Supplementary Information

Biodegradable Polycaprolactone Metallopolymer-Antibiotic Bioconjugates Containing Phenylboronic Acid and Cobaltocenium for Antimicrobial Application

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Materials. Chloro-ε-caprolactone, benzyl alcohol, and Sn(II) 2-ethylhexanoate ($Sn(Oct)_2$), sodium azide, and penicillin-G sodium salts were purchased from VWR. CuI, 3-(2-propynylaminocarbonyl)phenylboronic acid pinacol ester, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from Sigma-Aldrich and used as received. Ethynyl cobaltocenium hexafluorophosphate was synthesized according to a previous report.[1]

Characterization. Fourier-transform infrared (FTIR) spectroscopy was performed using a Perkin-Elmer spectrum with an Attenuated Total Reflectance (ATR) mode. ^1H NMR (300 MHz) spectra were recorded on a Varian Mercury 400 spectrometer with tetramethylsilane (TMS) as an internal reference. UV-vis spectra were recorded on a Shimadzu UV 2450 spectrophotometer. Thermogravimetric analysis (TGA) was conducted on a TA Instruments Q5000 with a heating rate of 10 °C/min from 40 to 800 °C under constant nitrogen flow at a rate of 20 mL/min. A Zeiss UltraPlus Field-Emission Scanning Electron Microscopy (FESEM) was used to observe bacterial morphologies. Samples were first coated with gold using Denton Dest II Sputter Coater for 120s and then observed by FESEM. A Zeiss LSM 410 Confocal Laser Scanning Microscope (CLSM) was used for fluorescent imaging to evaluate LIVE/DEAD bacterial viability.

Synthesis of Poly(α-chloro-ε-caprolactone) by Ring-Opening Polymerization. The synthesis was followed a procedure reported earlier.[2] α-Chloro-ε-caprolactone (1.0 g, 6.7 mmol), benzyl alcohol (14.5 mg, 0.134 mmol), and $Sn(Oct)_2$ (5.4 mg, 0.0134 mmol) were dissolved in dry toluene (1.0 mL) in a Schlenk flask, and the flask was purged

with nitrogen for 10 min. Then, the polymerization was carried out in a preheated oil bath at 120 ºC for 12 h under continuous stirring. After polymerization, the solution was diluted with dichloromethane and precipitated in cold methanol. The PCL-Cl polymer was finally recovered by centrifugation (8000 rpm at 25 ºC for 15 min) and dried in a vacuum oven until constant weight. The conversion of monomers was almost 100%, as determined by ^{1}H NMR. ^{1}H NMR ($CDCl_3$, δ): 4.25-4.17 (t, -CHClCO-), 4.17-4.07 (t, $-OCH_2-$), 2.07-1.16 (broad, $-CH_2CH_2CH_2-$).

Synthesis of Poly(α-azide-ε-caprolactone). PCL-Cl (1.0 g, 6.7 mmol azide units) was dissolved in DMF (10 mL) in a round bottomed flask, followed by the addition of sodium azide (2.2 g, 33.5 mmol). The mixture was stirred at room temperature overnight. After evaporation of DMF, 10 mL of toluene was added, and the insoluble salt was removed by centrifugation. The PCL-N_3 polymer was recovered by solvent evaporation. ^{1}H NMR ($CDCl_3$, δ): 4.28-4.13 (t, $-OCH_2-$), 3.90-3.80 (t, $-CHN_3CO-$), 1.95-1.23 (broad, $-CH_2CH_2CH_2-$).

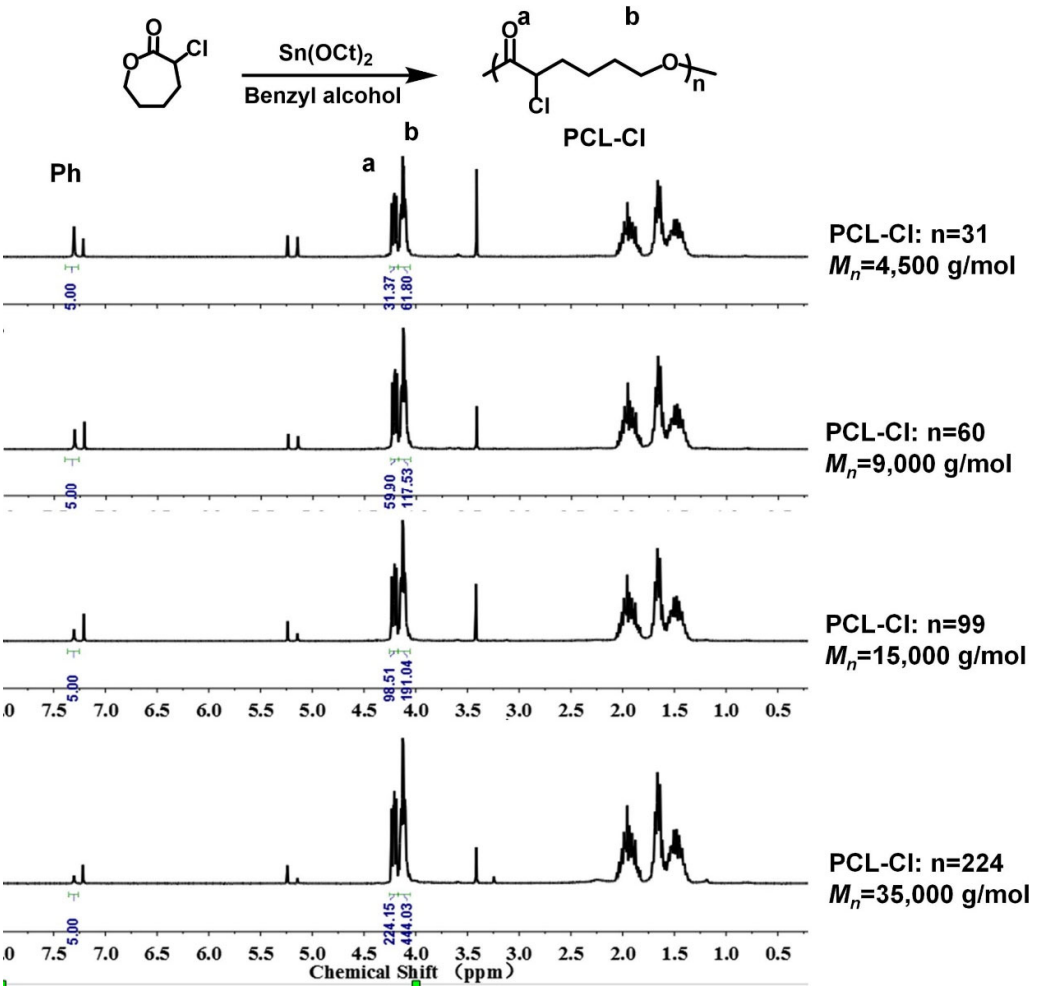


Figure S1. ^{1}H NMR spectra of poly(α-chloro-ε-caprolactone) (PCL-Cl, $CDCl_3$) with different molecular weight.

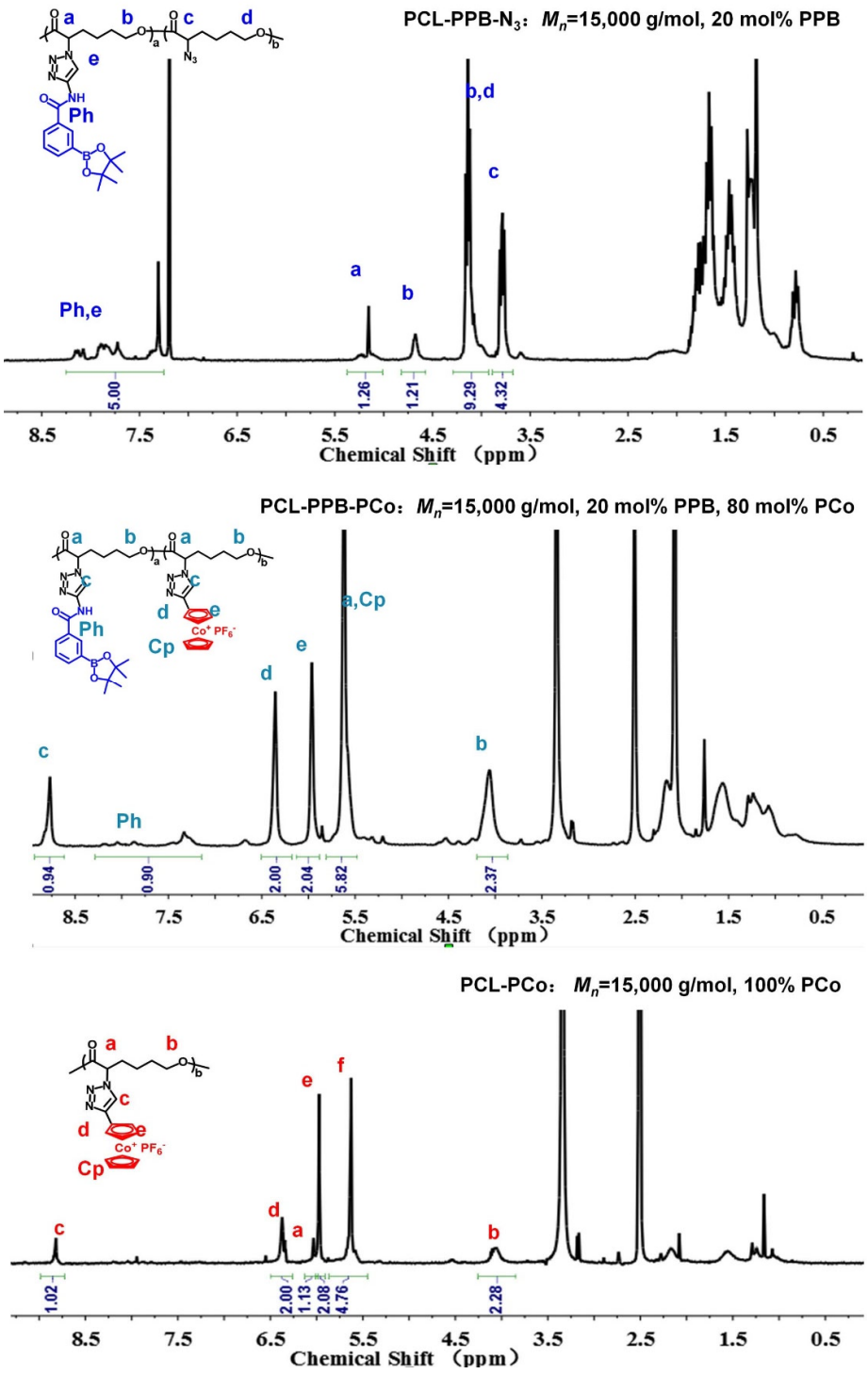
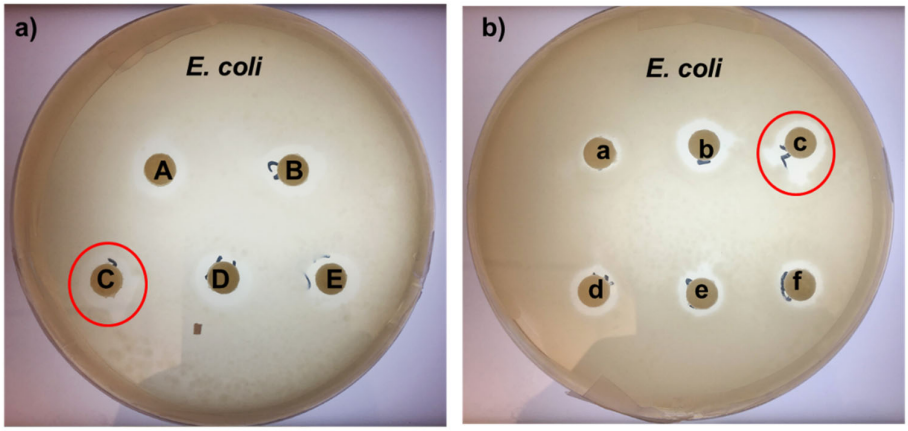


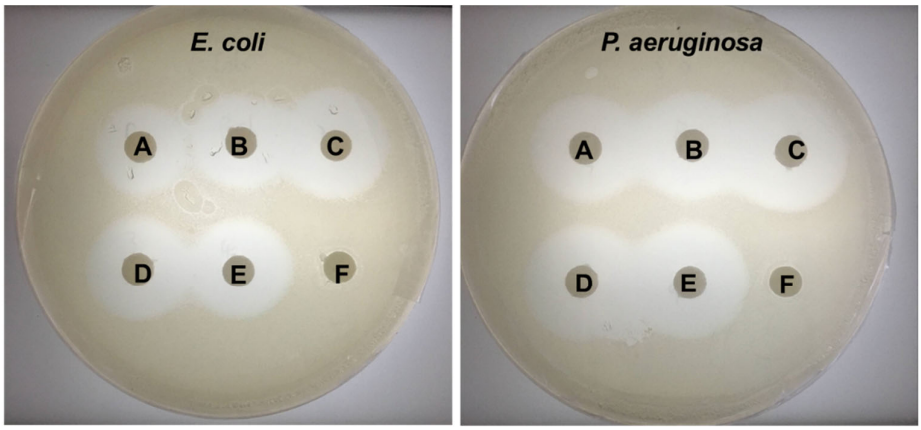
Figure S2. ^{1}H NMR spectra of PCL-PPB-N$_3$ (M_n = 15,000 g/mol, 20 mol% PPB), PCL-PPB-PCo (M_n = 15,000 g/mol, 20 mol% PPB, 80 mol% PCo), and PCL-PCo (M_n = 15,000 g/mol, 100% PCo).



A:PCL-PPB-PCo, 20%PPB, Mn=4,500
B:PCL-PPB-PCo, 20%PPB, Mn=9,000
C:PCL-PPB-PCo, 20%PPB, Mn=15,000
D:PCL-PPB-PCo, 20%PPB, Mn=25,000
E:PCL-PPB-PCo, 20%PPB, Mn=35,000
using100 μg copolymers

a:PCL-PPB, Mn=15,000,100%PPB
b:PCL-PPB-PCo, Mn=15,000,10%PPB
c:PCL-PPB-PCo, Mn=15,000,15%PPB
d:PCL-PPB-PCo, Mn=15,000,20%PPB
e:PCL-PPB-PCo, Mn=15,000,30%PPB
f:PCL-PCo, Mn=15,000,0%PPB

Figure S3. Antimicrobial effect of different PCL-PPB-PCo copolymers (100 μg) against Gram-negative bacterium *E. coli* using disk-diffusion assays.



A:PCL-PPB-PCo-1, 20% PPB, *Mn*=4,500; **B:**PCL-PPB-PCo-2, 20% PPB, *Mn*=9,000
C:PCL-PPB-PCo-3, 20% PPB, *Mn*=15,000; **D:**PCL-PPB-PCo-4, 20% PPB, *Mn*=25,000
E:PCL-PPB-PCo-5, 20% PPB, *Mn*=35,000; **F: CuI, 2 μg in 30 μL H_2O**
A-E: Using 250 μg PCL-PPB-PCo copolymers in 30 μL H_2O

Figure S4. Antimicrobial effect of different PCL-PPB-PCo copolymers (250 μg, containing 2 μg CuI tested by ICP-MS) and CuI (2 μg) against Gram-negative bacteria *E. coli* and *P. aeruginosa* using disk-diffusion assays.

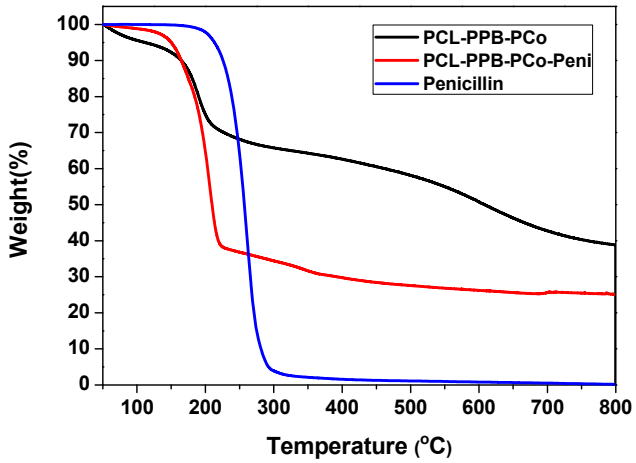


Figure S5. TGA thermograms of PCL-PPB-PCo-7 copolymer, PCL-PPB-PCo-Peni bioconjugate, and penicillin-G. High antibiotic loading capacity (38 wt%) was obtained.

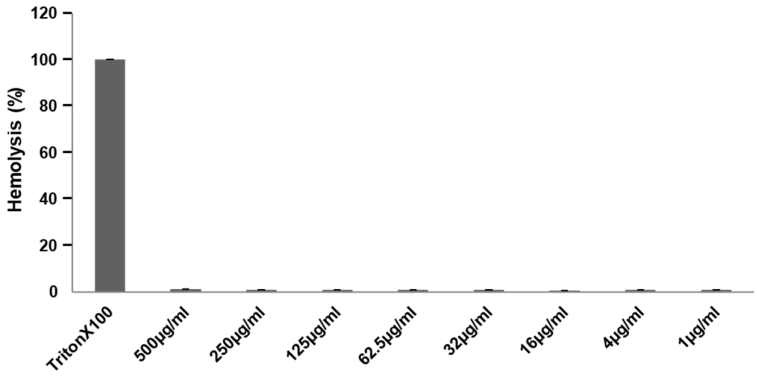


Figure S6. Hemolysis test of PCL-PPB-PCo-7 copolymers against red blood cells (RBCs). TritonX100 as the negative control.

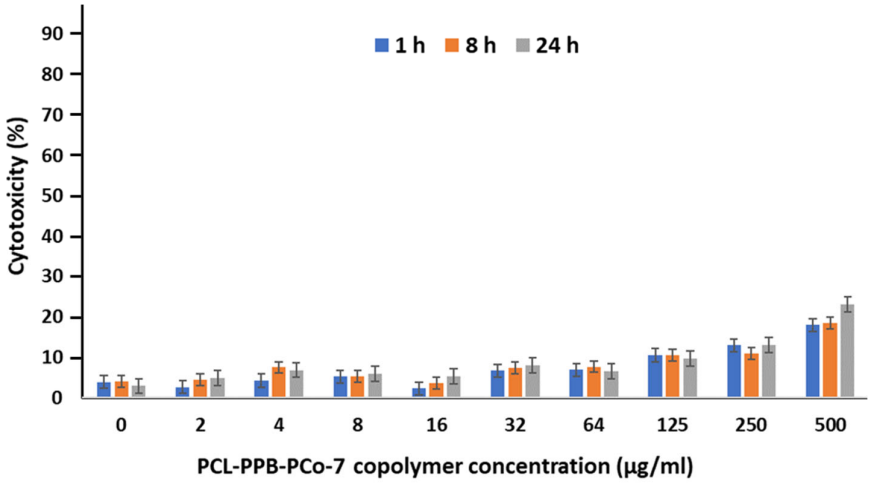


Figure S7. Toxicity test of PCL-PPB-PCo copolymer (PCL-PPB-PCo-7) on human primary umbilical vein endothelia cells (HUVEC).

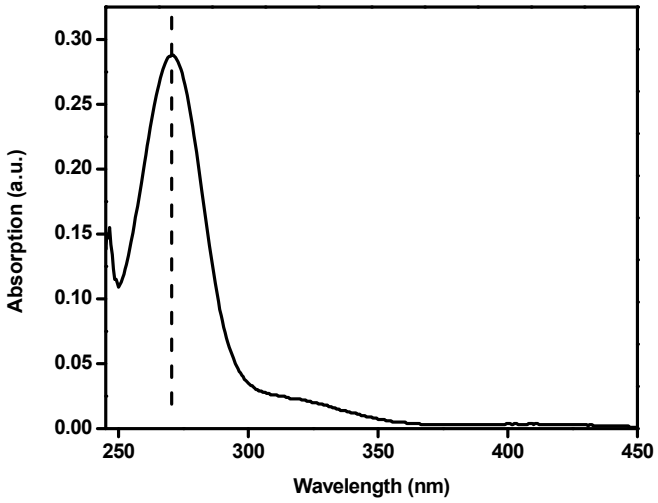


Figure S8. UV-vis absorption spectrum of cobaltocenium (Absorption peak at 270 nm).

REFERENCES

1. Yan, Y.; Zhang, J.; Qiao, Y.; Tang, C. *Macromol. Rapid Commun.* **2014**, *35*, 254-259.
2. Yao, K.; Wang, J.; Zhang, W.; Lee, J. S.; Wang, C.; Chu, F.; He, X.; Tang, C., Degradable rosin-ester–caprolactone graft copolymers. *Biomacromolecules* **2011**, *12*, 2171-2177.