameter spot on this paper (ESI †).

The wax-based barriers and the silicone barriers formed from either P1 or P2A/P2B were exposed to several solvents to establish differences in their barrier properties. Like the surfactant solutions, 15 µL of a given solvent was placed within the hollow circles. Neither the siloxane nor wax-based barriers were capable of containing solvents with very low surface tension e.g., toluene (Table 1). However, the silicone barriers were capable of containing both DMSO and DMF: P2A/P2B-derived silicone barriers could even contain 1,4-dioxane. Several seconds after adding dioxane, the solvent would slowly creep into the P2A/P2B barrier, but would not breach it even after addition of a second 15 µL of solvent. Wax barriers could not contain such surfactants/solvents and AKD is reported only to withstand liquids with surface tensions >35 mN/m.[10] Thus, siloxane inks form more robust hydrophobic barriers than either wax or AKD.

Table 1: Solvents tested against wax and siloxane based barriers (P1 and P2A/P2B) to determine if they are contained (Y), not contained (N) or partially contained (P)[a]. Surface tension values are reported at 25 °C.[33]

Solvent	Wax	Silicone barrier	Surface Tension (mN/m)
Toluene	N	N	29.46
Dioxane	N	P[a]	32.75
DMF	N	Y	35.74
DMSO	N	Y	42.94
Water	Y	Y	71.99

[a] The P1 silicone barriers could not contain the solvent, whereas the P2A/P2B derived ones could.

The wax and silicone barriers were characterized using a variety of techniques. Fluorescent images were taken after exposure to either B-PER (Figure 3A, B) or SDS (Figure 3C,D) CPR solutions. The B-PER/CPR surfactant solution breaches the wax barrier (black line) by creeping along the cellulose fibers. Creep of B-PER/CPR along the fibers on both sides of the wax barrier and across the barrier itself was visualized using fluorescence (Figure 3A, B). Surfactant migration is more likely associated with wax dissolution and/or its lower hydrophobicity than insufficient coating, based on the permeability of even 4 coatings of wax (Figure 2). By contrast, creep of the surfactant solutions was not observed with the P1 (ESI †) and P2A/P2B (Figure 3C, D) silicone barriers and was well contained.

The behavior of silicone barriers printed on only one face of the paper (P1) was different from barriers printed on both faces (P2A/P2B). The former showed a wide corona upon exposure to aqueous solution (ESI †), suggesting the ink spread horizontally as well as vertically through the paper, similar to the rhombus-like printed shape described by Li et al.[34] The more resilient silicone barriers provided by the P2A/P2B ink, with 2 face printing, showed only a very small corona after exposure to CPR surfactant solution, likely due to small printing misalignments, gradients in hydrophobization, etc. (Figure 3C, D). The small disadvantage associated in needing to control alignment in multiple printing passes can be overcome,[35] and is also compensated by the ease and low cost of using siloxane barriers.

The hydrophobization of paper typically involves grafting a hydrophobic layer on the paper surface via chemical modification, physical deposition on the fibers or physical blocking of the paper pores.[34] Wax physically deposits on the cellulose fibers without blocking the pores of Whatman #1 paper (Figure 3F, ESI †). Only at high magnifications can the physical deposition of wax be noticed – discrete fibrils cannot be observed. Higher magnification images of the silicone barriers on the Whatman #1 paper, however, indicate individual cellulose fibers were modified as shown by the 'blurring' of striations arising from fibrils (Figure 3G, ESI †), in contrast to MSQ barriers, which operate by pore-filling.[20]

When hydrosilane, alkoxysilane, cellulose and $B(C_6F_5)_3$ are in contact on the paper surface, where the alcohol concentration is comparatively high both from cellulose and the solvent (MeOH + IPA, ESI † Table 1S), competitive $B(C_6F_5)_3$-catalyzed reactions occur between the hydrosilane and either the alkoxysilane or the alcohols: the partition between the reaction pathways is unknown. Based on reaction rates in solution, it is expected that chemical anchoring of the silicone resin to the paper via silyl ether formations (in analogy with direct binding found with AKD) is less likely than a layer of silicone resin coating the exposed fibers during a PR reaction (ESI †, Figure 1S†), consistent with the SEM observations (Figure 3E – G).

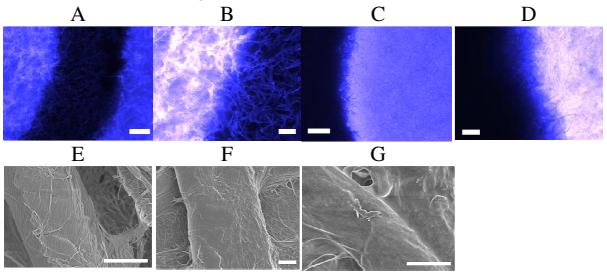

Figure 3: Fluorescent images of CPR surfactant solutions were added to wax (A, B) and to the P2A/P2B (C, D) barriers. Images B and D are magnifications of A,C, respectively (see also ESI †). SEM images of Whatman #1 paper: unmodified (E), wax-modified (F) and siloxane-modified (G). Scale bars = 500 µm (A,C), 200 µm (B, D), 5 µm (E,F,G)

Hossain et al. previously reported a paper-based sensor for total coliform detection.[29] The sensor relied on the colorimetric detection of a commonly used marker for total coliform, the intracellular enzyme β-galactosidase. The test strips contained a yellow substrate, chlorophenol red β-galactopyranoside, which in the presence of β-galactosidase, was hydrolyzed into a red-magenta product, chlorophenol red. The color change from yellow to red-magenta was thus indicative of the presence of β-

galactosidase and, therefore, of coliform. A chemical lysing step was performed prior to the assay to release the enzyme, as β-galactosidase is an intracellular enzyme. The lysed samples were then assayed via a lateral flow (LF) format.

As part of optimization studies, the strip design was modified to replace the LF format by a direct drop format. After deposition onto paper within a hydrophobized circle, a purple color change was observed for the presence of β-galactosidase. When the circles were formed by a wax barrier, the sample leached outside of the sensing zone, rendering the results hard to analyze and quantify (Figure 4A). The P2A/P2B silicone barriers, when used in lieu of wax, allowed the containment of the assay in a well-defined zone on the paper and did not interfere with the assay. The cell lysate did not leach outside of the circle and an intense color change was readily observed (Figure 4B, C) demonstrating the potential of a silicone barrier to be utilized for a biologically relevant assay.

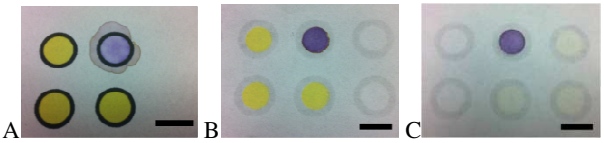

Figure 4: Inkjet printed β-galactosidase sensor (yellow) within wax barriers (A) and P2A/P2B-barrier (B, C) with the addition of lysed *E. Coli* cells in a 10% B-PER solution (purple) for total coliform detection. Images B and C show both printed sides of the paper. Scale bars = 1 cm.

Silicone barriers offer advantages over conventional barriers derived from wax or alkyl ketene dimers in challenging assays on paper based microfluidic devices, and they are more easily created than lower surface energy Teflon barriers.[21] Localization of the assays into specific locations is important both because migration leads to dilution of colors needed for assay development and, in the worst case, will lead to mixing of reagents/analytes in adjacent spots. The silicone barriers are able to resist migration of the surfactants commonly found in biological assays. The silicone barriers also offer advantages over MSQ in terms of compatibility with inexpensive printers and more rapid curing. Various hydrophobic patterns can be printed using an inexpensive inkjet printer, and allows printing to be done on-site in resource-limited settings.

Conclusions

Siloxane-based hydrophobizing agents are readily ink-jet printed onto Whatman paper and, after cure, give hydrophobic barriers that resist water, a variety of aqueous surfactant solutions, and even some organic solvents. The most efficient barrier was prepared from the PR condensation of QM^{H}_{4} with DMTMDS catalyzed by $B(C_6F_5)_3$. This facile, low cost approach lends itself to rapid prototyping of assays, including in resource challenged locations.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Full experimental detail including synthesis, survey experiments with all formulations, printer protocols, SEM, fluorescent images and EDS spectra of silicone barriers, biological assay protocol. ,See DOI: 10.1039/b000000x/

Different inks need to be formulated for paper of different porosity.†

▽ In principle, a 'color ink' cartridge would allow on to separate siloxanes from catalyst. However, the Canon Pixma printer has an automatic (uncontrollable) cleaning routine that led to nozzle contamination.

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