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Microfluidic Paper-based Analytical Devices Fabricated by Low-cost Photolithography and Embossing of Parafilm®

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Supplementary data

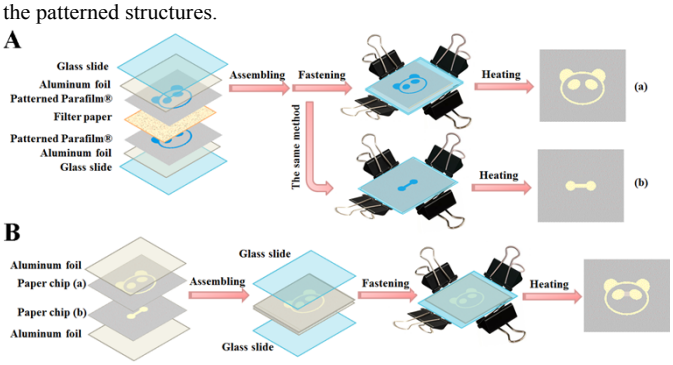
Materials

Whatman filter paper #1, Uric acid, horseradish peroxidase (HRP) and 3,3′,5,5′-Tetramethyl benzidine (TMB) were purchased from Sigma-Aldrich (St. Louis, USA). Uricase was from Sangon Biotech (China). Polyvinylidene difluoride (PVDF) transfer membrane for Western blotting (pore size: 0.2 μm) was from Millipore, methomyl was from Aladin (China), while cholinesterase (ChE), acetylcholine (ACh), dithiodipropionic nitrobenzene acid (DTNB) were ordered from LvShang laboratory apparatus (China). Nylon66 membrane filter for organic solvent filtration (diameter: 50 mm, pore size: 0.45 μm) was from Tianjin Jinteng Experiment Equipment Co., Ltd (China). Printed circuit broad (PCB) UV photosensitive inks was obtained from IC Machinery Equipment Group (China). Concentrated food coloring in green was purchased from Jiangsu World Chemical Co., Ltd. (China). All other chemicals used in this study are analytical grade. The deionized (DI) water used in all experiments was produced by PURELAB flex system, ELGA Corporation.

Method

Fabrication of 3-D microfluidic paper-based analytical devices

To fabricate a 3-D microfluidic paper-based analytical devices (μPDA), a modified procedure was used to make 2-D μPDA. As shown in sFig.1A, paper was sandwiched between carefully aligned two pieces identical photoresist-patterned Parafilm® (patterned Parafilm®-paper- patterned Parafilm® sandwich). Following the same procedure of assembling with aluminium foil and glass, the structures were heated at 120°C for ~5 min. To assemble the 3-D fluidic system, 2-D μPDA chips with different structure were simply aligned vertically and fastened between two pieces of glass for heating (sFig.1B). Because both side of the paper were covered by parafilm, it is very easy to form firmly adhesion between pieces with heating at 100°C for ~5s and effectively avoid damaging to the patterned structures.

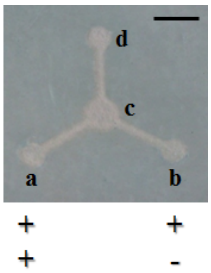


sFig.1 Fabrication of 3-D microfluidic paper-based analytical devices (μPDA) A. preparing of 2-D μPDA: paper is sandwiched between two identical photoresist-patterned Parafilm® and fastened for heating at 120°C for ~5 min. B. 2-D μPDA with different structures were vertically aligned and fastened for healing at 100°C for ~2-5 s.

Detection of uric acid with microfluidic paper-based analytical devices

To achieve uric acid detection, uricase (2 μL, 12U/mL), TMB (1 μL, 2 mg/mL) and HRP (0.5 μL, 10 mg/mL) were drop casted on to desired area (***a***: HRP+TMB, ***b***: TMB, ***c***: uricase, sFig.2) To avoid liquid confusion caused cross-contamination, 0.5 μL reagent was casted each

time. The left amount of reagent was added once the paper dry. During uric acid detection, 10 μL sample was dropped on the sampler reservoir (***d***) and migrated through channel to ***a*** and ***b***. After 5 min of reaction, the chips were imaged and the grey intensity (G.I) at reservoir ***a*** and ***b*** was quantified by NIH ImageJ software. Since every three-channel system has a negative control, the uric acid induced color change can be calculated as signal (reservoir ***a***)/noise (reservoir ***b***) ratio. All experiment was repeated



TMB	+	+
HRP	+	-

sFig.2 preparation of 2-D μPDA for uric acid detection: pre-loading of HRP and TMB at reservoir ***a***, TMB only on ***b*** and uricase at ***c.*** The sample is dropped at ***d***. HRP: horseradish peroxidase; TMB: tetramethyl benzidine. The scale bar denotes 5 mm.