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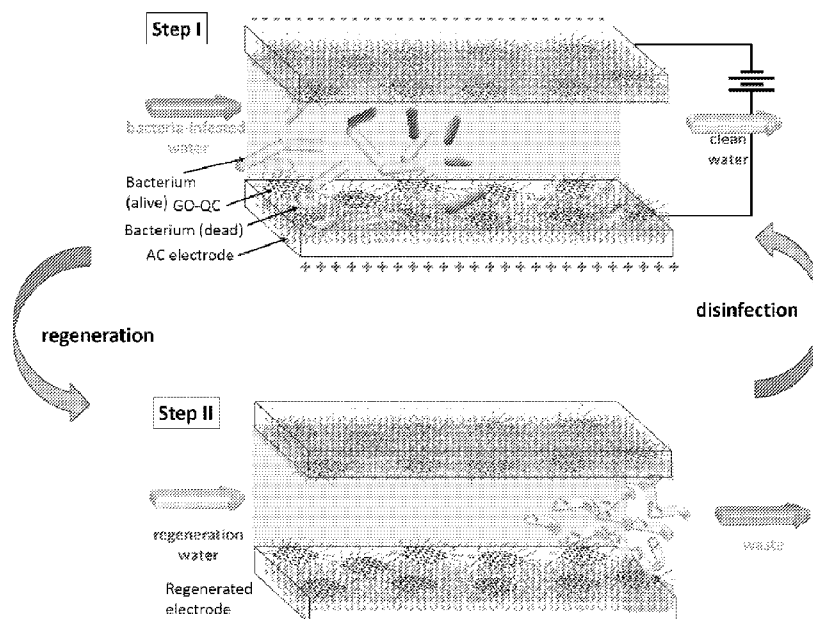
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(54) Title: ELECTRODE CONTAINING A HYBRID NANOMATERIAL OF GRAPHENE OXIDE NANOMATERIAL AND CATIONIC QUATERNIZED CHITOSAN

FIG. 1B



(57) Abstract: An electrode comprising an electrically conductive support and a layer of a hybrid nanomaterial attached to a surface of the electrically conductive support is provided. The hybrid nanomaterial consists of a graphene oxide nanomaterial covalently conjugated to cationic quaternized chitosan. An electrochemical cell comprising the electrode, and use of the electrode or electrochemical cell in a capacitive deionization process for killing microorganisms are also provided.



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ELECTRODE CONTAINING A HYBRID NANOMATERIAL OF GRAPHENE OXIDE NANOMATERIAL AND CATIONIC QUATERNIZED CHITOSAN

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of Singapore patent application No. 10201505863U filed on 28 July 2015, the content of which is incorporated herein by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] Various embodiments refer to an electrode, an electrochemical cell comprising the electrode, and use thereof for killing microorganisms in applications such as water disinfection.

BACKGROUND

[0003] The 2015 Global Risk Report published by the World Economic Forum (WEF) has identified scarcity of clean water to be the most serious risk facing the world, where approximately 750 million people worldwide do not have access to clean water.

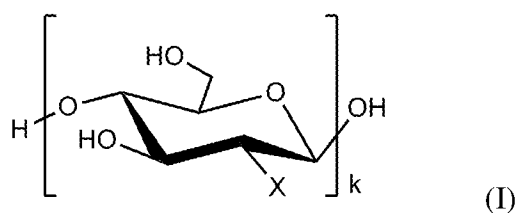
[0004] There is therefore a great demand for improved and effective water disinfection technologies. Although state of the art water disinfectant agents are able to annihilate pathogenic microorganisms in drinking water, they are generally toxic or produce side products which are harmful to human health. For example, disinfectants such as chlorine, phenol, peroxides and silver nanoparticles, which generally work by solvation into the bio-contaminated water, are toxic. Strong oxidizing agents such as hypochlorite and ozone which are used in water disinfection produce harmful by-products which may be carcinogenic, mutagenic or teratogenic. To aggravate the situation, multi-drug resistant bacteria which are difficult to treat are on the rise. At present, there is great demand for disinfection techniques which meet higher safety standards but are still effective towards a broad spectrum of pathogenic microbes. It has, however, been challenging to develop novel agents which effectively kill a broad spectrum of bacteria with zero or negligible contamination to the bio-contaminated water.

[0005] In view of the above, there remains a need for an improved disinfecting technology which may be used for water treatment that eliminates or at least alleviates one or more of the above-mentioned problems.

SUMMARY

[0006] In a first aspect, an electrode is provided. The electrode comprises

- a) an electrically conductive support; and
- b) a layer of a hybrid nanomaterial attached to a surface of the electrically conductive support, the hybrid nanomaterial consisting of graphene oxide nanomaterial covalently conjugated to cationic quaternized chitosan, wherein the cationic quaternized chitosan is represented by formula (I)



wherein

each X is independently selected from $-\text{NH}-\text{C}(\text{O})-\text{CH}_3$, $-\text{N}(\text{R}^1)(\text{R}^2)$ and $-\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$, provided that at least one X is $-\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$, R^1 , R^2 , R^3 , R^4 , and R^5 are independently selected from H and C_{1-18} alkyl, and k is an integer from 3 to 3000.

[0007] In a second aspect, an electrochemical cell comprising an electrode according to the first aspect is provided.

[0008] In a third aspect, use of an electrode according to the first aspect or an electrochemical cell according to the second aspect in a capacitive deionization process for killing microorganisms is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0010] **FIG. 1A** depicts chemical structure of quaternized chitosan (QC) and synthesis schematic of graphene oxide-quaternized chitosan (GO-QC) nanohybrid by amine coupling

reaction in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS).

[0011] **FIG. 1B** is a schematic diagram showing capacitive deionization disinfection (CDID) and regeneration of the graphene oxide-quaternized chitosan/activated carbon (GO-QC/AC) electrode for the continuous disruption of the anionic microbial envelopes which leads to microbe cell death.

[0012] **FIG. 2** depicts synthesis schematic of quaternized chitosan (dimethyldecylammonium chitosan).

[0013] **FIG. 3** is a graph showing gel permeation chromatograph (GPC) spectrum of synthesized quaternized chitosan (QC, specifically dimethyldecylammonium-chitosan).

[0014] **FIG. 4A** shows field emission scanning electron microscopy (FESEM) images of GO and GO-QC (1:5). Scale bar in the figure denotes 1 μm .

[0015] **FIG. 4B** shows atomic force microscopy (AFM) images of GO and GO-QC (1:5) with thickness determination. Scale bar in the figure denotes 500 nm.

[0016] **FIG. 4C** shows Fourier transform infrared spectroscopy (FTIR) spectra of GO, QC and GO-QC (1:5).

[0017] **FIG. 4D** shows thermogravimetric analysis (TGA) curves of (i) GO, (ii) GO-QC (1:5) and (iii) QC at a heating rate of 10 $^{\circ}\text{C}$ per min under nitrogen protection.

[0018] **FIG. 5A** is a scanning electron microscopy (SEM) image of GO. Scale bar in the figure denotes 1 μm .

[0019] **FIG. 5B** is a graph showing size distribution of GO. At least 100 nanoflakes were measured for each sample to obtain the average size and distribution.

[0020] **FIG. 6A** is a scanning electron microscopy (SEM) image of GO-QC (1:5). Scale bar in the figure denotes 1 μm .

[0021] **FIG. 6B** is a graph showing size distribution of GO-QC (1:5). At least 100 nanoflakes were measured for each sample to obtain the average size and distribution.

[0022] **FIG. 7** is a graph showing nuclear magnetic resonance spectroscopy (^1H NMR) of negatively charged chitosan (nCS) in D_2O .

[0023] **FIG. 8A** shows a microscope image of GO-QC flake conjugated with Texas-red dye under darkfield imaging. Scale bar in the figure denotes 10 μm .

[0024] **FIG. 8B** shows a microscope image of GO-QC flake conjugated with Texas-red dye under fluorescence imaging. Scale bar in the figure denotes 10 μm .

[0025] **FIG. 9A** is a graph depicting antimicrobial activity (1 h) of GO, QC and GO-QC (1:5) ($100\ \mu\text{g mL}^{-1}$) vs concentration of sodium chloride (NaCl).

[0026] **FIG. 9B** is a graph depicting antimicrobial activity (1 h) of GO, QC and GO-QC (1:5) ($100\ \mu\text{g mL}^{-1}$) vs concentration of potassium chloride (KCl).

[0027] **FIG. 9C** is a graph depicting antimicrobial activity (1 h) of GO, QC and GO-QC (1:5) ($100\ \mu\text{g mL}^{-1}$) vs concentration of calcium chloride (CaCl_2).

[0028] **FIG. 9D** is a graph depicting antimicrobial activity (1 h) of GO, QC and GO-QC (1:5) ($100\ \mu\text{g mL}^{-1}$) vs concentration of magnesium chloride (MgCl_2).

[0029] **FIG. 10A** is an optical micrograph of a mixture of *S. aureus* and QC.

[0030] **FIG. 10B** is an optical micrograph of a mixture of *S. aureus* and GO. Arrows point to bacteria in suspension.

[0031] **FIG. 11A** is an optical micrograph of *S. aureus* attachment to a piece of GO-QC. A larger piece of GO-QC (chosen for better visualization) is shown with the attached bacteria.

[0032] **FIG. 11B** is an optical micrograph of *S. aureus* attachment to a different piece of GO-QC from that in **FIG. 11A**.

[0033] **FIG. 11C** is a fluorescence micrograph of *S. aureus* attachment to the same piece of GO-QC as that in **FIG. 11B**. Bacteria staining with BacLight bacterial viability kit indicates that the attached bacteria on GO-QC were dead (indicated by red color).

[0034] **FIG. 12A** is a FESEM image of *E. coli* cells immediately (0 min) after contact with GO-QC (1:5).

[0035] **FIG. 12B** is a FESEM image of *E. coli* cells after 1 h contact with GO-QC (1:5). As can be seen, the morphology of *E. coli* cells was changed.

[0036] **FIG. 12C** is a FESEM image of *E. coli* cells, wherein dead cells were separated from the GO-QC nanohybrids by centrifugation. Major damages to bacteria cell walls are indicated with arrows.

[0037] **FIG. 13A** is a graph showing fraction of microbes detached from pre-challenged GO-QC (1:5) determined by measuring the optical density of detached microbes after centrifuging at different Relative Centrifugation Forces (RCF). The absorbance is normalized to the initial concentration of microbes added to GO-QC.

[0038] **FIG. 13B** is a graph showing Zeta potentials of the various microbes.

[0039] **FIG. 14A** shows atomic force microscopy (AFM) image of GO in small size, the height curve and lateral dimension histograms of GO in small size. The scale bar denotes $1\ \mu\text{m}$.

[0040] **FIG. 14B** shows AFM image of GO in medium size, the height curve and lateral dimension histograms of GO in medium size. The scale bar denotes 1 μm .

[0041] **FIG. 14C** shows AFM image of GO in large size, the height curve and lateral dimension histograms of GO in large size. The scale bar denotes 1 μm .

[0042] **FIG. 14D** is a graph showing average size value of GO in small/medium/large size. (30 pieces of GO were measured to calculate the average size and standard deviation for each sample.)

[0043] **FIG. 15A** is a graph showing % kill vs. concentration of GO-QC for *S. aureus* for the three different sizes of GO-QC and polystyrene-quaternized chitosan (PS-QC).

[0044] **FIG. 15B** shows AFM of the different particles, GO-QC (Small), GO-QC (Medium), GO-QC (Large) and PS-QC, as well as their effect on bacteria morphology. Scale bar represents 1 μm and the totally lysed bacteria are outlined in black.

[0045] **FIG. 16** is a graph showing TGA of (i) polystyrene spheres and (ii) PS-QC.

[0046] **FIG. 17A** is a schematic diagram showing probability of GO-QC (Small) in bacteria contact at concentrations lower than the minimum bactericidal concentrations (MBC).

[0047] **FIG. 17B** is a schematic diagram showing probability of GO-QC (Large) in bacteria contact at concentrations lower than the minimum bactericidal concentrations.

[0048] **FIG. 18A** is a X-ray photoelectron spectroscopy (XPS) wide scan of GO.

[0049] **FIG. 18B** is a XPS wide scan of GO-QC.

[0050] **FIG. 18C** is a XPS high resolution C1s spectra of GO.

[0051] **FIG. 18D** is a XPS high resolution C1s spectra of QC.

[0052] **FIG. 18E** is a XPS high resolution C1s spectra of GO-QC.

[0053] **FIG. 18F** is a XPS high resolution O1s spectra of GO.

[0054] **FIG. 18G** is a XPS high resolution O1s spectra of QC.

[0055] **FIG. 18H** is a XPS high resolution O1s spectra of GO-QC.

[0056] **FIG. 18I** is a XPS high resolution N1s spectra of QC.

[0057] **FIG. 18J** is a XPS high resolution N1s spectra of GO-QC.

[0058] **FIG. 19A** is a graph showing killing rate of microbes after incubation with GO, QC and GO-QC series ($100 \mu\text{g mL}^{-1}$) for 1 h at 10^8 CFU mL^{-1} .

[0059] **FIG. 19B** is a graph showing minimum bactericidal concentrations (MBC).

[0060] **FIG. 19C** is a graph showing antimicrobial activity of GO-QC (1:5) ($100 \mu\text{g mL}^{-1}$) in deionized (DI) water and NaCl solution (100 mg L^{-1} and 200 mg L^{-1}).

[0061] **FIG. 19D** is a graph showing selectivity (HC_{50}/MBC).

[0062] **FIG. 19E** is a graph showing *in vitro* cytotoxicity study of fibroblast cells cultured with $100\text{ }\mu\text{g mL}^{-1}$ GO-QC (1:5). The cell viability was determined by Cell Counting Kit-8 (CCK-8) assay; tissue culture polystyrene (TCPS) was used as control. ($p>0.05$, no significant difference).

[0063] **FIG. 19F** shows LIVE/DEAD assay (using calcein-AM and ethidium homodimer-1) of fibroblast cells cultured (i) without and (ii) with $100\text{ }\mu\text{g mL}^{-1}$ GO-QC (1:5) for 7 days.

[0064] **FIG. 20** is a graph showing time dependence of antimicrobial activity (killing curve) was investigated by varying the incubation time of *E. coli* with GO, QC or GO-QC (1:5) dispersions ($100\text{ }\mu\text{g mL}^{-1}$) from 0.5 h to 24 h. The % kill of *E. coli* by GO, QC and GO-QC increased monotonically with incubation time. QC and GO-QC (1:5) produced high % kill quickly and reached nearly 100 % after 4 h. The % kill of GO increased more gradually and plateaued beyond 12 h in the range of 70 % kill.

[0065] **FIG. 21** is a schematic diagram of the continuous water purification and disinfection (Step I) and regeneration (Step II) of the CDID system.

[0066] **FIG. 22A** is a FESEM image for a pristine AC electrode (control).

[0067] **FIG. 22B** is a FESEM image for QC/AC electrode according to an embodiment of fabricated disinfecting nanohybrid electrode disclosed herein.

[0068] **FIG. 22C** is a FESEM image for GO-QC/AC electrode according to an embodiment of fabricated disinfecting nanohybrid electrode disclosed herein.

[0069] **FIG. 22D** is a FESEM image for GO-QC/AC electrode according to a further embodiment of fabricated disinfecting nanohybrid electrode disclosed herein.

[0070] **FIG. 22E** is a FESEM image for high magnification junctions of GO-QC to AC bound by binder (PVDF) according to an embodiment of fabricated disinfecting nanohybrid electrode disclosed herein.

[0071] **FIG. 22F** is a FESEM image for high magnification junctions of GO-QC to AC bound by binder (PVDF) according to a further embodiment of fabricated disinfecting nanohybrid electrode disclosed herein.

[0072] **FIG. 23A** depicts GO-QC/AC CDID cell performance, where a disinfection performance plot of AC (reference) and GO-QC/AC electrodes is shown. *E. coli* concentration is 10^6 CFU mL^{-1} .

[0073] **FIG. 23B** depicts GO-QC/AC CDID cell performance, where regeneration efficiency of GO-QC/AC electrode is shown. *E. coli* concentration is 10^6 CFU mL⁻¹.

[0074] **FIG. 23C** depicts GO-QC/AC CDID cell performance, where disinfection performance plot of GO-QC/AC electrode with lower *E. coli* concentration of 10^4 CFU mL⁻¹ is shown.

[0075] **FIG. 23D** depicts GO-QC/AC CDID cell performance, where desalination capacity of AC and GO-QC/AC electrode in NaCl solution (100 mg L^{-1} and 200 mg L^{-1}) is shown. *E. coli* concentration is 0 CFU mL⁻¹.

[0076] **FIG. 23E** depicts GO-QC/AC CDID cell performance, where disinfection performance plot of CDID with GO-QC/AC electrodes in DI water and NaCl solution (100 mg L^{-1} and 200 mg L^{-1}) is shown. *E. coli* concentration is 10^6 CFU mL⁻¹.

[0077] **FIG. 23F** depicts GO-QC/AC CDID cell performance, where regeneration efficiency of CDID with GO-QC/AC electrode in DI water and NaCl solution (100 mg L^{-1} and 200 mg L^{-1}) is shown. *E. coli* concentration is 10^6 CFU mL⁻¹.

[0078] **FIG. 24** is a graph showing UV-vis absorption of water exiting out of the GO-QC/AC CDID cell after 10 min and 1 h. Reference used is GO (concentration is 0.15 mg L^{-1}).

[0079] **FIG. 25A** shows morphology of *E. coli* for (i) untreated control, treated with (ii) QC, (iii) GO and (iv) GO-QC (1:5) at $100\text{ }\mu\text{g mL}^{-1}$ for 1 h.

[0080] **FIG. 25B** shows adenosine triphosphate (ATP) leakage induced by QC and GO-QC (1:5).

[0081] **FIG. 25C** shows Zeta potential of GO, QC and GO-QC series.

[0082] **FIG. 25D** depicts a fluorescence study, in which the bacteria (*E. coli*) pellet separated by centrifugation was stained after contact with QC solution, but not stained after contact with GO-QC. Both GO-QC and QC molecules were conjugated with a fluorescence dye (Texas Red-X).

[0083] **FIG. 26A** is a FESEM image of the GO-QC/AC electrode after disinfection process according to an embodiment.

[0084] **FIG. 26B** is a FESEM image of the GO-QC/AC electrode after disinfection process according to an embodiment.

[0085] **FIG. 26C** is a FESEM image of the GO-QC/AC electrode after regeneration process according to an embodiment.

[0086] **FIG. 26D** is a FESEM image of the GO-QC/AC electrode after regeneration process according to an embodiment.

DETAILED DESCRIPTION

[0087] Various embodiments refer in a first aspect to an electrode. The electrode comprises an electrically conductive support and a layer of a hybrid nanomaterial attached to a surface of the electrically conductive support. The hybrid nanomaterial consists of graphene oxide nanomaterial which is covalently bonded to cationic quaternized chitosan. Advantageously, the hybrid nanomaterial possesses a synergistic effect where antimicrobial efficacy of the hybrid nanomaterial is superior to its constituent components of graphene oxide and quaternized chitosan. The hybrid nanomaterial has demonstrated a broad-spectrum antimicrobial activity for microorganisms, such as Gram-negative bacteria, Gram-positive bacteria, and fungi. Further, the chitosan groups confer good biocompatibility properties on the hybrid nanomaterial as demonstrated by its reduced hemolytic activity.

[0088] The hybrid nanomaterial is able to confer surface electrical conductivity to the electrode even though cationic quaternized chitosan is electrically non-conductive, as the graphene oxide nanomaterial component of the hybrid nanomaterial is electrically conductive. The electrode may be used in an electrochemical cell, such as in a capacitive deionization process for killing microorganisms such as Gram-negative bacteria, Gram-positive bacteria, and fungi. In embodiments disclosed herein, the electrode has demonstrated at least 99.9999 % killing (i.e. 6-log reduction) of *E. coli* in water flowing continuously through a capacitive deionization disinfection cell.

[0089] Advantageously, as the hybrid nanomaterial is attached to the electrically conductive support, risks of contamination due to leaching of the hybrid nanomaterial to the fluid stream is mitigated. Use of the electrode or electrochemical cell disclosed herein in a capacitive deionization process for killing microorganisms provides an economical method towards ultrafast, contaminant-free, and continuous killing of pathogens in bio-contaminated water, and offers a new approach for a novel green *in-situ* disinfectant system.

[0090] As used herein, the term “hybrid nanomaterial” refers to a nanoscale material formed from at least two components that are connected to one another by one or more chemical bonds, and having a functional and/or a structural property that is different from that of the individual

components. Nanomaterial, otherwise termed herein as nanoscale material, refers to a material having at least one dimension that is in the nanometer range.

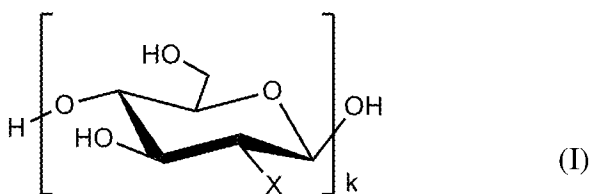
[0091] The hybrid nanomaterial consists of graphene oxide nanomaterial that is covalently conjugated to cationic quaternized chitosan.

[0092] The term “graphene” as used herein refers generally to a form of graphitic carbon, in which carbon atoms are covalently bonded to one another to form a two-dimensional sheet of bonded carbon atoms. The carbon atoms may be bonded to one another via sp^2 bonds, and may form a 6-membered ring as a repeating unit, and may further include a 5-membered ring and/or a 7-membered ring. In its crystalline form, two or more sheets of graphene may be stacked together to form multiple stacked layers. Generally, the side ends of graphene are saturated with hydrogen atoms.

[0093] Graphene oxide (GO) refers to oxidized forms of graphene, and may include an oxygen-containing group such as a hydroxyl group, an epoxide group, a carboxyl group, and/or a ketone group. The term “graphene oxide” also includes reduced graphene oxide, which are reduced forms of graphene oxide, such as graphene oxide that has been subjected to a reduction process, thereby partially or substantially reducing it.

[0094] The graphene oxide nanomaterial is covalently conjugated to cationic quaternized chitosan, wherein the cationic quaternized chitosan is represented by formula (I)

[0095]



[0096] wherein each X is independently selected from $-NH-C(O)-CH_3$, $-N(R^1)(R^2)$ and $-N^+(R^3)(R^4)(R^5)$, provided that at least one X is $-N^+(R^3)(R^4)(R^5)$, R^1 , R^2 , R^3 , R^4 , and R^5 are independently selected from H and C_{1-18} alkyl, and k is an integer from 3 to 3000.

[0097] The term “chitosan”, also referred to as poly-D-glucosamine or polyglucosamine, refers to a biopolymer derived from chitin that consists of β -1,4-glykosidic linked glucosamine and, optionally, N-acetylglucosamine residues (2-acetamido-2-desoxy- β -D-glukopyranose residues), wherein the ratio of glucosamine to N-acetylglucosamine residues is greater than 1,

i.e. the ratio of monomers with $X = -N(R^1)(R^2)$ and $-N^+(R^3)(R^4)(R^5)$ to those with $X = -NH-C(O)-CH_3$ is greater than 1.

[0098] Chitosan has good biodegradability, biocompatibility, and antimicrobial activity, which render its usefulness for biomedical applications. Quaternized chitosan, also referred to herein as quaternary ammonium chitosan, refers to a derivative of chitosan that is prepared by introducing a quaternary ammonium group on a dissociative hydroxyl group or amino group of the chitosan. As a consequence of the quaternization of the amino group, quaternized chitosan possess a permanent positive charge on the polysaccharide backbone. Due to this permanent positive charge, quaternized chitosan may also be termed as cationic quaternized chitosan.

[0099] Referring to formula (I), each X in the formula is independently selected from $-NH-C(O)-CH_3$, $-N(R^1)(R^2)$ and $-N^+(R^3)(R^4)(R^5)$, provided that at least one X is $-N^+(R^3)(R^4)(R^5)$, R^1 , R^2 , R^3 , R^4 , and R^5 are independently selected from H and C_{1-18} alkyl, and k is an integer from 3 to 3000.

[00100] The term " C_1-C_{18} alkyl" refers to a fully saturated aliphatic hydrocarbon having 1 to 18 carbon atoms, e.g. it means that the alkyl group comprises 1 carbon atom, 2 carbon atoms, 3 carbon atoms etc. up to and including 18 carbon atoms. The C_1-C_{18} alkyl group may be straight chain or branched chain, and may be substituted or unsubstituted. Exemplary substituents include, but are not limited to, C_{1-6} aliphatic group, hydroxy, alkoxy, cyano, halogen group, nitro, silyl, and amino, including mono- and di-substituted amino groups. Specific exemplary substituents include C_1-C_{10} alkoxy, C_5-C_{10} aryl, C_5-C_{10} aryloxy, sulfhydryl, C_5-C_{10} aryl, thio, halogen such as F, Cl, Br, I, hydroxyl, amino, sulfonyl, nitro, cyano, and carboxyl. Examples of alkyl groups may be, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, t-butyl, n-hexyl, n-heptyl, n-octyl, n-nonyl or n-decyl and the like.

[00101] Referring to formula (I), k is an integer from 3 to 3000. For example, k may be an integer from 3 to 2500, 3 to 2000, 3 to 1500, 3 to 1000, 3 to 500, 100 to 2500, 500 to 3000, 500 to 2000, 1000 to 3000, 1000 to 2000, 1500 to 3000, 2000 to 3000, or 2500 to 3000.

[00102] In various embodiments, R^1 and R^2 are selected from H and C_{1-18} alkyl, preferably H; and R^3 , R^4 , and R^5 are each independently C_{1-10} alkyl. In specific embodiments, R^1 and R^2 are H, and R^3 , R^4 , and R^5 are each independently C_{1-10} alkyl.

[00103] In various embodiments, R^3 and R^4 are methyl and R^5 is C_{1-10} alkyl, preferably methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl or n-decyl. In specific embodiments, R^3 and R^4 are methyl and R^5 is selected from the group consisting of methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and n-decyl.

[00104] The ratio of monomers with $X = -N(R^1)(R^2)$ and $X = -N^+(R^3)(R^4)(R^5)$ to monomers with $X = -NH-C(O)-CH_3$ may be in the range of 2:1 to 5:1, preferably about 4:1. For example, the ratio of monomers with $X = -N(R^1)(R^2)$ and $X = -N^+(R^3)(R^4)(R^5)$ to monomers with $X = -NH-C(O)-CH_3$ may be in the range of about 2:1 to about 4:1, about 2:1 to about 3:1, about 3:1 to about 4:1, or about 3:1 to about 5:1; about 2:1, about 3:1, about 4:1, or about 5:1. In specific embodiments, the ratio of monomers with $X = -N(R^1)(R^2)$ and $X = -N^+(R^3)(R^4)(R^5)$ to monomers with $X = -NH-C(O)-CH_3$ is about 4:1.

[00105] The ratio of monomers with $X = -N(R^1)(R^2)$ to monomers with $X = -N^+(R^3)(R^4)(R^5)$ is in the range of 1:4 to 4:1, preferably about 1:2 to 1:1. For example, the ratio of monomers with $X = -N(R^1)(R^2)$ to monomers with $X = -N^+(R^3)(R^4)(R^5)$ may be in the range of about 1:3 to about 4:1, about 1:2 to about 4:1, about 1:2 to about 3:1, about 1:2 to about 2:1, about 1:2 to about 1:1; about 1:2, or about 1:1. In specific embodiments, the ratio of monomers with $X = -N(R^1)(R^2)$ to monomers with $X = -N^+(R^3)(R^4)(R^5)$ is in the range of about 1:2 to about 1:1.

[00106] The graphene oxide nanomaterial is covalently conjugated to the cationic quaternized chitosan. The term “covalently conjugated” refers to formation of one or more covalent bonds between the graphene oxide nanomaterial and the cationic quaternized chitosan. Cationic quaternized chitosan of formula (I) may, for example, be reacted with graphene oxide nanomaterial in the presence of a coupling reagent to covalently bond the cationic quaternized chitosan to the graphene oxide nanomaterial.

[00107] The coupling reagent may be any suitable compound that is able to covalently bind the cationic quaternized chitosan to the graphene oxide nanomaterial. In various embodiments, the coupling reagent is a carbodiimide compound. The term “carbodiimide compound” as used herein refers to a water-soluble organic compound having at least one carbodiimide functional group of formula $-N=C=N-$. In specific embodiments, the coupling reagent comprises 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

[00108] Any suitable amount of carbodiimide compound that allows covalently binding of the cationic quaternized chitosan to the graphene oxide nanomaterial may be used. In various embodiments, concentration of the carbodiimide compound is in the range of about 100 mM

to about 2000 mM, such as about 100 mM to about 1500 mM, about 100 mM to about 1000 mM, about 100 mM to about 500 mM, about 500 mM to about 2000 mM, about 500 mM to about 1500 mM, about 500 mM to about 1000 mM, about 1000 mM to about 2000 mM, about 1000 mM to about 1500 mM, about 500 mM to about 1500 mM, or about about 1000 mM to about 1500 mM.

[00109] In embodiments where the coupling reagent comprises 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, the reaction to covalently bind cationic quaternized chitosan to the graphene oxide nanomaterial may be carried out in the presence of N-hydroxysuccinimide. Concentration of the N-hydroxysuccinimide may be in the range of about 100 mM to about 2000 mM. Advantageously, N-hydroxysuccinimide acts as a stabilizer to stabilize active intermediates that are formed during the reaction. The coupling agent comprising 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide may react with N-hydroxysuccinimide to form an amide bond between the cationic quaternized chitosan and the graphene oxide nanomaterial.

[00110] As mentioned above, antimicrobial efficacy of the hybrid nanomaterial is superior to its constituent components of graphene oxide and quaternized chitosan. By conjugating graphene oxide with cationic quaternized chitosan, antimicrobial efficacy of pristine graphene oxide is improved. Without wishing to be bound by theory, it is postulated that cationic charge on the quaternized chitosan results in an electrostatic driven contact with the microbial cell envelope which is anionic. Attraction between the cationic hybrid nanomaterial and the anionic microbial cells promote incidence of contact or collision of the hybrid nanomaterial with the microbial cells. In so doing, membrane of the microbial cells may be ruptured by the sharp edges of the graphene oxide nanomaterial. The loss of membrane integrity and leakage of inner components lead to eventual cell death, and result in improved antimicrobial efficacy of the hybrid nanomaterial. Moreover, the quaternized chitosan confers good biocompatibility properties on the hybrid nanomaterial.

[00111] The improvement in antimicrobial efficacy may also be effected by improvements in killing efficacy and stability of the hybrid nanomaterial due to increase in grafting density of quaternized chitosan on the graphene oxide nanomaterial. The increase in grafting density may be attributed to a large surface to volume ratio of the graphene oxide nanomaterial. Furthermore, the hybrid nanomaterial in its particulate form may be more effective than state

of the art antimicrobial agents in solution form as charges on the particulate hybrid nanomaterial are concentrated.

[00112] In one embodiment, the cationic quaternized chitosan comprises or consists essentially of dimethyldodecylammonium chitosan having general formula (II)

[00113]



[00114] wherein R is selected from the group consisting of $-\text{CH}_2(\text{CH}_2)_8\text{CH}_3$ and $-\text{CH}_3$; and ratio of m:n:p is 3:5:2.

[00115] The weight ratio of the graphene oxide nanomaterial to the cationic quaternized chitosan in the hybrid nanomaterial may be in the range of about 1:1 to about 1:10. For example, the weight ratio of graphene oxide nanomaterial to cationic quaternized chitosan in the hybrid nanomaterial may be in the range of about 1:2 to about 1:3, about 1:2 to about 1:2.8, about 1:2 to about 1:2.5, about 1:2 to about 1:2.3, about 1:2.05 to about 1:2.25, or about 1:2.05 to about 1:2.2, about 1:2.07, about 1:2.1, about 1:2.15, or about 1:2.2. In specific embodiments, the weight ratio of graphene oxide nanomaterial to cationic quaternized chitosan in the hybrid nanomaterial is in the range of about 1:2.05 to about 1:2.2.

[00116] The hybrid nanomaterial is present as a layer attached to a surface of an electrically conductive support. The electrically conductive support may be a porous support. Generally, the electrically conductive support may be formed of any electrically conductive material. In various embodiment, the electrically conductive support comprises or is formed entirely of a material selected from the group consisting of activated carbon, graphite, conductive carbon black, conductive carbon fibers, intrinsically conductive polymers such as polypyrrole or polyaniline, and combinations thereof.

[00117] In specific embodiments, the electrically conductive support comprises activated carbon. Advantageously, activated carbon is electrically conductive and possesses a high surface area. In applications such as capacitive deionization, the high surface area of activated carbon functions advantageously to allow adsorption of ions of salt thereon.

[00118] The electrically conductive support comprising activated carbon may be prepared by dispersing activated carbon and a binder in a solvent such as anhydrous N,N-

dimethylformamide (DMF) to form a slurry, and removing the solvent from the slurry to obtain the electrically conductive support.

[00119] The term “binder” as used herein refers to a substance that is capable of holding or attaching two or more materials together. By dispersing the activated carbon with the binder in a solvent to form a slurry, the activated carbon particles may be held together by the binder to form a porous support. Examples of a binder that may be used include, but are not limited to, polyvinylidene fluoride (PVDF), styrene-butadiene rubber (SBR), carboxymethyl cellulose (CMC), polytetrafluoroethylene (PTFE), poly(vinyl alcohol), poly(vinyl acetate), combinations thereof, and copolymers thereof. One or more of the above-mentioned binders may, for example, be further modified into a copolymer for crosslinking to render it non-water soluble after heat treatment.

[00120] Choice of binder may depend on the intended application of the electrode, such as temperature and pH of the environment at which the electrode is placed. Type and amount of binder used may in turn affect the electrochemical characteristics and surface structures of the resulting electrode. In various embodiments, the binder comprises or is formed entirely of polyvinylidene fluoride (PVDF). Advantageously, PVDF provides good binding properties as well as good electrochemical stability.

[00121] Removal of the solvent from the slurry may be carried out by drying. In some embodiments, the slurry is physically agitated by stirring, for example, to improve on uniformity in composition of the slurry. The slurry may be applied on a substrate surface prior to solvent removal to form the electrically conductive support.

[00122] The hybrid nanomaterial may be attached to a surface of the electrically conductive support by a binder. Examples of suitable binder have already been discussed above. In various embodiments, the binder comprises or is formed entirely of polyvinylidene fluoride (PVDF). Weight ratio of the hybrid nanomaterial to the binder may be in the range of about 9:1 to about 4:1. In specific embodiments, weight ratio of the hybrid nanomaterial to the binder is about 9:1.

[00123] In various embodiments, the hybrid nanomaterial is formed as a layer on the electrically conductive support by dispersing the hybrid nanomaterial with a binder in a solvent such as N,N-dimethylformamide (DMF) to form a slurry, applying the slurry on a surface of the electrically conductive support to form a layer, and removing solvent from the applied slurry.

[00124] Advantageously, physical dimensions of the hybrid nanomaterial may be laterally large such that it is able to form a particulate coating on the electrically conductive support without penetrating into the voids of the support to cause malfunctioning of the electrode. By grafting the hybrid nanomaterial to the electrically conductive support to form the electrode, it also eliminates or at least alleviates issues during application such as in water treatment faced by conventional antimicrobial agents which release biocides such as Ag ions and phosphonium salts into the treated water, where presence of the biocides render them unsuitable for disinfecting water and where they pose environmental hazards in long-term use. Grafting of the hybrid nanomaterial to the electrically conductive support also avoids issues arising due to nano-scale thickness of GO-based hybrid nanomaterials which makes their suspension in water relatively stable and their recovery from water highly challenging. For example, it may be impractical to recover GO-based hybrid nanomaterials after their dispersion into large water volumes by filtration or centrifugation.

[00125] Generally, the electrode disclosed herein may be of any suitable size or dimension. In various embodiments, the electrode has an elongated shape, such as a bar shape. To obtain the elongated shape of the electrode, the electrically conductive support comprised in the electrode may have a corresponding elongated shape or bar shape having a first surface and an opposing second surface. The term “opposing” is used herein to describe an orientation of the second surface with respect to the first surface, whereby the second surface is positioned on a reverse or opposite side to the first surface.

[00126] The layer of a hybrid nanomaterial may be attached to the first surface of the electrically conductive support. Thickness of the electrode comprising the electrically conductive support and layer of hybrid nanomaterial may be in the range of about 100 μm to about 200 μm . For example, thickness of the electrode may be about 120 μm to about 200 μm , about 150 μm to about 200 μm , about 100 μm to about 180 μm , about 100 μm to about 150 μm , about 120 μm to about 180 μm , or about 150 μm .

[00127] The layer of hybrid nanomaterial attached to the first surface of the electrically conductive support may have a thickness in the range of about 5 μm to about 50 μm , such as about 10 μm to about 50 μm , about 20 μm to about 50 μm , about 5 μm to about 30 μm , about 5 μm to about 20 μm , about 10 μm to about 40 μm , about 15 μm to about 25 μm , or about 20 μm .

[00128] In some embodiments, the electrode further comprises a second layer of a hybrid nanomaterial, which may be arranged on and attached to the opposing second surface of the electrically conductive support. The hybrid nanomaterial attached to the first surface and the second surface of the electrically conductive support may be the same or different. In some embodiments, the hybrid nanomaterial attached to the first surface of the electrically conductive support is the same as the hybrid nanomaterial attached to the second surface of the electrically conductive support.

[00129] In embodiments wherein the electrode comprises two layers of the hybrid nanomaterial, each layer being arranged on opposing sides of the electrically conductive support, the thickness ranges indicated above may refer to the combined thickness of the two layers of the hybrid nanomaterial.

[00130] In a second aspect, an electrochemical cell comprising an electrode according to the first aspect is provided.

[00131] The term “electrochemical cell” or “cell” refers to a device that converts chemical energy into electrical energy, or electrical energy into chemical energy. Generally, electrochemical cells have two or more electrodes and an electrolyte, wherein electrode reactions occurring at the electrode surfaces result in charge transfer processes. Examples of electrochemical cells include, but are not limited to, batteries and electrolysis systems.

[00132] The electrochemical cell comprises an anode and a cathode. The terms “anode” and “negative electrode” are used interchangeably, and refer to the electrode having the lower of electrode potential in an electrochemical cell (i.e. lower than the positive electrode). Conversely, the terms “cathode” and “positive electrode” are used interchangeably, and refer to the electrode having the higher of electrode potential in an electrochemical cell (i.e. higher than the negative electrode).

[00133] The electrode according to the first aspect may independently be an anode and/or a cathode of the electrochemical cell. By the term “independently”, it is meant that the anode and the cathode may be the same or different. For example, the anode and the cathode may have the same or a different composition and/or dimensions.

[00134] In some embodiments, the anode and the cathode of the electrochemical cell disclosed herein are the same. Accordingly, the anode and the cathode in the electrochemical cell may be termed as symmetric electrodes, whereby the term “symmetric electrodes” refers two electrodes having the same composition, i.e. they are made from the same materials. Such

electrochemical cells may also be termed as symmetrical electrochemical cells. In various embodiments, the electrochemical cell is a symmetrical electrochemical cell.

[00135] The electrode according to the first aspect, or the electrochemical cell according to the second aspect may be used in a capacitive deionization process for killing microorganisms.

[00136] The terms “microorganism” and “microbe” are used interchangeably herein, and refer to an organism that is unicellular or lives in a colony of cellular organisms such as bacteria, fungi, protest, or archea. For example, the microorganisms may be selected from the group consisting of Gram-positive bacteria, Gram-negative bacteria, fungus, and combinations thereof.

[00137] The term “gram-positive bacteria” refers to bacterial cells which stain violet (positive) in the Gram stain assay. The Gram stain binds peptidoglycan which is abundant in the cell wall of gram-positive bacteria. In contrast thereto, the cell wall of “gram-negative bacteria” is low in peptidoglycan, thus gram-negative bacteria adopt the counterstain in the gram stain assay. The bacteria may, for example, be of the genus *Acinetobacter*, *Actinomyces*, *Aeromonas*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Campylobacter*, *Chlamydia*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Erwinia*, *Escherichia*, *Francisella*, *Haemophilus*, *Helicobacter*, *Klebsiella*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Veillonella*, *Vibrio* or *Yersinia*. In specific embodiments, the bacteria is selected from the group consisting of *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Klebsiella pneumonia*, *Aeromonas hydrophila*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Escherichia coli*.

[00138] The fungus may be of the species *Candida albicans*, *Candida tropicalis*, *Candida (Clasvispora) lusitaniae*, *Candida (Pichia) guilliermondii*, *Lodderomyces elongisporus*, *Debaryomyces hansenii*, *Pichia stipitis*, *Asperigillus fumigatus*, *Blastomyces dermatitidis*, *Cladophialophora bantiana*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Fusarium spp.*, *Microsporum spp.*, *Penicillium marneffeii* or *Trichophyton spp.*

[00139] In specific embodiments, the microorganisms are selected from the group consisting of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and combinations thereof.

[00140] As mentioned above, the electrode and/or the electrochemical cell disclosed herein may be used in a capacitive deionization process for killing microorganisms.

[00141] The term “capacitive deionization process” as used herein refers generally to a process involving continuous flow of a liquid between two oppositely charged electrodes. It may be used in water desalination, for example, to remove ions from an aqueous solution by ion electrosorption on the electrodes, where a low electrical potential of about 2 V may be applied across the electrodes for capture of the ions. To regenerate the electrodes, polarity of the electrodes may be reversed so that the adsorbed ions are desorbed from the electrodes, and which may be taken by the liquid flowing between the electrodes. By alternating between electrosorption and desorption, the capacitive deionization process is able to function as a continuous process, different from a batch process that requires system downtime for regeneration of the electrodes, for example.

[00142] In embodiments disclosed herein, the electrode and/or the electrochemical cell disclosed herein are used in a capacitive deionization process for killing microorganisms. As mentioned above, membrane of the microbial cells may be ruptured by the sharp edges of the graphene oxide nanomaterial upon contact with the hybrid nanomaterial. This forms a type of contact-active disinfecting mechanism in which microorganisms are killed on contact by physical disruption of the anionic microbe cytoplasmic membrane, and where the loss of membrane integrity and leakage of inner components leads to eventual cell death.

[00143] Accordingly, by using an electrode or an electrochemical cell disclosed herein in a capacitive deionization process, microorganisms such as bacteria having negatively charged surfaces may be adsorbed on the positive electrode. Upon contact with the layer of hybrid nanomaterial present on a surface of the electrode, the microorganisms may be killed. This provides an improvement over state of the art water treatment processes involving capacitive deionization, whereby the microorganisms are merely adsorbed on the electrode and are not killed.

[00144] As more and more microorganisms are adsorbed on the electrode, the electrode may become saturated with the microorganisms. The point at which the electrode is saturated with microorganisms may be detected by presence of microorganisms in the outflow liquid. To regenerate the electrode, voltage applied across the electrodes may be reversed or turned off, while water continues to flow through the system between the electrodes. As a result, the microorganisms which are adsorbed on a surface of the positive electrode may be washed off, hence removed, from the electrode, thereby regenerating it.

[00145] Advantageously, the capacitive deionization process disclosed herein provides a continuous disinfection process with low power requirement and high regeneration potential. Furthermore, it does not require use of a membrane, and non-hazardous chemical are used in the pre-/post-treatment stages. It has also been demonstrated herein that the hybrid nanomaterial has ability to retain antimicrobial activity in the presence of salt, which renders it suitable for use in ionic environments.

[00146] Another advantageous feature of the electrode and/or electrochemical cell disclosed herein relates to its reusable nature. It has been demonstrated herein that the hybrid nanomaterial is able to exert biocidal effect repeatedly, as it is prevented from absorption by the microbes. Since the hybrid nanomaterial is not absorbed by the microbes, it does not precipitate together with the bacteria cells, and is able to retain its antimicrobial efficacy after repeated use.

[00147] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including”, “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00148] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00149] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups,

those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[00150] Various embodiments relate to a novel contact-active graphene oxide-*graft*-quaternized chitosan (GO-QC) coated activated carbon (AC) electrode for ultra-high disinfection performance *via* capacitive deionization disinfection (CDID) process (**FIG. 1A** and **FIG. 1B**).

[00151] Firstly, GO-QC cationic nanohybrid particles were synthesized by covalently grafting cationic QC, specifically dimethyldecyl-ammonium chitosan (DMDC), onto GO (**FIG. 1A**). The GO-*graft*-cationic polysaccharide demonstrated both good antimicrobial and good hemolytic properties (**TABLE 1**).

[00152] **TABLE 1** Minimum bactericidal concentrations (MBC) and hemolytic activities.

No.	Materials	A GO:QC ratio determined by TGA	B MBC ($\mu\text{g mL}^{-1}$)/Selectivity*			C HC ₅₀ ($\mu\text{g mL}^{-1}$)
			<i>E. coli</i> (Gram -)	<i>S. aureus</i> (Gram +)	<i>C. albicans</i> (Fungi)	
1	QC (DMDC)	--	60/250	30/500	16/938	15000
2	GO	--	>5000/<0.4	>5000/<0.4	>5000/<0.4	1250
3a	GO-QC (1:1) [†]	1: 0.54	310/8	160/16	160/16	2500
3b	GO-QC (1:2.5)	1: 1.04	80/63	40/125	20/250	5000
3c	GO-QC (1:5)	1: 2.07	30/333	10/1000	5/2000	10000
3d	GO-QC (1:10)	1: 2.20	30/333	10/1000	5/2000	10000
4	nCS	--	>2500	>2500	>2500	>3850

[†] Design ratios. *Selectivity = HC₅₀/MBC

[00153] The GO-QC in water showed broad spectrum antimicrobial activity against three clinically significant pathogens and even lower minimum bactericidal concentrations (MBCs of 5 to 30 $\mu\text{g mL}^{-1}$) than the QC polymer alone (16 to 60 $\mu\text{g mL}^{-1}$) or GO (greater than 5000 $\mu\text{g mL}^{-1}$) alone. The mechanism of antimicrobial action of the GO-QC was studied by field emission scanning electron microscopy (FESEM) and adenosine triphosphate (ATP) leakage tests.

[00154] Secondly, by coating a thin particulate layer of GO-QC on top of an AC electrode, the CDID process disclosed herein displays an ultrafast, contact-active, efficacious microbicidal effect. Under test conditions of ultra-high *E. coli* loading (10^6 CFU mL^{-1}) in flowing water, the CDID was able to achieve more than 99.9999% (*i.e.* 6-log reduction)

bacteria killing. Without the disinfecting GO-QC coating layer, the AC electrode alone cannot kill the bacteria and adsorbs a much smaller fraction ($< 82.8 \pm 1.8 \%$) of *E. coli* from the same bio-contaminated water or bio-contaminated water having a moderate bacteria loading (10^4 CFU mL⁻¹), *i.e.* with much lower efficiency at less than 1-log reduction. Noteworthy, the AC electrode coated with QC alone in a continuous film form is non-conductive and cannot be used for the CDID process.

[00155] The inventors have also shown that the GO-QC/AC electrode has very good disinfection capacity for bio-contaminated water with different sodium chloride (NaCl) concentrations (100 mg L⁻¹ and 200 mg L⁻¹) in the range typically found in brackish water. After some saturation with bacteria, the GO-QC/AC electrode may be desorbed of killed bacteria by flushing the electrodes with pure water under 0 V cell potential in between the disinfection cycles (**FIG. 1B**). The CDID cell may be run for at least 10 consecutive cycles of bacteria killing alternated by bacteria desorption for the ultra-high bacteria loading of (10^6 CFU mL⁻¹) with both bio-contaminated DI water and NaCl solutions (100 and 200 mg L⁻¹), while maintaining its ultra-high microbiocidal efficacy. With a lower bacteria loading (10^4 CFU mL⁻¹) that is representative of typical brackish water bio-contamination, the GO-QC/AC electrode may continuously achieve 99.99 % bacteria killing, such as *E. coli* in water for at least 5 hours.

[00156] The GO-QC coated layer showed some electrical surface conductivity with finite measurable resistivity of $4.83 \pm 0.78 \text{ M}\Omega \text{ sq}^{-1}$ in contrast to the insulating QC polymer alone (with infinity resistivity), so that the GO-QC coated AC electrode construct is conductive enough to be used as an electrode. Also, the large surface/volume ratio of GO facilitates its fixing on the electrode with a small amount of binder, which prevents contamination of the water flowing through the CDID cell.

[00157] The CDID process according to embodiments disclosed herein involves alternating cycles of water disinfection followed by electrode regeneration, each of a few minutes duration, so that this water disinfection process may be continuous. Also, it is energy-efficient as it uses only a small electrode voltage (2 V). Further, the disinfecting GO-QC is securely attached on the AC electrode surface so that it is non-contaminating to water, unlike many other chemicals used today. The GO-QC nanohybrids themselves have excellent intrinsic antimicrobial properties in suspension form, with MBCs against representative bacteria and fungi (*i.e.* *E. coli*, *S. aureus* and *C. albicans*) in the range of 5-30 $\mu\text{g mL}^{-1}$. The inventors have shown that the GO-QC/AC electrode may be used to realize an energy-efficient and continuous process to

disinfect bio-contaminated water with no observable leaching into or contamination of the water.

[00158] **Example 1: Synthesis of quaternized chitosan (dimethyldecylammonium chitosan) and determination of quaternization degree**

[00159] Quaternized chitosan (QC, specifically dimethyldecylammonium chitosan (DMDC)) was synthesized by *N*-alkylation of chitosan (82 % deacetylated) with decanal, and then methylated with methyl iodide (**FIG. 2**).

[00160] Briefly, 1 g of chitosan (6.2 mmol monosaccharide) was dissolved in 100 mL of 1 % acetic acid, followed by addition of 0.97 g of decanal and stirring for 1 h at room temperature. The pH was then adjusted to 4.5 using sodium hydroxide (NaOH) (1 M). Sodium borohydride (9.3 mmol) was then added and the reacting mixture was stirred for another 1.5 h. The pH of this mixture was then increased to 10 by adding NaOH (1 M). The precipitate was filtered and washed with distilled water until neutrality. Soxhlet extraction using ethanol and diethyl ether mixture was performed to remove unreacted reagents. 1 g of the resulting *N*-decyl chitosan was then added to NMP (50 mL) and NaOH solution (1.5 M, 15 mL).

[00161] After 30 min of stirring at 50 °C, methylation was performed by addition of 1.08 g sodium iodide (7.2 mmol) and 11.2 g iodomethane (78.7 mmol) and stirred for 24 h. The solution was then suction filtered. After dropping the filtrate into acetone (400 mL), the precipitate obtained was filtered and then dried under vacuum to yield the product.

[00162] Quaternization degree of the chitosan derivative was estimated from elemental analysis and calculated from the relation:

$$\text{Quaternization degree} = \frac{\frac{C}{N} \text{mol\%}(\text{chito san derivative}) - \frac{C}{N} \text{mol\%}(\text{chito san})}{\frac{C}{N} \text{mol\%}(\text{chito san})} \times 100\%$$

[00163] The synthesized QC had a quaternization degree of 56 % and molecular weight of 38 kDa (**FIG. 3**).

[00164] **Example 2: Preparation and Characterization of GO-QC Nanohybrid**

[00165] Separately, GO nanoflakes were prepared using a modified Hummers method involving chemical exfoliation of natural graphite powder, which introduced abundant oxidized functional groups, including carboxyl groups to the GO nanoflakes.

[00166] A series of GO-QC derivatives was synthesized with designed weight ratios of GO to QC varying from 1:1 to 1:10 (w/w) (**TABLE 1**) *via* the reaction of –COOH on GO and the

residual -NH_2 on QC (**FIG. 1A**). Briefly, GO-QC was prepared by a coupling reaction using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma) as a coupling reagent together with *N*-hydroxysuccinimide (NHS) illustrated in **FIG. 1A**. After reaction, the suspension was centrifuged ($20000 \times g$, 2 h) to remove the unreacted GO nanoflakes, then filtered with polyamide membrane ($0.2 \mu\text{m}$, Sartorius) and the filter cake was repeatedly rinsed with deionized water to remove all unreacted reagents. The solid residue was dispersed in water, dialyzed using a cellulose membrane (Sigma, MWCO 14000) for 3 days and then freeze dried. The synthesized GO-QC was characterized by Fourier transform infrared spectroscopy (FTIR, Nicolet 5700) and thermogravimetric analysis (TGA, Netzsch STA 409).

[00167] To prepare different sizes of GO-QC (small, medium and large), pristine GO was first ultrasonicated for 8 h, 3 h and 15 min respectively using a probe sonicator prior to conjugation with QC. To prepare QC conjugated onto polystyrene spheres (PS-QC), polystyrene spheres with carboxylic groups (carboxyl latex, 4 %, 500 nm, Life Technologies) were similarly prepared by conjugating QC using EDC/NHS.

[00168] The GO nanoflakes had an average thickness of about 1 nm and average diameter of $746 \pm 308 \text{ nm}$ (**FIG. 4A to 4B**, **FIG. 5A to 5B**, **FIG. 6A to 6B**).

[00169] **Example 3: Determination of the content of carboxylic acid groups of GO and GO-QC by acid-base titration**

[00170] The carboxylic acid groups content of GO and GO-QC was determined by an acid–base titration method. 50 mg GO or GO-QC was mixed with 10 mL NaOH solution (0.1 M) under sonication for 30 min, then stirred for 2 days. The suspension was then dialyzed using a dialysis membrane (Sigma, MWCO 14000) until the pH of the dialysate reached neutral. The combined dialysate was rotary-evaporated to reduce its volume and then titrated with 0.1 M HCl to neutrality ($\text{pH} = 7.00$). The amount of carboxylic acid groups was estimated by the amount of NaOH consumed by GO or GO-QC.

[00171] For pristine GO, the determined content of carboxylic acid groups was about 5.1 mmol g^{-1} ; for GO-QC (1:5) and (1:10) the content was 0.6 mmol g^{-1} and 0.5 mmol g^{-1} respectively. The small amount of carboxylic acid groups left on GO-QC nanohybrids may be masked by the grafted QC molecules, thus preventing further grafting reaction.

[00172] **Example 4: Preparation of negatively-charged chitosan (nCS)**

[00173] nCS was prepared as follows. Briefly, chitosan acetate salt was formed by dissolving chitosan in 1 % acetic acid. Insoluble particles were removed by centrifugation at $4000 \times g$ for

20 min, after which the supernatant was lyophilized. 1 g of chitosan acetate salt was dissolved in 150 mL of deionized water, followed by addition of 0.5 g of succinic anhydride within 10 min. The reaction mixture was stirred for 1 h at room temperature, and then the pH was adjusted to about 8 to 9 with 0.2 M NaHCO₃ and stirred overnight at room temperature. After that, the pH was adjusted to about 10 to 11 by adding 1 M NaOH, and the solution was dialyzed in deionized water (MWCO 10,000) for one week. The nCS product was obtained by freeze-drying.

[00174] ¹H NMR (300 MHz, D₂O) (**FIG. 7**), δ /ppm: 1.95 (s, CH₃), 2.23-2.76 (m, CH₂CH₂), 3.30-3.90 (m, backbone of CS), 4.85 (s, CH).

[00175] **Example 5: Preparation of microbial cells**

[00176] *Escherichia coli* (ATCC8739), *Staphylococcus aureus* (ATCC6538), *Candida albicans* (ATCC10231) were obtained from American Type Culture Collection. All broths and agar media were purchased from Becton Dickinson Company (Franklin Lakes, US). For bacteria, a single colony was inoculated in Mueller Hinton (MH) broth and cultured at 37 °C overnight, shaking at 200 rpm. Bacteria were harvested at the *mid*-logarithmic phase by centrifugation (1,000 ×g, 10 min), and washed with phosphate buffered saline (PBS) solution to remove the residual nutrition. Fungi were inoculated in Yeast-Malt (YM) broth and cultured at 28 °C for 2 days, then harvested, centrifuged and washed in the same ways as the bacteria cells.

[00177] **Example 6: Antimicrobial assay for GO and GO-QC dispersions**

[00178] Bacteria or fungi cells were centrifuged and the pellet was re-suspended in water and diluted to the desired concentration. 10⁸ CFU cells were inoculated into 1 mL GO/GO-QC dispersions, then incubated at 37 °C (28 °C for fungi) under shaking conditions at 200 rpm for the desired time. The cell numbers were determined by the plate colony counting method.

[00179] Briefly, 100 μL of 10-fold dilutions were pipetted into a 10 cm culture plate and spread with 50 °C MH agar (YM agar for fungi). The plates were incubated at 37 °C (28 °C for fungi) overnight for colony formation. The number of colonies was counted and the percentage kill determined using the equation below. This experiment was performed in triplicates.

$$[00180] \quad \% \text{ kill} = \frac{\text{Cell count of control} - \text{Survivor count on sample}}{\text{Cell count of control}} \times 100\%$$

[00181] **Example 7: Minimum bactericidal concentration (MBC) determination**

[00182] The minimum bactericidal concentration (MBC) of GO, QC and GO-QC was determined using a nutrient-free protocol to eliminate the replication of bacteria. A two-fold dilution series of 100 μL antimicrobial reagent solution was made in a 96-well microplate, and then 100 μL of bacterial/fungal suspensions (10^6 CFU mL^{-1}) was added to each well. The microplate was incubated at 37 °C (28 °C for fungi) for 6 h. After incubation the sample treated bacterial/fungal suspensions were plated using MH/YM agar. MBC was determined as the lowest concentration for which no bacterium/fungus growth occurred on the nutrition plates. The test was performed twice independently.

[00183] **Example 8: Nanohybrid CDID electrode fabrication**

[00184] The AC electrode was fabricated similarly to that for a normal CDI electrode. To fabricate the carbon support layer for cationic disinfecting CDI electrode, a mixture of activated carbon (90 wt. %) and polyvinylidene fluoride as a binder (10 wt. %) was dissolved in a proper amount of anhydrous N,N-Dimethylformamide (DMF). A uniform carbon slurry was obtained by mixing the mixture using a high speed planetary mixer at 20,000 rpm for 20 min. The produced slurry was cast onto a graphite foil using a micrometer adjustable applicator with a specified film thickness 150 μm and was dried at 50 °C for 2 h at atmospheric pressure and then under vacuum at 50 °C overnight to remove the remaining organic solvent.

[00185] To prepare the disinfecting GO-QC layer, a mixture of GO-QC (90 wt. %) and polyvinylidene fluoride (PVDF, 10 wt. %) was dissolved in N,N-dimethylformamide (DMF) solvent and mixed into a uniform slurry. The cationic disinfecting GO-QC thin film was coated onto the carbon layer and dried at 50 °C for 2 h. The GO-QC/PVDF slurry was cast on top of an AC electrode (10 cm $\times$ 10 cm). After solvent drying, the electrode was soaked in doubled distilled water to remove the air bubbles which may be present inside the coating layer, and is then ready for use. The final thickness of the cationic GO-QC layer after drying was approximately 20 ± 3 μm , and the mass of the disinfecting material in the carbon electrode was 1.3 mg cm^{-2} . The active electrode area was $10 \times 10 \text{ cm}^2$.

[00186] **Example 9: % killing and log reduction calculation of CDID process**

[00187] The *E. coli* was cultured in MHB solution for 6 h at 37 °C in an incubator. 1 mL of the solution was centrifuged and the pellet was re-suspended in water and diluted to the desired concentration. One mL 10^8 CFU cells was diluted into 100 mL DI water. The fresh *E. coli* (10^6 - 10^7 CFU mL^{-1}) solution (DI water) was prepared as the starting bio-contaminated water. The

CDID outflow solution was collected at specific time intervals for determination of cell numbers by the plate colony counting method.

[00188] Briefly, 100 μL of 10 , 10^2 and 10^3 -fold dilutions were pipetted into 4 inch culture plates and spread with 50 °C LB agar. The plates were incubated at 37 °C overnight for colony formation. The number of colonies was counted and the percentage kill determined using the equation below. This experiment was performed in triplicates.

$$[00189] \quad \% \text{ kill} = \frac{\text{Cell count of control} - \text{Survivor count on sample}}{\text{Cell count of control}} \times 100\%$$

$$[00190] \quad \log \text{ reduction} = \log \frac{\text{Cell count of starting colution}}{\text{Servior count in solution at outlet}}$$

[00191] **Example 10: Surface Resistivity Measurements**

[00192] The surface resistivity of AC, GO-QC/AC and QC/AC electrodes was measured by a four-point probe station (KEITHLEY 2636A). The electrodes were kept at 80 °C in an oven overnight and the current-voltage curve was measured with a driving electrodes sweeping from 0.1 V to 0.2 V with 0.01 V steps.

[00193] **Example 11: Microbe Morphology Study**

[00194] The morphology changes of microorganisms induced by QC, GO and GO-QC were examined with Field Emission Scanning Electron Microscopy (FESEM, JEOL JSM-6701F).

[00195] Microbe cells were incubated with QC, GO and GO-QC at 100 $\mu\text{g mL}^{-1}$ for 1 h. After incubation, the microbes were collected by centrifugation (1000 $\times g$, 10 min), and were then fixed with 2.5 % glutaraldehyde for 4 h, and with 1% osmium tetroxide solution for another 4 h at 4 °C. The fixed microbes were then dehydrated in ethanol series solution with graded concentrations from 20 % to 100 % each for 15 min, after which the samples were dried under a nitrogen flow. After the samples were vacuum dried and coated with platinum, they were observed with FESEM for microbe morphology changes.

[00196] **Example 12: ATP leakage Assay**

[00197] The membrane disruption activity was also characterized with adenosine triphosphate (ATP) leakage assay. ATP released from the bacterial cells was determined with BacTiter-Glo microbial cell viability assay kit (Promega, US) and a luminometer (GloMax 20/20, Promega, US).

[00198] Briefly, mid-log phase *E. coli* was harvested by centrifugation (1000 $\times g$, 10 min) and washed with PBS three times. The bacterial suspension was diluted to $1\text{--}1.5 \times 10^6$ CFU mL^{-1} in PBS, and the antimicrobial reagent was added with a concentration of 100 $\mu\text{g mL}^{-1}$. At

desired time points, 50 μL samples were collected and the released ATP concentration was determined with BacTiter-Glo kit and luminometer.

[00199] **Example 13: Zeta Potential Measurement**

[00200] The charge states of GO, nCS, QC and GO-QC at a concentration of 100 $\mu\text{g mL}^{-1}$ in water at pH 7 were determined with a zeta potential analyzer (ZetaPALS, Brookhaven Instruments Corporation, US).

[00201] To determine the surface charge of the different microbe species (*E. coli*, *S. aureus* and *C. albicans*), 1 mL of each microbial species were centrifuged to remove the culture media, washed with water twice, and re-suspended in water for measurement with the same zeta potential analyzer.

[00202] **Example 14: Hemolysis Assay**

[00203] Human erythrocytes were collected by centrifugation (at 1,000 $\times g$ for 10 min) of 5 mL fresh blood from a healthy donor (male, age 25). The separated erythrocytes were washed three times with Tris buffer, then diluted to a concentration of 5% (v/v) with Tris buffer. GO and GO-QC solutions (50 μL) with different concentrations were added to a 96-well microplate, and the same volume of erythrocytes suspension was added to each well. Positive and negative control wells received, respectively, 0.1% Triton X-100 in Tris buffer and Tris buffer instead of the GO or GO-QC solutions.

[00204] The microplate was incubated in a shaking incubator at 37 $^{\circ}\text{C}$ for 1 h at a shaking speed of 150 rpm. After incubation, the contents of the microplate wells were centrifuged (at 1000 $\times g$) for 10 min. After centrifugation, 80 μL of the supernatant from each well was pipetted to the wells of a new 96-well microplate, and an equal volume of Tris buffer was added to the wells to get a final volume of 160 μL . The absorbance at 540 nm was determined with a microplate spectrophotometer (BIO-RAD Benchmark Plus, US). The percentage of hemolysis was determined from the following equation:

[00205]
$$\text{Hemolysis (\%)} = [(A_p - A_b)/(A_t - A_b)] \times 100\%$$

[00206] where A_p is the absorbance value for the GO or GO-QC containing sample, A_t is the absorbance value for the positive control, and A_b is the absorbance value for the negative control.

[00207] **Example 15: In vitro Biocompatibility Study**

[00208] Human foreskin fibroblasts (FibroGROTM, Millipore) were seeded into the 24-well culture plates at the density of 0.5×10^5 cells cm^{-2} . The culture medium was supplemented with

100 $\mu\text{g mL}^{-1}$ GO-QC (1:5). On specified days, cells were analyzed with CCK-8 kit (Sigma, US) by the absorbance at 450 nm to determine the cell viability. Cells in TCPS wells without GO-QC (1:5) were used as control. The viability of fibroblasts was also examined with the LIVE/DEAD assay.

[00209] After specified cell culture time periods, the cells were stained with LIVE/DEAD kit (Invitrogen, US) containing 2 mM calcein AM and 4 mM Ethidium homodimer-1 in PBS. After 45 min incubation the LIVE/DEAD dye was removed by PBS rinsing, the cell morphology was observed in fluorescence with an inverted optical microscope (Zeiss, Germany).

[00210] **Example 16: Conjugation of Texas red ® dye onto QC and GO-QC**

[00211] 10 mg mL^{-1} of Texas Red®-X, Succinimidyl Ester (Life Technologies) was incubated with a solution of QC or a suspension of GO-QC for 1 hour at 25 °C. The excess unreacted dye was removed by dialyzing for 3 days. To confirm the conjugation of QC on to GO-QC, dark field microscopy and fluorescence microscopy was used to image the GO-QC-dye hybrid. The results in **FIG. 8A** and **FIG. 8B** show that the red fluorescence took the shape of the GO-QC nanohybrid, indicating the successful conjugation of the dye onto GO-QC.

[00212] **Example 17: Optical Microscopy for visualization of microbes attachment**

[00213] 10 μl of suspensions of GO, QC, GO-QC and different microbes were placed on a glass slide and covered with a coverslip before viewing under a confocal microscope (Olympus BX51, Germany) and images were taken using the software, analySIS (Olympus, Germany). To assess the viability of the attached bacteria, the bacteria-GO-QC suspension was first incubated with BacLight bacterial viability kit (L13152, Invitrogen) for 30 min at room temperature and then placed on to glass slides and viewed under a fluorescence microscope.

[00214] **Example 18: Antimicrobial activity in the presence of NaCl, KCl, MgCl_2 and CaCl_2**

[00215] The combination of QC with GO in a nanohybrid enhanced the microbiocidal potency synergistically. GO-QC (1:5) and QC also retained their antimicrobial activities, unlike GO, in the presence of physiologically important salts, including NaCl and KCl, CaCl_2 and MgCl_2 (**FIG. 9A** to **FIG. 9D**). The GO-QC nanohybrid was salt-insensitive, like pristine QC, because the microbial killing action did not depend on secondary structures of the QC molecule but on its cationic charge.

[00216] The antimicrobial activity of GO declined with increasing KCl concentration between 0 mM and 150 mM (**FIG. 9B**). The antimicrobial activities of GO-QC and QC were unaffected by KCl concentration up to 150 mM. Biological concentrations of divalent ions such as Mg^{2+} and Ca^{2+} were much lower than those of monovalent ions; the effect of these divalent ions was tested over the concentration range 0 to 5 mM. The antimicrobial activity was not affected by adding Mg^{2+} or Ca^{2+} up to 5 mM, while the % kill of GO for *E. coli* decrease slightly along with the increasing divalent ion concentration (**FIG. 9C** and **FIG. 9D**).

[00217] **Example 19: Detailed antimicrobial mechanism study of (I) QC and GO individually and (II) GO-QC nanohybrid**

[00218] Example 19.1: QC and GO individually

[00219] The inventors hypothesized that free QC in solution and grafted QC in GO-QC both penetrate the cell surface but with different modes. Free QC molecules in solution form were absorbed into the bacterial cell wall and electrostatically bind with the anionic cell envelope so that they cannot be removed by centrifugation (**FIG. 10A** and **FIG. 10B**).

[00220] Optical microscopy for visualization of microbes attachment was carried out. Briefly, 10 μ L of suspensions of GO, QC, GO-QC and different microbes were placed on a glass slide and covered with a coverslip before viewing under a confocal microscope (Olympus BX51, Germany) and images were taken using the software, analySIS (Olympus, Germany). To assess the viability of the attached bacteria, the bacteria-GO-QC suspension was first incubated with BacLight bacterial viability kit (L13152, Invitrogen) for 30 min at room temperature before being placed onto glass slide and viewed under a fluorescence microscope.

[00221] Example 19.2: GO-QC nanohybrid

[00222] Contact angle measurements were carried out. A suspension of microbes was drop casted onto glass slides and a drop of either water or 10 mg mL⁻¹ QC solution was deposited on top of the slides. The contact angles were then measured using a contact angle analysis system (FTA200, First Ten Angstroms Inc.)

[00223] Example 19.3: Discussion

[00224] **FIG. 11A** shows bacteria clustering together and sticking onto the planes of the GO-QC nanohybrids rather than internalizing them. **FIG. 11C** further shows that these bacteria sticking to GO-QC nanohybrids are dead. These images corroborate the inventors' hypothesis that GO-QC nanohybrids were not internalized by microbes due to their relatively larger size, although the tethered QC likely penetrate partially into the microbe envelope. A FESEM image

of the mixture of GO-QC with bacteria (**FIG. 8B**) shows that the bacteria surfaces are fuzzy, suggesting that they are covered with GO-QC nanohybrid.

[00225] The inventors qualitatively distinguished the attachment forces between GO-QC (1:5) and different microbes by adapting the recovery process with low and variable centrifugal force and measuring the relative fractions of microbes that detached from pre-challenged nanohybrids. The amounts of microbes detached were determined by pelleting the free microbes by centrifugation, and then re-suspending and measuring the optical density.

[00226] For all the microbes, a consistent trend is seen in which the fraction of microbes pelleted, *i.e.* detached from GO-QC as indicated by the optical density, increases with the relative centrifugal force applied (**FIG. 13A**). The Gram negative bacterium (*E. coli*) has the highest fraction of microbes dislodged, followed by Gram positive bacteria *S. aureus* and finally the fungus *C. albicans* which has the lowest fraction dislodged. This trend mirrors the MBC values (**TABLE 1**): the Gram negative bacteria which are the most poorly killed by the GO-QC nanohybrid (*i.e.* they have the highest MBCs) have the smallest attachment force to the nanohybrids, while the fungi with the lowest MBC values have the highest attachment force. It appears that high attachment force correlates to high killing efficacy and low MBC values.

[00227] Zeta potentials of the various microbes were also measured (**FIG. 13B**) and it appears that the Gram positive *S. aureus* was more negatively charged than the Gram negative *E. coli*. Comparing the Gram positive and Gram negative bacteria, **FIG. 13A** and **FIG. 13B** indicated a correlation between the magnitude of the microbe negative charge and the strength of the bacterium/GO-QC adhesion. It appears that more negative bacterial charge and higher attachment force correlated with lower MBCs for the Gram positive bacterium *S. aureus* as compared to the Gram negative bacterium *E. coli* (**TABLE 1**). Increased electrostatic attraction between the anionic bacterial envelope and cationic GO-QC may explain why GO-QC is more effective against *S. aureus* than it is against *E. coli*.

[00228] The inventors further hypothesized that compositional compatibility of the QC polymer disclosed herein with bacterial cell wall also contributed to microbes membrane disruption. Bacteria have cell walls that are rich in polysaccharides; the peptidoglycan cell wall layer is made from a polysaccharide of poly(muramic acid-*co*-glucosamine). Fungi, on the other hand, have a cell wall rich in chitin, which is very similar to the chitosan backbone of the QC disclosed herein.

[00229] The inventors qualitatively characterized the compatibility of QC with microbe cell wall by measuring the wettability by water and QC solution of glass slides coated with microbes. Results were summarized in **TABLE 2**.

[00230] **TABLE 2** Compatibility study of the various microbes surfaces with QC by contact angle analysis at 25 °C. (No. of samples per datum = 10).

	Contact angle (°)	
	Medium	
Surface	Water	QC solution
<i>S. aureus</i>	15.5 ± 4.0	10.5 ± 0.6
<i>E. coli</i>	12.8 ± 0.8	9.7 ± 1.1
<i>C. albicans</i>	12.7 ± 0.9	6.5 ± 1.5
Glass slide	25.5 ± 1.7	26.8 ± 1.3

[00231] The results in **TABLE 2** show that QC solutions have lower contact angles than the water medium, indicating that they wet the microbes better than water. Also, the lowest contact angle of QC solution ($6.5 \pm 1.5^\circ$) was achieved with *C. albicans* film, indicating that the QC tethered to the GO-QC nanohybrid probably has the highest compatibility with the *C. albicans* cell wall. *C. albicans* is also the most vulnerable of the microbes to GO-QC (**TABLE 1**, see **Sample 3c**). Although *C. albicans* is much less anionic than the bacterial species (**FIG. 13B**), the MBC value against it is the lowest, suggesting that cell wall compatibility is a major contributor to the adhesion of *C. albicans* cells to GO-QC (**FIG. 13A**) and to the high killing efficacy of GO-QC against this microbe. The inventors hypothesized that increased compatibility of QC with microbe cell wall enhances the adsorption of the grafted cationic QC on the surface of the microbe cell wall which in turn increases charge density near the cytoplasmic membrane to enhance disruption.

[00232] A unique feature of the GO-QC disclosed herein is the employment of polysaccharide as the compatibilizing component, unlike other contact-active nanoparticles that employ hydrophobic polymers to enhance the interaction with membrane lipids. Others have found that hydrophobicity of the cationic polymer enhanced its ability to penetrate the cell membrane to avoid the aqueous environment but this entailed significant toxicity to mammalian cells as well as to microbial pathogens. The polymer according to embodiments

disclosed herein is tuned to be compositionally similar to microbe cell wall so as to improve microbe selectivity and reduce toxicity to mammalian cells.

[00233] Further, the QC appeared to “blunt” the GO edge in the nanohybrid so that GO-QC was less hemolytic than GO. The inventors also tested a series of three different GO-QC sizes which have different edge lengths per unit area of the nanohybrid; they found that all the different sizes (from small to medium and to large sizes) have low hemolysis corroborating that mechanical cutting due to the particle edges is not prominent in the GO-QC action (see **FIG. 14A to 14D, FIG. 15A and 15B, FIG. 16, FIG. 17, and TABLE 3**).

[00234] **TABLE 3** Minimum bactericidal concentrations (MBC), % kill, vs. concentration, hemolytic activities and ζ -potentials for the three different sizes of GO-QC and PS-QC

Materials	MBC ($\mu\text{g mL}^{-1}$)	% kill			HC ₅₀ ($\mu\text{g mL}^{-1}$)	ζ potential (mV)
		0.5*MB C	0.25*MB C	0.125* MBC		
GO-QC (Small)	31.3	99.7	99.7	99.4	10000	42.3 $\pm$ 2.2
GO-QC (Medium)	31.3	99.7	97.0	71.5	10000	20.9 $\pm$ 5.7
GO-QC (Large)	31.3	69.2	48.6	27.8	10000	17.5 $\pm$ 6.9
PS-QC	8.1	99.9	99.8	98.9	650	48.1 $\pm$ 0.7

[00235] **Example 20: Antimicrobial activity of different nanotemplates of GO-QC (Small), GO-QC (Medium), GO-QC (Large) and polystyrene (PS)-QC**

[00236] To investigate if the GO-QC nanohybrid kills bacteria by mechanical cutting due to the edge or charge concentration, different sizes of GO-QC were prepared. The small GO-QC nanohybrid had greater edge length per unit area but also more polymer molecules conjugated per unit area as the functional groups on the GO for polymer attachment were concentrated at the edge defects.

[00237] Small, medium and large GO-QC nanohybrids were prepared by varying the GO sonication duration prior to functionalization with QC (**FIG. 14A to 14D and TABLE 3**). The average lateral dimensions of GO-QC (Small), GO-QC (Medium) and GO-QC (Large) were approximately 100 nm, 500 nm and 1 μm , respectively (**FIG. 14A to 14D**). At sub-MBC, the three sizes have different rates of killing with GO-QC (Small) having the highest rate (**FIG. 15A and 15B, and TABLE 3**); for example, at 25 % MBC, the % kill for GO-QC (Small), GO-QC (Medium) and GO-QC (Large) were 99.7 %, 97.0 %, and 48.6 % respectively (**FIG. 15A**).

At MBC, GO-QC (Small) also produces the most extensive damage over the bacterial surface, as shown by AFM analysis of challenged bacterial cells (**FIG. 15B**). From only these sub-MBC data, the inventors were not able to differentiate between the contributions due to the charge concentration of QC *versus* mechanical cutting by GO edges on bacterial killing efficacy; with the small GO-QC, either the higher QC concentration leading to increased electrostatic killing or the higher edge length leading to increased mechanical cutting could increase the killing efficacy for these smaller sizes.

[00238] The “mechanical cutting” *versus* “charge concentration” interpretations may be tested by consideration of nanohybrid size effect on hemolysis. The hemolysis values for all three sizes were found to be the same, $10,000 \mu\text{g mL}^{-1}$ (**TABLE 3**). From the fact that GO was rather hemolytic but GO-QC was significantly less hemolytic (**TABLE 1**), the inventors inferred that hemolysis was mainly due to the sharp GO edges and not the QC tethered at the edges. Though there was more edge length per unit area in GO-QC (Small), it was also not more hemolytic than GO-QC (Large), which indicated that mechanical edge cutting effects were not a significant contributor to the killing action of these nanohybrids. The inventors inferred that QC made the GO-QC relatively less-hemolytic compared to GO because of edge blunting and made the antibacterial activity greater because of higher charge density per unit area. Zeta potential measurements (**TABLE 3**) corroborated this interpretation, with potential increasing as the GO-QC nanohybrid size decreased, strongly so for the smallest sized nanohybrid.

[00239] The inventors also conjugated QC to polystyrene (PS) spheres with diameter of 500 nm. The PS-QC spheres were more hemolytic since QC was tethered over the entire surface and not primarily at the edges, and so the areal density of QC was high. QC coated spheres were highly hemolytic in spite of having no sharp edges. This showed that high areal density of cationic charge, above that present in GO-QC, may be harmful to Red Blood Cells. The high areal density of QC on PS-QC also led to the low normalized MBC value for PS-QC (for normalization, the inventors multiplied the measured MBC value of PS-QC by the approximate weight fraction of QC measured by TGA which was about 14 % (**FIG. 16**).

[00240] The MBC values of the different sizes of GO-QC were found to be similar (at $31.3 \mu\text{g mL}^{-1}$, **TABLE 3**) but the smaller nanohybrids were more effective at killing bacteria at sub-MBC. Large GO-QC nanohybrids were less numerous and fewer large GO-QC nanohybrids may attach per bacterium. Conversely, the smaller sizes permit higher counts of GO-QC

(Small) nanohybrids to adhere onto the typical bacterium leading to more complete killing (**FIG. 17A and 17B**). Statistically, for the small GO-QC nanohybrids which are smaller in size than the average bacterium size, at concentrations lower than the MBC, a smaller fraction of the bacterial cells will have fewer than the critical number of GO-QC nanohybrids needed for killing, as compared to GO-QC (Large). This explains the sharp rise but non-100 % kill at slightly lower than the MBC values for GO-QC (Small).

[00241] For the large GO-QC nanohybrids, there was not such a steep increase in the killing rate with increase in concentration as the probability of a bacterium not contacting with GO-QC (Large) nanohybrids is higher at a given concentration. The behavior of the % kill for GO-QC (Medium) was intermediate between that of (Small) and (Large).

[00242] **Example 21: Discussion of results**

[00243] **FIG. 1B** shows the architecture and application of the novel GO-QC/AC electrode disclosed herein. Two QC-QC/AC electrodes each with a disinfecting surface coating of GO-QC nanohybrid were placed parallel to each other and assembled into a CDID cell. Bacteria-contaminated water was pumped through the space between the two GO-QC/AC electrodes. With the application of a small potential (2 V), the bacteria in the flowing water was electrically attracted onto the positive electrode due to the negative charges on their cell envelope and then killed by the GO-QC nanohybrid coating when in physical contact. The electrodes may be regenerated and cleared of bacteria by switching off the voltage and passing non-bio-contaminated water through the cell for rinsing.

[00244] **FIG. 4A, FIG. 5A to 5B, and FIG. 6A to 6B** show that both GO and GO-QC nanohybrids are individual nanoflakes. AFM imaging of the GO-QC (1:5) (**FIG. 4B**) indicates that the average thickness is increased to about 2 nm whilst the average diameter of the GO-QC (1:5) nanohybrid is not much changed (573 ± 160 nm) by the QC grafting.

[00245] **FIG. 4C** displays FITR spectra of pristine GO, QC and a typical GO-QC (1:5). The peak at 1535 cm^{-1} in the GO-QC spectrum, which is absent in the GO spectrum, corresponds to the newly formed -NHCO- bond between GO and QC, corroborating that QC molecules have been grafted onto the GO through an amide linkage. In the spectrum of QC, there is also a weak peak at 1535 cm^{-1} which is due the incomplete deacetylation of chitosan. In addition, XPS analysis further confirmed the covalent bonding of QC onto GO (**FIG. 18A to 18J**).

[00246] Thermal Gravimetric Analysis (TGA) (**FIG. 4D**) of GO indicated 4 % of its weight is lost below $100\text{ }^{\circ}\text{C}$, which is attributable to evaporation of adsorbed water. The major weight

loss of GO was observed between 165 °C to 215 °C, which is likely due to pyrolysis of labile oxygen-containing groups. In contrast, QC has less than 2 % weight loss below 100 °C but 35 % weight loss between 210 °C to 250 °C, which is due to the cleavage of substituent groups and decomposition of glucopyranose rings.

[00247] The weight loss curve of GO-QC has two stages which may be attributed respectively to the major losses of GO (165 – 195 °C, Stage I) and QC (210 – 250 °C, Stage II). From the weight losses in these two main regimes, the actual weight ratios of GO to QC can be inferred and these values differ from the GO:QC design ratios (**TABLE 1**).

[00248] For the design GO:QC reactant weight ratios of 1:1 to 1:5, the measured GO:QC ratios were respectively 1:0.54 to 1:2.07, indicating that around 50 % of design QC molecules were successfully grafted onto GO nanoflakes. However, for the design GO:QC weight ratio of 1:10, the measured GO:QC ratio determined by TGA was 1:2.20, indicating that only 22 % of QC was grafted onto GO nanoflakes. The measured GO:QC ratio plateaus at around the design ratio of 1:5. This may be attributed to the limited availability of carboxyl groups on GO nanoflakes. The near complete reaction of carboxyl groups on GO with high QC design ratio was confirmed by acid-base titration as mentioned in **Example 3**.

[00249] Three clinically significant microbes, specifically *E. coli* (Gram-negative bacterium), *S. aureus* (Gram-positive bacterium), and *C. albicans* (fungus) were chosen as model pathogens to investigate the antimicrobial activity of GO and GO-QC.

[00250] **FIG. 19A** shows that GO had poor bacteria and fungi killing efficacy of < 40%. The QC polymer itself had excellent antimicrobial activities towards both bacteria and fungi, with respective % kills for these three microbes of $97.5 \pm 2.1\%$, $96.9 \pm 2.5\%$ and $98.8 \pm 1.2\%$. Among the GO-QC nanohybrid series, dramatically higher antimicrobial activities against the three model pathogens compared to pristine GO were observed for the two formulations with higher QC contents (**FIG. 19A**).

[00251] With GO-QC (1:5), the respective % kills for the three microbes are $93.6 \pm 4.2\%$, $97.8 \pm 1.8\%$ and $99.3 \pm 0.4\%$, similar to those of QC itself ($p > 0.05$, no significant difference). For GO-QC (1:10), the corresponding % kills are $93.7 \pm 1.9\%$, $98.4 \pm 1.5\%$ and $98.2 \pm 1.7\%$ which are similar to those of GO-QC (1:5) ($p > 0.05$, no significant difference). It appears that the antimicrobial activities plateaued beyond GO-QC (1:5), corroborating the plateauing of the actual GO:QC ratios inferred from TGA measurements (**TABLE 1**).

[00252] The time dependence of the killing of *E. coli* was also investigated (**FIG. 20**). The % kill by QC and GO-QC reached nearly 100% after 4 h, while the % kill of GO increased more gradually and plateaued beyond 12 h at around 70+ % kill.

[00253] The minimum bactericidal concentrations (MBCs) of GO, QC and GO-QC were also determined (**FIG. 19B** and **TABLE 1**). The control materials, pristine GO and negatively charged chitosan (nCS), did not show bactericidal activity at concentrations up to 5000 and 2500 $\mu\text{g mL}^{-1}$, respectively. The QC solution showed good antibacterial activity with MBCs of 60 $\mu\text{g mL}^{-1}$, 30 $\mu\text{g mL}^{-1}$ and 16 $\mu\text{g mL}^{-1}$ for *E. coli*, *S. aureus* and *C. albicans* respectively. GO-QC (1:5) nanohybrid exhibited improved MBCs in the range of 5 – 30 $\mu\text{g mL}^{-1}$, which were lower than those of QC polymer alone.

[00254] The MBC results of the GO-QC nanohybrids were superior to those of QC polymer solution alone, which is contrary to what is usually achieved with surface immobilization of polymer. The MBCs plateaued with GO-QC (1:5) and (1:10), corresponding to the behavior of the % kill. The combination of QC with GO in a nanohybrid enhances the microbicidal potency synergistically.

[00255] The time dependence of killing curve of GO-QC of *E. coli* in DI water and NaCl (100 mg L^{-1} and 200 mg L^{-1}) was also investigated (**FIG. 19C**). The % kill by GO-QC (1:5) reached nearly 100% after 1 h in both DI water and NaCl solutions. The presence of salt ions has little effect on the *E. coli* killing, indicating the possible utility of GO-QC for disinfection of brackish water. The antimicrobial activity in the presence of other NaCl concentrations and other salts, including KCl, MgCl_2 and CaCl_2 can be found in **FIG. 9**.

[00256] The GO-QC series generally have 50 % hemolysis concentrations (HC_{50}) (**TABLE 1**) which are intermediate between those of the two individual components, QC and GO. Both high QC-content formulations of GO-QC (1:5 and 1:10) and QC show high HC_{50} values of 10,000 $\mu\text{g mL}^{-1}$ and 15,000 $\mu\text{g mL}^{-1}$ respectively, much higher than that of pristine GO (1250 $\mu\text{g mL}^{-1}$). As shown in **FIG. 19D**, the selectivity (defined as $\text{HC}_{50}/\text{MBC}$) of GO-QC (1:5) for *S. aureus* and *C. albicans* (1000 and 2000) are significantly improved compared to QC alone (500 and 938) and are much improved compared to GO. The significant hemolytic activity of GO is due to the sharp edges of pristine GO which are expected to be harmful to mammalian cells. Chitosan derivatives, such as QC in this report, have low hemolytic activity and low toxicity to mammalian cells. The QC molecules surrounding the GO-QC nanohybrid likely function as a biocompatible protection layer which lowers the frequency of physical damage

to mammalian cells by the GO substrate so that GO-QC shows a much improved HC_{50} compared with pristine GO. The inventors have also found GO-QC to have good *in vitro* biocompatibility. Human fibroblast cells (FibroGRO™, Millipore) exposed to GO-QC (1:5) ($100 \mu\text{g mL}^{-1}$) *in vitro* are as viable as on tissue culture polystyrene (TCPS) dish control (**FIG. 19E**, $p > 0.05$, no significant difference). The *in vitro* live/dead test shows many live cells after lengthy exposure to GO-QC (1:5) (**FIG. 19F**).

[00257] A schematic diagram of our CDID process for disinfection of bacteria-contaminated water and regeneration of GO-QC/AC electrodes is shown in **FIG. 21**. The bio-contaminated solution was pumped into the CDID cell where a small voltage (2 V) was applied onto the parallel GO-QC/AC electrodes (Step I, **FIG. 21**). As the solution passed through the space between the electrodes, the ions were absorbed onto the electrodes with the opposite potential; bacteria, in this example *E. coli*, were attracted to the positive electrode because of their negatively charged cell envelopes. On contact with the GO-QC, the *E. coli* were killed. As more suspension passed through the cell, the electrodes became saturated with bacteria. Electrode saturation was detected by the presence of bacteria in the outflow liquid. Before that point, the regeneration process was started and the electrode polarity was turned off and regeneration water was pumped into the CDID cell to remove the dead bacteria accumulated on the GO-QC/AC electrode surface (Step II, **FIG. 21**). After regeneration, the cell was then switched back to disinfection mode to treat more bio-contaminated water.

[00258] **FIG. 22A** shows a FESEM of the surface of an AC electrode (the control). The inventors also attempted to use a QC coating on top of the AC electrode to effect disinfection. However, the QC formed a dense continuous layer on the AC electrode and blocked all the pores between the individual AC particles (**FIG. 22B**), making the QC/AC composite insulating and non-functional as an electrode (**TABLE 4**).

[00259] **TABLE 4** Resistivity of CDID electrodes with different coating layer

No.	Samples	Average ($\Omega \text{ sq}^{-1}$)	Standard deviation
1	AC electrode (control)	48	12
2	GO-QC/AC electrode	4,834,500	778,331
3	QC/AC electrode	∞ (non-conductive)	not applicable

[00260] GO-QC nanohybrids form a porous surface coating on top of the AC electrode (**FIG. 22C** and **22D**), which allows the solution to reach the AC and current to pass between the electrodes. The GO-QC layer is itself also somewhat conductive with finite surface resistivity of $4.83 \pm 0.78 \text{ M}\Omega \text{ sq}^{-1}$ so that the GO-QC/AC composite can function as an electrode (**TABLE 4**). A small amount (10 % based on total solid content) of PVDF binder was mixed into the GO-QC to prevent dislodgement of the nanohybrid from the electrodes into the water which is highly undesirable. Only a small quantity of binder was needed as the large GO surface enables the GO-QC to be securely bound by PVDF to the AC particles below (**FIG. 22E** and **22F**) and the GO-QC nanohybrid appears to be still freely available for killing bacteria.

[00261] The GO-QC/AC electrode was first applied for fast, ultra-high log reduction of microbes in bio-contaminated deionized water. Even with ultra-high bacteria loading (10^6 CFU mL^{-1}), the GO-QC/AC electrode (**FIG. 23A**) can maintained high log-reduction of 6.68 ± 0.15 (i.e. >99.9999% kill) continuously without any regeneration, in the first 20 min; no live *E. coli* was detected at the outlet despite the high loading of bacteria at the inlet ($>10^6 \text{ CFU mL}^{-1}$). *E. coli* solution was pumped into the CDID cell at the flow rate of 1 mL min^{-1} and the voltage was only 2 V. *E. coli* were mostly captured onto the surface of GO-QC/AC electrode and clean water flowed out of the CDID cell. After 60 min, the log reduction was still 2.45 ± 1.37 , which meant the % kill was $98.5 \pm 1.4 \%$. However, with uncoated AC electrode, the best log reduction of CDID was only 0.76 ± 0.05 (i.e. % removal was $82.8 \pm 1.8 \%$) with the same bacteria loading; the bacteria stuck to the AC electrodes were not dead when we cultured them on Agar plates. Compared with the typical uncoated AC electrodes used normally for the CDI process, the GO-QC/AC CDID electrodes have an excellent % kill of *E. coli*. The superior disinfection achieved with our CDID process is due to the thin GO-QC layer.

[00262] For a typical CDI process to continuously remove ionic species, the electrodes need to be continuously regenerated and usually the ion adsorption and electrode regeneration are conducted alternately. Recyclability of our GO-QC/AC CDID electrode was also investigated by alternating the voltage from 0 V to 2 V at the start of the disinfection step and then switching from 2 V to 0 V at the start of the regeneration step. As shown in **FIG. 23B**, the CDID cell maintained its high microbicidal activity with microbe log reduction maintaining at around 6.68 ± 0.15 order for over 10 consecutive cycles of alternate disinfection followed by electrode regeneration even with the ultra-high bacteria loading (10^6 CFU mL^{-1}). Separately, it is noteworthy to mention that no UV peaks characteristic of GO were detected in the exit water

after it was flushed into the cell for 1 h (**FIG. 24**) confirming durable attachment of the GO-QC coating to the electrode.

[00263] The inventors next evaluated the lifetime of the CDID cell with a moderate bacteria loading (10^4 CFU mL⁻¹) that is relevant to brackish water. By running the cell with alternating cycles of 4 min bacteria capture followed by 6 min bacteria desorption, the CDID cell can maintain > 99.99% kill (*i.e.* 4-log reduction) of *E. coli* for at least 5 hours (**FIG. 23C**).

[00264] The inventors also evaluated the salt removal efficiency of their GO-QC/AC CDID cell (**FIG. 23D**). Their GO-QC/AC electrode still retains the initial interconnected porosity of AC with a highly disinfecting GO-QC layer at the top. The laterally large GO-QC nanohybrids form a particulate coating atop the regular porous activated carbon electrode without penetration into the voids to cause malfunctioning of the electrode. Their GO-QC/AC electrode still has a reasonable salt removal efficiency of 66% and 63% respectively in non-infected NaCl solution (with 100 mg L⁻¹ and 200 mg L⁻¹) compared to AC CDID cell controls with 73 % and 69 % efficiency, respectively (**FIG. 23D**). The high salt removal efficiency confirmed that the GO-QC/AC electrode may be successfully used for bio-contaminated brackish water.

[00265] The disinfection of salty water with their CDID cell was also carried out in 100 mg L⁻¹ and 200 mg L⁻¹ NaCl solutions with ultra-high bacteria loading (10^6 CFU mL⁻¹). With Na⁺ and Cl⁻ ions, the time that the cell can achieve 99.9999% *E. coli* killing without any regeneration decreased slightly from 20 min to 16 min (**FIG. 23E**). The accumulation of Cl⁻ ions near the GO-QC layer may reduce its effective cationicity and decrease the killing efficiency. However, with the regeneration cycle included (**FIG. 23F**), the microbicidal activity of the cell was maintained at almost 10 cycles like for deionized water (**FIG. 23B**), as the salts could probably be successfully removed.

[00266] The inventors firstly studied the intrinsic killing mechanism of GO-QC in suspension. FESEM of the morphological changes of *E. coli* cells after contact with QC, GO and GO-QC (1:5) solution/dispersion (100 µg mL⁻¹) indicate that these materials kill the bacteria by contact-active physical disruption (**FIG. 25A**, and **FIG. 12A** to **12C**).

[00267] QC-treated *E. coli* cells (**FIG. 25A(ii)**) show wrinkled cell surfaces compared to the smooth surfaces of untreated control cells (**FIG. 25A(i)**). GO-treated *E. coli* cells, on the other hand, are found to have physical defects at the two ends of the cell (arrows in **FIG. 25A(iii)**), which is likely to be produced by the sharp edges of GO nanoflakes. GO-QC (1:5)-treated cells show even more drastic morphological changes compared to those treated with QC or GO:

distinct areas of damage (or holes) on the cells can be clearly seen (arrows in **FIG. 25A(iv)**), and the cell envelopes appear severely collapsed, suggesting physical damage as well as loss of cell contents into the environment.

[00268] Disruption of the cell membrane may be verified by detecting ATP released into the extracellular environment, stained with BacTiter-Glo luminescence kit. As shown in **FIG. 25B**, there is significant increase in luminescence after contact with QC and GO-QC, corroborating the FESEM observation of wrinkled cell surfaces that is suggestive of membrane disruption and cell content leakage. Thus the FESEM study and ATP leakage assay support that the GO-QC nanohybrid effectively disrupt microbial membranes causing cell death.

[00269] The inventors hypothesized that the improved MBC (and selectivity) of the GO-QC nanohybrid compared to either GO or QC is contributed by the higher areal charge density that QC immobilized on GO presents to the cell wall, compared with what exposure to solution QC can produce. Zeta potential measurements were used to evaluate the charge of the materials (**FIG. 25C**). GO and nCS are negatively charged (-45.8 ± 1.9 mV and -4.0 ± 1.5 mV respectively) but the GO-QC nanohybrid is cationic.

[00270] The zeta potentials of GO-QC (1:5) and (1:10) (43.4 ± 1.2 mV and 44.5 ± 1.0 mV respectively) are even higher than that of QC (20.0 ± 4.2 mV), confirming the hypothesis that the QC molecule is present at higher areal concentration on the GO-QC surface compared to being evenly distributed in QC solution. By comparing the zeta potentials of the different GO-QC nanohybrid (**FIG. 25C**) to their respective MBCs (**TABLE 1**), it is apparent that the MBC improves with increased positive charge, with the best MBC achieved with GO-QC (1:5 and 1:10) possessing the highest positive charge of around +45 mV. The high QC areal concentration on the GO-QC nanohybrid makes electrostatic induced disruption of the microbe cytoplasmic membrane more effective, thereby decreasing the MBC values to below those of pure QC.

[00271] **FIG. 25D** show *via* fluorescence study that there are significant differences in the penetration modes of the two materials (QC *versus* GO-QC) into bacterial cells (*E. coli*). Both GO-QC and QC were conjugated with a fluorescence dye (Texas Red-X), and then the labeled materials were incubated with bacteria. The conjugation of GO-QC with Texas-Red was confirmed by dark field and fluorescence microscopy (**FIG. 8A** and **8B**). After 1 h incubation, the bacteria were separated by centrifugation (2000 ×g, 10 min) and observed under

fluorescence microscopy. Bacteria incubated with QC-Texas Red were stained red with the fluorescence dye, while the GO-QC-Texas Red did not stain the cells (**FIG. 25D**).

[00272] The inventors hypothesized that both the free QC in solution and the grafted QC in GO-QC penetrate the cell surface but with different modes. Free QC molecules in solution form were absorbed into the bacterial cell wall and electrostatically bind with the anionic cell envelope so that they cannot be removed by centrifugation. The 2D GO-QC nanohybrids have lateral dimensions comparable to the size of a bacterium and are too large to be absorbed into the cell wall. Other detailed studies of the killing mechanism of the suspended GO-QC can be found in **FIG. 10A** and **10B**, **FIG. 11A** to **11C**, **FIG. 14A** to **14D**, **FIG. 15A** and **15B**, **FIG. 16**, **FIG. 17A** and **17B**, and related text.

[00273] The inventors also studied the killing mechanism of GO-QC when it is part of the composite CDID electrode. **FIG. 26A** and **26B** show killed *E. coli* covering the surface of disinfecting GO-QC/AC electrode after the disinfection step. The wrinkled cell surface of dead *E. coli* is apparent (**FIG. 26B**) which corroborates that GO-QC physically damages the *E. coli* envelope. After the regeneration process, no *E. coli* bacteria can be observed on the electrode surface (**FIG. 26C** and **26D**) which suggests excellent regeneration and recyclability of the GO-QC/AC electrode.

[00274] GO-QC exploits the synergistic effects of nanoscale thickness, micron-scale lateral size, and the (partial) conductivity of GO and high charge density due to QC that the grafting procedure disclosed herein produces. Grafting of the GO nanomaterial with cationic QC serves various functions. Firstly, modification of GO with water soluble cationic QC improves the dispersion of the resulting nanohybrid in aqueous environment, thus overcoming the aggregation tendencies of GO in solution. Secondly, the mechanical cytotoxicity of GO nanoflakes to mammalian cells is reduced by grafting with the biocompatible chitosan derivative at their sharp edges. Thirdly, functionalization of GO with cationic quaternized chitosan turns the charge state of GO from anionic to cationic and endows the GO-QC nanohybrid with net positive charge, so that electrostatic attraction between the cationic GO-QC and the anionic bacterial cell cytoplasmic membrane can electrostatically disrupt the bacteria membrane. Thirdly, GO has large surface area, which enhances its adhesion to the underlying AC electrodes and it has sufficient conductivity for the GO-QC/AC electrode to function in the CDID process. With GO-QC coated AC electrodes and a high initial bacteria loading of $>10^6$ CFU mL⁻¹, the % kill of bacteria achievable is $>99.9999\%$ and the regeneration

potential is at least 10 cycles in both DI water and NaCl solution (100 mg L^{-1} and 200 mg L^{-1}). The continuous CDID process with the novel GO-QC/AC electrode disclosed herein offers a novel strategy that provides safe, effective, non-contaminating, energy-efficient and ultrafast contact disinfection of water.

[00275] The single CDID cell disclosed herein employing the GO-QC/AC electrode was able to kill more than 99.9999% *E. coli* in DI water in the first 20 min of the disinfection step with ultra-high bacteria loading (10^6 CFU mL^{-1}); regeneration flushes the dead *E. coli* from the GO-QC/AC electrode and the more than 99.9999% killing can be maintained for 10 cycles (about 200 min). The ultra-high *E. coli* concentration in the loading solution (*i.e.* 1×10^6 to $1 \times 10^7 \text{ CFU mL}^{-1}$) was much higher than that commonly found in brackish water which typically have bacteria loading of about 10^4 CFU mL^{-1} . The inventors have also shown that with a moderate bacteria loading (10^4 CFU mL^{-1}), the CDID cell was able to disinfect water continuously for at least 5 hours. In the prototype, the GO-QC nanohybrid was simply casted onto the AC layer but there is much room for optimization of the specific surface area and thickness of the GO-QC layer and the 3D structure of GO-QC. Further, the surface area of our cell is about 100 cm^2 , and up to 25 or 50 such cells could be connected in stacks or in series. The parallel stacking of hundreds of CDI cells is a common way to increase the processing capacity of salty water in industry; this is also a good way to improve the flow rate and killing time of our CDID cell. Considering these factors (cell morphology, geometry and stacking), the flowrate and durable lifetime of the CDID cell may each potentially be increasable by a factor of 100 or more. Optimization of cell parameters may improve the performance of CDI cells for desalination.

[00276] There are at present numerous technologies that have been developed to disinfect bio-contaminated water for drinking, such as ultrafiltration, distillation and reverse osmosis (RO). However, there is still lacking a decontamination process that is continuous, non-contaminating and yet able to effectively kill and remove bacteria. For example, with RO, chlorine often has to be added upstream of the RO process since bacteria cause biofouling and destroy the membrane, and the current practice of adding chlorine is not ideal for drinking water. The CDID process disclosed herein may be easily integrated with other such continuous processes.

[00277] **TABLE 5** Minimum bactericidal concentrations (MBC), hemolytic activities and ζ -potentials for the PS-QC with three different PS:QC ratio

Materials	MBC ($\mu\text{g mL}^{-1}$)	HC ₅₀ ($\mu\text{g mL}^{-1}$)	ζ potential (mV)
PS-QC (1:1)	312.5	>10000	9.8 ± 1.7
PS-QC (1:5)	78	5000	23.3 ± 0.49
PS-QC (1:10)	8.1	650	48.1 ± 0.7

[00278] In summary, as disclosed herein, a unique disinfecting cationic GO-QC nanohybrid material has been synthesized and explored as part of a novel CDID electrode. The GO-QC nanohybrid forms a thin and porous microbicidal layer on the surface of an AC electrode. The GO-QC/AC electrode may achieve ultra-high killing, *i.e.* 99.9999% (6-log reduction), of 10^6 CFU mL^{-1} *E. coli* in water flowing continuously through the CDID cell. Further, the electrodes may be easily regenerated: with ultra-high bacteria loading, for at least 10 times even with salt water. With moderate (10^4 CFU mL^{-1}) *E. coli* loading, the CDID was able to remove and kill at least > 99.99% bacteria continuously for at least 5 hours. There was no observable GO-QC contamination to the water. The strategy presented herein outperformed all traditional water disinfection processes, and demonstrated impressive features: no observable residual was left in the purified water, and the GO-QC/AC electrode contact killing process was shown to be ultra-fast, continuous, energy-efficient (uses only low voltage of 2 V), and may be easily applied without expensive equipment.

[00279] The GO-QC nanohybrid killed bacteria by contact; it demonstrated excellent MBC and selectivity values against representative Gram-negative, Gram-positive, and fungi species, even compared with the individual components (GO or QC alone). The single atomic layer GO with high functionality enabled dense surface grafting of the cationic QC and low mass contribution of the GO carrier, so that the MBC of this nanohybrid was outstanding. The GO also contributed conductivity to the GO-QC CDID electrode and enabled the hybrid to be bound securely by the binder to the AC electrode. This CDID process with GO-QC nanohybrid modified electrode that is highly microbicidal, fast-acting, non-contaminating and energy-efficient, demonstrated outstanding disinfection application prospects in biomedical, environmental, personal care and other fields.

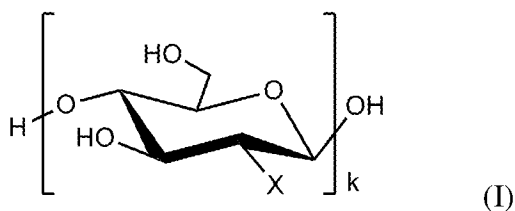
[00280] While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

CLAIMS

1. An electrode comprising

a) an electrically conductive support; and

b) a layer of a hybrid nanomaterial attached to a surface of the electrically conductive support, the hybrid nanomaterial consisting of graphene oxide nanomaterial covalently conjugated to cationic quaternized chitosan, wherein the cationic quaternized chitosan is represented by formula (I)



wherein

each X is independently selected from $-\text{NH}-\text{C}(\text{O})-\text{CH}_3$, $-\text{N}(\text{R}^1)(\text{R}^2)$ and $-\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$, provided that at least one X is $-\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$,

R^1 , R^2 , R^3 , R^4 , and R^5 are independently selected from H and C_{1-18} alkyl, and k is an integer from 3 to 3000.

2. The electrode according to claim 1, wherein R^1 and R^2 are selected from H and C_{1-18} alkyl, preferably H; and R^3 , R^4 , and R^5 are each independently C_{1-10} alkyl.

3. The electrode according to claim 1 or 2, wherein R^3 and R^4 are methyl and R^5 is C_{1-10} alkyl, preferably methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl or n-decyl.

4. The electrode according to any one of claims 1 to 3, wherein ratio of monomers with $\text{X} = -\text{N}(\text{R}^1)(\text{R}^2)$ and $\text{X} = -\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$ to monomers with $\text{X} = -\text{NH}-\text{C}(\text{O})-\text{CH}_3$ is in the range of 2:1 to 5:1, preferably about 4:1.

5. The electrode according to any one of claims 1 to 4, wherein ratio of monomers with $X = -N(R^1)(R^2)$ to monomers with $X = -N^+(R^3)(R^4)(R^5)$ is in the range of 1:4 to 4:1, preferably about 1:2 to 1:1.

- 5 6. The electrode according to any one of claims 1 to 5, wherein the cationic quaternized chitosan comprises dimethyldecylammonium chitosan having general formula (II)



wherein

- 10 R is selected from the group consisting of $-\text{CH}_2(\text{CH}_2)_8\text{CH}_3$ and $-\text{CH}_3$; and ratio of m:n:p is 3:5:2.

7. The electrode according to any one of claims 1 to 6, wherein weight ratio of the graphene oxide nanomaterial to the cationic quaternized chitosan in the hybrid nanomaterial is in the range of about 1:1 to about 1:10.

8. The electrode according to any one of claims 1 to 7, wherein the cationic quaternized chitosan is covalently bonded to the graphene oxide nanomaterial via an amide bond.

- 20 9. The electrode according to any one of claims 1 to 8, wherein the electrically conductive support is a porous support.

10. The electrode according to any one of claims 1 to 9, wherein the electrically conductive support comprises activated carbon.

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11. The electrode according to any one of claims 1 to 10, wherein the hybrid nanomaterial is attached to a surface of the electrically conductive support by a binder.

12. The electrode according to claim 11, wherein the binder is selected from the group consisting of polyvinylidene fluoride, styrene-butadiene rubber, carboxymethyl

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cellulose, polytetrafluoroethylene, poly(vinyl alcohol), poly(vinyl acetate), combinations thereof, and copolymers thereof.

13. The electrode according to claim 11 or 12, wherein weight ratio of the hybrid
5 nanomaterial to the binder is in the range of about 9:1 to about 4:1.

14. An electrochemical cell comprising an electrode according to any one of claims 1 to
13.

10 15. The electrochemical cell according to claim 14, wherein the electrode is an anode
and/or a cathode of the electrochemical cell.

16. Use of an electrode according to any one of claims 1 to 13, or an electrochemical cell
according to claims 14 or 15 in a capacitive deionization process for killing
15 microorganisms.

17. Use according to claim 16, wherein the microorganisms are selected from the group
consisting of Gram-positive bacteria, Gram-negative bacteria, fungus, and
combinations thereof.

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18. Use according to claim 16 or 17, wherein the microorganisms are selected from the
group consisting of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and
combinations thereof.

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FIG. 1A

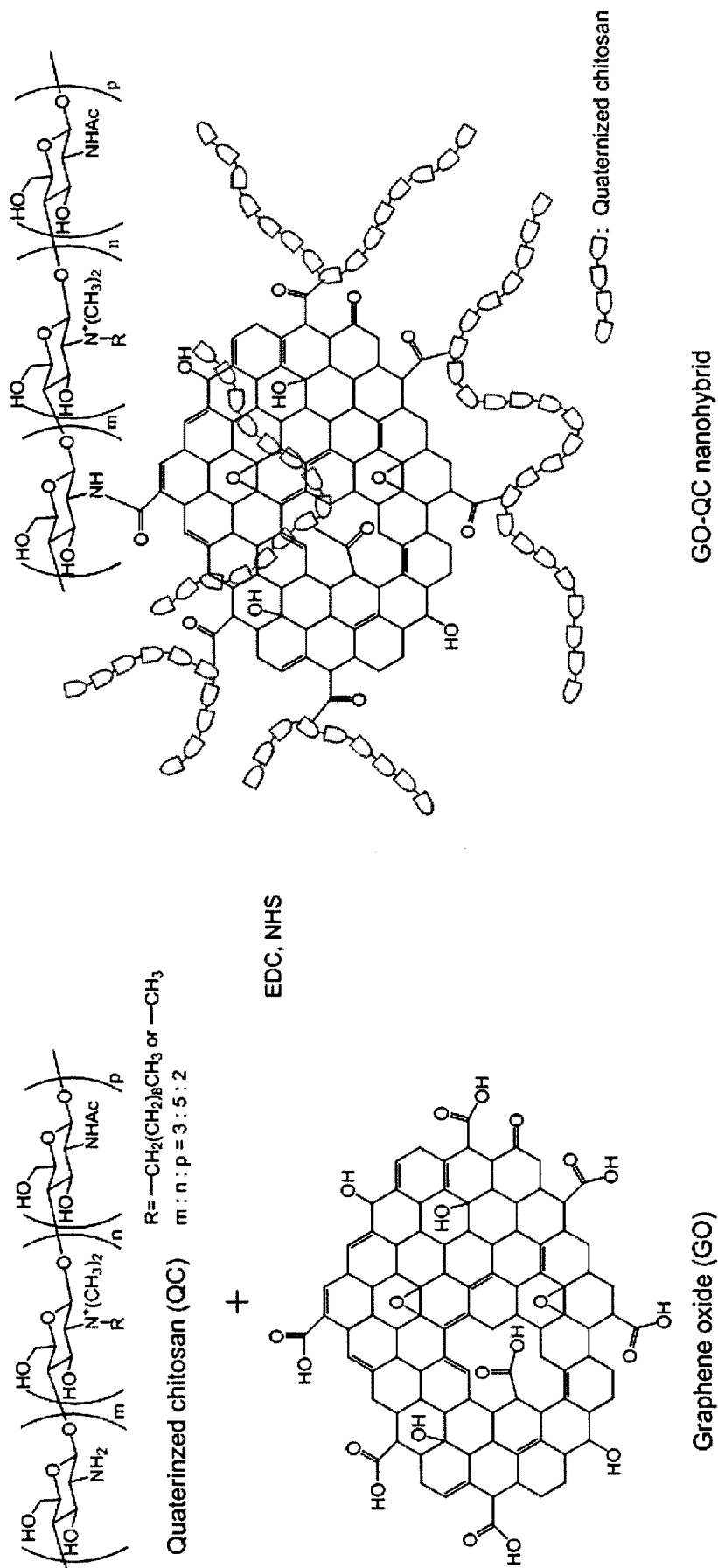


FIG. 1B

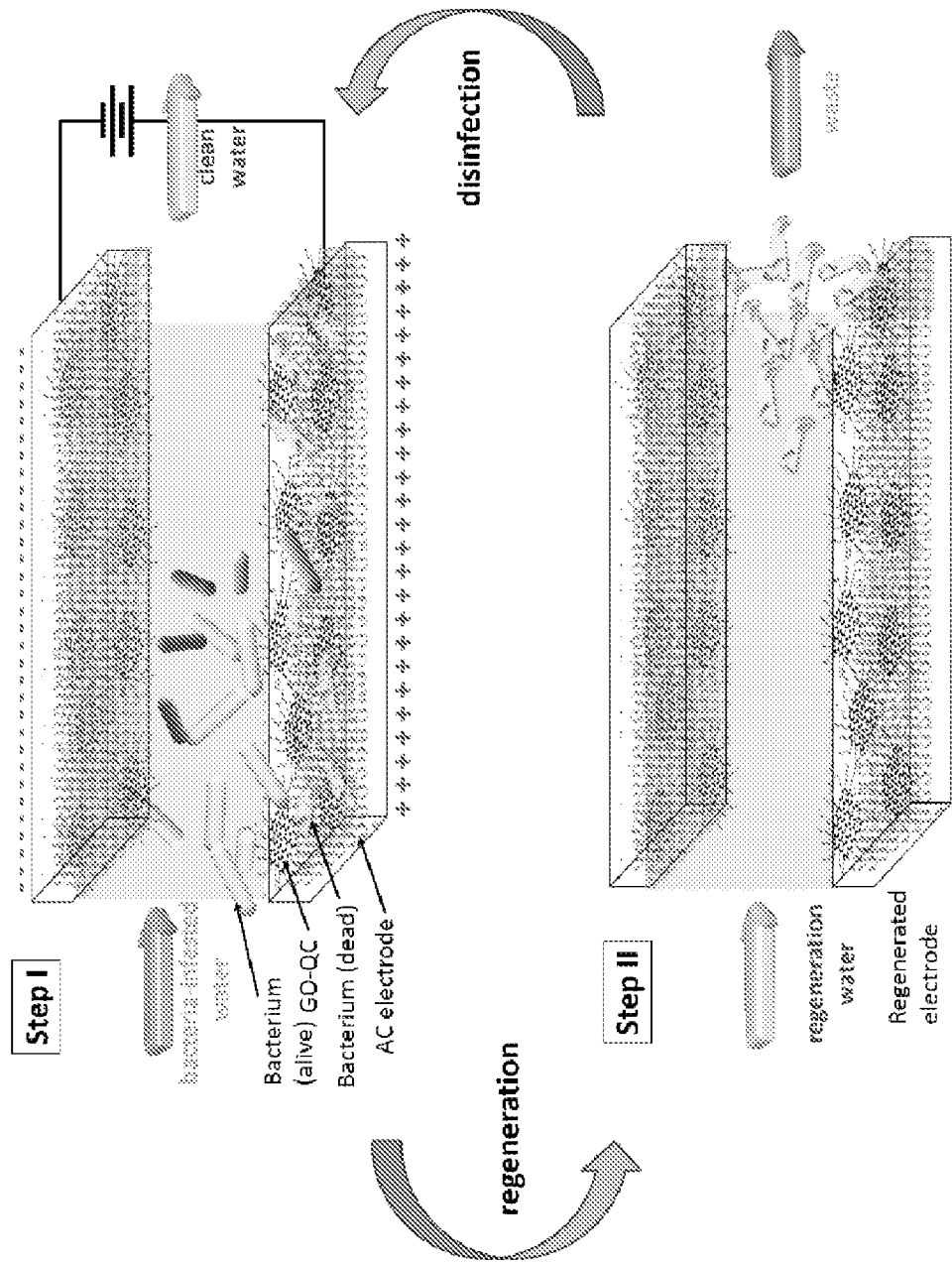


FIG. 2

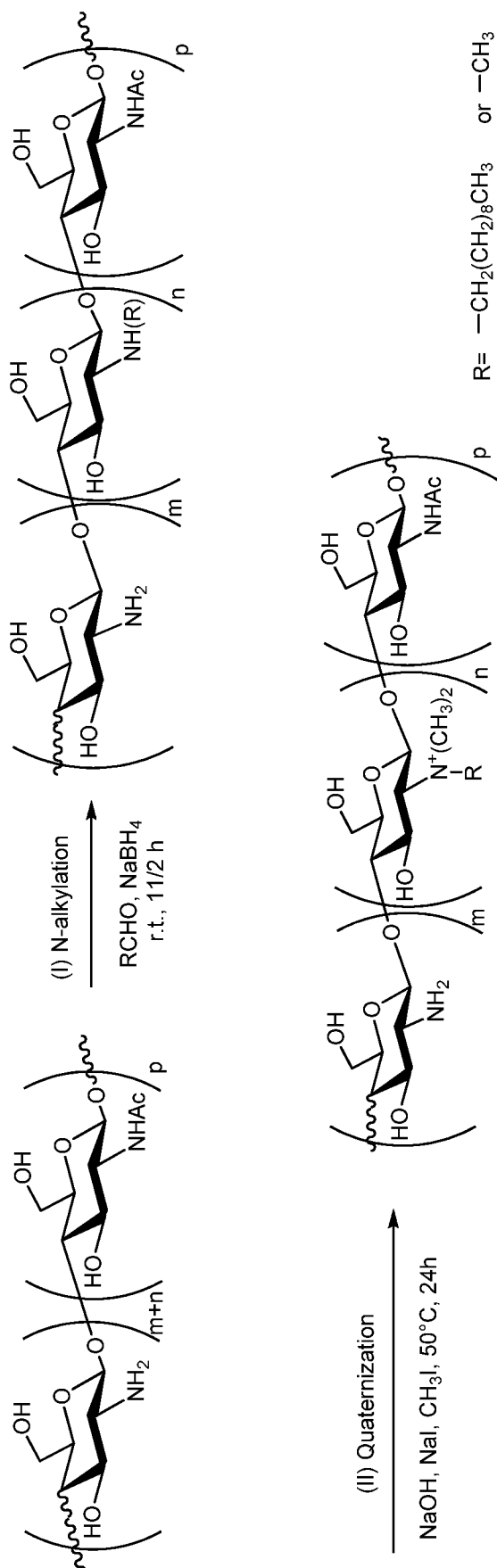


FIG. 3

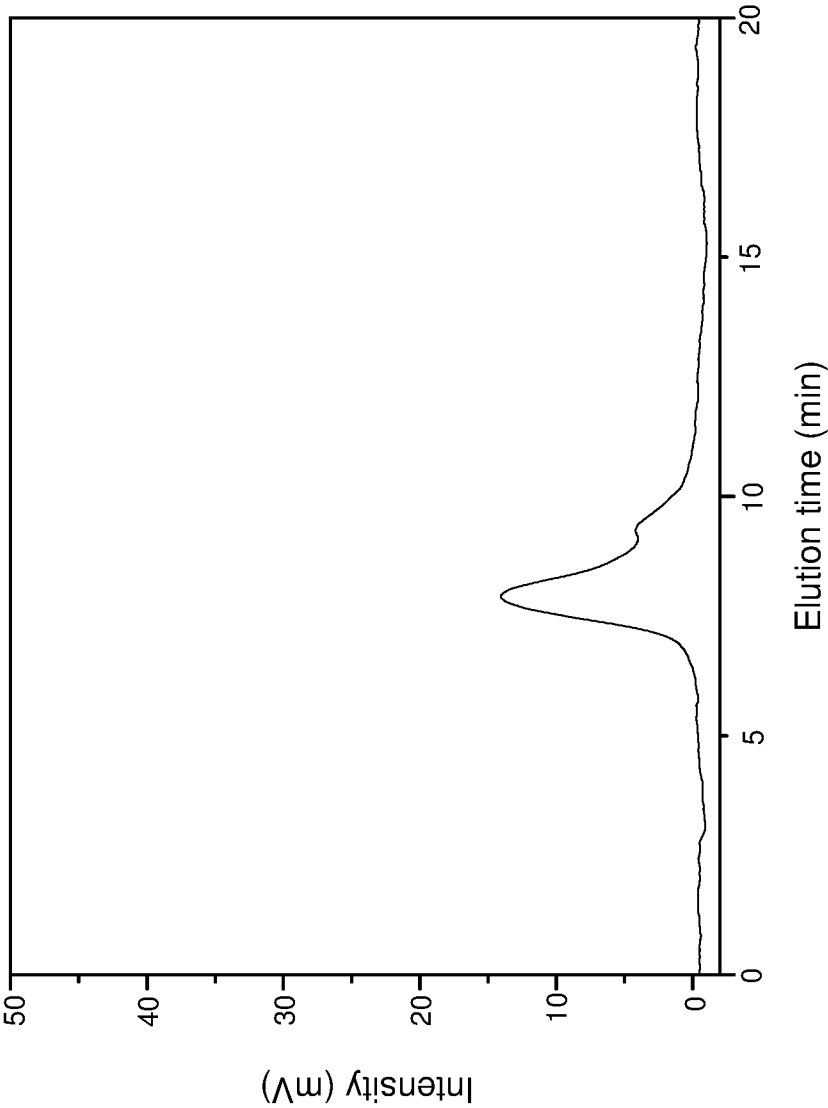


FIG. 4A

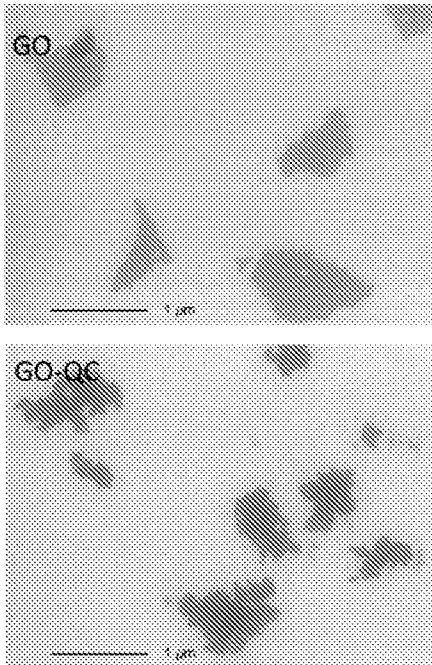


FIG. 4B

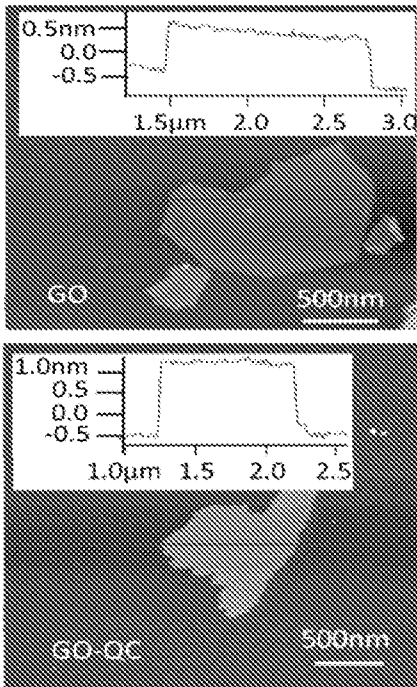


FIG. 4C

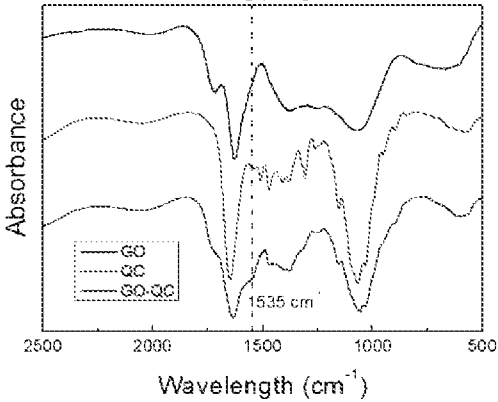
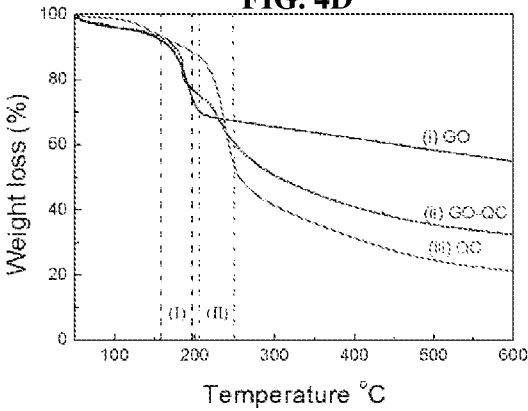
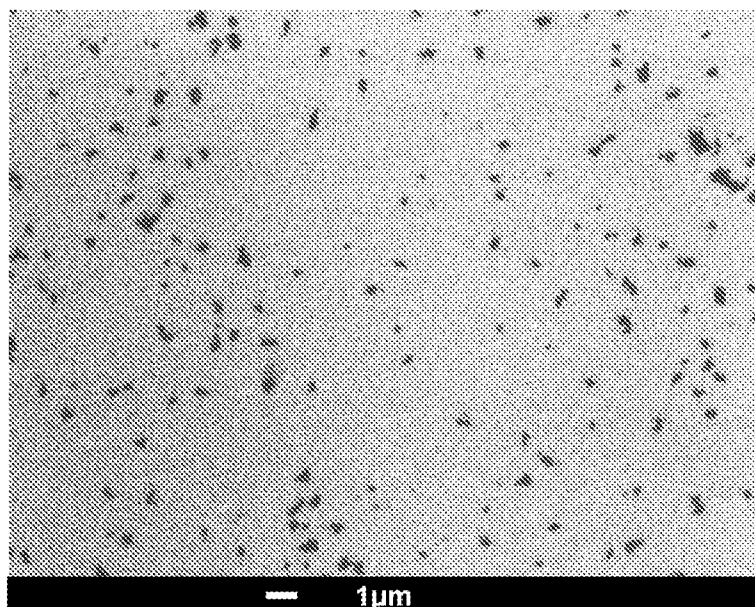
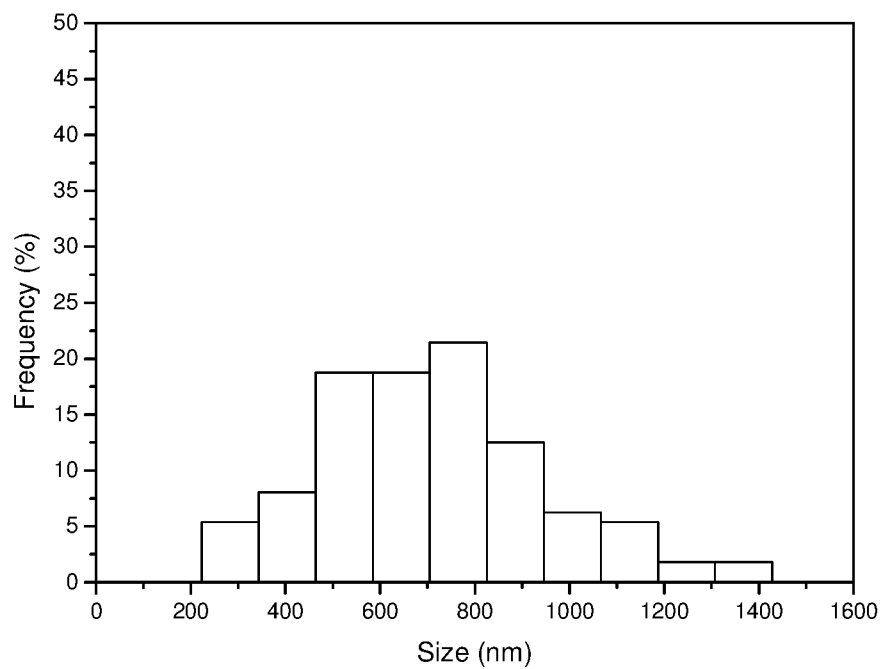


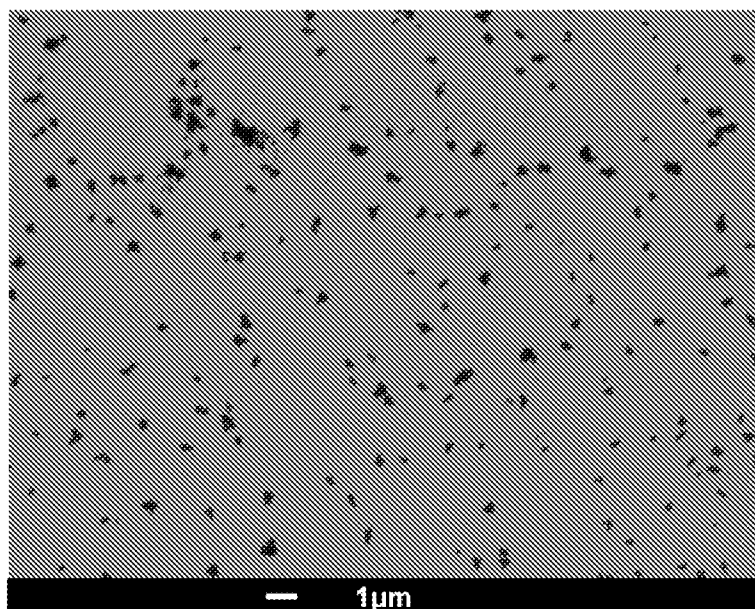
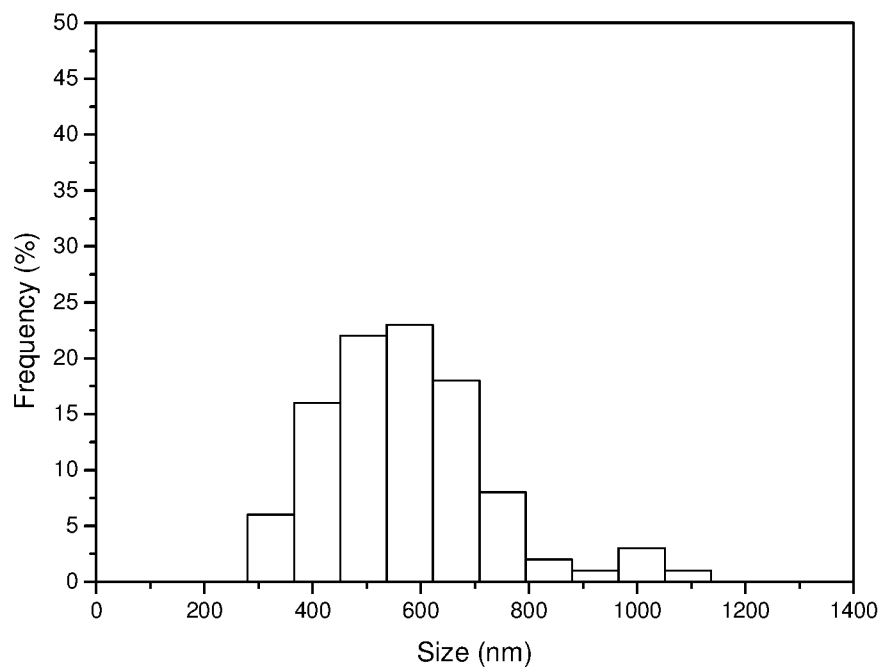
FIG. 4D

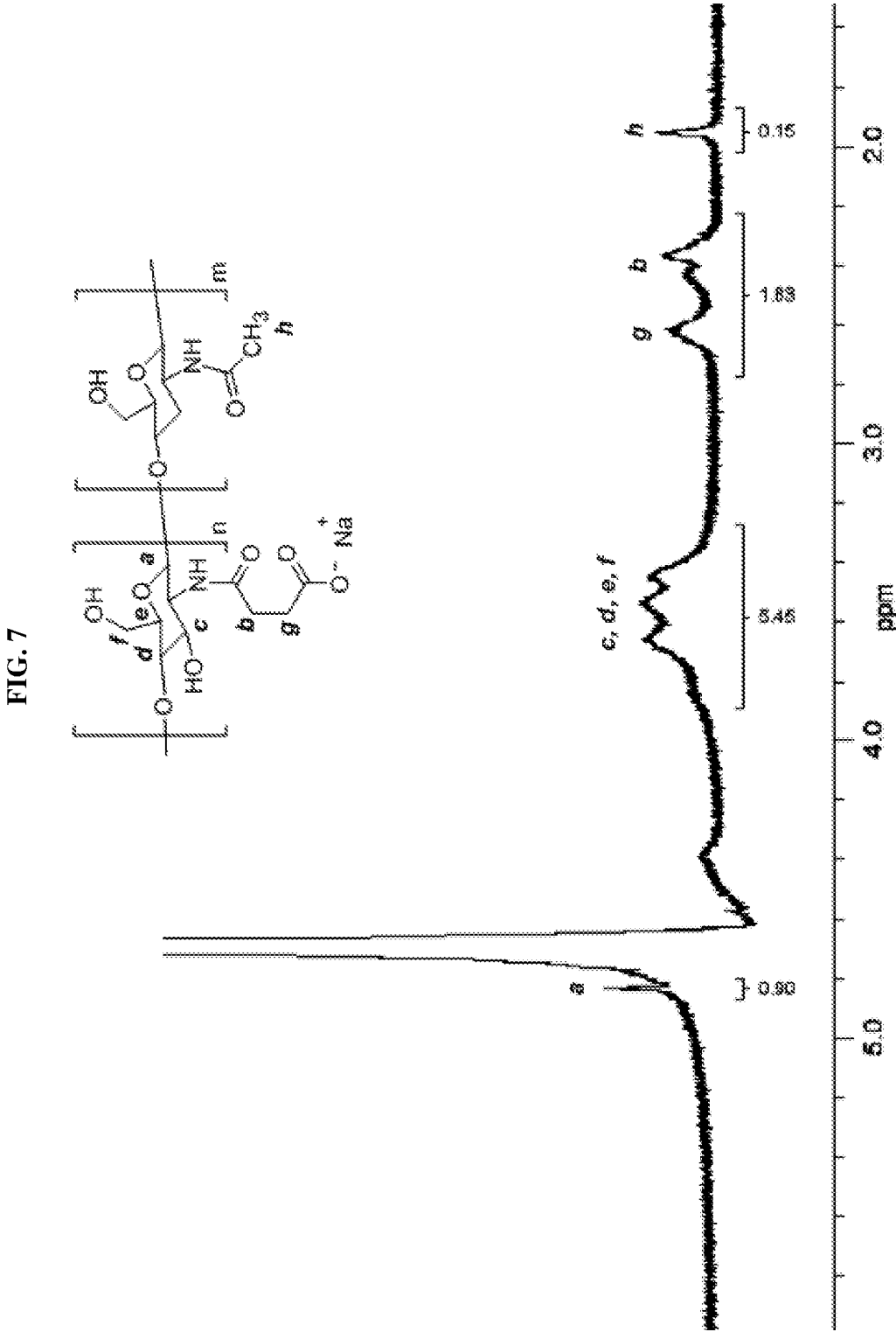


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FIG. 5A**FIG. 5B**

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FIG. 6A**FIG. 6B**



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FIG. 8A

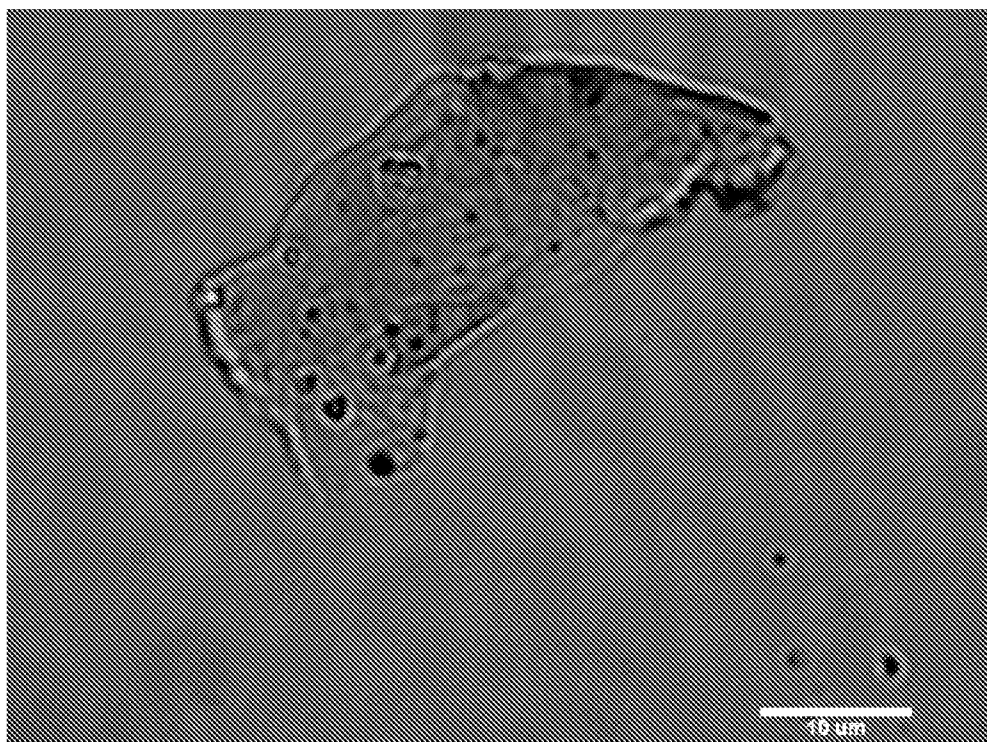
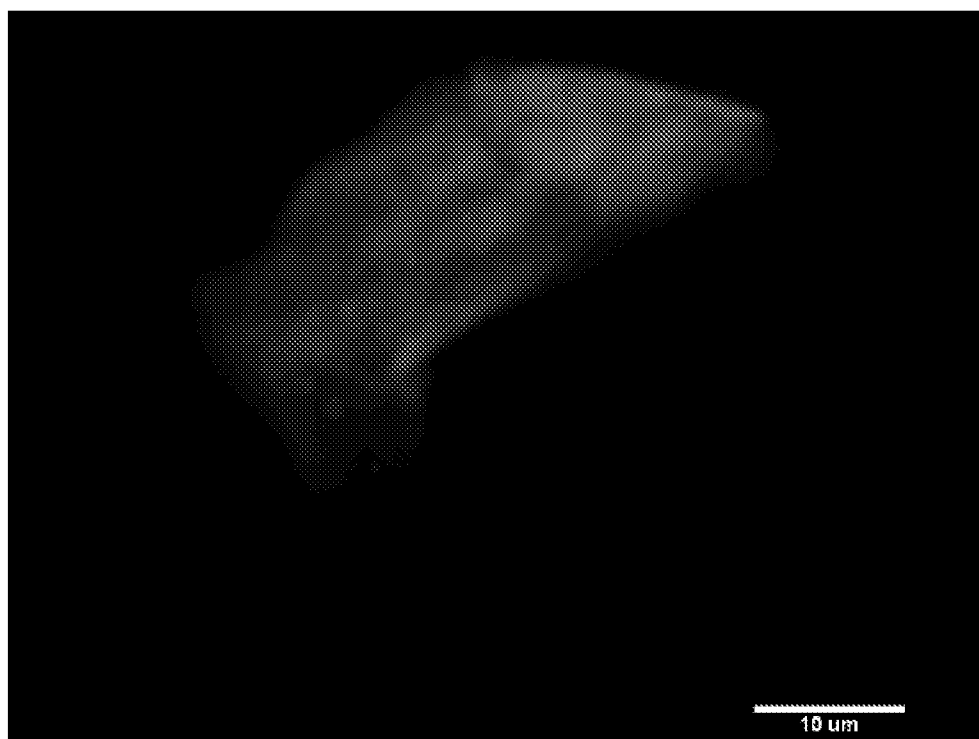


FIG. 8B



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FIG. 9A

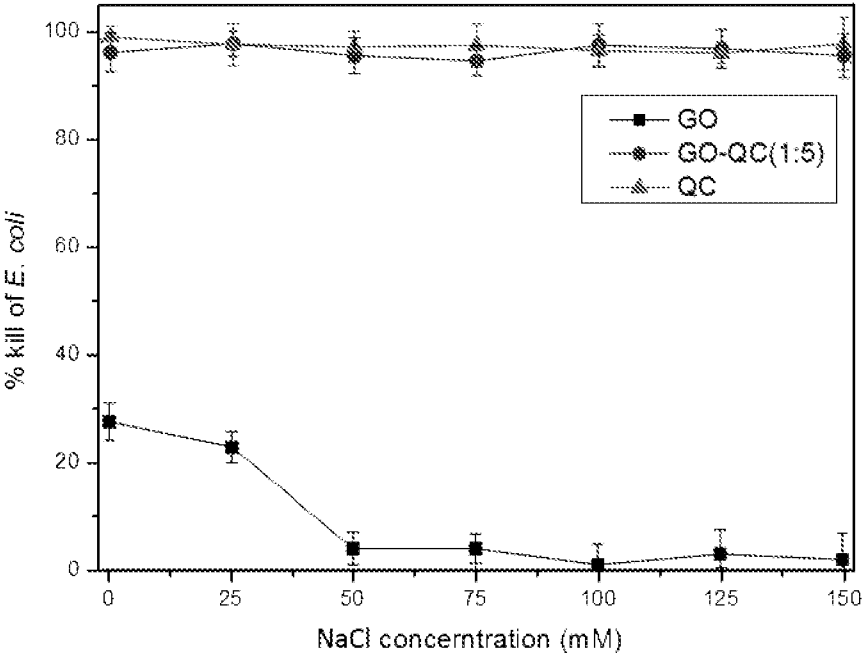


FIG. 9B

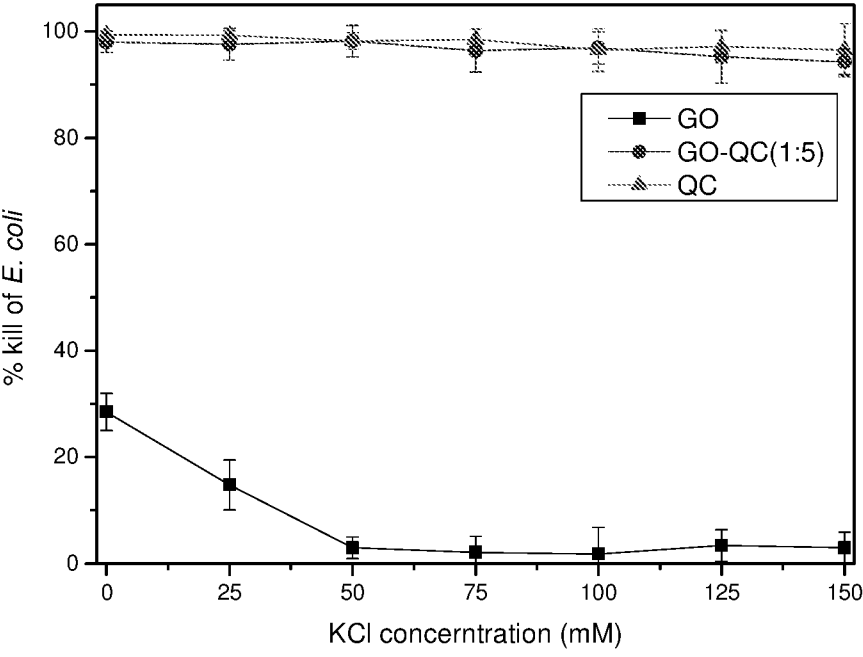


FIG. 9C

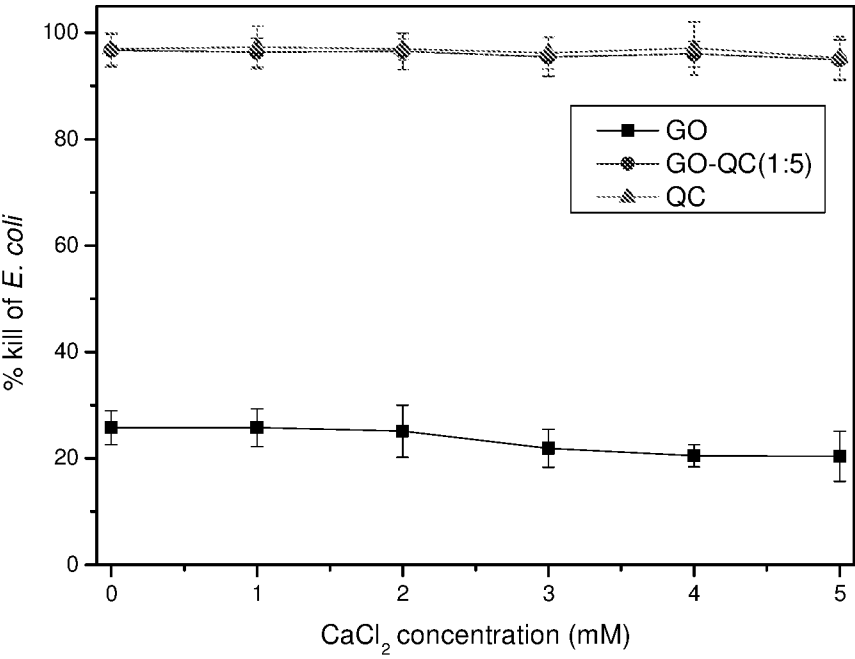


FIG. 9D

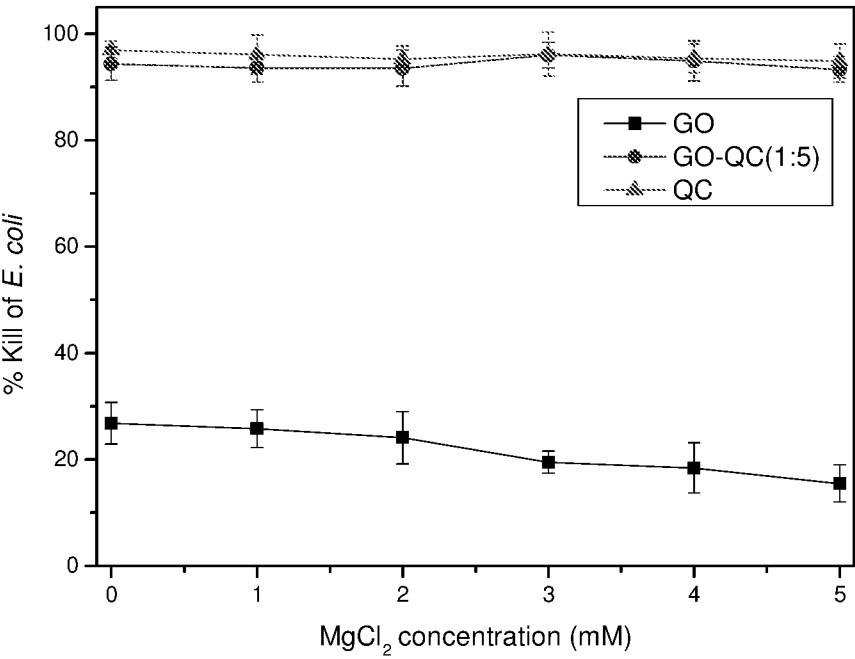


FIG. 10B

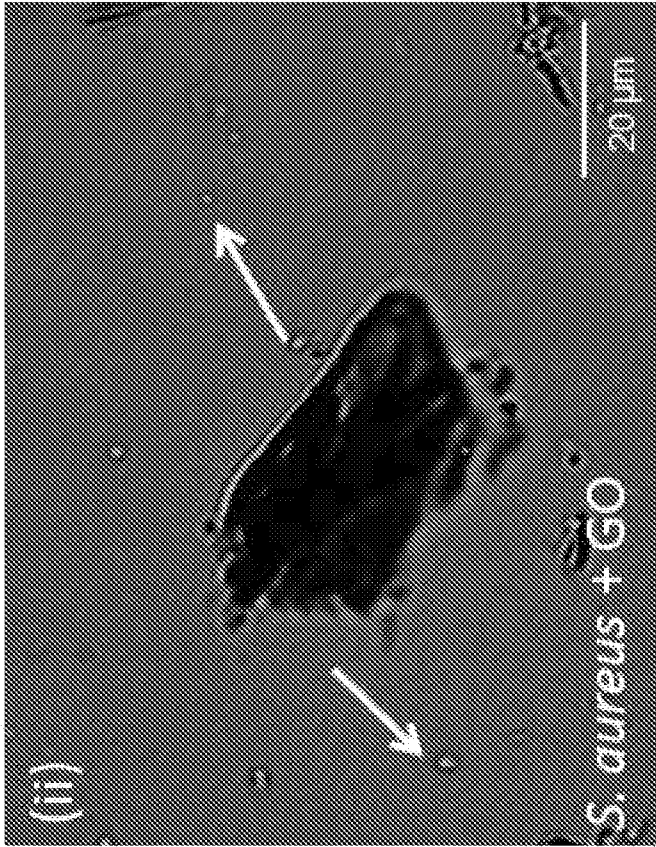


FIG. 10A

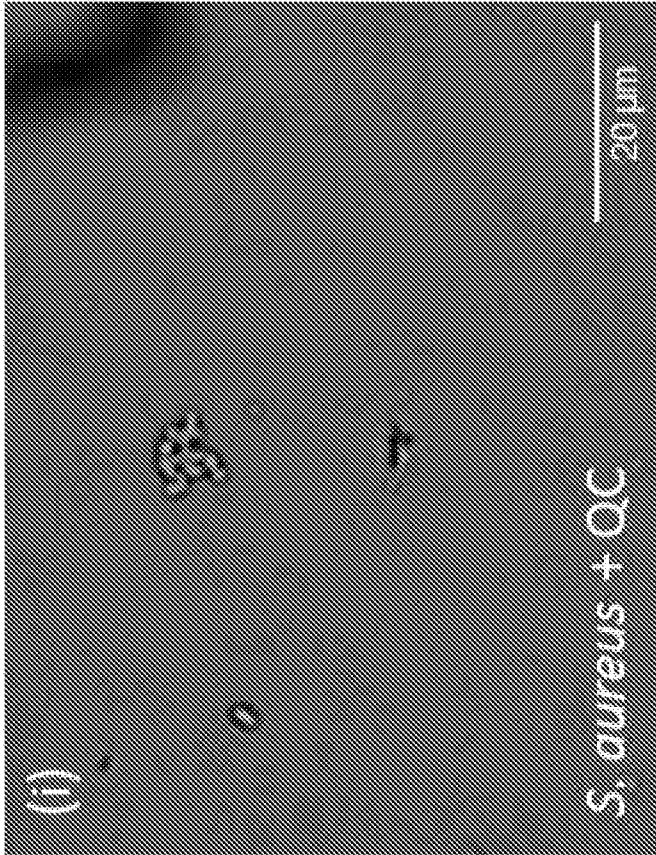


FIG. 11A

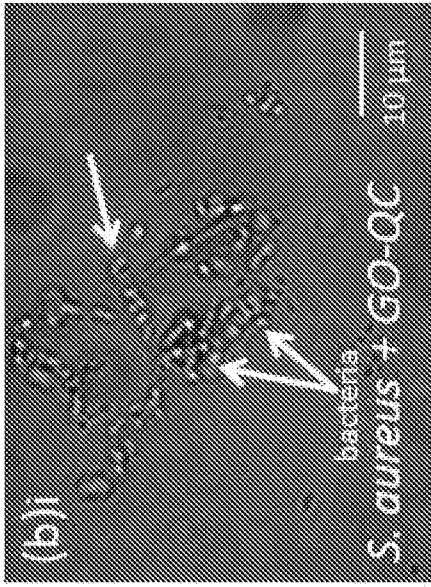
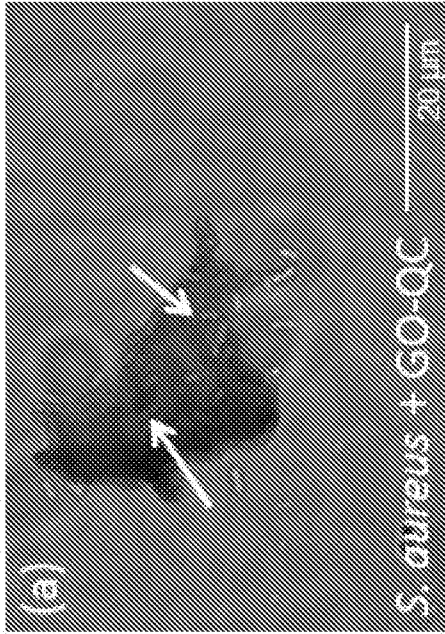


FIG. 11B

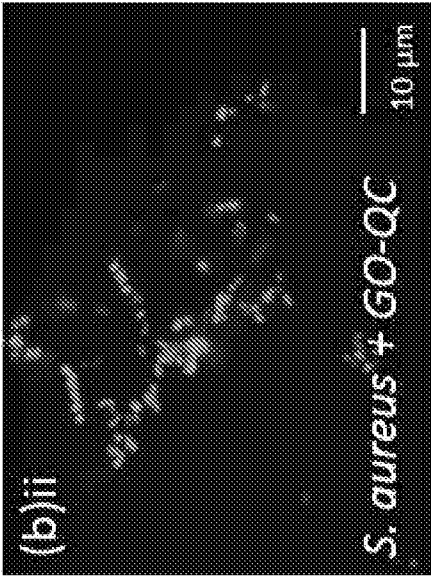


FIG. 11C

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FIG. 12A

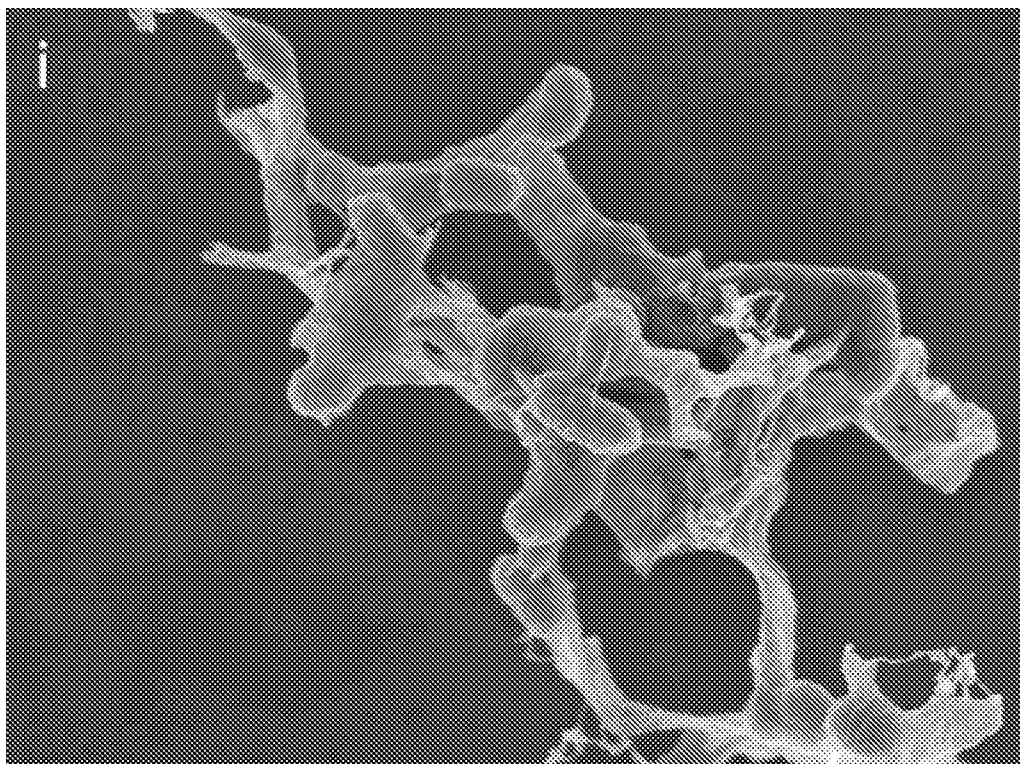
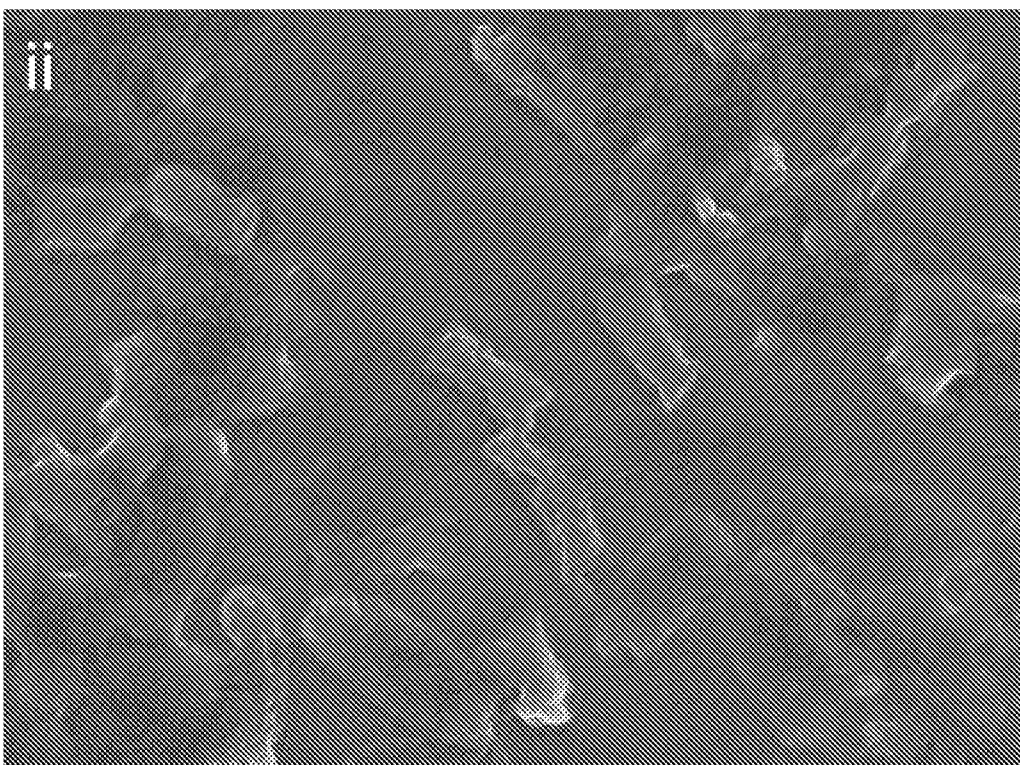


FIG. 12B



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FIG. 12C

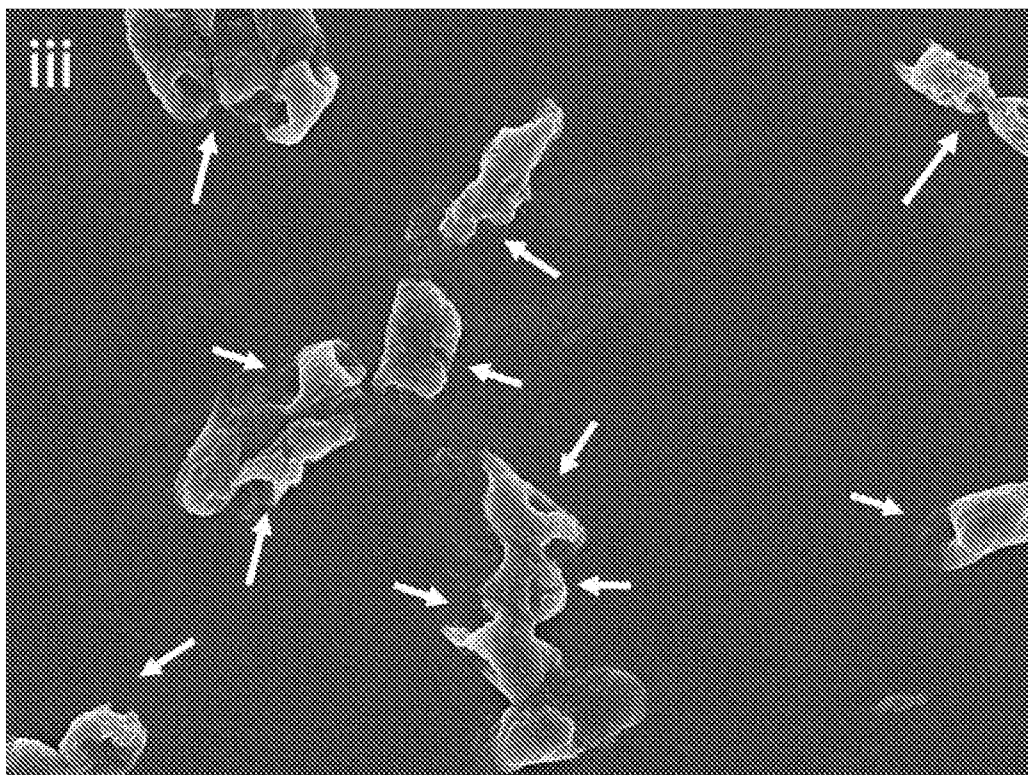


FIG. 13B

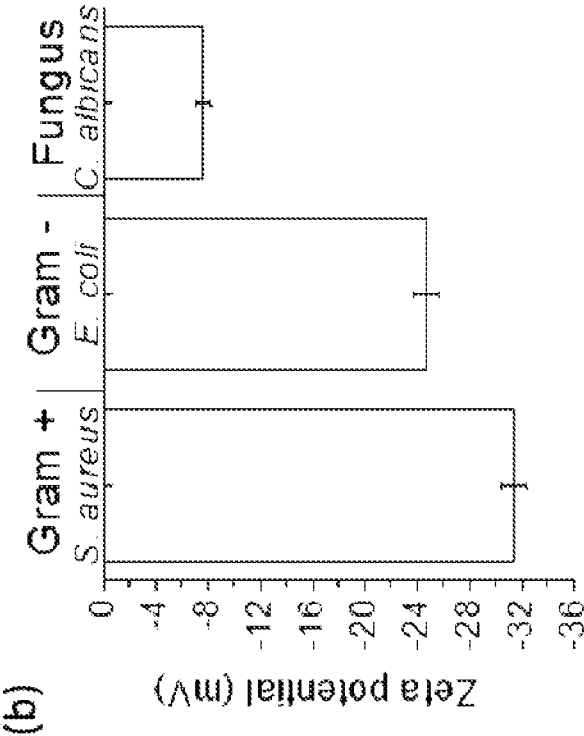


FIG. 13A

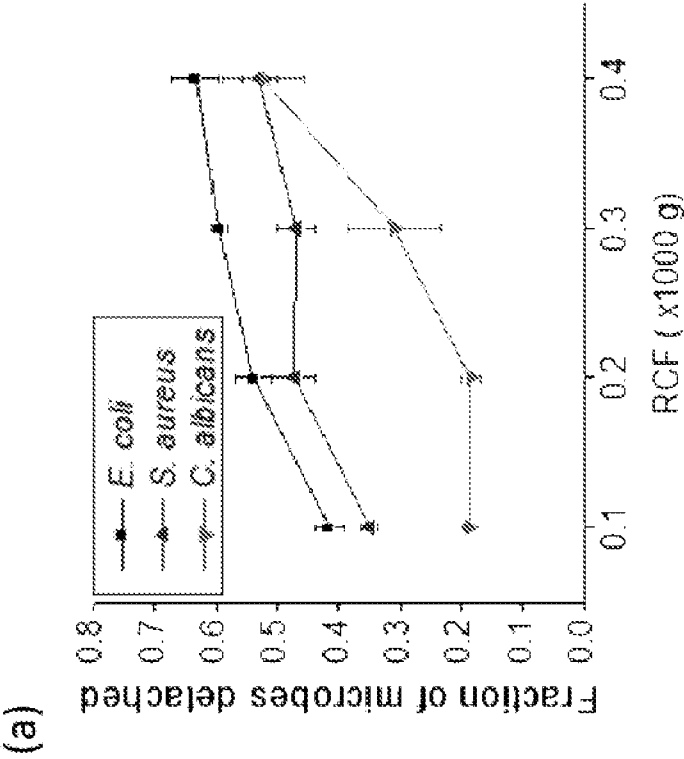


FIG. 14C
GO (large)

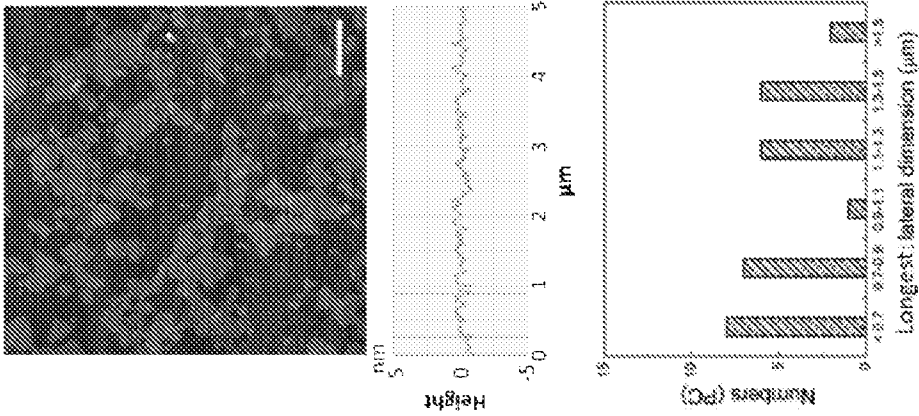


FIG. 14B
GO (Medium)

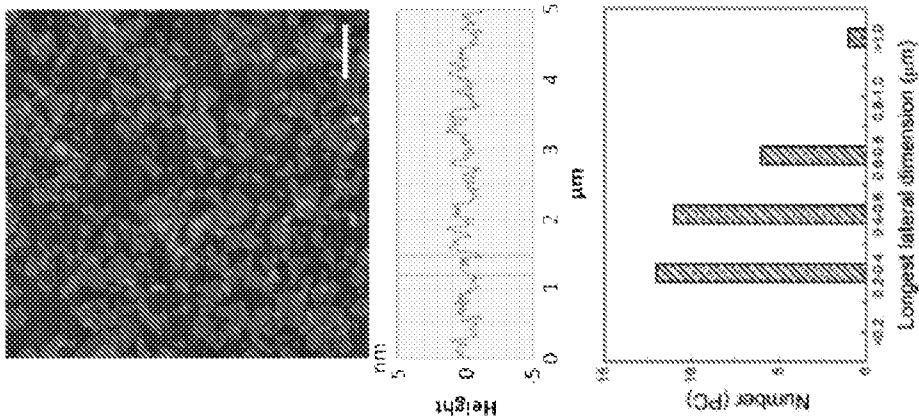


FIG. 14A
GO (small)

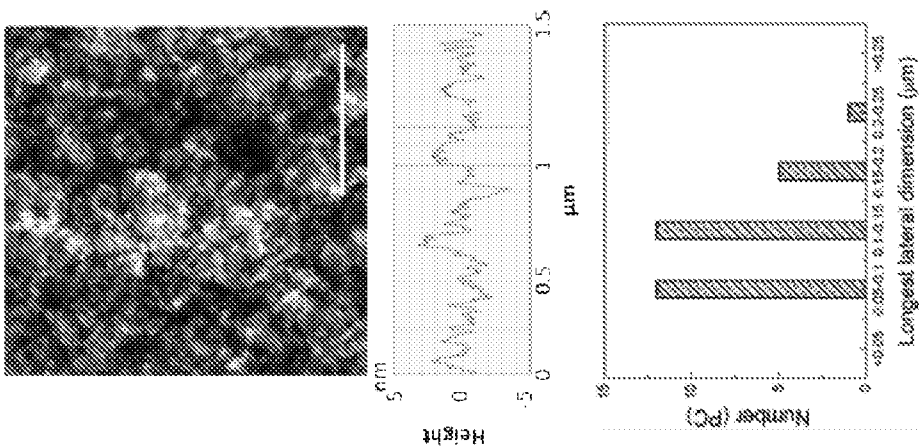


FIG. 14D

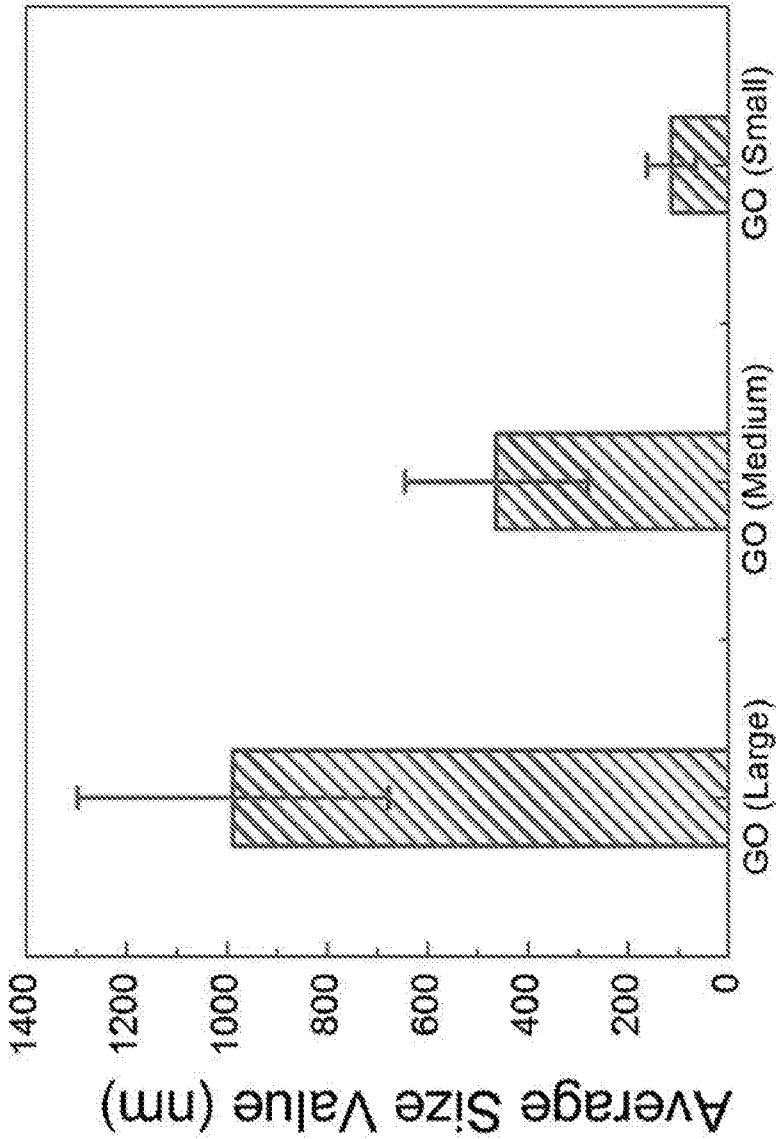


FIG. 15A

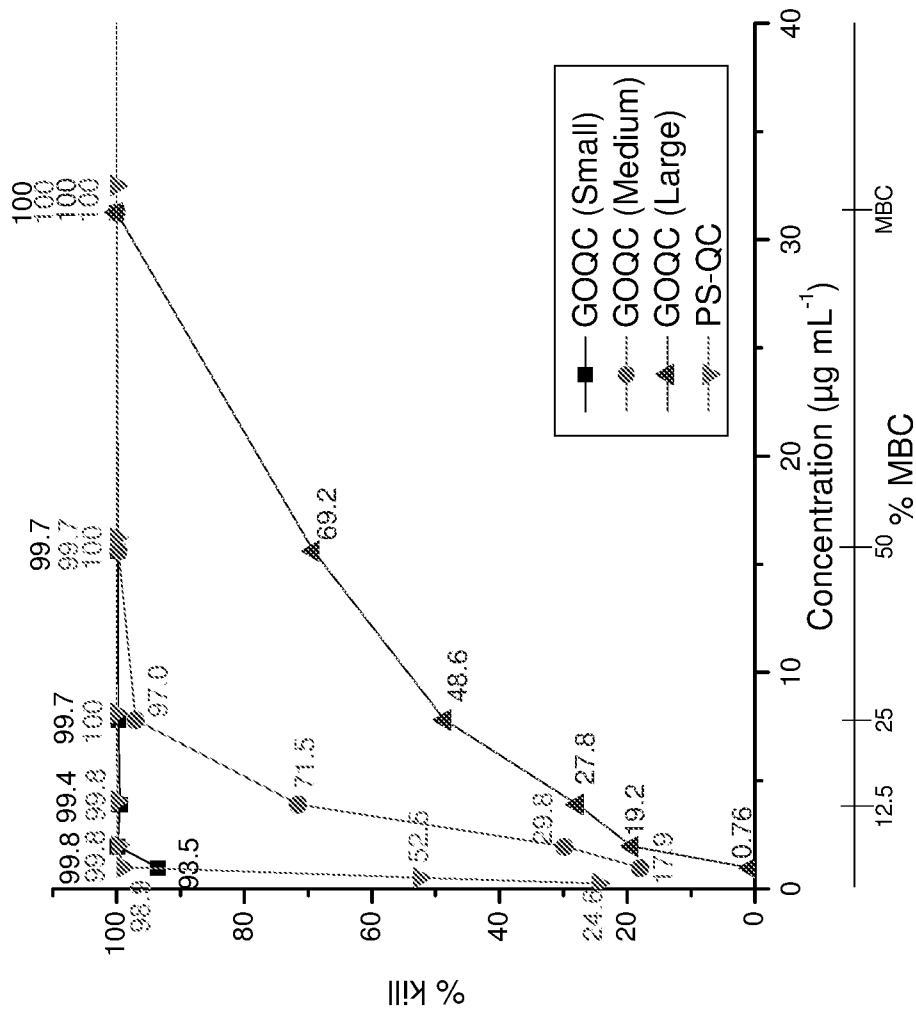


FIG. 15B

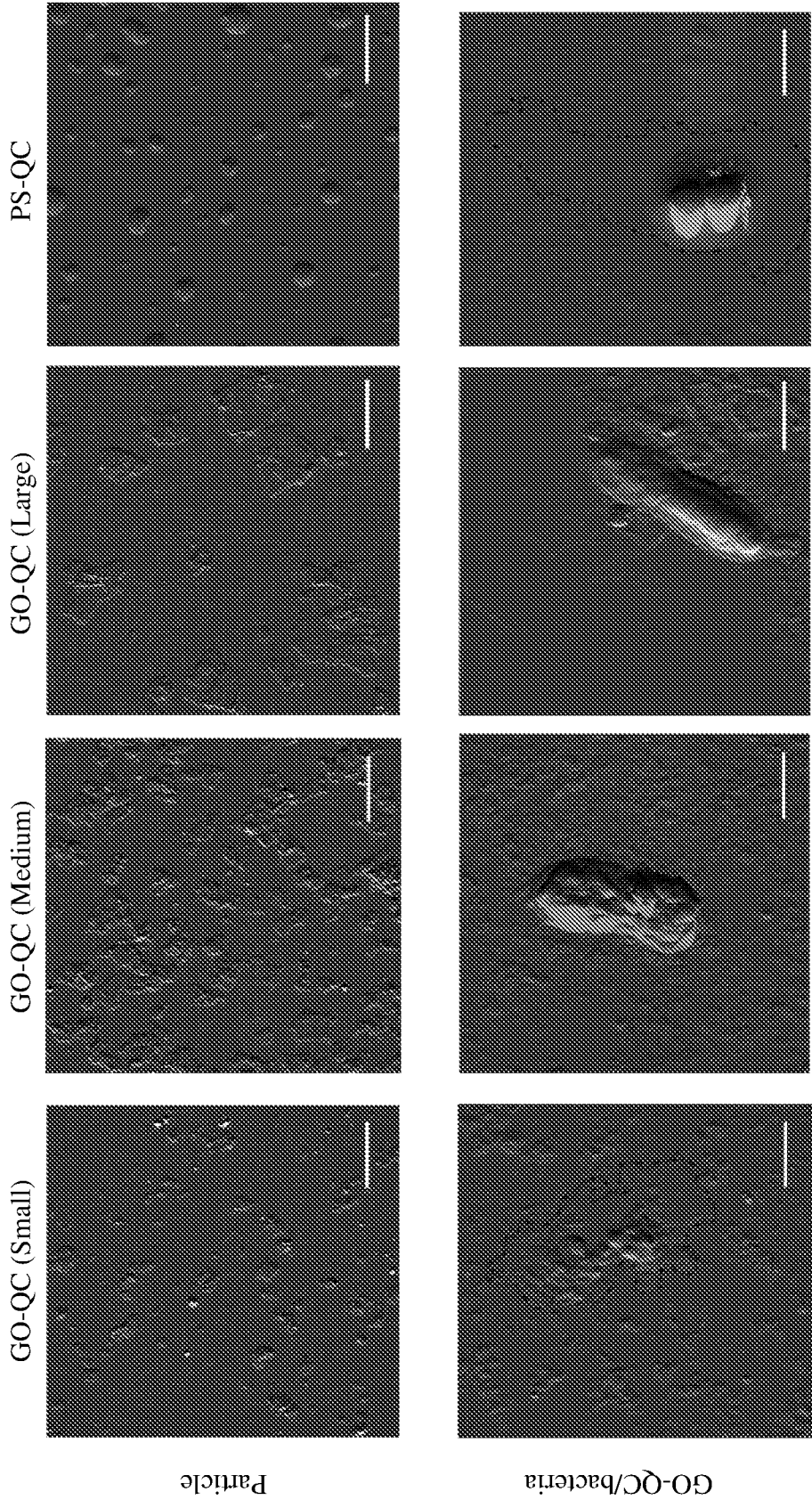


FIG. 16

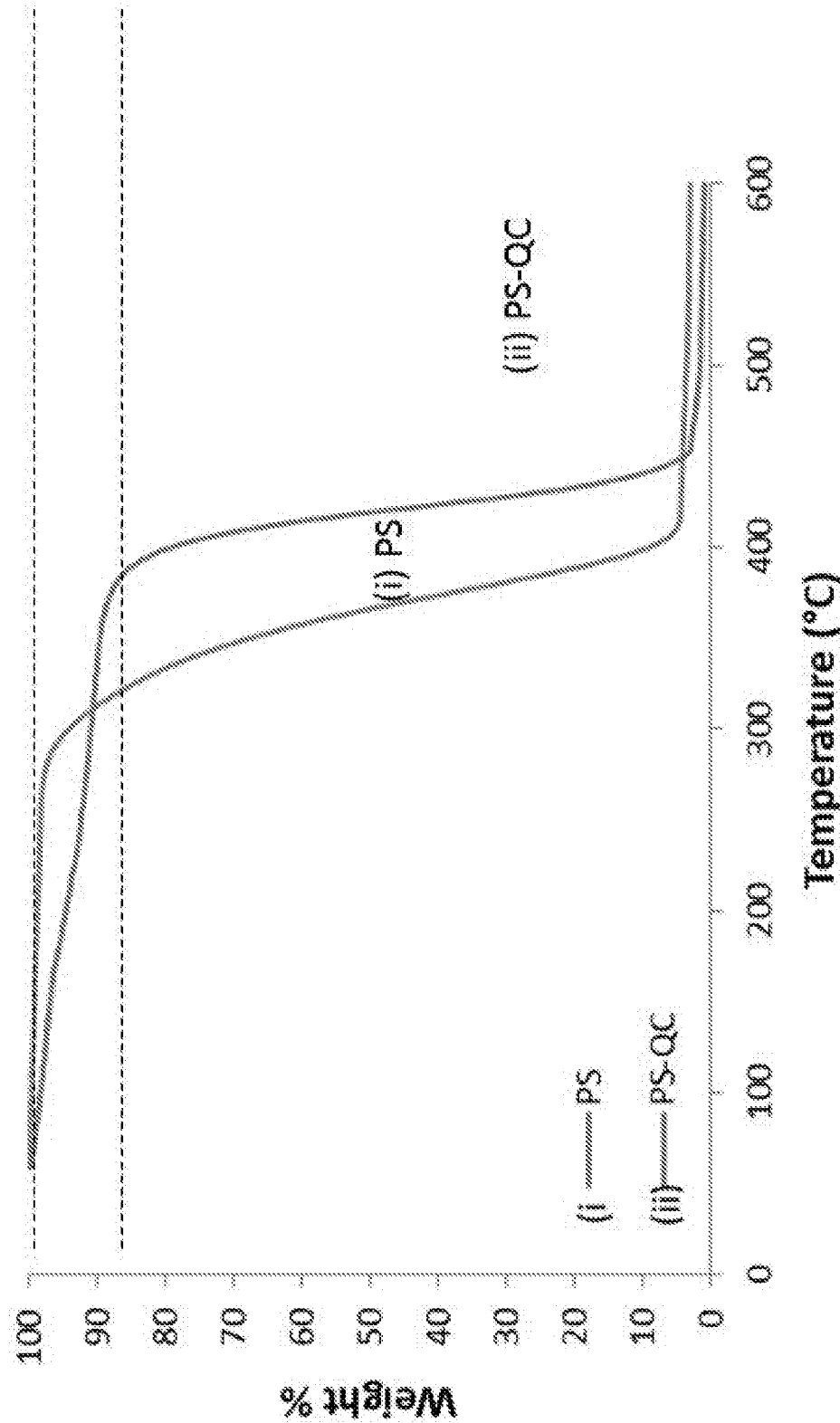


FIG. 17B
GO-QC (Large)

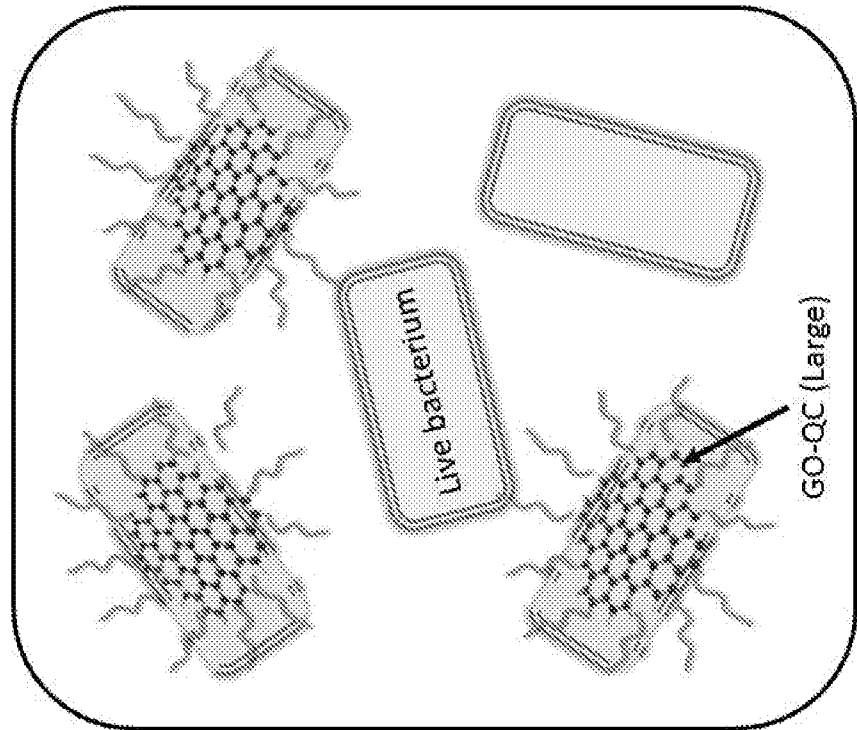


FIG. 17A
GO-QC (Small)

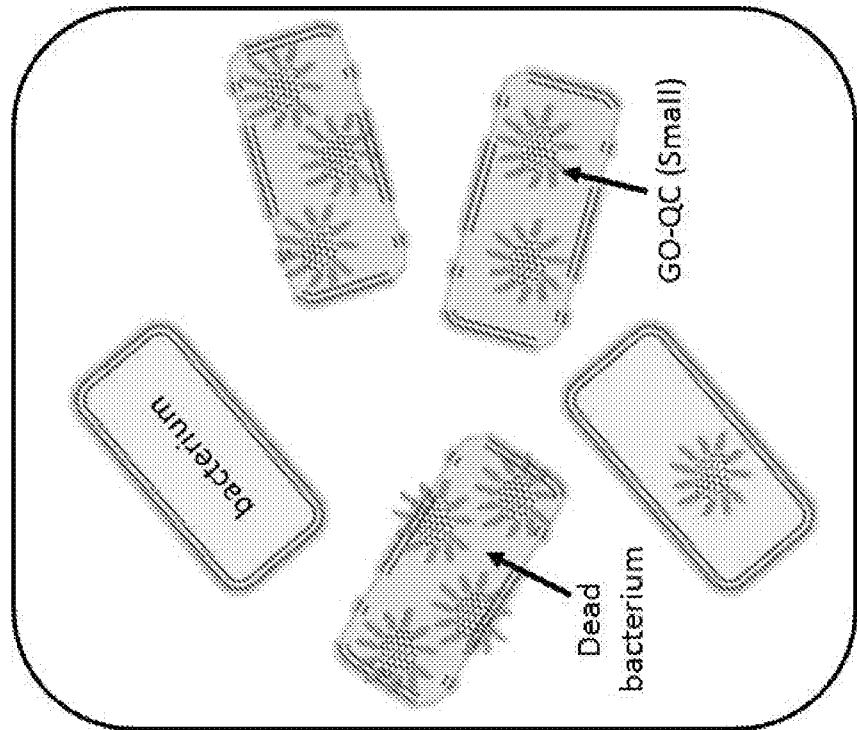


FIG. 18B

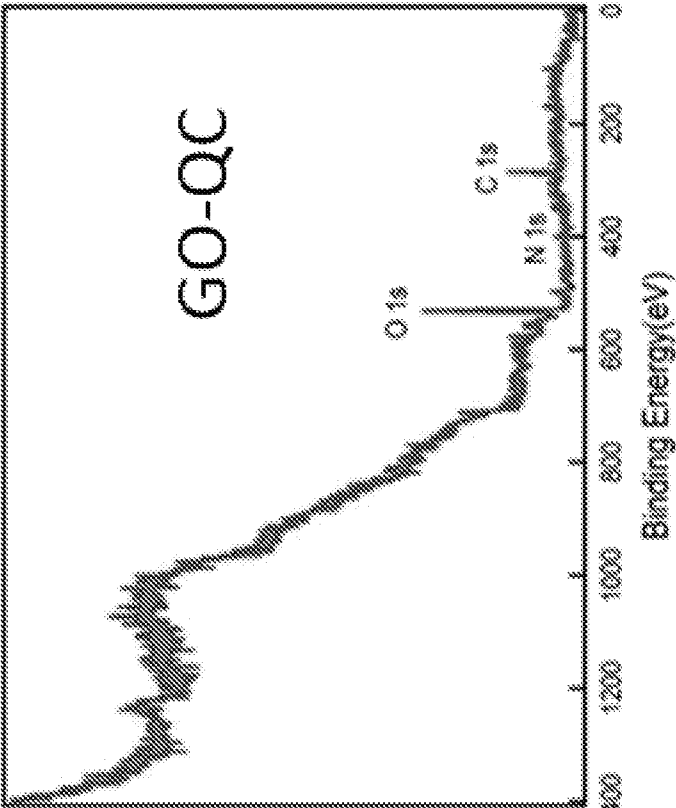


FIG. 18A

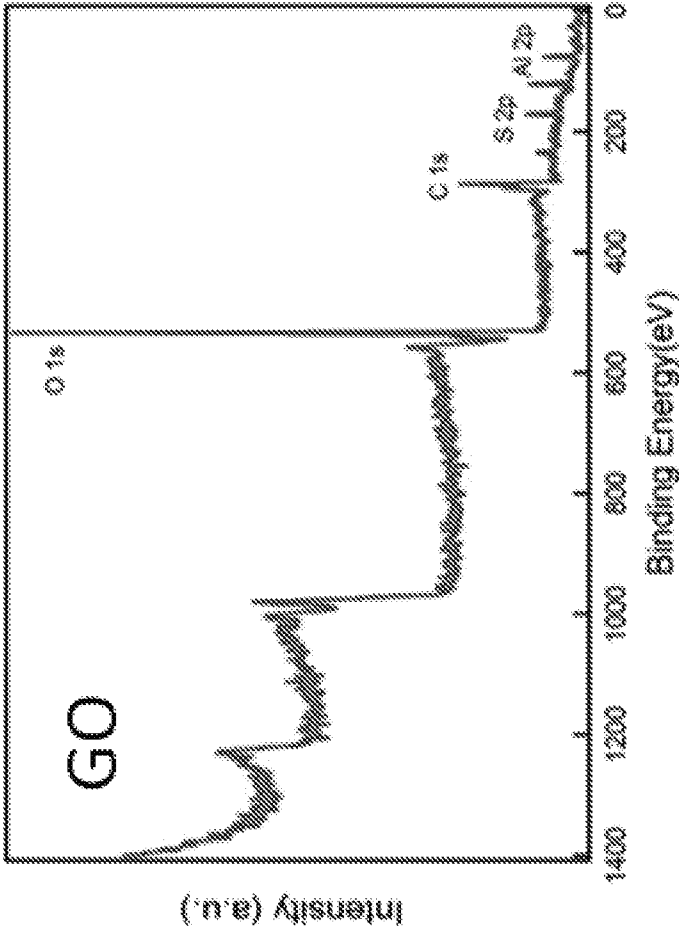


FIG. 18C

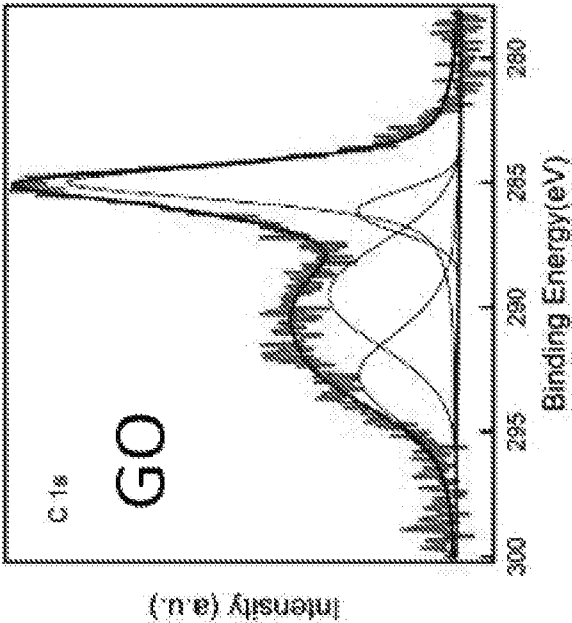


FIG. 18D

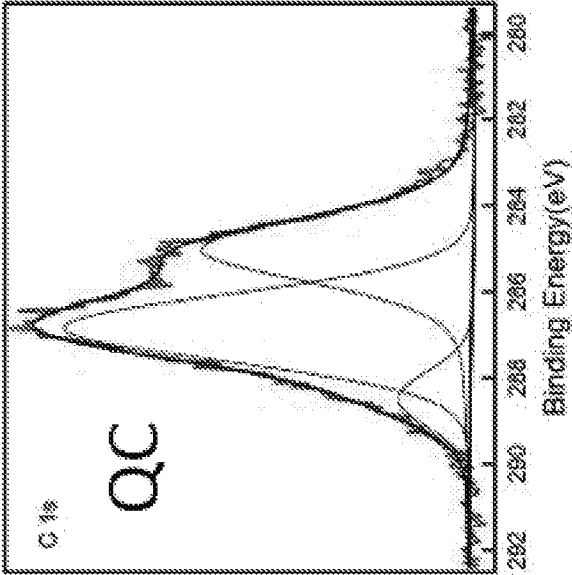


FIG. 18E

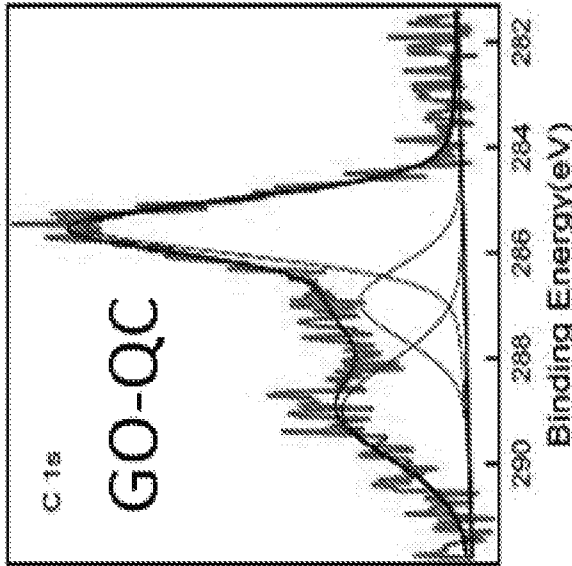


FIG. 18F

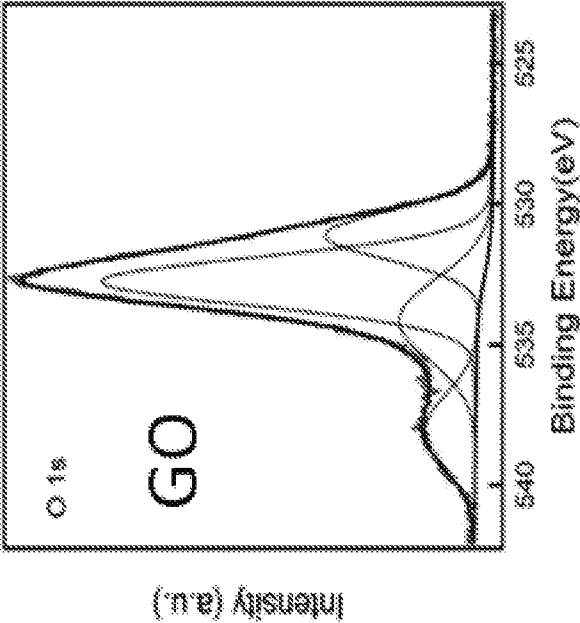


FIG. 18G

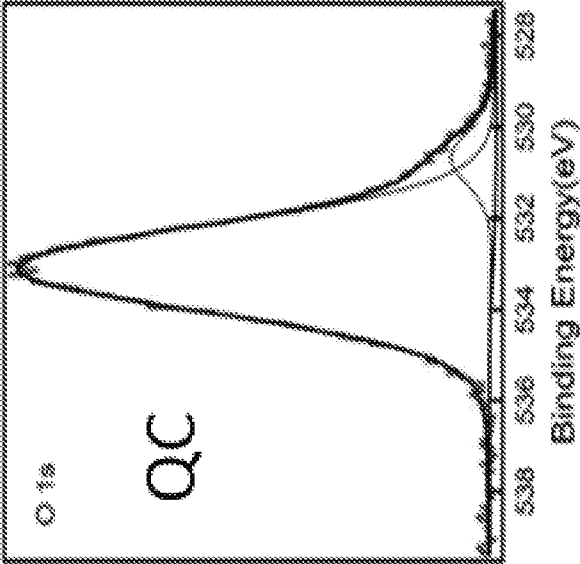


FIG. 18H

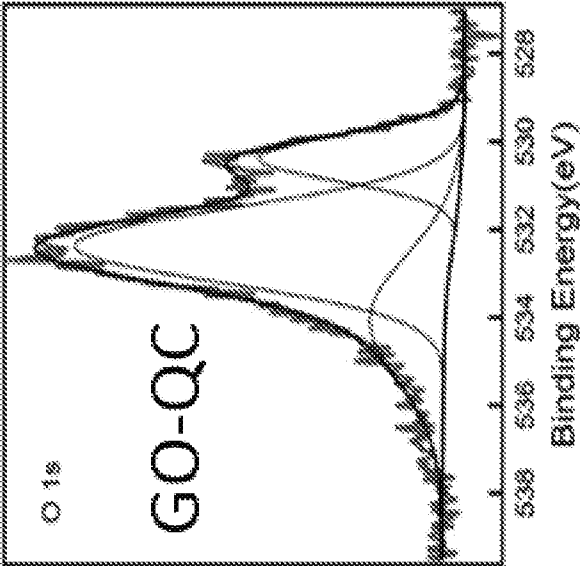


FIG. 18I

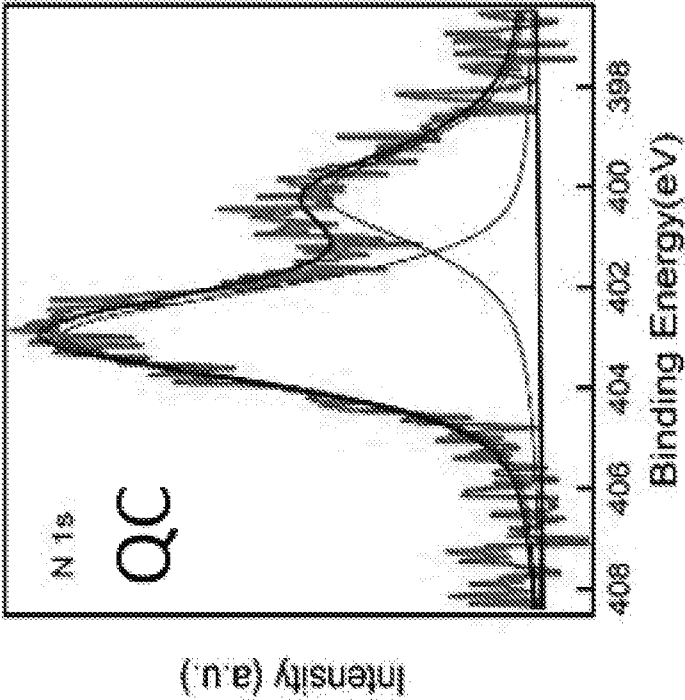


FIG. 18J

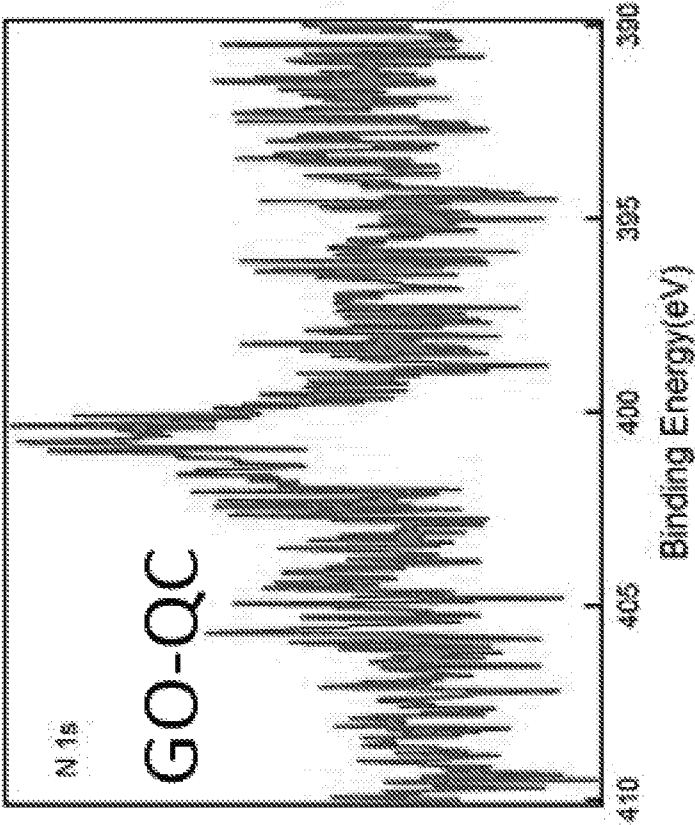


FIG. 19A

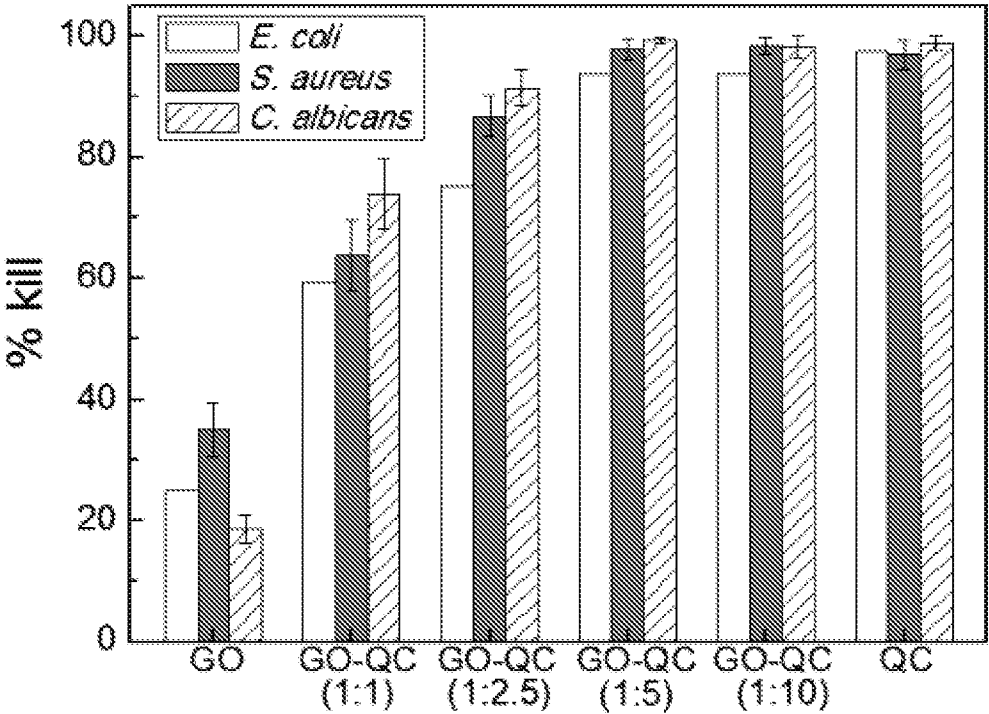


FIG. 19B

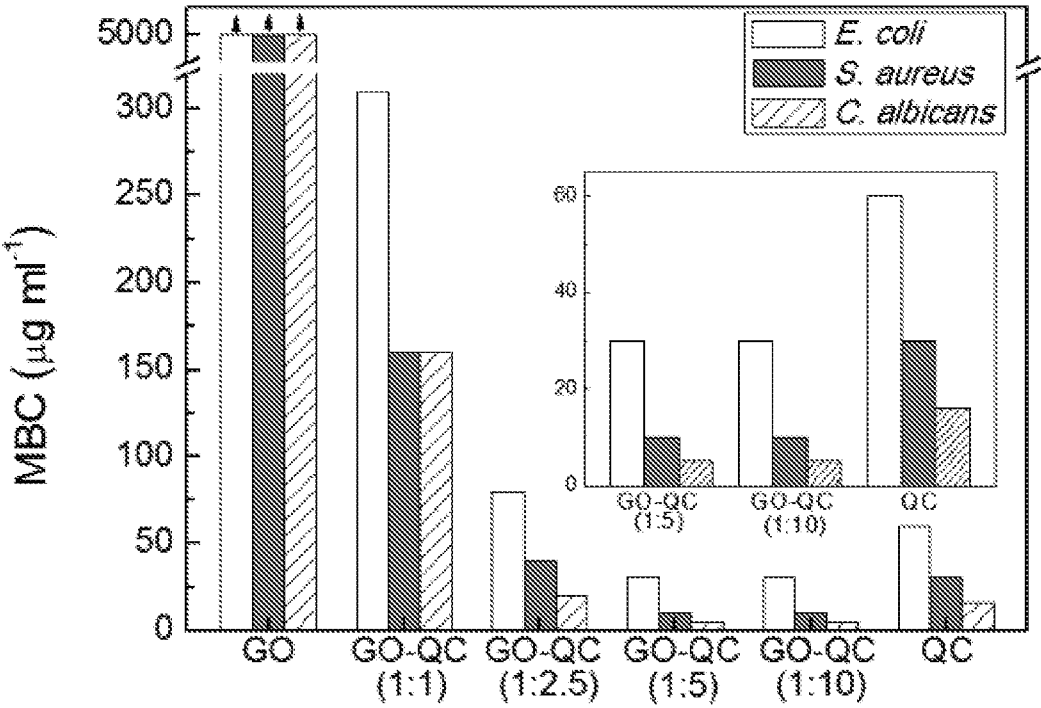


FIG. 19C

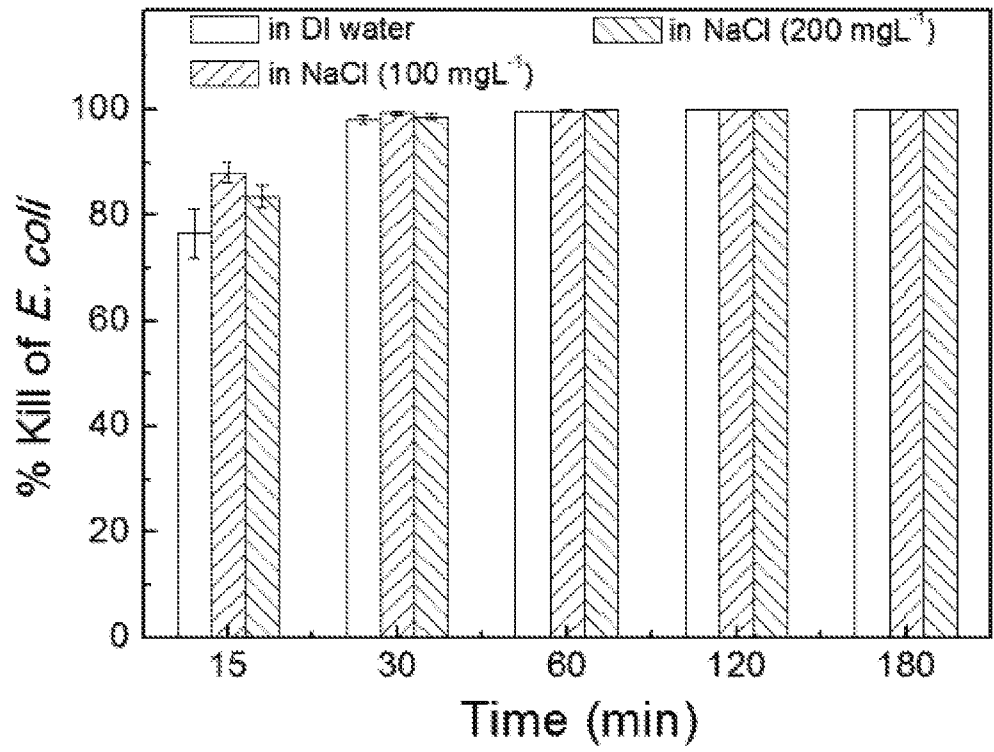
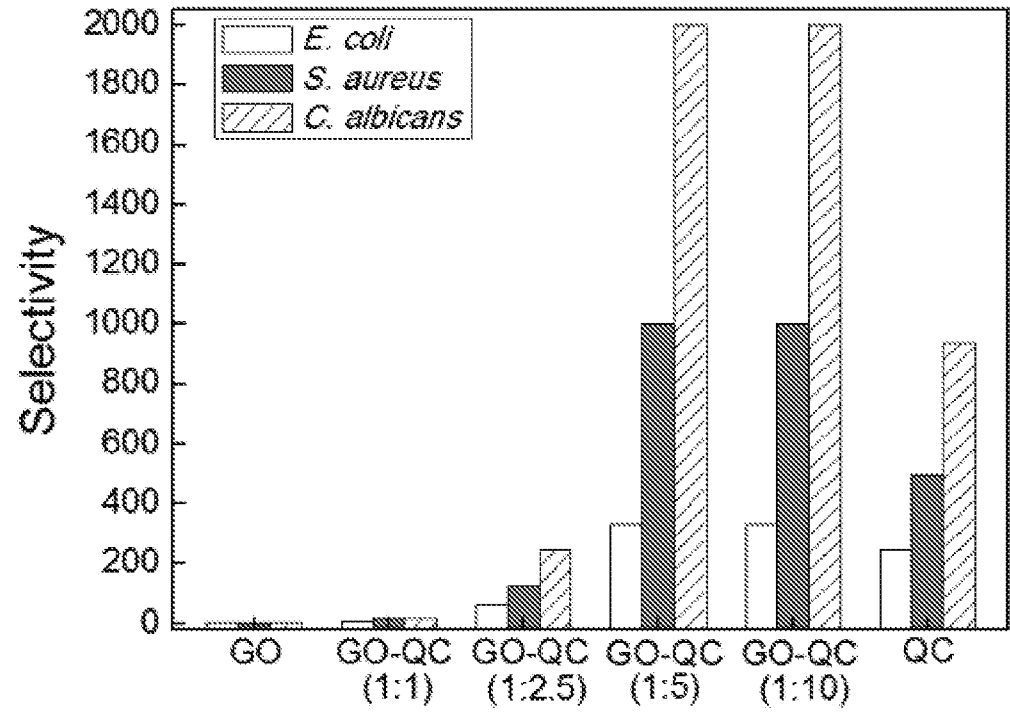


FIG. 19D



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FIG. 19E

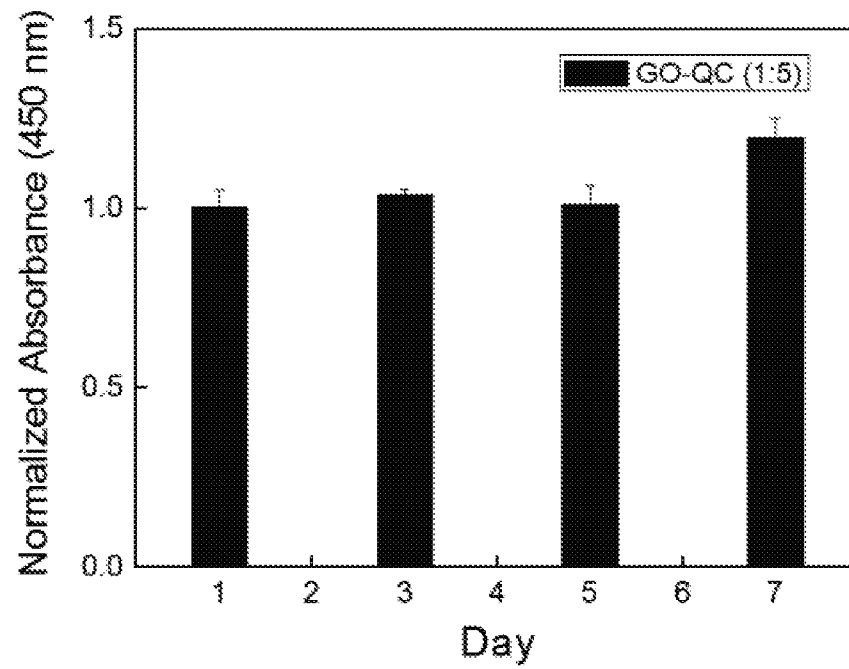


FIG. 19F

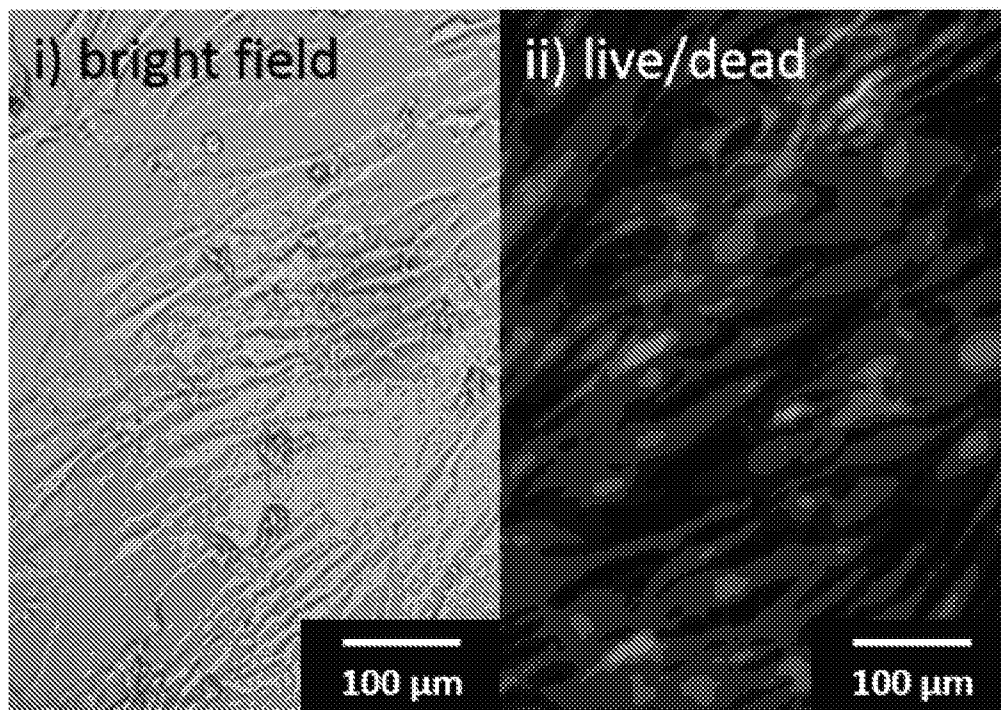


FIG. 20

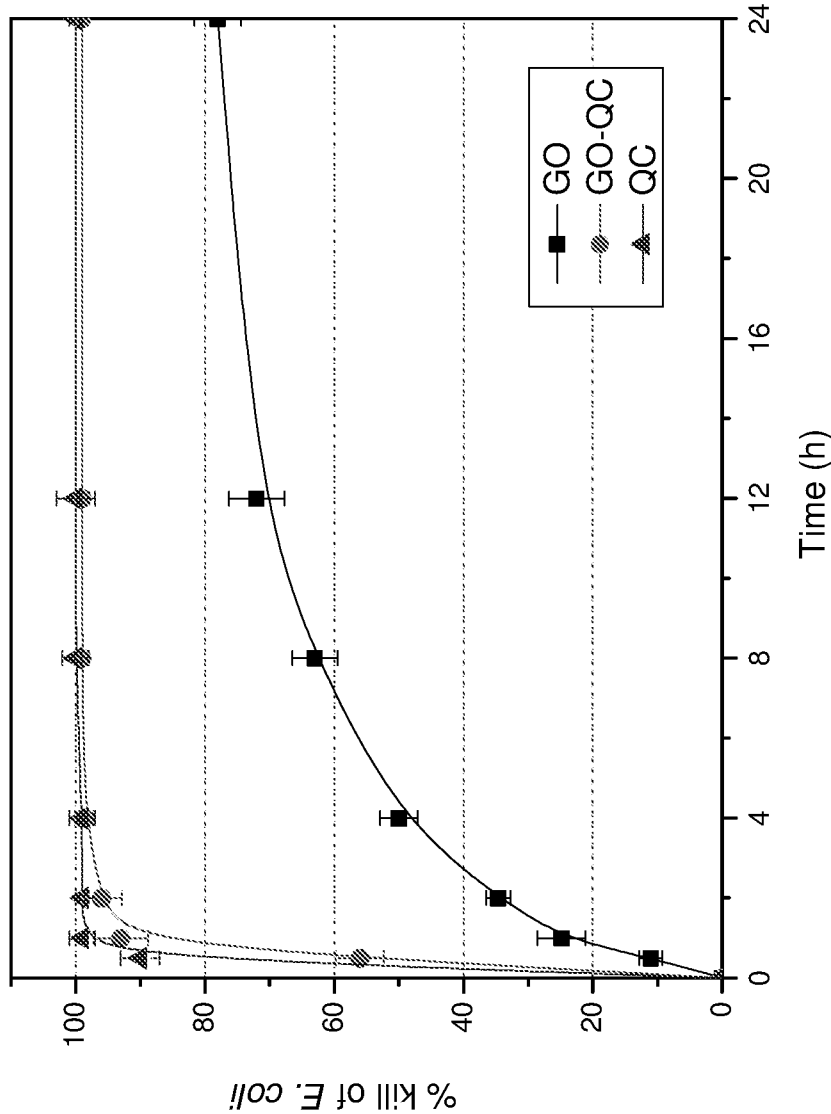
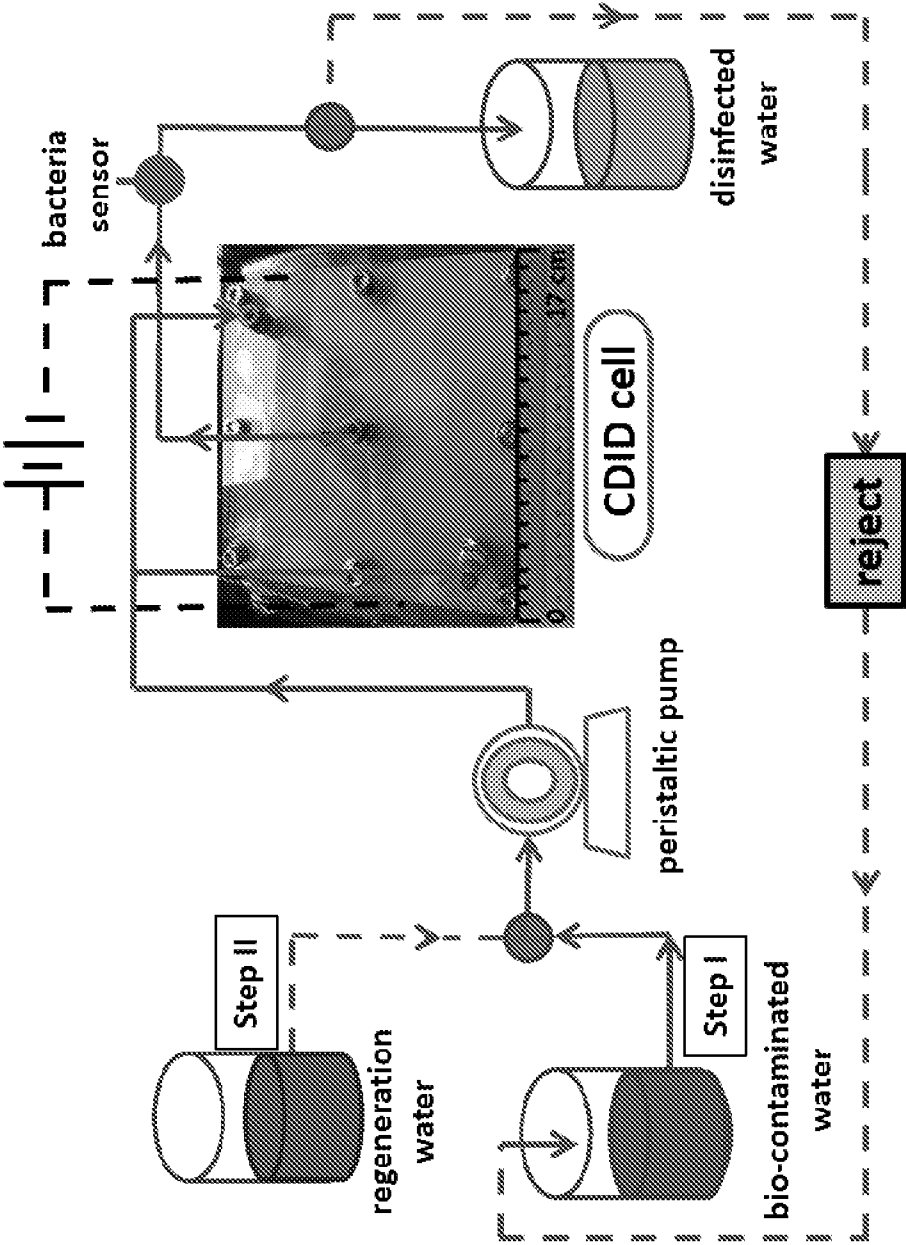


FIG. 21



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FIG. 22A

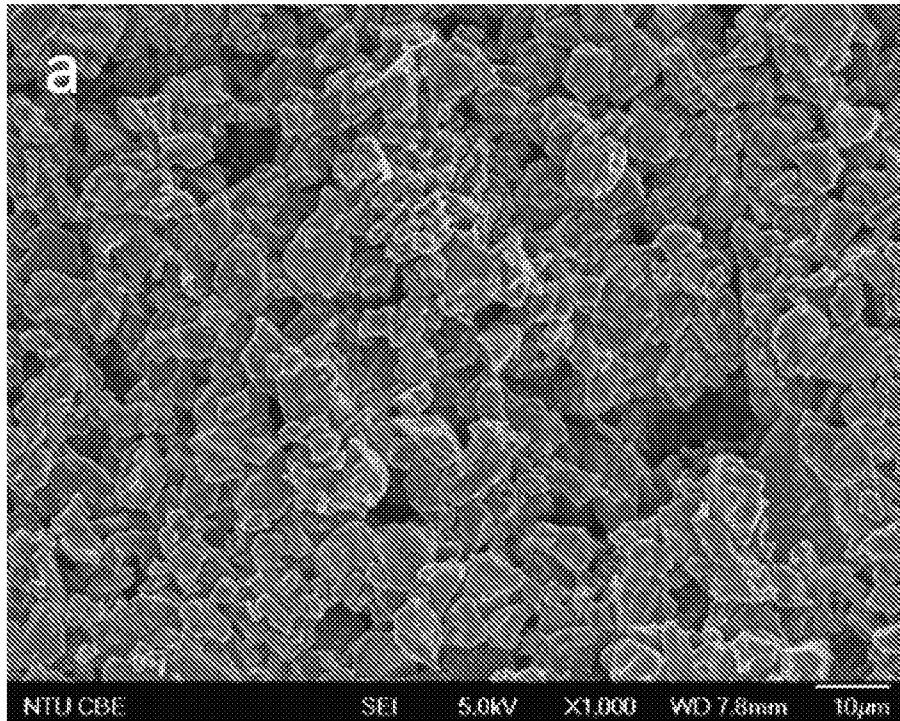
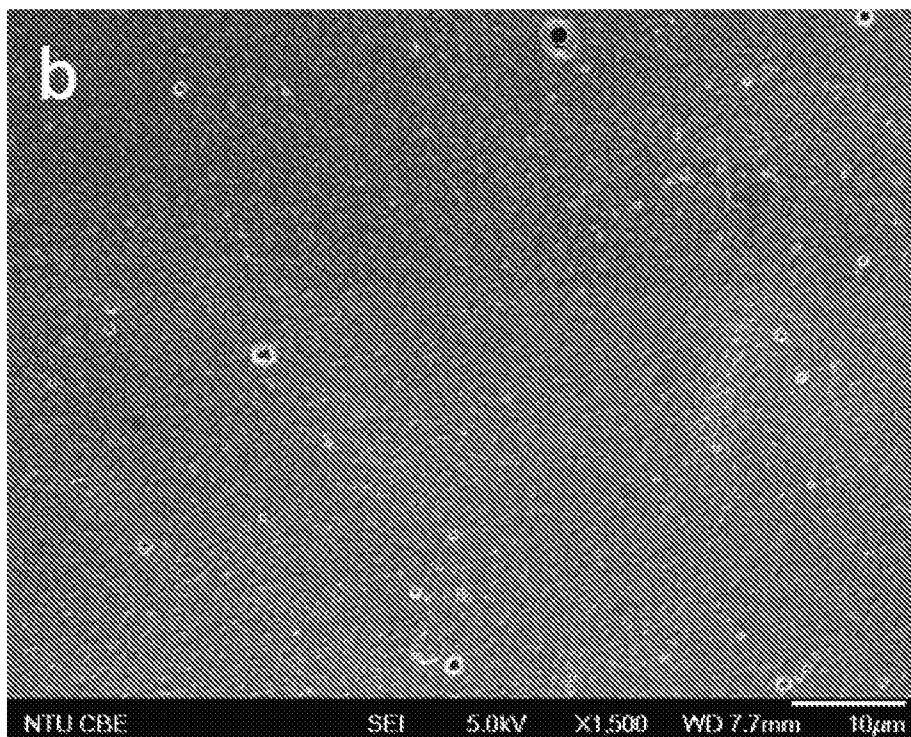


FIG. 22B



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FIG. 22C

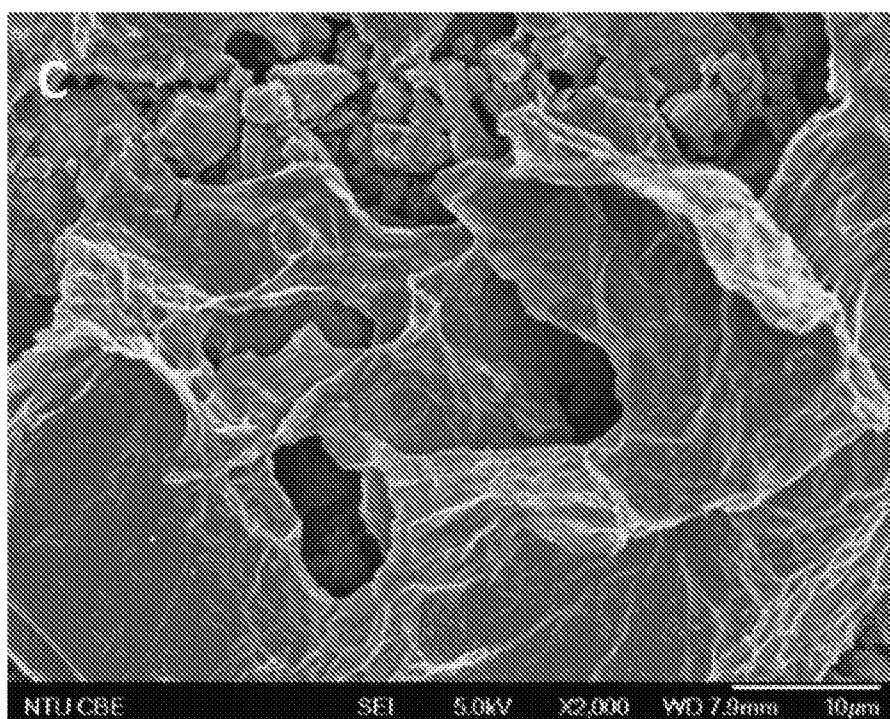
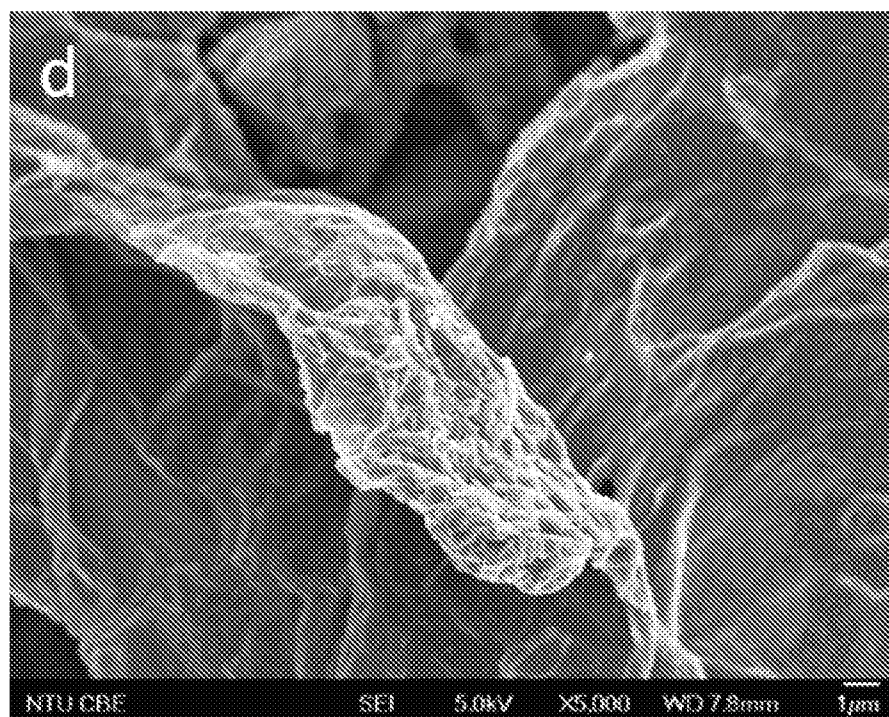


FIG. 22D



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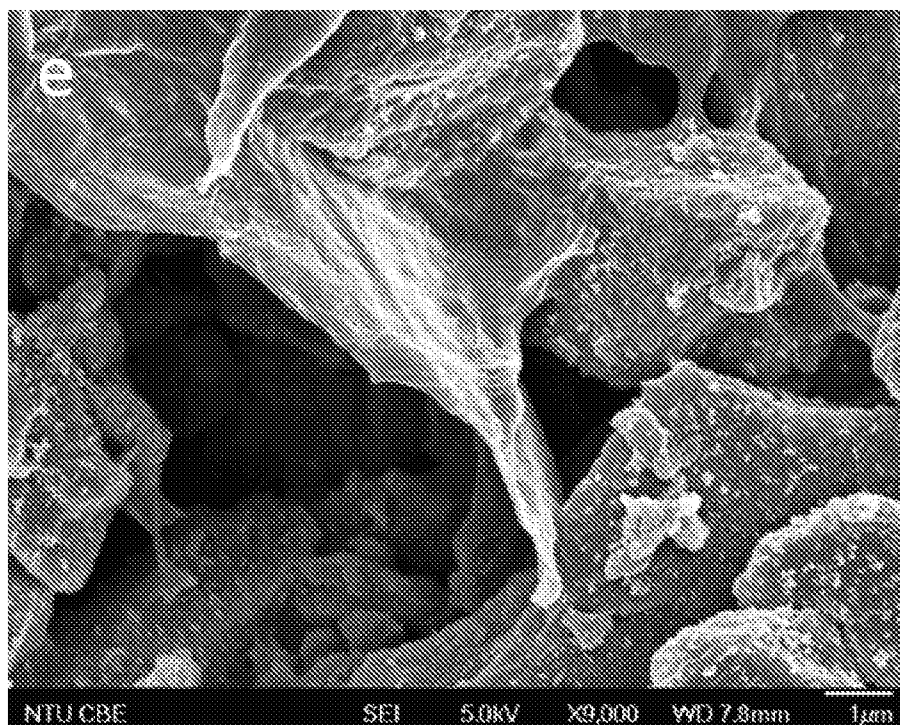
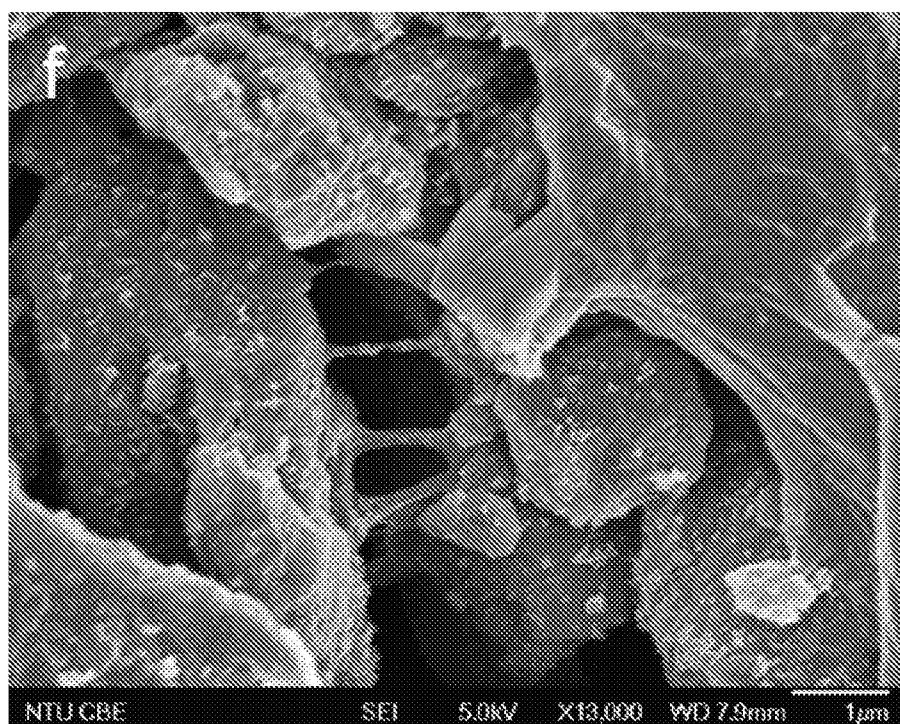
FIG. 22E**FIG. 22F**

FIG. 23A

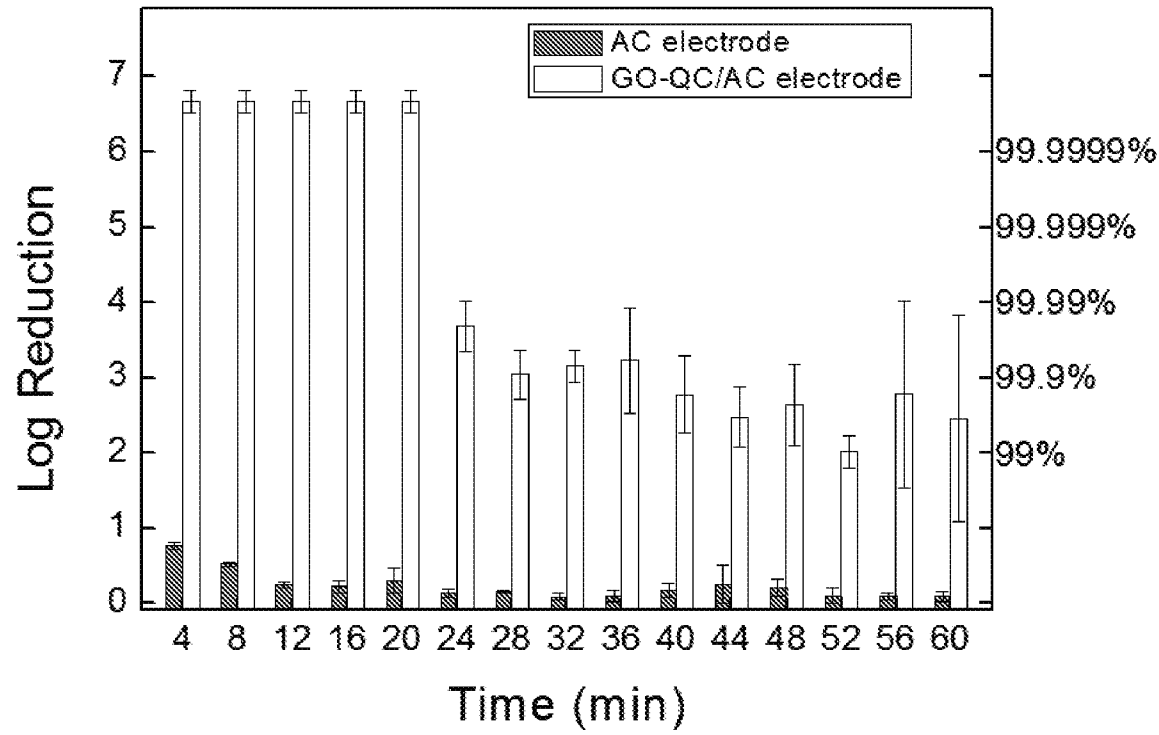
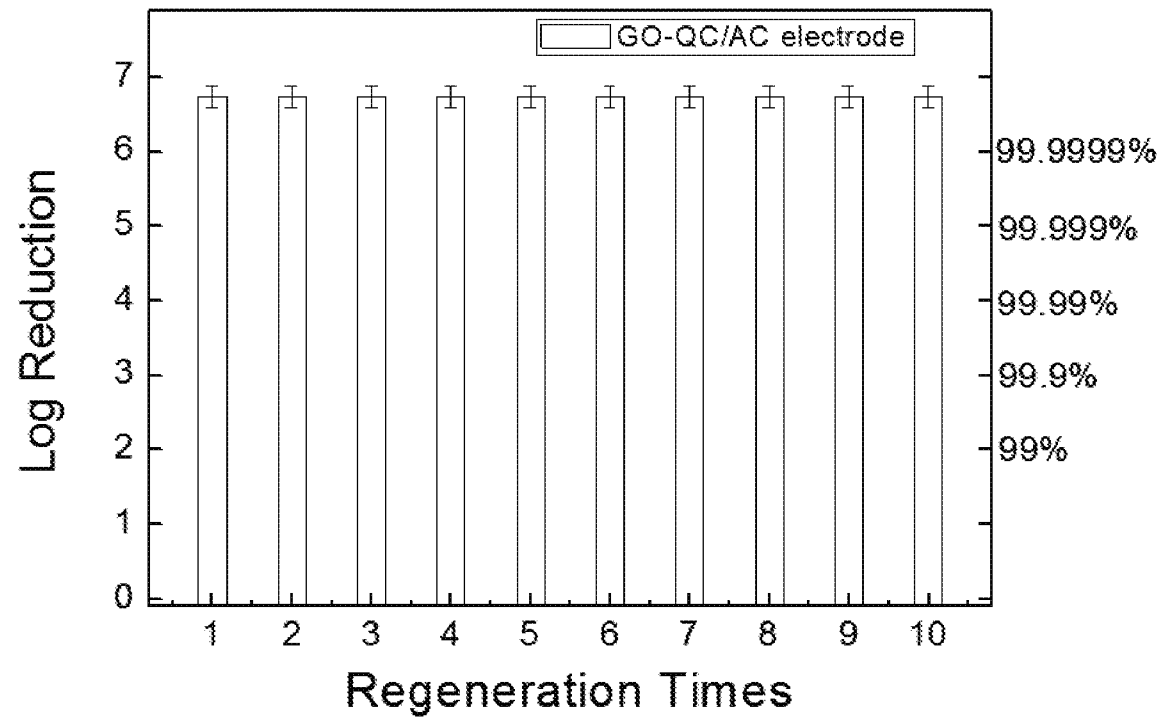


FIG. 23B



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FIG. 23C

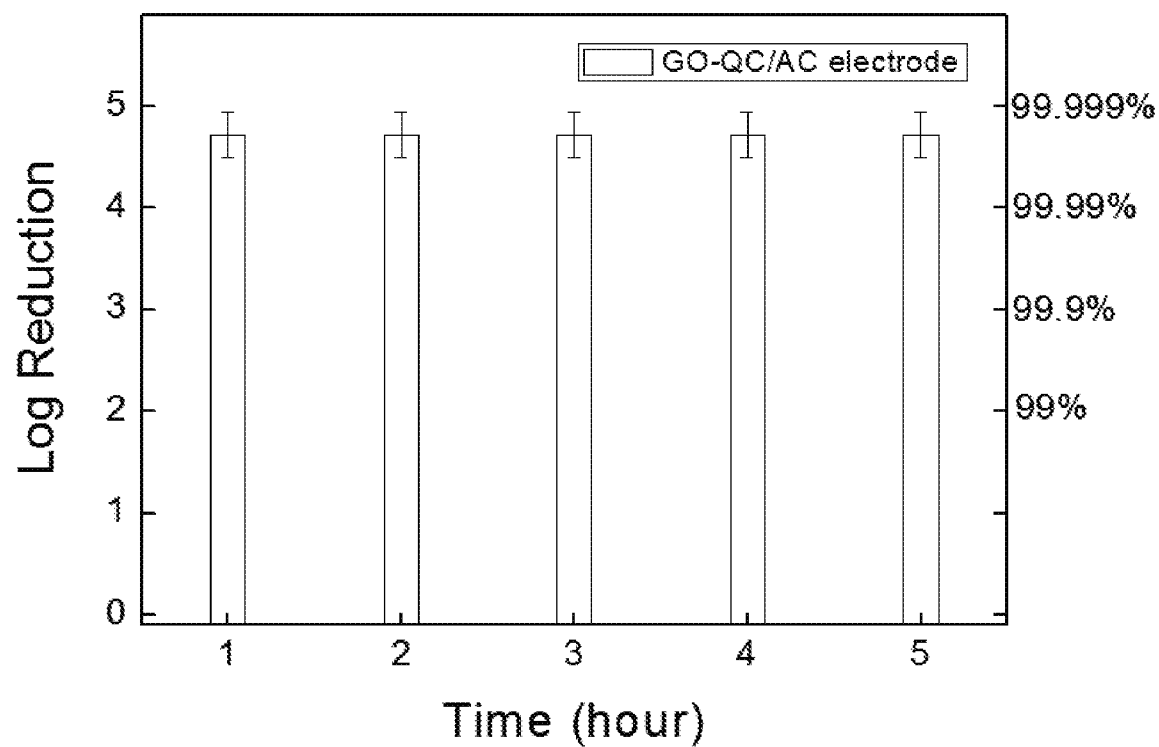


FIG. 23D

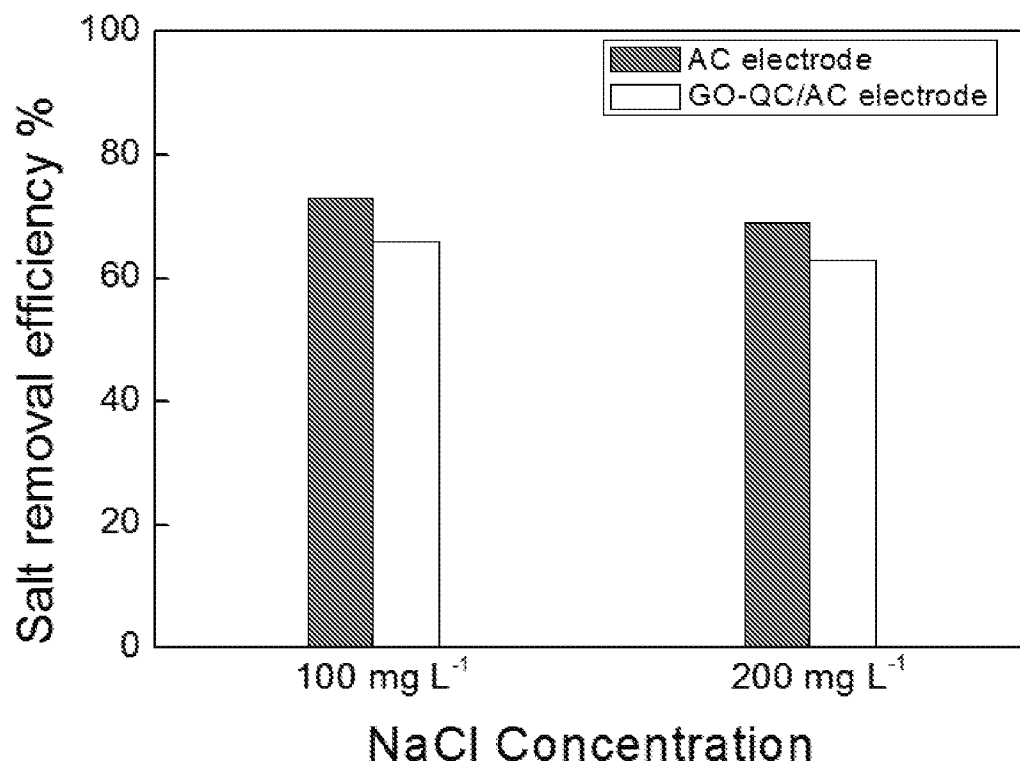


FIG. 23E

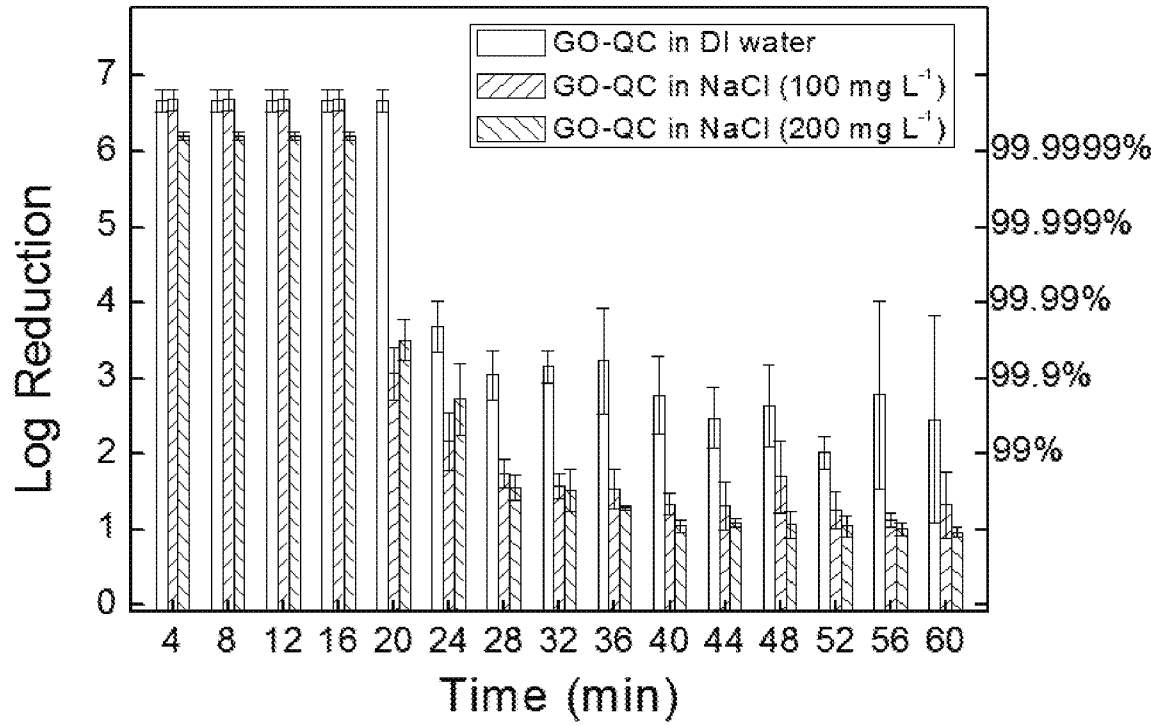
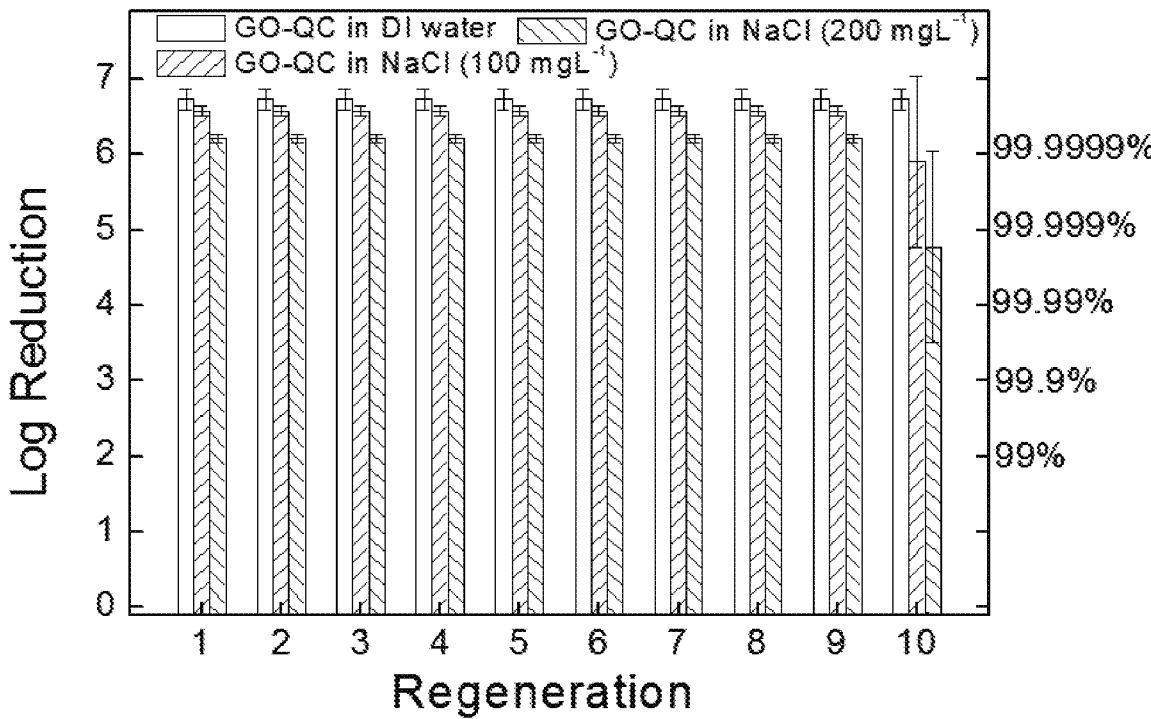


FIG. 23F



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FIG. 24

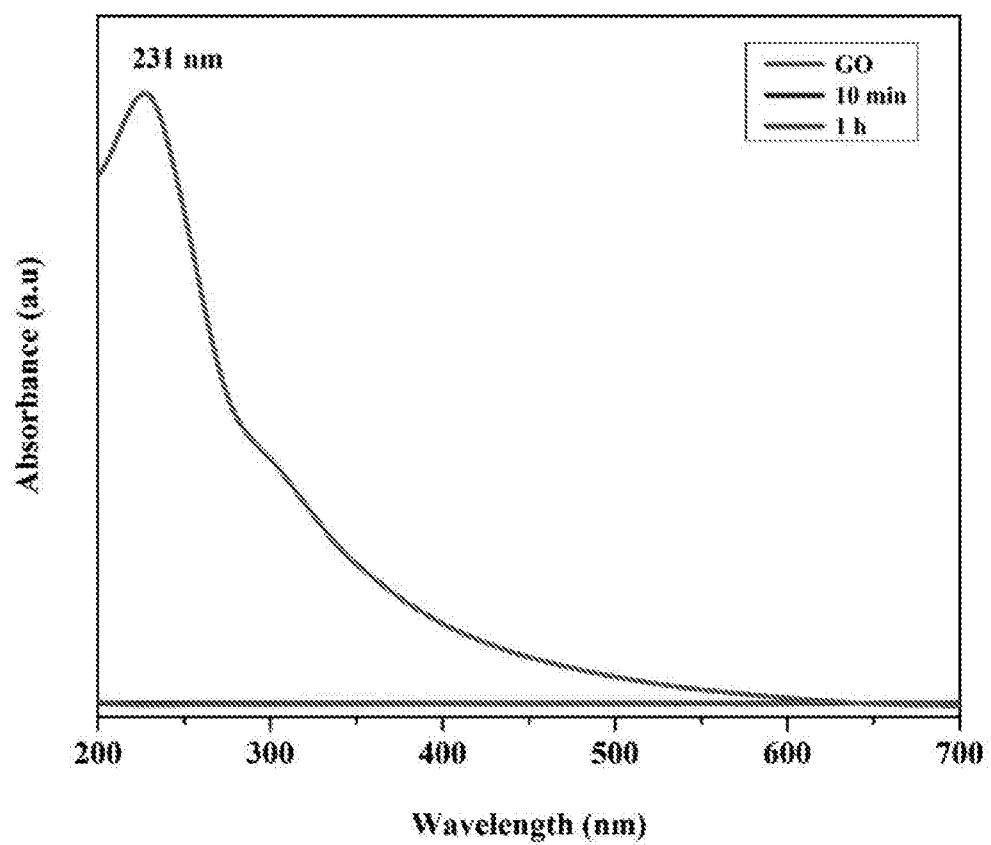


FIG. 25A

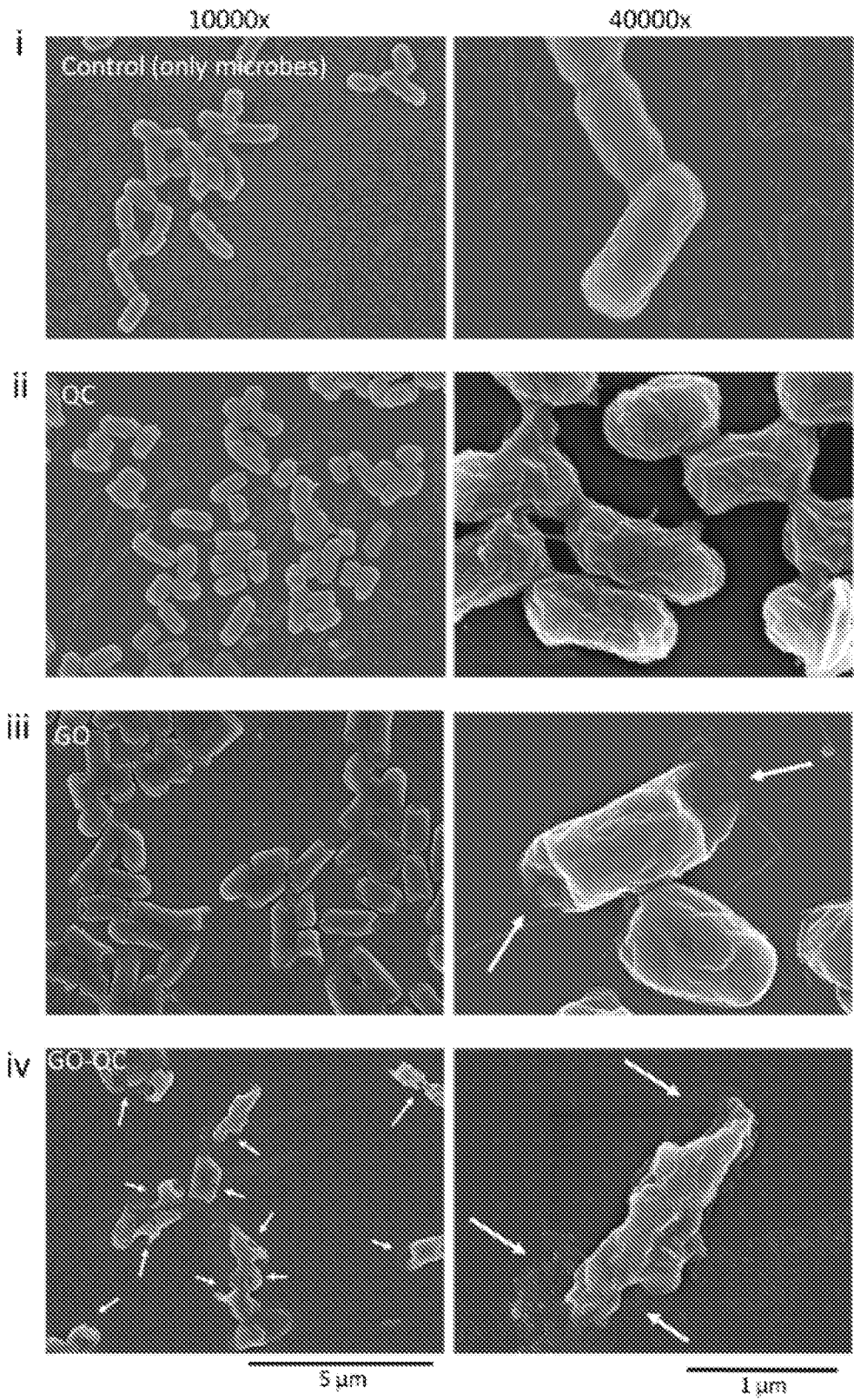


FIG. 25B

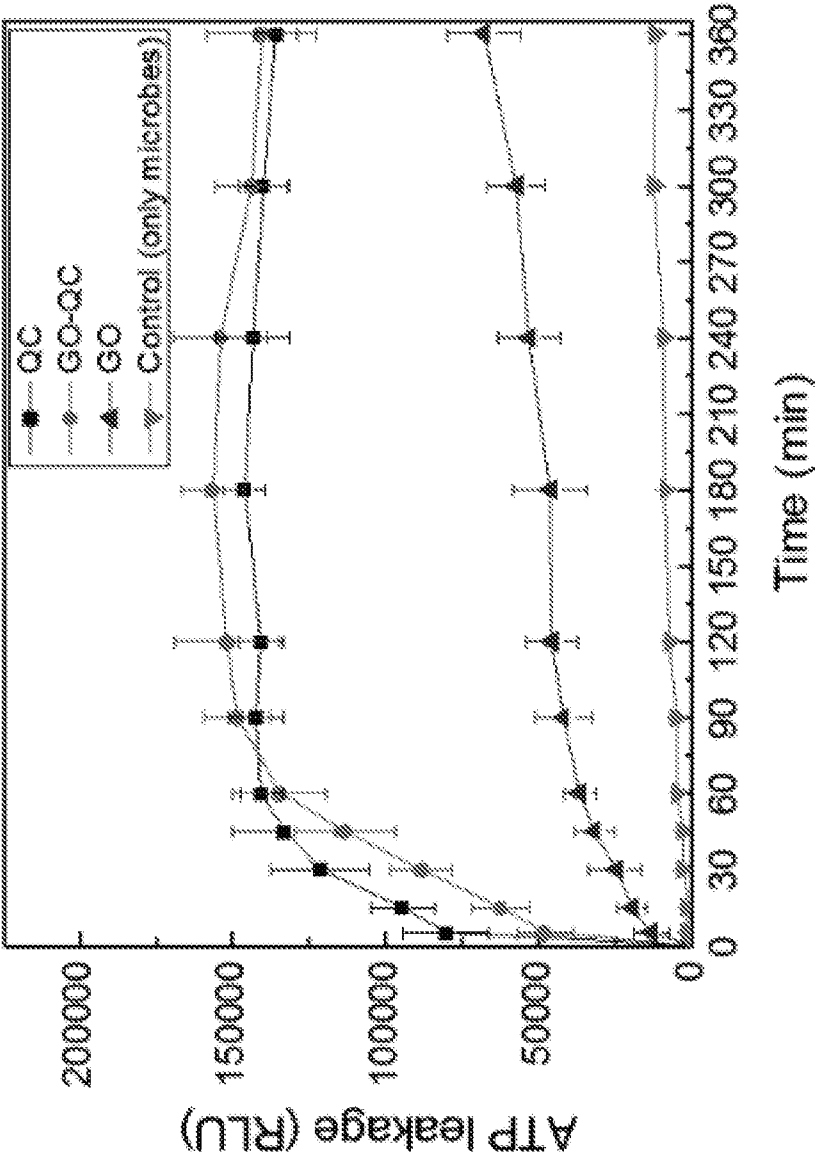


FIG. 25C

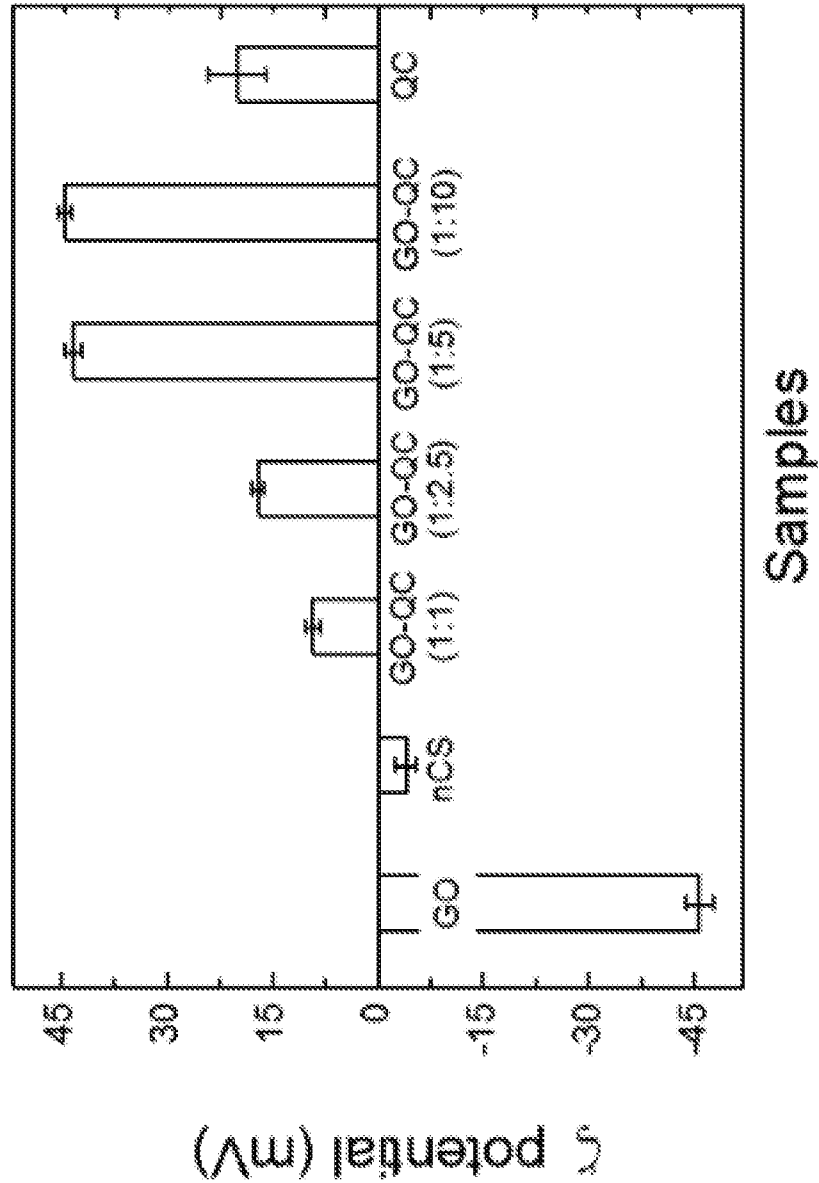


FIG. 25D

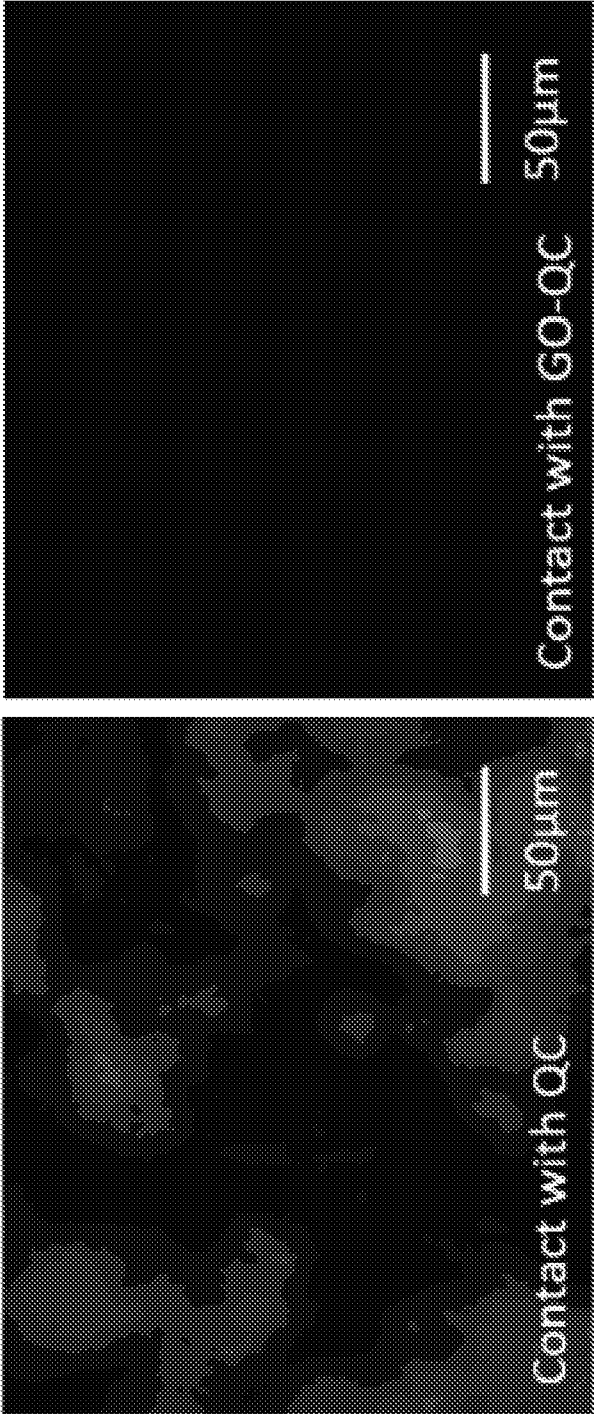


FIG. 26B

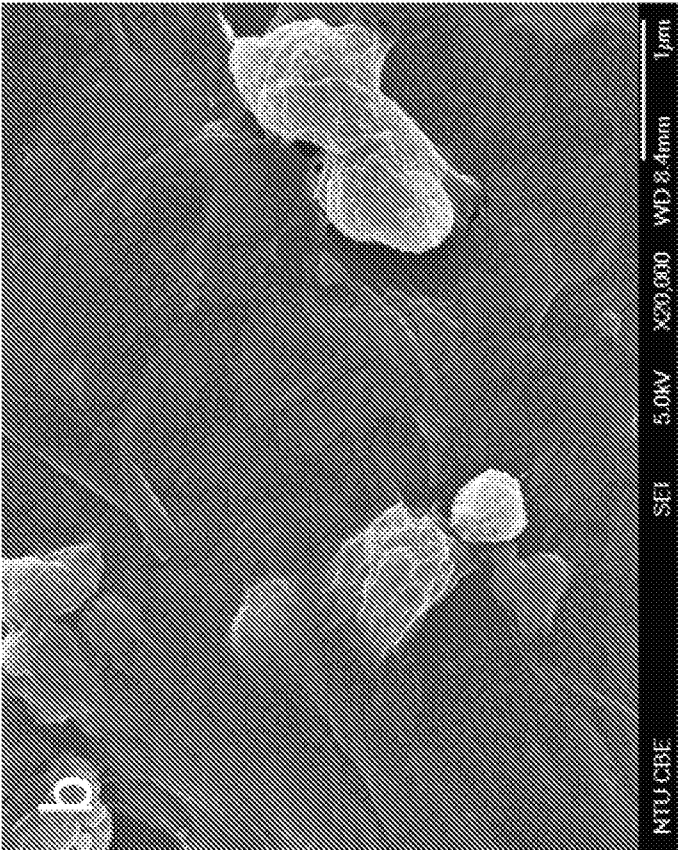


FIG. 26A

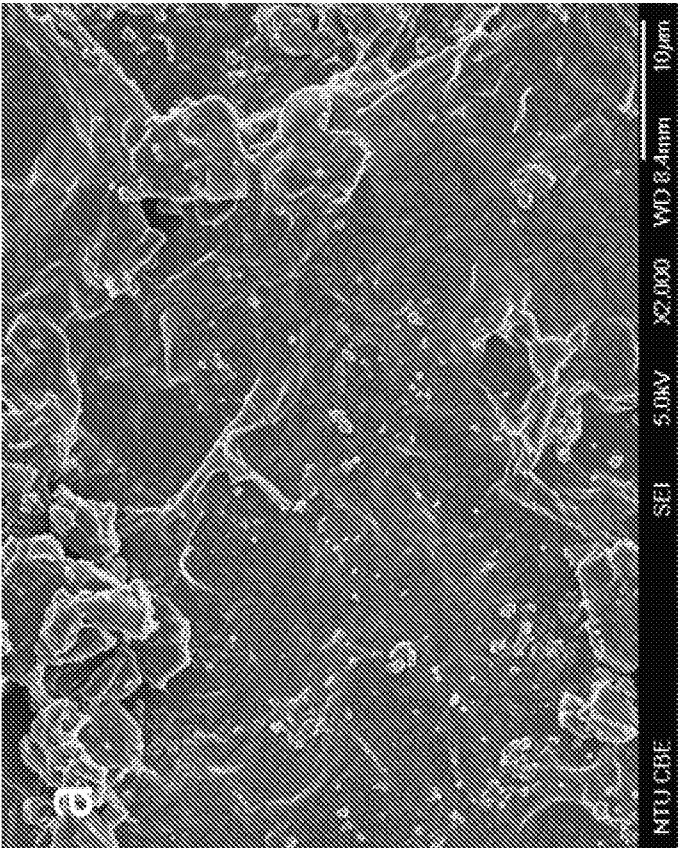


FIG. 26D

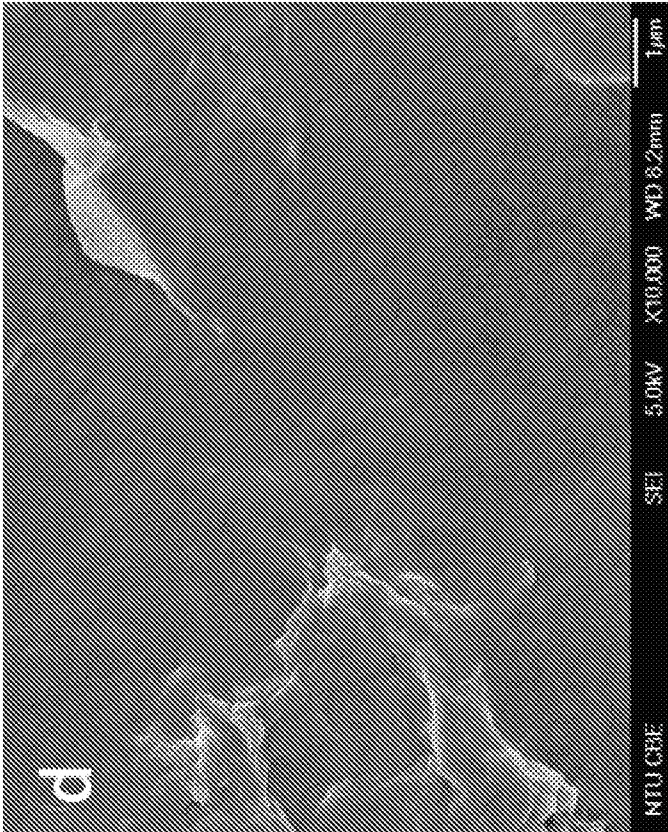
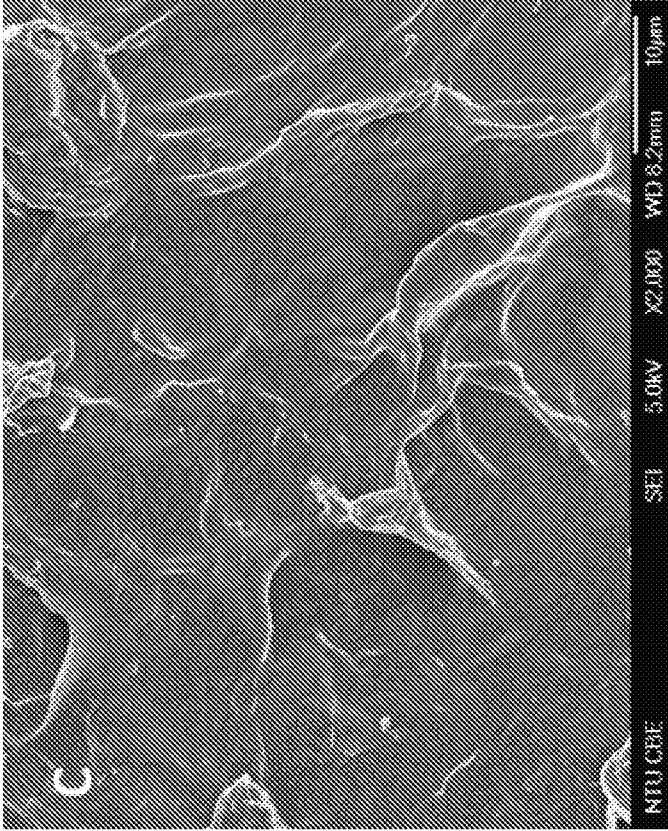


FIG. 26C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2016/050363

A. CLASSIFICATION OF SUBJECT MATTER

See Supplemental Box

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

H01M, C01B, C08B, C08L, C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAplus, Biosis, Embase, Medline, FAMPAT: Applicant and Inventor Search; Keywords such as graphene oxide, graphitic oxide, graphitic acid, chitosan, poliglusam, electrode, battery, capacitive deionization and similar terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/142757 A1 (NANYANG TECHNOLOGICAL UNIVERSITY) 18 September 2014 Abstract; para [0003, 0004, 0007, 0009-0015, 0052, 0058, 0071-0084, 0094-00107]; experimental section; examples; figures	1-18
Y	YADAV S. K. ET AL., Mechanically Robust, Electrically Conductive Biocomposite Films Using Antimicrobial Chitosan-Functionalized Graphenes. <i>Part. Part. Syst. Charact.</i> , 20 June 2013, Vol. 30, No. 8, pages 721-727 [Retrieved on 2016-09-20] <DOI: 10.1002/ppsc.201300044> Whole Document	1-8
Y	TERZOPOULOU Z. ET AL., Recent Advances in Nanocomposite Materials of Graphene Derivatives with Polysaccharides. <i>Materials</i> , 16 February 2015, Vol. 2015, No. 8, pages 652-683 [Retrieved on 2016-09-20] <DOI: 10.3390/MA8020652> Abstract; pages 654, Section 2.3	1-8, 14-18

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20/09/2016 (day/month/year)

Date of mailing of the international search report

26/10/2016 (day/month/year)

Name and mailing address of the ISA/SG

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Authorized officer

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IPOS Customer Service Tel. No.: (+65) 6339 8616

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2016/050363

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 103077766 A (QINGDAO ZHONG KE HAO TAI NEW MATERIAL SCIENCE & TECHNOLOGY CO. LTD.) 1 May 2013 Abstract; para [0010-0012]; Example 2; Figure 4 in the machine translation	1-13
Y	WO 2015/076586 A1 (SAMSUNG ELECTRONICS CO. LTD.) 28 May 2015 Abstract; pages 3-7; examples; claims in the machine translation	1-18
A	CN 103626151 A (FUDAN UNIVERSITY) 12 March 2014 Abstract; example 1; page 4; claims in the machine translation	
A	CN 103240063 A (SHANXI UNIVERSITY OF SCIENCE & TECHNOLOGY) 14 August 2013 Abstract; examples in the machine translation	
A	CN 104307491 A (WUHAN UNIVERSITY OF TECHNOLOGY) 28 January 2015 Abstract; examples in the machine translation	
P,X	WANG Y. ET AL., High-Performance Capacitive Deionization Disinfection of Water with Graphene Oxide- <i>graft</i> -Quaternized Chitosan Nanohybrid Electrode Coating. <i>ACS Nano</i> , 21 September 2015, Vol. 9, No. 10, pages 10142-10157 [Retrieved on 2016-09-19] <DOI: 10.1021/ACSNANO.5B03763> Whole document	1-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2016/050363

Supplemental Box

(Classification of Subject Matter)

Int. Cl.

H01M 4/60 (2006.01)

C01B 32/198 (2017.01)

C08B 37/00 (2006.01)

C08L 5/08 (2006.01)

C02F 1/469 (2006.01)

C02F 1/461 (2006.01)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2016/050363

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014/142757 A1	18/09/2014	CN 105793190 A SG 11201506641R A US 2016022827 A1	20/07/2016 29/09/2015 28/01/2016
CN 103077766 A	01/05/2013	-	-
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CN 103626151 A	12/03/2014	-	-
CN 103240063 A	14/08/2013	-	-
CN 104307491 A	28/05/2015	-	-