Supporting Information

Catalyst-mediated yet catalyst-free hydrogels formed by interfacial chemical activation

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1. Materials

Dopamine hydrochloride, 3,4-dihydroxyhydrocinnamic acid (HCA), hematin porcine, *N*-hydroxysuccinimide (NHS), *N,N*-diisopropylethylamine (DIPEA), 1-methyl-2-pyrrolidone (NMP), and hydrogen peroxide were purchased from Sigma-Aldrich (Milwaukee, WI). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was purchased from TCI-SU (Tokyo, Japan). 4-arm-PEG-amine was purchased from Sunbio (Anyang, Korea). Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) were purchased from Novabiochem. Chitosan (M.W. 100 kDa, 70% deacetylated) was purchased from Heppe Medical Chitosan GmbH (Halle (Saale), Germany). All commercially available chemicals were used as-received without further purification. Distilled and deionized water (DDW) was used for the all washing procedures.

2. Instruments

The UV–vis spectra of the samples were obtained using an UV–vis absorption spectrometer (Agilent 8453, USA). The X-ray photoelectron spectroscopy (XPS) was performed with a Sigma Probe (Thermo VG Scientific, England) with a microfocused monochromator X-Ray source. Surface compositions were determined by the area under each spectrum with consideration of elemental sensitivity factors. The radical generation of the flow-through solution was determined using an electron paramagnetic resonance (EPR, Bruker EMX/Plus, Germany) spectrometer operating at the X-band frequency (~9.6 GHz). The iron metal concentration of the flow-through solution was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Varian Vista MPX, Varian, Palo Alto, CA, USA). The oscillatory rheometry was performed with a rotating rheometer (Bohlin Advanced Rheometer, Malvern Instruments, UK) to moniter the mechanical properties of the chitosan-catechol hydrogels.

3. Methods

3.1 Poly(dopamine) (pDA) coating and hematin immobilization on the surface

Dopamine hydrochloride was dissolved in 10 mM Tris buffer (pH 8.5) at a concentration of 0.1 wt % (1 mg/mL), and the dopamine solution was filled in a syringe for pDA coating (overnight approximately 18 hrs). The surface color changed to dark brown. To immobilize hematin on the pDA-coated surfaces, hematin porcine (4.1 mg, 0.0065 mmol) was dissolved in a co-solvent of DDW and DMF (1:1 v/v), and then 1 M NaOH was added to adjust the pH to 12. After reducing the solution pH to 8.5, EDC (5 mg, 0.026 mmol) and NHS (0.75 mg, 0.0065 mmol) were added to the dissolved hematin solution, and then the solution was poured into the pDA-coated syringes. UV-Vis spectrophotometry and XPS were performed to confirm the surface functionalization at each step.

3.2 Synthesis of 4-arm-PEG-catechol

For the preparation of 4-arm-PEG-catechol, HCA (364 mg, 2 mmol) and BOP (888 mg, 2 mmol) were added to a stirring solution of 4-arm-PEG-amine (1 g, 0.1 mmol) (M_w = 10 kDa) in 10 mL of a co-solvent of NMP and methanol (1:1 v/v), and the solution was allowed to react overnight at room temperature with stirring. After the reaction was completed, the solution was dialyzed (MWCO = 6,000-8,000 Da) four times under acidified water (a few drops of 5 M HCl was added to the dialysis water, and each dialysis step was performed for 4-6 hrs or overnight). A white powder was obtained by lyophilization.

3.3 Characterization of the catalytic activity of immobilized hematin by EPR, ICP-OES, and UV-Vis measurements

To test the catalytic activity of immobilized-hematin, a 4-arm-PEG-catechol solution was prepared. 4-arm-PEC-catechol (0.2 wt %) was dissolved in 1X PBS (pH 7.0) solution, and hydrogen peroxide (H_2O_2, 100 mM) was added to the 4-arm-PEG-catechol solutions. After the solution passed through the hematin-immobilized syringe (< 3 min), the flow-through solution, which is expected to contain the quinone/semiquinone free radical (SFR), was subjected to EPR measurement. The EPR parameters were as follows: temperature of 20 K; microwave frequency of 9.64 GHz; microwave power of 0.94 mW; modulation amplitude of 10 G; modulation frequency of 100 kHz; and five scans. The flow-through solution was measured using ICP-OES to detect Fe ions at two different wavelengths. The ICP-OES conditions were as follows: temperature of 23 °C; relative humidity of 50 %; wavelengths of 238.2 nm and 259.9 nm. The 4-arm-PEG-catechol solution was placed in contact with the hematin-immobilized surfaces during the first 3 min to form the quinone/SFR, and the flow-through solution was incubated for 0 min, 30 min, 1.5 hrs, 6 hrs and 12 hrs. The UV-Vis absorption was monitored at 330 nm.

3.4 Synthesis of catechol-modified chitosan (CHI-C)

For the preparation of CHI-C, chitosan (1 g, 6.5 mmol for the monomer stoichiometry) was dissolved in a HCl solution (100 mL, pH 2), and the pH was adjusted to 5.0. EDC (1.24 g, 6.5 mmol) and HCA (1.18 g, 6.5 mmol) were dissolved in a co-solvent of DDW and ethanol (1:1 v/v), and then both were added to the chitosan solution. The reaction time was 12 hrs, and the pH was maintained between 4.5 and 5 during the entire reaction time. The final product was dialyzed by a membrane (MWCO: 12,000-14,000 Da) against 10 mM NaCl solutions (pH 2) for 2 days and was then further dialyzed followed by DDW for 8 hrs. The degree of catechol substitution was 12.4 %, as determined by ^{1}H-NMR (Bruker Avance, 400 MHz).

3.5 Preparation of CHI-C hydrogels

To prepare the CHI-C hydrogels, CHI-C (2 wt %) was dissolved in 1X PBS (pH 7.0) solution, and hydrogen peroxide (H_2O_2, 100 mM) was added to the CHI-C solutions. The mixture solution was filled and mixed by repeating a piston motion in the hematin-immobilized syringe or a petri-dish for model reactions. The volume ratio of CHI-C and H_2O_2 was fixed at 9:1 (v/v), and the mixing time of the CHI-C solution in the syringe was limited to 1 min. Gelation was confirmed by a vial-inverting method.

3.6 Rheological analysis of the CHI-C hydrogels

The gelation behavior was monitored by a rotating rheometer. A parallel plate measuring system was used, and the gap between the two parallel plates was 150 μm. To verify the gelation time of the CHI-C hydrogels, frequency sweep methods were employed as a function of time (0, 10, 20, 30, and 60 min) after injection of the CHI-C solution. The elastic (G') and viscous (G'') modulus values were collected as a function of frequency (from 0.1 to 10 Hz), and the stress was fixed at 100 Pa for all measurements.

3.7 *In vitro* cell viability test for CHI-C hydrogels

In vitro cytotoxicity of CHI-C hydrogels was evaluated using CCK assay (cell counting kit-8, Dojindo Laboratories, Japan) using extracts of CHI-C hydrogels. The extracts (10 mL) of CHI-C hydrogels were obtained by incubation of hydrogels (1 g) in separate media for 24 hrs and sterilized by filtration (0.45 μm, Millipore, USA). NIH 3T3 cells were seeded onto 96-well plates (5,000 cells/well), and the plates were incubated for 24 hrs. The extracts (200 μL) were added to the 96-well plates. After the incubation, CCK solution was added to the well plates, and the plates were incubated for 2 hrs at 37 °C. The absorbance of the well plates at 450 nm was measured by a microplate reader to quantify the viability of the cells (BioRad,

Model 550, Hercules, CA, USA). All experiments were performed in quadruplicates. The control indicates the cells without any exposure to the hydrogels.

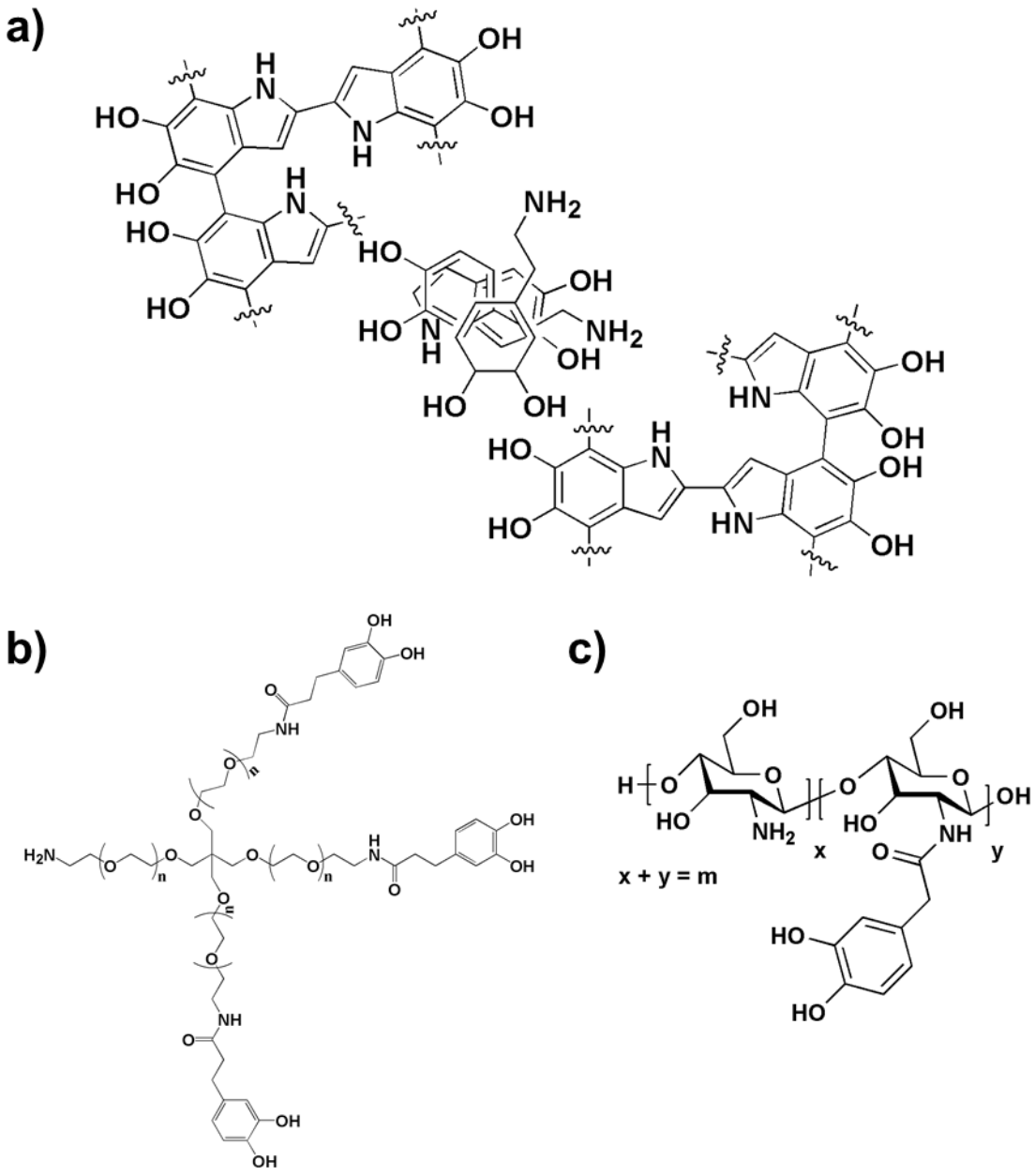

Figure S1. Chemical structures of a) polydopamine (pDA) b) 4-arm-PEG-catechol c) chitosan-catechol

Figure S2.

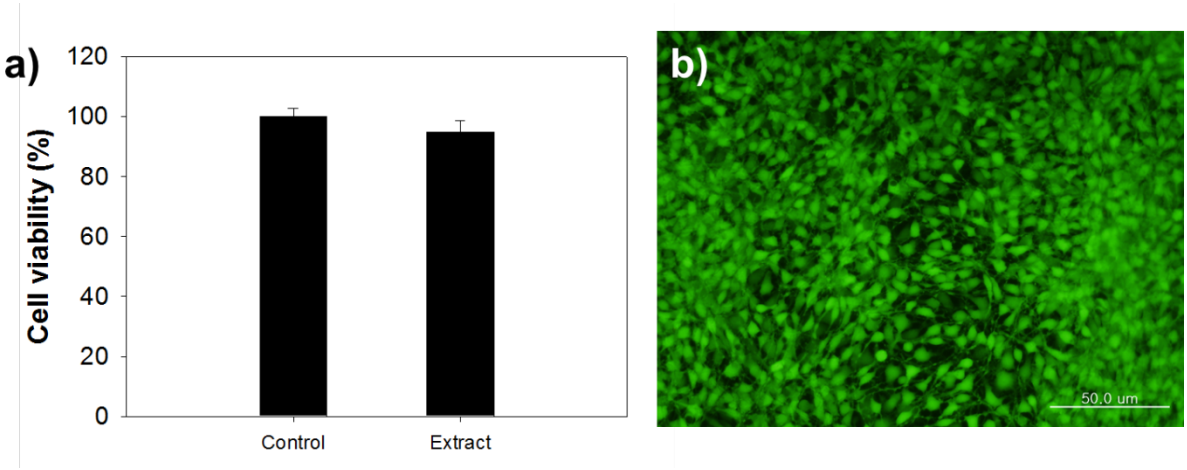

Figure S2. Cytotoxicity of CHI-C hydrogels. A) Quantitative CCK assay results. B) The fluorescence microscope image of the NIH3T3 cells exposed to the hydrogels. The green color indicates living cells.