DISSOLVABLE FLUIDIC TIME DELAYS FOR AUTOMATED PAPER DIAGNOSTICS

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ABSTRACT

Lateral flow tests (LFTs) are fundamentally limited to assay chemistries that can be reduced to a single chemical step. In contrast, most laboratory diagnostic assays rely on multiple timed steps carried out by a human or a machine. Here, we show multi-step assays can be programmed by using shaped paper networks and fluidic time delays created by drying sugar on the paper. Solutions of sucrose (the main component of table sugar) dried on the membrane created time delays that were tunable from minutes to nearly an hour, and errors (range 11-24%) were comparable to untreated paper (12%). We created a folding card format to automate a four-step fluidic process initiated by a single user activation step (folding the card); delivery of four fluids showed errors (9-19%) comparable to those in simple strips. This device was used to perform a signal-amplified sandwich immunoassay for a malarial diagnostic biomarker. Signals in the amplified card were ~3x larger than cards using gold nanoparticles alone (the conventional LFT endpoint).

KEYWORDS

point-of-care (POC) diagnostics, lateral flow test (LFT), rapid diagnostic test (RDT), immunochromatographic test (ICT), two-dimensional paper network (2DPN)

INTRODUCTION

Lateral flow tests (LFTs) are ingenious tools for rapid diagnosis at the point of care (POC). The material has built-in wicking power to move fluids, and small pores result in fast diffusion and provide high surface area for detection of colorimetric labels. However, as a class of devices, LFTs are limited to assays that can be reduced to a single chemical step. Thus, multi-step protocols typical of nearly all laboratory assays, such as signal amplification, are not possible unless the steps are carried out manually by the user. A device that is as simple to operate as an LFT but is capable of automatically controlling fluid steps would enable more sophisticated chemical tests at the POC.

We present a paper device that automatically carries out a multi-step signal amplification protocol. Timing mechanisms are created by adding a dissolvable material to each "leg" of a shaped piece of wicking paper, which we call a two-dimensional paper network (2DPN)[1-5]. We chose to use sucrose because it is a well-known reagent-preservation matrix and is putatively benign to typical assay chemistries. When delays are added to different legs of a 2DPN, they can be used to control the delivery time of fluids to an assay. The design goal for the 2DPN is simply to coordinate the sequence of "on" and "off" times to deliver a sequence of fluids, much like the timed pipetting steps used in many laboratory assay protocols.

METHODS

Devices were fabricated from common LFT materials cut to shape by a laser cutter (Universal Laser Systems, Scottsdale, AZ). LFT materials included plastic-backed nitrocellulose as the assay strip (Mylar-backed HiFlow Plus 135, HF13504, Millipore, Billerica, MA), cellulose as an absorbent pad (C083, Millipore), and glass fiber pads as fluid sources (Ahlstrom, Helsinki, Finland). Assay devices were fabricated by sticking components to folding cards made from 0.25 mm thick Mylar film with adhesive coating on one side (10 mil, Fraylock Inc., San Carlos, CA).

Stock sugar solutions were prepared by mixing excess sucrose (#84097, Sigma-Aldrich) in distilled water at room temperature to create a saturated solution; dilutions were prepared in distilled water (10%-70% of saturation). Sugar was applied by inserting strips into the sugar solution, then blotting excess sugar solution on tissue paper and drying in a desiccator for several days prior to experiments.

Timing experiments used phosphate buffered saline with 0.05% Tween 20 (PBST) to which food coloring was added. Fluid fronts were tracked manually from web camera videos (Logitech, Fremont CA) using ImageJ. Strips were enclosed in a plastic housing (modified CD jewel case) to reduce evaporation.

2DPN devices for flow visualization and the amplified malaria assay were fabricated in a folding card format. Devices were run in a Petri dish containing wet paper towels to reduce evaporation. The time of fluid arrival was calculated using ImageJ by creating a stack from the videos, selecting a region of interest (a line across the strip), and performing a "reslice" along the time dimension. For the malaria cards, a sandwich immunoassay format with signal amplification was used to detect *Plasmodium falciparum* histidine-rich-protein-2 (PfHRP2), a diagnostic biomarker for malarial infection. Fetal bovine serum (FBS, Certified, One Shot, Invitrogen, Carlsbad, CA) was used as a simulated serum matrix; it was spiked with recombinant PfHRP2 antigen (CTK Biotech, San Diego, CA). The sample was mixed with monoclonal anti-PfHRP2 antibody (Immunology Consultants Laboratory, Portland, OR) conjugated to gold nanoparticles (40 nm Immunogold, BBI, Cardiff, UK). A gold enhancement solution (040260, Nanoprobes, Yaphank, NY) was used for signal amplification, and wash steps used Tris-buffered saline with 0.1% Tween 20 (TBST). Signal intensity was measured from videos recorded with the web camera.

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RESULTS AND DISCUSSION

The wet-out flow through dry paper strips can be slowed by pre-drying sugar solutions onto the paper. Figure 1A describes the preparation of dried sugar delay zones and the method for measuring the delay time. Paper strips were dipped in sucrose solutions of different concentrations (up to 70% saturation at room temperature) and dried in a desiccator. The set of dry strips, including an untreated strip and a blank (0% sugar), were dropped simultaneously into a trough with dyed PBST, and the arrival time at the end of the delay zone ("finish line") was measured from videos. Figure 1B shows an image during the experiment. As expected, the time to reach the finish line increases with increasing concentration of the applied sugar solution. Figure 1C plots the arrival time for each treated strip. For the strips used here, arrival times were tunable from minutes to nearly an hour, which corresponds to a nearly 80-fold increase over untreated strips. The errors in arrival time range from 11%-24%, which are comparable to errors for the strip without sugar (12%).

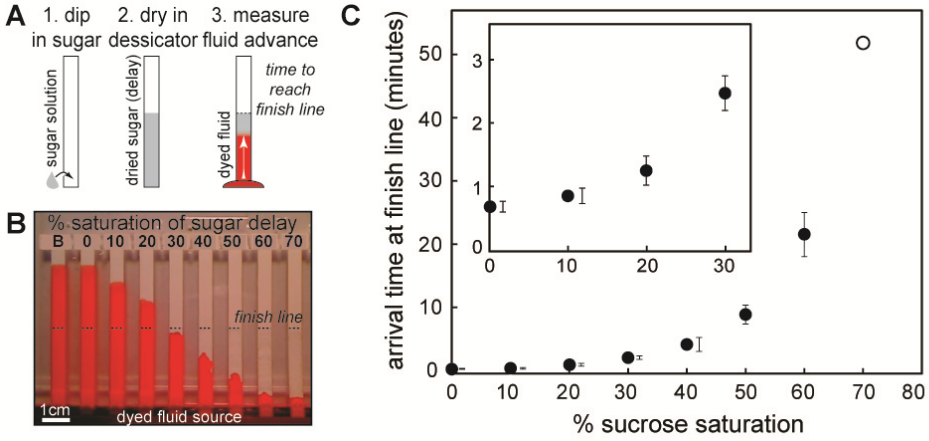

Figure 1. Time delays created by sugar solutions of different concentration dried on paper strips. Dry strips were dipped into dyed PBST, and the time required to reach the end of the sugar-treated region (finish line) was measured. "B" indicates an untreated strip, and "0%" indicates a strip dipped in DI water (no sugar). Error bars are from three experiments (no replicate data for the unfilled point).

Figure 2 shows a 2DPN designed for sequential delivery of four fluids to a common detection zone. The shape is reminiscent of the branched paper devices pioneered by the Whitesides group[6], but it is "upside down" with respect to flow direction, and its purpose is different. Rather than splitting a sample among multiple detection zones, this structure delivers multiple fluids to a common leg in a timed sequence. Sequential delivery is based on two principles: 1) flow from each leg is "turned on" at a specific time based on the sugar delay, and 2) each fluid flows until it is "turned off" when the source fluid is depleted. We created a folding card format that allows the user to initiate the test by a single folding step (Figure 2A), thus eliminating user timing errors that would arise with multiple fluid applications. Each leg of the 2DPN was treated with a different sugar concentration (including 0% for the first fluid leg), and the device was dried before testing (Figure 2B). Dyed PBST was added to each reagent pad, and the card was folded to initiate the timed sequence; no user intervention was required after the folding step. Figure 2C shows images corresponding to the arrival of each fluid at the detection zone, and Figure 2D shows the time course of fluid arrival at the detection zone across the full strip width. Each fluid is displaced effectively by the subsequent fluid, leading to distinct transitions between each fluid (important for wash steps) and providing uniform treatment of the detection zone by each fluid. Errors in arrival times were 9-19% (n=5 replicate devices), which is comparable to errors in simple strips.

The folding device was used to carry out a multi-step amplified immunoassay for a malaria diagnostic biomarker, PfHRP2. The amplified assay uses gold nanoparticles common in LFTs, but the 2DPN adds wash steps and application of a gold plating solution that increases the light absorbance of bound gold nanoparticles by increasing their size. This enhancement chemistry (as well as silver deposition on gold) requires several sequential steps. It has been used to increase signal in LFTs *via* manual assay steps carried out by the user[7], and we have automated this chemistry in 2DPN devices based on leg length delays[1, 5].

Figure 3A shows the location of fluids applied to pads in the 2DPN. The sample was mixed with the gold-anti-PfHRP2 conjugate (gold-Ab), and this solution was applied to one pad (red); other pads received gold enhancement solution (GE, purple) or wash buffer (light blue). Figure 3B shows the component stack for the PfHRP2 assay, and Figure 3C shows the signal at the detection line at two stages of the assay. After the first two steps (capture of gold-Ab-PfHRP2 complex and wash), the faint pink line represents the endpoint of a typical LFT (no amplification). After the final two steps (gold signal enhancement and final wash), the line is darkened with typical purple-grey color due to increased nanoparticle size. The signal enhancement was ~3-fold, which is modest, but is comparable to the enhancement we observe using the gold signal amplification in other 2DPN devices and manually-operated strip tests[1, 5]. Other amplification chemistries, such as silver enhancement of gold nanoparticles or enzymatic signal amplification used in ELISA, are likely to yield greater sensitivity, and the device in Figure 3 is capable of carrying out the steps for those reactions.

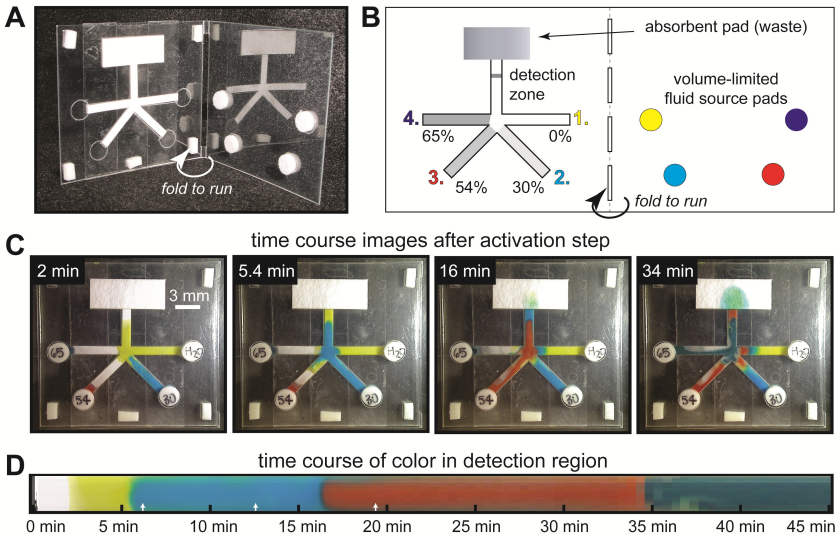

Figure 2. Folding card design for timed sequential delivery using a 2DPN with sugar time delays and volume-limited fluid source pads. A) Photo of the folding card with sugar-treated legs and reagent pads. B) The sugar solution dried on each leg is indicated as a % saturation. Dyed PBST was added to each pad (11 microliters), and the card was activated by folding. C) Time sequence of dye delivery to the detection zone (upper vertical leg). Adhesive pads (white) are located around the card perimeter to hold the card closed. D) Time course of color at the detection zone.

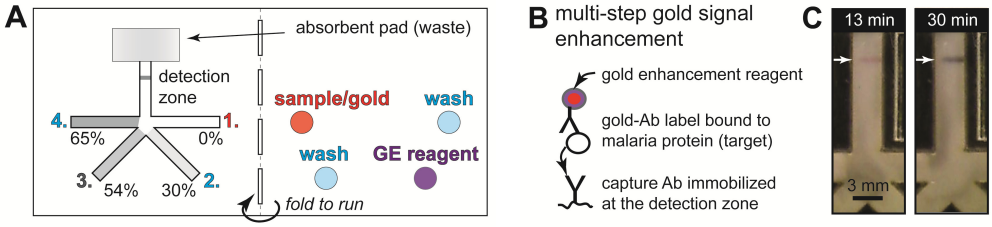

Figure 3. Amplified immunoassay for a malarial protein. Reagents were added to pads; folding the card initiated the automated assay. The gold enhancement solution plates gold on the bound gold nanoparticles, creating a darker band.

CONCLUSIONS

Sugar-based time delays were combined with paper networks; this allowed timed fluidic sequences to be programmed into simple devices. As performed here, the experiments required loading of four pads with separate liquid reagents. A more practical device would include reagents stored dry on each pad, such that the user would only be required to add water and sample. In addition, we are developing methods to automate the pad loading step so that no controlled pipetting steps are required. The programming tools for 2DPNs can be applied to translate other multi-step assay chemistries, such as ELISA or nucleic acid tests, to a point-of-care format.

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