

A TWO-DIMENSIONAL PAPER NETWORK FOR COMPREHENSIVE DENGUE DETECTION AT THE POINT OF CARE

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ABSTRACT

The two-dimensional paper network (2DPN) is an inexpensive technology based on the strengths of one-dimensional lateral flow rapid diagnostic tests. The 2DPN allows complex (bio)chemical processing to be performed without the need for external instrumentation, which is ideally suited to point-of-care testing for infectious disease in low resource settings. We have been exploring the use of 2DPNs for sensitive multiplexed immunoassays. We present progress in the development of 2DPN modules to be used in a multiplexed amplified test for dengue virus proteins and two types of antibodies against them.

KEYWORDS: paper network, rapid diagnostic test, dengue, immunoassay

INTRODUCTION

With current attention on the twin goals of reducing the costs of healthcare and, simultaneously, increasing access to that care, there is a great deal of interest in reducing the equipment required to implement microfluidic solutions to chemical detection problems; this is particularly true when applied to the determination of the cause of infectious disease. When the detection must be carried out in a low-resource setting, both low cost and simple operation are vital for successful implementation of any new technology. One such solution that has been commercialized for a few decades has been to use capillary flow in strips of paper and paper-like materials to avoid the need for pumping fluids. Recent work from the Whitesides laboratory showed the potential for two- and three-dimensional paper networks to be used to great effect for simultaneously performing multiple medically-important bioassays [1-3]. Complementary work in our laboratory has been aimed at developing the 2DPN, which utilizes converging paper “legs” to sequence multiple reagent steps for single or multiplexed assays [4-7]. The 2DPNs are being developed to reduce user actions to an absolute minimum, as in existing lateral flow tests.

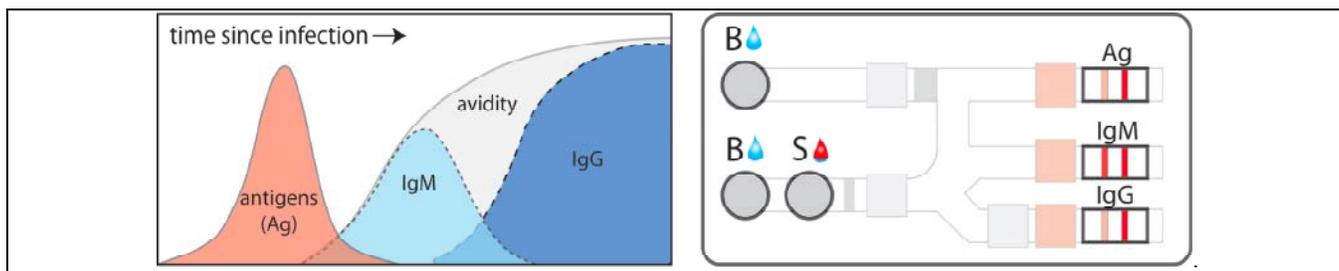


Figure 1.:Left: Schematic of progression of analytes present in blood as a dengue infection progresses (concentration vs. time). Right: Schematic of 2DPN-based assay for antigen, IgG and IgM that requires only addition of blood and buffer.

We are engaged in a set of projects aimed at developing the 2DPN as a robust platform for multiplexed sandwich immunoassays with enhanced sensitivity compared to existing one-dimensional lateral flow tests, but with required user activities at an absolute minimum. One assay we have been designing is a test for diagnosis of dengue fever. Like many viral diseases, there is a progression of analytes found in blood that could indicate that the cause of symptoms is the dengue virus, but over the course of a few days the predominant species change, so that any definitive (immuno)assay must measure viral proteins, IgM and IgG (Fig. 1 left). The ultimate test will be similar to the scheme shown at the right of Fig. 1, in which the user only adds two fluids.

Described here are experimental results with modular components of the complete test. Since IgG and IgM both bind to the antigen, and therefore interfere with the surface binding assay, a critical step to get an accurate determination of IgM is to remove IgG upstream of the IgM detection zone. Timing of reagent flow is controlled by placing dissolvable barriers of sugar to temporarily delay flow through regions of the 2DPN. If pads for holding reagents (which may be stored in dry form on those pads or in the downstream network) are placed on different parts of the 2DPN from the channels meant to connect them, flows can be initiated by folding wetted pads onto nitrocellulose channels; we call this control process “microfluidic origami”. By combining processes, we have demonstrated assay amplification.

MATERIALS

The nitrocellulose membrane substrate (the “paper” of the 2DPNs) was Hi-Flow 135 (Millipore, Billerica, MA). Polyester and glass fiber pads (Ahlstrom) were used as source pads for sample application and cellulose for wicking pads. Paper network housings were fabricated of Mylar film (Fraylock). Materials were cut to appropriate shapes on a CO₂ laser system

(Universal Laser Systems, Scottsdale, AZ). A web camera (Logitech, Fremont, CA) was used to acquire image data. Mock samples were created by spiking recombinant NS1 antigen into fetal bovine serum (Invitrogen).

RESULTS AND DISCUSSION

Pretreatment. IgG mop-up. A 20 μ l sample containing F-IgG (0.17 mg/ml in PBS + 0.5% casein at pH 7.4) was loaded onto the sample pad and allowed to flow down the membrane. The absorbent pad at the distal end acted as a sink. After sample addition, the device was washed 2x with 20 μ l PBS. Images were collected throughout the experiment for analysis. The control membrane was a native nitrocellulose membrane. The majority of the F-IgG binding occurs near the sample entry point. The control membrane showed no significant non-specific binding of the F-IgG (red). Images were quantified in ImageJ by summing the pixel intensity over the entire membrane and sample pad. The raw intensity values (I_R) were normalized (I_N) to the maximum intensity measured for a 20 μ l sample of F-IgG (I_{max}) and minimum intensity from a buffer-filled membrane (I_{min}). Approximately 80% of the F-IgG in the sample was removed by the protein-G-coated membranes.

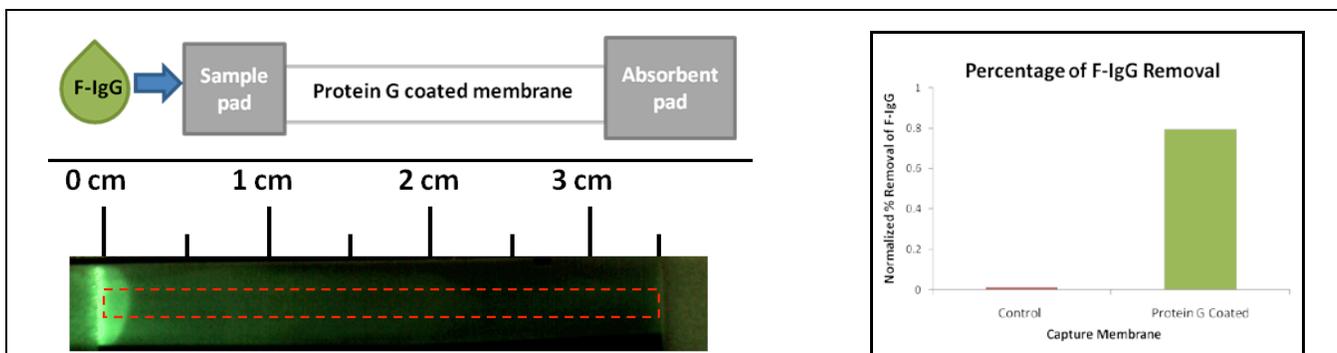


Figure 2: IgG removal module. a) Schematic of the experimental setup. b) Paper devices were 0.5 cm x 4 cm. Image of protein-G-coated membrane after all wash steps were completed. The red box indicates the measurement area. c) Integrated concentration profile measurement of membrane-bound F-IgG (green).

Sugar barriers for integrated card. Washburn flow is the anticipated linear dependence of progression of the wet-out front of a solution through a porous matrix on the square root of time. Addition of sugars like sucrose to the nitrocellulose, followed by drying the sugar in place, produces a zone that transiently slows the progression of the advancing fluid front through that zone, after which normal Washburn flow returns. As shown in Fig. 3, the delay is a function of the amount of dried sugar deposited per unit area, and the range of delays possible is very large. This allows one to sequence flows in a 2DPN in a way not possible using the length of paper segments alone.

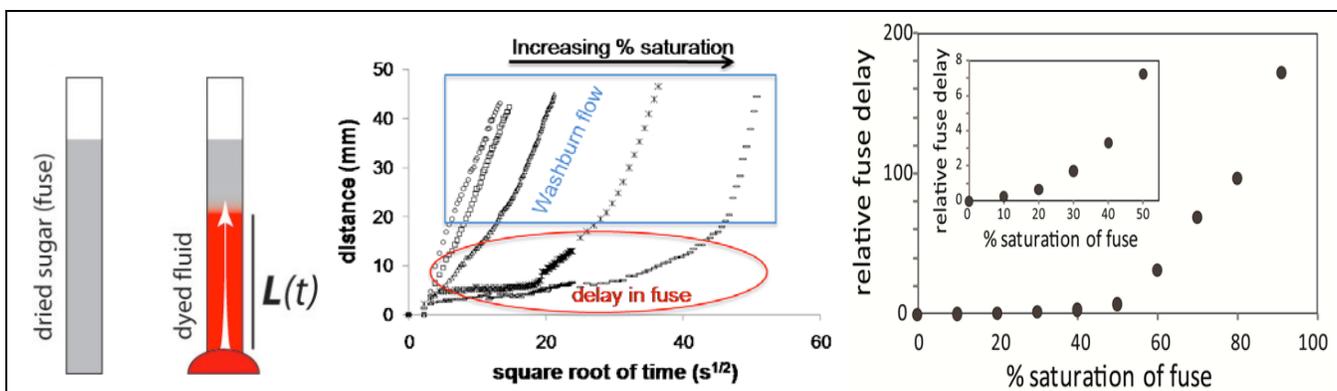


Figure 3: Schematic of a dried sugar barrier (or fuse) that can be used as a “turn-on” valve (left). The plot shows the distance traveled by the fluid front vs. the square root of time for sugar barriers made of varying % saturation sucrose solutions (center). The fluid is delayed while in the barrier and then resumes Washburn flow after exiting the barrier region. The concentration of the applied sucrose solution can be used to tune the delay time (right). A relative delay time of “100”=55 min.

Microfluidic origami and signal amplification. As mentioned, folding devices to create connected 2DPNs (from originally disconnected segments) allows simple user initiation of coordinated start times for a set of reactions; once the 2DPN is formed by folding, the delays between sequential steps are controlled by the contour length of nitrocellulose segments, or by

sugar barriers, or both. An example that combines all these features is shown in Fig. 4. In this case, a well-known sandwich immunoassay for human chorionic gonadotropin (hCG), a protein antigen, produces a signal using conventional secondary antibodies labeled with gold nanoparticles, producing a pink signal. After suitable rinsing and delay by a sucrose barrier in a side channel, a solution that contains a reducing solution of gold ions is allowed to flow across the capture zone, further darkening the gold nanoparticles and enhancing the sensitivity of the assay.

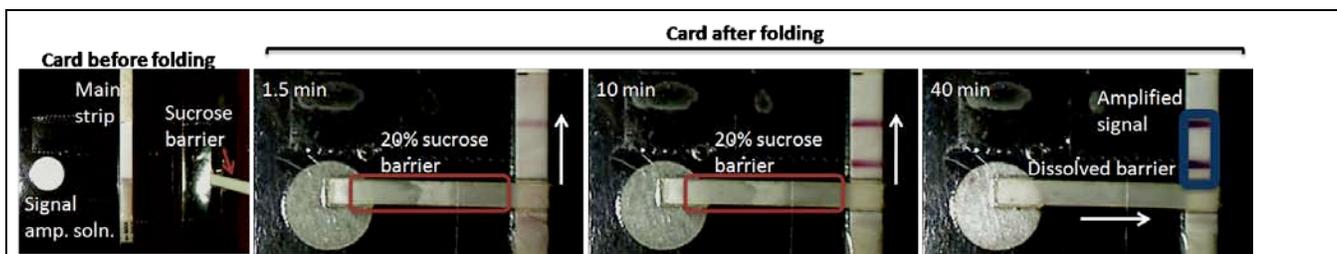


Figure 4: Use of a sucrose barrier to create a delay in an origami device. This demonstration is in a model system (hCG). A 20% sucrose solution was dried onto the horizontal strip to create a dissolvable barrier. The user adds gold enhancement solution to the source pad and sample to the sample pad, then folds the device (left). The sucrose barrier delays delivery of the gold enhancement solution to the capture zone until after much of the sample has left the detection region.

NS1 antigen card. With multiple inlet legs it is possible to eliminate the sugar delays. Shown in Figures 5 and 6 is an assay for the dengue NS1 antigen. Polyclonal anti-NS1 antibody (ICL) was patterned onto the nitrocellulose to serve as the capture species. Recombinant NS1 antigen (Meridian) and a monoclonal anti-NS1 antibody (ICL) conjugated to colloidal gold particles (Arista) were premixed and 10 μ l (1.5 μ g/mL of NS1) added to the source pad nearest the wicking pad. A 10 μ l volume of rinse buffer of phosphate buffered saline at pH 7.4 was added to the middle source pad. A volume of 25 μ l of gold enhancement solution (Nanoprobes) was added to the source pad farthest from the wicking pad. The device was folded closed to activate it, and amplification of the immunoassay was demonstrated (Fig. 6).



Figure 5: 2DPN origami card with three inlet legs. The user adds different reagents to three pads on the right (or adds water to pre-dried reagents), then folds the card to activate an automated flow sequence. The longer the leg from the wicking pad, the longer the delay before the next reagent arrives at the detection zone (just before the wick).

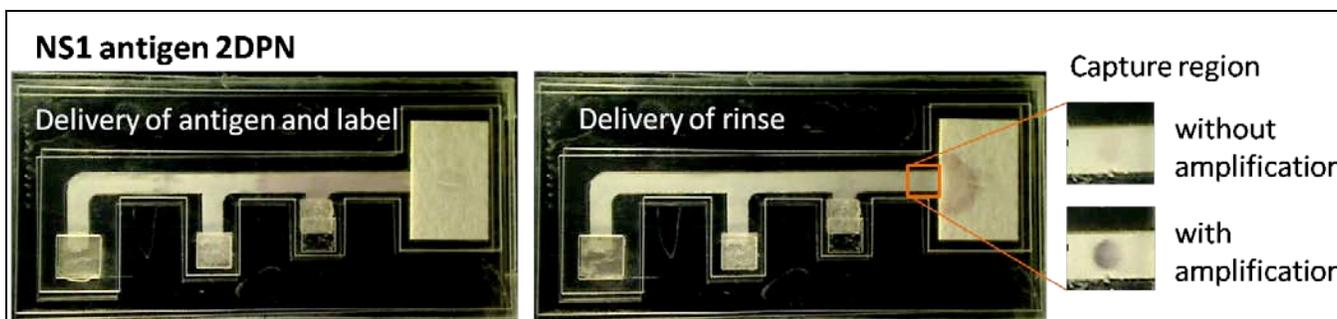


Figure 6: 2DPN NS1 detection origami card in operation. Reagents are staged in the main channel of the device for delivery to the detection region (left). The rinse is delivered to the detection region (right). The close-up images depict the detection region at 15 min. without amplification, and at 40 min, after amplification. The signal is significantly darkened after amplification.

CONCLUSION

We have shown that the key modules of the proposed multiplexed dengue 2DPN function. We are now working to integrate them into a complete 2DPN for detection of all three proposed analytes.

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