Supplementary Information

Facile fabrication of microsphere-polymer brush hierarchically three-dimensional (3D) substrates for immunoassays

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Experiment section

Materials. Cycloolefin polymer (COP) Zeonex 690R granules and sheets (1 mm thickness, inject molding) were obtained from ZEON Corp.. 2-Hydroxyethyl methacrylate (HEMA) and 2-carboxyethyl acrylate (CEA) were purchased from Sigma-Aldrich. Benzophenone (BP), N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were obtained from Aladin. Alexa Fluor 555 labeled Goat anti-rabbit IgG (A555-Go-anti-Ra IgG), rabbit anti-avidin IgG and Alexa Fluor 488 labeled avidin (A488-avidin) were purchased from Abcam. PS microspheres (~3 μm in diameter) were obtained from Suzhou Nano-Micro Corp..

Immobilization of PS microspheres. COP supports were cleaned by ethanol and dried with nitrogen stream. An ethanol solution containing PS microspheres (0.02

wt%) and BP (1 wt%) was dropped onto the COP supports, dried at the atmosphere, then put between two quartz plates and subjected to UV irradiation for 8 min, followed by thorough rinsing with water and drying in gentle N_2 flow. The as-prepared substrates were denoted as COP-PS.

Growth of polymer brush on the COP surfaces. The virgin COP supports and COP-PS supports were immersed into an ethanol solution of BP (1 wt%) for 0.5 h and then dried at ambient temperature. The supports were put on a quartz plate and coated with an aqueous solution containing CEA or HEMA monomer (10 vol%), followed by covering with another quartz plate. For the graft of p(HEMA-co-CEA) brush, the mixture of CEA and HEMA (1:1, v/v) was used. For microarray substrates, photomasks were placed on the support surfaces. The sandwich system was subsequently exposed to UV light (high-pressure mercury lamp, 400 W, main wavelength 380 nm) for 6 min. All the supports were thoroughly rinsed with water and ethanol for 24 h and then dried in a N_2 flow. The amount of grafting carboxyl groups was determined by colorimetric method using Toluidine Blue (TB) staining and the molar ratio between carboxyl groups and TB was supposed to be 1:1. In detail, the samples were immersed into the 0.5 mM TB solution (pH = 10) for 5 h with continuous stirring and then rinsed thoroughly with 0.1 M NaOH solution to remove the non-complexed TB. Finally, the samples were incubated in 50 vol% acetic acid aqueous solution for 30 min to remove the complexed TB molecules and the optical density of the solution was detected at 632 nm by a UV-VIS spectrophotometer (Synergy H1, BioTek Instruments, Inc.).

Antibody immobilization. For COP-g-p(HEMA-*co*-CEA) and COP-PS-g-p(HEMA-*co*-CEA) supports, carboxyl groups were activated with a 0.1 M MES solution containing 0.4 M EDC and 0.1 M NHS at 4 °C for 1 h, followed by rinsing with MES solution and drying in a N_2 flow. The activated substrates were then incubated in a PBS solution containing A555 Go-anti-Ra IgG (50 μg/mL) at 37 °C for 2 h. The samples were then thoroughly rinsed with PBS and dried in a N_2 flow. For COP and COP-PS supports, antibodies (50 μg/mL) were physically adsorbed onto the supports at 37 °C for 2 h.

Nonspecific protein absorption test. For nonspecific protein adsorption test, the samples were incubated in a PBS solution containing A488-Fib (100 μg/mL) at 4 °C for 12 h, followed by rinsing, drying and finally examined by fluorescence scanning.

Antigen recognition. The antibody-immobilized samples were blocked with a BSA solution (10 mg/mL) at 37 °C for 1 h. After rinsing with PBS, they were incubated with antigen (A488-avidin) solution at 4 °C for 12 h. After rinsing with PBS solution, the samples were then tested by fluorescence scanning.

Surface characterization. The ATR-FTIR spectra were obtained from a Bruker Vertex 70 Fourier transform infrared spectroscopy (FTIR) with an attenuated total reflection (ATR) mode. The surface morphology was determined via atomic force microscopy in contact mode (AFM; SPA300HV with a SPI 3800 controller, Seiko Instruments Industry). Then the surface morphology images and root-mean-square (RMS) were obtained by AFM analysis. Fluorescence was detected through a confocal laser scanning microscope (Zeiss, LSM 700). Target biomolecules labeled

with A488 and A555 were respectively excited by an argon ion laser at 488 nm and 555 nm.

Experimental results

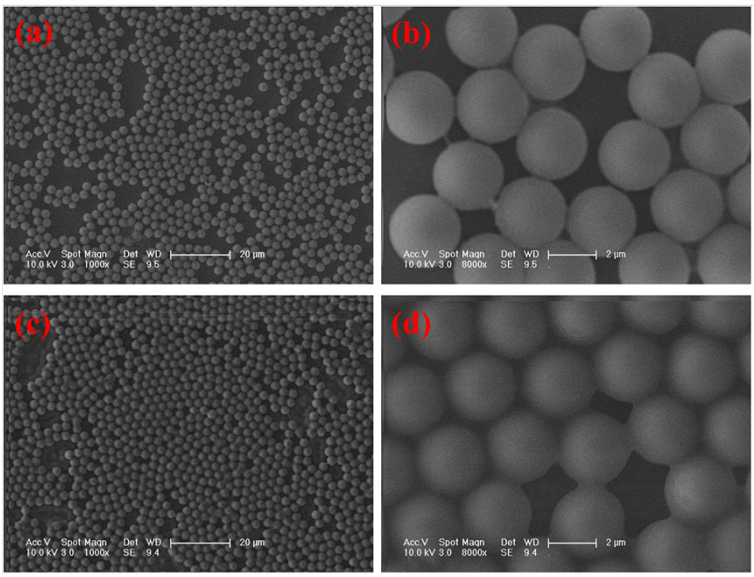

Fig. S1 SEM images of the top view for COP-PS supports (a, b) before and (c, d) after surface-initiated polymerization.

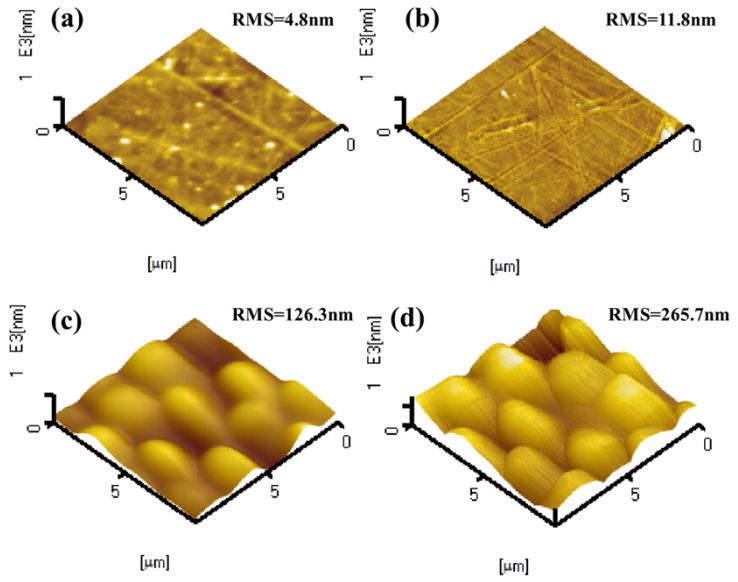

Fig. S2 AFM images of (a) COP, (b) polymer brush-modified COP, (c) COP-PS, (d) polymer brush-modified COP-PS.

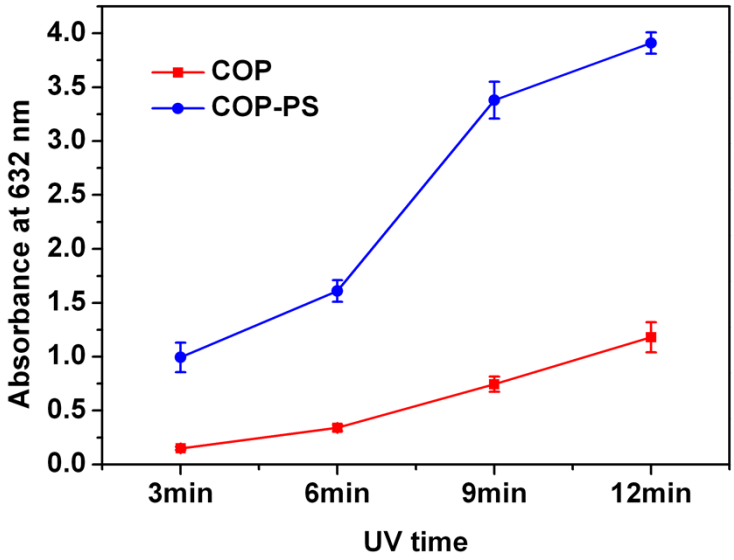

Fig. S3 Optical absorption of TB solution versus photografting time for the polymer brush-modified COP and COP-PS supports.

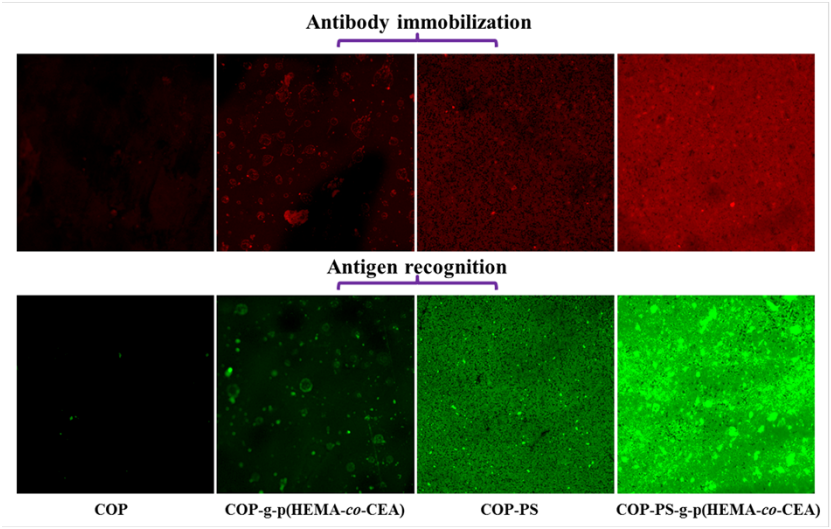

Fig. S4 Fluorescence images for antibody immobilization and antigen recognition on the substrates.

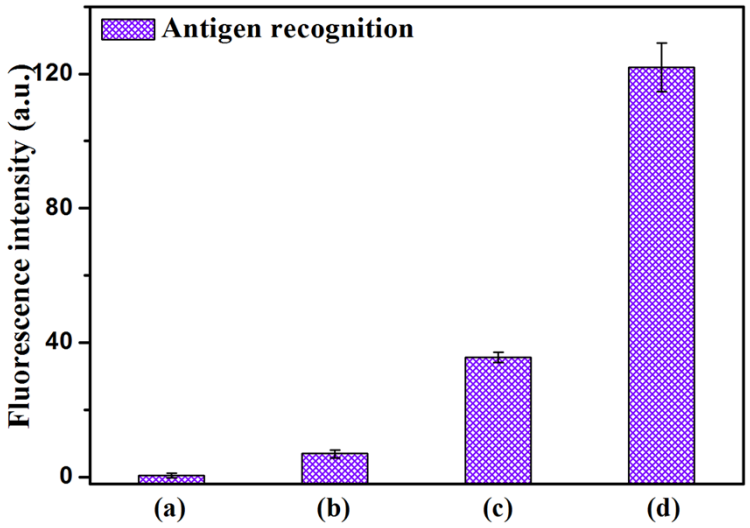

Fig. S5 The mean fluorescence intensity for antigen recognition on the substrates.(a) COP, (b) COP-g-p(HEMA-*co*-CEA), (c) COP-PS, (d) COP-PS-g-p(HEMA-*co*-CEA).

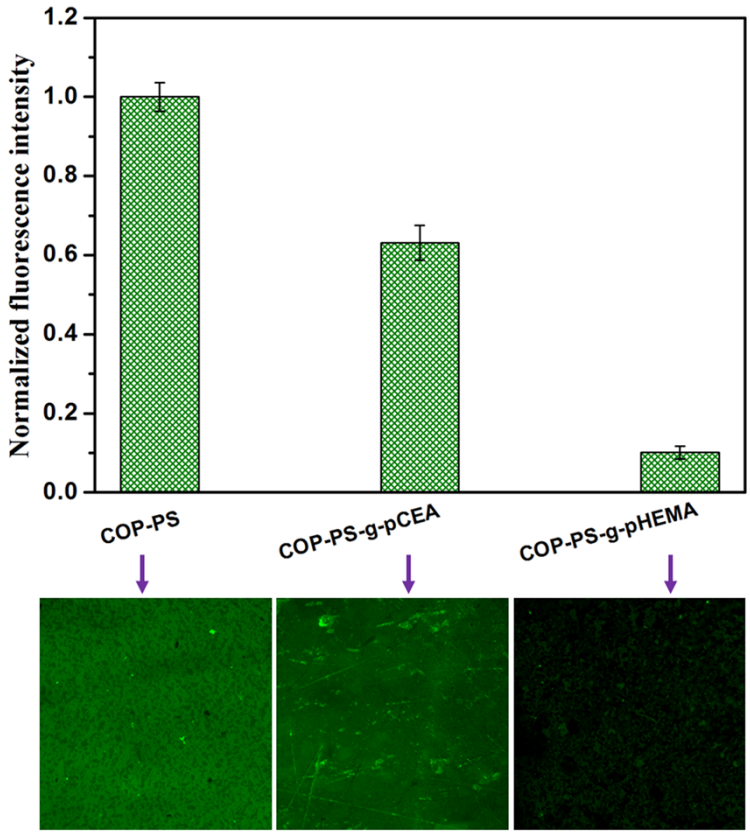

Fig. S6 Normalized A488-fib absorption on the samples and their corresponding fluorescence images.