High antimicrobial effectiveness with low hemolytic and cytotoxic activity for PEG/quaternary copolyoxetanes.

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Supplemental Information

Section 1. ^{1}H-NMR spectra for P[(BBOx-50)(ME2Ox)-50-50-4].

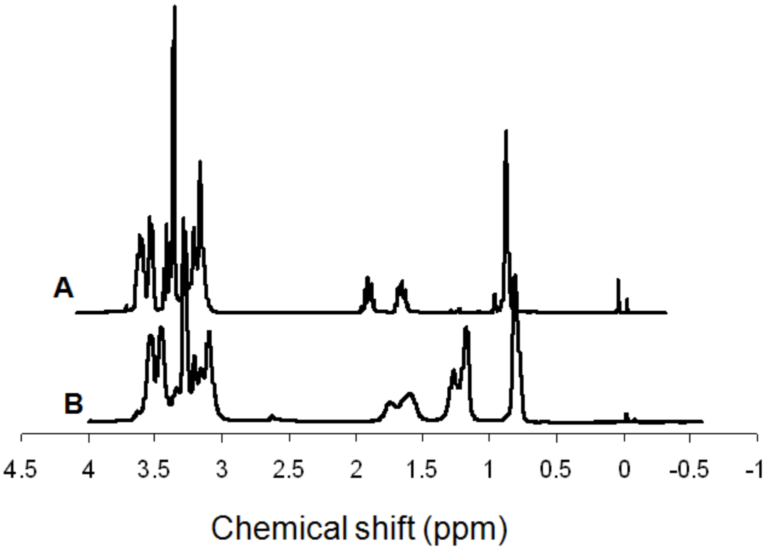

Figure S1. ^{1}H-NMR spectra: **A**, P[(BBOx)(ME2)-50:50-4] and **B**, C8-50.

A. Figure S1 shows the ^{1}H-NMR spectrum of P[(BBOx)(ME2Ox)-50-50-4] ($CDCl_3$): δ 0.91 ppm (-C**H**$_3$, 3H,s), 1.68 ppm (Side chain –OCH_2CH_2C**H**$_2CH_2Br$, –C**H**$_2$-for BBOx, 2H), 1.92 ppm (-OCH_2C**H**$_2CH_2CH_2Br$, –C**H**$_2$-for BBOx, 2H), 3.19 ppm (main chain –C**H**$_2$-, 4H,m), δ3.30 ppm (-C**H**$_2$, 2H, s), 3.38 ppm (-OC**H**$_3$ 3H, s), 3.4 ppm (-C**H**$_2$Br-, 2H), 3.55 ppm (-OC**H**$_2CH_2O$-, 4H, m), 3.64 ppm (-OCH_2C**H**$_2$O-, 4H, m).

B. Assignments and analysis details for the ^{1}H-NMR spectrum of P[(BBOx)(ME2Ox)-50-50-4] copolyoxetane. (Figure S1). The ^{1}H-NMR peak at 0.91 ppm corresponds to the -C**H**$_3$ side chains on each repeat unit, while the peaks at 1.68 and 1.92 ppm are the middle methylene groups, -O-CH_2C**H**$_2$C**H**$_2CH_2$-Br, in the BBOx side chain. Considering ME2Ox as 'x' and BBOx as 'y', Equation 1a describes the relationship of these peak areas:

$$\frac{Area_{0.91ppm}}{Area_{1.68,1.92ppm}} = \frac{3x+3y}{4y} \qquad \text{Equation 1a}$$

Where $Area_{1.68,1.92\text{ppm}}$ = area of the peaks between 1.68 and 1.92 ppm, and $Area_{0.91\text{ ppm}}$ = area of the peak at 0.91 ppm. From Equation 1a we obtain:

$$\frac{x}{y} = \frac{4Area_{0.91ppm} - 3Area_{1.68,1.92ppm}}{3Area_{1.68,1.92ppm}} \qquad \text{Equation 1b}$$

From Equation 1b, a value for x:y is obtained. Using Equation 1c, the value of '1-m' is calculated and likewise the value of m is obtained.

$$1-m = \frac{x}{x+y} = \frac{4Area_{0.91ppm} - 3Area_{1.68,1.92ppm}}{4Area_{0.91ppm}} \qquad \text{Equation 1c}$$

The integrals for the peaks at 0.91 ppm [$-C\mathbf{H}_3$ from ME2Ox plus BBOx] and 1.68 and 1.92 ppm [$-O-CH_2(C\mathbf{H}_2)_2CH_2-Br$] are, respectively, 15.65 (= 3x+3y) and 10.45 (=4y). From Equation 1b, the value for x/y is 0.996. Similarly, from Equation 1c, the value of '1-m' is 0.498 and 'm' is 0.501. The composition of the copolyoxetane thus obtained is P[(BBOx)(ME2Ox)-50-50]. The ratio of (BBOx)/(ME2Ox) determined by ^{1}H-NMR (1:1) compared to the feed ratio of 1:1.22 indicates that BBOx is incorporated preferentially into the copolyoxetane.

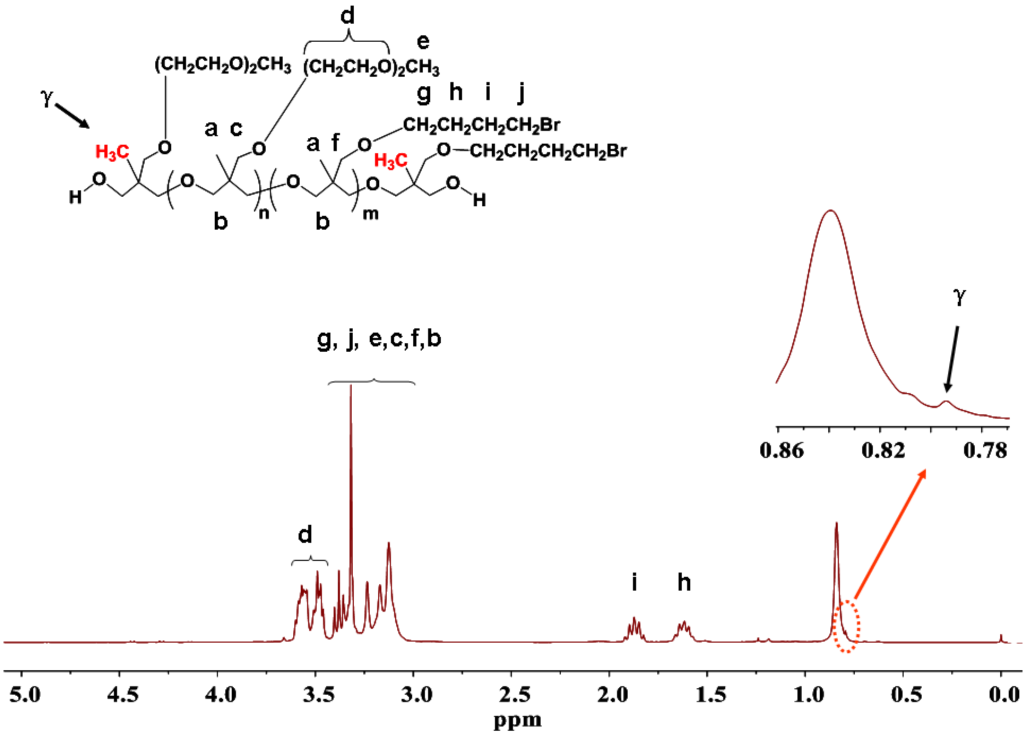

Figure S2. ^{1}H NMR spectrum for P[(BBOx)(ME2Ox)-50-50)-4] (600 MHz). The small γ-peak upfield from the 3-methyl group is assigned to terminal 3-methyl groups.

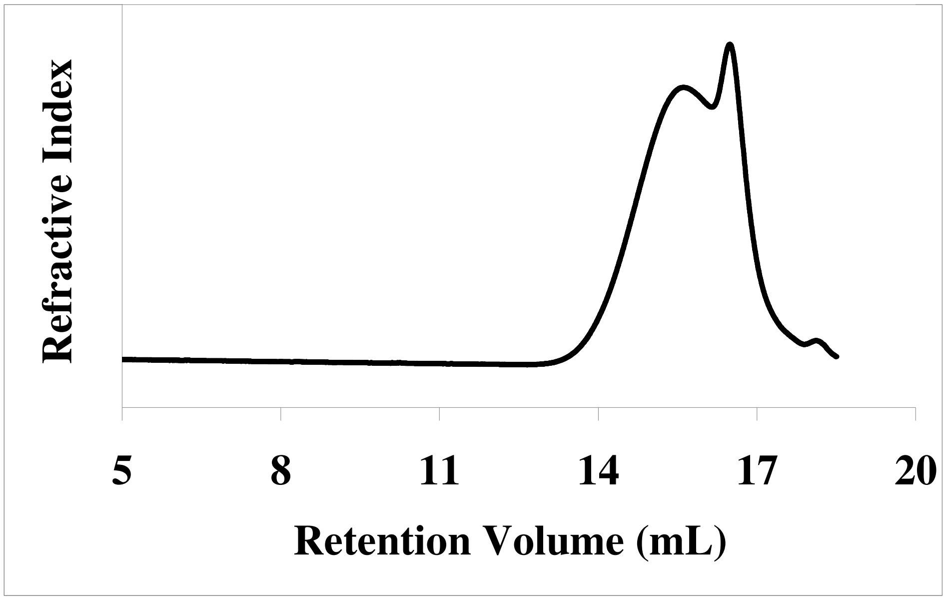

Figure S3. Representative GPC for P[BBOx)(ME2Ox)-50-50-4]. By integration, the peak at longer retention time, assumed to be cyclic tetramer, is 12%.

C. End group analysis. Figure 1 shows the ^{1}H-NMR spectrum for P[(BBOx)(ME2Ox)-50-50] after trifluoroacetic anhydride addition. The downfield shift of the γ peak with concomitant separation from the main chain polyoxetane methyl peak was used for determination of M_n. The appearance of α and β peaks for oxetane and butoxy methylene protons was also used for the determination of M_n and provided confirmation of assignments and a measure of internal consistency.

1. Equations 2a and 2b describe the relationship of methyl peak areas for determination of M_n.

$$\frac{4m}{3(n+m)} = \frac{S_{-CH_2CH_2-}}{S_{-CH_3}} \qquad \text{Eq 2a}$$

$$\frac{3DP}{6} = \frac{S_{-CH_3}}{S_\gamma} \qquad \text{Eq 2 b}$$

Where:

S represents integral areas
m and n are the number of BBOx and ME2Ox units, respectively
DP is the degree of polymerization
γ is for $-CH_3$ end group

The results for this analysis are shown in Table 2.

2. Equations 2c and 2d describe the relationship of end group methylene peak areas for determination of M_n.

$$\frac{4m}{3(n+m)} = \frac{S_{-CH_2CH_2-}}{S_{-CH_3}} \qquad \text{Eq 2c}$$

$$\frac{3DP}{4} = \frac{S_{-CH_3}}{S_\alpha + S_\beta} \qquad \text{Eq 2d}$$

Where S, m, n, and DP are as above, α is for the oxetane methylene group and β is for the terminal butoxy methylene group (Figure 1).

Where S, m, n and D_p are integral area, unit number of BBOx, ME2Ox, degree of polymerization, respectively. α is for end group of backbone and β is for BD in P[(ME2Ox)(BBOx)-50-50]-TFAA.

The results for this analysis are shown in Table 2.

Section 2. ^{1}H-NMR analysis for Cx-50 copolyoxetanes.

A. Figure S1 shows the ^{1}H-NMR spectrum for C8-50. ^{1}H-NMR chemical shifts for C8-50 are: ^{1}H-NMR ($CDCl_3$): δ 0.91 ppm (-C**H**$_3$, 6H,s), 1.2~1.4 (-CH_2(C**H**$_2$)$_6$$CH_3$, 12H), 1.68~ 1.92 ppm(-$OCH_2$C**H**$_2$C**H**$_2$$CH_2$N-, 4H, broad), 3.19 ppm (main chain – C**H**$_2$-, 6H,m), 3.30 ppm (-C**H**$_2$, 2H, s), 3.38 ppm (-OC**H**$_3$ 3H, s), 3.4 ppm (-C**H**$_2$N- 2H, C**H**$_3$-N-C**H**$_3$,6H), 3.55 ppm (-OC**H**$_2$C**H**$_2$O-, 4H, m), 3.64 ppm (-OC**H**$_2$C**H**$_2$O-, 4H, m). Equations used for calculating the molar ratios and percent substitution from ^{1}H-NMR spectra are provided below.

B. ^{1}H-NMR spectroscopy was used to establish that substitution of C-Br by the amine was complete (Example, Figure S1). This was ascertained by the absence of the characteristic BBOx peak at 2 ppm. The Cx/ME2Ox ratio for P[(C12-m)(ME2Ox)] copolyoxetanes was also confirmed by ^{1}H-NMR. An example of ^{1}H-NMR analysis of C8-50 copolyoxetane follows.

The ^{1}H-NMR peak at 0.91 ppm corresponds to -C**H**$_3$ side chains on the 2-position of each repeat unit plus the -C**H**$_3$ group at the end of the quaternary alkyl chain. The peaks between 1.2 and 1.4 ppm are unique to the C8 quaternary side chain. These peaks are due to the following (**bolded**) protons: -CH_2(C**H**$_2$)$_6$$CH_3$. Considering ME2Ox as ‘x’ and C8 as ‘y’, Equation 2a describes the relationship of these peak areas:

$$\frac{Area_{0.91ppm}}{Area_{1.2,1.4ppm}} = \frac{3x+6y}{12y} \qquad \text{Equation 2a}$$

Where $Area_{1.2,1.4\ \mathrm{ppm}}$ = area of the peaks between 1.2 and 1.4 ppm, and $Area_{0.91\ \mathrm{ppm}}$ = area of the peak at 0.91 ppm. From Equation 1 we obtain:

$$\frac{x}{y} = \frac{12Area_{0.91ppm} - 6Area_{1.2,1.4ppm}}{3Area_{1.2,1.4ppm}} \qquad \text{Equation 2b}$$

From Equation 2, a value for x:y is obtained. Using Equation 3, we calculate 'm' and obtain 1-m.

$$1 - m = \frac{x}{x + y} = \frac{12Area_{0.91ppm} - 6Area_{1.2,1.4ppm}}{12Area_{0.91ppm} - 3Area_{1.2,1.4ppm}} \qquad \text{Equation 2c}$$

Figure S1 shows the ^{1}H-NMR spectrum of P[(BBOx)(ME2Ox)-50-50] and the C8-50. Here, 'a' = peaks for [-O-$CH_2(C\mathbf{H}_2)_2CH_2$-Br], 'b' = peak for [-$C\mathbf{H}_3$ from ME2Ox plus BBOx], 'c' = peak for [-$CH_2(C\mathbf{H}_2)_{10}CH_3$ for C12] and 'd' = peaks for [-$C\mathbf{H}_3$ from ME2Ox plus C12].

The integrals for the peaks at 0.91 ppm [-$C\mathbf{H}_3$ from ME2Ox plus C8] and between 1.2 to 1.4 ppm [-$CH_2(C\mathbf{H}_2)_6CH_3$ for C8] are, respectively, 13.08 (= 3x+6y) and 17.42 (=12y). From Equation 2b, the value of x/y is 1.00. Similarly, from Equation 2c, the value of '1-m' is 0.5 and m is 0.5. The composition of the copolyoxetane thus obtained is C8-50. The ^{1}H-NMR analyses for the other substituted copolyoxetanes are also done and are listed in Table 1.

Table S1. Log reductions of *S. aureus* for C8-50 and selected polycations.

Polycation	Main paper reference	MIC (MIC multiplier) (µg/ml)	Challenge (cfu/ml)	Log reduction				
				1 hr	2 hr	3 hr	4 hr	8 hr
C8-50	This work	2 (5)	10^7	7	---	---	---	---
PGON, **11**	52	4	10^5	5	---	---	---	---
A copoly-acrylate biguanide	38	100-330 (0.3-0.09)	3.2 x 10^5	3.1	5.5	---	---	---
CSA-13, **10**	40	1 (10)	3.2 x 10^8	0.9	2.1	3.2	3.8	4.8

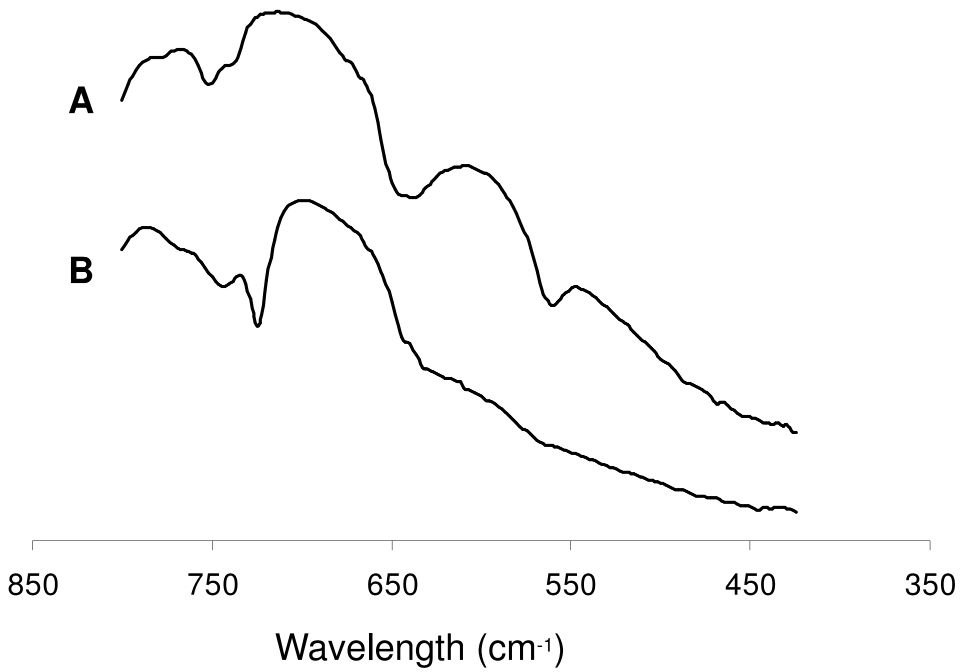

Figure S4. FT-IR spectra for **A** – P[(ME2Ox)(BBOx)-50:50] and **B** – C8-50