Electronic Supplementary Information

Paper-based Analytical Device for Point-of-care Nucleic Acid Quantification Combining CRISPR/Cas12a and Personal Glucose Meter

Yohei Tanifuji, Guodong Tong, Yuki Hiruta, Daniel Citterio*

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku,

Yokohama 223-8522, Japan. E-mail: citterio@applc.keio.ac.jp; Tel: +81 45 566 1568

*To whom correspondence should be addressed.

Email: citterio@applc.keio.ac.jp

Table of Contents

Assay with freeze-dried MB conjugates in microtubes

2 µL of 10 mg/mL MB conjugate in buffer E with 0 or 40 mg/mL of trehalose was added in a 0.5 mL microtube, followed by freeze-drying for 60 min (snap freezing by liquid nitrogen followed by vacuum application). During drying, a solution containing 100 nM Cas12a and 200 nM crRNA in buffer D with 0 or 40 mg/mL of trehalose was pre-incubated for 15–30 min, 3 µL of which was then added to the microtube with freeze-dried MB conjugates. After addition of 3 µL of buffer D and 2 µL of 0 or 100 nM tgDNA in autoclaved water, the mixture was incubated on a rotator for 60 min. Subsequently, 4 µL of supernatant was separated using a magnetic stand and added to 8 µL of sucrose solution (6 µL of 1 M sucrose, 1.2 µL of buffer F, and 0.8 µL of autoclaved water). After additional incubation for 60 min, the concentration of produced glucose was measured by the PGM.

Table 1 Sequences of nucleic acids used in this report

Name	Sequence (5′→3′)
tgDNA (double-stranded)	TTTTTTTTTTAGTACATTGCAAGATACTAAATGTGAGGTACCA
crRNA	UAAUUUCUACUAAGUGUAGAUGUACAUUGCAAGAUACUAAA
ssDNA	Biotin-TTATTATTATTATTATTATTATTATTA-disulfide-$(CH_2)_6$-CH_3

Table S2 Estimated material costs to fabricate the paper-based device

Material	Price (USD per device)
MB conjugate*	0.824
Cas12a protein	0.037
crRNA	0.005
Sucrose	<0.001
Filter paper	0.013
OHP film	<0.001
Lamination film	<0.001
Double-sided tape	<0.001
Total	0.984
PGM electrode**	0.866

*Calculated based on cost of invertase, crosslinker, ssDNA, and streptavidin-coated MBs.

**PGM electrodes were bought from LifeScan Japan (Tokyo). The market price depends on the seller.

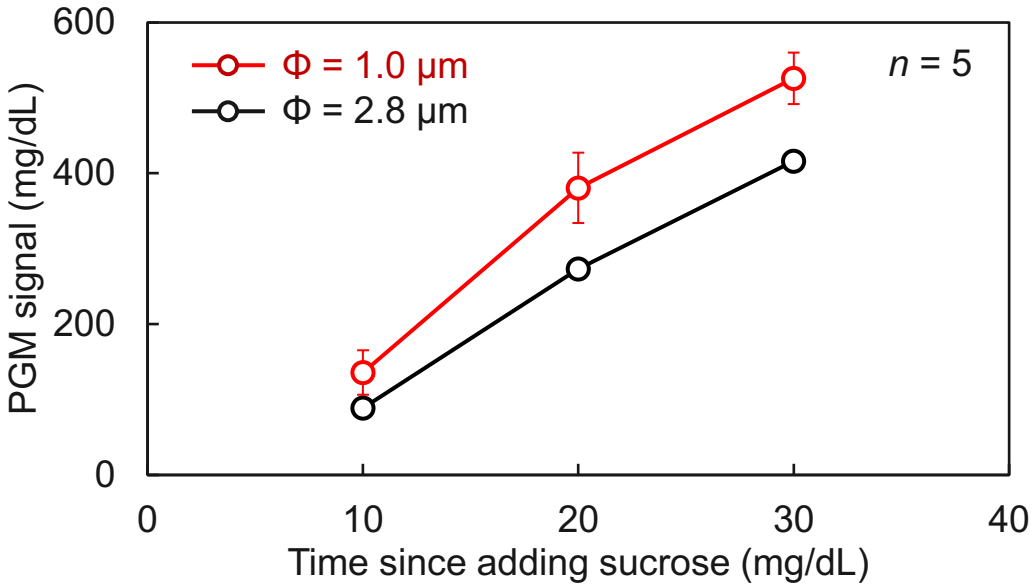

Fig. S1 Changes of PGM signal over time from adding 8 μL of sucrose solution (6 μL of 1 M sucrose in autoclaved water, 1.2 μL of buffer F, and 0.8 μL of autoclaved water) to 4 μL of 10 mg/mL MB conjugate; error bars represent the mean values ± 1σ (*n* = 5). Increase of the PGM signal indicates the successful immobilization of invertase on the surface of MBs, and indirectly indicates the amount of immobilized invertase.

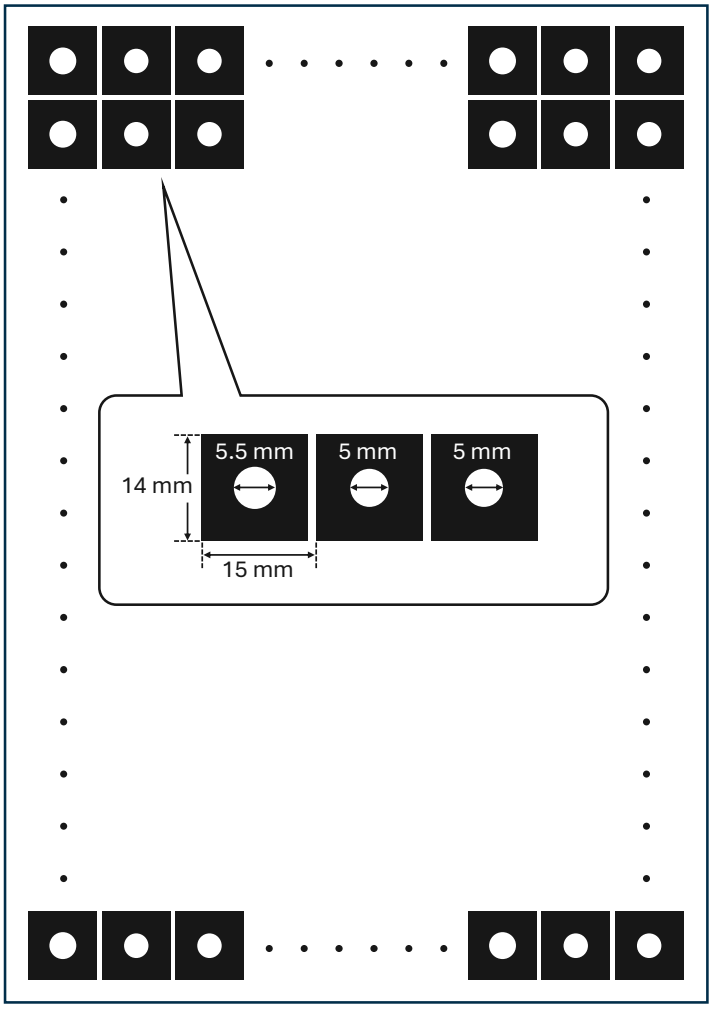

Fig. S2 The wax printing pattern designed using Adobe Illustrator CC software. Black areas were printed using the wax-printer.

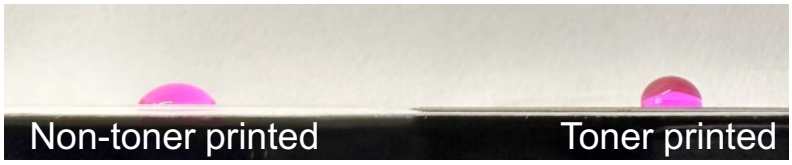

Fig. S3 Comparison of water droplet behaviour on non-toner modified transparency film (left) and toner printed film (right). The picture indicates a larger contact angle for the toner-modified film, proving the successful hydrophobic treatment of the transparency film by toner printing.

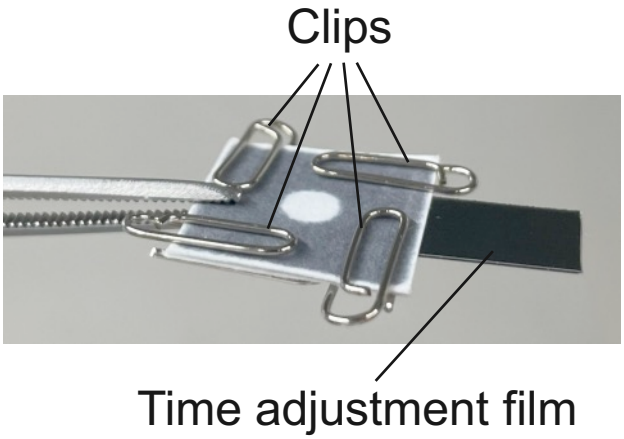

Fig. S4 Photograph of the PAD used for basic evaluation.

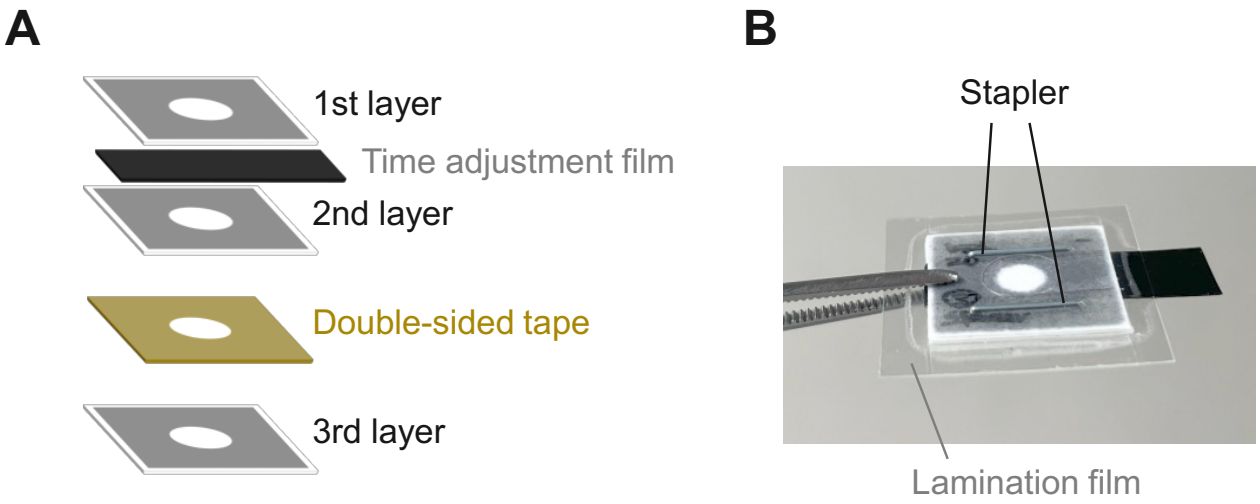

Fig. S5 (A) Illustration of the sandwich structure consisting of three paper-layers, time adjustment film, and double-sided tape. (B) Photograph of the fully integrated device.

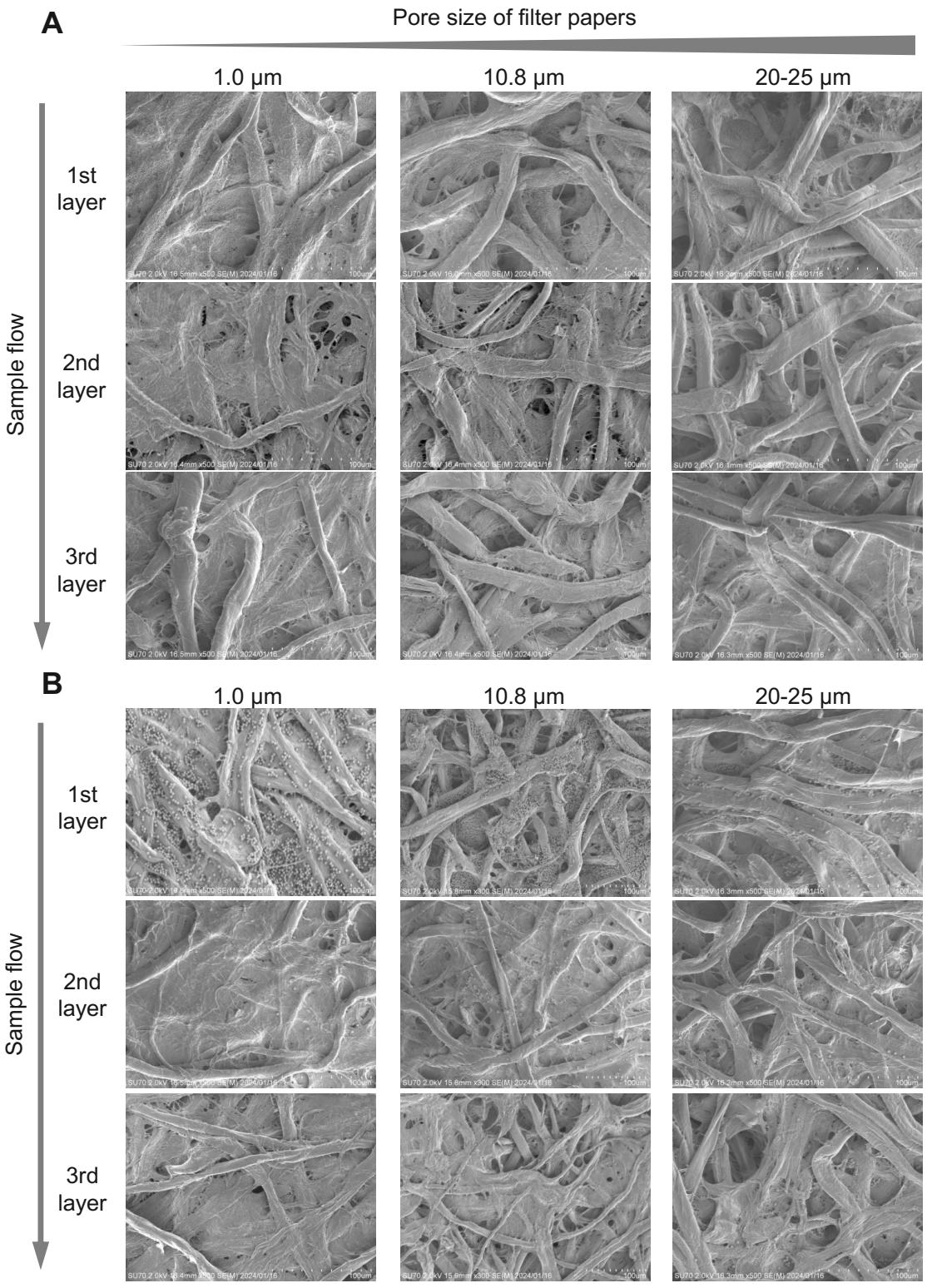

Fig. S6 SEM images of the front side of each paper layer after assays without adding sucrose solution with using different pore size of filter papers and (A) 1.0 and (B) 2.8 μm of MBs. It can be confirmed that the number of MBs on 2nd and 3rd layer is decreased as MBs size increases and pore size of filter papers decreases, suggesting successful filtration of MBs by filter papers.

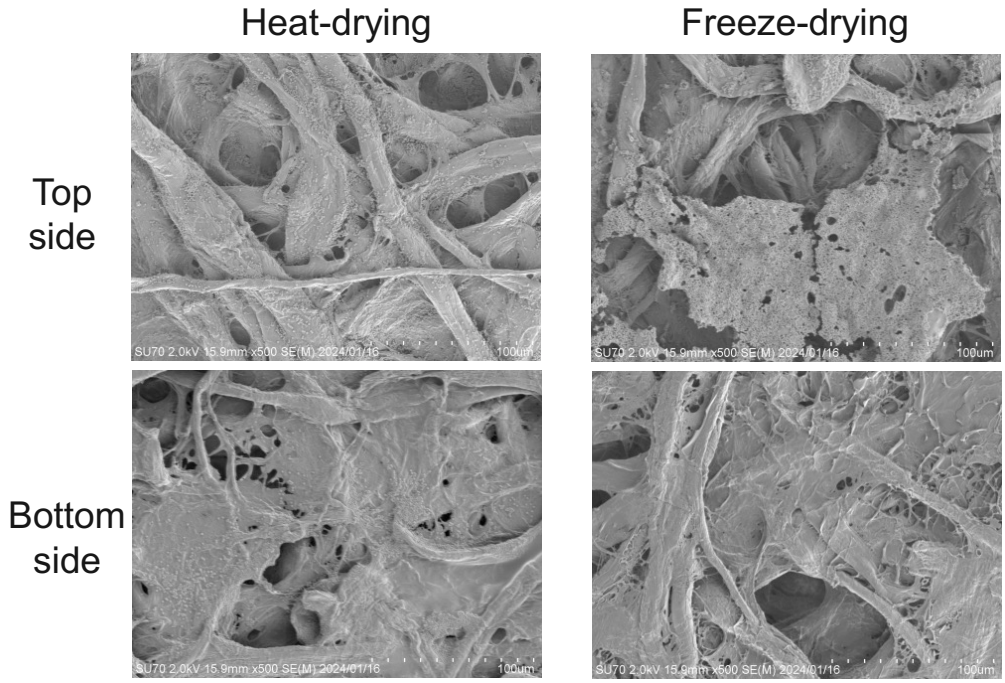

Fig. S7 SEM images of the top and bottom sides of paper substrates (A5C) on which MB conjugates and Cas12a-crRNA complex have been (left) heat-dried or (right) freeze-dried. The presence of MB conjugates was confirmed on both sides of the paper substrate in the case of heat-drying, while they were observed only on the top side when freeze-dried.

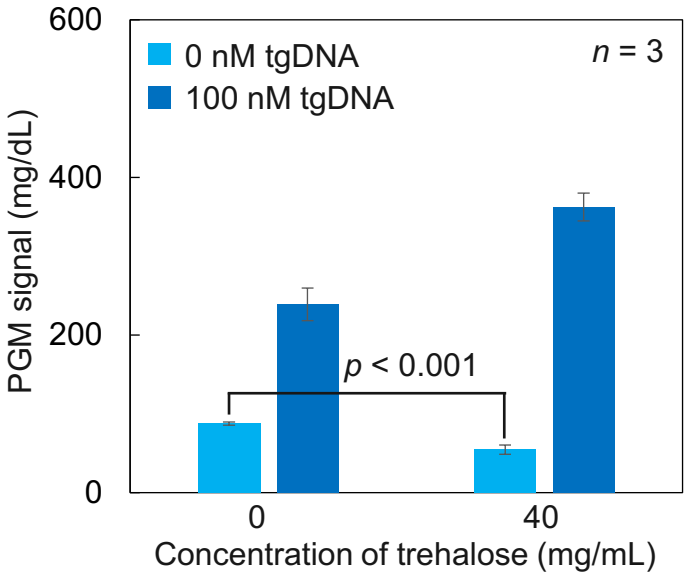

Fig. S8 Influence of presence of trehalose during freeze-drying of MB conjugates in microtubes; error bars represent the mean values ± 1σ (n = 3). In the case of experiments performed in microtubes, the addition of trehalose during freeze-drying had a positive influence on both the blank and the tgDNA-positive signals. This contrasts with the observations on the paper substrate (Fig. 3A of the main text), where the tgDNA-positive signal did not increase upon addition of trehalose, presumably due to the enzymes being already sufficiently stabilized by BSA used for paper blocking.

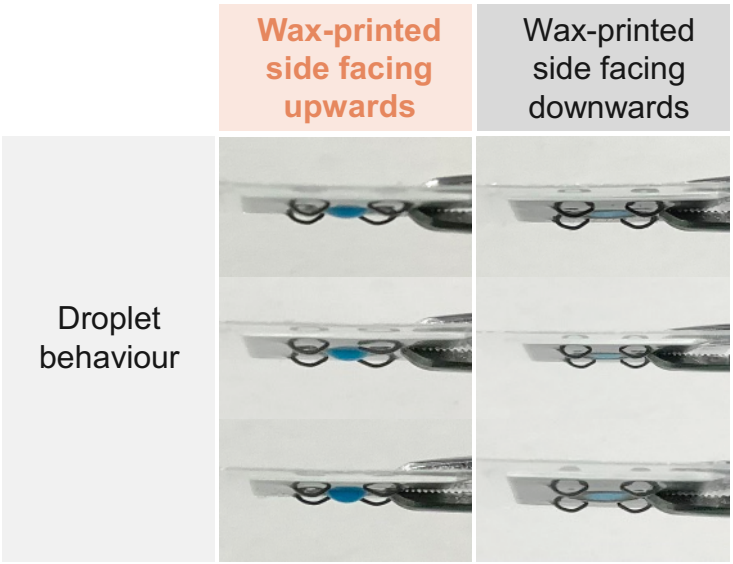

Fig. S9 Dependence of liquid dye droplet formation on orientation of wax-printed paper side in a fully integrated multilayer paper device: (A) wax-printed side facing upwards and (B) wax-printed side facing downwards.

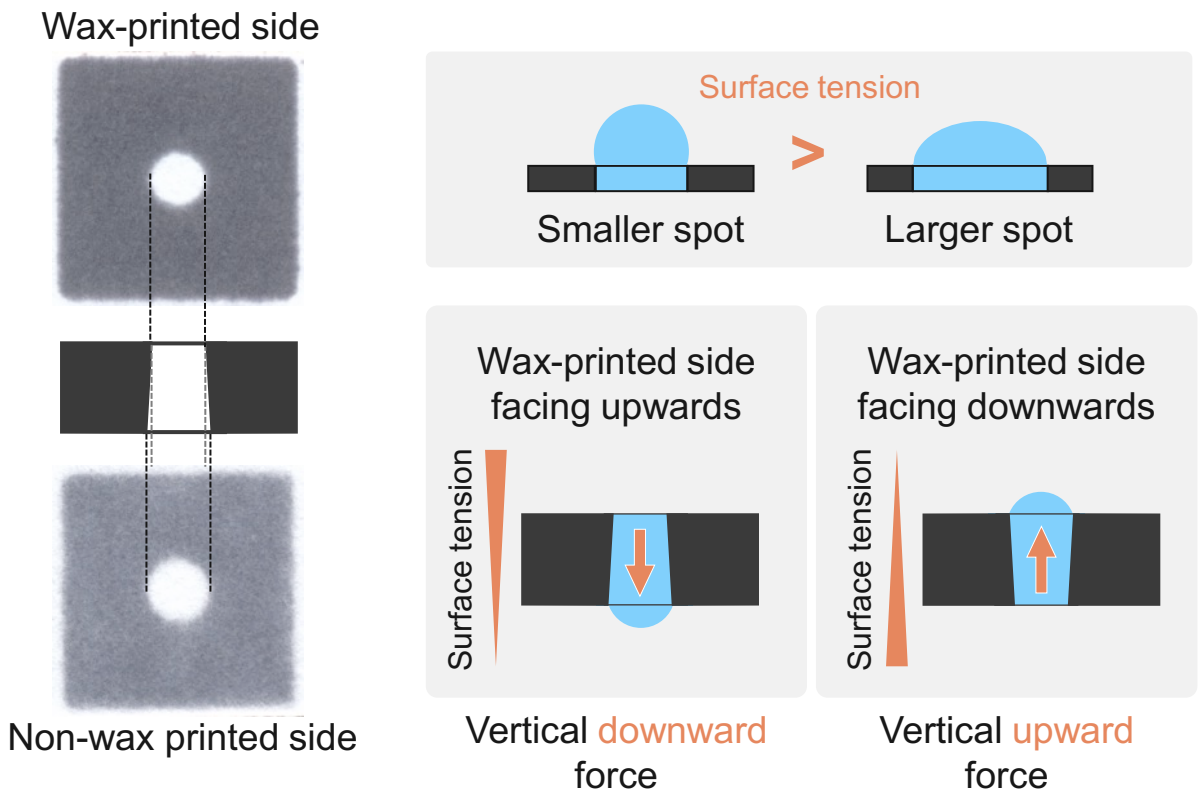

Fig. S10 Schematic of the dependence of wax-printed paper orientation on vertical sample flow caused by the conical frustum shape of a wax-patterned circular spot.