Supporting Information

Modification of a Conventional Polyurethane Composition Provides Significant Anti-Biofilm Activity Against *Escherichia coli*

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Materials

Di-tert-butyl dicarbonate was purchased from Oakwood Chemical. Live/dead BacLight Bacterial Viability Kit, and Pierce LDH Cytotoxicity Assay Kit were purchased from Thermo Fisher Scientific. 4N HCl in 1,4-dioxane, dibutyltin dilaurate, 1,4-butanediol, 4,4'-methylenebis(cyclohexyl isocyanate), sodium phosphate dibasic, potassium phosphate monobasic, potassium chloride, calcium chloride, Trizma® base, poly(tetramethylene oxide) (average M_n ~2000), and Triton X-100 were purchased from Sigma-Aldrich. Diethanolamine and 6-aminohexanoic acid were purchased from Alfa Aesar. Thionyl chloride was purchased from TCI America. Trypticase Soy Broth (TSB) was purchased from Becton-Dickinson. Defibrinated sheep blood was purchased from Hardy Diagnostics. Penicillin/streptomycin, fetal bovine serum, and DMEM were purchased from HyClone. M9 minimal salts, 5x was purchased from DIFCO Laboratories. *Escherichia coli* (ATCC 25922) and NIH-3T3 fibroblast cells (ATCC CRL-1658) were purchased from ATCC. Anhydrous methanol was purchased from EMD Millipore. Unless otherwise stated, all reagents were used as received. Dichloromethane was dried by distilling over calcium hydride. Boc protected amine functionalized diol was synthesized according to a reported procedure.[1]

Analytical Methods

NMR spectra were recorded on a Varian NMRS 300 MHz instrument. ^{1}H NMR chemical shifts are reported in ppm relative to the solvent's residual ^{1}H signal. Size exclusion chromatography (SEC) analysis in DMF was performed on an HLC-8320 GPC from TOSOH equipped with an RI detector using PS as the standard. Contact angle measurements were performed on a rame-hart model 500 advanced goniometer with the DROPimage software. Absorbance and fluorescence spectroscopy were performed using a BioTek Synergy H1 plate reader. The morphology of the bacteria was characterized by scanning electron microscopy (SEM) using JEOL-JSM-7401F. Fluorescence imaging was performed on a U-TV1X-2 fluorescence microscope (Olympus Co., Japan) under 100x and 400x magnification with standard FITC/TRITC filter set. Solid-state NMR experiments were carried out by BRUKER Avance 300 equipped with a 4 mm double resonance VT CP/MAS probe. The ^{1}H carrier frequency is 300.1 MHz. Samples were packed in a 4mm Zirconia MAS rotor with a Kel-F drive cap and were measured with a MAS frequency of 10 kHz at 298 K. The CH_3 signal of TMS was set to 0.2 ppm as an external chemical shift reference. ^{1}H 90deg pulse was adjusted to 2.5 us. Recycle delay was 2 s and accumulation number was 16.

Synthesis of Tecoflex®

In a round-bottom flask equipped with a magnetic stir bar, 4,4'-methylenebis(cyclohexyl isocyanate) (MDI) (0.524 g, 2.00 mmol), poly(tetramethylene oxide) (PTMO) (2.021 g, 1.00 mmol), and 15 mL of anhydrous CH_2Cl_2 were added. Then, 10 µL of dibutyltin dilaurate (DBTDL) was added and the reaction was carried out at 50 °C for 4 hours. Then, a solution of 1,4-butanediol (0.097 g, 1.05 mmol) in 4 mL of anhydrous CH_2Cl_2 as well as 10 µL of DBTDL were added to the reaction solution and stirred at room temperature for 24 hours. The product was purified by precipitation in diethyl ether 3 times and dried under vacuum.

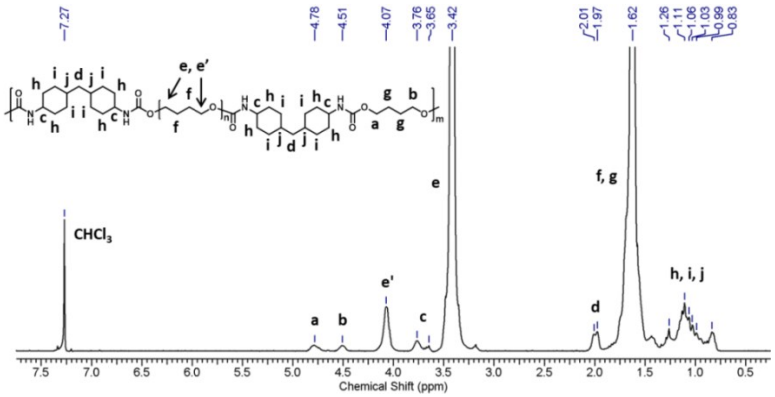

Figure S1. ^{1}H NMR spectra of Tecoflex®

Synthesis of Tecoflex-NH_3^+

In a round-bottom flask equipped with a magnetic stir bar, 4,4'-methylenebis(cyclohexyl isocyanate) (MDI) (0.524 g, 2.00 mmol), poly(tetramethylene oxide) (PTMO) (2.021 g, 1.00 mmol), and 15 mL of anhydrous CH_2Cl_2 were added. Then, 10 µL of dibutyltin dilaurate (DBTDL) was added and the reaction was carried out at 50 °C for 4 hours. Then, a solution of Boc protected amine functionalized diol (0.334 g, 1.05 mmol) in 4 mL of anhydrous CH_2Cl_2 as well as 10 µL of DBTDL were added to the reaction solution and stirred at room temperature for 24 hours. The resulting polymer was purified by precipitation in diethyl ether 3 times and dried under vacuum.

In a round-bottom flask equipped with a magnetic stir bar, the Boc protected amine functionalized polyurethane (0.5 g) was dissolved in 6 mL of anhydrous CH_2Cl_2. Then, 3 mL of 4N HCl in 1,4-dioxane was added and the reaction was carried out at room temperature for 45 minutes. After that, the solvent was removed under vacuum to the final product.

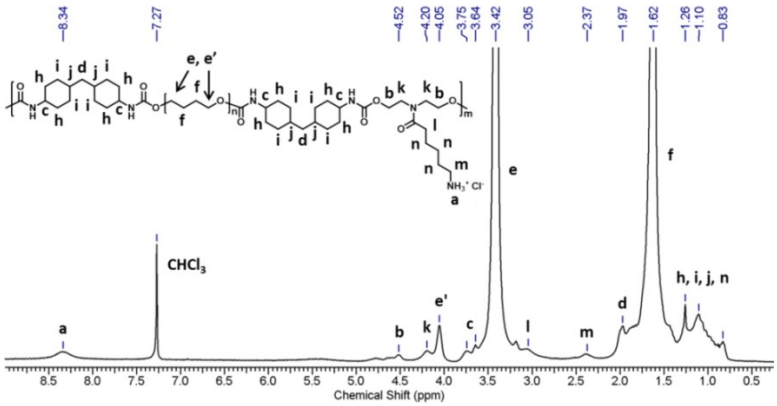

Figure S2. ^{1}H NMR spectra of Tecoflex-NH_3^+

Table S1. Molecular weight information of the polymers

polymer	M_n (kDa)[a]	M_w (kDa)[a]	Đ (M_w/M_n)[a]
Tecoflex®	41.5	65.2	1.6
Tecoflex-NH_3^+	30.7[b]	46.7[b]	1.5[b]

[a]Determined by SEC using DMF with 25 mM LiBr as the eluent. [b]Determined before deprotection.

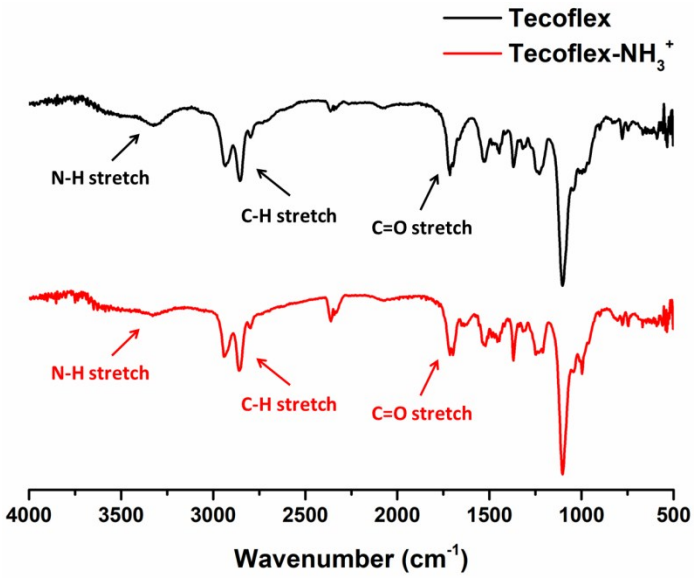

Figure S3. IR spectra of Tecoflex® and Tecoflex-NH_3^+

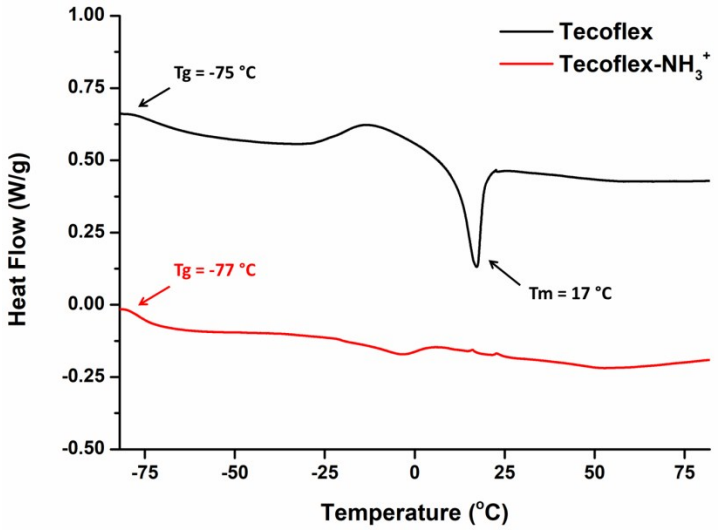

Figure S4. DSC curves of Tecoflex® and Tecoflex-NH_3^+

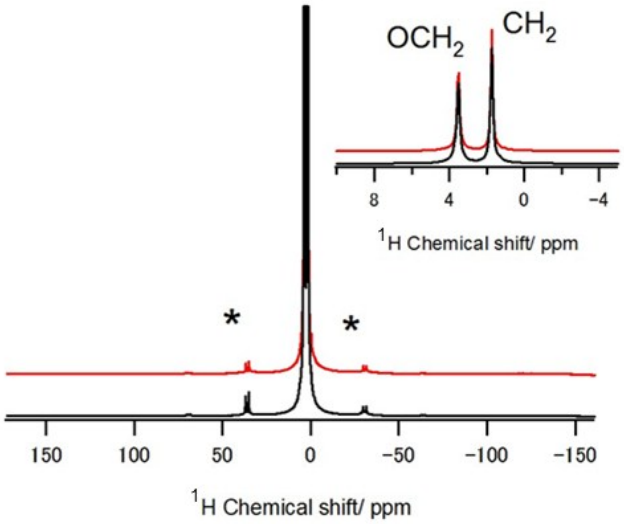

Figure S5. Solid-state ^{1}H MAS NMR spectra of Tecoflex® (black) and Tecoflex-NH_3^+ (red) recorded at 298K. The peaks at 3.5 ppm and 1.7 ppm (inset) correspond to the -OCH_2 and CH_2 peaks of PTMO, respectively. The narrow peaks show that the polymers are amorphous in nature. The full spectrum (150 to -150 ppm) shows no evidence of crystalline structure at the test conditions. Asterix show the spinning side bands.

Bacteria Culture

An overnight culture of *E. coli* (ATCC 25922) was used to inoculate 3 mL of TSB and incubated at 37 °C on a shaker until the culture reached an OD_{600nm} of 0.4-0.8. The suspension was diluted to OD_{600nm} of 0.1 and subsequently diluted 100 times in M9 minimal medium to give a suspension of approximately 1×10^6 colony forming units (CFU)/mL. Then, 2 mL of the resulting bacteria suspension was added to each well of a 12-well plate with either Tecoflex® or Tecoflex-NH_3^+ coated coverslips. The well plate was incubated in an incubator at 37 °C for 1 day, 3 days or 5 days, and the M9 minimal medium was refreshed every 24

hours. The bacteria attached on the polymer coated coverslips were characterized by fluorescence microscopy or scanning electron microscopy (SEM).

Bactericidal Activity of Polymer Coatings on Planktonic Bacteria

An *E. coli* (ATCC 25922) suspension in M9MM with approximately 1×10^6 CFU/mL was prepared. The polymer coated coverslips and the blank coverslips were placed into a 12-well plate, and 2 mL of the bacteria suspension was added to each well and incubated at 37 °C for 24 hours. After that, the bacteria were serially diluted in PBS, spread onto trypticase soy agar (TSA) plates, and incubated at 37 °C overnight to determine the number of viable bacteria.

Disk Diffusion Assays

An *E. coli* (ATCC 25922) suspension in M9MM with approximately 1×10^6 CFU/mL was prepared and 100 μL of the bacteria suspension was spread onto TSA plates. The polymer coated coverslips and the blank coverslips were placed on the inoculated agar surface and incubated at 37 °C for 24 h, the inhibition zone diameters were determined.

SEM Study of Bacteria on the Polymer Coated Coverslips

An *E. coli* (ATCC 25922) suspension in M9MM with approximately 1×10^6 CFU/mL was prepared, and 2 mL of the bacteria suspension was added to each well and incubated at 37 °C for 1 day, 3 days and 5 days. The M9 minimal medium was refreshed every 24 hours. At each time interval, the coverslips were removed from the media and gently washed with PBS 3 times. Then, the bacteria were fixed with 4% formaldehyde in PBS for 2 hours. After that, the coverslips were washed with DI water 3 times and dehydrated in a gradient ethanol/water mixture (from 50% to 100%). The samples were air-dried overnight before SEM study.

Hemolysis Assays

The sheep blood was centrifuged at 500 x g for 10 min at 5 °C. The supernatant was aspirated carefully and the red blood cells were washed with phosphate-buffered saline (PBS; pH 7.4). This process was repeated until the supernatant no longer contained any observable hemoglobin. Then, the suspension was diluted 1:50 in PBS to yield the working suspension of sheep blood. 1 mL of the suspension was added into a 24-well plate with the polymer coated coverslips and the well plate was incubated at 37 °C for 1 h. For the experiment, the wells contained blank coverslips were used as the 0% hemolysis control, and the wells contained 1% Triton X-100 were used as the 100% hemolysis control. After incubation, the plate was centrifuged at 500 x g for 10 min at 5°C. The supernatant was removed and the percentage of hemolysis was quantified by measuring the absorbance at 450 nm.

LDH Assays

Mouse fibroblast NIH-3T3 cells were cultured using DMEM medium supplemented with 10% FBS and 1% penicillin streptomycin. The cells were grown at 37 °C in 5% CO_2 until reaching ~90% confluence. The cells were harvested using 0.25% trypsin solution and seeded onto the polymer coated coverslips and the blank

coverslips in a 24 well plate at a density of 5000 cells/cm^2. The cells were incubated for 24 and 72 hours before determining viability using a Pierce LDH Cytotoxicity Assay Kit according to the instructions of the manufacturer.

References

(1) Gokhale, S.; Xu, Y.; Joy, A. *Biomacromolecules* **2013**, *14*, 2489.