SUPPLEMENTARY INFORMATION

Two-dimensional Paper‡ Networks: programmable fluidic disconnects for multi-step processes in shaped paper

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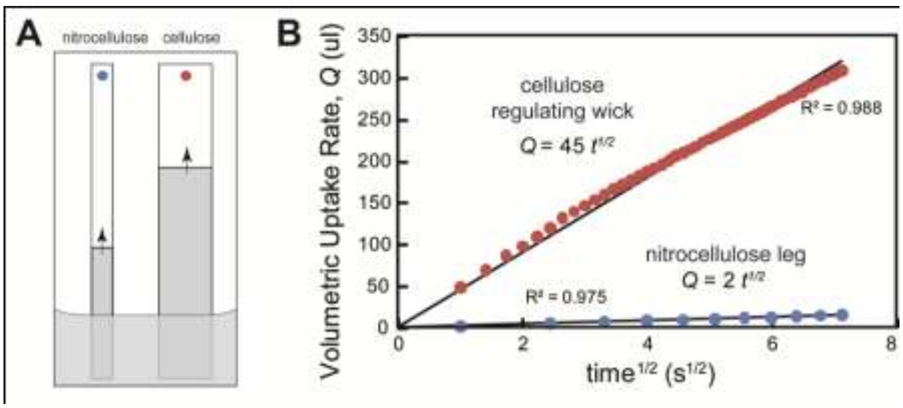

Figure S1. Fluid wicking into cellulose and nitrocellulose strips. The Lucas-Washburn relationship is modified to include the capacity for the cellulose (C=7.3 µL/mm and W=37 mm^2/s for a 1 cm wide strip) and the nitrocellulose (C=0.6 µL/mm and W=9.4 mm^2/s for a 5 mm wide strip). Specifically, the volumetric uptake rate, $Q=C*L=C*(Wt)^{1/2}$, for the cellulose regulating wick was about 22x larger than for a single nitrocellulose strip (ratio of $C*W^{1/2}$ for the two materials), or ~7x larger than three nitrocellulose strips combined.

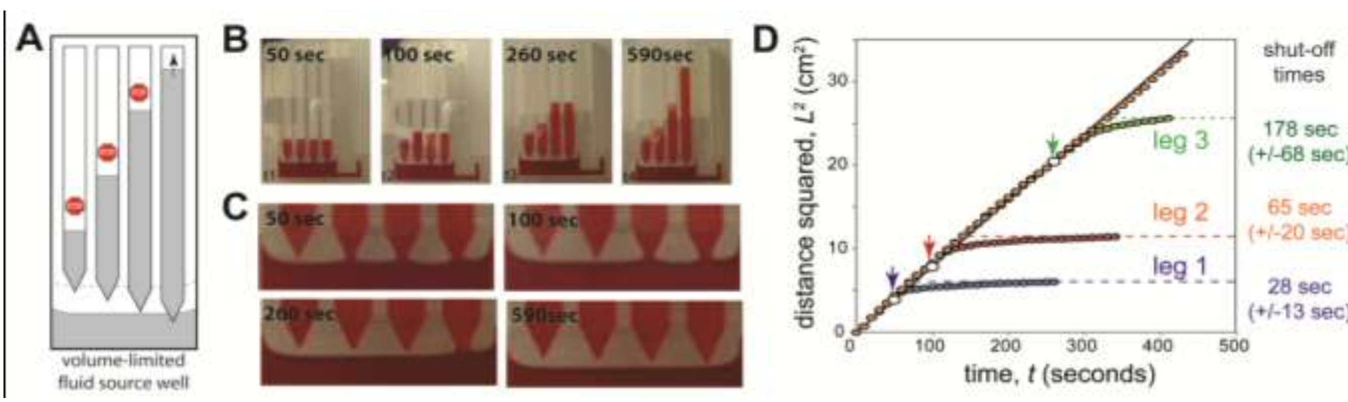

Figure S2. Fluid shut off from a volume-limited source well without a regulating wick. Nitrocellulose legs are immersed to different depths in the common fluid well. To achieve shut of times comparable to those in Figure 1 (with a regulating wick), the difference between leg immersion depths is very small (immersion depths 1.2 mm, 1.5 mm, 1.9 mm, and 2.2 mm), and the well was made very thin (0.25 mm) and narrow (closely-spaced legs). Fabrication error can be significant for such small differences in immersion depth, and the error is reflected in the shut off times. Pointed leg tips shown here had less error than blunt-ended leg tips, yet the error is still larger than using a regulating wick with blunt-ended leg tips. Fluid: Tris-buffered saline with 0.01% Tween 20 (TBST) and red food coloring. Nitrocellulose legs: 0.5 cm wide, W=9.5 mm^2/s (with TBST). Well dimensions: 3 cm wide x 0.25 mm thick (10 mil Mylar spacer).

When a regulating wick is not used, the fluid level drops much more slowly. In order to achieve shut off times similar to those in Figure 1, the system without a regulating wick must be designed to allow shut off using very small volume changes. This can be done by 1) reducing the well cross-sectional area, and/or 2) reducing the differential between leg immersion depths. In **Figure S2**, the well cross-sectional area was reduced by making the well very thin (250 microns) and spacing adjacent legs close together (to reduce the well width). This design

Electronic Supplementary Material (ESI) for Lab on a Chip

sometimes led to irreproducible capillary bridges between adjacent legs and between the legs and the well surface. We found that reproducibility of capillary effects was improved somewhat by adding surfactant to the buffer (we normally use surfactant in our assay buffers), but the capillary bridging was not cured. Further, in **Figure S2**, the differential in leg immersion depths was reduced to be 1.2 mm, 1.5 mm, 1.9 mm, and 2.2 mm for the four legs (compared to 3.5 mm, 4.5 mm, and 5.5 mm for the legs in **Figure 1**). The small differentials in immersion depth are more susceptible to fabrication error. We found that using pointed leg tips improved the reproducibility compared to blunt-ended legs, however replicate experiments of **Figure S2** gave shut off times with relatively large error: 28 ± 68 sec (±47%), 66 ± 20 sec (31%), and 178 ± 13 (38%). Using the regulating wick addressed all of these problems and resulted in improved reproducibility. The results of **Figure 1** show that error was significantly reduced by including a regulating wick, even without pointed leg tips. Adding pointed leg tips may further improve reproducibility when using a regulating wick.

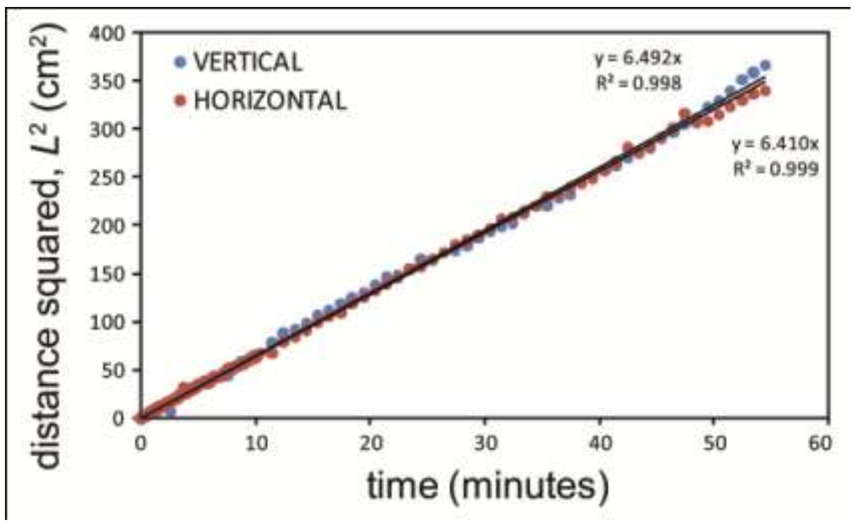

Figure S3. "Washburn plot" for wicking into nitrocellulose strips in vertical and horizontal orientations to test for gravity effects on wicking. Nitrocellulose (HF12004) was mounted in an enclosed cartridge with cellulose humidification strips running on both sides of the nitrocellulose. The cellulose strips were allowed to wick fluid at least overnight to humidify the cartridge, then wicking was initiated in the nitrocellulose. A lack of gravitational effects is indicated by 1) the linearity of the L^2 versus t plots (gravity effects would be observed as a curvature toward a horizontal asymptote), and 2) the similar results for the horizontal and vertical orientations. These test strips were much longer (20 cm) than the 2DPN devices (~2 cm).

In upright devices, gravity could cause the flow rate to deviate from pure Lucas-Washburn wicking behavior, which would be observed as a curvature toward the horizontal asymptote in L^2 versus t plots (*i.e.*, when gravitational forces balance capillary forces, wicking stops). We measured wicking into long nitrocellulose strips (20 cm) and compared the L^2 versus t plots (**Figure S3**). Both arrangements gave linear plots and there was no observable difference between wicking in vertical and horizontal arrangements. Thus, gravity does not have a significant impact on wicking for nitrocellulose devices up to at least 20 cm tall; 2DPN devices used here are only a few centimeters tall, thus gravity effects were not significant.