Electronic Supplementary Information

Tunable Coverage of Immobilized Biomolecules for Biofunctional Interface Design

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Experimental Details

Surface modification

To prepare the density-varied poly(4-carboxylic acid pentafluorophenol ester-*p*-xylyene-*co*-*p*-xylylene) coating (PFP ester coating), a dual-sourced CVD system was utilized during the copolymerization process, in which each source contained independent sublimation and pyrolysis zones, and both sources were connected to the deposition chamber. For the CVD copolymerization process, the starting materials (dimers), 4-carboxylic acid pentafluorophenol ester-[2.2]paracyclophane was synthesized following previous report,[1] and [2.2]paracyclophane was obtained commercially from Sigma Aldrich (USA). The dimers with molar ratios of 1:1, 1:20, and 1:80 in each source and were simultaneously transferred to the pyrolysis zone maintained at temperatures of 600 °C and 670 °C, respectively. The pyrolytically formed monomers were transferred into the chamber and then copolymerized onto a rotating holder. Throughout the CVD process, a deposition rate of approximately 0.5 A^{-1} and a reduced pressure of 100 mTorr were maintained to optimize the polymerization, and a cooled holder maintained at 15 °C was applied to ensure the formation of uniform coatings.

Immobilizations

Cyanine 3-labeled aptamer (GGTTGGTGTGGTTGG) (Cy3-aptamer) (Protech Technology, Taiwan), cyanine 5-labeled bovine serum albumin (Cy5-BSA) (Yao-Hong Biotechnology Inc., Taiwan), O-(2-aminoethyl)polyethylene glycol (amine-PEG) (M.W. 5000, Sigma Aldrich, USA) and fibronectin from human plasma (Sigma Aldrich, USA) were obtained commercially. Cy3-aptamer and Cy5-BSA were used to react with substrates deposited with a PFP ester coating via amine-PFP ester conjugation at room temperature for 24 hours. After rinsing with deionized water three times, the samples were analyzed with a microarray scanner (GeneTool, USA). Similarly, conjugation of amine-terminated PEGs at a concentration of 300 $mg \cdot mL^{-1}$ and fibronectin was performed on gold quartz crystal microbalance (QCM) chips and cell culture plates that were previously deposited with PFP ester coatings at room temperature for 24 hours, followed by washing with deionized water three times to remove unbound reagents. The samples were then used for QCM analysis and cell culture study.

Surface characterization

Infrared reflection absorption spectroscopy (IRRAS) spectra were recorded by utilizing a spectrum 100 FT-IR spectrometer (PerkinElmer, USA) equipped with an advanced grazing angle specular reflectance accessory (AGA, PIKE Technologies, USA) and a liquid nitrogen-cooled MCT detector; the recorded spectra were corrected for any residual baseline drift. The samples were mounted in a chamber with purged nitrogen to eliminate noise due to CO_2 and H_2O. X-ray photoelectron spectroscopy (XPS) data were recorded using a Theta Probe X-ray photoelectron spectrometer (Thermal Scientific, UK) with monochromatized AlKα as the X-ray source at an X-ray power of 150 kW. Pass energies of 200.0 eV and 20.0 eV were used for the survey scan and the high-resolution C_{1s} elemental scan, respectively. The XPS atomic analysis results were reported as atomic concentrations (%) and were compared with the theoretical values calculated based on structure. For the QCM analysis, a QCM instrument (ANT Technologies, Taiwan) equipped with a flow injection analysis (FIA) device and continuous frequency variation recording device was also used for characterization. The sensing element of the instrument was an AT-cut piezoelectric quartz disc with a 9-MHz resonant frequency and a 0.1-cm^2 total sensing area. Deposition of the density-varied PFP ester coating was performed on the crystals of the QCM system, and subsequently, the amine-PEGs were allowed to immobilize onto such modified crystals. The resulting crystal samples were used in the QCM system to examine the substrates' antifouling properties. PBS (pH = 7.4) was used as a carrier solution for the QCM system and was continuously delivered into the flow channel until a stable frequency response was obtained. A 10% fetal bovine serum (FBS) (Biological Industries, Israel) solution was then injected into the flow system, and the time-dependent change in frequency was continuously monitored with the flow rate maintained 35.0 $\mu g \cdot mL^{-1}$ while detecting FBS adsorption.

Cell culture and osteoinduction analysis

Mouse embryonic fibroblast (3T3-L1) cells were obtained commercially (ATCC, USA). The cells were seeded at a density of 1.5 × 104 cells·cm-2 on modified cell culture plates (48-well, Corning, USA), where amine-PEG or fibronectin were immobilized in pure tissue culture polystyrene (TCPS) plates (48-well, Corning, USA), which served as control surfaces. 3T3-L1 cells were then cultured in Dulbecco's modified Eagle's medium (Biological

Industries, Israel) containing 10% FBS and 1% P/S/A (Biological Industries, Israel), and the culturing conditions were maintained at 37 °C with 5% CO2 and 100% humidity. After 24 hours of incubation, the cells were fixed with 10% formalin (Macron Fine Chemicals, USA) for 30 minutes and then stained with 1 μg·mL-1 4',6-diamidino-2-phenylindole (Life technologies, USA) for 5 minutes. The resulting samples were observed and photographed using an inverted microscope (Olympus, Japan). The number of adhered cells on the different surfaces was determined by counting the fluorescent nuclei in the images using software (Image J, USA) analysis according to the reported procedures.[2] All experiments were conducted in quintuplicate.

Table S1. Calculated/characterized concentrations of PFP ester groups for the density-varied PFP ester coatings.

Molar ratio of PEP ester	Concentrations of PEP ester groups [no.·cm^{-2}]		
	ChemBio3D Ultra	UV absorption	QCM Analysis
1:0	1.76×10^{14}	$2.51 \pm 0.11 \times 10^{15}$	$9.99 \pm 0.92 \times 10^{14}$
1:1	8.82×10^{13}	$1.26 \pm 0.20 \times 10^{15}$	$5.00 \pm 0.78 \times 10^{14}$
1:20	8.40×10^{12}	$1.20 \pm 0.09 \times 10^{15}$	$4.76 \pm 0.62 \times 10^{13}$
1:80	2.18×10^{12}	$3.10 \pm 0.46 \times 10^{13}$	$1.23 \pm 0.25 \times 10^{13}$
0:1	0	0	0

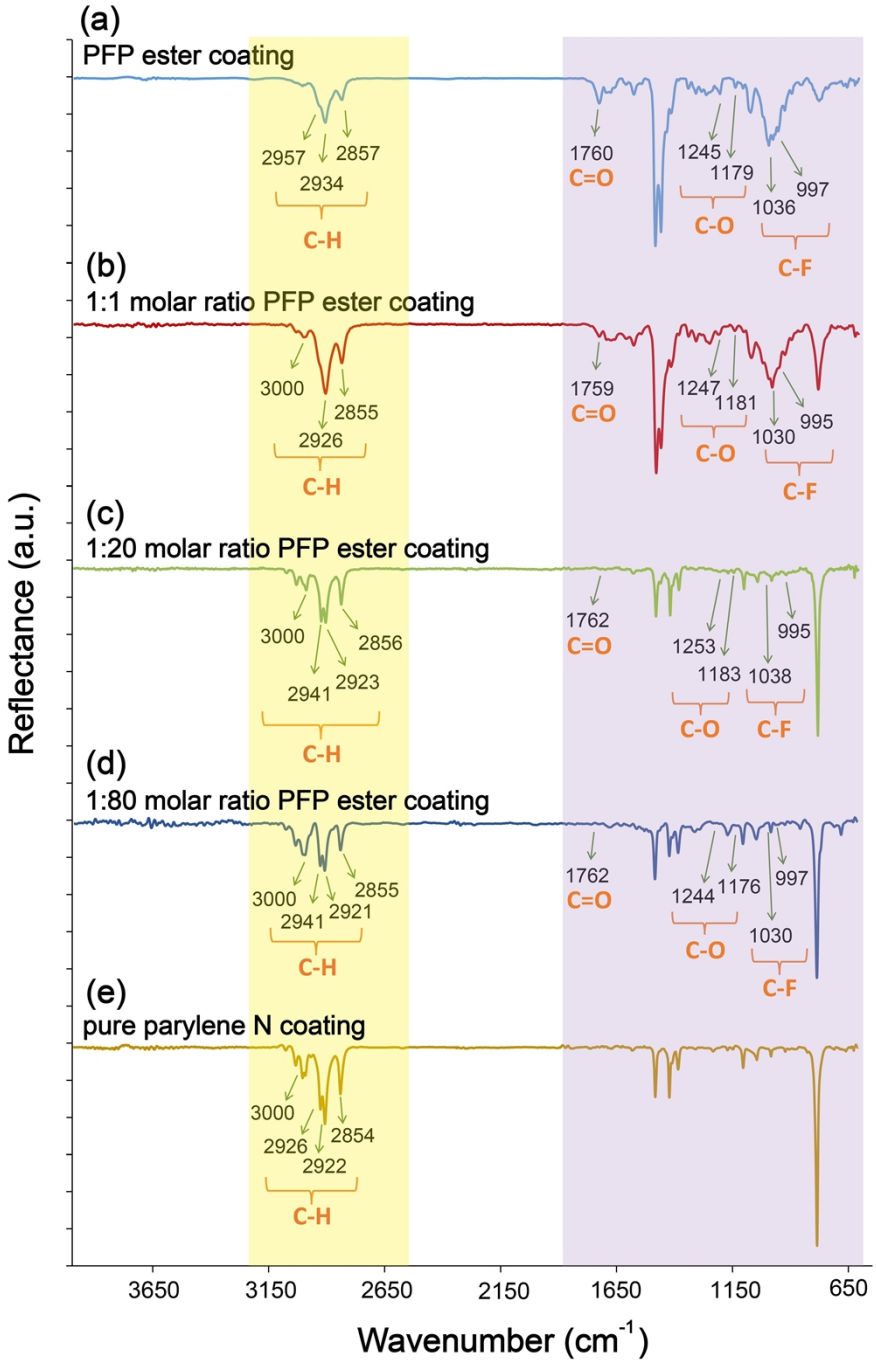

Figure S1. IRRAS characterization of the density-varied PFP ester coatings. The feeding ratio of PFP ester-[2.2]paracyclophane and nonsubstituted-[2.2]paracyclophane (1:0, 1:1, 1:20, 1:80, 0:1) copolymerized during the CVD process was varied to produce PFP ester coatings composed of the corresponding molar ratio of PFP ester motif: a) pure PFP ester coating (1:0); b) 1:1 molar ratio PFP ester coating; c) 1:20 molar ratio PFP-ester coating; d) 1:80 molar ratio PFP ester coating; e) pure parylene N coating (0:1). Peaks at approximately 1760 cm^{-1}, which are attributed to the PFP ester C=O bond; peaks between 1253 and 1176 cm^{-1}, indicative of C–O stretching of the PFP ester; and peaks between 1038 and 995 cm^{-1}, resulting from the C–F bond of the PFP ester, were detected for all of the density-varied PFP ester coatings, and the reflectance decreased as the density of PFP ester decreased. The absorption bands observed between 3000 and 2850 cm^{-1} in all the groups represent symmetric and asymmetric C–H stretching bands.

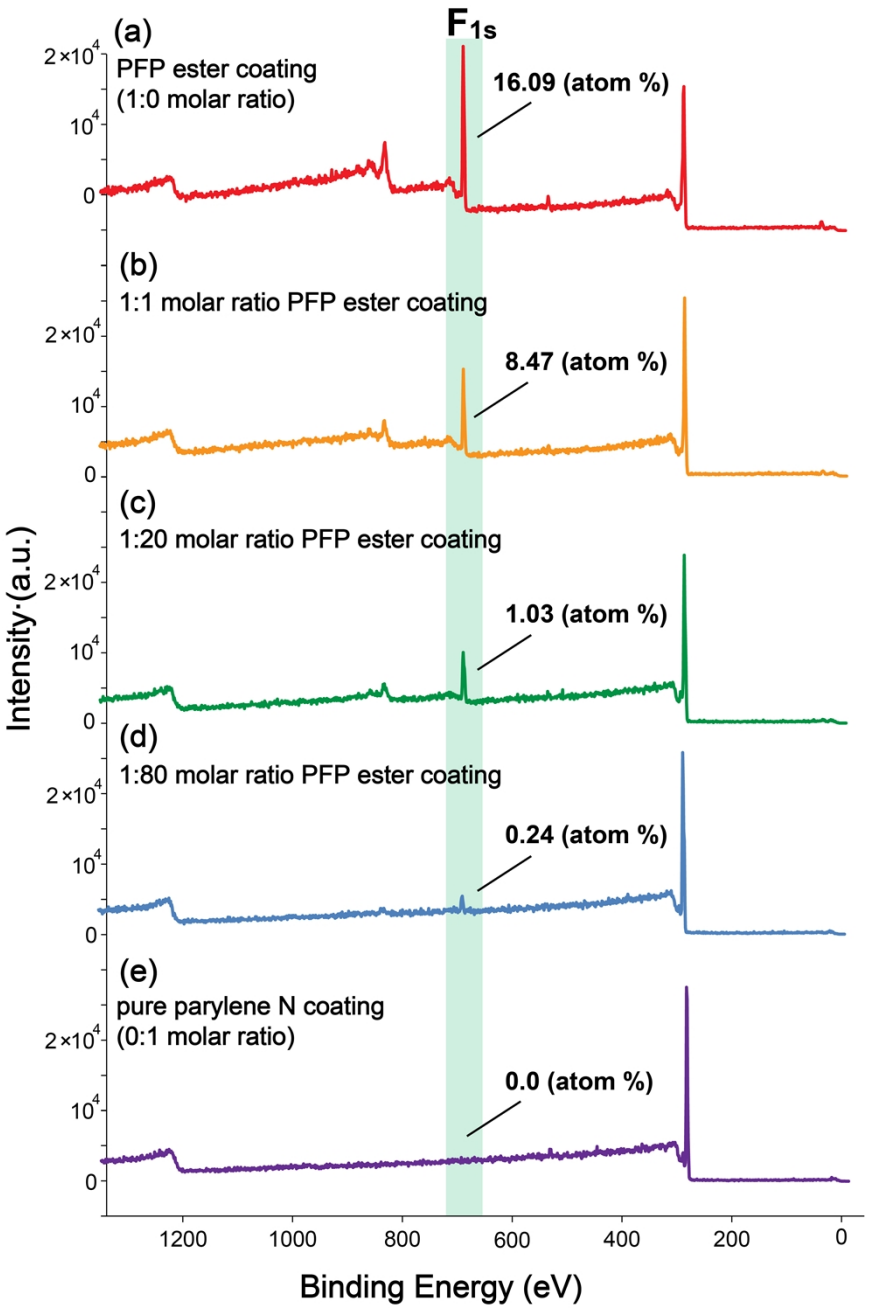

Figure S2. XPS survey spectra were obtained for PFP ester coatings with various molar ratios of PFP ester groups (1:0, 1:1, 1:20, 1:80, 0:1, with respect to a nonsubstituted unit). The peak detected at approximately 690 eV is assigned to the fluorine element of PFP ester, and a decreased dependence of intensity of the fluorine peaks and the fluorine atom% (a) 16.09 %, b) 8.47 %, c) 1.03 %, d) 0.24 %, and e) 0.0 %) is observed with decreasing molar ratio.

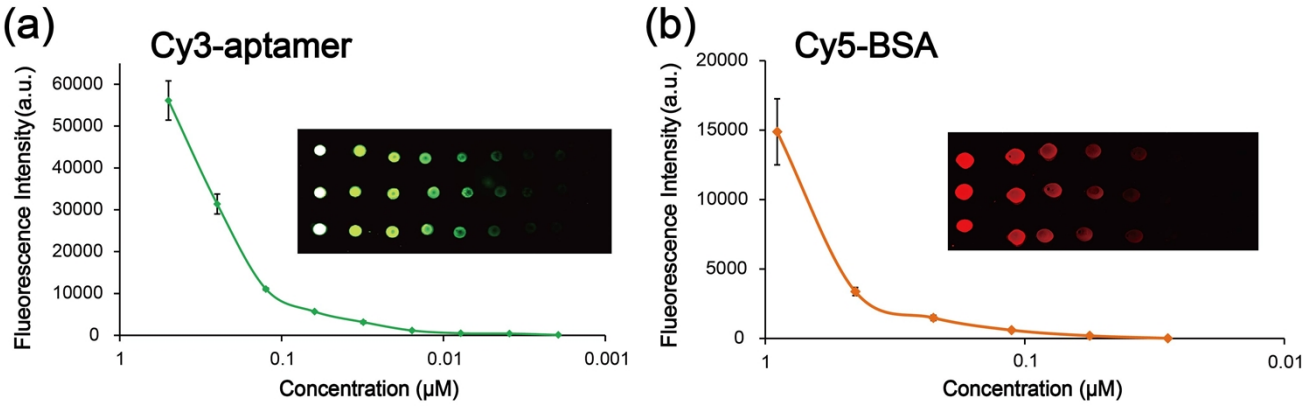

Figure S3. Calibration curves of the fluorescence-labeled molecules, including a) Cy3-aptamer and b) Cy5-BSA. The fluorescence signals were recorded using a fluorescence scanner. Each experiment was performed in triplicate.

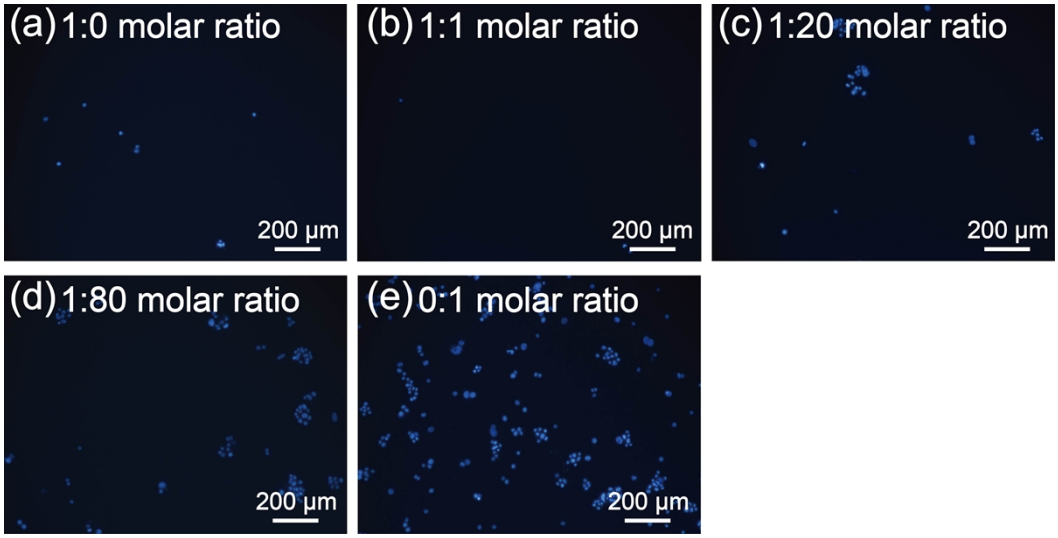

Figure S4. Fluorescence micrographs of nuclei-stained 3T3-L1 cells attached to the modified surfaces with various ratio of PEG immobilizations: (a) pure PFP ester coating/PEG, (b) 1:1 molar ratio PFP ester coating/PEG, (c) 1:20 molar ratio PFP ester coating/PEG, (d) 1:80 molar ratio PFP ester coating/PEG, and (e) pure parylene N coating (no PEG). Each experiment was performed in quintuplicate.

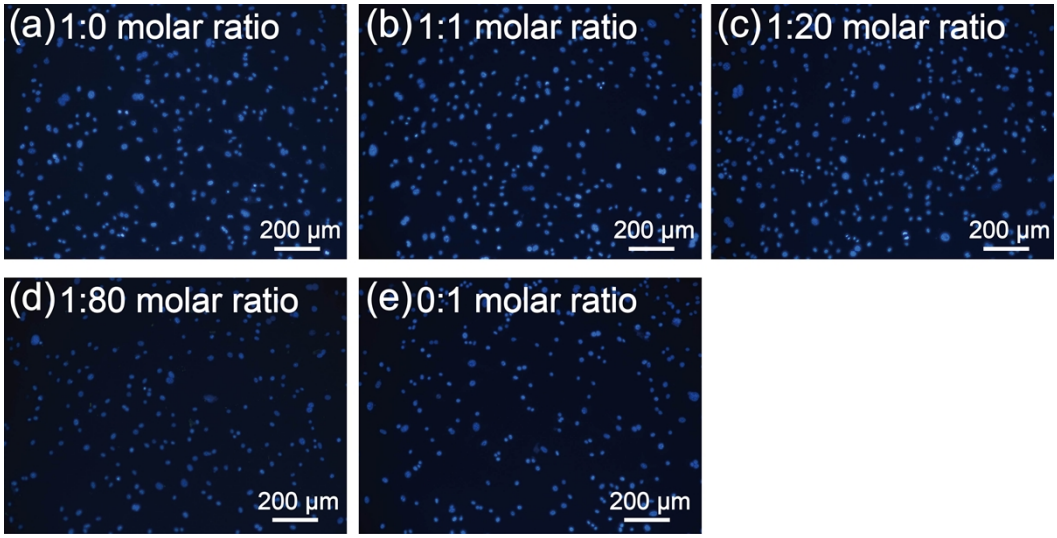

Figure S5. Fluorescence micrographs of nuclei-stained 3T3-L1 cells attached to the modified surfaces with various ratios of fibronectin immobilizations: (a) pure PFP ester coating/fibronectin, (b) 1:1 molar ratio PFP ester coating/fibronectin, (c) 1:20 molar ratio PFP ester coating/fibronectin, (d) 1:80 molar ratio PFP ester coating/fibronectin, and (e) pure parylene N coating (no fibronectin). Each experiment was performed in quintuplicate.

Reference

1 J. Lahann, I. S. Choi, J. Lee, K. F. Jensen and R. Langer, *Angewandte Chemie International Edition*, 2001, **40**, 3166.

2 C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat Meth*, 2012, **9**, 671.