Electronic Supplementary Material (ESI) for Nanoscale.

SUPPORTING INFORMATION

Enzymatic Film Formation of Nature-Derived Phenolic Amines

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CONTENTS

Experimental Section

Materials. Tyrosinase from mushroom (>1000 U mg^{-1}, Sigma), DL-tyrosine (99%, Sigma), 3,4-dihydroxy-L-phenylalanine (L-DOPA, >98%, Sigma), dopamine hydrochloride (Sigma), DL-norepinephrine hydrochloride (>97%, Sigma), tyramine hydrochloride (>98%, Sigma), pyrocatechol (>99%, Sigma), pyrogallol (>98%, Sigma), 3,4-dihydroxybenzaldehyde (Sigma), tannic acid (Sigma), gallic acid (>97.5%), Rosell Park Memorial Institute 1640 medium (RPMI 1640, with L-glutamine and 26 mM HEPES, Welgene), fetal bovine serum (FBS, Welgene), penicillin-streptomycin (P/S, 5,000 U mL^{-1} of penicillin and 5,000 mg/mL of streptomycin, Welgene), LIVE/DEAD® viability/cytotoxicity kit for mammalian cells (Life Technologies), polystyrene microparticles (PS-R-5.0, d=4.97 μm, PS-R-3.0, d=3.03 μm, Microparticles), phosphate-buffered saline (PBS, pH 7.4, Welgene), yeast extract peptone dextrose (YPD) agar (Duchefa Biochemistry), YPD broth (Duchefa Biochemistry), fluorescein diacetate (FDA, Sigma), propidium iodide (PI, Sigma), trypan blue (Sigma), poly-L-lysine-FITC labeled (FITC-PLL, Mw: 30000-70000, Sigma), polyethylenimine branched (PEI, Mw: 25000), tetramethyl orthosilicate (TMOS, Sigma), (3-Mercaptopropyl)trimethoxysilane (MPTMS, Sigma), 2-bromoisobutyryl bromide (Sigma), triethylamine (Junsei), 2-(Dimethylamino)ethyl methacrylate (DMAEMA, sigma), copper(II) bromide ($CuBr_2$, Sigma), 2,2'-bipyridine (bpy, Sigma), and ascorbic acid (Sigma) were used as received. Deionized water (DI water, 18.3 MΩ·cm) from Human Ultrapure System (Human Corporation) was used. Jurkat cells (Jurkat clone E6-1, No. 40152, Korean Cell Line Bank) and *Saccharomyces cerevisiae* (baker's yeast) were used for cell-surface engineering.

Tyrosinase-Catalyzed Film Formation. *(a) Flat substrates and mircoparaticles:* Gold substrates were prepared with thermal evaporation of 5 nm of titanium and 100 nm of gold onto silicon wafers (99.9999%). Prior to use, gold substrates were washed with ethanol and acetone for 15 min each with sonication. Tyrosine was diluted in PBS solution (0.4 mg mL^{-1}), followed by stirring at 50 ºC for complete dissolution and cooling down to room temperature (25 ºC). Washed gold substrates were immersed in 2 mL of the tyrosine solution, to which was added 2 μL of tyrosinase solution (10 kU mL^{-1} in PBS). After predetermined time of incubation (10 min to 6 h), the gold substrates were washed with DI water. The same reaction protocol was applied to other substrates, such as metal and plastic flat substrates and microparticles. The same monomer concentration (0.4 mg mL^{-1}) was used for other phenolic amines. *(b) Living cells.* Jurkat clone was suspended in a T75 cell culture flask with the 15-mL RPMI 1640 medium containing 10% FBS and 1% P/S (the cell culture medium for Jurkat cells), and cultured in a humidified incubator at 37 ºC under 5% CO_2. After reaching 80% confluency, the cells were sub-cultured in another T75 cell culture flask in 1/15 scale. After collecting by centrifugation and washing with RPMI 1640 medium, Jurkat cells were placed in a tyrosine solution (0.4 mg/mL in a cell culture medium for Jurkat cells) with gentle pipetting, followed by addition of the tyrosinase solution (2 μL). After 6 h, the coated jurkat cells were collected and washed with RPMI 1640 medium. For polydopamine coating, a dopamine solution (2 mg mL^{-1}) was prepared with RPMI 1640 medium, and pH was adjusted to 8.5 with 1 M NaOH. A single colony of yeast from YPD agar plate was picked and cultured in YPD broth media with gentle shaking at 33 ºC for 30 h. After washing with PBS, the yeast samples were placed in tyrosine solution (0.4 mg

mL^{-1} in PBS), followed by the addition of the tyrosinase solution (2 μL). After 6 h, the cells were collected and washed with PBS for further characterization.

Post-Functionalization of Films. *(a) Micropattern generation:* Poly-L-lysine (PLL) micropatterns were fabricated by microcontact printing (μCP). Poly(dimethylsiloxane) (PDMS) elastomer stamps with line patterns (width: 50 μm, spacing: 150 μm) were washed with 2-propanol, and treated with oxygen plasma for 15 min. Then, 1 mg mL^{-1} of the FITC-PLL aqueous solution was spread on the stamps for 15 min, and dried with Ar gas. The stamps were placed on eumelanin-coated gold (Au) substrates for 15 min, resulting in the generation of PLL-patterned substrates. *(b) Bioinspired silicification:* PEI was dissolved in the Tris-HCl buffer solution (2 mg mL^{-1}, pH: 8.5), and eumelanin-coated Au substrates were immersed in the PEI solution for 2 h, and washed with DI water. For bioinspired silicification, the PEI-grafted substrate was placed in a 100 mM silicic acid derivative solution, which had been made by hydrolyzing TMOS (1 M) and MPTMS (1 M), respectively, in an aqueous HCl solution (1 mM) at room temperature for 30 min and adding the resulting solutions to a phosphate buffer (50 mM, pH 5.8) with 25:75:900 (v/v/v) ratio. After 30 min, the substrate was washed with DI water and dried with Ar gas. (c) *Surface-initiated polymerization:* 2-Bromoisobutyryl bromide and triethylamine were dissolved in degassed dichloromethane (DCM, 100 mM each). For initiator priming, eumelanin-coated Au substrates were immersed in the initiator solution for 2 h, and washed with DCM. For surface-initiated, atom transfer radical polymerization (SI-ATRP), initiator-primed substrates were immersed in aqueous ATRP solution containing DMAEMA (2 M), $CuBr_2$ (1 mM), bpy (2 mM), and ascorbic acid (10 mM) for 3 h. Polymer-grafted substrates were washed with DI water.

Charaterizations. Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded with a nitrogen-purged Thermo Nicolet Nexus FT-IR spectrophotometer. FT-IR spectra were equalized by adding approximately 4000 scans for background and each sample. The ellipsometric thickness was measured with Gaertner L116s ellipsometer (Gaertner Scientific Corporation). A refractive index of 1.46 was used for all samples. Five different points of each sample were measured, and average values were recorded. Field-emission scanning electron microscopy (FE-SEM) imaging was performed with an FEI Inspect F50 microscope (FEI) with an accelerating voltage of 10 kV, after sputter-coating with platinum. TEM imaging was performed with a LEO 912AB microscope (Carl Zeiss). Samples were fixed with glutaraldehyde and osmium tetroxide, and then dehydrated in ethanol. UV-visible absorption spectra were measured with a UV-2550 spectrophotometer (Shimadzu). Atomic force microscopy (AFM) images were obtained with an INNOVA-LABRAM HR800 (Horiba Jobin & Bruker). The scans were performed in the contact mode using a CONT20A-CP tip (Bruker). Scan rate of 1 Hz and resolution of 256 × 256 were used to obtain the topographical images of 10 μm × 10 μm. Root-mean-square roughness (R_{rms}) was calculated by NanoScope Analysis and AtomicJ software. X-ray photoelectron spectroscopy (XPS) spectra were acquired with a multi-purpose X-ray photoelectron spectroscope (Sigma Probe, Thermo VG Scientific) with Al-Kα (1486.6 eV) X-ray source. The pass energy was 50.0 eV (in wide scan) and 20.0 eV (in individual narrow scan). Contact angle measurements were performed using a Phoenix 300 goniometer (Surface Electro Optics Co.) equipped with a video

camera. The static contact angle of a 3-μL water droplet was measured at four different locations on each sample. Zeta potentials and dynamic light scattering (DLS) particle sizes were measured with Zetasizer Nano ZS (Malvern) and calculated based on the Smoluchowski model. The viability of Jurkat cells was determined with LIVE/DEAD® viability/cytotoxicity kit (calcein acetoxymethyl (calcein AM): 1.6 μM, ethidium homodimer-1: 4 μM in PBS). The cells were incubated with the kit for 20 min, washed with PBS, and characterized by confocal laser-scanning microscopy (CLSM, LSM 700 Confocal Microscope, Carl Zeiss). The viability of yeast cells was investigated with FDA and PI; 5 μL of the FDA solution (10 mg mL^{-1} in acetone) and 2 μL of aqueous PI solution (1 mg mL^{-1}) were added to 1 mL of yeast sample, incubated for 20 min, washed with PBS, and characterized by CLSM.

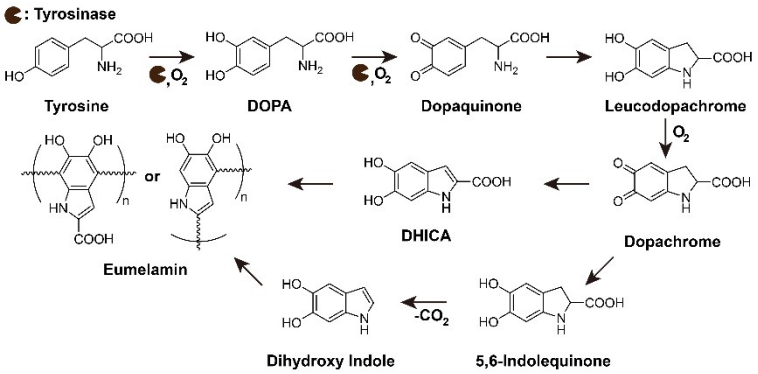

Fig. S1 Reaction pathway of Raper-Mason melanogenesis.

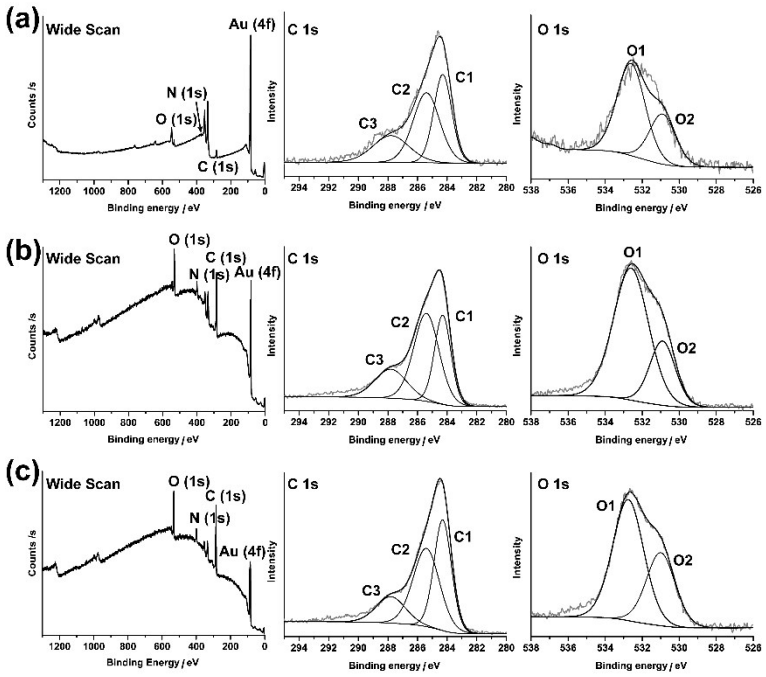

Fig. S2 XPS analysis of gold substrates after coating with different reaction times: (left) survey scans, (middle) high-resolution atomic scans of carbon, and (right) high-resolution atomic scans of oxygen. (a) 1 h, (b) 3 h, and (c) 6 h. C1: C-C and C-H; C2: C-O and C-N; C3: C=O and C=N; O1: O-H; O2: C=O.

Coating Time	1h	3h	6h
Au (substrate)	36.17	4.69	2.37
C	47.45	65.42	67.72
O	12.72	17.76	18.27
N	3.66	12.14	11.28
sum	100	100.01	99.64
C:O ratio	**3.730**	**3.684**	**3.707**

Fig. S3 XPS atomic ratio of films with different coating times.

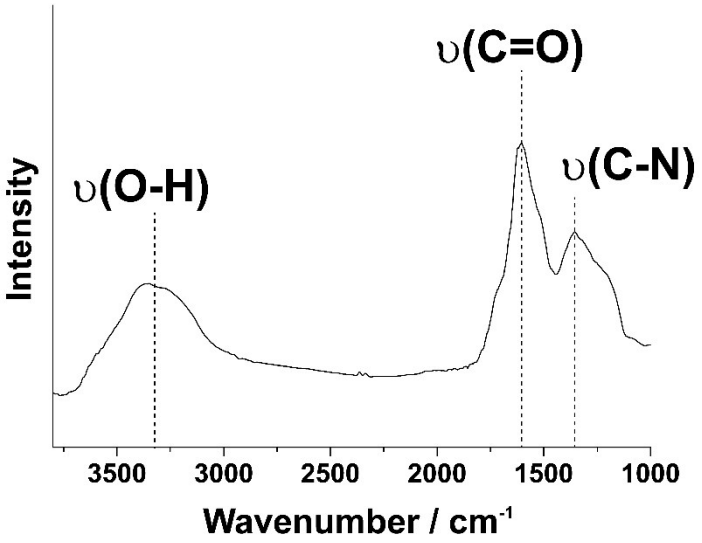

Fig. S4 FT-IR spectrum of a gold substrate after 6 h of enzymatic coating.

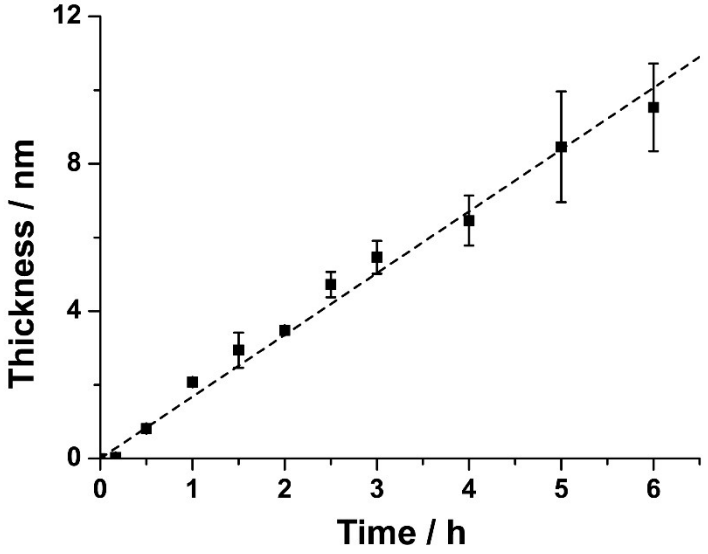

Fig. S5 Ellipsometric thickness after polydopamine deposition in Tris buffer (pH 8.5).

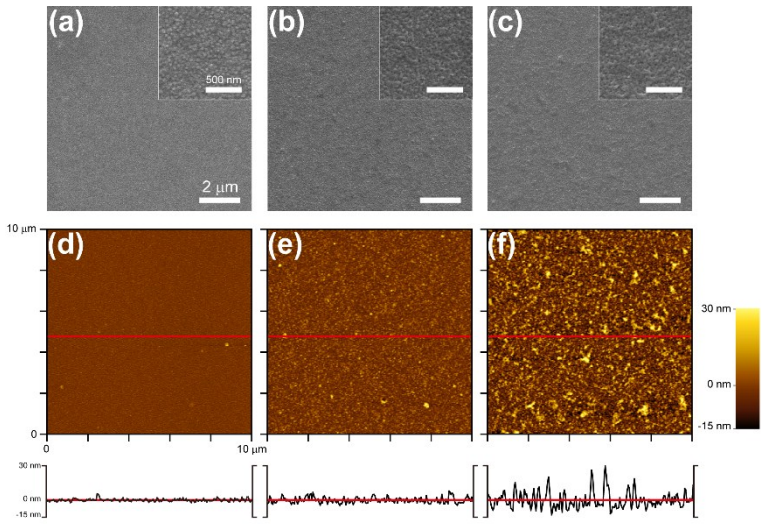

Fig. S6 (a-c) SEM and (d-f) AFM images of gold substrates after enzymatic coating of (left) 1 h, (middle) 3 h, and (right) 6 h. Root-mean-square roughness (R_{rms}): 1.44 nm (after 1 h of reaction), 3.79 nm (after 3 h of reaction), and 8.16 nm (after 6 h of reaction).

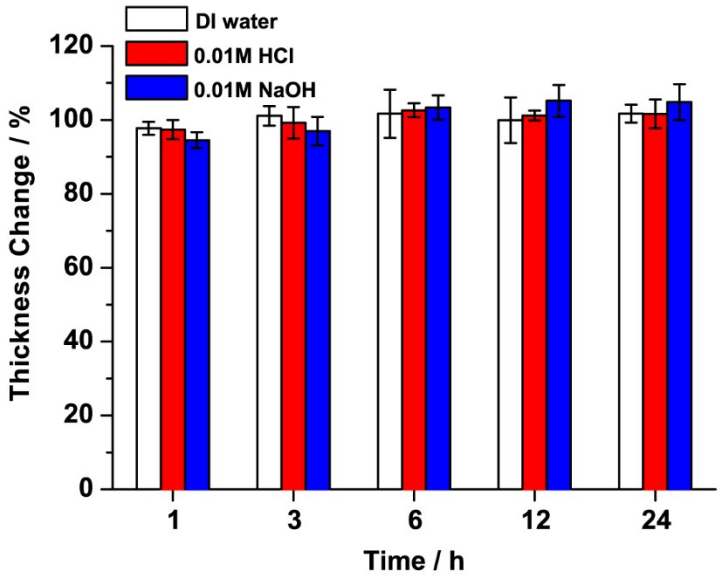

Fig. S7 Film stability test. The ellipsometric thickness was measured at the predetermined time after immersion and divided by the initial film thickness (before immersion) to obtain the value of thickness change. Film-coated gold substrates were immersed in DI water, 0.01M HCl solution, or 0.01M NaOH solution.

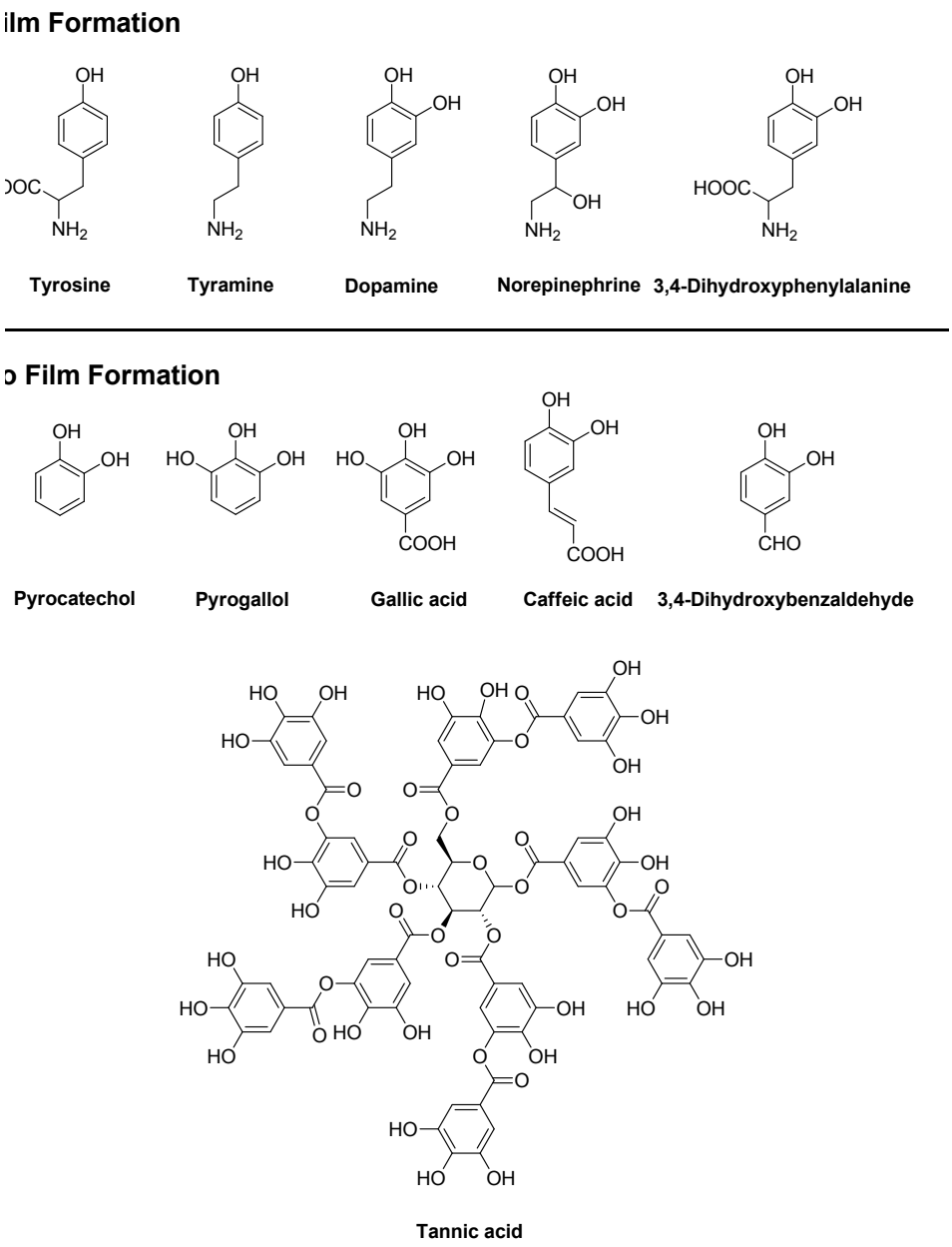

Fig. S8 Structures of phenolic molecules screened in this study.

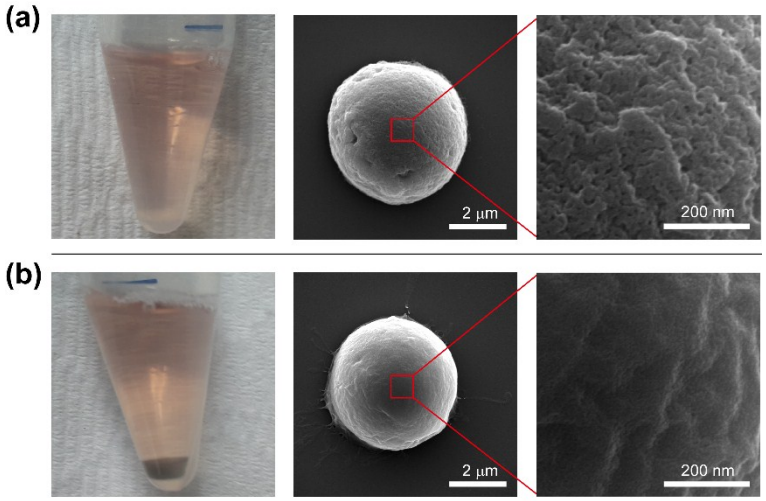

Fig. S9 Optical SEM images of Jurkat cells (a) before and (b) after enzymatic coating of 6 h.

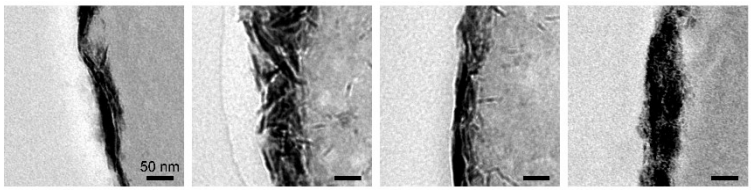

Fig. S10 High-resolution TEM images of Jurkat cells after enzymatic coating.

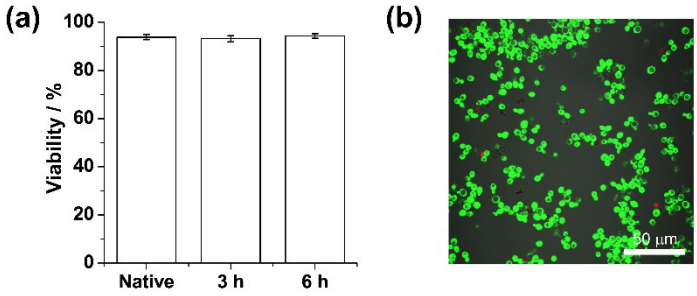

Fig. S11 (a) Viability of yeast cells after coating. (b) CLSM image of yeast cells after coating of 6 h. The cells were treated with FDA and PI for viability test.

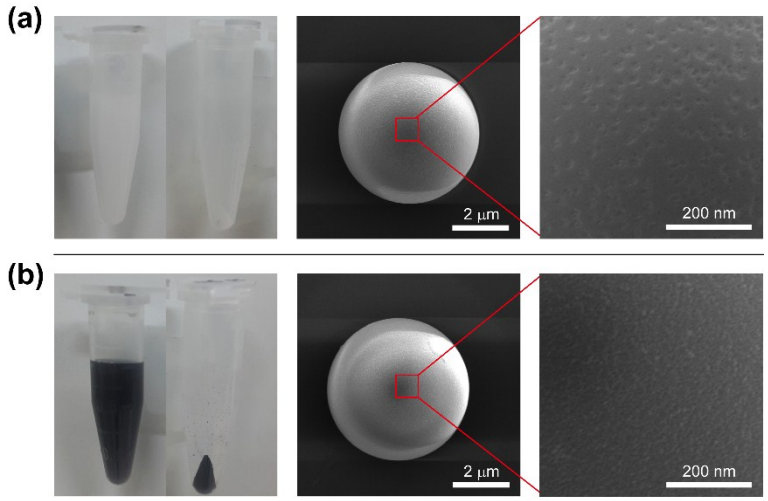

Fig. S12 Optical and SEM images of PS microparticles (diameter: 5 µm) (a) before and (b) after enzymatic coating of 6 h.

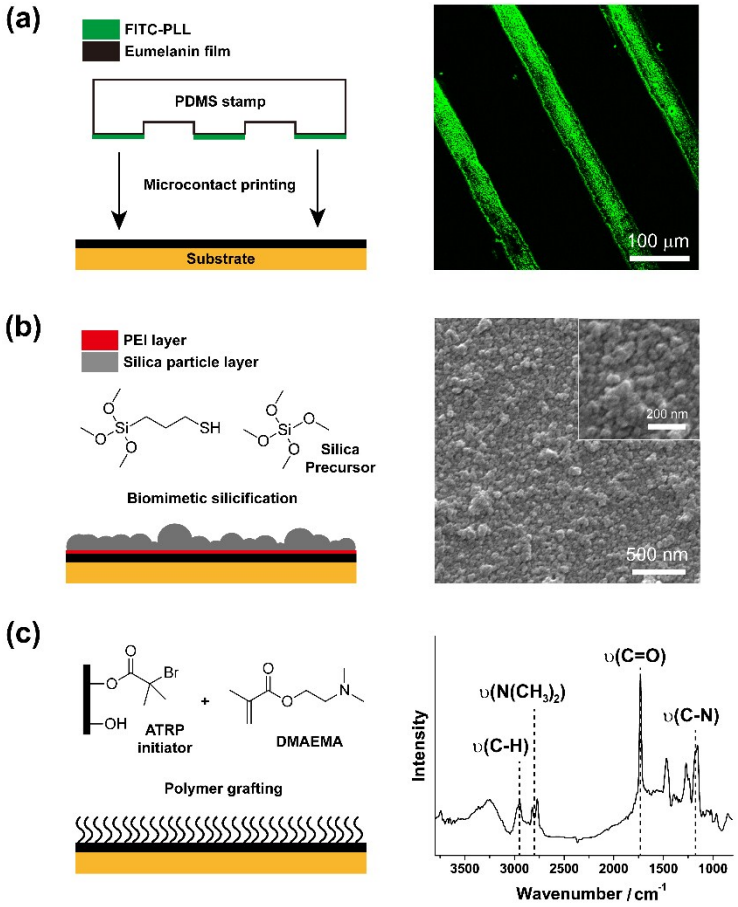

Fig. S13 Post-functionalization of the films. (a) CLSM image of the FITC-poly-L-lysine pattern generated by microcontact printing (µCP). (b) SEM images of the substrate after bioinspired silicification. (c) FT-IR spectrum of the substrate after surface-initiated, atom transfer radical polymerization (SI-ATRP) with DMAEMA. The films were formed on Au substrates. The details on experimental procedures are found in the experimental section.