Electronic Supplementary Information (ESI)
Experimental details for the communication

"Biological Self-assembly of Injectable Hydrogel as Cell Scaffold via Specific Nucleobase Pairing"

Huaping Tan*, Chao Xiao, Jinchen Sun, Dangsheng Xiong, Xiaohong Hu

Materials: Thymine, adenine, oleic acid, 2-(dimethylamino) ethanethiol hydrochloride, 11-bromo-1-undecene, thiolacetic acid, azobis(isobutylnitrile), adenosine 3'-monophosphate (AMP), dimethylformamide (DMF), *N*-ethylmaleimide, human insulin and dexamethasone were purchased from Sigma-Aldrich. Maleimide terminated four-arm PEG (PEG-Mal) (MW 10 KDa and 20 KDa) was purchased from NOF Corporation, Japan. Trypsin–EDTA solution, streptomycin, penicillin were purchased from Invitrogen Co. (Carlsbad, CA). VEGF was purchased from Cell Signaling Technology, Inc., Danvers, MA. CyQuant Cell Proliferation Assay Kit was purchased from Invitrogen, Eugene, Oregon, USA. All other reagents were used as received.

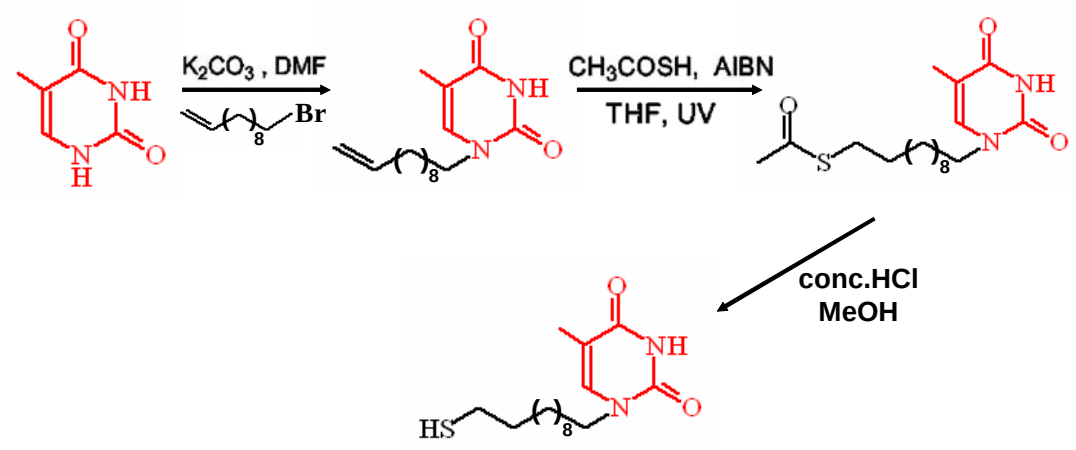

Fig. 1 Outline of the procedure of synthesis of T-SH.

Synthesis of T-SH and A-SH: Thymine (5 mmol) was dissolved in 50 mL DMF and K_2CO_3 (5 mmol) was added to the solution, followed by stirring at 80 ^oC for 1 h. Subsequently, 11-bromo-1-undecene (5 mmol) was dropped into the resulting mixture. After storage at 80 ^oC overnight, DMF was removed under vacuum. The residues were dissolved in 100 mL CH_2Cl_2 and washed twice with 60 mL water. The organic layer was collected and dried by Na_2SO_4. The removal of CH_2Cl_2 yielded a white powder, followed by purification of column chromatography with acetate/hexane (1:4). Thiolacetic acid (3 mmol) and azobis(isobutylnitrile) (0.3 mmol) were added into a solution of the white powder (3 mmol) in dehydrated THF (50 mL). After irradiation in a photochemical reactor for 22 h under nitrogen, the reaction mixture was concentrated and purified by column chromatography with ethyl acetate/hexane

(1:2), leading to a compound. 2.5 mL HCl (30 mmol) was added into a solution of the formed compound (3 mmol) in methyl alcohol (50 mL), followed by reflux for 20 h. After washing with water, the organic layers were collected and dried using Na_2SO_4. When the organic layer was removed, followed by purification by column chromatography with ethyl acetate/hexane (1:1), T-SH was produced as a white powder (Fig. 1). M.p. 101–103 ^oC; ^1H NMR ($CDCl_3$) δ: 8.85 (s, 1H), 6.91 (s, 1H), 1.85 (m, 3H), 1.60 ~ 1.51 (m, 4H), 1.30 ~ 1.20 (m, 15H) ppm.
Following the similar procedure to synthesize T-SH, A-SH was prepared as a white powder (Fig. 2). M.p. 132–134 ^oC; ^1H NMR ($CDCl_3$) δ: 8.37 (s, 1H), 7.78 (s, 1H), 5.67 (s, 2H), 1.90 ~ 1.87 (m, 3H), 1.61 ~ 1.59 (m, 2H), 1.36 ~ 1.25 (m, 14H) ppm.

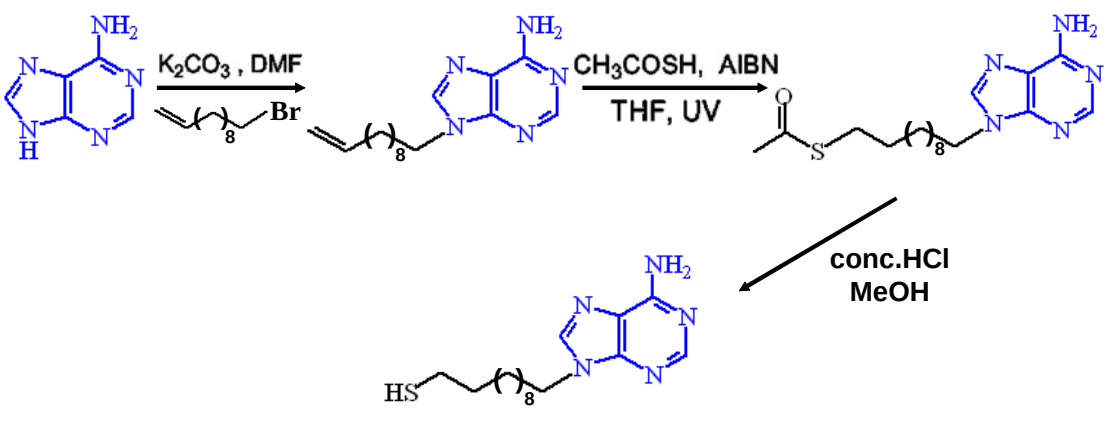

Fig. 2 Outline of the procedure of synthesis of A-SH.

Synthesis of Four-Arm PEG-T and PEG-A: Four-arm PEG-Mal (0.25 mmol) (Fig. 3) was dissolved in DMF. T-SH or A-SH (10 mmol) was dissolved in 100 mL DMF, followed by dropwise addition of four-arm PEG-Mal solution, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then quenched by adding a solution of *N*-ethylmaleimide (12 mg) in DMF (0.4 mL). Subsequently, DMF was distilled off in vacuo, and the moist residue was dissolved in water and kept in an ice-box for about 1 h to precipitate unreacted T-SH or A-SH. The PEG-T and PEG-A were respectively purified via dialysis against nanopure water (MWCO 3,500) and lyophilized to give white foams.

Self-assembly of Hydrogels: PEG-T and PEG-A were dissolved in PBS (pH 7.4) separately to form precursor solutions at a concentration of 10 or 20 wt%, respectively. For self-assembly of hydrogel, solutions of PEG-T and PEG-A were sufficiently mixed by pipetting in various mole ratios of ligands to ensure homogeneity and resulted in the formation of self-supporting hydrogels (Fig. 4). For preparation of GFs loaded hydrogels, insulin, dexamethasone and VEGF were dissolved in 1 mL PEG-T or PEG-A solution, respectively. The precursor solutions containing GFs were sufficiently mixed by pipetting to resulted in hydrogels at 37 ^oC. The loading concentrations of GFs were fixed with 50 μg/mL.

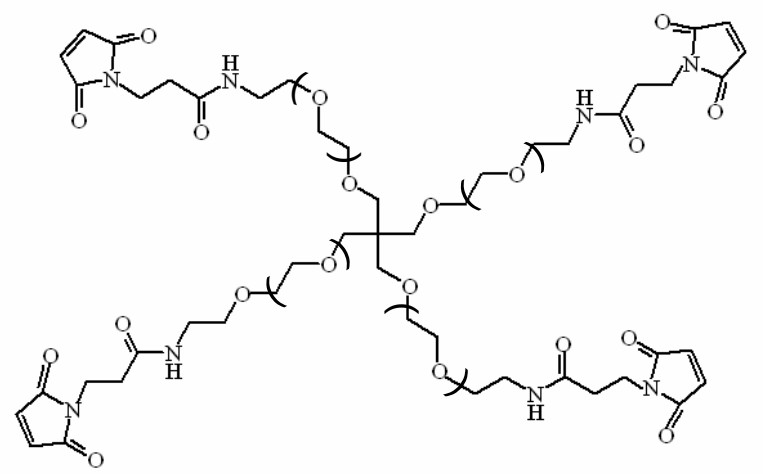

Fig. 3 Chemical structure of maleimide terminated four-arm PEG (PEG-Mal).

Characterization of Hydrogels: The rheological analysis of mixtures of PEG-T/PEG-A solutions was investigated on a strain-controlled rheometer (AR2000, TA instrument, USA) using a parallel plate (diameter, 40 mm) at 37 ^{o}C. The angular frequency ω was set at 6.28 rad/s. Degradation of hydrogel was examined with respect to weight loss under aqueous conditions. Weight loss of initially hydrogels (W_0) was monitored as a function of incubation time in PBS at 37 ^{o}C. At specified time intervals, hydrogels were removed from the PBS and weighed (W_t). The weight loss ratio was defined as $100\%\times(W_0-W_t)/W_0$. The weight remaining ratio was defined as $1-100\%\times(W_0-W_t)/W_0$. For swelling experiment, the hydrogels were immersed in PBS. The swollen hydrogels were removed and immediately weighed with a microbalance after the excess of water lying on the surfaces was absorbed with a filter paper. The swelling ratio was calculated using the following equation of SR = (Ws–Wd)/Wd, where Ws and Wd are the weights of the hydrogels at the swelling state and at the dry state, respectively. For GFs release experiment, 100 μL of gels were suspended in 1 mL of PBS at 37 ^{o}C, provided a reservoir into which GFs could be released from the gel complex and subsequently measured during culture. At predetermined intervals, a 200 μL sample of release medium was extracted from the sample vials and replenished with 200 μL fresh release medium to maintain a constant volume. The release of dexamethasone was analyzed spectrophotometrically at 242 nm. The release of insulin and VEGF were determined by the ELISA kit assays (Invitrogen, USA).

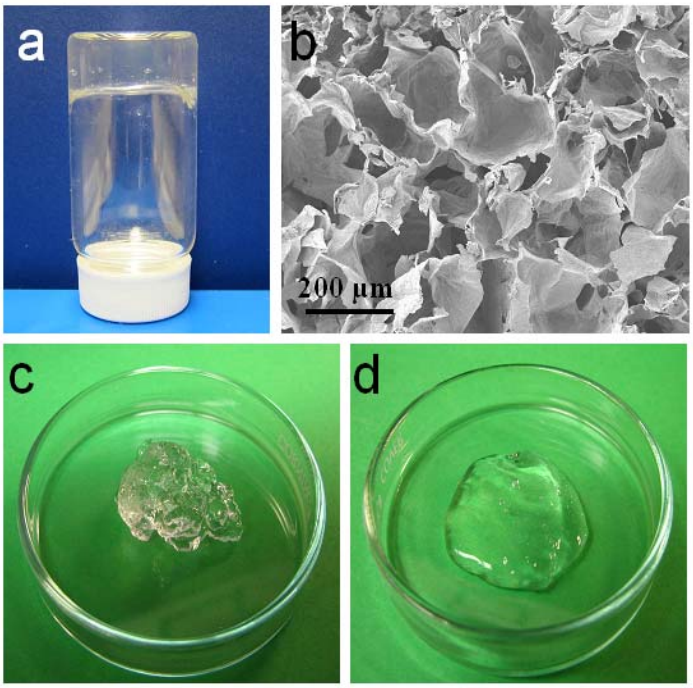

Fig. 4 (a) Photograph image illustrates a formed hydrogel in PBS at 37 ^{o}C. (b) SEM photograph of the freeze-dried hydrogel. (c) The hydrogel at 20 ^{o}C. (d) The hydrogel at 50 ^{o}C.

Cell Culture: Human adipose-derived stem cells (ASCs) were isolated from human adipose tissue obtained from elective cosmetic surgery procedures. The fat tissues were minced with scissors in the collagenase solution consisted of Hanks' balanced salt solution (3.0 mL/g of fat) (Sigma-Aldrich), bovine serum albumin (fatty acid free, pH 7.0, 3.5 g/100 mL Hanks') (Intergen Company, Purchase, NY) and 1% type II collagenase (3.0 mg/g of fat) (Worthington Biochemical Corporation, Lakewood, NJ). The centrifuge tubes were shaken at 100 rpm for 50 min at 37 °C. Following digestion, the content of each tube was filtered through double-layered sterile gauze. The filtrates were then centrifuged at 1000 rpm for 10 min at 37 °C, and a three layer suspension, consisting of a fatty layer on the top, a serum layer in the middle, and a cellular pellet at the bottom of each tube, was obtained. The fatty layer and most of the supernatant was aspirated off, leaving the pellet intact at the bottom. The pellet in each tube was then suspended in 10 mL of erythrocyte lysis buffer (pH = 7.4), vortexed, and centrifuged again at 1000 rpm for 10 min at 37 °C. The pellets were suspended in the plating medium consisted of DMEM/F12 with 10% heat-inactivated fetal bovine serum, 1% penicillin/streptomycin and 1% Fungizone (all products obtained from Gibco, Invitrogen Corporation, Carlsbad, CA). Adherent ASCs were expanded for a period of 5–8 days at 37 °C, and the medium was changed every other day until the cells achieved 80% confluence.

PEG-T and PEG-A foams were sterilized under germicidal UV irradiation for 1 h and respectively dissolved in sterile PBS to obtain 20 wt% solutions. For adhesion assay, 300 μL PEG-T/PEG-A mixtures (1:1) were added into 24-well culture plate, which

were then incubated at 37 ^{o}C for 10 min to self-assemble hydrogels. 1mL DMEM/F12/10%FBS culture medium containing 50,000 cells was added into each well. After 24 h, the number of ASCs attached to the hydrogels was quantified using a CyQuant Cell Proliferation assay (Invitrogen, USA). For encapsulation assay, 1 mL PEG-T/PEG-A mixture was added into the ASCs containing vial. After sufficient mixing, the cell containing PEG-T/PEG-A solutions were injected into 48-well culture plate. The resulting mixture with cells was incubated at 37 ^{o}C for 10 min. The cell density was 5×10^{6}/mL hydrogel. Pre-warmed DMEM/F12/10%FBS culture medium was added into each well and changed daily. The DNA content was quantified using the CyQuant Cell Proliferation assay. The live/dead cells were observed by confocal laser scanning microscopy (CLSM, Olympus FV1000). The live cells were dyed with Cell Tracker Orange CMRA (Red) and all cell nuclei were dyed with Hoechst 33342 (Blue). Cell morphologies were observed by SEM (JEOL, JSM-6330F) after fixation, sequential dehydration, critical point drying and gold coating.