A POLYMER, A METHOD OF PREPARING THE POLYMER AND ITS USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to Singapore application number 10202400700R filed with the Intellectual Property Office of Singapore on 13 March 2024, the contents of which is hereby incorporated by reference.

TECHNICAL FIELD

The present invention relates generally to a topologically complex polymer and a method of preparing the topologically complex polymer as defined herein. The present invention also relates to a method of preparing a packaging material comprising the topologically complex polymer and a packaging material comprising the topologically complex polymer as defined herein.

BACKGROUND ART

Multidrug resistance (MDR) remains a menacing situation globally. Following the discovery of pathogenic microbes identified as the root causes of many infectious diseases, the search for remedies culminated with the golden age of antibiotics in the pharmaceutical industry between 1940s to 1960s, which has presented mankind with a number of resources that greatly improved our capacity to combat pathogens. However, the vogue of antibiotics was overshadowed by the appearance of widespread multidrug resistance microorganisms, and this situation was further worsened by a number of pharmaceutical companies shifting away from new antibiotic research due to reasons such as saturated and genericized marketplace, as well as the more stringent criteria required in clinic trials for new drug development. MDR also affected livestock production which traditionally relied on antibiotics (such as veterinary medicines or food supplements) to enhance growth. The excessive use of such antibiotics has also led to environmental and food contamination, further deteriorating the overall situation. The World Health Organization (WHO) has thus recommended the elimination of antibiotics used as feed supplements in livestock.

Furthermore, given the fact that origins of most marketed antibiotics are metabolites from various microbes or their derivatives, it is not difficult to understand that drug resistance coding genes have already existed in the environment even before the antibiotic era, and the improper use of these chemicals resulted in a selection pressure which encouraged the proliferation of drug resistance genes. To circumvent the existing drug resistance mechanisms, targeting different components of the pathogenic bacteria are desired to reduce the chance of drug resistance development.

Antimicrobial peptides (AMPs), also known as host defence peptides (HDPs) or cationic antimicrobial peptides (CAPs), are a sustainable alternative and is highly valued due to its high efficacy, minimum MDR development risk, biodegradability, and being more environmentally friendly. AMPs are biomacromolecules in multicellular organisms having antibacterial, antiviral, antifungal, anticancer, and immunoregulatory properties, which serve as key components of innate immune system. While AMPs were discovered as early as 1922 (eg. protein lysozyme, gramicidin etc.), more intensive investigations in this class of naturally sourced antibacterial reagents started in 1980s which resulted in the discoveries such as cecropins and magainins, extracted from pupae of silk moth *Hyalophora cecropia*, and African clawed frog *Xenopus laevis*, respectively. Natural AMPs are featured as cationic (having 2 to 9 positive charges) and amphiphilic short peptides (10 to 50 amino acids). Regardless of their origins, AMPs can be categorized into four groups based on their secondary structures namely, α-helical peptides, β-sheet peptides, extended peptides, and loop peptides.

In recent years, food safety concerns and spoilage prevention has been gaining more attention and active packaging technologies have become the spotlight in academic, industry and public communities. For instance, to significantly improve the food preservation during storage, there are ongoing research to include various functional materials. Since AMPs are materials derived from natural amino acids and exhibit antimicrobial activity, food packaging including AMPs as a component is regarded as a promising option to prevent microbial contamination and food deterioration or spoilage.

AMPs functions by targeting negatively charged components in the cell wall of Gram-positive (G+) or Gram-negative (G-) bacteria, which are essential infrastructures to maintain the integral

structure of bacteria and the matrix to hold various virulence factors, such as adhesins and types of protein secretion systems, dubbed as the "Achilles heels" of microorganisms. Early studies of AMPs were conducted based on naturally occurring compounds, which were obtained from natural samples, or recombinantly by molecular cloning technique. The advent of solid-phase peptide synthesis (SPPS) and its variations greatly facilitated the exploration of AMP's antibacterial study, in that it could grant peptides with new building blocks (ie. nonprotein amino acids, D-amino acids, pseudo amino acids), novel sequences, and with different topologies.

Recently, synthetic AMPs have been generated by mimicking their leads via the following strategies such as substitution, truncation, sliding window, and ligation. Unnatural amino acid (N-alkylglycine, and β-amino acid) derivatives are usually used as building blocks to synthesize peptidomimetics with excellent activity, improved stability, and acceptable cytotoxicity. Although many AMPs explored have reached clinical research stages, most of the peptides involved are canonical linear structure prepared via expensive SPPS protocols, and their applications are mostly limited to topical uses.

Therefore, there is a need to prepare more cost-effective antimicrobial AMPs with high performance that can be applied in various scenarios, especially in the sectors related to food production and preservation.

SUMMARY

In one aspect, the present disclosure relates to a topologically complex polymer comprising at least one functionalized macromolecule coupled to a plurality of antimicrobial peptides via a plurality of furcated linkers, wherein each antimicrobial peptide is bonded to each furcated terminal end of the furcated linker.

Advantageously, complex topologies of the polymer having a plurality of antimicrobial peptides covalently linked within a macrostructure in the polymer allows the minimum inhibiting concentration (MIC) to be lowered when compared with the isolated individual peptides. For instance, short peptides typically adopt tertiary/quaternary structures to exert their antimicrobial function, primarily at the target site (such as the bacterial membrane). However,

the complex topologies of the polymer, having the peptides covalently linked within the polymer, are able to overcome the minimum threshold required to form the tertiary/quaternary structures and therefore reducing the MIC. Further advantageously, the non-linear arrangements of the plurality of antimicrobial peptides may result in restricted conformations and thus allow for greater binding capacity to targets over their freely rotating linear counterparts. Advantageously, the topologically complex polymer may bring the plurality of antimicrobial peptides into close proximity, thereby producing synergetic effects within the macromolecules.

In another aspect, the present disclosure relates to a method of preparing the topologically complex polymer, the method comprising the steps of: a) reacting a mixture of at least one functionalized macromolecule precursor and a plurality of furcated linkers precursor in a solvent and subsequently deprotecting the resulting mixture to form an intermediate polymeric product; and b) polymerizing a mixture of the intermediate polymeric product and a plurality of antimicrobial peptides monomers in a buffer system to form the topologically complex polymer.

Advantageously, the method allows for a simple and straightforward preparation of the topologically complex polymer compared to the tedious biosynthesis and solid-phase peptide synthesis (SPPS) known in the art.

Advantageously, the buffer system allows for the polymerization (such as ring opening polymerization) to be conducted in a controlled manner such that the polymerization would undergo the selective normal amine ring opening polymerization (selective NA-ROP) (Fig. 2b) (where polymerization may only propagate at sites where primary amines are generated through the process) instead of the undesired activated monomer ring opening polymerization (AM-ROP) (where chain growth may occur randomly at any sites as any monomers may initiate polymerization), and thereby allowing the plurality of antimicrobial peptides to be formed uniformly in the final topologically complex polymer.

In another aspect, the present disclosure relates to a method of preparing a packaging material, the method comprising integrating the topologically complex polymer into a material to form the packaging material.

In another aspect, the present disclosure relates to a packaging material comprising the topologically complex polymer.

DEFINITIONS

The following words and terms used herein shall have the meaning indicated:

As used herein, the term "topologically complex polymer" refers to a polymer having a plurality of antimicrobial peptides chemically linked within a macrostructure, wherein the plurality of antimicrobial peptides is in a non-linear arrangement within a complex network and thereby restricting the resulting conformations of the polymer.

As used herein, the term "dendrimeric macromolecule" refers to the macromolecule having highly branched monomers emerging radially from a core or the macromolecule having a branching pattern and with repeat units that each contribute a branch point.

As used herein, the term "furcated linker" refers to the compound or molecule having two or more chemical units branching out from a central chemical group.

As used herein, the term "alkyl group" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-

dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, and the like.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

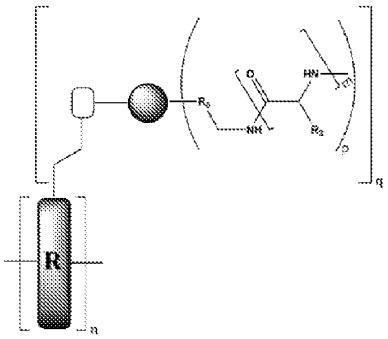
DETAILED DESCRIPTION OF OPTIONAL EMBODIMENTS

Exemplary, non-limiting embodiments of a topologically complex polymer having antimicrobial properties will now be disclosed. The topologically complex polymer comprises at least one functionalized macromolecule coupled to a plurality of antimicrobial peptides via a plurality of furcated linkers, wherein each antimicrobial peptide is bonded to each furcated terminal end of the furcated linker.

Advantageously, complex topologies of the polymer having a plurality of antimicrobial peptides covalently linked within a macrostructure in the polymer allows the minimum

inhibiting concentration (MIC) to be lowered when compared with the isolated individual peptides. For instance, short peptides typically adopt tertiary/quaternary structures to exert their antimicrobial function, primarily at the target site (such as the bacterial membrane). However, the complex topologies of the polymer, having the peptides covalently linked within the polymer, are able to overcome the minimum threshold required to form the tertiary/quaternary structures. Further advantageously, the non-linear arrangements of the plurality of antimicrobial peptides may result in restricted conformations and thus allows for greater binding capacity to targets over their freely rotating linear counterparts. Advantageously, the topologically complex polymer may bring the plurality of antimicrobial peptides into close proximity, thereby producing synergetic effects within the macromolecules.

The topologically complex polymer may be defined by the general formula (I):



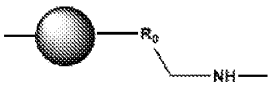
(I)

wherein,

R may independently represent the at least one functionalized macromolecule;

n may represent an integer selected from ≥ 3;

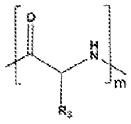
 may independently represent a chemical moiety formed between the at least one functionalised macromolecule and the plurality of furcated linkers;



may independently represent each of the plurality of furcated linkers

wherein  may independently represent a central chemical group of each furcated linker from which two or more chemical units branches out therefrom and R_0 may independently represent an alkyl with or without heteroatoms;

p may represent an integer selected from 1 to 10;



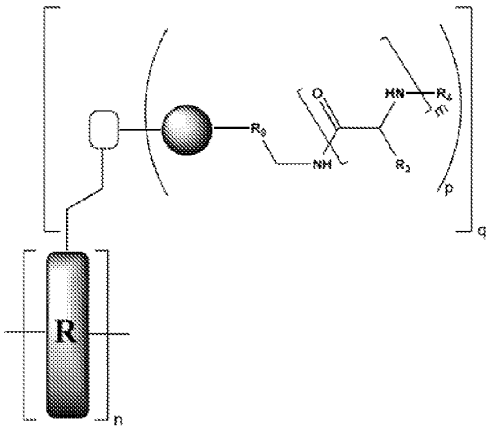
may independently represent each of the plurality of antimicrobial peptides bonded to each furcated terminal end of the furcated linker;

R_3 may independently represent each functional group of an amino acid;

m may represent an integer between 2 to 1000; and

q may represent an integer of more than 1 depending on the number of available functionalized groups on the functionalized macromolecule available for the furcated linker to bind to.

The topologically complex polymer may further comprise a plurality of hydrophilic, hydrophobic and/or amphiphilic components coupled to the terminal ends of the plurality of antimicrobial peptides to form a modified topologically complex polymer. The modified topologically complex polymer may be defined by the general formula (II):



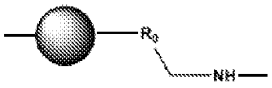
(II)

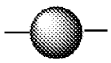
wherein,

R may independently represent the at least one functionalized macromolecule;

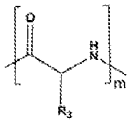
n may represent an integer selected from ≥ 3;

 may independently represent a chemical moiety formed between the at least one functionalised macromolecule and the plurality of furcated linkers;

 may independently represent each of the plurality of furcated linkers

where  may independently represent a central chemical group of each furcated linker from which two or more chemical units branches out and R_0 may independently represent an alkyl with or without heteroatoms;

p may represent an integer selected from 1 to 10;



may independently represent each of the plurality of antimicrobial peptides bonded to each furcated terminal end of the furcated linker;

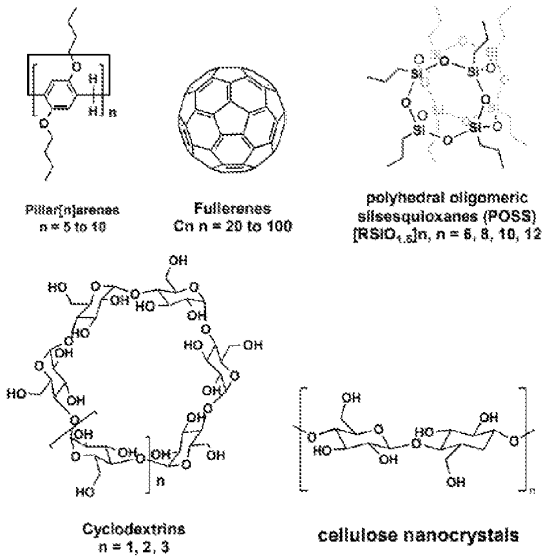
R_3 may independently represent each functional group of an amino acid;

m may represent an integer between 2 to 1000;

q may represent an integer more than 1 depending on the number of available functionalized groups on the functionalized macromolecule available for the furcated linker to bind to; and

R_4 may independently represent each of the plurality of hydrophilic, hydrophobic and/or amphiphilic components bonded to each terminal end of the plurality of antimicrobial peptides.

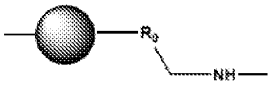
The at least one macromolecule defined by R may independently be selected from the group consisting of pillararenes, fullerenes, polyhedral oligomeric silsesquioxanes (POSS), cyclodextrins and cellulose nanocrystals, as shown below.

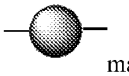


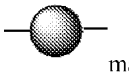
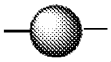
The at least one functionalized macromolecule R may independently be the macromolecules as defined herein functionalised with functional groups selected from azide, amide, acid, acid

halide, isocyanate, isothiocyanate, hydroxyl, amine, thiol, or alkyl halide but not limited thereto. The at least one macromolecule R may be Pillar[5]arenes functionalised with terminal azide groups. The at least one functionalized macromolecule R may be Pillar[5]arene-10N_3.

 may independently represent the chemical moiety formed from the chemical reaction between the at least one functionalised macromolecule and the plurality of furcated linkers. The chemical moiety formed may be dependent upon the chemical reaction between the at least one functionalised macromolecule and the plurality of furcated linkers. The chemical moiety formed may be amide, ether, thioether, ester, carbamate, thiocarbamate, amine, ammonium or triazole group but not limited thereto. For example, when the chemical reaction is a click reaction between an azide group on the at least one functionalised macromolecule and a terminal alkyne on the plurality of furcated linkers, the chemical moiety formed may be a triazole group. Therefore, the chemical reaction may be, but not limited to, click reactions, Williamson Ether Synthesis, esterification, carbamate formation, ammonia reaction or amide coupling well known in the art.  may represent a triazole group.



may independently represent each of the plurality of furcated linkers where  may independently represent a central chemical group of each furcated linker from which two or more chemical units branches out. The central chemical group  where the linkers branch out from may be ring/cyclic structures and/or may be acyclic structures. The ring/cyclic structures may be selected from, but not limited thereto, single ring, bridged-rings, fused rings, and/or spiro rings. The acyclic structures may comprise atoms selected from carbon, nitrogen, or phosphine but not limited thereto. In an example, the central chemical group  may be a single ring selected from N-alkyl benzamide or alkyl-ester

benzoic. The central chemical group  may be a N-methyl benzamide. In another example, the central chemical group  may comprise a Newkome-type structure derived from TRIS.

R_0 may independently represent an alkyl with or without heteroatoms.

R_3 may independently represent each functional group of an amino acid and be dependent upon the amino acid. The amino acid may be natural or synthetic/artificial amino acids. The amino acids may be selected from the group consisting of methionine, threonine, lysine, tryptophan, histidine, isoleucine, leucine, valine, glycine, alanine, glutamate, proline, and N-alkylglycine and a derivative thereof. R_3 may be lysine.

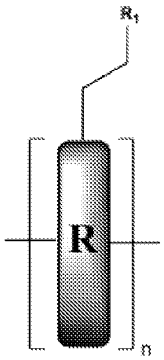
R_4 may independently represent each of the plurality of hydrophilic, hydrophobic and/or amphiphilic components bonded to each terminal end of the plurality of antimicrobial peptides. R_4 may independently be selected from the group consisting of a linear or branched C_2 to C_{40} alkyl, a linear or branched polyethylene glycol (PEG), polyethylene oxide (PEO) having repeating units of 2 to 40 and a combination thereof.

The topologically complex polymer or the modified topologically complex polymer may have a polydispersity index of about 1 to 2, or about 1.5 to 2.

Exemplary, non-limiting embodiments of a method of preparing the topologically complex polymer will now be disclosed. The method comprises the steps of: a) reacting a mixture of at least one functionalized macromolecule precursor and a plurality of furcated linkers precursor in a solvent and subsequently deprotecting the resulting mixture to form an intermediate polymeric product; and b) polymerizing a mixture of the intermediate polymeric product and a plurality of antimicrobial peptides monomers in a buffer system to form the topologically complex polymer.

Advantageously, the method allows for a simple and straightforward preparation of the topologically complex polymer compared to the tedious biosynthesis and solid-phase peptide synthesis (SPPS) known in the art.

The at least one functionalized macromolecule precursor in step a) may be defined by the general formula (Ia):



(Ia)

wherein,

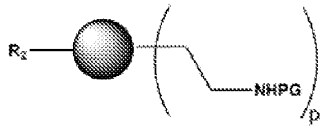
R may independently represent the at least one functionalized macromolecule;

n may represent an integer selected from ≥3; and

R_1 may independently be selected from an azide, amide, acid, acid halide, isocyanate, isothiocyanate, hydroxyl, amine, thiol, or alkyl halide but not limited thereto.

The functionalized macromolecule R may be as defined above. R_1 may independently represent any functional group capable of undergoing chemical reaction with the plurality of furcated linkers. The chemical reactions may be, but not limited to, click reaction, Williamson Ether Synthesis, esterification, carbamate formation, ammonia reaction or amide coupling that is well known in the art. R_1 may be selected from an azide, amide, acid, acid halide, isocyanate, isothiocyanate, hydroxyl, amine, thiol, or alkyl halide but not limited thereto. The functionalized macromolecule may couple to the plurality of furcated linkers via R_1.

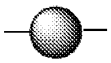
The plurality of furcated linkers precursor in step a) may be defined by the general formula (Ib):

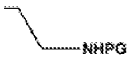


(Ib)

wherein,

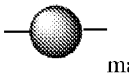
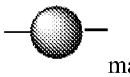
R_2 may be independently selected from a terminal alkyne, hydroxyl, amine, thiol, halide or a terminal alkene but not limited thereto;

 may independently represent the central chemical group from which two or more chemical units branches out;

 may independently represent an alkyl amine capped with a protecting group (PG); and

p may represent an integer selected from 1 to 10.

R_2 may independently represent any functional group capable of undergoing chemical reaction with the at least one functionalised macromolecule. The chemical reactions may be, but not limited to, click reaction, Williamson Ether Synthesis, esterification, carbamate formation, ammonia reaction or amide coupling that is well known in the art. R_2 may be independently selected from a terminal alkyne, hydroxyl, amine, thiol, halide or a terminal alkene but not limited thereto. The plurality of furcated linkers may couple to the functionalized macromolecule via R_2.

 may independently represent the central chemical group from which two or more chemical units branches out.  may be as defined above.

 may independently represent an alkyl amine capped with a protecting group (PG). PG may be any protecting group capable of masking the reactivity of the amine. PG may be

selected from the group consisting of tert-butyloxycarbonyl (Boc) group, carbobenzyloxy (Cbz) group, 9-Fluorenylmethyloxycarbonyl (Fmoc) group, benzyl (Bn) group and allyloxycarbonyl (Alloc) group.

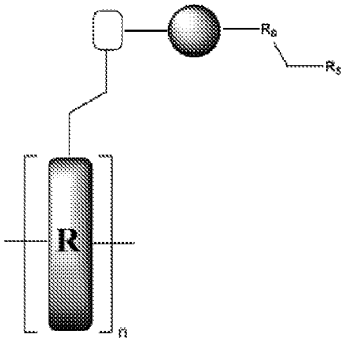
Step a) may further comprise a catalyst depending on the requirement of the chemical reactions to form the chemical moiety as stated above. When the reaction is a click chemical reaction, the catalyst in step a) may be selected from the group consisting of $CuSO_4$, CuI, CuBr, CuCl, CuOAc, $CuOTf{\cdot}C_6H_6$, $[Cu\text{-}(NCCH_3)_4][PF_6]$, $CuIP(OEt)_3$, $Cu(CH_3CN)_4PF_6$, $CuBr(PPh_3)_4$, Cu(tris(2-diocta-decylaminoethyl)amine)Br), $Cu(PPh_3)_2OAc$, $Cu(P(OMe)_3)_3Br$, $Cu(PPh_3)_3Br$ and a combination thereof.

Step a) may further comprise a reducing agent depending on the requirement of the chemical reactions to form the chemical moiety as stated above. When the reaction in step a) is a click chemical reaction, the reducing agent used may be selected from the group consisting of sodium ascorbate (NaAsc), hydrazine, 3',3''- phosphanetriyltripropanoic acid (TCEP) and a combination thereof.

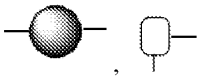
The solvent used in step a) may be aprotic organic solvents, such as, but not limited to tetrahydrofuran (THF), acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), toluene or combinations thereof.

The deprotecting in step a) may involve adding an acid in a solvent to form the intermediate polymeric product. The acid may be selected from hydrochloric acid (HCl), trifluoracetic acid CF_3COOH, phosphoric acid (H_3PO_4), nitric acid (HNO_3), sulfuric acid (H_2SO_4), tosylic acid (TsOH), methanesulfonic acid ($MeSO_3H$), Aluminum chloride ($AlCl_3$), Tin (IV) chloride ($SnCl_4$), Boron trifluoride etherate ($BF_3{\cdot}Et_2O$), Tin (II) trifluoromethanesulfonate ($Sn(OTf)_2$), Zinc bromide ($ZnBr_2$), or combinations thereof. The solvent used may be selected from dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), tetrahydrofuran (THF), acetonitrile (CH_3CN), acetone, dichloroethane ($ClCH_2CH_2Cl$), chlorofoam ($CHCl_3$), dioxane, EtOAc, water or combinations thereof.

The intermediate polymeric product resulting from the deprotecting may be defined by the general formula (Ic):



(Ic)

wherein R, R_0,  and n may be as defined above and R_5 may independently represent a cation generated as a result of the deprotecting. R_5 may be an ammonium cation.

The reacting step a) may be conducted for a duration of between about 1 hour to about 120 hours, about 1 hour to about 100 hours, or about 1 hour to about 80 hours at a temperature between about -10°C to about 90°C, preferably from about 20 °C to about 50 °C. The deprotecting in step a) may be conducted for a duration of between about 1 hour to about 120 hours, about 1 hour to about 100 hours, or about 1 hour to about 80 hours at a temperature between about -10°C to about 90°C, preferably from about 20 °C to about 30 °C.

The reacting or deprotecting in step a) may involve stirring the mixture/resulting mixture by method known in the art. The reacting step a) may involve before deprotecting, the step a1) of dispersing the mixture in an organic solvent, washing the mixture with an aqueous solvent and drying the resulting mixture. The organic solvent used in step a1) may be selected from dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), tetrahydrofuran (THF), acetonitrile (CH_3CN), or combinations thereof. The aqueous solvent used in step a1) may be selected from ethylenediaminetetraacetic acid (EDTA), water, sodium chloride solution or

combinations thereof. The drying method may be a method known in the art, such as, but not limited to rotary evaporation.

The plurality of antimicrobial peptides monomers may be amino acid N-carboxyanhydrides (NCAs). The amino acid N-carboxyanhydrides (NCAs) may be derived from natural or synthetic amino acids. The plurality of antimicrobial peptides monomers may be selected from the group consisting of methionine N-carboxyanhydrides (NCAs), threonine lysine N-carboxyanhydrides (NCAs), tryptophan N-carboxyanhydrides (NCAs), histidine N-carboxyanhydrides (NCAs), isoleucine N-carboxyanhydrides (NCAs), leucine N-carboxyanhydrides (NCAs), valine N-carboxyanhydrides (NCAs), glycine N-carboxyanhydrides (NCAs), alanine N-carboxyanhydrides (NCAs), glutamate N-carboxyanhydrides (NCAs), proline N-carboxyanhydrides (NCAs), and N-alkylglycine N-carboxyanhydrides (NCAs), and a derivative thereof. The plurality of antimicrobial peptides monomers may comprise protecting groups (PG) such as but not limited to tert-butyloxycarbonyl (Boc) group, carbobenzyloxy (Cbz) group, 9-Fluorenylmethyloxycarbonyl (Fmoc) group, benzyl (Bn) group or allyloxycarbonyl (Alloc) group.

The buffer system in polymerizing step b) may comprise an organic solvent and a salt formed from an organic base and an acid. The salt may be selected from the group consisting of propylamine hydrochloride, m-phenylenediamine dihydrochloride, 1,4-phenylenediamine dihydrochloride, *N*-(1-naphthyl)ethylenediamine dihydrochloride, pyridine hydrochloride, pyridine hydrobromide, triethylamine hydrochloride, trimethylamine hydrochloride, ethylenediamine dihydrochloride, ethylenediamine dihydroiodide, tetrabutylammonium hydrogensulfate, methylenediamine dihydrochloride, hexamethylenediamine dihydrochloride, o-xylylenediamine dihydrochloride and a combination thereof.

The organic solvent may be selected from acetone, acetonitrile, chloroform, 1,2-dichloroethane, diethyl ether, diglyme (diethylene glycol dimethyl ether), 1,2-dimethoxy-ethane (glyme, DME), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), 1,4-dioxane, ethyl acetate, hexamethylphosphoramide (HMPA), hexamethylphosphorous triamide (HMPT), methyl *tert*-butyl ether (MTBE), methylene chloride, N-methyl-2-pyrrolidinone (NMP), nitromethane, tetrahydrofuran (THF) or combinations thereof.

The acid may be an organic acid or an inorganic acid.

Advantageously, the buffer system allows for the polymerization (such as ring opening polymerization) to be conducted in a controlled manner such that the polymerization would undergo the selective normal amine ring opening polymerization (selective NA-ROP) (Fig. 2b) (where polymerization may only propagate at sites where primary amines are generated through the process) instead of the undesired activated monomer ring opening polymerization (AM-ROP) (where chain growth may occur randomly at any sites as any monomer may initiate polymerization), and thereby allowing the plurality of antimicrobial peptides to be formed uniformly in the final topologically complex polymer.

The polymerizing step b) may be conducted for a duration of between about 1 hour to about 120 hours, about 1 hour to about 100 hours, or about 1 hour to about 80 hours at a temperature between about 0°C to about 50°C, preferably from about 20 °C to about 30 °C.

The method may further comprise after polymerising step b), a step c) of modifying the resulting topologically complex polymer by mixing the resulting topologically complex polymer, an activated acid, a solvent and a base under reaction conditions to form a modified topologically complex polymer.

The activated acid in modifying step c) may be an acid bearing corresponding hydrophilic, hydrophobic and/or amphiphilic component(s) to be added and may be selected from the group consisting of acid anhydride, acid chloride, NHS ester, PFP ester, and a combination thereof. The corresponding hydrophilic, hydrophobic and/or amphiphilic component(s) may be as defined above.

The base in modifying step c) may be selected from triethylamine, diisopropylethylamine, pyridine, or combinations thereof. The solvent in modifying step c) may be mid-polar or polar aprotic organic solvents selected from acetone, acetonitrile, chloroform, 1,2-dichloroethane, diethyl ether, diglyme (diethylene glycol dimethyl ether), 1,2-dimethoxy-ethane (glyme, DME), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), 1,4-dioxane, ethyl acetate,

hexamethylphosphoramide (HMPA), hexamethylphosphorous triamide (HMPT), methyl *tert*-butyl ether (MTBE), methylene chloride, N-methyl-2-pyrrolidinone (NMP), nitromethane, tetrahydrofuran (THF) or combinations thereof.

The modifying step c) may be conducted for a duration of between about 1 hour to about 120 hours, about 1 hour to about 100 hours, or about 1 hour to about 80 hours at a temperature between about 0°C to about 90°C, or from about 20 °C to about 30 °C.

When the plurality of antimicrobial peptides monomers comprises protecting groups, the polymerizing step b) or the modifying step c) may further comprise the step of deprotecting the resulting topologically complex polymer or the modified topologically complex polymer. The deprotecting step may be as described above.

Exemplary, non-limiting embodiments of a method of preparing a packaging material comprising the topologically complex polymer will now be disclosed. The method may comprise integrating the topologically complex polymer into a material to form the packaging material. The topologically complex polymer may be as defined herein or produced according to the method defined herein.

Exemplary, non-limiting embodiments of a packaging material comprising the topologically complex polymer will now be disclosed. The topologically complex polymer may be as defined herein or produced according to the method defined herein. The packaging material may be useful for packaging objects (such as food, health care products, therapeutics, veterinary medicine and feed complement) that is susceptible to spoilage due to pathogens (such as food-borne pathogens). Advantageously, the topologically complex polymer may provide antimicrobial peptides with high antimicrobial activities to combat the pathogens.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

Fig. 1

Fig. 1 shows a schematic diagram of the synthetic routes to form the topologically complex peptides.

Fig. 2a

Fig. 2a shows a schematic diagram of the reaction pathways that usually results from the ring opening polymerization which undergoes two different routes; Normal Amine Ring Opening Polymerization (NA-ROP) (top reaction pathway) and Activated Monomer Ring Opening Polymerization (AM-ROP) (bottom reaction pathway).

Fig. 2b

Fig. 2b shows a schematic diagram of the reaction pathway taken by the ring opening polymerization of the present invention which undergoes selective Normal Amine Ring Opening Polymerization to produce a uniform polymer product.

DETAILED DESCRIPTION OF THE DRAWINGS

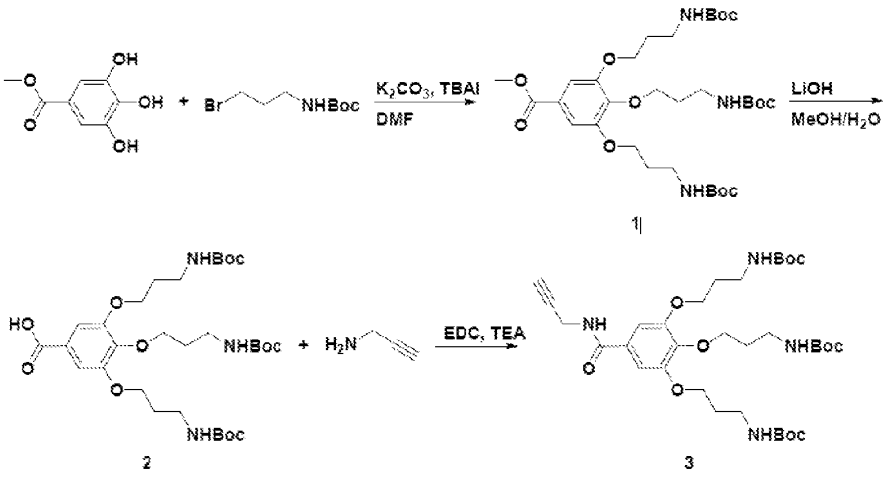
Fig. 1 shows a schematic diagram of the synthetic routes [steps (1) to (3)] to form the topologically complex peptides. Step (1) is the step of reacting a mixture of at least one functionalized macromolecule precursor, a plurality of furcated linkers precursor, a catalyst, a reducing agent in a solvent and subsequently deprotecting the resulting mixture to form an intermediate polymeric product; step (2) involves polymerizing a mixture of the intermediate polymeric product and a plurality of antimicrobial peptides monomers in a buffer system to form the topologically complex polymer; and step (3) involves modifying the resulting topologically complex polymer by mixing the resulting topologically complex polymer, an activated acid, a solvent and a base under reaction conditions to form a modified topologically complex polymer.

EXAMPLES

Non-limiting examples of the invention will be further described in greater detail by reference to specific examples, which should not be construed as in any way limiting the scope of the invention.

Example 1: Preparation of pillar[5]arene-30AMPs (topologically complex polymer)

Preparation of trifurcate dendron 3 (furcated linker)



Methyl gallate (5.0 g, 27.2 mmol), 3-(Boc-amino)propyl bromide (19.8 g, 82 mmol), potassium carbonate (22.5 g, 163 mmol), and tetrabutylammonium iodide (TBAI, 1.0 g, 0.27 mmol) were added to round bottom flask containing anhydrous dimethylformamide (100 mL), and the reaction was heated at 60 °C for 20 hours, when the completion of the reaction was indicated by analytical thin layer chromatography (TLC, see below) showing the disappearance of starting materials and presence of a new spot. The reaction mixture was then cooled down to room temperature, and diluted by diethyl ether (200 mL), and the mixture was then washed with DI water (200 mL × 2) and saturated NaCl aqueous solution (150 mL), the organic layer was then subject to rotary evaporator to afford crude product which was then purified by silica flash chromatography to afford the target product.

Analytical TLC was performed with Merck pre-coated TLC plates, silica gel 60 F-254, layer thickness 0.25 mm. Flash chromatography separations were performed on Merck 60 (0.040-0.0063 mm) mesh silica gel. Chemical yield refers to pure isolated substances. 1H NMR spectra were recorded on Bruker AscendTM 400 spectrometer. The solvent signal of $CDCl_3$ was referenced at δ=7.26 ppm. The solvent signal of DMSO-*d6* was referenced at δ= 2.50 ppm. The solvent signal of D_2O was referenced at δ= 4.79 ppm. Coupling constants (*J* values) are

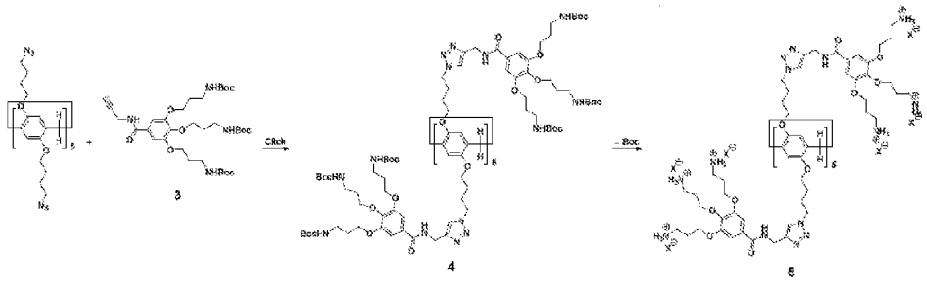
reported in Hertz (Hz). Mass spectra (ESI) were obtained on a Shimadzu LCMS-2020 spectrometer.

1 as white solid (14.2 g, yield, 80%). 1H NMR (400 MHz, DMSO-*d6*): δ 7.21 (s, 2H), 6.89 (t, *J* = 5.52, 2H), 6.74 (t, *J* = 5.44, 1H), 4.05 – 3.95 (m, 6H), 3.82 (s, 3H), 3.14 – 3.08 (m, 6H), 1.85 (qu, *J* = 6.44, 4H), 1.76 (qu, *J* = 6.69, 2H), 1.37 (brs, 27H). MS (ESI) calculated for C32H53N3NaO11 [M+Na]+: 678.36, found: 678.25. **1** (10.8 g, 16.5 mmol) and $LiOH·H_2O$ (3.5 g, 83 mmol) in DI water (40 mL) were added to round-bottom flask containing methanol (85 mL), the resulting reaction was stirred at room temperature for 10 hours, when the completion of the reaction was indicated by TLC showing the disappearance of starting materials and presence of a new spot. MeOH was removed by rotary evaporation, then DI water (150 mL) was added, and pH was adjusted to around 3 with 10% citric acid at 0 °C, white precipitate was collected by filtration to afford **2** (10.06 g, yield, 95%). 1H NMR (400 MHz, DMSO-*d6*): δ 7.19 (s, 2H), 6.89 (t, *J* = 3.88, 2H), 6.74 (t, *J* = 6.00, 1H), 4.01 – 3.95 (m, 6H), 3.13 – 3.08 (m, 6H), 1.85 (qu, *J* = 6.41, 4H), 1.75 (qu, *J* = 6.72, 2H), 1.37 (brs, 27H).

2 (10.06 g, 15.7 mmol), propargyl amine (1.2 g, 22 mmol), and 4-(dimethylamino)pyridine (2.9 g, 23.5 mmol) were added to round-bottom flask containing anhydrous dichloromethane (100 mL), the resulting reaction mixture was then cooled down to 0 °C, followed by the addition of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (4.5 g, 23.5 mmol) at 0 °C. The reaction was allowed to warm to room temperature slowly and stirred for 15 hours. Dichloromethane (100 mL) was added to the reaction mixture, and the resulting solution was washed with DI water (150 mL × 2) and saturated NaCl aqueous solution (100 mL), the organic layer was evaporated by rotary evaporator and the crude was then purified by silica flash chromatography to afford desired product.

3 (9.5 g, yield, 89%). 1H NMR (400 MHz, DMSO*d6*): δ 8.84 (t, *J* = 5.50, 1H), 7.17 (s, 2H), 6.87 (t, *J* = 5.44, 2H), 6.73 (t, *J* = 5.50, 1H), 4.05 – 3.99 (m, 6H), 3.93 (t, *J* = 6.20, 2H), 3.14 – 3.08 (m, 7H), 1.85 (qu, *J* = 6.47, 4H), 1.75 (qu, *J* = 6.63, 2H), 1.37 (brs, 27H). MS (ESI) calculated for $C_{34}H_{54}N_4NaO_{10}$ [M+Na]+: 701.37, found: 701.35.

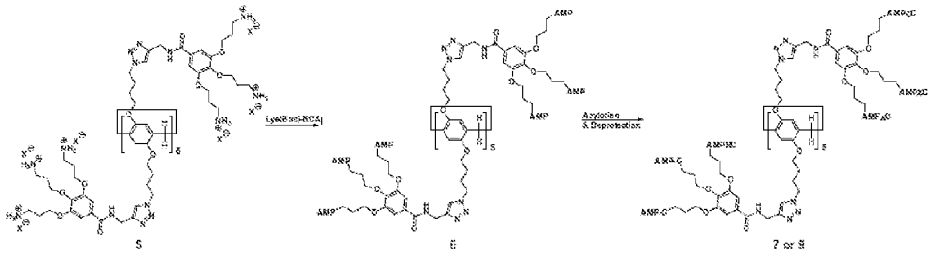
Preparation of amine decorated pillar[5]arene – Pillar[5]arene-30NH_2 (intermediate polymeric product)



Pillar[5]arene-10N_3 (50 mg, 0.03 mmol), **3** (206 mg, 0.3 mmol), CuSO4 (20 mg, 0.13 mmol), and sodium ascorbate (NaAsc, 125 mg, 0.63 mmol) were added to round-bottom flask containing anhydrous THF (4 mL) and water (0.25 mL), the resulting mixture was stirred at room temperature for 2 days. The reaction mixture was then dispersed in dichloromethane (20 mL), and washed with EDTA aqueous solution (2%, 20 mL × 2), and saturated NaCl aqueous solution (20 mL), the organic layer was then concentrated by rotary evaporation to afford the crude **4** for next step. 1H NMR (400 MHz, DMSO-*d6*): δ 8.95 (brs, 10H), 8.00 (s, 10H), 7.17 (s, 20H), 6.82 (t, *J* = 5.32, 20H), 6.73 (brs, 10H), 6.69 (t, *J* = 5.44, 10H), 4.47 – 4.41 (m, 40H), 3.95 – 3.88 (m, 70H), 3.73 – 3.55 (m, 0H), 3.13 – 3.06 (m, 60H), 2.10 – 2.01 (m, 20H), 1.86 – 1.70 (m, 80H), 1.35 (brs, 90H), 1.33 (brs, 180H).

4 (100 mg) was added to round-bottom flask containing anhydrous dichloromethane (3 mL), the reaction was then cooled to 0 °C, followed by the addition of trifluoroacetic acid (1 mL) and water (200 μL) at 0 °C, the reaction was allowed to warm to room temperature slowly and stirred for 5 hours. Then the reaction mixture was concentrated by rotary evaporator to afford the target compound **5** (98 mg). 1H NMR (400 MHz, D2O): δ 7.86 (s, 10H), 7.06 (s, 20H), 6.29 (s, 10H), 4.56 – 4.46 (m, 20H), 4.26 – 4.05 (m, 80H), 3.49 (brs, 20H), 3.26 – 3.17 (m, 70H), 2.21 – 2.06 (m, 60H), 1.73 – 1.61 (m, 20H), 1.38 (brs, 20H).

Preparation of pillar[5]arene-30AMPs (topologically complex polymer/ modified topologically complex polymer)



5 (7.0 mg, 1 μmol), Lys(Boc)-NCA (408 mg, 1.5 mmol) were added to round-bottom flask containing anhydrous DMF (6 mL), followed by the addition of DIEA (6.4 μL), the resulting reaction mixture was then allowed to react at room temperature for 3 days. After that, half of the reaction mixture was added to diethyl ether (30 mL), and white precipitate was formed and collected by filtration, and dried by vacuum to afford AMPs **6** (236 mg). To the other half reaction mixture was added DIEA (65 μL), DMAP (5 mg), and valeric anhydride (24 μL) or decanoyl chloride (25 μL) or NHS ester of 2,5,8,11-tetraoxatridecan-13-oic acid (60 μg) successively, and the resulting reaction mixture was allowed to further react for 15 hours. Then the reaction mixture was added to diethyl ether (30 mL), and white precipitate was formed and collected by filtration, and dried by vacuum to afford AMPs **6-C** (256 mg).

6 (80 mg) was added to round-bottom flask containing TFA (2 mL), and the reaction mixture was stirred at room temperature for 15 hours. Then the reaction mixture was poured into diethyl ether (20 mL), white precipitate was formed and collected by filtration, which was further purified by dialysis, and the target AMPs **7** (49 mg) was obtained after freeze-drying. 1H NMR (400 MHz, D_2O) for P[5]-Lysine: δ 8.45 (brs, 40H, protons from the core), 4.35 – 4.31 (m, 1731H, protons from polylysine), 3.87 (t, J = 6.72, 60H, protons from the core), 3.05 – 3.02 (m, 3471H, protons from polylysine), 1.87 – 1.38 (m, 10577H, protons from polylysine). 1H NMR (400 MHz, D2O) for P[5]-Lysine-C5: δ 8.37 (brs, 40H, protons from the core), 4.26 – 4.22 (m, 1441H, protons from polylysine), 2.97 – 2.93 (m, 2848H, protons from polylysine),

2.26 – 2.20 (m, 71H, protons from valeroyl group), 1.87 – 1.38 (m, 8561H, protons from polylysine), 0.84 – 0.80 (m, 106H, protons from valeroyl group).

Example 2: Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentration (MIC) of AMPs was tested by broth microdilution method. Briefly, 3 to 5 individual colonies of E. coli were inoculated into sterilised Nutrient medium and incubated at 37 °C for 15 hours to log growth phase, with the OD600 reaching ~0.6, corresponding to a concentration of ~5 × 108 CFU/mL). The bacteria solution was then centrifuged (5000 rpm for 5 minutes), harvested, washed and diluted in Nutrient medium to ~5 × 105 CFU/mL as the working suspension. The AMP PBS solution was diluted to the desired concentration in PBS by two-fold serial dilution and added into a 96-well plate. 100 µL bacterial suspension was added into each well, which was then incubated at 37 °C. The MIC was defined as the lowest concentration at which there was no microbial growth observed with naked eyes and the microplate reader. Nutrient media containing microbial cells alone was used as the negative control.

Table 1. Table showing the minimum inhibitory concentration (MIC) of AMPs

AMP	MIC (µg/mL)	AMP	MIC (µg/mL)
P[5]-Lys	7	P[5]-Arg	5
P[5]-Lys-C5	6	P[5]-Arg-C5	50
P[5]-Lys-C10	6	P[5]-Arg-C10	50
P[5]-Lys-PEG3	6	P[5]-Arg-PEG3	3

P[5]-Lys, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polylysine.

P[5]-Lys-C5, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polylysine with the terminal amine linked to valeroyl group.

P[5]-Lys-C10, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polylysine with the terminal amine linked to decanoyl group.

P[5]-Lys-PEG3, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polylysine with the terminal amine linked to 2,5,8,11-tetraoxatridecan-13-oyl group.

P[5]-Arg, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polyarginine.

P[5]-Arg-C5, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polyarginine with the terminal amine linked to valeroyl group.

P[5]-Arg-C10, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polyarginine with the terminal amine linked to decanoyl group.

P[5]-Arg-PEG3, refer to the AMP obtained from P[5]arene derived initiator and 30 arms of polyarginine with the terminal amine linked to 2,5,8,11-tetraoxatridecan-13-oyl group.

INDUSTRIAL APPLICABILITY

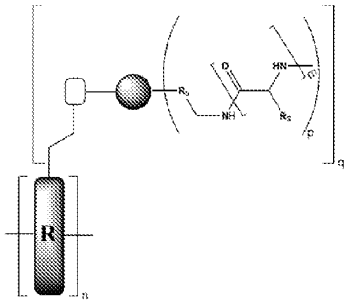
The disclosed topologically complex polymer may be used as an antimicrobial packaging material which is applicable to industries such as medical, pharmaceutical, food and veterinary.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A topologically complex polymer comprising at least one functionalized macromolecule coupled to a plurality of antimicrobial peptides via a plurality of furcated linkers, wherein each antimicrobial peptide is bonded to each furcated terminal end of the furcated linker.

2. The topologically complex polymer of claim 1, having the general formula (I):



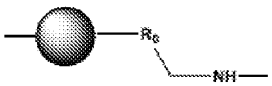
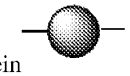
(I)

wherein,

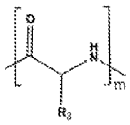
R independently represents the at least one functionalized macromolecule;

n represents an integer selected from ≥ 3;

 independently represents a chemical moiety formed between the at least one functionalised macromolecule and the plurality of furcated linkers;

 independently represents each of the plurality of furcated linkers wherein  independently represents a central chemical group of each furcated linker from which two or more chemical units branches out therefrom and R_0 independently represents an alkyl with or without heteroatoms;

p represents an integer selected from 1 to 10;



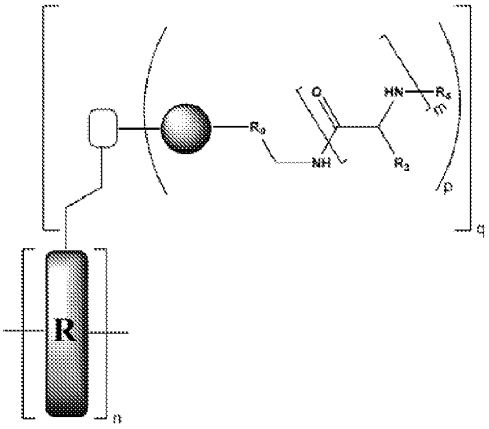
represent each of the plurality of antimicrobial peptides bonded to each furcated terminal end of the furcated linker;

R_3 independently represents each functional group of an amino acid;

m represents an integer between 2 to 1000; and

q represents an integer of more than 1.

3. The topologically complex polymer of claim 1, further comprising a plurality of hydrophilic, hydrophobic and/or amphiphilic components coupled to the terminal ends of the plurality of antimicrobial peptides to form a modified topologically complex polymer, the modified topologically complex polymer having the general formula (II):



(II)

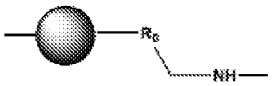
wherein,

R independently represents the at least one functionalized macromolecule;

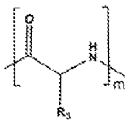
n represents an integer selected from ≥ 3;



independently represents a chemical moiety formed between the at least one functionalised macromolecule and the plurality of furcated linkers;



independently represents each of the plurality of furcated linkers where  independently represents a central chemical group of each furcated linker from which two or more chemical units branches out and R_0 independently represents an alkyl with or without heteroatoms;



represent each of the plurality of antimicrobial peptides bonded to each furcated terminal end of the furcated linker;

R_3 independently represents each functional group of an amino acid;

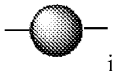
m represents an integer between 2 to 1000;

p represents an integer selected from 1 to 10;

q represents an integer of more than 1; and

R_4 independently represents each of the plurality of hydrophilic, hydrophobic and/or amphiphilic components bonded to each terminal end of the plurality of antimicrobial peptides.

4. The topologically complex polymer of any one of claims 1 to 3, wherein the at least one functionalized macromolecule R is independently selected from the group consisting of pillararenes, fullerenes, polyhedral oligomeric silsesquioxanes (POSS), cyclodextrins and cellulose nanocrystals.

5. The topologically complex polymer of any one of claims 1 to 4, wherein the central chemical group  is ring/cyclic structures and/or acyclic structures.

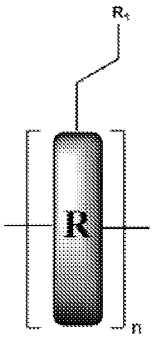
6. The topologically complex polymer of claim 3, wherein R_4 is independently selected from the group consisting of a linear or branched C_2 to C_{40} alkyl, a linear or branched

polyethylene glycol (PEG), polyethylene oxide (PEO) having repeating units of 2 to 40 and a combination thereof.

7. The topologically complex polymer of any one of claims 1 to 6, wherein the polydispersity index of the topologically complex polymer is about 1 to 2.

8. A method of preparing the topologically complex polymer of any one of claims 1 to 7, the method comprising the steps of: a) reacting a mixture of at least one functionalized macromolecule precursor and a plurality of furcated linkers precursor in a solvent and subsequently deprotecting the resulting mixture to form an intermediate polymeric product; b) polymerizing a mixture of the intermediate polymeric product and a plurality of antimicrobial peptides monomers in a buffer system to form the topologically complex polymer.

9. The method of claim 8, wherein the at least one functionalized macromolecule precursor in step a) is defined by the general formula (Ia):



(Ia)

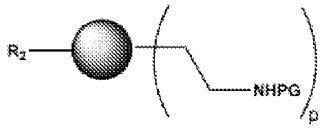
wherein,

R independently represents the at least one functionalized macromolecule;

n represents an integer selected from ≥3; and

R_1 is independently selected from an azide, amide, acid, acid halide, isocyanate, isothiocyanate, hydroxyl, amine, thiol, or alkyl halide.

10. The method of any one of claims 8 or 9, wherein the plurality of furcated linkers precursor in step a) is defined by the general formula (Ib):

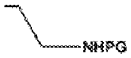


(Ib)

wherein,

R_2 is independently selected from a terminal alkyne, hydroxyl, amine, thiol, halide or a terminal alkene;

 independently represents the central chemical group from which two or more chemical units branches out;

 independently represents an alkyl amine capped with a protecting group (PG); and

p represents an integer selected from 1 to 10.

11. The method of claim 10, wherein the protecting group (PG) is selected from the group consisting of tert-butyloxycarbonyl (Boc) group, carbobenzyloxy (Cbz) group, 9-Fluorenylmethyloxycarbonyl (Fmoc) group, benzyl (Bn) group and allyloxycarbonyl (Alloc) group.

12. The method of any one of claims 8 to 11, further comprising a catalyst, a reducing agent or both the catalyst and the reducing agent.

13. The method of any one of claims 8 to 12, wherein the solvent used in step a) is selected from tetrahydrofuran (THF), acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), toluene or a combination thereof.

14. The method of any one of claims 8 to 13, wherein the deprotecting in step a) comprises adding an acid in a solvent to form the intermediate polymeric product.

15. The method of any one of claims 8 to 14, wherein the plurality of antimicrobial peptides monomers is an amino acid N-carboxyanhydrides (NCAs).

16. The method of any one of claims 8 to 15, wherein the buffer system in polymerizing step b) comprises an organic solvent and a salt formed from an organic base and an acid.

17. The method of any one of claims 8 to 16, further comprising after step b), a step c) of modifying the resulting topologically complex polymer by mixing the resulting topologically complex polymer, an activated acid, a solvent and a base under reaction conditions to form a modified topologically complex polymer.

18. The method of claim 17, wherein the activated acid is selected from the group consisting of acid anhydride, acid chloride, NHS ester, PFP ester, and a combination thereof.

19. A method of preparing a packaging material, the method comprising integrating the topologically complex polymer of any one of claims 1 to 7 or the topologically complex polymer prepared according to any one of claims 8 to 18 into a material to form the packaging material.

20. A packaging material comprising the topologically complex polymer of any one of claims 1 to 7 or the topologically complex polymer prepared according to any one of claims 8 to 18.