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(19) **United States**(12) **Patent Application Publication****Jiang et al.**(10) **Pub. No.: US 2010/0247614 A1**(43) **Pub. Date: Sep. 30, 2010**(54) **HEMOSTATIC WOUND DRESSING****Related U.S. Application Data**(75) Inventors: **Shaoyi Jiang**, Redmond, WA (US);
Hong Xue, Seattle, WA (US); **Gang Cheng**, Akron, OH (US)

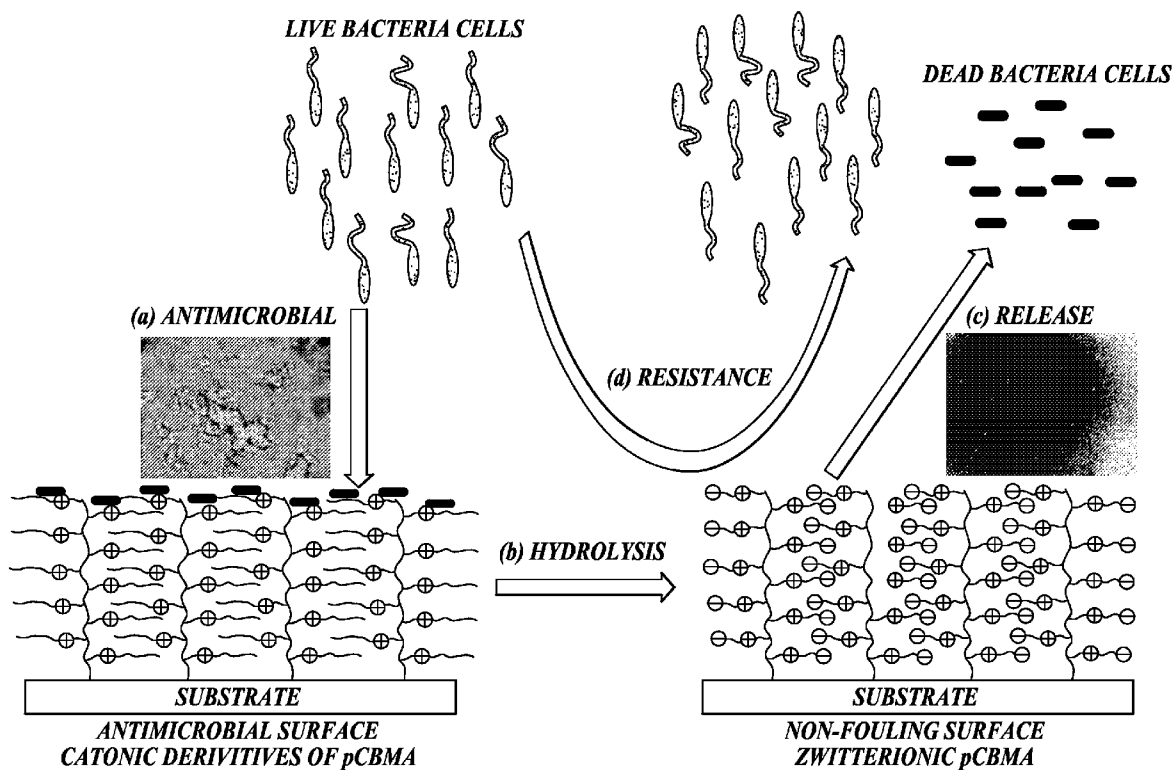
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(60) Provisional application No. 60/989,073, filed on Nov. 19, 2007.

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Seattle, WA 98101-2347 (US)**Publication Classification**(51) **Int. Cl.****A61K 31/785** (2006.01)**A61P 17/02** (2006.01)**A61K 47/48** (2006.01)(52) **U.S. Cl. 424/445; 424/78.06; 525/54.1**(57) **ABSTRACT**

Hemostatic wound dressings that include cationic polymers and related hydrogels, methods for making and using the wound dressings.

(73) Assignee: **WASHINGTON, UNIVERSITY OF**, Seattle, WA (US)(21) Appl. No.: **12/780,194**(22) Filed: **May 14, 2010**

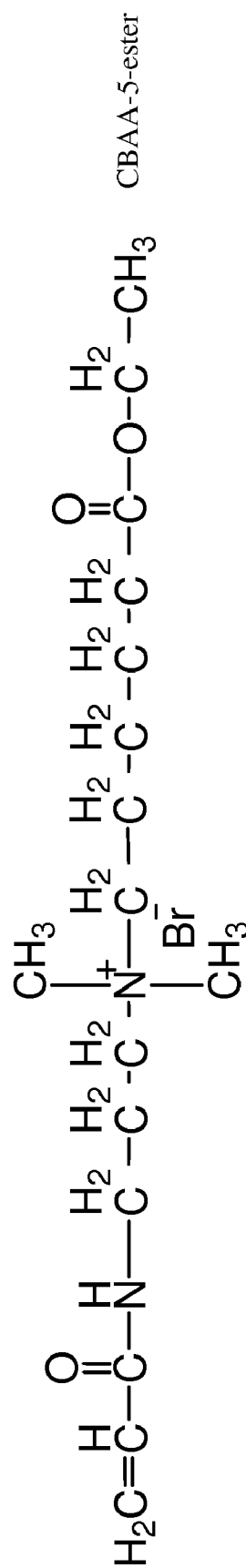
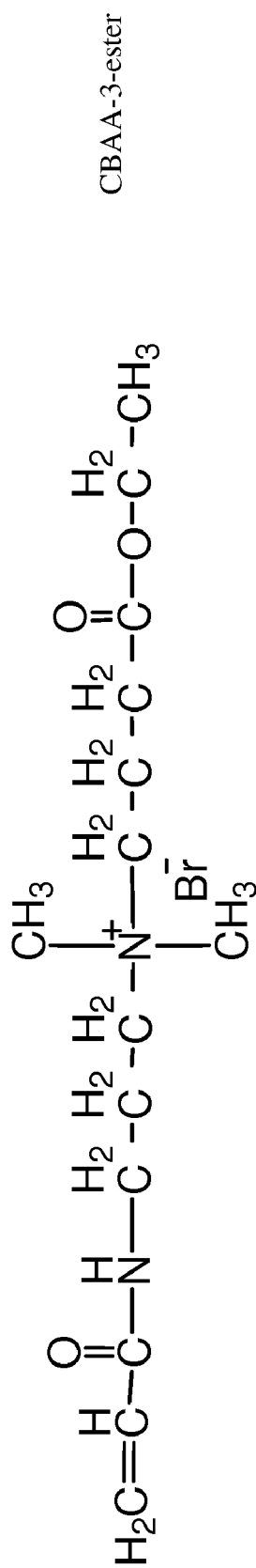
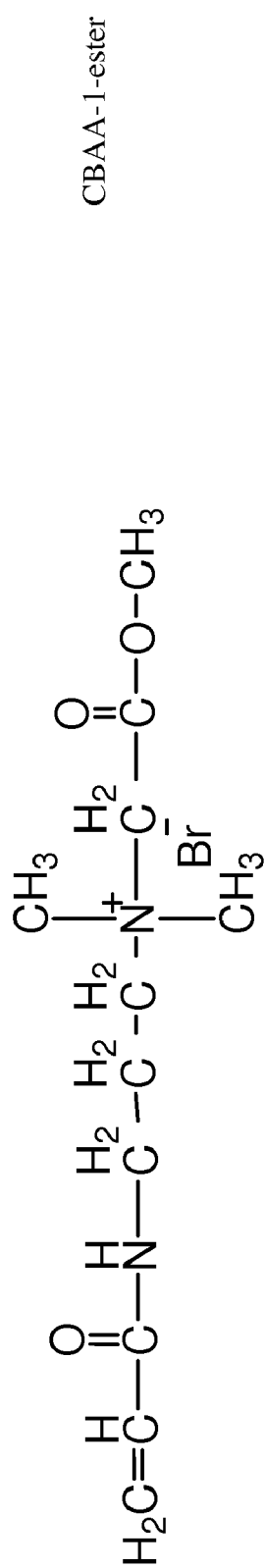


FIG. 1.

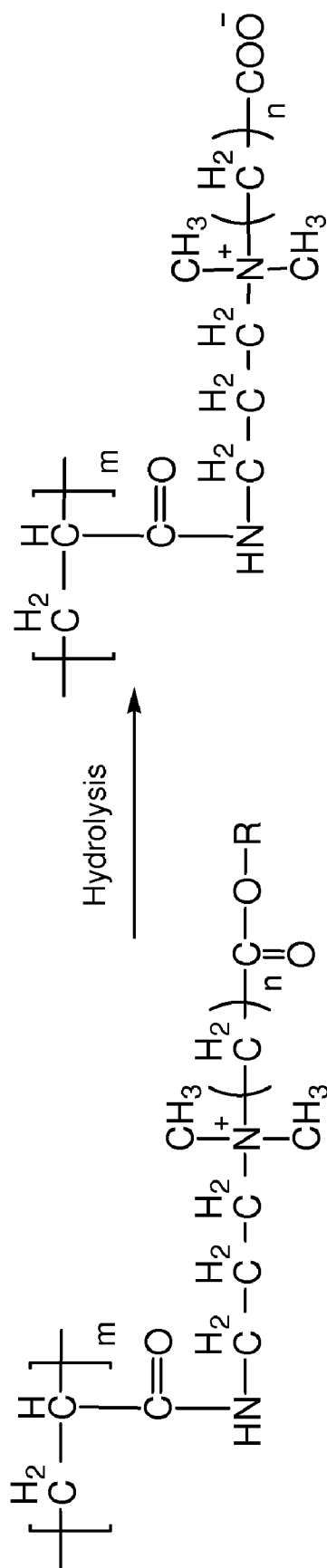
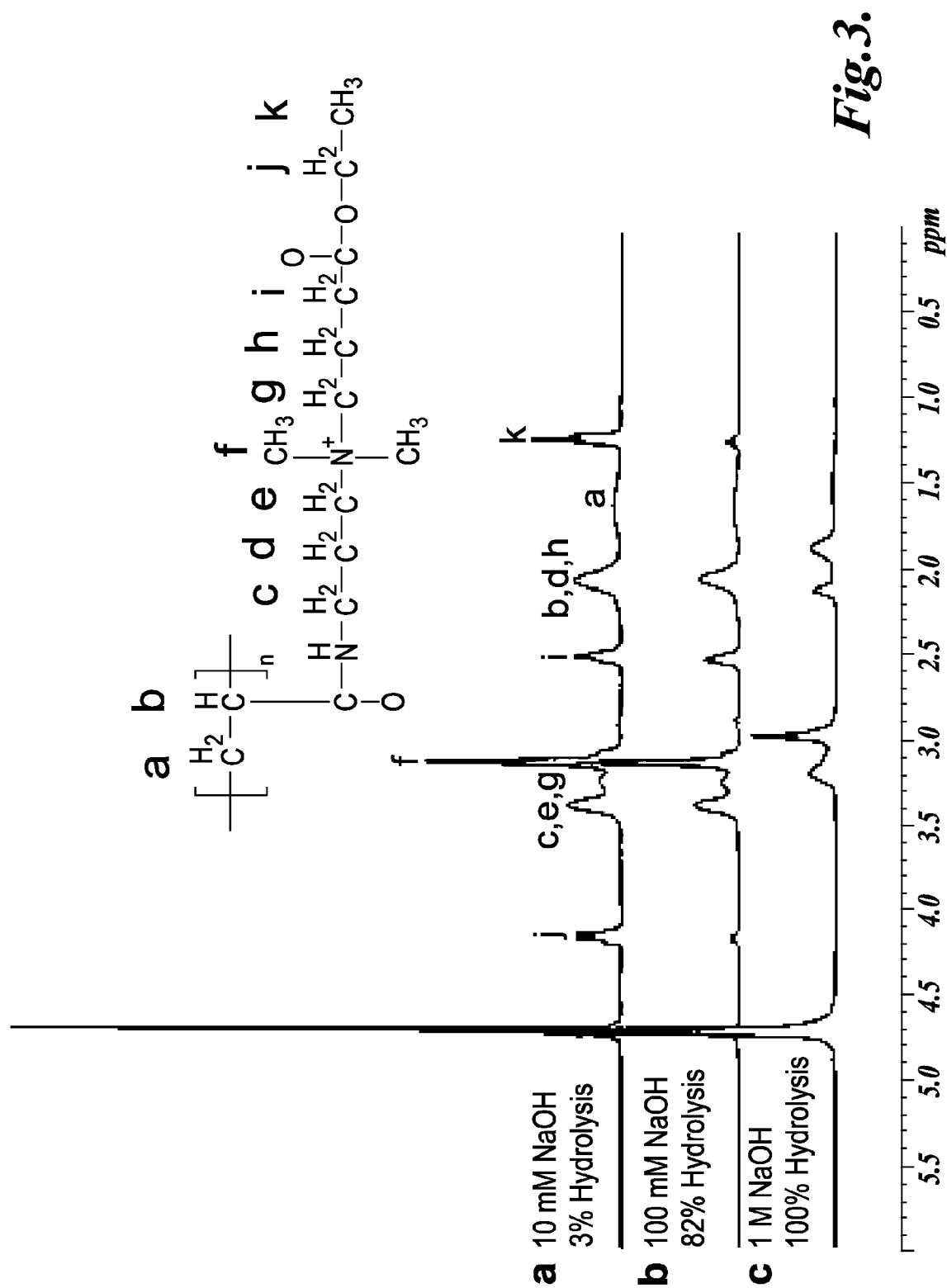
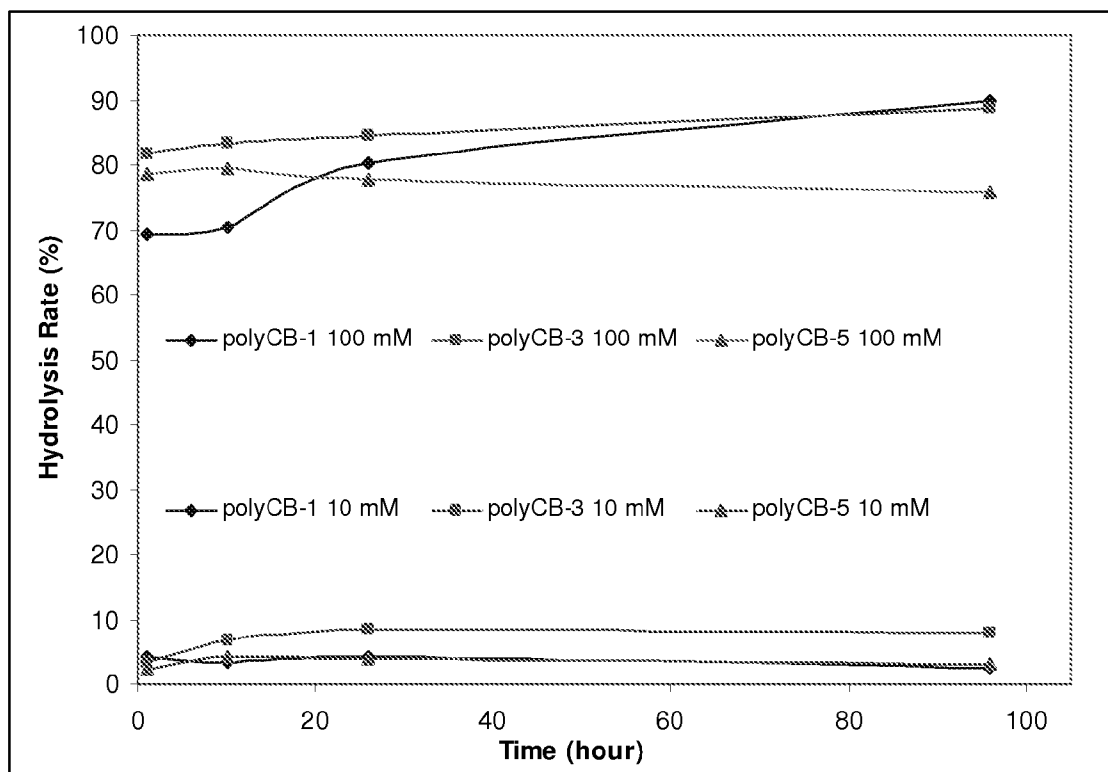
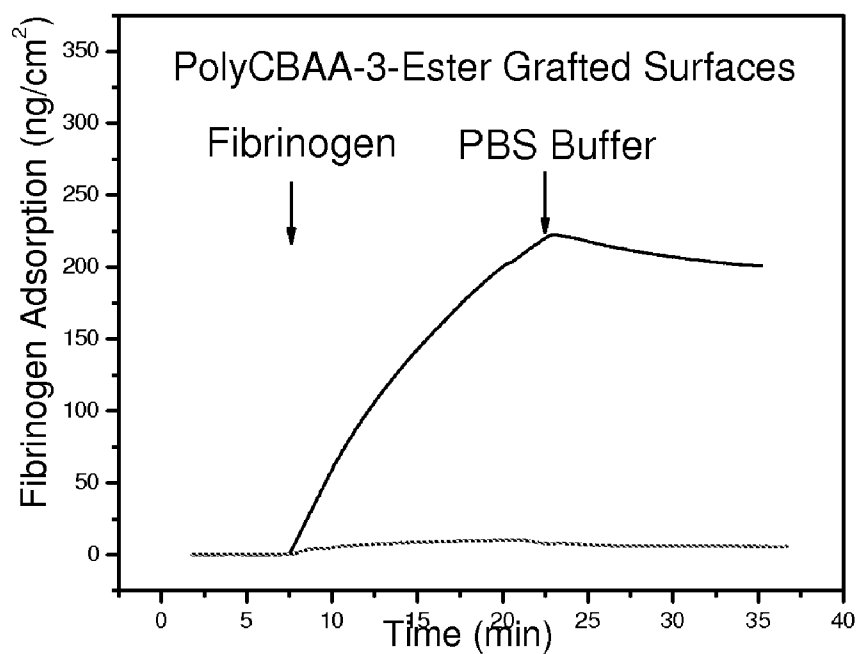
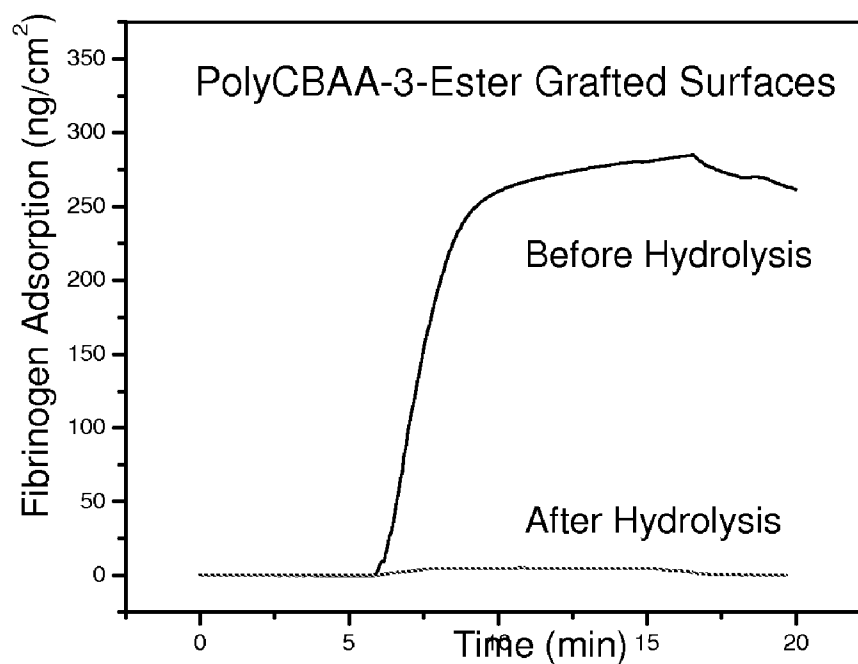
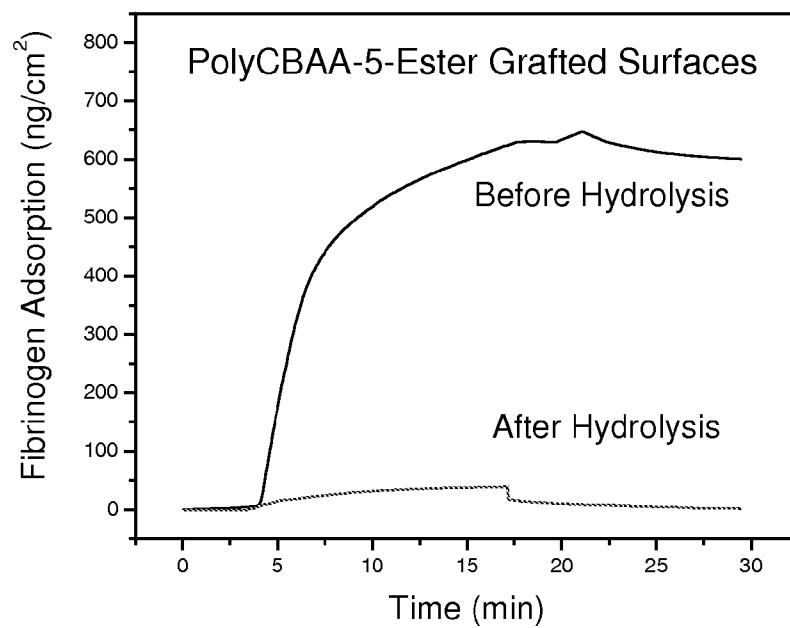
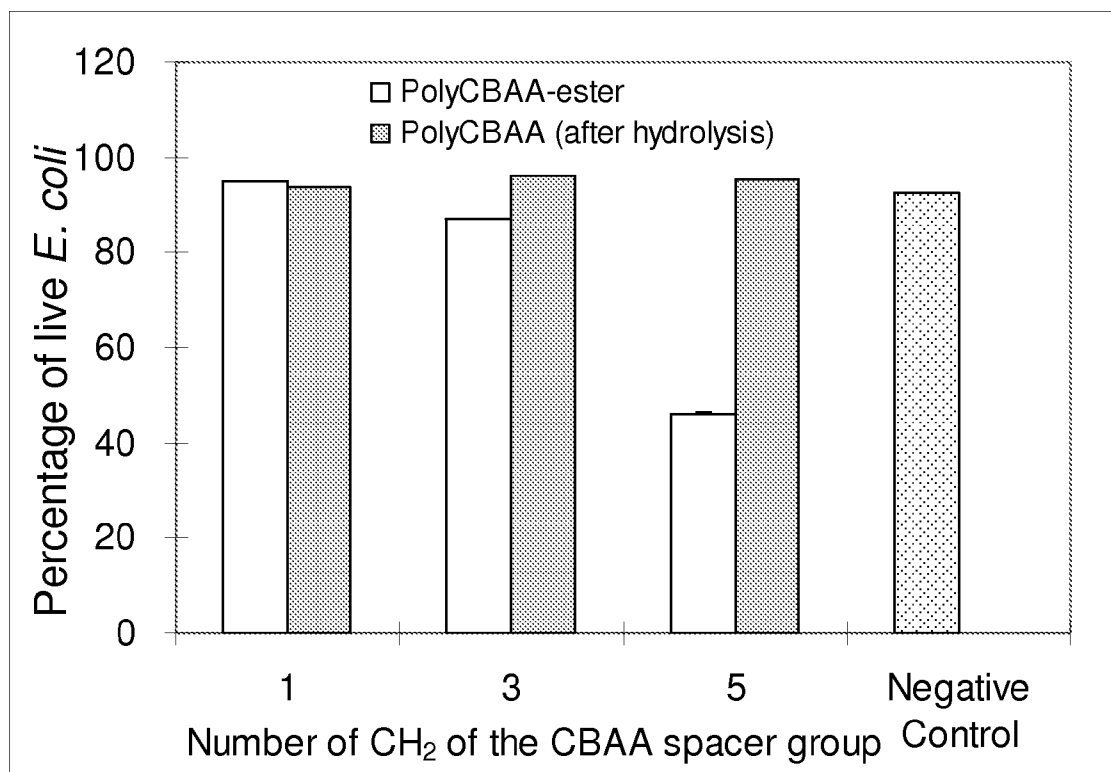


FIG. 2.



**FIG. 4.**

**FIG. 5A.****FIG. 5B.**

**FIG. 5C.****FIG. 6.**

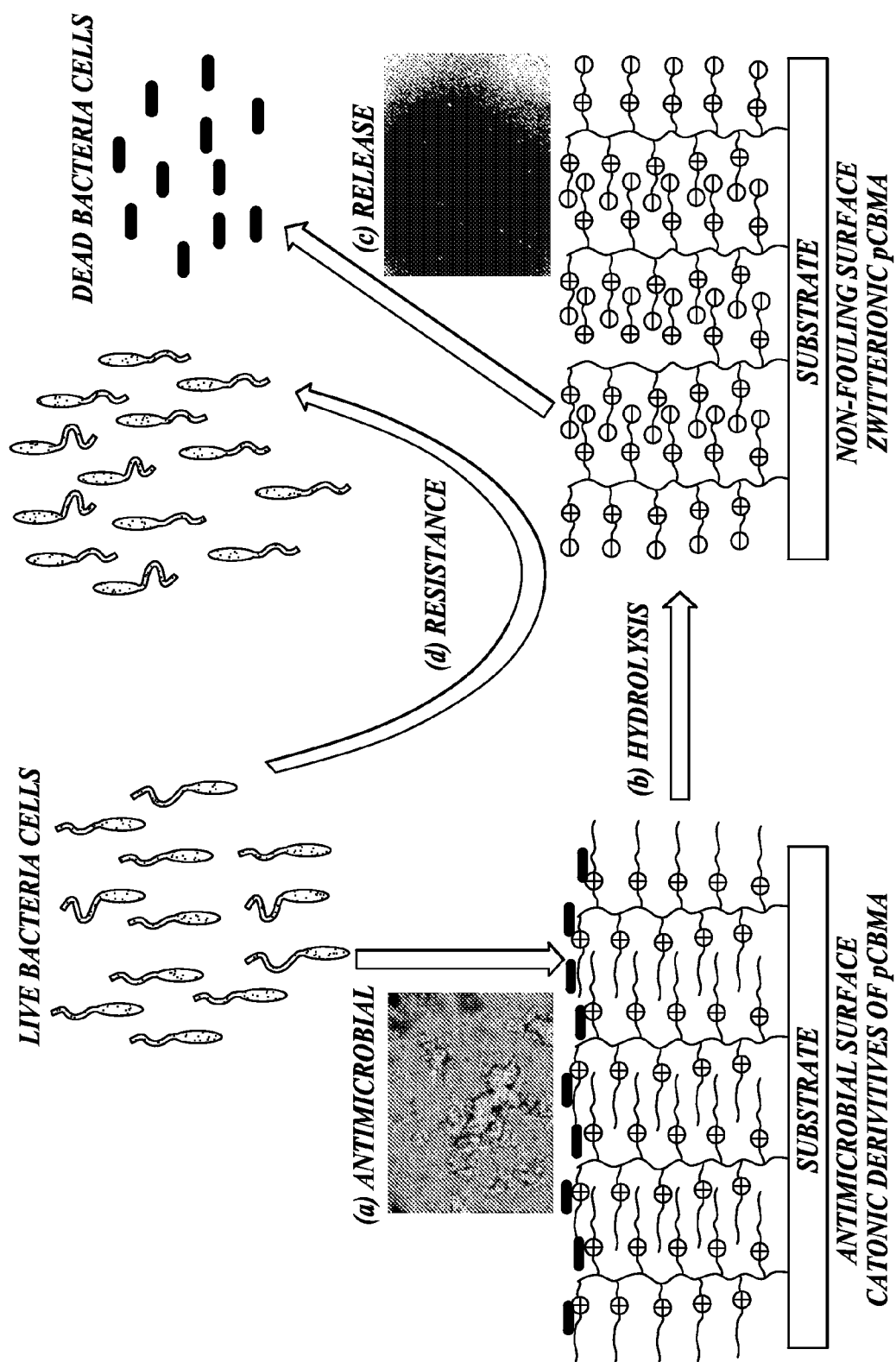


Fig. 7.

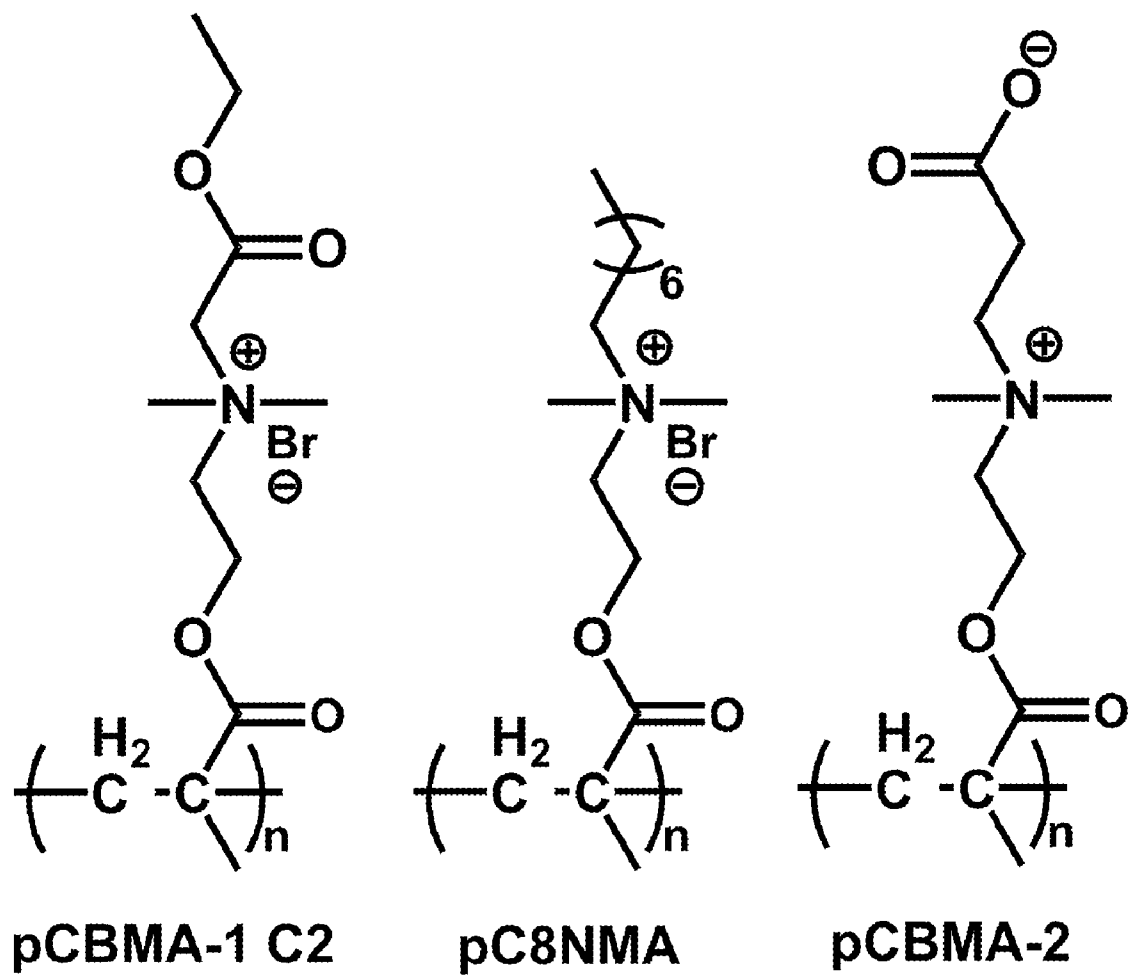


FIG. 8.

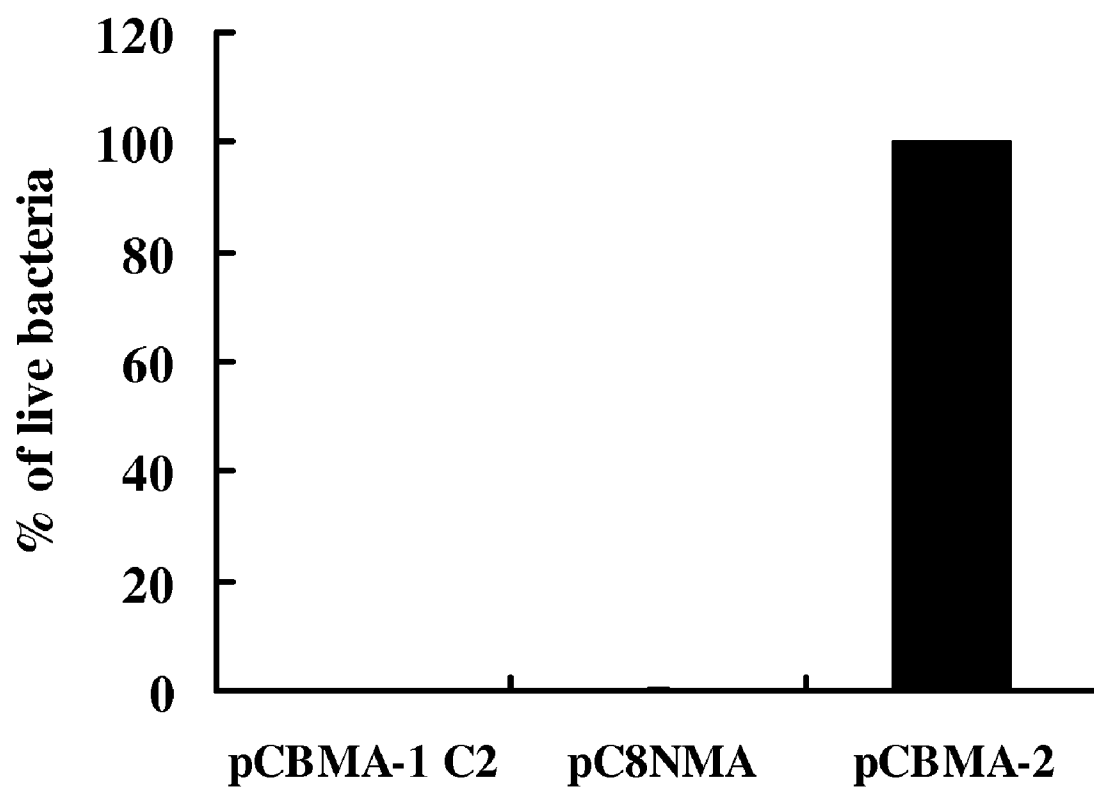
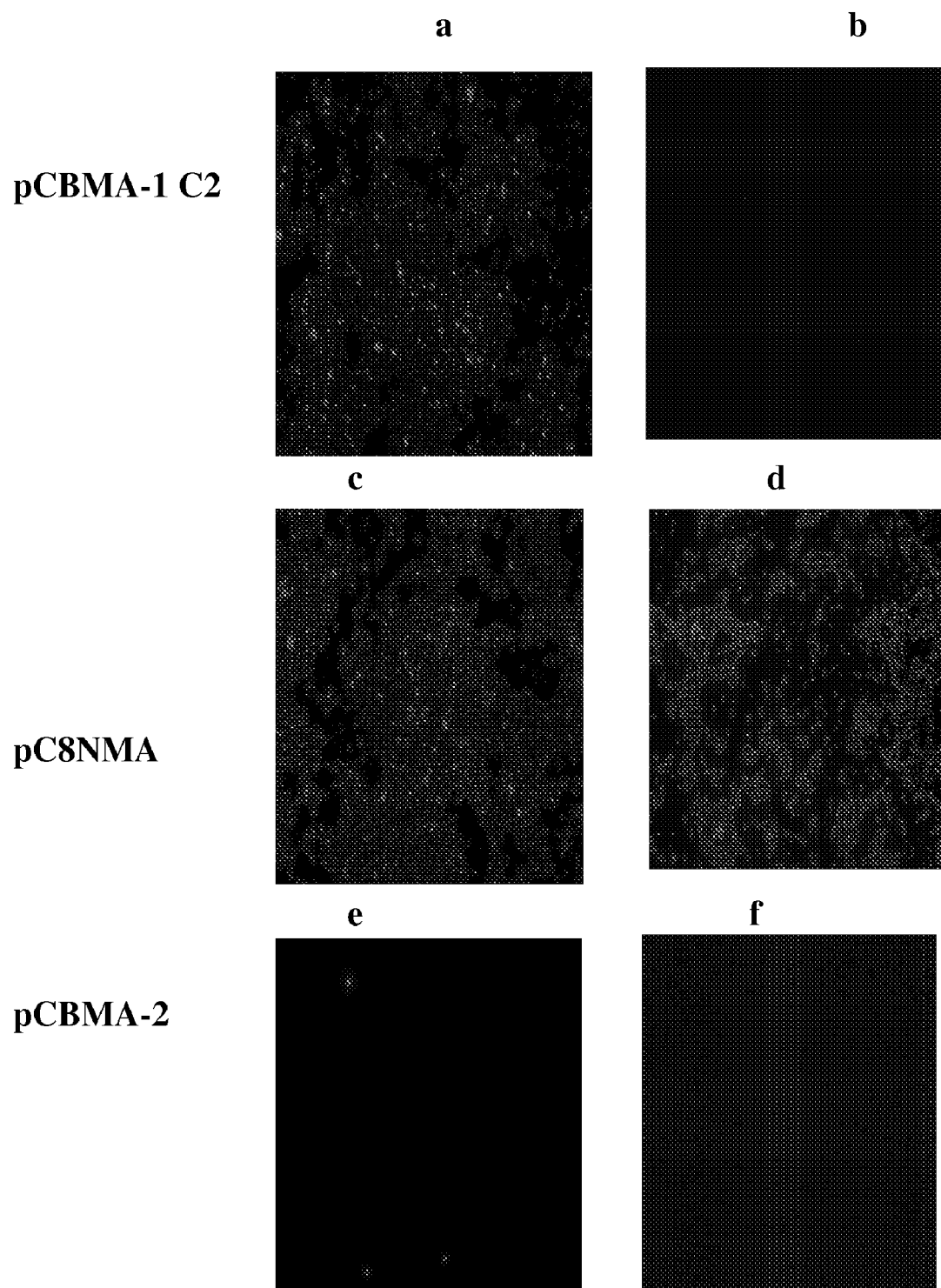


FIG. 9.



FIGS. 10A-10F.

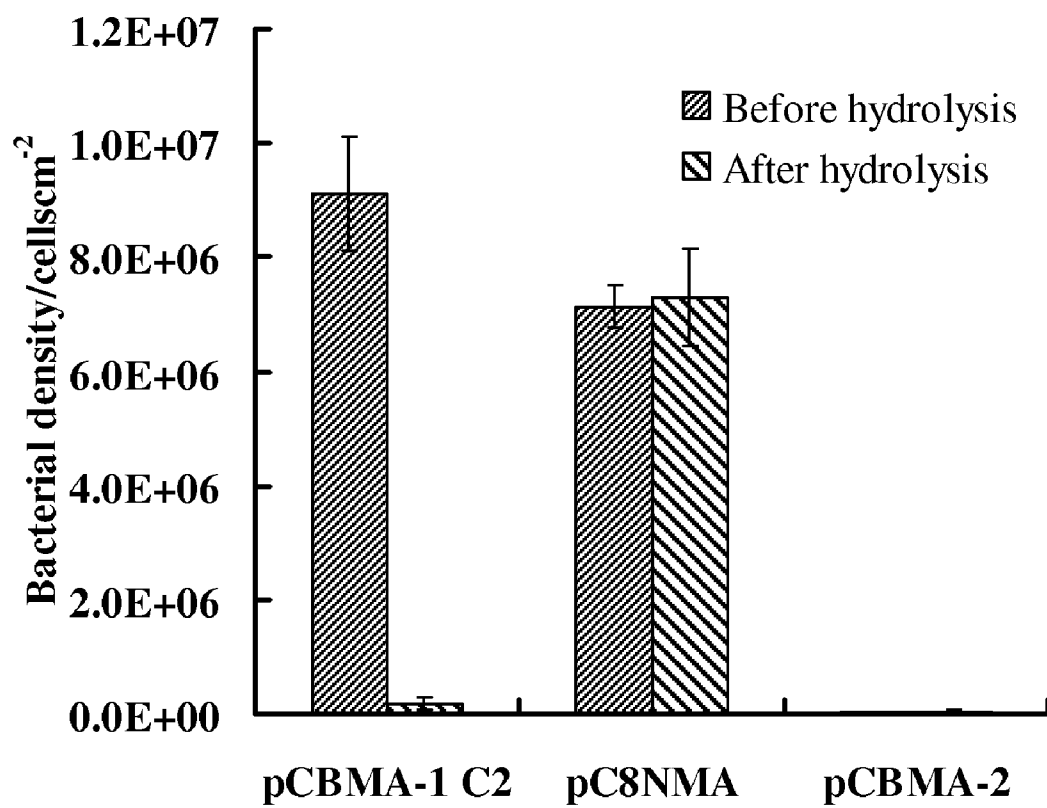
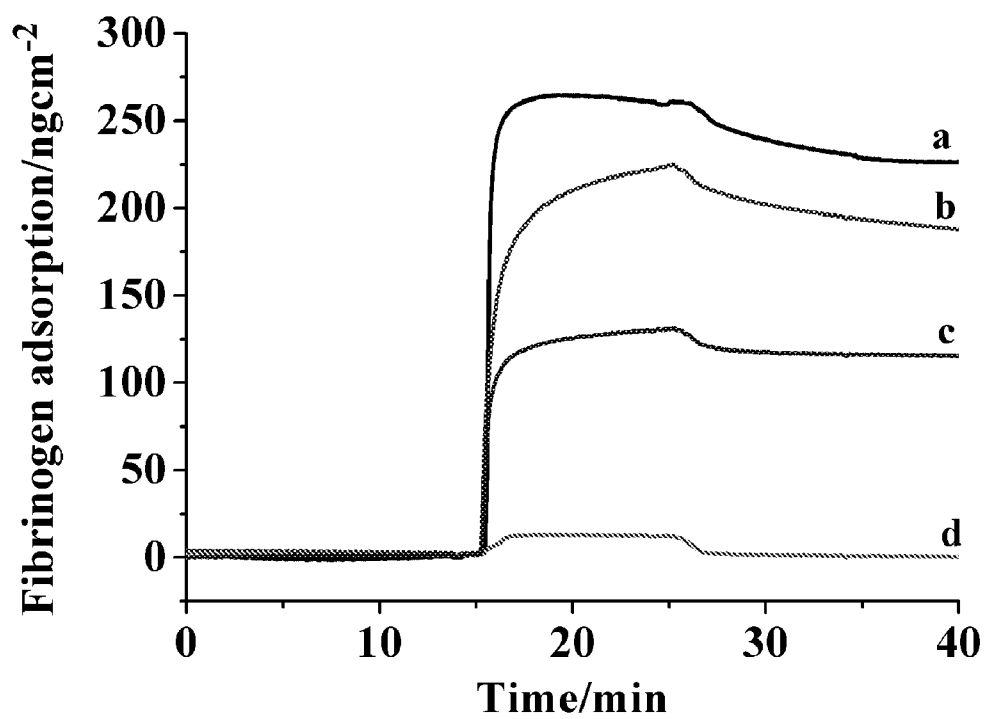
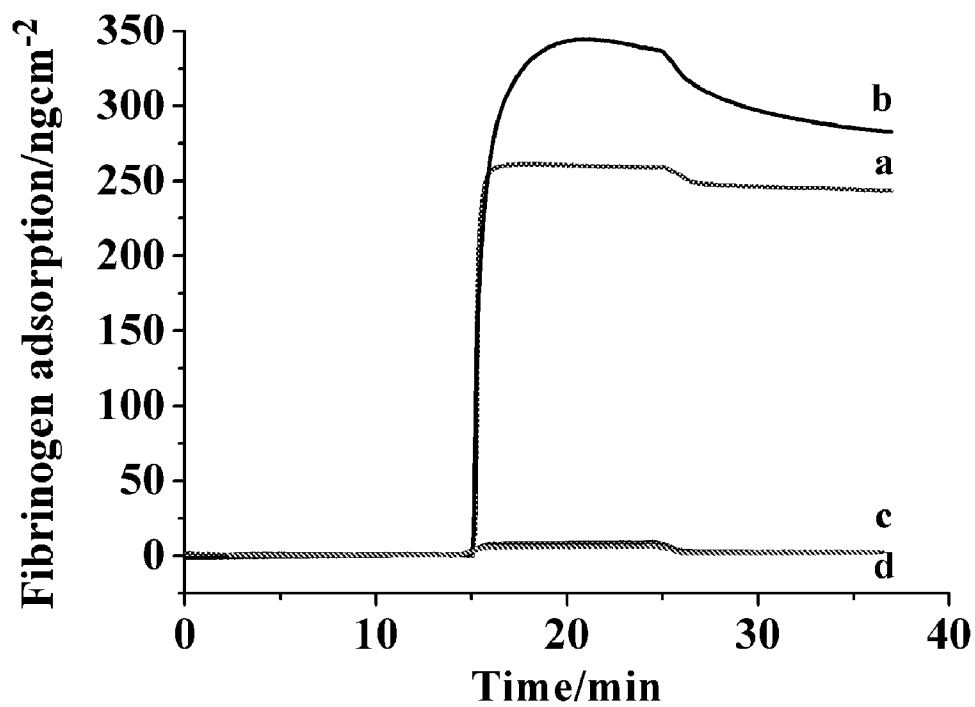
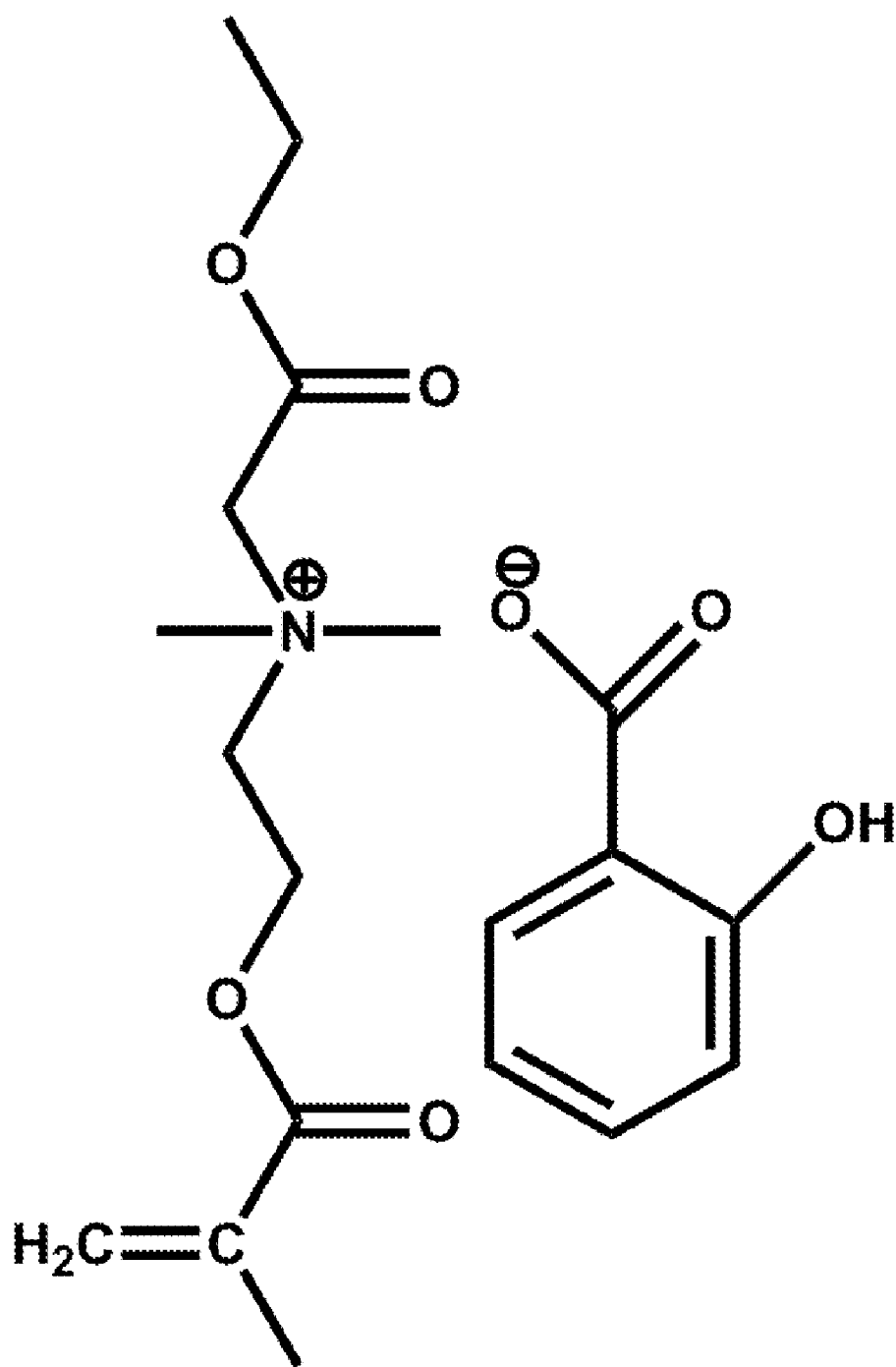


FIG. 11.

*FIG. 12A.**FIG. 12B.*



CBMA-1 C2 SA

FIG. 13.

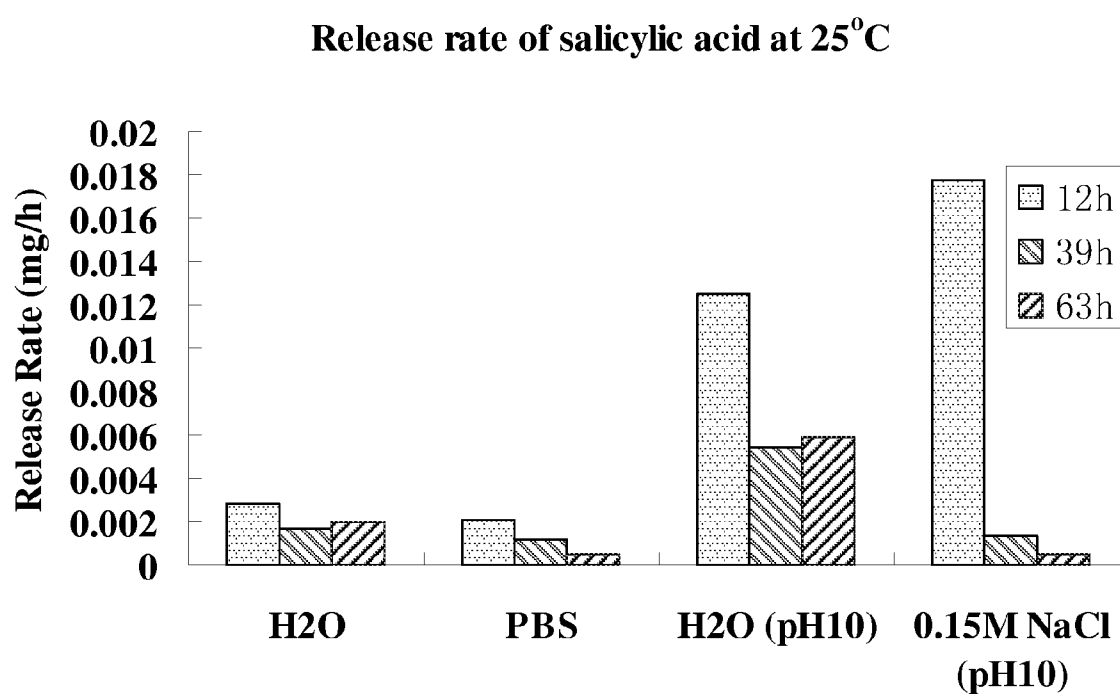


FIG. 14.

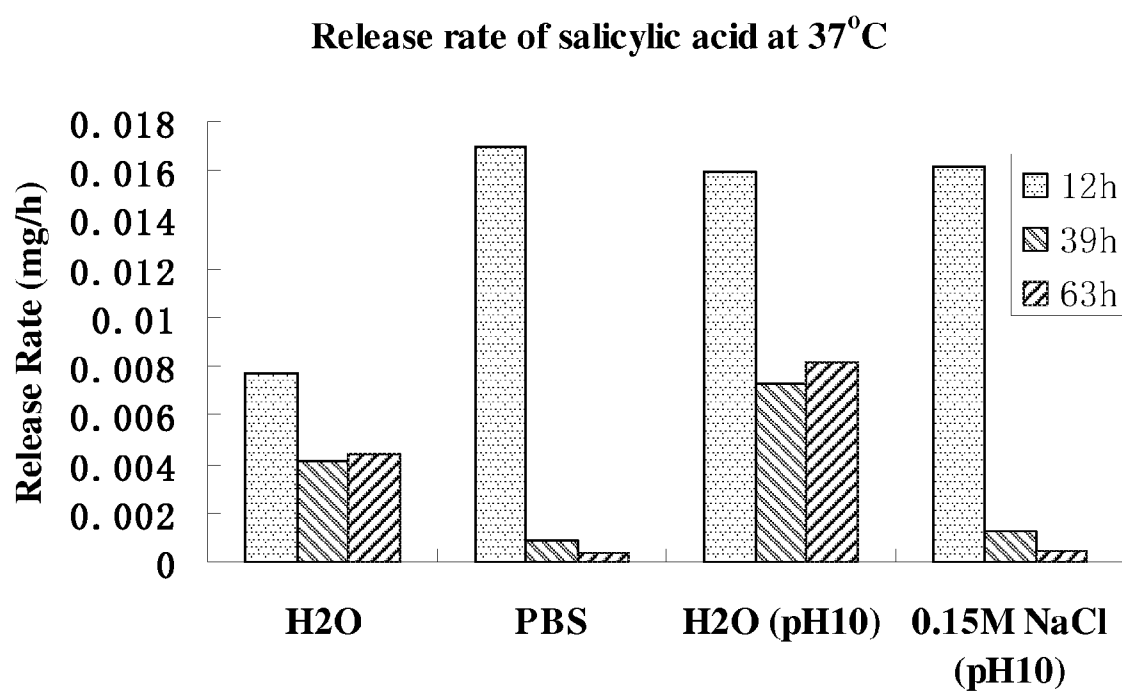


FIG. 15.

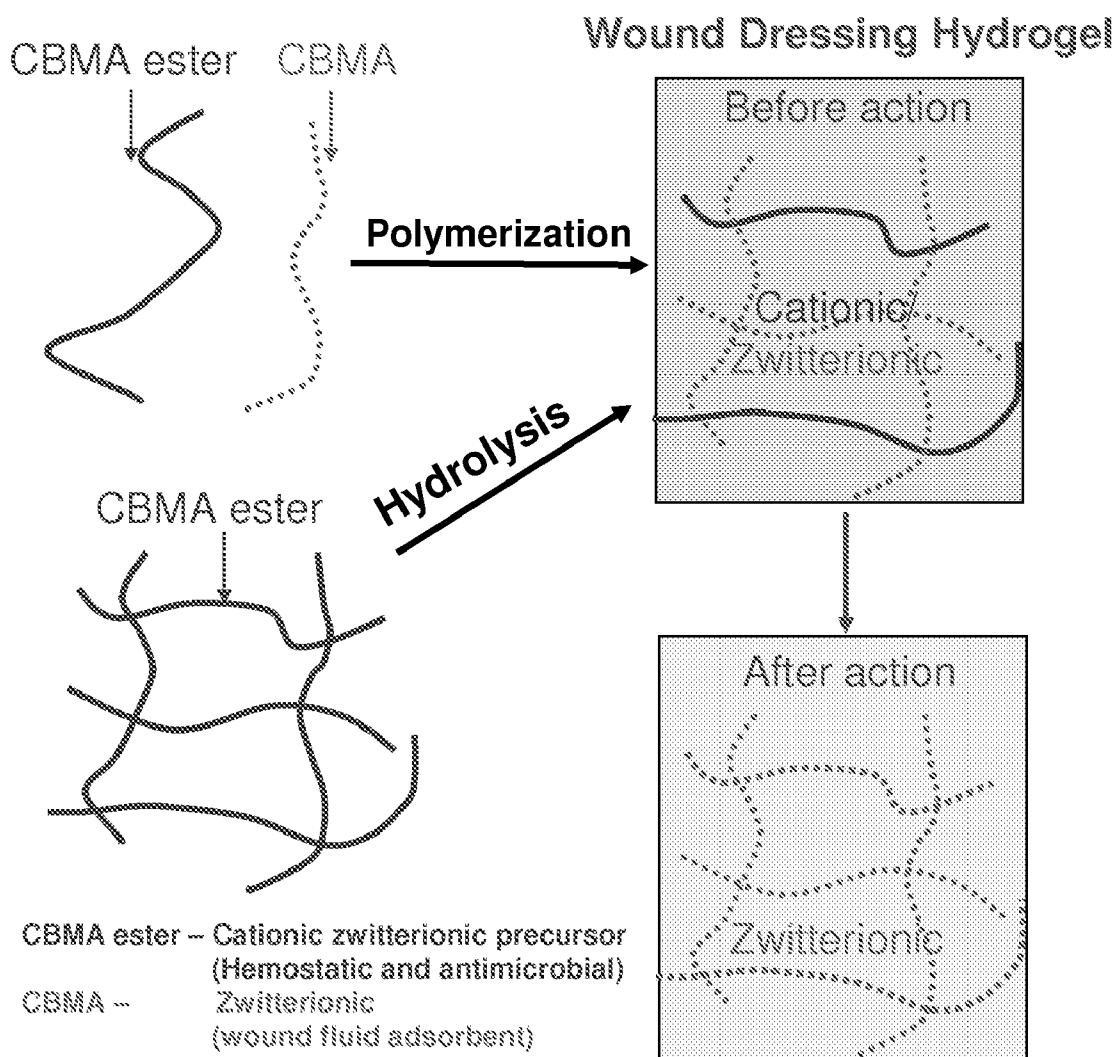


FIG. 16.

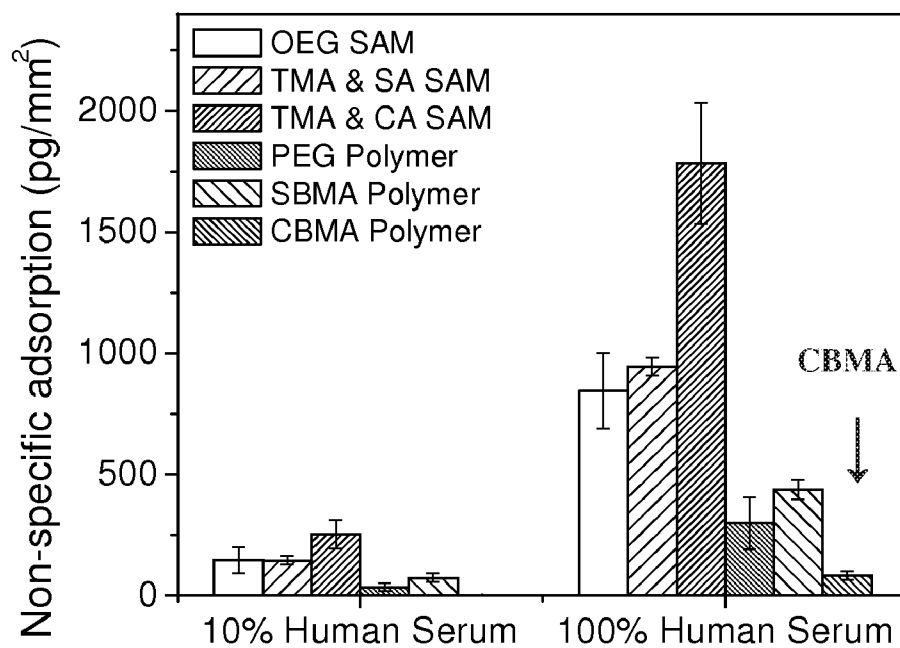


FIG. 17A.

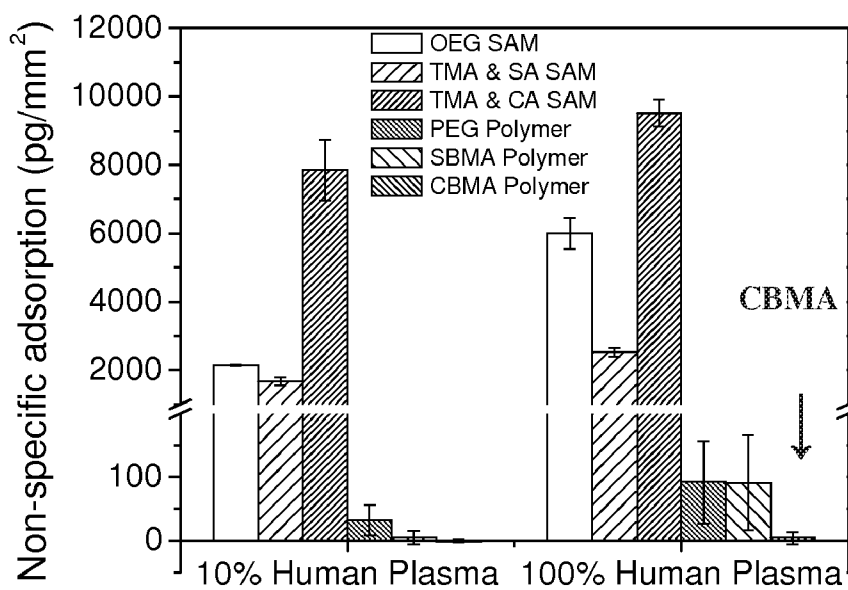
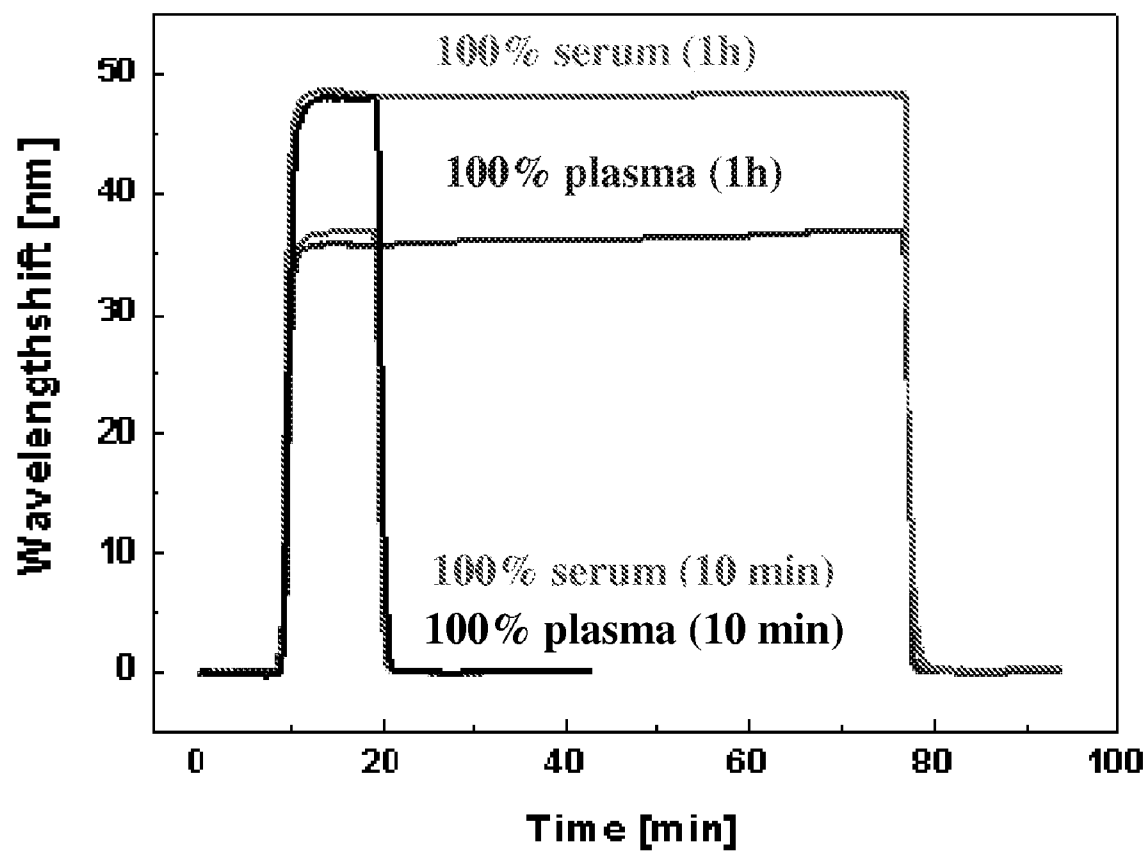


FIG. 17B.

**FIG. 18.**

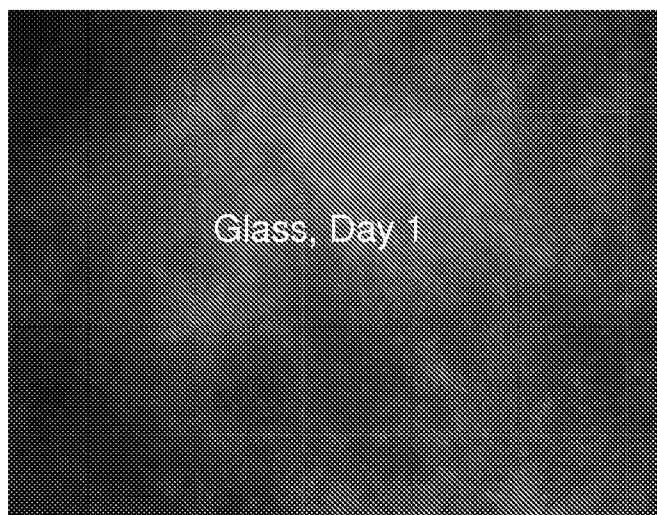


FIG. 19A.

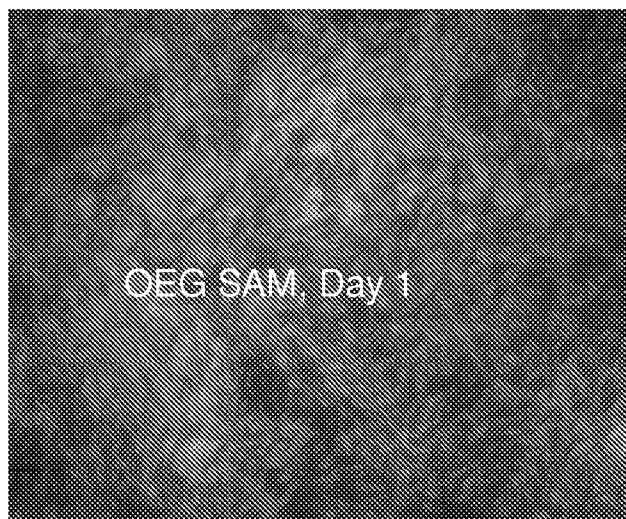


FIG 19B.

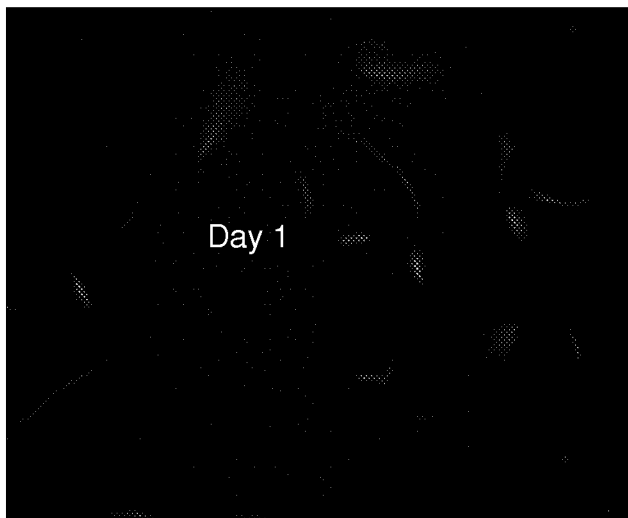


FIG. 19 C.

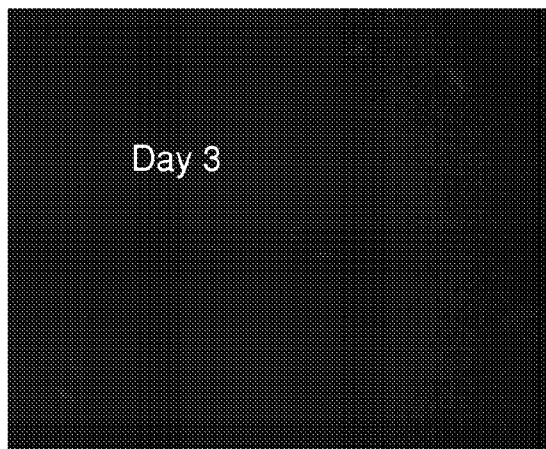


FIG. 19D.

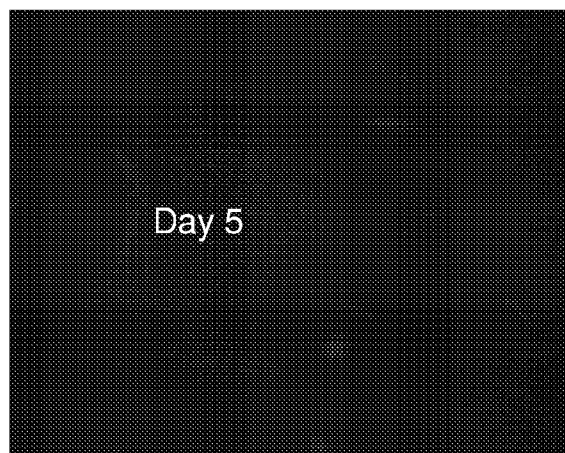


FIG. 19E.

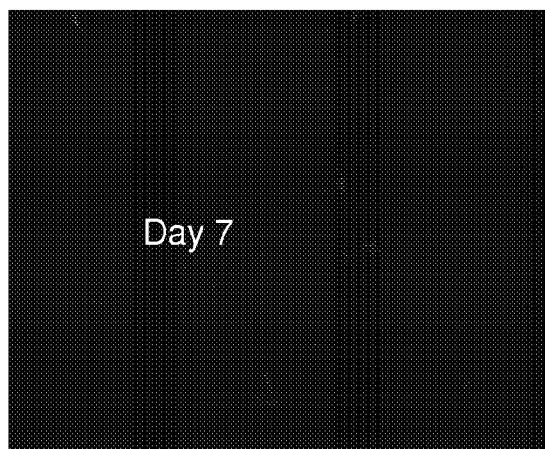


FIG. 19F.

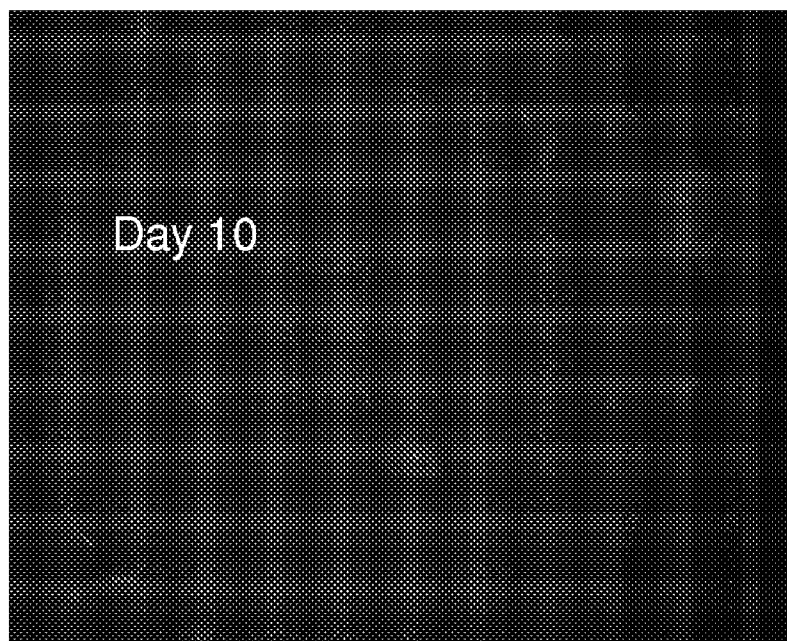


FIG. 19G.

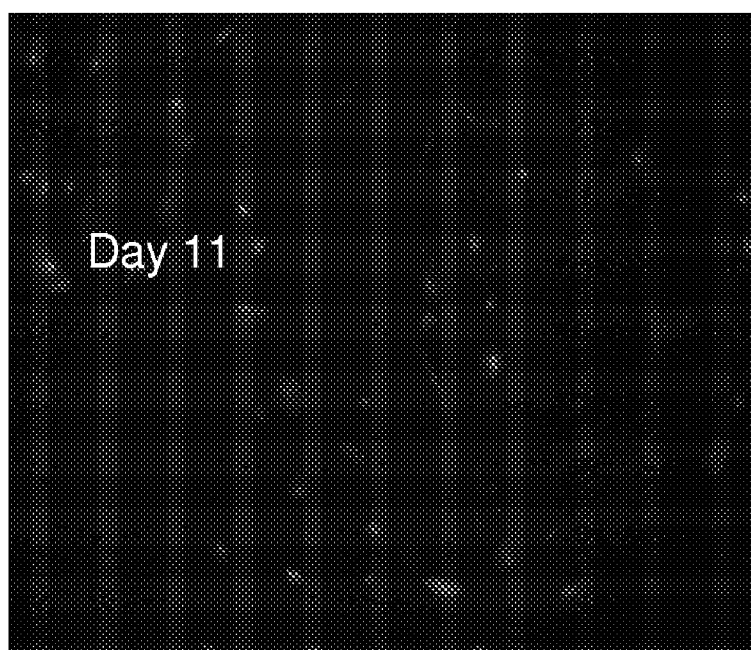


FIG. 19H.

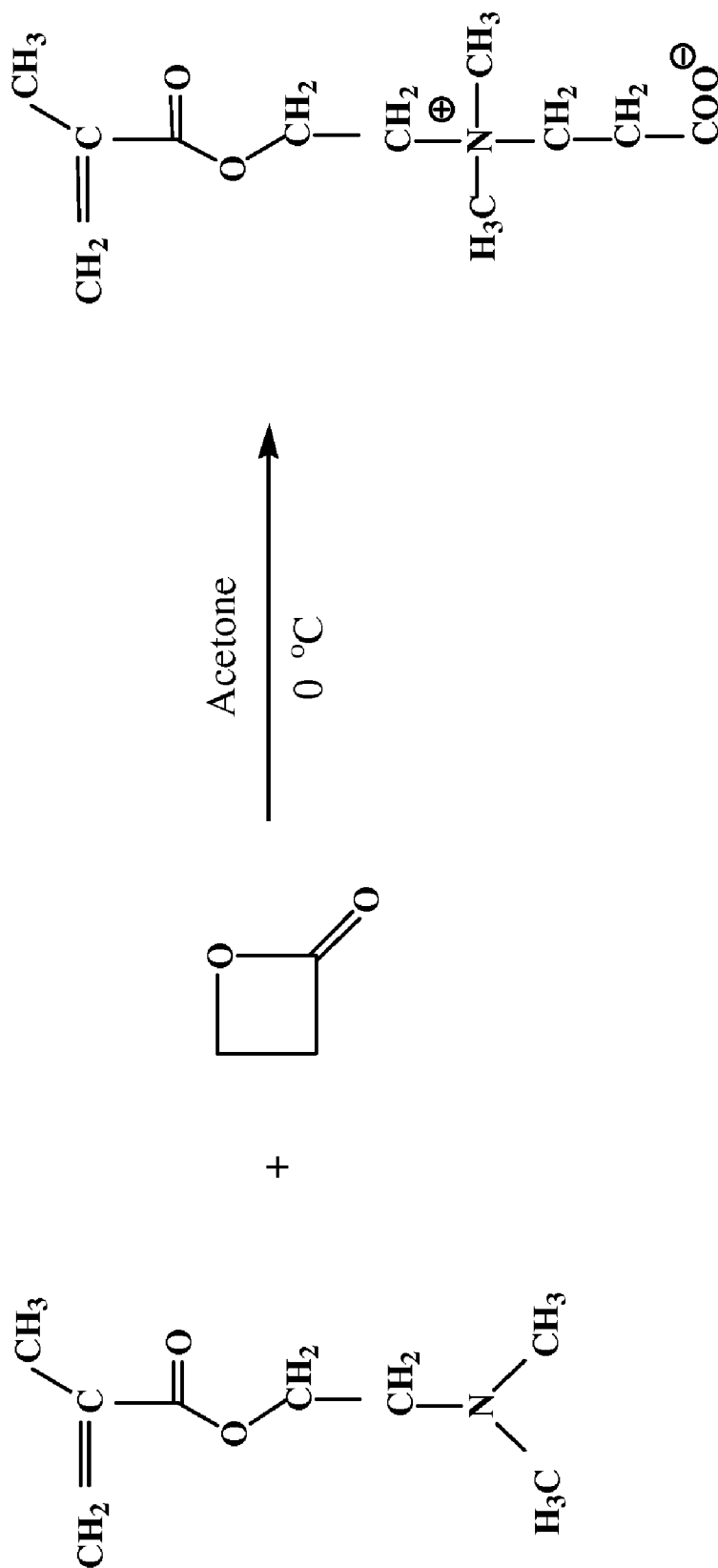


FIG. 20.

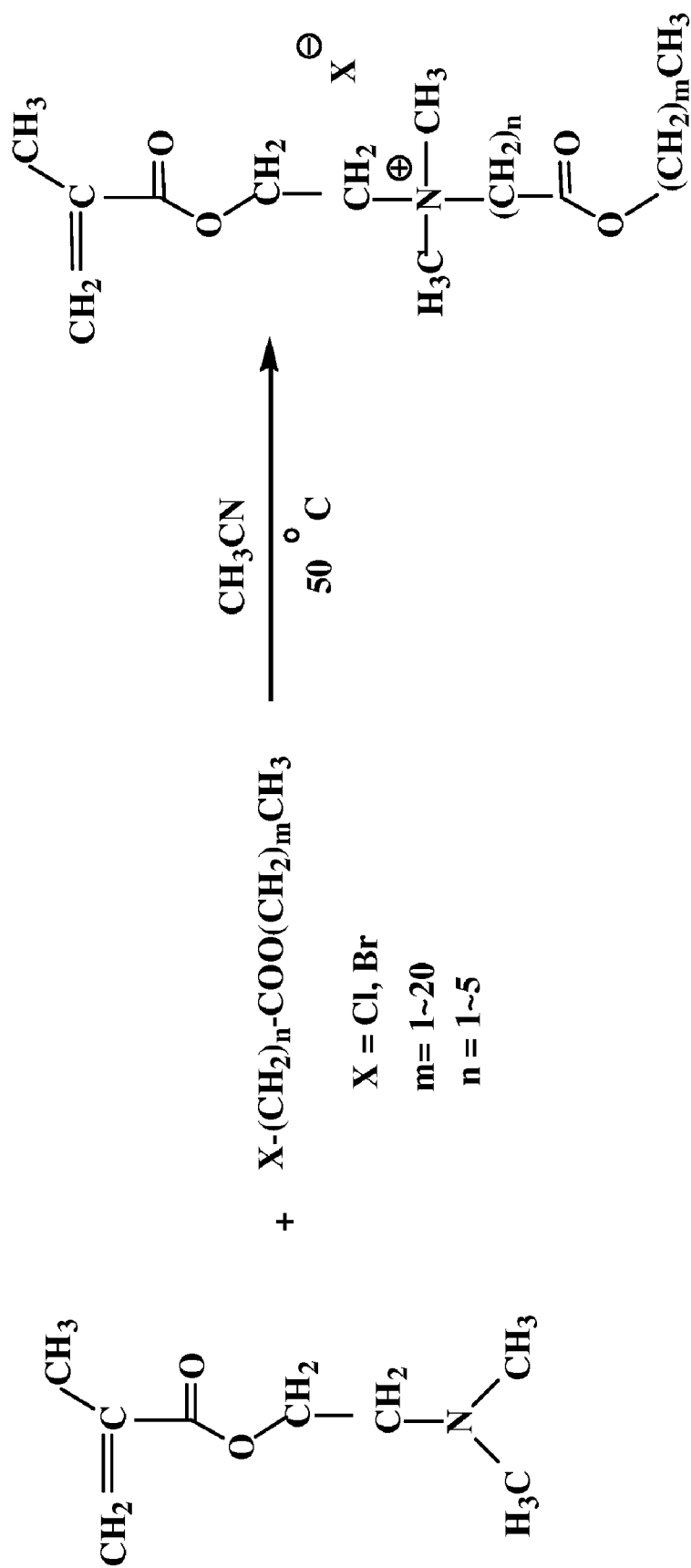


FIG. 21.

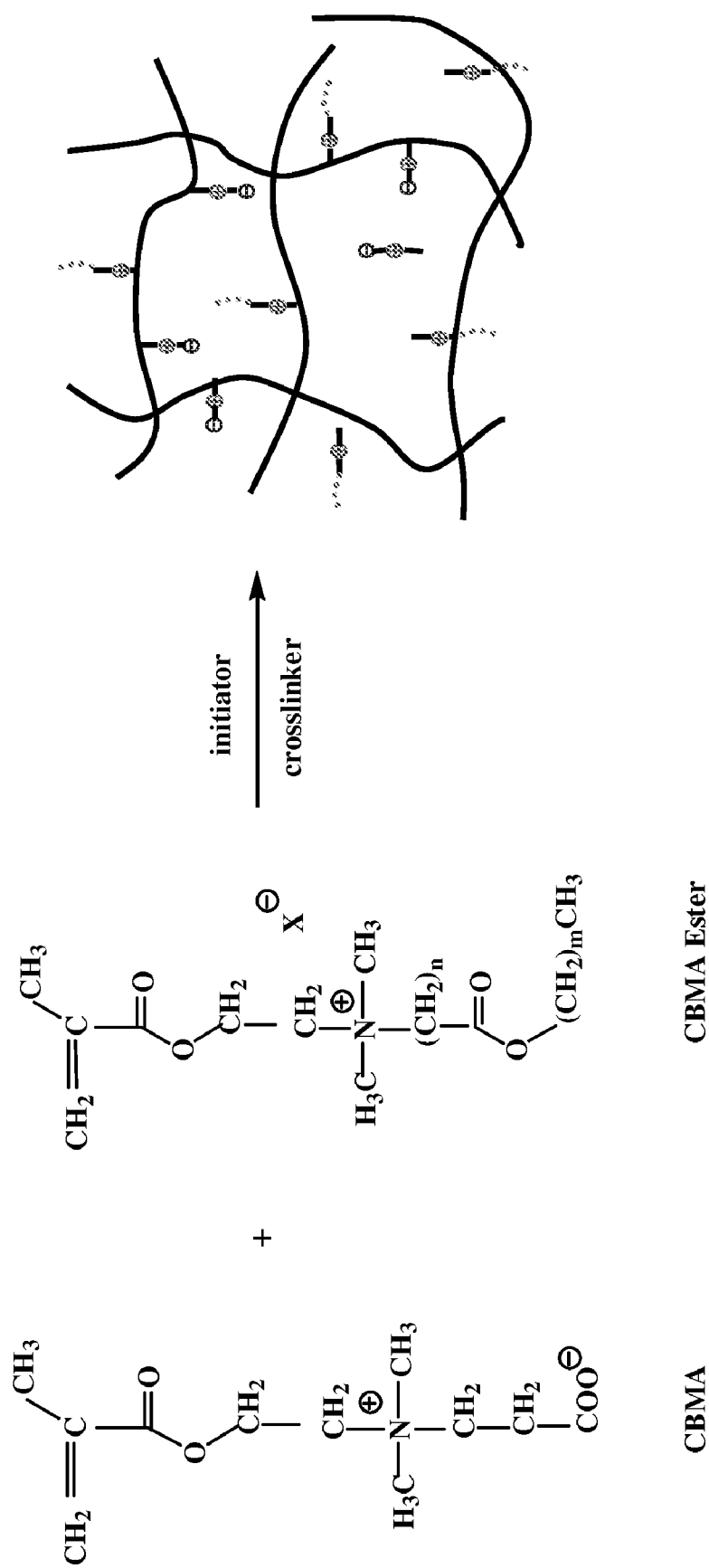


FIG. 22.

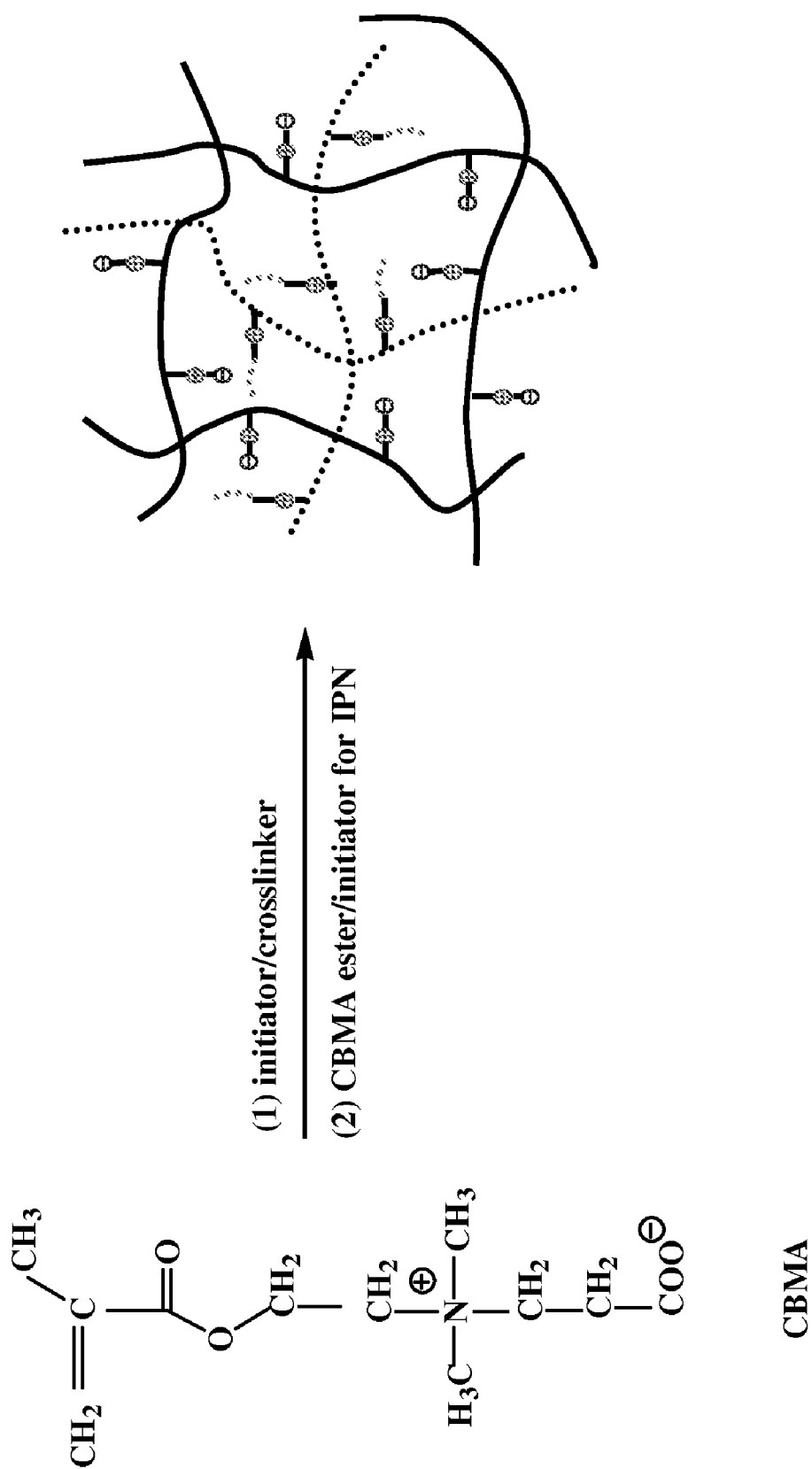


FIG. 23.

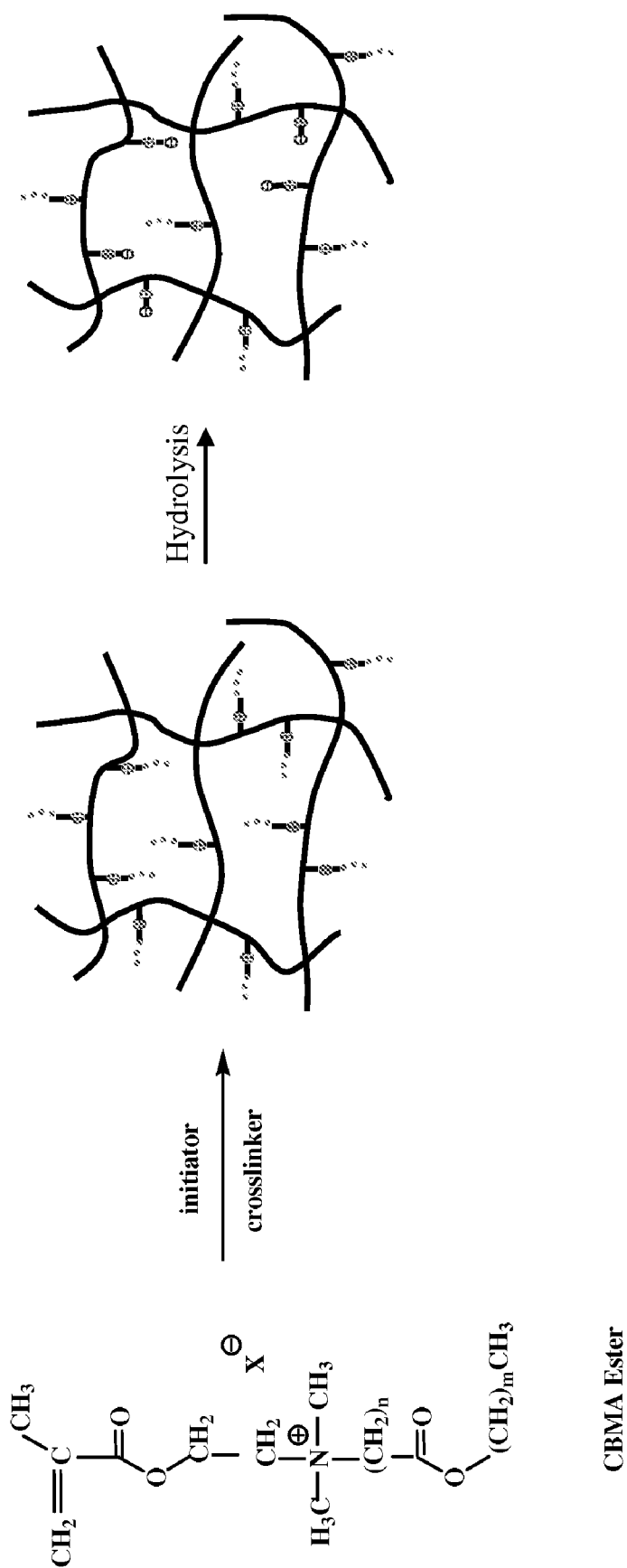
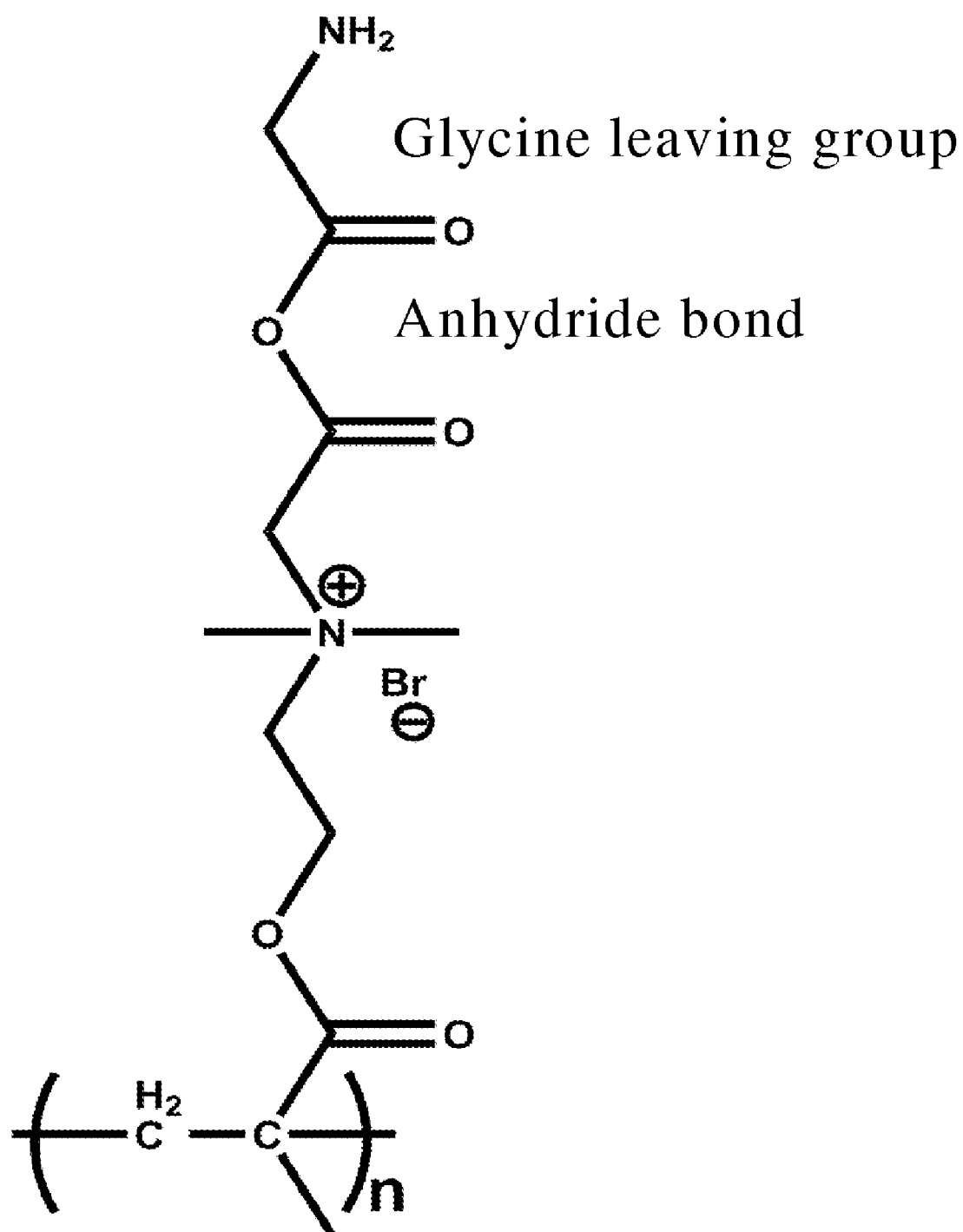


FIG. 24.

**FIG. 25.**

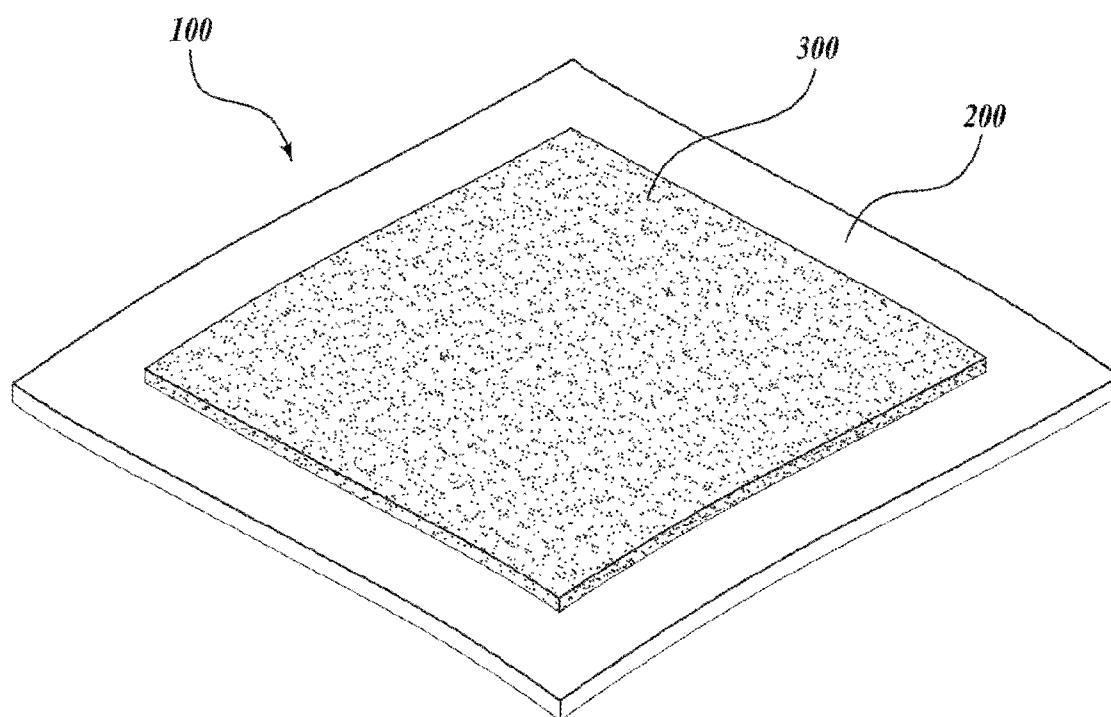


FIG. 26.

HEMOSTATIC WOUND DRESSING

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation of International Patent Application No. PCT/US2008/084099, filed Nov. 19, 2008, which claims the benefit of U.S. Provisional Application No. 60/989,073, filed Nov. 19, 2007. Each application is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with Government support under Grant Nos. N00014-04-1-0409 and N00014-07-1-1036 awarded by the Office of Naval Research, Grant No. AB06BAS759 awarded by the Defense Threat Reduction Agency, and Grant No. DMR0705907 awarded by the National Science Foundation. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Uncontrolled bleeding remains the leading cause of preventable death in the battlefield and effective control of hemorrhage can improve outcome. Early and effective hemorrhage control is obviously important and can save more lives than any other measure. The use of effective hemostatic dressings will benefit most combat injuries. Furthermore, the nature of combat injuries is such that bacterial contamination is frequently present in traumatic wounds. Infections that develop in traumatic and surgical wounds remain a major problem. There has been an increased effort to develop better hemostatic agents and dressings, some of which also have antimicrobial capability. Over the last decade, many hemostatic agents and dressings such as collagen, dry fibrin thrombin, chitosan, quaternary amine-containing compound, silver, and antimicrobial-loaded poly(ethylene glycol) (PEG) polymer have been tested with a variable degree of success. While some of them do not adequately prevent and limit bleeding, hemorrhaging, or bacterial infection, others may present side effects or antimicrobial-resistance. Some dressings can also be very expensive (e.g., \$ 1,000 per fibrin dressing).

[0004] Two hemostatic products designed to control severe hemorrhage are used by the military and commercially available: (1) chitosan-based HemCon® (HC) Bandage (HemCon Inc., Tigard, Oreg.) and (2) granular zeolite, QuickClot® (QC) (Z-Medica, Newington, Conn.). The chitosan dressing is a deacetylated complex carbohydrate derived from the naturally occurring substance chitin (N-acetyl D-glucosamine). Because chitosan has a positive charge, it attracts negatively charged red blood cells and offers an antibacterial barrier. Both dressings were judged to be effective based on study findings to date, but the Committee on Tactical Combat Casualty Care (COTCCC) was not able to identify a clear winner based on efficacy. According to the committee's recommendations, all the bleeding wounds should initially be treated with standard of care (e.g., pressure dressings and tourniquets). If the bleeding continues, application of HemCon® with manual compression should be the next step. Finally, if bleeding still does not stop, then HemCon® should be removed and QuickClot® applied with manual compression for 5 minutes as a lifesaving measure. There is no single perfect hemostatic dressing. Each has its drawbacks and ben-

efits. While QuickClot® is effective, granular zeolite becomes markedly exothermic in blood and may result in thermal injury to tissues. Chitosan-based wound dressings also have several issues: (a) HC chitosan has limited solubility; (b) chitosan-based wound dressings are not as efficient as hydrogel-based counterparts and have limited capability to absorb wound fluids, wound fluids are absorbed by other means before HC bandages are applied; (c) HC chitosan is prepared and compressed on a pad and is a fairly rigid wafer and, as a result, these HC dressings work well on planar surfaces, but some investigators have reported difficulties in conforming them to deep, narrow wounds or wounds of more irregular shapes; (d) the amines in chitosan are not as effective as quaternary amines against bacterial infection; (e) the difficulties in production (e.g., batch to batch variability) need to be resolved.

[0005] Despite the advancement of hemostatic dressings, there exists a need for new hemostatic wound dressings having improved properties. The present invention seeks to fulfill this need and provides further related advantages.

SUMMARY OF THE INVENTION

[0006] The invention provides hemostatic wound dressings that include cationic polymeric materials hydrolyzable to zwitterionic polymeric materials. Methods for making and using the cationic polymeric materials and wound dressings are also provided.

[0007] In one aspect, the invention provides a wound dressing that includes a cationic polymer comprising:

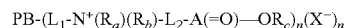
[0008] (a) polymer backbone;

[0009] (b) a plurality of cationic centers, each cationic center covalently coupled to the polymer backbone by a first linker;

[0010] (c) a counter ion associated with each cationic center; and

[0011] (d) a hydrolyzable group covalently coupled to each cationic center through a second linker, wherein the hydrolyzable group is hydrolyzable to an anionic center to provide a zwitterionic polymer having the anionic center covalently coupled to the cationic center through the second linker.

[0012] In one embodiment, the polymer has the formula:



wherein PB is the polymer backbone having n pendant groups $L_1-N^+(R_a)(R_b)-L_2-A(=O)-OR_c$; N^+ is the cationic center; R_a and R_b are independently selected from hydrogen, alkyl, and aryl; $A(=O)-OR_c$ is the hydrolyzable group, wherein A is selected from the group consisting of C, S, SO, P, or PO, and R_c is an alkyl, aryl, acyl, or silyl group that may be further substituted with one or more substituents; L_1 is a linker that covalently couples the cationic center to the polymer backbone; L_2 is a linker that covalently couples the cationic center to the hydrolyzable group; X^- is the counter ion associated with the cationic center; and n is an integer from about 10 to about 10,000.

[0013] In one embodiment, the counter ion is a hydrophobic organic counter ion. Representative counter ions include C1-C20 carboxylates and C1-C20 alkylsulfonates.

[0014] In one embodiment, the counter ion is a therapeutic agent. Representative therapeutic counter ions include antimicrobial, antibacterial, and antifungal agents.

[0015] In certain embodiments, the counter ion is an amino acid, protein, or peptide.

[0016] In one embodiment, the hydrolyzable group releases a hydrophobic organic group on hydrolysis. Representative hydrophobic groups include C1-C20 carboxylates.

[0017] In one embodiment, the hydrolyzable group releases a therapeutic agent on hydrolysis. Representative therapeutic agents include antimicrobial, antibacterial, and antifungal agents.

[0018] In certain embodiments, the cationic center is selected from ammonium, imidazolium, triazolium, pyridinium, morpholinium, oxazolidinium, pyrazinium, pyridazinum, pyrimidinium, piperazinum, and pyrrolidinium groups.

[0019] In one embodiment, R_a and R_b are independently selected from C1-C10 straight chain and branched alkyl groups.

[0020] In one embodiment, L_1 is selected from the group consisting of $-C(=O)O-(CH_2)_n-$ and $-C(=O)NH-(CH_2)_n-$, wherein n is an integer from 1 to 20.

[0021] In one embodiment, L_2 is $-(CH_2)_n-$, where n is an integer from 1 to 20.

[0022] In one embodiment, A is selected from the group consisting of C, SO, and PO.

[0023] In one embodiment, R_c is C1-C20 alkyl. In another embodiment, R_c is an amino acid.

[0024] In certain embodiments, X^- is selected from halides, carboxylates, alkylsulfonates, sulfate; nitrate, perchlorate, tetrafluoroborate, hexafluorophosphate, trifluoromethylsulfonate, bis(trifluoromethylsulfonyl)amide, lactate, and salicylate.

[0025] In one embodiment, the cationic polymer is a hydrogel. Representative hydrogels include chemical hydrogels and interpenetrating network hydrogels.

[0026] In one embodiment, the hydrogel comprises first and second polymers, wherein the first polymer is a cationic polymer hydrolyzable to provide a zwitterionic polymer, and wherein the second polymer is a zwitterionic polymer. In one embodiment, the first and second polymers are crosslinked.

[0027] In one embodiment, the hydrogel is prepared by copolymerizing a first cationic monomer having a hydrolyzable group and second zwitterionic monomer, optionally with a crosslinking agent.

[0028] In one embodiment, the hydrogel is prepared by polymerizing the first monomer to provide a cationic polymer having hydrolyzable groups, optionally with a crosslinking agent; adding the second monomer to the cationic polymer, and polymerizing the second monomer in the presence of the cationic polymer, optionally with a crosslinking agent. In another embodiment, the hydrogel is prepared by polymerizing the second monomer to provide a zwitterionic polymer, optionally with a crosslinking agent; adding the first cationic monomer having a hydrolyzable group to the zwitterionic polymer, and polymerizing the first monomer in the presence of the zwitterionic polymer, optionally with a crosslinking agent.

[0029] In one embodiment, the hydrogel is prepared by polymerizing a first cationic monomer having a hydrolyzable group, optionally with a crosslinking agent, to provide a cationic polymer having hydrolyzable groups, and hydrolyzing at least a portion of the hydrolyzable groups of the cationic polymer.

[0030] In another aspect of the invention, a method for treating a wound is provided. In one embodiment, the method includes applying a wound dressing of the invention to a wound.

DESCRIPTION OF THE DRAWINGS

[0031] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0032] The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

[0033] FIG. 1 illustrates the structures of three representative cationic monomers useful for making cationic polymers useful in the invention: three acrylamide monomers with different carboxybetaine ester groups; CBAA-1-ester, CBAA-3-ester, and CBAA-5-ester.

[0034] FIG. 2 illustrates the hydrolysis of a representative cationic polymer of the invention: hydrolysis of a cationic polycarboxybetaine ester to zwitterionic polycarboxybetaine.

[0035] FIG. 3 compares the 1H NMR spectra of the hydrolysis of a representative cationic polymer of the invention, polyCBAA-3-ester, after one-hour treatment in a solution with the sodium hydroxide concentration of (a) 10 mM (3% hydrolysis), (b) 100 mM (82% hydrolysis), and (c) 1 M (100% hydrolysis).

[0036] FIG. 4 compares the hydrolysis rates of representative cationic polymers useful in the invention at 10 mM and 100 mM aqueous sodium hydroxide.

[0037] FIGS. 5A-5C are SPR sensorgrams for fibrinogen adsorption on the surfaces grafted with representative polymers useful in the invention: polycarboxybetaine esters before and after hydrolysis; (a) polyCBAA-1-ester, (b) polyCBAA-3-ester, and (c) polyCBAA-5-ester. The surfaces with polymer brushes were hydrolyzed with a 100 mM NaOH solution for 1-2 h.

[0038] FIG. 6 is a graph comparing antimicrobial activities of three representative cationic polymers useful in the invention, polyCBAA-esters, before and after hydrolysis. *E. coli* (10^8 cells/mL) was incubated with each polymer solution (repeat unit molar concentration: 2 mM) for 30 min. PBS buffer (pH 7.4 and 150 mM) is used as a negative control.

[0039] FIG. 7 is a schematic illustration of a representative surface of the invention coated with a cationic polymer. The surface switches from an antibacterial surface to a non-fouling surface upon hydrolysis: (a) antimicrobial cationic pCBMA-1 C2 effectively kills bacteria, (b) pCBMA-1 C2 is converted to non-fouling zwitterionic pCBMA-1 upon hydrolysis, (c) killed bacteria remaining on the surface is released from non-fouling zwitterionic pCBMA-1 demonstrating that (d) zwitterionic pCBMA-1 itself is highly resistant to bacterial adhesion.

[0040] FIG. 8 illustrates the chemical structures of a representative cationic polymer of the invention, switchable pCBMA-1 C2; antimicrobial cationic pC8NMA; and non-fouling zwitterionic pCBMA-2.

[0041] FIG. 9 is a graph comparing bactericidal activity of pCBMA-1 C2 and pC8NMA against *E. coli* K12. The percentage of live *E. coli* K12 colonies that grew on the surfaces

coated with antimicrobial polymers is relative to the number of colonies that grew on the pCBMA-2 control ($n=3$).

[0042] FIGS. 10A-10F are fluorescence microscopy images of attached *E. coli* K12 cells (red color) from a suspension with 10^{10} cells/mL for one-hour exposure to the surfaces covered with various polymers: (a), (c), and (e) are for pCBMA-1 C2, pC8NMA and pCBMA-2, respectively, before hydrolysis and (b), (d), and (f) are for the same polymers, respectively, after hydrolysis. Hydrolysis was for 8 days with 10 mM CAPS (pH 10.0).

[0043] FIG. 11 is a graph comparing the attachment of *E. coli* K12 from a suspension with 10^{10} cells/mL for one-hour exposure to pCBMA-1 C2, pC8NMA, and pCBMA-2 before and after hydrolysis ($n=3$).

[0044] FIG. 12A compares SPR sensorgrams showing the adsorption of 1 mg/mL fibrinogen in PBS buffer on the surfaces grafted with pCBMA-1 C2 via ATRP (a) before hydrolysis, and (b), (c) and (d) after 24 hr hydrolysis with water, 10 mM CEHS at pH 9.0, and 10 mM CAPS at pH 10.0, respectively; FIG. 12B compares SPR sensorgrams showing the adsorption of 1 mg/mL fibrinogen in PBS buffer on the surfaces grafted with pC8NMA (a) before and (b) after 24 hr incubation with 10 mM CAPS at pH 10.0, and on the surfaces grafted with pCBMA-2 (c) before hydrolysis and (d) after 24 h of hydrolysis with 10 mM CAPS at pH 10.0.

[0045] FIG. 13 illustrates the structure of a representative cationic monomers useful for making cationic polymers useful in the invention: CBMA-1 C2 SA, the ethyl ester of CBMA-1 having a salicylate counter ion.

[0046] FIG. 14 compares the release rate (mg/h) of salicylic acid over time (12 h, 39 h, and 63 h) at 25° C. under four conditions from hydrogels prepared by polymerizing CBMA-1 C2 SA: (a) water, neutral pH; (b) phosphate buffered saline (PBS); (c) water, pH 10; and (d) 0.15 M aqueous sodium chloride, pH 10.

[0047] FIG. 15 compares the release rate (mg/h) of salicylic acid over time (12 h, 39 h, and 63 h) at 37° C. under four conditions from hydrogels prepared by polymerizing CBMA-1 C2 SA: (a) water, neutral pH; (b) phosphate buffered saline (PBS); (c) water, pH 10; and (d) 0.15 M aqueous sodium chloride, pH 10.

[0048] FIG. 16 illustrates schematic representative wound dressing hydrogels of the invention containing both zwitterionic and cationic zwitterionic precursor polymers. The cationic zwitterionic precursor polymers are useful for hemostatic and antimicrobial actions and the zwitterionic polymers are useful for wound fluid adsorbents. After action, cationic zwitterionic precursor polymers are converted to nontoxic, non-sticky, and biocompatible zwitterionic polymers by hydrolysis. These wound dressing hydrogels can be prepared from two monomers (e.g., CBMA and CBMA ester) via polymerization or from just one monomer (CBMA ester) by partially hydrolyzing the corresponding pCBMA ester hydrogel.

[0049] FIGS. 17A-17B illustrate the SPR response for non-specific adsorption of 10% human serum in PBS and 100% human serum (17A) and non-specific adsorption of 10% human plasma in PBS and 100% human serum (17B). Error bars represent the standard error of the mean. An adsorbed protein monomer is equivalent to 2,500 pg/mm².

[0050] FIG. 18 illustrates the resistance of a representative zwitterionic polymer, CBAA-2, to nonspecific protein adsorption from 100% blood plasma and 100% serum (<0.3 ng/cm² adsorbed proteins).

[0051] FIGS. 19A-19H compares microscopy images of accumulated *P. aeruginosa* on surfaces treated with pCBMA (FIGS. 19C-19H, days 1, 3, 5, 7, 10, and 11, respectively) with untreated (FIG. 19A) and OEG SAM-modified glass substrates (FIG. 19B) (as references) in growth medium over an 11-day growth period.

[0052] FIG. 20 is a schematic illustration of the preparation of a zwitterionic CBMA monomer.

[0053] FIG. 21 is a schematic illustration of the preparation of representative cationic polymers useful in the invention, CBMA ester monomers ($m=1-20$ and $n=1-5$).

[0054] FIG. 22 is a schematic illustration of the preparation of representative hydrogels of the invention from the zwitterionic monomer illustrated in FIG. 20 and the cationic monomer illustrated in FIG. 21: pCBMA ester/pCBMA chemical hydrogels.

[0055] FIG. 23 is a schematic illustration of the preparation of representative IPN hydrogels of the invention from the zwitterionic monomer illustrated in FIG. 20 and the cationic monomer illustrated in FIG. 21: CBMA ester/pCBMA IPN hydrogels.

[0056] FIG. 24 is a schematic illustration of the preparation of representative hydrogels of the invention from the cationic monomer illustrated in FIG. 21 by partial hydrolysis: partially hydrolyzed pCB ester hydrogels.

[0057] FIG. 25 illustrates the chemical structure of a representative cationic polymer of the invention having a glycine leaving group: pCBMA with a glycine leaving group.

[0058] FIG. 26 illustrates a representative wound dressing of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0059] The invention provides hemostatic wound dressings that include cationic polymeric materials hydrolyzable to zwitterionic polymeric materials. Methods for making and using the cationic polymeric materials and wound dressings are also provided.

[0060] In one aspect of the invention, hemostatic wound dressings that include cationic polymeric materials hydrolyzable to zwitterionic polymeric materials are provided. The cationic polymers useful in the invention include hydrolyzable groups that can be hydrolyzed to provide zwitterionic polymers. Zwitterionic polymers are polymers having a balance of positive and negative charge. Zwitterionic polymers can be highly resistant to protein adsorption and bacterial adhesion. Due to their biomimetic nature, zwitterionic polymers, such as phosphobetaine, sulfobetaine, and carboxybetaine polymers, exhibit high biocompatibility.

[0061] Controlled Hydrolysis. The variation of the structural features of the cationic polymers allows for their controlled hydrolysis and the control of the biological, chemical, and mechanical properties. The rate of hydrolysis can be significantly affected by and controlled by the selection of the nature of the hydrolyzable group (e.g., for esters, —OR).

[0062] As described below, in certain embodiments, the cationic polymers useful in the invention advantageously release functional groups on hydrolysis. For example, for cationic esters of the invention, hydrolysis ester releases an —OR group. In these embodiments, the released group can be a therapeutic agent (e.g., an antimicrobial, antibacterial, an antifungal agent). Similarly, in certain embodiments, the cationic polymers can release their counter ions (X^-), which can also be therapeutic agents (e.g., nucleic acids, amino acids, peptides, proteins, and salicylate).

[0063] For applications as antimicrobial agents, antimicrobial cationic polymers can be converted to zwitterionic polymers, leaving no toxic residues in the environment or no killed microbes on a surface.

[0064] It will be appreciated that the hydrolyzable group can be cleaved not only by hydrolysis, but also by cleavage (e.g., degradation or erosion) that occurs by other means. The cationic polymers can be converted to their corresponding zwitterionic polymers by environmental changes due to enzymatic catalysis, redox, heat, light, ionic strength, pH, and hydrolysis, among others.

[0065] Representative cationic polymers useful in the invention and their corresponding zwitterionic polymer counterparts are described below.

[0066] Cationic Polymers

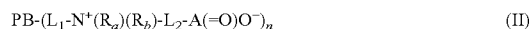
[0067] The cationic polymers useful in the invention include hydrolyzable groups that, when hydrolyzed, provide anionic groups that render the polymer zwitterionic. In each polymer, the number of hydrolyzable groups is substantially equal to the number of cationic groups such that, when the hydrolyzable groups are hydrolyzed, in the resulting polymer is zwitterionic. As used herein, the term "zwitterionic polymer" refers to a polymer having substantially equal numbers of cationic groups and anionic groups.

[0068] Representative cationic polymers useful in the invention have formula (I):



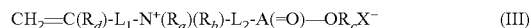
wherein PB is the polymer backbone having n pendant groups (i.e., $\text{L}_1-\text{N}^+(\text{R}_a)(\text{R}_b)-\text{L}_2-\text{A}(=\text{O})-\text{OR}_c$); N^+ is the cationic center; R_a and R_b are independently selected from hydrogen, alkyl, and aryl groups; $\text{A}(=\text{O})-\text{OR}_c$ is the hydrolyzable group, wherein A is C, S, SO, P, or PO, and R_c is an alkyl, aryl, acyl, or silyl group that may be further substituted with one or more substituents; L_1 is a linker that covalently couples the cationic center to the polymer backbone; L_2 is a linker that covalently couples the cationic center to the hydrolyzable group; X^- is the counter ion associated with the cationic center; and n is from about 10 to about 10,000. The average molecular weight of the polymers of formula (I) is from about 1 kDa to about 1,000 kDa.

[0069] Hydrolysis of the cationic polymer of formula (I) provides zwitterionic polymer having formula (II):



wherein PB, L_1 , N^+ , R_a , R_b , L_2 , A, and n are as described above, and $\text{A}(=\text{O})\text{O}^-$ is the anionic group.

[0070] In this embodiment, the polymer of formula (I) includes n pendant groups and can be prepared by polymerization of monomers having formula (III):



wherein L_1 , N^+ , R_a , R_b , $\text{A}(=\text{O})\text{OR}_c$, and L_2 , and X^- are as described above, R_d is selected from hydrogen, fluorine, trifluoromethyl, C1-C6 alkyl, and C6-C12 aryl groups.

[0071] In formulas (I) and (II), PB is the polymer backbone. Representative polymer backbones include vinyl backbones (i.e., $-\text{C}(\text{R}')(\text{R}'')-\text{C}(\text{R}''')(\text{R}'''')-$, where R' , R'' , R''' , and R'''' are independently selected from hydrogen, alkyl, and aryl) derived from vinyl monomers (e.g., acrylate, methacrylate, acrylamide, methacrylamide, styrene). Other suitable backbones include polymer backbones that provide for pendant cationic groups that include hydrolyzable groups that can be converted to zwitterionic groups, and backbones that include cationic groups and that provide for pendant hydrolyzable

groups that can be converted to zwitterionic groups. Other representative polymer backbones include peptide (polypeptide), urethane (polyurethane), and epoxy backbones.

[0072] Similarly, in formula (III), $\text{CH}_2=\text{C}(\text{R}_d)-$ is the polymerizable group. It will be appreciated that other polymerizable groups, including those noted above, can be used to provide the monomers and polymers of the invention.

[0073] The following is a description of the polymers and monomers of formulas (I)-(III) described above.

[0074] In formulas (I)-(III), N^+ is the cationic center. In certain embodiments, the cationic center is a quaternary ammonium (N bonded to L_1 ; R_a , R_b , and L_2). In addition to ammonium, other useful cationic centers include imidazolium, triazolium, pyridinium, morpholinium, oxazolidinium, pyrazinium, pyridazinium, pyrimidinium, piperazinium, and pyrrolidinium.

[0075] R_a and R_b are independently selected from hydrogen, alkyl, and aryl groups. Representative alkyl groups include C1-C10 straight chain and branched alkyl groups. In certain embodiments, the alkyl group is further substituted with one of more substituents including, for example, an aryl group (e.g., $-\text{CH}_2\text{C}_6\text{H}_5$, benzyl). In one embodiment, R_a and R_b are methyl. Representative aryl groups include C6-C12 aryl groups including, for example, phenyl. For certain embodiments of formulas (I)-(III), R_2 or R_3 is absent.

[0076] L_1 is a linker that covalently couples the cationic center to the polymer backbone. In certain embodiments, L_1 includes a functional group (e.g., ester or amide) that couples the remainder of L_1 to the polymer backbone (or polymerizable moiety for the monomer of formula (III)). In addition to the functional group, L_1 can include an C1-C20 alkylene chain. Representative L_1 groups include $-\text{C}(=\text{O})\text{O}-(\text{CH}_2)_n-$ and $-\text{C}(=\text{O})\text{NH}-(\text{CH}_2)_n-$, where n is 1-20 (e.g., 3).

[0077] L_2 is a linker that covalently couples the cationic center to the hydrolyzable group (or anionic group for the zwitterionic polymer of formula (II)). L_2 can be a C1-C20 alkylene chain. Representative L_2 groups include $-(\text{CH}_2)_n-$, where n is 1-20 (e.g., 1, 3, or 5).

[0078] The hydrophobicity and the rate of hydrolysis of the cationic polymers of formula (I) can be controlled by L_1 and/or L_2 . The greater the hydrophobicity of L_1 or L_2 , the slower the hydrolysis of the hydrolyzable group and the conversion of the cationic polymer to the zwitterionic polymer.

[0079] $\text{A}(=\text{O})-\text{OR}_c$ is the hydrolyzable group. The hydrolyzable group can be an ester, such as a carboxylic acid ester (A is C), a sulfinic acid ester (A is S), a sulfonic acid ester (A is SO), a phosphinic acid ester (A is P), or a phosphonic acid ester (A is PO). The hydrolyzable group can also be an anhydride. R_c is an alkyl, aryl, acyl, or silyl group that may be further substituted with one or more substituents.

[0080] Representative alkyl groups include C1-C30 straight chain and branched alkyl groups. In certain embodiments, the alkyl group is further substituted with one of more substituents including, for example, an aryl group (e.g., $-\text{CH}_2\text{C}_6\text{H}_5$, benzyl). In certain embodiments, R_c is a C1-C20 straight chain alkyl group. In one embodiment, R_c is methyl. In another embodiment, R_c is ethyl. In one embodiment, R_c is a C3-C20 alkyl. In one embodiment, R_c is a C4-C20 alkyl. In one embodiment, R_c is a C5-C20 alkyl. In one embodiment, R_c is a C6-C20 alkyl. In one embodiment, R_c is a C8-C20 alkyl. In one embodiment, R_c is a C10-C20 alkyl. For applications where relatively slow hydrolysis is desired, R_c is a C4-C20 n-alkyl group or a C4-C30 n-alkyl group.

[0081] Representative aryl groups include C6-C12 aryl groups including, for example, phenyl including substituted phenyl groups (e.g., benzoic acid).

[0082] Representative acyl groups ($-\text{C}(=\text{O})\text{R}_e$) include acyl groups where R_e is C1-C20 alkyl or C6-C12 aryl.

[0083] Representative silyl groups ($-\text{SiR}_3$) include silyl groups where R is C1-C20 alkyl or C6-C12 aryl.

[0084] In certain embodiments of the invention, the hydrolysis product R_cO^- (or R_cOH) is a therapeutic agent (e.g., an antimicrobial agent, such as salicylic acid (2-hydroxybenzoic acid), benzoate, lactate, and the anion form of antibiotic and antifungal drugs).

[0085] In certain other embodiments, the hydrolysis product R_cO^- (or R_cOH) is lactate, glycolate, or an amino acid.

[0086] The rate of hydrolysis of the cationic polymers of formula (I) can also be controlled by R_c . The slower the hydrolysis of the hydrolyzable group due to, for example, steric and/or kinetic effects due to R_c , the slower the conversion of the cationic polymer to the zwitterionic polymer.

[0087] X^- is the counter ion associated with the cationic center. The counter ion can be the counter ion that results from the synthesis of the cationic polymer of formula (I) or the monomers of formula (III) (e.g., Cl^- , Br^- , I^-). The counter ion that is initially produced from the synthesis of the cationic center can also be exchanged with other suitable counter ions to provide polymers having controllable hydrolysis properties and other biological properties.

[0088] The rate of hydrolysis of the cationic polymers of formula (I) can be controlled by the counter ion. The more hydrophobic the counter ion, the slower the hydrolysis of the hydrolyzable group and the slower the conversion of the cationic polymer to the zwitterionic polymer. Representative hydrophobic counter ions include carboxylates, such as benzoic acid and fatty acid anions (e.g., $\text{CH}_3(\text{CH}_2)_n\text{CO}_2^-$ where $n=1-19$); alkyl sulfonates (e.g., $\text{CH}_3(\text{CH}_2)_n\text{SO}_3^-$ where $n=1-19$); salicylate; lactate; bis(trifluoromethylsulfonyl)amide anion ($\text{N}^-(\text{SO}_2\text{CF}_3)_2$); and derivatives thereof. Other counter ions also can be chosen from chloride, bromide, iodide, sulfate; nitrate; perchlorate (ClO_4); tetrafluoroborate (BF_4); hexafluorophosphate (PF_6); trifluoromethylsulfonate (SO_3CF_3); and derivatives thereof.

[0089] Other suitable counter ions include hydrophobic counter ions and counter ions having therapeutic activity (e.g., an antimicrobial agent, such as salicylic acid (2-hydroxybenzoic acid), benzoate, lactate, and the anion form of antibiotic and antifungal drugs).

[0090] For the monomer of formula (III), R_d is selected from hydrogen, fluoride, trifluoromethyl, and C1-C6 alkyl (e.g., methyl, ethyl, propyl, butyl). In one embodiment, R_d is hydrogen. In one embodiment, R_d is methyl. In another embodiment, R_d is ethyl.

[0091] The variation of the structural features of the cationic polymers allows for their controlled hydrolysis and the control of the biological, chemical, and mechanical properties. The structural features of the cationic polymers noted above that can be varied to achieve the desired controlled hydrolysis of the polymer include L_1 , L_2 , R_a , R_b , A , R_c , and X^- . In general, the more hydrophobic the polymer or the noted structural feature, the slower the hydrolysis of the cationic polymer to the zwitterionic polymer.

[0092] Homopolymers, Random Copolymers, Block Copolymers. The cationic polymers useful in the invention include homopolymers, random copolymers, and block copolymers.

[0093] In one embodiment, the invention provides cationic homopolymers, such as defined by formula (I), prepared by polymerizing a cationic monomer, such as defined by formula (III). It will be appreciated that the advantageous properties associated with cationic polymers useful in the invention including those polymers defined by formula (I) can be imparted to other polymeric materials.

[0094] In one embodiment, the invention provides random copolymers prepared by copolymerizing two different cationic monomers of formula (III).

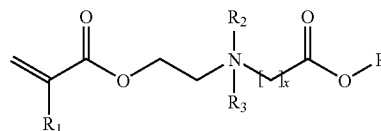
[0095] In another embodiment, the invention provides random copolymers that include cationic repeating units prepared by copolymerizing one or more cationic monomers of the invention defined by formula (III) with one or more other monomers (e.g., hydrophobic monomers, anionic monomers, or zwitterionic monomers, such as phosphorylbetaine, sulfo-betaine, or carboxybetaine monomers).

[0096] In one embodiment, the invention provides block copolymers having one or more blocks comprising cationic repeating units and one or more other blocks. In this embodiment, the one or more blocks that include cationic repeating units include only cationic repeating units (e.g., homo- or copolymer prepared from cationic monomers of formula (III)). Alternatively, the one or more blocks that include cationic repeating units include cationic repeating units and other repeating units (e.g., hydrophobic, anionic, zwitterionic repeating units).

[0097] Other Suitable Polymers

[0098] The invention also provides the following polymers.

[0099] In one embodiment, the cationic polymer has the following structure:



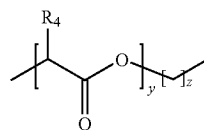
[0100] $\text{R}_1 = \text{H}$, $-\text{CH}_3$, $-\text{C}_2\text{H}_5$

[0101] $\text{R}_2 = \text{no atom}$, $-\text{H}$, $-\text{CH}_3$, $-\text{C}_2\text{H}_5$

[0102] $\text{R}_3 = \text{H}$, $-\text{CH}_3$, $-\text{C}_2\text{H}_5$

[0103] $x=1-8$.

[0104] $\text{R} = \text{any alkyl chain, aromatic or lactate or glycolate}$



[0105] $\text{R}_4 = \text{H}$, $-\text{CH}_3$, $-\text{C}_2\text{H}_5$

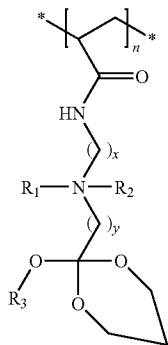
[0106] $\text{Y}=1-10$

[0107] $\text{Z}=0-22$

[0108] or $\text{C}(=\text{O})\text{R}'$

[0109] $\text{R}' = \text{any alkyl chain or aromatic group}$.

[0110] In another embodiment, the cationic polymer has the following structure:



[0111] $n > 5$

[0112] $x = 1-5$

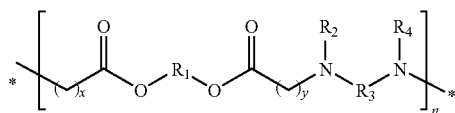
[0113] $y = 1-5$

[0114] $R_1 = \text{H, or alkyl chain}$

[0115] $R_2 = \text{no atom, H, or alkyl chain}$

[0116] $R_3 = \text{alkyl chain.}$

[0117] In another embodiment, the invention provides a polymer having the following structure:



[0118] R_1 is any alkyl chain

[0119] R_3 is any alkyl chain

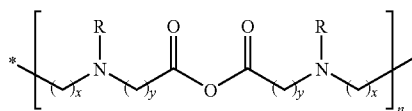
[0120] R_2, R_4 is any alkyl chain

[0121] $x = 1-18$

[0122] $y = 1-18$

[0123] $n > 3$.

[0124] In another embodiment, the invention provides a polymer having the following structure:



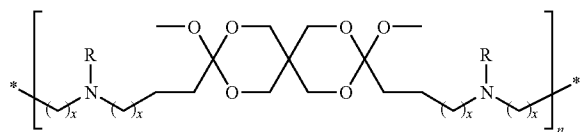
[0125] R is alkyl chain

[0126] $x = 1-18$

[0127] $y = 1-18$

[0128] $n > 3$.

[0129] In another embodiment, the invention provides a polymer having the following structure:

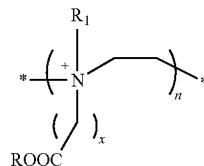


[0130] $R = \text{any alkyl chain}$

[0131] $x = 0-11$

[0132] $n > 3$.

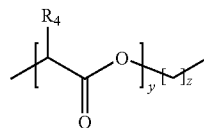
[0133] In another embodiment, the invention provides a polymer having the following structure:



[0134] $n > 3$

[0135] $x = 1-10$

[0136] $R = \text{any alkyl chain, aromatic or lactate or glycolate.}$



[0137] $R_4 = \text{—H, —CH}_3, \text{—C}_2\text{H}_5$

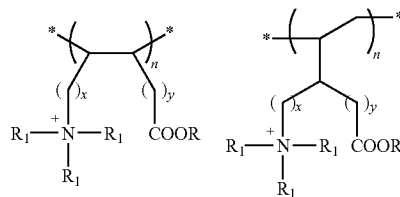
[0138] $y = 1-10$

[0139] $z = 0-22$

[0140] or C(=O)R'

[0141] $R' = \text{any alkyl chain, aromatic group.}$

[0142] In another embodiment, the invention provides polymers having the following structure:

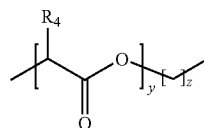


[0143] $n > 3$

[0144] $x = 1-6$

[0145] $y = 0-6$

[0146] $R = \text{any alkyl chain, aromatic or lactate or glycolate)}$



[0147] $R_4 = \text{—H, —CH}_3, \text{—C}_2\text{H}_5$

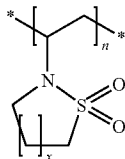
[0148] $y = 1-10$

[0149] $z = 0-22$

[0150] or C(=O)R'

[0151] $R' = \text{any alkyl chain, aromatic group.}$

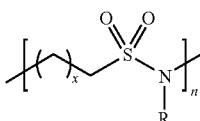
[0152] In another embodiment, the invention provides a polymer having the following structure:



[0153] $n > 5$

[0154] $x = 0-5$.

[0155] In another embodiment, the invention provides a polymer having the following structure:

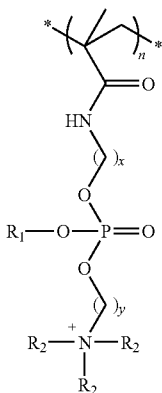


[0156] $x = 0-17$

[0157] $n > 5$

[0158] $R = H$ or alkyl chain.

[0159] In another embodiment, the invention provides a polymer having the following structure:

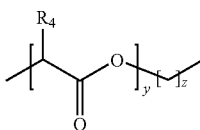


[0160] $n > 5$

[0161] $R_2 = H$ or any alkyl chain, e.g., methyl

[0162] $x, y = 1-6$

[0163] $R_1 = \text{any alkyl chain,}$

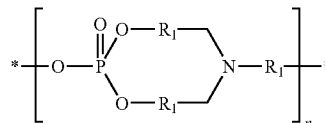


[0164] $R_4 = H, -CH_3, -C_2H_5$

[0165] $y = 1-10$

[0166] $z = 0-22$

[0167] In another embodiment, the invention provides a polymer having the following structure:



[0168] $n > 3$

[0169] $R_1 = \text{any alkyl chain.}$

[0170] Three representative cationic monomers of formula (III) useful for making cationic polymers of formula (I), and ultimately the zwitterionic polymers of formula (II) are illustrated in FIG. 1. Referring to FIG. 1, three positively charged polyacrylamides having pendant groups that bear cationic carboxybetaine ester groups are illustrated. The three monomers have different spacer groups ($L_2: -CH_2)_n-$) between the quaternary ammonium groups (cationic center) and the ester (hydrolyzable) groups: CBAA-1-ester ($n=1$); CBAA-3-ester ($n=3$); and CBAA-5-ester ($n=5$). Polymerization of the monomers provides the corresponding cationic polymers. The three monomers were polymerized using free radical polymerization to form linear polymers, or using surface-initiated ATRP to prepare polymer brushes on SPR sensors. The polymers with different spacer groups (L_2) and ester groups were expected to have different chemical, physical and biological properties. The synthesis of the three monomers and their polymerizations are described in Example 1.

[0171] For the linear polymers polymerized via free radical polymerization, their molecular weights were measured using gel permeation chromatography (GPC) in aqueous solutions. PolyCBAA-1-ester, polyCBAA-3-ester, and polyCBAA-5-ester had average molecular weights of 14 kDa, 13 kDa, and 9.6 kDa, respectively

[0172] Hydrolysis of the cationic polymers provides the zwitterionic polymers (i.e., zwitterionic polycarboxybetaines). The hydrolysis of representative cationic polymer of the invention is described in Example 2 and illustrated schematically in FIG. 2. In FIG. 2, n is 1, 3, or 5 (corresponding to polyCBAA-1-ester, polyCBAA-3-ester, and polyCBAA-5-ester, respectively). The three carboxybetaine ester polymers were dissolved under different sodium hydroxide concentrations and their hydrolysis behavior was studied. After a period of time, the hydrolysis rate of the polymers was analyzed by measuring the retaining ester groups on the polymer using 1H NMR. All the three polymers are stable in water and no evident hydrolysis was detected after four days. The concentration of NaOH is crucial for the hydrolysis of the carboxybetaine ester polymers. FIG. 3 illustrates the NMR spectra of polyCBAA-3-ester after a one-hour treatment with three different concentrations of NaOH. For NaOH solution with a concentration of 10 mM, only slightly hydrolysis was detected (ca. 3%). For 100 mM NaOH solution, about 82% polymer was hydrolyzed. For the NaOH concentration of 1 M, the polymer was totally hydrolyzed in one hour. FIG. 4 graphs the hydrolysis rate under 100 mM or 10 mM NaOH as a function of time. Referring to FIG. 4, under these two NaOH concentrations, most hydrolysis happens in the first hour. After that, the hydrolysis rate changes only slightly with the time.

[0173] As noted above, the hydrolysis rate of the cationic polymers useful in the invention can be controlled by modi-

ifying their structures. To obtain the different hydrolysis behavior, cationic polymers having varying structure parameters such as ester groups (hydrolyzable groups), spacer groups (L_1 and L_2), and counter ions (X^-). Hydrolysis behavior can also be controlled by varying polymer molecular weight or copolymerizing with other monomers. Hydrolyzable ester groups (such as *t*-butyl and alkyl substituted silyl) or anhydride groups can be easily hydrolyzed under acidic or basic condition. Changing spacer groups (L_2 : $-\text{CH}_2)_n-$) between the quaternary ammonium groups (cationic center) and the ester (hydrolyzable) groups also can tune the hydrolysis rate. Short spacers can increase the hydrolysis rate. In addition, counter ions, such as hydrophilic anions (e.g., Cl^- , Br^- , I^- , SO_4) also increase the hydrolysis rate, and low polymer molecular weight and copolymerization with other hydrophilic monomers also help to increase the hydrolysis rate.

[0174] Protein Adsorption

[0175] The hydrolyzable cationic polymers useful in the invention can advantageously be used as materials effective in reducing protein adsorption to surfaces treated with the polymers. The cationic polymers can be used to prepare low-fouling surfaces. These surfaces can be advantageously employed for devices in environments where the protein adsorption to device surfaces are detrimental.

[0176] To demonstrate the utility of representative cationic polymers useful in the invention in providing surfaces having low protein adsorption, polymer brushes were prepared from representative cationic polymers as described in Example 3 and their protein adsorption measured.

[0177] The three monomers (CBAA-1-ester, CBAA-3-ester, and CBAA-5-ester) were grafted on the surfaces of a SPR sensor using surface-initiated ATRP. The polymer brushes had a thickness of 5-20 nm estimated from XPS analysis. Protein adsorption from a 1 mg/mL fibrinogen solution on the three polymer brushes was measured using SPR. Fibrinogen is a sticky protein and plays an important role in platelet aggregation and blood clotting on biomaterials. Fibrinogen adsorption was 195 ng/cm², 255 ng/cm², and 600 ng/cm² for polyCBAA-1-ester, polyCBAA-3-ester, and polyCBAA-5-ester, respectively (see FIGS. 5A-5C). All three polymers have evident protein adsorption due to their positive charges. PolyCBAA-1-ester had relatively lower fibrinogen adsorption due to its higher hydrophilicity compared to the other two esters having more hydrophobic L_2 (i.e., C3 and C5, respectively). With the increase in L_2 from methylene to propylene to pentylene, the hydrophobicity of the polymer increases, leading to higher fibrinogen adsorption.

[0178] After hydrolysis at 100 mM for 1-2 hours, surface properties were dramatically changed. FIGS. 5A-5C illustrate that the surfaces grafted with each of the three polymers were converted to surfaces that were highly resistant to fibrinogen adsorption. On the surfaces with hydrolyzed polyCBAA-1-ester and hydrolyzed polyCBAA-3-ester, fibrinogen adsorption is less than 0.3 ng/cm², which is the detection limit of the SPR. Fibrinogen adsorption on hydrolyzed polyCBAA-5-ester was about 1.5 ng/cm². By controlling hydrolysis, the polymer-grafted surfaces can change their properties from high protein adsorption to strongly resistant to protein adsorption. Moreover, resulting surfaces with zwitterionic polymers after hydrolysis are biocompatible and highly resistant to nonspecific protein adsorption from blood plasma/serum and bacterial adhesion/biofilm formation.

[0179] Antimicrobial Properties

[0180] The hydrolyzable cationic polymers useful in the invention exhibit antimicrobial properties. The evaluation of antimicrobial properties of representative cationic polymers useful in the invention is described in Example 4.

[0181] To evaluate the antimicrobial properties of the cationic polycarboxybetaine esters, polymer solutions of polyCBAA-1-ester, polyCBAA-3-ester, and polyCBAA-5-ester were incubated with *E. coli*. It was found that at a concentration of 2 mM (repeat unit molar concentration), polyCBAA-1-ester, polyCBAA-3-ester, and polyCBAA-5-ester present a live cell percentage of 95%, 87.3%, and 46.2%, respectively (see FIG. 6). Antimicrobial activities appears to increase with the increase in the length of L_2 . After hydrolysis, the zwitterionic polymers, polyCBAA-1, polyCBAA-3, and polyCBAA-5, exhibit a live cell percentage of 93.7%, 96.3% and 95.3%, respectively, indicating that the antimicrobial activity decreases with the hydrolysis of the cationic polymers (i.e., polycarboxybetaine esters) to the zwitterionic polymers (i.e., polycarboxybetaines).

[0182] Several amphiphilic polycations have been investigated for their antibacterial activities. The alkyl pendent chain length of the polycations was studied to compare the bactericidal efficiency of different polycations. It is found that the polymers with quaternary amine groups and longer hydrophobic pendant chains have better antimicrobial activities due to higher hydrophobicity. Small molecular quaternary ammonium compounds (QMCs) with carboxybetaine esters were found to have rapid bactericidal action when they have longer hydrocarbon groups. These QMCs could bind to the outer membrane and cytoplasmic membrane of enterobacteria and permeate into the bacterial membranes. The antimicrobial effect is increased with increasing the spacer length (L_2) of the cationic polymers (e.g., polycarboxybetaine esters) of the invention.

[0183] The antimicrobial efficacy of the polyCBAA-5-ester is comparable to that of other quaternized polymers with similar alkyl chain length. Higher antimicrobial efficacy can be achieved with longer alkyl chain lengths (e.g., C1-C20).

[0184] For conventional antimicrobial coatings, the killed microbes and adsorbed proteins usually accumulate on the surfaces and dramatically decrease their antimicrobial activities. In contrast, antimicrobial coatings made from the cationic polymers useful in the invention are hydrolyzed to zwitterionic polymers to provide surfaces that are highly resistant to the adsorption of various biomolecules. These zwitterionic polymers are nontoxic, biocompatible, and nonfouling, both as bulk materials and surface coatings.

[0185] Representative crosslinked zwitterionic polymers useful in the invention, polycarboxybetaines hydrogels, were non-cytotoxic and contain less than 0.06 units (EU)/mL of endotoxin using a *Limulus Amebocyte* Lysate (LAL) endotoxin assay kit (Cambrex Bioscience, Walkersville, Md.). The polycarboxybetaine hydrogels were implanted subcutaneously within mice for up to four weeks. The results showed that the polycarboxybetaines have in vivo biocompatibility comparable to that of poly(2-hydroxyethyl methacrylate) (polyHEMA) hydrogels, a well-accepted model biomaterial for implantation. The nontoxic properties of the zwitterionic polymers convert the toxicity of their cationic polymer precursors and further provide nonfouling properties that can prevent dead microbes and adsorbed proteins from accumulating on the surface.

[0186] Switchable Polymer Coatings and their Use in Wound Dressings

[0187] The cationic polymers useful in the invention, hydrolyzable to zwitterionic polymers, can be advantageously incorporated into hemostatic wound dressings. The cationic polymers useful in the invention provide switchable biocompatible polymer surfaces having self-sterilizing and nonfouling capabilities.

[0188] FIG. 7 is a schematic illustration of a switchable biocompatible polymer surfaces having self-sterilizing and nonfouling capabilities. Referring to FIG. 7, antimicrobial surface (a) is a surface coated with a representative cationic polymer of the invention (i.e., pCBMA-1 C2, see FIG. 8) that effectively kills bacteria. On hydrolysis (b) the representative cationic polymer is converted to a nonfouling zwitterionic polymer (i.e., pCBMA-1, the carboxylate corresponding to pCBMA-1 C2 ester) and dead bacteria remaining on the surface are released (c) from the nonfouling zwitterionic polymer (i.e., pCBMA-1) to provide a surface coated with the zwitterionic polymer, which is highly resistant to bacterial adhesion (d).

[0189] The materials of the invention (e.g., polymers, hydrogels) are advantageously used to coat surfaces to provide biocompatible, antimicrobial, and nonfouling surfaces. Accordingly, in another aspect, the invention provides wound dressings and related materials having a surface (i.e., one or more surfaces) to which have been applied (e.g., coated, covalently coupled, ionically associated, hydrophobically associated) one or more materials of the invention. Representative wound dressings and related materials devices are advantageously treated with a material of the invention, modified to include a material of the invention, or incorporate a material of the invention.

[0190] Microbial adhesion onto implanted biomaterials and the subsequent formation of biofilms is one of the major causes of biomedical device failure. The use of antimicrobial and nonfouling coatings are two strategies for the prevention of the attachment and spreading of microorganisms on the surfaces of implantable materials. Antimicrobial surfaces containing covalently linked quaternary ammonium compounds (QACs) have proved to be able to efficiently kill a variety of microorganisms. A major problem with QAC surfaces is the attachment of dead microorganisms remaining on antimicrobial coatings, which can trigger an immune response and inflammation, and block its antimicrobial functional groups. In addition, such antimicrobial coatings can not fulfill the requirements of nonfouling and biocompatibility as implantable biomaterials. Poly(ethylene glycol) (PEG) derivatives or zwitterionic polymers have been extensively used as nonfouling materials to reduce bacterial attachment and biofilm formation. However, the susceptibility of PEG to oxidation damage has limited its long-term application in complex media. Zwitterionic materials such as poly(sulfobetaine methacrylate) (pSBMA) are able to dramatically reduce bacterial attachment and biofilm formation and are highly resistant to nonspecific protein adsorption, even from undiluted blood plasma and serum. Although zwitterionic coatings can reduce the initial attachment and delay colonization of microbes on surfaces, there is a possibility of introducing pathogenic microbes into the patient during implantation operations and catheter insertions, which results in the failure of implanted devices; the use of antimicrobial agents will then be necessary to eliminate these microbes. Surface-responsive materials have been developed for a broad spectrum of appli-

cations, but it is still a great challenge to develop biocompatible materials that have both antimicrobial and nonfouling capabilities.

[0191] As noted above, in one embodiment, the present invention provides a switchable polymer surface coating that combines the advantages of both nonfouling surface and that can kill greater than 99.9% of *Escherichia coli* K12 in one hour, with 98% of the dead bacterial cells released when the cationic derivatives are hydrolyzed to nonfouling zwitterionic polymers. pCBMA-1-C2 (cationic polymer of formula (I) where L_1 is $—C(=O)OCH_2CH_2—$, L_2 is $—CH_2—$, R_c is CH_2CH_3 , and X^- is Br^-) control coatings were grafted by surface-initiated atom transfer radical polymerization (ATRP) onto a gold surface covered with initiators. The thicknesses of the obtained polymer coatings, as measured by atomic force microscopy (AFM), were 26-32 nm (Table 1).

TABLE 1

Film thicknesses (av $\pm$ std dev.) of pCBMA-1 C2, pC8NMA, and pCBMA-2 grafted onto gold-coated glass slides by ATRP and fibrinogen adsorption on these surfaces measured by SPR before and after hydrolysis under different conditions.			
	pCBMA-1 C1	pC8NMA	pCBMA-2
polymer brush thickness (nm)	(31.2 $\pm$ 2.4)	(27.8 $\pm$ 2.8)	(26.1 $\pm$ 2.5)
protein adsorption (ng cm ⁻²)			
0 h	229.2	243.4	1.5
24 h H ₂ O	189.9	—	—
24 h CHES (pH 9.0)	114.9	—	—
24 h CAPS (pH 10.0)	0	285.1	0.7

[0192] The bactericidal activity of pCBMA-1 C2 surfaces was determined using *E. coli* K12, according to a modified literature procedure (Tiller et al., *Proc. Natl. Acad. Sci. USA* 98:5981, 2001). The permanently cationic poly(methacryloyloxyethyl-dimethyloctylammonium bromide) (pC8NMA, cationic control, (see FIG. 8) and the zwitterionic poly(2-carboxy-N,N-dimethyl-N-[2'-(methacryloyloxy)ethyl]ethanaminium) (pCBMA-2, zwitterionic control, see FIG. 8) were used as the positive and the negative control surfaces, respectively. The antimicrobial efficiency was defined as the amount of live cells on the tested surfaces relative to those on the pCBMA-2 surface. FIG. 9 shows that pCBMA-1 C2 and pC8NMA surfaces kill greater than 99.9% and 99.6%, respectively, of the *E. coli* in one hour relative to pCBMA-2 surfaces. The total number of live bacterial cells on the gold surface, which was also used as a negative-control surface, is similar to that on the pCBMA-2 surface.

[0193] The attachment and release of *E. coli* K12 were tested on the pCBMA-1 C2 surfaces before and after hydrolysis. Cationic pC8NMA and zwitterionic pCBMA-2 were used as the negative and the positive nonfouling control surfaces, respectively, and as the positive and the negative antimicrobial control surfaces, respectively. FIGS. 10A-10F show that large amounts of bacteria were attached to the cationic pCBMA-1 C2 and pC8NMA surfaces before hydrolysis, whereas very few bacterial cells were attached to the zwitterionic pCBMA-2 surface. In contrast to pC8NMA, pCBMA-1 C2 released the majority of cells after hydrolysis while pCBMA-2 remained nonfouling. FIG. 11 shows quantitative data for the amount of bacterial cells remaining on all three polymer surfaces before and after hydrolysis. There

were similar amounts of bacterial residues on both cationic pCBMA-1 C2 and pC8NMA surfaces before hydrolysis, while the amount of attached cells on the pCBMA-2 surface is less than 0.3% of that on both cationic pCBMA-1 C2 and pC8NMA surfaces. To test the release of bacterial residues, the three surfaces were incubated in N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer (10 mM, pH 10.0) at 37° C. for 8 days. The pCBMA-1 C2 surfaces were hydrolyzed to poly(N-(carboxymethyl)-N,N-dimethyl-2-[(2-methyl-1-oxo-2-propen-1-yl)-oxy]ethanaminium) (pCBMA-1) and 98% of the dead bacterial cells were released. In contrast, no release of the dead cells was observed on pC8NMA surfaces ($p > 0.1$) while pCBMA-2 surfaces retained very low bacterial adhesion.

[0194] The release of the attached bacterial cells is dependent on the conversion of cationic pCBMA-1 C2 into zwitterionic pCBMA-1. Hydrolysis rate of betaine esters is influenced by several factors, such as the length of the spacer (L_2) between the quaternary amine and the carboxyl groups, the nature of the hydrolyzable group, temperature,¹ and pH value. The majority of polymer chains of the ester group used were hydrolyzed. The hydrolysis rate of the betaine esters is also slower after bacterial cells and proteins are attached to the surface. pCBMA-1 C2, which has one methylene spacer (L_2), was chosen and the experimental temperature was set at 37° C. to achieve a fast hydrolysis rate and to provide a physiologically relevant temperature. The protein adsorption results (see Table 2) showed that the clean, cationic pCBMA-1 C2 surface was hydrolyzed into a nonfouling zwitterionic surface after only 24 h at 37° C. and pH 10.0, while it took 48 h to form a nonfouling surface and release bacterial residues after the attachment of bacteria from an *E. coli* K12 suspension of 10^7 cells mL^{-1} . When bacterial cells were attached to the pCBMA-1 C2 surface from a suspension of 10^{10} cells mL^{-1} , the release of attached bacteria took eight days under the same hydrolysis conditions.

[0195] Nonspecific protein adsorption on various surfaces was measured by a surface plasmon resonance (SPR) sensor to determine the nonfouling characteristics of the surfaces (see Table 2). Hydrolysis conditions for pCBMA-1 C2 and control surfaces were investigated in situ in the SPR sensor. FIGS. 12A and 12B show representative SPR sensorgrams for fibrinogen adsorption on pCBMA-1 C2 and control surfaces over time. The fibrinogen adsorption on pCBMA-1 C2 before hydrolysis was 229.2 ng cm^{-2} . After 24 h of incubation with CAPS buffer (pH 10.0), there was no measurable protein adsorption on the pCBMA-1 C2 surface, which indicated that pCBMA-1 C2 was completely hydrolyzed to nonfouling zwitterionic pCBMA-1. In contrast, hydrolysis of pCBMA-1 C2 was not complete after 24 h incubation in either water or N-cyclohexyl-2-aminoethanesulfonic acid (CEHS) buffer (pH 9.0). As shown in FIG. 12B, high fibrinogen adsorption was observed on the pC8NMA surface before and after the surface was incubated with CAPS buffer (pH 10.0) for 24 h at 37° C. However, under identical conditions, the pCBMA-2 surface still exhibited excellent nonfouling properties, with less than 2 ng cm^{-2} fibrinogen absorption. This result indicates that the obtained zwitterionic surfaces are highly resistant to protein adsorption and are qualified as ultralow fouling surfaces, which are required for the surface coatings of implantable medical devices.

[0196] In this embodiment, the invention provides a switchable polymer surface that integrates antimicrobial and non-fouling properties and is biocompatible. The representative

cationic polymer (i.e., precursor of pCBMA) is able to kill bacterial cells effectively and switches to a zwitterionic non-fouling surface and releases dead bacterial cells upon hydrolysis. Moreover, the resulting nonfouling zwitterionic surface can further prevent the attachment of proteins and microorganisms and reduce the formation of a biofilm on the surface. The switchable process from antimicrobial to non-fouling surfaces can be tuned through adjusting the hydrolysis rate of these polymers for specific requirements of applications.

[0197] As noted above, the cationic polymers useful in the invention can include a hydrophobic counter ion or a counter ion having therapeutic activity (e.g., antimicrobial or antibacterial activity). A representative polymer having a salicylate counter ion (polyCBMA-1 C2) can be prepared from the monomer illustrated in FIG. 13: CBMA-1 C2 (“1” indicates one carbon between two charged groups and “C2” indicates C2 ester). PolyCBMA-1 C2 hydrogel loaded with salicylic acid (SA) as its counter ion was prepared by copolymerizing 1 mM CBMA-1 C2 SA monomer (FIG. 13) with 0.05 mM tetraethylenglycoldimethacrylate in 1 ml of solvent (ethylene glycol:water:ethanol=1:2:1) at 65° C. for 2 hours. The resulting hydrogel was soaked in DI water for 12 hours. The hydrogel was cut into round disks with 1 cm diameter. The hydrogel disks were then transferred into solutions with different pH and ionic strength and incubated at 25° C. or 37° C. At different time points the aqueous phase was completely removed and new solutions were added. The release of SA into the aqueous phase was measured by high performance liquid chromatography (HPLC). The release rate of SA is defined as the amount of released SA divided by time (mg/h). The release rate of SA from pCBMA-1 C2 SA hydrogel depends on temperature, ionic strength, and pH. FIG. 14 and FIG. 15 indicated that higher pH promotes the release of SA and that increased ionic strength can slightly increase the release rate of SA. By comparing FIG. 14 and FIG. 15, it can be observed that the elevated temperature results in a faster release of SA in water and phosphate buffered saline (PBS). The release rate of SA decreases as a function of time for all the conditions.

[0198] Cationic Polymers and their Use in Wound Dressings

[0199] The cationic polymers useful in the invention, hydrolyzable to zwitterionic polymers, can be advantageously used as coatings for the surfaces of hemostatic wound dressings. In this embodiment, the cationic polymers useful in the invention provide switchable biocompatible polymer surfaces having self-sterilizing and nonfouling capabilities. These polymers are useful in hemostasis devices optionally in combination with one or more conventional hemostasis components.

[0200] As noted above, the present invention provides switchable biocompatible polymer surfaces having self-sterilizing and non-fouling capabilities based on the cationic polymers useful in the invention (e.g., pCBMA ester). See FIG. 7. Antimicrobial cationic pCBMA ester can prevent bacterial infection and is converted to biocompatible and water-absorbent zwitterionic pCBMA upon hydrolysis.

[0201] In one embodiment, the wound dressing includes a hydrogel. In this embodiment, the hydrogel includes both a zwitterionic polymer as well as a cationic polymer (i.e., a cationic zwitterionic precursor polymer). The cationic zwitterionic precursor polymers are used for hemostatic and antimicrobial actions while the zwitterionic polymers are used as

wound fluid adsorbents. After action, cationic zwitterionic precursor polymers are converted to nontoxic, non-sticky, and biocompatible zwitterionic polymers upon hydrolysis (controllable hydrolysis rates). In one embodiment, the wound dressing hydrogels can be prepared from two monomers (CBMA and CBMA ester) via polymerization or from just one monomer (CBMA ester) by partially hydrolyzing the pCBMA ester hydrogel.

[0202] A representative wound dressing hydrogel of the invention is schematically illustrated in FIG. 16.

[0203] In one embodiment, the invention provides wound dressings based on an integrated formulation containing both cationic (e.g., pCBMA ester) and zwitterionic (e.g., pCBMA) polymers. These wound dressings containing both cationic and zwitterionic polymers improve hemorrhage control and survival, promote wound healing, and remove bacteria. The advantages of the wound dressings include (a) multiple hemostatic, antimicrobial, wound fluid-absorbent, and wound healing functions with high efficacy since cationic polymers (e.g., pCBMA ester) can attract red blood cells and kill bacteria while the zwitterionic polymer (e.g., pCBMA) can absorb wound fluids; (b) add-on wound fluid adsorbent capability, non-sticky, biocompatibility, and nontoxicity even at high concentrations when cationic polymers are converted to zwitterionic polymers upon hydrolysis; (c) simplicity, reproducibility, and low-cost since they can be prepared from only one or two monomers (i.e., CBMA ester and CBMA). The wound dressings of the invention are based on cationic polymers hydrolyzable to zwitterionic polymers. The wound dressings include switchable polymer surfaces described above have both self-sterilizing and non-fouling/biocompatible capabilities. This cationic zwitterionic precursor polymer has unique performance as coatings on a surface or as additives to a solution. On hydrolysis, the cationic polymers are converted to zwitterionic polymers that are highly resistant to nonspecific protein adsorption from undiluted blood plasma and serum and bacterial adhesion/biofilm formation. The zwitterionic polymer hydrolysis products are also highly biocompatible with tissues from in vivo animal studies. The wound dressing includes a switchable polymer surface integrating antimicrobial and nonfouling/biocompatible properties. The antimicrobial cationic zwitterionic precursor surface can effectively kill bacterial cells and then switch to a non-fouling and biocompatible zwitterionic surface (see FIG. 7).

[0204] Six representative cationic polymers useful in the invention and SAM modifications were evaluated for their interactions with human serum and plasma. Human serum and plasma are components of human blood, comprised of a complex mixtures of hundreds of proteins. FIGS. 17A and 17B show sensor responses to human serum and human plasma, respectively. pCBMA had the best non-specific resistance to the test media. A pCBMA surface had an improved resistance to nonspecific protein adsorption from human plasma or serum over the poly[oligo(ethylene glycol) methacrylate] (pOEGMA) and poly(sulfobetaine methacrylate) (pSBMA) (See FIGS. 17A and 17B). While protein adsorption from 10% serum is generally low, the difference among several surfaces studied to resist nonspecific protein adsorption from 100% plasma and serum is enormous as shown in FIGS. 17A and 17B. With the optimization of film thickness and density, pCBMA can highly resist 100% blood plasma and serum to the level that is undetectable by SPR (See FIG. 18).

[0205] Microbial adhesion and the subsequent formation of biofilm are critical issues for many biomedical applications. Therefore, the development of surfaces that resist the initial adhesion of bacteria is the first step towards the effective prevention of long-term biofilm formation. The accumulation of *P. aeruginosa* on pSBMA modified glass chips formed with a silane initiator and surface-initiated ATRP indicates that pSBMA grafted surfaces show strong resistance to bacterial adhesion and biofilm formation for one day. *Pseudomonas aeruginosa* PAO1 with a GFP expressing plasmid was used for long-term adhesion and biofilm formation studies. There is an absence of attached bacteria after exposure to *P. aeruginosa* for a long period of time, while *P. aeruginosa* is readily attached to the unmodified portion of glass or oligo (ethylene glycol) (OEG) self-assembled monolayer (SAM) modified substrates. These studies were performed in a laminar flow chamber in situ.

[0206] FIGS. 19A-19H are representative microscopy images of the accumulated *P. aeruginosa* on pCBMA-treated surfaces in the growth medium over an 11-day growth period. On the bare and OEG-modified glass surfaces, very quick bacterial adhesion and subsequent biofilm formation of *P. aeruginosa* were observed (see FIGS. 19A and 19B, respectively). A confluent biofilm was formed by the second day on these two control surfaces. However, the surface concentration of adherent *P. aeruginosa* on the pCBMA-coated glass was very small ($<10^6$ cell/mm²). Over the 11-day growth mode experiments, there was no observed biofilm formation (see FIGS. 19C-19H). It is believed that the ability of zwitterionic pCBMA materials to resist protein adsorption and inhibit bacterial adhesion is due to its strong hydration via ionic solvation.

[0207] In one embodiment, the wound dressings of the invention include hydrogels containing cationic polymers (zwitterionic precursors) of the invention and zwitterionic polymers.

[0208] Dried hydrogels as wound dressings can be prepared in two ways: (1) forming a chemical or IPN gel from zwitterionic monomers (e.g., CBMA) and cationic monomers (e.g., CBMA ester monomers) (a more controllable way) and (2) forming a chemical gel from the cationic monomer (pCB ester monomer) first and then partially hydrolyzing the product hydrogel (e.g., pCBMA ester hydrogel) to mixed cationic and zwitterionic hydrogel (pCBMA ester/pCBMA hydrogel) (a more economic way).

[0209] A representative hydrogel can be prepared from carboxybetaine methacrylate (CBMA) and cationic zwitterionic precursor monomers. CBMA monomer can be synthesized by the reaction of 2-(N,N'-dimethylamino)ethyl methacrylate (DMAEMA) and β -propiolactone with anhydrous acetone as solvent at low temperature (see FIG. 20). The purity (>99%) can be monitored by nuclear magnetic resonance (NMR) and elemental analysis. Alternatively, carboxybetaine acrylamide (CBAA) can be similarly prepared and used. Similar to CBMA, CBAA monomer can be synthesized by the reaction of N-(3-dimethylaminopropyl)acrylamide (DMPAA) and β -propiolactone with anhydrous acetone as solvent at low temperature.

[0210] Cationic zwitterionic precursor monomer (i.e., CBMA ester) can be synthesized by quaternization reaction from DMAEMA and alkyl chloro (or bromo) carboxylates using anhydrous acetonitrile as solvent (see FIG. 21).

[0211] CBMA was synthesized by DMAEMA and β -propiolactone with anhydrous acetone as solvent at low temperature using ice-bath as cooling method (yield ~90%).

[0212] Representative hydrogels can be prepared from zwitterionic (e.g., CBMA) and cationic (e.g., CBMA ester) monomers. The polymerization of the monomers is illustrated schematically in FIG. 22. CBMA, CBMA ester and 4 mol % N,N'-methylene-bisacrylamide (MBA, as a cross-linker) are dissolved in deionized water ([M] 10 wt %). To this solution, 0.2 wt % ammonium peroxodisulfate and 1.0 wt % N,N,N',N'-tetramethylethylenediamine (TMEDA, as an accelerator) is added as redox initiators. TMEDA is chosen since it is an initiator for reaction at room temperature. Polymerization is performed at room temperature for 24 hr. After the gelation is completed, the gel is immersed in an excess amount of deionized water for 3 days to remove the residual unreacted monomers. Swollen polymer gels are dried at room temperature for 1 day, and these samples are further dried with freeze drying method or in vacuum oven for 2 days at 60° C.

[0213] Representative interpenetrating network (IPN) hydrogels can be prepared from zwitterionic (e.g., CBMA) and cationic (e.g., CBMA ester) monomers. The polymerization of the monomers is illustrated schematically in FIG. 23. As a mixture of two or more crosslinked networks that are dispersed or mixed at a molecular segmental level, IPNs can help improve the mechanical strength and resiliency of the polymer gels. An example of the preparation of such an IPN hydrogel is as follows. CBMA and 4 mol % MBA (as a cross-linker) are dissolved in deionized water ([M] 10 wt %). To this solution, 0.2 wt % ammonium peroxodisulfate and 1.0 wt % TMEDA (as an accelerator) are added as redox initiators. Polymerization is performed at room temperature for 24 hr. After the gelation is completed, the gel is immersed in an excess amount of deionized water for 3 days to remove the residual unreacted monomer and initiator. The degree of crosslinking is defined as the molar ratio of the crosslinking agent (MBA) to CBMA.

[0214] Representative interpenetrating network (IPN) hydrogels can be prepared from zwitterionic (e.g., pCBMA) and cationic (e.g., pCBMA ester) polymers. pCBMA/pCBMA ester IPN gels are prepared according to the following procedure. A pCBMA gel is immersed in 10 ml of the pCBMA ester monomer solution of the prescribed concentration containing redox initiators mentioned above, and left for 5 days at 4° C. to let pCBMA ester penetrate into pCBMA gel. pCBMA ester inside the pCBMA gel is polymerized at 30° C. for 24 hr to give the IPN gels comprising pCBMA ester linear polymer and pCBMA gel. After the polymerization, the gel is immersed in an excess amount of deionized water for 3 days to remove the residual unreacted monomer and initiator. These samples are dried with freeze drying method or in vacuum oven for 2 days at 60° C.

[0215] While the mixed (chemical or IPN) hydrogel from zwitterionic (e.g., CBMA) and cationic (e.g., CBMA ester) monomers can be prepared in a controllable way, it is possible to achieve mixed zwitterionic and cationic hydrogels from a single cationic (e.g., CBMA ester) monomer. This is done by preparing cationic (e.g., pCBMA ester) hydrogel first and then partially hydrolyzing the hydrogel to a mixed cationic/zwitterionic hydrogel (e.g., pCBMA ester/pCBMA).

[0216] Hydrophilic cationic (e.g., pCBMA ester) hydrogels can be used as antimicrobial wound dressing once they are partially hydrolyzed. The gels after partial hydrolysis are

a mixture of zwitterionic pCBMA and cationic pCBMA ester compounds, which integrates both hemostatic, antimicrobial, and water-adsorbent functions.

[0217] A representative procedure for preparing to a partially hydrolyzed cationic (e.g., pCBMA ester) hydrogel is described below and illustrated schematically in FIG. 24. CBMA and 4 mol % MBA as crosslinker) is dissolved in deionized water ([M] 10 wt %). To this solution, 0.2 wt % ammonium peroxodisulfate and 1.0 wt % TMEDA (as an accelerator) are added as redox initiators. Polymerization is performed at room temperature for 24 hr. After the gelation is completed, the gel is immersed in an excess amount of deionized water for 3 days to remove the residual unreacted monomer and initiator. Then, the gel is immersed in the buffer solution (pH 8~12) for 3 to 12 hr, then is washed with deionized water, and immersed in an excess amount deionized water and left for 5 days at 4° C. to remove the residual salts. These samples are dried with freeze drying method or in vacuum oven for 2 days at 60° C.

[0218] The hydrogels of the invention prepared as described above can be optimized and evaluated for their antimicrobial, hemostatic, water-adsorbent, and other physical/chemical properties in vitro by adjusting the degree of cross-linking, the ratio of cationic polymer (e.g., pCBMA ester to pCBMA, and hydrolysable groups. Hydrogel pastes are coated onto a polymeric pad to form wound dressing-pad assemblies (or bandages) for in vivo experiments and practical applications. Standard gauze dressing and commercial HC dressing will be used as negative and positive controls whenever is possible.

[0219] In one embodiment, the invention provides a wound dressing-pad assembly that includes the wound dressing of the invention. The wound dressing-pad assembly is sized and configured for easy manipulation by the caregiver's fingers and hand. The backing isolates a caregiver's fingers and hand from the dressing gels. The backing permits the dressing matrix to be handled, manipulated, and applied at the tissue site, without adhering or sticking to the caregiver's fingers or hand. The backing can include low-modular meshes and/or films and/or weaves of synthetic and naturally occurring polymers. For temporary external wound applications, the backing includes a fluid impermeable polymer material, e.g., polyethylene (3M 1774T polyethylene foam medical tape, 0.056 cm thick). Other polymers may be used, including cellulose polymers, polyethylene, polypropylene, metalocene polymers, polyurethanes, polyvinylchloride polymers, polyesters, polyamides or their combinations. The backing can be attached or bonded by directed adhesion with a top layer of the wound dressing gel. The dried hydrogel samples can be moistened with 1~3 wt % of sterile physiological saline, which can turn into a sticky paste and have sufficient adhesive properties for attaching to the backing materials. If needed, an adhesive such as 3M 9942 Acrylate Skin Adhesive, or fibrin glue, or cyanoacrylate glue can be employed to enhance the adhesion between the wound dressing and the pad. The wound dressing matrix is desirably vacuum packaged before use with low moisture content, preferably 5% moisture or less, in an air-tight heat sealed foil-lined pouch. The antimicrobial wound dressing pad assembly is subsequently terminally sterilized within the pouch by the use of gamma irradiation. Once removed from the pouch, the wound dressing pad assembly is immediately ready to be adhered to the targeted tissue site. These wound dressings are non-irritating and non-sticky to the wound, absorb wound

exudate, enhance the sterile environment around the wound, stem blood loss, and promote wound healing. The wound dressing gels can be removed without leaving any gel residue on the wound due to the nonfouling properties of these wound dressing hydrogels.

[0220] A representative wound dressing of the invention is illustrated in FIG. 26. Referring to FIG. 26, representative wound dressing 100 includes backing 200 and gel 300.

[0221] The hydrolysis rate of the cationic polymers (e.g., esters) is influenced by several factors, such as the length of the spacer between the quaternary amine and the carboxyl group, the type of the hydrolyzable group, temperature, and pH. For a representative cationic polymer, pCBMA ester with $n=1$, the ester is an ethyl ester that produces ethanol on hydrolysis. A limited amount of ethanol will be generated upon hydrolysis, some of which will be trapped within the hydrogel, and some ethanol will be released, but will not affect wound healing. Alternatively, in one embodiment, the cationic polymer has a glycine leaving group (hydrolyzable bond is an anhydride) as illustrated in FIG. 25.

[0222] The following examples are provided for the purpose of illustrating, not limiting, the claimed invention.

EXAMPLES

Example 1

The Synthesis and Characterization of Representative Cationic Polymers

[0223] Materials. N-(3-dimethylaminopropyl)acrylamide (>98%) was purchased from TCI America, Portland, Oreg. Methyl bromoacetate (97%), ethyl 4-bromobutyrate ($\geq 97.0\%$), ethyl 6-bromohexanoate (99%), copper (I) bromide (99.999%), bromoisobutyl bromide (BIBB 98%), 11-mercapto-1-undecanol (97%), and 2,2'-bipyridine (BPY 99%), and 2,2'-azobis(2-methylpropionitrile) (AIBN 98%) were purchased from Sigma-Aldrich. Fibrinogen (fraction I from bovine plasma) and phosphate buffer saline (PBS, pH 7.4, 0.15 M, 138 mM NaCl, 2.7 mM KCl) were purchased from Sigma Chemical Co. Ethanol (absolute 200 proof) was purchased from AAPER Alcohol and Chemical Co. Water used in experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 M Ω .cm.

[0224] ω -Mercaptoundecyl bromoisobutyrate (1) was synthesized through reaction of BIBB and 2 using a method described in Ilker, M. F.; Nuesslein, K.; Tew, G. N.; Coughlin, E. B., "Tuning the Hemolytic and Antibacterial Activities of Amphiphilic Polynorbornene Derivatives," *Journal of the American Chemical Society* 126(48):15870-15875, 2004). ¹H NMR (300 MHz, CDCl₃): 4.15 (t, J=6.9, 2H, OCH₂), 2.51 (q, J=7.5, 2H, SCH₂), 1.92 (s, 6H, CH₃), 1.57-1.72 (m, 4H, CH₂), and 1.24-1.40 (m, 16H, CH₂).

[0225] Cationic Monomer Syntheses

CBAA-1-ester: (2-carboxymethyl)-3-acrylamidopropyl dimethylammonium bromide, methyl ester.

[0226] N-(3-dimethylaminopropyl)acrylamide (25 mmol), methyl bromoacetate (37.5 mmol), and acetonitrile (25 mL) were added into a 100-mL round-bottom flask. The mixture was stirred under a nitrogen atmosphere for 6 hr at room temperature. The precipitate was collected, washed with ca 500 mL of anhydrous acetone. The solvent was removed on a rotary evaporator to get a white powder (96% yield). ¹H NMR (300 MHz, D₂O): 2.02 (m, 2H, —CH₂—), 3.25 (s, 6H,

N⁺(CH₃)₂), 3.37 (t, 2H, CH₂—N⁺), 3.58 (m, 2H, CH₂—N), 3.79 (s, 3H, O—CH₃), 4.29 (s, 2H, CH₂—C=O), 5.77 (m, 1H, CH=C—CON—trans); 6.19 (m, 1H, CH=C—CON—cis), 6.23 (m, 1H, =CH—CON—).

[0227] CBAA-3-ester: (4-carboxypentyl)-3-acrylamidopropyl dimethylammonium bromide, ethyl ester.

[0228] N-(3-dimethylaminopropyl)acrylamide (50 mmol), ethyl 4-bromobutyrate (60 mmol), and acetonitrile (25 mL) were added into a 100-mL round-bottom flask. The mixture was stirred under a nitrogen atmosphere for three days at room temperature. The solvent was removed on a rotary evaporator to get a colorless oil (92% yield). ¹H NMR (300 MHz, D₂O): 1.22 (t, 3H CH₃), 2.00 (m, 4H, C—CH₂—C), 2.47 (t, 2H, CH₂—C=O), 3.06 (s, 6H, N⁺(CH₃)₂), 3.28-3.35 (6H, CH₂—N and CH₂—N⁺—(CH₂), 4.14 (q, 2H, O—CH₂), 5.75 (m, 1H, CH=C—CON—trans); 6.19 (m, 1H, CH=C—CON—cis), 6.26 (m, 1H, =CH—CON—).

[0229] CBAA-5-ester: (6-carboxypentyl)-3-acrylamidopropyl dimethylammonium bromide, ethyl ester.

[0230] N-(3-dimethylaminopropyl)acrylamide (50 mmol), ethyl 6-bromohexanoate (55 mmol), and acetonitrile (25 mL) were added into a 100-mL round-bottom flask. The mixture was stirred under a nitrogen atmosphere for five days at 45° C. The solvent was removed on a rotary evaporator to get a slightly yellowish oil (87% yield). ¹H NMR (300 MHz, D₂O): 1.20 (t, 3H CH₃), 1.34 (m, 2H, C—C—CH₂—C—C), 1.60-1.72 (4H, C—CH₂—C—CH₂—C), 2.00 (m, 2H, N—C—CH₂—C—N), 2.34 (t, 2H, CH₂—C=O), 3.04 (s, 6H, N⁺(CH₃)₂), 3.24-3.37 (6H, CH₂—N and CH₂—N⁺—CH₂), 4.12 (q, 2H, O—CH₂), 5.75 (m, 1H, CH=C—CON—trans); 6.20 (m, 1H, CH=C—CON—cis), 6.24 (m, 1H, =CH—CON—).

[0231] Representative Cationic Polymer Syntheses

[0232] Surface-Initiated ATRP. Three monomers, CBAA-1-ester, CBAA-3-ester, and CBAA-5-ester, were grafted onto gold-coated SPR sensor chips or gold-coated silicon chips using surface-initiated ATRP. The preparation and characterization of the polymer brushes is described in Zhang, Z.; Chen, S.; Chang, Y.; Jiang, S., Surface Grafted "Sulfobetaine Polymers Via Atom Transfer Radical Polymerization as Superlow Fouling Coatings," *Journal of Physical Chemistry B* 110(22):10799-10804, 2006, and Zhang, Z.; Chen, S.; Jiang, S., "Dual-Functional Biomimetic Materials: Nonfouling Poly(carboxybetaine) With Active Functional Groups for Protein Immobilization," *Biomacromolecules* 7(12):3311-3315, 2006. previous publications. Briefly, CuBr (1 mmol) and a SPR chip or a gold disk with a Br-thiol SAM was placed in a nitrogen-purged reaction tube. Degassed solution (pure water and methanol in a 1:1 volume ratio, 10 mL) with CBAA ester (6.5 mmol), and BPY (2 mmol, in 5 mL degassed methanol) were transferred to the tube using a syringe. After reaction for more than 1 hour under nitrogen, the SPR chip or gold disk was removed and rinsed with ethanol, water and PBS solution. The samples were stored in PBS solutions before testing.

[0233] Polymer Synthesis and Characterization

[0234] CBAA-1-ester solution of ca. 0.3 M in methanol was purged with nitrogen for 30 min. The polymerization was then performed at 60° C. for ca 48 hours under nitrogen using 3 mol % AIBN as an initiator to provide polyCBAA-1-ester. Similar methods were applied for preparation of polyCBAA-3-ester or polyCBAA-5-ester using ethanol as a solvent. The polymers were washed with ethyl ether and the solvent was then removed. The structures of the polymers were confirmed

by NMR. ¹H NMR (300 MHz, D₂O): polyCBAA-1-ester: 1.62 (br, 2H), 2.05 (br, 3H), 3.25-3.32 (br, 8H), 3.62 (br, 2H), 3.83 (s, 3H), 4.38 (s, 2H); polyCBAA-3-ester 1.21 (t, 3H), 1.61 (br, 2H), 2.04 (br, 5H), 2.50 (t, 2H), 3.37 (br, 6H), 3.12 (s, 6H), 4.14 (q, 2H); polyCBAA-5-ester: 1.22 (t, 3H), 1.37 (m, 2H), 1.62-1.80 (br m, 6H), 2.01 (br, 3H), 2.39 (t, 2H), 3.03 (s, 6H), 3.24 (br m, 6H), 4.12 (q, 2H).

[0235] The molecular weight of linear polyCBAA was estimated using a Waters Alliance 2695 Separations Module equipped with a Waters Ultrahydrogel 1000 column and detected with a Waters 2414 Reflex Detector. The mobile phase was an aqueous solution at a flow rate of 0.5 mL/min. The instrument and column were calibrated with poly(ethylene oxide) standards from Polymer Laboratories. All measurements were performed at 35° C. The molecular weight of the polymer was calculated using Empower Pro from Waters.

Example 2

Representative Cationic Polymer Hydrolysis

[0236] The cationic polymers prepared as described in Example 1 were dissolved in NaOH solutions with different concentration (10 mM, 100 mM, and 1 M) in a concentration of 50 mg/mL. After an appropriate time interval, the polymer solutions were neutralized with dilute HCl solution and the water was removed by vacuum. ¹H NMR spectroscopy (D₂O) was performed to measure the degradation rate by determining the amount of intact ester groups and comparing with other non-hydrolyzable pendant groups as inner standards. The results are illustrated in FIG. 3.

Example 3

Representative Cationic Polymer Protein Adsorption and Release

[0237] The cationic polymers prepared as described in Example 1 were evaluated for protein adsorption by surface plasmon resonance (SPR).

[0238] Protein adsorption was measured with a custom-built SPR sensor, which is based on wavelength interrogation. A SPR chip was attached to the base of the prism, and optical contact was established using refractive index matching fluid (Cargille). A dual-channel flow cell with two independent parallel flow channels was used to contain liquid sample during experiments. A peristaltic pump (Ismatec) was utilized to deliver liquid sample to the two channels of the flow cell. Fibrinogen solution of 1.0 mg/mL in PBS (0.15M, pH 7.4) was flowed over the surfaces at a flow rate of 0.05 mL/min. A surface-sensitive SPR detector was used to monitor protein-surface interactions in real time. Wavelength shift was used to measure the change in surface concentration (mass per unit area). The results are illustrated in FIGS. 5A-5C.

Example 4

Representative Cationic Polymer Antimicrobial Properties

[0239] The cationic polymers prepared as described in Example 1 were evaluated for their antimicrobial properties.

[0240] *E. coli* K12 were first cultured in separate pure cultures overnight at 37° C. on LB agar plates, which was then incubated with shaking at 37° C. for 24 h. Cultures on agar plates can be used for two weeks, if kept at 4° C. Several colonies were used to inoculate 25 ml of LB (20 g/L). These

initial cultures were incubated at 37° C. with shaking at 100 rpm for 18 hours and were then used to inoculate a second culture of each species in 200 ml of appropriate medium. When the second suspended culture reached an optical density of 1.0 at 600 nm, bacteria were collected by centrifugation at 8,000×g for 10 min at 4° C. Cell pellets were washed three times with sterile phosphate buffered saline (PBS, pH 7.4) and subsequently suspended in PBS to a final concentration of 10⁸ cells/mL.

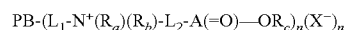
[0241] Exposure of bacterial cells to representative polymer solutions was started when the culture containing bacterial cells was added to above polymer suspension which was pre-equilibrated and shaken at 30° C., and the mixture were incubated at room temperature for 30 min. The final solution contains ca. 10⁸ cells/mL *E. coli* and 2 mM repeat unit concentration, which is the molar concentration of the repeat unit of the polymers (ca. 0.6-0.76 mg/mL based on molecular weight of CBAA and CBAA-esters). Bacteria were stained with Live/Dead BacLight™ (Invitrogen, USA), and bacterial suspension was subsequently filtered through a polycarbonate membrane filter with 0.2 μm pore size (Millipore, USA), and observed directly with a CCD-CoolSNAP camera (Roper scientific, Inc., USA) mounted on Nikon Eclipse 80i with 100× oil lens. The number of live and dead cells was determined, respectively, through FITC and Rhodamine filters with the same microscope described in Cheng, G.; Zhang, Z.; Chen, S.; Bryers, J. D.; Jiang, S., "Inhibition of Bacterial Adhesion and Biofilm Formation on Zwitterionic Surfaces," *Biomaterials* 28(29):4192-4199, 2007. The results are illustrated in FIG. 6.

[0242] While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

1. A wound dressing, comprising a cationic polymer comprising:

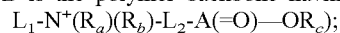
- (a) polymer backbone;
- (b) a plurality of cationic centers, each cationic center covalently coupled to the polymer backbone by a first linker;
- (c) a counter ion associated with each cationic center; and
- (d) a hydrolyzable group covalently coupled to each cationic center through a second linker, wherein the hydrolyzable group is hydrolyzable to an anionic center to provide a zwitterionic polymer having the anionic center covalently coupled to the cationic center through the second linker.

2. The wound dressing of claim 1, wherein the polymer has the formula:



wherein

PB is the polymer backbone having n pendant groups



N⁺ is the cationic center;

R_a and R_b are independently selected from hydrogen, alkyl, and aryl;

A(=O)—OR_c is the hydrolyzable group, wherein A is selected from the group consisting of C, S, SO, P, or PO, and R_c is an alkyl, aryl, acyl, or silyl group that may be further substituted with one or more substituents;

L₁ is a linker that covalently couples the cationic center to the polymer backbone;

- L_2 is a linker that covalently couples the cationic center to the hydrolyzable group;
 X^- is the counter ion associated with the cationic center; and
 n is an integer from about 10 to about 10,000.
3. The wound dressing of claim 1, wherein the counter ion is a hydrophobic organic counter ion.
4. The wound dressing of claim 1, wherein the counter ion is selected from the group consisting of C1-C20 carboxylates and C1-C20 alkylsulfonates.
5. The wound dressing of claim 1, wherein the counter ion is a therapeutic agent.
6. The wound dressing of claim 1, wherein the counter ion is selected from the group consisting of an antimicrobial, an antibacterial, and an antifungal agent.
7. The wound dressing of claim 1, wherein the counter ion is selected from the group consisting of amino acids, proteins, and peptides.
8. The wound dressing of claim 1, wherein the hydrolyzable group releases a hydrophobic organic group on hydrolysis.
9. The wound dressing of claim 1, wherein the hydrolyzable group releases a C1-C20 carboxylate on hydrolysis.
10. The wound dressing of claim 1, wherein the hydrolyzable group releases a therapeutic agent on hydrolysis.
11. The wound dressing of claim 1, wherein the hydrolyzable group releases an antimicrobial, an antibacterial, or an antifungal agent on hydrolysis.
12. The wound dressing of claim 1, wherein the cationic center is selected from the group consisting of ammonium, imidazolium, triazolium, pyridinium, morpholinium, oxazolidinium, pyrazinium, pyridazinium, pyrimidinium, piperazinium, and pyrrolidinium.
13. The wound dressing of claim 2, wherein R_a and R_b are independently selected from the group consisting of C1-C10 straight chain and branched alkyl groups.
14. The wound dressing of claim 2, wherein L_1 is selected from the group consisting of $-C(=O)O-(CH_2)_n-$ and $-C(=O)NH-(CH_2)_n-$, wherein n is an integer from 1 to 20.
15. The wound dressing of claim 2, wherein L_2 is $-(CH_2)_n-$, where n is an integer from 1 to 20.
16. The wound dressing of claim 2, wherein A is selected from the group consisting of C, SO, and PO.
17. The wound dressing of claim 2, wherein R_c is C1-C20 alkyl.

18. The wound dressing of claim 2, wherein R_c is an amino acid.
19. The wound dressing of claim 2, wherein X^- is selected from the group consisting of halide, carboxylate, alkylsulfonate, sulfate; nitrate, perchlorate, tetrafluoroborate, hexafluorophosphate, trifluoromethylsulfonate, bis(trifluoromethylsulfonyl)amide, lactate, and salicylate.
20. The wound dressing of claim 1, wherein the cationic polymer is a hydrogel.
21. The wound dressing of claim 20, wherein the hydrogel is a chemical hydrogel.
22. The wound dressing of claim 20, wherein the hydrogel is an interpenetrating network hydrogel.
23. The wound dressing of claim 20, wherein the hydrogel comprises first and second polymers, wherein the first polymer is a cationic polymer hydrolyzable to provide a zwitterionic polymer, and wherein the second polymer is a zwitterionic polymer.
24. The wound dressing of claim 23, wherein the first and second polymers are crosslinked.
25. The wound dressing of claim 23, wherein the hydrogel is prepared by copolymerizing a first cationic monomer having a hydrolyzable group and second zwitterionic monomer.
26. The wound dressing of claim 24, wherein the hydrogel is prepared by copolymerizing a first cationic monomer having a hydrolyzable group and second zwitterionic monomer.
27. The wound dressing of claim 25, wherein the hydrogel is prepared by polymerizing the first monomer to provide a cationic polymer having hydrolyzable groups, adding the second monomer to the cationic polymer, and polymerizing the second monomer in the presence of the cationic polymer.
28. The wound dressing of claim 25, wherein the hydrogel is prepared by polymerizing the second monomer to provide a zwitterionic polymer, adding the first monomer having a hydrolyzable group to the zwitterionic polymer, and polymerizing the first monomer in the presence of the zwitterionic polymer.
29. The wound dressing of claim 20, wherein the hydrogel is prepared by polymerizing a first cationic monomer having a hydrolyzable group to provide a cationic polymer having hydrolyzable groups, and hydrolyzing at least a portion of the hydrolyzable groups of the cationic polymer.
30. A method for treating a wound, comprising applying the wound dressing of claim 1 to a wound.

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