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(54) **BIOMIMETIC PARTICLES AND FILMS FOR PATHOGEN CAPTURE AND OTHER USES**

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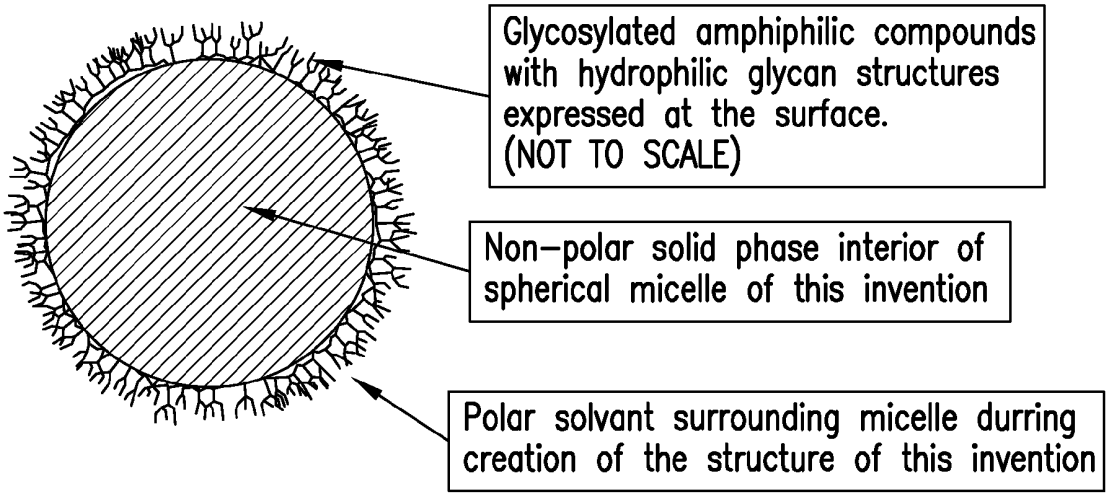
(57) **ABSTRACT**

The identification and immobilization of glycosylated molecules having biomimetic properties, more particularly naturally-occurring, tissue-derived, non-immunological glycan sequences or functional equivalents thereof, on solid state surfaces and films or on membranes arising at the interface between non-polar and polar materials is described herein. The biomimetic glycosylated films and particles constructed therefrom have industrial, environmental, diagnostic and/or therapeutic utility in the binding, capture, and/or extraction of pathogens, toxins and/or contaminants, in vivo, in vitro or in situ. The present invention further extends to the use of such biomimetic films and particles for the delivery of other therapeutic molecules as well as in the construction of body contacting devices having enhanced biocompatibility and reduced immunogenicity.



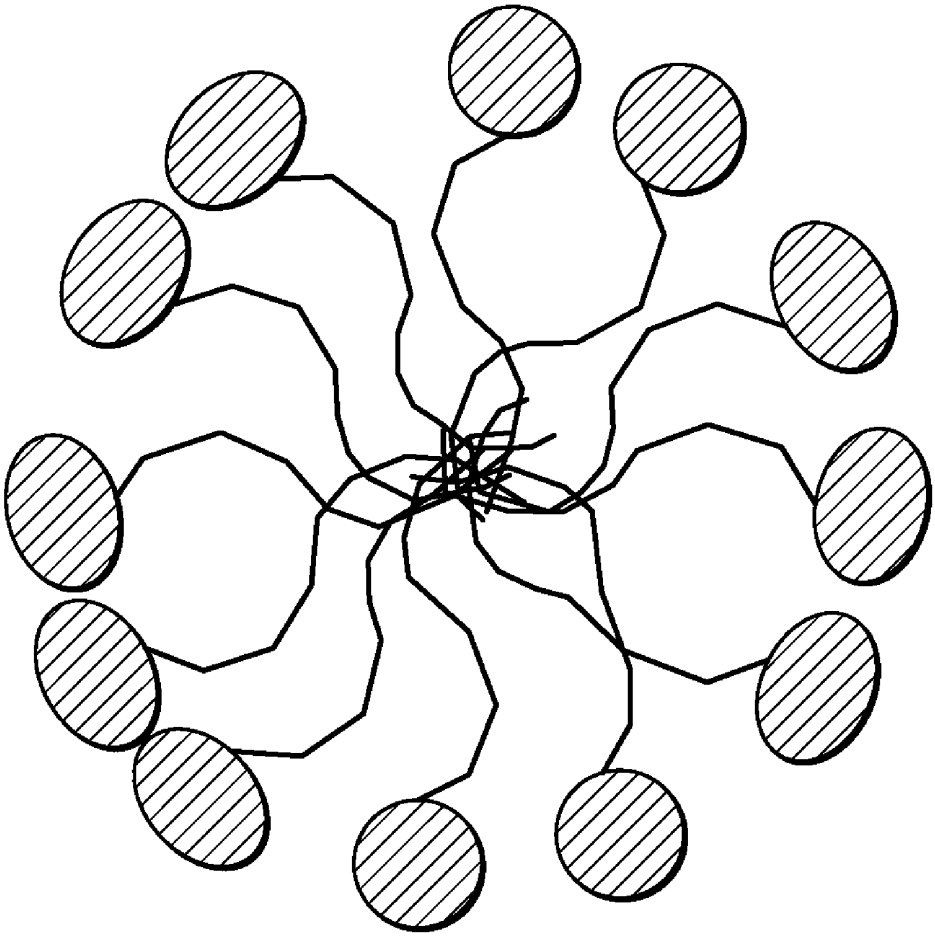


FIG. 1
PRIOR ART

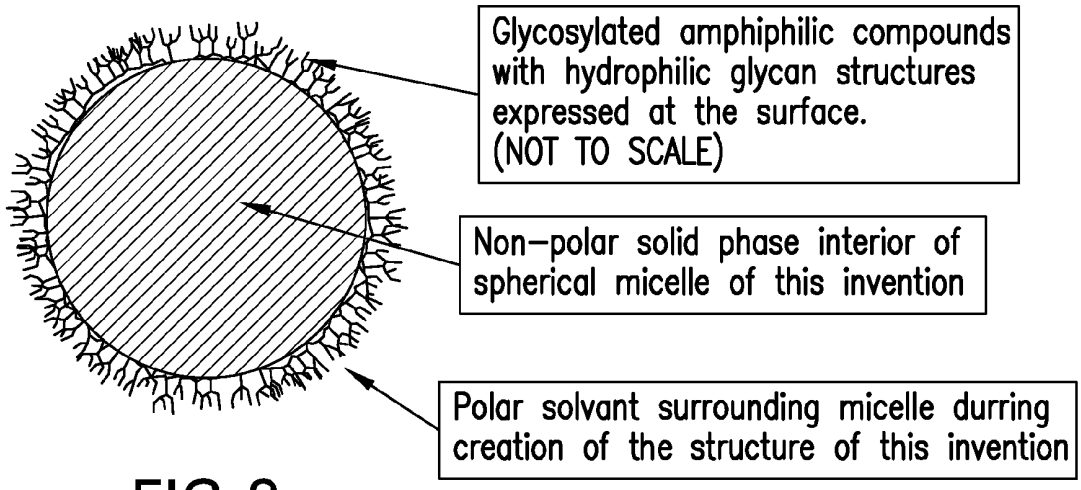

Glycosylated amphiphilic compounds with hydrophilic glycan structures expressed at the surface. (NOT TO SCALE)
Non-polar solid phase interior of spherical micelle of this invention
Polar solvant surrounding micelle durring creation of the structure of this invention

FIG.2

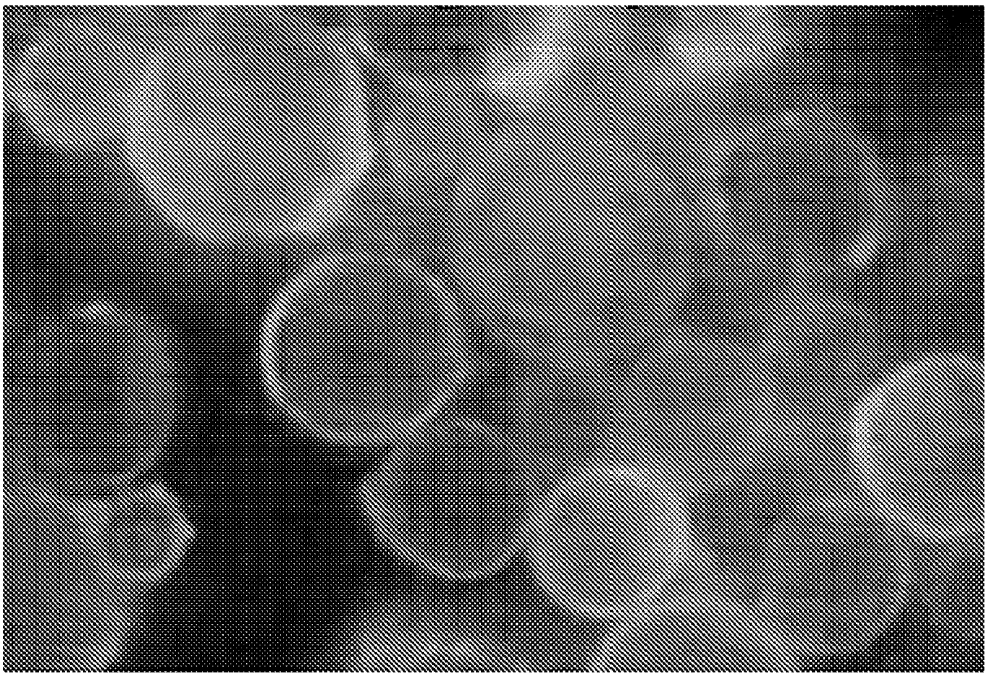


FIG. 3

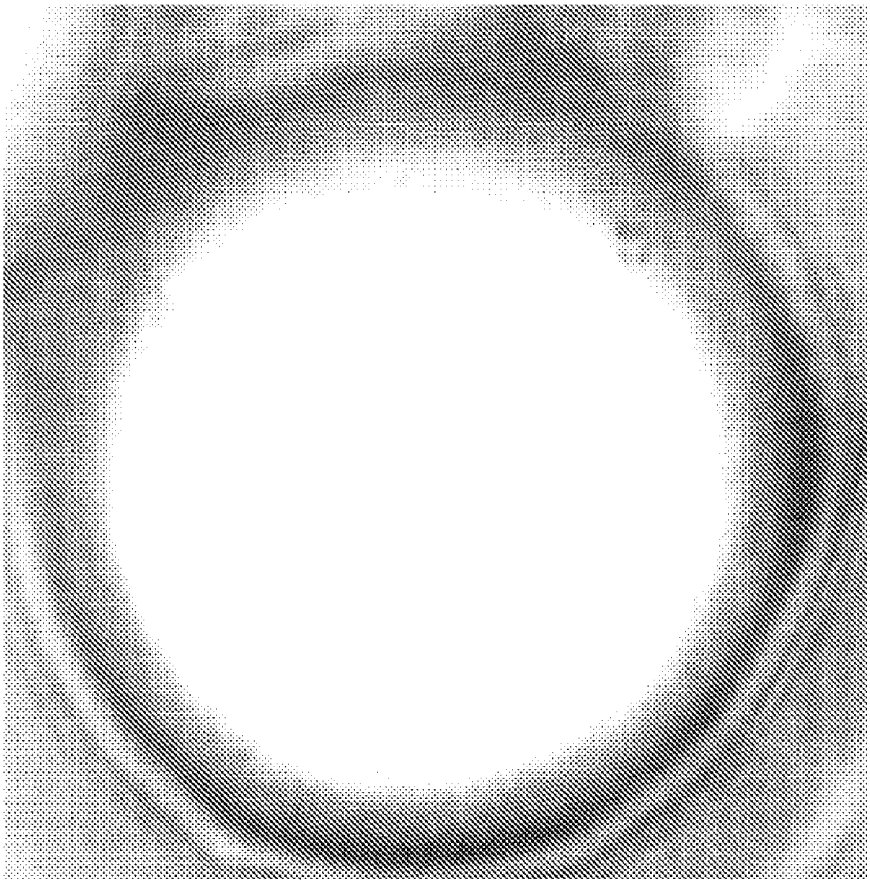


FIG.4

BIOMIMETIC PARTICLES AND FILMS FOR PATHOGEN CAPTURE AND OTHER USES

PRIORITY

[0001] This application claims the benefit of U.S. Utility patent application Ser. No. 11/761,045 filed Jun. 11, 2007, referred to hereinafter as the '045 Application, the contents of which are incorporated by reference herein in its entirety. This application further claims the benefit of U.S. Provisional Patent Application Nos. 61/027,375 filed Feb. 8, 2008 and 61/044,710 filed Apr. 14, 2008, referred to hereinafter as the '375 and '710 applications respectively, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of glycomics and the identification and immobilization of glycosylated molecules having biomimetic properties, more particularly naturally-occurring, tissue-derived, non-immunological glycan sequences or functional equivalents thereof, on solid state surfaces and films or on membranes arising at the interface between non-polar and polar materials. The present invention also relates to the industrial, environmental, diagnostic and/or therapeutic use of such biomimetic films and particles constructed therefrom in the binding, capture, and/or extraction of pathogens, toxins and/or contaminants, in vivo, in vitro or in situ. The present invention further extends to the use of such biomimetic films and particles for the delivery of other therapeutic molecules as well as in the construction of body contacting devices having enhanced biocompatibility and reduced immunogenicity.

BACKGROUND OF THE INVENTION

[0003] Most pathogens have some degree of host and tissue specificity. For example, many of the most virulent pathogens exhibit a degree of tissue tropism, wherein the pathogen recognizes and responds to tissue present in the most advantageous environment for the pathogens to thrive. Consequently, a pathogen which causes gastro-intestinal distress may fail to bind tissues outside this region, for example, mucosal tissues of the nose and throat of the same host, and thus be relatively benign in these other regions. In a similar fashion, a pathogen that is highly contagious in birds may have a limited virulence in humans. This host and tissue tropism is often mediated through glycan recognition.

[0004] Pathogen binding to host tissue is, in most cases, a prerequisite for the successful infection of the host. This binding is typically achieved by means of adherence factors or "adhesins" expressed on the pathogen surface which are specific to glycans or glycan sequences presented on the surfaces of the cells of the tropic host tissue. These endogenous glycan signatures are often unique to a specific area of the body and utilized by the host to regulate and induce tissue specific biological processes. Glycan signatures can also serve to designate the cell or tissue as "self", thereby insulating it from the immune system recognition. In contrast, the surface features of the pathogen (often referred to as surface antigens or antigenic fragments) are recognized by the host immune system as "non-self", thereby generating an antigen specific immune response, namely the production of pathogen/antigen specific antibodies or immunoglobulins. A similar process known as phage display may be used to produce synthetic adhesins to pathogenic antigens. Commercial quantities of antibodies and synthetic adhesins may be economically produced, thereby facilitating their routine use in laboratory scale capture, concentration and detection techniques.

[0005] The specificity of an antibody or its synthetic analog derived from phage display techniques can extend to a point where two pathogens of equivalent pathogenicity but with minor molecular differences in surface features will not be both recognized by the same antibody. Consequently, immunological techniques constitute powerful tool for the capture and identification of pathogens. However, these techniques are limited in their ability to capture unexpected, uncharacterized, rapidly evolving or engineered pathogens. Accordingly, one aspect of the present invention addresses this need, filling an important gap in the field of capture, concentration and detection of pathogens. More particularly, the present invention provides biomimetic films and particles that utilize non-immunological, glycosylated molecules harvested or derived from a relevant biological tissue of interest, particularly a pathogen specific host tissue, immobilized to a solid state support or substrate. By harnessing the host and tissue specificity of the glycan signature, the present invention is able to provide improved biomimetic films and particles uniquely suited to the capture of a wide range of pathogens and toxins based on tissue tropism. As discussed in detail below, these improved biomimetic films and particles find both diagnostic and therapeutic applications.

[0006] The alimentary tract of biological organisms (humans, animals, birds, fish, etc.) is a preferred tropism for many microorganisms. While many of these microorganisms are symbiotic in nature, others are pathogenic. Ingested pathogenic organisms are the known cause of major health issues in many parts of the world and some are now being implicated in diseases which had previously been thought not to be microorganism based. For example, *Helicobacter pylori*, is now thought to be a factor in development of gastritis and peptic ulcers, where previously these conditions were thought to be solely a result of diet, stress or genetic predisposition. The immune system of the human body has evolved to be effective in combating many of these pathogens; thus, whether through design or neglect, the most common treatment of pathogenic infection often involves simply letting the disease run its course. However, this option is often unacceptable when dealing with infection in the very young, the very old, in individuals whose immune system is impaired or compromised or when dealing with particularly virulent strains against which the body may not be able to mount an adequate defense.

[0007] In certain instances, antibiotics may be recommended. However, antibiotic therapy has its limitations. For example, antibiotics are ineffective against viral pathogens as well as the growing number of antibiotic resistant strains, the onset of which many scientists link to the overuse of antibiotics. In addition, antibiotics are not always readily available, particularly in economically disadvantaged locations in the world. Moreover, antibiotic therapy is not always advisable, even in the treatment of bacterial infections. For example, certain types of bacterial pathogens, such as Shiga toxin-producing *Escherichia Coli* (STEC), particularly 0157:H7 and its variations, exert their deleterious effects through the production and release of toxic chemicals. Studies have shown that 5-10% of individuals infected by STEC will develop hemolytic-uremic syndrome (HUS), and, of those, half will have renal damage and one in ten will die or have renal failure.[1] However, in vivo treatment of shiga toxin-

producing bacteria using antibiotics can be dangerous because the bacteria have been shown to produce more toxin when stressed.[2,3]

[1] Cheleste M Thorpe, Shiga toxin-producing *Escherichia coli* infection, Clin Infect Dis. 2004 May 1; 38 (9):1298-303

[2] Wong, C, M. D., et al. "The Risk of the Hemolytic-Uremic Syndrome after Antibiotic Treatment of *Escherichia Coli* O157:H7 Infection", The New England Journal of Medicine, Jun. 29, 2000, Vol. 342, No. 26, pp. 1930-1936.

[3] Mulvey G.; Rafter D. J.; Armstrong G. D., Potential for using antibiotics combined with a Shiga toxin-absorbing agent for treating O157:H7 *Escherichia coli* infections, Canadian Journal of Chemistry, Volume 80, Number 8, August 2002, pp. 871-874(4)

[0008] Accordingly, since antibiotics are not always advisable, available or even effective, alternative therapeutic treatments for pathogenic infections are heartily sought. The present invention addresses this need by providing therapeutically administrable, more preferably ingestible, biocompatible pathogen-binding films and particles having a size or geometric configuration so as not to be absorbed by the relevant tissues, said particles capable of removing both the pathogen and its associated toxin, without producing the undesirable side effect of inducing additional toxin production associated with antibiotic therapy. The technology of the present invention extends to the treatment of infection associated with numerous pathogens, including both natural and engineered disease producing agents, and thus constitutes a marked improvement in the treatment of infection by toxin-producing pathogens.

[0009] In the current art of immunologically based pathogen capture and/or detection in diagnostic samples, molecules such as antibodies may be immobilized to suitable solid substrates that serve as platforms to either allow recovery of the platform, including any attached pathogens, such as in the context of antibodies conjugated to magnetic beads, or to act as stationary capture surfaces, such as with microarrays or capture filters having antibodies attached thereto. Accordingly, by affixing or immobilizing an antibody to a support structure or substrate, one can provide an extremely specific means for capturing anticipated pathogens and facilitating detection.

[0010] The immobilization of molecules on the surface of a solid substrate is well-studied science and techniques of value can be utilized from many fields. Current antibody immobilization procedures utilize conventional molecular conjugating techniques, which are principally based on conjugation of molecules using chemical binding techniques, such as with biotintylation or binding with various polymers, often block copolymers. Immobilization of antibodies can also be achieved by means of adsorption on a properly charged surface. Common techniques for conjugation molecules of biologic origin and other molecules, natural and synthetic, include, but are not limited to reduction amination, diazo coupling, use of isothiocyanates, amidation, use of hom-bifunctional reagents, cycloadditions, maleimide addition thioether linkages, oxime conjugation, stauding ligation olefin metathesis, biotintylation and PEGylation. Block copolymers are often used to facilitate such conjugations. Molecules of interest can also be incorporated directly into a material matrix which constitutes the body of the substrate or which is applied as a coating on a substrate. This later method, also known to those skilled in the art, is less efficient in the expression of active moieties at the surface since the molecules of interest may be embedded in a manner where the active moieties are not exposed at the surface.

[0011] Glycan signatures and other membrane bound molecules tend to be amphiphilic in nature. This feature can be utilized effectively in the context of the present invention for the creation of a biomimetic pathogen-binding films and particles. Accordingly, another aspect of the present invention is to provide novel techniques for immobilizing glycosylated molecules, particularly amphiphilic glycosylated molecules, to solid state supports.

[0012] Amphiphilic molecules with both hydrophobic and hydrophilic ends tend to self align to form a membrane at the interface of a polar solvent and a non-polar liquid or a solid. When the amphiphilic molecules are in the presence of a polar solvent and a non-polar liquid, they form micelles or micelle-like structures in which the hydrophobic end of the molecule is embedded in the non-polar liquid. When the amphiphilic molecules are in the presence of a polar solvent and a solid, the hydrophobic ends of the amphiphilic compound tend to align against the solid with the hydrophilic end presented to the solvent. In both these situations, when the polar solvent is removed, the electrical forces which maintain the alignment of the amphiphilic compounds are also removed and the membrane structure fails.

[0013] These types of structures, often referred to interchangeably as micelles, or vesicles, are well known in science and nature, with liposomes being a specific subset of these structures. Biological cell membranes consist of lipid bilayers in which amphiphilic phospholipids and related compounds align with their hydrophobic ends against a lipid layer and their hydrophilic ends facing surrounding or interior sides of the membrane; the resultant construct is a double wall of phospholipids.

[0014] A useful rendition of these structures in conjunction with non-polar liquids has been achieved through the use of biological amphiphilic compounds to form micelles and micelle-like structures. In particular, lipo-glycoprotein membranes and micelles formed therefrom are described in U.S. Pat. Nos. 5,824,337, 6,528,092 and 7,148,031, and U.S. Patent Publication US2007/0141694, all to Elaine Mullen, the entire contents of which are incorporated herein by reference. In these patents, the unique and important contribution of Mullen in the creation of the lipo-glycoprotein membranes over other micelle-like constructs is the presentation of important glycans and other biological structures at the surface of the membrane in a manner that can be beneficially used. The presence of these glycans allows for the transport of substances that can be dissolved or suspended in lipids as well as the capture and concentration of biological and inorganic entities that naturally bind to the selected glycan structures.

[0015] Amphiphilic compounds have been used in the emulsion polymerization process to produce synthetic rubber and some grades of plastics (PVC, polystyrene, PMMA, polyvinylidene fluoride and PTFE). The emulsion polymerization process is designed to produce small polymer particles that can remain in suspension in products such as paint or other emulsions.

[0016] The immobilization of amphiphilic molecules on latex based polymer particles is described in U.S. Pat. No. 4,929,662, to Hogenmuller, et. al., and in U.S. Pat. Nos. 4,952,622 and 5,109,038, both to Chauvel, et. al., the entire contents of which are incorporated herein by reference. The techniques described in these patents are applicable to one aspect of the present invention in the creation and construction of biomimetic films and particles comprising amphiphilic moieties, particularly glycolsylated molecules, harvested from tissue, wherein the hydrophobic tails of said molecules are bound to the surface of a latex film or particle

through hydrophobic interaction. The construct of the amphiphilic biomimetic molecules hydrophobically bound to the surface of said latex film or particle may be heated to the glass transition temperature of the latex and then cooled in a manner which results in the hydrophobic tails of the amphiphilic compound becoming embedded in the latex material.

[0017] In U.S. Pat. No. 5,919,408, the entire contents of which are incorporated herein by reference, Muller, et. al., teach a process for the production of pseudolatices and micro- or nanoparticles with embedded pharmaceutical preparations. The techniques of forming said particles are also of use in creation and construction of amphiphilic biomimetic surfaces, films, and particles disclosed herein.

[0018] Previous work done in the field combining polymer substrata with biological amphiphilic compounds at the surface has been focused on production of micelle-like structures, without attention to preserving or utilizing biological surface properties of any surfactants used to form biomimetic constructs with utility in pathogen capture. Accordingly, the present invention constitutes a marked improvement not only in the area of micelle technology by providing for the secure immobilization of an amphiphilic moiety of interest, thereby enabling long term storage and facilitated transport, but also in the area of pathogen capture, by utilizing amphiphilic biomimetic structures, particularly unique glycosylated amphiphiles which mimic the pathogen activity of native host cells and tissues.

SUMMARY OF THE INVENTION

[0019] Thus, in view of the foregoing, it is an object of the present invention to provide novel biomimetic films and particles suitable for pathogen capture and other uses, as well as methods of making and using same.

[0020] It will be understood by those skilled in the art that one or more aspects of this invention can meet certain objectives, while one or more other aspects can meet certain other objectives. Each objective may not apply equally, in all its respects, to every aspect of this invention. As such, the following objects can be viewed in the alternative with respect to any one aspect of this invention.

[0021] A primary objective of the present invention is to provide novel biomimetic films and particles for pathogen capture. In particular, the present invention provides a solid state film with biomimetic properties comprised of a solid, preferably non-polar, substrate having a plurality of biomimetic glycosylated molecules immobilized thereon. Of particular interest are endogenous glycan sequences expressed at the surface of cells, particularly epithelial cells, that represent the principal binding sites for pathogens at the initiation of a host-tropism type infection. These same glycan signatures may also constitute "self antigens" that can be used to mask the foreign nature of the film, thereby providing it with reduced immunogenicity. The unique glycan signatures can be either harvested directly from host tissues of interest or derived therefrom, using phage display and recombinant techniques. Functional mimics of these glycan signatures are also useful in the context of the present invention.

[0022] Accordingly, it is an object of the present invention to construct biomimetic films, surfaces, and particles suitable for pathogen capture for diagnostic, therapeutic or other uses, such surfaces composed of a solid substrate having glycosylated molecules expressed on its surface, with the glycans or glycan sequences representative of non-immunological, glycosylated molecules. Molecules of particular interest are those which might be expressed by cells of the potential target host, or mimics of the glycans structures of said molecules or functional analogues. Sialated molecules are of also of particular value in the context of the present invention.

[0023] It is a further object of the present invention to utilize the biomimetic films and particles for pathogen capture, for example by exposing said surface to a fluid media which may or may not contain pathogens which exhibit the appropriate tissue tropism. It is also an object of the present invention that the construct can subsequently be subjected to analysis techniques to determine the presence and/or nature of any organisms binding to the surface.

[0024] It is yet another object of the present invention to produce the biomimetic films and particles in an appropriate particulate form and use it in the context of an agglutination assay.

[0025] It is yet another object of the present invention to affix non-immunological, glycosylated molecules, particularly molecules from biological tissue, to magnetic beads, and expose such magnetic beads with these moieties to a solution which may or may not contain pathogens which exhibit the appropriate tissue tropism. The magnetic beads can be subsequently subjected to analysis procedures familiar to those skilled in the art to determine the presence and/or nature of material which bound to the beads in solution.

[0026] It is yet another object of the present invention to provide the biomimetic films and particles of the present invention with one or more type of non-immunological, glycosylated molecules, examples of which include, but not limited to, glycoproteins (eukaryotic glycoproteins, proteoglycans, glycomucins), and glycolipids, including natural, synthetic, and recombinant versions thereof as well as homologues, analogues, and functional equivalents thereto.

[0027] It is yet another object of the present invention that the non-immunological, glycosylated molecules include synthetic molecules with part or all of the glycans structure of naturally occurring glycoproteins (eukaryotic glycoproteins, proteoglycans, glycomucins), and glycolipids, including molecules which are conjugates of different materials.

[0028] It is yet another object of the present invention to construct the biomimetic films and particles of the present invention with a substrate of a polymer, wax and other hydrocarbon-based substrate, ceramic, glass, metal or other solid material or combinations thereof.

[0029] It is yet another object of the present invention to immobilize the non-immunological, glycosylated molecules to a substrate which further includes a semi-conductor device, examples of which include, but not limited to, lab-on-a-chip type devices.

[0030] It is yet another object of the present invention to provide various methods for constructing embodiments of the present invention.

[0031] A second objective of the present invention is to provide novel biocompatible particles formed from a biomimetic film analogous to that disclosed above, comprised of a solid state film having a plurality of glycosylated molecules immobilized thereon, wherein the particle is of a size or geometric configuration that prevents it from being absorbed by the biological tissue or organs of a living system. In this manner, the biocompatible particles find utility as ingestible pharmaceutical compositions for the treatment of pathogenic infection, particularly infection with a toxin-producing pathogen. Such particles can also be introduced to other areas

of the body as well, according to other conventional administration protocols, such that they can later be removed or expelled.

[0032] Accordingly, it is an object of the present invention to provide therapeutic biocompatible particles with a minimum nominal diameter sufficient to inhibit absorption of a majority of the particles by the surrounding tissue, the diameter being greater than 20 nanometers, preferably greater than 50 nanometers, more preferably greater than 500 nanometers.

[0033] It is further an object of the present invention to provide therapeutic biocompatible particles in the form of micelles, such as wax micelles, or fibers or worm micelles (also nanowires), with or without embedded substrate materials. The fibers or worm micelles can be part of a construct of entangled fiber-like particles which in concert provide an effective diameter or configuration to impede absorption into surrounding tissue. Alternatively, the particles can be in the form of a free-standing film as long as at least one dimension is sufficient to prevent absorption of a majority of the particles by the surrounding tissue.

[0034] It is an object of the present invention to provide therapeutic biocompatible particles expressing surface molecules comprising pathogen binding glycosylated molecules. In a preferred embodiment, the pathogen binding moieties include glycans or glycan sequences, natural or synthetic, which mimic pathogen binding sites in the host body, for example, glycan sequences expressed in glycoproteins (eukaryotic glycoproteins, proteoglycans, glycomucins) or glycolipids (glycosphingolipids), including natural, synthetic, and recombinant versions thereof as well as homologues, analogues, and functional equivalents thereto.

[0035] The pathogen binding glycosylated molecules may be isolated, harvested, or derived from endogenous pathogen host tissues or surrogates thereof (such as tissue cultures). Alternatively, they may be recombinantly or synthetically produced. The population of glycosylated molecules may be homogeneous or heterogeneous.

[0036] The pathogen binding moieties can comprise transmembrane molecules isolated or derived from host tissues or bacterial intimin binding molecules or mimics as expressed by attaching and effacing lesions. They may also include molecules or mimics of molecules harvested, or otherwise derived, from translocated intimin receptor (TIR) or its interaction with epithelial cells.

[0037] It is further an object of the present invention to provide a the substrate of natural or synthetic polymers, natural or synthetic waxes, ceramics, metals, materials of biological origin or combinations thereof.

[0038] It is a further object of the present to formulate the therapeutic particle as a pharmaceutical composition suitable for ingestion or administration by other means. Such pharmaceutical compositions may optionally include additional

[0039] It is a further object of the present invention to provide a therapeutic dose that consists of a plurality of particles of the present invention and that those particles can include a range of sizes, shapes, substrate materials, and types of surface molecules.

[0040] It is yet another object to provide unique methods of making and using the therapeutic biocompatible particles and films of the present invention.

[0041] A final objective of the present invention is to provide functionally active solid state films of glycosylated amphiphilic molecules. In particular, it is an objective of the present invention to provide a non-polar solid surface or film, for example of a polymer, plastic or wax, that has embedded in it or conjugated or chemically bound to it the hydrophobic end(s) of a glycosylated amphiphilic molecule, such that its opposing hydrophilic end(s) having useful functional properties are expressed at or above the surface of the solid. These properties may be viable either in air or when a polar solvent, such as water, is present.

[0042] The functionally active solid state films of glycosylated amphiphilic molecules of the present invention are useful in imbuing various surface properties of organic cells onto the surface of solid materials.

[0043] The present invention represents an improvement in the art that utilized similar membranes formed between a polar solvent and a non-polar liquid. Structures of such membranes traditionally include micelles and liposomes wherein the hydrophobic groups align at the interface between a polar solvent and a non-polar liquid. The solid state films of the present invention differ from conventional micelles and liposomes in that the hydrophobic groups are affixed to or anchored in a solid in such a way that the properties of the hydrophilic groups are preserved on the surface for advantageous use. In conventional membranes between a polar solvent and a non-polar liquid, if the polar solvent is removed, the alignment of the hydrophobic and hydrophilic ends of the amphiphilic compounds fails and the membrane is destroyed. However, with the solid state films of the present invention, the polar solvent can be removed and then later reintroduced or then another polar solvent introduced with maintenance of the properties of the hydrophilic groups on the surface.

[0044] Accordingly, it is an object of the present invention to provide a solid state film having at least one functionally active surface, the film composed of a non-polar substrate having a homogenous or heterogeneous population of glycosylated amphiphilic molecules affixed thereto, each glycosylated amphiphilic molecule composed of a hydrophobic tail end and a hydrophilic head end provided with one or more functional groups such that the hydrophobic tail ends are embedded in or chemically or mechanically linked to the non-polar substrate and the functional groups of the hydrophilic tail ends project from the substrate so as to provide at least one surface of said film with functional activity.

[0045] It is a further object of the present invention to provide a solid state micelle or micelle-like structure with a surface film of the present invention, wherein the non-polar substrate and hydrophobic tail ends are sequestered in the interior of the micelle while the hydrophilic head ends are present on the outer surface of the micelle.

[0046] It is yet a further object of the present invention to provide a method for making a film of the present invention, including the following steps:

[0047] (a) providing a polar solvent having sufficient quantities of one or more glycosylated amphiphilic molecules dissolved therein;

[0048] (b) exposing the polar solvent to a non-polar liquid, the non-polar liquid being immiscible in the polar solvent;

[0049] (c) allowing the glycosylated amphiphilic molecules to align so as to form a membrane that separates the polar solvent from the non-polar liquid; and

[0050] (d) inducing transformation of the non-polar liquid to a corresponding non-polar solid having upper and lower surfaces, wherein the hydrophobic tail ends of the amphiphilic molecule are embedded in or chemically or mechanically linked to the upper surface of the non-

polar substrate and the hydrophilic tail ends project from the upper surface into the polar solvent so as to yield a film having functional activity.

[0051] It is a further object of the present invention to provide a method of extracting a target molecule of interest from a sample, including the following steps:

[0052] (a) exposing the sample to a solid state membrane of the instant invention, wherein the functional groups present at the hydrophilic tail end of the glycosylated amphiphilic molecules have a binding affinity for a target molecule;

[0053] (b) allowing sufficient time for the functional groups to bind target molecules present in the sample; and

[0054] (c) removing or separating the membrane, to which the target molecules are bound, from the sample.

[0055] It is yet another object of the present invention to provide a method of enhancing the biocompatibility of a medical device including the step of coating a solid state membrane of the present invention onto one or more exposed surfaces of the device. Medical devices having such membrane coatings are also provided herein.

[0056] It is a further object of the present invention to provide economical solid surfaces which can be imbued with useful properties of glycosylated amphiphilic compounds such as glycoproteins. Such surfaces are important in the fields of glycomics and proteomics for the study of how sugars and proteins react with various biological compounds. The surfaces are also useful in the field of pathogen capture, concentration and detection. Such surfaces capture the pathogen by presenting the same or similar sugars that the specific pathogen, etc. binds to in the body. A surface with this characteristic can concentrate the pathogen to a point where it can be detected through conventional detection means. Accordingly, it is an object of the present invention to utilize the glycosylated amphiphilic membranes of the present invention, in the form of a solid surface film or a film coating, to isolate and extract target compounds from a particular environment or sample.

[0057] It is a further object of the present invention to provide compositions and methods for preventing an immune response in a mammal. In that vein, it is an object of the present invention to utilize the glycosylated amphiphilic membranes of the present invention in the fabrication of or as a film coating for artificial organs, implants, and transplant materials, including both living and nonliving tissue. It is well-accepted that a material that mimics the surface properties of a biological cell or tissue is less likely to generate an immune response. Accordingly, through the selection of an appropriate biocompatible glycosylated amphiphilic compound, one can provide a biomimetic membrane which would be useful in the formation of or as a film coating for various body contacting, penetrating or implanted devices. Such glycosylated amphiphilic membranes of the present invention, embedded with molecules that mimic biological molecules or express a variety of biological properties, can also prove useful in wound dressings and drug delivery.

[0058] Within the context of biocompatibility, the use of lipids and waxes from a biocompatible entity as the non-polar substrate is anticipated as a means to further insure compatibility or achieve synergistic benefits. For example, the non-polar substrate may comprise a natural wax or fat isolated from an antigen-matched human donor.

[0059] These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the invention and the following detailed description are of a preferred embodiment and not restrictive of the invention or other alternate embodiments of the invention. In particular, while the invention is described herein with reference to a number of specific embodiments, it will be appreciated that the description is illustrative of the invention and is not constructed as limiting of the invention. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the invention, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present invention will be apparent from this summary and certain embodiments described below, and will be readily apparent to those skilled in the art having knowledge of various pathogen binding glycan motifs, amphiphilic compounds, self-assembly techniques and peptide synthesis. Such objects, features, benefits and advantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0060] Various aspects and applications of the present invention will become apparent to the skilled artisan upon consideration of the brief description of the figures and the detailed description of the present invention and its preferred embodiments which follows:

[0061] FIG. **1** depicts a classic micelle structure, in which the hydrophilic "head" regions present outward to contact the surrounding solvent while the hydrophobic "tail" regions are sequestered in the micelle centre.

[0062] FIG. **2** is a schematic of a glycoprotein micelle of the present invention, particularly depicting the spontaneous aggregation and specific alignment of glycoproteins to form a membrane at the interface between polar and non-polar solutions.

[0063] FIG. **3** depicts the successful binding of fluorescent lectins to the sugars of the glycoproteins presented on the surface of the paraffin beads (10-100 microns in diameter). The lectins are glowing under the light of an epi-fluorescent microscope. This type of binding is analogous to the binding of toxins from a fluid sample.

[0064] FIG. **4** depicts the successful binding of *Salmonella* to the sugars of the glycoproteins presented on the surface of the wax beads (5-50 microns in diameter) with the surface moieties of a biomimetic film derived from glycosylated molecules harvested from porcine small intestine.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0065] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. However, before the present materials and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols herein described, as these may vary in

accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0066] I. Elements of the Present Invention:

[0067] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. However, in case of conflict, the present specification, including definitions, will control. Accordingly, in the context of the present invention, the following definitions apply:

[0068] As used herein and in the appended claims, the singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "molecule" is a reference to one or more molecules and equivalents thereof known to those skilled in the art, and so forth.

[0069] In the context of the present invention, the term "biomimetic" refers to a material which exhibits surface properties, including but not limited to molecular structures such as amino acid and carbohydrate sequences, which provide the surface with characteristics, and in particular molecular binding or biological recognition features, which are in common with or provide functional analogues with biological features of biological materials such as tissue, and in particular cells, which the surface is intended to represent. The term biomimetic in the context of the present invention does not require that the surface duplicate all functions or binding modalities of the biological material being mimicked. Examples of preferred structures to be mimicked include pathogen binding proteins and immune recognition sequences (e.g., glycan signatures). Whether a particle moiety possesses the requisite biomimetic activity may be routinely assayed using conventional techniques known to those skilled in the art. For example, one may utilize well known immunoassay techniques, such as ELISA, to assay the binding activity of a proposed pathogen-binding biomimic as compared to endogenous host tissue. Likewise, one may utilize conventional immune response assays, such the multiplexed chemokine and cytokine assays available through Meso Scale Discovery (MSD) (Gaithersburg, Md.), to assess the risk and assay immunogenic potential of a proposed biomimic as compared to native tissue.

[0070] The present invention makes reference to glycan signatures and glycosylated molecules, and in certain instances "non-immunological, sialated, glycosylated molecules". As noted above, examples of suitable glycosylated molecules of interest in this invention include, but are not limited to glycoproteins (eukaryotic glycoproteins, proteoglycans, glycomucins), and glycolipids, including natural, synthetic, and recombinant versions thereof as well as homologues, analogues, and functional equivalents thereto.

[0071] As used herein, the term "glycosylation" refers to the addition of a carbohydrate moiety, typically a sugar, to either a biological molecule or a biocompatible synthetic molecule, such as a block copolymer. In the former context, glycosylation frequently arises as a result of co-translational or post-translational modification. In the latter context, the glycosylating moiety is frequently selected for its ability to mimic the surface properties of a biological cell or tissue and, in turn, reduce the immunogenic character of the block copolymer.

[0072] In the context of the present invention, a glycosylated lipid (or "glycolipid") is a biological molecule composed of a lipid and a carbohydrate, typically an oligosaccharide, whereas a glycosylated protein (or "glycoprotein") is a biological molecule composed of a protein and an oligosaccharide. In the context of glycoproteins, the addition of sugar chains to a protein occurs either at an asparagine moiety (referred to herein and elsewhere in the art as "N-glycosylation") or at a hydroxylysine, hydroxyproline, serine or threonine moiety (referred to herein and elsewhere in the art as "O-glycosylation"). In the context of the self-assembled films of present invention, glycoproteins with N-linked glycoyslations may provide advantages in forming self-assembled films without the assistance of other amphiphilic molecules.

[0073] Monosaccharides commonly found in eukaryotic glycoproteins include glucose, N-acetylglucosamine, galactose, N-acetylgalactosamine, mannose, fucose, xylose and N-acetylneuraminic acid (also known as sialic acid). The sugar group(s) often assist in protein folding or improve proteins' stability. Furthermore, the carbohydrate moieties of glycoproteins and glycolipids are often key components in various intercellular recognition processes, particularly immune responses. Accordingly, presentation of glycoproteins on the exposed surface of a micelle, liposome or membrane can be chosen to target appropriate tissue, inhibit uptake by a particular tissue, and/or induce endocytosis of the micelle. Antibodies, apoproteins, and opsonins are examples of glycoproteins that mediate such responses. Soluble glycoproteins are found in a wide range of biological fluids. For example, the glycoproteins "ovotransferrin", "ovalbumin" and "ovomucoid" are found in certain egg whites (albumen) and the glycoprotein "Glyocophorin A" is commonly found in blood plasma, Similarly, kappa-Casein is found in cow's milk, and the "Tamm-Horsfall" protein is found in the urine of mammals. A wide array of glycoproteins are also found in fruit juice and other plant fluids. Other glycoproteins suitable for use in the context of the present invention include, but are not limited to, human immunoglobulins, such as IgG1; hormones, such as pituitary hormones lutropin (LH), thyrotropin, and pro-opiomelanocortin; proteoglycans, and derivatives thereof. Further examples of suitable glycoprotein hormones include, but are not limited to, follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), human chorionic gonadotropin (hCG), alpha-fetoprotein, and erythropoietin (EPO).

[0074] Proteoglycans are a special class of heavily glycosylated glycoproteins comprised of a core protein with one or more glycosaminoglycan (GAG) chains. The GAG chains are long, linear carbohydrate polymers that are negatively charged under physiological conditions, due to the occurrence of sulphate or uronic acid groups. Proteoglycans are categorized by the nature of their GAG chains, examples of which include, but are not limited to, chondroitin sulfate and dermatan sulfate, heparin and heparin sulfate, keratin sulfate, etc. Examples of large proteoglycans include aggrecan, the major proteoglycan in cartilage, and versican, present in many adult tissues including blood vessels and skin. Small leucine rich repeat proteoglycans (SLRPs) include decorin, biglycan, fibromodulin and lumican.

[0075] In the context of the present invention, the term glycosylated transmembrane molecule, or transmembrane

molecule, refers to any molecule produced by a biological organism (either through natural processes or as a result of recombinant techniques) which is amphiphilic in nature, possessing a glycosylated hydrophilic end and a hydrophobic end; these molecules typically, but not necessarily project into or across cell membranes.

[0076] In the context of the present invention, the term "harvested", as in, for example 'harvested from biological tissues or cells', refers to any process which separates molecular structures of interest from the tissue or cell membranes in a manner which preserves at least part of said molecular structure's biomimetic properties relative to the source tissue or cell.

[0077] In addition to being isolated from natural sources, glycoproteins suitable for use in the instant invention can also be "derived from" biological sources, for example, synthetically produced or produced by genetically engineered plants and animals, including bacteria and other microbes, in accordance with well-known and conventional techniques.

[0078] As used herein, the term "glycan" is synonymous with the term "polysaccharide" as referring to compounds composed of a large number of glycosidically linked monosaccharide units (typically ten or more).

[0079] As used herein, the term "glycoside" generically refers to a molecule in which a sugar moiety, typically through its anomeric carbon, is attached to another non-sugar moiety. When the anomeric carbon is attached via an oxygen atom, the resulting molecule is referred to as an "O-glycoside". Similarly, when the anomeric carbon is attached via an sulfur atom, the resulting molecule is referred to as an "S-glycoside" or "thioglycosides". When the anomeric carbon is attached via a nitrogen atom, the resulting molecule is preferably designated as a "glycosylamine" rather than an "N-glycoside". Illustrative examples of amphiphilic glycosides suitable for use in the context of the present invention include, but are not limited to, chitin, chitosan, cellulose, saponin, and derivatives thereof.

[0080] The present invention makes reference to the immobilization of non-immunological, often sialated, glycosylated molecules on solid substrates to create biomimetic films and particles suitable for pathogen capture. The substrate preferably consists of a relatively non-polar material. As used herein and in the appended claims, the term "non-polar" refers to a substance or mixture of substances that is relatively uncharged when compared to a polar solvent being used. The concept is also reflected in the references herein to systems of "differing" or "diverging" polarity. As such, the terms "relatively non-polar" "less-polar" can be interchangeably exchanged herein for the term "non-polar". The non-polar material is typically water insoluble (hydrophobic). A mixture of non-polar and polar substances can be used to form the non-polar material of this invention as long as the resulting combination supports the formation of an amphiphilic film when in the presence of a selected polar solvent.

[0081] As used herein and in the appended claims, the term "substrate" refers to the relatively non-polar material in the construct of forming a biomimetic film at the interface between a non-polar substrate and a polar solvent. The substrate may be used in conjunction with other materials to provide shape, structural support or other properties of interest. Biocompatible substrate materials are of particular interest in the context of this invention. Biocompatible materials include natural materials extracted from the intended host or appropriate surrogates. Biocompatible substrate materials may also comprise synthetic and other materials which have been found to be benign when introduced in the body. The distinct advantage to biocompatible materials in the context of this invention is that they minimize the potential for adverse reactions when introduced into the intended host.

[0082] The present invention makes reference to biomimetic surfaces or films composed of solid substrates that find utility in the field pathogen capture. As discussed in greater detail below, the biomimetic solid surfaces of the present invention are useful for binding target molecules. More particularly, films bearing glycosylated molecules exhibiting appropriate binding components (typically sugars) can be used to bind, isolate and extract target molecules from aqueous samples or solutions, particularly those containing disease-causing organisms or other harmful materials such as biotoxins and heavy metals. The aqueous sample assayed is not particularly limited and includes both environmental samples and biological samples.

[0083] As used herein and in the appended claims, the term "biological sample" includes body fluids, secretions and exudates, examples of which include, but are not limited to, blood, serum, saliva, sputum, urine, plasma, spinal fluid, amniotic fluid, fluids in the gastrointestinal tract, and fluids in the lungs, sweat, breast milk, tears or other lacrimal secretions, pus, and other bodily discharges associated with either normal or diseased conditions.

[0084] As discussed in greater detail below, the biomimetic films and particles of the present invention find utility in the field of diagnostic sensing and pathogen capture. Filters made of cellulose or synthetic fibers coated with or formed from the biomimetic films of the present invention are particularly useful for removing certain organisms or biotoxins from aqueous solution. Enzymes can be incorporated into the films of the present invention to catalyze a variety of chemical reactions in aqueous media. Sensors employing such films are also useful in the context of environmental sensing, finding utility in the inspection of foods and in forensic science, for example.

[0085] As used herein and in the appended claims, the term "target molecule" encompasses both endogenous biological entities, such as peptides, proteins, hormones, oligonucleotides, nucleic acid molecules, (e.g., RNA and/or DNA), cellular components, and particulate analytes, as well as foreign materials, including, but not limited to, pathogens, toxins, drugs, contaminants, pollutants, chemical substances, and analytes. In certain instances, the presence and/or level of target molecule in a sample will correlate with a particular disease or disorder (e.g., a bacterial infection, heavy metal poisoning, cancer, etc.).

[0086] As discussed in greater detail below, the biomimetic films and particles of the present invention find particular utility in the context of pathogen capture. As used herein and in the appended claims, the term "pathogen" is used to refer to an agent of disease or disease producer and encompasses any natural or bioengineered disease-producing agent, particularly viruses, bacteria, and other microorganisms (e.g., amoeba, protozoans, etc.). Accordingly, the term pathogen includes not only infectious organisms, such as bacteria (such as staph), viruses (such as HIV), and fungi (such as yeast), but also noninfectious agents of disease such as a toxins (including molecules of both biological, non-biological (natural or synthetic) origin).

[0087] The biomimetic films and particles of the present invention also find utility in the context of environmental

detection and detoxification, for example in the removal of heavy metals. As used herein and in the appended claims, the term "heavy metal" refers to a metal having a relatively high density (i.e., a specific gravity greater than 4.0, more preferably greater than 5) or a relatively high atomic weight (i.e., falling on the periodic table between copper and bismuth). Excessive levels of heavy metals are known to be detrimental to living organisms. Examples of heavy metals associated with serious illness (e.g., heavy metal poisoning) include, but are not limited to, lead, mercury, copper, cadmium, manganese, aluminum, beryllium, molybdenum, vanadium, strontium, zinc, and iron.

[0088] The present invention is directed, at least in part, to a solid state biomimetic film composed of a non-polar substrate having a surface to which a plurality of glycosylated molecules are adhered or affixed.

[0089] In the context of the present invention, the terms "film" and "membrane" are used interchangeably to refer to the thin (usually a molecule in depth), wall-like structure formed by the specific alignment of amphiphilic molecules in the presence of polar and non-polar media. The film or membrane of the present invention can consist of a mixture of different amphiphilic molecules. Within the concept of this invention, the membrane can assume any form that the interface between polar and non-polar media can assume, including curved of any radius (including constant and irregular radius (rough) spheres), flat or a random combination (rough) surfaces. The important property is that once the non-polar media is transformed to its solid state the hydrophobic ends of the amphiphilic molecules or the matrix that the molecules form become affixed to the solid's surface while the hydrophilic ends remain free. Mullen micelles can be formed in the manner taught in the relevant Mullen patents while the non-polar media is in a liquid state. In the context of the present invention, rapid cooling is one method for preserving the micelle form in the transition of the non-polar media to a solid state.

[0090] As noted above, the term "non-polar" refers to a substance that is relatively uncharged, typically water insoluble (hydrophobic). In the context of the self-assembled amphiphilic films of the present invention, the non-polar substrate must be capable of stable expression both as a liquid and a solid. In preferred embodiments, the non-polar substrate exists in solid state at a conventional room temperature (typically between 15 and 25° C.). However, bearing in mind that thermal degradation of many amphiphilic compounds is a function of time at elevated temperature and that the non-polar substrate needs to be in a liquid state for only a very short period of time to permit the hydrophobic tails of the amphiphilic compound to become bound in the surface, relatively high processing temperatures can indeed be used without causing substantial degradation of the amphiphilic compound.

[0091] Furthermore, in that pressure and temperature are inversely related, non-polar substrates with melting points above the boiling point of the desired polar solvent can be used if the films are formed while the liquids are under pressure. Examples of non-polar materials suitable for use in the context of the instant invention include, but are not limited to, resins, synthetic and natural waxes, synthetic plastics, polymers, and copolymers, including elastic, thermoplastic and vinyl polymers, nylons, polyethylene and the like. Any mixture of hydrocarbons that results in a non-polar liquid when heated and become solid or semi-solid when cooled are appropriate. Waxes such paraffin, microcrystalline, carnauba, beeswax, candelilla, ceresine, ozokerite, and various other animal, vegetable, and synthetic waxes and blends thereof are particularly suitable for use in the instant invention. Petroleum jelly and many animal and vegetable fats are other examples of appropriate non-polar substrate materials.

[0092] As used herein and in the appended claims, the term "amphiphilic" describes any of many organic and synthetic molecules that possess both hydrophilic and hydrophobic properties. It is used interchangeably herein with the term "amphipathic" to describe molecules that have a polar end that is attracted to water and a non-polar end that is repelled by it. Amphiphilic compounds suitable for use in the context of the instant invention may express a net charge at physiological pH, either a net positive or negative net charge, or may be zwitterionic.

[0093] The hydrophobic component of an amphiphilic molecule is typically a large hydrocarbon moiety, such as a long chain of the form $CH_3(CH_2)_n$, with n>4. The hydrophilic component is either comprised of charged groups (e.g., anionic entities such as carboxylates, sulfates, sulfonates, and phosphates, or cationic entities such as amines, amino acids, or peptides) or polar groups such as alcohols. Often, amphiphilic species have several hydrophobic parts, several hydrophilic parts, or several of both. Proteins and some block copolymers are such examples. The present invention includes both glycosylated biological amphiphiles and glycosylated synthetic molecules, such as block copolymers. Illustrative methods for preparing glycosylated block copolymers are disclosed in U.S. Pat. No. 7,109,280 (Kulkarni, et. al.), the entire contents of which are incorporated by reference herein. Block copolymers can be used to construct a hydrophobic tail with the same functionality as the hydrophobic amine based tails of glycoproteins.

[0094] Surfactants such as sodium dodecyl sulphate, benzalkonium chloride, octanol, and cocaminopropyl betaine are examples of amphiphilic compounds with known industrial uses. However, as noted above, the present invention is directed to glycosylated amphiphiles, examples of which include, but are not limited to, glycoproteins, glycolipids, glycosylated block copolymers and the like.

[0095] As discussed in greater detail below, the solid state biomimetic films of the present invention may be used to create useful micelles of novel construction. As used herein and in the appended claims, the term "micelle" refers to an aggregate of molecules dispersed in a liquid colloid in which hydrophilic polar components of the molecules orient themselves toward and interact with the aqueous component while hydrophobic, lipophillic and/or nonpolar components are sequestered in the micelle structure. In a typical micelle, the hydrophilic "head" regions contact the surrounding solvent while the hydrophobic "tail" regions are sequestered in the micelle centre. This type of micelle is referred to as a normal phase micelle (oil-in-water micelle). Inverse micelles have the "head" groups at the centre with the "tails" extending out (water-in-oil micelle). Micelles are generally spherical in shape, though other phases, including shapes such as ellipsoids, cylinders, bilayers and objects with irregular or planar surfaces are also possible. The shape and size of a micelle is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH, and ionic strength, as well as the magnitude of shear forces present during formation.

[0096] As discussed in greater detail below, films and particles of the present invention are useful for binding target molecules. More particularly, membranes or micelles bearing glycosylated molecules, particularly glycoproteins, exhibiting the appropriate binding components (typically sugars) can be used to bind, isolate and extract target molecules from aqueous samples or solutions, particularly those containing disease-causing organisms or other harmful materials such as biotoxins and heavy metals. The aqueous sample assayed is not particularly limited and includes both environmental samples and biological samples. As noted above, the term "biological sample" includes body fluids, secretions and exudates, examples of which include, but are not limited to, blood, serum, saliva, sputum, urine, plasma, spinal fluid, amniotic fluid, fluids in the gastrointestinal tract, and fluids in the lungs, sweat, breast milk, tears or other lacrimal secretions, pus, and other bodily discharges associated with either normal or diseased conditions.

[0097] As discussed in greater detail below, biomimetic films and particles find utility in the fields of diagnostic sensing and pathogen capture. Filters made of cellulose or synthetic fibers coated with the glycosylated amphiphilic membranes of the present invention are particularly useful for removing certain organisms or biotoxins from aqueous solution. Enzymes can be incorporated into the micelles of the present invention to catalyze a variety of chemical reactions in aqueous media. Sensors employing such membranes are also useful in the context of environmental sensing, finding utility in the inspection of foods and in forensic science, for example.

[0098] As used herein and in the appended claims, the term "target molecule" encompasses both endogenous biological entities, such as peptides, proteins, hormones, oligonucleotides, nucleic acid molecules, (e.g., RNA and/or DNA), cellular components, and particulate analytes, as well as foreign materials, including, but not limited to, pathogens, toxins, drugs, contaminants, pollutants, chemical substances, and analytes. In certain instances, the presence and/or level of target molecule in a sample will correlate with a particular disease or disorder (e.g., a bacterial infection, heavy metal poisoning, cancer, etc.).

[0099] As discussed in greater detail below, the biomimetic films and particles of the present invention find particular utility in the context of pathogen capture. The biomimetic films and particles of the present invention also find utility in the context of environmental detection and detoxification, for example in the removal of heavy metals. As used herein and in the appended claims, the term "heavy metal" refers to a metal having a relatively high density (i.e., a specific gravity greater than 4.0, more preferably greater than 5) or a relatively high atomic weight (i.e., falling on the periodic table between copper and bismuth). Excessive levels of heavy metals are known to be detrimental to living organisms. Examples of heavy metals associated with serious illness (e.g., heavy metal poisoning) include, but are not limited to, lead, mercury, copper, cadmium, manganese, aluminum, beryllium, molybdenum, vanadium, strontium, zinc, and iron.

[0100] As discussed in greater detail below, the biomimetic films and particles of the present invention find therapeutic utility, as pharmaceutical formulations suitable for in vivo administration. In a preferred embodiment, the biomimetic particle takes the form of an ingestible micelle, suitable for oral delivery. However, other methods well known to those skilled in the art may be used to administer the pharmaceutical composition of the present invention to patients, examples of which include, but are not limited to, intraarterial, intravenous, intramuscular or percutaneous injection or via intranasal, transbronchial, transurethral, peritoneal or oral administration. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select a suitable mode and method of administration.

[0101] As discussed in greater detail below, the biomimetic films and particles of the present invention find utility as coatings for medical devices. In the context of the instant invention, the term "medical device" encompasses both devices intended for limited introduction (for example angioplasty catheters) as well as devices intended for long term insertion (for example cardiac pacemakers). More particularly, as used herein and in the appended claims, the term "medical device" refers to any apparatus, appliance, instrument, implement, material, machine, contrivance, implant, in vitro reagent, or other similar or related article including a component party or accessory which is intended for the diagnosis, prevention, monitoring, treatment or alleviation of disease, injury or handicap. It further encompasses any article intended to affect the structure or function of the body of humans or other animals, and which does not achieve its principal intended action in or on the body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

[0102] II. Non-Immunological Biomimetic Films for Pathogen Capture:

[0103] As noted previously, it is an object of the present invention to immobilize non-immunological glycosylated molecules with biomimetic properties on the surface of solid substrates for the purpose of pathogen, capture, concentration and/or detection. Central to the instant invention is the discovery that the pathogens and, to a certain extent, toxins exhibit tissue tropism based on adhesins which bind to specific combinations, sequences and/or orientations of glycans expressed on molecules at the surface of tissues in a prospective host organism. These molecules can be harvested from said tissues, or cultures of said tissues, or homologues, analogues or functional equivalents thereof through any number of techniques for rupturing the cell membrane known to those skilled in the art. These molecules can also be produced by other cells or organisms through recombinant techniques. In certain instances these molecules are also accessible in bodily fluids. The resultant structures are unique in their ability to capture pathogens and toxins on the basis of tissue tropism.

[0104] A. Identifying and Isolating Suitable Biomimetic Molecules:

[0105] One aspect of the present invention relates to the extraction of glycosylated structures from tissue and tissue analogues (e.g., glycan signatures), particularly those suitable for pathogen capture or immune system modulation. The present invention is unique in the use of a variety of non-immunological molecules which mimic the glycans signatures of epithelial and other cells of a pathogens target host, such that these molecules are immobilized on solid surfaces such that they can affect pathogen binding to the surfaces. The present invention is also unique in the method of using the resulting constructs. Other unique aspects of the present invention include the use of a variety of molecules from a body tissue to form biomimetic surfaces.

[0106] The present invention provides a unique method for creating biomimetic films, particles, and constructs thereof utilizing glycosylated molecules harvested, or otherwise derived, from tissues of interest. Consequently, it is further an object of the present invention to teach both the constructs and the method for utilizing molecules obtained from tissues from a biological organism. The molecules can be extracted from tissues obtained from the potential target organism of an infection, the host. The host tissues of particular interest are from the specific body area of potential infection, thus taking advantage of the same properties which permit pathogens to demonstrate host tropism. For example, a gastro-intestinal infection may exhibit adhesins to glycoproteins (particularly glycomucins) present in a specific section of the small intestine. In the context of the methods of the present invention, glycoproteins extracted from tissue in the affected region of the intestine can be immobilized on a solid substrate which, in turn, maybe be used for pathogen capture.

[0107] In addition to extracting molecules of interest from tissue obtained directly from the host organism, the present invention also provides for molecules of interest to be obtained from cultures of tissues derived or extracted from the host organism, from biological surrogates, or from other organisms genetically engineered to produce tissue mimics or functional analogues. For example, porcine tissue is often used as a biological surrogate for human tissue. Avian eggs have been demonstrated to produce glycosylated molecules of interest. There are a wide range of other potential surrogates and biological sources.

[0108] Glycosylated molecules with the desired glycans or glycans sequences can also be constructed through chemical synthesis, and are therefore also part of the present invention. Recombinant techniques and genetic engineering can also be used to induce other organisms, such as yeast and bacteria, to produce the glycosylated molecules (and particularly sialated glycosylated molecules) of interest in this invention in commercial quantities. [4]

[4] Hamilton et al., Humanization of Yeast to Produce Complex Terminally Sialylated Glycoproteins Science 8 Sep. 2006: 1441-1443 DOI: 10.1126/science.1130256

[0109] Glycosylated molecules of interest can also be harvested, or otherwise derived, from fluids present in or produced by an organism, including but not limited to body fluids such as mucous, blood, saliva, urine, synovial fluid, breast milk, tears, fluids of the reproductive system (including those related to birth). For example, urine contains the glycoprotein, Tamm-Horsfall Protein (THP), also known as Uromodulin (UMOD), and Tamm-Horsfall mucoprotein, THP exhibits a broad range of glycans structures useful in the capture of potential urinary tract infections. Blood contains albumin, which is widely known to bind to a variety of pathogens and is readily available in the form of Bovine Serum Albumin (BSA).

[0110] Glycosylated molecules in bodily fluids may be present as individual molecules and readily available for extraction and immobilization on a surface. In many other situations, the molecules of interest may be membrane bound, as with the glycosylated transmembrane molecules which populate cell walls (this can be true in both tissues and in bodily fluids). In those instances, the cell must be lysed or ruptured in a manner whereby the molecules of interest can be captured and immobilized. Methods for cell lysis are known to those skilled in the art and include, but are not limited to, mechanical means (such as sonication, freeze/thaw and high shear techniques), chemical means (for example, using detergents for whole cell lysis and cell fractionation) and biochemical means (for example, by means of enzymes and/or protease inhibitors).

[0111] Solutions of bodily fluids or solutions containing lysed cell material often include a wide variety of constituents, some of which are extraneous to the purposes of this invention. Many of the glycosylated molecules are amphiphilic in nature, particularly those which are produced as transmembrane molecules. These amphiphilic molecules tend to form micelles or micelle-like structures or to align at the interface between systems of divergent polarity, for example between a polar solvent and a relatively less polar material. In the context of the present invention, this trait can be used to extract molecules of interest from a solution of bodily fluids or solutions containing lysed cell material. In certain aspects of the present invention, the molecules can be immobilized directly on to the less-polar material to form the desired biomimetic constructs through the formation of self-aligning films and then the immobilization of those films through the transition of the less-polar material from a liquid to a solid. In other aspects of the present invention, the extracted molecules can be introduced into a system where they are conjugated to other molecules with appropriate binding affinities which are already immobilized on the substrates of this invention. In either case, when molecules of interest are extracted from bodily fluids using constructs of the present invention, additional molecules not of interest may be present in the final construct. The presence of these additional molecules can be complimentary or extraneous to the goal of the invention, which is to present a biomimetic selection of glycosylated molecules on a solid substrate. The unique advantage of the present invention in the procedure of extracting materials directly from bodily fluids or tissues is that the identity or nature of the glycosylated molecules of a tissue of interest need not be known to effectively use the molecules in the constructs of the present invention, this represents a significant advantage over techniques which seek to mimic a specific, defined glycans structure, since the broad range of potential pathogen affinities to tissue have not been defined.

[0112] B. Immobilizing Biomimetic Glycosylated Molecules:

[0113] Techniques for binding biological compounds to other materials are well-known to those skilled in the art. These techniques include means dependent on chemical, mechanical and electrical forces (including weak forces such as van der Waals forces). Techniques utilized for immobilizing antibodies on surfaces are also applicable to immobilization of the biomimetic molecules of the present invention. In addition, in that many of the biomimetic molecules utilized are either themselves amphiphilic in nature or can be conjugated with hydrophobic molecules to create an amphiphilic molecule, they may be immobilized using methods of the present invention for the creation of solid state films from amphiphilic molecules described in further detail below.

[0114] Substrates of interest suitable for use in the context of the present invention include, but are not limited to, polymers, plastics, resins, natural and synthetic waxes, metals, glasses, natural and synthetic fats, ceramics and combinations thereof. Particularly useful forms or configurations of interest include flat or formed surfaces, filter type membranes, fibers, and beads (including micelles and micelles-like structures).

[0115] Molecules of interest can readily be adsorbed on to any of these substrates given an appropriate electrical charge

difference between the substrate and the desired binding surface of the molecule. Substrate charge can be a result of induced voltage, inherent charge, embedded materials, or chemical or other surface treatment (such as plasma treatment). The relative charge of oil droplets in water can be adjusted through pH changes. The advantage of using adsorptive techniques is the relative simplicity of implementation and the minimization of disruption of the chemical properties of the molecule. The disadvantage is the potential of the bond to be disrupted when changing environments during use.

[0116] Alignment and immobilization through the use of surfactant forces is also of interest in the present invention. The creation and use micelle-like films and structures using amphiphilic molecules is known to those skilled in the art. The present invention expands on that art and provides novel constructs and methods based on immobilization of glycosylated amphiphiles on solid substrates. Techniques of relevance to the creation of constructs of interest in the present invention have also been taught in the use of surfactants to create solid lipid nanoparticles (SLN). The solid lipid nanoparticles tend to be micelle-like particles between 10 nanometers and 1 micrometer (sometimes 10 micrometers) in size.

[0117] In Published U.S. Patent Application No. 2006/0083781, the entire contents of which are included herein by reference, Shastri, et. al., teach methods and constructs for solid lipid nanoparticles, with particular focus on delivery of active agents across the blood-brain barrier, across a cellular lipid bilayer and into a cell, and to a subcellular structure. The present invention extends the use of these methods in the creation of biomimetic surfaces through the immobilization of glycosylated molecules and the use thereof for pathogen and toxin capture.

[0118] Many of the techniques of value in the immobilization of glycosylated molecules of interest in the present invention are suggested in connection with the immobilization of other types of molecules, for use in other applications. The techniques are generally based on conjugation of proteins with polymers (particularly copolymers), other proteins or carbohydrates.

[0119] Techniques taught by others for the immobilization of bioactive peptides can also be utilized to immobilize biomimetic molecules derived from biological tissue. Heparin and heparin-like molecules can be usefully conjugated to the biomimetic molecules of the present invention and these conjugated constructs can then be further conjugated to other molecules to create an immobilization structure. Heparin binding of bioactive peptides and synthetic molecules has been taught by Zamora in U.S. Pat. No. 6,921,811 and U.S. Pat. No. 7,297,343 and patent application US 2006/0024347, the entire contents of which are included herein by reference. The use of heparin binding of bioactive peptides has also been taught by Stupp, et. al. in patent application US 2007/0277250, the entire contents of which are included herein by reference.

[0120] Conjugates of heparin with bioactive peptides and a hydrophobic component to create an amphiphilic peptide compound have been described by Zamora and Stupp (both reference above). These same techniques are useful in the conjugation of biomimetic materials harvested from biological tissue to heparin and a hydrophobic component to create amphiphilic molecules of use in the constructs of the present invention. These synthetic compounds are useful in creation of self-assembled structures. Stupp in the reference above has taught the formation of nanofibers, also worm micelles. The nanofiber structures consist solely of amphiphilic compounds aligning to exclude other material from the hydrophobic centerline of the fiber. These nanofibers can entangle to create a hydrogel structure. The hydrogel structure has proven useful for entrapping other materials. It is the object of the present invention to utilize these hydrogel structures to form a construct which does not readily absorb into surrounding biological tissue. Stupp also has taught the creation of amphiphilic peptide compounds through the conjugation of a hydrophobic component and a growth factor recognition product of a phage display process to a peptide in patent application US 2005/0209145, the entire contents of which are included herein by reference. It is an object of the present invention to utilize pathogen and toxin recognition products of a phage display process in a similar technique to form a construct of the present invention. Create of said constructs can either be through conjugation of said product of a phage display process with a hydrophobic molecule to form an amphiphilic molecule of use in the present invention, or it could be through direct conjugation of said product of a phage display process with surface molecules of substrates to form constructs of the present invention.

[0121] Yet others have taught the immobilization of bioactive species on cross-linked block copolymer surfactants, and as above it is the object of the present invention to use the same techniques to immobilize biomimetic molecules harvested from biological tissue. See, for example, U.S. Pat. No. 5,897,955 to Paul D. Drumheller, the entire contents of which are incorporated herein by reference for relevant techniques applied to bioactive peptides. In particular, Drumheller describes the attachment of bioactive species using a cross-linking compound to copolymer surfactants which are bound to a substrate.

[0122] Bhaskaran, et. al. in U.S. Pat. Appl. 2004/0136952, the entire contents of which are incorporated herein by reference, teaches methods of synthesizing polymer conjugates of growth factor proteins and other compounds while maintaining a high level of functionality of these biological compounds. These techniques also find utility in the context of the present invention in the context of using similar techniques to form conjugates with biomimetic molecules harvested from biological tissue.

[0123] Techniques known in the art for immobilization of bioactive molecules on metallic and polymeric surfaces by the process of adsorption can be applied in the immobilization the biomimetic molecules harvested from biological tissue of the present invention, and such application is an object of the present invention.[5] [6]

[5] Jennissen, H. P., Chatzinikolaidou, M, Rumpf, H. M., (2000) Modification of metal surfaces and biocoating of implants with bone-morphogenetic protein 2 (BMP-2), DVM Bericht 313:127-140.

[6] Schrier, J. A., DeLuca, P. P., Porous Bone Morphogenetic Protein-2 Microspheres: Polymer Binding and In Vitro Release, AAPS PharmsciTech 2001; 2 (3) Article 17.

[0124] Methods currently in use for the immobilization of antibodies on solid substrates are also of particular use in connection with the constructs of this invention. Antibodies are a specific type of immunological glycoprotein. For commercial purposes, they are typically immobilized on magnetic beads and on surfaces constructed of block copolymers. For analytical purposes, they can also be immobilized on glass slides. In the context of the present invention, it is an objective to immobilize antibodies on solid substrates which are of a size and configuration that is not absorbed by surrounding tissue such that an antibody bearing construct can bind to a

targeted pathogen or toxin and then the construct can be removed or expelled from the body.

[0125] Magnetic beads are readily available with a variety of available binding sites for the conjugation of molecules. Commercially available beads include Amine-terminated Beads, DADPA-terminated Beads, Carboxy-terminated Beads, Carboxy-terminated Beads, Epoxy-activated Beads, Epoxy-activated Beads, Aldehyde-modified Beads, Aldehyde-modified Beads, Hydrazide-modified Beads, IDA-modified Beads and Silica-modified Beads. Kits are readily available to conjugate a variety of molecules to these beads. Many of these materials and techniques are of applicable to the creation of the constructs of the present invention, as in the binding of to solid substrates of various biomimetic molecules harvested from biological tissue, and also in the binding of antibodies. A typical example involves the use of biotintylation to conjugate a glycoprotein to a magnetic bead with activin present on the surface.

[0126] Block copolymers have also been used to immobilize antibodies in solid surfaces. In work done at Purdue University, antibodies were immobilized on polycarbonate surfaces using Poly-L-Lysine and the resultant surfaces were used for pathogen capture to facilitate detection.[7] This type of immobilization is also applicable to biomimetic molecules harvested from biological tissue and the use in that context is an object of the present invention.

[7] Chen, W.-T. (Speaker), M. R. Ladisch, T. Geng, and A. K. Bhunia, Pathogen Capture and Concentration on Functionalized Polycarbonate Membrane Detection and Sample Preparation Based on Immuno-Filters, BIOT Division Paper 140, 227th ACS National Meeting, Section: High Throughput Screening/Genomics and Proteomics, Anaheim, Calif. (Mar. 30, 2004).

[0127] Block copolymers have been used to construct super-amphiphilic molecules and polymersomes. These constructs are described in U.S. Pat. Nos. 6,835,394 and 7,217,427 and U.S. Pat. Appl. No. 2007/0218123, all to Ennis E. Discher, et. al., the entire contents of which are incorporated herein by reference. The patents describe the use of such constructs in the formation of vesicles that enclose materials of biological interest which may then embedded or conjugated with carrier material. The vesicle walls are semi-permeable to permit the material of biological interest to diffuse into the surrounding environment. It is an object of the present invention that these same constructs and surface block copolymers can be used to immobilize the biomimetic glycosylated molecules harvested from biological tissue to form constructs of the present invention. Polyethylene glycol (PEG) is an example of a polymer that is applicable to forming conjugations with biomimetic molecules to create constructs of the present invention.

[0128] In sum, the science of forming conjugates of proteins, and other functional biological materials relevant to this patent, and other molecules, specifically other biological and synthetic compounds, is well understood by those skilled in the art. In fact, numerous methods for forming protein to protein conjugates are available and readily known to those skilled in the art.[8,9] These techniques are of use in the context of the present invention in the immobilization of biomimetic molecules harvested from biological tissue to substrates in the formation of constructs of the present invention. To the extent that some of these techniques can also be used to form amphiphilic conjugates they are also applicable to other embodiments of the present invention.

[8] Stowell, C. P. and Lee, Y. C., *Neoglycoproteins: The Preparation and Application of Synthetic Glycoproteins*, ADVANCES IN CARBOHYDRATE CHEMISTRY AND BIOCHEMISTRY (Vol 37), 1980, Academic Press, Inc., pp. 225-281

[9] Pozsgay, V., Kubler-Kielb, J., *Conjugation methods towards synthetic vaccines*, Carbohydrate-Based Vaccines, ACS Symposium Sieries, R. Roy, Editor, In press, 2006.

[0129] In U.S. Pat. No. 6,322,810, the entire contents of which are included herein by reference, Alkan-Onyuksel, et. al. teach the use of lipid-polymer conjugates in the creation of micelle structures followed by an incubation step and the subsequent incorporation of biologically active amphiphilic compounds into the <20 nanometer diameter construct. The present invention can utilize techniques presented by Alkan-Onyuksel. The present invention is unique in the creation of constructs which incorporate epithelial cell moieties and other pathogen binding site molecules and their functional analogues in lieu of the various hormonal peptides envisioned by Alkan-Onyuksel. Additionally, in the context of the therapeutic constructs described below, it is an object to provide for constructs which are large enough to not be readily absorbed by surrounding tissue this generally places the constructs of the present invention in a size range greater than the 20 nanometer diameter limit taught by Alkan-Onyuksel

[0130] C. Constructing Biomimetic Films for Pathogen Capture:

[0131] The present invention is not limited to a particular construction method. However, the following methods are suitable for constructing biomimetic films and particles of the present invention and are therefore provided for illustration purposes.

[0132] An illustrative method for making biomimetic films and particles of the present invention comprises the following steps, with resultant films on solid substrates:

[0133] a. providing a relatively polar solvent having sufficient quantities dissolved therein of one or more amphiphilic molecules which include glycosylated molecules with biomimetic properties, or conjugates of such, or molecules which can be conjugated with glycosylated molecules with biomimetic properties, or molecules with other pathogen binding moieties;

[0134] b. exposing the polar solvent to a relatively non-polar liquid, the non-polar liquid being immiscible in the polar solvent; the relatively non-polar liquid can optionally comprise a material which can reversibly experience a state change from solid to a relatively non-polar liquid under stimulus;

[0135] c. allowing the amphiphilic molecules to align so as to form a membrane which separates the polar solvent from the non-polar liquid; and

[0136] d. inducing or allowing transformation of the non-polar liquid to a corresponding non-polar solid having upper and lower surfaces, wherein the hydrophobic tail ends of the amphiphilic molecule are embedded in or chemically, electrically or mechanically linked to the upper surface of the non-polar substrate and the hydrophilic tail ends project from the upper surface into the polar solvent so as to yield a film having functional activity; and/or

[0137] e. if additional properties are required: adsorbing or conjugating molecules with biomimetic or other properties to the functional surface of the film if the desired functionality is not present from the preceding steps.

[0138] f. Optionally, remove said film from any surrounding liquid to form a dry construct.

[0139] The construction method may further include a step wherein the non-polar liquid and the liquid containing the amphiphilic molecules are manipulated prior to or during

transition of the non-polar liquid to a solid state in order to yield a film having a geometric form. An exemplary manipulation method can comprise the step of agitating the film so as to form micelles, worm micelles or micelle-like constructs with non-polar substrates.

[0140] In the context of the above-described construction method, the transformation of the non-polar liquid to a corresponding non-polar solid can be performed in a manner so as to maintain the approximate geometric form achieved by the non-polar liquid prior to or during the transition to a solid form, and that this transformation can be achieved through methods which include, but are not limited to, rapid cooling of or removal of pressure force on the non-polar liquid.

[0141] An alternate method for making biomimetic films and particles of the present invention comprises the following steps, with resultant films on solid substrates:

[0142] a. providing a liquid having sufficient quantities dissolved therein of one or more molecules with biomimetic properties or pathogen or toxin binding properties;

[0143] b. exposing said liquid to a solid with surface moieties appropriate for conjugation with said molecules;

[0144] c. allowing or inducing the conjugation of said molecules to said surface moieties in a manner which results in the biomimetic moieties or pathogen or toxin binding moieties expressed on the surface of the substrate.

[0145] d. Optionally, removing said film from any surrounding liquid to form a dry construct.

[0146] Biomimetic films from biological cell- or membrane-bound glycosylated molecules may be constructed according to the following steps:

[0147] a. Isolating biological tissue extracted from human, animal or plant tissue, a culture of human, animal or plant tissue, a biological surrogate for human, animal or plant tissue, a culture of a biological surrogate for human, animal or plant tissue or recombinant versions of human, animal or plant tissue or human, animal or plant tissue biological surrogates.

[0148] b. Subjecting the biological cell or membrane to a process which ruptures or lyses the cell or other membrane, including but not limited to, mechanical means (for example, but not limited to, sonication, freeze/thaw and high shear techniques), chemical means (for example, but not limited to, using detergents for whole cell lysis and cell fractionation) and biochemical means (for example, but not limited to, by means of enzymes and/or protease inhibitors), to form a liquid volume with biomimetic molecules present.

[0149] c. Optionally, filtering or otherwise selectively separating a fraction of interest containing glycosylated molecules with biomimetic properties, said fraction may or may not comprise a liquid or other molecules

[0150] d. Immobilizing said biomimetic molecules as part of a film on an appropriate substrate.

[0151] e. Optionally, removing said film from any surrounding liquid to form a dry construct.

[0152] The methods above are equally applicable when the biological tissue includes attaching and effacing (A/E) lesions or their functional analogues.

[0153] Alternatively, the biomimetic films from biological cell- or membrane-bound glycosylated molecules may be constructed as follows:

[0154] a. Biological tissue is isolated, the biological tissue, including, but not limited to, biological tissue extracted from human, animal or plant tissue, a culture of human, animal or plant tissue, a biological surrogate for human, animal or plant tissue, a culture of a biological surrogate for human, animal or plant tissue or recombinant versions of human, animal or plant tissue or human, animal or plant tissue biological surrogates. Examples of suitable biological tissues include, but are not limited to epithelium, connective tissue, muscle tissue, nerve tissue and associated bodily systems and organs and material associated with or contained in amniotic fluid surrounding a fetus, aqueous humour, blood and blood plasma, interstitial fluid, breast milk, mucus, pus, saliva, serum, tears, urine, cerebrospinal fluid, synovial fluid, intracellular fluid, aqueous humour and vitreous humour and other bodily fluids, material from avian species, including material from eggs and tissues from plant origins.

[0155] b. In the case where molecules of interest are bound in cell membranes of other constructs, the biological tissue may then be lysed or otherwise processed using techniques known to those skilled in the art to rupture cell membranes in manner where non-immunological, glycosylated molecules of cell membranes and fluids and surrounding tissue fluids are liberated into a solution.

[0156] c. The solution may then subjected to procedures to permit some or all of the non-immunological, glycosylated molecules to bind to a substrate to form the constructs of the present invention; this procedure can include, but does not have to include, intermediate steps of refinement or isolation of the molecules, or enrichment of the solution with other amphiphilic molecules to achieve critical micelle concentration.

[0157] In the context of the above method, non-immunological, glycosylated molecules of an amphiphilic nature can be extracted from solution prior to or during the binding process by a method that includes the steps of:

[0158] a. Exposing the solution containing biological tissue, which can comprise, in part, lysed or otherwise ruptured cell material containing molecules of interest in the present invention to a less-polar material;

[0159] b. If necessary, adding amphiphilic molecules to achieve critical micelle concentration, before or after exposing said solution to said less-polar material;

[0160] c. Allowing the amphiphilic compounds to form a film at the interface between said solution and said less-polar material;

[0161] d. Conducting any desired procedures to affix the molecules to the less-polar substrate beyond the hydrophobic forces inherent in the film; and

[0162] e. Removing or replacing some or all of said solution with another liquid.

[0163] The resulting biomimetic films resulting of the present invention can be utilized in the construction of a surface having enhanced biocompatibility with particular applicability to a medical device, in a manner analogous to that described in detail below. In that context, the biomimetic surface molecules can be obtained from the tissue of the intended medical device host organism (also recipient) or an antigen-matched donor thereof or from a culture of tissue from said recipient or donor.

[0164] The present invention further provides a method for using the biomimetic films for pathogen capture, the method comprising the steps of

[0165] a. exposing said film to a liquid sample which may or may not contain pathogens or toxins,

[0166] b. allowing sufficient time for said film to bind to target pathogens or toxins present in said sample;

[0167] c. optionally separating said film, to which said target pathogens or toxins may be bound, from said sample.

[0168] d. optionally, analyzing said film to detect the presence or type of pathogen or toxin, or subjecting said surface, to which said target pathogens or toxins may be bound, to an agent which effectively separates the pathogens or toxins from the film and subjecting said pathogen or toxins, or media containing such, to analysis techniques to detect presence or type.

[0169] The above method of use can further comprise the step of calculating the amount of target molecule (pathogen or toxin) present in the sample based on the amount of target molecule bound to the film.

[0170] In the context of the above method of use, the pathogen or toxin can be an agent of disease or disease producer and encompasses any natural or bioengineered disease-producing agent, particularly viruses, bacteria, other microorganisms (for example, but not limited to, amoeba and protozoans), fungi (for example, but not limited to yeast), and toxins (including molecules of both biological, non-biological (natural or synthetic) origin), additionally said toxin can comprise a heavy metal.

[0171] In the context of the above method of use, the analysis step can utilize a detection technology selected from, but not limited to, the group consisting of PCR, immunoassays, DNA microarrays, protein microarrays, spectral analysis, and laser based and other optical techniques.

[0172] In the context of the above method of use, the liquid which may or may not contain pathogens or toxins can be derived from a food product, the processing of a food product or a potable water supply.

[0173] In the context of the above method of use, the liquid which may or may not contain pathogens or toxins can be from an environmental water sample, process water sample, process effluent sample, wastewater sample or is from a water sample from an HVAC or air scrubber system.

[0174] In the context of the above method of use, the liquid sample can be a biological sample selected from the group consisting of blood, serum, saliva, sputum, urine, plasma, cerebrospinal fluid, amniotic fluid, fluids in the gastrointestinal tract, and fluids in the lungs, sweat, breast milk, tears or other lacrimal secretions, pus, and other bodily discharges associated with either normal or diseased conditions. It is also an object of the present invention that said method can be performed in vivo.

[0175] In the context of the above method of use, the liquid which may or may not contain pathogens or toxins can include sample preparation either before and/or after exposure to biomimetic films of the present invention, said sample preparation comprising none or one or more of the following steps:

[0176] a. Enrichment of the sample with a nutrient compound,

[0177] b. Incubation of the sample at a temperature between 35 degrees and 40 degrees centigrade for a period of time,

[0178] c. Incubation in an anaerobic environment.

[0179] In the context of the above method of use, the sample can comprise a continuous fluid stream and the vessel through which said fluid stream sample is passed can have a fluid stream contacting surface which comprises a biomimetic surface of the present invention.

[0180] In the context of the above method of use, the fluid sample which may or may not contain pathogens or toxins, can be subjected to a force which promotes contact between the pathogens or toxins and the biomimetic surface, said force selected from the group consisting of, but not limited to, agitation, mechanical acceleration, centrifugal force, electrical force, magnetic force, and hydraulic force.

[0181] III. Treatment for Infection by Toxin Producing Pathogens:

[0182] The pathogen and toxin capture constructs described above, as well as similar biocompatible particles constructed with other pathogen and toxin binding molecules, such as antibodies, lectins, and other molecules also have a therapeutic use. In particular, when said molecules are immobilized on a substrate which does not degrade in a host organism environment, including for example, wax, and when such constructs are of a size and/or geometric configuration where they are not readily absorbed into the surrounding tissue of the host organism. For roughly spherical particles, a minimum nominal diameter greater than something in the range of 50-500 nanometers is considered optimal, though a dosage can comprise a distribution of particle sizes, and it is considered advantageous, but not required, that the average nominal diameter be 500 nanometers or greater.

[0183] Thus, the present invention is directed to a therapeutic biocompatible particle or material (also referred to as a carrier or substrate) in a size and geometric configuration which is not readily absorbed into the biological tissue of the host organism being treated, wherein said biocompatible particle has affixed to its surface molecules which exhibit properties which bind to pathogens. Illustrative host organism biological tissue include, but are not limited to the walls or membranes (either inside or outside) of the organs of a body, alimentary tract (also gastrointestinal tract), urinary tract, pulmonary tract, blood vessels, amniotic sac, ocular sac, nervous system (for example, but not limited to, the brain or spine), membranes of the musculoskeletal system or cell walls. In the context of the present invention, a plurality of biocompatible particles and associated pharmaceutical formulations thereof can be ingested or introduced into a host for therapeutic benefit resulting from said pathogens binding to the constructs and that the constructs are expelled or removed from the host body with the effect of reducing the concentration of pathogen within the host body.

[0184] The present invention represents an improvement over anti-adhesion drugs of the prior art, wherein pathogen binding molecules in solution are introduced in an attempt to coat the pathogen and blind all the pathogen's tissue binding sites. In the absence of an immobilizing carrier structure, such molecules are of a size and often a nature where they are readily absorbed through the intestinal (or other system) wall and thus do not achieve the goal of pathogen removal. Additionally, to blind all the adhesins on a specific pathogen (i.e., to completely coat the pathogen), the binding molecules have to be introduced in high concentrations. The high concentration of said molecules can also have a dehydration effect on the host due to osmotic pressures (i.e., water from surrounding tissues moves through membranes to dilute the concentration of the introduced binding molecules); this dehydration

effect can exacerbate stress conditions on the host. The dehydration effect is particularly significant in the case of gastro-intestinal illnesses where dehydration is already an issue.

[0185] In the case of the present invention, where the pathogen binding moiety is immobilized on a substrate which in not absorbed by the tissues of the body and can readily be removed or expelled from the body, only a sufficient number of pathogen adhesin sites need to be engaged to bind the pathogen to the substrate, the pathogen does not need to be completely coated for there to be a therapeutic effect. Additionally, since in the present invention the binding molecules are immobilized on a substrate, they are not lost through absorption into surrounding tissue. Finally, the immobilization of the binding molecules on a substrate also dramatically reduces any osmotic pressure issues and thus treatment with the present invention does not exacerbate dehydration of the host as much as introducing non-immobilized molecules might.

[0186] In the context of therapeutic treatment for infection with a bacteria associated with toxin production, such as *E. coli* 0157:H7, the present invention also represents an improvement over the use of antibiotics which can trigger an immediate release of high concentrations of toxin. As mentioned in the New England Journal of Medicine article referenced above, the use of antibiotic treatment is not recommended in the case of *E. coli* 0157:H7 infection. Similar hazards may be present with infection by other toxin-producing bacteria, including strains of *Vibrio cholerae, Salmonella, Shigella, Clostridium, Helicobacter*, enteropathogenic *E. coli* (EPEC). and enteroaggregative *E. coli* (EAEC). The present invention binds the bacteria in a manner where they can be expelled, rather than stress the bacteria in a manner where toxin production is increased. The present invention also represents an improvement over antibiotic treatment in the ability of the technology to bind and remove both the bacteria producing the toxin, as well as the toxin itself.

[0187] Additionally, since in many instances, the molecular moieties represented on the surface of the present invention constitute glycans structures which are natural to the host body (and thus identified by the immune system as "self antigens"), the present invention has the potential to avoid adverse immune response effects on or reactions by the host body. There is also the potential that the structures of the present invention may have less of a detrimental effect on beneficial bacteria present in the gastro-intestinal or other bodily systems.

[0188] Of particular interest in the context of the present invention are the patents of Dr. G. D. Armstrong and various co-inventors, listed below, the entire contents of which are included herein by reference:

[0189] 5,484,773—Treatment of Antibiotic-Associated Diarrhea.

[0190] 5,620,858—Method of Removing Shiga-like Toxins from Biological Samples.

[0191] 5,627,163—Treatment of Traveller's Diarrhea (claims to binding *E. coli*).

[0192] U.S. Pat. No. 5,635,606—A Method of Removing Toxin A.

[0193] U.S. Pat. No. 5,637,576—Treatment of Traveller's Diarrhea.

[0194] U.S. Pat. No. 5,661,131—Treatment of Cholera.

[0195] U.S. Pat. No. 5,679,653—Diagnosis and treatment of bacterial dysentery.

[0196] U.S. Pat. No. 5,811,409—Treatment of Cholera.

[0197] U.S. Pat. No. 5,817,633—Treatment of Cholera.

[0198] U.S. Pat. No. 5,849,714—Treatment of bacterial dystentery.

[0199] U.S. Pat. No. 5,858,698—Methods for detection of enteropathogenic *e. coli.*

[0200] U.S. Pat. No. 5,891,860—Treatment of traveller's diarrhea.

[0201] U.S. Pat. No. 5,939,397—Treatment of cholera.

[0202] U.S. Pat. No. 5,955,449—Diagnosis and treatment of bacterial dysentery.

[0203] U.S. Pat. No. 5,962,423—Treatment of bacterial dysentery.

[0204] U.S. Pat. No. 6,013,635—Treatment of *C. difficile* toxin B associated conditions.

[0205] U.S. Pat. No. 6,069,137—Treatment of traveller's diarrhea.

[0206] U.S. Pat. No. 6,107,282—Treatment of *C. difficile* toxin B associated conditions.

[0207] U.S. Pat. No. 6,121,242—Treatment of bacterial dysentery.

[0208] U.S. Pat. No. 6,224,891—Compounds and methods for the treatment of bacterial dysentery using antibiotics and toxin binding oligosaccharide compositions.

[0209] U.S. Pat. No. 6,262,037—Pharmaceutical compositions for the amelioration of enteropathogenic *E. coli* infection.

[0210] U.S. Pat. No. 6,291,435—Treatment of diarrhea caused by enteropathogenic *Escherichia coli.*

[0211] U.S. Pat. No. 6,310,043—Treatment of bacterial infections.

[0212] U.S. Pat. No. 6,358,930—Treatment of *C. difficile* toxin B associated conditions.

[0213] U.S. Pat. No. 6,465,435—Treatment of *C. difficile* toxin B associated conditions.

Dr. Armstrong, et. al., describe the therapeutic use for gastro-intestinal infections of a monosaccharide or oligosaccharide sequence covalently attached through a non-peptidyl compatible linker arm to an inert substrate, such as diatomaceous earth or silica particles, wherein said monosaccharide or oligosaccharide sequence binds a specific toxin. The techniques and constructs taught by Armstrong, et. al. are applicable to certain aspects of the present invention. However the present invention is unique and represents a significant improvement to the above mentioned patents in various areas that will be apparent to those skilled in the art. Among those unique aspects of the present invention in the field of therapeutic use for treatment of infection in comparison to said patents are:

[0214] 1. In certain aspects, the present invention utilizes glycosylated molecules from potential host organism tissues or their homologues, analogues or functional equivalents. This permits the potential for binding pathogens and toxins by the same mechanisms which permit those entities to demonstrate tissue tropism. This also permits constructs of the present invention to bind pathogens and toxins by multiple mechanisms, even unanticipated or not previously understood ones. Additionally, synthetically produced glycans structures may lack important components or molecular orientations which are inherent in tissue produced glycosylated molecules.

[0215] 2. In other aspects of the present invention, the present invention teaches the use of amphiphilic compounds which self-align at the interface between a polar solvent and a non-polar surface to form a film which can

incorporate glycosylated moieties at the surface. These self-aligned surfaces can be immobilized on wax or polymer substrates to form economical constructs for therapeutic use from materials which are generally regarded as safe and which can incorporate a broad range of natural and synthetic glycosylated molecules. In certain instances, certain elements of the patents referenced above can be utilized in conjunction with the present invention to provide unique constructs with additional value.

[0216] 3. In certain aspects, the present invention is taught for use in cases beyond the oral treatment of gastro-intestinal infections. The present technology also has utility in areas, such as, but not limited to, the urinary system, ocular fluid, blood and other circulatory fluids, saliva and any other bodily fluid or system where the material of the present invention can be introduced and then extracted. The present invention is also taught for introduction into other areas of infections through other means, such as through catheters or surgically. The present invention is also taught for the removal of pathogens and toxins from bodily fluids which are extracted from and then returned into the source body.

[0217] 4. The present invention also teaches unique methods for extraction of glycosylated molecules from solutions, and particularly tissues and bodily fluids, for use in pathogen and toxin capture constructs.

[0218] A. Identifying Suitable Pathogen-Binding Molecules:

[0219] As noted previously, it is an objective of the present invention to provide a solid state biocompatible film or particle comprised of a substrate material having a homogenous or heterogeneous population of pathogen-binding molecules affixed thereto, the substrate material which is of a physical size and shape such that it can be readily introduced into a host through ingestion or the use of a device, such as a catheter but also such that the resulting film or particle is not readily absorbed by surrounding tissue.

[0220] In a preferred embodiment, the pathogen binding moieties are composed of glycans or glycan sequences, natural or synthetic, which mimic pathogen binding sites in the host body. Such pathogen binding moieties may be harvested or derived for a target tissue of interest (including a tissue culture), produced by organisms developed through recombinant techniques, or alternatively produced from phage display techniques.[10]

[10] Smith, George P., Petrenko, Valery A., Phage Display, Chem. Rev. 1997, 97, 391-410

[0221] Examples of illustrative glycan sequences of interest include, but are not limited to those expressed in glycoproteins (eukaryotic glycoproteins, proteoglycans, glycomucins) or glycolipids (glycosphingolipids), including natural, synthetic, and recombinant versions thereof as well as homologues, analogues, and functional equivalents thereto. The pathogen binding moieties further include, but are not limited to, glycosylated molecules harvested from biological tissue, including, but not limited to, biological tissue extracted from human, animal or plant tissue, a culture of human, animal or plant tissue, a biological surrogate for human, animal or plant tissue, a culture of a biological surrogate for human, animal or plant tissue or recombinant versions of human, animal or plant tissue or human, animal or plant tissue biological surrogates. Examples of suitable biological tissues include, but are not limited to epithelium, connective tissue, muscle tissue, nerve tissue, mucous or serous cells, and associated bodily systems and organs and material associated with or contained in amniotic fluid surrounding a fetus, aqueous humour, blood and blood plasma, interstitial fluid, breast milk, mucus, pus, saliva, serum, tears, urine, cerebrospinal fluid, synovial fluid, intracellular fluid, aqueous humour and vitreous humour and other bodily fluids, material from avian species, including material from eggs and tissues from plant origins.

[0222] Examples of suitable glycosylated molecules include but are not limited to those expressing the following moieties:

[0223] Galabiose structure Galα1-4Gal which has been shown to be important in binding of uropathogenic *Escherichia coli, Pseudomonas aeruginosa* (PA-I lectin), and *Streptococcus suis*. This same structure has been found to be important in the binding of bacterial enterotoxins, such as verotoxin and Shiga-like toxin 1 (Stx1), both from *E. coli* strains, and enterotoxin B from *Staphylococcus aureus;*[11]

[11] Noriko Suzuki, Kay-Hooi Khoo, Hao-Chia Chen, James R. Johnson, and Yuan C. Lee, Isolation and Characterization of Major Glycoproteins of Pigeon Egg White, J. Biol. Chem., Vol. 276, Issue 26, 23221-23229, Jun. 29, 2001

[0224] Lactosylceramide structures which are thought to be important in the binding of EHEC to human tissues;[12]

[12] Teneberg, Susann, Jonas Angstrom, Asa Ljungh, Carbohydrate recognition by enterohemorrhagic *Escherichia coli*: characterization of a novel glycosphingolipid from cat small intestine, Glycobiology vol. 14 no. 2 pp. 187±196, 2004

[0225] Galα1-4Galβ1-4Glc, which has been shown to be important in binding of Shiga-like toxin 2 (Stx2)[13]; and

[13] Tomoda H.; Arai M. 1; Koyama N.; Matsui H.; O mura S.; Obata R.; Lee Y. C., Purification of Shiga-like toxin 1 by pigeon egg white glycoproteins immobilized on Sepharose gels, Analytical Biochemistry, Volume 311, Number 1, December 2002, pp. 50-56(7)

[0226] Galα(1-4)Galβ(1-4)GlcNAc which has been shown to be important in binding of Shiga-like toxin 1 (Stx1),[*Ibid*]

[0227] Surface molecules of the present invention may also include one or more known pathogen target sequences, examples of which are set forth in Appendix A.

[0228] As noted above, the pathogen binding molecules may be moieties derived from or representative of potential binding sites on the tissue of a target host by a pathogen. However, in the context of therapeutic constructs of the present invention, more particularly ingestible biocompatible glycosylated particles, the pathogen binding glycosylated molecule is not limited to those that mimic endogenous pathogen binding structures. In fact, any number of glycosylated molecules may be utilized as the pathogen binding moiety, including antibodies and products of a phage display process. Moreover, the therapeutic biocompatible particles of the present invention may comprise more complex mixtures, incorporating more than one type of binding moiety, for example a phage display product for binding bacterial spores, a tissue biomimetic for binding bacteria expressing pili and a toxin binding moiety for binding bacterial toxins.

[0229] Accordingly, additional examples of pathogen binding molecules suitable for use in the instant invention include, but are not limited to, sialic acid in any of its various forms; glycosylated molecules present in pigeon eggs; antibodies, particularly those expressing a degree of specificity for spores of bacteria; lectins; lactoferrin (also lactotransferrin) and functional mimics or analogues thereof. These examples are described in further detail below.

[0230] In addition to tagging a pathogen for an immune system response, antibodies are also known to interfere with the pathogen's ability to bind to and infect host tissues. As noted previously, binding and infection of pathogen with host

tissue arises from the specific interaction between pathogen expressed adhesins (e.g., lectins) and glycan signatures exhibited by the host tissue. Anti-adhesion drugs represent a class of therapeutics in which molecules presenting specific glycan structures are introduced into an infected host body to preferentially bind to adhesins on the pathogens and consequently interfere with the pathogen's ability to bind to host tissue.[14] The same molecules which are used in the fabrication of anti-adhesion drugs are also of value in the context of the present invention, in the construction of ingestible biomimetic films and particles for pathogen immobilization and removal.

[14] Sharon N., Carbohydrates as future anti-adhesion drugs for infectious diseases, Biochim Biophys Acta. Apr. 17, 2006 (4):527-37.

[0231] For example, glycodendrimers, which have been reported as useful as anti-adhesion drugs, are of interest in pathogen capture in the context of the present invention because of reported increased affinity of target pathogens for these constructs.[15] [16] Krippner, et. al., in International Publication No. WO/2007/048190, the entire contents of which are incorporated herein by reference, describe the formation of glycodendrimer and protein constructs. The glycodendrimers in the Krippner constructs can possess biomimetic properties and the constructs can be utilized in novel ways in the formation of the ingestible biomimetic films and particles of the present invention. Glycodendrimer and protein constructs, also known as glycodendriproteins, developed by Rendle, et. al. at the University of Oxford, have been constructed in a manner which has been shown to have inhibitory effects on pathogens.[17] Accordingly, both glycodendrimers and glycodendriproteins may be used in the construction of the ingestible biomimetic films and particles of the present invention.

[15] Touaibia, Mohamed; Roy, Rene, Glycodendrimers as Anti-Adhesion Drugs Against Type 1 Fimbriated *E. coli* Uropathogenic Infections, Mini Reviews in Medicinal Chemistry, Volume 7, Number 12, December 2007, pp. 1270-1283

[16] Yiwen Li, Yiyun Cheng, Tongwen Xu, Design, synthesis and potent pharmaceutical applications of glycodendrimers: a mini review. Curr Drug Discov Technol. Dec. 4, 2007 (4):246-54

[17] Rendle, P. M., A. P. Seger, J. Rodrigues, N. J. Oldham, R. R. Bott, J. B. Jones, M. M. Cowan, B. G. Davis Glycodendriproteins: a Synthetic Glycoprotein Mimic Enzyme with Branched Sugar-display Potently Inhibits Bacterial Aggregation, J. Am. Chem. Soc. 2004, 126, 4750-4751

[0232] Antibodies are also of interest in the therapeutic aspects of the present invention due to their pathogen binding properties and the potential to immobilize them using techniques taught herein. Likewise, synthetically produced molecules, molecules from biological surrogates and molecules produced as a result of genetic (also recombinant) engineering of organisms and molecules may find utility in the context pathogen binding. One example of such technology is in the use of recombinant Factor C peptide as disclosed in U.S. Pat. No. 6,719,973 by Jeak L Ding, et. al., the entire contents of which are incorporated herein by reference. Ding describe the use of a recombinant peptide to induce bacteriostasis by binding of the lipopolysaccharide (LPS or endotoxin) generated by gram-negative bacteria with the LPS present either in the cell membrane or as a liberated molecule.

[0233] Pathogen binding can also be achieved through the use of lectins (also known as "agglutinins") The therapeutic use of lectins in the binding of pathogen receptors to diminish the pathogen's ability to bind to a host is described by Krivan, et. al. in U.S. patent application Ser. Nos. 11/413,826, 10/654,104, 10/097,409, 10/038,645, 08/861,596, 08/640,693, and 08/385,306, all of which are incorporated herein by reference in their entirety. Krivan, et. al. describe the use of a broad range of lectins to blind receptors for a range of pathogens and thereby prevent binding and subsequent infection. Accordingly, the present invention contemplates the use of Krivian's blinding lectins for pathogen binding in the construction of the ingestible biomimetic films and particles of the present invention.

[0234] Lactoferrin (also known as lactotransferrin) also has pathogen binding properties of interest. [18] [19] [20] [21] Studies have shown that some pathogens exhibit host tropism based on lactoferrin properties specific to a single host species.[22] A method for immobilization of lactoferrin on a substrate is described by Satyanarayan A. Naidu in U.S. Pat. No. 6,172,040, the contents of which is incorporated herein by reference in its entirety. Naidu describes the use of immobilized lactoferrin as a coating for beef carcasses during processing as a means of preventing bacterial adhesion to the beef tissue. The present invention contemplates a unique use of lactoferrin in the context of biomimetic films and particles of the present invention, in both the fields of pathogen capture for concentration and detection and pathogen capture in therapeutic uses.

[18] van der Strate B W, Beljaars L, Molema G, et al. (2002). "Antiviral activities of lactoferrin.". Antiviral Res. 52 (3): 225-39.

[19] Weinberg E D (2002). "Human lactoferrin: a novel therapeutic with broad spectrum potential.". J. Pharm. Pharmacol. 53 (10): 1303-10.

[20] Valenti P, Antonini G (2006). "Lactoferrin: an important host defence against microbial and viral attack.". Cell. Mol. Life. Sci. 62 (22): 2576-87.

[21] Ward P P, Paz E, Conneely O M (2006). "Multifunctional roles of lactoferrin: a critical overview.". Cell. Mol. Life. Sci. 62 (22): 2540-8.

[22] DHAENENS, L., F. SZCZEBARA, M. O. HUSSON, Characterization, and Immunogenicity of the Lactoferrin-Binding Protein from *Helicobacter pylori*, INFECTION AND IMMUNITY, Vol. 65, No. 2, February 1997, p. 514-518

[0235] In binding to the intestinal epithelium, Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) exhibit a broad range of binding motifs depending on the stage of the infection. Both EPEC and EHEC are thought to initiate attachment to the host cells through carbohydrate recognition/binding of glycolipids expressed by the intestinal mucosal cells.[23] Biomimetics of the mucosal cell carbohydrates and specifically these glycolipids (glycosphingolipids) are useful in the context of the biomimetic films and particles of the present invention. Specifically, the bacteria follow the initial binding with injection of species specific molecules, translocated intimin receptors (tir), into the cytoplasmic membrane of the epithelial cells. Tir is an enabling protein that initiates a complex change in the epithelium which results in the creation of attaching and effacing (A/E) lesions and ultimately the expression of transmembrane molecules to which the bacteria initiate a very secure intimin-based binding.[24] Such A/E lesions are typical of, but not limited to, EPEC and EHEC infections of the intestinal epithelium and the surface moieties include bacterial-initiated binding sites, and are known by those knowledgeable in the science of translocated intimin receptors (tir). Thus, the surface moieties of attaching and effacing (A/E) lesions may be harvested, for example by rupturing the cell membranes. Alternatively, synthetic equivalents of such surface expressing moieties may be synthetically derived, for example by means of phage display to develop an intimin recognition product. In either event, the resulting transmembrane and other surface expressing molecules or their synthetic equivalents may then be immobilized on a solid state support or substrate for use in the construction of the pathogen binding biomimetic films and particles of the present invention.

[23] Teneberg, Susann, Jonas Angstrom, Asa Ljungh, Carbohydrate recognition by enterohemorrhagic *Escherichia coli*: characterization of a novel glycosphingolipid from cat small intestine, Glycobiology vol. 14 no. 2 pp. 187±196, 2004

[24] Donnenberg M S, *Escherichia coli*: Virulence Mechanisms of a Versatile Pathogen. ed. San Diego: Academic Press (2002).

[0236] Trans-membrane molecules, as well as other glycan signature sequences, tend to be amphiphilic in nature and, as

discussed in greater detail below, that feature can be utilized effectively in the context of the present invention for the creation of a pathogen-binding surface.

[0237] Finally, the constructs of the present invention may include a number of divergent pathogen binding moieties as well as therapeutic molecules with other therapeutic purposes, for example, but not intended as limiting, a heterogeneous mixture of amphiphilic molecules or conjugates with pathogen and/or toxin binding properties is present in the film in a manner which provides for multiple pathogen and/or toxin binding mechanisms or moieties. In this manner, the resulting construct can provide functionality beyond what can be achieved with a homogeneous population. In instances wherein the construct contains a mixture of amphiphilic molecules, this mixture can be adjusted in a manner which effectively controls the surface density of a specific biomimetic molecule due to the presence of other displacing material in the surface film. Likewise, in instances wherein the pathogen binding film results from a mixture of amphiphilic molecules which self-align, the other amphiphilic molecules can be selected so as the mixture forms a more stable amphiphilic film than one of just biomimetic molecules.

[0238] B. Constructing Therapeutic Biocompatible Particles:

[0239] In the context of the present invention, therapeutic particle preferably has a minimum nominal diameter sufficient to inhibit absorption of a majority of the particles by the surrounding tissue; in some instances, such particles may have a minimum nominal diameter of greater than 20 nanometers, in other instances a minimum nominal diameter of 50 nanometers or greater may be required, in other instances where inhibiting absorption is of particular concern, a preferred minimum nominal diameter of 500 nanometers or greater may be desired. The maximum particle size is based on the constraint of what can be effectively delivered by ingestion or introduction through a catheter, however efficiency as measured by effective surface area to volume of substrate material decreases with increased particle size for solid particles (porous particles, which are also within the scope of the present invention, have a more complex relationship between particle size and effective surface area), and accordingly, smaller particle sizes may be advantageous.

[0240] The therapeutic biocompatible particles of the present invention may take the form of fibers or worm micelles (also nanowires) with or without embedded substrate materials. Worm micelles can have diameters as small as the length of two molecules as long as the effective length is sufficient to prevent absorption into the surrounding tissue. The fibers or worm micelles can be part of a construct of entangled fiber-like particles which in concert provide an effective diameter or configuration to impede absorption into surrounding tissue. Alternatively, the particles can be in the form of a free-standing film, so long as at least one dimension is sufficient to prevent absorption of a majority of the particles by the surrounding tissue.

[0241] The substrate for the construction of the therapeutic biocompatible particle can be of any material which will not substantially degrade in form or ability to immobilize the surface moieties when subjected to the environment present in the host. It is further an object of the present invention that the substrate can consist of natural or synthetic polymers, natural or synthetic waxes, ceramics, metals, materials of biological origin or combinations thereof.

[0242] The substrate may also be a vesicle or porous in nature, so long as the overall dimension of the particle remains such that the majority of particles will not be absorbed by the surrounding tissue. Use of a porous substrate or vesicle allows for inclusion of other materials in the substrate which are intended to be released in the host which have therapeutic benefit in concert with or independent of the substrate and substrate surface molecules of the present invention.

[0243] The present invention is not limited to a particular construction method. However, the following methods are suitable for constructing biocompatible particles of the present invention and are therefore provided for illustration purposes.

[0244] An illustrative method for making a biomimetic films and biocompatible particles of the present invention suitable for therapeutic use, comprises the following steps:

[0245] a. provide a relatively polar solvent having sufficient quantities dissolved therein of one or more amphiphilic molecules which include glycosylated molecules, or conjugates of such, or molecules which can be conjugated with glycosylated molecules, or molecules with other pathogen or toxin binding moieties;

[0246] b. expose the polar solvent to a relatively non-polar liquid, the non-polar liquid being immiscible in the polar solvent; the relatively non-polar liquid can optionally comprise a material which can reversibly experience a state change from solid to a relatively non-polar liquid under stimulus;

[0247] c. allow the amphiphilic molecules to align so as to form a membrane which separates the polar solvent from the non-polar liquid;

[0248] d. induce or allow transformation of the non-polar liquid to a corresponding non-polar solid having upper and lower surfaces, wherein the hydrophobic tail ends of the amphiphilic molecule are embedded in or chemically, electrically or mechanically linked to the upper surface of the non-polar substrate and the hydrophilic tail ends project from the upper surface into the polar solvent so as to yield a film having functional activity; optionally, prior to or during transition of the non-polar liquid to a solid state the substrate and film can be manipulated in order to yield a film having a geometric form, said manipulation can include, but not be limited to agitation so as to form micelles, worm micelles or micelle-like constructs; the transformation of the non-polar liquid to a corresponding non-polar solid can be performed in a manner so as to maintain the approximate geometric form achieved by the non-polar liquid prior to or during the transition to a solid form, for example, said transformation can be achieved through rapid cooling of or removal of pressure force on the non-polar liquid.

[0249] e. if additional properties are required: adsorb or conjugate molecules with pathogen and/or toxin binding moieties or other properties to the functional surface of the film if the desired functionality is not present from the preceding steps.

[0250] f. Optionally, remove said film from any surrounding liquid to form a dry construct.

[0251] An alternate method for making the biomimetic films, particles, and constructs thereof comprising the following steps:

[0252] a. provide a liquid having sufficient quantities dissolved therein of one or more glycosylated or other molecules with pathogen or toxin binding properties;

[0253] b. expose said liquid to a solid with surface moieties appropriate for conjugation with said molecules;

[0254] c. allow or induce the conjugation of said molecules to said surface moieties in a manner which results in the pathogen or toxin binding moieties bound on the surface of the substrate.

[0255] d. Optionally, remove said film from any surrounding liquid to form a dry construct.

[0256] An illustrative method for using the biomimetic films, particles, and constructs thereof may comprise the following steps:

[0257] a. Administer one or multiple doses of a plurality of particles of said constructs to a suspected host of a pathogen or toxin infection through a catheter or similar device or through other means of physical placement into a region of suspected infection or a point in the host where the particles will migrate to a region where pathogens and or toxins may be present, or one or multiple oral doses of a plurality of particles of said constructs to a suspected host to a pathogen or toxin infection.

[0258] b. Allow the particles of the present invention to contact those areas of the host where pathogens and/or toxins may be present.

[0259] c. Allow the host organism to discharge the particles through defecation, urination, vomiting, mucosal flow, expectoration or other means of natural or induced discharge or remove the particles from the host through a catheter or other device.

[0260] Another method of use for the biomimetic films, particles, and constructs thereof comprising the following steps:

[0261] a. Extract a portion of bodily fluid of a suspected host to a pathogen or toxin infection, including, but not limited to amniotic fluid, aqueous or vitreous humour, blood and blood plasma, bone marrow fluids, cerebrospinal fluid, interstitial fluid, lymph fluids and pleural fluid

[0262] b. Induce contact of said bodily fluid to one or more surfaces with the pathogen and toxin binding moieties of the present invention.

[0263] c. Remove said surfaces from contact with said bodily fluid.

[0264] d. Optionally, reintroduce said bodily fluid with pathogens/toxins removed into the organism.

[0265] e. Optionally, subject said surfaces of the present invention to analysis techniques to determine presence and/or type of pathogens or toxins which may have bound to the surface.

[0266] As noted previously, the present invention relates to the creation of therapeutic materials which bind to pathogens and toxins and facilitate their removal and expulsion from an infected body. Of value in the therapeutic aspects of the present invention are particles of a biocompatible nature which are large enough (or have appropriate geometric configuration) so as not to be absorbed into surrounding tissue at the point of therapeutic application. The minimum particle size to avoid adsorption is dependent in part on the properties of the surrounding tissues. It is the object of the present invention that the minimum particle size of constructs of the present invention for therapeutic use be such that a majority of particles are not absorbed into surrounding tissue. As a general rule, for roughly spherical particles, this may require particles with nominal diameters greater than 20 nanometers. To produce particles which can pass through the gastro-intestinal tract without being absorbed through the intestinal wall, studies suggest that at a greater than 50 nanometer particle size, less than 50% are absorbed through the intestinal wall, and as a general rule particles of 500 nanometers and larger do not pass into epithelia of the gastrointestinal tract.[25][26][27][28] This presumes a roughly spherical shape, though a cylindrical shape with a length longer than that threshold might be absorbed if oriented properly. Additionally, the properties of the intestinal wall will vary from host to host and also vary based on condition of health of the intestinal wall tissue.

[25] Jani P, Halbert G W, Langridge J, Florence A T. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J Pharm Pharmacol. 1990 December; 42(12):821-6.

[26] Desai M P, Labhasetwar V, Amidon G L, Levy R J. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res. 1996 December; 13(12):1838-45.

[27] Hussain N, Jaitley V, Florence A T. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv Drug Deliv Rev. 2001 Aug. 23; 50(1-2):107-42.

[28] Francis M, Cristea M, Winnik F M. Polymeric micelles for oral drug delivery: Why and how Pure Appl. Chem. 2004; 76:1321-1335.

[0267] The probability that a cylinder with a length greater than 50 nanometers is absorbed decreases with increasing length. Even film or thread-like constructs with diameters less than 50 nanometers will not easily be absorbed if the length is significantly greater than that. Very thin constructs, having diameters far smaller than 50 nanometers, are also of value in the present invention so long as the length is sufficient to impair absorption. Accordingly, constructs such as nano-thickness films and worm micelles[29] (also nanofibers) are of use in the constructs of the present invention. Worm micelle constructs which are interwoven or entangled in a random manner can form aggregate structures (also hydrogels) with properties of larger constructs than the individual worm micelles. These aggregate structures can also have the appearance of tangled balls of yarn and in some cases these aggregate structures can appear similar to some depictions of casein micelles, but this analogy is not meant to be limiting. Aggregate structures of worm micelles are of value in the constructs of the present invention since the resultant structure can have the equivalent shape and size of a particulate construct of sufficient size so as not to be absorbed into surrounding tissue even though the individual components might not be that large.

[29] Vijayan, Kandaswamy, Discher, Dennis E., Block Copolymer Worm Micelles in Dilution: Mechanochemical Metrics of Robustness as a Basis for Novel Linear Assemblies Journal of Polymer Science Part B: Polymer Physics, Volume 44, Issue 24 (p 3431-3433)

[0268] One preferred embodiment of the therapeutic biocompatible particle is the micelle, more particularly a wax or worm micelle as discussed above. A micelle of the present invention may be formed from the glycosylated films previously described, preferably having a sufficient size and/or a geometric configuration so as not to be readily absorbed into biological tissue of an organism, including but not limited to the walls or membranes (either inside or outside) of the organs of a body, alimentary tract (also gastrointestinal tract), urinary tract, pulmonary tract, blood vessels, amniotic sac, ocular sac, nervous system (for example, but not limited to, the brain or spine), membranes of the musculoskeletal system or cell walls; Suitable glycosylated pathogen binding moieties include, but are not limited to lactosylceramide structures, which are thought to be important in the binding of EHEC to human tissues; Galα1-4Galβ1-4Glc, which has been shown to be important in binding of Shiga-like toxin 2 (Stx2); Galα(1-4)Galβ(1-4)GlcNAc, which has been shown to be important in binding binding of Shiga-like toxin 1

(Stx1); or any one or more of the pathogen binding sequence set forth in Appendix A included herewith. Synthetic equivalents thereof are also contemplated.

[0269] In a preferred embodiment, the glycosylated molecules are themselves amphiphilic in nature or conjugated to hydrophobic moieties to form an amphiphilic molecule. In this manner, these molecules can self-align through hydrophobic interaction to form films and structures of the present invention, particularly in conjunction with non-polar or less-polar surfaces. The conjugates can include synthetic moieties, such as the products of chemical synthesis to produce combinations of molecules in a predetermined structure. Said conjugates can also include moieties known for their binding properties, such as block copolymers, or more specifically polyethylene glycol (PEG), or natural binding molecules, such as heparin or its derivatives.

[0270] In the context of the micelles with self-assembled amphiphilic films, it is preferable to utilize substrates that are relatively non-polar material, with particular value in materials that can transition from a relatively non-polar liquid or gel to a solid, often crystalline structure. Substrate materials of value in the context of the present invention include, but are not limited to natural wax and synthetic wax, natural and synthetic plastics, polymers or hydrocarbon mixtures. Substrate materials comprising natural and synthetic fats and resins are also of interest as substrate materials in the present invention.

[0271] As noted above, it is an object that the amphiphilic molecules self-align due to hydrophobic forces to form the film. It is a further object of the present invention that such constructs of film and substrate can form a micelle or micelle-like structure, wherein the non-polar substrate and hydrophobic tail ends are sequestered in the interior of the micelle while the hydrophilic head ends are present on the outer surface of the micelle. The glycosylated molecules presented by the wax micelle may consist of a heterogeneous population of amphiphilic molecules or conjugates such that the combination of different amphiphilic molecules or conjugates in a surface provides functionality beyond what can be achieved with a homogeneous population. Further, said heterogeneous population of amphiphilic molecules:

[0272] a. can contain a mixture of glycosylated amphiphilic molecules with pathogen and/or toxin binding properties and other amphiphilic molecules which together form a more stable amphiphilic film than one of just said glycosylated molecules,

[0273] b. can contain a mixture of amphiphilic molecules in a manner which effectively controls the surface density of a specific glycosylated molecule due to the presence of other displacing material in the surface film, and/or

[0274] c. can contain a heterogeneous mixture of amphiphilic molecules or conjugates with pathogen and/or toxin binding properties present in the film in a manner which provides for multiple pathogen and/or toxin binding mechanisms or moieties.

[0275] Another preferred embodiment of the therapeutic biomimetic film is the worm micelle. The design and construction as well as the delivery the worm micelles of the present invention is analogous to that set forth above.

[0276] C. Formulation and Delivery of Biocompatible Pathogen Binding Particles:

[0277] It is an object of the present invention that the biocompatible pathogen binding particles and associated pharmaceutical formulations thereof be designed for use, recommended for use, or administered to the intended host in a manner consistent with the first dose being provided to the host organism before the pathogen or toxin has been identified. This use is in contrast with the therapeutic use of antibiotics in, for example, the case of a gastro-intestinal infection, wherein it is recommended that the bacteria be identified before antibiotics are administered, to avoid the chance of increased bacterial release of toxins. However, it is also noted that the particles and formulations of the present invention can be provided to a host organism at any point in the cycle of an infection.

[0278] The particles may be formulated as a pharmaceutical composition suitable for administration to human or animal subjects in need thereof. Preferred subjects are those having a complex gastro-intestinal system, including but not limited to humans, livestock, for example hoofed animals, poultry and fish, domestic pets and animals at zoos and game parks, and wild species. In the context of the present invention, a suitable therapeutic dose may include a plurality of biocompatible particles, those particles can include a range of sizes, shapes, substrate materials, and types of surface molecules.

[0279] A pharmaceutical composition of the present invention can be formulated in a liquid or gel matrix, which may optionally include other molecules of therapeutic, nutritional or psychological (such as those which provide taste or texture properties) benefit. Other molecules of therapeutic benefit include materials designed to facilitate ingestion of the matrix, including such that might reduce the possibility of vomiting. It may also be desirable to include as well as agents known to be of value in oral rehydration therapy (also ORT), such as oral rehydration salts or solutions and electrolytes, to synergistically treat the dehydration that is often a component of gastrointestinal infections. It may also be desirable to include sources of iron with the formulation, as the production of toxin in enterohemorrhagic *E. coli* is thought to be enhanced under iron deficient conditions.[30] [31] The formulation may also include antibiotics or probiotics.

[30] Todar, Kenneth, Todar's Online Textbook of Bacteriology, 2008, http://www.textbookofbacteriology.net/e.coli.html

[31] Roth, R. I., Panter, S. S., Zegna, A. I., Arellano, F. A., Levin, J., Effects of iron on bacterial endotoxin. Journal of Endotoxin Research 4:44, 273-278, 1997.

[0280] The additional therapeutic agents noted above can either be formulated with the therapeutic particles of the present invention, administered in a delivery matrix or administered as a separate dose. The benefit of simultaneous treatment both with the present invention and with antibiotics is that the present invention has the potential to capture some of the additional toxins which might be expelled by the pathogens in response to the antibiotic treatment. This may allow destruction of some of the bacteria by antibiotics without the resulting short-term rise in toxin production causing unacceptable stress on the host's tissues.

[0281] The biocompatible particles and subsequent pharmaceutical formulations thereof may be administered in any conventional manner, including oral delivery, percutaneous administration, intravenous administration, intranasal administration and the like. In a preferred embodiment, the particles are formulated for ingestion, although introduction through a medical device, such as a catheter or hypodermic needle, or introduction during a surgical procedure, or as an irrigation therapy procedure such as might be used to flush pathogens or toxins from an organ or bodily system, is also contemplated.

[0282] The therapeutic pathogen binding particles of the present invention can be introduced into a biological organism, with or without other molecules and in a liquid or solid form, and subsequently removed or expelled from said biological organism. The particles of the present invention can be optionally be recovered after expulsion or removal from the host and subsequently subjected to diagnostic techniques known by those skilled in the art to detect the presence and optionally the type of pathogen bound to the particle as part of a medical diagnostic procedure.

[0283] Also contemplated is the inclusion of indicator molecules detectable by medical imaging techniques, to provide indication of presence of particles of the present invention in a treated the host. Such indicator molecules may be provided on the substrate itself or conjugated to the therapeutic particle. Indicator molecules suitable for indicating presence, location or density of constructs of the present invention within a biological organism include, but are not limited to, a fluorescent label, a radioactive label, a dye and a compound which enhances magnetic resonance imaging. Labels which are detectable by external instruments, such as a radioactive label, could permit identification of areas in the host organism where constructs of the present invention are bound to pathogens which are securely bound to tissue in the host.

[0284] The particles and formulations of the present invention can be produced and stored in a dried form for later rehydration. The particles may further be provided with a solid matrix which provides benefits for transport and storage, further wherein the matrix may include other molecules of therapeutic, nutritional or psychological benefit (similar to those projected for liquid matrices).

[0285] The particles of the present invention can also be provided with an exterior coating layer that protects the particles of the present invention from the environment for a particular period of time or until a specific area of the host is encountered, for example as would be the case if particles of the present invention are enclosed in a capsule, tablet or other matrix to be ingested. Formulation and time release delivery of materials of therapeutic value through means of a protective capsule is well known by those skilled in the art, and the utilization of such techniques are an object here.

[0286] An illustrative treatment method suitable for use with the therapeutic particles and formulations of the present invention may include the following steps:

[0287] 1. Administering one or multiple oral doses of a plurality of particles of the present invention to the host.

[0288] 2. Allowing the particles of the present invention to contact areas of the alimentary system of the host where pathogens may be present.

[0289] 3. Allowing the host organism to discharge the particles through defecation, vomiting, expectoration or other means of natural or induced discharge.

[0290] An alternative therapeutic treatment comprises the steps of:

[0291] 1. Administering one or multiple doses of a plurality of particles of the present invention to the host through a catheter or similar device or through other means of physically placement into a region of suspected infection or a point in the host where the particles will migrate to a region where pathogens may be present.

[0292] 2. Allowing the particles of the present invention to contact those areas of the host where pathogens may be present.

[0293] 3. Allowing the host organism to discharge the particles through defecation, urination, vomiting, mucosal flow, expectoration or other means of natural or induced discharge or removing the particles from the host through a catheter or other device.

[0294] Another alternative method for therapeutic treatment or diagnostic using the particles of the present invention is as follows:

[0295] 1. Extraction of a bodily fluid of an organism, including, but not limited to bone marrow fluids, amniotic fluid, aqueous or vitreous humour, blood and blood plasma, interstitial fluid, lymph fluids and pleural fluid

[0296] 2. Inducing contact of said bodily fluid to one or more surfaces with the pathogen and toxin binding moieties of the present invention.

[0297] 3. Optionally, reintroducing of the bodily fluid into the organism.

[0298] 4. Optionally, subjecting said surfaces of the present invention to analysis techniques to determine presence and/or type of pathogens or toxins which may have bound to the surface.

[0299] IV. Solid State Films from Glycosylated Amphiphilic Molecules:

[0300] The present invention further relates to are glycosylated amphiphilic molecules composed of hydrophobic "tails" and hydrophilic "heads" that self align to form a membrane at the interface of a polar solvent and a non-polar liquid or a solid. In this aspect, the present invention is directed to a solid state membrane, typically a thin film, composed of a non-polar solid material having the hydrophobic "tail" of a glycosylated amphiphilic molecule embedded in or linked to its surface such that the hydrophilic "head" protrudes from the solid surface and presents useful properties to the surrounding environment. A membrane or film in accordance with the present invention is produced when a non-polar liquid, in the presence of a polar solvent and an amphiphilic biological compound, undergoes a transformation from liquid to solid, through thermal, chemical or radiative means, with the resultant effect that the amphiphilic molecule is affixed or "locked" to the surface, more particularly the hydrophobic ends of the amphiphilic compounds are mechanically or chemically linked to or embedded in the non-polar solid. The membrane and micelles produced therefrom remain stable even in the absence of the polar solvent, thereby allowing the hydrophilic components of the amphiphilic compounds to present useful properties at the surface thereof.

[0301] This aspect of the present invention arose with the discovery that many amphiphilic molecules, particularly glycolipids and glycoproteins endogenous to plant and animal tissue fluids, spontaneously aggregate at the interface between certain aqueous solutions and certain non-polar liquids to form a flexible membrane. The lipophilic components of the molecules adhere, through non-covalent hydrophobic interactions, to molecules of oil or other non-polar liquids and present their hydrophilic moieties toward the aqueous phase to produce a membrane, which, when agitated (e.g., rolled, shaken, or forced through a filter), stretches and breaks into closed vesicles or micelles. This technology is discussed in detail in U.S. Pat. Nos. 5,824,337 (Mullen), 6,528,092 (Mullen), and 7,148,031 (Mullen), the contents of which are incorporated by reference herein in their entirety. Accordingly, micelles that embody this technology are often referred to herein as "Mullen micelles".

[0302] In many instances the glycoprotein membrane and the micelles produced by association of non-polar liquids with glycoproteins are mechanically and chemically more stable than those formed from phospholipids. For example, unlike phospholipids based liposomes and micelles, the polar surfaces of the glycoprotein micelles do not fuse easily and are quite stable in aqueous media. They are able to retain their shape and hold their contents and are very resistant to destruction. For example, they can be selectively extracted using physical means such as reparatory funnel or syringe which, in turn, enables the creation of micelle populations that are relatively uniform in size. In addition, the glycoprotein micelles may be used as carriers for substances that are not, when used alone, capable of forming relatively stable micelles (e.g., lipids, lipophilic, and lipid-like moieties).

[0303] Depending upon the amphiphilic compound utilized, the Mullen micelles can be kept for months at room temperature in sterile aqueous solution and indeed in some instances can, under very restrictive conditions, be dried and subsequently rehydrated. However, the dried membranes tend to lose their elasticity and are quite fragile. If fact, when the aqueous solvent is evaporated, only a diaphanous, brittle monolayer of glycoprotein remains. It is accordingly an object of the present invention to provide a means for stabilizing the micelle membrane, so as to facilitate the long term storage and convenient transport of both membranes and micelles formed therefrom. To that end, the present invention utilizes a non-polar material, first in a liquid form to first initiate membrane formation, then, in a solid form in which the hydrophobic "tails" of the amphiphilic molecule are embedded in or linked to its surface while the hydrophilic "heads" protrude therefrom so as to present useful properties to the surrounding environment. The transformation from liquid to solid, either through thermal, chemical or radiative means, results in "locking" of the amphiphilic molecule to non-polar material. More particularly, the hydrophobic ends of the amphiphilic compounds are affixed to the surface of the solid, either through mechanical, chemical or physical means. The resulting membrane or film remains stable even if the polar solvent is removed, thereby allowing the hydrophilic components of the amphiphilic compounds to present useful properties at the surface of the solid material.

[0304] The amphiphilic molecules that form the surface of the membranes and micelles of the present invention can vary in form and source. The amphiphilic molecules need only to act as surfactants in the presence of the selected polar solvent and the fluid state of the non-polar substrate, be it wax, plastic, fat, polymer or other hydrocarbon. In the context of stable films of amphiphilic molecules that self-assembly as a result of on hydrophobic forces, in many cases it is necessary to achieve a threshold density of molecules with amphiphilic properties in the polar solution. This concentration is known as the critical micelle concentrations (CMC). In a situation where biological molecules of interest are not present in a solution at or above the critical micelle concentration, it may be necessary to have present in the solution (through nature or design) other amphiphilic molecules which may or may not be glycosylated in order to achieve a sufficient concentration of amphiphilic molecules to form a stable self-assembled film which includes the biological molecules of interest.

[0305] The substrate material can also vary greatly. The substrate needs to be non-polar (or sufficiently non-polar) in the fluid form to permit formation of micelle or micelle-like alignment of the amphiphilic compounds at the interface with the polar solvent. Upon solidification, the solid material must provide a mechanical or chemical bond such that the amphiphilic material remains oriented with the hydrophilic end presented to the surrounding environment and thus exhibiting useful properties.

[0306] In preparation of the constructs of this invention, the solid or solid-film material may be allowed to solidify without agitation, resulting in a relatively flat or contour conforming surface. If the non-polar material, for example a wax or plastic polymer, when in liquid form is agitated in the polar solvent in the presence of an amphiphilic compound at the proper concentration and temperature, then micelle structures spontaneously will form. These structures can be preserved in the transition of the non-polar materials from liquid to a solid state.

[0307] As with fluid micelles, the size of the solid micelle-like structures can be influenced during formation through the degree of agitation, concentration of amphiphilic compound and selection of amphiphilic compound.

[0308] Accordingly, a typical method for a making a glycosylated amphiphilic membrane or film of the present invention generally includes the following steps:

[0309] (a) providing a polar solvent having sufficient quantities of one or more glycosylated amphiphilic molecules dissolved therein;

[0310] (b) exposing the polar solvent to a non-polar liquid, the non-polar liquid being immiscible in said polar solvent;

[0311] (c) allowing the glycosylated amphiphilic molecules to align so as to form a membrane that separates the polar solvent from the non-polar liquid; and

[0312] (d) inducing transformation of the non-polar liquid to a corresponding non-polar solid, for example, through the application of thermal, chemical or radiative stimulus, such that the hydrophobic tail ends of the amphiphilic molecule become embedded in or chemically or mechanically linked to the non-polar substrate while the hydrophilic tail ends project from the substrate to provide the membrane with a functionally active surface.

[0313] The polar solvent may optionally be removed so as to yield a dry solid state film. As mentioned above, depending upon the desired membrane form, shape and structure, it may be further desirable to manipulate the reactants prior to, during or subsequent to transition. For example, by controlling the temporal component of the transition process (for example, speeding up the transition process through rapid cooling of the non-polar liquid), one can drive the production of micelle constructs having particular size or geometry. Alternatively, agitating the film prior to or during transition will result in the formation of micelle-like constructs.

[0314] The structures of the present invention have been shown to have a level of stability after removal of the non-polar solvent. This is considered a very useful property in allowing preservation of the material of the invention. Lack of moisture will decrease the likelihood of biological attack during storage. Additionally, removal of moisture allows for storage of the material at reduced temperature without the risk of ice crystals damaging the biological compounds.

[0315] Fluid micelles and micelle-like structures have an inherent problem of reduced dimensional stability when exposed to high-shear conditions such as in a turbulent non-polar solvent flow. The solid micelles and micelle-like structures of this invention are more stable in those conditions and

thus are more suited to applications involving turbulent flow, for example in the context of environmental filters.

[0316] V. Utilities of the Films, Particles, and Constructs of the Present Invention:

[0317] The biomimetic films and particles, especially the micelle particles, of the present invention have a wide range of utilities, ranging from environmental sensing to pathogen capture and therapeutic applications. The membranes of the present invention are particularly useful for extracting target molecules, for example, contaminating substances such as pathogens, toxins, and the like, from a particular sample based on tissue tropism. Selectivity based on tissue tropism provides a significant advantage over current selectivity filters which are either very specific to a particular serotype of bacteria, where only bacteria which are anticipated can be found, or very general as with a sized-based filter, where potentially harmful bacteria could get lost in the biological clutter. Selectivity based on tissue tropism permits capture of bacteria and toxins which have the potential to bind to the host tissue and thus at least have the potential to represent an infectious agent. This selectivity permits the present invention to potentially capture unexpected, emerging or engineered pathogens, providing a powerful tool in the early detection which is so critical in containing the spread of communicable disease.

[0318] Because the pathogen and toxin binding membranes can be immobilized on relatively stable, benign, and economical surfaces of a size and configuration which will generally not be absorbed by surrounding tissue, the constructs of the present invention can be utilized for therapeutic treatment in the early stages of an infection. This is particularly beneficial in the case of toxin producing gastrointestinal infections in which the use of antibiotics in contraindicated. Constructs of the present invention can be administered as an oral dose, bind to free pathogens or toxins in the intestines and subsequently be expelled by the body. When a substrate such as wax is utilized, this material is generally regarded as safe and is usually considered environmentally benign.

[0319] A typical method for extracting a target molecule of interest from a particular sample using a glycosylated amphiphilic membrane or film of the present invention generally includes the following steps:

[0320] (a) exposing the sample to a solid state membrane or membrane coated implement or device;

[0321] (b) allowing a sufficient time for the functional groups present on the hydrophilic tail end of the glycosylated amphiphile to bind target molecules present in the sample; and

[0322] (c) removing or separating the membrane, having target molecules bound thereto, from the sample.

[0323] In certain instances, it may be desirable to subject the sample to a force which promotes contact between the target molecule and the membrane. Illustrative examples of such forces include, but are not limited to, mechanical acceleration, centrifugal force, electrical force, magnetic force, hydraulic force and various other means for agitation.

[0324] Upon removal from the sample, the membrane may then be analyzed for the presence of target molecule using any number of commercially available detection and measurement technologies (e.g., PCR, immunoassays, DNA microarrays, protein microarrays, etc.). In certain instances, the amount of target molecule bound to the membrane may be used to estimate the amount of target molecule present in the sample. Such levels can also be used to diagnose the presence of a disease condition (e.g., a bacterial infection, cancer, etc.)

[0325] Since the technology can exhibit both the properties of binding to pathogens and physical manifestation in a microscopic form (micelle construct), the instant technology also finds utility as an alternative to Hemagglutination, wherein pathogens are detected through their ability to bind to multiple red blood cells at once, resulting in visible agglutination of the cells.

[0326] In that the films and particles of the instant invention find application in a number of divergent environments, the structure and form of the inventive film is not particularly limited. For example, the films of the present invention may take the form of a biological or environmental filter or sensor, such as a microarray or biochip sensor having specificity (via the functional ends of the embedded glycosylated amphiphile) for one or more proteins, antibodies, tissues, or chemical substances. Alternatively, in the context of pathogen capture and filtration, it may be beneficial to coat a vessel or pipe or filter media contained within with a biomimetic film of the present invention having binding specificity for one or more target molecules, for example glycoproteins exhibiting sugars specific to a particular class of pathogens, with the intent of placing a liquid sample of interest in the pipe in contact with said membranes. Accordingly, in addition to serving as a source for micelle construction, the biomimetic films of the present invention, surface-embedded or associated with glycosylated amphiphiles, find utility both as singular solid state materials and as film coatings on other materials or for other devices.

[0327] In the context of the instant invention, the "other" material on which the biomimetic film is disposed may serve as structural or geometric support or, alternatively, may address thermal or density issues. Alternatively, the "other" material may comprise a useful device or implement, for example, a sample containing vessel, pipe, tube, or the like. For example, in those instances where the sample is in the form of a continuous fluid stream, a glycosylated amphiphilic membrane of the present invention may be anchored or adhered to a stream contacting surface of a vessel through which the fluid stream sample is passed. In other embodiments, it may be desirable to adhere a membrane coating of the instant invention to the walls of a centrifuge, such that contaminating substances present in the sample are accelerated towards and then captured by the hydrophilic moieties present on the surface of the film, the particular hydrophilic moieties having binding specificity for target contaminants of interest. In further embodiments, the useful implement may comprise a medical device that would benefit from biological properties displayed by the glycosylated amphiphilic membrane of the present invention.

[0328] Medical devices, particularly implantable medical devices, often fail as a result of a biological organism's reaction to its introduction. In many instances, the reaction arises from the identification of the introduced device as "foreign" and involves subsequent protective attempts by the organism's immune system to remove, sequester or destroy the perceived injurious stimuli and initiate the healing process. An example of such a reaction is transplant rejection, which occurs when the immune system of a recipient of a transplant attacks the transplanted organ or tissue. In addition, nonspecific binding (NSB) of proteins at medical implant surfaces is believed to be at least partially responsible for triggering the foreign body response, which in turn can lead to

device failure or rejection. This "biofouling" is also blamed for device infection incidence, thrombosis, and sensor deterioration over time in vivo.

[0329] Accordingly, in the context of medical devices, it is highly advantageous to be able to mask the foreign nature of the device so as to prevent the rejection process. One means to achieve this goal involves coating the medical device with a biocompatible material that minimizes or substantially eliminates such negative reactions. In addition, functionally inert surface coatings for medical implant devices can effectively limit host rejection of the medical implant device through attached ligands, incorporated drugs, and reduced NSB responsible for biofouling of such devices in or ex vivo. The glycosylated amphiphilic membranes of the instant invention find particular utility as such biocompatible coatings. In that many glycosylated moieties are native to living systems, they are less likely to trigger immune response. Accordingly, solid state membranes expressing such endogenous glycosylated amphiphiles (or functionally active fragments or derivatives thereof) will mimic endogenous tissues and thus be recognized by the immune system as "self" rather than "foreign". Such "biomimetic" membranes are particularly useful as antigenicity reducing coatings for medical devices.

[0330] In certain instances, in the interest of further reducing potential immune response, it may be desirable to construct a biomimetic film of the present invention using a glycosylated amphiphile isolated, extracted, harvested or otherwise derived from an intended recipient or, alternatively, an antigen-matched donor. While an ideal donor would be an HLA-identical sibling, alternative donors include an HLA-phenotypically matched unrelated donor (MUD), a partially mismatched related donor (PMRD) or a cord blood donor (CBD), who can be a phenotypically matched or mismatched related or unrelated donor.

[0331] Medical devices that would benefit from such antigenicity reducing coatings include both temporary implants (i.e., devices intended for limited introduction, for example angioplasty catheters) and more permanent implants (i.e., devices intended for long term insertion, for example cardiac pacemakers). Examples of medical devices contemplated by the instant invention include, but are not limited to, needles, catheters (e.g., intravenous, urinary, and vascular catheters), stents, shunts (e.g., hydrocephalus shunts, dialysis grafts), tubes (e.g., myringotomy tubes, tympanostomy tubes), implants (e.g., breast implants, intraocular lens), prosthetics, and artificial organs, as well as cables, leads, wires, and electrodes associated therewith (e.g., leads for pace makers and implantable defibrillators, bipolar and monopolar RF electrodes, vascular guidewires). Also contemplated are devices such as wound dressings, sutures, staples, anastomosis devices, vertebral disks, bone pins, suture anchors, hemostatic barriers, clamps, screws, plates, clips, vascular implants, tissue adhesives and sealants, tissue scaffolds, various types of dressings, bone substitutes, intraluminal devices, vascular supports, and other body contacting devices that may benefit from enhanced biocompatibility.

[0332] In addition to enhancing the biocompatibility of medical devices, the solid state films and membranes of the instant invention, more particularly the functionally active glycosylated moieties provided on the surface thereof, may possess analytic, diagnostic and/or therapeutic utility. For example, the glycosylated amphiphilic molecule selected may have a binding specificity for a target molecule of biological or medical interest. Glycosylated amphiphilic membranes so fabricated can be used to capture and extract such materials, either in vivo or ex vivo. Examples of target molecules of biological or medical interest include, but are not limited to, biological entities such as hormones, proteins, nucleic acid molecules, (e.g., RNA and/or DNA), circulating cells, and particulate analytes of biological origin that serve as diagnostic indicia or biomarkers of disease, for example cancer. Also contemplated are foreign materials, particularly biohazardous materials, such as contaminants, drugs, toxins, heavy metals, pathogens and the like.

[0333] In certain instances, the glycosylated amphiphilic membranes of the present invention (as well as medical devices formed therewith) can serve not only to detect the presence of the target molecule but also effectively remove it from (i.e., detoxify) the sample. For example, when the target molecule of interest is a heavy metal, such as lead, mercury, copper, or the like, the selected glycosylated amphiphile may act as a chelating agent that binds the heavy metal and effectively eliminates it from circulation.

[0334] The glycosylated amphiphilic membranes of the instant invention also find utility in the areas of environmental detection and detoxification. For example, the inventive biomimetic films may take the form of a filter for capturing target molecules that may be present in an environmental sample, such an entering or exiting water source (e.g., an agricultural feed trough or runoff ground water). By positioning a filter with a functionally active surface in contact with a stationary or flowing water source, target molecules present in the source become bound to the hydrophilic ends of the glycosylated amphiphiles. In this manner, the target molecules can effectively removed from the source and safely transported to a laboratory setting. Alternatively, the filters may be provided with a means for real-time detection and measurement of target molecule binding. Real-time detection may involve analysis of a change in a readily measurable parameter, for example changes in optical spectra (e.g., fluorescence, color change), electrical activity, electrical field (e.g., conductance), or magnetic field.

[0335] Depending upon the detection methodology utilized, it may be desirable to transport the biomimetic films to a separate location, such as a laboratory setting, for analysis. In that the target molecule is firmly bound to the membrane, it no longer constitutes a danger to either the surrounding environment or to the analyst (i.e., safe handling through conjugation). Accordingly, a technician may opt to either assess the target molecule in situ, while bound to the membrane or, alternatively, may opt to unbind it from the filter, for example with the help of a lysing solution or the like. In the context of hazardous materials, such as pathogens, the lysing solution is likely to effectively kill the pathogen, thereby eliminating the dangers associated with conventional detection methods that utilize live pathogens.

[0336] Hereinafter, the present invention is described in more detail by reference to the Examples. However, the following materials, methods and examples only illustrate aspects of the invention and in no way are intended to limit the scope of the present invention. As such, methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

EXAMPLES

Example 1

[0337] Porcine small intestine was treated in a tissue disruptor and then sonicated to release the membrane bound

glycosylated compound. The resultant solution was sterile filtered. The glycoprotein rich filtrate was heated along with food grade paraffin wax to 60 degrees Celsius. The resulting mixture was agitated and then pumped through a zone excited by sonication. The mixture was then sprayed through a small orifice into a continuous stream of cold water. The resulting particles in the 50 nm to 1000 nm diameter size range were concentrated and washed. The particles were then added to an LB broth solution with active *Salmonella Montevideo* present and rotated for 20 minutes at 37 degrees Celsius. The particles were removed from the LB broth solution and then rinsed. One sample was stained and observed under a microscope. *Salmonella* were observed bound to the surface of the particles. A second sample was processed for PCR analysis techniques known to those skilled in the art. The wax was easily removed early in the process since when it melted, it separated to the top of the vessel, and then upon cooling the wax was easily removed, and the bacterial remnants of lysing remained in the liquid below. The PCR analysis gave positive results for *Salmonella*.

[0338] The porcine small intestine wax micelles were demonstrated for research and analytical use in this case. However, the particles were created with material which is generally regarded as safe, and consequently would also be suitable in the context of an ingestible biomimetic particle, for as an oral therapy for pathogens that would bind to the small intestine. Porcine intestine is used as an analogue for human intestine in this case.

Example 2

[0339] Bovine Serum Albumin (BSA) in solution was heated to 55 degrees Celsius. Food grade paraffin wax, also at 55 degrees was added to the solution. The resulting mixture was agitated at high shear using a food processor and then rapidly quenched in a volume of 25 degree Celsius water. The resultant micelle-like particles in the range of 500 nm to 5000 nm were allowed to rise to the surface and then collected. The particles were then rediluted and separated by rise time in the fluid over three successive dilutions to yield relative size separations. Samples of the various size fractions were successfully demonstrated as agglutination assay in trials on a mutant *Escherichia Coli* strain which is always fimbirated.

Example 3

[0340] Shaw, et. al., [32] demonstrated that Tir can be translocated into red blood cell (RBC) membranes, and that the resulting transmembrane molecules (described by Race, et. al.[33]) which are expressed can result in Tir-intimin binding of other serotypes than those initially secreting the Tir. Accordingly, red blood cells are exposed to Tir producing bacteria per the procedures used by Shaw, et. al. (*E. coli* strain CVD206 was used in that case, but the technique is applicable to other strains as well). The resulting sample of infected red blood cells is then subjected to cell rupture techniques which both destroy the bacteria as well as liberate the transmembrane molecules of Tir, which according to Shaw are in the 78 kDa size range. In this example, the transmembrane Tir, with expressed intimin receptors is immobilized directly into a solid substrate based on amphiphilic properties, along with the other transmembrane glycoproteins of RBC using self-aligning film techniques. It is also envisioned that Tir molecules can also be selectively extracted from the solution using size based fractionation techniques, including but not limited to size-exclusion chromatography and dialysis, and then the selected particles can be added to a crafted solution of selected amphiphilic molecules prior to immobilization. The Tir transmembrane solution is sterile filtered. The glycoprotein rich filtrate is heated to 54 degrees Celsius along with food grade paraffin wax which has a melting point of 52 degrees Celsius. The paraffin includes a radioactive tag. The resulting mixture is agitated and then pumped through a zone excited by sonication. The mixture is then sprayed through a small orifice into a continuous stream of water at 25 degrees Celsius. The resulting particles in the 50 nm to 1000 nm diameter size range are allowed to rise to the surface and then collected. The particles are added to an Oral Rehydration Therapy solution of a mixture of sugar and salt and known to those skilled in the art. The solution is provided to an individual with diarrhea and a suspected enteropathogenic *Escherichia coli* infection. During the course of treatment, the intestinal area of the individual are scanned at regular intervals to detect location and accumulation levels of radioactively tagged paraffin particles, to determine points and gross quantities of intimin expressing bacteria.

[32] Shaw, Robert K., Sarah Daniell, Gad Frankel and Stuart Knutton, Enteropathogenic *Escherichia coli* translocate Tir and form an intimin-Tir intimate attachment to red blood cell membranes, Microbiology (2002), 148, 1355-1365.

[33] Race, Paul R., Jeremy H. Lakey, and Mark J. Banfield Insertion of the Enteropathogenic *Escherichia coli* Tir Virulence Protein into Membranes in Vitro, J. Biol. Chem., Vol. 281, Issue 12, 7842-7849, Mar. 24, 2006

Example 4

[0341] A hydrogel is prepared using techniques taught by Stupp, et. al. in US patent application 2005/0209145. In this application Stupp teaches the creation of growth factor binding hydrogels based on self-assembling peptide amphiphiles which have been configured through phage display techniques to bind to growth factors. The same phage display techniques are used in this example to develop peptide epitopes with binding affinity for *Clostridium difficile* spores and to create the associated hydrogels. The hydrogels are further tagged with a radioactive tag. The hydrogels are introduced by catheter into the colon of an individual with suspected residual *C. difficile* infection in the form of spores. The colon is then irrigated to flush unbound hydrogel from the system. The colon is then scanned for the radioactive tag to identify areas of potential spore infestation.

Example 5

[0342] Paraffin wax (100 ml) was melted at a temperature of 70° C. Pigeon egg white (50 ml) was dissolved in distilled water (400 ml) and the resultant mixture was filtered and then heated to 70° C. The melted wax was added to the egg white mixture, which was then agitated and quickly cooled to a temperature below 40° C. through the addition of chilled water. Glycoproteins recovered from pigeon egg whites acted as a surfactant and became embedded in the surface of paraffin wax in a manner that the wax formed micelles in the diameter range of 0.001 to 4 mm. The resultant spherical structures (i.e., micelles) were dried, rewetted, and exposed to fluorescent lectins. The lectins were found to successfully bind to the sugars on the embedded glycoproteins. The glycoproteins of pigeon egg white express Gal(1-4)Galβ(1-4) GlcNAc, which has been shown to bind to Shiga-like Toxin 1.[34] This glycans structure also has potential to bind to *Streptococcus suis* which can cause meningitis humans and has also been implicated in serious porcine diseases.[35] Accord-

ingly, the material can be a component in an orally administered therapeutic treatment for these two types of infections.

[34] Tomoda, Hiroshi; Masayoshi Arai, Nobuhiro Koyama, Hidenori Matsui, Satoshi mura, Rika Obata and Yuan C. Lee, Purification of Shiga-like toxin 1 by pigeon egg white glycoproteins immobilized on Sepharose gels, Analytical Biochemistry Vol 311, Issue 1, 1 Dec. 2002, pp 50-56

[35] Haataja S, Tikkanen K, Liukkonen J, François-Gerard C, Finne J., Characterization of a novel bacterial adhesion specificity of *Streptococcus suis* recognizing blood group P receptor oligosaccharides, J Biol. Chem. 1993 Feb. 25; 268(6):4311-7.

[0343] Said spherical structures are mixed with an Oral Rehydration Therapy mixture, which also contains antibiotics known to be effective for *E. coli* O157:H7. The spherical constructs of the present invention are intended to capture and immobilize some of the increased toxin loading caused by the use of antibiotics.

Example 6

[0344] A solution of yeast invertase and low density polyethylene (LDPE) with a melting point of 105 degrees Celsius was heated in an agitated pressure reactor to a temperature of 120° Celsius and a pressure of 15 psig. The solution was then cooled through rapid depressurization of the reactor followed by the addition of chilled water. LDPE micelles having surface embedded glycoproteins recovered from yeast invertase were thus formed, the micelles having a diameter ranging from 500 nm to 2,000 nm. The resultant micelles were dried, rewetted, and exposed to fluorescent lectins. The fluorescent lectins were observed to have successfully bound to the sugars on the embedded glycoproteins. The same procedure is used with genetically modified yeast which produces glycoproteins with sialic acid decorations.[3637] The resulting particles provide diagnostic, research and therapeutic particles for application to influenza, particularly potential mutations of the avian influenza virus to human virulence, as well as other pathogens and toxins whose tropism may, in part, be based on sialic acid.

[36] Hamilton, Stephen R., et. al., Humanization of Yeast to Produce Complex Terminally Sialylated Glycoproteins, Science 8 Sep. 2006: Vol. 313. no. 5792, pp. 1441-1443

[37] Amano, Koh, et. al., Engineering of mucin-type human glycoproteins in yeast cells, PNAS | Mar. 4, 2008, vol. 105, no. 9, pp. 3232-3237

Example 7

[0345] A solution containing Tamm-Horsfall glycoproteins (also uromodulin) and microcrystalline wax was heated to 98° C., until the wax melted the resulting mixture was agitated and then pumped through a zone excited by sonication. The mixture was then sprayed through a small orifice into a continuous stream of cold water. The resulting particles in the 50 nm to 1000 nm diameter size range were concentrated and washed. The particles are then suspended in a irrigation solution, which is used to irrigate an infected human bladder.

Example 8

[0346] Paraffin wax particles of the present invention having a surface embedded with the glycoproteins of yeast invertase are exposed to two mutant strains of *Escherichia Coli* CTF073 in LB broth at 37 degrees Celsius for 20 minutes. The first strain was genetically modified to always express type 1 fimbriae and the second strain was modified to not produce type 1 fimbriae. The particles in solution were then rinsed, stained using crystal violet, and observed under the microscope. In the case of the mutant *E. coli* which expressed type 1 fimbriae, binding was observed. No binding was observed with in the case of the bacteria with no ability to produce type 1 fimbriae.

Example 9

[0347] Wax particles with surface moieties glycosylated molecules of porcine intestine were created using techniques of the present invention. The particles were exposed to *Salmonella Kentucky* for 30 minutes at 37 degrees Celsius. The particles were then rinsed three times with sterile water. A lysing compound (InstaGene by BioRad) was introduced into the solution with the particles to rupture the bacteria and liberate the DNA. The particle solution was heated to 56 degrees Celsius for 15 minutes, vortexed and then raised to 100 degrees Celsius for eight minutes and then vortexed again. The solution was allowed to cool. The wax separated and formed a solid film at the top of the vial containing the solution. A pipette was used to puncture a hole in the wax and extract DNA containing solution from the vial. A PCR analysis correctly identified the *Salmonella*. Control samples with no bacteria, properly indicated that case also.

Example 10

[0348] Glycoproteins are harvested from a culture of porcine brain tissue which has been lysed to disrupt the cell membranes. These glycoproteins are conjugated to a hydrophobic block copolymer utilizing PEG-tethering to form an amphiphilic conjugate. Other glycoproteins been shown to retain bioactivity after covalent bonding with heterobifunctional acrylate-N-hydroxysuccinimide poly(ethylene glycol) (PEG).[38] A polar solution containing this material is agitated. A predominantly non-polar mixture of monounsaturated and saturated fatty acids which are at a temperature above their melting point are added to the solution under agitation to form micelles. The solution is then cooled to form semi-solid, but pliable micelles with glycosylation sites expressed on the surface. These micelles are used as an agglutination assay in the study of potential brain pathogens.

[38] Liu H W, Chen C H, Tsai C L, Lin I H, Hsiue G H, Heterobifunctional poly(ethylene glycol)-tethered bone morphogenetic protein-2-stimulated bone marrow mesenchymal stromal cell differentiation and osteogenesis, Tissue Eng. 2007 May; 13(5):1113-24.

Example 11

[0349] An amphiphilic compound is synthesized by PEGylating lactoferrin to a hydrophobic polymeric tail of poly (ethylethylene) utilizing techniques known to those skilled in the art. A polar solution of the PEGylatd lactoferrin amphiphiles is heated to 50 degrees Celsius and then food grade paraffin with a melting point of 48 degrees Celsius is added to the solution. The solution is agitated and then rapidly cooled by dilution to a temperature of 40 degrees Celsius. The resultant wax substrate micelles are washed and then placed in a sterile solution. The mixture is packaged in aseptic packaging as an oral treatment for amoebic dysentery caused by *Entamoeba histolytica*. These pathogens are known to bind to lactoferrin.

Example 12

[0350] Activin is surface immobilized on polymeric filter material. Biotinylated transferrin is then conjugated onto the

surface of the filter. The filter is then provided as a means of capture for *Neisseria meningitidis* in the spinal fluid for diagnostic, research or therapeutic purposes.[39]

[39] Evans & Oakhill Biochem. Soc. Trans. 30 (4): 705-7, 2002

INDUSTRIAL APPLICABILITY

[0351] The biomimetic films and particles of the present invention find utility in the capture, concentration and identification of pathogens and toxins in environmental, research and medical contexts, either in vivo, in vitro, or in situ. Constructs of the biomimetic particles also have therapeutic applications, as pharmaceutical formulations, ingestibles, and the like, having particular applicability in the early stages of an infection, before the infecting organism has been identified, when antibiotics tend to be contraindicated yet when therapeutic intervention has the highest potential for success. As such, the present invention has applicability to both civilian and military medical personnel, individuals responsible for public safety in regards to infectious diseases, food supply integrity, both potable and recreational water quality, livestock (including, but not limited to, hoofed animals, poultry, fish) and pet owners, travelers (including leisure, business, military and disaster relief personnel) subject to intestinal illnesses in visited locations, and researchers doing work on pathogen and toxin tissue tropism. The present invention has particular applicability to disaster relief scenarios where the public is at great risk to waterborne illness; emergency stocks of the present invention can be maintained in a dry form until needed and then administered as part of a hydration therapy to individuals showing symptoms of gastrointestinal illness. The present invention also has applicability to address emerging antibiotic resistant infections.

[0352] In addition, the solid state films and membranes of the present invention, formed from the spontaneous aggregation and specific alignment of glycosylated amphiphilic molecules at the interface between a polar solvent and a non-polar liquid, can be used to produce relatively robust and stable micelles having improved long term storage capacity. In that the biomimetic films and particles of the present invention having surface embedded amphiphilic molecules present useful biological properties to the surrounding environment, they find particular industrial applicability in the areas of environmental sensing, pathogen capture, diagnostic assessment, and therapeutic detoxification.

[0353] All patents and publications mentioned herein are incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0354] While the invention has been described in detail and with reference to specific embodiments thereof, it is to be understood that the foregoing description is exemplary and explanatory in nature and is intended to illustrate the invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

[0355] Other advantages and features will become apparent from the claims filed hereafter, with the scope of such claims to be determined by their reasonable equivalents, as would be understood by those skilled in the art. Thus, the invention is intended to be defined not by the above description, but by the following claims and their equivalents.

APPENDIX A

Known Pathogen Target Sequences

(b1-3)Gal

(b1-4)Gal

Fuc

Fuc(a1-2)[Gal(a1-3)]Gal(b1-3)GalNAc(b1-4)[Gal(b1-4)Glc(b1-1)Cer]NeuAc(a1-3)

Fuc(a1-2)[Gal(a1-3)]Gal(b1-3)GalNAc(b1-4)[NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-2)[Gal(a1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer]NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)

Fuc(a1-2)[Gal(a1-3)]Gal(b1-4)GlcNAc(b1-6)[NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-2)[Gal(a1-3)Gal(b1-3)]GlcNAc[Fuc(a1-4)]

Fuc(a1-2)[GalNAc(a1-3)Gal(b1-3)]Fuc(a1-4)[GlcNAc]

Fuc(a1-2)[GalNAc(a1-3)Gal(b1-3)]GlcNAc

Fuc(a1-2)[GalNAc(a1-3)Gal(b1-3)]GlcNAc[Fuc(a1-4)]

Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]Gal

Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-2)Gal(b1-3)Fuc(a1-4)[GlcNAc]

Fuc(a1-2)Gal(b1-3)GalNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-2)Gal(b1-3)GlcNAc

Fuc(a1-2)Gal(b1-4)GlcNac

Fuc(a1-3)[Gal(b1-4)]GlcNAc(b1-6)[Gal(b1-3)]GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-3)[Gal(b1-4)]GlcNAc(b1-6)[GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer]Gal(b1-3)

Fuc(a1-3)[NeuAc(a1-3)Gal(b1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-3)[NeuAc(a1-3)Gal(b1-4)]GlcNAc(b1-3)Gal(b1-4)[GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer]Fuc(a1-3)

Fuc(a1-3)[NeuAc(a1-3)Gal(b1-4)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-3)[NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-4)[Fuc(a1-2)Gal(b1-3)]Gal

Fuc(a1-4)[Fuc(a1-2)Gal(b1-3)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Fuc(a1-4)[Gal(b1-3)]GlcNAc(b1-3)Gal(b1-4)Glc

Fuc(a1-4)[Gal(b1-3)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal

Gal(a1-3)[Fuc(a1-2)]Gal(b1-3)GalNAc(b1-4)[Gal(b1-4)Glc(b1-1)Cer]NeuAc(a1-3)

APPENDIX A-continued

Known Pathogen Target Sequences

Gal(a1-3)[Fuc(a1-2)]Gal(b1-3)GalNAc(b1-4)[NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer]NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)

Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)GlcNAc(b1-6)[NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(a1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(a1-3)Gal(b1-3)[Fuc(a1-2)]Fuc(a1-4)[GlcNAc]

Gal(a1-3)Gal(b1-3)[Fuc(a1-2)]GlcNAc[Fuc(a1-4)]

Gal(a1-3)Gal(b1-4)Glc

Gal(a1-3)Gal(b1-4)Glc(b1-1)

Gal(a1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(a1-3)Gal(b1-4)GlcNAc

Gal(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(a1-3)Gal(b1-4)GlcNAc(b1-6)[NeuAc(a2-3)Gal(b1-4)Glc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)

Gal(a1-4)Gal

Gal(a1-4)Gal(b

Gal(a1-4)Gal(b1-1)

Gal(a1-4)Gal(b1-1)Cer

Gal(a1-4)Gal(b1-1)Glc

Gal(a1-4)Gal(b1-4)Glc

Gal(a1-4)Gal(b1-4)Glc(b1-1)

Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(a1-4)Gal(b1-4)Glc(b1-1)Me

Gal(a1-4)Gal(b1-4)GlcNAc

Gal(a1-4)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(a1-4)Glc(b

Gal(b

Gal(b1-1)Cer

Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc

Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)[NeuAc(a2-3)]GalNAc(b1-4)Gal(b1-4)[NeuAc(a2-3)Glc(b1-1)Cer

Gal(b1-3)[NeuAc(a2-3)]GalNAc(b1-4)Gal(b1-4)Glc

APPENDIX A-continued

Known Pathogen Target Sequences

(b1-1)Cer

Gal(b1-3)Gal

Gal(b1-3)GalNAc

Gal(b1-3)GalNAc(b1-3)[NeuAc(a2-8)NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)

Gal(b1-3)GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)[NeuAc(a1-3)]Gal(b1-3)GalNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)[NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)[NeuAc(a1-8)NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-3)]Gal

Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)

Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-8)NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)

Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-8)NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)[NeuGc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GalNAc(b1-4)Gal(b1-4)Glc

Gal(b1-3)GalNAc(b1-4)Gal(b1-4)Glc(b1-1)

Gal(b1-3)GalNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)Glc(b1-3)Gal(b1-4)Glc

Gal(b1-3)GlcNAc

Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc

Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)]GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer]Gal(b1-3)

Gal(b1-4)[NeuAc(a2-3)]GlcNAc(b1-3)Gal(b1-4)GlcNAc

Gal(b1-4)GalNAc

Gal(b1-4)GalNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-4)Glc

Gal(b1-4)Glc(b1-1)Cer

Gal(b1-4)GlcNAc

APPENDIX A-continued

Known Pathogen Target Sequences

Gal(b1-4)GlcNAc(b1-3)Gal

Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)

Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

GalNAc

GalNAc(a1-3)Gal(b1-3)[Fuc(a1-2)]Fuc(a1-4)[GlcNAc]

GalNAc(a1-3)Gal(b1-3)[Fuc(a1-2)]GlcNAc

GalNAc(a1-3)Gal(b1-3)[Fuc(a1-2)]GlcNAc[Fuc(a1-4)]

GalNAc(a1-3)GalNAc(a1-3)Gal(a1-4)Gal(b1-4)Cer

GalNAc(a1-3)GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

GalNac(b

GalNAc(b1-3)[Gal(a1-3)]Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-3)Gal

GalNAc(b1-3)Gal(a1-3)Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)

GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-3)GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-4)[NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-4)[NeuAc(a1-8)NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-4)[NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)

GalNAc(b1-4)[NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)Cer

GalNAc(b1-4)Gal

GalNAc(b1-4)Gal(b1-4)Glc(b1-1)

GalNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

Glc(b1-1)Cer

GlcNAc

GlcNAc(b1-3)Gal

GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

GlcNAc(b1-4)GlcNAc

Lac(-)Cer

Man

Man(a1-2)Man

Man(a1-2)Man(a1-6)Man(a1-6)

Man(a1-3)[Man(a1-6)]Man(a1-6)[Man(a1-2)]Man(a1-3)Man

APPENDIX A-continued

Known Pathogen Target Sequences

Man(a1-3)[Man(a1-6)]Man(a1-6)[Man(a1-3)Man]Man(a1-2)

Man(a1-3)[Man(a1-6)]Man(a1-6)Man(a1-4)Man

Man(a1-3)Man(a1-4)GalNAc

Man(a1-3)Man(a1-6)Man

Man(a1-6)[Man(a1-3)]Man(a1-6)[Man(a1-2)]Man(a1-3)Man

Man(a1-6)[Man(a1-3)]Man(a1-6)[Man(a1-3)Man]Man(a1-2)

Man(a1-6)[Man(a1-3)]Man(a1-6)Man(a1-4)Man

NeuAc

NeuAc(a1-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)[GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal

NeuAc(a1-3)Gal(b1-3)GalNAc(b1-4)[NeuAc(a1-8)NeuAc(a1-3)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)

NeuAc(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer]Fuc(a1-3)

NeuAc(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)[NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-6)[NeuAc(a1-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-6)Gal

NeuAc(a1-8)NeuAc(a1-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-8)NeuAc(a1-3)[GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a1-8)NeuAc(a1-3)[NeuAc(a1-3)Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

APPENDIX A-continued

Known Pathogen Target Sequences

NeuAc(a2-3)

NeuAc(a2-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)[Gal(b1-4)]GlcNAc(b1-3)Gal(b1-4)GlcNAc

NeuAc(a2-3)[GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)[GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)[NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)]Gal(b1-3)Glc(b1-1)

NeuAc(a2-3)[NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal

NeuAc(a2-3)Gal(b1-3)[NeuAc(a2-6)]GalNAc

NeuAc(a2-3)Gal(b1-3)[NeuAc(a2-6)]GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-3)GalNAc

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-3)]Gal(b1-3)Glc(b1-1)

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-3)NeuAc(2-8)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-8)NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)[NeuAc(a2-8)NeuAc(a2-3)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-4)GalNAc

NeuAc(a2-3)Gal(b1-4)Glc

NeuAc(a2-3)Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-4)Glc(b1-3)[Gal(a1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)Gal(b1-4)GlcNAc

NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-3)[NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

APPENDIX A-continued

Known Pathogen Target Sequences

NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-4)Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-4)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)

NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-6)[NeuAc(a2-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-3)Lac

NeuAc(a2-3)NeuAc(2-8)[NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-6)[NeuAc(a2-3)Gal(b1-3)]GalNAc

NeuAc(a2-6)[NeuAc(a2-3)Gal(b1-3)]GalNAc(b1-3)Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-6)Gal

NeuAc(a2-6)Gal(b1-3)GlcNAc(b1-4)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-6)Gal(b1-4)GlcNAc

NeuAc(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-6)Gal(b1-4)GlcNAc(b1-4)Gal(b1-4)Glc(b1-1)

NeuAc(a2-8)

NeuAc(a2-8)NeuAc(a2-3)[Gal(b1-3)GalNAc(b1-3)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-8)NeuAc(a2-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)

NeuAc(a2-8)NeuAc(a2-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuAc(a2-8)NeuAc(a2-3)[NeuAc(a2-3)Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)

NeuAc(a2-8)NeuAc(a2-3)Gal(b1-4)Glc(b1-1)

NeuAc(a2-8)NeuAc(a2-3)Gal(b1-4)Glc(b1-1)Cer

NeuGc(a1-3)[Gal(b1-3)GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuGc(a1-3)[GalNAc(b1-4)]Gal(b1-4)Glc(b1-1)Cer

NeuGc(a1-3)Gal(b1-4)Glc(b1-1)Cer

NeuGc(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuGc(a2-3)Gal(b1-4)Glc

NeuGc(a2-3)Gal(b1-4)Glc(b1-1)

NeuGc(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer

NeuNAc(a2-3)Gal(b

NeuNAc(a2-3)Gal(b1-4)Glc

1-97. (canceled)

98. A solid state film with biomimetic properties having at least one functionally active surface, said film comprising a substrate having a plurality of glycosylated molecules immobilized therein, wherein said glycosylated molecules are either harvested or derived from biological fluid or tissue, or comprise a functional mimic of a component of said biological fluid or tissue.

99. The biomimetic film of claim **98**, wherein the glycosylated molecules comprise endogenous cell-surface glycan signatures harvested or derived from biological fluid or tissue or functional mimics thereof.

100. The biomimetic film of claim **99**, wherein said glycan signature are selected from the group consisting of pathogen binding moieties and self antigens that masks the foreign nature of the film and thereby reducing its immunogenic potential.

101. The biomimetic film of claim **98**, wherein the glycosylated molecules are selected from the group consisting of glycoproteins, glycolipids, glycosides, glycosylated transmembrane proteins, glycodendrimers, glycodendriproteins, and non-immunological sialated glycosylated molecules.

102. The biomimetic film of claim **98**, wherein the glycosylated molecules include surface moieties of attaching and effacing (A/E) lesions or functional mimics thereof.

103. The biomimetic film of claim **99**, wherein said glycan signatures are harvested or derived from the inner or outer walls or membranes of the organs of the gastrointestinal tract, urinary tract, pulmonary tract, blood vessels, amniotic sac, ocular sac, nervous system, muscoskeletal system.

104. The biomimetic film of claim **99**, wherein said glycan signatures are isolated from body fluids selected from the group consisting of mucous, blood, blood plasma, saliva, urine, synovial fluid, breast milk, tears, fluids of the reproductive system, aqueous or vitreous humor, bone marrow, and cerebrospinal fluids.

105. The biomimetic film of claim **98**, wherein said substrate is non-polar or exhibits hydrophobic surface properties and is selected from the group consisting of resin, natural wax, synthetic wax, natural and synthetic plastics, polymers, hydrocarbon mixtures, natural and synthetic fats, ceramics, glass, diatomaceous earth, and metal.

106. The biomimetic film of claim **98**, wherein said substrate further comprises a detectable label selected from the group consisting of a fluorescent label, a radioactive label, a dye, and a compound that enhances magnetic resonance imaging.

107. The solid state biomimetic film of claim **98**, wherein said film comprises a relatively non-polar substrate and said plurality of glycosylated molecules are immobilized to the upper surface of said substrate, further wherein one or more amphiphilic molecules having hydrophobic tail ends and hydrophilic head ends are embedded in or chemically, electrically or mechanically linked via said hydrophobic tail ends to the relatively non-polar substrate, further wherein the amphiphilic molecules either themselves comprise biomimetic moieties or have such biomimetic moieties attached or conjugated to their hydrophilic head ends such that the biomimetic moieties project from said upper surface so as to provide at least one surface of said film with functional activity.

108. The solid state biomimetic film of claim **107**, wherein a heterogeneous mixture of amphiphilic molecules or conjugates with pathogen and/or toxin binding properties is present in the film in a manner which provides for multiple pathogen and/or toxin binding mechanisms or moieties.

109. A therapeutic biocompatible particle formed from the solid state biomimetic film of claim **1**, said particle of a size or geometric configuration that prevents its absorption by the biological tissue of an organism.

110. The therapeutic particle of claim **109**, wherein said biological tissue is selected from the group consisting of biological tissue extracted from human, animal or plant tissue; a culture of human, animal or plant tissue; a biological surrogate for human, animal or plant tissue; a culture of a biological surrogate for human, animal or plant tissue or a recombinant version of human, animal or plant tissue or a human, animal or plant tissue biological surrogate, further wherein said biological tissues are selected from the group consisting of epithelium, connective tissue, muscle tissue, nerve tissue, material associated with or contained in amniotic fluid surrounding a fetus, aqueous humour, blood, blood plasma, interstitial fluid, breast milk, mucus, pus, saliva, serum, tears, urine, cerebrospinal fluid, synovial fluid, intracellular fluid, aqueous humour, vitreous humour and other bodily fluids.

111. The therapeutic particle of claim **109**, wherein the substrate comprises a nonbiodegradable material capable of maintaining the immobilization of said glycosylated molecules when ingested by a living organism.

112. The therapeutic particle of claim **109**, wherein the particle is in the form of a wax micelle.

113. The therapeutic particle of claim **109**, wherein the particle is in the form of a worm micelle.

114. A pharmaceutical composition comprising the therapeutic particle of claim **109** formulated for introduction into a living organism.

115. The pharmaceutical composition of claim **114**, wherein said composition further comprises one or more additional agents selected from the group consisting of antibiotic agents, probiotics, vitamin supplements such as iron, or therapeutic agents for oral rehydration therapy such as oral rehydration salts, solution, and electrolytes.

116. A method for making the biomimetic film of claim **98** comprising the following steps, with the resultant films being disposed on solid substrates:

a. providing a relatively polar solvent having sufficient quantities dissolved therein of one or more amphiphilic molecules which include glycosylated molecules with biomimetic properties, or conjugates of such, or molecules which can be c conjugated with glycosylated molecules with biomimetic properties, or molecules with other pathogen binding moieties;

b. exposing the polar solvent to a relatively non-polar liquid, the non-polar liquid being immiscible in the polar solvent; the relatively non-polar liquid can optionally comprise a material which can reversibly experience a state change from solid to a relatively non-polar liquid under stimulus;

c. allowing the amphiphilic molecules to align so as to form a membrane which separates the polar solvent from the non-polar liquid; and

d. inducing or allowing transformation of the non-polar liquid to a corresponding non-polar solid having upper and lower surfaces, wherein the hydrophobic tail ends of the amphiphilic molecule are embedded in or chemically, electrically or mechanically linked to the upper surface of the non-polar substrate and the hydrophilic

tail ends project from the upper surface into the polar solvent so as to yield a film having functional activity.

117. The method of claim **116**, further comprising the step of adsorbing or conjugating molecules with biomimetic or other properties to the functional surface of the film if the desired functionality is not present from the preceding steps.

118. A method of forming the biomimetic film of claim **98** from biological cell- or membrane-bound glycosylated molecules comprising the following steps:

a. isolating biological tissue extracted from human, animal or plant tissue, a culture of human, animal or plant tissue, a biological surrogate for human, animal or plant tissue, a culture of a biological surrogate for human, animal or plant tissue or recombinant versions of human, animal or plant tissue or human, animal or plant tissue biological surrogates;

b. subjecting the biological cell or membrane to a process which ruptures or lyses the cell or other membrane, including but not limited to, mechanical means (for example, but not limited to, sonication, freeze/thaw and high shear techniques), chemical means (for example, but not limited to, using detergents for whole cell lysis and cell fractionation) and biochemical means (for example, but not limited to, by means of enzymes and/or protease inhibitors), to form a liquid volume with biomimetic molecules present;

c. optionally filtering or otherwise selectively separating a fraction of interest containing glycosylated molecules with biomimetic properties, said fraction may or may not comprise a liquid or other molecules;

d. immobilizing said biomimetic molecules as part of a film on an appropriate substrate; and

e. optionally removing said film from any surrounding liquid to form a dry construct.

119. The method of claim **118**, wherein the step of selectively separating a fraction of interest containing glycosylated molecules with biomimetic properties comprises the following steps for molecules which are amphiphilic in nature:

a. exposing the solution containing biological tissue, which can comprise, in part, lysed or otherwise ruptured cell material containing molecules of interest in the present invention to a less-polar material;

b. if necessary, adding amphiphilic molecules to achieve critical micelle concentration, before or after exposing said solution to said less-polar material;

c. allowing the amphiphilic compounds to form a film at the interface between said solution and said less-polar material;

d. conducting any desired procedures to affix the molecules to the less-polar substrate beyond the hydrophobic forces inherent in the film; and

e. removing, or replacing with another liquid, some or all of said solution.

120. The method of claim **118**, wherein the transformation is achieved through cooling of the non-polar liquid.

121. A method of using of biomimetic film of claim **98** comprising the steps of:

a. exposing said surface to a liquid sample which may or may not contain pathogens or toxins;

b. allowing sufficient time for said surface to bind to target pathogens or toxins present in said sample;

c. optionally separating said surface, to which said target pathogens or toxins may be bound, from said sample; and

d. optionally, analyzing said surface to detect the presence or type of pathogen or toxin, or subjecting said surface, to which said target pathogens or toxins may be bound, to an agent which effectively separates the pathogens or toxins from the surface and subjecting said pathogen or toxins, or media containing such, to analysis techniques to detect presence or type.

122. A method of using the biomimetic film of claim **98**, comprising the steps of:

a. extracting of a bodily fluid of an organism, including, but not limited to amniotic fluid, aqueous or vitreous humour, blood and blood plasma, bone marrow fluids, cerebrospinal fluid, interstitial fluid, lymph fluids and pleural fluid;

b. inducing contact of said bodily fluid to one or more surfaces with the pathogen and toxin binding moieties of the present invention;

c. optionally, reintroducing of the bodily fluid into the organism; and

d. optionally, subjecting said surfaces of the present invention to analysis techniques to determine presence and/or type of pathogens or toxins that may have bound to the surface.

123. A method of using the therapeutic particle of claim **109**, comprising the steps of:

a. administering one or multiple oral doses of a plurality of particles of constructs of the present invention to the host;

b. allowing the particles of the present invention to contact areas of the alimentary system of the host where pathogens may be present; and

c. allowing the host organism to discharge the particles through defecation, vomiting, expectoration or other means of natural or induced discharge.

124. A method of using the therapeutic particle of claim **109**, comprising the steps of:

a. administering one or multiple doses of a plurality of particles of the present invention to the host through a catheter or similar device or through other means of physically placement into a region of suspected infection or a point in the host where the particles will migrate to a region where pathogens may be present;

b. allowing the particles of the present invention to contact those areas of the host where pathogens may be present; and

c. allowing the host organism to discharge the particles through defecation, urination, vomiting, mucosal flow, expectoration or other means of natural or induced discharge or removing the particles from the host through a catheter or other device.

125. A medical device with enhanced surface biocompatibility formed from or coated with the biomimetic film of claim **98**.

126. The device of claim **125**, wherein said biomimetic glycosylated molecules are harvested or derived from tissue of an intended host organism of said medical device or an antigen-matched donor thereof or from a culture of tissue from said recipient or donor.

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专利名称(译)	用于病原体捕获和其他用途的仿生颗粒和薄膜		
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申请号	US12/663904	申请日	2008-06-11
[标]申请(专利权)人(译)	SPEDDEN RICHARD ^ h		
申请(专利权)人(译)	SPEDDEN RICHARD ^ h		
当前申请(专利权)人(译)	SPEDDEN RICHARD ^ h		
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摘要(译)

具有仿生性质的糖基化分子，更特别是天然存在的，组织衍生的，非免疫学聚糖序列或其功能等同物在固态表面和膜上或膜上的鉴定和固定在非极性和极性材料。由其构建的仿生糖基化膜和颗粒在体内，体外或原位结合，捕获和/或提取病原体，毒素和/或污染物中具有工业，环境，诊断和/或治疗效用。本发明进一步延伸到这种仿生膜和颗粒用于递送其它治疗分子以及具有增强的生物相容性和降低的免疫原性的身体接触装置的构建中的用途。