Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Trehalose coated nanocellulose to inhibit the infections by *S. aureus*

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1. Experimental

1.1 Materials

Microcrystalline cellulose (MCCs; Sigma-Aldrich), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; 98%, Sigma-Aldrich), sodium bromide (NaBr; ≥99%, Sigma-Aldrich), sodium hypochlorite solution (NaOCl; chlorine 8 - 12%, Sigma-Aldrich), sodium hydroxide (NaOH; ≥97%, Sigma-Aldrich), hydrochloric acid (HCl; 32%, Sigma-Aldrich), ethanol (95%), potassium hydroxide (KOH; 85%, Sigma-Aldrich), magnesium sulfate ($MgSO_4$; ≥99.5%, Sigma-Aldrich), deuterated chloroform ($CDCl_3$; 100%, Sigma-Aldrich), deuterium oxide (D_2O; Sigma-Aldrich), dimethyl sulfoxide-*d6* (DMSO-d_6; 100%, Sigma-Aldrich), dichloromethane (DCM; Sigma-Aldrich), anhydrous dichloromethane (DCM; ≥99.8%, Sigma-Aldrich), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC ▪ HCl; Sigma-Aldrich), anhydrous trehalose (Thermo Scientific), trimethylsilyl chloride (Merck), pyridine anhydrous (99.8%, Sigma-Aldrich), triethyl amine (TEA, Sigma Aldrich), Amberlyst® 15 (Sigma-Aldrich), hexane (95%, Sigma-Aldrich), ethyl acetate (Sigma-Aldrich), *N,N*-dimethylformamide (DMF, Sigma-Aldrich), methanol, cyclohexyl isocyanide (98%, Sigma-Aldrich), 2-hydroxyethyl acrylate (HEA; Sigma-Aldrich), 1-amino-3,3-diethoxypropane (≥97%, Sigma-Aldrich), acetic anhydride (Chem-Supply), ciprofloxacin (>98%, Sigma-Aldrich) were used as received. Dialysis tubing was purchased from Thermo Scientific. 2,2-Azobis(isobutyrinitrile) (AIBN; 98%, Fluka) was purified by recrystallization from methanol. The cell lines were obtained from the CellBank Australia.

1.2 Analyses

Nuclear magnetic resonance (NMR) spectrometry. NMR data was obtained on a Bruker Avance III 300 MHz NMR spectrometers. The raw data was analyzed by using Bruker TOPSPIN 3.7.2 software. Chemical shifts are

reported in ppm relative to tetramethylsilane (δ = 0 ppm).

Size Exclusion Chromatography (SEC). The molecular weight and dispersity of polymers were studied by sized exclusion chromatography. The samples were prepared in *N*,*N*-dimethylformamide (DMF) and analyzed using a Shimadzu modular system comprising a degasser (DGU-12A), pump (LC-20AT), autoinjector (SIL-20AD), column oven (CTO-20A) and refractive index detector (RID-20A). Samples were separated by a guard column (50 × 7.8 mm) followed by three linear columns (300 × 7.8 mm) with 10^3, 10^4, 10^5 Å pore size and 5 μm particle size, respectively. The solvent system was *N,N*-dimethylformamide (HPLC grade, 0.05% w/v BHT, 0.03% w/v LiBr). The flow rate was set to 1 mL min^{-1} at 50 °C. The calibration was performed using polystyrene standards (0.5-1000 kDa, Polymer Laboratories) with narrow dispersity.

To provide the solubility of $PTre_{18}$ in DMF, the hydroxyl groups were acetylated prior the SEC analysis using acetic anhydride as follows: 15 μL of an acetic anhydride/pyridine mixture (v/v: 1/1) and 90 μL of DMF (containing 0.03% w/v LiBr) were added to ~2 mg of the polymer sample and heated at 70 °C for 2 h; the resulting clear solution was diluted with 900 μL of DMF (0.03% w/v LiBr), filtered through a syringe filter (0.45 μm), and analyzed.

Ultraviolet-Visible (UV-Vis) Spectroscopy. Cary 50 Bio UV-Vis spectrophotometer (Varian Australia Pty Ltd) was used to measure polymers and polymer-grafted CNFs. The samples dispersed in DPBS or Milli-Q water were measured using baseline correction. The peaks of interest were 305 nm for synthetic polymers and 277 nm for ciprofloxacin.

Dynamic Light Scattering (DLS). Zetasizer Nano ZS (Malvern) was used with a He-Ne laser (4 mV) operating at 632 nm and detection was at an angle of 173°. The temperature was kept constant at 25 °C. Samples was dissolved in Milli-*Q* water at concentration of less than 1 mg mL^{-1} to determine the hydrodynamic diameter and size distribution.

Transmission Electron Microscopy (TEM). TEM micrographs were obtained using JEOL 1400 transmission electron microscope operating at accelerating voltages up to 120 kV. TEM samples were prepared by dropping 5 μL particle solution (less than 1 mg mL^{-1}) on a formvar-coated copper grid and leave for 1 min. Excess solution was blotted away and samples were left to air dry overnight. The samples subsequently subjected to negative staining with Uranyl

acetate (UA, 2% aqueous solution). UA droplet was placed into the sample grids for 2 min, followed by blotting away the excess UA and washing with water. As dried with filter paper to remove water, the TEM samples were left to dry in the oven overnight.

1.2 Synthesis

Synthesis of TEMPO-oxidized CNFs

TEMPO-oxidized CNFs were synthesized based on literature procedure.[1] Microcrystalline cellulose (MCCs; 2 g) was suspended in Milli-Q water (200 mL) and stirred at room temperature. 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; 35 mg) and sodium bromide (NaBr; 0.58 g) were added into the suspension. Sodium hypochlorite (NaOCl; 8 mL) was added dropwise after TEMPO fully dissolved. The pH of reaction was monitored and adjusted to the range of 10 to 11 by adding an appropriate amount of NaOH solution (0.5 M). Following the consumption of NaOCl in the reaction, a second aliquot of NaOCl (8 mL) was added into the suspension and the addition of NaOCl was repeated until the overall volume of NaOCl close to 23 mL. The reaction was stirred for 24 h at room temperature and then quenched by the addition of ethanol (3 mL). The resulting mixture was dialyzed against Milli-Q water (6-8 kDa MWCO) for 2 days. The suspension was subsequently diluted with Milli-Q water to allow the concentration of CNF close to 0.2%, following with ultrasonication at room temperature for 15 min using BRANSON solid/liquid digital sonifier (450, amplitude= 30%). As the suspension became clear, CNFs-COO^- was treated with 1 M HCl solution for 30 min which resulted in the gel-like nanomaterial CNF-COOH. This product was washed with Milli-Q water and purified *via* centrifugation to remove excess HCl. The cycle of washing and centrifugation was conducted for 4 times to allow the supernatant to reach a neutral pH. The resulting CNFs gel was stored at 4 °C.

Synthesis of RAFT agent BSPA

3-(Benzylsulfanylthiocarbonylsulfanyl)-propionic acid (BSPA) was synthesised using a previously reported method.[2] 3-Mercapto propionic acid was added (2.12 g, 20 mmol) to K_3PO_4 (4.24 g, 20 mmol) in acetone (40 mL), followed by stirring for 10 min. CS_2 (4.56 g, 60 mmol) was added to the solution. After 10 min, benzyl bromide (3.40 g, 20 mmol) was added. The solvent was removed after 15 min under reduced pressure and the crude was added to a saturated solution of brine (200 mL) and extracted with CH_2Cl_2 (2 x 200 mL) and washed with saturated brine solution (3 x 200 mL). After drying the organic extracts over anhydrous $MgSO_4$, the solvent was removed under vacuum to give a yellow

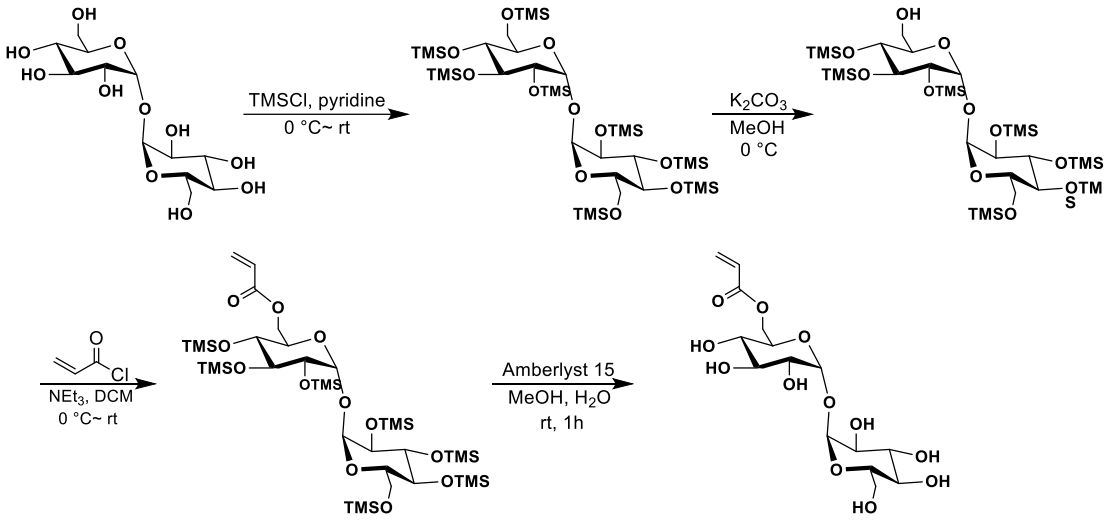
solid (5.4 g, 20 mmol, yield > 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.29-7.39 (m, *J* = 2.9 Hz, 5H, Ph), 4.63 (s, 2H, CH_2-Ph), 3.64 (t, *J* = 6.9 Hz, 2H, S-C*H*$_2$-CH_2), 2.87 (t, *J* = 6.9 Hz, 2H, S-CH_2-C*H*$_2$).

Synthesis of *N*-(2-formylethyl)-3-(benzylthiocarbonothioylthio)-propionamide (aldehyde-CTA)

3-(benzylthiocarbonothioylthio) propanoic acid (BSPA; 1.5 g, 5.5 mmol) and *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC▪HCl; 1.5 g, 7.8 mmol) was dissolved in anhydrous DCM (5 mL). The mixture was cooled down to 0 °C and stirred for 30 min, followed by the dropwise addition of a solution of 1-amino-3,3-diethoxypropane (0.81 g, 5.5 mmol) and 4-(dimethylamino)pyridine (0.2 g, 1.6 mmol) in anhydrous DCM (2 mL). After stirring for 1 h at 0 °C, the mixture was further reacted at room temperature for 22 h. The solution was diluted with DCM (30 mL) and washed with water and brine. The organic phase was dried with $MgSO_4$ and then concentrated under vacuum. The resulting product was dissolved in acetone (1 mL), followed by the dropwise addition of 10% HCl solution (1 mL) to liberate the aldehyde group. The solution was stirred for 30 min at room temperature, followed by dilution with DCM (30 mL) and washing with water and brine. The organic phase was dried with $MgSO_4$ and then concentrated under vacuum. The crude product was purified with column chromatography with a gradient hexane/ethyl acetate mixture (hexane: ethyl acetate 10:1 → 1:2) as eluent to yield the product as yellow solid (0.25g, 38%). ^{1}H NMR (300 MHz, $DMSO_{d6}$) δ 9.63 (t, *J*= 1.6 Hz, 1 H), 8.06 (t, *J*= 5.0 Hz, 1H), 7.41-7.29 (m, 5H), 4.67(s, 1H), 3.54 (t, *J*= 6.9 Hz, 2H), 3.29 (~t, *J*= 6.3 Hz, 2H), 2.58-2.53 (m, 2H), 2.50 (~t, *J*= 1.8Hz, 2 H).

Synthesis of 6-*O*-acryloyl-*α,α'*-D-trehalose (Tre-A)

Trehalose acrylate monomer was prepared according to the literature procedure[3] as depicted in **Scheme S1**.



Scheme S1. Synthesis of monomer Tre-A.

Synthesis of 2,3,4,6,2',3',4',6'-okta-O-trimethylsilyl-α,α'-D-trehalose (T_{MS}Tre)

Anhydrous trehalose (7.5 g, 21.9 mmol) was dissolved in anhydrous pyridine (80 mL) and cooled with an ice-water bath. Trimethylsilyl chloride (39.4 g, 362.8 mmol) was added dropwise under stirring. The mixture was then stirred overnight at room temperature. The solution was diluted with ethyl acetate and washed with water, following with dried with $MgSO_4$ and concentrated under vacuum. The crude product was evaporated twice with toluene to yield product as white crystals (19.7 g, 98%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.91 (d, *J* =3.2 Hz, 2H), 3.89 (t, *J* =9.0 Hz, 2H), 3.81-3.76 (ddd, *J* =9.5, 4.1, 2.3 Hz, 2H), 3.71-3.64 (m, 4H), 3.44 (t. *J* =9.1 Hz, 2H), 3.38 (dd, *J* =9.3, 3.2 Hz, 2H), 0.14, 0.14, 0.12, 0.10(4× s, 72H).

Synthesis of 2,3,4,2',3',4',6'-hepta-O-trimethylsilyl-α,α'-D-trehalose (T_{MS}Tre-6-OH)

T_{MS}Tre (3.95 g, 4.3 mmol) was dissolved in methanol (270 mL) and cooled with an ice-water bath. K_2CO_3 (0.57 g, 4.1 mmol) was added into the solution. After stirring for 18 min, the solution was diluted with hexane and washed twice with water. The organic phase was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified with silica gel flash chromatography, using hexane/ethyl acetate (98:2) as the eluent to afford products as colorless oil (1.71 g, 47%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.94 (d, *J* =3.1 Hz, 1H), 4.89 (d, *J* =3.1 Hz, 1H), 3.92 (t, *J* =9.0 Hz, 1H), 3.89 (t, *J* =9.0 Hz, 1H), 3.84 (dt, *J* =9.5, 3.5 Hz, 1H), 3.79 (ddd, *J* =9.5, 4.7, 2.0 Hz, 1H), 3.74-3.63 (m, 4H), 3.46 (t. *J* =9.1 Hz, 2H), 3.44-3.38 (m, 3H), 0.16, 0.14, 0.14, 0.14, 0.12, 0.11, 0.10 (7× s, 63H).

Synthesis of 6-O-acryloyl-2,3,4,2',3',4',6'-hepta-O-trimethylsilyl-α,α'-D- trehalose (T_{MSO}Tre-6-A)

T_{MS}Tre-6-OH (6.70 g, 7.9 mmol) and TEA (1.80 g, 17.8 mmol) were added into anhydrous DCM (30 mL) and cooled with an ice-water bath. A solution of acryloyl chloride (1.08 g, 11.9 mmol) in anhydrous DCM (20 mL) was added dropwise. The mixture was stirred and allowed to warm up to room temperature. After stirring for 2 h, the solution was diluted with DCM and washed twice with water. The organic phase was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified using silica gel flash chromatography, using hexane/ethyl acetate (98:2) as eluent to afford product as colorless oil (5.26 g, 74%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.44 (dd, *J* =17.3, 10.4 Hz,1H), 6.18 (dd, *J* =17.3, 10.4 Hz,1H), 5.84 (dd, *J* = 10.4,1.4 Hz,1H), 4.94 (d, *J* =3.1 Hz, 1H), 4.90 (d, *J* =3.1 Hz, 1H), 4.37, (dd, *J* =12.0, 2.4 Hz,1H), 4.16 (dd, *J* =12.0, 4.4 Hz,1H),4.05 (ddd, *J* =9.6, 4.4, 2.4 Hz, 1H), 3.94-3.86 (m, 2H), 3.77 (m, 2H), 3.70-3.64 (m, 2H), 3.51 (dd, *J* = 9.5, 8.6 Hz, 1H), 3.47-3.42 (m, 2H), 3.40 (dd, *J* =9.3, 3.1 Hz, 1H),0.15, 0.14, 0.13, 0.12, 0.08 (5× s, 63H).

Synthesis of 6-O-acryloyl-α,α'-D- trehalose (Tre-A)

T_{MSO}Tre-6-A (2.25 g, 2.5 mmol) was dissolved in the mixture of methanol (330 mL) and water (30 mL), following by the addition of Amberlyst® 15 (8.8 g). The mixture was stirred for 1 h at room temperature. Amberlyst® 15 was then filtered off and the filtrate was washed with hexane to remove protected monomer. The aqueous phase was concentrated under vacuum at 30 °C to 15 mL. The resulting solution was filtered using a syringe filter (0.45 μm) and subsequently lyophilized to afford the product as white solid (0.97 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.32 (dd, *J* = 17.3, 1.7 Hz, 1H), 6.17 (dd, *J* = 17.3, 10.3 Hz, 1H), 5.94 (dd, *J* = 10.3, 1.7 Hz, 1H), 5.09 (d, *J* = 5.3 Hz, 1H), 4.89-4.85 (m, 2H), 4.84 (d, *J* = 3.6 Hz,1H), 4.76 (d, *J* = 5.2 Hz, 1H), 4.74 (d, *J* = 4.9 Hz, 1H), 4.69 (d, *J* = 6.0 Hz, 1H), 4.67 (d. , *J* = 6.3 Hz, 1H), 4.34 (t, *J* = 5.9 Hz, 1H), 4.29 (dd, *J* = 11.8, 2.1 Hz,1H), 4.16 (dd, *J* = 11.8, 5.7 Hz, 1H), 3.96 (ddd, , *J* = 10.1, 5.6, 2.1 Hz, 1H), 3.64 (ddd, *J* = 9.9, 4.9, 2.3 Hz , 1H), 3.60-3.50 (m, 3H), 3.47 (ddd, *J* = 11.4, 6.2, 5.0 Hz, 1H), 3.30-3.21 (m, 2H), 3.21-3.08 (m, 2H).

Representative Synthesis of Aldehyde-terminated polymer: synthesis of Poly(trehalose acrylate) ($PTre_{18}$)

The monomer TreA (198 mg, 0.5 mmol), aldehyde-CTA (8.2 mg, 25.0 μmol) and ACVA (0.7 mg, 2.5 μmol) was dissolved in DMSO (0.5 mL) in a 4 mL glass vial. The mixture was degassed by purging with nitrogen for 1h at room temperature. The solution was polymerized at 70 °C for 10 h at a monomer concentration of

1M, then stopped by cooling the solution with an ice bath and introducing air. Polymers were purified by dialysis against Milli-Q water for 24 h (3.5 kDa MWCO), followed by lyophilization to yield the product as yellow solid.

Representative Synthesis of Polymer Grafted CNFs *via* Passerini Reaction

The surface modification *via* the Passerini reaction was carried out using a literature procedure.[1] $PTre_{18}$ (9 mg, 1.2 μmol) was dissolved in Milli-Q water (0.1 mL) and mixed with CNF-COOH gel (1.72% w/w; 319 mg, 11.2 μmol COOH), followed by the addition of cyclohexyl isocyanide (1.2 mg, 11.0 μmol). The mixture was stirred for 24 h at room temperature. The resulting gel was diluted with Milli-Q water (2 mL) and then dialyzed against Milli-Q water for 2 days (12-14 kDa MWCO). PTre-grafted CNFs was stored as a solution at 4 °C.

1.3 Methods

Determination of the Amount of Carboxyl Acid Groups on CNFs (Titration). The amount of COOH groups on CNFs was determined using an established method.[1] CNF-COOH gel was freeze dried and then 2 mg of CNFs was dispersed in Milli-Q water (10 mL). Whilst stirring, 5 μL of 0.1M NaOH solution was added to the CNF solution with the pH change monitored using a pH meter until a pH value of 10 was reached.

Quantification of Grafting efficiency. A Calibration curve was established by measuring the UV-Vis absorption at 305 nm of the polymers at various concentrations based on a literature work.[1] Polymer grafted CNFs were then measured by UV-Vis spectroscopy at concentrations in the range of 0.4 – 1.3 mg mL^{-1}. Grafting efficiency was then calculated using the calibration curve.

Drug Loading. Ciprofloxacin was suspended in the mixture of H_2O and acetone (1/1) at a concentration of 0.2 mg mL^{-1}. The suspension was sonicated for 5 min followed by the dropwise addition of 0.1 M HCl to adjust the pH to 6.6-6.7 resulting in a clear stock solution of ciprofloxacin (0.2 mg mL^{-1}). 1.429 mL stock solution of CNF-PTre (2.8 mg mL^{-1}) was mixed with 3.36 mL stock solution of ciprofloxacin (0.02 mg mL^{-1}) in a 20 mL glass vial. The mixture was sealed and stirred at room temperature for 8 h. After this, 1 mL Milli-Q water was added into the mixture and then the mixture was stirred with an open lid for 16 h at room temperature to allow the evaporation of acetone. The drug loaded CNF-PTre was collected by centrifugation at 14000 rpm for 5 min and redispersed in Milli-Q water three times. The supernatant was combined and measured by UV-

Vis spectroscopy to determine the drug loading efficiency.

***In vitro* Cell Culture.** Mouse macrophages RAW 264.7 and human umbilical vein endothelial cells (HUVEC) were cultured in T75 cell culture flasks with 5% CO_2 at 37 °C. RAW 264.7 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 2% glutamax, whilst HUVECs were cultured in commercial endothelial cell growth medium. After reaching 70% confluence, the cells were passaged by treatment with trypsin/EDTA mixture. Cells were collected by centrifuge at 180*g* for 5 min. Notably, the HUVECs used for experiments were between passage 3 to passage 7. Bacteria *Staphylococcus aureus* 38 and *Pseudomonas aeruginosa* PAO1 were grown in Tryptic Soy Broth (TSB) overnight at 37 °C prior to treatment with particles. The bacteria were collected by centrifuge at 3000*g* for 10 min. These were then analyzed for using a microplate reader (FLUOstar Omega) where an OD_{600nm} of 0.1 indicated the density was 10^8 CFU mL^{-1} (CFU: colony forming units).

Cytotoxicity Assay. The cytotoxicity of functional CNFs against RAW 264.7 and HUVECs were evaluated by WST-1 assay. RAW 264.7 were seeded at a density of 4×10^3 cells per well, whilst HUVEC were seeded at a density of 10^4 cells per well in 96-well cell culture plates respectively. Cells were cultured with 5% CO_2 at 37 °C for 1 day with 100 µL of medium per well. The solutions of functional CNFs were serially diluted (2 × dilution) with sterile Milli-Q water to gradient concentrations. The medium in each well was then replaced by 100 µL 2 × concentrated DMEM medium and mixed with 100 µL of the sample solution. After incubation of 48 h, the cells were washed with DPBS twice, followed by 100 µL of fresh warm DMEM medium along with 5 µL WST-1 added into each well. The plates were further incubated for 3.5 h, followed by the microplate reader measurements. The absorbance of samples against the background control was measured on a Benchmark Microplate Reader (Bio-Rad) at wavelength 450 nm with a reference wavelength of 655 nm.

Inhibition Assay by Flow Cytometry. The inhibition assay was performed *via* flow cytometry based on our previous work.[4]

Determination of Minimum Inhibitory Concentration (MIC). Stock solution of samples was sterilized by UV irradiation for 30 min in a biosafety cabinet prior to series dilution. Bacteria was cultured overnight and diluted to a suspension at bacterial density of 10^6 CFU mL^{-1} in Mueller-Hinton broth (MHB)

II medium. The sample solution was serially diluted (2 × dilution) with MHB II medium to gradient concentrations. 50 μL of each dilution was added into each well of 96-well plate, followed by adding 50 μL of bacterial suspension. Non-treated bacteria and medium were included as positive control and blank, respectively. The plate was incubated in a shaking incubator (180 rpm) at 37 °C. After 18 h, the plate was observed with naked eyes. Clear solution indicated the absence of bacteria growth while cloudy solution indicated growth. MIC was recorded as the lowest concentration of polymer that prevented visible turbidity in the medium. The tests were conducted at least twice and repeated in three independent experiments.

***In vitro* Drug Release Study.** The drug release study was conducted in either acetate buffer (0.1 M, pH 5.5) or phosphate buffer solution (0.1M, pH=7.4). 0.5 mL stock solution of ciprofloxacin-loaded CNF-PTre (CNF-PTre-Cip; 2 mg mL^{-1}) was placed in a dialysis membrane (3.5 kDa) and dialyzed against the buffer solution (14.5 mL) at 37 °C. To determine the drug release, 0.7 mL buffer solution was taken for measurement using UV-Vis spectroscopy at multiple time points. The sample volume was replaced by adding fresh buffer to the solution.

2. Characterization

2.1 TEMPO-oxidized CNFs

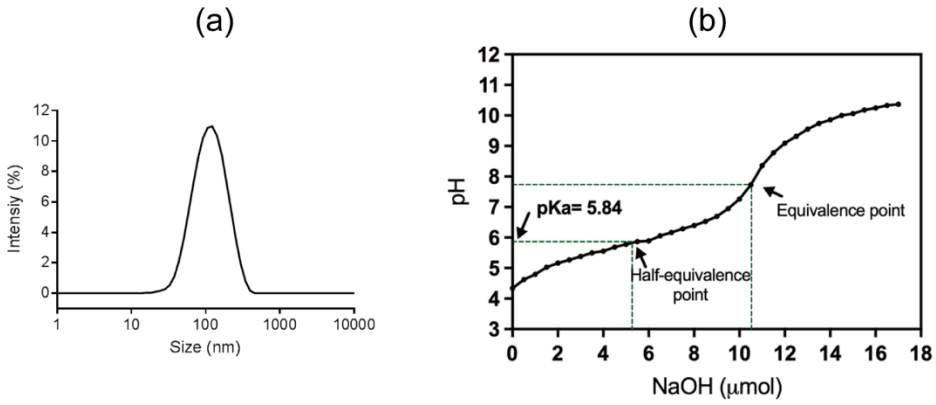


Figure S1. (a) Size distribution of CNF-COONa (pH=10) by DLS study. (b) Titration curve of CNFs after acid treatment using 0.1 M NaOH solution (moles of COOH= 2.24 mmol g^{-1} of CNFs; pK_a=5.84).

2.2 Trehalose monomer and aldehyde-terminated polymers

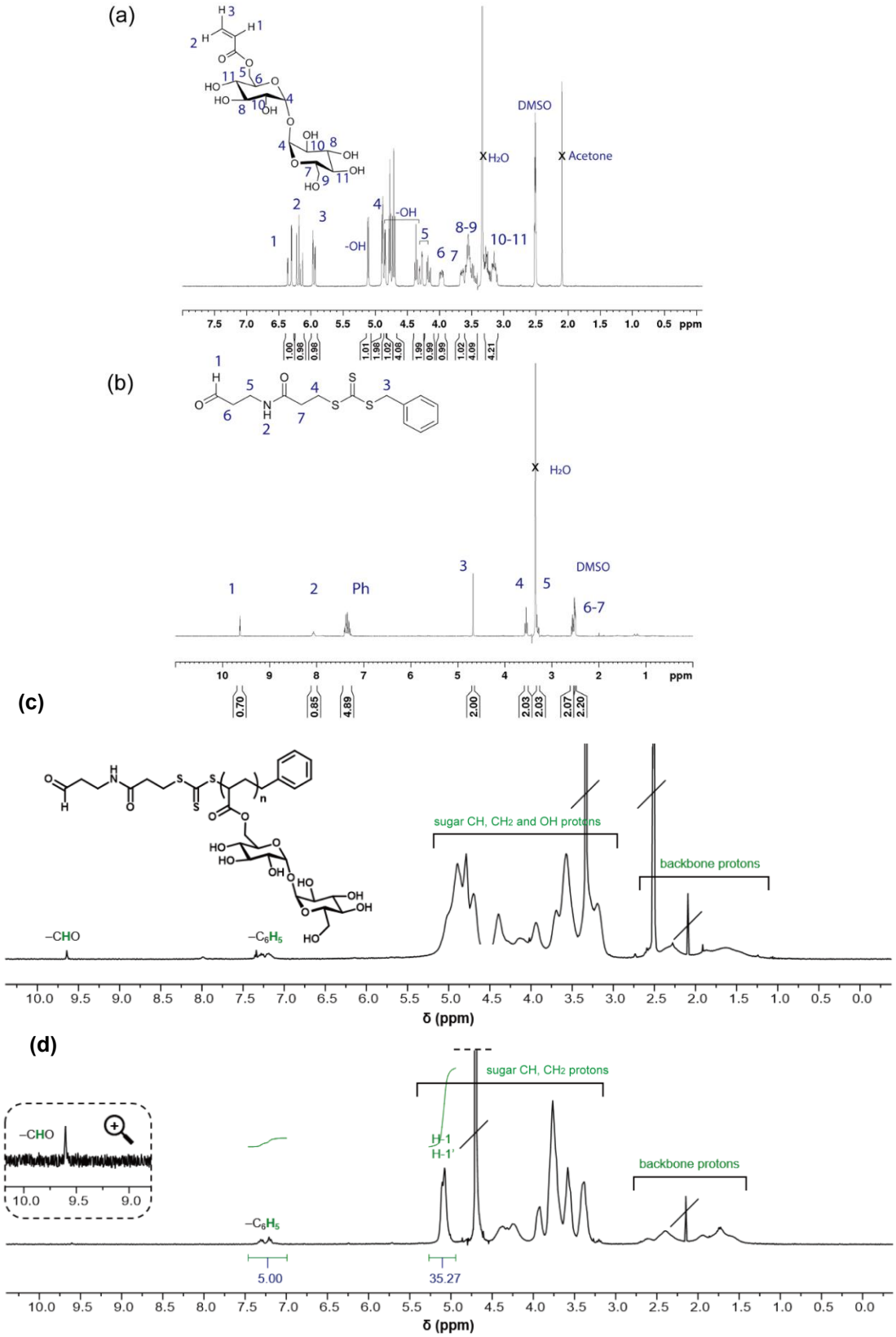


Figure S2. ^{1}H-NMR spectra of (a) monomer Tre-a (300 MHz, $DMSO_{d6}$); (b) aldehyde-CTA (300 MHz, $DMSO_{d6}$); (c) polymer $PTre_{18}$ (300 MHz, $DMSO_{d6}$) and (d) $PTre_{18}$ (300 MHz, D_2O)

Table S1. Summary of aldehyde-terminated polymers by RAFT polymerization, [M]:[R]:[I] represents [monomer]:[Aldehyde-CTA]:[AIBN].

Polymer	[M]:[R]:[I]	Reaction Time (h)	Conversion	$M_{n(NMR)}$/ g mol^{-1} [b]	$M_{n(SEC)}$/ g mol^{-1} [c]	$Đ$ (SEC)
$PTre_{18}$	20:1:0.2	10	88%	12400 [a]	10800 [a]	1.07
$PHEA_{16}$	30:1:0.1	2	53%	2200	5100	1.10

[a] Trehalose polymer was protected with acetyl groups prior to SEC measurements in DMF.

[b] Calculated from conversion by analyzing ^{1}H-NMR spectra.

[c] Determined with DMF-SEC.

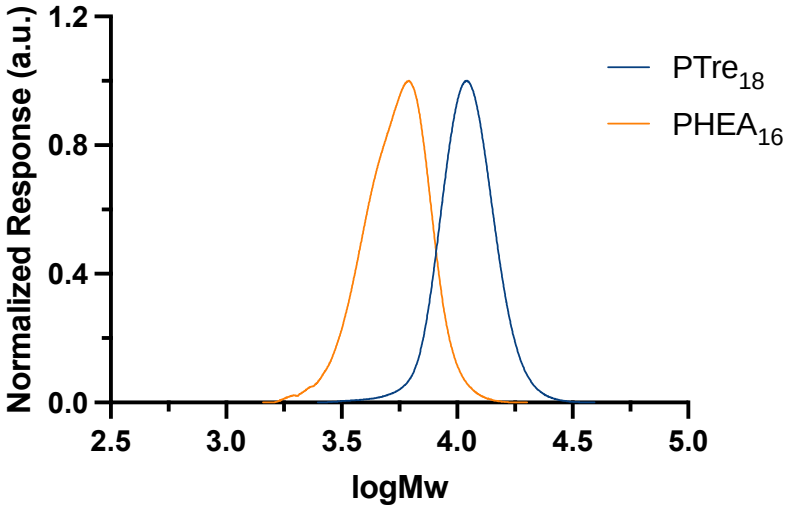


Figure S3. SEC traces of acetylated $PTre_{18}$ and $PHEA_{16}$ in DMF

2.3 Surface modification via Passerini reaction

Table S2 Hydrodynamic diameter D_h of the various polymer coated CNF

	D_h (nm)	PDI
CNF	296	0.28
CNF-PTre	337	0.40
CNF-PHEA	494	0.51

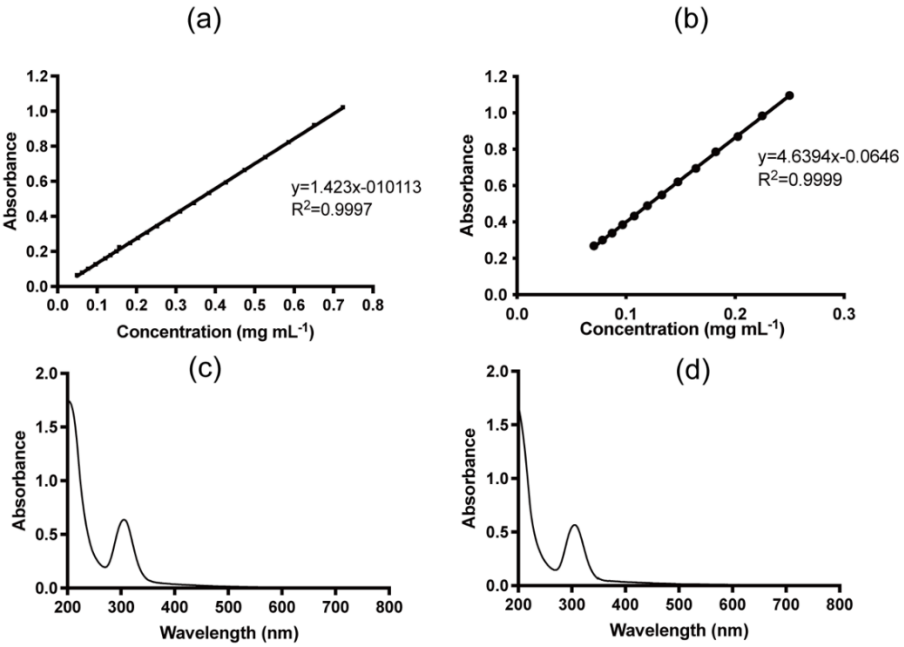


Figure S4. Grafting densities determined using UV-Vis spectroscopy. Calibration curve of (a) $PTre_{18}$ and (b) $PHEA_{16}$ in water determined by UV-Vis spectroscopy. UV-Vis spectra of (c) CNF-PTre (1.3 mg mL^{-1}) in water (A= 0.637 at 305 nm) and (d) CNF-PHEA (0.5 mg mL^{-1}) in water (A= 0.566 at 305 nm).

3. Anti-adhesion assay

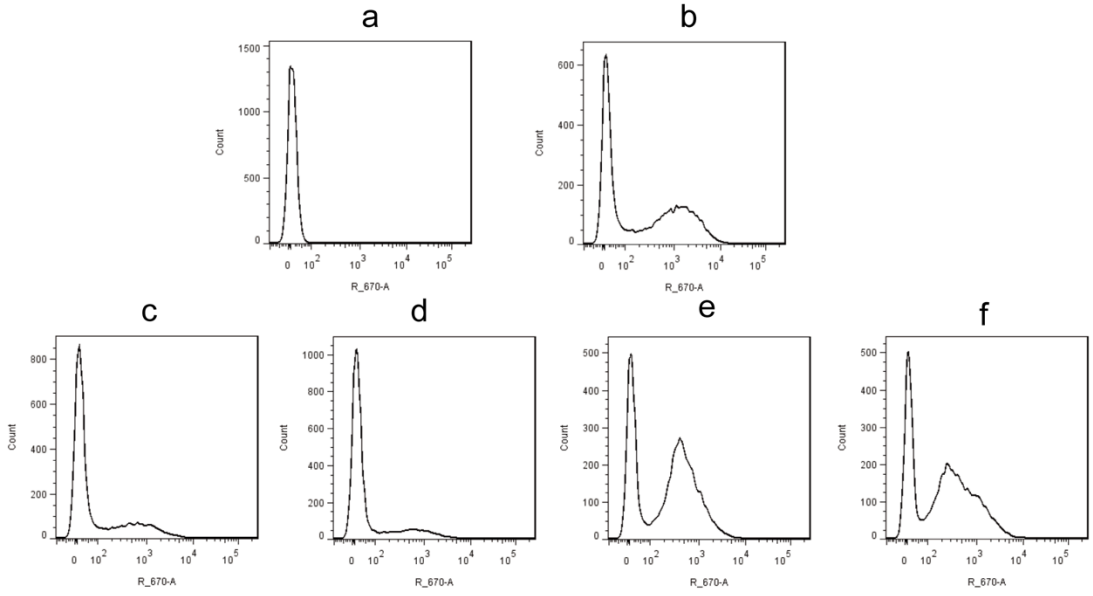


Figure S5. Representative flow cytometry results at 670 nm excitation (DiD channel) analysed by Flowjo. (a) Non-infected HUVECs; (b) Non-treated infective HUVECs; infective HUVECs treated with (c) CNF-PTre (0.1 mg mL^{-1}) and (d) CNF-PTre (0.2 mg mL^{-1}); infective HUVECs treated with (e) CNF-PHEA (0.1 mg mL^{-1}) and (f) CNF-PHEA (0.2 mg mL^{-1}).

Table S3. Relative infection rate determined by flow cytometry and molar concentration of grafting polymers used in this experiment.

	CNF-PTre (μg mL^{-1})		CNF-PHEA (μg mL^{-1})	
	100	200	100	200
Relative infection (%)	27.0±0.9	20.4±0.2	57.3±1.3	46.2±2.5
[$PTre_{18}$] (μM)	4.7	9.3		
[$PHEA_{16}$] (μM)			12.3	24.6

4. Ciprofloxacin loading

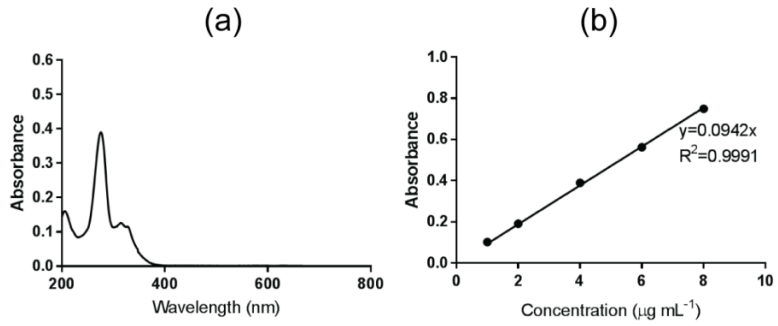


Figure S6. (a) An example of UV-Vis spectrum of ciprofloxacin (4 μg mL^{-1}) in Milli-Q water. (b) calibration curve of ciprofloxacin determined by UV-Vis spectroscopy.

Equation of drug loading efficiency:

$$Loading\ efficiency\ (\%) = \frac{Amount\ of\ drug\ loaded}{Amount\ of\ drug\ added\ initially} \times 100\%$$

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