New Journal of Chemistry

Supporting Information

Fabrication of carbohydrate microarrays on poly(2-hydroxyethyl methacrylate)-cyanuric chloride-modified substrates for analysis of carbohydrate-lectin interactions

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1. Preparation of HPA-CC-modified glass slides

The APTMS-modified slides were immersed in a 1:1 mixed solution of HPA (113 mM) in ethanol and EDC (219 mM) in water for 3 h. After reaction, the slides were washed three times with ethanol and water, and then dried under a N_2 stream. The HPA-modified glass slides were then immersed in acetone solution of CC (100 mM) and DIPEA (100 mM) for at least 8 h at 4 °C. The slides were then washed three times with acetone and dried under a N_2 stream for carbohydrate microarray fabrication.

2. Procedures of fluorescence wash-off measurements

2-NBDG aqueous solution was spotted on pHEMA-CC-modified substrates, each spot was around 1.8 mm diameter. The slides were dried in the air and imaged by array scanner. After reaction at dark in a humid chamber at room temperature for 10 h, the slides were washed by water, dried by N_2 stream and imaged again by array scanner. The intensity from each spot was compared with that before washing.

3. Calculation of $K_{D,surf}$ values

For the binding affinity determination of mannose with ConA-FITC on pHEMA-CC- and HPA-CC-modified substrates, the binding intensity was plotted against the concentration of ConA-FITC by fitting curves to equation (S1) [1]

$$F = \frac{F_{max}[P]}{[P]+K_{D,surf}} \qquad \text{(S1)}$$

where F_{max} denotes the maximum fluorescence intensity; [P] is the concentration of ConA-FITC in solution; $K_{D,surf}$ is the equilibrium dissociation constant for immobilized mannose and ConA-FITC.

4. Test of anti-nonspecific adsorption ability of different substrates

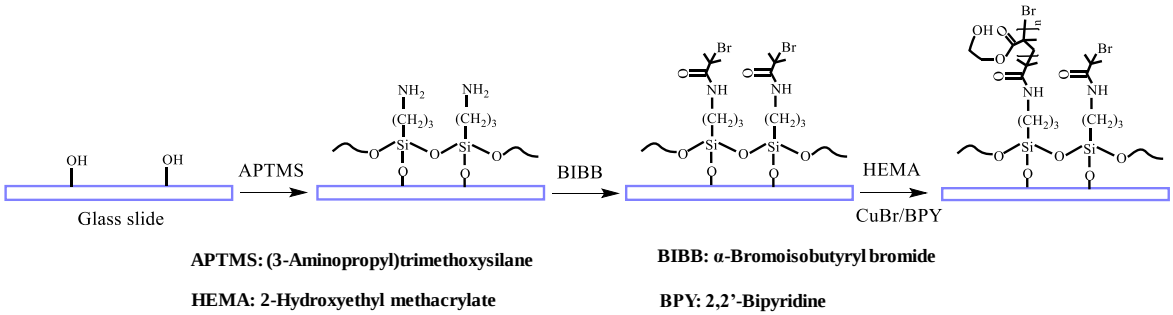
The anti-nonspecific adsorption ability of carbohydrate microarrays fabricated on different substrates (pHEMA-modified, pHEMA-CC-modified and HPA-CC-modified

substrates) were evaluated by the background signals resulted from ConA-FITC nonspecific adsorption. Each test was carried out in dual incubation channels on same chip. One channel was used to incubate with ConA-FITC (100 μg/mL) and another one was used for reference (ConA-FITC, 0 μg/mL). The background signals on each substrate resulted from nonspecific adsorption of ConA-FITC were then obtained, respectively.

5. Cytopathic effect (CPE) inhibition assay

The CPE assay was performed as previously reported [2]. MDCK cells in 96-well plates were firstly infected with IAV, and then treated with ManA2 in triplicate after removal of the virus inoculum. After 48 h incubation, the cells were fixed with 4% formaldehyde for 20 min at room temperature. After removal of the formaldehyde, the cells were stained with 0.1% crystal violet for 30 min. The plates were then washed and dried followed by solubilization of the dye with methanol, and the intensity of crystal violet staining for each well was measured at 570 nm. The concentration required for ManA2 to reduce the CPE of IAV by 50% (IC_{50}) was determined.

6. Schematic illustration of pHEMA modification procedures



Scheme S1 Preparation of pHEMA-modified surface *via* SI-ATRP.

7. Characterization of pHEMA-modified glass

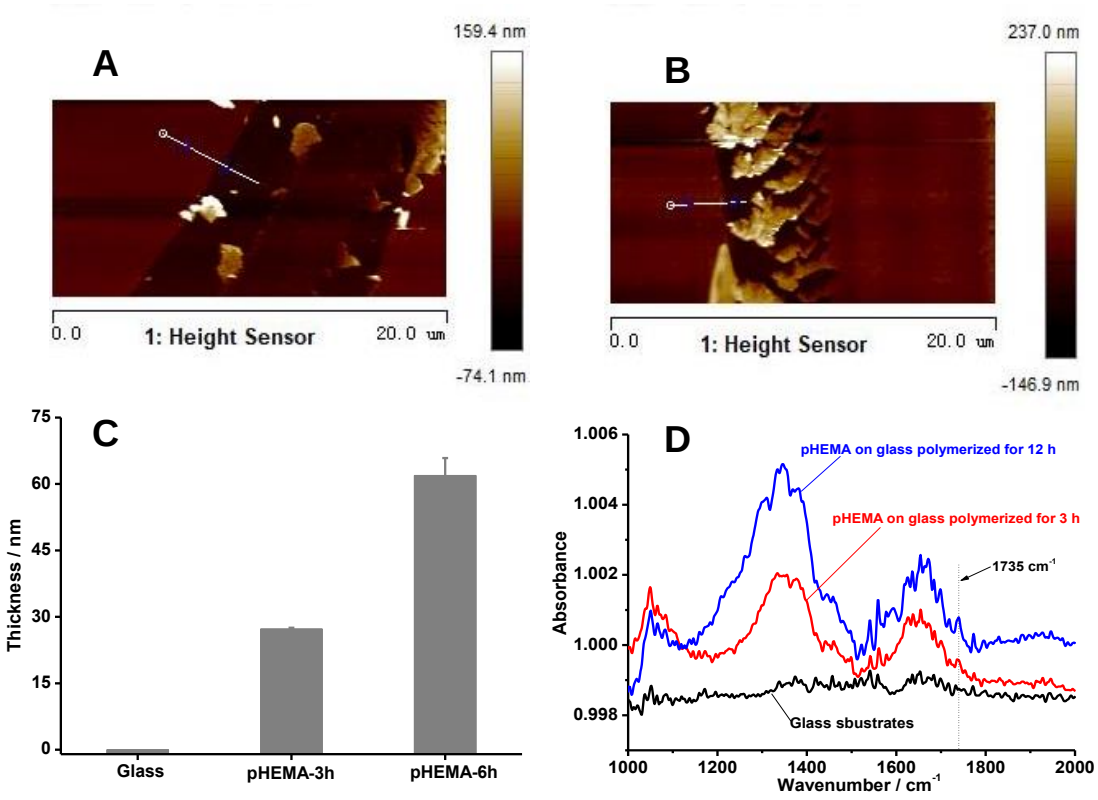


Figure S1 (A, B) Typical AFM images of pHEMA-modified glass substrates during thickness measurement, and the polymerization time of pHEMA was (A) 3 h and (B) 6 h, respectively. The white lines represented interested areas. (C) The pHMEA thickness on glass (n=3). (D) The ATR-FIIR spectrogram of pHEMA grafted from glass.

8. Fluorescence wash-off measurement of 2-NBDG

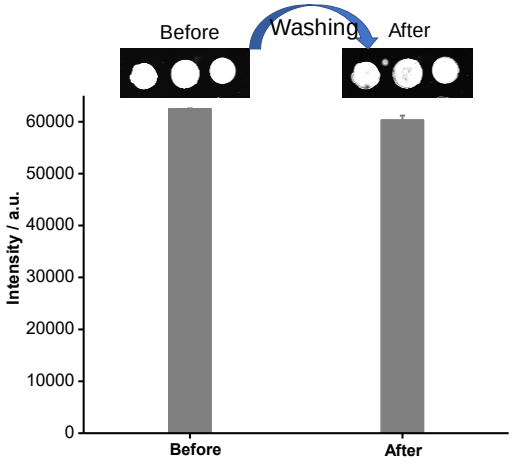


Figure S2 Fluorescence intensity of 2-NBDG (100 μM) spotted on pHEMA-CC-modified substrates before and after washing. Insets were corresponding fluorescence images.

9. Background signals on pHEMA-modified and pHEMA-CC-modified substrates

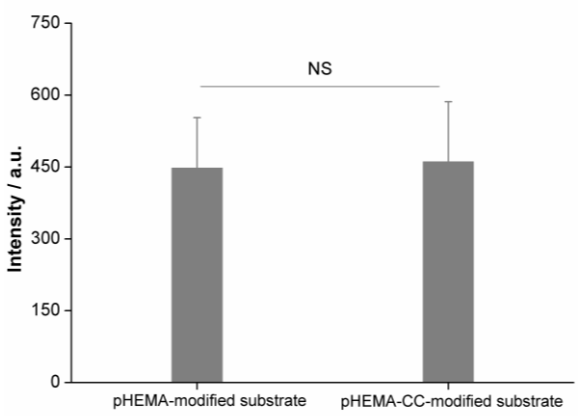


Figure S3 Nonspecific adsorption signals obtained from pHEMA- and pHEMA-CC-modified substrates after treating with ConA-FITC under same condition. The data represent mean ± SD, and determined by t-test (n=10). NS, not significant.

10. LOD of ConA-FITC in solution

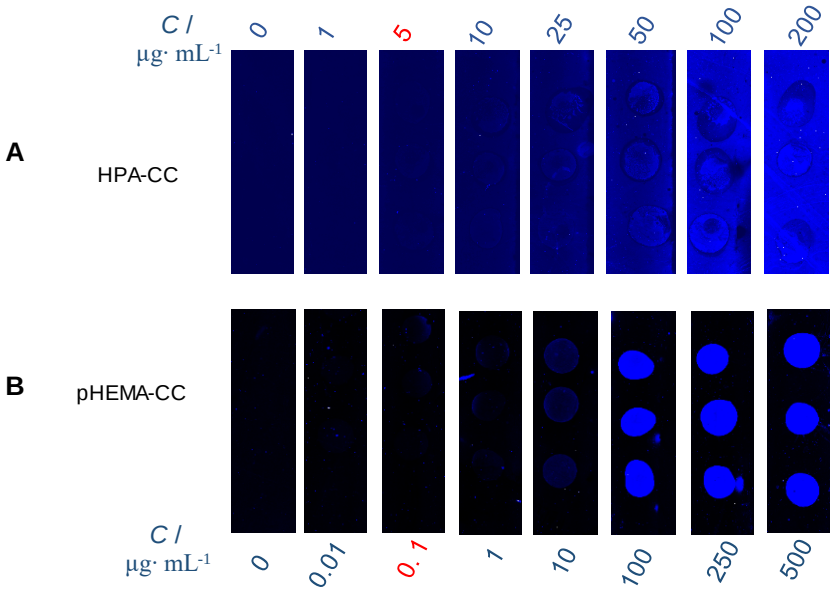


Figure S4 Typical fluorescence images of mannose (spotting concentration 100 mM) immobilized on (A) HPA-CC-modified and (B) pHEMA-CC-modified substrates after recognition with a series of ConA-FITC solutions in multi-channel incubation mode.

11. Dynamic range of ConA-FITC

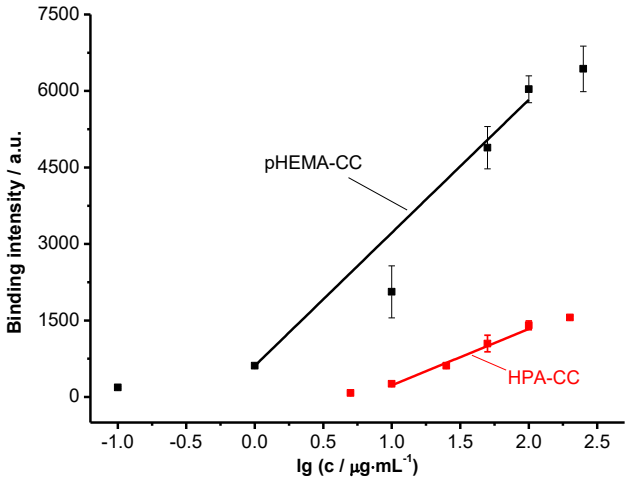


Figure S5. Mannose and ConA-FITC recognition. Relationship of fluorescence binding signals *vs* logarithm of ConA-FITC concentration; spotting concentration of mannose was 100 mM on pHEMA-CC- and HPA-CC-modified substrates.

12. Incubation time

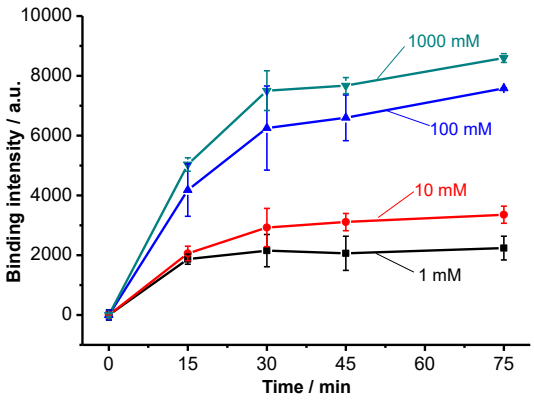


Figure S6 Fluorescence binding intensity of mannose at different spotting concentration (1, 10, 100, and 1000 mM) with ConA-FITC in different incubation time.

13. Fitting curves of mannose and ConA-FITC recognition

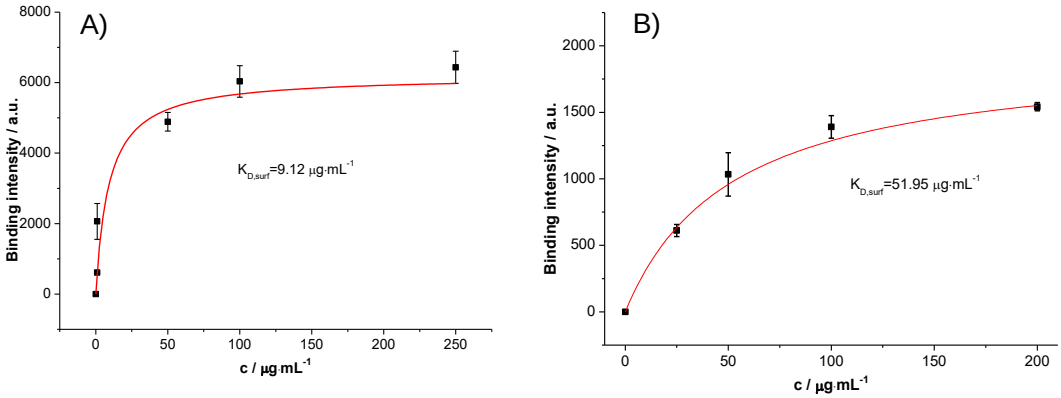


Figure S7 Fluorescence binding intensity of mannose (spotting concentration, 100 mM) immobilized on (A) pHEMA-CC-modified and (B) HPA-CC-modified substrates after recognition with a series of ConA-FITC solution, fitting curves were obtained by fitting to Langmuir isotherms (equation S1).

14. Stability of pHEMA-CC-based microarray

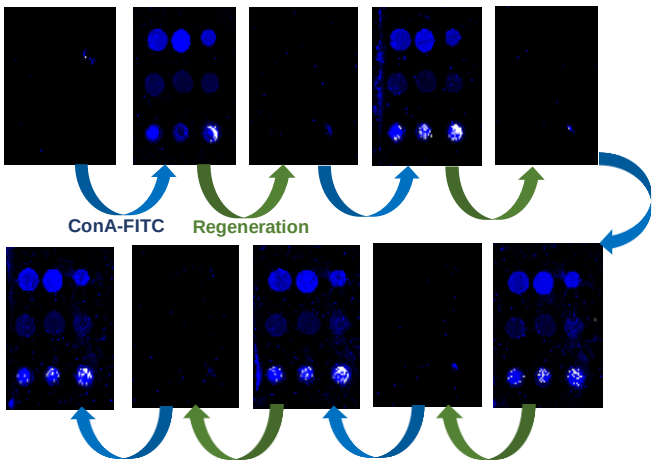


Figure S8 Fluorescence images of pHEMA-CC-based microarrays containing mannose, maltose, and dextran (from top to bottom) during ConA-FITC (928 nM) recognition-regeneration (0.1 M H_3PO_4) cycles.

Reference

1. P. H. Liang, S. K. Wang and C. H. Wong, *Journal of the American Chemical Society*, 2007, **129**, 11177-11184.
2. W. Wang, J. Wu, X. Zhang, C. Hao, X. Zhao, G. Jiao, X. Shan, W. Tai and G. Yu, *Scientific Reports*, 2017, **7**.