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SUPPORTING INFORMATION

Versatile Antifouling Coatings Based on Self-Assembled Oligopeptides for Engineering and Biological Materials

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1 Experimental

1.1 Reagents and Solutions

AK-VIII was synthesized by China Peptides Co., Ltd. (Shanghai, China). FITC (fluorescein isothiocyanate)-BSA (bovine serum albumin) and FITC-LYZ (lysozyme) were acquired from China Peptides Co., Ltd. (Shanghai, China). PDMS base and curing agent (Sylgard 184 silicone elastomer kit) were purchased from Dow Corning (Midland, MI). PS sheets were purchased from Head Biotechnology Co., Ltd. (Beijing, China). ELISA microtiter plates (flat bottom, non-treated) were purchased from Guangzhou Jet Bio-Filtration CO., Ltd. The human CEA kit with all the necessary components, mouse monoclonal capture CEA antibodies (Cap Ab), horseradish peroxidase (HRP)-labelled CEA antibody (anti-CEA Ab), 3,3′,5,5′-tetramethylbenzidine (TMB), and peroxide solution were purchased from Zhengzhou Biocell Biotechnology Co. Ltd. (China). Other reagents were purchased from xi'an suppliers. Deionized water (DIW) used to prepare all aqueous solutions was obtained from a Millipore filtration system (Z18 MΩ, Milli-Q, Direct).

Solutions of 1.0 mg/mL AK-VIII, 1% (v/v) BSA, and 1:3000 of anti-CEA Ab were prepared with 10 mM phosphate buffer (PB, pH 7.4) and 20.0 μg/mL Cap Ab was prepared with 50 mM sodium carbonate-sodium bicarbonate (Na_2CO_3-$NaHCO_3$, pH 9.6).

1.2 Fabrication of Microchips

PDMS microfluidic chips were prepared following our previous works.[1-3] PDMS base (3.0 g) and curing agent (0.3 g) were blended uniformly and degassed with vacuum for 2 min. Then injected them to master and heated in an oven at 80 °C for 40 min. Then the PDMS replica was peeled from the master and punched to obtain the reservoirs, and irreversibly bonded to a microscope glass or PS slide using air plasma to form a PDMS/Glass or PDMS/PS microchip with a simple cross channel of 100 μm width and 30 μm depth.

1.3 Preparation of PDMS and PS Surface Specimens

PDMS and PS sheets (1.0 cm $\times$ 1.0 cm $\times$ 2 mm) were sonicated in 1.0 M NaOH and DIW for 30 min and dried with nitrogen gas. Then, they were immersed in 1.0 mg/mL AK-VIII in 10 mM PB buffer at room temperature (RT) for 1 h. Then, the above sheets were took out and dried at RT, following washed with a lot of DIW and dried again with N_2.

1.4 Surface Characterization of PDMS and PS Specimens

WCA measurements were performed using an OCA 20 optical contact angle meter (Dataphysics, Inc., Stuttgart, Germany). All the data was acquired at six different positions on PDMS and PS surfaces. XPS analyses were performed on an Axis Ultra X-ray photoelectron spectrometer (Kratos Analytical Ltd, Manchester, UK) with an Al X-ray source operating at 150 W (15 kV, 10 mA). During XPS measurements, the main chamber was kept in vacuum. The specimens were analyzed at an electron take-off angle of 45°to the surface plane. General survey scans (binding energy range 0-1200 eV, pass energy 80 eV), and high-resolution spectra (pass energy 75 eV) in the C 1s, O 1s, and N 1s regions were recorded for all modified PDMS and PS surfaces. The binding energies (BEs) were referenced to the C 1s binding energy at 284.6 eV. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was determined by a Tensor 27 infrared spectrometer (Bruker, Billerica, MA) with a wedged germanium crystal of attenuated total reflectance accessory.

1.5 Characterization of Biofouling Resistance

Protein adsorption assay was performed following our previous report.[1-3] Briefly, the pristine and AK-VIII-coated PDMS and PS microchannels were filled with 1.0 mg/mL FITC-BSA and FITC-LYZ in 10 mM PB (pH 7.4) and incubated at 37 °C for 1 h. Afterward, BSA and LYZ solution were washed away and the channels were dried under vacuum at RT. After being washed with a lot of DIW and dried under, the obtained microchannels with possible protein contamination were photographed using an inverted microscope with a CCD camera. Thrombus testing was carried out in vitro blood flow model.[4-6] The pristine and the modified PDMS and PS microchannels were subject to the whole blood collected from healthy human, and each experimental process lasted 30 min with 100 μL human whole blood. Then the microchannels were washed with plenty of 10 mM PB (pH 7.4) and dried at RT. The images of PDMS and PS microchannels were acquired using a CCD camera.

1.6 Comparison of BSA Blocking and AK-VIII Blocking in ELISA

For ELISA tests, each microtiter plate was firstly treated with oxygen plasma for 3 min, following by overnight incubation with 100 μL of 20.0 μg/mL Cap Ab at 4 °C and then washed five times with PB. [7,8] Secondly, the pristine PS microtiter plates and Cap Ab-immobilized PS microtiter plates were separately incubated with 10 mM PB (as the controls), 1% (v/v) BSA in 10 mM PB, and 0.1% AK-VIII in 10 mM PB for 30 min at RT, following by thoroughly washing with 10 mM PB for five times. Thirdly, the CEA solutions of 0–30 ng/mL were added into the above PS microtiter plates at 37 °C for 60 min, and immediately washed them with PB for five times. Afterward, anti-CEA Ab, at a dilution of 1:3000, was incubated in the above microtiter plates for 1 h at 37 °C, and subsequently washed five times with 10 mM PB. Finally, 50 μL of the working solution, containing equal amounts of TMB (1.25 g/L) and peroxide solution (0.05% urea peroxide), was added to all the microtiter plates and incubated for 10 min at RT, and the reaction was stopped by addition 50 μL of 2 M H_2SO_4 (as per the manufacturer's recommendations). The absorbance was recorded using Shimadzu UV-1800 spectrophotometer (Beijing Purkinje, China) at 450 nm. All the data were conducted in triplicate. The control for this study was 0 ng/mL CEA, and the absorbance of the control was subtracted from all the assay values.

1.7 Ethics statement

Normal human serum was obtained from Hospital of Shaanxi Normal University. This study complied with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China (1988) and the Guidelines on the Humane Treatment of Laboratory Animals (2006). All experimental procedures with serum were approved by Human and Animal Research Ethics Board of Shaanxi Normal University (NO. 20150323). All participants provided written informed consent.

2 Tables and Figures

Table S1 Water Contact Angles on Surfaces Coated with AK-VIII

Coating additives	WCAs, degrees[a]	
	PDMS	PS
No additives	119.8 ± 0.2	85.7 ± 0.5
1.0 mg/mL AK-VIII	114.0 ± 0.8	91.7 ± 0.4

[a]Data are reported as the mean ± standard error (n = 6).

Table S2 Elemental Composition of Uncoated and Coated Surfaces Determined by XPS

Substrates	Atomic Concentration (%)				Ratio
	C1s	O1s	Si2p	N1s	O/C
PDMS	50.66	22.88	26.46	0.00	0.45
AK-VIII/PDMS	53.18	23.70	21.00	2.12	0.45
PS	89.49	10.51	0.00	0.00	0.12
AK-VIII/PS	85.77	11.54	0.00	2.69	0.13

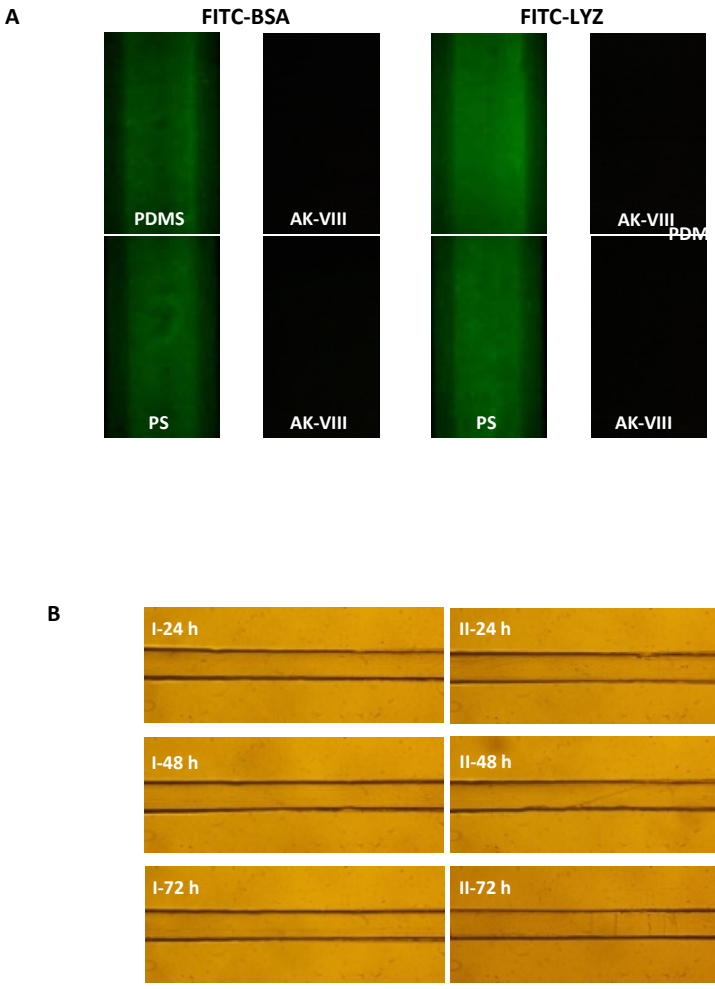

Fig. S1. (A).Fluorescent micrographs of the pristine and coated PDMS and PS microchannels after incubation at 37 °C for 1 h with 1.0 mg/mL FITC-BSA and FITC-LYZ in phosphate buffer (10 mM, pH 7.4) followed by washing with copious DIW. (B). Optical photos of the AK-VIII-coated-PDMS (I) and PS (II) microchannels in whole blood flow tests repeated at different time.

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