Electronic Supplementary Information

Nanofibers as precursors for the rapid formation of hydrogels

Pichapak Srikamut, Man Theerasilp and Daniel Crespy*

Department of Materials Science and Engineering, School of Molecular Science and Engineering, Vidyasirimedhi Institute of Science and Technology (VISTEC), Rayong 21210, Thailand

*Corresponding Author.

E-mail address: daniel.crespy@vistec.ac.th (D. Crespy).

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1. Experimental Section

1.1 Materials

Dextran (Dex, M_r ~ 70,000 Da, Sigma-Aldrich) was dried at 60 °C for 12 h in a vacuum oven before use. Methacrylic anhydride (94%, Aldrich), triethylamine (≥ 99%, Merck), Lithium Chloride Anhydrous (LiCl, >98%, TCI), ammonium persulfate (APS, 98%, Carlo Erba), *N,N,N',N'*-tetramethyl ethylenediamine (TEMED, 98%, TCI), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (IC2959, >98%, HPLC, TCI), tetraethyl orthosilicate (TEOS, Acros Organic, 98%), sodium oleate (TCI, 98%), hexadecane (HD, Acros Organic, 99%), octenidine·2HCl (OCT, TCI, >98%), tetracycline hydrochloride (TC, TCI, 98%), calcium chloride ($CaCl_2$, Carlo Erba, 90%), hydrochloric acid (HCl, Carlo Erba, 37%), chloroform ($CHCl_3$, Carlo Erba, 99%), 4-hydroxybenzaldehyde (99%, Acros Organics), dimethylformamide (DMF, 99.8%, RCI Labscan), dimethyl sulfoxide-D6 (DMSO-d6, 99.8%, Merck) were used as received. Deionized water was used throughout this work.

1.2 Synthesis of dextran methacrylate (DexM)

The procedure was adapted from a previous report.[1] Dextran (3.07 g, 57 mmol −OH groups) was dissolved in 30 mL of a 10wt% LiCl solution in DMF in a 50 mL round-bottom flask at 90 °C and then cooled at 60 °C. Afterwards, 0.0025, 0.005, 0.01, and 0.015 equivalents (to −OH groups of dextran) of triethylamine were added into the solution, which was stirred for 15 min at 60 °C. Then, 0.25, 0.5, 1.0, or 1.5 equivalents of methacrylic anhydride were slowly dropped in the mixture, which was stirred for 24 h at 60 °C. Afterwards, the products were purified by dialysis for 3 days and were recovered by freeze-drying to provide a white solid. ^{1}H NMR spectroscopy (600 MHz, DMSO-d6): δ 6.11, 5.69 (m, 1H), 4.91, 4.84, 4.69, 4.49 (s, 1H), 3.95-3.03 (m, 1H), 1.90 (s, 3H) (Figure S1). The degree of substitution (DS) of DexM was calculated by comparing the integrals of the signal of anomeric protons of dextran at δ 4.69 ppm in the ^{1}H NMR spectra with the signals of alkene protons of methacrylic anhydride at δ 6.11 and 5.69 ppm.

1.3 Preparation of nanofibers loaded with redox initiators

DexM (4.8 g) was dissolved in an aqueous solution of 192 mg TEMD in 4.8 g water while Dex (4.0 g) was dissolved in an aqueous solution of 123 mg APS in 4.0 g water. The two different mixtures were transferred in different 10 mL plastic syringes equipped with stainless steel 21G needles (0.8 mm). Then the two mixtures were separately co-spun at ~ 25 °C and 55–60% humidity at a controlled flow rate of 0.4 mL/h with a syringe pump. The distance between needle and collector was 10 cm and the applied voltage was −10/+14 kV.

1.4 Preparation of nanofibers loaded with photoinitiators

501.4 mg DexM was dissolved into a solution of 3.5 mg IC2959 in 500 mg water. The mixture was transferred in a 2.5 mL plastic syringe with stainless steel 21G needles (0.8 mm). Then the

syringe was assembled to a tubeless spinneret holder. Nanofibers were fabricated by electrospinning at ~ 25 °C and 60–65% humidity at a controlled flow rate of 0.5 mL/h with a syringe pump. The distance between needle and collector was 10 cm and the applied voltage was −10/+15 kV.

1.5 Quantification of initiators loadings in fibers

27.73 mg nanofibers loaded with TEMED or 17.27 mg nanofibers loaded with IC2959 or 16.12 mg nanofibers loaded with IC2959 after 30 days were dissolved separately in DMSO-d6 containing 4-hydroxybenzaldehyde as internal standard. The solutions were then measured by ^{1}H NMR spectroscopy (Bruker, 600 MHz) at 30 °C. To calculate the amount of TEMED or IC2959 in nanofibers, the integrals of the signals at 2.25 and 8.19 ppm associated to signals of TEMED and IC2959 were compared with the signal of the internal standard (9.77 ppm).

1.6 Preparation of hydrophobic ion pair with tetracycline (HIP)

The preparation of hydrophobic ion pair with tetracycline was carried out following a previous report.[2] Briefly, 20 mL of a 2.2wt% aqueous solution of TC and 10 mL of a 2.2wt% aqueous solution of $CaCl_2$ were mixed and stirred for 15 min, followed by the addition of 25 mL of a 2.4 wt% of aqueous solution of sodium oleate and further stirring for 3 h. The product was centrifuged at 7616 rcf for 30 min at 10 °C and extracted with water three times to eliminate non-reacted compounds followed by freeze-drying to provide a yellow solid (yield 67%).

1.7 Entrapment of HIP complexes in silica nanocapsules (SiO_2NCs-HIP)

The procedure was adapted from a previous report.[2] 2 g TEOS and 125 mg HD were dissolved in 1 g $CHCl_3$ containing 23.5 mg of the aforementioned HIP. Then, 30 mL of a 0.38wt% aqueous solution of OCT was added. The mixture was subsequently stirred at 540 rpm for 10 min and processed by ultrasonication (3 min, 50% amplitude in a pulsed regime with 3 s on and 1 s off) under ice cooling. The resulting miniemulsions were stirred for 20 h at 25 °C and then further stirred for 6 h at 40 °C to obtain silica nanocapsules.

1.8 Preparation of nanofibers loaded with redox initiators and SiO_2NCs-HIP

DexM (4.8 g) was dissolved into an aqueous solution of 192 mg TEMD in a mixture of 2.4 g water and 2.4 g SiO_2NCs-HIP dispersion while Dex (4.0 g) was dissolved into an aqueous solution of 123 mg APS in a mixture of 2.0 g water and 2.0 g SiO_2NCs-HIP dispersion. The two different mixtures were then transferred in separated 10 mL plastic syringes equipped with stainless steel 21G needles (0.8 mm). Then the two mixtures were separately co-electrospun with a dual-nozzle electrospinning set-up at ~ 25 °C and 57–60% humidity at a controlled flow rate of 0.5 mL/h with a syringe pump. The distance between needle and collector was 10 cm and the applied voltages was −10/+14 kV.

1.9 Preparation of hydrogels

Known amounts of DexM-T fibers and Dex-A fibers or DexM-IC fibers or DexM-T-Dex-A fibers were dissolved in water (Table S1). The aqueous solutions of DexM-T fibers and Dex-A

fibers or DexM-T-Dex-A fibers were mixed with a vortex (WiseMix, VM-10) at 1200 rpm. The solution of DexM-IC fibers was mixed by vortex (WiseMix, VM-10) at 1200 rpm and cured with a UV-LED lamp (UV intensity = 94 mW/cm^2) for 150 s with a maximum wavelength of 365 nm.

1.10 Release of TC and OCT from the hydrogels

The release of TC and OCT from DexM-T-DexA hydrogels were monitored by UV-vis spectroscopy. The maximum absorbance wavelengths of TC and OCT in phosphate buffer at pH 7.4 were 360 nm and 283 nm respectively. The calibration curves for the determination of TC and OCT concentrations at pH 7.4 are shown in Figure S2. To study the release of TC and OCT, 100 mg of DexM-TA fiber with embedded SiO_2NCs-HIP was dissolved in 488 mg of water using a vortex (WiseMix, VM-10) at 1200 rpm to form a hydrogel. A mixture containing 8.1wt% DexM, 6.78wt% Dex, 7.14wt% SiO_2NCs-HIP dispersion, 0.32wt% TEMED, 0.21wt% APS, and 77.46wt% water was vortex-mixed at 1200 rpm to form the hydrogels. Another mixture containing 8.72wt% DexM, 7.30wt% Dex, 0.002wt% TC, 0.03wt% OCT, 0.34wt% TEMED, 0.22wt% APS, and 83.39wt% water was vortex-mixed at 1200 rpm to form hydrogels containing physically entrapped TC and OCT. Then, the hydrogel was placed in 1.5 mL phosphate buffer at pH 7.4 and were shaken at 150 rpm and 30 °C. Aliquots (1 mL) of the media were taken at various time intervals and placed back in the release media after UV-vis spectroscopy measurements.

1.11 Analytical tools

^{1}H NMR spectra were measured with a 600 MHz nuclear magnetic resonance spectrometer at 30 °C (Avance III HD, Bruker). Infrared spectra of of DEX, DEX-MET and HIP were recorded by

attenuated total reflection fourier transform infrared spectroscopy with 32 scans across 4000 – 400 cm^{-1} and a resolution of 1 cm^{-1} (ATR-FTIR, PerkinElmer Frontier FTIR, Universal-ATR). Nanofibers were fabricated by electrospinning (TL-Pro-BM, Tong Li Tech) equipped with a syringe pump (TL-F6, Tong Li Tech). Surface morphology of nanofibers and silica nanocapsules was observed with a scanning electron microscope (SEM, JSM-7610F, JEOL), and the internal fiber structure was monitored with a transmission electron microscope (TEM, JEOL-ARM200F, JEOL). The release of TC and OCT were measured using a UV-vis spectrometer (Cary100, Agilent). Rheological properties of 1.8 mm thick hydrogels were determined with a rheometer (DHR-20, TA instruments) using a parallel plate geometry (diameter of 20 mm). Storage moduli (G′) and loss moduli (G″) were recorded over an angular frequency from 0.1 to 10 $rad \cdot s^{-1}$ with 1.0% strain at 25 °C. Frequency sweep measurements were performed to determine the linear equilibrium modulus plateau of the hydrogels.

2. Supplementary Figures and Tables

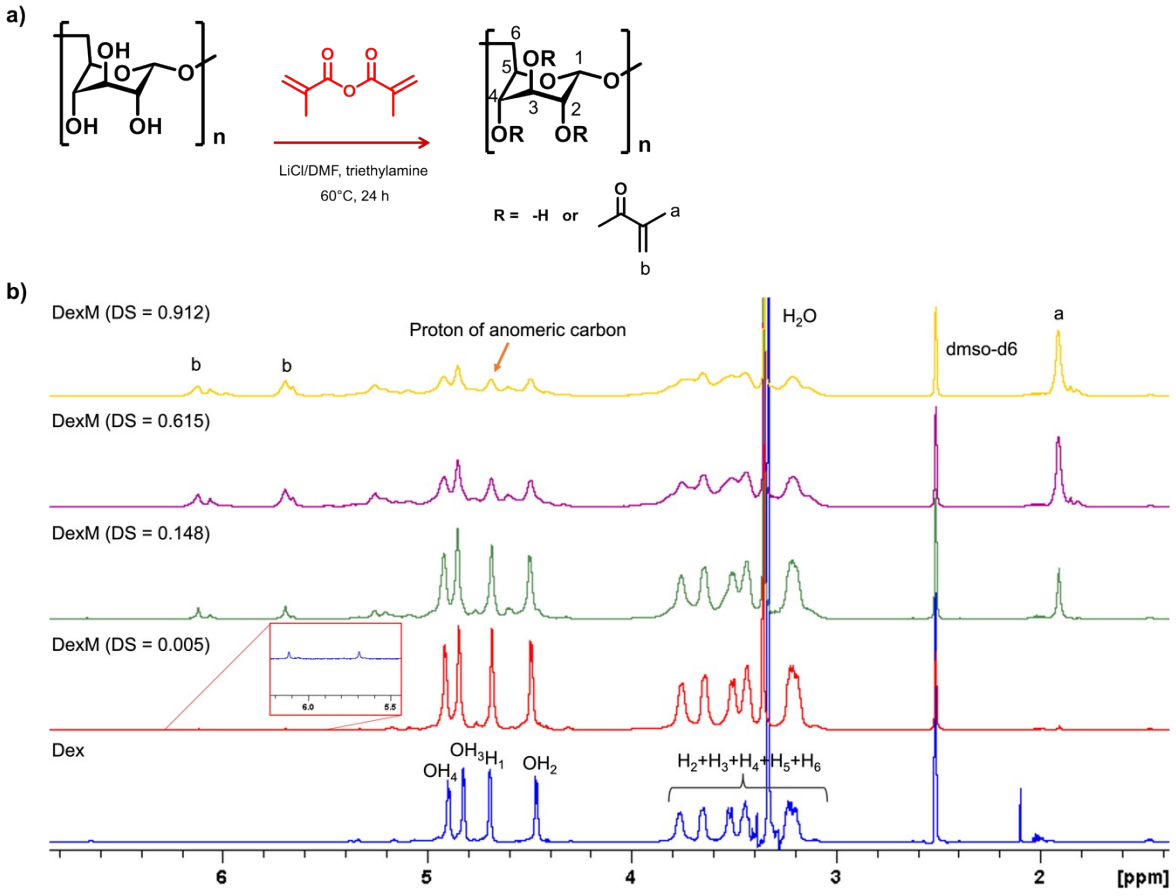

Figure S1. a) Synthetic procedure for the formation of dextran methacrylate (DexM) and b) ^{1}H NMR spectra of dextran (Dex) and DexM with various degrees of substitution in DMSO-d6.

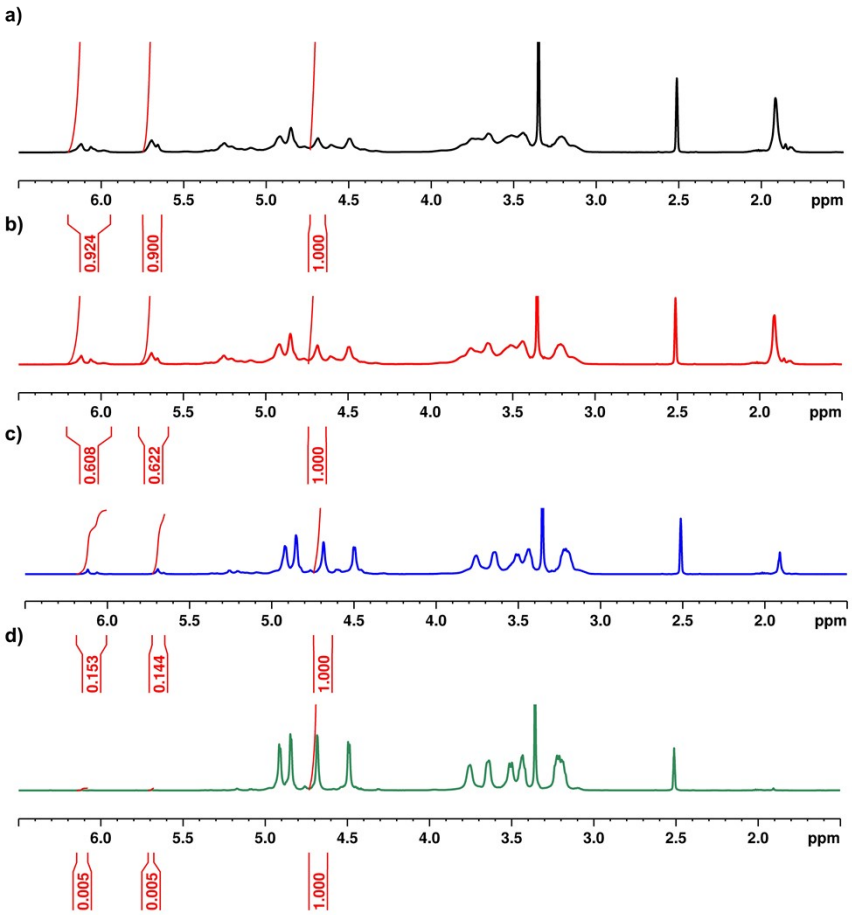

Figure S2. ^{1}H NMR spectra of DexM with various degrees of substitution a) 0.912, b) 0.615, c) 0.148, d) 0.005 in DMSO-d6.

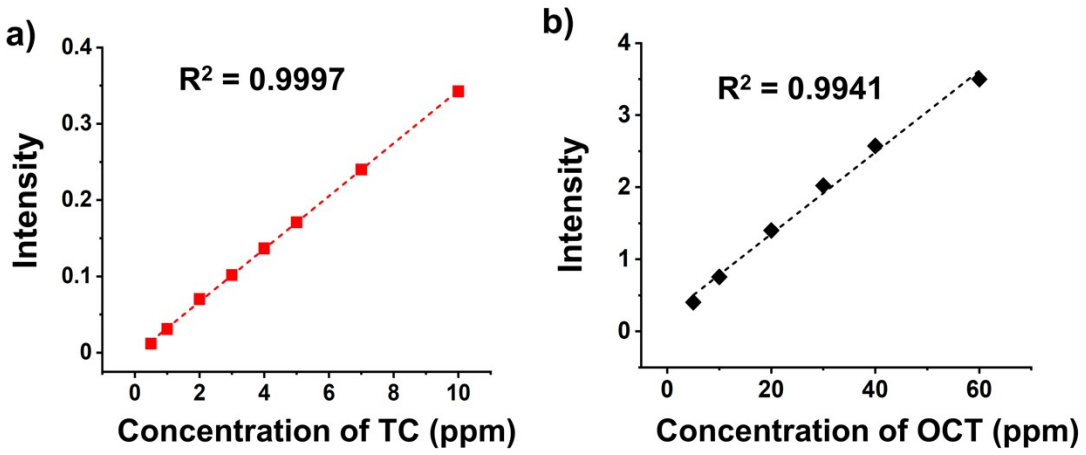

Figure S3. Calibration curves relating the absorption intensity of a) TC at 360 nm and b) OCT at 283 nm to the concentration of TC or OCT prepared with various dilutions in phosphate buffer pH 7.4 solution.

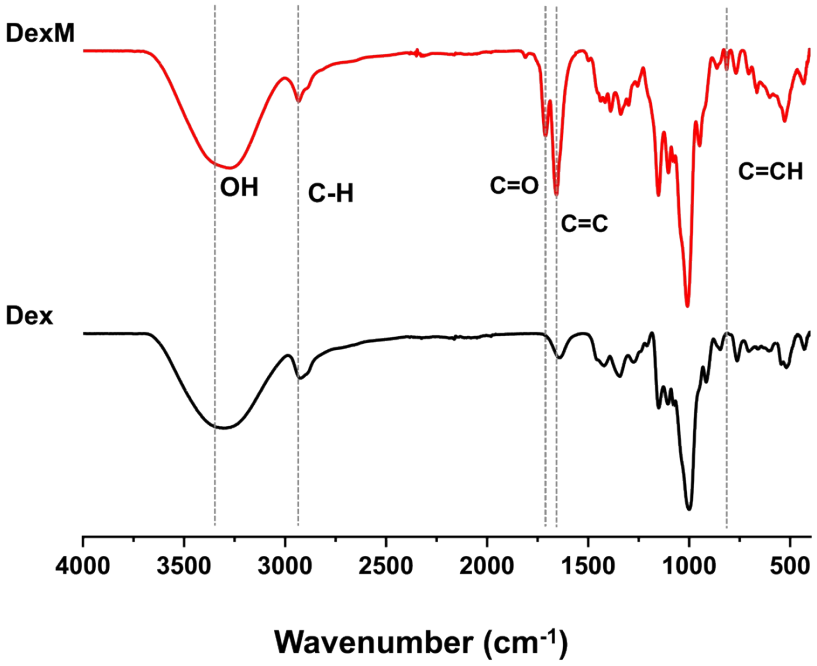

Figure S4. ATR-FTIR spectra of dextran (Dex) and dextran methacrylate (DexM) with DS = 0.148.

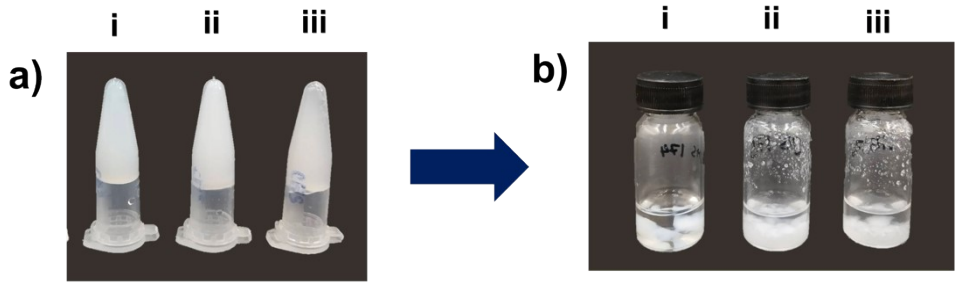

Figure S5. Photographs of dextran methacrylate (DexM) hydrogel/gels with degrees of substitution of i) DS = 0.148, ii) DS = 0.615, and iii) DS = 0.912 before (a) after (b) addition of water.

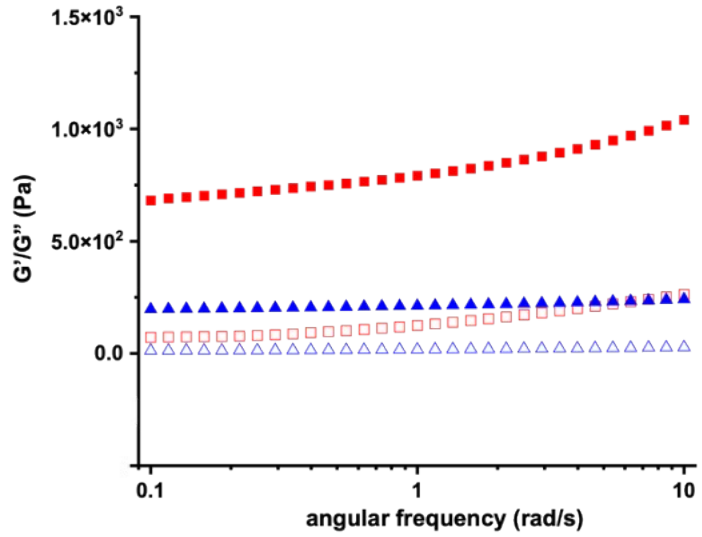

Figure S6. Frequency sweep measurement of G′ (filled symbols) and G″ (open symbols) of ▲, △, DexM-TA hydrogels and ■, □, DexM-IC hydrogels.

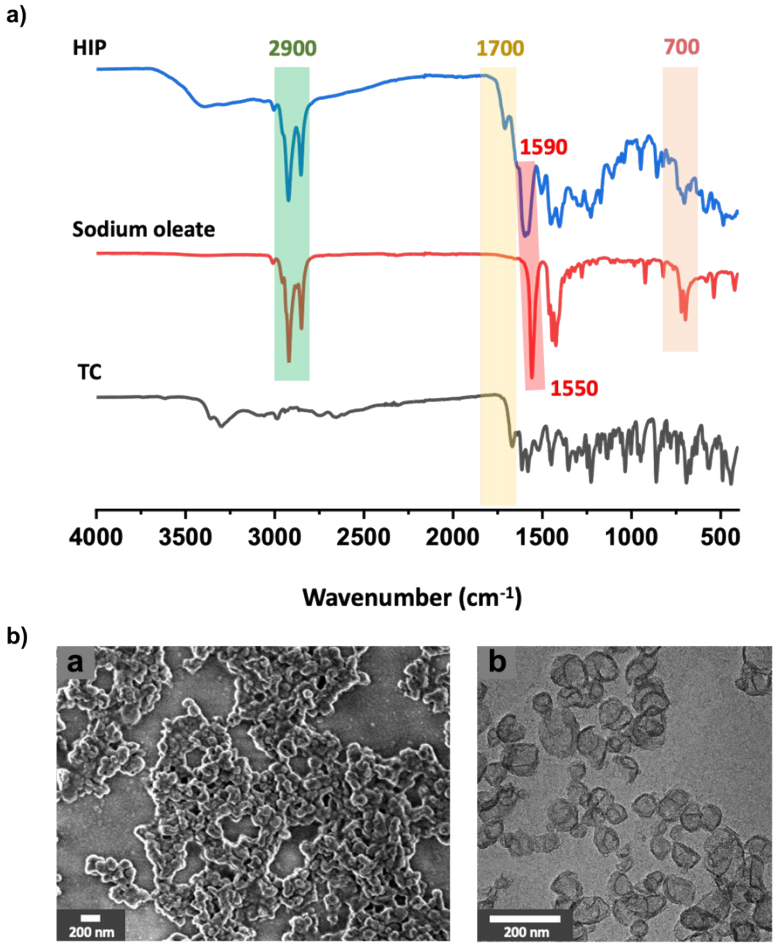

Figure S7. a) ATR-FTIR spectra of HIP, sodium oleate, and TC. b) SEM and TEM micrographs of SiO_2NCs-HIP.

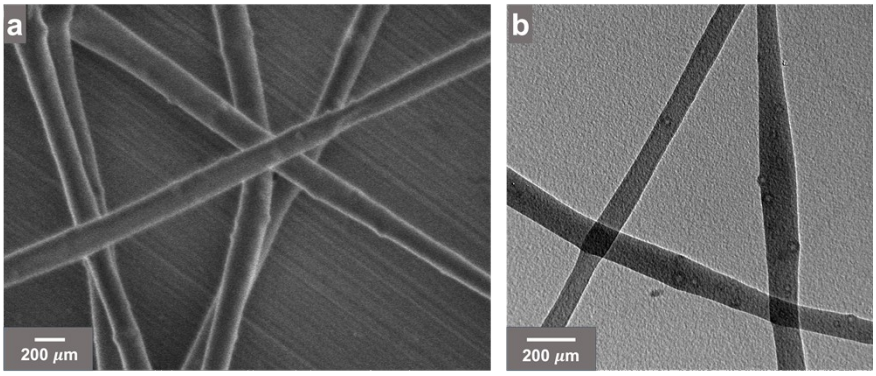

Figure S8. a) SEM and b) TEM micrographs of DexM-TA fibers embedding SiO_2NCs-HIP.

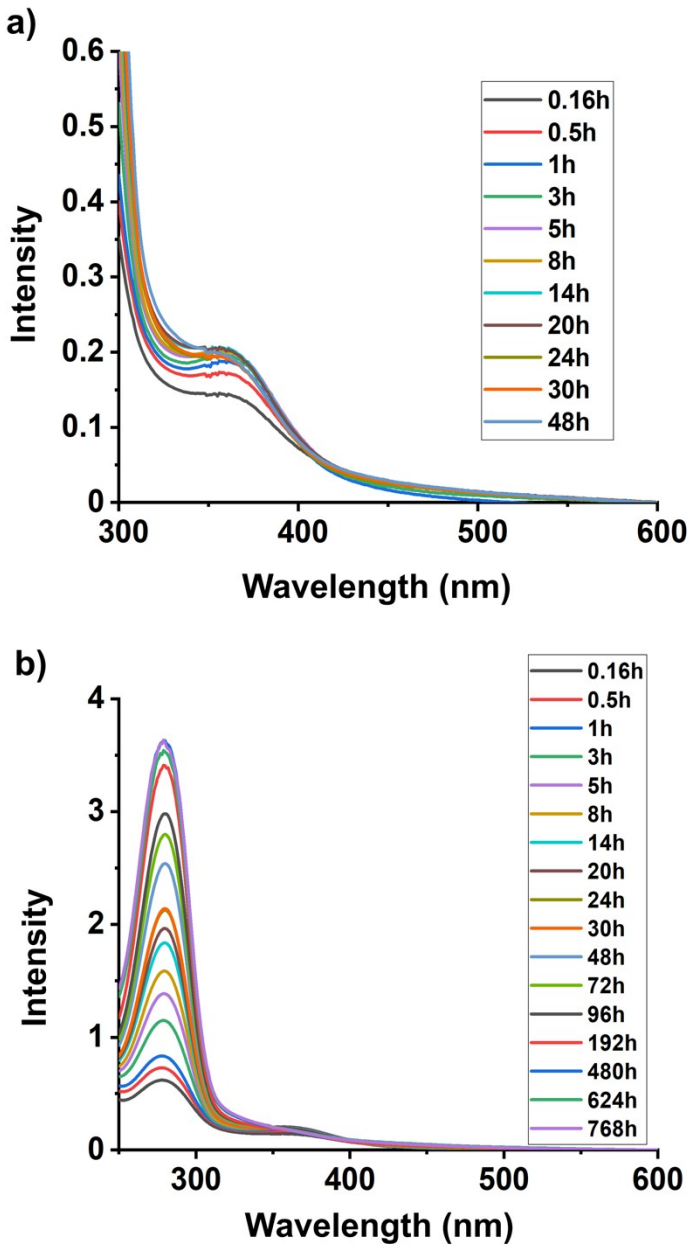

Figure S9. Temporal evolution of the absorption spectra of a) TC and b) OCT released from SiO_2NCs-HIP embedded in DexM-TA hydrogel in phosphate buffer at pH 7.4.

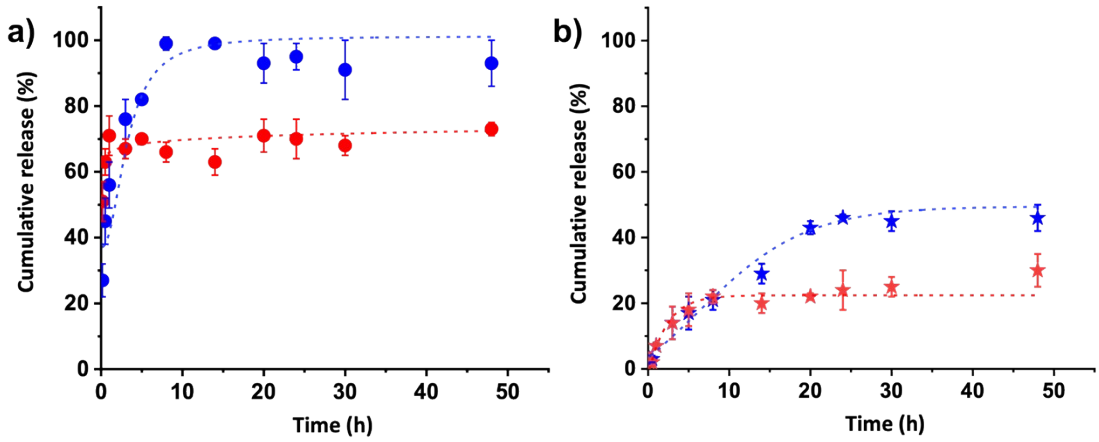

Figure S10. Release profiles of a: tetracycline hydrochloride (cycle shape) and b: octenidine (star shape) from DexM-TA physically embedding TC and OCT hydrogel (blue line). DexM-TA embedding SiO_2NCs-HIP hydrogel (red line) at pH 7.4.

Table S1. Compositions of the hydrogel precursors.

Entry	DexM-T fibers [wt%]	Dex-A fibers [wt%]	DexM-T-Dex-A fibers [wt%]	DexM-IC fibers [wt%]	Water [wt%]
1	9.55	8.05	0	0	82.40
2	0	0	17.00	0	83.00
3	0	0	0	12.20	87.80

References

1. S.-H. Kim and C.-C. Chu, *J. Biomed. Mater. Res.*, 2000, **49**, 517-527.
2. A. Jobdeedamrong, M. Theerasilp, N. Wongsuwan, N. Nasongkla and D. Crespy, *ChemComm*, 2020, **56**, 12725-12728.