

BIODEGRADABLE POLYION COMPLEX NANOPARTICLES OF CATIONIC ANTIMICROBIALS FOR ACTIVATABLE ANTIBACTERIAL THERAPY

Field of Invention

The current invention relates to an antimicrobial composite material, a pharmaceutical composition comprising the antimicrobial composite material, a method of treating a subject suffering from a microbial and/or fungal infection comprising the steps of administering to the subject a therapeutically effective amount of the antimicrobial composite material or the pharmaceutical composition, use of the antimicrobial composite material in the manufacture of a medicament to treat a microbial and/or fungal infection in a subject in need thereof, the antimicrobial composite material or the pharmaceutical composition for use in the treatment of a microbial and/or fungal infection, and a method of manufacture of the antimicrobial composite material.

Background

The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Rapid emergence of antimicrobial resistance (AMR) and sluggish development of new antibiotic candidates have created the imminent danger of heading to a “post-antibiotic era”, in which even a common infection or a minor injury can be life-threatening (Kwon, J.H., *Science* **2021**, 373, 471; Reardon, S., WHO warns against ‘post-antibiotic’ era, *Nature* **2014**; Leonard, C.T. et al., *Lancet*, **2017**, 389, 803). Alternative antibacterial strategies such as cationic polymeric systems (Lam, S.J. et al., *Nature Microbiology*, **2016**, 1, 16162; Ilker, M.F. et al., *JACS*, **2004**, 126, 15870; Tew, G.N. et al., *PNAS*, **2002**, 99, 5110; Zhou, C. et al., *Biomacromolecules*, **2010**, 11, 60; Li, P. et al., *Adv. Mater.*, **2012**, 24, 4130), bacteriolytic enzymes (Loeffler, J.M. et al., *Science*, **2001**, 294, 2170; Kerr, D.E. et al., *Nature Biotechnology*, **2001**, 19, 66) and stimuli-responsive materials (Kalelkar, P.P. et al., *Nature Reviews Materials*, **2022**, 7, 39; Mura, S. et al., *Nature Materials*, **2013**, 12, 991) to combat AMR have received extensive interest. In particular, cationic antibacterial agents are promising candidates that have demonstrated wide-spectrum antimicrobial activity and, more importantly, rare occurrence of resistance owing to their physical interaction mechanism with bacterial membrane (Fjell, C.D. et al., *Nature Reviews Drug Discovery*, **2012**, 11, 37; Zasloff, M. *Nature*, **2002**, 415, 389; Xu, J. et al., *Polymer Chemistry*, **2020**, 11, 6632; Pu, L. et al.,

Biomaterials Science, **2016**, 4, 871). However, clinical translation of cationic antimicrobials has been held back by their *in vivo* toxicity, such as the nonspecific interaction with abundant negatively charged surface components on human cells (Bacalum, M. & Radu, M., *International Journal of Peptide Research and Therapeutics*, **2015**, 21, 47; Askari, P. et al., *BMC Pharmacology and Toxicology*, **2021**, 22, 42; Stratton, T.R. et al., *Biomacromolecules*, **2009**, 10, 2550; Dathe, M. et al., *FEBS Letters*, **2001**, 501, 146; Jiang, Z. et al., *Peptide Science*, **2008**, 90, 369; Matsuzaki, K., *Biochimica et Biophysica Acta (BBA) – Biomembranes*, **2009**, 1788, 1687; Brogden, K., *Nat. Rev. Microbiol.*, **2005**, 3, 238). Intense efforts have been made to develop stimuli-responsive systems whose cationic charges become available only in specific bacterial infection microenvironments for subsequent bactericidal actions (Zhang, B. et al., *Biomaterials Science*, **2023**, 11, 356). Bacterial invasion and colonization can form unique microenvironments, mainly resulting from pathogen-secreted metabolites, toxins, and infection induced-inflammation. Reactive oxygen species (ROS), pH, and bacteria or host-secreted enzymes (e.g., lipase and hyaluronidase) that are often at aberrant levels during infection can also serve as triggers to activate the antimicrobial activity (Liu, Y. et al., *ACS Applied Materials & Interfaces*, **2019**, 11, 26590; Ma, J. et al., *Macromolecular Rapid Communications*, **2021**, 42, 2100255; Radovic-Moreno, A.F. et al., *ACS Nano*, **2012**, 6, 4279; Yu, J. et al., *Advanced Functional Materials*, **2022**, 32, 2202857).

Lipase is a virulence factor secreted by a variety of bacteria strains including *Staphylococcus aureus* that does not only hydrolyze its natural substrate glycerol esters, but also synthetic materials like poly(ϵ -caprolactone) (PCL) (Gan, Z. et al., *Polymer Degradation and Stability*, **1997**, 56, 209; Hu, C. et al., *Biochemical and Biophysical Research Communications*, **2012**, 419, 617). The selective degradation by lipase has enabled the use of PCL as drug carrier for the targeted delivery of small molecule antibiotics (Xiong, M.-H. et al., *JACS*, **2012**, 134, 4355; Xiong, M.-H. et al., *Advanced Materials*, **2012**, 24, 6175; Wang, A. et al., *Advanced Functional Materials*, **2021**, 31, 2011165), and loading of cationic quaternary ammonium salt in an implant coating (Wang, A. et al., *Advanced Functional Materials*, **2021**, 31, 2011165).

Summary of Invention

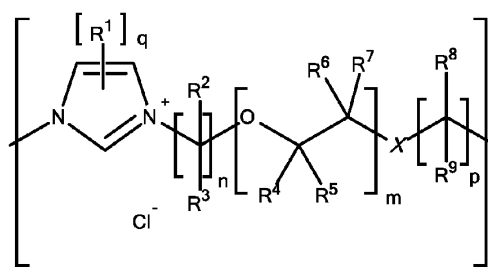
Disclosed herein is a strategy in which cationic antimicrobials are “caged” in nanoparticles by block copolymers with a polyethylene glycol (PEG) stealth block and an anionic lipase-degradable block (see FIG. 1).

Through the formation of polyion complexes, the positive charges of cationic antimicrobials were successfully neutralized and the complex showed minimal toxicity to mammalian cells.

The complex is degradable by bacterial enzyme, thereby liberating free cationic antimicrobials to kill enzyme-secreting strains. It is envisioned that this complexation-based caging strategy could be a generally applicable formulation strategy to address the toxicity issue of cationic antimicrobials.

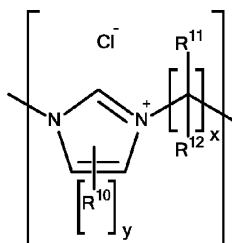
Aspects and embodiments of the invention will now be described by reference to the following numbered clauses.

1. An antimicrobial composite material, comprising:
a cationic antimicrobial compound; and
a bacterial-enzyme-degradable anionic copolymer, wherein
the bacterial-enzyme-degradable anionic copolymer and the cationic antimicrobial compound are attached to one another through electrostatic interaction.
2. The antimicrobial composite material according to Clause 1, wherein the cationic antimicrobial compound is selected from one or more of the group consisting of a polyimidazolium, an oligoimidazolium, and a cationic antimicrobial peptide.
3. The antimicrobial composite material according to Clause 2, wherein the cationic antimicrobial peptide is selected from one or more of the group Cecropin A, Cecropin B, Cecropin P1, Dermaseptin 1, Indolicidin, Lactofermicin B, Magainin I, Magainin II, Melittin, Polistes Mastoparan, Tachyplesin I, optionally wherein the cationic antimicrobial peptide is Melittin.
4. The antimicrobial composite material according to Clause 2, wherein the polyimidazolium has a repeating unit of formula (I):



(I)

or is a copolymer comprising the repeating unit of formula (I) and a repeating unit of formula (II):



(II)

wherein:

R^1 and R^{10} , when present, independently represent C_{1-6} alkyl;
 each R^2 to R^8 and R^{11} independently represent H or C_{1-6} alkyl;
 each R^9 and R^{12} independently represents H, C_{1-6} alkyl or CO_2R^{13} ;
 R^{13} represents H or C_{1-6} alkyl;
 X represents $CR^{14}R^{15}$, O or S;
 R^{14} and R^{15} independently represent H, C_{1-6} alkyl or CO_2R^{13} ;
 m is a number selected from 0 to 5;
 n is a number selected from 2 to 10;
 p is a number selected from 0 to 5;
 q is a number selected from 0 to 3;
 x is a number selected from 2 to 10;
 y is a number selected from 0 to 3; and
 solvates thereof,

provided that, when the polyimidazolium is a copolymer, the repeating unit of formula (I) and the repeating unit of formula (II) are not the same.

5. The antimicrobial composite material according to Clause 4, wherein:

R^1 and R^{10} , when present, independently represent C_{1-3} alkyl;
 each R^2 to R^8 and R^{11} independently represents H or methyl;
 each R^9 and R^{12} independently represents H, C_{1-3} alkyl or CO_2R^{13} ;
 R^{13} represents H or C_{1-3} alkyl;
 X represents $CR^{14}R^{15}$ or O;
 R^{14} and R^{15} independently represent H, C_{1-3} alkyl or CO_2R^{13} ;
 m is a number selected from 0 to 4;
 n is a number selected from 2 to 8;

p is a number selected from 0 to 3;
q is a number selected from 0 to 1;
x is a number selected from 2 to 8; and
y is a number selected from 0 to 1.

6. The antimicrobial composite material according to Clause 4 or Clause 5, wherein:
each R^2 to R^8 and R^{11} independently represents H;
each R^9 and R^{12} independently represents H, methyl or CO_2H ;
X represents $\text{CR}^{14}\text{R}^{15}$ or O;
 R^{14} and R^{15} independently represent H, methyl or CO_2H ;
m is a number selected from 0 to 3;
n is a number selected from 2 to 7;
p is a number selected from 0 to 2;
q and y are 0; and
x is a number selected from 2 to 7.

7. The antimicrobial composite material according to any one of Clauses 4 to 6, wherein:
each R^2 to R^8 and R^{11} independently represents H;
each R^9 and R^{12} independently represents H, methyl or CO_2H ;
X represents $\text{CR}^{14}\text{R}^{15}$ or O;
 R^{14} and R^{15} independently represent H, methyl or CO_2H ;
m is a number selected from 0 to 2;
n is a number selected from 2 to 7;
p is a number selected from 0 to 2;
q and y are 0; and
x is a number selected from 2 to 7.

8. The antimicrobial composite material according to any one of Clauses 4 to 7, wherein
the number average molecular weight is from 500 to 7,000 Daltons.

9. The antimicrobial composite material according to any one of Clauses 4 to 8, wherein
the polyimidazolium has the repeating unit of formula (I).

10. The antimicrobial composite material according to Clause 9, wherein:
each of R^2 to R^8 are H;
each R^9 represents H, methyl or CO_2H ;
X represents CH_2 or O;

m is a number selected from 0 to 2;

n is a number selected from 2 to 6;

p is a number selected from 0 to 2;

q is 0.

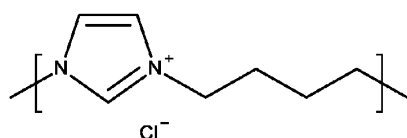
11. The antimicrobial composite material according to any one of Clauses 4 to 10, wherein one or both of the following apply:

(ia) when X is O, p is 1 or 2; and

(ib) the polyimidazolium is terminated by amino (NH₂) groups.

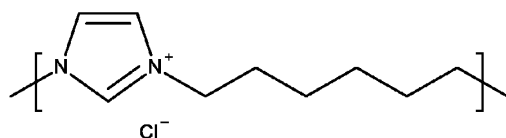
12. The antimicrobial composite material according to any one of Clauses 4 to 11, wherein the repeating unit is selected from the group consisting of:

(i)



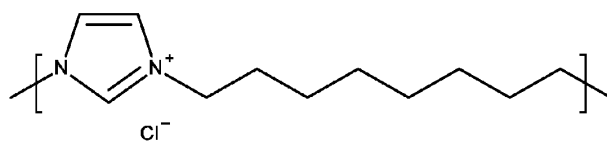
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(ii)



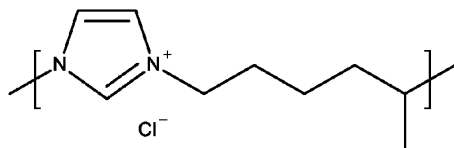
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(iii)



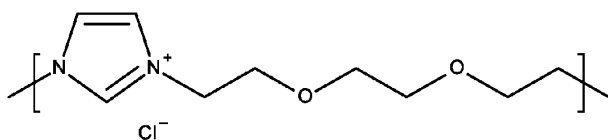
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(iv)



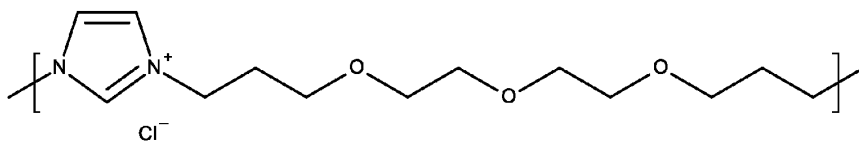
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,000 Daltons;

(v)



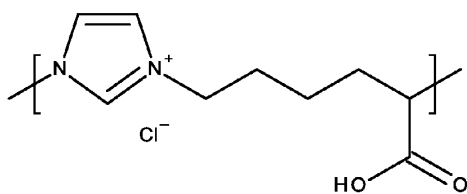
, optionally wherein the number average molecular weight of the polymer is from 4,000 to 5,500 Daltons;

(vi)



, optionally wherein the number average molecular weight of the polymer is from 4,000 to 5,000 Daltons; and

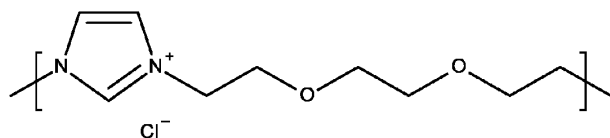
(vii)



, optionally wherein the number average molecular weight of the polymer is from 500 to 2,000 Daltons.

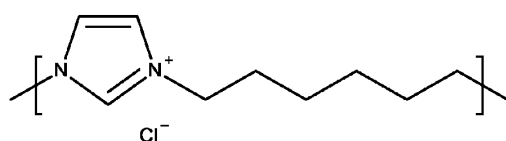
13. The antimicrobial composite material according to any one of Clauses 4 to 8, wherein the repeating unit of formula (I) and formula (II) are selected from the group consisting of:

(i)



as the repeating unit of

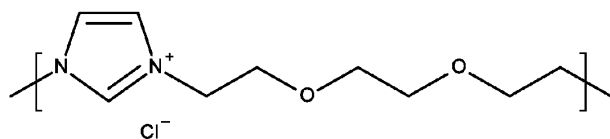
formula (I) and



as the repeating unit of formula (II) ,

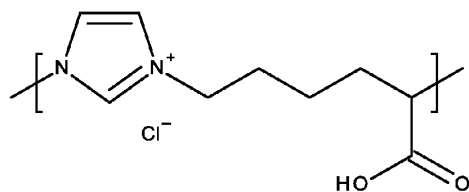
optionally wherein the number average molecular weight of the copolymer is from 1,000 to 5,000 Daltons; and

(ii)



as the repeating unit of

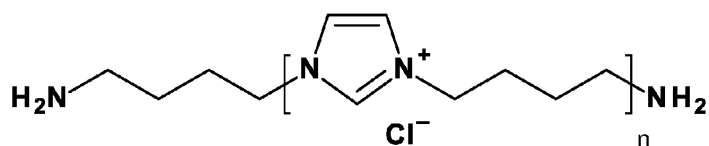
formula (I) and



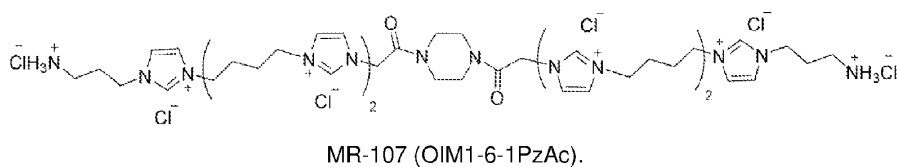
as the repeating unit of formula (II) ,

optionally wherein the number average molecular weight of the copolymer is from 1,000 to 5,000 Daltons.

14. The antimicrobial composite material according to Clause 2, wherein the polyimidazolium is:

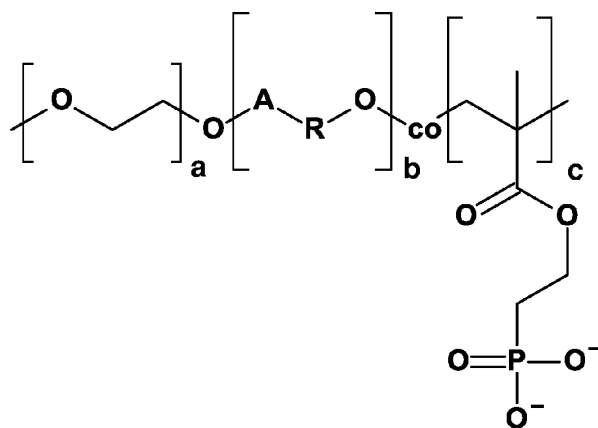


15. The antimicrobial composite material according to Clause 2, wherein the oligoimidazolium is MR-107 (OIM1-6-1PzAc):



16. The antimicrobial composite material according to any one of the preceding clauses, wherein the bacterial-enzyme-degradable anionic copolymer comprises a repeating unit that bears a phosphonate group.

17. The antimicrobial composite material according to any one of the preceding clauses, wherein the bacterial-enzyme-degradable anionic copolymer has the formula (III):



(III)

where:

R is a C₁ to C₁₀ linear or branched alkyl chain;

A represents C=O or P(OR_a)=O;

R_a represents a linear or branched C₁ to C₆ alkyl group;

a represents a block polyethylene glycol unit having a number average molecular weight of from 500 to 10,000 Daltons;

b and c together represent a random copolymer block, where b and c each independently have a value of from 8 to 100.

18. The antimicrobial composite material according to Clause 17, wherein one or more of the following apply:

(a) a represents a block polyethylene glycol unit having a number average molecular weight of about 2,000 Daltons;

(b) b and c each independently have a value of from 10 to 50, such as 15 to 20, such as 18;

(c) A represents C=O; and

(d) R represents $-(CH_2)_5-$.

19. The antimicrobial composite material according to any one of Clauses 16 to 18, wherein the bacterial-enzyme-degradable anionic copolymer has a number average molecular weight of from 6,000 to 10,000 Daltons, such as from 7,000 to 8,000 Daltons.

20. The antimicrobial composite material according to any one of the preceding clauses, wherein the bacterial-enzyme-degradable anionic copolymer is degradable by a bacterial-secreted lipase or a bacterial-secreted phosphoesterase, such as a bacterial-secreted lipase.

21. The antimicrobial composite material according to any one of the preceding clauses, wherein the weight to weight ratio of the cationic antimicrobial compound and the bacterial-enzyme-degradable anionic copolymer is selected to provide a material with a zeta potential of from +10 to -10 mV, such as from +6 to -6 mV, such as from 0 to -5.3 mV.

22. The antimicrobial composite material according to any one of the preceding clauses, wherein the weight to weight ratio of the cationic antimicrobial compound and the bacterial-enzyme-degradable anionic copolymer is from 1:5 to 5:1, such as from 1:2 to 2:1, such as about 1:1.7.

23. A pharmaceutical composition comprising an antimicrobial composite material according to any one of Clauses 1 to 22 and one or more of a pharmaceutically acceptable excipient and carrier.

24. An antimicrobial composite material according to any one of Clauses 1 to 22, or a pharmaceutical composition as described in Clause 23, for use as a medicament.

25. A method of treating a subject suffering from a microbial and/or fungal infection comprising the steps of administering to the subject a therapeutically effective amount of an antimicrobial composite material according to any one of Clauses 1 to 22, or a pharmaceutical composition as described in Clause 23, such that the infection is treated.

26. Use of an antimicrobial composite material according to any one of Clauses 1 to 22, or a pharmaceutical composition as described in Clause 23, in the manufacture of a medicament to treat a microbial and/or fungal infection in a subject in need thereof.

27. An antimicrobial composite material according to any one of Clauses 1 to 22, or a pharmaceutical composition as described in Clause 23, for use in the treatment of a microbial and/or fungal infection.

28. A method of manufacture of an antimicrobial composite material according to any one of Clauses 1 to 22, the method comprising the steps of:

- (i) providing a solution of a cationic antimicrobial compound and providing a solution of a bacterial-enzyme-degradable anionic copolymer as described in any one of Clauses 1 to 22; and
- (ii) mixing the solutions together to form a mixture and agitating the mixture for a period of time to form the antimicrobial composite material.

Drawings

FIG. 1 depicts the schematic illustration of the self-assembly of the cationic antimicrobials with the caging polymers to form cationic antimicrobial-caged complexes, and the release of free cationic antimicrobials upon degradation by bacterial enzyme.

FIG. 2 depicts the synthesis route of the caging copolymer PEG-*b*-(PCL-*co*-PPMA). (A) Synthesis of MA-DMOPE (phosphonic monomer) from methacryloyl chloride (MA-Cl) and dimethyl (2-hydroxyethyl) phosphonate (DMOPE); reaction condition: with triethanolamine

(TEA), in tetrahydrofuran (THF), under nitrogen (N_2) atmosphere, reaction time of 16 h. (B) Synthesis of PEG-*b*-(PCL-*co*-PPMA_{AdM}) copolymer by reacting phosphonic monomer with monomethoxy poly(ethylene glycol) (mPEG-OH) and ϵ -caprolactone; reaction condition: with $tBuP_4$ (1-tert-Butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)-phosphoranylidene-amino]-2 λ 5,4 λ 5-catenadi(phosphazene)), in THF, 60 °C, reaction time of 18 h. (C) Synthesis of PEG-*b*-(PCL-*co*-PPMA) copolymer from PEG-*b*-(PCL-*co*-PPMA_{AdM}) copolymer, reaction condition: step (1) with bromotrimethylsilane (TMSBr), in dichloromethane (DCM), reaction time of 24 h, step (2) in methanol (MeOH), reaction time of 24 h. See Example 1 for more details.

FIG. 3 depicts the design of the polyion complex nanoparticle formed by the self-assembly of polyimidazolium (PIM) and caging polymer PEG-*b*-(PCL-*co*-PPMA) for selective antimicrobial activity.

FIG. 4 (A) depicts 1H nuclear magnetic resonance (NMR) spectrum of the phosphonic monomer MA-DMOPE, recorded in $CDCl_3$, at 298 K, 400 MHz; (B) depicts 1H NMR spectrum of the methyl group-protected caging copolymer PEG-*b*-(PCL-*co*-PPMA_{AdM}), recorded in D_2O , at 298 K, 400 MHz; (C) depicts 1H NMR spectrum of the methyl group-protected caging copolymer PEG-*b*-(PCL-*co*-PPMA_{AdM}) (top) and the deprotected PEG-*b*-(PCL-*co*-PPMA) (bottom), recorded in D_2O , at 298 K, 400 MHz.

FIG. 5 depicts the gel permeation chromatography in dimethylformamide (DMF GPC) spectrum of PEG-*b*-(PCL-*co*-PPMA).

FIG. 6 depicts the characterization of the self-assembled polyion PIM-caged PEG-*b*-(PCL-*co*-PPMA) complex. (A) Zeta potential of complexes with different mixing ratios. (B) Transmission electron microscopy (TEM) image of complexes before and after lipase treatment. (C) Structures of the fluorescent dye-modified caging copolymers. (D) Emission spectra of the dye-complexes before and after lipase treatment. (E) Quantitative data of the fluorescence intensity of the Cyanine3 (Cy3) peak to Cyanine5 (Cy5) peak ratio.

FIG. 7 depicts the hydrodynamic diameter of (A) PIM-caged PEG-*b*-(PCL-*co*-PPMA) complex; (B) PIM-caged PEG-*b*-(PCL-*co*-PPMA) complex treated by lipase from *Pseudomonas Cepacia*; and (C) lipase alone ($n = 3$).

FIG. 8 depicts the synthesis route of the non-degradable copolymer PEG-*b*-(PPMA-*co*-PPMA).

FIG. 9 depicts the ^1H NMR spectrum of PEG-*b*-(PPMA-*co*-PPMA_{AdM}), recorded in CDCl_3 at 298K, 400 MHz.

FIG. 10 depicts the comparison of ^1H NMR spectrum of PEG-*b*-(PPMA-*co*-PPMA_{AdM}) (top) and the deprotected PEG-*b*-(PPMA-*co*-PPMA). The later was recorded in D_2O at 298K, 400 MHz.

FIG. 11 depicts the zeta potential of complex formed by different ratios of PEG-*b*-(PPMA-*co*-PPMA) and PIM.

FIG. 12 depicts the synthesis route for MR-107 (OIM1-6-1PzAc).

FIG. 13 depicts (A) the zeta potential of complex formed by different ratios of PEG-*b*-(PCL-*co*-PPMA) and MR-107; (B) the hydrodynamic diameter of MR-107-caged PEG-*b*-(PCL-*co*-PPMA) complex; and (C) TEM image of MR-107-caged PEG-*b*-(PCL-*co*-PPMA) complex.

FIG. 14 depicts (A) schematic drawing of the structure of melittin (reference: Terwiliger, T.C. & Eisenberg, D., *The Journal of Biological Chemistry*, **1982**, 257, 6016) (B) the zeta potential of complex formed by different ratios of PEG-*b*-(PCL-*co*-PPMA) and melittin; (C) the hydrodynamic diameter of melittin-caged PEG-*b*-(PCL-*co*-PPMA) complex; and (D) TEM image of melittin -caged PEG-*b*-(PCL-*co*-PPMA) complex.

FIG. 15 depicts (A) the synthesis route of the Cy3 and Cy5-conjugated copolymer 1-6; (B) the comparison of ^1H NMR spectra between copolymer 1-4, bottom inset is the downfield of the NMR spectra showing the aromatic H signals in Cy3 and Cy5 (Nuhn, L. et al., *Angewandte Chemie International Edition*, **2018**, 57, 10760), spectra were recorded in recorded in CDCl_3 , at 298K, 400 MHz; (C) Comparison of ^1H NMR spectra between dye-conjugated copolymer 3-6, spectra of copolymer 3 and 4 were recorded in recorded in CDCl_3 , at 298K, 400 MHz, spectra of copolymer 5 and 6 were recorded in recorded in D_2O , at 298K, 400 MHz.

FIG. 16 depicts (A) the excitation and emission spectrum of copolymer 3-6, the shadow indicates spectrum overlap between the emission of donor and excitation of acceptor; (B) the schematic illustration of the complexation of 1:1 mixed dye-conjugated copolymer with PIM, step (1) is the 1:1 mixing of dye-conjugated copolymers, step (2) is the mixing of dye-conjugated copolymers and PIM to form complex; and (C) the zeta potential of dye-labelled complex with different mixing ratio, the dye-conjugated copolymer alone, and 1:1 mixture of the dye-conjugated copolymers.

FIG. 17 depicts the tributyrin agar test of 4 strains of bacteria to confirm their lipase producibility. The arrows indicate the clear halo formed resulting from lipase activity.

FIG. 18 depicts the *in vitro* selective antibacterial efficacy. Inhibition curve of the PIM-caged PEG-b-(PCL-co-PPMA) complex, PIM and vancomycin on (A) methicillin-resistant *Staphylococcus aureus* (MRSA) and (B) *Enterococcus faecalis* (*E. faecalis*). (C) Inhibition curve of the complex formed by undegradable copolymer PEG-b-(PMMA-co-PPMA) and PIM on MRSA. The inset in (C) is the chemical structure of main-chain-undegradable PEG-b-(PMMA-co-PPMA). (D) Inhibition curve of the complex formed by undegradable copolymer and PIM on *E. faecalis*. (E) Colony forming units (CFUs) of MRSA after treated by different concentration of complex. The concentrations are shown in multiple of minimal inhibitory concentration (MIC). (F) SYTO9/PI staining of MRSA after treatment with different concentrations of PIM-caged PEG-b-(PCL-co-PPMA) complex. The live bacteria with intact membrane were stained by SYTO 9, green in colour (such as in "Control"). The dead MRSA was stained by propidium iodide (PI, red in colour) resulting from the compromised membrane integrity.

FIG. 19 depicts the inhibition curve of the PIM-caged PEG-b-(PCL-co-PPMA) complex and PIM on (A) *B.subtilis* and (B) *E.coli*.

FIG. 20 depicts the inhibition curves of the MR-107-caged PEG-b-(PCL-co-PPMA) complex and MR-107 on MRSA, *Pseudomonas aeruginosa* (*P.aeruginosa*) PAO1, *Pseudomonas aeruginosa* BAA-2797, *Klebsiella pneumonia* SGH-10, and *E. faecalis*.

FIG. 21 depicts the inhibition curves of the melittin-caged PEG-b-(PCL-co-PPMA) complex and melittin on MRSA and *E. faecalis*.

FIG. 22 depicts the evaluation of *in vitro* and *in vivo* safety of the PIM-caged PEG-b-(PCL-co-PPMA) complex. (A) Cytotoxicity to 3T3 cells of PIM, complex with near neutral charge, complex with positive charge, and caging copolymer alone. (B) Calcein AM/EthD-1 staining of 3T3 cells treated with PIM and complex. (C-E) The body weight changes of mice after being treated with single dose of PIM, phosphate-buffered saline (PBS, and repetitive dose of complex, respectively (n=5). The arrows in (E) indicates the days that complex was injected.

FIG. 23 depicts the body weight change of mice after being treated with single dose of (A) 10 g/mL and (B) 12 mg/mL complex, respectively.

FIG. 24 depicts the level of blood biochemical markers (A) alanine aminotransferase (ALT) and (B) aspartate aminotransferase (AST), and the level of kidney function markers (C) blood urea nitrogen (BUN) and (D) phosphorous (P) after treatment with PIM-caged PEG-b-(PCL-co-PPMA) complex, at set times (n=5).

FIG. 25 depicts the evaluation of *in vitro* selective biocompatibility of the MR-107-caged PEG-b-(PCL-co-PPMA) complex – evaluation of cytotoxicity to 3T3 cells of MR-107 alone and MR-107-caged PEG-b-(PCL-co-PPMA) complex after (A) 24 h, (B) 48 h, and (C), 72 h.

FIG. 26 depicts the evaluation of the *in vivo* safety of the MR-107-caged PEG-b-(PCL-co-PPMA) complex. The body weight changes of mice after being treated with (A) multiple dose intraperitoneal (I.P.) injection of MR-107 alone or complex (n=2, 15 mg/kg); and (B) single dose intravenous (I.V.) injection of complex (n=3). The arrows in (A) indicate the days that MR-107 was injected, one of the mice died on day 2 and the other mouse died on day 3. For single dose I.V. injection of MR-107 alone, all mice died immediately after injection. The mice remained unaffected after multiple dose I.P. injection of complex in (A) and after single dose I.V. injection of complex in (B).

FIG. 27 depicts the evaluation of *in vitro* selective biocompatibility of the melittin-caged PEG-b-(PCL-co-PPMA) complex – evaluation of cytotoxicity to 3T3 cells and MCF-7 cells of melittin alone, PEG-b-(PCL-co-PPMA) copolymer alone, and melittin-caged PEG-b-(PCL-co-PPMA) complex.

FIG. 28 depicts the *in vivo* therapeutic efficacy of the complex. (A) Schematic illustration of the experimental procedure of the therapeutic efficacy analysis, Group (1) – recording survival status and body weight for 14 days, Group (2) – dissection, organ collection, bacteria count. (B) Survival rate and (C) change in body weight of the MRSA-infected mice after different treatments (n=6). (D-G) MRSA count in I.P. fluid, liver, spleen, and kidney, respectively after 24-h treatment. Data are represented as the mean $\pm$ SD. (*) $p < 0.05$ and (* *) $p < 0.01$ (one-way ANOVA with Tukey's post hoc test; n = 6).

FIG. 29 shows a schematic illustration of the acute murine lung infection model.

Description

The presence of hydrophobic and anionic segments of the copolymer provides the driving force for self-assembly to package the cationic antimicrobial into the complex core (Meka, V.S. et al., *Drug Discovery Today*, **2017**, 22, 1697), thus minimizing the cellular and *in vivo* toxicity of the cationic antimicrobial before it reaches the infection site. The hydrophilic block of the copolymer provides stealth property and endows the system with colloidal stability in physiological environment. The above arrangement blocks the antimicrobial properties of the cationic antimicrobial and reduces the toxicity of the cationic antimicrobial to mammalian cells. The potency of the cationic antimicrobial is recovered at sites of infection by enzyme-secreting bacteria due to the degradation of the enzyme-degradable moiety, which leads to the dissociation of the complex. Consequently, the cationic antimicrobial is released and "activated" to interact with negatively charged bacterial membrane, and selectively kill the enzyme-producing bacteria (e.g. MRSA). Compared to free cationic antimicrobial alone, cationic-antimicrobial-caged complex exhibited minimal toxicity but similar antimicrobial capability in an *in vivo* murine model of systemic MRSA infection.

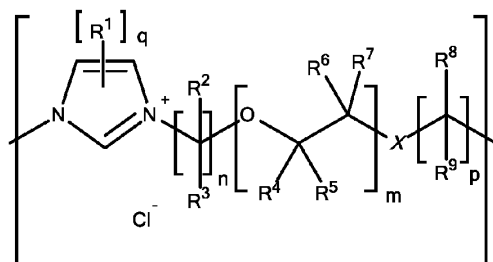
Thus, in a first aspect of the invention, there is provided an antimicrobial composite material, comprising:

- a cationic antimicrobial compound; and
- a bacterial-enzyme-degradable anionic copolymer, wherein the bacterial-enzyme-degradable anionic copolymer and the cationic antimicrobial compound are attached to one another through electrostatic interaction.

Any suitable cationic antimicrobial compound may be used herein. For example, the cationic antimicrobial compound may be selected from one or more of the group consisting of a polyimidazolium, an oligoimidazolium, and a cationic antimicrobial peptide.

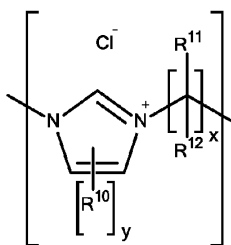
Any suitable cationic antimicrobial peptide may be used herein. For example, the cationic antimicrobial peptide may be selected from one or more of the group Cecropin A, Cecropin B, Cecropin P1, Dermaseptin 1, Indolicidin, Lactofermicin B, Magainin I, Magainin II, Melittin, Polistes Mastoparan, Tachyplesin I, optionally wherein the cationic antimicrobial peptide is Melittin.

Any suitable polyimidazolium may be used herein. For example, the polyimidazolium may have a repeating unit of formula (I):



(I)

or is a copolymer comprising the repeating unit of formula (I) and a repeating unit of formula (II):



(II)

wherein:

R¹ and R¹⁰, when present, independently represent C₁₋₆ alkyl;

each R² to R⁸ and R¹¹ independently represent H or C₁₋₆ alkyl;

each R⁹ and R¹² independently represents H, C₁₋₆ alkyl or CO₂R¹³;

R¹³ represents H or C₁₋₆ alkyl;

X represents CR¹⁴R¹⁵, O or S;

R¹⁴ and R¹⁵ independently represent H, C₁₋₆ alkyl or CO₂R¹³;

m is a number selected from 0 to 5;

n is a number selected from 2 to 10;

p is a number selected from 0 to 5;

q is a number selected from 0 to 3;

x is a number selected from 2 to 10;

y is a number selected from 0 to 3; and

solvates thereof,

provided that, when the polyimidazolium is a copolymer, the repeating unit of formula (I) and the repeating unit of formula (II) are not the same.

In embodiments of the polyimidazolium:

R^1 and R^{10} , when present, independently represent C_{1-3} alkyl;

each R^2 to R^8 and R^{11} independently represents H or methyl;

each R^9 and R^{12} independently represents H, C_{1-3} alkyl or CO_2R^{13} ;

R^{13} represents H or C_{1-3} alkyl;

X represents $CR^{14}R^{15}$ or O;

R^{14} and R^{15} independently represent H, C_{1-3} alkyl or CO_2R^{13} ;

m is a number selected from 0 to 4;

n is a number selected from 2 to 8;

p is a number selected from 0 to 3;

q is a number selected from 0 to 1;

x is a number selected from 2 to 8; and

y is a number selected from 0 to 1.

Additionally or alternatively, the polyimidazolium may be one in which:

each R^2 to R^8 and R^{11} independently represents H;

each R^9 and R^{12} independently represents H, methyl or CO_2H ;

X represents $CR^{14}R^{15}$ or O;

R^{14} and R^{15} independently represent H, methyl or CO_2H ;

m is a number selected from 0 to 3;

n is a number selected from 2 to 7;

p is a number selected from 0 to 2;

q and y are 0; and

x is a number selected from 2 to 7.

Additionally or alternatively, the polyimidazolium may be one in which:

each R^2 to R^8 and R^{11} independently represents H;

each R^9 and R^{12} independently represents H, methyl or CO_2H ;

X represents $CR^{14}R^{15}$ or O;

R^{14} and R^{15} independently represent H, methyl or CO_2H ;

m is a number selected from 0 to 2;

n is a number selected from 2 to 7;

p is a number selected from 0 to 2;

q and y are 0; and

x is a number selected from 2 to 7.

Additionally or alternatively, the polyimidazolium may be one in which the number average molecular weight may be from 500 to 7,000 Daltons.

Additionally or alternatively, the polyimidazolium may be one in which the polyimidazolium has the repeating unit of formula (I). For example, the polyimidazolium may be one in which:

each of R^2 to R^8 are H;

each R^9 represents H, methyl or CO_2H ;

X represents CH_2 or O;

m is a number selected from 0 to 2;

n is a number selected from 2 to 6;

p is a number selected from 0 to 2;

q is 0.

Unless otherwise stated, the term "alkyl" refers to an unbranched or branched, acyclic, saturated or unsaturated (so forming, for example, an alkenyl or alkynyl) hydrocarbyl radical, which may be unsubstituted or substituted (with, for example, one or more halo atoms). Where the term "alkyl" refers to an acyclic group, it is preferably C_{1-10} alkyl and, more preferably, C_{1-6} alkyl (such as ethyl, propyl (e.g. n-propyl or isopropyl), butyl (e.g. branched or unbranched butyl), pentyl or, more preferably, methyl).

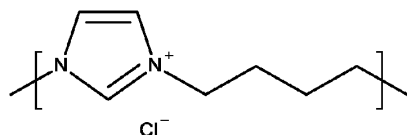
Additionally or alternatively, the polyimidazolium may be one in which one or both of the following apply:

(ia) when X is O, p is 1 or 2; and

(ib) the polyimidazolium is terminated by amino (NH_2) groups.

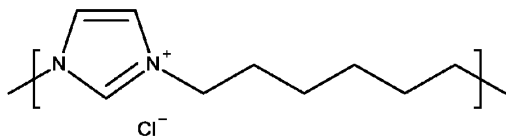
Additionally or alternatively, the polyimidazolium may be one in which the repeating unit is selected from the group consisting of:

(i)



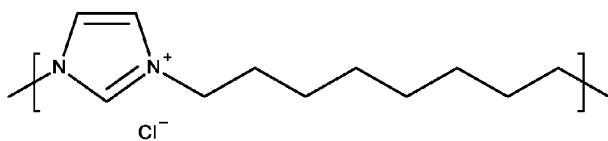
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(ii)



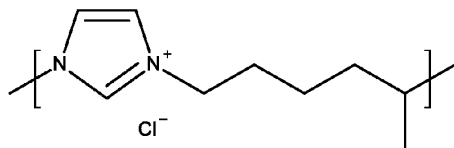
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(iii)



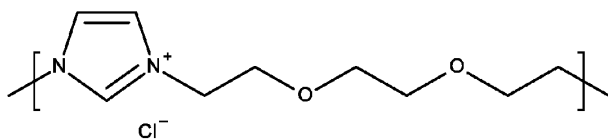
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(iv)



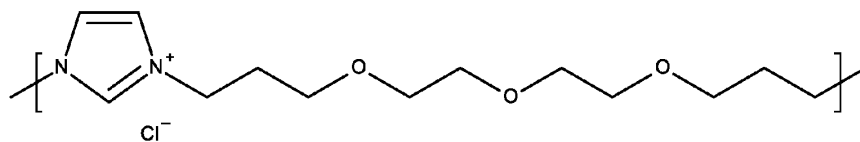
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,000 Daltons;

(v)



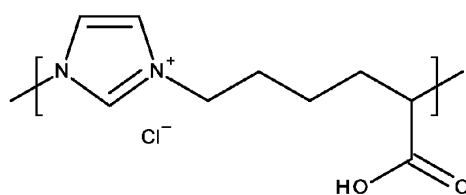
, optionally wherein the number average molecular weight of the polymer is from 4,000 to 5,500 Daltons;

(vi)



, optionally wherein the number average molecular weight of the polymer is from 4,000 to 5,000 Daltons; and

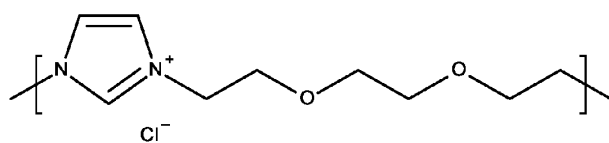
(vii)



, optionally wherein the number average molecular weight of the polymer is from 500 to 2,000 Daltons.

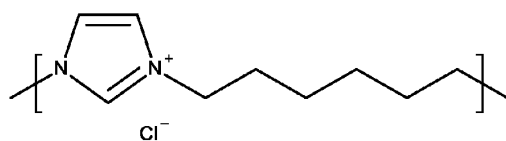
Additionally or alternatively, the polyimidazolium may be one in which the repeating unit of formula (I) and formula (II) are selected from the group consisting of:

(i)



as the repeating unit of

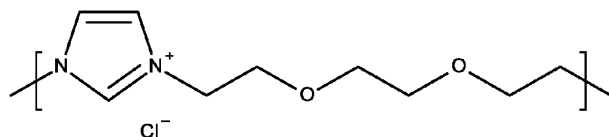
formula (I) and



as the repeating unit of formula (II) ,

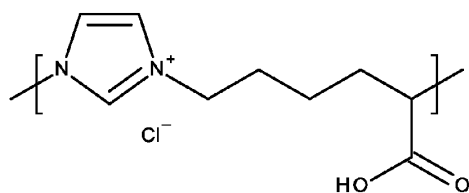
optionally wherein the number average molecular weight of the copolymer is from 1,000 to 5,000 Daltons; and

(ii)



as the repeating unit of

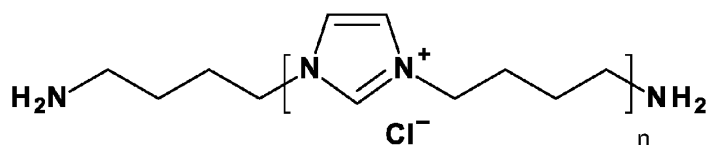
formula (I) and



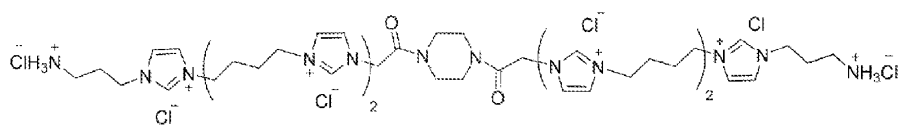
as the repeating unit of formula (II),

optionally wherein the number average molecular weight of the copolymer is from 1,000 to 5,000 Daltons.

In certain embodiments, the polyimidazolium may be:



In certain embodiments, the cationic antimicrobial compound present in the antimicrobial composite material of the present invention is an oligoimidazolium. In certain exemplary embodiments, the oligoimidazolium is MR-107 (OIM1-6-1PzAc), which has the chemical structure shown below (also see FIG. 12).



MR-107 (OIM1-6-1PzAc)

In certain embodiments, the cationic antimicrobial compound present in the antimicrobial composite material of the present invention may be a polymer or oligomer or a

pharmaceutically acceptable solvate thereof as defined according to the numbered clauses §1 to §9 below, or a molecule or a pharmaceutically acceptable solvate thereof as defined according to numbered clauses §10 to §15 below:

§1. A polymer or oligomer or a pharmaceutically acceptable solvate thereof comprising a first repeating unit comprising an imidazolium group and a biodegradable chain connected to an adjacent repeating unit.

§2. The polymer or oligomer according to §1, wherein the only repeating unit is the first repeating unit.

§3. The polymer or oligomer according to §1, wherein the polymer or oligomer further comprises a second repeating unit comprising an imidazolium group and a non-biodegradable alkyl chain or a further biodegradable alkyl chain connected to an adjacent repeating unit, optionally wherein the polymer or oligomer further comprises a second repeating unit comprising an imidazolium group and a non-biodegradable alkyl chain connected to an adjacent repeating unit.

§4. The polymer or oligomer according to §3, wherein one or more of the following apply:

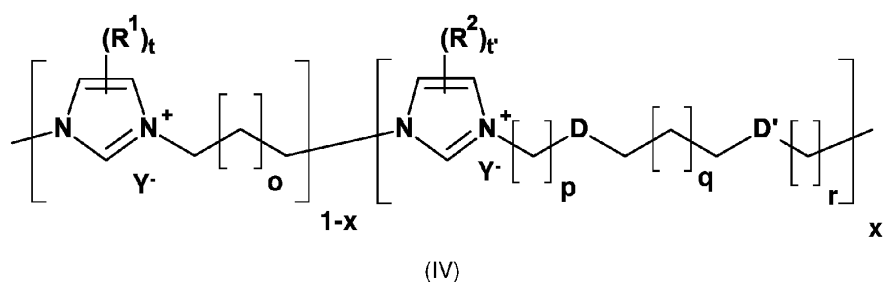
- (a) the polymer or oligomer comprises from 1 to 75 mol%, such as from 5 to 60 mol%, such as from 10 to 50 mol%, such as from 20 to 30 mol% of the first repeating unit; and
- (b) the repeating units of the polymer or oligomer are randomly distributed or the repeating units are formed as blocks, optionally wherein the repeating units of the polymer or oligomer are randomly distributed.

§5. The polymer or oligomer according to any one of §1 to §4, wherein the biodegradable chain in the first repeating unit comprises one or more biodegradable functional groups, where the one or more biodegradable functional groups are selected from one or more of the group consisting of urea, carbamate, acetal, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone, optionally wherein:

- (ai) the one or more biodegradable functional groups are selected from one or more of the group consisting of amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone;
- (aii) the one or more biodegradable functional groups are selected from one or more of the group consisting of carbamate or, more particularly, amide, ester and carbonate ester; or
- (aiii) the one or more biodegradable functional groups are amide.

§6. The polymer or oligomer according to any one of §1 to §5, wherein the number average molecular weight is from 800 to 10,000 Daltons, such as from 900 to 5,000 Daltons, such as from 1,000 to 3,000 Daltons, such as from 1,000 to 2,000 Daltons.

§7. The polymer or oligomer according to any one of §1 to §6, wherein the polymer or oligomer has the formula (IV):



wherein:

x is from 0.01 to 1.0;

Y⁻ is a counterion;

o is from 0 to 10;

p is from 1 to 12;

q is from 0 to 14;

r is from 0 to 12;

D is a biodegradable functional group;

D' is a biodegradable functional group or a bond;

each R¹ is a branched or unbranched C₁₋₃ alkyl or derivatives thereof;

each t is 0, 1 or 2;

each t' is 0, 1 or 2;

each R² is a branched or unbranched C₁₋₃ alkyl or derivatives thereof;

or a pharmaceutically acceptable solvate thereof.

§8. The polymer or oligomer according to §7, wherein one or more of the following apply:

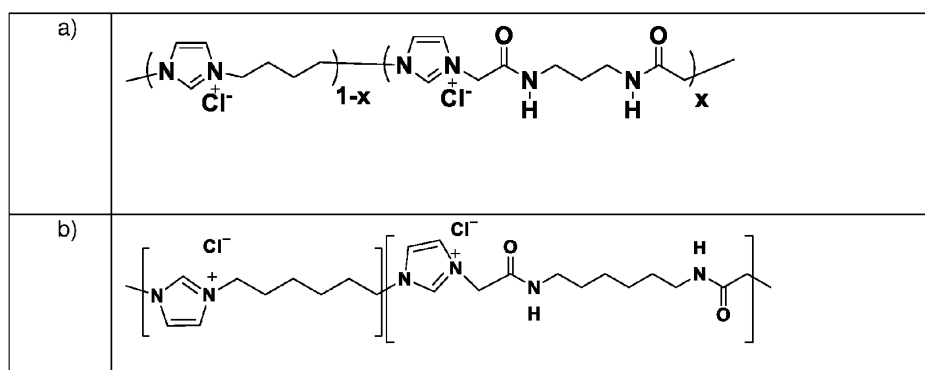
(bi) each D is selected from urea, carbamate, acetal, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone, optionally wherein:

(aa) each D is selected from one or more of the group consisting of amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone;

(ab) each D is selected from one or more of the group consisting of carbamate or, more particularly, amide, ester and carbonate ester; or

- (ac) each D is selected from one or more of the group consisting of carbonate ester and amide (e.g. each D is an amide);
- (bii) each D' is selected from a bond, urea, carbamate, acetal, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone, optionally wherein:
- (ad) each D' is selected from one or more of the group consisting of a bond, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone;
- (ae) each D' is selected from one or more of the group consisting of a bond, amide, ester, carbamate and carbonate ester;
- (af) each D' is selected from one or more of the group consisting of a bond and amide;
- (ag) each D' is selected from one or more of the group consisting of amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone;
- (ah) each D' is selected from one or more of the group consisting of amide, ester, carbamate and carbonate ester;
- (ai) each D' is an amide;
- (biii) Y⁻ is selected from one or more of the group consisting of halo, acetate, phosphate, sulfonate, and bis((trifluoromethyl)sulfonyl)imide (N(Tf)₂), optionally wherein Y⁻ is selected from one or more of the group consisting of chloro, acetate, phosphate, sulfonate, and bis((trifluoromethyl)sulfonyl)imide (N(Tf)₂);
- (biv) x is from 0.01 to 1.0, such as from 0.025 to 0.75, such as from 0.05 to 0.6, such as from 0.1 to 0.5, such as from 0.2 to 0.3;
- (bv) t and t' are 0;
- (bvi) p is from 1 to 6; and
- (bvii) r is from 1 to 6.

§9. The polymer or oligomer according to any one of §1 to §8, wherein the polymer is selected from the group consisting of:



c)	
d)	
e)	
f)	
g)	
h)	
i)	

§10. A molecule or a pharmaceutically acceptable solvate thereof comprising:

a first block of oligomeric repeating units, where each repeating unit comprises an imidazolium group and a non-biodegradable alkyl chain connected to an adjacent repeating unit;

a second block of oligomeric repeating units, where each repeating unit comprises an imidazolium group and a non-biodegradable alkyl chain connected to an adjacent repeating unit; and

a linking group connecting the first block and the second block together, wherein the linking group comprises one or more biodegradable functional groups.

§11. The molecule according to §10, wherein the one or more biodegradable functional groups are selected from one or more of the group consisting of urea, carbamate, acetal, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone, optionally wherein:

(ci) the one or more biodegradable functional groups are selected from one or more of the group consisting of amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone;

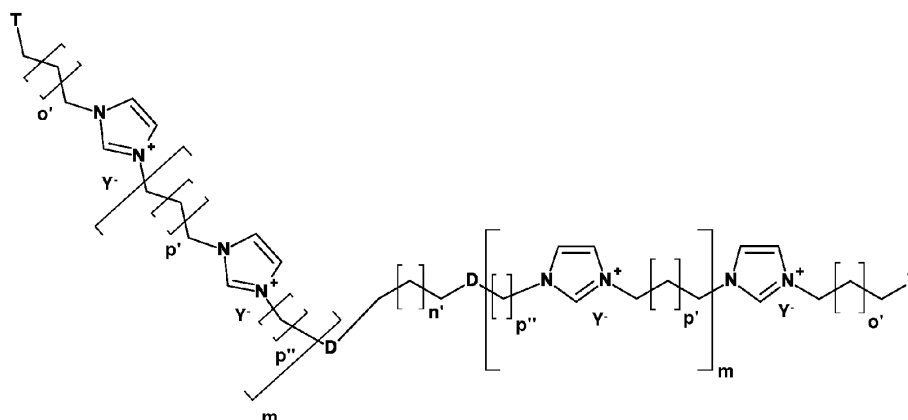
(cii) the one or more biodegradable functional groups are selected from one or more of the group consisting of carbamate or, more particularly, amide, ester and carbonate ester;

(ciii) the one or more biodegradable functional groups are selected from one or both of amide and carbonate ester; or

(civ) the one or more biodegradable functional groups are amide.

§12. The molecule according to §10 or §11 wherein, the molecular weight is from 1,000 Daltons to 5,000 Daltons, optionally wherein the molecular weight is from 1,000 Daltons to 4,000 Daltons.

§13. The molecule according to any one of §10 to §12, wherein the molecule has the formula (V):



(V)

wherein:

each m is independently from 1 to 8;

each Y^- is a counterion;

n' is from 0 to 12;

each o' is independently selected from 0 to 20;

each p' is independently selected from 0 to 12;

each p'' is independently selected from 0 to 12;

each T is independently a terminal functional group selected from amine, ammonium, guanidinium, bisguanidinium, alkyl, and aryl;

each D is a biodegradable functional group, or a pharmaceutically acceptable solvate thereof.

§14. The molecule according to §13, wherein one or more of the following apply:

(di) each D is independently selected from urea, carbamate, acetal, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone, optionally wherein:

(ba) each D is independently selected from one or more of the group consisting of amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone;

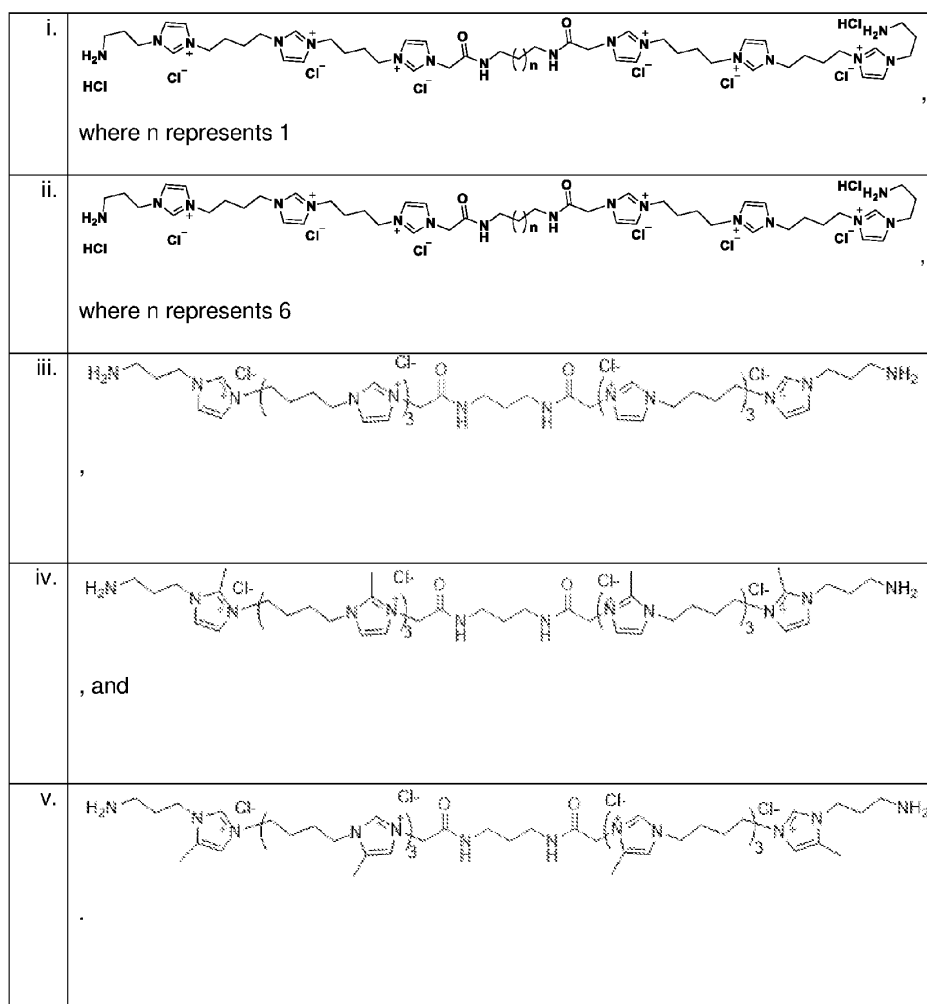
(bb) each D is independently selected from one or more of the group consisting of carbamate or, more particularly, amide, ester and carbonate ester; or

(bc) each D is amide;

(dii) Y^- is selected from one or more of the group consisting of halo, acetate, phosphate, sulfonate, and bis((trifluoromethyl)sulfonyl)imide ($N(Tf)_2^-$), optionally wherein Y^- is selected from one or more of the group consisting of chloro, acetate, phosphate, sulfonate, and bis((trifluoromethyl)sulfonyl)imide ($N(Tf)_2^-$); and

(dii) p'' is 0 to 6.

§15. The molecule according to any one of §10 to §14, wherein the molecule is selected from the group consisting of:



In clauses §1 to §15 above, when used herein, the term “biodegradable chain” refers to a linking group that connects one imidazolium group to another. This biodegradable chain may comprise one or more biodegradable functional groups. Any suitable biodegradable functional group may be used herein. When used herein, the term biodegradable functional group is intended to refer to a functional group that can be cleaved in the environment and/or *in vivo* either by chemical or biological materials present in the ambient environment in which an

oligomer, polymer or molecule of the current invention may find itself in. Non-limiting examples of biodegradable functional groups that may be mentioned herein include urea, carbamate, acetal, amide, ester, carbonate ester, urethane, disulfide, anhydride, and hydrazone. Said functional groups may be susceptible to cleavage by chemicals or biological materials in the ambient environment (e.g. esters may be cleaved due to acidic or basic conditions of the environment, or due to the presence of enzymes). This cleavage may take place *in vivo* or *ex vivo*, depending on the way that the materials disclosed herein are used and/or disposed of. Examples of functional groups that may not be biodegradable include ether linkages. In addition, when used herein, the term "C₁₋₃ alkyl" may refer to, for example, ethyl, propyl, (e.g. n-propyl or isopropyl), or more preferably, methyl. Derivatives of C₁₋₃ alkyl may refer to substituted C₁₋₃ alkyl groups. Examples of substituted C₁₋₃ alkyl groups that may be mentioned herein include, but are not limited to halo (e.g. Br, Cl or, more particularly F). A particular derivative that may be mentioned herein is CF₃.

In clauses §1 to §9 above, it will be appreciated that D and D' may be the same or different. In particular embodiments of the invention, D' may be a biodegradable functional group, such that the biodegradable chain has two biodegradable functional groups. However, in other embodiments (e.g. when D is carbamate), D' may be a bond.

In clauses §1 to §9 above, when the polymer or oligomer contains two repeating units, the amount of the repeating unit that contains the one or more biodegradable functional groups may be from 1 to 99 mol%, such as from 5 to 95 mol%, such as from 10 to 90 mol%, such as from 20 to 80 mol%, such as from 25 to 75 mol%, such as 50 mol%. For example, the amount of the repeating unit that contains the one or more biodegradable functional groups may be from 20 to 30 mol%.

In certain embodiments, in polymers (a) and (b) shown in the table in clause §9 above, the repeating unit that contains the one or more biodegradable functional groups may be present in an amount of 50 mol%.

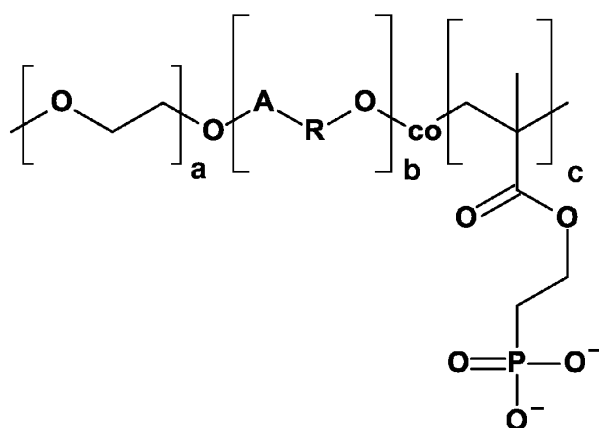
In certain embodiments, the polymers shown in the table in clause §9 above have a number average molecular weight of from 960 to 3,000 Daltons, such as from 966 to 2,800 Daltons.

Methods for preparing the polymer or oligomer or a pharmaceutically acceptable solvate thereof as defined according to the numbered clauses §1 to §9 above, or the molecule or a pharmaceutically acceptable solvate thereof as defined according to numbered clauses §10

to §15 above, are disclosed in PCT/SG2021/050290, which is incorporated herein by reference.

In embodiments of the invention, the bacterial-enzyme-degradable anionic copolymer may comprise a repeating unit that bears a phosphonate group.

In embodiments of the invention, the bacterial-enzyme-degradable anionic copolymer may have the formula (III):



(III)

where:

R is a C₁ to C₁₀ linear or branched alkyl chain;

A represents C=O or P(OR_a)=O;

R_a represents a linear or branched C₁ to C₆ alkyl group;

a represents a block polyethylene glycol unit having a number average molecular weight of from 500 to 10,000 Daltons;

b and c together represent a random copolymer block, where b and c each independently have a value of from 8 to 100.

In certain embodiments of the invention, the bacterial-enzyme-degradable anionic copolymer of formula III may be one in which one or more of the following apply:

(a) a represents a block polyethylene glycol unit having a number average molecular weight of about 2,000 Daltons;

(b) b and c each independently have a value of from 10 to 50, such as 15 to 20, such as 18;

(c) A represents C=O; and

(d) R represents $-(CH_2)_5-$.

In certain embodiments of the invention that may be mentioned herein, the bacterial-enzyme-degradable anionic copolymer may have a number average molecular weight of from 6,000 to 10,000 Daltons, such as from 7,000 to 8,000 Daltons.

In certain embodiments of the invention that may be mentioned herein, the bacterial-enzyme-degradable anionic copolymer may be degradable by a bacterial-secreted lipase or a bacterial-secreted phosphoesterase, such as a bacterial-secreted lipase.

In certain embodiments of the invention that may be mentioned herein, the weight to weight ratio of the cationic antimicrobial compound and the bacterial-enzyme-degradable anionic copolymer may be selected to provide a material with a zeta potential of from +10 to -10 mV, such as from +6 to -6 mV, such as from 0 to -5.3 mV.

In certain embodiments of the invention that may be mentioned herein, the weight to weight ratio of the cationic antimicrobial compound and the bacterial-enzyme-degradable anionic copolymer may be from 1:5 to 5:1, such as from 1:2 to 2:1, such as about 1:1.7.

The antibacterial composite material disclosed herein may be used in a pharmaceutical composition. Thus, in a second aspect of the invention, there is provided a pharmaceutical composition comprising an antimicrobial composite material as described herein and one or more of a pharmaceutically acceptable excipient and carrier.

Antimicrobial composite materials according to the current invention may be administered by any suitable route, but may particularly be administered orally, intravenously, intramuscularly, cutaneously, subcutaneously, transmucosally (e.g. sublingually or buccally), rectally, transdermally, nasally, pulmonarily (e.g. tracheally or bronchially), topically, by any other parenteral route, in the form of a pharmaceutical preparation comprising the compound in a pharmaceutically acceptable dosage form. Particular modes of administration that may be mentioned include oral, intravenous, cutaneous, subcutaneous, nasal, intramuscular or intraperitoneal administration.

Antimicrobial composite materials according to the current invention will generally be administered as a pharmaceutical formulation in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, which may be selected with due regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutically acceptable

carriers may be chemically inert to the active compounds and may have no detrimental side effects or toxicity under the conditions of use. Suitable pharmaceutical formulations may be found in, for example, Remington *The Science and Practice of Pharmacy*, 19th ed., Mack Printing Company, Easton, Pennsylvania (1995). For parenteral administration, a parenterally acceptable aqueous solution may be employed, which is pyrogen free and has requisite pH, isotonicity, and stability. Suitable solutions will be well known to the skilled person, with numerous methods being described in the literature. A brief review of methods of drug delivery may also be found in e.g. Langer, *Science* (1990) 249, 1527.

Otherwise, the preparation of suitable formulations may be achieved routinely by the skilled person using routine techniques and/or in accordance with standard and/or accepted pharmaceutical practice.

The amount of the antimicrobial composite material according to the current invention in any pharmaceutical formulation used in accordance with the present invention will depend on various factors, such as the severity of the condition to be treated, the particular patient to be treated, as well as the compound(s) which is/are employed. In any event, the amount of the antimicrobial composite materials according to the current invention in the formulation may be determined routinely by the skilled person.

For example, a solid oral composition such as a tablet or capsule may contain from 1 to 99 % (w/w) active ingredient (i.e. the antimicrobial composite material according to the current invention); from 0 to 99% (w/w) diluent or filler; from 0 to 20% (w/w) of a disintegrant; from 0 to 5% (w/w) of a lubricant; from 0 to 5% (w/w) of a flow aid; from 0 to 50% (w/w) of a granulating agent or binder; from 0 to 5% (w/w) of an antioxidant; and from 0 to 5% (w/w) of a pigment. A controlled release tablet may in addition contain from 0 to 90 % (w/w) of a release-controlling polymer.

A parenteral formulation (such as a solution or suspension for injection or a solution for infusion) may contain from 1 to 50 % (w/w) active ingredient; and from 50% (w/w) to 99% (w/w) of a liquid or semisolid carrier or vehicle (e.g. a solvent such as water); and 0-20% (w/w) of one or more other excipients such as buffering agents, antioxidants, suspension stabilisers, tonicity adjusting agents and preservatives.

Depending on the disorder, and the patient, to be treated, as well as the route of administration, the antimicrobial composite materials according to the current invention may be administered at varying therapeutically effective doses to a patient in need thereof.

As will be appreciated, the antimicrobial composite material and its pharmaceutical composition (formulations) may be used in medicine. Thus, in a further aspect of the invention, there is provided an antimicrobial composite material or a pharmaceutical composition as described hereinbefore for use as a medicament.

In a further aspect of the invention, there is provided a method of treating a subject suffering from a microbial and/or fungal infection comprising the steps of administering to the subject a therapeutically effective amount of an antimicrobial composite material or a pharmaceutical composition as described hereinbefore, such that the infection is treated.

In a further aspect of the invention, there is provided a use of an antimicrobial composite material or a pharmaceutical composition as described hereinbefore, in the manufacture of a medicament to treat a microbial and/or fungal infection in a subject in need thereof.

In a further aspect of the invention, there is provided an antimicrobial composite material or a pharmaceutical composition as described hereinbefore, for use in the treatment of a microbial and/or fungal infection.

In a further aspect of the invention, there is provided a method of manufacture of an antimicrobial composite material as described hereinbefore, the method comprising the steps of:

- (i) providing a solution of a cationic antimicrobial compound and providing a solution of a bacterial-enzyme-degradable anionic copolymer as described hereinbefore; and
- (ii) mixing the solutions together to form a mixture and agitating the mixture for a period of time to form the antimicrobial composite material.

For the avoidance of doubt, in the context of the present invention, the term "*treatment*" includes references to therapeutic or palliative treatment of patients in need of such treatment, as well as to the prophylactic treatment and/or diagnosis of patients which are susceptible to the relevant disease states.

The terms "patient" and "patients" include references to mammalian (e.g. human) patients. As used herein the terms "subject" or "patient" are well-recognized in the art, and, are used interchangeably herein to refer to a mammal, including dog, cat, rat, mouse, monkey, cow, horse, goat, sheep, pig, camel, and, most preferably, a human. In some embodiments, the subject is a subject in need of treatment or a subject with a disease or disorder. However, in

other embodiments, the subject can be a normal subject. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered.

The term "effective amount" refers to an amount of a compound, which confers a therapeutic effect on the treated patient (e.g. sufficient to treat or prevent the disease). The effect may be objective (i.e. measurable by some test or marker) or subjective (i.e. the subject gives an indication of or feels an effect).

However, the dose administered to a mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the mammal over a reasonable timeframe. One skilled in the art will recognize that the selection of the exact dose and composition and the most appropriate delivery regimen will also be influenced by *inter alia* the pharmacological properties of the formulation, the nature and severity of the condition being treated, and the physical condition and mental acuity of the recipient, as well as the potency of the specific compound, the age, condition, body weight, sex and response of the patient to be treated, and the stage/severity of the disease.

Administration may be continuous or intermittent (e.g. by bolus injection). The dosage may also be determined by the timing and frequency of administration. In the case of oral or parenteral administration the dosage can vary from about 0.01 mg to about 1000 mg per day of an antimicrobial composite material according to the current invention.

In any event, the medical practitioner, or other skilled person, will be able to determine routinely the actual dosage, which will be most suitable for an individual patient. The above-mentioned dosages are exemplary of the average case; there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Advantages associated with the aspects and embodiments of the fabrication of "caged" complex in the invention include, but are not limited to:

- (i) being generalizable;
- (ii) enabling design of formulations for the safe delivery of synthetic and natural cationic antimicrobials;
- (iii) not requiring sophisticated chemical modification;
- (iv) being mild to infected tissues by controlling the degradation profile of the complex; and
- (v) improving the metabolic and chemical stability of conventional cationic antimicrobials.

Further aspects and embodiments of the invention will now be described by reference to the following non-limiting examples.

Examples

Materials

Methacryloyl chloride, dimethyl (2-hydroxyethyl) phosphonate, triethanolamine (TEA), monomethoxy poly(ethylene glycol) (mPEG-OH), 1-tert-Butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)-phosphoranylideneamino]-2λ5,4λ5-catenadi(phosphazene) (tBuP₄), bromotrimethylsilane (TMSBr), tert-butyl methacrylate (tBMA), trifluoroacetic acid (TFA), N,N'-Dicyclohexylcarbodiimide (DCC), 4-Dimethylaminopyridine (DMAP), methyl methacrylate (MMA), mucin from porcine stomach, Muller Hinton Broth (MHB), Luria Bertani (LB) broth, lipase from *Pseudomonas cepacia*, methoxy polyethylene glycol bromide (mPEG-Br), Copper (II) bromide (CuBr₂), penicillin, streptomycin, trypsin, and vancocycin were purchased from Sigma-Aldrich. Cyanine3 amine (Cy3-NH₂), Cyanine5 amine (Cy5-NH₂) were purchased from Lumiprobe, PIM was synthesized as previously reported (Zhong, W. et al., *Proceedings of the National Academy of Sciences*, **2020**, 117, 31376).

Dulbecco's Modified Eagle Medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Gibco. The Cell Counting Kit-8 (CCK-8) was purchased from GLPBIO. Melittin was purchased from GenScript.

Enterococcus faecalis ATCC 29212 (*E. faecalis*), Methicillin-resistant *Staphylococcus aureus* ATCC BAA38 (MRSA), *Bacillus subtilis* (*B. subtilis* BR151), *Escherichia coli* (*E. coli* K12), *Pseudomonas aeruginosa* PAO1, and NIH/3T3 cell line were obtained from American Type Culture Collection (ATCC) and handled according to the protocols. *Klebsiella Pneumoniae* SGH-10 was obtained from Singapore General Hospital. ICR mice were purchased from InVivos Pte Ltd and handled according to protocols.

General Information for Characterisations of Compounds

Nuclear Magnetic Resonance (NMR) Measurements

Nuclear magnetic resonance (NMR) spectra were recorded by a Bruker AVIII with BBFO Probe.

Transmission Electron Microscopy (TEM) Measurements

Transmission electron microscopy (TEM) images were captured by a JEM-1400Flash Electron Microscope. The size and zeta potential of the samples were measured by a Malvern Nano-ZS Zetasizer.

Dynamic Light Scattering (DLS) for Polydispersity (PD) Measurements

The size and zeta potential of the samples were measured via dynamic light scattering with a Malvern Nano-ZS Zetasizer at 25 °C. The measurement angle was 173°backscatter and the dispersant was water. To test the lipase degradability, the complex was mixed with 1 mg/mL lipase from *Pseudomonas cepacia* for 6 h at 37 °C with shaking at 200 rpm.

Fluorescence Spectroscopy Measurements

Fluorescence spectrum was recorded by a HORIBA Jobin Yvon Fluoromax-3 Spectrofluorometer.

DMF GPC

Gel permeation chromatography was conducted by an Agilent 1260 Infinity GPC/SEC System using PLgel 5 µm MIXED-C Column, and DMF as mobile phase.

Optical Density (OD) Measurements

The optical density (OD) of bacterial suspensions were measured by a PerkinElmer EnSpire Multimode Plate Reader.

Confocal Laser Scanning Microscopy (CLSM) Measurements

Confocal laser scanning microscopy (CLSM) pictures were taken by a Zeiss LSM 800 Confocal Microscope.

Analysis of Blood Biochemical Markers

The blood biochemical markers were analysed by an MNCHIP Pointcare V2 Biochemistry Analyzer.

Synthesis of MR-107 (OIM1-6-PzAc)

The synthesis route for MR-107 (OIM1-6-PzAc) is shown in Figure 12.

Materials and Methods

All chemicals and solvents were obtained from Fischer Scientific UK, Sigma-Aldrich, Merck Millipore, TCI Chemicals, and BLDpharm, and were used without further purification. TLC was

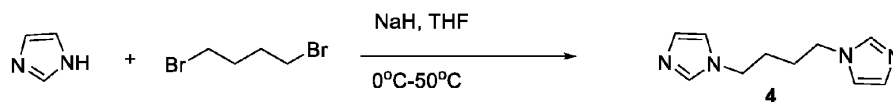
performed using Merck TLC Silica gel 60 Å F254 plates. TLC plate visualizations were conducted under UV light (256 & 366 nm). Column chromatography was carried out using Davisil® LC60A 40 – 63 micron chromatographic silica (pore size 60 Å, 0.040-0.063 mm). NMR spectroscopies were recorded on either a Bruker Avance DPX 300 (1H and 13C NMR at 300 MHz and 75.47 MHz respectively) or a Bruker Avance III 400 (1H and 13C NMR at 400.13 MHz and 101.62 MHz respectively). The data was processed using TopSpin (version 4.1.3), which referenced the spectra to those of the residual solvents. Chemical shifts (δ) were quoted in parts per million (ppm) and coupling constants (J) were reported to the nearest 0.01 Hz for 1H NMR and 0.1 Hz for 13C NMR along with peak multiplicities using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; qu, quintet; sext, sextet; m, multiplet, and br, broad. Mass spectrometry analysis was recorded on a ABI 4800 Proteomics Analyzer MALDI TOF/TOF mass spectrometer (Applied Biosystems). Oligomeric products were characterized by Waters Gel Permeation Chromatography (GPC) using a water phase ultra hydrogel column as the stationary phase and sodium acetate/acetic acid buffer (pH = 4.5) as the mobile phase. All samples were dissolved in the buffer solution with approx. 1 mg/mL final concentration and filtered through a 0.22 μ m microfilter before sample analysis. Elemental analysis for anion exchange was achieved by performing X-ray photoelectron spectroscopy (XPS) using an AXIS Supra spectrometer (Kratos Analytical, UK) equipped with a hemispherical analyzer and a monochromatic Al K-alpha source (1487 eV) operated at 15 mA and 15 kV. The XPS spectra were acquired from an area of 700 x 300 μ m² with a take-off angle of 90°. Pass energy of 160 eV and 20 eV were used for the survey and high-resolution scans, respectively. A 3.1-volt bias was applied to the sample to neutralize charge build-up on the sample surface.

Benzyl (3-(1H-imidazol-1-yl)propyl)carbamate (2)

To a solution of 3-(1H-imidazol-1-yl)propan-1-amine (10 gm, 1.0 equiv.) in DCM under an ice bath was added Et₃N (1.05 equiv.) and CBzCl (1.1 equiv.) portion wise. The reaction mixture was allowed to room temperature and stirred overnight. After completion of the reaction shown by TLC, the reaction mixture was transferred into separating funnel and wash with 0.05 N HCl (aq) and distilled water (twice) and the DCM layer was concentrated under reduced pressure onto silica and purified by silica gel column chromatography eluting with 50-100% EtOAc in hexane to afford the desired product. ¹H NMR (300 MHz, DMSO-d₆) δ 7.63 (s, 1H), 7.50 – 7.24 (m, 6H), 7.17 (s, 1H), 6.90 (s, 1H), 5.04 (s, 2H), 3.97 (t, J = 6.9 Hz, 2H), 2.98 (q, J = 6.3 Hz, 2H), 1.84 (p, J = 6.7 Hz, 2H). ¹³C NMR (75 MHz, DMSO-d₆) δ 156.1, 137.2, 137.1, 128.3, 127.7, 119.3, 65.3, 43.4, 37.4, 31.0. MALDI-TOF (HCCA matrix, Reflector mode): *m/z* calculated (M+H)⁺ for C₁₄H₁₇N₃O₂ = 260.1321; measured = 260.0104.

1-(3-(((benzyloxy)carbonyl)amino)propyl)-3-(4-bromobutyl)-1H-imidazol-3-ium bromide (3)

1,4 dibromo butane (3.45ml, 2.5 equiv.) was added to a stirred solution of the required starting material (**2**, 3.00 g, 11.5 mmol) (1.0 equiv.) in dry MeCN (1 mmol/mL) under argon. The reaction mixture was heated under reflux overnight and then cooled to rt. The reaction mixture was concentrated under rotary evaporation and purified by silica gel chromatography eluting with 0-15% MeOH in EtOAc to afford the desired alkylated bromo-product **3** as a colorless gum (4.12 g, 8.67 mmol, 75%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.39 (s, 1H), 7.88 (d, *J* = 3.4 Hz, 2H), 7.58 – 7.21 (m, 6H), 5.02 (s, 2H), 4.24 (q, *J* = 7.2 Hz, 4H), 3.56 (t, *J* = 6.4 Hz, 2H), 3.02 (q, *J* = 6.0 Hz, 2H), 2.05 – 1.86 (m, 4H), 1.86 – 1.72 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 156.2, 137.0, 136.2, 128.3, 127.76, 127.70, 122.45, 122.40, 65.3, 47.9, 46.5, 36.9, 34.1, 29.7, 28.7, 28.1. MALDI-TOF (HCCA matrix, Reflector mode): *m/z* calculated (M-Br)⁺ for C₁₈H₂₅Br₂N₃O₂ = 394.1125; measured = 394.1405.

Bis-imidazole butane (4)

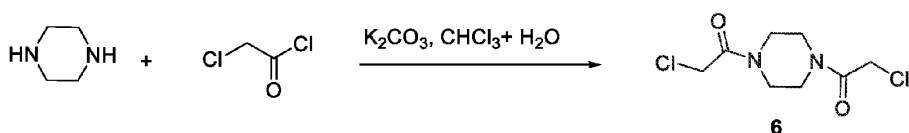
Imidazole (8.00 g, 117.5 mmol 1.0 equiv.) was dissolved in THF (0.5 mmol/mL) and stirred in an ice bath for 10 min. NaH (2.5 equiv.) was added portion-wise to the reaction mixture and the reaction mixture was removed from the ice bath and stirred for 1 hr. 1,4-Dibromobutane (0.5 equiv.) was added, and the reaction mixture was stirred at 50°C overnight. The resultant mixture was cooled to rt, and filtered through a pad of celite with THF washings. The filtrate was dried under rotary evaporation and subsequently dissolved in methanol. The methanol layer was washed three times with hexane and concentrated under rotary evaporation to afford desired product without the need for further purification. ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.61 (s, 2H), 7.14 (br s, 2H), 6.89 (br s, 2H), 3.98 – 3.73 (m, 4H), 1.64 – 1.59 (m, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 137.27, 128.45, 119.31, 45.34, 27.73. MALDI-TOF (HCCA matrix, Reflector mode): *m/z* calculated (M+H)⁺ for C₁₀H₁₄N₄ = 191.1219; measured = 191.1296.

1-(4-(1H-imidazol-1-yl)butyl)-3-(4-(1-(3-(((benzyloxy)carbonyl)amino)propyl)-1H-imidazol-3-ium-3-yl)butyl)-1H-imidazol-3-ium dibromide (5)

The required bromo-starting material **3** (3, gm, 1 equiv.) in dry MeCN (0.5 mmol/mL) was added dropwise to a stirred solution of the required bis-imidazole starting material **4** (2.44 gm) in dry MeCN (2 mmol/mL) at 80 °C. The resulting mixture was stirred under reflux for overnight. After completion of the reaction monitored by ¹H NMR, the reaction mixture was concentrated under rotary evaporation, and the residue was treated with a 1:1 mixture of water and 3:1

chloroform: isopropanol and transferred into a separating funnel. The organic layer was removed and the aqueous layer was further washed with 3:1 chloroform: isopropanol five times. The aqueous layer was concentrated under reduced pressure to afford the desired product **5** as a colorless hygroscopic gum (3.07 g, 4.61 mmol, 73%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.45 – 9.29 (m, 2H), 7.88 – 7.78 (m, 4H), 7.72 (s, 1H), 7.48 – 7.28 (m, 6H), 7.20 (s, 1H), 6.92 (s, 1H), 5.02 (s, 2H), 4.20 (t, *J* = 6.9 Hz, 8H), 4.02 (t, *J* = 6.5 Hz, 2H), 3.05-2.99 (m, 2H), 2.02 – 1.88 (m, 2H), 1.80-1.72 (m, 8H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 155.0, 136.0, 135.8, 135.1, 134.7, 127.2, 127.0, 126.68, 126.60, 121.3, 121.2, 118.1, 64.0, 47.0, 46.9, 45.4, 44.0, 43.8, 35.7, 28.5, 26.0, 25.3, 24.8. MALDI-TOF (HCCA matrix, Reflector mode): *m/z* calculated (M-2Br-H)⁺ for C₂₈H₃₉Br₂N₇O₂ = 504.3082; measured = 504.3814.

PzAc linker (6)



Add 100 mL of saturated K₂CO₃ to a solution of piperazine (5.00 g, 58.14 mmol) in CHCl₃ (200 mL). Stir the reaction mixture and cool to 0 °C in an ice bath. Add 2-chloroacetyl chloride (19.71 g, 174.44 mmol) in CHCl₃ dropwise over 1 hour. Allow the reaction mixture to warm to room temperature and stir for 2 hours. Wash the organic phase with HCl (2 x 100 mL, 1 M) and water (2 x 100 mL). Dry the CHCl₃ layer and evaporate in vacuo to obtain the product as white solid (12.5 gm, 90%). ¹H NMR (400 MHz, CDCl₃) δ 4.09 (s, 4H), 3.78 – 3.49 (m, 8H). ¹³C NMR (101 MHz, CDCl₃) δ 165.3, 45.9 42.8, 40.7.

Synthesis of OIM1-6-1PzAc (MR107)

The title compound was synthesized following the method described in general procedure 5 using compound **5** (1.0 gm, 1.57 mmol) and PzAc linker (**6**) (185 mg, 0.75 mmol) in acetonitrile (20 ml). The reaction mixture was heated to 90 °C for 18 hr. The reaction mixture was allowed to cool to rt. The resulting gum was separated, and further triturated three times with MeCN/EtOAc (8:2), and the resulting precipitate/gum was isolated and treated with a solution of HBr in acetic acid (33%) (10 equiv.) and the resulting mixture was stirred at rt for 2 hr. The reaction mixture was treated with excess EtOAc and the resulting precipitate/gum was isolated. The gum was further triturated with EtOAc. The gum was dissolved in water to give a final concentration of 50-60 mM and was passed through a glass column containing ion-exchange resin Amberlyst® A-26 for exchanging counterion to chloride. The product-containing eluent was transferred to a dialysis bag with an Mw cut-off of 1000 Da and dialyzed against 1 mL HCl in 1 L of deionized water over 1 hr, with a single dialysis water change at *t* = 0.5 hr. The

resulting solution was concentrated under rotary evaporation and subjected to lyophilization to afford the desired product (0.744 gm, 75%). ^1H NMR (400 MHz, D_2O) δ 8.91 – 8.73 (m, 6H), 7.48 (ddt, J = 15.7, 10.6, 1.6 Hz, 12H), 5.35 (d, J = 3.8 Hz, 4H), 4.28 (ddd, J = 25.0, 23.1, 11.6 Hz, 20H), 3.81 – 3.55 (m, 8H), 3.07 – 2.89 (m, 4H), 2.23 (dt, J = 15.2, 7.7 Hz, 4H), 1.89 (dd, J = 11.0, 8.3 Hz, 16H). ^{13}C NMR (101 MHz, D_2O) δ 165.6, 137.1, 135.5, 124.2, 122.5, 122.1, 50.3, 49.3, 48.6, 46.5, 43.8, 41.9, 36.4, 27.3, 26.4, 25.9. MALDI-TOF (HCCA matrix, Reflector mode): m/z calculated $(\text{M}-6\text{H}-8\text{Cl})^+$ for $\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_2$ = 905.2140; measured = 905.6682. GPC (Water Phase) M_n = 1290, M_w = 1290, M_p = 1296, PDI = 1.005.

Example 1. Caging Copolymers – Synthesis, Characterisation, and Formation of Complexes with Cationic Antimicrobials

Hybrid copolymerization catalysed by tBuP_4 allows the readily design of biodegradable polymers of cyclic monomers such as lactide and ϵ -CL with functional pedant groups (Herzberger, J. et al., *Chemical Reviews*, **2016**, *116*, 2170; Yang, H. et al., *Science China Chemistry*, **2013**, *56*, 1101). Here, using poly(ethylene glycol) methyl ether (mPEG) as initiator, copolymerization of ϵ -CL and 2-(dimethoxyphosphoryl)ethyl methacrylate (MA-DMOPE, a vinyl phosphonate monomer) was conducted, followed by the deprotection of phosphate units by bromotrimethylsilane (TMSBr), leading to PEG-*b*-(PCL-*co*-PPMA) with phosphonic acid groups (FIG. 2).

Synthesis of 2-(Dimethoxyphosphoryl)ethyl Methacrylate (MA-DMOPE)

Dimethyl (2-hydroxyethyl) phosphonate (DMOPE, 3.4 g, 0.022 mol) and TEA (2.22 g, 0.022 mol) were dissolved in THF in a 100 mL round bottom flask. Then the flask was placed in ice bath, and allowed to cool down for 10 min. Methacryloyl chloride (MA-CL, 2.08 g, 0.2 mol) was dissolved in THF and were added dropwise in 10 min to the round bottom flask. The reaction mixture was warmed up to room temperature and was stirred for 16 h. The salt precipitation was removed by vacuum filtration and subsequently the THF was removed by a rotary vacuum evaporator. The resultant content was again dissolved in DCM, washed by saturated NaHCO_3 and brine sequentially. The organic phase was collected and dried over anhydrous MgSO_4 , concentrated and purified by silica column chromatography with DCM/MeOH (15:1, v:v) as eluent. The fraction with R_f as 0.56 was collected and the collected fraction was concentrated and obtained as MA-DMOPE.

*Synthesis of PEG-*b*-(PCL-*co*-PPMA*AdM*) Copolymer*

0.2 g mPEG-OH was added to a 25 mL Schlenk flask, and the flask was heated to 110 °C for 30 min to remove residual water. Then, 0.2 mL ϵ -caprolactone (ϵ -CL, 1.87 mmol) and 0.8 g

MA-DMOPE was quickly added to the flask by syringe. After 3 cycles of freeze-pump-thaw to remove oxygen, 0.2 mL (0.25 mmol) $t\text{-BuP}_4$ catalyst was added to the bottom of the flask with a syringe under vigorous N_2 flow. Subsequently, the flask was heated to 60 °C to initiate the polymerization. After 18 h, the reaction was terminated by adding 400 μL acetic acid, and the mixture was precipitated in 10 folds of cold hexane and re-dissolved in THF for 2 times. The final product of PEG-*b*-(PCL-*co*-PPMAdM) was obtained after lyophilisation.

*Synthesis of PEG-*b*-(PCL-*co*-PPMA) Copolymer*

PEG-*b*-(PCL-*co*-PPMAdM) obtained from the previous step was dissolved in anhydrous DCM and was added with bromotrimethylsilane (TMSBr) (molar ratio of phosphonate group to TMSBr was 1:5). The solution was stirred at room temperature for 24 h, after which equivoluminal of MeOH was added and stirred for another 24h. The product was purified by dialysis with 3500 MWCO (molecular weight cut-off) membrane in deionised water (DI H_2O) for 7 days. The final product of PEG-*b*-(PCL-*co*-PPMA) was obtained after lyophilization.

*Synthesis of PEG-*b*-(PMMA-*co*-PPMA) Copolymer*

PEG-*b*-(PMMA-*co*-PPMAdM) were synthesized via the activator regenerated by electron transfer (ARGET) atom transfer radical polymerization (ATRP) with slight modification of a published method (Lei, Q. et al., *ACS Omega*, **2018** 3, 15996). Briefly, 0.2 g mPEG-Br, 0.187 g MMA, 0.8 g MA-PMODE, 5 mg CuBr_2 , 35 mg bipyridyl, and 4 mL anhydrous toluene was added to a 25 mL Schlenk flask. After 3 cycles of freeze-pump-thaw, 38.7 mg of ascorbic acid was added to the flask under vigorous N_2 , which was then heated to 70 °C to initiate the polymerization. After 18 h, the mixture was precipitated in cold hexane and lyophilized to give the product PEG-*b*-(PMMA-*co*-PPMAdM).

Deprotection of the methyl group in PEG-*b*-(PMMA-*co*-PPMAdM) to give PEG-*b*-(PMMA-*co*-PPMA) was achieved by reacting with TMSBr and MeOH, as described in previous section for the synthesis of PEG-*b*-(PCL-*co*-PPMA) copolymer.

Complexation of Cationic Antimicrobial with Copolymer

When the cationic antimicrobial is PIM: The fixed volume of PIM (1 mg/mL) were added to different volume of copolymer PEG-*b*-(PCL-*co*-PPMA) solution (1 mg/mL) dropwise. Then the mixture was stirred for 16 h under room temperature to allow the electrostatic interaction to take place.

Similar protocol can be adopted for other cationic antimicrobial and copolymer combinations, such as when PIM is replaced with melittin or MR-107 (OIM1-6-1PzAc); or when copolymer PEG-*b*-(PCL-*co*-PPMA) is replaced with copolymer PEG-*b*-(PMMA-*co*-PPMA).

Lipase Degradation of Complex

Complex with concentration 1 mg/mL was added to 1 mg/mL lipase from *Pseudomonas cepacia*. The mixture was then placed in a 37 °C incubator for 6 h to allow the degradation process to occur.

Results and Discussions

Main-chain cationic 1-amino poly(1-butyl imidazole-3-ium) amine (hereafter denoted as PIM) (FIG. 3) has demonstrated potent broad-spectrum antibacterial activity against many drug-resistant strains and invasive clinical isolates, with a representative minimal inhibitory concentration (MIC) of ~2 µg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA) (Zhong, W. et al., *Proceedings of the National Academy of Sciences*, **2020** 117, 31376; Mon, K.K.Z. et al., *Antimicrobial Agents and Chemotherapy*, **2022**, 66, e00597). Zhong et al. also demonstrated *in vivo* therapeutic efficiency of PIM-1 against pan-antibiotic-resistant *P. aeruginosa* PAER (Zhong, W. et al., *Proceedings of the National Academy of Sciences*, **2020** 117, 31376). PIM compounds bind to bacterial cell membranes by electrostatic and hydrophobic interactions, then enter cells to impart bactericidal effects. However, the highly potent non-degradable PIM (Wang, A. et al., *Advanced Functional Materials*, **2021**, 31, 2011165) lacks sufficient selectivity for targeting infection-causing microbes, resulting in severe *in vivo* systemic toxicity, as evidenced by the significant weight loss of mice after intraperitoneal (I.P.) delivery (Zhong, W. et al., *Proceedings of the National Academy of Sciences*, **2020** 117, 31376).

The designed block copolymer with a poly(ethylene glycol) (PEG) block and an anionic biodegradable block electrostatically interacts with the PIM to self-assemble into polyion complex nanoparticles, in which PIM is caged in the core with PEG chains extending to cover the surface (FIG. 3). The block copolymer is synthesized using *t*-BuP₄-catalyzed hybrid polymerization of cyclic ϵ -caprolactone (ϵ -CL) and phosphonic acid-containing methacrylate (PMA) initiated by a PEG macroinitiator, leading to PEG-*b*-(PCL-*co*-PPMA).

Composition of the block copolymers can be controlled through the length of the mPEG initiators and the feeding ratio and reaction time of the copolymerization (FIG. 2 and FIG. 4). PEG-*b*-(PCL-*co*-PPMA) with PEG of 2000 Da and polymerization degree of 18 for both ϵ -CL (x) and phosphonic acid monomer (y) was selected to form nanoscale polyion complexes. The

number-average molecular weight (M_n) of the deprotected PEG-*b*-(PCL-*co*-PPMA) was determined to be 7330 g/mol by size exclusion chromatography (SEC), as shown in FIG. 5 and Table 1. This composition of caging polymer not only offered excellent water-solubility and strong interaction with the PIM, but also allows for selective rapid biodegradation by lipase, all of which are critical for the efficient caging and reactivation of the cationic PIM.

Table 1. Molecular Weight and Polydispersity (PD) of Copolymer PEG-*b*-(PCL-*co*-PPMA) determined by DMF GPC

Mp	M _n	M _w	PD
7980	7330	7931	1.08

It is well-known that oppositely charged polyelectrolytes can undergo self-assembly through electrostatic interaction to form polyion complexes (Thünemann, A.F. et al., *Polyelectrolytes with Defined Molecular Architecture II*, **2004**, 113). Through this means, the positive charge of PIM can be shielded by complexation with the PEG-*b*-(PCL-*co*-PPMA) block copolymer, which was achieved by simply mixing aqueous solutions of the two polymers as illustrated in FIG. 3. Since the surface charge is a vital parameter that may affect both antibacterial activity and cytotoxicity (Dathe, M. et al., *FEBS Letters*, **2001**, 501, 146; Jiang, Z. et al., *Peptide Science*, **2008**, 90, 369), the stoichiometric ratio between oppositely-charged PIM and PEG-*b*-(PCL-*co*-PPMA) was varied to obtain complexes with different net charges (FIG. 6A).

When adding different amounts of copolymer to a fixed amount of PIM, it was found that increasing the ratio of the “caging” copolymer to PIM progressively decreased the surface charge of the assemblies, suggesting a ratio-dependent “caging” effect of the copolymer (FIG. 6A). The complex with a zeta potential of -5.3 mV assembled from the mixture using a mass ratio of 1:1.7 (PIM: copolymer) was selected for further study, as this was the highest PIM content formulation for which the cytotoxic positive charge of PIM was completely shielded. At the ratio of 1:1.7, the self-assembly of PIM and copolymer primarily formed spherical nanoparticles with an average diameter of 76 nm, as confirmed by transmission electron microscopy (TEM, FIG. 6B), which was in line with the hydrodynamic sizes measured by dynamic light scattering (DLS, FIG. 7A). The charge density of polymers plays a key role in the stability of self-assembled polyion complexes, as stronger interaction between oppositely charged chains occurs for polymers with higher charge density (Insua, I. et al., *European Polymer Journal*, **2016**, 81, 198). The use in our system of phosphonic acid, with lower pKa (~1.3) and two net charges per molecule, affords a stronger interaction with PIM, and thus a higher stability of the polyion complex in complex environments, compared to

weaker acids that carry a single charge, such as carboxylic acids (Franz, R.G., *AAPS PharmSci*, **2001**, 3, 10; Sevrain, C.M. et al., *Beilstein J. Org. Chem.*, **2017**, 13, 2186). The complexation cages the cationic PIM compounds in the nanoparticles; the lipase-degradability of these PIM-containing complex is a crucial function as it allows liberation of the “caged” PIM for antibacterial activity towards pathogens. In our design, this function was provided by incorporating degradable PCL into the copolymer backbone. As shown in FIG. 6B, nanoparticles disappeared after lipase treatment, after which only amorphous structures were observed. This lipase-triggered degradation of the complex was further confirmed by DLS (FIG. 7B-C), as the hydrodynamic diameter of the complex decreased to 6.5 nm after 6 h treatment with bacterial lipase.

We also designed a non-degradable copolymer, in which polymethyl methacrylate (PMMA) was introduced in the polymer backbone instead of the degradable PCL in the original copolymer. This copolymer, PEG-*b*-(PMMA-*co*-PPMA), was synthesized by atom transfer radical polymerization (ATRP) (FIG. 8), and the corresponding characterization of its structure is shown in FIG. 9 and FIG. 10. The increment in the copolymer-PIM ratio decreases the surface charge of the complex, indicating the shielding of PIM's positive charge (FIG. 11).

Another example of a synthetic cationic antimicrobial is MR-107 (OIM1-6-1PzAc). FIG. 13A shows the zeta potential of the complex formed by different mixing ratio of MR-107 and the copolymer. FIG. 13B shows the hydrodynamic size of the complex formed by MR-107 and the copolymer at mixing ratio of 1:1.167 (copolymer-MR-107 ratio), and FIG. 13C shows a TEM image of the complex formed by MR-107 and the copolymer at mixing ratio of 1:1.167 (copolymer-MR-107 ratio).

Melittin, an example of natural cationic antimicrobials, is a main component and the major pain producing substance of honeybee. The N terminal of this polypeptide has 4⁺ charges, whereas its C terminal has 2⁺ charges at physiological pH (FIG. 14A).

FIG. 14B shows the zeta potential of the complex formed by different mixing ratio of melittin and the copolymer. FIG. 14C shows the hydrodynamic size of the complex formed by melittin and the copolymer at mixing ratio of 1:0.35 (melittin-copolymer ratio), and FIG. 14D shows the TEM image of the complex formed by melittin and the copolymer at mixing ratio of 1:0.35 (melittin-copolymer ratio).

Example 2. Verification of the Formation and the Lipase-induced Dissociation of The Cationic-antimicrobial-caged Complex

Cyanine 5 (Cy5) and Cyanine 3 (Cy3), a common pair of fluorophores for FRET, were conjugated onto the caging copolymer with carboxylic acid groups *via* esterification (FIG. 6C and FIG. 15). Fluorescence resonance energy transfer (FRET) was used to verify the formation and the lipase-induced dissociation of the complex.

Synthesis of Cy3 and Cy5-conjugated Copolymers

Briefly, 0.2 g mPEG-OH, 0.2 mL ϵ -CL, 0.8 g MA-DMOPE, 0.034 g tert-butyl methacrylate (tBMA) (molar ratio of MA-DMOPE to tBMA was equaled to 1:15), and 4 mL THF were added to a 25 mL Schlenk flask. t-BuP₄ catalyst was added in vigorous N₂ protection after 3 cycles of freeze-pump-thaw. The flask was then placed in 60 °C oil bath for 18 h and the reaction was terminated by adding 400 μ L acetic acid. The product PEG-*b*-(PCL-*co*-PPMAdM-*co*-PBMA) was obtained after precipitation in cold hexane for 2 times and lyophilisation.

Deprotection of the tert-butyl group of PEG-*b*-(PCL-*co*-PPMAdM-*co*-PBMA) to give PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMA) was achieved by reacting with TFA in anhydrous DCM for 24 h (molar ratio of tert-butyl group to TFA was equaled to 1:3). The residual TFA was removed by lyophilisation.

PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMA) was then conjugated to fluorescence dyes. Briefly, 0.15g PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMA) was dissolved in DCM and mixed with 1.6 mg DCC and 0.2 mg DMAP. The mixture was then added by 5 mg of Cy3-NH₂ or Cy5-NH₂ to yield PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMCy3) or PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMCy5). The reaction mixture was stirred at room temperature in dark for 24 h, and the products were obtained after precipitation in cold hexane for two times, dialysis with 3500 MWCO membrane in H₂O/MeOH (v/v=1:1) for 3 days, and lyophilisation.

Deprotection of the methyl group in PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMCy3) and PEG-*b*-(PCL-*co*-PPMAdM-*co*-PMCy5) were carried out similar to that of PEG-*b*-(PCL-*co*-PPMAdM). Briefly, the two polymers were dissolved in anhydrous DCM, respectively, and react with TMSBr for 24 h and subsequently, MeOH for another 24 h. The product of PEG-*b*-(PCL-*co*-PPMA-*co*-PMCy3) and PEG-*b*-(PCL-*co*-PPMA-*co*-PMCy5) were purified by dialysis with 3500 MWCO membrane in H₂O for 3 days and lyophilisation.

Results and Discussions

There is a significant spectral overlap between the excitation and emission spectra of Cy3- and Cy5-conjugated copolymers (FIG. 16A), making it possible for FRET to occur when donor

(Cy3) and receptor (Cy5) are in close proximity (Hou, S. et al., *Advanced Materials*, **2029**, 32, 1906475). The Cy5- and Cy3-conjugated caging polymers were mixed in 1:1 molar ratio to complex with PIM (FIG. 16B), leading to the neutralization of the PIM compound (FIG. 16C), similar to the case using dye-free copolymer. The resulting complex that gave a nearly neutral surface charge, denoted "Cy3/Cy5 complex," was selected for the FRET study. FIG. 6D shows the Cy3/Cy5 complex had a decreased donor emission and an increased acceptor emission compared to the complex formed by the Cy3- or Cy5-conjugated copolymer only (denoted "Cy3 complex" and "Cy5 complex," respectively), substantiating the formation of self-assembled complex nanoparticles. After incubation with lipase, the fluorescence of acceptor decreased while that of donor increased, and the ratio of Cy3/Cy5 emission increased by a factor of 3 after 24h (FIG. 6E), verifying the dissociation of the complex after lipase treatment, which supported the size and morphology changes as discussed above.

Example 3. *In Vitro* Antibacterial Activity of the Cationic-antimicrobial-caged Complexes

To evaluate the antibacterial efficacy and selectivity of the complex, bacteria strains including MRSA (ATCC BAA38), *Enterococcus faecalis* (*E. faecalis* ATCC 29212), *Bacillus subtilis* (*B. subtilis* BR151), *Escherichia coli* (*E. coli* K12), *Pseudomonas aeruginosa* (*P. aeruginosa*) PAO1 and BAA-2797, *Klebsiella pneumonia* (*K. pneumonia* SGH-10) were selected as models of lipase-producing and lipase-non-producing strains, respectively. Their lipase production was confirmed by the tributyrin agar test, in which tributyrin can be degraded by lipase (Wu, H.-S. & Tsai, M.-J., *Enzyme and Microbial Technology*, **2004**, 35, 488), thus forming a plainly visible halo around lipase-producing bacterial colonies (FIG. 17). The bacteria were incubated with different concentrations of (1) cationic antimicrobial and (2) the charge-neutralized cationic-antimicrobial-caged complex, and/or (3) vancomycin as a positive control.

In Vitro Bacterial Growth Inhibition Assay

A colony of MRSA, *B. subtilis*, or *E. coli* was inoculated in LB Broth at 37 °C overnight with shaking at 280 rpm. 10 μ L of bacterial suspension from overnight culture was diluted 100 times with fresh broth and incubated in 37 °C for another 4 h to reach mid-exponential phase. Then the bacteria suspension were diluted by MHB to obtain an OD₆₀₀ = 0.0004 (optical density at 600 nm). The PIM, the complex, and vancomycin were serially diluted in MHB, and mixed with the diluted bacteria with volume 1:1 in the 96-well plates. The plates were placed in a 37 °C incubator for 24 h. Then OD₆₀₀ was measured by a microplate reader. The growth inhibition rate was calculated as the equation below (Eq. 1).

$$\text{Growth Inhibition (\%)} = \left(1 - \frac{OD_{x-blank}}{OD_{ctrl-blank}}\right) \times 100\% \quad (\text{Eq. 1})$$

Where OD_x is the OD_{600} of each well, OD_{ctrl} is the OD_{600} of untreated bacterial culture (negative control), and blank is the OD_{600} of broth only.

Verification of Lipase Productibility

The lipase productibility was verified by tributyrin agar test. Briefly, tributyrin agar was dissolved in water as guided, followed by autoclaving at 121 °C for 15 min. The agar was then added by neutral tributyrin while hot. The mixture was mixed thoroughly by vortex and ultrasound to emulsify completely. After the agar was cooled down, different bacteria culture were inoculated in the plate and incubated at 37 °C until desired phenomenon was observed.

In Vitro Bacterial Killing Efficacy Assay

Log phase MRSA were suspended in MHB to obtain 6×10^5 CFU/ mL and mixed with equal volume of complex MHB solution. An additional group without complex was included as control. Then, bacteria suspensions were incubated in 37 °C with shaking at 280 rpm for 24 h. 20 μ L of each sample were withdrawn from all cultures and were serially diluted in PBS. The diluted aliquots were plated on LB agar and incubated 37 °C for 24 h and CFUs was counted. Data from triplicate plate counts were taken as an average.

For bacterial membrane permeability staining, after treatment of complex, the bacteria were stained by the LIVE/DEAD™ BacLight™ bacterial viability kit (Thermo Fisher) in the dark for 15 min and washed with PBS twice (4000 rcf, 5 min) before imaging by a confocal laser scanning microscope.

Results and Discussions

As demonstrated in FIG. 18A, the complex formed by PEG-*b*-(PCL-co-PPMA) exhibited significant inhibition toward the lipase-secreting MRSA. It is noteworthy that the minimum inhibitory concentration (MIC) of the complex on MRSA was higher than that of PIM alone (Table 2). This discrepancy could be attributed to incomplete or delayed degradation of the nanoparticles. In contrast, even at the highest concentration we tested, the complex exhibited negligible effects on the growth of *E. faecalis*, which does not secrete lipase (FIG. 18B). Importantly, external addition of lipase externally restored the ability of the complex to inhibit the growth of *E. faecalis*. These data establish the soundness of our design: the lipase produced by certain strains degrades the PCL of the polyanions in the complex, weakening

the multivalent electrostatic interactions between the polyelectrolytes (Insua, I. et al., *European Polymer Journal*, **2016**, 81, 198) and leading to the dissociation of cationic PIM from the particles, releasing it to exert its antibacterial effect. But for strains that cannot secrete lipase, the complex remains intact in the culture medium, and no bactericidal PIM is released. The results for two other strains of bacteria, lipase-secreting *Bacillus subtilis* BR151 and non-secreting *Escherichia coli* K12, further support this conclusion (FIG. 17, FIG. 19 and Table 2).

Table 2. MIC of Different Treatment on Various Bacteria Strains, Shown in PIM Concentration ($\mu\text{g/mL}$).

Bacterial Strain	Lipase Producibility	PIM	Complex	Complex+Lipase
MRSA BAA38	Yes	2	8	-
<i>B. subtilis</i> BR151	Yes	2	4	-
<i>E. faecalis</i> ATCC 29212	No	2	>64	4
<i>E. coli</i> K12	No	4	>64	16

To further confirm the selective antibacterial effect of the complex formed by PIM and degradable copolymer PEG-*b*-(PCL-*co*-PPMA), we also designed a complex formed by PIM and non-degradable copolymer PEG-*b*-(PMMA-*co*-PPMA), which serves as the control group for the comparison. When MRSA and *E. faecalis* were incubated with different concentrations of the complex formed by PIM and PEG-*b*-(PMMA-*co*-PPMA), this complex exhibited no inhibitory effect (FIG. 18C-D) on both lipase-producing MRSA and lipase-non-producing *E. faecalis* at the same concentration range as that in FIG. 18A-B, further corroborating our design and our hypothesis of how it works.

We investigated the killing efficacy of the complex by counting the colony forming units (CFU) of MRSA cultured with different concentrations of the complex. As shown in FIG. 18E, although MRSA proliferation was stopped at MIC, there were still live bacteria in the culture (~ 105 CFUs). When the concentration reached 4 times MIC, all the bacteria were killed by the complex. This result was further confirmed by a membrane integrity staining as shown in FIG. 18F. The dead MRSA could be stained by propidium iodide (PI, in red colour) resulting from the compromised membrane integrity, while live bacteria with intact membrane were stained by SYTO 9, shown in green colour. Confocal laser scanning microscopy (CLSM) images demonstrated progressive increase of dead bacteria with increasing complex concentration, with complete bacterial kill at 4 times MIC.

The antimicrobial effect of MR-107 alone and MR-107-caged complex is demonstrated by the data in FIG. 20 and Table 3.

Table 3. Minimum Inhibitory Concentration (MIC; $\mu\text{g/mL}$) on Various Bacteria Strains using MR-107 alone and as a caged complex according to the current invention.

Bacterial Strain	Lipase Producibility	MR-107	Complex
MRSA BAA38	Yes	2	8
<i>P. aeruginosa</i> PAO1	Yes	4	32
<i>P. aeruginosa</i> BAA2797	Yes	4	16
<i>K. pneumoniae</i> SGH-10	Yes	2	8
<i>E. faecalis</i>	No	32 (with hemin)	>256

As shown in FIG. 21, the melittin-caged complex also had a selective antimicrobial activity to lipase-secreting MRSA.

Example 4. *In Vitro* and *In Vivo* Safety Profile of the Cationic-antimicrobial-caged Complexes

The primary aim of our design is to address the toxicity issue of cationic antimicrobials. Thus, after verifying their *in vitro* antibacterial efficacies we evaluated the safety profile of the cationic-antimicrobial-caged complexes.

To analyse the *in vitro* cytotoxicity, mammalian 3T3 cells and MCF-7 cells were treated with cationic antimicrobial alone, the cationic-antimicrobial-caged complexes, and the "caging" PEG-*b*-(PCL-*co*-PMMA) copolymer alone. Cell viability was studied *via* the Cell Counting Kit-8 (CCK-8) assay.

As PIM was found to induce acute systemic toxicity when delivered to mice by I.P. injection (Zhong, W. et al., *Proceedings of the National Academy of Sciences*, **2020** 117, 31376), we studied whether the complex could alleviate the *in vivo* toxicity of PIM. Mice were treated with PIM or different concentrations of the complex by I.P. injection, and their body weights were recorded continuously for 14 days. The experiments would be terminated if the body weight loss reached 20% as humane endpoints.

In Vitro Mammalian Cell Cytotoxicity Assay with 3T3 Cell Line

The mouse embryonic fibroblast 3T3 cell line and MCF-7 Cell Line were used as a mammalian cell models to test the cytotoxicity of the complex. Each cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin), at 37 °C with 5% CO₂. After the 80% confluence was reached, the cells were collected by trypsin and counted by a hemocytometer. Then, the cells were seeded to 96-well plates with 5×10^3 cells per well and cultured for 24 h before adding different concentration of compounds. After another 24-h incubation, the cell viability was tested by Cell Counting Kit-8 (CCK-8).

For mammalian cell membrane permeability staining, the cells were treated with different concentrations of PIM or complex for 6 h (PIM concentrations were both 100 µg/mL), then stained by a LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes) in dark for 30 min. After washing with PBS twice, the cells were imaged by a confocal laser scanning microscope.

Similar protocol was adopted for other cationic antimicrobials, such as when PIM was replaced with MR-107 or melittin.

In Vivo Toxicity Assessment of PIM-caged Complex

ICR mice (4-5 weeks of age) were housed for 7 days in a 12 h dark-light cycle at room temperature before any handling. The ICR mice were treated with single dose of PBS, single dose of PIM (6 mg/kg), and repetitive dose of complex (concentration of PIM was equaled to 6 mg/kg) with I.P. injection. Their body weights were recorded daily for 14 days. The mice would be euthanized if 20 % of weight loss was reached as humane endpoints.

To measure the blood biochemical markers after various treatment, the mice were treated with single dose of PBS, PIM (6 mg/kg), and complex (concentration of PIM was equaled to 6 mg/kg) with I.P. injection. Blood was collected from the submandibular vein before drug injection (day 0), and on 1st, 3rd, and 7th day after the administration of drugs. The withdrawn blood was kept in a blood collection tube with heparin lithium coating inside, after which the biochemical markers was analyzed by the Pointcare V2 Blood Chemistry Analyzer (MNCHIP).

In Vivo Toxicity Assessment of MR-107-caged Complex

The method described above for the PIM-caged complex was to test the MR-107-caged complex. In brief, mice were injected I.V. (10 mg/kg per dose) or I.P. (15 mg/kg per dose) with MR-107 alone and MR-107-caged complex. The I.P. injections of MR-107-caged complex

were repeated daily for 4 days, all other treatments were single dose. Then the body weight of the mice were recorded daily for the following 14 days.

Results and Discussions

As illustrated in FIG. 22A, PIM exhibited a dose-dependent toxicity to 3T3 cells, which could result from its non-specific interaction with the negatively charged components on cell surfaces. In contrast, the neutral charged complex (formulated with a mixing ratio of 1:1.7 as shown in FIG. 6A) minimally affected the cell viability, which is attributable to the complete shielding of the positive charges of PIM by the biocompatible anionic copolymer. In contrast, a comparison positively charged complex, in which the PIM charges were only partially neutralized (a mixing ratio of 1:1 as shown in FIG. 6A) impaired the cell viability at higher concentrations. Mammalian cell membrane permeability staining was also conducted (FIG. 22B). For cells treated with PIM, although the cellular morphology remained intact, the membrane was significantly permeabilised as evidenced by the intense intracellular ethidium homodimer-1 (EthD-1) signals, whereas the complex did not influence the cellular membrane integrity owing to its neutralization of the PIM charge. These findings support our interpretation that the positive charge of PIM is the source of its cytotoxicity, and our approach to minimization of PIM toxicity through neutralization of this charge.

As shown in FIG. 22C-D, single dose of PIM at 6 mg/kg led to a rapid decrease in body weight within 7 days compared to mice treated with just PBS, indicating acute systemic toxicity. In sharp contrast, repeated I.P. injection of complex (every other day) at a PIM dose of 6 mg/kg only caused fluctuations within $\pm 10\%$ or an overall gain of the body weight (FIG. 22E). We further increased the concentration of the complex to determine a safety window. When the concentration was elevated to 10 mg/kg (PIM concentration, single dose), the body weight still experienced an increasing trend as shown in FIG. 23A, suggesting a minimal adverse effect of the complex on the body. Evidence of toxicity was observed when the concentration was further increased to 12 mg/kg with single dose, in which a drop in the body weight was recorded (FIG. 23B).

To obtain more information on the murine toxicology of PIM and the complex, blood was collected from the submandibular vein before (day 0), and on day 1, 3, and 7 after different treatments. Blood biochemical markers were then analysed, using alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as standard markers for drug-induced liver injury, and blood urea nitrogen (BUN) and phosphorus level (P level) as markers of kidney function (Campion, S. et al., *Expert Opin Drug Metab Toxicol*, **2013**, 9, 1391). As shown in FIG. 24A-D, elevated levels of all four biomarkers were recorded in mice treated with PIM alone,

indicating damage to liver and kidney. In contrast, the levels of the biomarkers fluctuated only slightly after treatment with the complex. These results show that the systemic toxicity of PIM can be greatly mitigated by complexation with PEG-*b*-(PCL-*co*-PPMA).

As shown by FIG. 25, compared to MR-107 alone, the MR-107-caged complex exhibited minimized toxicity to mammalian 3T3 cells.

Table 4. Half Maximal Inhibitory Concentration (IC50; µg/mL) of 3T3 Cell Line using MR-107 alone and as a caged complex as according to the current invention.

3T3 Cell Line	MR-107	Complex
24 h	>256	>256
48 h	256	>512
72 h	64	>512

The mice received single I.P injection of MR-107 alone experienced a sharp drop in their bodyweight. In comparison, 4 doses of the MR-107-caged complex based on the same MR-107 concentration did not significantly affect the body weight (FIG. 26A). Furthermore, 3 mice received single I.V. injection of MR-107 alone (10 mg/kg) died immediately after the injection, while those I.V. injected by the complex survived and their bodyweight remained stable during the 14-day experiment (FIG. 26B). These result indicated an improved safety profile of the MR-107-caged complex compared to MR-108 alone.

As shown in FIG. 27, melittin alone displayed toxicity towards both 3T3 and MCF-7 cell lines, indicating that melittin itself has no selective properties. In comparison, both the caging copolymer and the melittin-caged complex exhibited minimal adverse effects to the cells. This verifies the toxicity of cationic antimicrobial can be greatly reduced by complexation with the caging copolymer.

Example 5. *In Vivo* Therapeutic Efficacy of the PIM-Caged Complex

We then examined the capability of the complex at its safe dosage to treat systemic MRSA infection, which was established by I.P. injection of bacterial suspension to mice. MRSA was selected for this study as it is a WHO high priority pathogen and is a frequent source of nosocomial infection (World Health Organisation, *2021 Antibacterial Agents in Clinical and Preclinical Development: An Overview and Analysis*, 2022).

In Vivo Antibacterial Efficacy

Each ICR mouse was challenged with 300 μ L of MRSA intraperitoneally (3×10^7 CFU/mL in 5% mucin PBS solution). At 2 hours post-infection, the mice were intraperitoneally injected with PBS (negative control), PIM (6 mg/kg), complex (concentration of PIM was equaled to 6 mg/kg). After 26 hours post-infection, the intraperitoneal fluids (I.P. fluids), livers, kidneys, and spleens were collected. Briefly, to collect I.P. fluids, 3 mL PBS was injected intraperitoneally, and 1 mL of which was collected after 10 seconds' massage in the stomach. The organs were collected after the mice were euthanized and were subsequently homogenized by a tissue homogenizer. Finally, the bacterial loads in I.P. fluids, livers, spleens, and kidneys were measured by agar plate count method. A different set of mice was used to study the survival status. Briefly, at two hours post-infection, the mice were intraperitoneally injected with PBS (negative control), PIM (6 mg/kg), complex (concentration of PIM was equaled to 6 mg/kg). Their survival condition and body weight were recoded daily. Complex was injected for 4 times on day 0, 2, 4, and 6. The animal studies were carried out in accordance with the protocols A20029 and A21037 approved by the NTU Institutional Animal Care and Use Committee (NTU-IACUC).

Results and Discussions

The infected mice were given different treatments, and their survival status and body weights were recorded daily for 14 days for those in Group 1 (FIG. 28A, group 1). As shown in FIG. 28B, all mice treated with PBS and PIM died within 2 and 5 days, respectively. It should be noted that both actual death and over 20% loss of body weight were considered as endpoints, which were recorded as "death". Although PIM has potent *in vitro* antibacterial efficacy, its toxicity resulted in no beneficial effect on the survival of infected mice. Owing to its good biocompatibility, the complex was injected, every other day, 4 times (the arrows in FIG. 28B-C), and all mice survived the 14-day duration of the experiment. For the group treated with the complex, although a sharp drop of body weight was observed at the beginning, body weight gradually recovered to normal level (FIG. 28C). To gain more insight on the therapeutic efficacy, another set of mice in Group 2 was used in a shorter duration study and the bacterial loads in organs were determined (FIG. 28A) after 24 hours of treatment. As shown in FIG. 28D-G, MRSA counts in all the tested organs, *i.e.*, I.P. fluid, liver, spleen, and kidney were significantly reduced for mice treated with PIM and the complex, compared to the PBS-treated group.

Animal studies showed the complex to be safe to mice compared to PIM alone, and to have antibacterial efficacy against systemic infection similar to that of PIM. The complex alleviated

the bacterial loads in systemically infected mice and contributed to their survival at its safe dosage. As clinical isolates of MRSA have already been found to evolve resistance to the last-resort drug vancomycin (Chang, S. et al., *N. Engl. J. Med.*, **2003**, 348, 1342), the development of this cationic antimicrobial-caged complex could be a promising alternative for the treatment of this bacterial infection.

Example 6. *In Vivo* Therapeutic Efficacy of the MR-107-caged Complex

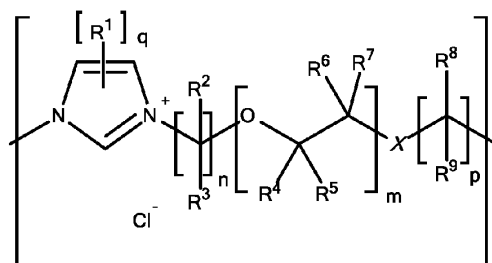
As shown in FIG. 29, mice were injected with bacteria intranasally followed by 2 doses of treatment at 2 h and 4 h post-infection, respectively. After 24 h, mice were sacrificed and their organs were collected for bacterial count.

Results and Discussions

Both MR-107 and the complex were able to alleviate the bacterial loads in the infected lungs and livers. The bacterial counts between the group of MR-107 and complex showed no statistical difference, indicating they exhibited similar *in vivo* therapeutic efficacy in the model tested (see FIG. 29B-C).

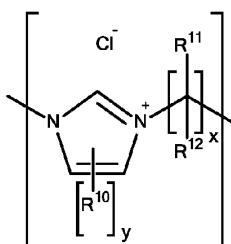
Claims

1. An antimicrobial composite material, comprising:
a cationic antimicrobial compound; and
a bacterial-enzyme-degradable anionic copolymer, wherein
the bacterial-enzyme-degradable anionic copolymer and the cationic antimicrobial compound are attached to one another through electrostatic interaction.
2. The antimicrobial composite material according to Claim 1, wherein the cationic antimicrobial compound is selected from one or more of the group consisting of a polyimidazolium, an oligoimidazolium, and a cationic antimicrobial peptide.
3. The antimicrobial composite material according to Claim 2, wherein the cationic antimicrobial peptide is selected from one or more of the group Cecropin A, Cecropin B, Cecropin P1, Dermaseptin 1, Indolicidin, Lactofermicin B, Magainin I, Magainin II, Melittin, Polistes Mastoparan, Tachyplesin I, optionally wherein the cationic antimicrobial peptide is Melittin.
4. The antimicrobial composite material according to Claim 2, wherein the polyimidazolium has a repeating unit of formula (I):



(I)

or is a copolymer comprising the repeating unit of formula (I) and a repeating unit of formula (II):



(II)

wherein:

R^1 and R^{10} , when present, independently represent C_{1-6} alkyl;

each R^2 to R^8 and R^{11} independently represent H or C_{1-6} alkyl;

each R^9 and R^{12} independently represents H, C_{1-6} alkyl or CO_2R^{13} ;

R^{13} represents H or C_{1-6} alkyl;

X represents $CR^{14}R^{15}$, O or S;

R^{14} and R^{15} independently represent H, C_{1-6} alkyl or CO_2R^{13} ;

m is a number selected from 0 to 5;

n is a number selected from 2 to 10;

p is a number selected from 0 to 5;

q is a number selected from 0 to 3;

x is a number selected from 2 to 10;

y is a number selected from 0 to 3; and

solvates thereof,

provided that, when the polyimidazolium is a copolymer, the repeating unit of formula (I) and the repeating unit of formula (II) are not the same.

5. The antimicrobial composite material according to Claim 4, wherein:

R^1 and R^{10} , when present, independently represent C_{1-3} alkyl;

each R^2 to R^8 and R^{11} independently represents H or methyl;

each R^9 and R^{12} independently represents H, C_{1-3} alkyl or CO_2R^{13} ;

R^{13} represents H or C_{1-3} alkyl;

X represents $CR^{14}R^{15}$ or O;

R^{14} and R^{15} independently represent H, C_{1-3} alkyl or CO_2R^{13} ;

m is a number selected from 0 to 4;

n is a number selected from 2 to 8;

p is a number selected from 0 to 3;

q is a number selected from 0 to 1;

x is a number selected from 2 to 8; and
y is a number selected from 0 to 1.

6. The antimicrobial composite material according to Claim 4 or Claim 5, wherein:
each R^2 to R^8 and R^{11} independently represents H;
each R^9 and R^{12} independently represents H, methyl or CO_2H ;
X represents $\text{CR}^{14}\text{R}^{15}$ or O;
 R^{14} and R^{15} independently represent H, methyl or CO_2H ;
m is a number selected from 0 to 3;
n is a number selected from 2 to 7;
p is a number selected from 0 to 2;
q and y are 0; and
x is a number selected from 2 to 7.

7. The antimicrobial composite material according to any one of Claims 4 to 6, wherein:
each R^2 to R^8 and R^{11} independently represents H;
each R^9 and R^{12} independently represents H, methyl or CO_2H ;
X represents $\text{CR}^{14}\text{R}^{15}$ or O;
 R^{14} and R^{15} independently represent H, methyl or CO_2H ;
m is a number selected from 0 to 2;
n is a number selected from 2 to 7;
p is a number selected from 0 to 2;
q and y are 0; and
x is a number selected from 2 to 7.

8. The antimicrobial composite material according to any one of Claims 4 to 7, wherein
the number average molecular weight is from 500 to 7,000 Daltons.

9. The antimicrobial composite material according to any one of Claims 4 to 8, wherein
the polyimidazolium has the repeating unit of formula (I).

10. The antimicrobial composite material according to Claim 9, wherein:
each of R^2 to R^8 are H;
each R^9 represents H, methyl or CO_2H ;
X represents CH_2 or O;
m is a number selected from 0 to 2;
n is a number selected from 2 to 6;

p is a number selected from 0 to 2;

q is 0.

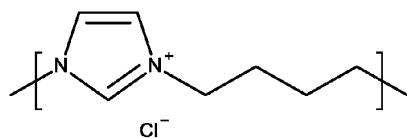
11. The antimicrobial composite material according to any one of Claims 4 to 10, wherein one or both of the following apply:

(ia) when X is O, p is 1 or 2; and

(ib) the polyimidazolium is terminated by amino (NH_2) groups.

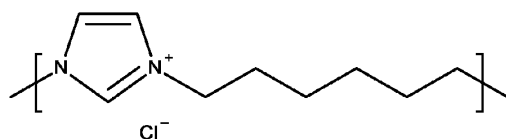
12. The antimicrobial composite material according to any one of Claims 4 to 11, wherein the repeating unit is selected from the group consisting of:

(i)



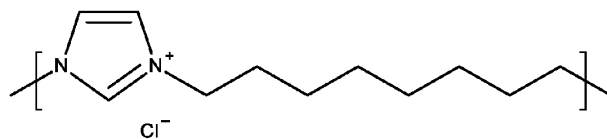
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(ii)



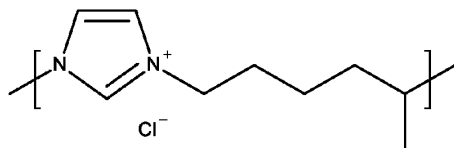
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(iii)



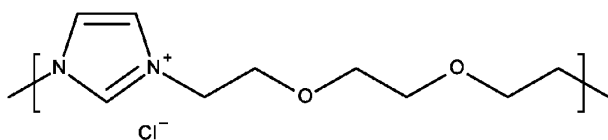
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,500 Daltons;

(iv)



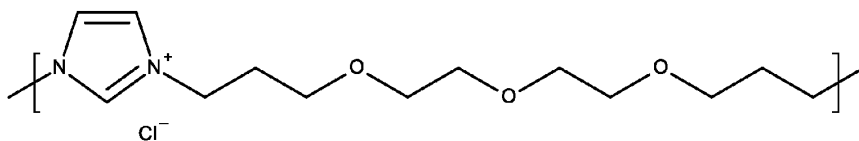
, optionally wherein the number average molecular weight of the polymer is from 500 to 2,000 Daltons;

(v)



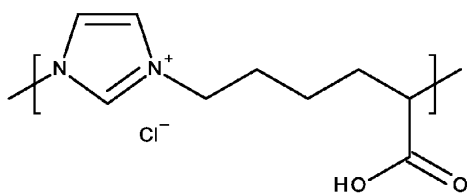
, optionally wherein the number average molecular weight of the polymer is from 4,000 to 5,500 Daltons;

(vi)



, optionally wherein the number average molecular weight of the polymer is from 4,000 to 5,000 Daltons; and

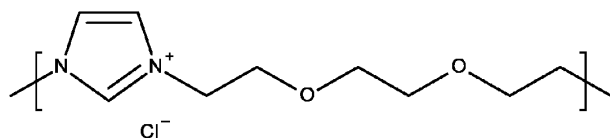
(vii)



, optionally wherein the number average molecular weight of the polymer is from 500 to 2,000 Daltons.

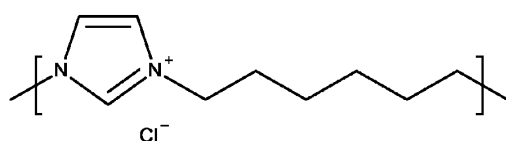
13. The antimicrobial composite material according to any one of Claims 4 to 8, wherein the repeating unit of formula (I) and formula (II) are selected from the group consisting of:

(i)



as the repeating unit of

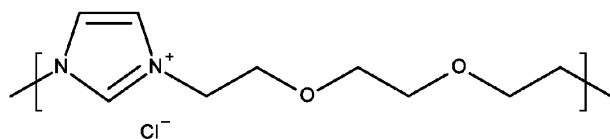
formula (I) and



as the repeating unit of formula (II) ,

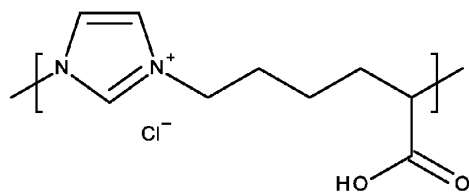
optionally wherein the number average molecular weight of the copolymer is from 1,000 to 5,000 Daltons; and

(ii)



as the repeating unit of

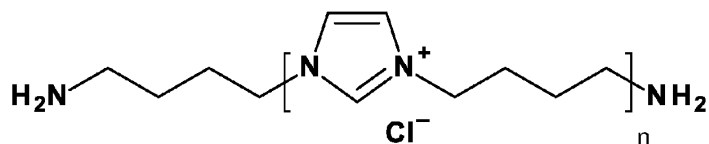
formula (I) and



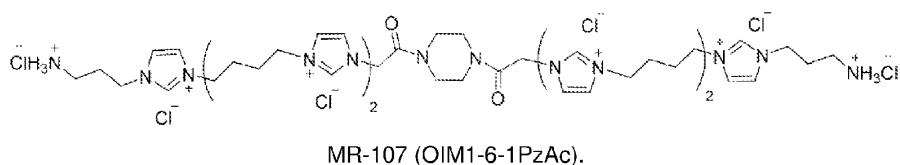
as the repeating unit of formula (II) ,

optionally wherein the number average molecular weight of the copolymer is from 1,000 to 5,000 Daltons.

14. The antimicrobial composite material according to Claim 2, wherein the polyimidazolium is:

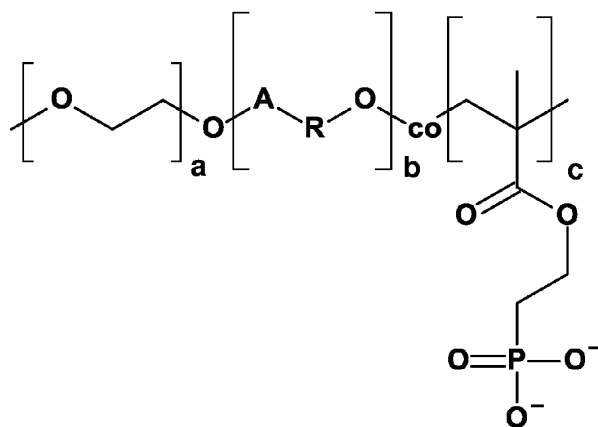


15. The antimicrobial composite material according to Claim 2, wherein the oligoimidazolium is MR-107 (OIM1-6-1PzAc):



16. The antimicrobial composite material according to any one of the preceding claims, wherein the bacterial-enzyme-degradable anionic copolymer comprises a repeating unit that bears a phosphonate group.

17. The antimicrobial composite material according to any one of the preceding claims, wherein the bacterial-enzyme-degradable anionic copolymer has the formula (III):



(III)

where:

R is a C₁ to C₁₀ linear or branched alkyl chain;

A represents C=O or P(OR_a)=O;

R_a represents a linear or branched C₁ to C₆ alkyl group;

a represents a block polyethylene glycol unit having a number average molecular weight of from 500 to 10,000 Daltons;

b and c together represent a random copolymer block, where b and c each independently have a value of from 8 to 100.

18. The antimicrobial composite material according to Claim 17, wherein one or more of the following apply:

(a) a represents a block polyethylene glycol unit having a number average molecular weight of about 2,000 Daltons;

(b) b and c each independently have a value of from 10 to 50, such as 15 to 20, such as 18;

(c) A represents C=O; and

(d) R represents $-(CH_2)_5-$.

19. The antimicrobial composite material according to any one of Claims 16 to 18, wherein the bacterial-enzyme-degradable anionic copolymer has a number average molecular weight of from 6,000 to 10,000 Daltons, such as from 7,000 to 8,000 Daltons.

20. The antimicrobial composite material according to any one of the preceding claims, wherein the bacterial-enzyme-degradable anionic copolymer is degradable by a bacterial-secreted lipase or a bacterial-secreted phosphoesterase, such as a bacterial-secreted lipase.

21. The antimicrobial composite material according to any one of the preceding claims, wherein the weight to weight ratio of the cationic antimicrobial compound and the bacterial-enzyme-degradable anionic copolymer is selected to provide a material with a zeta potential of from +10 to -10 mV, such as from +6 to -6 mV, such as from 0 to -5.3 mV.

22. The antimicrobial composite material according to any one of the preceding claims, wherein the weight to weight ratio of the cationic antimicrobial compound and the bacterial-enzyme-degradable anionic copolymer is from 1:5 to 5:1, such as from 1:2 to 2:1, such as about 1:1.7.

23. A pharmaceutical composition comprising an antimicrobial composite material according to any one of Claims 1 to 22 and one or more of a pharmaceutically acceptable excipient and carrier.

24. An antimicrobial composite material according to any one of Claims 1 to 22, or a pharmaceutical composition as described in Claim 23, for use as a medicament.

25. A method of treating a subject suffering from a microbial and/or fungal infection comprising the steps of administering to the subject a therapeutically effective amount of an antimicrobial composite material according to any one of Claims 1 to 22, or a pharmaceutical composition as described in Claim 23, such that the infection is treated.

26. Use of an antimicrobial composite material according to any one of Claims 1 to 22, or a pharmaceutical composition as described in Claim 23, in the manufacture of a medicament to treat a microbial and/or fungal infection in a subject in need thereof.

27. An antimicrobial composite material according to any one of Claims 1 to 22, or a pharmaceutical composition as described in Claim 23, for use in the treatment of a microbial and/or fungal infection.

28. A method of manufacture of an antimicrobial composite material according to any one of Claims 1 to 22, the method comprising the steps of:

- (i) providing a solution of a cationic antimicrobial compound and providing a solution of a bacterial-enzyme-degradable anionic copolymer as described in any one of Claims 1 to 22; and
- (ii) mixing the solutions together to form a mixture and agitating the mixture for a period of time to form the antimicrobial composite material.