Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

Toward instrument-free quantitative measurements: a three-dimensional microfluidic device fabricated in a single sheet of paper by double-sided printing and lamination†

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In this paper, we studied the relationship between the printed features of a wax pattern and the dimensions of the hydrophobic patterns in paper because the formation of vertical-, upper-, and lower-channels strongly depends on controlling the heights of the wax-patterns. We have derived several basic equations to calculate prerequisite conditions for the formation of a 3D-microfluidic network in a single sheet of paper (Fig. S1).

First, the formation of a docking-wax-barrier requires that the total sum of H_{hf} and H_{hb} should be larger than P_{Th}. This condition can be expressed as in equation (1):

$$(H_{hf} + H_{hb}) > P_{th} \tag{1}$$

Secondly, a lower-channel is formed by an independent upper-heated-wax (Fig. S1(B)). H_{LC} is determined by the following simple equation (2):

$$H_{LC} = P_{th} - H_{hf} \tag{2}$$

A lower-channel exists when $H_{LC} > 0$. Thus, it can be expressed as in equation (3):

$$H_{hf} < P_{th} \tag{3}$$

Thirdly, an upper-channel is formed by an independent lower-heated-wax (Fig. S1(C)). H_{UC} is determined by the following equation:

$$H_{UC} = P_{th} - H_{hb} \tag{4}$$

An upper-channel exists when $H_{UC} > 0$. Therefore, it can be expressed as in equation (5):

$$H_{hb} < P_{Th} \tag{5}$$

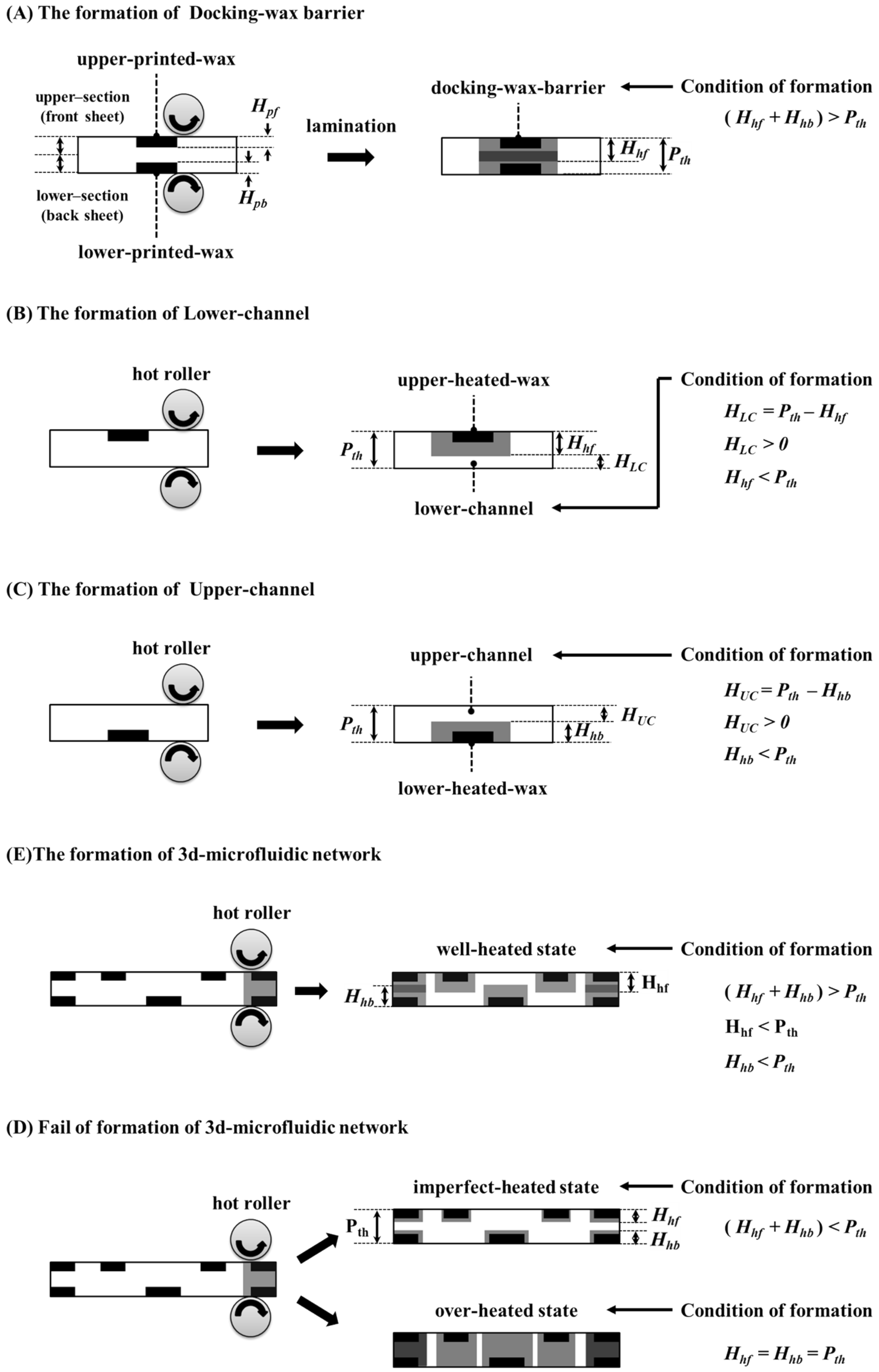

Fig. S1. Conditions for the formation of a docking-wax-barrier and lower- and upper-channels. (A) A docking-wax-barrier forms when an upper-heated-wax makes contact with a lower-heated-wax. The condition for forming a docking-wax-barrier is that the height of paper thickness (P_{th}) is smaller than the total of the heated height of an upper-heated-wax (H_{hf}) and the heated height of a lower-heated-wax (H_{hb}). (B) The lower-channel (H_{hf}) should be lower than the thickness of paper (P_{th}). The height of a lower-channel (H_{LC}) is equal to P_{th} minus the heated height of an upper-heated-wax (H_{hf}) on the front of the sheet ($H_{LC} = P_{th} – H_{hf}$). (C) An upper-channel is formed when the height of a lower-heated-wax (H_{hb}) is lower than thickness of paper. The height of an upper-channel (H_{UC}) is equal to P_{th} minus the heated height of a lower-heated-wax (H_{hb}) on the back of the sheet ($H_{UC} = P_{th} – H_{hb}$). (D) A 3D microfluidic network is formed when both H_{hb} and H_{hf} are lower than thickness of the paper (P_{th}), and total of H_{hb} and H_{hf} should be lower than P_{th}. (E) A 3D microfluidic network is not formed when total of H_{hb} and H_{hf} are larger than P_{th} (imperfect-heated state) or when H_{hb} and H_{hf} reach P_{th} (over-heated state). In the case of an imperfect-heated state, there is no docking-wax-barrier to prevent horizontal out flow. In the case of an over-heated state, there are no upper- or lower-channels to flow fluid flow horizontally and vertically. The printed height of an upper-printed-wax (H_{pf}) and the printed height of a lower-printed-wax (H_{pb}) were changed to the heated height of an upper-heated-wax (H_{hf}) and the heated height of a lower-heated-wax (H_{hb}), respectively. The change in the height of the waxes resulted in the formation of a docking-wax-barrier and channels.

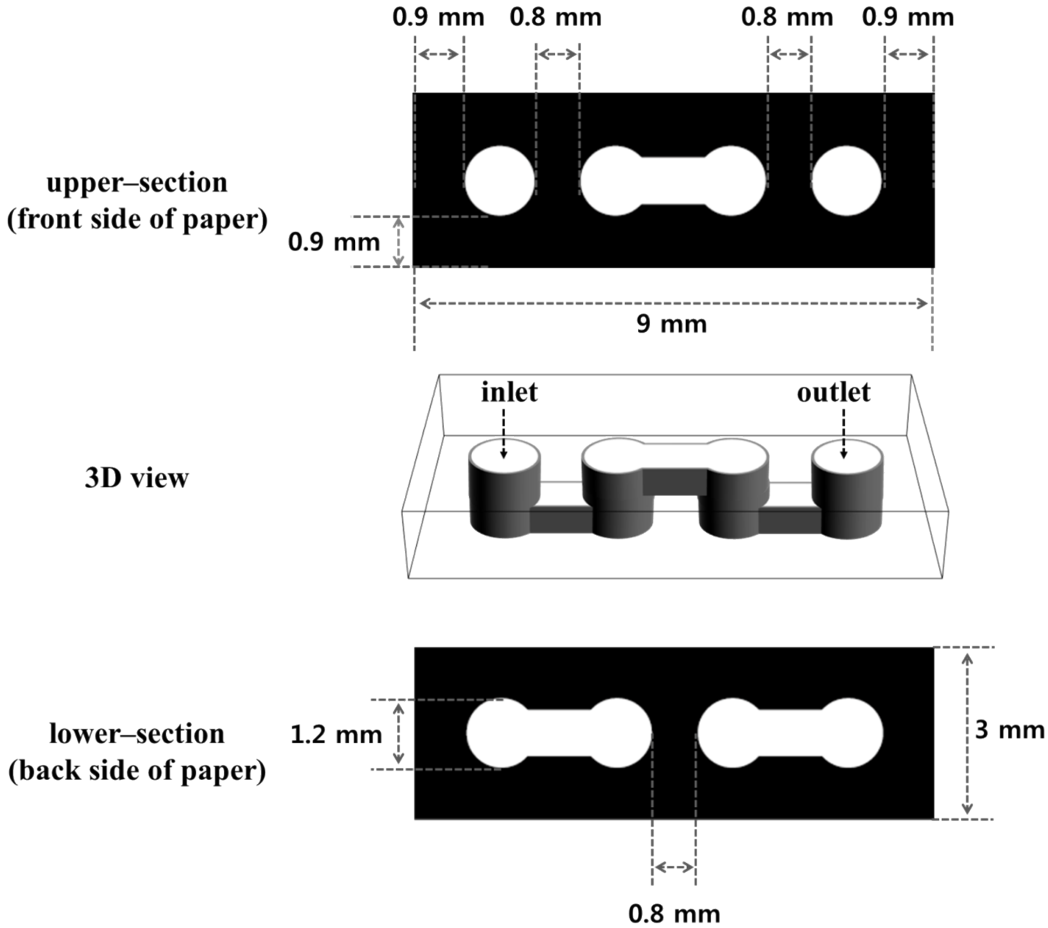

Fig. S2. Design of a 3D-microfluidic network in a single sheet of paper.

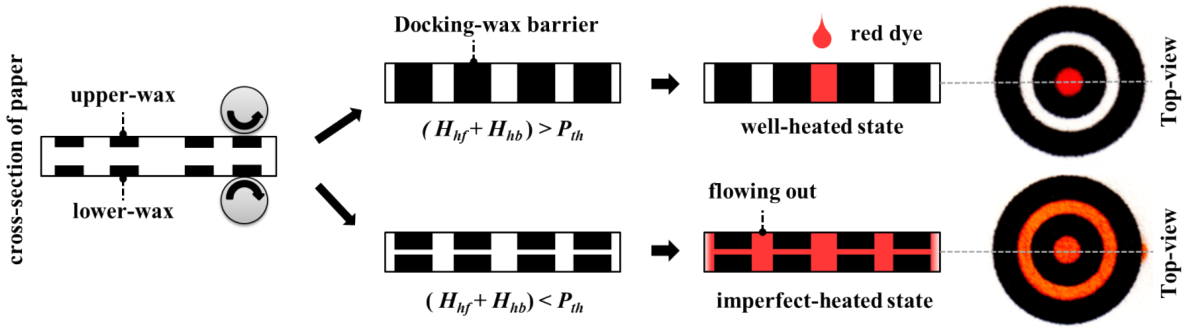

Fig. S3. Leaking-test as a function of the docking-wax-barrier. We loaded a red dye solution into the inner ring. The illustration shows the formation of a docking-wax-barrier in accordance with the applied heating condition. When a docking-wax-barrier was formed in the well-heated state, there was no leaking of fluid. In the case of imperfect formation of the docking-wax-barrier, the fluid leaked out. The two wax-patterns of the ring were printed on both-sides of the paper and then heated in a laminator for various times (0.08~0.40 sec). The wax-patterns of the ring consisted of an inner ring and an outer ring. The inner ring has different nominal widths (W_n) to investigate the effect of nominal width on docking-wax-barrier formation. The outer ring plays a role in preventing the leaking of fluid to an adjacent ring. The results of the leaking test are below.

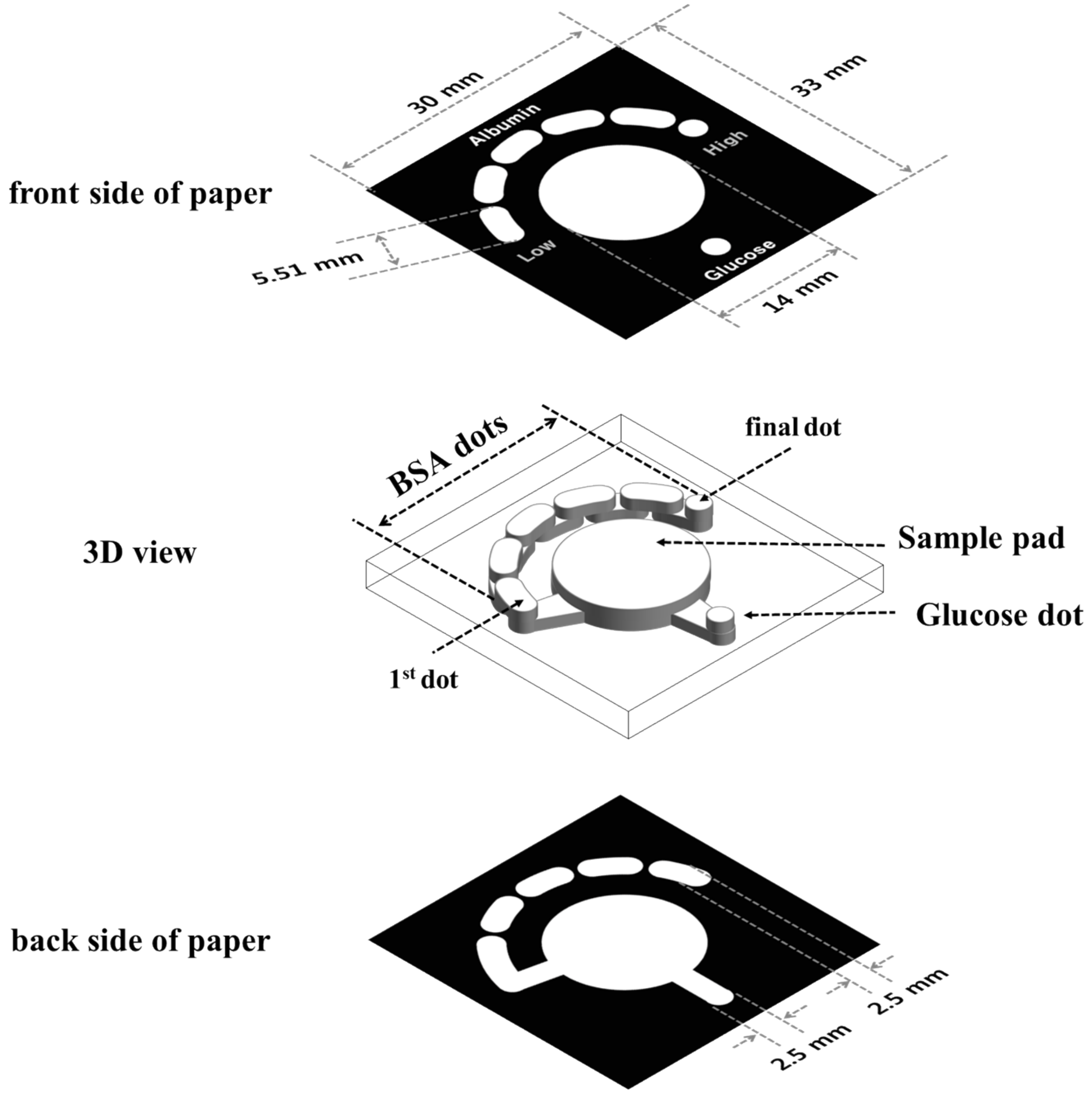

Fig. S4. Design of an instrument-free 3D paper-based diagnostic device for multiplexed digital assays. It has (1) sample pad, (2) BSA dots, and (3) a glucose dot.

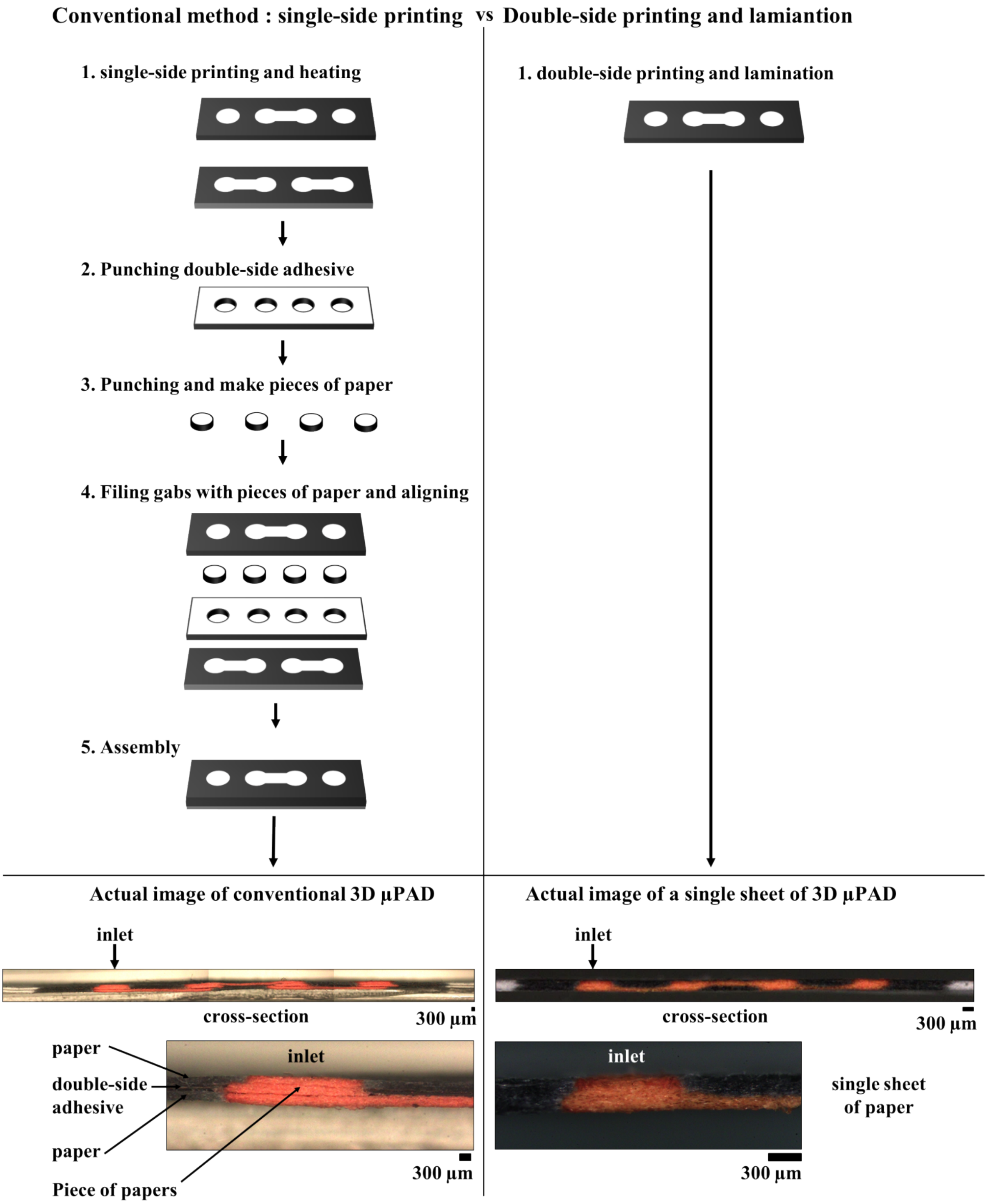

Fig. S5. Comparison between the conventional method of fabrication for 3D-μPADs and the new method based on double-side printing and heating using a laminator.

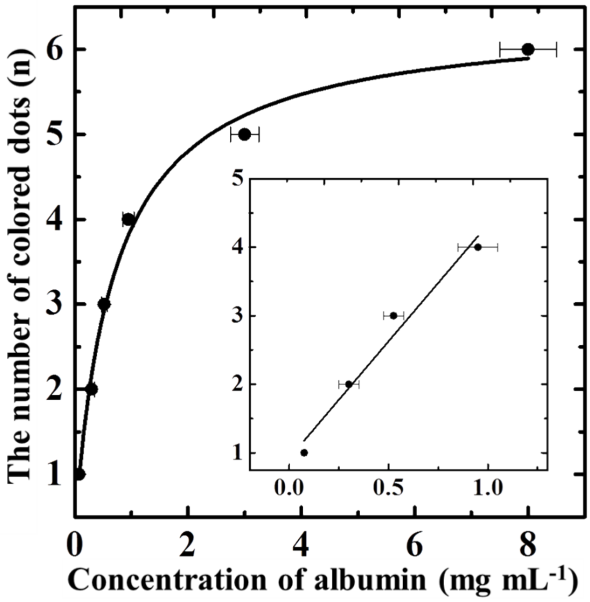

Fig. S6. Relationship between the number of colored dots in 3D-μPAD and the concentration of albumin in a sample. The inset shows linear dynamic range (0.1 to 1.0 mg mL^{-1}).

Fig. S6 indicates a good linear relationship between the number of colored dots and the concentration of bovine serum albumin (BSA). As a proof-of-concept assay, the dynamic detection range is 0.1 - 3.0 mg mL^{-1} BSA and the limit of detection is 0.1 mg mL^{-1}. Conventional dipstick analysis is used in most outpatient settings to semi-quantitatively measure the urine protein concentration. The results are graded as negative (less than 0.1 mg mL^{-1}), trace (0.1 to 0.2 mg mL^{-1}), 1+ (0.3 mg mL^{-1}), 2+ (1 mg mL^{-1}), and 3+ (3 mg mL^{-1}).[1, 2] Therefore, our paper chip accommodates detection range in conventional analysis.

Table S1. Assessment of the processing time required for the fabrication of a 3D-microfluidic network in a single sheet of paper (A4 size) using double side printing and lamination.

	Time
Double-side printing	4 sec
Lamination (Double-side heating)	31.5 sec
Total process time	35.5 sec

The described fabrication method is rapid (< 36 sec for A4 paper), cheap, easy to manipulate, and programmable (Table S1).

Reference

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2. J. S. Cnossen, R. K. Morris, G. ter Riet, B. W. Mol, J. A. van der Post, A. Coomarasamy, A. H. Zwinderman, S. C. Robson, P. J. Bindels and J. Kleijnen, Can. Med. Assoc. J., 2008, **178**, 701-711.