**Supporting information**

# An easy-to-use antimicrobial hydrogel effectively kills bacteria, fungi, and influenza virus

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## 1. Synthesis and characterization of quaternary polyethyleneimine derivative (QPEINH-$C_6$)

### 1.1. Synthesis of *N*-hexyl-2-bromoethanamide (4)

Hexyl amine (6 g, 60 mmol) was dissolved in DCM (100 mL) and then it was added to an aqueous solution (100 mL) containing $K_2CO_3$ (12.4 g, 90 mmol). The mixture was cooled to 4−5 °C. Then bromo-acetyl bromide (18 g, 90 mmol) dissolved in DCM (75 mL), was added to the mixture drop-wise over a period of 30 min by using a dropping funnel. The reaction mixture was kept at the room temperature (RT) for 12 h for completion of reaction. Finally, DCM layer was collected, and aqueous layer was washed further with DCM (2 × 50 mL). DCM solutions were then combined and washed with water three times (100 mL each time). Finally, DCM layer was passed through anhydrous sodium sulfate ($Na_2SO_4$) and solvent was removed to obtain the colourless liquid product with a yield of 86 %. FT-IR, $^1$H-NMR, and HR-MS were used to characterize the product.[1]

FT-IR (KBr): $\bar{\nu}$ = 3285 cm$^{-1}$ (−NH− str.), 2857-2954 cm$^{-1}$ (-C-H str.), 1650 cm$^{-1}$ (Amide I, C=O str.), 1554 cm$^{-1}$ (amide II, NH ben.), 1438-1461 cm$^{-1}$ (-C-H bend), 1308 cm$^{-1}$ (C-N str.). $^1$H NMR ($CDCl_3$, 400 MHz) δ/ppm: 0.86-0.89 (t, $CH_3CH_2$−, 3H), 1.3 (s, $CH_3(CH_2)_3$-, 6H), 1.51 (t, −$CH_2CH_2$−, 2H), 3.28 (m, −$CH_2CH_2$NH-, 2H), 3.87 (s, −CO$CH_2$Br, 2H). HRMS (ESI, [M+H$^+$]): m/z calculated. for $C_8H_{16}BrNO$ 222.04, found 222.0488.

### 1.2. Synthesis of linear polyethyleneimine [PEI (2)]

Market available polyethyl(2-ethyl-2-oxazoline) (PEOZ, molecular weight 50 kDa) was subjected to acid-catalysed hydrolysis to obtain the linear deacylated PEIs (2). 10.0 g of the PEOZ was hydrolysed using 400 mL of 24 % (w/v) HCl and refluxed for 96 h. Within 2 h PEOZ crystals dissolved and after 3 h a white precipitate appeared. After 96 h collection of the precipitate was done. The collected precipitate was redissolved in 50 mL distilled water. Then 6 (M) KOH was added dropwise until the pH of the solution reached ~11 to obtain the deprotonated polyethyleneimine (PEI). The deprotonated PEI was repeatedly washed with distilled water to remove the excess base. Washing is continued until it reached pH ~7. 22 kDa

linear deprotonated PEI was obtained. FT-IR was used to characterize the product. (yield = 98 %)

FT-IR (KBr): $\bar{\nu}$ = 3274 $cm^{-1}$ (−NH− str.), 2974-2839 $cm^{-1}$ (-C-H str.), 1619 $cm^{-1}$, 1427-1467 $cm^{-1}$ (-C-H bend).

**1.3. Synthesis of *N*-methyl polyethyleneimine [*N*-methyl PEI (3)]**

Deprotonated linear polyethyleneimine (2) of 22 kDa (5 g, 116 mmol) was transferred to a round-bottom flask. After that, 90 % formic acid (24.5 mL, 0.48 mol) was added followed by 37 % formaldehyde (29.3 mL, 0.36 mol) and 20 mL of water into that round bottom flask. Then the reaction mixture was refluxed at 90 °C for 60 h. After cooling down to room temperature, 8 M KOH was used to adjust the pH to 11. The deprotonated *N*-methyl PEI (3) was extracted by chloroform wash, and the entire organic part was also washed with water. Chloroform was evaporated to obtain the *N*-methyl PEI with 75 % yield. The products were characterized by FT-IR and $^1$H-NMR spectroscopy.[2]

FT-IR (KBr): $\bar{\nu}$ = 2934-2761 $cm^{-1}$ (-C-H str.), 1452 $cm^{-1}$ (-C-H bend). $^1$H NMR (400 MHz, $CDCl_3$) δ/ppm: 2.22 (s, -NHCH$_2$*CH$_3$*, 3H), 2.48 (s, -NH*CH$_2$*CH$_3$, 2H).

**1.4. Synthesis of quaternary polyethyleneimine derivative [QPEINH-$C_6$ (5)]**

Linear *N*-methyl PEI (1 g, 17.5 mmol/repeating unit) was dissolved in 50 mL of chloroform in a screw-top pressure tube, and *N*-hexyl-2-bromoethanamide (4) (12 g, 53 mmol) were added to it. The reaction mixture was stirred at 85 °C for 96 h. After the reaction, the solvent was removed, and ether was poured in excess to get the precipitation. The precipitate was dissolved in $CHCl_3$, and ether was added to reprecipitate for further purification. Finally, the excess solvent was decanted and collected product was dried for further usage. FT-IR and NMR spectroscopy was used to characterize the product. (yield = 67 %)

FT-IR (KBr): $\bar{\nu}$ = 3230 $cm^{-1}$ (−NH− str.), 2951 $cm^{-1}$ (-$CH_2$- assym. Str.), 2861 $cm^{-1}$ (-$CH_2$- sym. Str.), 1660 $cm^{-1}$ (Amide I, -C=O str.), 1560 $cm^{-1}$ (Amide II, -NH bend), 1458 $cm^{-1}$ (-C-H bend). $^1$H NMR: (400 MHz, $D_2O$) δ/ppm: 0.87 (s, *CH$_3$*CH$_2$−, 3H), 1.30 (s, CH$_3$(*CH$_2$*)$_3$-, 6H), 1.53 (s, −CH$_2$*CH$_2$*−, 2H), 3.00-3.59 (m, −N$^+$CH$_2$*CH$_3$*, 2H), 4.16-4.45 (m, −N$^+$*CH$_2$*CH$_3$, 2H).

**2. Antimicrobial assay of QPEINH-$C_6$**

**2.1. Antibacterial assay**

The antibacterial assay of the compound against different bacteria was performed by following our previously reported protocol.[1] Single bacterial colony was added to 3 mL of nutrient broth and incubated in shaking condition for 6 h to obtain mid-log phase bacteria ($10^8$ CFU/mL) cells.

$10^5$ CFU/mL bacteria cells were prepared by diluting the bacterial culture in MHB and used for antibacterial efficacy determination. Stock solution (10.24 mg/ml) of compound was prepared in Milli-Q water and 2-fold serially diluted in 96 well plate in triplicate (upto 10 times-11th well). The 20 µL aqueous solutions of the compound was added to 96 well plate. 180 µL of bacterial suspension in MHB was added to the 20 µL compound solution (working concentration of compounds from 1024 µg/mL to 1 µg/mL). At 37 °C for 24 h, plates were incubated in static condition. Optical density (OD) of bacterial suspension was then measured at 600 nm using TECAN Plate Reader.[3]

### 2.2. Antifungal assay

To grow the fungi cells (*C. albicans* AB226 and *C. albicans* AB399) single colony of the fungi was added to 3 mL of the YPD (Yeast Extract–Peptone–Dextrose) media and incubated for 10 h to obtain $10^8$ CFU/mL fungi cells. To prepare $10^5$ CFU/mL cells, the initial fungi culture ($10^8$ CFU/mL) was diluted in the RPMI media. Stock solution (10.24 mg/ml) of compound was prepared in Milli-Q water and 2-fold serially diluted in 96 well plate in triplicate (upto 10 times-11th well). The 20 µL aqueous solutions of the compound was added to 96 well plate. 180 µL of fungal suspension in RPMI was added to 20 µL compound solution (working concentration of compound was from 1024 µg/mL to 1 µg/mL). The plates were then incubated at 37 °C for 48 h in static condition. After that TECAN Plate Reader was used to measure the optical density of the compound treated fungal solution at 600 nm.

## 3. Toxicity towards human red blood cells

Red blood cells (RBCs) collected from a healthy donor were isolated by centrifugation at 3500 rpm for 5 min. After that RBCs were resuspended in PBS (5 vol%). 50 µL compound was taken in 96-well plate (working concentration of compounds from 1024 µg/mL to 1 µg/mL) and 150 µL of cell suspension was added to solutions of serially diluted solution. One without the compounds (only 50 µL PBS) and the other with 0.1 vol% solution of Triton X-100 (TRX, 50 µL), were the two controls. After compound treatment the plate was incubated for 1 h at 37 °C. Next, RBCs were centrifuged at 3500 rpm for 5 minutes and 100 µL supernatants from the wells were transferred to a new 96-well plate to measure the absorbance of the supernatants at 540 nm. Hemolysis percentage was calculated using this equation, $(A–A_o)/(A_{total}–A_o) \times 100$, where absorbance for the RBC suspension with the test samples and wells contained RBC suspension with only PBS are denoted as A and $A_o$, respectively. $A_{total}$ is absorbance of fully lysed cells (wells with TRX), all at 540 nm.

## 4. Time-kill kinetics of QPEINH-$C_6$

### 4.1. Bactericidal kinetics against planktonic bacteria

Mid-log phase of bacterial culture ($10^8$–$10^9$ CFU/mL, MRSA ATCC 33591 and VRSA 1) were suspended in MHB to obtain $10^5$-$10^6$ CFU/mL. This bacterial suspension (180 µg/mL) was treated with 640 µg/mL and 1280 µg/mL of QPEINH-$C_6$ solution (20 µL) (final concentration obtained 64 µg/mL and 128 µg/mL) followed by incubation at 37 °C. 20 µL saline was used as a negative control instead of any antimicrobial compound. 20 µL of aliquots from the wells were diluted 10-fold serially in saline at 0 h, 1 h, 3 h, and 6 h time points. After dilutions, 20 µL were spot plated on nutrient agar plate and incubated for 24 h in 37 °C to count viable bacteria cells.

### 4.2. Fungicidal kinetics

$10^8$ CFU/mL of fungal culture (*C. albicans* AB226 and *C. albicans* AB399) was suspended in RPMI to obtain ~$10^5$ CFU/mL cells. This fungal suspension (180 µg/mL) was treated with 1280 µg/mL and 2560 µg/mL of QPEINH-$C_6$ solution (20 µL) (final concentration obtained 128 µg/mL and 256 µg/mL) followed by incubation at 37 °C. 20 µL saline was used as a negative control instead of any antimicrobial compound. 20 µL of aliquots from the wells were diluted 10-fold serially in saline at 0 h, 1 h and 3 h time points. After dilutions, 20 µL were spot plated on YPD agar plate and incubated for 24 h in 37 °C for counting viable cells. The detection limit for this experiment was 50 CFU/mL.

### 4.3. Bactericidal kinetics of QPEINH-$C_6$ against stationary phase bacteria

5 µL of mid-log bacterial solution was cultured in 5 mL nutrient broth in a 50 mL falcon tube for 16 h to get stationary phase bacteria. After that stationary phase bacterial cultures (MRSA ATCC 33591 and VRSA 1) were diluted in MHB and 180 µL of $10^5$–$10^6$ CFU/mL bacterial suspension was treated with 640 µg/mL and 1280 µg/mL of QPEINH-$C_6$ solution (20 µL) (Final concentration obtained 64 µg/mL and 128 µg/mL) followed by incubation at 37 °C. 20 µL saline was used as a negative control instead of any antimicrobial compound. 20 µL of aliquots from the wells were diluted 10-fold serially in saline at 0 h, 1 h, 3 h, and 6 h time points. After dilutions, 20 µL were spot plated on nutrient agar and incubated for 24 h at 37 °C for counting viable cells.

### 5. Antibiofilm activity of QPEINH-$C_6$

Antibiofilm activity of QPEINH-$C_6$ was assessed against drug-resistant biofilm forming deadly pathogens (MRSA ATCC 33591 and VRSA 1).[4] Glass coverslips with diameter of 18 mm were sterilized using ethanol and placed in wells of 6-well plate after drying it in presence of flame. These sterile coverslips were allowed to cool to room temperature for 10-15 minutes. After that, 2 mL of mid-log phase bacterial suspension (~$10^5$ CFU/mL suspended in nutrient broth supplemented with 1 % (w/v) NaCl and 1 % (w/v) glucose) was added to the wells containing coverslips. Biofilms were allowed to form on coverslips under stationary condition for 24 h at 37 ºC. At the end of the required time, biofilms

containing coverslips were taken out carefully and washed with saline. After that, they were placed into 6-well plate and treated with 2 mL biofilm media containing 128 μg/mL and 256 μg/mL QPEINH-$C_{6.}$ Then biofilms were incubated for 24 h at 37 °C. After 24 h coverslips were taken out from the media and placed in another 6-well plate, then 1mL trypsin-EDTA + Saline (1:9, v/v) solution was used in each plate to detach the biofilm from the coverslip. The biofilms were scratched and collected. Solution was serially diluted and plated on agar plate to count viable bacterial burden in the biofilms.

**6. Mechanism of antibacterial action through membrane depolarization assay**

Planktonic bacteria culture (MRSA ATCC33591) was centrifuged (3500 rpm, 5 min) to collect the bacterial pellet, which was rinsed with 5 mM HEPES buffer and then suspended in 1:1:1 of 5 mM HEPES buffer, 5 mM glucose, and 100 mM KCl solution. DiSC 3 (5) (3,3′-Dipropylthiadicarbocyanine iodide) was added to this bacterial suspension and the final concentration of dye was maintained at 2 μM. Bacterial suspension with this dye (180 μL) was put into each well of clear bottom 96-black well plate which was further incubated for 1 h in dark condition. After incubation, fluorescence intensity of dye containing bacterial suspensions was measured for 4 min duration at excitation wavelength of 670 nm and emission wavelength of 622 nm. 640 μg/mL and 1280 μg/mL of QPEINH-$C_6$ solution (20 μL, final concentration obtained was 64 μg/mL and 128 μg/mL) was kept with bacterial suspension (180 μL) and monitored for 20 min. 20 μL of Millipore water with the bacterial suspension was considered as negative control.

**7. Live-dead assay of compound against microbes**

1 mL of the $10^8$ CFU/mL bacteria cells (MRSA ATCC 33591) and fungi cells (*C. albicans* AB226) were pelleted down by centrifuging (3500 rpm for 5 min). Then the media was discarded, and the pellet was washed with saline. Then the bacteria/ fungi cells were resuspended in 1 mL saline and treated with QPEINH-$C_6$ compound solution was put into the bacterial cell suspension to attain the final concentration of 128 μg/mL. Compound treated bacteria/fungi suspension was incubated for 6 h at 37 °C and afterthat bacteria/fungi cells were pelleted down by centrifugation (5 min at 3500 rpm). The supernatant was removed, and bacteria/ fungi pellet was again re-suspended in saline. Into that solution SYTO-9 and PI were added to obtain a final concentration of 3 μM and 15 μM, respectively. This bacterial solution was incubated with dyes for 30 min in dark condition. The excess dye was discarded by centrifugation followed by rinsing with saline. After that on the glass slide 5 μL solution was placed and Leica DM2500 microscope at 40X objective was captured the fluorescence images. 450-490 nm wavelength band-pass filter and 515-560 nm wavelength band-pass filter were used for SYTO-9 and PI respectively.[3]

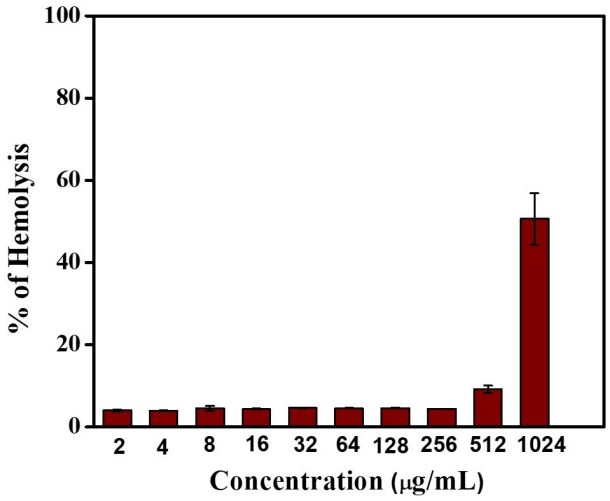


**Fig. S1** Percentage of hemolysis of QPEINH-$C_6$ at different concentration.

## References


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