Supporting Information

Dynamically Crosslinked Polymer Nanocomposites to Treat Multidrug-Resistant Bacterial Biofilms

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Synthesis and Characterization of Crosslinker 1,3,5,7-Tetrakis(4-formylphenyl)adamantane (ATA)

Synthesis of 1

To a 150 mL three-neck flask equipped with a reflux condenser, a calcium chloride drying tube, and magnetic stir-bar, 1-bromoadamantane (5.0 g, 23.0 mmol, 1.0 eq), tert-butyl bromide (6.3 g, 46.0 mmol, 2 eq), and benzene (50 ml, 560 mmol, 560.0 eq) was added and allowed to stir at 55°C for 10 min. Then, aluminum chloride (0.6 1g, 4.60 mmol, 0.2 eq) was added slowly and the reaction solution was heated and stirred under vigorous reflux for 1 hour. Afterwards, the system was then poured into ice water and ether was then added into the mixture while stirring. The resulting undissolved substance was filtered and vacuum dried followed by Soxhelt purification.
in chloroform for 48 hours. After vacuuming dry, 1 was obtained as white powder and carried directly to the next step.

**Synthesis of 2**

Following a modified literature procedure, to a 250 mL three-neck round-bottom flask equipped with a magnetic stir-bar, 1 (3.8 g, 8.6 mmol, 1.0 eq) and 150 mL dichloromethane was added. Under a continuous flow of nitrogen, the mixture was stirred rapidly and cooled to -10°C with an ice/salt bath. Afterwards, titanium tetrachloride (19.0 mL, 172.4 mmol, 20.0 eq) was added slowly to the mixture and stirred at -10°C for 30 min. Then, dichloromethyl methylether (12.5 mL, 137.9 mmol, 16.0 eq) was subsequently added dropwise to the mixture. The reaction was held at -10°C for 3 hours and then allowed to warm to room temperature and stirred overnight. The mixture was poured into 300 mL ice-water, and 100 mL of 1 M HCl was added and allowed to stir for 30 minutes. The two-phase mixture was separated, and the aqueous phase was washed twice with 100 mL DCM. The combined organic phases were successively washed with saturated aqueous NaHCO₃ and saturated NaCl and then dried with Na₂SO₄. The solution was filtered, and the solvent removed with a rotavapor. The resultant yellow solid was purified by column chromatography and then recrystallized from dioxane to give ATA as white crystals.
Figure S1. $^1$HNMR spectrum of ATA with CDCl₃ as the solvent. $^1$H NMR (400 MHz, CDCl₃): $\delta$ (ppm) 10.02 (s, 4H), 7.91 (d, 8H), 7.67 (d, 8H), 2.26 (s, 12H).

![Figure S1](image)

Figure S2. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectrum of ATA. ATR-FTIR (cm$^{-1}$): 3029 (Phenyl, C-H); 2928 (Adamantyl, CH₂); 2852, 2738 (CHO, CH); 1697 (C=O); 1601, 1571 (Phenyl ring).

Synthesis and Characterization of dynamically crosslinked polymer nanocomposites (DCPN)

Freeze-dried samples of DCPN along with the non-crosslinked counterparts (NonPN) as the control samples were analyzed using ATR-FTIR.
Figure S3. ATR-FTIR spectra of DCPN and NonPN. The results show that the aldehyde groups (2852, 2738 cm\(^{-1}\)) from crosslinker ATA are lost, and imine bonds (C=N,1605 cm\(^{-1}\); C-N,1173cm\(^{-1}\)) formed within DCPN.

Dynamic Light Scattering (DLS) of DCPNs

Figure S4. DLS curves monitoring imine – oxime displacement in PBS. DCPNs were incubated with the imine-displacing reagent hydroxylamine (HA) for the indicated durations. DCPNs size is compromised in the presence of HA, indicating PONI-GAT no longer provides stability and results in composite aggregation.
Figure S5. Variation of DLS curves detected after incubated half an hour in serum media for DCPN with different crosslinker content in oil core. The results show that the DCPN stability increases with the crosslinker content until 5wt%, so the 5wt% was chosen as the optimal crosslinker content in this study.

Figure S6. DLS curves monitoring the shelf life of DCPN and the NonPN control. Both the DCPN and NonPN were prepared as stated in main text and allowed to stand for 6months within our studied range, The DLS curves were obtained by detecting the same sample after 24h and 6 months, respectively. The results illustrate that DCPN has significantly improved storage stability than the non-crosslinked analog.
Figure S7. SEM micrographs of pathogenic planktonic bacteria. *E. coli* and *S. aureus* were incubated either with M9 only (control) or 4 v/v% DCPNs (treated) for 3 h. Treated samples show a reduction in bacteria population and compromised bacterial cell walls/membranes. Scale bars are 10µm and 5µm, respectively.

Figure S8. Antibacterial effect of DCPNs against CD-2 biofilms for the duration of the time indicated. Experiments were performed in triplicates and the error bars indicate standard deviation.
**Figure S9.** Remaining biomass of CD-2 biofilms after treated with DCPNs for 3 hr. After DCPN treatment, biofilms were stained with crystal violet, washed, and analyzed. Experiments were performed in triplicates and the error bars indicate standard deviation.

**Figure S10.** Antibacterial mechanism of DCPNs using Propidium Iodide (PI) kinetic assay. Negative controls (DCPN + PI and Bacteria + PI), along with ciprofloxacin, a non-membrane disrupting antibiotic, indicate no significant adverse fluorescence. Fluorescence from PI intercalation is shown when bacteria are subjected to DCPNs, indicating DCPNs activity is through membrane disruption.
Minimum Inhibition Concentrations (MIC) of DCPNs Against Bacterial Strains

Table S1. MICs of the 5wt%DCPN against different strains of bacteria

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>DCPN MICs (v/v%)</th>
<th>(mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em> - Pathogenic</td>
<td>CD-1006</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Acinetobacter</em> species - Pathogenic</td>
<td>CD-575</td>
<td>4.0</td>
</tr>
<tr>
<td><em>E. coli</em> - Pathogenic</td>
<td>CD-2</td>
<td>2.0</td>
</tr>
<tr>
<td><em>E. cloacae</em> complex - Pathogenic</td>
<td>CD-1412</td>
<td>2.0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> - Non-Pathogenic</td>
<td>ATCC-19660</td>
<td>2.0</td>
</tr>
<tr>
<td><em>S. aureus</em> - MRSA - Pathogenic</td>
<td>CD-489</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table S2. MICs of DCPN and antibiotic control against quality control strain *P. aeruginosa* (ATCC-27583) in M9 and CAMHB media. DCPNs indicated no increase in MIC when introduced into a protein-rich media (CAMHB). Alternatively, colistin’s MIC increased 4-fold in M9 as compared to CAMHB.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>M9 Media</th>
<th>DCPN</th>
<th>Colistin</th>
<th>CAMHB Media</th>
<th>DCPN</th>
<th>Colistin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC-27583</td>
<td>468.70</td>
<td>1.0</td>
<td>468.70</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P. aeruginosa*