Supplementary Material (ESI) for Soft Matter

Supplementary Information

Cyclodextrin/dextran based in situ hydrogel formation: a carrier for hydrophobic drugs

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Experimental Section

Materials. Maleic anhydride, thiourea, *N*,*N*′-dicyclohexylcarbodiimide (DCC), all-*trans* retinoic acid (RA) and p-toluene sulfonic acid monohydrate were obtained from Fluka. Triphenylphosphine and 4-dimethylaminopyridine were obtained from Aldrich. β-cyclodextrin hydrate and iodine were obtained from Acros. All these chemicals were used as received. 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS) was synthesized from 4-(dimethylamino)-pyridine and p-toluenesulfonic acid monohydrate and recrystallized from toluene.[1] Dextran ($M_n \sim 10000$, Pharmacia Fine Chemicals, Sweden) was dried in the vacuum oven for several days prior to use. *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were dehydrated with molecular sieves. Water used in all experiments was purified through deionization and filtration with a Millipore purification apparatus.

Synthesis of maleimide functionalized dextran (Dex-mal). Dex-mal was prepared through DCC mediated esterification of the hydroxyl group of the dextran with *N*-maleoylamino acid, which was obtained through a procedure reported previously.[2] Typically, *N*-maleoylamino acids (0.96 g, 6.2 mmol) was dissolved in 30 mL DMSO followed by addition of DPTS (0.29 g, 0.9 mmol) and DCC (1.92 g, 9.3 mmol). Dextran (1.84 g, 10.3 mmol anhydroglucose (AHG) units) was dissolved in 10 mL DMSO and added to the reaction mixture slowly. After stirring for 24 h at room temperature, the formed *N,N'*-dicyclohexylurea salt was removed by filtration, and the crude product was obtained by precipitation in cold ethanol. The precipitate was collected by filtration and washed with ethanol and then dissolved in water and purified

by ultrafiltration (MWCO 3500). The product was recovered by freeze drying; yield: 1.48 g (80 %). ^{1}H NMR (400 MHz, D_2O): δ 3.2-4.0 (m, dextran glucosidic protons), 4.4 (s, maleimide), 4.9 (s, dextran anomeric proton), 6.9 (s, maleimide).

The degree of substitution (DS) of dextran is defined as the number of maleimide groups per 100 AHG units. The DS of Dex-mal was calculated from the ^{1}H NMR spectra based on the protons of the maleimides (δ 6.9) and the glucosidic protons of dextran (δ 3.2 - 4.0, 5.1 and 5.3). In the case mentioned above, DS was determined to be 17. Using a different feeding ratio of *N*-maleoylamino acids to dextran and different reaction time, a series of Dex-mal with different DS were obtained; Dex-mal (DS = 26) was prepared by changing feed ratio from 0.6 to 0.9 and Dex-mal (DS = 10) was prepared by shorting the reaction time to 12 hours.

Synthesis of thiol functionalized β-cyclodextrin. Per-6-thio-β-cyclodextrin sodium salt (β-CDS) was prepared following a reported procedure.[3] Triphenylphosphine (20.20 g, 77.1 mmol) was dissolved in 80 mL dry DMF, I_2 (20.30 g, 79.7 mmol) was added to this solution carefully with vigorous stirring. Then vacuum oven dried β-cyclodextrin hydrate (5.40 g, 4.7 mmol) was added to this dark brown solution and stirred overnight at 70 °C under N_2 atmosphere. DMF was removed under reduced pressure and pH of the resulted solution was adjusted to 9–10 by adding 3 M sodium methoxide in methanol. The solution was allowed to cool down to room temperature and was kept at room temperature for 30 min. Then the reaction mixture was poured into 400 mL methanol to form a precipitate. The precipitate was filtered, washed with methanol and further purified by Soxhlet extraction with methanol. The product per-6-iodo-β-cyclodextrin was dried in a vacuum oven for 1 week; yield 5.37 g (60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.2–3.4 (m, 21H), 3.5–3.7 (m, 14H), 3.8 (d, 7H), 4.9 (d, 7H), 5.9 (d, 7H), 6.05 (d, 7H).

Per-6-iodo-β-cyclodextrin (0.97 g, 0.5 mmol) was dissolved in 10 mL dry DMF and then thiourea (0.30 g, 4.0 mmol) was added to this solution slowly. The resulted mixture was stirred overnight at 70 °C under N_2 atmosphere. Then DMF was removed under reduced pressure and yellow oil was obtained. 50 mL water and 0.26 g sodium hydroxide were added. The mixture was heated to a gentle reflux under N_2 atmosphere for 1 hour. After the suspension was acidified with 2 M HCl (2 mL), the resulted precipitate was collected by filtration and washed thoroughly with distilled water. The product was dried in a vacuum oven for 2 days; yield 0.56 g (58 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.1 (t, 7H), 2.7 (m, 7H), 3.2 (br m, 7H), 3.4–3.7 (m, 28H), 4.9 (d, 7H), 5.83 (s, 7H), 5.95 (d, 7H).

Obtained per-6-thio-β-cyclodextrin (0.10 g) was suspended in 10 mL H_2O and one equivalent of sodium hydroxide (0.23 g) to the thiols was added. After stirring for 30 min, excess NaOH was neutralized with 1 M HCl by monitoring the pH of the solution. Water soluble per-thio-β-cyclodextrin sodium salt was recovered by freeze-drying with a quantitative yield.

Hydrogel Formation. Hydrogels were obtained by mixing solutions of Dex-mal and β-CDS. Typical procedure was following: 200 μL Dex-mal (60 mg) and 200 μL β-CDS (8 mg) in phosphate buffered saline (PBS) solutions were mixed by vortexing. The gelation time was determined by the vial tilting method. When there was no flow of the sample within 5 seconds, it was regarded as a gel. In order to keep the ratio of cross-linkable groups at 1:1, 10% excess thiols to maleimide groups were used due to the fact that thiol groups may be oxidized or form disulfide bonds. Due to the high abundance of thiol groups on β-CDS, it gives amber solution and it turns into red after the reaction with Dex-mal.

Characterizations of the Hydrogel. Sample for visco-elastic measurements was prepared by mixing two solutions of Dex-mal (0.18 g in 800 μL PBS) and β-CDS (24.0 mg in 400 μL PBS). A small sample of the hydrogel to be analyzed was manually applied to a rheometer (Ares AR-G2 TA) with plate-plate geometry (40 mm in diameter) and a 400 microns gap distance. Prior to the measurements, the strain sweep tests were performed on the sample to determine the maximum limit of the linear viscoelastic regime. Data acquisition started when steady state was reached, as indicated by normal forces. Frequency sweeps were done between 0.1 and 100 Hz in the linear response regime at 25 °C.

Scanning electron microscopy (SEM) was used to study the structure of the hydrogel. It was conducted on a Nova NanoSEM (FEI) with an accelerating voltage of 10 kV and spot size of 3.5. Sample was prepared by freeze-drying from 400 μL of the freshly made hydrogel. Fractured pieces were mounted onto an aluminum stub and coated with carbon before measurements.

Drug loading and release. All-*trans* retinoic acid (RA) (1.0 mg, 3.3 μmol) was dissolved in acetone (100 μL) and then this solution was added to 200 μL β-CDS (8.0 mg, 5.7 μmol) PBS solution. Acetone was removed by vacuum evaporation. Upon the addition of 200 μL Dex-mal (60.0 mg) in PBS to the mixture, the hydrogel was formed immediately. Before releasing experiment, the obtained hydrogel was aged overnight at room temperature. The RA carrying

hydrogel was put into a dialysis tube (MWCO 1000) with 5 mL of PBS and the tube was placed in 100 mL PBS solution. The surrounding PBS was stirred at 50 rpm at room temperature and refreshed every 24 hours. The amount of RA released from the hydrogel was detected by the HPLC (Shimadzu) using UV detection at 345 nm equipped with an auto-injector (Shimadzu, SIL-10AD) and a dC_{18} column (Atlantis, Waters; 150 × 46 mm; 5 μm particle size) with a 21 min linear gradient from 100% solvent A (methanol/acetonitrile/THF, 62:33:5, v/v/v) to 75% solvent A with 25% solvent B (acetic acid/H_2O, 2:98, v/v). For calibrations, a methanol solution of RA (5 mg/L) was used. In order to prevent decomposition of RA during the release experiment, the sample was kept in dark. It has also been confirmed that RA did not react with thiol functionalized cyclodextrin, with ^1H NMR in a DMSO solution.

References:

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