Supporting Information

Vapor-Based Synthesis of Maleimide-Functionalized Coating for Biointerface Engineering

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Experimental Section

Materials.

The following materials were obtained commercially and used as received, unless otherwise noted: [2.2]paracyclophane (98%, Jiangsu Miaoqiao Synthesis Chemical Co., Ltd.), titanium (IV) chloride (98%, Fluka,), α,α-dichloromethyl methyl ether (98%), anhydrous $MgSO_4$ (99.5%, J.T. Baker), $NaBH_4$ (98%), triphenylphosphine (98.5%, Fluka), DIAD (94%, Alfa Aesar), maleimide (99%), fluorescein-conjugated cysteine (95%, Kelowna International Scientific Inc., Taiwan), thiol-PEG M.W. 5000 (Nanocs Inc.), fibrinogen from human plasma, Alexa Fluor-546 conjugate (Life Technologies Corporation), Sulfo-LC-SPDP (Thermo Fisher Scientific Inc.), RRRGD (95%, Yao-Hong biotechnology Inc., Taiwan), CREDV (95%, Yao-Hong biotechnology Inc., Taiwan), PMMA (Taifonacrylic Co., Taiwan), polystyrene (Taifonacrylic Co., Taiwan), glass (FEA, Germany), silicon wafer (Goldeninent Inc., Taiwan). Silver substrates were fabricated on a 4-in silicon wafer using thermal evaporation method, in which a 300 Å thick titanium layer was formed, followed by the formation of a 700 Å thick silver layer (Kao Duen Technology Co., Taiwan). Similarly, titanium substrates were prepared on a 4-in silicon wafer via thermal evaporation

with the formation of a 700 Å thick titanium layer (Kao Duen Technology Co., Taiwan). Gold substrates were also fabricated using thermal evaporation on a 4-in silicon wafer; a titanium layer of 300 Å thickness was formed, followed by the formation of a gold layer of 700 Å thickness (Kao Duen Technology Co., Taiwan)

Synthesis of 4-N-maleimidomethyl-[2,2]paracyclophane.

Titanium (IV) chloride (8.4 mL, 77 mmol) was added slowly to an ice-cooled solution of [2,2]paracyclophane **1** (8.0 g, 38 mmol) in anhydrous CH_2Cl_2 (400 mL) under nitrogen environment. The mixture was stirred for 20 min, followed by the dropwise addition of *α,α*-dichloromethyl methyl ether (4.0 mL, 44 mmol). The reaction mixture was stirred at room temperature for 6 h and was subsequently poured into water and stirred for another 2 h (200 mL). The organic layer was washed with 3 M HCl (2 × 300 mL) and then with water (2 × 300 mL), and dried over $MgSO_4$. After filtration and removal of the solvent, the crude product was purified on silica gel using hexane/dichloromethane (5/1) as eluent to yield 4-formyl-[2,2]paracyclophane **2** as white crystals (6.6 g, 83%). The crystals of 4-formyl-[2,2]paracyclophane **2** were then dissolved in a mixture of methanol (200 mL) and anhydrous Tetrahydrofuran (THF) (10 mL). To this solution, $NaBH_4$ (2.1 g, 28 mmol) was added carefully and the mixture was stirred at room temperature for 3 h. The excess $NaBH_4$ was then decomposed by careful addition of water. The solution was then diluted by ethyl acetate (200 mL), washed with 3 M HCl (3 × 200 mL) and water (2 × 200 mL), and dried over $MgSO_4$. After filtration and removal of the solvent, 4-(hydroxymethyl)-[2,2]paracyclophane **3** was obtained as white crystals (6.0 g, 75%), which was used without further purification. Next, 4-(hydroxymethyl)-[2,2]paracyclophane **3** (6.0 g) and triphenylphosphine (13.1 g) were dissolved in anhydrous THF (200 mL), to which DIAD (10 mL) was added carefully and the mixture was stirred at room temperature for 20 min. Then a previously prepared maleimide solution (4.9 g maleimide in 30 mL anhydrous THF) was added to the mixture and stirred at room temperature for 24 h. The solution was then diluted with dichloromethane (200 mL), washed with 3 M HCl (3 × 200 mL) and water (2 × 200 mL), and dried over $MgSO_4$. The crude product was purified on silica

gel using hexane/ethyl acetate (5/1) to yield 4-N-maleimidomethyl-[2,2]paracyclophane **4** as white crystals (5.2 g, 65%). Product analysis revealed the following parameters: ^{1}H NMR (500 MHz, $CDCl_3$, TMS): $\boldsymbol{\delta}$ = 6.74 (2d, J = 7.9 Hz, 1.8 Hz, 1H), 6.51 (s, 2H), 6.50-6.41 (m, 5H), 6.21 (s, 1H), 4.50 (2d, J = 85.6 Hz, 14.9 Hz, 2H), 3.57 (m, 1H), 3.18 (m, 1H), 3.07-2.15 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): $\boldsymbol{\delta}$ = 33.3, 34.5, 35.0, 35.2, 39.8, 129.1, 132.1, 132.3, 133.1, 133.4, 133.8, 134.1, 134.7, 135.1, 137.7, 139.3, 139.5, 140.2, 170.4; EI-MS [70 eV, m/z (%)]: 317 (75) [M^+], 117 (19) [$C_9H_9^+$], 104 (100) [$C_8H_8^+$], 77 (6) [$C_6H_5^+$]; FT-IR (ATR): 3461(vw), 3094 (w), 2946 (w), 2924 (w), 2854 (w), 1768 (vw), 1732 (m), 1705 (vs), 1593 (w), 1541 (vw), 1498 (w), 1434 (s), 1404 (s), 1389 (m), 1346 (m), 1327 (m), 1276 (w), 1157 (w), 1139 (s), 1089 (m), 1036 (m), 896 (s), 873 (s), 836 (s), 827 (s), 795 (s), 717 (s), 693 (vs), 618 (s), 575 (s), 515 (vs), 479 (s), 464 (s), 413 (s) cm^{-1}.

CVD polymerization.

Coating **5** was prepared from 4-N-maleimidomethyl-[2,2]paracyclophane **4** using a home-built CVD polymerization system. Throughout the CVD polymerization process, a constant argon flow rate of 10 sccm and a system pressure of 75 mTorr were maintained. The pyrolysis temperature was set at 580°C, and the sublimation temperature was between 110°C and 120°C. Under these conditions, CVD polymerization occurred spontaneously on substrates that were placed on a rotating, cooled (15°C) sample holder. A deposition rate of ~0.3 Å/s was monitored on the basis of in situ quartz crystal microbalancing analysis (STM-100/MF, Sycon Instruments, USA). The resulting coating thickness was recorded using a EP^3-SW ellipsometer (Nanofilm Technologie GmbH, Germany), and the data revealed that the thickness of the polymer films deposited were in the range of 60–80 nm.

Surface characterization.

IRRAS spectra were recorded using a Thermo Nicolet NEXUS 470 FT-IR spectrometer that is equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector, and the spectra were corrected

for any residual baseline drift. The samples were mounted in a nitrogen-purged chamber. CVD coatings were modified on silver-coated silicon substrates (Ag/Si) for the measurement, and a blank Ag/Si substrate was used as a reference during the measurement. XPS data were recorded with a Theta Probe X-ray photoelectron spectrometer (Thermal Scientific, UK) and using a monochromatized AlKα as the X-ray source, at an X-ray power of 150 kW. Pass energies were 200.0 and 20.0 eV for survey spectra and C_{1s} high-resolution elemental spectra, respectively. XPS atomic analysis was reported based on atomic concentrations (%) and was compared to the theoretical values calculated on the basis of the structure of Coating **5**.

Immobilization of biomolecules.

Biomolecules used in the study were immobilized by confining them to selected areas via μCP technique, using PDMS stamps to conduct the printing. All reactions were carried out at room temperature (25°C) and at a humidity of 55%. Before conducting the printing, PDMS stamps were treated with 10 W oxygen plasma for 2 min to render the surface hydrophilicity. For the immobilization of fluorescein-conjugated cysteine, a stamp having features of square arrays, with a side length of 50 μm and a center–center spacing of 100 μm, was inked with fluorescein-conjugated cysteine in deionized water (5 mM), and then was printed on Coating **5** for 2 h. The resulting sample was then washed with phosphate-buffered saline (PBS, pH = 7.4; contains Tween 20, Sigma) three times and again with PBS (pH=7.4, Sigma) one more time, and was finally rinsed with deionized water. Immobilization of fluorescein-labeled RRRGD was performed in a similar way, using μCP with stamps having features of square arrays with a side length of 50 μm and a center–center spacing of 100 μm. The stamps were inked with Sulfo-LC-SPDP solution (with 30 mM TCEP at pH = 8.5) and printed on Coating **5**. After 2 h, the sample was rinsed with deionized water. The resulting sample was then incubated with fluorescein-conjugated RRRGD in deionized water (5 mM) for 4 h. For protein adsorption, PDMS stamps were treated with 10 W oxygen plasma for 2 min and then inked with 400 mg/mL solution of thiol-PEG in

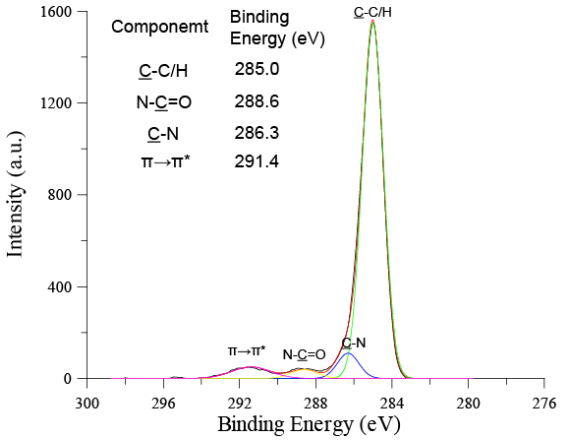
deionized water. The stamps were then printed on Coating **5** for 2 h. Alexa Fluor 546-conjugated fibrinogen was used to study the fouling property of surface-modified substrates. Protein solutions were prepared at a concentration of 100 μg/mL. The patterned substrates were then incubated with the protein solution for 5 min, and the resulting sample was washed three times with PBS (pH=7.4; contains Tween 20), and once more with PBS (pH=7.4) to rinse off the excess adsorbed proteins. The resulting samples were examined by a fluorescence microscope (NikonTE2000-U). For the first step of μCP, a stamp consisting of square patterns with a side length of 300 μm and a center–center spacing of 600 μm was used. The stamp was inked with 400 mg/mL thiol-PEG and then printed on the samples from first μCP for 2 h. A featureless flat stamp was inked with CREDV (4 mg/mL) and then printed on Coating **5**-modified polystyrene substrates for 2 h. The resulting samples were washed thoroughly with deionized water to remove any unreacted CREDV.

Cell culture.

Polystyrene substrates were modified with Coating **5** and then used for cell culture study. BAECs were obtained from Academia Sinica, Institute of Biomedical Science, and were cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). The modified surfaces were then fixed at the prepared wells and briefly maintained in DMEM (Hyclone, SH30003.02) without FBS (Biolegend, 420201) until cells were ready to be seeded. Cells, after resuspending in DMEM without FBS, were added on to the modified surfaces, allowing them to adhere onto the substrates for 2 h at 37°C, and observed with phase-contrast microscopy. After this initial 2 h of adhesion, the medium was changed to FBS-containing DMEM. The substrates were then incubated for an additional 48 h and observed without washing. After analyzing with microscopy, samples were washed with PBS (pH = 7.4, Aldrich) and fixed with 3.7% formaldehyde (Acros) solution for 10 min for staining. DAPI (0.29×10^{-3} M, Aldrich) and Alexa Fluor 546 phalloidin (6.6 μM, Molecular Probes) solution were used to detect the adhesion and morphology of the cells on the substrates. Samples were incubated with DAPI and phalloidin solution

simultaneously at room temperature for 30 min, and then washed with PBS for the removal of extra staining solution. The stained samples were analyzed with fluorescence microscopy (Nikon TE2000-U).

(a)



(b)

Chemical State/ Element	Binding Energy (eV)	Coating **5**
C-C/H	285.0	74.5 (75.0)
C-N	286.3	5.0 (4.2)
N-C=O	288.6	7.7 (8.3)
π→π*	291.4	2.1 (-)
O	527.0	7.6 (8.3)
N	396.0	3.1 (4.2)

Figure S1. (a) XPS high-resolution C_{1s} spectra of for poly[(4-N-maleimidomethyl-*p*-xylylene) -*co*-(*p*-xylylene)] (Coating **5**). (b) A table compares the experimental values of XPS survey and high-resolution spectra with theoretical data. Experimental values are compared with calculated values (in the bracket). Values of 89.3 atom% of carbon, 7.6 atom% of oxygen, and 3.1 atom% of nitrogen were found in close accordance with the theoretical values of 87.5 atom% of carbon, 8.3 atom% of oxygen, and 4.2 atom% of nitrogen, respectively. The signal at 285.0 eV is assigned to aliphatic and aromatic carbons (C–C, C–H), and the intensity of 74.5 atom% compared well with the theoretical concentration of 75.0 atom%. The C–N bond was detected with 5.0 atom%, which compared well with the theoretical value of 4.2 atom%. The peak at 288.6 eV is assigned to the N–C=O group of maleimide (7.7 atom%) and was in good agreement with the theoretical value of 8.3 atom%. A signal at 291.4 eV (2.1 atom%) indicates π→π* transitions, which were characteristic of aromatic polymers and previously reported for similar poly-*p*-xylylenes.[1-3] The monomeric ratio of Coating **5** has m:n = 1:1, as suggested by XPS characterization.

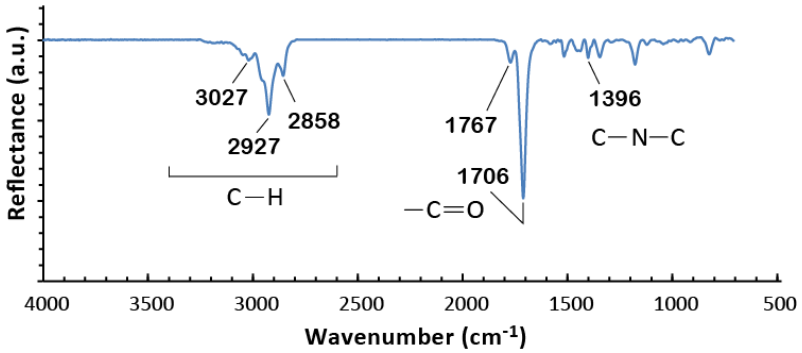


Figure S2. IRRAS characterization of as-deposited poly[(4-N-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)] (Coating **5**) on a gold-coated silicon substrate. Two significant peaks at 1767 and 1706 cm^{-1}, attributed to the asymmetric stretching bands of maleimide –C=O, and a peak at 1396 cm^{-1}, attributed to the symmetric C–N–C stretch of maleimide, were detected. The absorption bands recorded at 3027, 2927, and 2858 cm^{-1} were due to the C–H symmetric and asymmetric stretching bands.

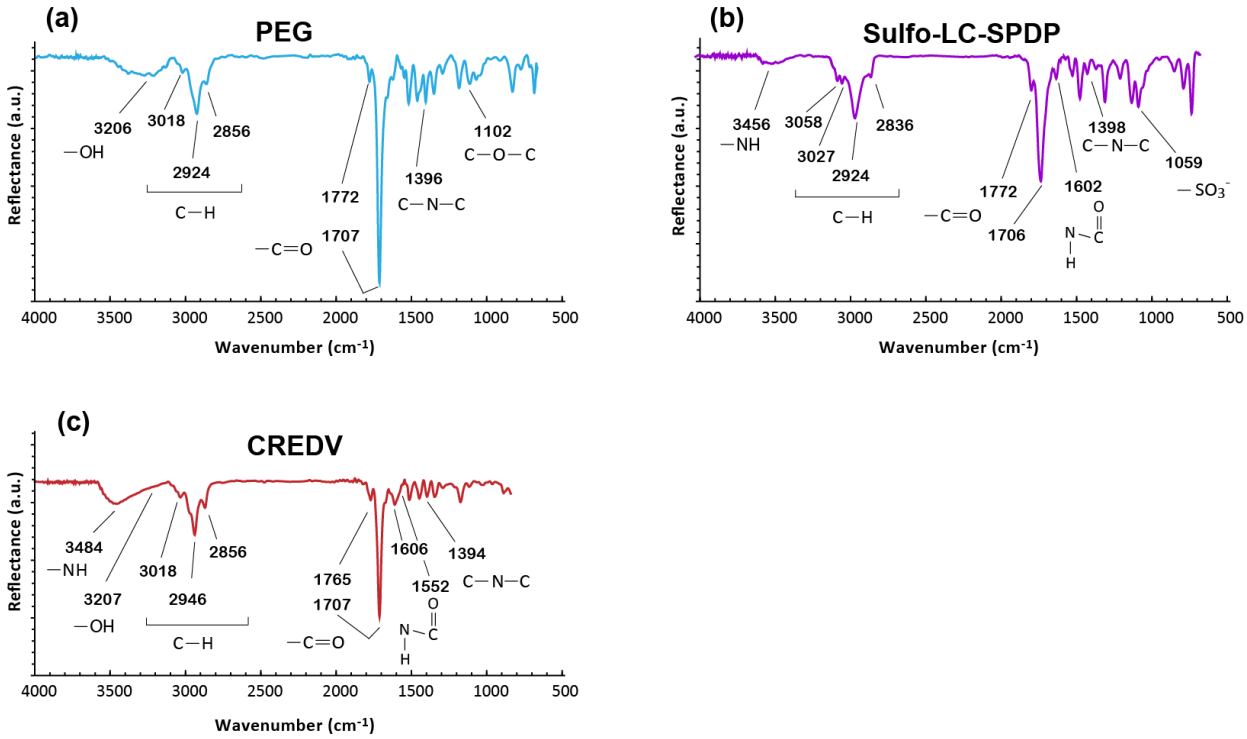


Figure S3. IRRAS characterizations of immobilized (a) thiol-PEG, (b) Sulfo-LC-SPDP, and (c) CREDV peptide, on poly[(4-N-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)] (Coating **5**). Two significant peaks at 1102 cm^{-1} (C–O–C) and 3206 cm^{-1} (–OH), attributed to the characteristic bands of PEG, were detected in (a). Stretching bands of Sulfo-LC-SPDP at 1059 cm^{-1} ($–SO_3^-$), 1602 cm^{-1} (–C(=O)NH–), and 3456 cm^{-1} (–NH), were detected in (b). Characteristic peaks at 1552 cm^{-1} and 1606 cm^{-1} (–C(=O)NH–), 3207 cm^{-1} (–OH), and 3484 cm^{-1} (–NH), attributed to the characteristic absorption bands of CREDV peptide, were detected in (c). All immobilizations were performed by μCP using a flat PDMS stamp at a temperature of 25°C and a humidity of 55%.

Reference

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2. H. Nandivada, H.-Y. Chen and J. Lahann, *Macromolecular Rapid Communications*, 2005, **26**, 1794-1799.
3. H. Nandivada, H.-Y. Chen, L. Bondarenko and J. Lahann, *Angewandte Chemie International Edition*, 2006, **45**, 3360-3363.