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(54) BIODETECTION ARTICLES

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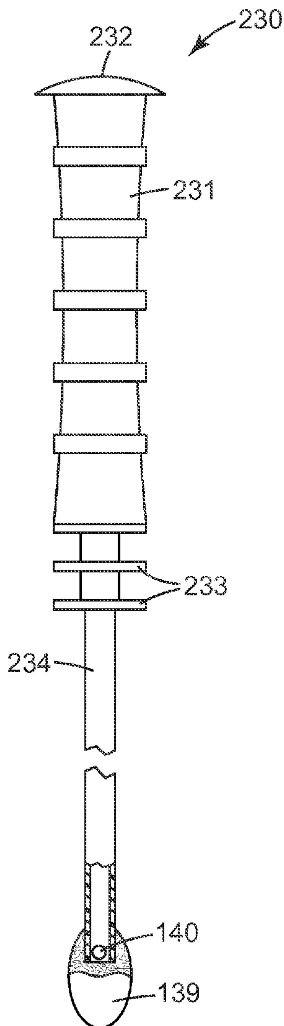
(52) **U.S. Cl.** **435/6.1**; 435/287.1; 435/287.2; 435/34; 435/283.1; 435/7.1; 435/39

(57) **ABSTRACT**

Articles (**610**) are provided for the detection of cells in a sample. The articles include a hydrogel (**640**) comprising a cell extractant. Methods of use are also disclosed.



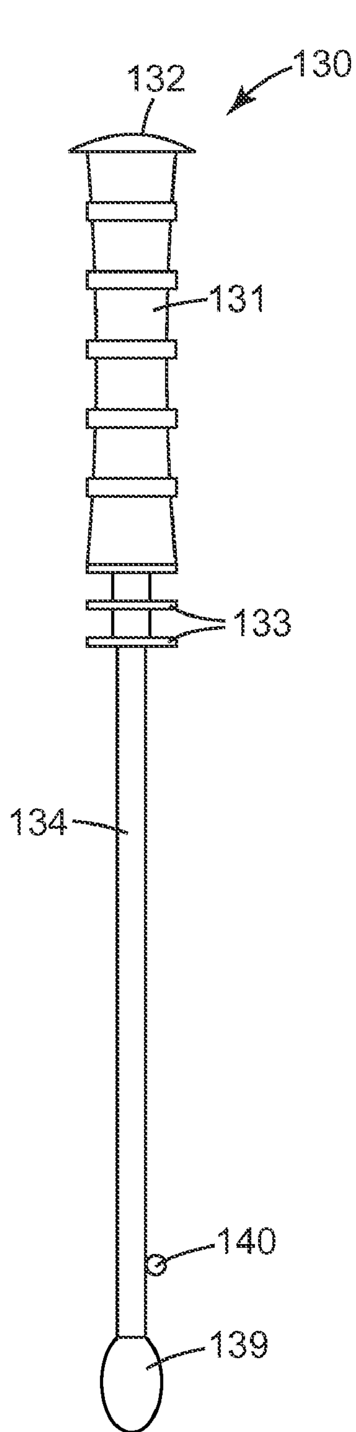


Fig. 1

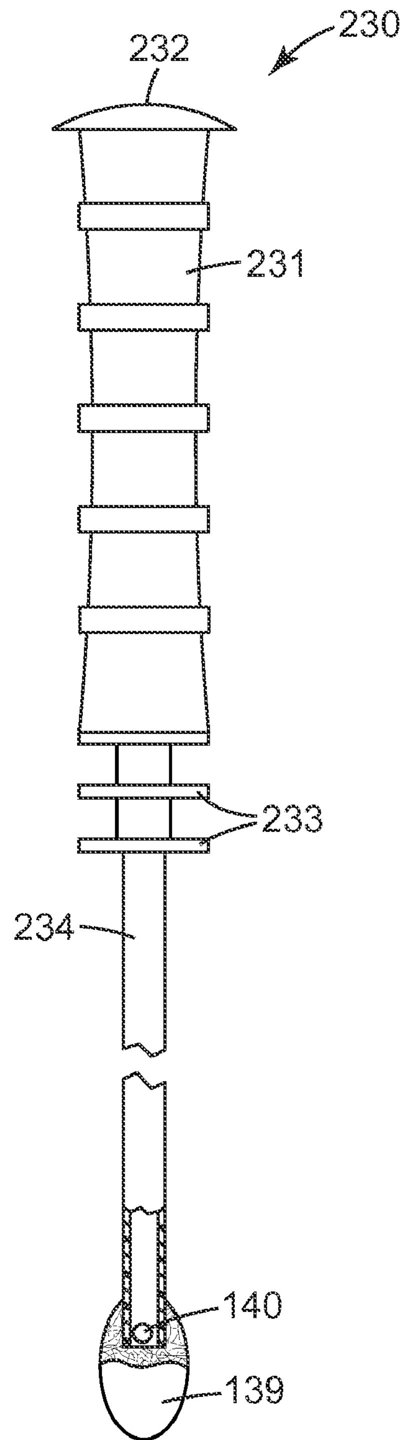


Fig. 2

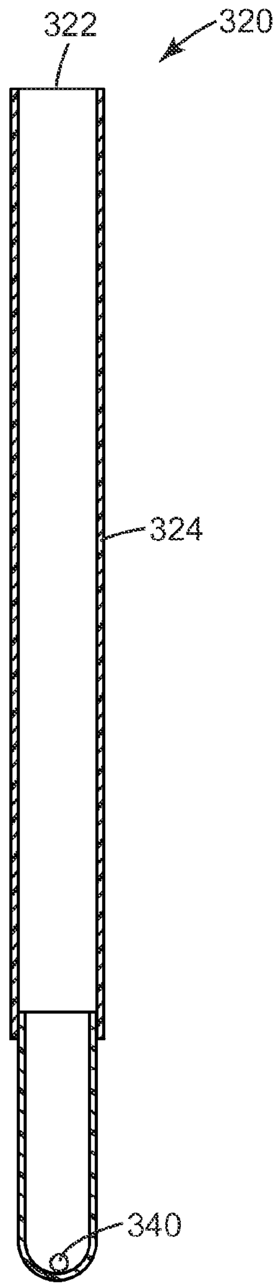


Fig. 3

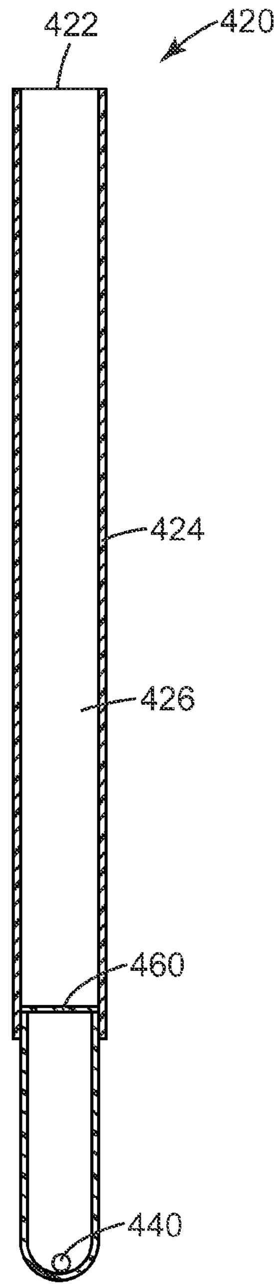


Fig. 4

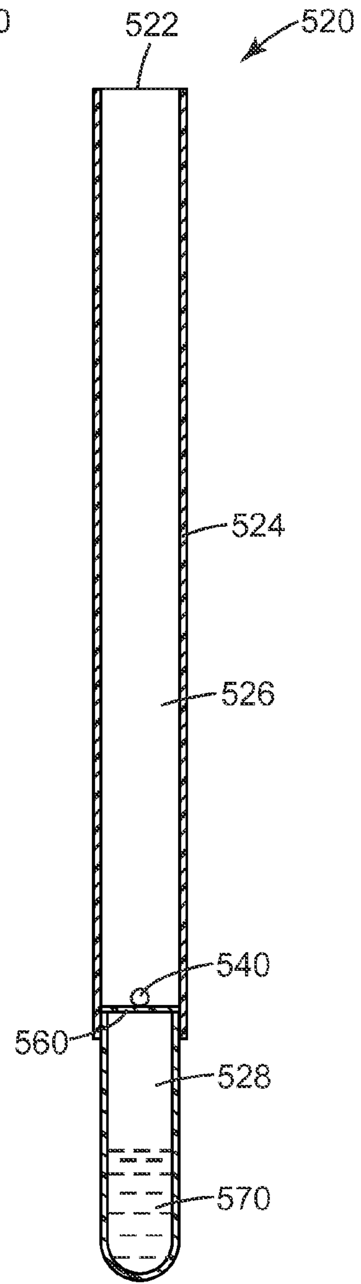


Fig. 5

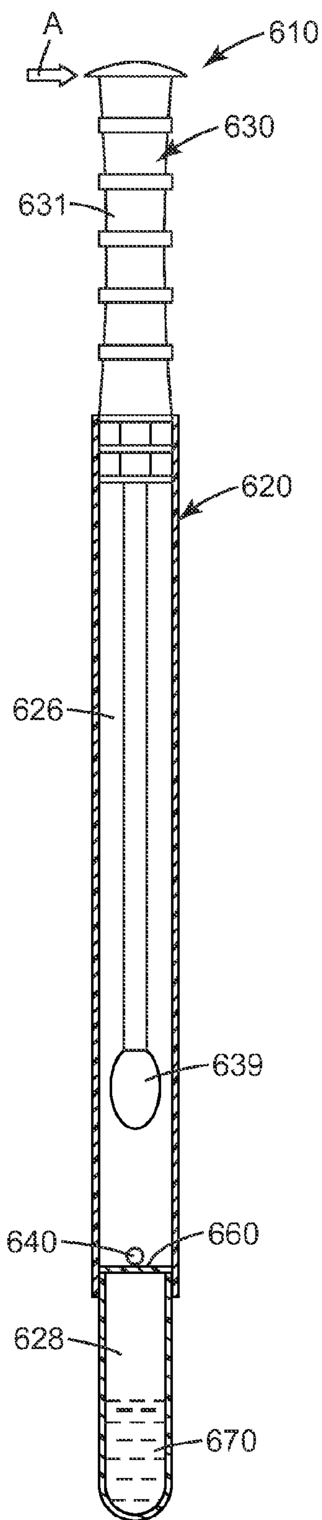


Fig. 6A

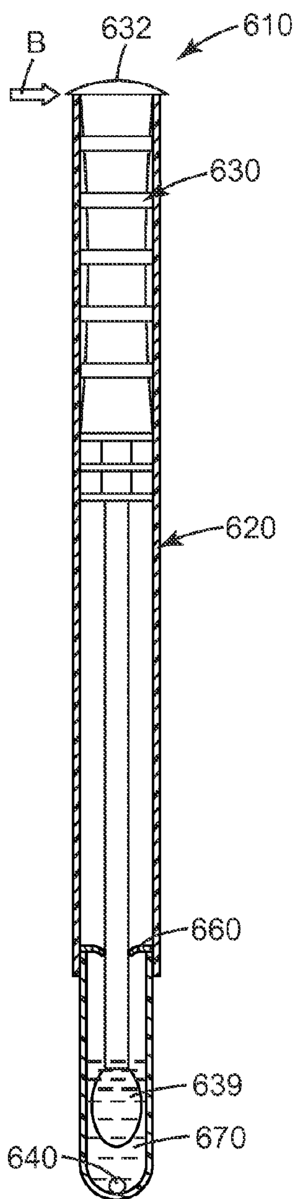


Fig. 6B

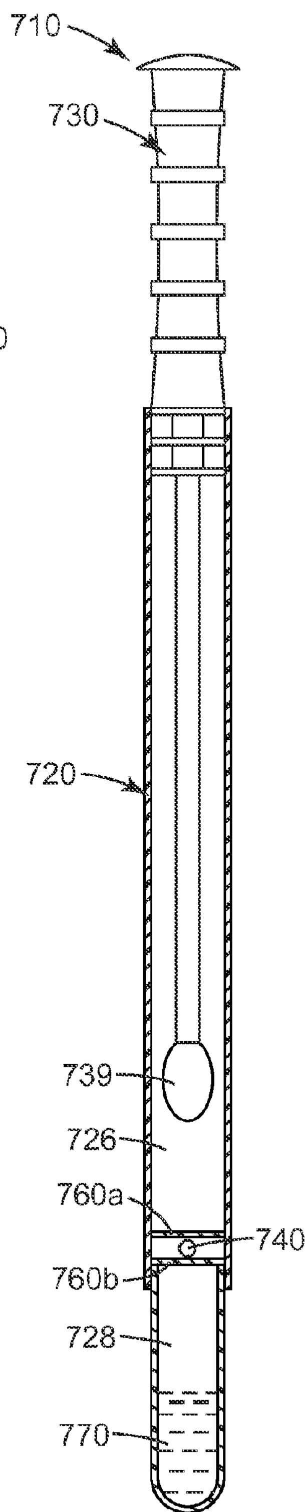


Fig. 7

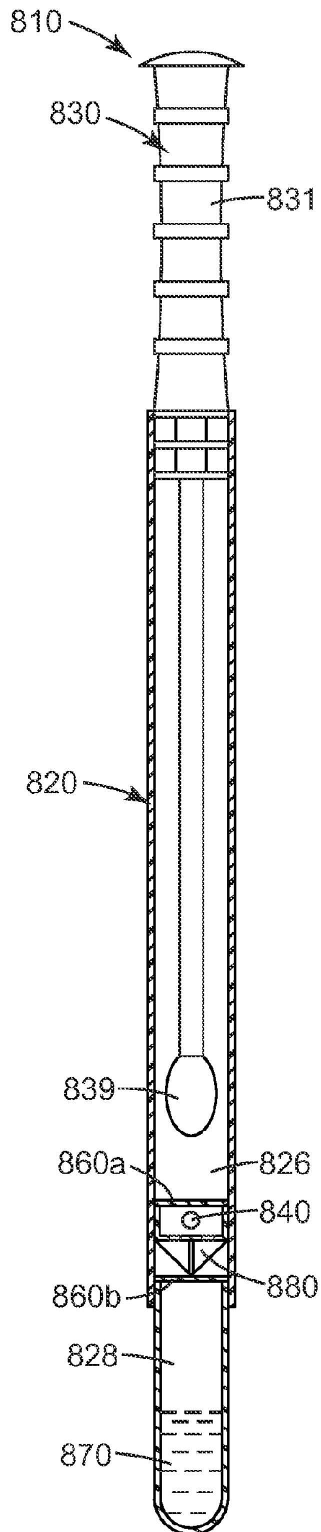


Fig. 8

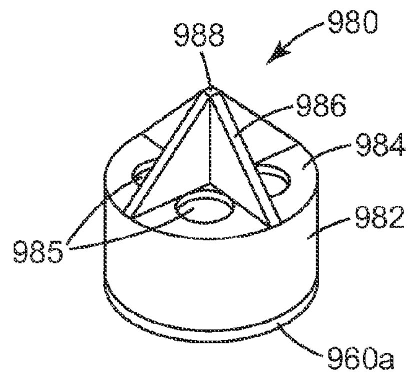


Fig. 9

BIODETECTION ARTICLES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Ser. Nos. 61/101,546 and 61/101,563, both filed Sep. 30, 2008.

BACKGROUND

[0002] Various tests are available that can be used to assess the presence of biological analytes in a sample (e.g. surface, water, air, etc). Such tests include those based on the detection of ATP using the firefly luciferase reaction, tests based on the detection of protein using colorimetry, tests based on the detection of microorganisms using microbiological culture techniques, and tests based on detection of microorganisms using immunochemical techniques. Surfaces can be sampled using either a swab device or by direct contact with a culture device such as an agar plate. The sample can be analyzed for the presence of live cells and, in particular, live microorganisms.

[0003] Results from these tests are often used to make decisions about the cleanliness of a surface. For example, the test may be used to decide whether food-processing equipment has been cleaned well enough to use for production. Although the above tests are useful in the detection of a contaminated surface, they can require numerous steps to perform the test, they may not be able to distinguish quickly and/or easily the presence of live cells from dead cells and, in some cases, they can require long periods of time (e.g., hours or days) before the results can be determined.

[0004] The tests may be used to indicate the presence of live microorganisms. For such tests, a cell extractant is often used to release a biological analyte (e.g., ATP) associated with living cells. The presence of extracellular material (e.g., non-cellular ATP released into the environment from dead or stressed animal cells, plant cells, and/or microorganisms) can create a high "background" level of ATP that can complicate the detection of live cells.

[0005] In spite of the availability of a number of methods and devices to detect live cells, there remains a need for a simple, reliable test for detecting live cells and, in particular, live microbial cells.

SUMMARY

[0006] In general, the present disclosure relates to articles and methods for detecting live cells in a sample. The articles and methods make possible the rapid detection (e.g., through fluorescence, chemiluminescence, or a color reaction) of the presence of cells such as bacteria on a surface. In some embodiments, the inventive articles are "sample-ready", i.e., the articles contain all of the necessary features to detect living cells in a sample. In some aspects, the inventive articles and methods provide a means to distinguish a biological analyte, such as ATP or an enzyme, that is associated with eukaryotic cells (e.g., plant or animal cells) from a similar or identical biological analyte associated with prokaryotic cells (e.g., bacterial cells). Furthermore, the inventive articles and methods provide a means to distinguish a biological analyte that is free in the environment (i.e., an acellular biological analyte) from a similar or identical biological analyte associated with a living cell. Methods of the present disclosure allow an operator instantaneously to form a liquid mixture containing a sample and a hydrogel comprising a cell extractant. In some embodiments, the methods provide for the operator to, within a predetermined period of time after the liquid mixture is formed, measure the amount of a biological analyte in the mixture to determine the amount of acellular biological analyte in the sample. In some embodiments, the methods provide for the operator to, after a predetermined period of time during which an effective amount of cell extractant is released from the hydrogel into the liquid mixture, measure the amount of a biological analyte to determine the amount of biological analyte from acellular material and live cells in the sample. In some embodiments, the methods provide for the operator, within a first predetermined period of time, to perform a first measurement of the amount of a biological analyte and, within a second predetermined period of time during which an effective amount of cell extractant is released from the hydrogel, perform a second measurement of the amount of biological analyte to detect the presence of live cells in the sample. In some embodiments, the methods can allow the operator to distinguish whether biological analyte in the sample was released from live plant or animal cells or whether it was released from live microbial cells (e.g., bacteria). The present invention is capable of use by operators under the relatively harsh field environment of institutional food preparation services, health care environments and the like.

[0007] In one aspect, the present disclosure provides an article for detecting cells in a sample. The article can comprise an enclosure containing a hydrogel wherein the hydrogel comprises a cell extractant.

[0008] Articles of the present disclosure can comprise a sample acquisition device wherein the sample acquisition device comprises the enclosure.

[0009] Articles of the present disclosure can comprise a housing wherein the housing comprises the enclosure.

[0010] In another aspect, the present disclosure provides a sample acquisition device with a hydrogel comprising a cell extractant disposed thereon.

[0011] A hydrogel comprising a cell extractant can be coated on a solid substrate.

[0012] In another aspect, the present disclosure provides a kit. The kit can comprise a housing that includes an opening configured to receive a sample acquisition device and a hydrogel comprising a cell extractant. Optionally, the kit can further comprise a sample acquisition device.

GLOSSARY

[0013] "Biological analytes", as used herein, refers to molecules, or derivatives thereof, that occur in or are formed by an organism. For example, a biological analyte can include, but is not limited to, at least one of an amino acid, a nucleic acid, a polypeptide, a protein, a polynucleotide, a lipid, a phospholipid, a saccharide, a polysaccharide, and combinations thereof. Specific examples of biological analytes can include, but are not limited to, a metabolite (e.g., staphylococcal enterotoxin), an allergen (e.g., peanut allergen(s), a hormone, a toxin (e.g., *Bacillus* diarrheal toxin, aflatoxin, etc.), RNA (e.g., mRNA, total RNA, tRNA, etc.), DNA (e.g., plasmid DNA, plant DNA, etc.), a tagged protein, an antibody, an antigen, and combinations thereof.

[0014] "Sample acquisition device" is used herein in the broadest sense and refers to an implement used to collect a liquid, semisolid, or solid sample material. Nonlimiting

examples of sample acquisition devices include swabs, wipes, sponges, scoops, spatulas, pipettes, pipette tips, and siphon hoses.

[0015] As used herein, the term "hydrogel" refers to a polymeric material that is hydrophilic and that is either swollen or capable of being swollen with a polar solvent. The polymeric material typically swells but does not dissolve when contacted with the polar solvent. That is, the hydrogel is insoluble in the polar solvent. The swollen hydrogel can be dried to remove at least some of the polar solvent.

[0016] "Cell extractant", as used herein, refers to any compound or combination of compounds that alters cell membrane or cell wall permeability or disrupts the integrity of (i.e., lyses or causes the formation of pores in) the membrane and/or cell wall of a cell (e.g., a somatic cell or a microbial cell) to effect extraction or release of a biological analyte normally found in living cells.

[0017] "Detection system", as used herein, refers to the components used to detect a biological analyte and includes enzymes, enzyme substrates, binding partners (e.g. antibodies or receptors), labels, dyes, and instruments for detecting light absorbance or reflectance, fluorescence, and/or luminescence (e.g. bioluminescence or chemiluminescence).

[0018] The words "preferred" and "preferably" refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the invention.

[0019] The terms "comprises" and variations thereof do not have a limiting meaning where these terms appear in the description and claims.

[0020] As used herein, "a," "an," "the," "at least one," and "one or more" are used interchangeably. Thus, for example, a housing that comprises "a" detection reagent can be interpreted to mean that the housing can include "one or more" detection reagents.

[0021] The term "and/or" means one or all of the listed elements or a combination of any two or more of the listed elements.

[0022] Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

[0023] The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The invention will be further explained with reference to the drawing figures listed below, where like structure is referenced by like numerals throughout the several views.

[0025] FIG. **1** shows a side view of one embodiment of a sample acquisition device with a hydrogel disposed thereon.

[0026] FIG. **2** shows a partial cross-section view of one embodiment of a sample acquisition device comprising an enclosure containing a hydrogel.

[0027] FIG. **3** shows a cross-section view of one embodiment of a housing with a hydrogel disposed therein.

[0028] FIG. **4** shows a cross-section view of the housing of FIG. **3**, further comprising a frangible seal.

[0029] FIG. **5** shows a cross-section view of one embodiment of a housing containing a hydrogel, a frangible seal, and a detection reagent.

[0030] FIG. **6**A shows a cross-section view of one embodiment of a detection device comprising the housing of FIG. **5** and side view of a sample acquisition device disposed in a first position therein.

[0031] FIG. **6**B shows a partial cross-section view of the detection device of FIG. **6**A with the sample acquisition device disposed in a second position therein.

[0032] FIG. **7** shows a partial cross-section view of one embodiment of a detection device comprising a housing, a plurality of frangible seals with a hydrogel disposed there between, and a sample acquisition device.

[0033] FIG. **8** shows a partial cross-section view of one embodiment of a detection device comprising a housing, a carrier comprising a hydrogel, and a sample acquisition device.

[0034] FIG. **9** shows a bottom perspective view of the carrier of FIG. **8**.

DETAILED DESCRIPTION

[0035] All patents, patent applications, government publications, government regulations, and literature references cited in this specification are hereby incorporated herein by reference in their entirety. In case of conflict, the present description, including definitions, will control.

[0036] Biological analytes can be used to detect the presence of biological material, such as live cells in a sample. Biological analytes can be detected by various reactions (e.g., binding reactions, catalytic reactions, and the like) in which they can participate.

[0037] Chemiluminescent reactions can be used in various forms to detect cells, such as bacterial cells, in fluids and in processed materials. In some embodiments of the present disclosure, a chemiluminescent reaction based on the reaction of adenosine triphosphate (ATP) with luciferin in the presence of the enzyme luciferase to produce light provides the chemical basis for the generation of a signal to detect a biological analyte, ATP. Since ATP is present in all living cells, including all microbial cells, this method can provide a rapid assay to obtain a quantitative or semiquantitative estimate of the number of living cells in a sample. Early discourses on the nature of the underlying reaction, the history of its discovery, and its general area of applicability, are provided by E. N. Harvey (1957), A History of Luminescence: From the Earliest Times Until 1900, Amer. Phil. Soc., Philadelphia, Pa.; and W. D. McElroy and B. L. Strehler (1949), *Arch. Biochem. Biophys.* 22:420-433.

[0038] ATP detection is a reliable means to detect bacteria and other microbial species because all such species contain some ATP. Chemical bond energy from ATP is utilized in the bioluminescent reaction that occurs in the tails of the firefly *Photinus pyralis*. The biochemical components of this reaction can be isolated free of ATP and subsequently used to detect ATP in other sources. The mechanism of this firefly bioluminescence reaction has been well characterized (DeLuca, M., et al., 1979 *Anal. Biochem.* 95:194-198).

[0039] The inventive articles and methods of the present disclosure provide simple means for conveniently controlling

the release of biological analytes from living cells in order to determine the presence, optionally the type (e.g., microbial or nonmicrobial), and optionally the quantity of living cells in an unknown sample. The articles and methods include a hydrogel comprising a cell extractant. Methods of the present invention are disclosed in U.S. Patent Application Ser. No. 61/101,563, filed on Sep. 30, 2008 and entitled "BIODETECTION METHODS", which is incorporated herein by reference in its entirety.

Hydrogels:

[0040] Articles of the present disclosure include a hydrogel. Suitable hydrogels include crosslinked hydrogels, swollen hydrogels, and dried or partially-dried hydrogels.

[0041] Suitable hydrogels of the present disclosure include, for example, the hydrogels, and polymeric beads made there from, described in International Patent Publication No. WO 2007/146722, which is incorporated herein by reference in its entirety.

[0042] Other suitable hydrogels include polymers comprising ethylenically unsaturated carboxyl-containing monomers and comonomers selected from carboxylic acids, vinyl sulfonic acid, cellulosic monomer, polyvinyl alcohol, as described in U.S. Patent Application Publication No. US2004/0157971; polymers comprising starch, cellulose, polyvinyl alcohol, polyethylene oxide, polypropylene glycol, and copolymers thereof, as described in U.S. Patent Application Publication No. US 2006/0062854; polymers comprising multifunctional poly(alkylene oxide) free-radically polymerizable macromonomer with molecular weights less than 2000 daltons, as described in U.S. Pat. No. 7,005,143; polymers comprising silane-functionalized polyethylene oxide that cross-link upon exposure to a liquid medium, as described in U.S. Pat. No. 6,967,261; polymers comprising polyurethane prepolymer with at least one alcohol selected from polyethylene glycol, polypropylene glycol, and propylene glycol, as described in U.S. Pat. No. 6,861,067; and polymers comprising a hydrophilic polymer selected from polysaccharide, polyvinylpyrolidone, polyvinyl alcohol, polyvinyl ether, polyurethane, polyacrylate, polyacrylamide, collagen and gelatin, as described in U.S. Pat. No. 6,669,981, the disclosures of which are all herein incorporated by reference in their entirety. Other suitable hydrogels include agar, agarose, polyacrylamide hydrogels, and derivatives thereof.

[0043] The present disclosure provides for articles and methods that include a shaped hydrogel. Shaped hydrogels include hydrogels shaped into, for example, beads, sheets, ribbons, and fibers. Additional examples of shaped hydrogels and exemplary processes by which shaped hydrogels can be produced are disclosed in U.S. Patent Application Publication No. 2008/0207794 A1, entitled POLYMERIC FIBERS AND METHODS OF MAKING and U.S. Patent Application Ser. No. 61/013,085, entitled METHODS OF MAKING SHAPED POLYMERIC MATERIALS, both of which are incorporated herein by reference in their entirety.

[0044] Hydrogels of the present disclosure can comprise a cell extractant. Hydrogels comprising a cell extractant can be made by two fundamental processes. In a first process, the cell extractant is incorporated into the hydrogel during the synthesis of the hydrogel polymer. Examples of the first process can be found in International Patent Publication No. WO 2007/146722 and in Preparative Example 1 described herein. In a second process the cell extractant is incorporated into the hydrogel after the synthesis of the hydrogel polymer. For example, the hydrogel is placed in a solution of cell extractant and the cell extractant is allowed to absorb into and/or adsorb to the hydrogel. An example of the second process is described in Preparative Example 5 below. A further example of the second process is the incorporation of an ionic monomer into the hydrogel, such as the incorporation of a cationic monomer into the hydrogel, as described herein in Preparative Example 2.

[0045] Hydrogels of the present disclosure may comprise a detection reagent system, such as an enzyme or an enzyme substrate. Such hydrogels can be used conveniently to store and/or deliver the detection reagent to a liquid mixture, comprising a sample and a cell extractant, for the detection of live cells in the sample.

[0046] An enzyme can be incorporated into a hydrogel during the synthesis of the hydrogel polymer. For example, luciferase can be incorporated into a hydrogel during the synthesis of the polymer, as described in Preparative Example 4 below. An enzyme can be incorporated into a hydrogel after the synthesis of the hydrogel. For example, luciferase can be incorporated into a hydrogel as described in Preparative Example 8 below.

[0047] An enzyme substrate can be incorporated into a hydrogel during the synthesis of the hydrogel polymer. For example, luciferin can be incorporated into a hydrogel during the synthesis of the polymer, as described in Preparative Example 3 below. An enzyme substrate can be incorporated into a hydrogel after the synthesis of the hydrogel. For example, luciferin can be incorporated into a hydrogel as described in Preparative Example 7 below.

[0048] A protein, such as an enzyme, can be incorporated into a hydrogel. For example, the incorporation of an enzyme (luciferase) into a hydrogel during the synthesis of the hydrogel is described in Preparative Example 4 below. Although proteins may be incorporated into the hydrogel during the synthesis of the hydrogel polymer, chemicals and or processes (e.g., u.v. curing processes) used in the polymerization process can potentially cause the loss of some biological activity by certain proteins (e.g. certain enzymes or binding proteins such as antibodies). Proteins can also be incorporated into a hydrogel after the hydrogel has been synthesized, as described in Preparative Example 8 below. Incorporation of the protein into the hydrogel after synthesis of the hydrogel can lead to improved retention of the protein's biological activity.

[0049] In some applications, it may be desirable that the hydrogel containing a cell extractant or detection reagent is in a dry or partially-dried state. Swollen hydrogels can be dried, for example, by methods known to those skilled in the art, including evaporative processes, drying in convection ovens, microwave ovens, and vacuum ovens as well as freeze-drying. When the dried hydrogel is exposed to a liquid or aqueous solution, the cell extractant or detection reagent can diffuse from the hydrogel. The cell extractant or detection reagent can remain essentially dormant in the bead until exposed to a liquid or aqueous solution. That is, the cell extractant can be stored within the dry hydrogel until the bead is exposed to a liquid. This can prevent the waste or loss of the cell extractant or detection reagent when not needed and can improve the stability of many moisture sensitive cell extractants or detection reagents that may degrade by hydrolysis, oxidation, or other mechanisms.

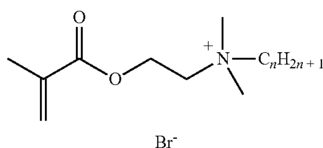
Cell Extractants:

[0050] Hydrogels of the present disclosure can comprise a cell extractant. Chemical cell extractants include biochemi-

cals, such as proteins (e.g., cytolytic peptides and enzymes). In some embodiments, the cell extractant increases the permeability of the cell, causing the release of biological analytes from the interior of the cell. In some embodiments, the cell extractant can cause or facilitate the lysis (e.g., rupture or partial rupture) of a cell.

[0051] Cell extractants include a variety of chemicals and mixtures of chemicals that are known in the art and include, for example, surfactants and quaternary amines, biguanides, surfactants, phenolics, cytolytic peptides, and enzymes. Typically, the cell extractant is not avidly bound (either covalently or noncovalently) to the hydrogel and can diffuse out of the hydrogel when the hydrogel is contacted with an aqueous liquid. In some embodiments, the precursor composition from which the hydrogel is made can contain an anionic or cationic monomer, such as described in WO 2007/146722 incorporated herein by reference, which is incorporated into the hydrogel and, as such can retain cell extractant activity. In some embodiments, the anionic or cationic monomers can be crosslinked to the surface of a hydrogel. Hydrogel beads or fibers can be dipped into a solution of the cationic monomers briefly, then quickly removed and cross-linked using actinic radiation (UV, E-beam, for example). This will result in the cationic monomer chemically bonding to the outer surface of the hydrogel beads or fibers.

[0052] Surfactants generally contain both a hydrophilic group and a hydrophobic group. The hydrogel may contain one or more surfactants selected from anionic, nonionic, cationic, ampholytic, amphoteric and zwitterionic surfactants and mixtures thereof. A surfactant that dissociates in water and releases cation and anion is termed ionic. When present, ampholytic, amphoteric and zwitterionic surfactants are generally used in combination with one or more anionic and/or nonionic surfactants. Nonlimiting examples of suitable surfactants and quaternary amines include TRITON X-100, Nonidet P-40 (NP-40), Tergitol, Sarkosyl, Tween, SDS, Igepal, Saponin, CHAPSO, benzalkonium chloride, benzethonium chloride, 'cetrimide' (a mixture of dodecyl-, tetradecyl- and hexadecyl-trimethylammoium bromide), cetylpyridium chloride, (meth)acrylamidoalkyltrimethylammonium salts (e.g., 3-methacrylamidopropyltrimethylammonium chloride and 3-acrylamidopropyltrimethylammonium chloride) and (meth)acryloxyalkyltrimethylammonium salts (e.g., 2-acryloxyethyltrimethylammonium chloride, 2-methacryloxyethyltrimethylammonium chloride, 3-methacryloxy-2-hydroxypropyltrimethylammonium chloride, 3-acryloxy-2-hydroxypropyltrimethylammonium chloride, and 2-acryloxyethyltrimethylammonium methyl sulfate). Other suitable monomeric quaternary amino salts include a dimethylalkylammonium group with the alkyl group having 2 to 22 carbon atoms or 2 to 20 carbon atoms. That is, the monomer includes a group of formula $—N(CH_3)_2(C_nH_{2n+1})^+$ where n is an integer having a value of 2 to 22. Exemplary monomers include, but are not limited to monomers of the following formula



where n is an integer in the range of 2 to 22.

[0053] Non-limiting examples of suitable biguanides, which include bis-biguanides, include polyhexamethylene biguanide hydrochloride, p-chlorophenyl biguanide, 4-chloro-benzhydryl biguanide, alexidine, halogenated hexidine such as, but not limited to, chlorhexidine (1,1'-hexamethylene-bis-5-(4-chlorophenyl biguanide), and salts thereof.

[0054] Non-limiting examples of suitable phenolics include phenol, salicylic acid, 2-phenylphenol, 4-t-amylphenol, Chloroxylenol, Hexachlorophene, 4-chloro-3,5-dimethylphenol (PCMX), 2-benzyl-4-chlorophenol, triclosan, butylated hydroxytoluene, 2-Isopropyl-5-methyl phenol, 4-Nonylphenol, xylenol, bisphenol A, Orthophenyl phenol, and Phenothiazines, such as chlorpromazine, prochlorperazine and thioridizine.

[0055] Non-limiting examples of suitable cytolytic peptides include A-23187 (Calcium ionophore), Dermaseptin, Listerolysin, Ranalexin, Aerolysin, Dermatoxin, Maculatin, Ranateurin, Amphotericin B, Direct lytic factors from animal venoms, Magainin, Rugosin, Ascaphin, Diptheria toxin, Maxymin, Saponin, *Aspergillus haemolysin*, Distinctin, Melittin, *Staphylococcus aureus* toxins, (α, β, χ, δ), Alamethicin, Esculetin, Metridiolysin, Streptolysin O, Apolipoproteins, Filipin, Nigericin, Streptolysin S, ATP Translocase, Gaegurin, Nystatin, Synexin, Bombinin, GALA, Ocellatin, Surfactin, Brevinin, Gramicidin, P25, Tubulin, Buforin, Helical erythrocyte lysing peptide, Palustrin, Valinomycin, Caerin, Hemolysins, Phospholipases, Vibriolysin, Cereolysin, Ionomycin, Phylloxin, Colicins, KALA, Polyene Antibiotics, Dermadistinctin, LAGA, Polymyxin B.

[0056] Non-limiting examples of suitable enzymes include lysozyme, lysostaphin, bacteriophage lysins, achromopeptidase, labiase, mutanolysin, streptolysin, tetanolysin, a-hemolysin, lyticase, lysing enzymes from fungi, cellulase, pectinase, Diselase® Viscozyme® L, pectolyase.

[0057] In some embodiments, various combinations of cell extractants can be used in the precursor composition (from which the hydrogel is synthesized) or sorbate (which is loaded into the hydrogel after synthesis of the hydrogel). Any other known cell extractants that are compatible with the precursor compositions or the resulting hydrogels can be used. These include, but are not limited to, chlorhexidine salts such as chlorhexidine gluconate (CHG), parachlorometaxylenol (PCMX), triclosan, hexachlorophene, fatty acid monoesters and monoethers of glycerin and propylene glycol such as glycerol monolaurate, Cetyl Trimethylammonium Bromide (CTAB), glycerol monocaprylate, glycerol monocaprate, propylene glycol monolaurate, propylene glycol monocaprylate, propylene glycol moncaprate, phenols, surfactants and polymers that include a (C12-C22) hydrophobe and a quaternary ammonium group or a protonated tertiary amino group, quaternary amino-containing compounds such as quaternary silanes and polyquaternary amines such as polyhexamethylene biguanide, transition metal ions such as copper containing compounds, zinc containing compounds, and silver containing compounds such as silver metal, silver salts such as silver chloride, silver oxide and silver sulfadiazine, methyl parabens, ethyl parabens, propyl parabens, butyl parabens, octenidene, 2-bromo-2-nitropropane-1,3 diol, or mixtures of any two or more of the foregoing.

[0058] Suitable cell extractants also include dialkyl ammonium salts, including N-(n-dodecyl)-diethanolamine; cationic ethoxylated amines, including 'Genaminox K-10', Genaminox K-12, 'Genamin TCL030', and 'Genamin

C100'; amidines, including propamidine and dibromopropamidine; peptide antibiotics, including polymyxin B and nisin; polyene antibiotics, including nystatin, amphotericin B, and natamycin; imidazoles, including econazole, clotramizole and miconazole; oxidizing agents, including stabilized forms of chlorine and iodine; and the cell extractants described in U.S. Pat. No. 7,422,868, which is incorporated herein by reference in its entirety.

[0059] Cell extractants are preferably chosen not to inactivate the detection system (e.g., a detection reagent such as luciferase enzyme) of the present invention. For microbes requiring harsher cell extractants (e.g., ionic detergents etc.), modified detection systems (such as luciferases exhibiting enhanced stability in the presence of these agents, such as those disclosed in U.S. Patent Application Publication No. 2003/0104507, which is hereby incorporated by reference in its entirety) are particularly preferred.

[0060] Methods of the present invention provide for the release of an effective amount of cell extractant from a hydrogel to cause the release of biological analytes from a live cell. The present disclosure includes a variety of cell extractants known in the art and each of which may be released form the hydrogel at a different rate and may exert its effect on living cells at a different concentration than the others. The following will provide guidance concerning the factors to be considered in selecting the cell extractant and the in determining an effective amount to include in the hydrogel.

[0061] It is known in the art that the efficacy of any cell extractant is determined primarily by two factors—concentration and exposure time. That is, in general, the higher the concentration of a cell extractant, the greater the effect (e.g., permeabilization of the cell membrane and/or release of biological analytes from the cell) it will have on a living cell. Also, at any given concentration of cell extractant, in general, the longer you expose a living cell to the cell extractant, the greater the effect of the cell extractant. Other extrinsic factors such as, for example, pH, co-solvents, ionic strength, and temperature are known in the art to affect the efficacy of certain cell extractant. It is known that these extrinsic factors can be controlled by, for example, temperature controllers, buffers, sample preparation, and the like. These factors, as well as the cell extractant, can also have effects on the detection systems used to detect biological analytes. It is well within the grasp of a person of ordinary skill to perform a few simple experiments to determine an effective amount of cell extractant to produce the articles and perform the methods of the present disclosure. Further guidance is provided in the Examples described herein.

[0062] Initial experiments to determine the effect of various concentrations of the cell extractant on the cells and/or the detection system can be performed. Initially, the hydrogel comprising a cell extractant can be screened for its effect on the biological analyte detection system. For example, the hydrogel can be placed into an ATP assay (without bacterial cells) similar to that described herein in Example 19. The assay can be run with solutions of reagent-grade ATP (e.g. from about 0.1 to about 100 picomoles of ATP) and the amount of bioluminescence emitted by the luciferase reaction in the sample with hydrogel can be compared to the amount of bioluminescence emitted by a sample without hydrogel. Preferably, the amount of bioluminescence in the sample with hydrogel is greater than 50% of the amount of bioluminescence in the sample without the hydrogel. More preferably, the amount of bioluminescence in the sample with hydrogel is greater than 90% of the amount of bioluminescence in the sample without the hydrogel. Most preferably, the amount of bioluminescence in the sample with hydrogel is greater than 95% of the amount bioluminescence in the sample without the hydrogel.

[0063] Additionally, the effect of the hydrogel on the release of the biological analyte from the cells can be determined experimentally, as described in Example 19. For example, liquid suspensions of cells (e.g., microbial cells such as *Staphylococcus aureus*) are exposed to relatively broad range of concentrations of a cell extractant (e.g., BARDAC 205M) for a period of time (e.g. up to several minutes) in the present of a detection system to detect biological analytes from a cell (e.g., an ATP detection system comprising luciferin, luciferase, and a buffer at about pH 7.6 to 7.8). The biological analyte is measured periodically, with the first measurement usually performed immediately after the cell extractant is added to the mixture, to determine whether the release of the biological analyte (in this example, ATP) from the cells can be detected. The results can indicate the optimal conditions (i.e., liquid concentration of cell extractant and exposure time) to detect the biological analyte released from the cells. As shown in Table 24, the results can also indicate that, at higher concentrations of cell extractant, the cell extractant may be less effective and/or may interfere with the detection system (i.e., may absorb the light or color generated by the detection reagents).

[0064] After the effective amount of cell extractant in liquid mixtures is determined, consideration should be given to the amount of cell extractant to incorporate into the hydrogel by the methods described herein. When the hydrogel comprising a cell extractant forms a liquid mixture (e.g., a sample suspected of containing live cells in an aqueous suspension) the cell extractant diffuses out of the hydrogel until a concentration equilibrium of the cell extractant, between the hydrogel and the liquid, is reached. Without being bound by theory, it can be assumed that, until the equilibrium is reached, a concentration gradient of cell extractant will exist in the liquid, with a higher concentration of extractant present in the portion of the liquid proximal the hydrogel. When the concentration of the cell extractant reaches an effective concentration in a portion of the liquid containing a cell, the cell releases biological analytes. The released biological analytes are thereby available for detection by a detection system.

[0065] Achieving an effective concentration of cell extractant in the liquid containing the sample can be controlled by several factors. For example, the amount of cell extractant loaded into the hydrogel can affect final concentration of cell extractant in the liquid at equilibrium. Additionally, the amount of hydrogel and the amount of surface area of the hydrogel in the liquid mixture can affect the rate of release of the cell extractant from the hydrogel and the final concentration of cell extractant in the liquid at equilibrium. Furthermore, the temperature of the aqueous medium can affect the rate at which the hydrogel releases the cell extractant. Other factors, such as the ionic properties and or hydrophobic properties of the cell extractant and the hydrogel may affect the amount of cell extractant released from the hydrogel and the rate at which the cell extractant is released from the hydrogel. All of these factors can be optimized with routine experimentation by a person of ordinary skill to achieve the desired parameters (e.g., manufacturing considerations for the articles and the time-to-result for the methods) for detection of cells in a sample. In general, it is desirable to incorporate at

least enough cell extractant into the hydrogel to achieve the effective amount (determined by the experimentation without hydrogels) when the cell extractant reaches equilibrium between the hydrogel and the volume of liquid comprising the sample material. It may be desirable to add a larger amount of cell extractant to the hydrogel (than the amount determined by experimentation without hydrogels) to reduce the amount of time it take for the hydrogel to release an effective amount of cell extractant.

[0066] In some embodiments, achieving an effective concentration of cell extractant can comprise using size-selected hydrogel compositions. For example, hydrogel beads can be loaded (e.g., by absorption and/or adsorption) with a cell extractant (e.g., a 50% (w/v) aqueous solution of BARDAC 205M; or a 10%, 17.5%, or 25% (w/v) aqueous solutions of benzalkonium chloride). The hydrogel beads may be size-selected (for example, by sieving the beads through different fine series mesh sizes, such as No. 10 (2.0 mm), No. 12 (1.7 mm), No. 14 (1.4 mm), No. 16 (1.18 mm) and No. 18 (1.0 mm) 8" Round Test Sieves available from Glison Company, Lewis Center, Ohio) to obtain uniform size beads. The hydrogel beads can be size-selected before and/or after they are loaded with the cell extractant. In some embodiments, the average diameter of the size-selected hydrogel beads may be about 1.0 mm, about 1.18 mm, about 1.4 mm, about 1.7 mm, or about 2.0 mm. In some embodiments, the average diameter of the size-selected hydrogel beads may be less than 1.0 mm. In some embodiments, the average diameter of the size-selected hydrogel beads may be greater than 2.0 mm. Advantageously, the size-selected hydrogel beads can provide better control of the amount of time it takes for the hydrogel to release an effective amount of cell extractant.

[0067] In some embodiments, a selected amount of the size-selected hydrogel beads can be used in a detection device. For example, in some embodiments, about 2.5 mg to about 4 mg of hydrogel beads containing BARDAC 205M can be used in a detection device. In some embodiments, about 5 mg to about 10 mg of hydrogel beads containing BARDAC 205M can be used in a detection device. In some embodiments, about 11 mg to about 14 mg of hydrogel beads containing BARDAC 205M can be used in a detection device.

[0068] The cell extractant can diffuse into the hydrogel, diffuse out of the hydrogel, or both. The rate of diffusion should be controllable by, for example, varying the polymeric material and the crosslink density, by varying the polar solvent in which the hydrogel is made, by varying the solubility of the cell extractant in the polar solvent in which the hydrogel is made, and by varying the molecular weight of the cell extractant. The rate of diffusion can also be modified by varying the shape, size, and surface topography of the hydrogel.

[0069] The hydrogel can be contacted with the liquid sample material either statically, dynamically (i.e., with mixing by vibration, stirring, aeration or compressing, for example), or a combination thereof. Example 16 shows that mixing can effect a faster release of an effective amount of cell extractant from a hydrogel. Example 17 shows that compressing the hydrogel can effect a faster release of an effective amount of cell extractant from hydrogel. Compressing the hydrogel can include, for example, pressing the hydrogel against a surface and/or crushing the hydrogel. Thus, in some embodiments, mixing can advantageously provide a faster release of cell extractant and thereby a faster detection of biological analytes (e.g., from live cells) in a sample. In some embodiments, compressing the hydrogel (e.g., by exerting pressure against the hydrogel using a sample acquisition device such as a swab or a spatula, a carrier (described below) or some other suitable implement) can advantageously provide a faster release of cell extractant and thereby a faster detection of biological analytes in a sample. Additionally, the step of compressing the hydrogel can be performed to accelerate the release of the cell extractant at a time that is convenient for the operator. In some embodiments, static contact can delay the release of an effective amount of cell extractant and thereby provide additional time for the operator to carry out other procedures (e.g., reagent additions, instrument calibration, and/or specimen transport) before detecting the biological analytes. In some embodiments, it may be advantageous to hold the mixture statically until a first biological analyte measurement is taken and then dynamically mix the sample to reduce the time necessary to release an effective amount of cell extractant.

[0070] It is fully anticipated that the most preferred concentration(s) or concentration range(s) functional in the methods of the invention will vary for different microbes and for different cell extractants and may be empirically determined using the methods described herein or commonly known to those skilled in the art.

Samples and Sample Acquisition Devices:

[0071] Articles and methods of the present disclosure provide for the detection of biological analytes in a sample. In some embodiments, the articles and methods provide for the detection of biological analytes from live cells in a sample. In certain preferred embodiments, the articles and methods provide for the detection of live microbial cells in a sample. In certain preferred embodiments, the articles and methods provide for the detection of live bacterial cells in a sample.

[0072] The term "sample" as used herein, is used in its broadest sense. A sample is a composition suspected of containing a biological analyte (e.g., ATP) that is analyzed using the invention. While often a sample is known to contain or suspected of containing a cell or a population of cells, optionally in a growth media, or a cell lysate, a sample may also be a solid surface, (e.g., a swab, membrane, filter, particle), suspected of containing an attached cell or population of cells. It is contemplated that for such a solid sample, an aqueous sample is made by contacting the solid with a liquid (e.g., an aqueous solution) which can be mixed with hydrogels of the present. Filtration of the sample is desirable in some cases to generate a sample, e.g., in testing a liquid or gaseous sample by a process of the invention. Filtration is preferred when a sample is taken from a large volume of a dilute gas or liquid. The filtrate can be contacted with hydrogels of the present disclosure, for example after the filtrate has been suspended in a liquid.

[0073] Suitable samples include samples of solid materials (e.g., particulates, filters), semisolid materials (e.g., a gel, a liquid suspension of solids, or a slurry), a liquid, or combinations thereof. Suitable samples further include surface residues comprising solids, liquids, or combinations thereof. Non-limiting examples of surface residues include residues from environmental surfaces (e.g., floors, walls, ceilings, fomites, equipment, water, and water containers, air filters), food surfaces (e.g., vegetable, fruit, and meat surfaces), food processing surfaces (e.g., food processing equipment and cutting boards), and clinical surfaces (e.g., tissue samples, skin and mucous membranes).

[0074] The collection of sample materials, including surface residues, for the detection of biological analytes is known in the art. Various sample acquisition devices, including spatulas, sponges, swabs and the like have been described. The present disclosure provides sample acquisition devices with unique features and utility, as described herein.

[0075] Turning now to the Figures, FIG. **1** shows a side view of one embodiment of a sample acquisition device **130** according to the present disclosure. The sample acquisition device **130** comprises a handle **131** which can be grasped by the operator while collecting a sample. The handle comprises an end **132** and, optionally, a plurality of securing members **133**. Securing members **133** can be proportioned to slideably fit into a housing (such as housing **320** or housing **420** shown in FIGS. **3** and **4**, for example). In some embodiments, the securing members **133** can form a liquid-resistant seal to resist the leakage of fluids from a housing.

[0076] The sample acquisition device **130** further comprises an elongated shaft **134** and a tip **139**. In some embodiments, the shaft **134** can be hollow. The shaft **134** comprises a tip **139**, positioned near the end of the shaft **134** opposite the handle **131**. The tip **139** can be used to collect sample material and can be constructed from porous materials, such as fibers (e.g., rayon or Dacron fibers) or foams (e.g., polyurethane foam) which can be affixed to the shaft **134**. In some embodiments, the tip **139** can be a molded tip as described in U.S. Patent Application No. 61/029,063, filed on Dec. 5, 2007 and entitled, "SAMPLE ACQUISITION DEVICE", which is incorporated herein by reference in its entirety. The construction of sample acquisition devices **130** is known in the art and can be found, for example, in U.S. Pat. No. 5,266,266, which is incorporated herein by reference in their entirety.

[0077] Optionally, the sample acquisition device **130** can further comprise a hydrogel **140** comprising a cell extractant. In some embodiments, the hydrogel **140** is positioned in or on the sample acquisition device **130** at a location other than the tip **139** that is used to collect the sample (e.g., on the shaft **134**, as shown in FIG. **1**). The hydrogel **140** can be coated onto shaft **134** as described herein or it can be adhered to the shaft **134** by, for example, a pressure-sensitive adhesive or a water-soluble adhesive (not shown). The adhesive should be selected for its compatibility with the detection system used to detect a biological analyte from live cells (i.e., the adhesive should not significantly impair the accuracy or sensitivity of the detection system).

[0078] In use, the tip **139** of a sample acquisition device **130** is contacted with a sample material (e.g., a solid, a semisolid, a liquid suspension, a slurry, a liquid, a surface, and the like) to obtain a sample suspected of containing cells. The sample acquisition device **130** can be used to transfer the sample to a detection system as described herein.

[0079] FIG. **2** shows a partial cross-sectional view of another embodiment of a sample acquisition device **230** according to the present disclosure. In this embodiment, the sample acquisition device **230** comprises a handle **231** with an end **232**, optional securing members **233** to slideably fit within a housing (not shown), a hollow elongated shaft **234**, and a tip **239** comprising porous material. The sample acquisition device **230** further comprises a hydrogel **240**, which comprises a cell extractant, disposed in the interior portion of the shaft **234**. Thus, the sample acquisition device **230** provides an enclosure (shaft **234**) containing the hydrogel **240**. The material comprising the tip **239** is porous enough to permit liquids to flow freely into the interior of the shaft **234** without permitting the hydrogel **240** to pass through the material and out of the tip **239**.

[0080] In use, sample acquisition device **230** can be used to contact surfaces, preferably dry surfaces, to obtain sample material. After the sample is obtained, the tip **239** of the sample acquisition device **230** is moistened with a liquid (e.g. water or a buffer; optionally, including a detection reagent such as an enzyme and/or an enzyme substrate), thereby permitting an effective amount of the cell extractant to be released from the hydrogel **240** and to contact the sample material. The release of an effective amount of cell extractant from hydrogel **240** permits the sample acquisition device **230** to be used in methods to detect biological analytes from live cells as described herein.

[0081] Another embodiment (not shown) of a sample acquisition device including a hydrogel comprising a cell extractant can be derived from the "Specimen Test Unit" disclosed by Nason in U.S. Pat. No. 5,266,266 (hereinafter, referred to as the "Nason patent"). In particular, referring to FIGS. 7-9 of the Nason patent, the handle of the sample acquisition devices described herein can be modified to embody Nason's functional elements of the housing base 14 (which forms reagent chamber 36) and the seal fitting 48, which includes central dispense passage 50 (optional, with housing cap 30) connected to the hollow swab shaft 22. The central passage 50 of the seal fitting 48 can be closed by a break-off nib 52 in the form of an extended rod segment 54 connected to the seal fitting 48 at the inboard end of the passage 50 via a reduced diameter score 56. Thus, in one embodiment of the present disclosure, the sample acquisition device handle comprises a reagent chamber, as described by Nason. The reagent chamber located in the handle of the sample acquisition device of this embodiment includes hydrogel particles (e.g., beads) comprising a cell extractant. Thus, the sample acquisition device of this embodiment provides an enclosure (reagent chamber 36) containing the hydrogel. In this embodiment, the hydrogel particles are not suspended in a liquid medium than causes the release of the cell extractant from the hydrogel. The hydrogel particles are proportioned and shaped to allow free passage of the individual particles into and through the central passage 50 and the hollow shaft 22.

[0082] In use, the sample acquisition device comprising a handle including a reagent chamber can be used to obtain a sample as described herein. If the sample is a liquid, the break-off nib 52 can be actuated, as described in the Nason patent, enabling the passage of the hydrogel through the shaft to contact the liquid sample in the swab tip, thereby forming a liquid mixture comprising the sample and the hydrogel. The liquid mixture comprising the sample and the hydrogel can be used for the detection of a biological analyte associated with a live cell, as described herein. If the sample is a solid or semi-solid, the tip of the sample acquisition device can be contacted or submersed in a liquid solution and the break-off nib 52 can be actuated, as described in the Nason patent, enabling the passage of the hydrogel through the shaft to contact the liquid sample in the swab tip, thereby forming a liquid mixture comprising the sample and the hydrogel. The liquid mixture comprising the sample and the hydrogel can be used for the detection of a biological analyte associated with a live cell, as described herein.

Detection Devices:

[0083] FIG. **3** shows a cross-sectional view of one embodiment of a housing **320** of a detection device according to the

present disclosure. The housing **320** comprises an opening **322** configured to receive a sample acquisition device and at least one wall **324**. Disposed in the housing **320** is a hydrogel **340** comprising a cell extractant. Thus, the housing **320** provides an enclosure containing the hydrogel **340**.

[0084] In FIG. **3**, the hydrogel **340** is a shaped hydrogel, in the form of a generally spherical bead. It will be appreciated that a bead is just one example of a variety shaped hydrogels disclosed herein that are suitable for use in housing **320**.

[0085] In some embodiments (not shown), the hydrogel **340** can be coated onto a solid substrate (e.g., the wall **324** of the housing **320**). Nonlimiting examples of other suitable solid substrates (not shown) onto which hydrogels **340** of the present disclosure can be coated include a polymeric film, a fiber, a nonwoven, a ceramic particle, paper, and a polymeric bead. Solid substrates can be coated with hydrogel **340** by a variety of processes including; for example, dip coating, knife coating, curtain coating, spraying, kiss coating, gravure coating, offset gravure coating, and/or printing methods such as screen printing and inkjet printing can be used to apply the hydrogel composition onto the substrate in a pattern if desired. The choice of the coating process will be influenced by the shape and dimensions of the solid substrate and it is within the grasp of a person of ordinary skill in the appropriate art to recognize the suitable process for coating any given solid substrate.

[0086] It should be recognized that in this and all other embodiments (for example, the illustrated embodiments of FIGS. **1**, **2**, **4**, **5**, **6**A-B, **7**, and **8**), the hydrogel (e.g., hydrogel **340**) may include a plurality (for example, at least 2, 3, 4, 5, up to 10, up to 20, up to 50, up to 100, up to 500, up to 1000) of hydrogel bodies such as beads, fibers, ribbons, coated substrates, or the like. For example, hydrogel **340** can comprise up to 2, up to 3, up to 4, up to 5, up to 10, up to 20, up to 50, up to 100, up to 500, up to 1000 or more hydrogel bodies.

[0087] The wall **324** of the housing **320** can be cylindrical, for example. It will be appreciated that other useful geometries, some including a plurality of walls **324**, are possible and within the grasp of one of ordinary skill in the appropriate art. The housing **320** can be constructed from a variety of materials such as plastic (e.g., polypropylene, polyethylene, polycarbonate) or glass. Preferably, at least a portion of the housing **320** is constructed from materials that have optical properties that allow the transmission of light (e.g., visible light). Suitable materials are well known in devices used for biochemical assays such as ATP tests, for example.

[0088] Optionally, housing **320** can comprise a cap (not shown) that can be shaped and dimensioned to cover the opening **322** of the housing **320**. It should be recognized that other housings (for example, housings **420** and **520** as shown in FIGS. **4** and **5**, respectively and described herein) can also comprise a cap.

[0089] In some embodiments, the housing **320** can be used in conjunction with a sample acquisition device (not shown). Optionally, the sample acquisition device may comprise a hydrogel, such as, for example, sample acquisition devices **130** or **230** shown in FIGS. **1** and **2**, respectively, and described herein. The hydrogel in the sample acquisition device can comprise the same composition and/or amount of cell extractant as hydrogel **340**. The hydrogel in the sample acquisition device can comprise a different composition and/or amount of cell extractant than hydrogel **340**. In some embodiments, the sample acquisition device can comprise a somatic cell extractant and the housing **320** can comprise a microbial cell extractant. In some embodiments, the sample acquisition device can comprise a microbial cell extractant and the housing **320** can comprise a somatic cell extractant. It should be recognized that other housings (for example, housings **420** and **520** as shown in FIGS. **4** and **5**, respectively and described herein) can similarly comprise a sample acquisition device that may optionally include a hydrogel.

[0090] The housing **320** can be used in methods to detect live cells in a sample. During use, the operator can form a liquid (e.g., an aqueous liquid or aqueous solutions containing glycols and/or alcohols) mixture in the housing **320**, the mixture comprising a liquid sample and the hydrogel **340**. In some embodiments, the mixture can further comprise a detection reagent. The liquid mixture comprising the sample and the hydrogel **440** can be used for the detection of a biological analyte associated with a live microorganism.

[0091] FIG. **4** shows a partial cross-section view of one embodiment of a housing **420** of a detection device according to the present disclosure. The housing **420** comprises a wall **424** with an opening **422** configured to receive a sample acquisition device. A frangible seal **460** divides that housing **420** into two portions, the upper compartment **426** and the reaction well **428**. Disposed in the reaction well **428** is a hydrogel **440**. Thus, the housing **420** provides an enclosure containing the hydrogel **440**.

[0092] The frangible seal **460** forms a barrier between the upper compartment **426** (which includes the opening **422** of the housing **420**) and the reaction well **428**. In some embodiments, the frangible seal **460** forms a water-resistant barrier. The frangible seal **460** can be constructed from a variety of frangible materials including, for example polymer films, metal-coated polymer films, metal foils, dissolvable films (e.g., films made of low molecular weight polyvinyl alcohol or hydroxypropyl cellulose (HPC) and combinations thereof.

[0093] Frangible seal **460** may be connected to the wall **424** of the housing **420** using a variety of techniques. Suitable techniques for attaching a frangible seal **460** to a wall **424** include, but are not limited to, ultrasonic welding, any thermal bonding technique (e.g., heat and/or pressure applied to melt a portion of the wall **424**, the frangible seal **460**, or both), adhesive bonding, stapling, and stitching. In one desired embodiment of the present invention, the frangible seal **460** is attached to the wall **424** using an ultrasonic welding process.

[0094] The housing **420** can be used in methods to detect cells in a sample. Methods of the present disclosure include the formation of a liquid mixture comprising the sample material and the hydrogel **440** and include the detection of a biological analyte, as described herein.

[0095] If the sample is a liquid sample (e.g., water, juice, milk, meat juice, vegetable wash, food extracts, body fluids and secretions, saliva, wound exudate, and blood), the liquid sample can be transferred (e.g., poured or pipetted) directly into the upper chamber **426**. A detection reagent can be added to the sample before the sample is transferred to the housing **420**. A detection reagent can be added to the sample after the sample is transferred to the housing **420**. A detection reagent can be added to the sample while the sample is transferred to the housing **420**. The frangible seal **460** can be ruptured (e.g., by piercing it with a pipette tip or a sample acquisition device) before the liquid sample is transferred to the housing **420**. The frangible seal **460** can be ruptured after the liquid sample is transferred to the housing **420**. The frangible seal **460** can be ruptured while the liquid sample is transferred to the housing **420**. When the liquid sample is in the housing **420** and the

frangible seal is ruptured, a liquid mixture comprising the sample and the hydrogel **440** is formed. The liquid mixture comprising the sample and the hydrogel **440** can be used for the detection of a biological analyte associated with a live microorganism.

[0096] If the sample is a solid sample (e.g., powder, particulates, semi-solids, residue collected on a sample acquisition device, air filter), the housing **420** can advantageously be used as a vessel in which the sample can be mixed with a liquid suspending medium such as, for example, water or a buffer. Preferably, the liquid suspending medium is substantially free of microorganisms. More preferably, the liquid suspending medium is sterile. Before, after or during the process of mixing the solid sample with the liquid suspending medium, a detection reagent can be added to the liquid suspending medium. Either before, after, or during the process of mixing the solid sample with the liquid suspending medium, the frangible seal **460** can be ruptured (e.g., by piercing with a pipette tip or a swab), thus forming a liquid mixture comprising the sample and the hydrogel **440** comprising a cell extractant. The liquid mixture comprising the sample and the hydrogel **440** can be used in a method for the detection of a biological analyte associated with a live cell.

[0097] FIG. **5** shows a partial cross-section view of one embodiment of a housing **520** of a detection device according to the present disclosure. The housing **520** comprises a wall **524** with an opening **522** configured to receive a sample acquisition device. A frangible seal **560** divides the housing **520** into two portions, the upper compartment **526** and the reaction well **528**. Disposed in the upper compartment **526** is a hydrogel **540** comprising a cell extractant. The reaction well **528** further includes a detection reagent **570**.

[0098] In FIG. **5**, the hydrogel **540** is positioned on the frangible seal **560**, in the upper chamber **526** of the housing **520**. Thus, the housing **520** provides and enclosure containing the hydrogel **540**. In some embodiments (not shown), the hydrogel **540** may be coupled to the frangible seal **560** or wall **524** of the upper chamber **526**. For example, the hydrogel **540** may be adhesively coupled (e.g., via a pressure-sensitive adhesive or water-soluble adhesive) or coated onto one of the surfaces (e.g., the frangible seal **560** and/or the wall **524**) that form a portion of the upper chamber **526** of the housing **520**.

[0099] The reagent well **528** of housing **520** comprises a detection reagent **570**. Optionally, the detection reagent **570** can comprise a detection reagent (i.e., a detection reagent may be dissolved and/or suspended in the detection reagent **570**). In other embodiments (not shown), the reagent well **528** can comprise a dry detection reagent (e.g., a powder, particles, microparticles, a tablet, a pellet, and the like) instead of the detection reagent **570**.

[0100] The housing **520** can be used in methods to detect cells in a sample. Methods of the present disclosure include the formation of a liquid mixture comprising the sample material and the hydrogel **440** and include the detection of a biological analyte, as described herein.

[0101] If the sample is a liquid sample (e.g., water, juice, milk, meat juice, vegetable wash, food extracts, body fluids and secretions, saliva, wound exudate, and blood), the liquid sample can be transferred (e.g., poured or pipetted) directly into the upper compartment **526**, thus forming a liquid mixture comprising the sample and the hydrogel **540**. Before, after or during the transfer of the sample into the housing **520**, a detection reagent can be added to the liquid sample. Before, after, or during the transfer of the liquid sample to the housing **520**, the frangible seal **560** can be ruptured (e.g., by piercing with a pipette tip or a swab). The liquid mixture comprising the sample and the hydrogel **540** can be used for the detection of a biological analyte associated with a live microorganism before and/or after the frangible seal **560** is ruptured.

[0102] If the sample is a solid sample (e.g., powder, particulates, semi-solids, residue collected on a sample acquisition device), the housing **520** can advantageously be used as a vessel in which the sample can be mixed with a liquid suspending medium such as, for example, water or a buffer. Preferably, the liquid suspending medium is substantially free of microorganisms. More preferably, the liquid suspending medium is sterile.

[0103] Mixing the solid sample with a liquid suspending medium forms a liquid mixture comprising the sample and the hydrogel **540**. Before, after or during the process of mixing the solid sample with the liquid suspending medium, a detection reagent can be added to the liquid suspending medium. Before, after, or during the process of mixing the solid sample with the liquid suspending medium, the frangible seal **560** can be ruptured (e.g., by piercing with a pipette tip or a swab). The liquid mixture comprising the sample and the hydrogel **540** can be used for the detection of a biological analyte associated with a live microorganism, as described herein.

[0104] FIGS. **6**A-**6**B show partial cross-section views of a detection device **610** according to the present disclosure. Referring to FIG. **6**A, the detection device **610** comprises a housing **620** and a sample acquisition device **630**, as described herein. The housing **620** includes a frangible seal **660**, a hydrogel **640** comprising a cell extractant disposed in the upper compartment **626**, and an optional detection reagent **670** disposed in the reagent well **628**. Thus, the housing **620** provides an enclosure containing the hydrogel **640**. The detection reagent **670** may further comprise a detection reagent.

[0105] The sample acquisition device **630** comprises a handle **631** which can be grasped by the operator while collecting a sample. The sample acquisition device **630** is shown in FIG. **6**A in a first position "A", with the handle **631** substantially extending outside the housing **620**. Generally, the handle **631** will be in position "A" during storage of detection device **610**. During use, the sample acquisition device **630** is withdrawn from the housing **620** and the tip **629** is contacted with the area or material from which a sample is to be taken. After collecting the sample, the sample acquisition device is reinserted into the housing **620** and, typically, while the housing **620** is held in place, the end **632** of the handle **631** is urged (e.g., with finger pressure) toward the housing **620**, moving the sample acquisition device **630** approximately into position "B" and thereby causing the tip **639** to pass through the frangible seal **660** and into the detection reagent **670**, if present, in the reaction well **628** (as shown in FIG. **6**B). As the tip **639** ruptures the frangible seal **660**, the hydrogel **640** is also moved into the reaction well **628**. This process forms a liquid mixture that includes a sample and a hydrogel **640**. The liquid mixture comprising the sample and the hydrogel **640** can be used for the detection of a biological analyte associated with a live cell, as described herein.

[0106] FIG. **7** shows a cross-sectional view of a detection device **710** comprising a housing **720** and a sample acquisition device **730**, as described herein. The housing **720** is divided into an upper chamber **726** and a reaction well **728** by frangible seals **760***a* and **760***b*. Positioned between frangible

seals **760***a* and **760***b* is hydrogel **740** comprising a cell extractant. Thus, the housing **720** provides an enclosure containing the hydrogel **740**. Reaction well **728** comprises a detection reagent **770**.

[0107] In use, the tip **739** of a sample acquisition device **730** is contacted with a sample material (e.g., a solid, a semisolid, a liquid suspension, a slurry, a liquid, a surface, and the like), as described above. After collecting the sample, the sample acquisition device **730** is reinserted into the housing **720** and the handle is urged into the housing **720**, as described above, thereby causing the tip **739** to pass through frangible seals **760***a* and **760***b* and into the detection reagent in the reaction well **728**. As the tip **739** passes through frangible seals **760***a* and **760***b*, the hydrogel **740** is also moved into the detection reagent **770** in the reaction well **728**. This process forms a liquid mixture that includes a sample and a hydrogel **740**. The liquid mixture comprising the sample and the hydrogel **40** can be used for the detection of a biological analyte associated with a live microorganism, as described herein.

[0108] FIG. **8** shows a partial cross-section view of a detection device **810** according to the present disclosure. The detection device **810** comprises a housing **820** and a sample acquisition device **830**, both as described herein. A frangible seal **860***b*, as described herein, divides the housing into two sections, the upper compartment **826** and the reagent chamber **828**. The reagent chamber **828** includes a detection reagent **870**, which may be a liquid detection reagent **870** (as shown) or a dry detection reagent as described herein. Slideably disposed in the upper compartment **824**, proximal the frangible seal **860***b*, is a carrier **880**. The carrier **880** includes a hydrogel **840** comprising a cell extractant and an optional frangible seal **860***a*. Thus, the carrier **880** provides an enclosure containing the hydrogel **840**. The carrier **880** can be, for example, constructed from molded plastic (e.g., polypropylene or polyethylene). In the illustrated embodiment, the frangible seal **860***a* functions to hold the hydrogel **840** (shown as a hydrogel bead) in the carrier **880** during storage and handling. In some embodiments, the hydrogel **840** is coated onto the carrier **880** and the frangible seal **860***a* may not be required to retain the hydrogel **840** during storage and handling.

[0109] In use, the sample acquisition device **830** is removed from the detection device **810** and a sample is collected as described herein on the tip **839**. The sample acquisition device **830** is reinserted into the housing **820** and the handle **831** is urged into the housing **820**, as described for the detection device in FIG. **6**A-B. The tip **839** of the sample acquisition device **830** ruptures frangible seal **860**A, if present, and pushes the carrier **880** through frangible seal **860***b*. The carrier **880** drops into the detection reagent **870** as the tip **839** comprising the sample contacts the detection reagent **870**, thereby forming a liquid mixture including the sample and a hydrogel comprising a cell extractant. The liquid mixture comprising the sample and the hydrogel **840** can be used for the detection of a biological analyte associated with a live cell, as described herein.

[0110] FIG. **9** shows a bottom perspective view of one embodiment of the carrier **980** of FIG. **8**. The carrier **980** comprises a cylindrical wall **982** and a base **984**. The wall **982** is shaped and proportioned to slideably fit into a housing (not shown). The carrier **980** further comprises optional frangible seal **960***a*. The base **984** comprises holes **985** and piercing members **986**, which form a piercing point **988**. The piercing point **988** can facilitate the rupture of a frangible seal in a housing (not shown)

Methods of Detecting Biological analytes from Live Cells:

[0111] Methods of the present disclosure include methods for the detection of biological analytes that are released from live cells including, for example, live microorganisms, after exposure to an effective amount of cell extractant.

[0112] The detection of the biological analytes involves the use of a detection system. Detection systems for certain biological analytes such as a nucleotide (e.g., ATP), a polynucleotide (e.g., DNA or RNA) or an enzyme (e.g., NADH dehydrogenase or adenylate kinase) are known in the art and can be used according to the present disclosure. Methods of the present disclosure include known detections systems for detecting a biological analyte. Preferably, the accuracy and sensitivity of the detection system is not significantly reduced by the cell extractant. More preferably, the detection system comprises a homogeneous assay.

[0113] In some embodiments, the detection system comprises a detection reagent. Detection reagents include, for example, dyes, enzymes, enzyme substrates, binding partners (e.g., an antibody, a monoclonal antibody, a lectin, a receptor), and/or cofactors. In some embodiments, the detection system comprises an instrument.

[0114] Nonlimiting examples of detection instruments include a spectrophotometer, a luminometer, a plate reader, a thermocycler, an incubator.

[0115] Detection systems are known in the art and can be used to detect biological analytes colorimetrically (i.e., by the absorbance and/or scattering of light), fluorescently, or lumimetrically. Examples of the detection of biomolecules by luminescence are described by F. Gorus and E. Schram (Applications of bio- and chemiluminescence in the clinical laboratory, 1979, Clin. Chem. 25:512-519).

[0116] An example of a biological analyte detection system is an ATP detection system. The ATP detection system can comprise an enzyme (e.g., luciferase) and an enzyme substrate (e.g., luciferin). The ATP detection system can further comprise a luminometer. In some embodiments, the luminometer can comprise a bench top luminometer, such as the FB-12 single tube luminometer (Berthold Detection Systems USA, Oak Ridge, Tenn.). In some embodiments, the luminometer can comprise a handheld luminometer, such as the NG Luminometer, UNG2 (3M Company, Bridgend, U.K.).

[0117] Methods of the present disclosure include the formation of a liquid mixture comprising a sample suspected of containing live cells and a hydrogel comprising a cell extractant. Methods of the present disclosure further include detecting a biological analyte. Detecting a biological analyte can further comprise quantitating the amount of biological analyte in the sample.

[0118] In some embodiments, detecting the biological analyte can comprise detecting the analyte directly in a vessel (e.g., a tube, a multi-well plate, and the like) in which the liquid mixture comprising the sample and the hydrogel comprising a cell extractant is formed. In some embodiments, detecting the biological analyte can comprise transferring at least a portion of the liquid mixture to a container other than the vessel in which the liquid mixture comprising the sample and the hydrogel comprising a cell extractant is formed. In some embodiments, detecting the biological analyte may

comprise one or more sample preparation processes, such as pH adjustment, dilution, filtration, centrifugation, extraction, and the like.

[0119] In some embodiments, the biological analyte is detected at a single time point. In some embodiments, the biological analyte is detected at two or more time points. When the biological analyte is detected at two or more time points, the amount of biological analyte detected at a first time (e.g., before an effective amount of cell extractant is released from a hydrogel to effect the release of biological analytes from live cells in at least a portion of the sample) point can be compared to the amount of biological analyte detected at a second time point (e.g., after an effective amount of cell extractant is released from a hydrogel to effect the release of biological analytes from live cells in at least a portion of the sample). In some embodiments, the measurement of the biological analyte at one or more time points is performed by an instrument with a processor. In certain preferred embodiments, comparing the amount of biological analyte at a first time point with the amount of biological analyte at a second time point is performed by the processor.

[0120] For example, the operator measures the amount of biological analyte in the sample after the liquid mixture including the sample and the hydrogel comprising a cell extractant is formed. The amount of biological analyte in this first measurement (T_0) can indicate the presence of "free" (i.e. acellular) biological analyte and/or biological analyte from nonviable cells in the sample. In some embodiments, the first measurement can be made immediately (e.g., about 1 second) after the liquid mixture including the sample and the hydrogel comprising a cell extractant is formed. In some embodiments, the first measurement can be at least about 5 seconds, at least about 10 seconds, at least about 20 seconds, at least about 30 seconds, at least about 40 seconds, at least about 60 seconds, at least about 80 seconds, at least about 100 seconds, at least about 120 seconds, at least about 150 seconds, at least about 180 seconds, at least about 240 seconds, at least about 5 minutes, at least about 10 minutes, at least about 20 minutes after the liquid mixture including the sample and the hydrogel comprising a cell extractant is formed. These times are exemplary and include only the time up to that the detection of a biological analyte is initiated. Initiating the detection of a biological analyte may include diluting the sample and/or adding a reagent to inhibit the activity of the cell extractant. It will be recognized that certain detection systems (e.g., nucleic acid amplification or ELISA) can generally take several minutes to several hours to complete.

[0121] The operator allows the sample to contact the hydrogel comprising the cell extractant for a period of time after the first measurement of biological analyte has been made. After the sample has contacted the hydrogel for a period of time, a second measurement of the biological analyte is made. In some embodiments, the second measurement can be made up to about 0.5 seconds, up to about 1 second, up to about 5 seconds, up to about 10 seconds, up to about 20 seconds, up to about 30 seconds, up to about 40 seconds, up to about 60 seconds, up to about 90 seconds, up to about 120 seconds, up to about 180 seconds, about 300 seconds, at least about 10 minutes, at least about 20 minutes, at least about 60 minutes or longer after the first measurement of the biological analyte. These times are exemplary and include only the interval of time from which the first measurement for detecting the biological analyte is initiated and the time at which the second measurement for detecting the biological analyte is initiated. Initiating the detection of a biological analyte may include diluting the sample and/or adding a reagent to inhibit the activity of the cell extractant.

[0122] Preferably, the first measurement of a biological analyte is made about 1 seconds to about 240 seconds after the liquid mixture including the sample and the hydrogel comprising a cell extractant is formed and the second measurement, which is made after the first measurement, is made about 1.5 seconds to about 540 seconds after the liquid mixture is formed. More preferably, the first measurement of a biological analyte is made about 1 second to about 180 seconds after the liquid mixture is formed and the second measurement, which is made after the first measurement, is made about 1.5 seconds to about 120 seconds after the liquid mixture is formed. Most preferably, the first measurement of a biological analyte is made about 1 second to about 5 seconds after the liquid mixture is formed and the second measurement, which is made after the first measurement, is made about 1.5 seconds to about 10 seconds after the liquid mixture is formed.

[0123] The operator compares the amount of a biological analyte detected in the first measurement to the amount of biological analyte detected in the second measurement. An increase in the amount of biological analyte detected in the second measurement is indicative of the presence of one or more live cells in the sample.

[0124] In certain methods, it may be desirable to detect the presence of live somatic cells (e.g., nonmicrobial cells). In these embodiments, the hydrogel comprises a cell extractant that selectively releases biological analytes from somatic cells. Nonlimiting examples of somatic cell extractants include nonionic detergents, such as non-ionic ethoxylated alkylphenols, including but not limited to the ethoxylated octylphenol Triton X-100 (TX-100) and other ethoxylated alkylphenols; betaine detergents, such as carboxypropylbetaine (CB-18), NP-40, TWEEN, Tergitol, Igepal, commercially available M-NRS (Celsis, Chicago, Ill.), M-PER (Pierce, Rockford, Ill.), CelLytic M (Sigma Aldrich). Cell extractants are preferably chosen not to inactivate the analyte and its detection reagents.

[0125] In certain methods, it may be desirable to detect the presence of live microbial cells. In these embodiments, the hydrogel can comprise a cell extractant that selectively releases biological analytes from microbial cells. Nonlimiting examples of microbial cell extractants include quaternary ammonium compounds, including benzalkonium chloride, benzethonium chloride, 'cetrimide' (a mixture of dodecyl-, tetradecyl- and hexadecyl-trimethylammoium bromide), cetylpyridium chloride; amines, such as triethylamine (TEA) and triethanolamine (TeolA); bis-Biguanides, including chlorhexidine, alexidine and polyhexamethylene biguanide Dialkyl ammonium salts, including N-(n-dodecyl)-diethanolamine, antibiotics, such as polymyxin B (e.g., polymyxin B1 and polymyxin B2), polymyxin-beta-nonapeptide (PMBN); alkylglucoside or alkylthioglucoside, such as Octyl-β-D-1-thioglucopyranoside (see U.S. Pat. No. 6,174,704 herein incorporated by reference in its entirety); nonionic detergents, such as non-ionic ethoxylated alkylphenols, including but not limited to the ethoxylated octylphenol Triton X-100 (TX-100) and other ethoxylated alkylphenols; betaine detergents, such as carboxypropylbetaine (CB-18); and cationic, antibacterial, pore forming, membrane-active, and/or cell wall-active polymers, such as polylysine, nisin, magainin, melittin, phopholipase A_2, phospholipase A_2 activating pep-

tide (PLAP); bacteriophage; and the like. See e.g., Morbe et al., Microbiol. Res. (1997) vol. 152, pp. 385-394, and U.S. Pat. No. 4,303,752 disclosing ionic surface active compounds which are incorporated herein by reference in their entirety. Cell extractants are preferably chosen not to inactivate the biological analyte and/or a detection reagent used to detect the biological analyte.

[0126] In certain alternative methods to detect the presence of live microbial cells in a sample, the sample can be pretreated with a somatic cell extractant for a period of time (e.g., the sample is contacted with a somatic cell extractant for a sufficient period of time to extract somatic cells before a liquid mixture including the sample and a hydrogel comprising a microbial cell extractant is formed). In the alternative embodiment, the amount of biological analyte detected at the first measurement will include any biological analyte that was released by the somatic cells and the amount of additional biological analyte, if any, detected in the second measurement will include biological analyte from live microbial cells in the sample.

EXAMPLES

[0127] The present invention has now been described with reference to several specific embodiments foreseen by the inventor for which enabling descriptions are available. Insubstantial modifications of the invention, including modifications not presently foreseen, may nonetheless constitute equivalents thereto. Thus, the scope of the present invention should not be limited by the details and structures described herein, but rather solely by the following claims, and equivalents thereto.

Preparative Example 1

Incorporation of Cell Extractant into Hydrogel Beads During Polymerization of the Hydrogel

[0128] Beads were made as described in example 1 of International Patent Publication No. WO 2007/146722, in which the deionized water was replaced with the desired loading solution. A homogeneous precursor composition was prepared by mixing 40 grams of 20-mole ethoxylated trimethylolpropane triacrylate (EO_{20}-TMPTA) (SR415 from Sartomer, Exeter, Pa.), 60 grams deionized (DI) water, and 0.8 grams photoinitiator (IRGACURE 2959 from Ciba Specialty Chemicals, Tarrytown, N.Y.). The precursor composition was poured into a funnel such that the precursor composition exited the funnel through a 2.0 millimeter diameter orifice. Precursor composition fell along the vertical axis of a 0.91 meter long, 51 millimeter diameter quartz tube that extended through a UV exposure zone defined by a light shield and a 240 W/cm irradiator (available from Fusion UV Systems, Gaithersburg, Md.) equipped with a 25-cm long "H" bulb coupled to an integrated back reflector such that the bulb orientation was parallel to falling precursor composition. Below the irradiator, polymeric beads were obtained. The entire process was operated under ambient conditions

[0129] The BARDAC 205 M and 208M (blends of quaternary ammonium compounds and alkyl dimethyl benzyl ammonium chloride; Lonza Group Ltd., Valais, Switzerland) hydrogel beads were prepared by mixing 20 grams of EO_{20}-TMPTA, 30 grams of the BARDAC 205M or 208M solution and 0.4 grams of Irgacure 2959 and exposed to UV light to prepare beads as described in example 1 in International Patent Publication No. WO 2007/146722. The beads were prepared using 12.5% and 25% (w/v) solutions of BARDAC 205M and 208M in deionized water. After recovering the beads, they were stored in a jar at room temperature. The beads were designated as shown below:

25% 205M solution bead	205M-1s
12.5% 205M solution bead	205M-2s
25% 208M solution bead	208M-1s
12.5% 208M solution bead	208M-2s

Preparative Example 2

Incorporation of Cationic Monomers into Hydrogel Beads During Polymerization of the Hydrogel

[0130] Polymeric beads with cationic monomers were prepared as described in Example 30 to 34 of International patent WO2007/146722. The precursor composition used for making beads is indicated in Table 1. The various components of the precursor compositions were stirred together in an amber jar until the antimicrobial monomer dissolved.

[0131] DMAEMA-C_8Br was formed within three-neck round bottom reaction flask that was fitted with a mechanical stirrer, temperature probe and a condenser. The reaction flask was charged with 234 parts of dimethylaminoethylmethacryalte, 617 part of acetone, 500 parts 1-bromoethane, and 0.5 parts of BHT antioxidant. The mixture was stirred for 24 hours at 35° C. At this point, the reaction mixture was cooled to room temperature and a slightly yellow clear solution was obtained. The solution was transferred to a round bottom flask and acetone was removed by rotary evaporation under vacuum at 40° C. The resulting solids were washed with cold ethyl acetate and dried under vacuum at 40° C. DMAEMA-C_{10}Br and DMAEMA-C_{12}Br were formed using a similar procedure in which the 1-bromooctane was replaced by 1-bromodecane and 1-bromododecane, respectively.

[0132] The 3-(acryloamidopropyl)trimethylammonium chloride was obtained by Tokyo Kasei Kogyo Ltd (Japan). Ageflex FA-1080MC was obtained from Ciba Specialty Chemicals.

TABLE 1

Beads with antimicrobial Monomer					
Bead	Cationic monomer	Antimicrobial monomer	Propylene Glycol	SR415	Irgacure 2959
C_8-1s	DMAEMA-C_8Br	1.86 g	7.44 g	13.02 g	0.30 g
C_{10}-1s	DMAEMA-C_{10}Br	1.91 g	7.60 g	13.30 g	0.30 g
C_{12}-1s	DMAEMA-C_{12}Br	1.92 g	7.68 g	13.44 g	0.31 g

TABLE 1-continued

Beads with antimicrobial Monomer					
Bead	Cationic monomer	Antimicrobial monomer	Propylene Glycol	SR415	Irgacure 2959
ATAC-1s	3-(acryloamidopropyl) trimethylammonium chloride	2.34 g	9.38 g	17.50 g	0.40 g
Ageflex-1s	Ageflex FA-1Q80MC	2.50 g	10.00 g	17.50 g	0.40 g

Preparative Example 3

Incorporation of Luciferin into Hydrogel Beads During Polymerization of the Hydrogel

[0133] Hydrogel beads containing luciferin were made similarly by mixing 20 parts of EO_{20}-TMPTA with 30 parts of luciferin (2 mg in 30 ml of 14 mM of phosphate buffer, pH 6.4) and 0.4 parts photoinitiator (IRGACURE 2959) and exposed to UV light to prepare beads as described in example 1 in International Patent Publication No. WO 2007/146722 A1. The beads were then stored in a jar at 4° C. and designated as Luciferin-1s.

Preparative Example 4

Incorporation of Luciferase into Hydrogel Beads During Polymerization of the Hydrogel

[0134] Hydrogel beads containing luciferase were made by mixing 20 parts of polymer with 30 parts of luciferase (150 µl of 6.8 mg/ml in 30 ml of 14 mM of phosphate buffer, pH 6.4) and 0.4 parts photoinitiator (IRGACURE 2959) and exposed to UV light to prepare beads as described in example 1 in International Patent Publication No. WO 2007/146722 A1. The beads were then stored in a jar at 4° C. and designated as Luciferase-1s.

Preparative Example 5

Incorporation of Cell Extractant into Hydrogel Beads after Polymerization of the Hydrogel

[0135] Hydrogel beads were prepared as described in example 1 International Patent Publication No. WO 2007/146722. Active beads were prepared by drying as described in example 19 and then soaking in active solution as described in example 23 of International Patent Publication No. WO 2007/146722. One gram of beads was dried at 60° C. for 2 h to remove water from the beads. The dried beads were soaked in 2 grams of BARDAC 205M for at least 3 hrs to overnight at room temperature. After soaking, the beads were poured into a Buchner funnel to drain the beads and then rinsed with 10 to 20 ml of distilled water. The excess water was removed from the surface of the beads by blotting them with a paper towel. The beads were prepared using 10%, 12.5%, 20%, 25%, 50% and 100% (w/v) aqueous solutions of BARDAC 205M, 5%, 10%, 12.5%, 25% and 50% solutions of 208M, 20% solution of Triclosan (Ciba Specialty Chemicals,), 1% and 5% solutions of chlorohexidine digluconate (CHG; Sigma Aldrich, St. Louis, Mo.) and 0.25% and 0.5% solutions of Cetyltrimethylammoniumbromide (CTAB; Sigma Aldrich). The beads were then stored in a jar at room temperature. The beads were designated as shown below.

100% 205M solution bead	205M-1p
50% 205M solution bead	205M-2p
25% 205M solution bead	205M-3p
20% 205M solution bead	205M-4p
12.5% 205M solution bead	205M-5p
10% 205M solution bead	205M-6p
50% 208M solution bead	208M-1p
25% 208M solution bead	208M-2p
12.5% 208M solution bead	208M-3p
10% 208M solution bead	208M-4p
5% 208M solution bead	208M-5
20% Triclosan solution bead	Triclosan-1p
1% CHG solution bead	CHG-1p
5% CHG solution bead	CHG-2p
0.25% CTAB solution bead	CTAB-1p
0.5% CTAB solution bead	CTAB-2p

[0136] Hydrogel beads of VANTOCIL (Arch Chemicals, Norwalk, Conn.), CARBOSHIELD (Lonza) and a blend of Vantocil and CarboShield were prepared similarly. The dried hydrogel beads were soaked in 50% solution (in distilled water) of VANTOCIL or 100% solution of CARBOSHIELD 1000 or 1:1 mixture of 50% Vantocil and 100% Carboshield solutions. The beads with the mixture of VANTOCIL and CARBOSHIELD resulted in 25% Vantocil and 50% Carboshield beads. The beads were then stored in a jar at room temperature and designated as follows

50% Vantocil solution bead	Van-1p
100% Carboshield solution bead	Carbo-1p
25% Vantocil and 50% Carboshield solution bead	Van-Carbo-1p

Preparative Example 6

Incorporation of Cell Extractant into Hydrogel Fibers after Polymerization of the Hydrogel

[0137] Polymeric fibers were made as described in example 1 of US Patent Application Publication No. US2008/207794. A homogeneous precursor composition was prepared that contained about 500 grams of 40 wt-% 20-mole EO_{20}-TMPTA (SR415 from Sartomer) and 1 wt-% photoinitiator (IRGACURE 2959 from Ciba Specialty Chemicals) in deionized water. The precursor composition was processed as described in example 1 of US Patent Application Publication No. US2008/207794 to make the polymeric fibers.

[0138] One gram of fibers was dried at 60° C. for 2 h to remove water from the fibers. The dried fibers were soaked in 2 grams of 50% solution of BARDAC 205M for at least 3 hrs to overnight at room temperature. After soaking, the fibers

were poured into a Buchner funnel to drain the fibers and then rinsed with 10 to 20 ml of distilled water. The excess water was removed from the surface of the fibers by blotting them with a paper towel. The fibers were then stored in a jar at room temperature.

Preparative Example 7

Incorporation of Luciferin into Hydrogel Beads after Polymerization of the Hydrogel

[0139] Hydrogel beads (1× gram) were dried at 60° C. for 2 h and soaked in 2× grams of luciferin solution (2 mg in 30 ml of 14 mM of phosphate buffer, pH6.4) for at least 16 h at 4° C. After soaking, the beads were poured into a Buchner funnel to drain the beads and then rinsed with distilled water. The excess water was removed from the surface of the beads by blotting them with a paper towel. The beads were then stored in a jar at 4° C. and designated as Lucifein-1p

Preparative Example 8

Incorporation of Enzymes into Hydrogel Beads after Polymerization of the Hydrogel

[0140] Hydrogel beads (1× gram) were dried at 60 C for 2 h and soaked in 2× grams of luciferase solution (150 µl of 6.8 mg/ml luciferase in 30 ml of 14 mM of phosphate buffer, pH6.4) for at least 16 h at 4° C. After soaking, the beads were poured into a Buchner funnel to drain the beads and then rinsed with distilled water. The excess water was removed from the surface of the beads by blotting them with a paper towel. Hydrogel beads containing lysozyme or lysostaphin were prepared similarly by soaking in 2× grams of 50 mM TRIS pH 8.0 solution containing 0.5 mg/ml lysozyme or 50 µg/ml lysostaphin. The beads were then stored in a jar at 4° C. and designated as Luciferase-1p, Lysozyme-1p and Lysostaphin-1p.

Preparative Example 9

Size Selection of Hydrogel Beads after Polymerization of the Hydrogel and Incorporation of Cell Extractant into Hydrogel Beads

[0141] Hydrogel beads were prepared as described in example 1 International Patent Publication No. WO 2007/146722. The hydrogel beads were sieved through different fine series mesh sizes No. 10 (2.0 mm), No. 12 (1.7 mm), No. 14 (1.4 mm), No. 16 (1.18 mm) and No. 18 (1.0 mm) (8" Round Test Sieves, Glison Company, Lewis Center, Ohio) to obtain uniform size beads. The beads were sieved using a Model AS200 shaker (Retsch, Inc., Newtown, Pa.) set at 1.00 mm/"g" for a 15 second interval. Total shaking time for each batch was 10 minutes. Active beads from various size selected beads were prepared as described in Preparative Example 5. Some beads were prepared using 50% (w/v) aqueous solutions of BARDAC 205M. Other beads were prepared using 10%, 17.5%, or 25% (w/v) aqueous solutions of bezalkonium chloride (BAC; Alfa Aesar, Ward Hill, Mass.). The beads were then stored in an amber jar at room temperature. The beads were designated as shown below.

Disinfectant Solution	Bead Diameter	Designation
50% 205M solution bead	(1.7 to 2.0 mm)	205M-7p
50% 205M solution bead	(1.4 to 1.7 mm)	205M-8p
50% 205M solution bead	(1.18 to 1.4 mm)	205M-9p
50% 205M solution bead	(1.0 to 1.18 mm)	205M-10p
10% BAC solution bead	(1.4 to 1.7 mm)	BAC-1p
10% BAC solution bead	(1.18 to 1.4 mm)	BAC-2p
17.5% BAC solution bead	(1.18 to 1.4 mm)	BAC-3p
25% BAC solution bead__	(1.18 to 1.4 mm)	BAC-4p

Example 1

Effect of BARDAC 205M Disinfectant-Loaded Hydrogel Beads on the Release of ATP from *S. aureus* and *E. coli* Cells

[0142] The microbial species used in the examples (Table 2) were obtained from ATCC (Manassas, Va.). 3M™ Clean-Trace™ Surface ATP system and NG Luminometer UNG2 were obtained from 3M Company (St. Paul, Minn.). Rayon-tipped applicators were obtained from Puritan Medical Products (Guilford, Me.). Beads containing BARDAC 205M were made according to Preparative Example 5.

TABLE 2

Microorganisms used in examples

Microorganism	ATCC No.
Candida albicans	MYA-2876
Candida albicans	10231
Corynebacterium xerosis	373
Enterococcus faecalis	49332
Enterococcus faecalis	700802
Enterococcus faecium	6569
Enterococcus faecium	700221
Escherichia coli	51183
Kocuria kristinae	BAA-752
Micrococcus luteus	540
Pseudomonas aeruginosa	9027
Salmonella enterica subsp. *enterica*	4931
Staphylococcus aureus	6538
Staphylococcus epidermidis	14990
Streptococcus pneumoniae	6301

[0143] Pure cultures of the bacterial strains were inoculated into tryptic soy broth and were grown overnight at 37° C. Swabs from some of the Clean-Trace surface ATP hygiene tests, which include microbial cell extractants, were replaced with sterile rayon-tipped applicators, which do not include microbial cell extractants. Various amounts (approximately 10^6, 10^7 and 10^8, colony-forming units (CFU) per milliliter, respectively) of bacteria were suspended in Butterfield's buffer and cell suspensions were added directly to the Clean-Trace surface ATP swabs (10 microliters) or the rayon-tipped applicators (100 microliters). Each swab or applicator was activated by pushing it into the reagent chamber according to the manufacturer's instructions. The test unit was immediately inserted into the reading chamber of a NG Luminometer, UNG2 and an initial (T_0) measurement of Relative Light Units (RLUs) was recorded. One BARDAC 205M-containing hydrogel bead, 205M-1p, was added to some of the test units and subsequent RLU measurements were recorded at 20 sec interval using the "Unplanned Testing" mode of the luminometer until the number of RLUs reached a plateau. The data

were downloaded using the software provided with the NG luminometer. 205M-1p beads were able to lyse bacteria and release ATP from cells, as shown by the data in Table 3. The relative light units (RLU) increased over time with BARDAC 205M beads, while without beads the background did not increase. Experiments using the Clean-Trace surface ATP swabs showed that the RLU reached maximum within 20 seconds and then began to decrease.

[0146] The hydrogel beads, containing individual disinfectants or a disinfectant mixture, extracted ATP from the *S. aureus* cells and the ATP reacted with the ATP-detection reagents of the Clean-Trace surface ATP units, as shown in Table 4. The relative light units (RLU) increased over time in the tubes that received the disinfectant-loaded beads, while the tubes without beads did not show a significant increase in RLU over time.

TABLE 3

Detection of ATP from microbial cells exposed to microbial cell extractants released from hydrogels.

	S. aureus						*E. coli*					
	10^5 CFU			10^6 CFU			10^5 CFU			10^6 CFU		
Time (sec)	RA 0 bead	RA 1 bead	CT 0 bead	RA 0 bead	RA 1 bead	CT 0 bead	RA 0 bead	RA 1 bead	CT 0 bead	RA 0 bead	RA 1 bead	CT 0 bead
0	64	226	1175	1183	1647	8140	28	308	1235	228	338	7557
20	71	236	1183	1161	1709	8215	29	310	1243	230	345	7684
40	84	288	1185	1175	2042	8262	30	317	1250	243	656	7764
80	92	301	1166	1179	2158	8053	31	326	1251	245	763	7772
120	NR	334	NR	NR	2237	NR	30	343	1249	244	973	7781
160	NR	463	NR	NR	2955	NR	28	353	NR	246	1463	7504
200	NR	643	NR	NR	5612	NR	31	428	NR	243	2036	NR
240	NR	776	NR	NR	6807	NR	NR	531	NR	NR	2570	NR
280	NR	852	NR	NR	6919	NR	NR	629	NR	NR	3614	NR
320	NR	899	NR	NR	7050	NR	NR	639	NR	NR	4687	NR
360	NR	963	NR	NR	7303	NR	NR	633	NR	NR	5078	NR
400	NR	996	NR	NR	7345	NR	NR	NR	NR	NR	5288	NR

Values expressed in the table are relative light units (RLUs).
RA = rayon-tipped applicator,
CT = Clean-Trace surface ATP swab,
NR = not recorded.
BARDAC 205M beads, 205M-1p if present, were added to the sample immediately after the T_0 measurement was obtained.

Example 2

Effect of VANTOCIL and CARBOSHIELD Disinfectant-Loaded Hydrogel Beads on the Release of ATP From *S. aureus*

[0144] A *S. aureus* overnight culture was prepared as described in Example 1. Hydrogel beads containing VANTOCIL and/or CARBOSHIELD were prepared as described in Preparative Example 5. The luciferase/luciferin liquid reagent solution (300 μl) was removed from Clean-Trace surface ATP hygiene test units and transferred to 1.5 ml microfuge tubes. The bacterial culture was diluted to 10^7 CFU/ml in Butterfield's buffer and 10 microliters of the diluted suspension were added directly to individual microfuge tubes (i.e., approximately 10^5 CFU per tube). Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (FB-12 single tube luminometer, Berthold Detection Systems USA, Oak Ridge, Tenn.) and an initial (T_0) measurement of RLUs was recorded. The initial (and all subsequent luminescence measurements) were obtained from the luminometer using FB 12 Sirius PC software that was provided with the luminometer. The light signal was integrated for 1 second and the results are expressed in RLU/sec.

[0145] A hydrogel bead containing VANTOCIL (Van-1p), CARBOSHIELD (Carbo-1p), or both VANTOCIL and CARBOSHIELD (Van-Carbo-1p) was added to individual tubes and RLU measurements were recorded at 10 sec intervals until the number of RLUs reached a plateau (Table 3).

TABLE 4

Detection of ATP released from *S. aureus* cells after exposure to VANTOCIL- and/or CARBOSHIELD-loaded hydrogel beads.

Time (sec)	No Bead	VANTOCIL Bead (Van-1p)	CARBOSHIELD Bead (Carbo-1p)	VANTOCIL + CARBOSHIELD Bead (Van-Carbo-1p)
0	840	994	1354	5150
50	910	2809	2745	5202
100	940	5529	6868	6228
200	950	9246	12292	9243
300	920	13413	15110	14341
400	910	19723	17107	19337
600	780	35195	22725	29997
800	NR	50421	28719	38939
1000	NR	59389	32822	46965
1200	NR	59872	33252	51271
1600	NR	56717	33401	60293
1800	NR	52527	31483	63154

NR = not recorded. Beads containing extractants, if present, were added to the sample immediately after the T_0 measurement was obtained.

Example 3

Effect of the Number of Disinfectant-Loaded Beads on the Release of ATP from *S. aureus* and *E. coli* Cells

[0147] *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. 3M Clean-Trace surface

ATP system swabs were replaced with sterile rayon-tipped applicators, as described in Example 1. The bacterial suspensions were diluted to approximately 10^7 CFU/ml in Butterfield's buffer. One hundred-microliter aliquots of the suspension were added directly to the swabs. BARDAC 205M hydrogel beads were prepared as described in Preparative Example 5. Up to three hydrogel beads (i.e., 0 bead, 1 bead, or 3 beads) were added to individual test units and each applicator was inserted into a Clean-Trace surface ATP test unit to activate ATP detection according to the manufacturer's instructions. The test unit was immediately inserted into the reading chamber of a NG Luminometer, UNG2 and RLU measurements were recorded at 20 sec intervals using the "Unplanned Testing" mode of the luminometer until the number of RLUs reached a plateau. The results are shown in Table 5. The data indicate that the BARDAC 205M beads, 205M-1p, permeabilized the bacteria, causing release of ATP from cells. The relative light units (RLU) increased over time in the samples containing the BARDAC beads, with a larger increase observed in a short period of time with higher number of beads. In contrast, the samples without the beads did not show a similar increase in RLU.

TABLE 5

Detection of ATP from microbial cells exposed to various amounts of BARDAC 205M hydrogel beads.

	S. aureus			*E. coli*		
Time (sec)	0 bead	1 bead	3 beads	0 bead	1 bead	3 beads
10	1066	1647	3143	837	338	1651
20	1051	1709	4574	892	345	2031
40	1058	2042	5885	940	656	2524
80	1055	2158	6836	962	763	2956
120	1063	2237	7509	965	973	3368
160	1047	2955	8230	1020	1463	4263
200	1048	5612	8610	1052	2036	5048
240	1051	6807	8851	1067	2570	5695

TABLE 5-continued

Detection of ATP from microbial cells exposed to various amounts of BARDAC 205M hydrogel beads.

	S. aureus			*E. coli*		
Time (sec)	0 bead	1 bead	3 beads	0 bead	1 bead	3 beads
280	1043	6919	8993	1090	3614	6232
320	1039	7050	9117	1091	4687	6682
360	1033	7303	9164	1127	5078	6975
400	1025	7345	9171	1127	5288	7266

BARDAC 205M beads, 205M-1p, if present, were added to the sample immediately before the first measurement was obtained.

Example 4

Detection of ATP from Microbial Cells Exposed to Various Amounts of a Microbial Cell Extractant

[0148] *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. Immediately before use in these tests, the bacterial suspensions were diluted in Butterfield's buffer to concentrations of approximately 10^6 and 10^7 CFU per milliliter. Luciferase/luciferin reagent (300 μl) from Clean-Trace surface ATP system was removed and added to 1.5 ml microfuge tubes. Ten-microliter amounts of the bacterial suspensions were added directly to individual microfuge tubes containing the reagents. BARDAC 205M hydrogel beads were prepared as described in Preparative Example 5. Up to three hydrogel beads (i.e., 0 beads, 1 bead, 2 beads or 3 beads) were added to each tube. Relative Light Units (RLUs) were recorded at 10 sec interval in a bench top luminometer (FB-12 single tube luminometer with software), as described in Example 2. The results of the experiments are shown in Table 6. The results indicate that the BARDAC 205M beads, 205M-1p, were able to lyse bacteria and release ATP from cells. The relative light units (RLU) increased over time in tubes containing at least one BARDAC 205M bead, with a larger increase observed in a short period of time with higher number of beads. Tubes containing no beads did not show a significant increase in RLU's.

TABLE 6

Detection of ATP from microbial cells exposed to various amounts of BARDAC 205M hydrogel beads.

	S. aureus					*E. coli*								
	10^5 CFU					10^5 CFU					10^6 CFU			
Time (sec)	RA 0 bead	RA 1 bead	RA 2 beads	RA 3 beads	CT 0 bead	RA 0 bead	RA 1 bead	RA 2 beads	RA 3 beads	CT 0 bead	RA 0 bead	RA 1 beads	RA 2 beads	RA 3 beads
10	470	1770	2147	1888	21489	1371	3208	5537	8996	41489	1820	6646	12765	18981
20	500	2500	2528	4185	35610	1486	3330	11498	38219	45610	1865	9682	24253	136641
40	55	3315	4894	26452	50678	1495	5716	46091	60362	53111	1920	12470	69865	172179
80	571	5771	17148	41192	55568	1502	46047	53283	59372	55412	1980	30875	146756	179238
120	608	19088	32480	51329	48785	1500	51490	52499	59344	49655	1940	85141	187122	170591
160	596	39596	42698	55421	NR	1495	53915	51643	55508	NR	1895	150016	186277	148221
200	NR	44421	50054	56714	NR	NR	50884	50461	51048	NR	NR	165112	185182	136720
240	NR	49942	56378	55674	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
280	NR	50510	51713	54544	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Values expressed in the table are relative light units (RLUs).

RA = rayon-tipped applicator,

CT = Clean-Trace surface ATP swab,

NR = not recorded.

BARDAC 205M beads, 205M-1p, if present, were added to the sample immediately before the first measurement was obtained.

Example 5

Detection of ATP from Suspensions of Live and Dead Microbial Cells Exposed to Hydrogel Beads Containing BARDAC 205M Antimicrobial

[0149] *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One milliliter of the overnight culture in tryptic soy broth (approximately 10^9 CFU/ml) was boiled for 10 min to lyse the cells. Both the live and the dead cell suspensions were diluted to approximately 10^7 and 10^8 CFU/mL in Butterfield's buffer. 3M Clean-Trace surface ATP system swabs were replaced with sterile rayon-tipped applicators, as described in Example 1. Ten microliter amounts of live, dead, or mixtures of both live and dead bacterial suspensions were added directly to the rayon applicators or Clean-Trace surface ATP swabs. A BARDAC 205M hydrogel bead, 205M-1p, was added to the test units and each applicator or swab was inserted into a Clean-Trace surface ATP test unit to activate ATP detection according to the manufacturer's instructions. The test unit was inserted into a NG Luminometer, UNG2 instrument and RLU measurements were recorded at 15 sec intervals using the "Unplanned Testing" mode of the luminometer until the number of RLUs reached a plateau. The results are shown in Table 7. The RLU observed in samples containing dead cells reached maximum within about 30 sec and the addition of BARDAC beads did not result in a significant change in measurable RLUs. In samples containing both live and dead cells, the addition of BARDAC beads caused the RLU to increase relatively slowly over a period of several minutes, indicating that the beads caused the release of ATP from live cells. In contrast, tubes containing the Clean-Trace surface ATP swabs (which contain a cell extractant), showed an initial increase in RLU until a maximum was reached within about 30 seconds to 1 min.

TABLE 7

Detection of ATP from live and dead microbial cells exposed to BARDAC 205M hydrogel beads.

	S. aureus					*E. coli*				
Time (sec)	Dead RA	Mixture RA	Live RA	Dead CT	Mixture CT	Dead RA	Mixture RA	Live RA	Dead CT	Mixture CT
15	3255	3330	1570	3657	17763	6817	8035	2070	6983	11136
30	3267	3460	2216	3691	20681	6787	8200	2112	7112	11278
45	3294	4636	2771	3708	22099	6756	8351	2255	7221	11323
60	3285	5143	3369	3738	22834	6749	8794	2322	7280	11352
90	3291	6369	4138	3792	22678	6780	10422	2373	7479	11319
120	3298	9254	4531	3853	22603	6761	12584	2412	7584	11310
150	3252	10760	5360	3898	22472	6756	13755	2420	7726	11344
180	3229	11535	9135	3922	22180	6827	14407	2423	7833	11219
210	3197	12577	9484	3967	22035	6862	14599	2475	7928	11153
240	3205	12801	9564	3988	21565	6851	14712	2472	8020	11098

Values expressed in the table are relative light units (RLUs).
RA = rayon-tipped applicator,
CT = Clean-Trace surface ATP swab.
BARDAC 205M beads (205-1p), if present, were added to the sample immediately before the first measurement was obtained.

Example 6

Detection of ATP from Suspensions of Microbial Cells Exposed to Hydrogel Beads Containing BARDAC 205M Antimicrobial in the Presence of Added Pure ATP

[0150] *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. Immediately before use in these tests, the bacterial suspensions were diluted in Butterfield's buffer to concentrations of approximately 10^8 CFU per milliliter. Luciferase/luciferin reagent (300 μl) from Clean-Trace surface ATP system was removed and added to 1.5 ml microfuge tubes. 100 nM solution of ATP (Sigma-Aldrich) was prepared in sterile water. Ten-microliter of ATP solution was added to individual microfuge tubes containing the reagents. Ten-microliter of the bacterial suspensions was added to some tubes containing reagents and ATP. BARDAC 205M hydrogel beads were prepared as described in Preparative Example 5 and one bead, 205M-1p, was added to some tubes. Relative Light Units (RLUs) were recorded at 10 sec interval in a bench top luminometer (FB-12 single tube luminometer with software), as described in Example 2. The results of the experiments are shown in Table 8. The results indicate that the addition of bacteria to pure ATP containing solution gave increased signal in the presence of BARDAC 205M beads. The extractants from beads were able to release ATP from cells leading to increased ATP levels which contribute to increased signal over the pure ATP background. Tubes containing no beads and bacteria did not show a significant increase in RLU's over that of pure ATP alone.

TABLE 8

Detection of ATP from microbial cells exposed to BARDAC 205M hydrogel beads in the presence of added pure ATP.

	ATP 1 picomole No Bead		ATP 1 picomole 1 Bead	
Time (sec)	ATP alone	ATP + 10^6 CFU *S. aureus*	ATP alone	ATP + 10^6 CFU *S. aureus*
10	26985	28131	26890	31657
20	27223	28572	26823	32850
30	27423	28610	26931	124994
40	27325	28425	26980	184209
50	27030	28025	26640	243044
60	26995	27986	26525	340044
70	NR	NR	NR	466805

TABLE 8-continued

Detection of ATP from microbial cells exposed to BARDAC 205M hydrogel beads in the presence of added pure ATP.

Time (sec)	ATP 1 picomole No Bead: ATP alone	ATP 1 picomole No Bead: ATP + 10^6 CFU *S. aureus*	ATP 1 picomole 1 Bead: ATP alone	ATP 1 picomole 1 Bead: ATP + 10^6 CFU *S. aureus*
80	NR	NR	NR	561999
90	NR	NR	NR	600158
100	NR	NR	NR	631060

Values expressed in the table are relative light units (RLUs). NR = not recorded. BARDAC 205M beads, 205M-1p, if present, were added to the sample immediately before the first measurement was obtained.

Example 7

Detection of Live Microbial ATP in Milk

[0151] *S. aureus* overnight cultures were prepared as described in Example 1. BARDAC 205M beads were prepared as described in Preparative Example 5. Fresh, unpasteurized milk was obtained from a farm in River Falls, Wis. The milk was diluted with Butterfield's buffer (100-fold and 1000-fold). One hundred microliters of the diluted milk was mixed with 100 μl of luciferase/luciferin reagent from the Clean-Trace surface ATP system in a 1.5 ml tube and initial (T_0) luminescence measurements were recorded in a bench top luminometer (FB-12 single tube luminometer with software) as described in Example 2. After several measurements, one BARDAC 205M bead, 205M-1p, was added to milk and subsequent luminescence measurements were recorded at 10-second intervals. To other samples, *S. aureus* (approximately 10^5 cells in 10 μL Butterfield's buffer) was added and, after taking the initial luminescence measurements, one 205M-1p bead was added to the sample. Subsequent luminescence measurements were recorded at 10-second intervals. The results are shown in Table 9. The data indicate that BARDAC beads were able to lyse bacteria spiked into milk and release ATP from cells, resulting in higher luminescence readings. The samples without added bacteria did not show a similar increase in luminescence after the BARDAC beads were added.

TABLE 9

Detection of *S. aureus* in milk samples.

Time (sec)	1:100 (no bacteria)	1:100 (with bacteria)	1:100 (no bacteria)	1:100 (with bacteria)
0	19247	20015	7770	8600
10	19338	21230	7760	8810
20	19950	21460	7590	8330
30	19530	21000	7580	8200
40	18850	21140	7590	8810
50	19570	25390	7570	10800
60	21420	32190	8430	16420
70	21230	38250	8700	24090
80	21520	41876	8630	25180
90	21190	42910	8380	26310
100	21530	43830	8320	26580
110	21340	43840	8290	26880

BARDAC 205M bead, 205M-1p was added to the tubes immediately after the T_{40} measurement was obtained. All measurements are reported in relative light units (RLU's).

Example 8

Distinguishing Microbial ATP from Somatic ATP

[0152] CRFK feline kidney cells (CCL-94, ATCC) were grown Dulbecco's Modified Eagle's Medium (DMEM) with 8% serum under CO_2 atmosphere at 37° C. to achieve 70% confluency. The medium was removed from the bottles and the cell monolayers were washed and were trypsinized (0.25% trypsin) for about 5 min. The detached cells were diluted with fresh medium and centrifuged at 3K for 5 min. The cells were further washed twice and resuspended in phosphate-buffered saline (PBS). The cells were diluted with PBS to get the desired cell concentration. One hundred microliters of cells were mixed with 100 μl of luciferase/luciferin reagent from Clean-Trace surface ATP system in a 1.5 ml tube. In one experiment, the tube was placed into a bench-top luminometer (FB-12 single tube luminometer with software), as described in Example 2, and initial luminescence measurements were recorded. After several initial measurements, one BARDAC 205M bead, 205M-1p, was added to the cell suspension and the luminescence was monitored at 10 sec intervals. In another experiment, *S. aureus* (approximately 10^5 or 10^6 cells in 10 μL of Butterfield's buffer) was added to the tube before the luminescence measurements were started. The results are shown in Table 10. The data indicate that BARDAC beads were able to cause the release of ATP from both mammalian cells and bacterial cells, resulting in an increased luminescence after the beads were added. In another experiment, the luminescence was monitored in a sample containing CRFK cells and a BARDAC bead. After 3 minutes, *S. aureus* cells were added to the same sample and luminescence was monitored for additional two minutes. The results, shown in Table 10, indicate that the amount of luminescence increased upon addition of *S. aureus* cells.

TABLE 10

Detection of ATP from somatic and microbial cells exposed to BARDAC 205M hydrogel bead.

Time (sec)	Experiment 1 CRFK (10^4)	2 CRFK (10^5)	3 *S. aureus* (10^5)	4 *S. aureus* (10^6)	5 CRFK (10^4) + *S. aureus* (10^5)	6 CRFK (10^4) + *S. aureus* (10^5)	7 CRFK (10^5) + *S. aureus* (10^6)
0	31180	597030	1080	6030	33000	37769	583640
20	31870	593150	990	5990	33710	35757	585310
40	30100	585960	1090	6026	32790	33610	586920
60	31390	675559	3810	14413	32243	33130	868317
80	49970	678860	8450	23190	55110	49860	900480
100	49100	683520	10410	33890	80139	49150	918520
120	46380	697660	15889	45110	88900	47210	913270
140	45792	706010	32510	61800	100000	46025	903490
160	45691	714020	32950	80450	98450	45435	900860
180	NR	NR	NR	NR	NR	91048	NR
200	NR	NR	NR	NR	NR	101580	NR
220	NR	NR	NR	NR	NR	103230	NR
240	NR	NR	NR	NR	NR	99530	NR
260	NR	NR	NR	NR	NR	97403	NR
280	NR	NR	NR	NR	NR	97293	NR
300	NR	NR	NR	NR	NR	95340	NR

BARDAC 205M hydrogel bead, 205M-1p, was added to the tubes immediately after the T_{40} measurement was obtained.
Values expressed in the table are relative light units (RLUs).
In Experiment 6, the *S. aureus* cells were added immediately after T = 160 measurement.
NR = not recorded.

Example 9

Detection of ATP from Live Microbial Cells in Food Extracts

[0153] Various food extracts (Spinach, Banana, and ground turkey) were prepared by adding 10 g to 100 ml of PBS in a stomacher bag and stomaching the food samples in a stomacher. 100 μl of spinach and banana extract and 100 μl diluted turkey extract (10-fold and 100-fold) were mixed with 100 μl of luciferase/luciferin reagent from Clean-Trace surface ATP system in a 1.5 ml microfuge tube and background readings were taken in a bench top luminometer (20/20n single tube luminometer, Turner Biosystems, Sunnyvale, Calif.). The initial (and all subsequent luminescence measurements) were obtained from the luminometer using 20/20n SIS software that was provided with the luminometer. The light signal was integrated for 1 second and the results are expressed in RLU/sec. After several readings, one BARDAC 205M bead, 205M-1p was added to the food extract and ATP release was monitored at 10 sec interval. The background levels were very high with banana and turkey extract and the levels increased upon addition of BARDAC bead. After 2 minutes, *S. aureus* cells (10^5) were added to the same samples containing food extract and BARDAC bead and ATP release was monitored for additional four minutes. The ATP level increased upon addition of *S. aureus* cells (Table 11).

TABLE 11

Detection of ATP in food extracts.

Time (sec)	Spinach Extract	Banana Extract	Turkey Extract (1:100)	Turkey Extract (1:10)
0	1063	150260	132670	997953
30	1081	130724	158942	1168784
60	1079	117705	172726	1284126
90	1093	105374	176684	1320036
120	1288	114530	155486	1599607
150	1316	121609	156589	1656526
180	1325	128329	157589	1746661
210	1391	140298	159553	1798493
240	10925	173838	177211	1930924
270	14730	176112	200387	2010237
300	18046	178565	212250	2088844
330	19607	182871	222775	2135284
360	20349	186227	229216	2178602
390	20549	190752	233637	2216695
420	20603	193788	238308	2265087
450	20600	197347	241146	2297345

BARDAC 205M bead, 205M-1p, was added to the tubes immediately after the T_{100} measurement was obtained. *S. aureus* cells were added to the tubes immediately after the T_{220} measurement was obtained. All measurements are reported in relative light units (RLU's).

Example 10

Detection of ATP from Microbial Cells in Water

[0154] Overnight cultures of *S. aureus* were prepared as described in Example 1. Cooling tower water samples were obtained from two local cooling towers. One hundred microliters of water from each cooling tower was mixed with 100 μL of luciferin/luciferase reagent from Clean-Trace surface ATP system in individual 1.5 ml microfuge tubes. Luminescence was measured in a bench top luminometer (20/20n single tube luminometer with software) as described in Example 9, at 10-second intervals. After several measurements, one BARDAC 205M bead, 205M-1p, was added to the water sample and additional luminescence measurements were recorded to determine whether ATP was released from indigenous cells in the water samples. To other samples of cooling water from the same water towers, approximately 10^5 CFU of *S. aureus* (suspended in 10 microliters of Butterfield's buffer) were added into individual 1.5 ml tubes containing the luciferin/luciferase reagent. The luminescence was measured in a bench top luminometer (20/20n single tube luminometer). After taking background (T_0) readings, one BARDAC 205M bead, 205M-1p, was added to the sample and luminescence was recorded at 10 second intervals. The results are shown in Table 12. The data indicate that the BARDAC beads were able to lyse bacteria spiked into water and release ATP from cells, causing an increase in luminescence over time.

TABLE 12

Detection of *S. aureus* in coolin tower water.

Time (sec)	Cooling Tower 1	Cooling Tower 1 + *S. aureus*	Cooling Tower 2	Cooling Tower 2 + *S. aureus*
0	2652	3351	430	1211
10	2724	3387	427	1204
20	2768	3486	442	1202
30	2767	3525	440	1221
40	2922	3901	434	1270
50	2940	4371	621	2164
60	2997	5400	648	3151
70	3044	6586	666	4794
80	3110	7391	694	7809
90	3175	8014	725	10195
100	3214	8589	740	11972
110	3321	9228	772	13247

BARDAC 205M bead, 205M-1p, was added to the tubes containing cooling tower water samples immediately after recording to measurement. One 205M-1p bead was added to each tube containing cooling tower water spiked with *S. aureus* immediately after recording 40-second luminescence measurement. All measurements are reported in relative light units (RLU's).

Example 11

Detection of ATP from Suspensions of Live Microbial Cells Exposed to Aqueous Extractants and Hydrogel Beads Containing Extractants

[0155] BARDAC 205M and 208M beads were produced as described in Preparative Example 5.1 g of BARDAC 205M beads, 205M-1p, were added to 100 ml of distilled water and the water-soluble antimicrobial components were allowed to diffuse out of the beads and into the bulk solvent for 45 min. The beads were removed and the antimicrobial solution ("bead extract") was saved. The amount of quaternary ammonium chloride (QAC) released was estimated using LaMotte QAC Test Kit Model QT-DR (LaMotte Company, Chester town, Md.). The amount of QAC released at the end of 45 min was 240 ppm.

[0156] A lysis solution (0.07% w/v Chlorhexidine digluconate (CHG, Sigma Aldrich) and 0.16% w/v Triton-X 100, Sigma Aldrich) was prepared in distilled water. A *S. aureus* overnight culture was prepared as described in Example 1 and the cells were diluted in Butterfield's buffer. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to 1.5 ml microfuge tubes containing approximately 10^5 cells. The lysis solution (25 or 50 μl) or bead extract (25 or 50 μl) was added to one of the microfuge tubes and the resulting luminescence was monitored in a bench top luminometer (20/20n single tube luminometer with software) as described in Example 9. To another set of samples one BARDAC 205M or 208M bead was added

and the luminescence was monitored similarly. The results are shown in Table 13. The data indicate that the luminescence generated by the release of ATP from the bacteria was very gradual in samples that received the BARDAC beads. In contrast, samples that received either the lysis solution or the bead extract showed a rapid increase in luminescence, corresponding to a rapid release of ATP from the bacteria.

TABLE 13

Detection of ATP from cells exposed to a cell extractant contained in a hydrogel or in an aqueous solution.

Time (sec)	CHG Lysis Soln. (25 μL)	CHG Lysis Soln. (50 μL)	205M-1p bead	208M-1p bead	205M-1p Bead Extract (25 μL)	205M-1p Bead Extract (50 μL)
0	1650	2243	853	881	918	932
10	15445	18232	1579	1930	5502	15288
20	16067	18771	2206	3453	10133	22579
30	16222	19156	3119	4881	17951	25554
40	16449	19314	4034	6583	26698	25795
50	16578	19501	4821	8215	28928	25964
60	16810	19629	5550	9814	29397	25895
80	16940	19839	7538	12910	30943	26203
100	17162	19903	8738	14074	32032	26125
120	17251	20050	9690	15049	32854	26204
140	17413	20180	10363	16259	33441	26137
160	17375	20233	10919	16737	33647	26042
180	17330	20076	11190	17096	33663	26041

All measurements are reported in relative light units (RLU's).

Example 12

Detection of ATP from Suspensions of Microbial Cells Exposed to Hydrogel Beads Containing Various Amounts of Extractants

[0157] Hydrogel beads with various amounts of BARDAC 205M or 208M were prepared as described in Preparative Example 5. *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to individual 1.5 ml microfuge tubes containing approximately 10^5 CFU of one of the respective bacterial cultures. One bead or Clean-Trace surface ATP swab was added to each tube. Luminescence, resulting from the release of ATP from the cells, was recorded at 10 sec intervals in a bench top luminometer (20/20n single tube luminometer with software) as described in Example 9. The results are shown in Tables 14 and 15. The data indicate that ATP release was very gradual in the samples containing the beads. In contrast, samples containing the swabs (which contain a cell extractant solution) showed a very rapid release of ATP from the cells.

TABLE 14

Detection of *S. aureus* using hydrogel beads containing BARDAC 205M or BARDAC 208M antimicrobial mixtures.

Time (sec)	205M-1p bead	205M-2p bead	208M-1p bead	208M-2p bead	CT Swab
0	377	537	484	427	489
10	1126	1816	2055	951	17746
20	1215	2624	6585	1116	20330
30	1299	4738	15094	1474	21886
40	1492	8709	21706	2035	23172

TABLE 14-continued

Detection of *S. aureus* using hydrogel beads containing BARDAC 205M or BARDAC 208M antimicrobial mixtures.

Time (sec)	205M-1p bead	205M-2p bead	208M-1p bead	208M-2p bead	CT Swab
50	1870	13511	26156	2845	23444
60	2339	18283	29355	4013	23483
80	3622	29767	32316	10224	23580
100	4933	33298	32894	14878	23544
120	6434	34126	31614	19264	23389
140	8439	33810	30164	23320	23407
160	10420	31938	28664	27478	23282
180	13013	30078	27085	29058	23197

Hydrogel beads containing BARDAC mixtures were added to the tubes immediately after the T_0 measurement was recorded. All measurements are reported in relative light units (RLU's).

TABLE 15

Detection of *E. coli* using hydrogel beads containing BARDAC 205M or BARDAC 208M antimicrobial mixtures.

Time (sec)	205M-1p bead	205M-2p bead	208M-1p bead	208M-2p bead	CT Swab
0	484	508	635	685	886
10	699	1427	2507	1464	40823
20	717	1656	3038	1615	42986
30	728	1996	3888	1975	43125
40	770	2982	5681	2525	43274
50	936	5250	9546	3614	43275
60	1020	8762	15512	5606	43084
80	1321	17693	28302	14018	42869
100	1678	24646	39101	20923	42779
120	2185	27352	40693	27997	42677
140	2757	28165	40612	34621	42512
160	3436	28131	39926	36797	42360
180	4193	28010	38988	37846	42215

Hydrogel beads containing BARDAC mixtures were added to the tubes immediately after the T_0 measurement was recorded. All measurements are reported in relative light units (RLU's).

Example 13

Release of ATP from *S. aureus* Exposed to Various Antimicrobial-Loaded Hydrogel Beads

[0158] Hydrogel beads with various amounts of BARDAC 205M or 208M were prepared as described in Preparative Example 1. A *S. aureus* overnight culture was prepared as described in Example 1. Microfuge tubes (1.5 mL) were prepared by adding 100 microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system. 100 microliters of the diluted suspension were added directly to individual microfuge tubes (i.e., approximately 10^5 CFU per tube). Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. A hydrogel bead containing extractants was added to individual tubes and RLU measurements were recorded at 10 sec intervals until the number of RLUs reached a plateau or began to decrease (Table 16). The data indicate that all four of the bead formulations caused the release of ATP from the microbial cells.

TABLE 16

Release of ATP from *S. aureus* after exposure of the bacteria to antimicrobial-loaded hydrogels.

Time (sec)	205M-2s bead	208M-2s bead	205M-1s bead	208M-1s bead
0	1099	990	2053	1198
10	2025	2073	3573	1228
20	9442	3074	5921	1313
30	16070	4063	8757	1517
40	22844	5136	12056	1761
50	27610	6186	14748	2090
60	29653	7222	16417	2481
70	29906	8484	17095	2802
80	29453	9420	16979	3224
90	28449	10259	16524	3618
100	27396	11176	15723	3987
110	26152	11765	15062	4355
120	25039	12601	14586	4699

All data are reported in relative light units (RLU's). BARDAC hydrogel beads were added to the sample immediately after the T_0 measurement was obtained.

Example 14

Release of ATP from Various Microbial Cells Exposed to Antimicrobial-Loaded Hydrogel Beads

[0159] Hydrogel beads with various amounts of BARDAC 205M or 208M were prepared as described in Preparative Example 5. Cultures of *S. aureus, P. aeruginosa* and *S. epidermidis* were prepared as described in Example 1. Microfuge tubes (1.5 mL) were prepared by adding 100 microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system. 100 microliters of the diluted suspension were added directly to individual microfuge tubes (i.e., approximately 10^5 CFU per tube). Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. A hydrogel bead containing extractants was added to individual tubes and RLU measurements were recorded at 10 sec intervals until the number of RLUs reached a plateau or began to decrease (Table 17). The data indicate that all of the bead formulations caused the release of ATP from the microbial cells.

TABLE 17

Release of ATP from microbial cells after exposure of the bacteria to antimicrobial-loaded hydrogels.

	S. aureus				*P. aeruginosa*			
Time (sec)	205M-5p bead	208M-3p bead	205M-3p bad	208M-2p bead	205M-5p bead	208M-3p bead	205M-3p bad	208M-2p bead
0	1099	990	2053	1198	5799	2922	2523	1699
10	2025	2073	3573	1228	7426	3112	15190	11977
20	9442	3074	5921	1313	9107	3197	13717	11271
30	16070	4063	8757	1517	11267	3369	12320	10279
40	22844	5136	12056	1761	14585	3735	10884	8971
50	27610	6186	14748	2090	17849	4337	9583	7989
60	29653	7222	16417	2481	20063	4934	8343	6987
70	29906	8484	17095	2802	21050	5511	7325	6255
80	29453	9420	16979	3224	20662	5938	6423	5509
90	28449	10259	16524	3618	20369	6255	5262	4913
100	27396	11176	15723	3987	19632	6340	4677	4389

	S. epidermidis				*S. enterica* subsp. *enterica*			
Time (sec)	205M-5p bead	208M-3p bead	205M-3p bead	208M-2p bead	205M-5p bead	208M-3p bead	205M-3p bead	208M-2p bead
0	2273	2117	4378	445	1091	4265	5164	3142
10	3217	2383	6376	424	1080	8676	8570	5409
20	6444	4132	8468	444	1230	9309	9208	8460
30	11060	6840	10863	471	1701	9235	9244	9642
40	15496	10587	12958	480	2275	8658	8804	9708
50	19211	13437	14564	499	2879	8076	8248	9369
60	21743	14011	15991	532	3496	6929	7644	8971
70	23296	13493	16231	665	4057	6370	7109	8388
80	23954	12429	16383	917	4687	5922	6551	7471
90	24056	11839	16044	996	5966	5428	6121	6652
100	23700	11231	15708	1053	6247	5026	5759	6007

All data are reported in relative light units (RLU's). BARDAC hydrogel beads were added to the sample immediately after the T_0 measurement was obtained.

Example 15

Release of ATP from Various Microbial Cells Exposed to BARDAC 205M Hydrogel Beads

[0160] Hydrogel bead with 50% solution of BARDAC 205M was prepared as described in Preparative Example 5. Cultures of a number of different microorganisms were prepared as described in Example 1. Microfuge tubes (1.5 mL) were prepared by adding 100 microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system. 100 microliters of the diluted suspension were added directly to individual microfuge tubes (i.e., approximately 10^5 or 10^6 or 10^7CFU per tube). Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. A hydrogel bead made from 50% BARDAC 205M solution, 205M-2p, was added to individual tubes and RLU measurements were recorded at 10 sec intervals until the number of RLUs reached a plateau or began to decrease (Table 18). The data indicate that the hydrogel bead containing BARDAC 205M caused the release of ATP from a variety of microbial cells.

Example 16

Detection of ATP from Suspensions of Microbial Cells Exposed to BARDAC 205M Containing Hydrogel Beads with Continuous Mixing and No Mixing

[0161] Hydrogel bead with 50% solution of BARDAC 205M, 205M-2p, was prepared as described in Preparative Example 5. *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to individual 1.5 ml microfuge tubes containing approximately 10^5 or 10^6 CFU of one of the respective bacterial cultures. Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. One 205M-1p bead was added to each tube. One set of tubes were vortexed for 5 sec between each reading and luminescence resulting from the release of ATP from the cells, was recorded at 10 sec intervals. The other set of tubes were not vortexed, but allowed to sit for 5 sec between each readings. The results are shown in Table 19. The data indicate that ATP release was very rapid in tubes that were mixed and very gradual in the samples that were not mixed.

TABLE 18

Release of ATP from microbial cells after exposure of the bacteria to BARDAC 205M, 205M-2p, hydrogel beads.

	10^5 CFU							10^6 CFU			10^7 CFU	
Time (sec)	*C. albicans* MYA-2876	*C. albicans* 10231	*K. kristinae* BAA-752	*E. faecium* 6569	*E. faecium* 700221	*E. faecalis* 49332	*E. faecalis* 700802	*C. xerosis* 373	*S. pneumoniae* 6301	*S. aureus* 6538	*S. aureus* 6538	*M. luteus* 540
0	5161	4125	3762	145661	43780	1649	36858	3482	3306	1394	12079	44909
10	16987	11776	4000	153959	155023	14727	44451	16180	8462	2308	25805	52335
20	21356	21991	50137	170058	285666	29188	63119	24878	11582	3091	35028	57498
30	28823	44325	82543	209621	349995	50927	123300	31381	15338	5774	51282	66746
40	43630	67484	128571	260697	386112	77724	212171	37995	20488	10499	67125	93143
50	65132	86962	194474	301252	408647	107231	300942	47178	26440	17387	93337	142442
60	86624	105048	267643	333434	425346	134214	365570	60455	32002	27294	222624	199757
70	107992	124236	341048	358913	449577	157280	408127	75323	37780	38335	319768	257819
80	131101	144606	411850	379094	459167	176794	456630	90074	44291	49969	401580	317016
90	157399	166654	477954	395504	467340	192530	470824	104404	51766	61759	491648	378876
100	190305	187641	537507	408552	474223	216918	483047	120709	60659	73799	575341	444460
120	228718	209062	592031	419596	480639	226425	492008	138375	70014	85640	675291	506650
130	317509	232116	639725	428420	485000	235454	500264	157147	79893	96589	877112	559625
140	363503	255940	684210	436263	490791	243147	506623	175508	90109	106625	950544	602616
150	410236	277256	723642	441677	492648	250465	512667	191248	100082	115289	1020667	637557
160	460327	296434	758988	445742	493961	256537	517304	210910	110172	121803	1085813	664924
170	510335	313496	791605	449138	494542	264996	521471	215701	119760	126891	1131039	688074
180	559811	328745	819710	451383	494909	267500	524029	218404	129408	130700	1164549	707790

All data are reported in relative light units (RLU's). 205M-2p beads were added to the sample immediately after the T_0 measurement was obtained.

TABLE 19

Detection of *S. aureus* and *E. coli* using hydrogel beads containing BARDAC 205M antimicrobial mixtures.

	S. aureus				*E. coli*			
	10^5 CFU		10^6 CFU		10^5 CFU		10^6 CFU	
Time (sec)	Vortexing	No vortexing	Vortexing	No vortexing	Vortexing	No vortexing	Vortexing	No vortexing
0	305	498	2005	2091	463	580	1906	1823
10	1006	826	9195	3734	1445	1488	13743	6104
20	2864	1010	42528	12846	2197	1615	18709	6867
30	14239	1359	223585	23113	4232	1775	29616	7363
40	31719	2832	387510	54554	12623	2082	112254	10903
50	53347	6246	570830	107449	17823	2493	193775	12743
60	69178	11550	643850	182751	18410	5780	195720	14176
70	74075	19119	654632	258945	17600	7299	192598	16919
80	74932	27536	644469	327138	16939	10271	188490	22830
90	73404	35364	637619	407092	16507	12478	182579	35515
100	71450	42173	618062	475468	15725	15344	176829	55633
110	67412	49205	588024	563239	15062	18152	172002	77675
120	62889	55253	604301	613548	13983	20871	175739	103648
130	58353	61832	583681	678871	13893	23163	169994	147703
140	55479	67893	574342	754416	13204	24771	170803	174745
150	52797	72663	580001	829087	12325	26561	167078	193287
160	50302	76902	557410	878127	12565	27572	156931	223821

For vortexing experiment, the tubes were vortexed for 5 sec before each measurement. For no vortexing experiment, the tubes were allowed to sit for 5 sec before recording each measurement. All measurements are reported in relative light units (RLU's). BARDAC 205M bead, 205M-2p was added to the tubes immediately after the T_0 measurement was recorded.

Example 17

Detection of ATP from Suspensions of Microbial Cells Exposed to Crushed and Uncrushed BARDAC 205M Containing Hydrogel Beads

[0162] *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to individual 1.5 ml microfuge tubes containing approximately 10^5 or 10^6 CFU of one of the respective bacterial cultures. Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. One BARDAC 205M bead, 205M-2p was added to each tube and in one set of tubes the beads were crushed using the blunt end of a sterile cotton swab. Luminescence, resulting from the release of ATP from the cells, was recorded at 10 sec intervals. The results are shown in Table 20. The data indicate that the crushed beads rapidly released ATP from cells unlike uncrushed beads which showed a gradual increase in ATP levels.

TABLE 20

Detection of *S. aureus* and *E. coli* using hydrogel beads containing BARDAC 205M antimicrobial mixtures.

	S. aureus				*E. coli*			
	10^5 CFU		10^6 CFU		10^5 CFU		10^6 CFU	
Time (sec)	Uncrushed Bead	Crushed Bead	Uncrushed Bead	Crushed Bead	Uncrushed Bead	Crushed Bead	Uncrushed Bead	Crushed Bead
0	755	569	1434	1685	661	921	1547	1548
10	1717	23912	7813	90362	3065	20831	17151	95027
20	1826	44857	9584	160602	5298	23054	36596	165658
30	2106	50007	12476	211406	6841	23123	57201	205345
40	2582	49632	17628	255960	7973	22404	75033	245350
50	3504	47961	24936	278480	9347	21510	91107	282125
60	5103	45779	38234	281923	10930	20218	132156	235422
70	7299	43708	54223	276518	12637	18474	155682	202187
80	10201	41601	68084	266337	14294	16633	177966	175465
90	13371	39292	86533	253028	16003	14940	200829	152480
100	16581	37091	113368	237359	17748	NR	222834	NR
110	19865	NR	142674	NR	19399	NR	244224	NR
120	25670	NR	171218	NR	22426	NR	288422	NR
130	28060	NR	197768	NR	23497	NR	308373	NR
140	30086	NR	223874	NR	24529	NR	322147	NR
150	31676	NR	251004	NR	25028	NR	331004	NR

TABLE 20-continued

Detection of *S. aureus* and *E. coli* using hydrogel beads containing BARDAC 205M antimicrobial mixtures.

	S. aureus				*E. coli*			
	10^5 CFU		10^6 CFU		10^5 CFU		10^6 CFU	
Time (sec)	Uncrushed Bead	Crushed Bead	Uncrushed Bead	Crushed Bead	Uncrushed Bead	Crushed Bead	Uncrushed Bead	Crushed Bead
160	33231	NR	274659	NR	25531	NR	335211	NR
170	34626	NR	299417	NR	25843	NR	336701	NR
180	35942	NR	323084	NR	26050	NR	340089	NR
190	36809	NR	348944	NR	26157	NR	339987	NR
200	37804	NR	370478	NR	26469	NR	340442	NR
210	38582	NR	388143	NR	26451	NR	340842	NR
220	39364	NR	404821	NR	26615	NR	341181	NR
230	39905	NR	416442	NR	26824	NR	340627	NR
240	40344	NR	427181	NR	26766	NR	338149	NR

For crushed bead experiment the bead was crushed immediately after T_0 measurement with the blunt end of a sterile cotton swab. All measurements are reported in relative light units (RLU's). NR = Not recorded. BARDAC bead, 205M-2p was added to the tubes immediately after the T_0 measurement was recorded.

Example 18

Detection of ATP from Suspensions of Microbial Cells Exposed to Hydrogel Beads Containing Various Extractants

[0163] Hydrogel beads with various amounts of chlorhexidine digluconate (CHG) or Cetyl trimethylammonium bromide (CTAB) and Triclosan were prepared as described in Preparative Example 5. *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to individual 1.5 ml microfuge tubes containing approximately 10^6 CFU of one of the respective bacterial cultures. Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. One bead containing the extractant was added to each tube. Luminescence, resulting from the release of ATP from the cells, was recorded at 10 sec intervals. The results are shown in Tables 21. The data indicate that CHG, CTAB and Triclosan beads were able to release ATP from cells.

TABLE 21

Detection of *S. aureus* and *E. coli* using hydrogel beads containing various extractants.

Time	*S. aureus*					*E. coli*				
(sec)	CHG-1p	CHG-2p	CTAB-1p	CTAB-2p	Triclosan-1p	CHG-1p	CHG-2p	CTAB-1p	CTAB-2p	Triclosan-1p
0	532	1875	985	937	1425	1049	1211	844	1197	650
10	2950	10184	1244	1160	2906	2193	8234	911	1561	1594
20	5045	14078	1322	1259	3067	3038	14288	973	1584	1906
30	8615	17165	1492	1368	3201	4335	21251	989	1624	2265
40	10248	19891	1810	1476	3453	5894	30499	1036	1703	2756
50	11790	22362	1959	1609	3768	7690	40819	1117	1748	3424
60	13420	24836	2102	1734	4495	9548	65544	1177	1814	4145
70	15024	27116	2256	1866	4874	11558	79557	1242	1930	4868
80	16697	29353	2401	1986	5273	13641	93910	1327	1995	5656
90	18293	31330	2587	2131	5691	15757	108312	1391	2095	6415
100	19924	33402	2741	2279	6138	17862	122120	1608	2169	7150
110	21562	35642	2957	2400	6620	20219	135239	1685	2308	7944
120	23225	37478	3098	2596	7035	22528	159694	1792	2421	8738
130	24904	39402	3298	2768	7385	25077	170749	1875	2515	9425
140	26453	41107	3477	2971	7858	27749	181638	1984	2626	10142
150	28095	42896	3720	3158	8281	30435	191090	2087	2761	10924
160	29673	44621	3925	3322	8688	33344	200990	2314	2873	11619
180	31203	46286	4353	3545	9111	36249	210178	2423	3026	12374
190	32666	47911	4598	3779	9585	39263	218677	2538	3158	13198
200	34244	49513	4854	4006	10310	42268	226765	2625	3280	13868
210	35728	51022	5084	4239	10779	45511	234148	2742	3436	14597
220	37071	52500	5318	4480	11286	48709	241269	2868	3603	15161
230	38586	53808	5571	4691	11761	51994	247902	3042	3755	15938
240	40048	55096	6135	4933	12147	55230	254243	3138	3933	16572

Beads containing extractants were added to the tubes immediately after the T_0 measurement was recorded. All measurements are reported in relative light units (RLU's).

Example 19

Detection of ATP from Suspensions of Microbial Cells Exposed to Hydrogel Beads Containing Cationic Monomers

[0164] Hydrogel beads with cationic monomers were prepared as described in Preparative Example 2. *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to individual 1.5 ml microfuge tubes containing approximately 10^6 CFU of one of the respective bacterial cultures. Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. One bead containing the extractant was added to each tube. Luminescence, resulting from the release of ATP from the cells, was recorded at 10 sec intervals. The results are shown in Tables 22. The data indicate that beads containing cationic monomers were able to release ATP from cells.

TABLE 22

Detection of *S. aureus* and *E. coli* using hydrogel beads containing cationic monomers.

Time	*S. aureus*					*E. coli*				
(sec)	C_8-1s	C_{10}-1s	C_{12}-1s	ATAC-1s	Ageflex-1s	C_8-1s	C_{10}-1s	C_{12}-1s	ATAC-1s	Ageflex-1s
0	1632	1388	945	1379	1319	790	945	1056	760	982
10	2388	4136	1886	2540	3427	1596	1845	1974	1159	1527
20	2995	6939	2556	2661	3771	1831	1889	2518	1199	1539
30	3563	8198	2717	2784	3917	2009	1928	2898	1219	1555
40	4128	9390	2801	2902	4064	2091	3206	3059	1298	1597
50	4728	10603	2825	3073	4237	2206	3602	3187	1314	1618
60	5346	11779	2875	3192	4424	2256	3919	3241	1366	1648
70	5814	12945	2912	3340	4579	2284	4257	3319	1356	1686
80	6318	14071	2906	3535	4900	2321	4531	3337	1392	1705
90	6873	15223	2951	3696	5039	2364	5074	3405	1401	1741
100	7300	16414	2945	3836	5196	2396	5329	3369	1498	1737
110	7741	17496	2984	4009	5330	2402	5598	3334	1530	1764
120	8153	18497	3001	4135	5395	2450	5872	3327	1541	1790
130	8629	19601	3018	4261	5593	2468	6104	3279	1616	1824
140	8948	20727	3060	4439	5877	2498	6380	3242	1639	1840
150	9415	22776	3090	4592	5949	2545	6866	3229	1702	1864
160	9702	24009	3105	4692	6058	2545	7134	3197	1736	1881
170	10085	25035	3048	4880	6176	2548	7379	3102	1757	1893
180	10429	26159	NR	4995	6316	2568	7886	NR	1844	1956
190	10738	28310	NR	5109	6347	2514	8091	NR	1846	1956
200	11060	29416	NR	5257	6584	2499	8293	NR	1849	1967
210	11351	30469	NR	5394	6681	2457	8579	NR	1949	1999
220	11589	31536	NR	5563	6764	2475	8748	NR	1930	2030
230	12012	32585	NR	5617	6818	2474	8801	NR	2043	2061
240	12265	33561	NR	5739	6859	2461	9048	NR	2022	2080

Beads containing extractants were added to the tubes immediately after the T_0 measurement was recorded. All measurements are reported in relative light units (RLU's). NR = not recorded

Example 20

Detection of ATP from Suspensions of Microbial Cells Exposed to Hydrogel Fibers Containing Microbial Extractant

[0165] Hydrogel fibers were prepared as described in Preparative Example 6. *S. aureus* and *E. coli* overnight cultures were prepared as described in Example 1. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to individual 1.5 ml microfuge tubes containing approximately 10^5 or 10^6 CFU of one of the respective bacterial cultures. Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. About 5 mg of hydrogel fiber containing the extractant was added to each tube. Luminescence, resulting from the release of ATP from the cells, was recorded at 10 sec intervals. The results are shown in Table 23. The data indicate that fibers containing microbial extractant were able to release ATP from cells.

TABLE 23

Detection of *S. aureus* and *E. coli* using hydrogel fibers containing BARDAC 205M.

Time	*S. aureus*		*E. coli*	
(sec)	10^5 CFU	10^6 CFU	10^5 CFU	10^6 CFU
0	438	1167	533	1169
10	2381	8279	22776	13951
20	2677	8273	26139	16023

TABLE 23-continued

Detection of *S. aureus* and *E. coli* using hydrogel fibers containing BARDAC 205M.

Time	*S. aureus*		*E. coli*	
(sec)	10^5 CFU	10^6 CFU	10^5 CFU	10^6 CFU
30	3216	11174	26044	18415
40	4049	14556	25732	23670
50	4999	18989	25341	27481

TABLE 23-continued

Detection of *S. aureus* and *E. coli* using hydrogel fibers containing BARDAC 205M.				
Time	*S. aureus*		*E. coli*	
(sec)	10^5 CFU	10^6 CFU	10^5 CFU	10^6 CFU
60	6098	25040	24953	30280
70	7078	33423	24659	33077
80	8034	52090	24236	35107
90	8896	68418	23803	37464
100	9694	74989	23569	40172
110	10412	84991	23203	42787
120	10951	92328	22786	45949
130	11458	105210	22422	54125
140	11984	108477	22265	68429
150	12440	118505	21981	76109
160	12771	124434	21639	85564
170	13184	136390	21339	101311
180	13655	141752	21122	112249
190	13948	145560	20828	143322
200	14372	148799	20517	159694
210	14740	152368	20395	173869
220	15273	155312	20118	190660
230	15785	158528	19992	201130
240	16178	161061	19649	211916

About 5 mg of BARDAC 205M fibers were added to the tubes immediately after the T_0 measurement was recorded. All measurements are reported in relative light units (RLU's).

Example 21

Detection of ATP from Suspensions of Live Microbial Cells Exposed to Aqueous Extractant

[0166] BARDAC 205M was diluted in water to achieve 0.1%, 0.5%, and 1% solution in water. *S. aureus* and *E. coli* overnight culture was prepared as described in Example 1 and the cells were diluted in Buttefield's buffer. One hundred microliters of luciferin/luciferase reagent from Clean-Trace surface ATP system was added to 1.5 ml microfuge tubes containing approximately 10^5 cells. Immediately after adding the bacterial suspension, the tube was placed into a bench-top luminometer (20/20n single tube luminometer with software), as described in Example 9, and an initial (T_0) measurement of RLUs was recorded. One to 5 microliters of BARDAC 205M solution was added to each of the microfuge tubes and the resulting luminescence was monitored in a bench top luminometer (20/20n single tube luminometer). The results are shown in Table 24. The effective concentration of BARDAC 205M to achieve good signal was between 0.0025 to 0.005%.

TABLE 24

Detection of ATP from cells exposed to a cell extractant in an aqueous solution.												
Time	SA 6538 10^5 CFU						EC 51183 10^5 CFU					
(sec)	0.0005%	0.001%	0.0025%	0.005%	0.010%	0.025%	0.0005%	0.001%	0.0025%	0.005%	0.010%	0.025%
0	1061	1838	1865	1004	1955	1715	683	780	865	985	955	351
10	1664	3423	9589	63723	31953	6330	1778	3667	9463	43499	44723	347
20	1854	3594	15966	78217	43709	2533	1910	3864	11324	52764	46923	345
30	2361	3883	22870	80657	46535	1147	1990	4008	14362	53255	47778	323
40	3183	4222	28830	81722	47465	608	2116	4070	18241	53005	48015	319
50	4027	4484	38514	82869	47918	422	2164	4161	25951	52903	48099	321
60	4845	4781	42670	83720	47981	362	2244	4234	30264	52739	48339	324
70	5619	4948	46745	84321	47860	312	2414	4344	35780	52260	48322	307
80	6932	5174	50394	84670	47942	293	2562	4431	42146	52085	48216	307
90	7545	5324	53874	85074	47857	288	2761	4532	48528	51730	48074	293
100	8121	5554	56828	85322	47781	274	3065	4703	54169	51233	47980	280
110	8688	5708	59518	85672	47591	263	3374	4924	60955	50930	47664	281
120	9243	5881	61800	85793	47444	268	3668	5188	62269	50529	47462	283
130	9793	6096	63366	85831	47183	258	3976	5526	62616	50239	47205	273
140	10823	6201	64497	86128	47044	252	4325	5883	62454	49872	47078	286
150	11310	6426	65541	85879	46911	246	4711	6340	62410	49477	46867	281
160	11942	6566	66044	86177	46644	239	5111	6921	61817	49247	46774	264
180	12450	6798	66409	86130	46364	235	5523	7518	61184	48891	46615	261
190	12968	6923	66617	86088	46140	233	5990	8284	60715	48600	46344	263
200	13527	7141	66754	85984	46004	235	6450	9119	60374	48237	46235	254
210	14732	7383	66799	86055	45803	222	6957	10025	59894	48064	45890	253
220	15297	7657	66695	86080	45368	219	7498	11010	59486	47829	45880	240
230	15871	7860	66689	86117	45004	214	8018	12106	59272	47359	45724	244
240	16539	8024	66567	86118	44948	202	8577	13338	58471	47184	45411	226

About 1 to 5 microliter of BARDAC 205M solution was added to the tubes immediately after the T_0 measurement was recorded. All measurements are reported in relative light units (RLU's).

Example 22

Luciferin Hydrogel Beads

[0167] Hydrogel beads containing luciferin were made either using direct method (Preparative Example 3) or by post-absorption (Preparative Example 7).

[0168] Microfuge tubes were set up containing 100 μl of PBS, 10 μl of 1 μM ATP and 1 μl of 6.8 μg/ml luciferase. Background reading was taken in a bench top luminometer (20/20n single tube luminometer with software), as described in Example 9, and hydrogel beads containing luciferin were added to the tube and reading was followed at 10 sec interval. The post-absorbed beads were more active than the preparative beads (Table 24).

TABLE 25

ATP bioluminescence using luciferin hydrogel beads.

Time (sec)	Luciferin-1s bead	Luciferin-1p bead
0	135	114
10	33587	366562
20	32895	365667
30	32297	360779
40	31914	356761
50	31721	353358
60	31524	348912

Luciferin bead was added to the sample immediately after the T_0 measurement was obtained.

Example 23

Luciferase Hydrogel Beads

[0169] Hydrogel beads containing luciferase were made either using direct method (Preparative Example 4) or by post-absorption (Preparative Example 8).

[0170] Microfuge tubes were set up containing 100 microliter of luciferase assay substrate buffer (Promega Corporation, Madison, Wis.) Background reading was taken in a bench top luminometer (20/20n single tube luminometer with software, as described in Example 9) and hydrogel beads containing luciferase were added to the tube and reading was followed at 10 sec interval. Both types of beads showed good activity (Table 26).

TABLE 26

ATP bioluminescence using luciferase hydrogel beads.

Time (sec)	Luciferase-1s bead	Luciferase-1p bead
0	85	112
10	2757674	2564219
20	4790253	2342682
30	7079855	2201900
40	12865862	2142650
50	16588018	2048034
60	21054562	1958730
70	26456702	1886521

Luciferase bead was added to the sample immediately after the T_0 measurement was obtained.

[0171] In a similar experiment, effect of increasing number of post-absorbed luciferase beads was tested. Microfuge tubes containing 100 microliter of luciferase assay substrate buffer (Promega) were set up and luciferase hydrogel beads (1-4 beads per tube) were added. The luminescence was monitored immediately in a bench top luminometer (FB-12 single tube luminometer with software as described in Example 2). The experiment was done in triplicates. The results, shown in Table 27, indicate a generally linear relationship between the number of beads per tube and the amount of luciferase activity.

TABLE 27

Detection of luciferase activity in hydrogel beads.

	0 beads	1 bead	2 beads	3 beads	4 beads
Trial 1	1379	2148034	3302458	4734298	5130662
Trial 2	609	1858030	2975657	4364022	5090202
Trial 3	602	1788521	2806418	4144277	4831947
Average	863	1931528	3028178	4414199	5017604

Luciferase-1p beads containing luciferase enzyme were added to the tubes containing luciferase assay buffer and measurements were obtained. All measurements are reported in relative light units (RLU's).

Example 24

Detection of ATP from Microbial Cells Exposed to Different Size BARDAC 205M Loaded Hydrogel Beads

[0172] *S. aureus* overnight culture was prepared as described in Example 1.

[0173] Immediately before use in these tests, the bacterial suspensions were diluted in Butterfield's buffer to concentrations of approximately 10^8 CFU per milliliter. Luciferase/luciferin reagent (600 μl) from Clean-Trace surface ATP system was removed and added to 1.5 ml microfuge tubes. Ten-microliter amounts of the bacterial suspensions were added directly to individual microfuge tubes containing the reagents. Size selected BARDAC 205M hydrogel beads were prepared as described in Preparative Example 9. Three hydrogel beads from each size-selected group were added to the tube and the test was done in five independent tubes for each of the beads. Luminescence was measured in a bench top luminometer (20/20n single tube luminometer with software, as described in Example 9) at 10-second intervals. The results of the experiments are shown in Table 28 and 29. The weights shown in the table indicate the total mass of the beads in each respective tube. The results indicate that all size-selected BARDAC 205M beads were able to lyse bacteria and release ATP from cells.

TABLE 28

Detection of ATP from *S. aureus* (10^6 CFU) exposed to size selected BARDAC 205M hydrogel beads, 205M-7p and 205M-8p.

Time	205M-7p					205M-8p				
(sec)	9.9 mg	9.1 mg	9.2 mg	9.8 mg	10.8 mg	6.2 mg	5.1 mg	5.8 mg	5.6 mg	5.6 mg
10	5884	5786	4483	6008	5624	2308	2173	2228	2198	1928
20	6298	6109	4710	8593	6364	2701	2327	2597	2512	2122
30	7130	6344	5178	11247	7748	3091	2503	2886	3035	2550
40	8560	6529	5778	13817	11704	3637	2656	3682	3972	3505
50	9750	6835	6393	17568	16586	5015	2837	5032	5904	5668
60	11237	7080	7150	22835	24984	7175	3223	6777	8975	8593
70	13764	7329	8764	28803	35881	9373	3615	8565	12457	12534
80	16575	7810	11515	35563	47915	12064	5409	13226	16627	17035
90	19920	8483	15605	43238	60288	14614	7059	16638	20563	22632
120	31527	11528	30538	68073	93871	23746	12918	30256	39683	46852
150	45656	16352	53992	98221	121988	35120	24599	50687	69222	74181
180	58711	26266	72507	131722	155123	48201	35655	66329	90797	109371
210	71582	41569	90989	172147	179646	62010	50266	85849	110186	134157
240	83846	53251	109722	198732	206566	76626	60339	99440	127800	156112
270	96412	64820	134903	220626	232601	90879	73501	116759	143244	176096
300	108595	77350	153257	237546	256487	110087	83328	129736	157839	199860
330	120622	94038	170676	249095	275925	125157	96660	145214	170874	215885
360	134746	107818	184854	259270	291140	140259	106748	155467	182676	234142
390	146969	125701	196868	262917	304814	155479	120212	165205	193014	245509

Hydrogel beads were added to the sample immediately before the first measurement was obtained.

TABLE 29

Detection of ATP from *S. aureus* (10^6 CFU) exposed to size selected BARDAC 205M h ydro gel beads ,205M-9 pand 205M-10p .

Time	205M-9p					205M-10p				
(sec)	3.4 mg	3.8 mg	3.0 mg	2.8 mg	3.4 mg	2.8 mg	2.9 mg	2.8 mg	3.0 mg	2.5 mg
10	2469	3026	4441	2493	2782	3647	4395	4019	3592	3678
20	2681	4615	4683	2767	2980	3956	4751	4360	3984	4001
30	3003	7648	5216	3293	3395	4676	5822	5469	5633	4642
40	3587	12448	6620	4739	4605	6954	9814	9983	12513	6870
50	5097	17865	9358	7650	7884	12825	18515	19453	24941	19752
60	8300	24038	13355	11917	13591	21857	29404	30122	38367	28840
90	22445	54141	32278	35746	37157	50645	56301	58266	68301	50049
120	48798	78925	51842	54254	59219	75586	80239	81199	90649	73471
150	72730	107715	71919	73371	79927	98439	100948	101146	108378	93752
180	96158	126358	101114	95755	99418	118844	120988	119670	129868	118908
210	117329	148894	120442	123084	117306	137368	140589	137204	146680	136398
240	136718	164367	138671	141820	134761	155643	161570	154931	164721	158953
270	154479	178696	155639	159279	151630	173528	190227	173471	183401	175251
300	170882	192697	177296	176165	172754	191464	216437	192608	207573	197096
330	186663	209932	193111	191659	187815	209728	232774	210719	224254	212351
360	201237	220007	211971	206515	201431	227969	248849	226224	237204	225685
390	214630	229581	228533	220699	212361	245308	256972	238267	246774	237246

Hydrogel beads were added to the sample immediately before the first measurement was obtained.

Example 25

Detection of ATP from Microbial Cells Exposed to Different Size Benzalkonium Chloride Loaded Hydrogel Beads

[0174] *S. aureus* and *E. coli* overnight culture were prepared as described in Example 1. Immediately before use in these tests, the bacterial suspensions were diluted in Butterfield's buffer to concentrations of approximately 10^8 CFU per milliliter. Luciferase/luciferin reagent (600 μl) from Clean-Trace surface ATP system was removed and added to 1.5 ml microfuge tubes. Ten-microliter amounts of the bacterial suspensions were added directly to individual microfuge tubes containing the reagents. Size selected BAC hydrogel beads were prepared as described in Preparative Example 9. Six hydrogel beads (BAC-1p) or eight hydrogel beads (BAC-2p, BAC-3p and BAC-4-p) from each size-selected group were added to the tube and the test was done in several independent tubes for each of the beads. Luminescence was measured in a bench top luminometer (20/20n single tube luminometer with software, as described in Example 9) at 10-second intervals. The results of the experiments are shown in Tables 30 to 32. The weights shown in the table indicate the total mass of the beads in each respective tube. The results indicate that BAC loaded beads were able to lyse bacteria and release ATP from cells. The size-selected beads (1.4 to 1.7 mm and 1.18 to 1.4 mm beads) containing BAC gave consistent increase in signal across the replicates.

TABLE 30

Detection of ATP from *S. aureus* and *E. coli* exposed to BAC hydrogel beads, BAC-1p.

Time	BAC-1p									
	E. coli 10^6 CFU					*S. aureus* 10^6 CFU				
(sec)	12.8 mg	13 mg	11.8 mg	12.8 mg	13 mg	13.2 mg	13 mg	13.1 mg	13.2 mg	13.5 mg
10	3770	4086	6379	3686	5664	3731	3422	4480	4942	351
20	3886	4360	6608	4009	5932	3922	3730	4783	5370	3666
30	4094	4668	6850	4278	6271	4171	3980	5236	5683	3948
40	4689	5304	7338	4928	7051	4764	4383	6153	6171	4863
50	5563	6525	8406	6228	8689	5936	5360	7321	7403	5766
60	6264	7649	9337	7684	10575	7052	6825	8442	9218	6643
90	9412	11969	12845	11068	17068	11181	12722	13062	13605	12205
120	14801	21524	19147	16585	27558	17329	18929	20114	20501	19162
150	24171	32086	27555	23751	42396	25629	27528	28644	29060	26668
180	32291	43542	37207	31341	59667	34662	37251	37658	40400	34899
210	40978	56824	48267	39188	78298	43252	49714	46774	52385	44111
240	50463	71622	60075	46970	95367	52580	63412	56308	67552	54938
270	60650	87385	72642	55163	109474	62499	78491	66700	82357	66874
300	71622	104475	86107	63824	120129	73549	94056	76956	97347	79597
330	87128	120553	99482	73450	128215	84973	109312	87699	111424	92939
360	98670	133920	111849	83594	133178	96197	120969	97393	124774	105049
390	109284	145492	122719	93824	135941	107150	131360	106339	135883	115259

Six hydrogel beads were added to the sample immediately before the first measurement was obtained.

TABLE 31

Detection of ATP from *S. aureus* (10^6 CFU) exposed to BAC hydrogel beads.

Time	BAC-2p				BAC-3p				BAC-4p			
(sec)	10.2 mg	10.6 mg	11.7 mg	10.6 mg	10.5 mg	11.1 mg	10.3 mg	10 mg	11.2 mg	10.6 mg	10 mg	10.3 mg
10	7042	3583	4004	2623	4704	4745	5793	5067	8573	7847	7040	8727
20	8494	4472	4889	2953	5206	5361	6375	5556	10756	10126	10506	15975
30	9411	5188	5711	3369	5968	6416	6958	6116	14304	13413	19795	34229
40	10769	7942	7018	4070	7684	8462	8086	7011	21834	19975	39045	65078
50	12449	9721	8504	5327	9950	10789	10707	9053	36947	31260	69767	109758
60	14075	11274	9902	7210	12210	13735	14081	12359	58687	45234	112204	166743
90	21911	19237	15414	12448	27862	28289	30077	24866	149019	103148	279423	356984
120	34306	30664	23568	18245	48617	49981	57236	40989	255586	196409	445037	482771
150	46973	45323	35526	29626	71353	71938	86934	64723	366297	296021	538360	521795
180	61083	60493	50069	42465	95818	94901	118545	91017	458252	384365	569586	522438
210	75742	76988	65380	57235	122466	119556	151900	117694	512670	452522	569199	512185
240	90439	93625	81970	73092	150297	145173	186314	144844	532796	495511	559828	500483
270	105206	110574	98789	88982	179202	172751	224450	173219	533046	515236	547005	489084
300	120909	128552	116825	104849	208238	200011	259836	200157	525093	521088	533665	476571
330	136810	147276	134958	121186	233282	225802	289361	225212	513850	517335	519205	463345
360	153646	166789	154002	137528	252947	249808	310708	247577	501097	511306	503074	ND
390	171303	188231	173577	154868	267795	270789	325002	266339	487950	503360	487299	ND

Eight hydrogel beads were added to the sample immediately before the first measurement was obtained. ND = Not Determined

TABLE 32

Detection of ATP from *E. coli* (10^6 CFU) exposed to BAC hydrogel beads.

Time	BAC-2p				BAC-3p				BAC-4p		
(sec)	10 mg	10 mg	10.6 mg	10.2 mg	10.4 mg	9.5 mg	11.5 mg	10.2 mg	11 mg	9.3 mg	10.8 mg
10	2561	4601	7515	7160	4934	4259	6676	3937	8275	6423	6430
20	2754	4826	7600	7574	5196	4510	7074	4215	8737	6797	6906
30	2876	4979	7802	7753	5431	4743	7359	4363	8990	7077	7226
40	3186	5222	8031	8098	5846	5112	8073	4611	9555	7382	7667
50	3815	5630	8777	8234	6752	6047	9242	5175	10674	8370	7855
60	4444	6465	9511	8563	8205	7432	10617	6168	12483	10151	10818
90	6547	10811	12375	10807	13683	11995	16749	10955	20007	16614	20206
120	10272	16285	17332	15038	22221	18292	28896	17534	31101	26747	38339
150	14613	22353	22703	21789	32909	27225	49958	27263	46077	40230	68478

TABLE 32-continued

Detection of ATP from *E. coli* (10^6 CFU) exposed to BAC hydrogel beads.											
Time	BAC-2p				BAC-3p				BAC-4p		
(sec)	10 mg	10 mg	10.6 mg	10.2 mg	10.4 mg	9.5 mg	11.5 mg	10.2 mg	11 mg	9.3 mg	10.8 mg
180	19964	29477	28588	32683	45919	39955	74482	39230	62424	57028	96669
210	26393	37911	34167	47746	60309	56033	94041	52189	75687	74271	110966
240	34235	46963	39786	65116	73277	72145	104638	65451	84371	88973	114584
270	43009	56449	46836	83161	87169	86934	108333	77490	88023	99063	112607
300	52837	67288	53929	99852	97741	97944	108788	86166	88987	104461	109886
330	63453	78682	62428	113321	105571	105321	107529	91204	88267	105947	107174
360	73381	89173	71332	123086	110865	109152	106033	93855	ND	105954	103483
390	81489	98130	80183	129577	114289	110764	104024	95089	ND	104412	100569

Eight hydrogel beads were added to the sample immediately before the first measurement was obtained. ND = Not Determined

Example 26

Effect of the Number of Benzalkonium Chloride Loaded Beads on the Release of ATP from *S. aureus*

[0175] *S. aureus* overnight culture was prepared as described in Example 1. Immediately before use in these tests, the bacterial suspensions were diluted in Butterfield's buffer to concentrations of approximately 10^7 and 10^8 CFU per milliliter. Luciferase/luciferin reagent (600 μl) from Clean-Trace surface ATP system was removed and added to 1.5 ml microfuge tubes. Ten-microliter amounts of the bacterial suspensions were added directly to individual microfuge tubes containing the reagents. Size-selected BAC hydrogel beads were prepared as described in Preparative Example 9. Various amounts of hydrogel beads, BAC-3p, were added to the tube and the test was done in several replicates. Luminescence was measured in a bench top luminometer (20/20n single tube luminometer with software, as described in Example 9) at 10-second intervals. The results of the experiments are shown in Tables 33 and 34. The weights shown in the table indicate the total mass of the beads in each respective tube. The results indicate that BAC loaded beads were able to lyse bacteria and release ATP from cells. The size selected beads (1.18 to 1.4 mm beads) containing BAC gave consistent increase in signal across the replicates with different amount of beads.

TABLE 33

Detection of ATP from *S. aureus* (10^5 CFU) exposed to 17.5% BAC hydrogel beads.												
Time	6 Beads			8 Beads			10 Beads			12 Beads		
(sec)	7.2 mg	6.9 mg	7.7 mg	10.1 mg	9.8 mg	9.9 mg	12 mg	11 mg	11.3 mg	13.7 mg	14.3 mg	13.4 mg
10	859	994	1078	1574	1307	1426	1367	1491	1828	3850	1788	3518
20	846	1001	1072	1597	1328	1470	1397	1505	1971	4053	1979	3795
30	828	1022	1117	1668	1305	1487	1417	1575	2075	4233	2163	4189
40	840	1028	1099	1696	1335	1500	1454	1640	2209	4435	2680	4881
50	857	1040	1143	1734	1392	1548	1511	1721	2404	4953	3804	6721
60	886	1099	1172	1845	1496	1599	1654	1931	2788	6619	6961	11591
90	1045	1392	1521	2738	2190	2185	2679	4728	7498	21241	22931	27647
120	1275	1843	2087	5020	3368	4379	5778	11556	15478	27102	25903	29381
150	1521	2359	2786	8925	6272	7877	10997	15709	18652	27256	25765	29249
180	1923	2871	3494	13024	10112	11586	14707	16945	19259	26963	25646	28952
210	2434	3602	4507	16389	13606	14477	16445	17325	19400	26612	25271	28867
240	2978	4326	5652	18256	15999	16233	17077	17262	19209	26312	24707	28425
270	3643	5205	6859	19174	17300	17036	17223	17238	19128	25994	24258	27968
300	4516	6053	8077	19497	17873	17336	17133	17043	18921	25676	23682	27644
330	5276	6954	9509	19465	17978	17388	16866	17110	18679	25376	22899	27034
360	5890	7917	10818	19362	17958	17426	16676	16988	18403	25011	22305	26820
390	6490	8871	11890	19249	17721	17284	16409	17046	18232	24545	21602	26453

Various amount of hydrogel bead, BAC-3p were added to the sample immediately before the first measurement was obtained.

TABLE 34

Detection of ATP from *S. aureus* (10^6 CFU) exposed to 17.5% BAC hydrogel beads.												
Time	6 Beads			8 Beads			10 Beads			12 Beads		
(sec)	7 mg	7.6 mg	7.7 mg	9.9 mg	10 mg	9.5 mg	12.6 mg	11.9 mg	11.5 mg	14.3 mg	14.8 mg	15.1 mg
10	7538	7083	11794	6083	7417	7340	4887	3532	5423	3005	3709	3820
20	7746	7318	12232	6752	8363	7662	5212	3990	5945	3344	4470	4848

TABLE 34-continued

Detection of ATP from *S. aureus* (10^6 CFU) exposed to 17.5% BAC hydrogel beads.

Time	6 Beads			8 Beads			10 Beads			12 Beads		
(sec)	7 mg	7.6 mg	7.7 mg	9.9 mg	10 mg	9.5 mg	12.6 mg	11.9 mg	11.5 mg	14.3 mg	14.8 mg	15.1 mg
30	7939	7688	13238	7832	9076	8136	5905	4505	6613	3718	5510	6433
40	8159	8169	14049	8001	9961	8844	6771	5290	7346	4267	7086	9176
50	8594	8716	15204	10551	11255	9633	8274	6011	8150	5105	11398	16909
60	9058	9790	17329	12384	13561	10829	11394	6953	9482	7007	20723	32148
90	12028	15442	28000	24339	28134	22134	35926	18823	27555	24011	58447	83860
120	15931	23772	43640	55144	58560	48313	80885	54089	73731	48705	68972	94544
150	24266	34989	66931	93372	97000	94146	116194	92022	100365	60751	70740	95302
180	36017	49775	91041	123833	120494	132426	130804	108374	108015	64031	71949	95217
210	50914	67846	108046	132744	129303	148160	134920	113737	111445	65623	72043	93933
240	68276	87324	117705	137358	133289	153941	135103	116272	113662	65823	71416	92089
270	86083	104161	123175	140853	135853	156517	134748	117417	115953	65270	70676	89965
300	101891	115930	126798	142017	137066	158171	132900	117211	117069	64321	69053	87799
330	114217	123584	128925	142596	137563	159007	130985	117072	117705	63471	67603	85772
360	122472	128241	130917	142137	137000	159155	128615	115670	116859	62019	65924	83447
390	127837	131069	132109	141135	137016	158676	126104	114285	116155	60557	64748	81431

Various amount of hydrogel bead, BAC-3p were added to the sample immediately before the first measurement was obtained.

[0176] The present invention has now been described with reference to several specific embodiments foreseen by the inventor for which enabling descriptions are available. Insubstantial modifications of the invention, including modifications not presently foreseen, may nonetheless constitute equivalents thereto. Thus, the scope of the present invention should not be limited by the details and structures described herein, but rather solely by the following claims, and equivalents thereto.

1. An article for detecting cells in a sample, the article comprising a housing with an opening, a sample acquisition device, and a hydrogel comprising a cell extractant, wherein the housing is configured to receive the sample acquisition device.

2. The article of claim **1**, wherein the hydrogel is disposed in the housing.

3. The article of claim **1**, wherein the hydrogel is disposed in the sample acquisition device.

4. The article of claim **3**, wherein the sample acquisition device comprises a hollow shaft and wherein the hydrogel is disposed in the hollow shaft.

5. The article of claim **1** wherein the sample acquisition device comprises a reagent chamber.

6. The article of claim **5**, wherein the reagent chamber comprises a detection reagent.

7. The article of claim **6**, wherein the detection reagent is selected from the group consisting of an enzyme, an enzyme substrate, an indicator dye, a stain, an antibody, and a polynucleotide.

8. The article of claim **6**, wherein the detection reagent comprises a reagent for detecting ATP.

9. The article of claim **8**, wherein the detection reagent comprises luciferase or luciferin.

10. The article of claim **6**, wherein the detection reagent comprises a reagent for detecting adenylate kinase.

11. An article for detecting cells in a sample, the article comprising a housing with an opening configured to receive a sample, a sample acquisition device comprising a reagent chamber, and a hydrogel comprising a cell extractant; wherein the hydrogel is disposed in the reagent chamber.

12. The article of claim **1**, wherein the hydrogel is a shaped hydrogel.

13. The method of claim **12**, wherein the shaped hydrogel is a bead, a fiber, a ribbon or a sheet.

14. The article of claim **1**, wherein the hydrogel is coated on a solid substrate.

15. The article of claim **14**, wherein the solid substrate is selected from the group consisting of a polymeric film, a fiber, a nonwoven, a ceramic particle, and a polymeric bead.

16. The article of claim **1**, wherein the cell extractant is selected from the group consisting of a quaternary amine, a biguanide, a nonionic surfactant, a cationic surfactant, a phenolic, a cytolytic peptide, and an enzyme.

17. The article of claim **1**, where the cell extractant is a microbial cell extractant.

18. The article of claim **17**, further comprising a somatic cell extractant.

19. The article of claim **1**, wherein the housing further comprises a frangible barrier that forms a compartment in the housing.

20. The article of claim **19**, wherein the compartment comprises a detection reagent.

21. The article of claim **20**, wherein the detection reagent is selected from the group consisting of an enzyme, an enzyme substrate, an indicator dye, a stain, an antibody, and a polynucleotide.

22. The article of claim **20**, wherein the detection reagent comprises a reagent for detecting ATP.

23. The article of claim **22**, wherein the detection reagent comprises luciferase or luciferin.

24. The article of claim **19**, wherein the frangible barrier comprises the hydrogel.

25. The article of claim **18**, wherein the compartment comprises the hydrogel.

26. The article of claim **1**, wherein the hydrogel comprises a water-swollen hydrogel.

27. An article for detecting cells in a sample, the article comprising a housing with an opening, a sample acquisition device, and at least two types of hydrogels, wherein the hous-

ing is configured to receive the sample acquisition device, wherein one of the at least two hydrogel types comprises a cell extractant.

28. (canceled)

29. The article of claim **27** wherein at least one of the two hydrogel types comprises a detection reagent.

30. The article of claim **29**, wherein the detection reagent is selected from the group consisting of an enzyme, an enzyme substrate, an indicator dye, a stain, an antibody, and a polynucleotide.

31. The article of claim **29**, wherein the detection reagent comprises a reagent for detecting ATP.

32. The article of claim **31**, wherein the detection reagent comprises luciferase or luciferin.

33. An article for detecting cells in a sample, the article comprising a housing with an opening configured to receive a sample, a hydrogel comprising a cell extractant; and a detection reagent, wherein the hydrogel and the detection reagent are disposed in the housing.

34. The article of claim **33**, wherein the housing further comprises a compartment.

35. The article of claim **34**, where the hydrogel or the detection reagent is disposed in the compartment.

36. A sample acquisition device with a hydrogel disposed thereon, wherein the hydrogel comprises a cell extractant.

37. The sample acquisition device of claim **36**, wherein the cell extractant comprises a microbial cell extractant.

38. The sample acquisition device of claim **36**, wherein the cell extractant comprises a somatic cell extractant.

39. (canceled)

40. A kit comprising a housing that includes an opening configured to receive a sample, a hydrogel comprising a cell extractant, and a detection system.

41. The kit of claim **40**, further comprising a sample acquisition device, wherein the opening is configured to receive the sample acquisition device.

42. The kit of claim **40**, wherein the cell extractant is a microbial cell extractant.

43. The kit of claim **42**, further comprising a somatic cell extractant.

44. A method of detecting cells in a sample, the method comprising:

providing a hydrogel comprising a cell extractant and a sample suspected of containing cells;

forming a liquid mixture comprising the sample and the hydrogel; and

detecting an analyte in the liquid mixture.

45. A method of detecting cells in a sample, the method comprising:

providing a sample acquisition device, a housing that includes an opening configured to receive the sample acquisition device and a hydrogel comprising a cell extractant disposed therein;

obtaining sample material with the sample acquisition device;

forming a liquid mixture comprising the sample material and the hydrogel; and

detecting an analyte in the liquid mixture.

46. A method of detecting cells in a sample, the method comprising:

providing a sample acquisition device that includes a hydrogel comprising a cell extractant and a housing that includes an opening configured to receive the sample acquisition device;

obtaining sample material with the sample acquisition device;

forming a liquid mixture comprising the sample material and the hydrogel; and

detecting an analyte in the liquid mixture.

47. A method of detecting cells in a sample, the method comprising:

providing a sample acquisition device and a housing that includes

an opening configured to receive the sample acquisition device; and

a hydrogel comprising a cell extractant;

obtaining sample material with the sample acquisition device;

forming a liquid mixture comprising the sample material and the hydrogel; and

detecting an analyte in the liquid mixture.

48. The method of claim **44**, wherein detecting the analyte is indicative of the presence of a live cell.

49. The method of claim **44**, wherein detecting the analyte comprises using a detection system.

50. The method of claim **44**, wherein detecting an analyte comprises detecting an analyte associated with a microbial cell.

51. The method of claim **44**, further comprising the steps of providing a somatic cell extractant and contacting the sample with the somatic cell extractant.

52. The method of claim **44**, wherein detecting the analyte comprises quantifying an amount of the analyte.

53. The method of claim **52**, wherein the amount of the analyte is quantified two or more times.

54. The method of claim **53**, wherein the amount of analyte detected at a first time point is compared to the amount of analyte detected at a second time point.

55. The method of claim **44**, wherein detecting the analyte comprises detecting ATP from cells.

56. The method of claim **55**, wherein detecting the ATP comprises detecting ATP from microbial cells.

57. The method of claim **56**, wherein detecting the ATP comprises detecting ATP from bacterial cells.

58. The method of claim **44**, wherein detecting the analyte comprises detecting the analyte immunologically.

59. The method of claim **44**, wherein detecting the analyte comprises detecting the analyte genetically.

60. The method of claim **44**, wherein detecting the analyte comprises detecting an enzyme released from a live cell in the sample.

61. The method of claim **44**, wherein detecting the analyte comprises detecting colorimetrically, fluorimetrically, or lumimetrically.

62. The method of claim **44**, further comprising the step of compressing the hydrogel.

* * * * *

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摘要(译)

提供了文章（610）用于检测样品中的细胞。该制品包括含有细胞提取剂的水凝胶（640）。还公开了使用方法。