ADVANCED FUNCTIONAL BIOCOMPATIBLE POLYMERIC MATRIX USED AS A HEMOSTATIC AGENT AND SYSTEM FOR DAMAGED TISSUES AND CELLS

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See application file for complete search history.

ABSTRACT
A hemostatic tissue sealant sponge and a spray for acute wounds are disclosed. The sponge comprises hydrophobically modified polymers that anchor themselves within the membrane of cells in the vicinity of the wound. The seal is strong enough to substantially prevent the loss of blood inside the boundaries of the sponge, yet weak enough to substantially prevent damage to newly formed tissue upon recovery and subsequent removal of the sponge. In examples, the polymers inherently prevent microbial infections and are suitable for oxygen transfer required during normal wound metabolism. The spray comprises hydrophobically modified polymers that form solid gel networks with blood cells to create a physical clotting mechanism to prevent loss of blood. In an example, the spray further comprises at least one reagent that increases the mechanical integrity of the clot. In another example, the reagent prevents microbial infection of the wound.

3 Claims, 6 Drawing Sheets
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FIG. 1

FIG. 2
hydrophobes anchor and remain anchored in the bilayer of the tissue/cell membrane

FIG. 3
administering a polymer attached with a plurality of short hydrophobic substituents to a wounded tissue or a red blood cell
FIGURE 8
ADvanced functional biocompatible polymeric matrix used as a hemostatic agent and system for damaged tissues and cells

CROSS REFERENCE TO RELATED APPLICATION

The present application claims priority under 35 U.S.C. §119 to the U.S. Provisional Application Ser. No. 60/969,721, filed on Sep. 4, 2007, which are both herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention generally relates to the field of hydrophobically modified polymers and their use in promoting hemostasis in wounded tissues and cells.

BACKGROUND OF THE INVENTION

Currently, every year 21 million people worldwide suffer from serious injuries resulting in severe blood loss, and more than one third of these cases lead to death. (See Bledsoe, B. E., “The Golden Hour: Fact or Fiction.” Emergency Med. Serv. 6, 105 (2002), which is herein incorporated by reference in its entirety.) Among these patients are American soldiers in Iraq whose fatality rate with severe injuries is 90%. Uncontrolled hemorrhaging from these injuries is the leading cause of preventable combat deaths among U.S. soldiers in Iraq. According to the Marine Corps registry, 45-60% of combat casualty deaths are due to potentially preventable uncontrolled hemorrhage. (See Clarke, Patrick E., "Z-Medica’s Products Cited as Life Saving on Battlefield", which is herein incorporated by reference in its entirety.) Similarly, uncontrolled hemorrhage is the leading cause of potentially preventable deaths in the U.S. with 30-40% of all trauma deaths in the civilian population due to uncontrolled bleeding. In many cases, these deaths occur before the injured are able to be transported to medical treatment with approximately 40% of civilian and 90% of military casualties occurring before the patients reach a treatment facility. (See Kim, Seung-Ho M D; Stezoski, S; William; Safar, Peter M D; Capone, Antonio M D; Fisherman, Samuel M D. Journal of Trauma-Injury Infection & Critical Care. 42(2):213-222, February 1997, which is herein incorporated by reference in its entirety.) Many of these casualties can be prevented if an effective treatment is used to quickly stop the significant loss of blood.

Controlling hemorrhage is also a critical issue in medical facilities with 97 million patients experiencing surgical bleeding every year worldwide. (See B.S. Kheirabadi, E. M. Acheson, R. Deguzman, J. L. Sondeen, K. L. Ryan, A. Delgado, E. L. Dick, J. B. Holcomb, “Hemostatic efficacy of two advanced dressings in an aortic hemorrhage model in swine.” J. Trauma. (2005), which is herein incorporated by reference in its entirety.) Despite advances in medical technology, hemorrhage control is still a major problem in emergency medical care. In the first 48 hours of hospitalization, approximately 51% of deaths are due to hemorrhage (See F W Verheugt, M J van Enigbe, J C Res, M L Simoons, P W Serruys, F Vermeer, D C van Hoogenhuyze, P J Remme, C de Zwaan, and F Baer. Bleeding complications of intracoronary fibrinolytic therapy in acute myocardial infarction. Assessment of risk in a randomised trial, which is herein incorporated by reference in its entirety.) Improving the ability to control hemorrhage for injuries that are otherwise survivable would greatly reduce trauma mortality, and this knowledge has encouraged numerous advancements in hemostatic control; however, the currently available hemostatic bandages are not sufficiently resistant to termination in high blood flow and they do not have strong enough adhesive properties to stop severe blood flow for an adequate time period.

Today the application of continuous pressure using gauze bandages remains the primary technique used to staunch blood flow, particularly in severe bleeding wounds. This procedure neither successfully nor safely stops severe blood flow. As in the past, this method continues to be a major survival problem in the case of serious life-threatening bleeding. Other currently available hemostatic bandages, such as collagen wound dressings or dry fibrin thrombin wound dressings do not have strong enough adhesive properties to serve any realistic purpose in the staunching of severe blood flow. These hemostatic bandages are also fragile and are therefore liable to fall if damaged due to bending or application of pressure. (See Gregory, Kenton W. and Simon J. McCarthy. Wound dressings, apparatus, and methods for controlling severe, life-threatening bleeding, which is herein incorporated by reference in its entirety.)

Recent advancement in hemostatic bandages have targeted the immediate treatment of acute wounds, such as the prevention of casualties due to hemorrhage on the battlefield. Chitosan based bandages have been approved and used in numerous settings including battlefield use with success. Chitosan is an amino polysaccharide that is commercially produced from the deacetylation of chitin which is an abundant natural biopolymer that is found in the exoskeleton of crustaceans. Advantages of chitosan as a material for wet wound dressings include its ability to accelerate wound-healing, its hemostatic properties, its stimulation of macrophage activity, and its general anti-microbial impact which helps prevent infection at the wound site. Chitosan is used as a hemostatic agent because of its cationic nature. Since the surfaces of most biological cells are anionic, including red blood cells, chitosan strongly adheres to the cells of tissue at wound sites because of an electrostatic interaction and is able to initially halt blood flow. (See Dornard, Alain and Monique. “Chitosan: Structure-Properties Relationship and Biomedical Applications.” (2002), which is herein incorporated by reference in its entirety.)

Despite the advantages of using a chitosan-based dressing, there are also significant disadvantages. In a study conducted in 2005 by Kheirabadi et al, researchers caused injury to the aorta of pigs and attempted to control hemorrhage using the Hemostatic HemConA® Bandage. The results of the study showed that though the chitosan bandage effectively reached hemostasis immediately after its application, secondary bleeding resumed approximately 2 hours later and resulted in the death of the pigs. They found that the adherence between the bandage and the tissue increased as the bandaged became saturated with blood. (See B. S. Kheirabadi, E. M. Acheson, R. Deguzman, J. L. Sondeen, K. L. Ryan, A. Delgado, E. L. Dick, J. B. Holcomb, “Hemostatic efficacy of two advanced dressings in an aortic hemorrhage model in swine.” J. Trauma. (2005), which is herein incorporated by reference in its entirety.) In realistic situations in which patients are incapable of reaching adequate medical treatment, such as on the battlefield, this time period is too short and the Hemostatic HemConA® Bandage would be an insufficient hemostatic agent.

Wound physiology is difficult to manipulate due to the complexity of cell-to-cell signaling networks which communicate during wound healing, but fortunately for healthy individuals, severe wounds can be healed quite well by simply
disinfecting the wound area and stopping the loss of blood by suturing the open, damaged tissue. However, in many cases of acute wounds, internal or external, suturing is neither effective nor practical. In these cases, it is advantageous to use solid materials which adhere strongly enough to tissue such that they provide a seal upon pressing such materials onto the damaged tissue, thus preventing the loss of blood within the boundaries of the seal.

Additionally, blood clotting is a necessary aspect of wound physiology, and in many practical cases it is highly valuable for survival. However, for human beings and most mammals, the ability for blood to clot is limited because a significant increase in the ability of healthy mammalian organisms to form blood clots would most likely result in death of the organism as a result of any minor internal injury. In such cases, clots would form at the site of the injury, enter into the blood stream and eventually cause a stroke or heart failure due to blockage of important blood vessels. Because clotting ability must be limited under normal physiological conditions, in the case of acute wounds, which refers to a fast impact injury resulting in a high rate of blood loss, clotting is not a legitimate way to provide hemoostasis. Therefore, acute wounds typically drain blood banks of their stocks as large blood transfusions are required to keep patients alive during transport to treatment facilities and subsequently during the surgery required to close the wound.

Therefore, it would be desirable to provide a strongly adhesive hemostatic bandage that promotes increased adhesion to wounded tissue or cells. It would also be desirable to provide a strongly adhesive flowable spray solution or surgical sealant to stop minor bleeding and to seal tissues in surgical applications.

**SUMMARY OF THE INVENTION**

Accordingly, the present invention provides a novel hybrid composition of matter that provides a strongly adhesive hemostatic agent. In a preferred exemplary embodiment, the composition of matter is a film or solid state tissue sealant sponge that is composed of a hydrophobically modified biopolymer matrix capable of hydrophobically interacting with tissue, particularly hemostatic interaction with damaged tissue. It is contemplated that this sponge may be included as part of a bandage or other applicator form as may be known by those skilled in the art. The hemostatic interaction occurs through a process wherein a plurality of short hydrophobic substituents, that are attached with the polymer backbone, interact with and adhere to the tissue.

In a further preferred exemplary embodiment, the composition of matter is formulated in a liquid state as a spray solution. The solution is composed, at least in part, of the hydrophobically modified biopolymer matrix capable of hydrophobically interacting with tissue and/or gelling with cells, particularly a hemostatic interaction with tissue and/or red blood cells. Similar to the solid state film's interaction process, the hemostatic interaction of the solution occurs through a process wherein a plurality of short hydrophobic substituents, that are attached with the polymer backbone, interact with and adhere to the tissue and/or cells.

In other preferred exemplary embodiments, the current invention provides a system for delivering the novel solution including the functional capabilities of the hydrophobically modified polymer matrix. In one exemplary embodiment, the system includes a container for storing the solution and an ejection mechanism connected with the container to eject the solution to an environment outside the container. In an alternative exemplary embodiment the system includes two or more containers operationally connected through an ejection mechanism for ejecting a mixture of the solution and various other secondary components.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed. The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate an embodiment of the invention and together with the general description, serve to explain the principles of the invention.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The numerous advantages of the present invention may be better understood by those skilled in the art by reference to the accompanying figures in which:

FIG. 1 is a structural and graphical illustration representing a readily reactive, hydrophobically modified chitosan matrix (hm-Chitosan), wherein the hm-Chitosan “backbone” or “scaffold” is capable of binding with a plurality of short hydrophobic substituents and the resulting compound being formulated as a tissue sealant sponge (solid state) or solution (liquid state) in accordance with an exemplary embodiment of the present invention;

FIG. 2 is an illustration of a tissue sealant sponge showing the matrix including the plurality of short hydrophobic substituent that provide the hydrophobic interaction functional capability to the solid state and liquid state formulation of the current invention;

FIG. 3 is an illustration providing a representation of hydrophobic interaction between the short hydrophobic substituents of the modified polymer matrix and the bi-layer of tissue and/or cells;

FIG. 4 is a block diagram representation of a method of using the hydrophobically modified biopolymer matrix of the current invention;

FIG. 5 is a graph showing the Force required for removal of chitosan and hm-chitosan films vs. level of hydrophobic modification.

FIG. 6A is an illustration of 4-octadecoxylbenzaldehyde 2.5% modified hm-chitosan and blood solution when first mixed

FIG. 6B is an illustration of 4-octadecoxylbenzaldehyde 2.5% modified hm-chitosan and blood solution immediately after mixture with inverted test tube to show gelation of solution.

FIGS. 7A-7D are illustrations of dynamic rheology frequency sweeps. FIG. 7A shows a chitosan (0.75 wt %)+blood (heparinized); FIG. 7B shows 2.5% C12 mod hm-chitosan (0.75 wt %)+blood (heparinized); FIG. 7C shows 6% C12 mod hm-Chitosan (0.75 wt %)+blood (heparinized); and FIG. 7D shows 2.5% mod (C18-benz) hm-Chitosan (0.4 wt %)+blood (heparinized).

FIGS. 8A-8D are illustration of time to gelation. FIG. 8A shows a chitosan (0.75 wt %)+blood (heparinized); FIG. 8B shows 2.5% C12 mod hm-chitosan (0.75 wt %)+blood (heparinized); FIG. 8C shows 6% C12 mod hm-Chitosan (0.75 wt %)+blood (heparinized); and FIG. 8D shows 2.5% mod (C18-benz) hm-Chitosan (0.4 wt %)+blood (heparinized).

**DETAILED DESCRIPTION OF THE INVENTION**

Reference will now be made in detail to the presently preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings.
Referring generally to FIGS. 1 and 2, the current invention provides a novel composition of matter that is capable of promoting hemostasis and/or hemostatic response through hydrophobic interactions with tissue and/or cells. In preferred embodiments, the current invention provides a hydrophobically modified polymer matrix capable of hydrophobic interactions with various tissue and/or cells to promote hemostasis. The hydrophobically modified polymer provides a readily reactive matrix and is capable of maintaining its reactive nature through various formulations, such as a solid-state film or liquid state solution.

In a preferred embodiment, the current invention provides a solid state, hemostatic tissue sealant sponge that is composed of at least one polymer and a plurality of short hydrophobic substituents attached along the backbone of the polymer. The sponge extends the hemostatic lifespan of a polymer-based bandage due to anchoring of the hydrophobic grafts into the membranes of cells in the vicinity of the damaged tissue. As a result, the sponge is an effective hemostatic sealant device. The level of hydrophobic modification of the polymer as well as hydrophobic substituent type is substantially optimized to develop materials which adhere to tissue in a manner idealized for clinical applications: the material comprising the sponge adheres strongly enough to provide hemostasis for a long enough time period to allow for substantially full patient recovery, yet weakly enough such that newly formed tissue is substantially undamaged upon removal of the tissue sealant device after patient recovery.

In another preferred embodiment, a hemostatic spray solution (liquid state) is composed of the hydrophobically modified polymer matrix providing at least one water-soluble polymer and a plurality of short hydrophobic substituent attached along the backbone of the polymer. The hydrophobically modified polymer sprayed in aqueous solution is able to form solid networks upon interaction with blood, as the hydrophobic substituent are able to anchor themselves within the bilayers of blood cell. The result is a localized "artificial clot" which physically prevents further blood loss around the newly formed solid network. "Artificial clots" herein refer to physical networks of hydrophobically modified polymers, blood cells, and surrounding tissue cells which collectively act as a solid barrier to prevent further blood loss. Additionally, the level of hydrophobic modification of the polymer as well as hydrophobic substituent type can be substantially optimized to yield rapidly forming and mechanically robust artificial clots. In an example, the hydrophobically modified polymer spray solution is mixed with at least one water-soluble reagent that results in faster and more efficient healing of the wound.

The novel hemostatic agent of the current invention is suitable for use with any various tissues and cells. These agents may be used with the tissues and cells of mammals. As used herein, the term "mammals" means any higher class of vertebrates that nourish their young with milk secreted by mammary glands, e.g. humans, rabbits and monkeys.

The polymer that forms the backbone of this reactive matrix is of synthetic or natural origin, including for example, water-soluble polysaccharides and water-soluble polypeptides. In particularly preferred embodiments, the polymer is one or more hydrophobically modified polysaccharides, including but not limited to celluloses, chitosans and alginates, all of which are abundant, natural biopolymers. All three types of materials allow for the transfer of oxygen and moisture required to metabolize the wound healing physiology.

The natural origin of these polysaccharides varies, celluloses are found in plants, whereas chitosans and alginates are found in the exoskeleton or outer membrane of a variety of living organisms. Many of these naturally occurring polymers, in addition to being able to form long stable chains for forming the backbone of the current invention, have other benefits that may promote further advantages for their use in environments of damaged tissue, hemorrhaging, and/or exposed red blood cells. For instance, chitosan also has inherent anti-microbial properties; this is a crucial asset for materials covering open wounds because it eliminates the need to constantly change wound dressings in order to disinfect the wound manually between changes. Positive charges along the backbone of chitosan cause it to interact electrostatically with negatively charged blood cells, thus creating a sticky interface between a chitosan sponge and the wound. Chitosan provides hemostasis for an extended period of time when compared against standard bandages. For example, the sponge of the current invention provides substantially 30 minutes more absorption/adherence time before becoming saturated with blood cells and losing adherence to the wound site.

The form of the natural polymers used may vary to include standard states, derivatives and other various formulations. For example, the celluloses may include without limitation, hydroxethyl cellulose, hydroxpropyl cellulose, methyl cellulose, hydroxypropyl methyl cellulose, and/or hydroxethyl methyl cellulose. Chitosans may include without limitation, the following chitosan salts: chitosan lactate, chitosan salicylate, chitosan pyridinone carboxylate, chitosan itaconate, chitosan niacinate, chitosan formate, chitosan acetate, chitosan gallate, chitosan glutamate, chitosan maleate, chitosan aspartate, chitosan glycollate and quaternary amine substituted chitosan and salts thereof. Alginates may include without limitation, sodium alginate, potassium alginate, magnesium alginate, calcium alginate, and/or aluminum alginate. It is to be understood that various other forms of any of these natural polysaccharides that provide the proper functional capabilities may be employed without departing from the scope and spirit of the present invention.

In alternative embodiments of this invention the polymeric component of the current invention is a mixture of polysaccharides. For instance, the mixture may be of various different sub-classes of a single polymer class. Alternatively, the mixture may include two or more different classes of polymer, for instance a cellulose and a chitosan.

In a preferred embodiment, a matrix of the current invention is formed through the binding of numerous hydrophobically modified chitosan compounds, as shown in FIG. 1. These novel compounds consist of a biopolymer (e.g., chitosan) backbone that includes a hydrophilically reactive functional group (e.g., amino groups) that binds with the hydrophobically reactive head groups (e.g., carbonyl functional group) of an amphiphilic compound (e.g., aldehyde). The head group is further associated with a hydrophobic tail group. In the current embodiment, the hydrophobic tail may be for example a hydrocarbon. Thus, a hydrophobic tail is associated with the biopolymer's chitosan backbone providing the hydrophobic modification to the molecule that extends from the backbone and may interact with a surrounding environment in numerous ways, such as through hydrophobic interaction with other tissues, cells, molecules and/or structures. The hydrophobic interaction between the modified chitosan and the bi-layer of various tissues and/or cells occurs via the "insertion and anchoring" of the hydrophobic tail group of the short hydrophobic substituent into the bilayer membrane of the tissues or cells. The insertion process is driven by the generally understood hydrophobic interaction and those forces that are at work which tend to group like
molecules when they exist in a heterogenous environment. Thus, the hydrophobic effect or interaction is evidenced by the tendency of hydrophobic components to group together versus interacting or bonding with other molecules.

Typically, and for the purposes of the preferred embodiments of the instant application, these hydrophobically modified polymers (biopolymers) are referenced as being composed of a chitosan “backbone”, “scaffold”, and/or “lattice”. Thus, the backbone of the hydrophobically modified biopolymer film matrix of the preferred embodiments of the current invention is the biopolymer chitosan. Other biopolymers, including but not limited to the celluloses and alginites, which include similar characteristics of the chitosan backbone may be employed with departing from the scope and spirit of the instant invention.

Chitosan is a deacetylated derivative of chitin, wherein the degree of deacetylation (% DA) may range from 60-100% and determines the charge density. Chitosan is a linear polysaccharide composed of repeating β-(1-4)-linked D-glucosamine monomeric units.

These repeating monomeric units include a free amino group (functional group) and may make molecules or compounds containing chitosan or its derivatives readily reactive. The hydrophobic modification of the chitosan backbone is through the association of an amphiphilic compound with the amino group, such that the hydrophobic tail of the amphiphilic compound is bound with the hydrophobic backbone structure. As seen in FIGS. 1 and 2, this hydrophobically modified chitosan backbone (hm-Chitosan) may then be cast into a film. In the preferred embodiment of FIGS. 1 and 2, numerous hm-Chitosan backbones may fill a solution which may then be cast into a film forming the novel hm-Chitosan film of the current invention. This film matrix is a solid-state or dried film of the hm-Chitosan.

The formation or fabrication of the novel solid-state, hm-Chitosan matrix occurs through well known processes. The formation of the sponge of the current invention includes the formation step of freeze drying the solid state film of the matrix. Thus, a preferred embodiment of the current invention is a freeze dried, hm-Chitosan sponge or composition of matter which may be readily reactive with tissue, cells and additional molecules and/or compounds. The sponge composition of matter is prepared as a readily reactive, solid-state film matrix for application and use in damaged tissue adhesion and red blood cell gelling for promoting hemostasis. The sponge may be a bandage or included as part of a bandage that may be applied to a wounded area. However, various other implementation states of the current invention as may be contemplated by those of ordinary skill in the art are hereby assumed to fall within the scope of the current invention.

The sponge and spray solution of the current invention include at least one polymer and a plurality of short hydrophobic substituent attached along the backbone of the polymer. The short hydrophobic substituent preferably includes a hydrocarbon group having from about 8 to about 18 carbon atoms attached to the backbone of the at least one polymer. In preferred embodiments, the hydrocarbon group comprises an alkyl or arylalkyl group. As used herein, the term “arylalkyl group” means a group containing both aromatic and aliphatic structures. Examples of procedures for modifying polymers are as follows:

1. Alginates can be hydrophobically modified by exchanging their positively charged counterions (e.g. Na+) with tertiary-butyl ammonium (TBA+) ions using a sulfonated ion exchange resin. The resulting TBA-alginate is dissolved in dimethylsulfoxide (DMSO) where reaction occurs between alkyl (or aryl) bromides and the carboxylate groups along the alginate backbone.

2. Celluloses can be hydrophobically-modified by first treating the cellulose material with a large excess highly basic aqueous solution (e.g. 20 wt% sodium hydroxide in water). The alkali cellulose is then removed from solution and vigorously mixed with an emulsifying solution (for example, oleic acid) containing the reactant, which is an alkyl (or aryl) halide (e.g. dodecyl bromide).

3. Chitosans can be hydrophobically-modified by reaction of alkyl (or aryl) aldehydes with primary amine groups along the chitosan backbone in a 50/50 (v/v) % of aqueous 0.2 M acetic acid and ethanol. After reaction, the resulting Schiff bases, or imine groups, are reduced to stable secondary amines by dropwise addition of the reducing agent sodium cyanoborohydride.

The degree of substitution of the hydrophobic substituent on the polymer is from about 1 to about 30 moles of the hydrophobic substituent per mole of the polymer, which hydrophobic substitutions occur in up to 10% of available amines of the chitosan backbone, preferably between 1.5 and 4.5%. It is contemplated that more than one particular hydrophobic substituent is substituted onto the polymer, provided that the total substitution level is substantially within the ranges set forth above.

The short hydrophobic substituent is an amphiphilic compound meaning it is composed of a hydrophilic head group and a hydrophobic tail group. The head group binds with the polymer and positions the tail group to extend from the backbone of the polymer scaffold. This makes the hydrophobic tail group available for hydrophobic interactions. The tail group is preferably a hydrocarbon of various forms. As used herein, hydrocarbon(s) are any organic molecule(s) or compound(s) with a “backbone” or “skeleton” consisting entirely of hydrogen and carbon atoms and which lack a functional group. Thus, these types of compounds are hydrophobic in nature, unable to react hydrophilically, and therefore provide an opportunity for hydrophobic interaction. The hydrophobic interaction capability of the amphiphilic compound bound to the chitosan backbone may provide significant advantage to the current invention when compared to the prior art in that the interaction of the hydrophobically modified polymer matrix, whether chitosan, cellulose or alginate based, with the bi-layer membrane of tissue(s) and cell(s) is a self-driven, thermodynamic process requiring less energy input. Thus, regardless of any particular form of the Tail group of the short hydrophobic substituent (amphiphilic compound), so long as it provides the opportunity for hydrophobic interaction with the tissue(s), cell(s), or other hydrophobically active molecules and/or compounds it falls within the scope and spirit of the current invention.

Hydrocarbons, which are hydrophobic, may form into various types of compounds/molecules, such as gases (e.g.
methane and propane), liquids (e.g., hexane and benzene), waxes or low melting solids (e.g., paraffin wax and naphtha-
lene), polymers (e.g., polyethylene, polypropylene and poly-
styrene), or biopolymers. Currently, hydrocarbons may be
classified as follows:

1. Saturated Hydrocarbons (alkanes) are composed
totally of single bonds between the carbon and hy-
gen atoms and are denoted by (assuming non-cyclic
structures) the general formula $C_nH_{2n}$. These types
of compounds are the most simple of the hydrocarbons
and are either found as linear or branched species of
limited number.

2. Unsaturated Hydrocarbons include one or more multiple
bonds between carbon atoms of the compound, such as
double bonds (alkenes $C_nH_{2n-2}$) or triple bonds (alkynes
$C_nH_{2n-3}$). These multiple bonds create carbon atoms
which are also commonly referred to as hydrogenated
in that they are in need of the addition of further hydrogen
atoms.

3. Cycloalkanes consist of only carbon and hydrogen
atoms are cyclic or “ring-shaped” alkane hydrocarbons
denoted by the general formula $C_nH_{2n}$. Where
$n=$number of C atoms and $g=$number of rings in the
molecule. Cycloalkanes are saturated because there are
no multiple (double or triple) C—C bonds to hydroge-
nate (add more hydrogen to).

4. Aromatic Hydrocarbons, also known as arenes, are
hydrocarbons that contain at least one aromatic ring
and may be denoted by the formula $C_nH_{2n-6}$ wherein
at a minimum $n=6$. Aromatics (e.g., Benzene-$C_6H_6$) or
Aromatic Hydrocarbons include a molecular structure
which incorporates one or more planar sets of six carbon
atoms connected by delocalized electrons numbering
the same as if they consisted of alternating single
and double covalent bonds.

From this basic classification system there exist many deriv-
atives and further types of compounds that build therefrom.
For example, numerous and varied compounds include more
than one aromatic ring and are generally referred to as pol-
aryomatic hydrocarbons (PAH); they are also called polycy-
clic aromatic hydrocarbons and polynuclear aromatic hydro-
carbons. Various alternative/derivative forms of the saturated
or unsaturated cycloalkanes, and aromatic hydrocarbons as
are known and contemplated by those skilled in the art may
be employed with the current invention and should be read as
falling within the contemplated scope of the current inven-
tion.

Various types of other hydrophobic, organic compounds
may generally include hydrocarbon backbones but may also
include other types of atoms and/or incorporate/bind to other
compounds/molecular structures that incorporate other types
of atoms than just carbon and hydrogen. Thus, another clas-
sification system has developed by which organic compounds
with generally hydrocarbon backbones but bound with other
types of molecules may be separated, wherein such com-
ounds may be designated either aromatic or aliphatic. Thus,
compounds composed mainly, substantially or at least par-
tially, but not exclusively of carbon and hydrogen may be
divided into two classes:

1. aromatic compounds, which contain benzene and other
similar compounds, and

2. aliphatic compounds (G. alephar, fat, oil), which do not.

In aliphatic compounds, carbon atoms can be joined together
in straight chains, branched chains, or rings (in which case
they are called alicyclic). They can be joined by single bonds
(alkanes), double bonds (alkenes), or triple bonds (alkynes).
Besides hydrogen, other elements can be bound to the carbon
chain, the most common being oxygen, nitrogen, sulfur, and
chlorine. Those of ordinary skill in the art will recognize that
other molecules may also be bound to the carbon chains and
that compounds of such heteromomic structure are contempl-
ated as falling within the scope of the current invention.

The hydrophobic Tail group of the amphiphilic compound
bound to the polymer backbone of the current invention is
capable of branching and/or allowing the inclusion of side
chains onto its carbon backbone. This may promote the
hydrophobic interaction between the hydrophobically modi-
fied polymer matrix and damaged tissue and/or cell, as
discussed throughout the instant specification. It may be under-
stood that the strength of the hydrophobic interaction is based
upon the available amount of “hydrophobes” that may inter-
act amongst themselves or one another. Thus, it may further
promote the hydrophobic effect by increasing the amount of
and/or “hydrophobic” nature of the hydrophobic Tail group
that is interacting. For instance, a hydrophobic Tail group,
which in its original form may include a hydrocarbon chain,
may promote an increase in its hydrophobicity (ability to
hydrophobically bond and strength of hydrophobic interaction)
by having a hydrophobic side chain attach to one of
the carbons of its carbon backbone. In a preferred embodiment of
the current invention, this may include the attachment of
various polycyclic compounds, which may include for
instance various steroidal compounds and/or their derivatives
such as sterol type compounds, more particularly cholesterol.

In alternative embodiments, the current invention contem-
plates the use of various molecules and/or compounds that
may increase the hydrophobic interaction allowed between
the Tail group and the bi-layer membrane of tissues and cells.
The side chains may be linear chains, aromatic, aliphatic,
cyclic, polycyclic, or any various other types of hydrophobic
side chains as contemplated by those skilled in the art. Some
of the contemplated hydrophobic side chains may include the
following:

### I. Linear Alkanes

<table>
<thead>
<tr>
<th>Number of C</th>
<th>Formula</th>
<th>Common name</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>C$_1$H$_4$</td>
<td>Methane</td>
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<td></td>
<td></td>
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<td>Number of Carbon Atoms</td>
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### II. Cyclic Compounds

Cyclic compounds can be categorized:

<table>
<thead>
<tr>
<th>Cyclic compound</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alicyclic Compound</td>
<td>adamantane, amantadine, biperiden, metronidazole, rimantadine</td>
</tr>
<tr>
<td>Cycloalkane</td>
<td>Calixarenes, crown compounds, cycloedestrins, cycloparaffins, ethers, cyclic lactams, macrocyclic macrolides, peptides, cyclic tetrapyrroles, tris(ethylenediamine)triazine, acenaphthenes, anthraquinones, azulenes, benz[a]anthracenes, benz[j]cycloheptenes, fluorenes, indoles, naphthalenes, phenalenones, phanerothynes, pyrenes, spiro compounds</td>
</tr>
<tr>
<td>Cycloalkene</td>
<td>Polycyclic hydrocarbons, aromatic</td>
</tr>
</tbody>
</table>

### III. Polycyclic Compounds

Polycyclic compounds are cyclic compounds with more than one hydrocarbon loop or ring structures (Benzenic rings). The term generally includes all polycyclic aromatic compounds, including the polycyclic aromatic hydrocarbons, the heterocyclic aromatic compounds containing sulfur, nitrogen, oxygen, or another non-carbon atom, and substituted derivatives of these. The following is a list of some known polycyclic compounds.
The addition of the side chains may increase the stability and strength of the hydrophobic interaction between the Tail group and other hydrophobically active locations, such as a hydrophobic cavity in the bi-layer membrane of various biological structures including tissue and cell membrane structures. This increase in strength and stability may provide further advantages in the ability of the hydrophobically modified polymer matrix to self-assemble, such as providing increased or stabilized rates of reaction in the formation of the network film. The ability to adjust the side chain hydrophobicity may directly impact upon the tertiary and quaternary structure of the hydrophobically modified polymer matrix either as a reactive, solid-state matrix or as a liquid-state solution.

The molecular weight of the polymers comprising the tissue sealant sponge ranges from about 50,000 to about 500,000 grams per gram mole. It is contemplated that the molecular weight of the polymers in the sponge or solution formulations may be less than or greater than the range identified without departing from the scope and spirit of the current invention. For instance, the molecular weight of the polymers comprising the spray ranges from about 10,000 to about 200,000 grams per gram mole. As used herein, the term "molecular weight" means weight average molecular weight. In preferred examples, average molecular weight of polymers is determined by low angle laser light scattering (LLS) and Size Exclusion Chromatography (SEC). In performing low angle LLS, a dilute solution of the polymer, typically 2% or less, is placed in the path of a monochromatic laser. Light scattered from the sample hits the detector, which is positioned at a low angle relative to the laser source. Fluctuation in scattered light over time is correlated with the average molecular weight of the polymer in solution. In performing SEC measurements, again a dilute solution of polymer, typically 2% or less, is injected into a packed column. The polymer is separated based on the size of the dissolved polymer molecules and compared with a series of standards to derive the molecular weight.

The hydrophobically modified polymer spray solution is mixed with a variety of water-soluble reagents which results in faster and more efficient healing of the wound in alternative embodiments of the current invention. A first class of reagents that is mixed with the hydrophobically modified polymer is comprised of those reagents that contribute to the hemostatic integrity of the clot such as for example human thrombin, bovine thrombin, recombinant thrombin, and any of these thrombins in combination with human fibrinogen. Other examples of the first class of reagents include fibrinogen and Factor XIII. A second class of reagents that is mixed with the hydrophobically modified polymer is comprised of those reagents that prevent microbial infection such as norfloxacin, silver, ampicillin and penicillin. Reagents from both classes, e.g. recombinant thrombin and norfloxacin, or reagents from the same class, e.g. recombinant thrombin and fibronectin, may be mixed with the polymer. Various other reagents, catalysts, excipients, transporters, and/or penetrating agents as are known in the art may be employed by the current invention.

In another preferred embodiment of the current invention, the solution of the hydrophobically modified polymer matrix is formulated into a novel adhesive foam. Therefore, the current invention contemplates a method of preparing a foam of the hydrophobically modified polymer matrix. The foam formulation and development techniques employed may vary, including development by standard mechanical agitation means, freeze-dried foam, and various other techniques and formulations as may be contemplated by those of ordinary skill in the art. For instance, the foam may be produced by beating or otherwise agitating the polysaccharide polymer, including the plurality of short hydrophobic substituents, until it foams. It is also contemplated that depending on the polymer being used to prepare the foam, the foaming process takes place in an acidic solution or aqueous base.

It is contemplated that the foaming process may include the introduction of various other materials, such as various gases, into the solution that is being foamed. Different means of mixing the various other gases into the solution to provide a dispersion throughout the solution may be employed. Various foaming agents, modifiers, plasticizers and/or stabilizers may also be employed by the current invention to assist in foaming the solution. For instance, various ionic or non-ionic surfactants, cross-linkers or coagulant stabilizers may be used. It is also further contemplated that the various physical dimensions of and within the foam may be modified and/or controlled by various means as contemplated by those skilled in the art and such means may be employed without departing from the scope and spirit of the current invention.

The various forms of the novel composition of matter provided by the current invention may be used separately and independently. It is also contemplated that these various forms, whether sponge, solution and/or foam, may be employed in combination to provide their beneficial effect. It is also contemplated that one or more the different forms may be mixed and/or blended together for use as a combination product. It is contemplated that the different forms may include similar or different formulations of the novel matrix of the current invention. The interaction of the different forms may be promoted or affected through the use of various different agents as may be contemplated by those of ordinary skill in the art.

In FIG. 4 a method 400 of using a hydrophobically modified biopolymer matrix is provided. The method includes a step 410 of administering a polymer attached with a plurality of short hydrophobic substituent to a wounded tissue or a red blood cell, wherein the attached plurality of short hydrophobic substituent is capable, through hydrophobic interaction, of adhering with the wounded tissue or red blood cell. Administration may preferably occur through directly contacting the polymer matrix with the wounded tissue and/or cells and the application of pressure. The pressure is sustained thereby maintaining contact between the polymer matrix and the wounded tissue and/or cells. It is contemplated that the hydro-
The following methods and results provide an exemplary formulation of the matrix of the current invention and its efficacy in promoting hemostasis. It is understood that alternative formulations, such as those explicitly described in the instant specification as well as those that are contemplated by those of ordinary skill in the art, may be used and achieve similar results.

Chitosan was hydrophobically modified in order to increase its adhesion to tissue at the site of injury and to improve its hemostatic properties. Hydrophobes were attached along the backbone of the chitosan polymer. Based on previous work done by Lee et al., the working hypothesis for the present study is that hydrophobes will insert themselves into the bilayers of cells, thus providing chitosan with an added functionality in treating acute wounds. (See J. H. Lee, J. P. Gustin, T. Chen, G. F. Payne and S. R. Raghavan, “Vehicle-biopolymer gel: Network of surfactant vesicles connected by associating biopolymer.” Langmuir. (2005), which is herein incorporated by reference in its entirety.) If applied to a hemostatic bandage, this property could overcome the primary problem with the HemCon bandage while maintaining the advantages of using chitosan as the base polymer.

Due to these advanced properties of the hm-chitosan, the biopolymer is a viable option for usage as a strongly adhesive hemostatic bandage as well as a flowable spray or surgical sealant to stop minor bleeding and to seal tissues in surgical applications. In this study, the tissue adhesion relative to the level of hydrophobic modification was observed as well as the use of hm-chitosan to gel blood.

Materials and Methods; Hydrophobic Modification of Chitosan; Synthesizing hm-Chitosan Using Dodecaldehyde

Two grams of chitosan was dissolved in 100 mL of 0.2 M acetic acid by stirring for 30 minutes in a beaker covered with aluminum foil. The solution was filtered using a vacuum filter. Once the chitosan solution was poured from the flask into a 600 mL beaker, 100 mL of ethanol was added to the flask gradually and swirled around to remove the remaining chitosan on the sides of the flask. The ethanol and remaining chitosan was poured into the beaker with the rest of the chitosan. In a separate beaker, 20 mL of ethanol was added to dodecaldehyde (27.9 μL for the 1%, 69.7 μL for the 2.5%, 97.8 μL for the 3.5%, and 167 μL for the 6% modification), which was then slowly poured into the chitosan solution. Sodium cyanoborohydride, 0.78 g, was dissolved in 10 mL of ethanol and added to the chitosan solution. The sodium cyanoborohydride and ethanol solution was added twice more at 2 hour intervals. The mixture was stirred for 24 hours and the hm-chitosan was then precipitated from the solution by adding 0.2 M sodium hydroxide dropwise.

Synthesizing hm-Chitosan Using 4-octadeacyloxybenzaldehyde (2.5% Modification)

The same procedure to modify the chitosan using the dodecaldehyde is used, except 0.1178 g of 4-octadeacyloxybenzaldehyde was added instead of the dodecaldehyde. In this study, only a 2.5% modification was made using the 4-octadeacyloxybenzaldehyde.

Purification of hm-Chitosan Solutions

After precipitating the hm-chitosan, the solution was poured into centrifuge tubes, equilibrated in weight by adding ethanol, and centrifuged at 3,000 rpm for 10 minutes. The supernatant was removed and 20-25 mL of ethanol was added to each tube, stirred using a vortex, and centrifuged again. This process was repeated for a total of three times using ethanol and three additional times using deionized water.
Tissue Adhesion Experiments; Preparation of Films.

After the purification process, the hm-chitosan was removed from the centrifuge tubes into a beaker and an arbitrary amount of 0.2 M acetic acid so that the hm-chitosan was completely submerged. The solution was stirred for 2 hours and then poured into aluminum foil. It was vacuum dried to remove all of the water. Once dry, the hm-chitosan sheets were removed from the foil and dissolved in 0.2 M acetic acid to form a 2 wt % solution. Then 11 g of the hm-chitosan (or chitosan) was dropped into a 2.25" diameter plastic petri dish and left overnight to dry under the hood.


The dried films were removed from the petri dishes very carefully to prevent tearing the edges. Each film was cut in half and each piece weighed. All of the films were then further cut and reweighed until all of them were approximately the same weight. The films were then placed on bovine muscle tissue pressed down gently so that all of the film, except for the raised edges, was in contact with the tissue. For ten minutes, the films were allowed to adhere to the tissue. Then the bovine muscle tissue was inverted and weights were hung sequentially to the edge of the film until it was fully removed from the tissue.

Bovine Tissue Adhesion Tests — by Area.

Once the films were removed from the petri dishes, two rectangles measuring 1.5" by 0.75" each were cut from each film. With the film arranged vertically, a line was measured and drawn 0.5" from the bottom of the film with a thin permanent marker. The films were then placed on bovine muscle tissue so that the line was on the edge of the tissue with the majority of the film adhered to the tissue and the 0.5" by 0.75" rectangle hanging off the side. After being pressed gently into the tissue, the films were left to adhere to the tissue for ten minutes. The bovine muscle tissue was then held vertically so the films were perpendicular to the floor with the unattached portion of the film on the bottom. Weights were hung sequentially to the films until they were completely peeled from the tissue.

Texture Analyzer.

A TA.XT2i Texture Analyzer with a 5 kg load cell was used to perform this set of experiments. The instrument was set to the compression test mode. Small viles were filled approximately half full with 2 wt % solutions of hm-chitosan of varying levels of modification as well as the unmodified chitosan. A 1/8" diameter cylindrical probe was attached and aligned with the vile of solution. The settings were applied with a pre-test speed of 3 mm/s, a test speed of 2 mm/s, and a post test speed of 1 mm/s. The target mode was distance and the distance the probe traveled after touching the surface of the solution was set to 2 mm. As the probe was lowered into the vile, the force on the probe when it came in contact with the solution triggered the instrument to record the data. The resulting graphs produced were then used to calculate the adhesion of each solution.


When the human blood was drawn, it was put into test tubes whose interior walls were lined with heparin. A pipette was then used to measure cut 1 mL of blood, which was relocated to another test tube. Another pipette was used to add 1 mL of the chitosan (or hm-chitosan) to the blood. During the experiments, the 1%, 2.5%, and 3.5% modified hm-chitosan (modified using dodecaldehyde) was used as well as 2.5% modified hm-chitosan that was modified with the 4-octadecyloxybenzaldehyde. The time that it took for the mixture to gel was observed and recorded.

Rheology of Blood and Chitosan Solution.

A AR2000 advanced rheometer with a cone and plate geometry was used to measure the dynamic viscoelastic properties for these experiments. The cone had a 40 mm diameter with a 2 degree angle. In order to ensure that all the measurements were within the linear viscoelastic regions, first stress amplitude sweeps were performed. After the human blood was drawn into the test tubes with the heparin, a pipette was used to add 1 mL of blood onto the plate of the rheometer. Then 1 mL of the chitosan (or hm-chitosan) solution was added to the blood on the plate. Once the solutions were combines, the parameters for the rheometer were set up and the run was started. The cone lowered into contact with the solution and a sinusoidal strain was subjected to the subject with increasing frequency of oscillations. The elastic and viscous moduli were obtained over the frequency range of 0.01 to 10 Hz. Dynamic rheology experiments were performed using unmodified chitosan, 2.5% and 6% modified hm-chitosan (modified using dodecaldehyde), and 2.5% modified hm-chitosan modified using 4-octadecyloxybenzaldehyde.

Results and Discussion; Tissue Adhesion Experiments; Texture Analyzer.

In comparison of the varying hm-chitosan solutions, an obvious relationship was not observed in the relative adhesion calculations found using the texture analyzer. As shown in Table 1 below, the 1% and 3.5% modified hm-chitosan had the highest level of adhesion while the unmodified chitosan was the least adhesive.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Adhesion of hm-Chitosan Solutions - Texture Analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Modification</td>
<td>Adhesion (g · sec)</td>
</tr>
<tr>
<td>0%</td>
<td>3.126</td>
</tr>
<tr>
<td>1%</td>
<td>3.409</td>
</tr>
<tr>
<td>2.5%</td>
<td>3.200</td>
</tr>
<tr>
<td>3.5%</td>
<td>3.404</td>
</tr>
</tbody>
</table>

The difference between the highest level of adhesion (1% modification) and the lowest (0% modification) was 0.278 g·sec. The adhesion calculations found does not show a trend with the increasing level of modification with the 2.5% modified hm-chitosan being lower than the 1% and 3.5%; however, with the unmodified chitosan having the lowest adhesion, the data indicates that the hydrophobic modification of the chitosan does increase the adhesion of the solution.

Bovine Muscle Tissue; Standardized Film Weight.

The experiments in which the chitosan and hm-chitosan films were adhered to bovine muscle tissue and removed by sequentially hanging weights from the inverted tissue were repeated several times. As shown in FIG. 5, the average force needed to remove the films increased nearly linearly with the increasing level of hydrophobic modification. The standard deviation of force for each level of modification is fairly low with a high of 0.056 N. This precision in data significantly contributes to the credibility of the relationship between the adhesion and modification level.

In unmodified chitosan, the high adhesion to tissue is due to an electrostatic attraction between cationic chitosan and anionic cell surfaces. As previously mentioned, it is hypothesized that the increase in adhesion with increasing hydrophobic modification is due to the anchoring of the hydrophobes on the backbone of the chitosan into the bilayer of the tissue cells. In this study, the highest level of modification tested is 6% modified using dodecaldehyde, and this hm-chitosan produced the greatest adhesion to the bovine muscle.
tissue. Due to the hydrophobic nature of the modification, a percentage of modification higher than 6% would be difficult to dissolve into solution which would make it unusable in this study.

Though the results of the experiments provide a nearly linear relationship between the adhesion and the level of hydrophobic modification, the actual measurements of the force necessary to remove the films is negligible and the main focus is on the relationship. In this adhesion experiment, the standardization was administered by equalizing the weights of the films and by adhering all levels of modification to the same piece of bovine muscle tissue. With this method of standardization, the films were of varying surface area and thickness which could have had an effect on the adhesion.

Standardized Area of Contact Between Film and Tissue.

To vary the method of standardization of the films, additional adhesion experiments were performed using films with the same surface area in contact with the bovine muscle tissue. The films measured 0.75” by 1.5” with the portion in contact with the tissue measuring 0.75” by 1.00”.

### TABLE 2

<table>
<thead>
<tr>
<th>Weight required to remove the films of varying levels of hydrophobic modification</th>
<th>0%</th>
<th>1%</th>
<th>2.5%</th>
<th>3.5%</th>
<th>6%</th>
<th>2.5% (C18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>78.6451 g</td>
<td>50.6870 g</td>
<td>175.8375 g</td>
<td>194.4592 g</td>
<td>82.0132 g</td>
<td>249.0300 g</td>
</tr>
<tr>
<td>Trial 2</td>
<td>104.2506 g</td>
<td>112.8136 g</td>
<td>129.0033 g</td>
<td>144.8069 g</td>
<td>118.2246 g</td>
<td>171.9973 g</td>
</tr>
</tbody>
</table>

In addition to the five levels of modification of the hm-chitosan modified using dodecaldelyde that were used in the previous bovine muscle tissue adhesion experiments, a film composed of 2.5% modified solution of hm-chitosan that was modified using 4-octadecylxybenzaldehyde was also used. As shown in Table 2, the 2.5% 4-octadecylxybenzaldehyde modified hm-chitosan films required the greatest amount of force for removal which was notably higher than the amount required for the second most adhesive film. As the first experiment to test the adhesion of the 4-octadecylxybenzaldehyde modified hm-chitosan film, the results suggest that this modification causes an even higher increase in adhesive properties.

In blood gelling experiments, the blood that was drawn was mixed with heparin which is an anticoagulant. The presence of adding the heparin was to ensure that the change in blood viscosity and the blood gellation was due to the addition of the hm-chitosan solutions and not a result of the natural blood coagulation process. In the initial experiments, it was observed that the addition of unmodified chitosan to blood did not have a visually apparent increase in viscosity. The mixture was very fluidic and immediately flowed when the test tube was inverted. The blood, when mixed with 1%, 2.5%, and 3.5% modified hm-chitosan, became exceptionally viscous, though never gelled completely. A gel is characterized as a solution of infinite viscosity, and does not flow even when inverted in a test tube. In this experiment, the 1%, 2.5%, and 3.5% modified hm-chitosan, when mixed with blood, became so viscous that the solutions could remain intact for several seconds when the test tube was inverted; however, after a few seconds, the solutions slowly flowed down the side.

The 2.5% hm-chitosan solution that was modified using the 4-octadecylxybenzaldehyde completely gelled the blood almost instantaneously (less than 30 seconds) after being added. Figs. 6 shows a photo of the 2.5% modified hm-chitosan, modified using 4-octadecylxybenzaldehyde, mixed with the blood in a test tube and then another photo of the test tube completely inverted. The gelled solution remains in the bottom of the inverted test tube. The instantaneous gellation of the blood by the 2.5% modified hm-chitosan, modified using 4-octadecylxybenzaldehyde, is a significant characteristic and has promising future applications as a medical spray or surgical sealant.

**Rheology of Blood and hm-Chitosan; Frequency Sweep.**

The qualitative observations obtained in the initial blood gelling experiments were further quantified using an advanced rheometer. Dynamic rheology experiments ultimately yield plots of G' (elastic modulus) and G" (viscous modulus) as functions of frequency which are collectively called the frequency spectrum of the material. For our purposes of quantitatively defining the solutions of chitosan and
blood as viscous or elastic, the graphs shown in FIG. 7, identify each solution as liquid or gel (solid) form. For the unmodified chitosan and the 2.5% modified hm-chitosan modified using dodecylaldehyde, the results show that the viscous modulus is higher than the elastic modulus. In general terms, this data indicates that the solutions have stronger viscous properties than elastic and remain in liquid form. The graph of the 2.5% modified hm-chitosan (dodecylaldehyde) seems to show some visco-elastic properties with the intersection of the elastic and viscous modulus; however, for our purposes the viscous modulus is higher than the elastic modulus over most of the frequency range and only shows elastic behavior at high frequencies.

Both the graphs of the 6% modified hm-chitosan (dodecylaldehyde) and the 2.5% modified hm-chitosan (4-octadeclxybenzaldehyde) quantifies the solutions as gels since the elastic modulus remains above the viscous modulus throughout the frequency range. With the dodecylaldehyde 6% modified hm-chitosan and blood solution, the moduli are closer together than the 4-octadeclxybenzaldehyde 2.5% modified hm-chitosan and blood solution. This suggests that the elastic properties of the 4-octadeclxybenzaldehyde 2.5% modified hm-chitosan and blood solution are more dominant than with the dodecylaldehyde 6% modified hm-chitosan.

Dynamic Rheology—Time to Gelation

The previous frequency sweep graphs quantitatively show that blood, when mixed with two of the hm-chitosan solutions, form gels; however, the primary factor for these solutions' applicability as liquid medical sprays or surgical sealants lies within the time of gelation after the blood and hm-chitosan solutions mix. As shown in FIG. 8, the hm-chitosan and blood solutions experience negligible variation with time after the initial mixture. For both the blood and unmodified chitosan and the blood and 2.5% modified hm-chitosan (dodecylaldehyde) solutions that remained in liquid form, the elastic modulus and viscous modulus remain relatively constant over time. One unexplainable anomaly in the data is shown in the graph of the unmodified chitosan and blood solution with the initial nearly linear decrease in moduli. This is believed to be a result of an instrumental malfunction rather than a representation of the visco-elastic response of the solution; however, even with the initial decrease, the moduli quickly level out and remains constant with time.

In this experiment, the blood and hm-chitosan solutions are mixed together and the cone of the advanced rheometer immediately lowers into the solution and begins the oscillations of low frequency. As shown above in the graphs of the 6% modified hm-chitosan (dodecylaldehyde) and the 2.5% modified hm-chitosan (4-octadeclxybenzaldehyde) and blood solutions, the elastic modulus is higher than the viscous modulus for the first measurement which is determined only seconds after the solutions are mixed. This data corresponds with the initial blood gelation experiment results in showing the gelation time of only a few seconds. With nearly instantaneous gelation when it comes in contact with blood, the application of hm-chitosan liquid solutions could potentially mean a significant advancement in surgical sealants and sprays.

CONCLUSION

The motivation behind this study was to develop a new material with advanced properties for use as a hemostatic bandage for acute wounds and as a flowable spray or surgical sealant to stop minor bleeding and to seal tissues in surgical procedures. With our hydrophobically modified chitosan using dodecylaldehyde and 4-octadeclxybenzaldehyde, the experiments of this study focused on the impact of the modification on tissue adhesion in addition to the polymers interaction with blood. In the tissue adhesion experiments, it was concluded that tissue adhesiveness increases proportionally with the level of hydrophobic modification on the chitosan backbone. The experiments on the interaction of the modified chitosan with blood revealed that the 6% dodecylaldehyde modified hm-chitosan and the 4-octadeclxybenzaldehyde 2.5% modified hm-chitosan instantly forms a gel when mixed with blood. With these results showing the impact of the hydrophobic modification of chitosan in terms of tissue adhesion and interaction with blood, hm-chitosan has advanced qualities which give it a promising future as a hemostatic bandage and surgical sealant.

It is believed that the present invention and many of its attendant advantages will be understood by the foregoing description. It is also believed that it will be apparent that various changes may be made in the form, construction and arrangement of the components thereof without departing from the scope and spirit of the invention or without sacrificing all of its material advantages. The form herein before described being merely an explanatory embodiment thereof. It is the intention of the following claims to encompass and include such changes.

REFERENCES CITED

2. Clarke, Patrick E. “Z-Medica’s Products Cited as Life Saving on Battlefield”.
3. Kim, Seung-Ho M D; Staszoski, S. William; Safar, Peter M D; Capone, Antonino M D.

What is claimed is:
1. A method of using a hydrophobically modified chitosan matrix for treatment of wounds, comprising:
2. administering the hydrophobically modified chitosan matrix formulated as a solid state sponge that has been made from a solution having a concentration of about 1% to about 2.5% by weight relative to the total weight
of the solution of the hydrophobically modified chitosan and wherein hydrophobic substitutions occur in up to 10% of available amines of the chitosan backbone, to a damaged tissue or a red blood cell, wherein the hydrophobically modified chitosan matrix is capable of adhering to the damaged tissue or red blood cell via hydrophobic interactions; wherein the hydrophobic modifications comprise hydrocarbon substituents of eight to eighteen carbon residues in length covalently attached to chitosan.

2. The method of claim 1, wherein the solid state sponge is formulated by a process that comprises dehydration by at least one of a drying process, freezing process or a lyophilization process.

3. The method of claim 1, wherein hydrophobic substitutions occur in between 1.5 and 4.5% of available amines of the chitosan backbone.