Electronic Supplementary Information

Vapor-Based Tri-Functional Coatings

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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), dichloromethyl methyl ether (97%, TGI), anhydrous $MgSO_4$ (99.5%, J.T. Baker), $NaBH_4$ (Acros, 98%,), triphenylphosphine (98.5%, Fluka), DIAD (94%, Alfa Aesar), maleimide (99%, Sigma-Aldrich), Trifluoroacetic anhydride (99%, Acros), bromomethyl triphenylphosphonium bromide (98%, Acros), potassium tert-butoxide (98%, Acros), aluminum chloride (Alfa Aesar), benzoylchloride (99%, Alfa Aesar), fluorescein-conjugated cysteine (95%, Kelowna International Scientific Inc., Taiwan), Alexa Fluor 350-conjugated hydrazide (Molecular Probes), Alexa Fluor 555-conjugated azide (Molecular Probes), phosphate-buffered saline (PBS, pH 7.4) (Sigma-Aldrich), silicon wafer (Goldeninent Inc., Taiwan). Gold substrates were fabricated on a 4-in silicon wafer using a thermal evaporator (Kao Duen Technology Co., Taiwan) to deposit a titanium layer of 100 Å as an adhesion layer followed by the formation of a gold layer of 700 Å. A self-expanding stent (Abbott, RX Acculink Carotid Stent System, Ni−Ti) was used as the non-conventional substrate for CVD coating in the study. The stent has the dimension of a 7 mm I.D. on one end, a 5 mm I.D. on the other end, and a length of 40 mm. The stent substrate was cleaned using ethanol and deionized water before CVD deposition.

CVD copolymerization:

Poly[(4-ethynyl-*p*-xylylene)-*co*-(4-N-maleimidomethyl-*p*-xylylene)-*co*-(trifluoroacetyl-*p*-xylylene)-*co*-(*p*-xylylene)] (Coating 1) was prepared from 4-ethynyl-[2,2]paracyclophane, 4-N-maleimidomethyl-[2,2]paracyclophane, and trifluoroacetyl-[2.2]paracyclophane via CVD copolymerization using a home-built three-sourced CVD system. The starting materials, 4-ethynyl-[2,2]paracyclophane, 4-N-maleimidomethyl-[2,2]paracyclophane, and trifluoroacetyl-[2.2]paracyclophane, were sublimated at approximately 100 ºC, 115 ºC, and 80 ºC, respectively, in each source, and a 1:1:1 molar feed ratio of these three starting materials were controlled during the CVD copolymerization process. Distinguished pyrolysis temperatures were maintained at 650 ºC, 580 ºC, and 670 ºC, respectively, at the pyrolysis zone in each source. Finally, Coating 1 was spontaneously polymerized while rotating and cooled (15ºC) on a sample holder in the deposition chamber. The system pressure was maintained at 100 mTorr during the entire CVD copolymerization process. Deposition was controlled at a rate of ~0.3 Å/s as estimated in situ based on a quartz crystal microbalance (Sycon Instruments, USA). Mono-functional coatings of poly[(4-ethynyl-*p*-xylylene)-*co*-(*p*-xylylene)], poly[(4-N-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)], poly[(trifluoroacetyl-*p*-xylylene)-*co*-(*p*-xylylene)], and poly(4-benzoyl-*p*-xylylene-*co*-*p*-xylylene) were prepared via CVD polymerization following previous reported processes.[1-4] Silicon, gold-coated silicon (Au/Si), and stents were used as substrates to prepare these coatings.

Surface characterization:

Infrared reflection absorption spectroscopy (IRRAS) spectra were recorded using a NEXUS 470 FT-IR spectrometer (Thermo Nicolet, USA) equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector, and the spectra were corrected for any residual baseline drift. During IRRAS characterization, all samples were mounted in a nitrogen-purged chamber. X-ray photoelectron spectroscopy (XPS) data were recorded on a Theta Probe X-ray photoelectron spectrometer (Thermal Scientific, UK). During data acquisitions, X-ray power was adjusted to 150 kW, and pass energy was set to 200.0 eV for survey spectra and 20.0 eV for C_{1s}high-resolution elemental analysis.

Immobilization of biomolecules:

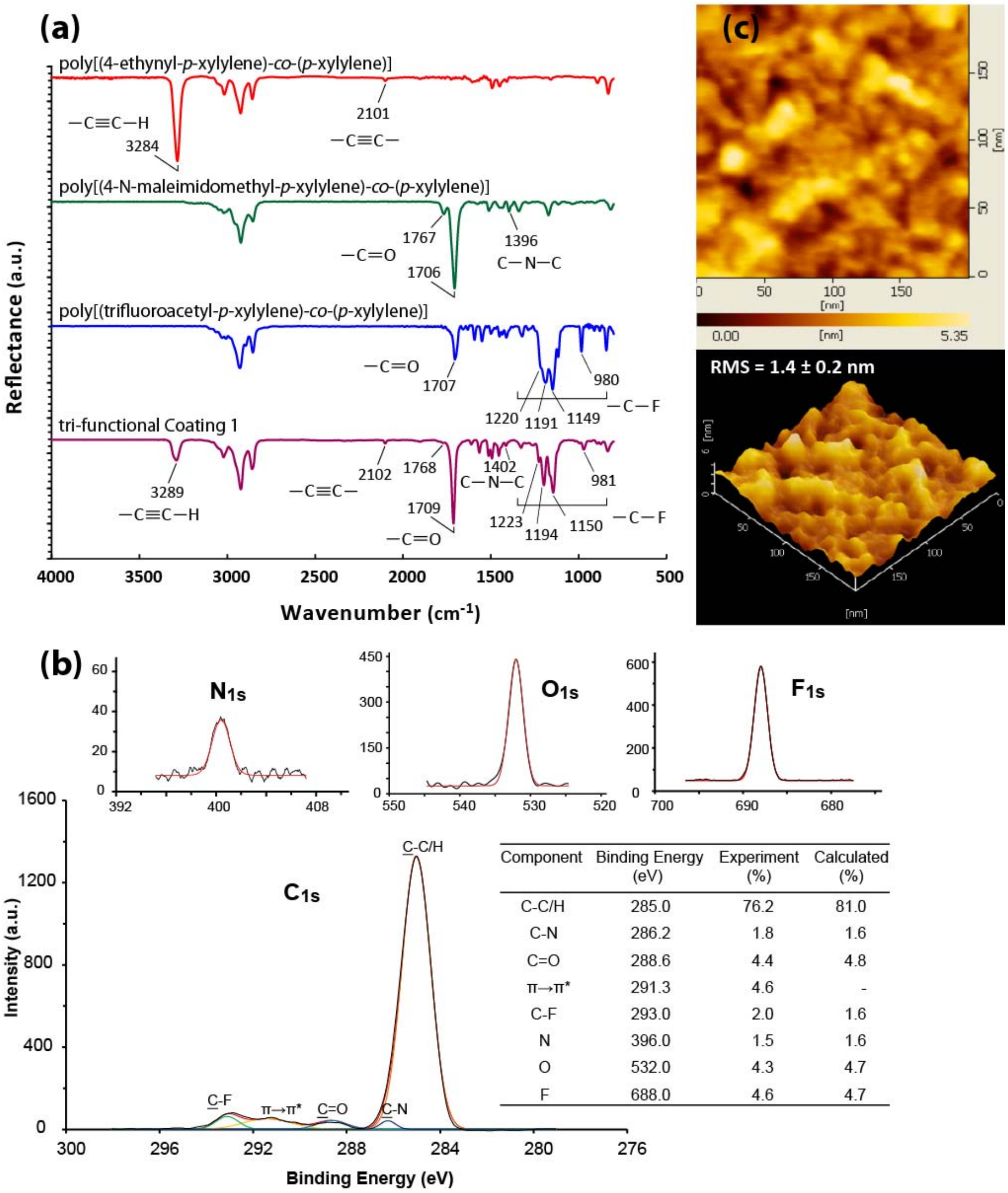
Immobilizations of biomolecules on flat substrates were performed by using μCP processes to confine the reactions at selected areas. All reactions were carried out at room temperature (25 °C) and at a humidity of 55%. Before conducting the printing, PDMS stamps were treated with 10 W oxygen plasma for 2 min to render the surface hydrophilic. The immobilization of Alexa Fluor 555-conjugated azide (Molecular Probes) was conducted by preparing an Alexa Fluor 555-conjugated azide (5 mM) inking solution in deionized water containing sodium ascorbate (20 mM) and $CuSO_4$ (10 mM). A stamp having features of square patterns, with a side length of 50 μm and a center–center spacing of 100 μm, was inked with the azide solution, and then was printed on Coating 1 for 2 h. The immobilization of fluorescein-conjugated cysteine (95%, Kelowna International Scientific Inc., Taiwan) was conducted by preparing the fluorescein-conjugated cysteine inking solution in deionized water (5 mM). A stamp having features of square patterns, with a side length of 300 μm and a center–center spacing of 600 μm, was inked with the cysteine solution, and then was printed on Coating 1 for 2 h. The immobilization of the Alexa Fluor 350-conjugated hydrazide (Molecular Probes) was conducted by preparing Alexa Fluor 350-conjugated hydrazide (5 mM) inking solution in deionized water at a pH of 5–6. A stamp having features of square patterns, with a side length of 300 μm and a center–center spacing of 600 μm, was inked with hydrazide solution, and then was printed on Coating 1 for 1 h. A washing procedure was performed between each μCP process using phosphate-buffered saline (PBS, pH = 7.4; containing Tween 20, Sigma-Aldrich) three times, PBS (pH=7.4, Sigma-Aldrich) one time, and finally, rinsed with deionized water. Immobilizations of biomolecules on stent substrates were performed by immersing the substrates subsequently in separate reagent solutions of Alexa Fluor 555-conjugated azide (5 mM, containing sodium ascorbate [20 mM] and $CuSO_4$ [10 mM]), fluorescein-conjugated cysteine (5 mM), and Alexa Fluor 350-conjugated hydrazide (5 mM, pH=5–6) for 6 h, 6 h, and 3 h, respectively. Between each immersing procedure, a washing procedure was performed three times using PBS containing Tween 20, one time with PBS (pH=7.4, Sigma-Aldrich), and finally, rinsed with deionized water. The resulting samples were then examined by fluorescence microscopy (Nikon TE-2000U).

Photolithography and microstructuring process:

A 70-nm-thick photoresist of poly(4-benzoyl-*p*-xylylene-*co*-*p*-xylylene)[5] was deposited on the stent substrates via CVD polymerization process. The microstructuring process on photoresist-coated stent substrates was performed using a Nikon TE-2000U microscope with a 10X N.A. 0.3 lens. A plastic mask (TKK, Taiwan) with a pre-defined pattern was placed on the field-stop plane of the microscope for projection photolithography. A 100-W HBO mercury lamp was used to serve as the UV source to initiate the reaction. The exposure time was 5-10 min, controlled by a VS25 shutter system (Uniblitz) and driven by a VMM-T1 shutter driver. A 90-nm-thick layer of tri-functional Coating 1 was then deposited on the stent sample via CVD copolymerization process. Finally, the resulting stent sample was developed in an agitated acetone bath for 10 min to remove non-crosslinked poly(4-benzoyl-*p*-xylylene-*co*-*p*-xylylene), and to render the desired microstructures.

XPS ANALYSIS:

Values of 89.0 atom% of carbon, 1.6 atom% of nitrogen, 4.7 atom% of oxygen, and 4.7 atom% of fluorine were found, which are in close accordance with the theoretical values: 89.0 atom% of carbon, 1.5 atom% of nitrogen, 4.3 atom% of oxygen, and 4.6 atom% of fluorine. The high-resolution C_{1s} spectrum of Coating1 offers further information on the copolymer composition. The signal at 285.0 eV could be assigned to aliphatic and aromatic carbons ($\underline{C}$–C, $\underline{C}$–H), and the intensity of 76.2 atom% compared well with the theoretical concentration of 81.0 atom%. A significant $\underline{C}$–N bond was detected (1.8 atom%), which compared well with the theoretical value of 1.6 atom%. The peak at 288.6eV could be assigned to the $\underline{C}$=O group (4.4 atom%) and was in agreement with the theoretical value of 4.8 atom%. A signal at 291.3eV (4.6 atom%) indicates $\pi\rightarrow\pi^*$ transitions, which are characteristic of aromatic polymers and previously reported for similar poly-*p*-xylylenes.[1, 6] A significant $\underline{C}$–F bond was detected (2.0 atom%), which compared well with the theoretical value of 1.6 atom%.



Component	Binding Energy (eV)	Experiment (%)	Calculated (%)
C-C/H	285.0	76.2	81.0
C-N	286.2	1.8	1.6
C=O	288.6	4.4	4.8
π→π*	291.3	4.6	-
C-F	293.0	2.0	1.6
N	396.0	1.5	1.6
O	532.0	4.3	4.7
F	688.0	4.6	4.7

Figure S1. (a) IRRAS spectra compare tri-functional Coating 1 with mono-substituted poly[(4-ethynyl-*p*-xylylene)-*co*-(*p*-xylylene)], poly[(4-N-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)], and poly[(trifluoroacetyl-*p*-xylylene)-*co*-(*p*-xylylene)]. (b) High-resolution XPS spectra of C_{1s}, N_{1s}, O_{1s}, and F_{1s} recorded for Coating 1. Experimental values are compared with calculated values based on an equimolar distribution of starting materials. (c) Surface roughness analysis by using SPM of Coating 1 with a surface roughness of RMS = 1.4 ± 0.2 nm.

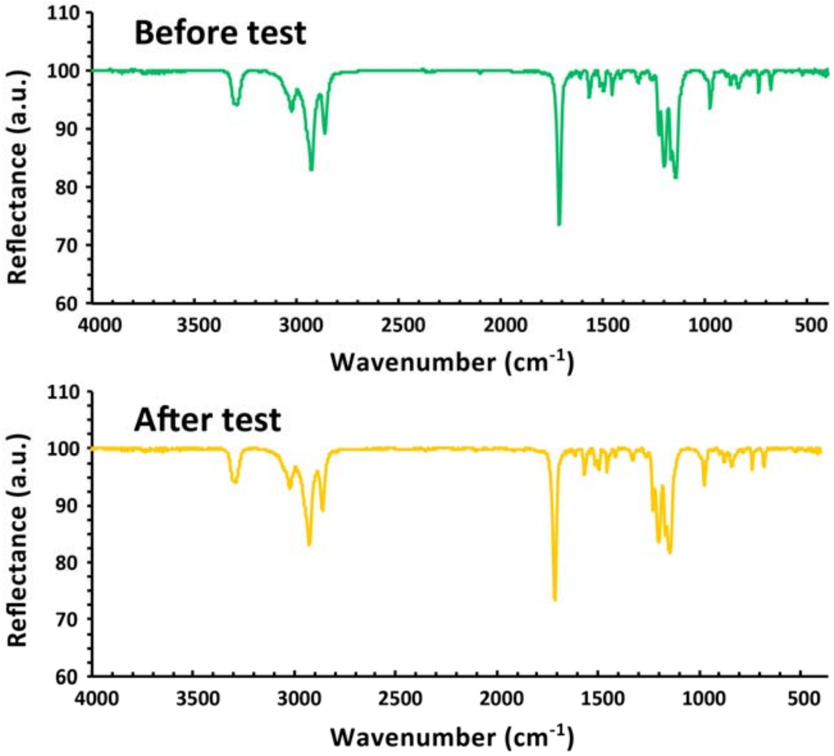


Figure S2. Testing the adhesion of Coating 1. A piece of Scotch tape was pressed onto the Coating 1, and the surface was observed before and after peeling off the tape. (a) IRRAS spectra of the surface before adhesion testing. (b) IRRAS spectra after testing. The result shows that the intact spectrum was preserved.

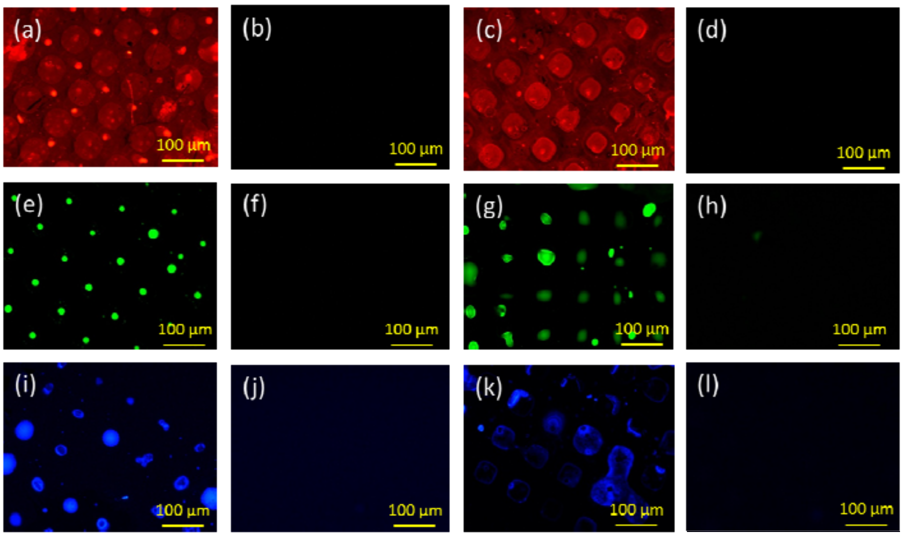


Figure S3. Control experiments to verify absence of cross-reactions. (a) Fluorescence micrograph showing adsorbed Alexa Fluor-555 azides on mono-functional coating of poly[(4-N-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)] using a µCP process for 2 h at 25 °C and at a humidity of 55%. Image was recorded before washing. (b) Fluorescent micrograph of the same sample from (a) after washing. (c) Fluorescence micrograph showing adsorbed Alexa Fluor-555 azides on mono-functional coating of poly[(trifluoroacetyl-*p*-xylylene)-*co*-(*p*-xylylene)] using a µCP process for 2 h at 25 °C and at a humidity of 55%. Image was recorded before washing. (d) Fluorescent micrograph of the same sample from (c) after washing. (e) Fluorescence micrograph showing adsorbed fluorescein-labeled cysteine on mono-functional coating of poly[(4-ethynyl-*p*-xylylene)-*co*-(*p*-xylylene)] using a µCP process for 2 h at 25 °C and at a humidity of 55%. Image was recorded before washing. (f) Fluorescent micrograph of the same sample from (e) after washing. (g) Fluorescence micrograph showing adsorbed fluorescein-labeled cysteine on mono-functional coating of poly[(trifluoroacetyl-*p*-xylylene)-*co*-(*p*-xylylene)] using a µCP process for 2 h at 25 °C and at a humidity of 55%. Image was recorded before washing. (h) Fluorescent micrograph of the same sample from (g) after washing. (i) Fluorescence micrograph showing adsorbed Alexa Fluor-350hydrazides on mono-functional coating of poly[(4-ethynyl-*p*-xylylene)-*co*-(*p*-xylylene)] using a µCP process for 1 h at 25 °C and at a humidity of 55%. Image was recorded before washing. (j) Fluorescent micrograph of the same sample from (i) after washing. (k) Fluorescence micrograph showing adsorbed Alexa Fluor-350hydrazides on mono-functional coating of poly[(4-N-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)] using a µCP process for 1 h at 25 °C and at a humidity of 55%. Image was recorded before washing. (l) Fluorescent micrograph of the same sample from (k) after washing. The washing process was performed three times using PBS containing Tween 20, one time with PBS, and finally, with deionized water.

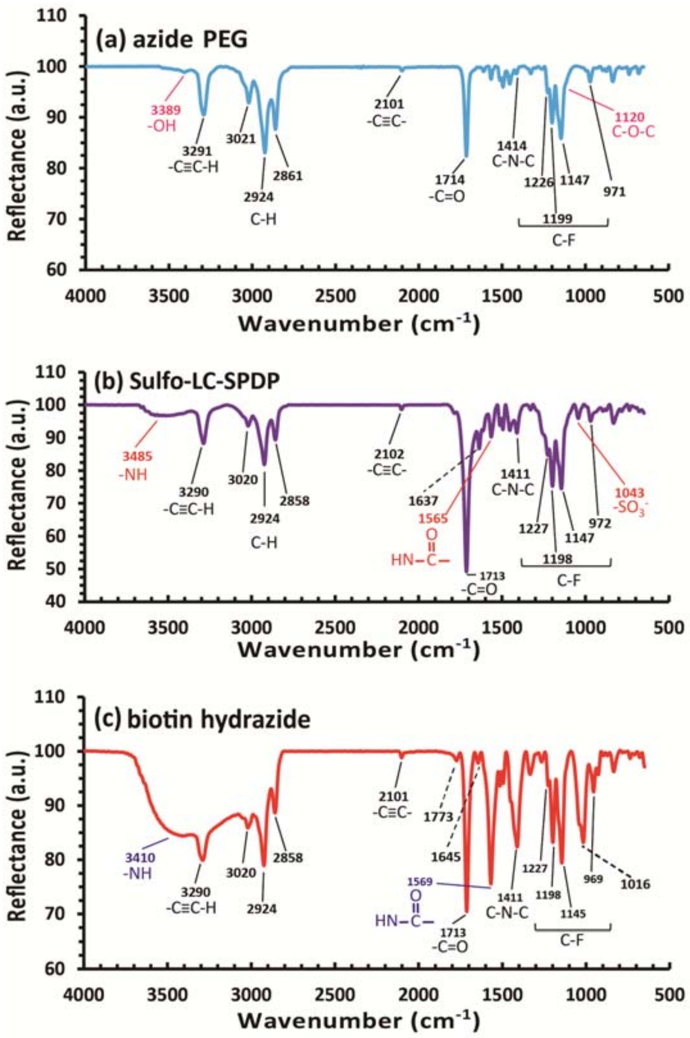


Figure S4. IRRAS spectra of immobilized (a) azide-PEG, (b) Sulfo-LC-SPDP, (c) biotin hydrazides, on Coating 1. Characteristic peaks at 1120 cm^{-1} (C–O–C) and 3389 cm^{-1} (–OH), associated with the characteristic bands of PEG, were detected in (a). Stretching bands of Sulfo-LC-SPDP at 1043 cm^{-1} (–SO_3^-), 1565 cm^{-1} (–C(=O)NH–), and 3485 cm^{-1}(–NH), were detected in (b). Two significant peaks at 1569 cm^{-1}(–C(=O)NH–) and 3410 cm^{-1}(–NH), attributable to biotin hydrazides, were detected in (c).

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