Supporting Information

Biodegradable waterborne gemini quaternary ammonium salts polyurethanes with antibacterial and biocompatible properties

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Synthesis and minimal inhibitory concentration of gemini quaternary ammonium (GQAS) chain extender (EGn).

The synthesis route of lysine-derivative GQAS chain extenders (EGn) was same with that of EG12 which has already been synthesized in our previous work \(^1\) (Scheme S1).

Scheme S1. The synthesis route of gemini quaternary ammonium (GQAS) chain extender EGn, n=8, 12, 16.

Synthesis of EG8

*Synthesis of* \(N, N, N', N'-\text{tetramethyl}-N, N'-\text{bisoctyl-2, 6-bis (ammonium bromide)-L-lysine ethyl ester (LG8).})*

\(N, N, N', N'-\text{tetramethyl-L-lysine ethyl ester (LE, 23.0 g, 0.1 mol) with 1-Bromooctane (20 \% excess) were dissolved in isopropanol with magnetic stirring under reflux at 84 \degree C for 48h. After cooled to room temperature, isopropanol was removed with a vacuum rotary evaporation at 50 \degree C, and the residue was dissolved in} \)
a small amount of ethyl acetate and then precipitated with petroleum ether three times. The products were dried with a vacuum rotary evaporator at 55 ºC for 6h to yield 51.4 g LG8 (yield: 83.3 %).

Mass spectra (MS, positive) theoretical: 616.60; observed ((m^2+-2Br)/2z): 228.34.

Synthesis of N, N, N', N'-tetramethyl-N, N'-bisocetyl-2, 6-bis (ammonium bromide)-L-lysine-(3-aminopropyl) amide (ALG8).

Under a nitrogen atmosphere, LG8 (43.4 g, 0.07 mol) and 51.9 g (0.7 mol) of 1, 3-propane diamine were heated around 60-65 ºC for 24 h with magnetic stirring. The surplus of 1, 3-propane diamine was removed with a vacuum rotary evaporation at 55 ºC. The remainder was dissolved with ethyl acetate and precipitated with petroleum ether and ethyl ether three times, and then the residual solvent in the products was removed with a vacuum rotary evaporation at 55 ºC for 8 h to yield 41.2 g ALG8 (Yield: 90.9%).

Mass spectrum (MS) (positive) theoretical: 644.65; observed ((m^2+Br)/2z): 242.27. Ms was acquired using an HP1100-LC/MSD with atmosphere pressure chemical ionization.

Synthesis of gemini quaternary ammonium salt chain extender with two primary amine groups (N, N, N', N'-tetramethyl-N, N'-bisocetyl-2, 6-bis (ammonium bromide)-L-lysine-(1', 3'-propylene diamide))-L-lysine (EG8)

Dicyclohexylcarbodiimide (DCC, 15.97 g, 0.077 mol) and N-hydroxysuccinimide (HOSU, 8.5 g, 0.074 mol) were added to a CH_2Cl_2 (135 mL) solution of Boc-lysine...
(24.37 g, 0.070 mol) and ALG8 (41.0 g, 0.064 mol) cooled by an ice water bath to 0 ºC. Then the mixture was stirred at room temperature for 48h. After completion of the reaction, dicyclohexylurea (DCU) was removed by filtration and the solution was orderly washed with 3M hydrochloric acid, saturated sodium bicarbonate solution, saturated salt water and deionized water, dried with anhydrous Na₂SO₄ for 24h and filtered. The solution was concentrated with a rotary evaporator to gain Boc-protected EG8 products. The Boc-protected amine groups of EG8 were converted to primary amine groups (EG8) by hydrogen chloride saturated ethyl acetate, then neutralized with dilute NaOH solution to pH=8. The solvents were removed by rotary evaporator at 50 ºC, then the residual was dissolved in anhydrous methanol. After the precipitate was filtered off, the methanol solution was dried with a rotary evaporator to gain the crude product (33.64 g, 0.038 mol), which was further purified with reverse silica gel (C18-RP) column chromatography with water/methanol eluent to yield pure EG8 (Yield: 89.6%). The synthesis route and structure are shown in Scheme S1. 

Ms (positive) theoretical: 772.83; observed ((m²+·2Br)/2z): 306.29.

¹H NMR (400MHz, DMSO-d6, δ): 0.85 (t, 6H), 1.25 (m, 24H), 1.48-1.93 (m, 12H), 2.15 (2H), 2.68 (t, 2H), 3.01 -3.24 (m, 23H), 4.43 (m, 1H), 8.04 (m, 2H). The peaks at 3.01 and 3.10 are assigned to characteristic peaks of -N⁺ (CH₃)₂, and 4.43 (m, 1H) ppm to chiral CH, respectively.

**Synthesis of EG12**

EG12 was synthesized in our previous work.¹

**Synthesis of EG16**
Synthesis of N, N', N'-tetramethyl-N, N'-biscetyl -2, 6-bis (ammonium bromide)-L-lysine ethyl ester (LG16)

Via a procedure similar to that for LG8, N, N, N', N'-tetramethyl-L-lysine ethyl ester (LE, 23 g, 0.1 mol) and 1-Bromohexadecane (20 % excess) were stirring under reflux at 84 ºC for 48h to yield 71.8 g LG16 (yield: 85.4 %). Mass spectra (MS, positive) theoretical: 841.02; observed ((m²⁺-2Br)/2z): 340.46.

Synthesis of N, N, N', N'-tetramethyl-N, N'-biscetyl-2, 6-bis (ammonium bromide)-L-lysine-(3-aminopropyl) amide (ALG16)

Via a procedure similar to that for ALG8, under a nitrogen atmosphere, LG16 (54.7 g, 0.065 mol) and 48.2g mL (0.65 mol) of 1, 3-propane diamine were heated at 60-65 ºC for 24 h with magnetic stirring to yield 40.5 g ALG16. (Yield: 71.6%).

Ms (positive) theoretical: 869.08; observed ((m²⁺-2Br)/2z): 354.45.

Synthesis gemini quaternary ammonium salt chain extender with two primary amine groups (N, N, N', N'-tetramethyl-N, N'-biscetyl-2, 6-bis (ammonium bromide) - L-lysine- (1', 3'-propylene diamide))-L-lysine (EG16)

Via a procedure similar to that for EG16 , Boc-lysine (15.3 g, 0.050 mol) and ALG16 (39.1 g, 0.045 mol) were reacted for 48h, then deprotection by hydrogen chloride saturated ethyl acetate to yield pure EG16 38.7g (Yield: 86.2%).

Ms (positive) theoretical: 997.25; observed ((m²⁺-2Br)/2z): 418.44.
$^1$H NMR (400MHz, DMSO-d$_6$, δ): 0.85 (t, 6H), 1.25 (m, 56H), 1.48-1.93 (m, 12H), 2.15 (2H), 2.74 (t, 2H), 3.01-3.24 (m, 23H), 4.43 (m, 1H), 8.04 (m, 6H). The peaks at 3.01-3.24 are assigned to characteristic peaks of -N$^+$ (CH$_3$)$_2$, and 4.43 (m, 1H) ppm to chiral CH, respectively.

**Figure. S1** The structure and $^1$H NMR spectrum of EGn recorded in DMSO-d$_6$, n= 8, 12, 16.

The gemini chain extenders (EGn) were successfully synthesized using a facile method as illustrated in scheme S1. The synthesis details and $^1$H NMR information (Fig. S1) are provided in the Supplementary Information. The $^1$H NMR spectra data of EGn show two characteristic peaks of -N$^+$ (CH$_3$)$_2$ and chiral CH at around 3.01ppm and 4.43ppm, respectively. The integrated peak areas at around 1.26 ppm correspond
to -CH<sub>2</sub>- of hydrophobic alkyl chains are expanded with the hydrophobic alkyl chains increasing. Peaks at around 0.85 ppm are assigned to the -CH<sub>3</sub> of hydrophobic alkyl chains.

### Table S1. The minimal inhibitory concentration results of EGn (µg/ml)

<table>
<thead>
<tr>
<th>Samples</th>
<th>S.aureus</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG8</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>EG12</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>EG16</td>
<td>8</td>
<td>64</td>
</tr>
</tbody>
</table>

2. Bulk and surface structures of waterborne polyurethanes (PCLPU<sub>n</sub>) samples.

![Figure. S2 The transmission FTIR spectra of PCLPU samples](image)

**Figure. S2** The transmission FTIR spectra of PCLPU samples
To confirm bulk structures of these obtained PCLPU samples, Transmission Fourier transform infrared (FTIR) spectra were recorded and shown in Fig. S2. FTIR spectra were obtained at room temperature on a Nicolet-6700 spectrophotometer (Thermo Electron Corporation) between 4000 and 600 cm\(^{-1}\) with the resolution of 4 cm\(^{-1}\). The samples of transmission FTIR spectra were prepared by casting of 2\% (w/v) PCLPU emulsions in dimethylacetamide directly onto KBr crystal plates in a 30 °C oven for 2 d, and then under vacuum at 60 °C for another 2 d prior to measurement.

In the amine adsorption region of all PCLPU FTIR spectra, the absorption bands at 3170-3500 cm\(^{-1}\) belong to N-H vibration in urethane and urea linkages. Hydrogen bonded N-H groups (\(\nu\) (N-H)) are observed at around 3330 cm\(^{-1}\). Free (non-hydrogen bonded) N-H group vibrations at approximately 3400-3500 cm\(^{-1}\) are weakly observed, indicating that most N-H groups of PCLPU are involved in hydrogen bonds. The bands assigned to \(\nu\) (C=O) of the carbonyl in urethane groups appear at around 1725 cm\(^{-1}\), and the adsorption peak at around 1654 cm\(^{-1}\) of carbonyl in urea groups belong to EGN and lysine are distinctly observed in Fig. S2. The strong bands at 1103 cm\(^{-1}\) are overlapped by the C-O-C stretching vibration adsorption of ether groups in PEG. The absorption bands at 2800-3010 cm\(^{-1}\) are correspond to C-H stretching vibrations, which are enhanced with the introduction of GQAS chain extender, and get larger with the increasing alkyl chain in GQAS chain extender. These results further confirm that the GQAS chain extender are introduced into the polyurethanes.
Figure. S3 The differential scanning calorimetry cures of PCLPU (second heating)

Study on thermal behavior of the waterborne PCLPUns was carried out using Differential scanning calorimetry (DSC). DSC was performed on a Netzsch STA 449C Jupiter (Germany) at a heating rate of 10 °C·min^{-1} in the range of -120 to 100 °C under a steady flow of nitrogen. The DSC results were fit using the combination of Lorentzian and Gaussian equations by Origin-pro 8.5. The change of the glass transition temperatures of soft segments (T_g) has been used as an indicator for the degree of microphase separation. The results were shown in Fig. S3. As can be seen, T_{g,soft} of all obtained waterborne PCLPUns ranged from -55.52 to -55.26°C, are higher than that of pure PCL (T_g = -58 °C) \(^2\). This might due to the strong polarity difference between soft segment and hard segment containing polar lysine and GQAS moieties causes a higher degree microphase separation in these waterborne polyurethanes. Additionally, T_{g,soft} of these GQAS PCLPUns have only tiny changes compared with
PCLPU0, which means that different hydrophobic alkyl chain lengths of GQAS can hardly change the aggregation state of these waterborne polyurethane samples. Again, these results demonstrate that these waterborne antibacterial polyurethanes have been successfully synthesized.

**Figure. S4** Time-related water contact angle curves of PCLPUn films surfaces

The surface hydrophilicity of various PCLPUns is determined by water contact angle (WCA) measurements. WCA measurements were carried out with a Drop Shape Analysis System DSA 100 (Krüss, Hamburg, Germany) and 3 μl of distilled water at room temperature. The results were the mean values of three replicates. The WCA of PCLPU0 changed from 76° to 35° during the observation 2s period (Fig. S4), due to the higher PEG content in polyurethanes and the hydrogen bonding interactions.
between PEG segments or carboxyl groups and water molecules aggregated on the surfaces after contact with water. The contact angles on the surfaces of GQAS PCLPUn surfaces are much lower than those of PCLPU0, indicating that the hydrophilicity of GQAS PCLPUn films surface are significantly increased with the incorporation of GQAS. The decreased time-related water contact angles and good hydrophilicity are attributed to the cationic nature of gemini quaternary ammonium polyurethanes on their surfaces.

Figure S5. Protein adsorption of PCLPUn films under shaking environment

The protein adsorption test was executed with bovine serum albumin (BSA, 1 mg/ml), dissolved in the isotonic PBS (pH=7.4). All PCLPUn films were equilibrated with PBS overnight before the test, and TPEU was used as a control. Then all samples were incubated in BSA solution at 37 °C, 100 rpm for 2h. After incubation, these films were gently rinsed with PBS and deionized water, then put into a washing solution (2 wt % sodium dodecyl sulfate (SDS), 0.05 M NaOH) at 37 °C for 1h under mechanical vibration to remove the adsorbing protein. At last, the protein was eluted
in the SDS solution, and the concentration of the adsorbed protein was quantified by a BCA protein assay reagent kit at 562 nm.\textsuperscript{3, 4} Independent measurements were carried out in triplicate samples, and the protein adsorption of each sample was calculated from the concentration of the standard BSA solution.

3. Cytotoxic of EGn and PCLPUn.

**Figure. S6** Cell viability measured by MTT assay after 24 h (A) and 72 h (B) of incubation with serial dilutions of GQAS chain extenders. Error bars represent means ± standard deviation for n=3. Statistical significance: p < 0.05
Figure S7 Cell viability measured by MTT assay after 24 h of incubation with different concentration of PCLPU degradation products. Error bars represent means ± standard deviation for n=3. Statistical significance: p < 0.05