Rapid fabrication of versatile omni-directional and long-distance three-dimensional flow paper-fluidic analytical devices using cut-and-insert method for biomedical applications

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1. Effect of PDMS mixing ratio

Three mixing ratios of PDMS mixture were used to determine the effect of PDMS mixture viscosity on barrier formation. The tested mixing ratios of mixtures were 1:15, 1:10, and 1:5 depending on the proportion of curing agent for silicone elastomer pre-polymer. Figure S1 (A) shows the average widths of the hydrophobic PDMS barrier depending on PDMS viscosity and vacuum time, and Figure S1 (B) shows the average widths of the hydrophilic fluid channel depending on PDMS viscosity and vacuum time. The graphs show that the higher proportion of the silicon elastomer pre-polymer tends to make thinner width of the hydrophobic channel barrier as well as larger width of the flow channel. On the order hand, the thicknesses of hydrophobic channel barriers are widened, and the widths of flow channels were narrowed, when the curing agent ratio increased. These results are explained from the lower viscosity of the mixture with higher ratio of curing agent which makes faster PDMS transport in the paper pad during vacuuming process.

![Graphs A and B](image)

Figure S1. Effect of mixture ratio and vacuuming time. (A) Effect on hydrophobic barrier thickness. (B) Effect on fluidic channel thickness.
2. **3D flow in bonded μ-PADs**

The characteristics of 3D fluid flow in the perpendicularly connected 3D μ-PADs constructed with plasma-enhanced PDMS bonding method was surveyed as shown in Figure S2. The dye solution flows from the inlet channel to the outlet channel through the joint area as shown in Figure S2 (A). Figure S2 (B) shows the mixing of two dye solutions at the junction area when two different solutions were injected in two inlets.

![Figure S2](image)

**Figure S2.** Fluid flow in the μ-PAD constructed by plasma-enhanced PDMS bonding method. (A) One directional 3D flow through the junction of two layers. (B) Mixing of two solutions at the junction area.
3. **RGB intensities of the parallel colorimetric sensing**

The each RGB values of the images for nitrite, glucose, and pH test are plotted in Figure S3. The recorded experimental images were converted into 24 bits color scale (RGB dimension) and then color changes of each bioassay were analyzed by ImageJ image analysis software (v1.47t, NIH, USA). The arithmetic mean pixel color intensity of each circular area within each test region was used to quantify the color intensity. Eventually, the blue color was selected to quantitate the concentrations of glucose and nitrite, as well as pH measurements.

![Figure S3. RGB intensities of the parallel colorimetric sensing. (A) Nitrite test. (B) Glucose test. (C) pH test.](image-url)
4. References