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(71) Applicant: **UNIVERSITY OF UTAH RESEARCH FOUNDATION** [US/US]; 615 Arapeen Drive, Suite 310, Salt Lake City, UT 84108 (US).

(72) Inventors; and
(71) Applicants: **GHANDAHARI, Hamid** [US/US]; Department of Bioengineering, University of Utah, Salt Lake City, UT 84108 (US). **OOTTAMASATHIEN, Siam** [US/US]; Dept. of Medicinal Chemistry and Center for Therapy, University of Utah, Salt Lake City, UT 84108 (US). **JESEN, Mark Martin** [US/US]; Department of Bioengineering, University of Utah, Salt Lake City, UT 84108 (US). **CAPPELLO, Joseph** [US/US]; Dept. of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84108 (US). **JAI, Wanjian** [US/US]; Dept. of Medicinal Chemistry and Center, for Therapeutic Biomaterials, Univesity of Utah, Salt Lake City, UT 84108 (US). **PRESTWICH, Glenn D.** [US/US]; College of Parmacy, University of Utah, Salt Lake City, UT 84108 (US).

(74) Agent: **VILLANUEVA, Lawrence A.**; Gardner Groff Greenwald & Villanueva, 2018 Powers Ferry Road, Suite 800, Atlanta, GA 30339 (US).

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(54) **Title:** IN SITU GELLING COMPOSITIONS FOR THE TREATMENT OR PREVENTION OF INFLAMMATION AND TISSUE DAMAGE

(57) **Abstract:** Described herein are in situ gelling compositions. The compositions include an anti-inflammatory polysaccharide and a gelling polymer, wherein the composition is a liquid prior to administration to a subject but converts to a gel upon administration to the subject. The compositions described herein have numerous applications with respect to the local treatment (reduction or prevention) of inflammation and/or tissue damage.

WO 2018/053111 A1

IN SITU GELLING COMPOSITIONS FOR THE TREATMENT OR PREVENTION OF INFLAMMATION AND TISSUE DAMAGE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority upon U.S. provisional application Serial Nos. 62/395,313 filed on September 15, 2016 and 62/458,618 filed February 14, 2017. These applications are hereby incorporated by reference in their entirety.

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BACKGROUND

The treatment of inflammation in patients has been the subject of extensive research. Conventionally accepted treatments of inflammation may involve UV phototherapy, corticosteroids and glucocorticoids, acitretin, cyclosporine, and methotrexate. However, each of these treatments may cause serious side effects ranging from immune suppression and liver disease to thinning skin and causing birth defects. Due to partial or complete ineffectiveness, these treatments often leave patients unsatisfied with their results.

One problem associated with the treatment of inflammation is the local delivery of an anti-inflammatory agent at the site of inflammation. An example of this is the delivery of anti-inflammatory agents to a body cavity in the subject where inflammation is present. In situations like this, it would desirable to deliver a solution composed of the anti-inflammatory agent into the body cavity where it subsequently forms a gel under physiological conditions. Thus, the gel would remain in the body cavity and locally deliver the anti-inflammatory agent at the site of inflammation. The compositions described herein address this approach.

SUMMARY

Described herein are *in situ* gelling compositions. The compositions include an anti-inflammatory polysaccharide and a gelling polymer, wherein the composition is a liquid prior to administration to a subject but converts to a gel upon administration to the subject. The compositions described herein have numerous applications with respect to the local treatment (reduction or prevention) of inflammation and/or tissue damage.

The advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. 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.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

Fig. 1 shows a schematic of an experimental treatment protocol in which mice are injected rectally with a solution of silk-elastinlike protein polymer (SELP 815K) and semi-synthetic glycosaminoglycan (GAG GM-0111) prior to treatment of the lower abdomen with radiation. The administration of this SELP 815K/GAG GM-0111 enema protects the rectum from radiation-induced damage.

Fig. 2 shows scanning electron micrographs of SELP 815K gels. Unsheared SELP 815K (right-hand photos, top and bottom) at two different weight percentages did not produce consistent samples due to phase separation during curing. Sheared SELP 815K gels (left-hand photos, top and bottom) at two different weight percentages formed consistent gels without macroscopic phase separation.

Fig. 3 shows SEM micrographs of SELP 815K gels containing GAG GM-0111. GAG on its own formed a thin, flaky film. The sheared SELP 815K gels formed fibrous networks with GAG films interpenetrating within the SELP. When the SELP was not sheared, deep cracks and pitting were observed and the fibers tended to form a less homogeneous matrix with interspersed zones of high fiber density.

Fig. 4 shows release of GAG GM-0111 from SELP 815K hydrogels. Increasing the concentration of SELP 815K reduced the burst release of GAG GM-0111 from hydrogels upon initial administration. Both 4% and 11% SELP 815K gels release their entire payloads within 24 hours in simulated intestinal fluid. (** indicates $p < 0.01$.)

Fig. 5A shows viscosity curves from 18 to 37 °C. Figure 5B shows a plot of the mean storage modulus ($n = 3$) as an indication of gel stiffness for each of the treatment groups over a 3 hour period. Figure 5C shows the storage modulus at 5 min as an indicator of gelation rate and at 3 hr as an indicator of peak stiffness. All vertical axes are on a log scale.

Fig. 6 shows representative fluorescence micrographs of rectal tissue samples. GAG accumulation is enhanced when delivered via a SELP matrix after 3 hours (left) compared to when delivered in phosphate buffered saline (PBS, center). An autofluorescent control is shown on the right. A GAG-Alexa633 construct is shown in red, while rectal tissue stained with Hoechst 33342 is shown in blue. The large amount of GAG in the leftmost images is an indication of enhanced accumulation due to administration with SELP 815K at 11 wt%.

Fig. 7 shows attenuation of pain response by SELP and GAG in an *in vivo* radiation-induced proctitis mouse model. The left-hand column shows mean response rates of mice to 0.04, 0.16, 0.4, 1, and 4 g von Frey filaments after 3, 7, and 21 days. The right-hand column shows response rate at 0.4 g of force for three different treatment groups (radiation only, radiation + SELP, and radiation + SELP and GAG). The normal baseline is composed of all the pretreatment tests for mice used in each of

the treatment groups (n = 36). Exposure to radiation sensitized the animals to pain (***, highly statistically significant at $p < 0.001$). SELP significantly reduced the pain sensitization of animals exposed to 35 Gy of radiation. SELP alone (see right-hand column) significantly reduced the pain sensitization due to radiation at 3 and 7 days but offered no amelioration at day 21 (†††, highly significant at $p < 0.01$ at d days, †††, very highly significant at $p < 0.001$ at 7 days). The addition of GAG to the SELP improved the efficacy of treatment (‡‡‡, very highly significant at 3, 7, and 21 days) and prolonged the efficacy to include the 21-day animals (†††, very highly significant at $p < 0.001$).

Fig. 8 displays light micrographs of representative tissue samples showing the epithelium of the rectum. Histological tissue samples were stained via hematoxylin and eosin (H & E). The "no treatment" animal shows complete breakdown of the crypts of Lieberkühn and lamina propria. GAG at 100 mg/mL in phosphate buffered saline (PBS) provided no discernible advantage over the "no treatment" group at 3 and 7 days. SELP alone provided some protection at 3 days, but no discernible advantage at 7 days. SELP with 100 mg/mL GAG displayed a protective effect as indicated by near normal histology at both 3 and 7 days. Edema was noted in all groups that received radiation.

DETAILED DESCRIPTION

Before the present materials, articles, and/or methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific compounds, synthetic methods, or uses, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

In the specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

It must be noted that, as used in the specification and appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an excipient" includes mixtures

of two or more excipients, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the pharmaceutical compositions described herein may optionally contain biologically active ingredients, where the biologically active ingredients may or may not be present.

As used herein, the term "about" is used to provide flexibility to a numerical range endpoint by providing that a given value may be "a little above" or "a little below" the endpoint without affecting the desired result.

Throughout this specification, unless the context dictates otherwise, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

A "subject" as used in the specification and concluding claims, refers to a human or non-human animal. For example, the subject is a non-human animal (domesticated, wild, farm) such as, for example, a horse, cat, dog, cow, pig, sheep, goat, mouse, rabbit, chicken, rat, or guinea pig.

A "residue" of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. For example, hyaluronan that contains at least one –OH group can be represented by the formula Y-OH, where the Y is the remainder (i.e., residue) of the hyaluronan molecule.

A "hydrogel" as used in the specification and concluding claims, refers to a semisolid composition constituting a substantial amount of water. A hydrogel can be formed from a network of polymer chains in which polymers or mixtures thereof are dissolved or dispersed. Hydrogels are composed of three dimensional polymer networks that will swell without dissolving when placed in water or other biological

fluids. A hydrogel is significantly more viscous than water or other similar liquid. Hence, for purposes herein, a hydrogel is generally a non-liquid form.

The term "treat" as used herein is defined as maintaining or reducing the symptoms of a pre-existing condition. The term "prevent" as used herein is defined as eliminating or reducing the likelihood of the occurrence of one or more symptoms of a disease or disorder. The term "inhibit" as used herein is the ability of the compounds described herein to completely eliminate the activity or reduce the activity when compared to the same activity in the absence of the compound.

The term "admixing" is defined as mixing two components together so that there is no chemical reaction or physical interaction. The term "admixing" also includes the chemical reaction or physical interaction between the two components. As an example, covalent bonding to reactive therapeutic drugs, e.g., those having nucleophilic groups, can be undertaken with respect to the compounds in the compositions disclosed herein. Second, non-covalent entrapment of a pharmacologically active agent in a cross-linked polysaccharide or cross-linked protein matrix is also possible. Third, electrostatic or hydrophobic interactions or physical constraint by the matrix can facilitate retention of a pharmaceutically-acceptable compound in the compositions disclosed herein.

References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight of component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

As used herein, a plurality of items, structural elements, compositional

elements, and/or materials may be presented in a common list for convenience. However, these lists should be construed as though each member of the list is individually identified as a separate and unique member. Thus, no individual member of any such list should be construed as a *de facto* equivalent of any other member of the same list based solely on its presentation in a common group, without indications to the contrary.

Concentrations, amounts, and other numerical data may be expressed or presented herein in a range format. It is to be understood that such a range format is used merely for convenience and brevity and thus should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range was explicitly recited. As an illustration, a numerical range of "about 1 to about 5" should be interpreted to include not only the explicitly recited values of about 1 to about 5, but also to include individual values and sub-ranges within the individual range. Thus, included in this numerical range are individual values such as 2, 3, and 4, the sub ranges such as from 1-3, from 2-4, from 3-5, etc., as well as 1, 2, 3, 4, and 5 individually. The same principle applies to ranges reciting only one numerical value as a minimum or maximum. Furthermore, such an interpretation should apply regardless of the breadth of the range or the characteristics being described.

Disclosed are materials and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed compositions and methods. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc., of these materials are disclosed, that while specific reference to each various individual and collective combination and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a class of silk-elastinlike proteins A, B, and C are disclosed, as well as a class of semi-synthetic glycosaminoglycans (GAGs) D, E, and F, and an example combination of A + D is disclosed, then even if each is not individually recited, each is individually and

collectively contemplated. Thus, in this example, each of the combinations A + E, A + F, B + D, B + E, B + F, C + D, C + E, and C + F is specifically contemplated and should be considered from disclosure of A, B, and C; D, E, and F; and the example combination of A + D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A + E, B + F, and C + E is specifically contemplated and should be considered from disclosure of A, B, and C; D, E, and F; and the example combination of A + D. This concept applies to all aspects of the disclosure including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed with any specific embodiment or combination of embodiments of the disclosed methods, each such combination is specifically contemplated and should be considered disclosed.

Described herein are *in situ* gelling compositions for the treatment or prevention of inflammation. Each component used to prepare the *in situ* gelling compositions as well as methods for preparing and using the compositions are described in detail below.

Anti-Inflammatory Polysaccharides

The compositions described herein include one or more anti-inflammatory polysaccharides. In one aspect, the polysaccharide is a glycosaminoglycan (GAG). Glycosaminoglycans can be sulfated or non-sulfated. A GAG is one molecule with many alternating subunits. For example, hyaluronan is (GlcNAc-GlcUA-)x. Other GAGs are sulfated at different sugars. Generically, GAGs are represented by the formula A-B-A-B-A-B, where A is an uronic acid and B is an amino sugar that is either O- or N-sulfated, where the A and B units can be heterogeneous with respect to epimeric content or sulfation. Any natural or synthetic polymer containing uronic acid can be used. Examples of glycosaminoglycans include, but are not limited to, chondroitin sulfate, dermatan, heparan, heparin, dermatan sulfate, and heparan sulfate.

In one aspect, the anti-inflammatory polysaccharide is a sulfated hyaluronan or the pharmaceutically acceptable salt or ester thereof. In one aspect, the sulfated hyaluronan has a degree of sulfation from 0.1 to 4.0 per disaccharide unit. In another

aspect, the sulfated hyaluronan has a degree of sulfation from 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, or 4.0 per disaccharide unit, where any value can be a lower and upper end-point of a range (e.g., 3.0 to 4.0, 3.2 to 3.8, etc.).

In another aspect, the average molecular weight of the sulfated hyaluronan is less than 1,000 kDa, less than 900 kDa, less than 800 kDa, less than 700 kDa, less than 600 kDa, less than 500 kDa, less than 400 kDa, less than 300 kDa, less than 200 kDa, less than 100 kDa, less than 50 kDa, less than 25 kDa, less than 10 kDa, or less than 5 kDa. In another aspect, the sulfated hyaluronan has an average molecular size from 0.5 kDa to less than 50 kDa, 2 Da to 20 kDa, or 3 kDa to 10 kDa. In a further aspect, the sulfated hyaluronan has an average molecular size from 0.5 kDa to 10 kDa or 1 kDa to 5 kDa. Depending upon reaction conditions, one or more different hydroxyl groups present in the low molecular hyaluronan or hyaluronan oligosaccharide can be sulfated. In one aspect, the primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue of the low molecular hyaluronan or hyaluronan oligosaccharide is sulfated. In another aspect, the primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue of hyaluronan and at least one C-2 hydroxyl proton or C-3 hydroxyl proton of a uronic acid residue or at least one C-4 hydroxyl proton of an N-acetyl-glucosamine residue is substituted with a sulfate group. In another aspect, the primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue of the low molecular hyaluronan or hyaluronan oligosaccharide and at least one C-2 hydroxyl proton and C-3 hydroxyl proton of a uronic acid residue and at least one C-4 hydroxyl proton of an N-acetyl-glucosamine residue is substituted with a sulfate group. In another aspect, 0.001%, 0.01%, 0.1%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or less than 100%, or any range thereof of hydroxyl protons present on the low molecular hyaluronan or hyaluronan oligosaccharide can be deprotonated and subsequently sulfated.

In another aspect, the sulfated hyaluronan has (1) 100% of the primary C-6 hydroxyl protons of the N-acetyl-glucosamine residue of the sulfated hyaluronan are substituted with a sulfate group, (2) a degree of sulfation from 3.0 to 4.0, and (3) an

average molecular weight from 1 kDa to 3 kDa.

The hyaluronan starting material used to produce the sulfated hyaluronan can exist as the free acid or the salt thereof. Derivatives of hyaluronan starting material can also be used herein. The derivatives include any modification of the hyaluronan prior to sulfation. A wide variety of molecular weight hyaluronans can be used herein for the depolymerization step. In one aspect, the hyaluronan has a molecular weight greater than 1,000 kDa prior to depolymerization. In another aspect, the hyaluronan can have a molecular weight of 10 kDa to 1,000 kDa prior to depolymerization. A wide variety of hyaluronan molecular weights can also be employed for the sulfation step. In one aspect, the hyaluronan starting material can be converted to low molecular hyaluronan or a hyaluronan oligosaccharide prior to sulfation to produce the partially or fully sulfated hyaluronan. As will be discussed in greater detail below, low molecular weight hyaluronan is hyaluronan that has been degraded with an acid or base. Alternatively, hyaluronan oligosaccharide is produced by degrading hyaluronan with an enzyme such as, for example, hyaluronan synthase or hyaluronidase in a controlled fashion. Subsequently, hyaluronan oligosaccharides having different molecular weights can be separated by GPC or ion exchange separation. Exemplary procedures for producing low molecular weight hyaluronan or hyaluronan oligosaccharide from hyaluronan are provided in WO 2011/156445.

In one aspect, the low molecular hyaluronan or hyaluronan oligosaccharide being sulfated has a molecular weight from 1 kDa to 2,000 kDa. In another aspect, the low molecular hyaluronan or hyaluronan oligosaccharide being sulfated has a molecular weight from 5 kDa to 500 kDa, 10 kDa to 200 kDa, or 20 kDa to 100 kDa. Exemplary procedures for preparing low molecular weight hyaluronan are provided in WO 2011/156445. As discussed above, the molecular weight of the hyaluronan can be modified by cleaving hyaluronan with an acid or base to produce lower molecular weight hyaluronan. In certain aspects, the hyaluronan starting material or a derivative thereof is not derived from an animal source. In these aspects, the hyaluronan can be derived from other sources such as bacteria. For example, a recombinant *B. subtilis* expression system can be used to produce the hyaluronan starting material.

After the low molecular hyaluronan or hyaluronan oligosaccharide has been treated with a base, it is reacted with a sulfating agent to produce the partially or fully sulfated hyaluronan. Sulfating agents commonly used in organic synthesis can be used herein. Examples of sulfating agents include, but are not limited to, pyridine-sulfur trioxide complex or the triethylamine-sulfur trioxide complex. In one aspect, low molecular hyaluronan or hyaluronan oligosaccharide can be converted to the tributylamine salt, lyophilized, resuspended in dimethylformamide, and subsequently treated with a sulfating agent (*e.g.*, pyridine-sulfur trioxide complex) to sulfate one or more hydroxyl protons.

In one aspect, when the sulfating agent is a pyridine-sulfur trioxide complex, a pyridinium adduct of the sulfated hyaluronan is produced, where pyridine is covalently attached to the sulfated hyaluronan. Not wishing to be bound by theory, when hyaluronan is reacted with the pyridine-sulfur trioxide complex in a solvent such as, for example, DMF, a small amount of acid is produced from traces of water present *in situ*, which causes partial depolymerization resulting in a free reducing end group. The hydroxyl group of the hemiketal can ultimately be sulfated to produce a sulfated intermediate, which subsequently reacts with free pyridine produced *in situ* to produce the pyridinium adduct. Thus, the sulfated hyaluronan used herein can include a mixture of sulfated hyaluronan that does not have pyridine covalently attached to the molecule and sulfated hyaluronan that does have pyridine covalently attached to the molecule. In one aspect, from 0.01% to 100%, 0.1% to 10%, or 0.15% to 2.5% of the sulfated hyaluronan has pyridine covalently attached to the molecule. In another aspect, the molecular weight of the pyridinium adduct of the sulfated hyaluronan is less than or equal to 10kDa. In other aspects, the molecular weight is 0.1 kDa, 0.5 kDa, 1 kDa, 2 kDa, 3 kDa, 4 kDa, 5 kDa, 6 kDa, 7 kDa, 8 kDa, 9 kDa, or 10 kDa, where any value can for the lower and upper end-point of a molecular weight range.

In another aspect, the anti-inflammatory polysaccharide is a modified hyaluronan or a pharmaceutically acceptable salt or ester thereof, wherein said hyaluronan or its pharmaceutically acceptable salt or ester comprises at least one sulfate group and at least one primary C-6 hydroxyl position of an N-acetyl-

glucosamine residue comprising an alkyl group or fluoroalkyl group (referred to herein as "SAGE").

In one aspect, at least one primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue of hyaluronan is substituted with an alkyl group. The term "alkyl group" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. In one aspect, the alkyl group is a C_1-C_{10} branched or straight chain alkyl group. In a further aspect, the alkyl group is methyl. The alkyl group can be unsubstituted or substituted. In the case when the alkyl group is substituted, one or more hydrogen atoms present on the alkyl group can be replaced with or more groups including, but not limited to, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, aralkyl, or alkoxy.

In another aspect, at least one primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue of hyaluronan is substituted with a fluoroalkyl group. The term "fluoroalkyl group" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, wherein at least one of the hydrogen atoms is substituted with fluorine. In certain aspects, the fluoroalkyl group includes at least one trifluoromethyl group. In other aspects, the fluoroalkyl group has the formula $-CH_2(CF_2)_nCF_3$, wherein n is an integer of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In one aspect, the fluoroalkyl group is $-CH_2CF_2CF_3$ or $-CH_2CF_2CF_2CF_3$.

Alkylated and fluoroalkylated hyaluronan useful herein as well as methods for making the same are provided in WO2009/124266. The hyaluronan starting material can exist as the free acid or the salt thereof. Derivatives of hyaluronan starting material can also be used herein. The derivatives include any modification of the hyaluronan prior to the alkylation or fluoroalkylation step. A wide variety of molecular weight hyaluronan can be used herein. In one aspect, the hyaluronan has a molecular weight greater than 10 kDa prior to alkylation or fluoroalkylation. In another aspect, the hyaluronan has a molecular weight from 25 kDa to 1,000 kDa, 100 kDa to 1,000 kDa, 25 kDa to 500 kDa, 25 kDa to 250 kDa, or 25 kDa to 100 kDa

prior to alkylation or fluoroalkylation. In certain aspects, the hyaluronan starting material or a derivative thereof is not derived from an animal source. In these aspects, the hyaluronan can be derived from other sources such as bacteria. For example, a recombinant *B. subtilis* expression system can be used to produce the hyaluronan starting material.

The hyaluronan starting material or derivative thereof is initially reacted with a sufficient amount of base to deprotonate at least one primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue. The selection of the base can vary. For example, an alkali hydroxide such as sodium hydroxide or potassium hydroxide can be used herein. The concentration or amount of base can vary depending upon the desired degree of alkylation or fluoroalkylation. In one aspect, the amount of base is sufficient to deprotonate at least 0.001% of the primary C-6 hydroxyl protons of the N-acetyl-glucosamine residue of the hyaluronan starting material or derivative thereof. In other aspects, the amount of base is sufficient to deprotonate from 0.001% to 50%, 1% to 50% 5% to 45%, 5% to 40%, 5% to 30%, 5% to 20%, 10% to 50%, 20% to 50%, or 30% to 50% of the primary C-6 hydroxyl protons of the N-acetyl-glucosamine residue of the hyaluronan starting material or derivative thereof. It is understood that the more basic the solution, the more likely are chain cleavage reactions and the higher the degree of alkylation/fluoroalkylation that can be achieved. For example, other hydroxyl groups present on hyaluronan (*e.g.*, 2-OH and/or 3-OH can be alkylated or fluoroalkylated). In one aspect, all of the hydroxyl groups present on hyaluronan can be alkylated or fluoroalkylated. In other aspects, 0.001%, 0.01%, 0.1%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or any range thereof of hydroxyl protons present on hyaluronan can be deprotonated and subsequently alkylated or fluoroalkylated.

After the hyaluronan starting material or derivative thereof has been treated with a base, the deprotonated hyaluronan is reacted with an alkylating agent or fluoroalkylating agent to produce the SAGE. Examples of alkylating agents include, but are not limited to, an alkyl halide. Alkyl bromides and iodides are particularly useful. Similarly, the fluoroalkylating agent can include a fluoroalkyl halide.

Alkylating agents and fluoroalkylating agents commonly used in organic synthesis can be used herein.

In certain aspects, it is desirable to sulfate the alkylated or fluoroalkylated SAGEs described above. In one aspect, the alkylated or fluoroalkylated SAGE is sulfated by reacting the alkylated or fluoroalkylated SAGE with a sulfating agent to produce a sulfated product. The degree of sulfation can vary from partial sulfation to complete sulfation. In general, free hydroxyl groups present on the alkylated or fluoroalkylated hyaluronan or a derivative thereof can be sulfated. In one aspect, at least one C-2 hydroxyl proton and/or C-3 hydroxyl proton is substituted with a sulfate group. In another aspect, the degree of sulfation is from 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 or any range thereof per disaccharide unit of the alkylated or fluoroalkylated SAGE. In one aspect, the alkylated or fluoroalkylated SAGE can be treated with a base to deprotonate one or more hydroxyl protons followed by the addition of the sulfating agent. The sulfating agent is any compound that reacts with a hydroxyl group or deprotonated hydroxyl group to produce a sulfate group. The molecular weight of the SAGE can vary depending upon reaction conditions. In one aspect, the molecular weight of the SAGE is from 2 kDa to 500 kDa, 2 kDa to 250 kDa, 2 kDa to 100 kDa, 2 kDa to 50 kDa, 2 kDa to 25 kDa, or from 2 kDa to 10 kDa.

In one aspect, the alkyl group of the SAGE is methyl and at least one C-2 hydroxyl proton and/or C-3 hydroxyl proton of hyaluronan is substituted with a sulfate group. In another aspect, the alkyl group of the SAGE is methyl, at least one C-2 hydroxyl proton and/or C-3 hydroxyl proton of hyaluronan is substituted with a sulfate group, and the compound has a molecular weight of 2 kDa to 200 kDa after alkylation.

Any of the sulfated and alkylated/fluoroalkylated hyaluronan useful herein can be the pharmaceutically acceptable salt or ester thereof. Pharmaceutically acceptable salts are prepared by treating the free acid with an appropriate amount of a pharmaceutically acceptable base. Representative pharmaceutically acceptable bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, zinc

hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, and the like. In one aspect, the reaction is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0 °C to about 100 °C such as at room temperature. The molar ratio of compounds of structural formula I to base used are chosen to provide the ratio desired for any particular salts. For preparing, for example, the ammonium salts of the free acid starting material, the starting material can be treated with approximately one equivalent of pharmaceutically acceptable base to yield a neutral salt.

Ester derivatives are typically prepared as precursors to the acid form of the compounds--as illustrated in the examples below--and accordingly can serve as prodrugs. Generally, these derivatives will be lower alkyl esters such as methyl, ethyl, and the like. Amide derivatives -(CO)NH_2, -(CO)NHR and -(CO)NR_2, where R is an alkyl group defined above, can be prepared by reaction of the carboxylic acid-containing compound with ammonia or a substituted amine. Also, the esters can be fatty acid esters. For example, the palmitic ester has been prepared and can be used as an alternative esterase-activated prodrug.

Gelling Polymers

The compositions described herein include a gelling polymer. The function of the gelling polymer is to permit the *in situ* gelling compositions described herein to transform from a liquid at room temperature (approximately 18-23 ^{o}C) to a gel at physiological temperature (approximately 37 ^{o}C). The viscosity and gelation rate of the *in situ* gelling composition can be adjusted by varying the selection and amount of the gelling polymer present in the *in situ* gelling composition.

In one aspect, the gelling polymer is an elastin like protein, a copolymer of N-isopropylacrylamide, alginate, a copolymer of poly-vinyl alcohol, a poloxomer, carboxymethyl cellulose, chitosan, amylum, gelatin, collagen memetic peptide, acrylates (e.g., methacrylates, methyl acrylate, ethyl acrylate, 2-chloroethyl vinyl

ether, 2-ethylhexyl acrylate, hydroxyethyl methacrylate, butyl acrylate, butyl methacrylate, trimethylolpropane triacrylate, cyanoacrylate, or any combination thereof), hydroxymethylropylmercapturic acid, polyethylene glycol, phosphazene, or any combination thereof.

In another aspect, the gelling polymer is a silk-elastinlike protein (SELP). SELPs are a class of genetically engineered protein polymers composed of repeating "blocks" of amino acids, referred to as "silk blocks" (Gly-Ala-Gly-Ala-Gly-Ser; SEQ ID NO. 1) and "elastin blocks"(Gly-Val-Gly-Val-Pro; SEQ ID NO. 2). By varying the number of silk and elastin blocks, the rheological properties of the *in situ* gelling composition can be modified to fit specific applications. For example, the silk to elastin ratio and the length of the silk and elastin block domains as well as the SELP concentration can be modified to optimize gelling upon administration of the *in situ* gelling composition to the subject.

Examples SELPs useful herein include, but are not limited to,

$[(VPGVG)_8(GAGAGS)_2]_{18}$ (SEQ ID NO. 3);

$[(GVGVP)_4(GAGAGS)_9]_{13}$ (SEQ ID NO. 4);

$[(VPGVG)_8(GAGAGS)_4]_{12}$ (SEQ ID NO. 5);

$[(VPGVG)_8(GAGAGS)_6]_{12}$ (SEQ ID NO. 6);

$[(VPGVG)_8(GAGAGS)_8]_{11}$ (SEQ ID NO. 7);

$[(VPGVG)_{12}(GAGAGS)_8]_8$ (SEQ ID NO. 8);

$[(VPGVG)_{16}(GAGAGS)_8]_7$ (SEQ ID NO. 9);

$[(VPGVG)_{32}(GAGAGS)_8]_5$ (SEQ ID NO. 10);

$[(GAGAGS)_{12}GAAVTGRGDSPASAAGY(GAGAGS)_5(GVGVGP)_8]_6$ (SEQ ID NO. 11);

$[(GAGAGS)_2(GVGVP)_4GKGVP(GVGVP)_3]_6$ (SEQ ID NO. 12);

$[(GAGAGS)_2(GVGVP)_4GKGVP(GVGVP)_3]_{12}$ (SEQ ID NO. 13);

$[(GAGAGS)_2(GVGVP)_4GKGVP(GVGVP)_3]_{18}$ (SEQ ID NO. 14);

$[(GAGAGS)_2(GVGVP)_4GKGVP(GVGVP)_3]_{17}(GAGAGS)_2$ (SEQ ID NO. 15);

$[(GAGAGS)_2$-$(GVGVP)_4$-(GKGVP)-$(GVGVP)_3$-$(GAGAGS)_2]_{1-3}$ (SEQ ID NO. 16);

$[GAGAGS(GVGVP)_4GKGVP(GVGVP)_3(GAGAGS)_2]_{12}$ (SEQ ID NO. 17);

$[(GVGVP)_4GKGVP(GVGVP)_{11}(GAGAGS)_4]_5(GVGVP)_4GKGVP($-$GVGVP)_{11}(GAGAGS)_2$ (SEQ ID NO. 18);

$[(GVGVP)_4(GKGVP)(GVGVP)_{11}(GAGAGS)_4]_7(GVGVP)_4GKGV$-$P(GVGVP)_{11}(GAGAGS)_2$ (SEQ ID NO. 19);

$[(GVGVP)_4GKGVP(GVGVP)_{11}(GAGAGS)_4]_9(GVGVP)_4GKGVP($-$GVGVP)_{11}(GAGAGS)_2$ (SEQ ID NO. 20);

$[GAGS(GAGAGS)_2(GVGVP)_4GKGVP(GVGVP)_{11}(GAGAGS)_5GA]_6$ (SEQ ID NO. 21);

$[(GAGAGS)_2(GVGVP)_1LGPLGP(GVGVP)_3GKGVP(GVGVP)_3]_{15}(GAGAGS)_2$ (SEQ ID NO. 22);

$[(GAGAGS)_2(GVGVP)_1GFFVRARR(GVGVP)_3GKGVP(GVGVP)_3]_{15}(GAGAGS)_2$ (SEQ ID NO. 23).

In one aspect, the SELP is 27k, 415K, pSE8Y, pS2E8Y, pS4E8Y, or any combination thereof. In another aspect, the SELP is

MDPVVLQRRDWENPGVTQLNRLAAHPPFASDPM$[GAGS(GA$ $GAGS)_2(GVGVP)_4GKGVP(GVGVP)_{11}(GAGAGS)_5GA]_6$GAMDPGRYQDLRSHHH HHH (815K; SEQ ID NO. 24) or

MDPVVLQRRDWENPGVTQLVRLAAHPPFASDPMGAGSGAGAGS$[(GVGVP)_4$ $GKGVP(GVGVP)_3(GAGAGS)_4]_{12}(GVGVP)_4GKGVP(GVGVP)_2(GAGAGS)_2$GAMD PGRYQDLRSHHHHHH (47K; SEQ ID NO. 25).

In another aspect, the gelling polymer can be a variant of a SELP. A "variant" with reference to a silk-like unit or elastin-like unit refers to a silk-like unit or elastin-

like unit that has an amino acid sequence that is altered by one or more amino acids. Typically, a unit sequence is altered by 1, 2 or 3 amino acids. The variant can have an amino acid replacement(s), deletions or insertions. For example, the variant can have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g. replacement of leucine with isoleucine). In some cases, a variant can have "nonconservative" changes (e.g., replacement of a glycine with a tryptophan). Similar minor variations can also include amino acid deletions or insertions, or both. In addition to the teaching herein, guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing bioactivity can be found using computer programs well known in the art, for example, DNASTAR software.

In another aspect, the gelling polymer is a SELP with one or more matrix metalloproteinase (MMP) cleavage sites. MMPs are a family of structurally related endopeptidases that exist in a dynamic balance with tissue inhibitors of metalloproteases (TIMPs) to control a myriad biological functions requiring ECM degradation. Proper function and regulation of MMPs is responsible for diverse biological functions such as angiogenesis, embryonic development, and wound healing. There are over 20 known specific MMPs, divided into subgroups based on their additional domains and known biological functions. The main classes of MMPs are collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other unclassified MMPs.

In one aspect, the MMP cleavage site is MMP-2, MMP-9, or a combination thereof. MMPs -2 and -9 are known as gelatinase type A and B, respectively, due to their known ability to degrade gelatin (denatured collagen). In normal situations, MMPs -2 and -9 contribute to several processes involving cell migration and signaling, for example angiogenesis and inflammation/innate immunity. However, these MMPs have also been shown to be overexpressed in certain disease states relative to their expression in healthy tissue.

In addition to MMP-2 and -9 cleavage sites, other protease cleavage sites may

be incorporated in the protein polymer (see Table 1). Protease-specific sites can be chosen to target a specific response in the desired microenvironment. The reorganization that occurs during wound healing, if predicted to be a normal response, will use known enzymes during various stages. The breakdown of the matrix will react to the enzymes released during a particular stage to have the most beneficial effect. This includes incorporation of specifically recognized cleavage sites for particular MMPs and other proteases. Sequences that are cleaved by specific enzymes used for extracellular matrix remodeling can be optimized for the release and degradation of recombinant protein hydrogels based on site preferences. The sequence will depend on the MMP or other proteases, regardless of the protein polymer used, and may be inserted in an advantageous location within the protein polymer.

Table 1

MMP Substrates and Cleavage Sequences Cleavage Substrate Sequence	
MMP-1, -8 (collagenases)	
Substrate	Cleavage Sequence
Type I collagen	APGQIAGQ (SEQ ID NO. 26)
Type II collagen	GPQGLAGQ (SEQ ID NO. 27)
Type III collagen	GPLGIAGI (SEQ ID NO. 28)
Aggrecan	IPENFFGV (SEQ ID NO. 29)
MMP-3 (stromelysins)	
Type IX collagen	MAASAKRE (SEQ ID NO. 30)
Fibronectin	PFSPLVAT (SEQ ID NO. 31)
MMP-2, 9 (gelatinases)	
Type IV collagen	GPQGIFGQ (SEQ ID NO. 32)
Cartilage link protein	RAIHIQAE (SEQ ID NO. 33)

MMP-7 (matrilysin)	
Laminin	GPLGIAGQ (SEQ ID NO. 34)
Elastin	GPQAIAGQ (SEQ ID NO. 35)

In another aspect, the gelling polymer is the sequence below, where the MMP-responsive sequence in indicated in bold.

$[GAGS(GAGAGS)_2(GVGVP)_3\mathbf{GVGGPQGIFGQPGKGVP}(GVGVP)_{11}(GAGAGS)_5GA]_6$ (SEQ ID NO. 36).

Methods for producing SELPs with one or more matrix metalloproteinase (MMP) cleavage sites is provided in WO 2013181471, which is incorporated by reference.

In one aspect, when the gelling polymer is a SELP, the SELP is sheared prior to formulating the *in situ* gelling composition. In one aspect, a solution of the SELP is introduced into a homogenizer through a needle valve at a pressure of from 1,500 psi to 17,000 psi. Exemplary methods for producing sheared SELPs is provided in Price et al., "Effect of shear on physicochemical properties of matrix metalloproteinase responsive silk-elastinlike hydrogels." *J. Control. Release* **195,** 92–98 (2014). Not wishing to be bound by theory, the shearing of the SELP solution breaks intramolecular hydrogen bonds between the silk-like motifs. Shearing linearizes the protein, which causes reduction in solution viscosity and increases the opportunity for the formation of intermolecular interactions between the silk-like domains of distinct SELP polymers. Shearing can ultimately increase the peak modulus and gelation rate of the gelling polymer. Increased intermolecular bonding enables the formation of a stiffer and more homogenous network.

In another aspect, the anti-inflammatory polysaccharide and gelling polymer can be admixed, and the resulting composition is sheared as described above. In this aspect, the composition of anti-inflammatory polysaccharide and gelling polymer is sheared, filled into a delivery device, packaged and stored frozen. The composition

would then be thawed at time of administration.

Preparation of *In Situ* Gelling Compositions

The anti-inflammatory polysaccharide and gelling polymer can be admixed by any method that is known to one of skill in the art such that the resulting mixture is a liquid solution containing the components therein. The term "admixing" is defined as mixing the two components together so that there is no chemical reaction or physical interaction. The term "admixing" also includes the chemical reaction or physical interaction between the two components. Third, electrostatic interactions, hydrophobic interactions, or physical entrapment can occur between the two components.

Typically, the components are admixed in a biocompatible solute or liquid such as, but not limited to, water, saline, phosphate buffered saline, SELP solution, tris(hydroxymethyl)methylamine (Tris), minimum essential medium (MEM) or other buffer, barium contrast, or isotonic aqueous solution. Typically, mixing occurs at temperatures less than 30 ^{o}C and most typically at room temperature of about or between about 18 ^{o}C to 25 ^{o}C, but could also be performed at temperatures ranging from 1 ^{o}C to 5 ^{o}C. Gentle mixing is generally desired. For example, the components can be combined at room temperature and the solution gently swirled or inverted periodically for a sufficient time to mix the components. The mixture or combination can be incubated together for at least 10 seconds, 30 seconds, 1 minute, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes or longer. Generally, incubation and mixing is performed in a sufficient time before the composition acquires a non-liquid form. Alternatively, components may be admixed and the solution frozen using liquid nitrogen, methanol or other volatile organic solvent chilled with dry ice, blast freezer, or other method for freezing liquids. The solution can then be stored at temperatures from -80 ^{o}C to -20 ^{o}C until use. The solution may then be thawed in saline, water, air, or other medium prior to administration. Exemplary procedures for producing the *in situ* gelling compositions are provided in the Examples.

In another aspect, the components may be admixed as powers and reconstituted as a solution using, but not limited to, water, saline, phosphate buffered saline, SELP solution, tris(hydroxymethyl)methylamine (Tris), minimum essential medium (MEM), barium contrast, organic solvents such as mineral oil or DMSO, or other buffer or isotonic aqueous solution. This may occur with a syringe preloaded with the powder mixture being used to draw up a solution prior to administration. Additionally, the powder may be reconstituted in a bowl, jar, vessel, bag, vial, or other container and subsequently loaded into a delivery device. In one aspect, mixing occurs at temperatures less than 30 °C, at room temperature of about or between about 18 °C to 25 °C, or in the alternative at a temperature ranging from 1 °C to 5 °C. Such a device may include, but is not limited to, a catheter, luer-lock syringe, bulb syringe, enema syringe, clyster syringe, "banana bag", "drip bag", tube, dropper, enema bottle, or other device for delivery.

The *in situ* gelling composition is a liquid at 18 to 23 °C that converts to a hydrogel at 37 °C. In one aspect, the composition has a viscosity of less than or equal to 2,500 cP, less than or equal to 2,000 cP, less than or equal to 1,500 cP, less than or equal to 1,000 cP, or less than or equal to 700 cP at 18 to 23 °C. Due to the fact that composition is a liquid at 18 to 23 °C, it can be injected using techniques known in the art.

In one aspect, when the gelling polymer is a SELP, the SELP is present in the composition at a weight percentage (wt %) of the *in situ* gelling composition of from 2% (w/w) to about 20% (w/w), from 2% (w/w) to 18% (w/w), from 2% (w/w) to 16% (w/w), from 2% (w/w) to 14% (w/w), from 2% (w/w) to 12% (w/w), from 4% (w/w) to 12% w/w, from 6% (w/w) to 12%, from 8% (w/w) to 12% (w/w), or from about 10% (w/w) to 12% (w/w). For example, the SELP is present in the *in situ* gelling composition at a weight percentage (wt %) of the composition of from 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15% or 20% (w/w).

It will be appreciated that the actual preferred amounts of the anti-inflammatory polysaccharide in a specified case will vary according to the specific

compound being utilized, the particular compositions formulated, the mode of application, and the particular situs and subject being treated. Dosages for a given host can be determined using conventional considerations, e.g. by customary comparison of the differential activities of the subject compounds and of a known agent, e.g., by means of an appropriate conventional pharmacological protocol. Physicians and formulators, skilled in the art of determining doses of pharmaceutical compounds, will have no problems determining dose according to standard recommendations (Physicians Desk Reference, Barnhart Publishing (1999).

In one aspect, the concentration of the anti-inflammatory polysaccharide within the *in situ* gelling composition can modify gelation kinetics, viscosity, and final modulus. In one aspect, when the anti-inflammatory polysaccharide is a sulfated hyaluronan (SAGE), the SAGE is at a weight percentage (wt %) of the *in situ* gelling composition of from 0.01% (w/w) to about 20% (w/w), 1% (w/w) to about 20% (w/w), from 0.01% (w/w) to 0.1% (w/w), from 0.01% (w/w) to 0.5% (w/w), from 0.01% (w/w) to 1% (w/w), from 0.01% (w/w) to 2% (w/w), from 0.01% (w/w) to 5% (w/w), from 0.01% (w/w) to 10% (w/w), from 1% (w/w) to 5% (w/w),from 1% (w/w) to 10% w/w, from 1% (w/w) to 12%, from 5% (w/w) to 15% (w/w), or from about 5% (w/w) to 20% (w/w) can be used herein. For example, the SAGE is present in the *in situ* gelling composition at a weight percentage (wt %) of the composition of from 0.01%, 0.1%, 0.5% 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15% or 20% (w/w), while the *in situ* gelling polymer may comprise from 1% to 20% of the remaining solution.

The *in situ* gelling compositions can also include one or more active ingredients used in combination with the *in situ* gelling compositions described herein. The resulting pharmaceutical composition can provide a system for sustained, continuous delivery of drugs and other biologically-active agents to tissues adjacent to or distant from the application site. The biologically-active agent is capable of providing a local or systemic biological, physiological or therapeutic effect in the biological system to which it is applied. For example, the agent can act to control

and/or prevent infection or inflammation, enhance cell growth and tissue regeneration, control tumor growth, act as an analgesic, promote anti-cell attachment, reduce alveolar bone and tooth loss, inhibit degeneration of cartilage and weight bearing joints, and enhance bone growth, among other functions. Additionally, any of the compounds described herein can contain combinations of two or more pharmaceutically-acceptable compounds. Examples of such compounds include, but are not limited to, further comprises an antioxidant (e.g., vitamin E), a mucoadhesive agent, an anti-inflammatory agent, an anti-pyretic agent, steroidal and non-steroidal drugs for anti-inflammatory use, a hormone, a growth factor, a contraceptive agent, an antiviral, an antibacterial, an antifungal, an analgesics, a hypnotic, a sedative, a tranquilizer, an anti-convulsant, a muscle relaxant, a local anesthetic, an antispasmodic, an antiulcer drug, a peptidic agonist, a sympathiomimetic agent, a cardiovascular agent, an antitumor agent, or an oligonucleotide.

In other aspects, the *in situ* gelling compositions can include a contrast agent that can be detected using techniques known in the art. Here, the contrast agent can be used to observe the location and duration of the gel once administered to the subject.

The *in situ* gelling composition, or components thereof, can be packaged as kits for preparing the composition. For example, the kit can include an anti-inflammatory polysaccharide and a gelling polymer. The kits can optionally include one or more components such as instructions for preparation and use, devices and additional reagents, and components, such as tubes, containers, syringes and other devices for delivering the composition to the subject. For example, the kit also can contain an aqueous solution such as a solvent or buffer for suspending or dissolving the components.

Applications of *In Situ* Gelling Compositions

The *in situ* gelling compositions described herein are liquids at room temperature but convert to hydrogels upon administration to the subject. The *in situ* gelling compositions are very useful in treating and/or prevention of inflammation in

a subject where the liquid *in situ* gelling composition can be readily administered to a subject. For example, *in situ* gelling composition can be administered intravenously, intramuscularly, transmucosally, or subcutaneously. In other aspects, the *in situ* gelling composition is applied to a mucosal tissue or membrane in the subject. Thus, in one aspect, the *in situ* gelling composition can be administered ophthalmically, vaginally, rectally, intranasally, or applied directly to the oral mucosa, gingival, or periodontal pocket.

The *in situ* gelling compositions described herein can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. When the *in situ* gelling composition is to be administered to a subject as a liquid, the composition can be formulated with sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles, if needed for collateral use of the disclosed compositions and methods, include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles, if needed for collateral use of the disclosed compositions and methods, include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In the case when *in situ* gelling composition is administered as a liquid, the composition can be administered by a syringe (with or without a needle), enema, catheter, or bulb syringe.

In other aspects, the *in situ* gelling composition can be formulated in a number of different formulations depending upon the application of the *in situ* gelling composition (e.g., ophthalmic, vaginal, rectal, intranasal, oral, or directly to the skin). The *in situ* gelling composition can be formulated as an ointment, lotion, cream, gel, drops, suppositories, sprays, liquids, powders, or an aerosol or dry micronized powder for inhalation. Conventional pharmaceutical carriers, aqueous, powder or oily bases,

thickeners and the like can be necessary or desirable.

The *in situ* gelling composition is useful in treating and/or preventing inflammation in a body cavity of a subject where the composition can be introduced into the cavity. In one aspect, the *in situ* gelling composition is useful in treating and/or preventing inflammation in the rectum of a subject. One source of inflammation in the rectum is radiation-induced proctitis (RIP). RIP is the most common clinical issue for patients receiving radiotherapy as part of the standard course of treatment for ovarian, prostate, colon, and bladder cancers. RIP limits radiation dosages, interrupts treatment, and reduces patient quality of life. More than 200,000 cancer patients receive abdominal or pelvic radiation therapy annually, and the number of cancer survivors with post-radiation intestinal dysfunction continues to grow. Acute RIP occurs in greater than 75% of patients receiving radiotherapy for prostate cancer and progresses to debilitating chronic RIP in 5-10% of cases. RIP is manifested by bleeding, pain, abdominal cramping, mucoid discharge, diarrhea, fecal urgency, and tenesmus.

During radiotherapy for prostate, cervical, ovarian, and bladder cancer, portions of the colon and rectum often fall within the radiation field due to anatomical proximity. Even with image-guided placement of the beams and protective shielding, irradiation of the cancerous tissue without also irradiating the rectum or other sensitive organs is not feasible.

The exact etiology and pathophysiology of RIP is not completely understood, but it is widely accepted that radiation-induced damage to lipids and DNA triggers mucosal atrophy, submucosal edema, and inflammation that cause RIP. Symptoms of RIP can emerge immediately or months after radiotherapy and can persist for 20 years post-diagnosis. Chronic RIP can lead to life-threatening complications including fistula formation, sepsis, perforation, and internal bleeding.

A prophylactic treatment that protects the gastrointestinal tract from the deleterious effects of radiotherapy would improve patient quality of life during and after treatment and would allow for higher doses of radiation to be administered more

regularly, leading to improved clinical outcomes. No effective clinical prophylactic treatment options exist for the prevention of RIP in spite of its prevalence and clinical significance. Current treatments for RIP are reactionary and typically administered only after the onset of RIP. Previously investigated treatments such as sucralfate, 5-amino-salicylic acid, short-chain fatty acids, sodium butyrate, hydrocortisone, vitamin E, epinephrine, hyaluronic acid, and topical application of formalin frequently fail to make significant improvement in patient quality of life and have even been shown to exacerbate the disease in rare cases. After the failure of pharmacological treatments, physicians utilize surgical interventions, including laser ablation, electrocauterization, sclerotic injections, argon plasma coagulation, and radiofrequency ablation, all of which carry significant risks of morbidity and mortality. These surgical interventions frequently increase rectal pain, diarrhea, tenesmus, ulcers, fistula, rectal stenosis, and anal strictures. The *in situ* gelling compositions described herein provide a much needed solution to this long-standing problem.

In addition to treating and/preventing inflammation in the rectum, the *in situ* gelling compositions described herein are useful in other applications as well. The *in situ* gelling compositions can be used in a variety of applications related to the treatment and/or prevention of inflammatory skin disorders, dental disorders, respiratory disorders, inflammatory eye disorders, burn injury healing, and tissue regeneration/engineering. In one aspect, the *in situ* gelling compositions can improve wound healing in a subject in need of such improvement. The *in situ* gelling compositions can be placed directly in or on any biological system. Examples of sites the partially sulfated hyaluronan can be placed include, but are not limited to, soft tissue such as muscle or fat; hard tissue such as bone or cartilage; areas of tissue regeneration; a void space such as periodontal pocket; surgical incision or other formed pocket or cavity; a natural cavity such as the oral, vaginal, rectal or nasal cavities, the joint space, the cul-de-sac of the eye, and the like; the peritoneal cavity and organs contained within, and other sites into or onto which the compounds can be placed including a skin surface defect such as a cut, scrape or burn area. It is contemplated that the tissue can be damaged due to injury or a degenerative condition

or, in the alternative, the *in situ* gelling compositions described herein can be applied to undamaged tissue to prevent inflammation and/or injury to the tissue.

In the case of inflammatory skin disorders such as psoriasis, acne, atopic dermatitis, rosacea or UV light dependent photo-aging (*i.e.*, photo-dermal ageing), the *in situ* gelling composition can be applied topically as part of an emollient to prevent or treat the intended condition. In the case of respiratory disorders such as asthma, chronic obstructive pulmonary disease, acute lung injury or cystic fibrosis, the *in situ* gelling compositions can be formulated as water-soluble isotonic vehicle compatible with airway lining fluid and delivered to the lung or nasal passages as an inhaled aerosol. Alternatively, the *in situ* gelling composition can be formulated into a micronized powder and inhaled into the lung as a dry powder. In the case of eye diseases, the *in situ* gelling composition can be formulated with an aqueous vehicle and applied to the eye topically as drops, or injected directly into the eye either by needle or using an implanted constant drug delivery device. In the case of dental disorders such as periodontal disease, the *in situ* gelling compositions can be formulated into creams or gingival packing materials to be applied directly to the gingival crevice.

In further aspects, the *in situ* gelling composition can be used in genitourinary applications (*e.g.*, prevention of urinary tract infection, treatment of the transitional cell cancer of the bladder and uroepithelial system; treatment of interstitial cystitis; and use as a vaginal lubricant/protective to prevent transmission of sexually transmitted diseases). In one aspect, the *in situ* gelling composition can be administered topically to the vagina in order to reduce or prevent bacterial, fungal, viral or mechanical or biologically-induced inflammation.

In another aspect, the *in situ* gelling composition can be used to treat a number of respiratory disorders including cystic fibrosis, bronchiectasis, rhinitis (both allergic and perennial), sinusitis, emphysema and chronic bronchitis (COPD), acute lung injury/adult respiratory distress syndrome, interstitial lung fibrosis, SARS, asthma, and respiratory syncytial virus. In other aspects, the partially or fully sulfated

hyaluronan can prevent and treat snoring and obstructive sleep apnea, prevent infection by common respiratory pathogens (*Stretococcus pneumoniae*, *Hemophilus influenzae*, *Staphylococcus*, *Mycoplasma pneumoniae*, Chlamydial pneumonia, Gram negative enteric infections) in immune suppressed hosts such as subjects who are HIV positive or who have hematopoietic malignancies, or prevent and treat otitis media.

The *in situ* gelling composition can be used in cardiovascular applications (*e.g.*, treating or preventing acute coronary syndrome or atherosclerosis); hematological/oncological applications (e.g., prevention and treatment of sickle cell anemia; prevention and treatment of metastatic disease; and prevention of hypercoagulable state of malignancy (Trousseau's syndrome)); treatment of infectious diseases (*e.g.*, cerebral vascular occlusive syndromes and nephritis in *Falciparum* malaria, Yellow fever, Denge fever, systemic sepsis, and adjunctive treatment of HIV to prevent viral fusion with and infection of target cells); treatment of gastrointestinal diseases (*e.g.*, ulcerative colitis, Crohn's disease of the bowel, Hemorrhoids, and the prevention of stress ulceration of the stomach and esophagus); treatment of rheumatological and immunological diseases (*e.g.*, prevention and treatment of osteoarthritis, rheumatoid arthritis, systemic lupus erythematosis, prevention and treatment of angioneurotic edema, Sjogren's syndrome, systemic sclerosis, systemic amyloidosis, and systemic mastocytosis); renal diseases (*e.g.*, prevention and treatment of diabetic nephropathy and glomerulonephritis); and neurologic diseases (*e.g.*, multiple sclerosis and Alzheimer's dementia).

In one aspect, the *in situ* gelling composition can be used to treat or prevent urological inflammation. The term "urological inflammation" as used herein is defined as inflammation associated with any part or region of the genitourinary system. Urological inflammation includes, but is not limited to, inflammation of the bladder, urethra, urothelium lining, kidney, prostate, vagina, uterus, or any combination thereof. In this aspect, the partially or fully sulfated hyaluronan can be injected parenterally, either intravenously, intramuscularly or subcutaneously, to treat or prevent systemic urological inflammatory disorders. Alternatively, the *in situ*

gelling composition can be administered by intravesical installation (*i.e.*, via a catheter).

In other aspects, *in situ* gelling composition can be used in ophthalmological applications such as, for example, in the treatment of age-related macular degeneration, diabetic retinopathy, dry eye syndrome and other inflammatory conjunctivitis, iritis, uveitis, allergic conjunctivitis, anti-inflammatory aid in surgery (e.g., cataract surgery), or in the prevention of inflammation and scarring (e.g., corneal). In one aspect, the *in situ* gelling composition can be administered intraocularly or directly to the surface of the eye.

In other aspects, *in situ* gelling composition can be used in the treatment and/or prevention of inflammation present in the ear. Thus, the composition can be directly administered into the sinuses or into the outer, middle or inner ear.

The *in situ* gelling composition can be administered prophylactically either immediately prior to exposing the subject to a source of inflammation and/or tissue damage (e.g., radiation). In one aspect, the *in situ* gelling composition is administered to the subject from 0.5 to 48 hours prior to exposing the subject to the source of inflammation and/or tissue damage. In addition to addition to be administered to a subject prior to inflammation insult, the *in situ* gelling composition can be administered prophylactically to the subject after the inflammation insult to prevent or reduce inflammation and/or tissue damage. The prophylactic pretreatment may be supplemented with daily, every other day, or weekly administration after the initial treatment.

The compositions described herein are useful in reducing or preventing tissue damage in a subject. In one aspect, the *in situ* composition can be administered to a subject prior to exposure to radiation or another source of inflammation in order reduce or prevent damage to a mucosal membrane or epithelium of the subject. In other aspects, the *in situ* composition can be used to reduce or prevent tissue damage in the thyroid of the subject.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. Numerous variations and combinations of reaction conditions (e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures, and other reaction ranges and conditions) can be used to optimize the product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

I. Materials and Methods

A. Materials

A silk-elastinlike protein polymer was synthesized with 6 repeats of blocks comprised of 8 silk-like units, 15 elastin-like units, and 1 lysine-substituted elastin-like unit (SELP 815K) according to a previously-published procedure. The SELP 815K was sheared at >17,000 psi to enhance homogeneity and improve material properties. A semi-synthetic glycosaminoglycan construct was generated from the sulfation of hyaluronic acid (GAG GM-0111), a sulfated HA meeting the specifications above and having a degree of sulfation from 3.5 – 4.0 and molecular size range from 1 kDa to 10 kDa. Poly(ethylene glycol) (PEG)-based suppositories were formulated and used as controls.

B. In vitro Release

The release of GAG from a SELP matrix was quantified using an Azure A base colorimetric assay. Samples of SELP 12% and GAG-GM-0111 (lyophilized

form) were mixed to create a 100 mg/mL GAG in SELP (final SELP concentrations of 4 wt% or 11 wt%) solution and loaded into 500 μL insulin syringes (obtained from Becton Dickenson and Company), and incubated overnight at 37 °C. The tip was severed from the syringe and the gel sectioned into 20 μL disks. The masses of the disks were measured and recorded. Samples were placed into test tubes (obtained from Bioexpress) containing 4 mL of simulated intestinal fluid without enzyme (Sigma Aldrich). Samples were incubated in a STEADYSHAKE™ 757 incubator (Amerex Instruments, Inc.) at 37 °C and 175 rpm. At 15 min, 30 min, 1 hr, 2 hr, 3 hr, 12 hr, and 24 hr, 100 μL of release media were combined with 190 μL of 0.025 mg/mL Azure A in a 96-well plate. Absorbance at 620 nm was measured on a Spectramax M2 spectrophotometer (Molecular Devices). The concentration of GAG in the solution was determined from an experimentally-obtained concentration curve.

C. Rheology

Rheological testing was performed using an AR550-Stress Controlled Rheometer (New Castle, DE). A cone-and-plate configuration with a 20 mm 4° cone geometry was used. An aluminum cover and mineral oil (Sigma Aldrich) were used to seal the environment and prevent dehydration of the samples during testing. Viscosity was measured from 18 to 37 °C (5.76 °C/min) using an oscillatory procedure at an angular frequency of 6.283 rad/s. This was immediately followed by a 3 hr oscillatory sweep at 37 °C using 0.1% strain and an angular frequency of 6.283 rad/s. Samples were prepared by thawing flash-frozen 12 wt% SELP solutions in room temperature water. The SELP was diluted with chilled solutions of phosphate buffered saline (PBS) containing GAG GM-0111 to achieve final concentrations of 4 wt% or 11 wt% SELP-815K and 100 mg/mL GAG GM-0111. All tests were performed in triplicate using separately prepared samples.

D. Scanning Electron Microscopy

Samples of the hydrogel matrices were examined using scanning electron microscopy performed on an FEI Quanta 600F. Samples were prepared from the same hydrogels used in Example I.B. and flash frozen in liquid nitrogen and then

lyophilized on a FreeZone 12 (Labconco) at ≤ -50 °C and ≤ mBar for 4 days. The lyophilized disks were mounted onto carbon tape and sputter coated with a 5 nm layer of gold palladium (Gatan 682 precision etching coating system) prior to imaging. Images were acquired using secondary electrons at 1000× magnification.

E. In vivo Accumulation

The accumulation of GAG GM-0111 within the rectum was evaluated by fluorescence microscopy. GAG GM-0111 was fluorescently labeled with ALEXA FLUOR®-633 (Thermo-Fisher Scientific) according to a published procedure. The labeled GAG GM-0111-Alexa633 was combined with non-labeled GAG at a ratio of 1:3 to generate sufficient material for the study. The GAG GM-0111-Alexa633 was combined with SELP immediately prior to administration. Female BDF-1 mice of 8-10 weeks in age were randomly assigned into three experimental arms (n = 6) GAG GM-0111-Alexa633 at 100 mg/mL in PBS, GAG GM-0111-Alexa633 at 100 mg/mL in an 11 wt% SELP 815K solution, and unlabeled GAG GM-0111 in an 11 wt% SELP-815K solution to serve as an auto fluorescence control. Mice were anesthetized with 600 µL of a 1.2% solution made from 2,2,2-tribromoethanol (Sigma Aldrich) and 2-methyl-2-butanol, tertiary amyl alcohol >99% (Sigma Aldrich) injected intraperitoneally. After anesthetization, a SILASTIC® 0.94 mm outer diameter, 0.51 mm inner diameter catheter (Dow Corning) was gently inserted 4 cm through the anus into the rectum of the mice. As the catheter was slowly withdrawn, 80-100 µL of the enema solution was injected and the animals were placed supine in their cages to recover. The mice were sacrificed at either 3 hr or 12 hr after the treatment was administered.

At necropsy, 1.5 cm of rectum was removed and fixed in 10% formalin (Ted Pella, CA) overnight at 4 °C. The tissue was then stained with Hoechst 33342b (Invitrogen) per the manufacturer's protocol. Standard dehydration methods were then used, employing serially increasing concentrations of ethanol (70%, 95%, 100%) and xylene. The fixed tissues were then embedded into paraffin blocks and sectioned into 0.5 µm thick slices. The samples were imaged on an Olympux BS40 fluoro-

microscope via an Infinity 3 amera (Lumenear Corp., Ottawa, Canada) at 350 ± 15 nm excitation and 460 ± 15 nm emission for Hoechst 33342 and 640 ± 15 nm excitation and 690 ± 15 nm emission for Alexa 633. The images were then composited in Infinity Analyze software (Lumenear Corp., Ottawa, Canada).

F. In vivo Efficacy

The efficacy of the SELP/GAG system was evaluated in a murine model of radiation-induced proctitis. Fig. 1 shows a schematic of an experimental treatment protocol in which mice are injected rectally with a solution of silk-elastin like protein polymer (SELP 815K) and semi-synthetic glycosaminoglycan (GM-0111) prior to treatment of the lower abdomen with radiation. The administration of this SELP 815K/GM-0111 enema protects the rectum from radiation-induced damage.

Female BDF-1 mice of 8-10 weeks in age were randomly assigned into 4 groups (n = 18): no treatment control, 100 mg/mL GAG GM-0111 in phosphate buffered saline (PBS), SELP 815K 11 wt% sheared, and SELP 815K 11 wt% with 100 mg/mL GAG GM-0111.

Within each treatment group, animals were designated for sacrifice after 3, 7, or 21 days (n = 6). The treatments were administered as described in Example I.E. Mice were then affixed to a steel plate in a supine position and a 6.35 mm thick lead plate with 1 cm by 4 cm windows was positioned such that the windows extended from the anus along the median plane of the mice, limiting the radiation exposure to just the lower abdomen. The mice were then placed in an RS 2000 X-Ray Irradiator (RAD SOURCE Technologies, GA, USA) set to level 3 for 16 min, 17 sec to receive 35 Gy of radiation. Following the procedure, mice were returned to their enclosures.

G. Behavioral Pain Assessment

The pain response of the mice was behaviorally assessed prior to treatment and prior to sacrifice on days 3, 7, and 21. The mice were placed into individual enclosures composed of a wire mesh floor and clear polycarbonate sides and allowed to acclimatize for at least 10 min prior to testing.

The degree of referred hyperalgesia and tactile allodynia were assessed using von Frey filaments corresponding to 0.04, 0.16, 0.4, 1, and 4 g, according to previously published protocols. The lower abdomen of each mouse was stimulated with each filament for approximately 1 second with at least 3 seconds between 10 successive tests. Care was taken to ensure that each stimulus was in a distinct location from previous stimuli to avoid pain wind-up effects. A sharp retraction of the abdomen, immediate licking or scratching of the stimulated area, or a jump was considered a positive indication of pain.

The response rate was taken as the proportion of positive indications out of 10 stimulations. The percent reduction of pain response for each treatment group was calculated from the average percent change of all 5 stimulus levels compared to the no treatment control.

H. Tissue Collection and Histology

Tissue was collected and fixed as described in Example I.E. Samples were then washed with distilled water and stained with hematoxylin solution (Ricca Chemical Company, TX, USA) for 45 seconds and rinsed for 5 minutes with running DI water. The samples were then rinsed with 95% ethanol (Decon Labs Corp., MA, USA) and counterstained with eosin solution for 1 min, 30 sec, dehydrated, embedded in paraffin blocks, and sectioned as described in Example I.E.

I. Statistical Analysis

A one-way paired student's T-test was used to compute statistical significance between samples and controls. A two-way ANOVA with a Bonferroni post-test was used to assess the significance of data containing greater than two experimental groups for comparison. Graphs and charts were created in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA). All data is reported as the mean ± standard deviation unless otherwise specified. A value of $p \leq 0.05$ (*) was considered statistically significant, $p \leq 0.01$ (**) highly significant, and $p \leq 0.0001$ (***) very highly significant.

J. Fabrication of GAG-Embedded SELP Hydrogels for Imaging and Release

Samples were fabricated to evaluate the effect of SELP concentration and shear processing on the release of GAG GM-0111. SELP 815K 12 % (w/v) in phosphate buffered saline (PBS) was sheared at >17,000 psi as described in Price, R., Poursaid, A., Cappello, J. & Ghandehari, H. Effect of shear on physicochemical properties of matrix metalloproteinase responsive silk-elastinlike hydrogels. *J. Control. Release* **195,** 92–98 (2014). Material from the same batch that was not subjected to shear processing was used to fabricate samples with unsheared SELP.

Syringes were prepared with homogeneous solutions of sheared and unsheared SELP 815K combined with GAG GM-0111 and PBS comprising 100 mg/mL GAG GM-0111 and 4 wt% or 11 wt% SELP 815K. The samples were then incubated overnight at 37 °C.

II. Results

A. Characterization of Sheared Hydrogels

Unsheared SELP 815K formed two distinct layers within a syringe, one a liquid and the other a gel. Sheared SELP 815K formed a single phase solid gel matrix. For analysis, gel specimens were sectioned into 20 μL disks. For unsheared specimens, the liquid phase was removed and the gel portion was analyzed (Fig. 2). Scanning electron microscopy as previously described was used to image the sheared and unsheared hydrogels.

SEM images of the gels showed higher density fiber networks in the SELP 11 wt% solutions compared to the SELP 4 wt% solutions, consistent with the notion that the fiber network was composed of SELP. The GAG formed localized deposits interspersed within the fibrous network of the SELP hydrogels in similar fashions among the various groups. Deep fissures and cracks were observed in the unsheared SELP samples but not within the sheared samples (Fig. 3). Due to the observed phase separation and mechanical instability, the unsheared samples were not tested further.

B. In vitro Release of GAG from SELP 815K Matrices

Release testing was carried out using gels produced with sheared 11 wt% and 4 wt% SELP 815K and 100 mg/mL GAG GM-0111. The 4 wt% gel released 39 ± 11% (n = 3) while the 11 wt% gels released only 10 ± 2% (n = 4) after 15 minutes ($p < 0.01$). In spite of the higher burst release from the 4 wt% gel, the 4 wt% and 11 wt% gels released 51 ± 9% and 54 ± 38%, respectively, after 1 hr (Fig. 4). Within 24 hr there was complete release of GAG from both the 11 wt% and 4 wt% hydrogels. No statistical difference was observed between the 11 wt% and 4 wt% groups after the 15 min time point, indicating that varying the concentration of SELP impacts only the initial burst release of GAG from the matrix, not the timing of total cumulative release.

C. Rheological Evaluation of in situ Gelling SELP Formulations

To determine the injectability of the *in situ* gelling system, solution viscosities were measured from 18 to 37 °C. The sheared 4 wt% SELP 815K and 11 wt% SELP 815K with 100 mg/mL of GAG were selected for rheological evaluation and compared to sheared SELP 815K at 4 wt% and 11 wt% without GAG, GAG dissolved in PBS at 100 mg/mL, and a PEG suppository. Flash frozen liquid stocks of sheared SELP 815K were used for the production of each sample. After thawing, the SELP 815K was combined with GAG GM-0111 to create the 4 wt% and 11 wt% SELP 815K solutions.

The addition of GAG to the SELP 11 wt% solution increased the viscosity at 25 °C from 140 ± 10 cP with SELP alone to 380 ± 250 cP. In the 4 wt% SELP samples at 25 °C, the viscosity changed from 53 ± 6 cP to 44 ± 16 cP. Even though the same amount of GAG was added to both the 11 wt% and 4 wt% SELP solutions, only the 11 wt% SELP solution increased substantially in viscosity. Even so, the observed viscosities of all solutions were within a range that could be easily administered by hand from typical enema administration devices. An upward trend in the viscosity of the SELP solutions was observed as the temperature increased above 33 °C, consistent with an SELP response to temperature (Fig. 5A). However, this trend was only statistically significant between 25 °C and 37 °C for SELP 815K 11

wt% ($p = 0.002$). The PEG suppository was solid until melting at 32-34 °C (Fig. 5A). After melting, the viscosity of the PEG suppository at 37 °C, 90 ± 27 cP, was less than either the viscosity of the SELP 815K 11 wt% or 4 wt% at 25 °C, but still nearly three times as viscous as the GAG in PBS, which was 34 ± 3 cP.

The transition from polymer solution to a three dimensional polymer network was evaluated rheologically using an oscillatory time sweep at 37 °C. One way to define the gelation point is the time at which the storage modulus (G′) exceeds the loss modulus (G″). All SELP containing solutions achieved a $G' > G''$ within 5 minutes of the start of the time sweep. This was not true for either the PEG suppository or the GAG dissolved in PBS, which exhibited a $G'' > G'$ at 37 °C throughout the entire 3 hour sweep.

The addition of GAG GM-0111 to SELP 815K accelerated the solution-gel transition and increased mechanical stiffness at the 5 min time point (Fig. 5C). After 5 min at 37 °C, SELP 815K 4 wt% and SELP 815K 11 wt% solutions with GAG had 3-fold and 5-fold greater stiffness, respectively, than the SELP solutions without GAG. The modulus continued to rise for the SELP gels ($p < 0.05$ for both the 4 wt% and 11 wt% SELP solutions). However, at 3 hr, GAG had different effects on the storage modulus of the solutions. GAG decreased the stiffness of the 11 wt% SELP 815K solution by 76.8% ($p < 0.05$) but increased the stiffness of the 4 wt% SELP 815K solution by 212% ($p < 0.05$). Both GAG in PBS and the PEG suppository maintained their liquid states, $G' < G''$, throughout the 37 °C sweep (Fig. 5C) and had storage moduli of 3 ± 4 Pa and 1.0 ± 0.4 Pa, respectively.

D. GAG Accumulation in Colorectal Tissue

To evaluate the accumulation of GAG within the rectum, fluorescently-labeled GAG was administered in two enema formulations, one representing a standard enema (PBS) and the other the *in situ* gelling enema (SELP enema) system. Large dark blue streaks were observed in the bedding material in treatment groups that received the GAG-GM-0111-Alexa633 in PBS 3 hours after administration. Similar streaking was not observed in groups receiving the SELP enema. During necropsy,

half of the mice treated with SELP enemas still had visible SELP gels within their rectums. There was no observable retention of SELP gels at 12 hours.

After histological preparation and staining with Hoechst 33342, the tissue samples were fluorescently imaged at 200× magnification for both Alexa 633 (shown in red in Fig. 6) and Hoechst 33342 (shown in blue in Fig. 6). All animals showed substantially greater accumulation of GAG-GM-0111-Alexa633 in the rectum when they received the SELP 815K 11 wt% enema compared to the PBS enema. After 12 hours, no SELP gels were observed in any of the tissues, consistent with SELP elimination from the bowels.

Fluorescent evaluation of the tissues harvested 12 hours post-administration showed significant amounts of GAG-GM-0111-Alexa633 remained in the tissue and had migrated further along the crypts of Lieberkühn all the way to the muscularis mucosae. This penetration was greater than observed for the GAG-GM-0111-Alexa633 administered in the SELP enema at 3 hours, indicating continued penetration. In the animals that received the PBS enema, no fluorescent indication of GAG-GM-0111-Alexa633 was observed at 12 hr. SELP 815K 11 wt% increased the accumulation of GAG-GM-0111 in the walls of the rectum and prolonged its residence time compared to a PBS enema.

E. In vivo Pain Response

To evaluate the ability of GAG and SELP enemas to alleviate pain, a mechanosensitivity-based assay using von Frey filaments was employed. An increased response rate at lower stimulation was indicative of increased hyperalgesia and malaise in mice. Baseline measurements were acquired for all the mice in the study and were used to establish a baseline response pattern. Filaments corresponding to 0.04 to 4 g were tested, but the filaments from 0.04 to 0.4 showed the greatest dynamic response as indicated by the slope seen between successive points (Fig. 7). For this reason, the response rate at 0.4 g stimulation was chosen for comparative treatments.

Radiation drastically sensitized the lower abdomen of the mice. At 0.4 g stimulus, the response rate of mice 3 days after receiving 35 Gy of radiation to the lower abdomen quadrupled from 18 ± 3% to 72 ± 7% ($p < 0.0001$). After 7 and 21 days, the response rate rose to 80 ± 4%. An even more profound change was observed at 0.04 g of stimulus where the mice became 8.3 times more sensitive after radiation (see orange traces in Fig. 7). These results indicate that the mice developed acute radiation-induced sensitization to mechanical stimuli, such as is observed with the onset of RIP.

GAG GM-0111 and SELP 815K enema reduced the pain response in mice exposed to 35 Gy of ionizing radiation. SELP 815K 11 wt% with and without GAG reduced the response rate at 0.4 g of stimulus from 72 ± 7% to 48 ± 5% and 65 ± 3%, respectively ($p < 0.001$ for SELP with GAG and $p < 0.01$ for SELP without GAG) 3 days after treatment. The mean inhibition of the pain response was 53 ± 4% for SELP with GAG and 24 ± 10% for SELP alone ($p < 0.001$).

Again, after 7 days, both SELP 815K and SELP 815K with GAG achieved significant reduction in response rate compared to the radiation only group ($p < 0.001$), with average inhibition of pain response being 19 ± 10% and 48 ± 10%, respectively ($p < 0.001$). The addition of GAG to the SELP significantly enhanced the therapeutic outcome compared to SELP alone ($p < 0.01$).

After 21 days, the SELP only treatment group was not statistically different from the radiation treatment group, while the SELP and GAG combination still inhibited the pain response by 12 ± 6% (average of all 5 stimulus levels). However, at least during this 21-day observation period, none of the treatments were able to restore the response rate to the levels observed in healthy mice (Fig. 7).

F. Histology

To test the hypothesis that GAG delivered from the SELP hydrogel can protect rectal tissue from radiation damage, the following four groups were examined: (1) no GAG and no SELP (control), (2) GAG in PBS, (3) SELP alone, and (4) GAG in SELP. The GAG concentration was 100 mg/mL in all applicable groups. Indications

of radiation-induced damage to the rectum include damage to the epithelial layer, mitotic arrest, damage to the crypts of Lieberkühn, damage to the mucosa, and inflammation (Fig. 8). 35 Gy radiation caused substantial damage to the rectum. This was demonstrated by the breakdown of the epithelial layer observed at days 3 and 7. The crypts of Lieberkühn and goblet cells were severely damaged in day 3 and day 7 images and were absent at 21 days. Significant edema, an indicator of inflammation, was observed in the tissue and at day 21, there was limited to no evidence of recovery from the observed histopathology.

Treatment with the SELP 815K 11 wt% enema substantially maintained the rectal epithelium, possibly by "holding" the lumen together, leading to reduced epithelial loss at 3 and 7 days compared to the GAG in PBS enema. The crypts, however, were moderately damaged, and edema was observed at day 3. By day 7, the crypts and goblet cells were virtually eliminated. The crypts recovered somewhat by day 21, showed dilation and formation of cryptic abscesses, and continued edema. SELP edema had little impact on inflammation when it was administered without GAG and the inflammatory response was similar to that observed in the GAG in PBS group. By day 21, moderate recovery was observed within the groups treated with GAG in PBS and SELP alone as evidenced by the reemergence of the crypts and goblet cells but continued moderate edema.

SELP 815K 11 wt% and GAG GM-0111 administered together had the greatest observed therapeutic effect. Mild edema was observed at day 3, but was reduced at days 7 and 21. The crypts of Lieberkühn exhibited moderate damage at day 3, but this damage was mostly ameliorated at day 7. Overall, the epithelial layers remained intact and the tissues demonstrated less inflammation. Less dilation and damage to the crypts of Lieberkühn were observed when compared to the other treatment groups. Edema was observable in the crypts at day 21, but not within the sub-mucosa, unlike the SELP only group.

Throughout this publication, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by

reference into this application in order to more fully describe the methods, compositions, and compounds herein.

Various modifications and variations can be made to the materials, methods, and articles described herein. Other aspects of the materials, methods, and articles described herein will be apparent from consideration of the specification and practice of the materials, methods, and articles disclosed herein. It is intended that the specification and examples be considered as exemplary.

What is claimed is:

1. A composition comprising an anti-inflammatory polysaccharide and a gelling polymer, wherein the composition is a liquid prior to administration to a subject but converts to a gel upon administration to the subject.
2. The composition of claim 1, wherein the anti-inflammatory polysaccharide comprises an glycosaminoglycan.
3. The composition of claim 1, wherein the anti-inflammatory polysaccharide comprises a sulfated hyaluronan or the pharmaceutically acceptable salt or ester thereof, or a combination thereof.
4. The composition of claim 3, wherein at least one primary C-6 hydroxyl proton of the N-acetyl-glucosamine residue is substituted with a sulfate group.
5. The composition of claim 3, wherein from 1% to 100% of the primary C-6 hydroxyl protons of the N-acetyl-glucosamine residue of hyaluronan are substituted with a sulfate group.
6. The composition of claim 3, wherein at least one C-2 hydroxyl proton and C-3 hydroxyl proton of a uronic acid residue and at least one C-4 hydroxyl proton of an N-acetyl-glucosamine residue is substituted with a sulfate group.
7. The composition of claim 3, wherein the compound has a degree of sulfation from 0.1 to 4.0 per disaccharide unit.
8. The composition of claim 3, wherein the partially or fully sulfated hyaluronan has an average molecular size of less than 20 kDa.
9. The composition of claim 3, wherein the partially sulfated hyaluronan has an average molecular size from 2 kDa to 10 kDa.
10. The composition of claim 3, wherein (1) 100% of the primary C-6 hydroxyl protons of the N-acetyl-glucosamine residue of the sulfated hyaluronan are substituted with a sulfate group, (2) the sulfated hyaluronan has a degree of

sulfation from 3.0 to 4.0, and (3) the sulfated hyaluronan has an average molecular weight from 1 kDa to 3 kDa.

11. The composition of claim 3, wherein the pharmaceutically acceptable ester is a prodrug.

12. The composition of claim 1, wherein the anti-inflammatory polysaccharide comprises a modified hyaluronan or a pharmaceutically acceptable salt or ester thereof, wherein said hyaluronan or its pharmaceutically acceptable salt or ester comprises at least one sulfate group and at least one primary C-6 hydroxyl position of an N-acetyl-glucosamine residue comprising an alkyl group or fluoroalkyl group.

13. The composition of claim 12, wherein the alkyl groups is an unsubstituted alkyl group.

14. The composition of claim 13, wherein the unsubstituted alkyl group is methyl.

15. The composition of claim 12, wherein the fluoroalkyl group comprises at least one trifluoromethyl group.

16. The composition of claim 12, wherein from 1% to 100% of the primary C-6 hydroxyl protons of the N-acetyl-glucosamine residue are substituted with an alkyl group or fluoroalkyl group.

17. The composition of claim 12, wherein the hyaluronan has a molecular weight from 10 kDa to 2,000 kDa prior to modification.

18. The composition of claim 12, wherein at least one C-2 hydroxyl proton and C-3 hydroxyl proton is substituted with a sulfate group.

19. The composition of claim 12, wherein the hyaluronan is sulfated at the C-4 hydroxyl position of the N-acetyl glucosamine moiety, the C-2 position of the glucuronic acid moiety, the C-3 position of the glucuronic acid, or any combination thereof.

20. The composition of claim 12, wherein the compound has a degree of sulfation from 0.5 to 4.0 per disaccharide unit.

21. The composition of claim 12, wherein the alkyl group is methyl and at least one C-2 hydroxyl proton and/or C-3 hydroxyl proton is substituted with a sulfate group.
22. The composition of claim 12, wherein the alkyl group is methyl, at least one C-2 hydroxyl proton and/or C-3 hydroxyl proton is substituted with a sulfate group, and the compound has a molecular weight of 2 kDa to 10 kDa.
23. The composition of claim 12, wherein the modified hyaluronan has an unsubstituted C_1-C_{10} alkyl group selected from the group consisting of methyl, ethyl, propyl and butyl; the sulfate group is at the C-2 or C-3 hydroxyl position of a glucuronic acid moiety having a degree of sulfation from 0.5 to 3.5 per disaccharide unit; and the modified hyaluronan has a molecular weight of 2 kDa to 10 kDa.
24. The composition of claim 3, wherein the pharmaceutically acceptable salt of the compound (a) or (b) comprises an organic salt, a metal salt, or a combination thereof.
25. The composition of claim 3, wherein the pharmaceutically acceptable salt of the compound (a) or (b) comprises a salt selected from the group consisting of NH_4^+, Na^+, Li^+, K^+, Ca^{+2}, Mg^{+2}, Fe^{+2}, Fe^{+3}, Cu^{+2}, Al^{+3}, Zn^{+2}, *2*-trimethylethanolammonium cation (choline), or a quaternary salt of isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, and histidine.
26. The composition of claim 1, wherein the gelling polymer comprises a silk-elastin like protein, an elastin like protein, a copolymer of N-isopropylacrylamide, alginate, a copolymer of poly-vinyl alcohol, a poloxomer, carboxymethyl cellulose, chitosan, or any combination thereof.
27. The composition of claim 1, wherein the gelling polymer is a silk-elastin like protein.
28. The composition of claim 27, wherein the silk-elastinlike protein has at least four silk units.

29. The composition of claim 27, wherein the silk-elastinlike protein is 47K, 815K, 27k, 415K, pSE8Y, pS2E8Y, pS4E8Y, or any combination thereof.
30. The composition of claim 27, wherein the gelling polymer comprises a silk-elastinlike protein with one or more matrix metalloproteinase (MMP) cleavage sites.
31. The composition of claim 30, wherein the one or more matrix metalloproteinase (MMP) cleavage sites comprise a cleavage site of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, or any combination thereof.
32. The composition in any one of claims 27-31, wherein the silk-elastinlike protein comprises sheared silk-elastin like protein.
33. The composition of claim 32, wherein the sheared silk-elastinlike protein is 815K.
34. The composition of claim 27, wherein the concentration of the silk-elastinlike protein in the composition is from 2% to 20% w/w.
35. The composition of claim 27, wherein the concentration of the silk-elastinlike protein in the composition is from 10% to 12% w/w.
36. The composition of claim 27, wherein the concentration of the silk-elastinlike protein in the composition is from 2% to 10% w/w.
37. The composition of claim 1, wherein the anti-inflammatory polysaccharide is modified hyaluronan, wherein the hyaluronan has at least one sulfate group and at least one primary C-6 hydroxyl position of an N-acetyl-glucosamine residue having a methyl group, the gelling polymer is sheared silk-elastinlike protein 815K, and the sheared silk-elastin like protein is from 3% to 12% w/w of the composition.
38. The composition of claim 1, wherein the composition further comprises an antioxidant, a mucoadhesive agent, an anti-inflammatory agent, an anti-pyretic agent, steroidal and non-steroidal drugs for anti-inflammatory use, a hormone, a growth factor, a contraceptive agent, an antiviral, an antibacterial, an antifungal, an analgesics, a hypnotic, a sedative, a tranquilizer, an anti-convulsant, a muscle relaxant, a local anesthetic, an antispasmodic, an

antiulcer drug, a peptidic agonist, a sympathiomimetic agent, a cardiovascular agent, an antitumor agent, or an oligonucleotide.

39. The composition of claim 1, wherein the composition further comprises a contrast agent.

40. The composition of claim 1, wherein the composition is an injectable composition from 18 to 23 °C.

41. The composition of claim 1, wherein the composition has a viscosity of less than or equal to or less than 2500 cP at 18 to 23 °C.

42. The composition of claim 1, wherein the composition has a viscosity of less than equal to 700 cP at 18 to 23 °C.

43. The composition of claim 1, wherein the composition is a hydrogel at 37 °C.

44. A method for reducing or preventing inflammation and/or tissue damage in a subject comprising administering to the subject the composition of claim 1.

45. The method of claim 44, wherein the composition is administered intravenously, intramuscularly, transmucosally, or subcutaneously.

46. The method of claim 44, wherein the composition is administered ophthalmically, vaginally, rectally, intranasally, directly into the sinuses, into the outer, middle or inner ear, or applied directly to the oral mucosa, gingival, periodontal pocket.

47. The method of claim 44, wherein the composition is administered rectally by an enema, suppository, catheter, needleless syringe, or bulb syringe.

48. The method of claim 44, wherein the inflammation is caused by proctitis, auto-immune disease, invading virus or bacterium, genetic factors, or poor diet.

49. The method of claim 44, wherein the inflammation is caused by radiation induced proctitis.

50. The method of claim 44, wherein the inflammation is produced by cancer, multiple sclerosis, osteoarthritis, rheumatoid arthritis, Alzheimer's beta amyloid peptide, periodontal disease, gingivitis, peri-implantitis, diabetic nephropathy, inflammatory bowel disease, asthma, rhinitis, rhinosinusitis,

chronic obstructive pulmonary disease, acute lung injury, cystic fibrosis, sickle cell anemia, a cardiovascular inflammatory disorder, a pulmonary inflammatory disorder, an ocular inflammatory disorder, a cerebral inflammatory disorder or an intestinal inflammatory disorder.

51. The method of claim 44, wherein the inflammation is produced by a skin disorder.

52. The method of claim 51, wherein the skin disorder comprises rosacea, atopic dermatitis (eczema), allergic contact dermatitis, psoriasis, dermatitis herpetiformis, acne, diabetic skin ulcers and other diabetic wounds, burns, sunburn, prevention of scarring, actinic keratoses, inflammation from insect bites, poison ivy, radiation-induced dermatitis/burn, interstitial cystitis, photo-dermal ageing, or seborrheic dermatitis.

53. The method of claim 44, wherein the inflammation is produced by an eye disorder.

54. The method of claim 52, wherein the eye disorder comprises the ophthalmic disorder is age-related macular degeneration, diabetic retinopathy, dry eye syndrome, conjunctivitis, iritis, uveitis, allergic conjunctivitis, inflammation caused by surgery, or corneal inflammation or scarring.

55. The method of claim 54, wherein the composition is administered intraocularly or to the surface of the eye.

56. The method of claim 44, wherein the inflammation is urological inflammation.

57. The method of claim 56, wherein the urological inflammation comprises inflammation of the bladder, urethra, urothelium lining, kidney, prostate, vagina, uterus, or any combination thereof.

58. The method of claim 56, wherein the composition is administered topically to the vagina to prevent or reduce bacterial, fungal, viral or mechanical or biologically-induced inflammation.

59. The method of claim 44, wherein the inflammation is orthopedic inflammation.

60. The method of claim 59, wherein the composition is administered into a joint of the subject.

61. The method of claim 44, wherein the inflammation is caused by a respiratory disorder.

62. The method of claim 61, wherein the respiratory disorder is cystic fibrosis, bronchiectasis, rhinitis, sinusitis, emphysema and chronic bronchitis (COPD), acute lung injury/adult respiratory distress syndrome, interstitial lung fibrosis, SARS, asthma, and respiratory syncytial virus.

63. The method of claim 61, wherein the inflammation is caused by a respiratory pathogen comprising *Stretococcus pneumoniae*, *Hemophilus influenzae*, *Staphylococcus*, *Mycoplasma pneumoniae*, Chlamydial pneumonia, or Gram negative bacteria.

64. The method of claim 44, wherein the inflammation is caused by otitis media.

65. The method of claim 44, wherein the inflammation is caused by a gastrointestinal disease or a bowel disease.

66. The method of claim 65, wherein the gastrointestinal disease is ulcerative colitis, Crohn's disease of the bowel, or hemorrhoids.

67. The method of claim 44, wherein the inflammation is caused by a periodontal disease.

68. A method for prophylactically treating inflammation and/or tissue damage comprising administration to the subject the composition of claim 1 prior to administration of a source of inflammation to the subject.

69. The method of claim 68, wherein the source of inflammation and/or tissue damage comprises exposing the subject to radiation.

70. The method of claim 68, wherein the composition is administered less than 1 week prior to the administration of the source of inflammation and/or tissue damage.

71. The method of claim 68, wherein the composition is administered less than 48 hours prior to the administration of the source of inflammation and/or tissue damage.

72. The method of claim 68, wherein the composition is administered less than 24 hours prior to the administration of the source of inflammation and/or tissue damage.

73. The method of claim 68, wherein the composition is administered less than 12 hours prior to the administration of the source of inflammation and/or tissue damage.

74. The method of claim 68, wherein the composition is administered less than 3 hours prior to the administration of the source of inflammation and/or tissue damage.

75. The method of claim 68, wherein the composition is administered immediately prior to the administration of the source of inflammation and/or tissue damage.

76. The method of claim 68, wherein the composition is administered to the rectum of the subject.

77. The method of claim 68, wherein inflammation and/or tissue damage in the thyroid of the subject is reduced and/or prevented.

78. The method of claim 68, wherein inflammation and tissue damage to the epithelium of the subject is reduced and/or prevented.

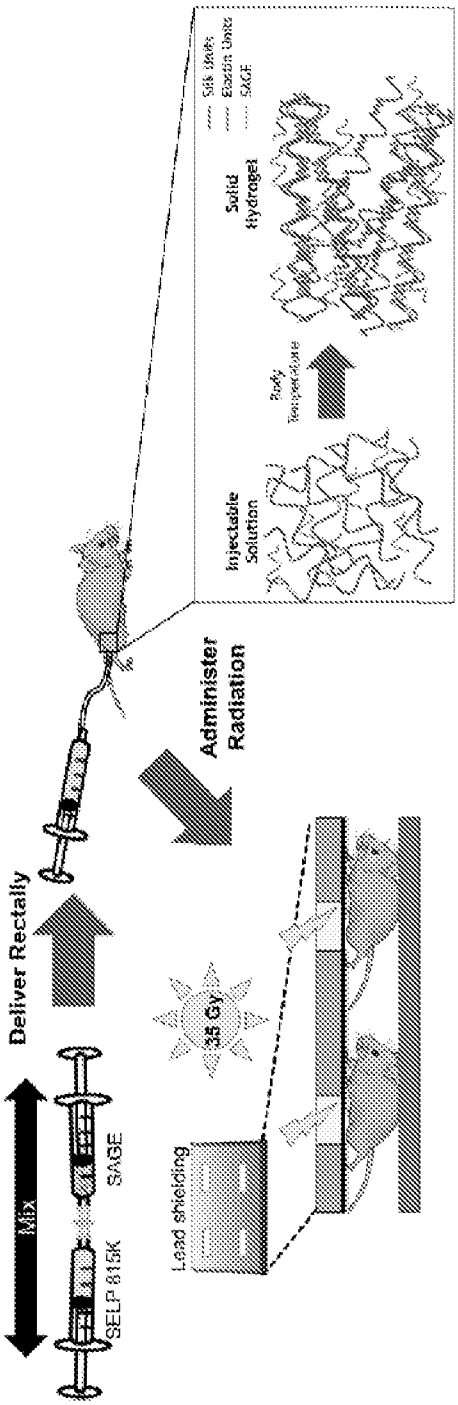

Silk Units
Elastin Units
SAGE
Solid Hydrogel
Injectable Solution
Body Temperature
Administer Radiation
35 Gy
Lead shielding
Deliver Rectally
Mix
SAGE
SELP 815K

FIGURE 1

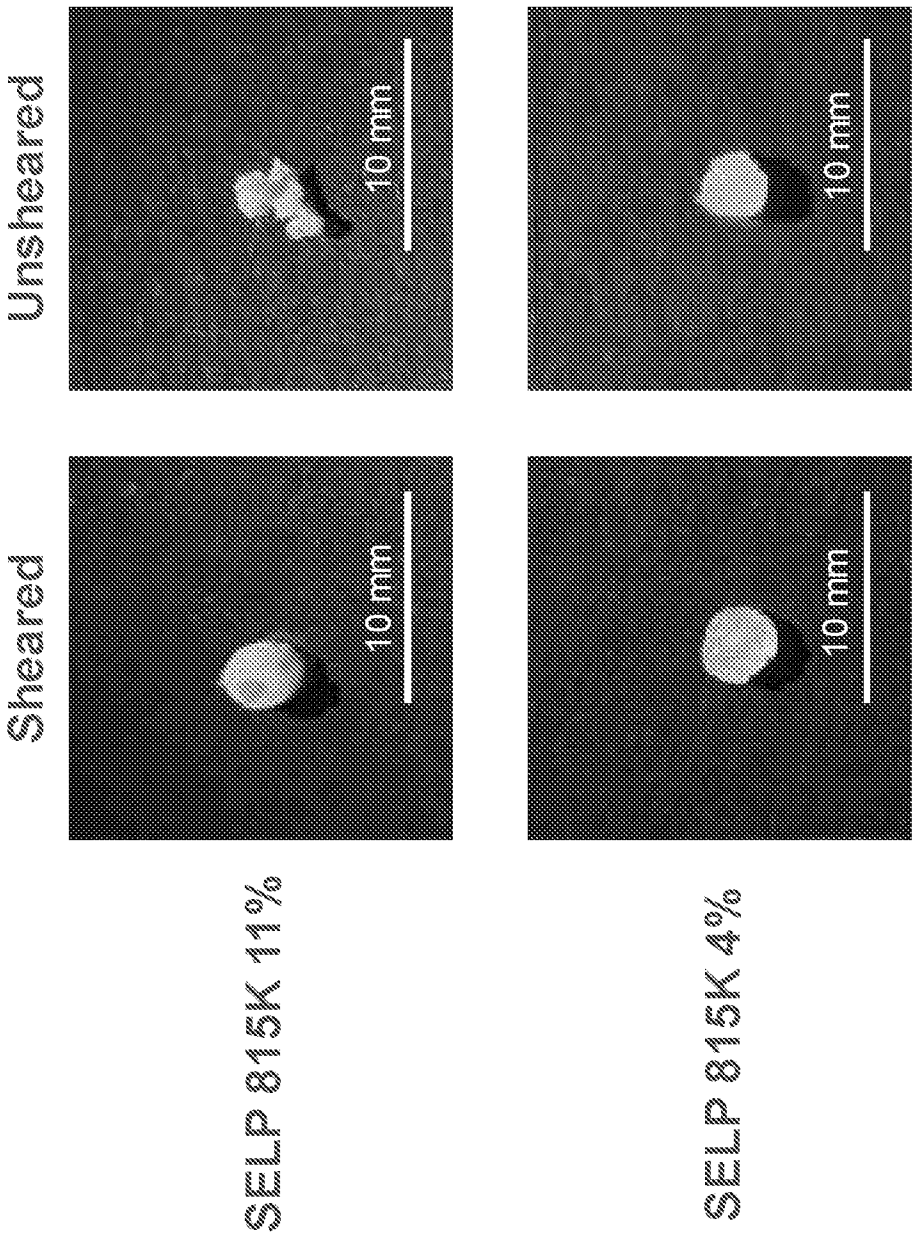

Unsheared
Sheared
10 mm
10 mm
10 mm
10 mm
SELP 815K 11%
SELP 815K 4%

FIGURE 2

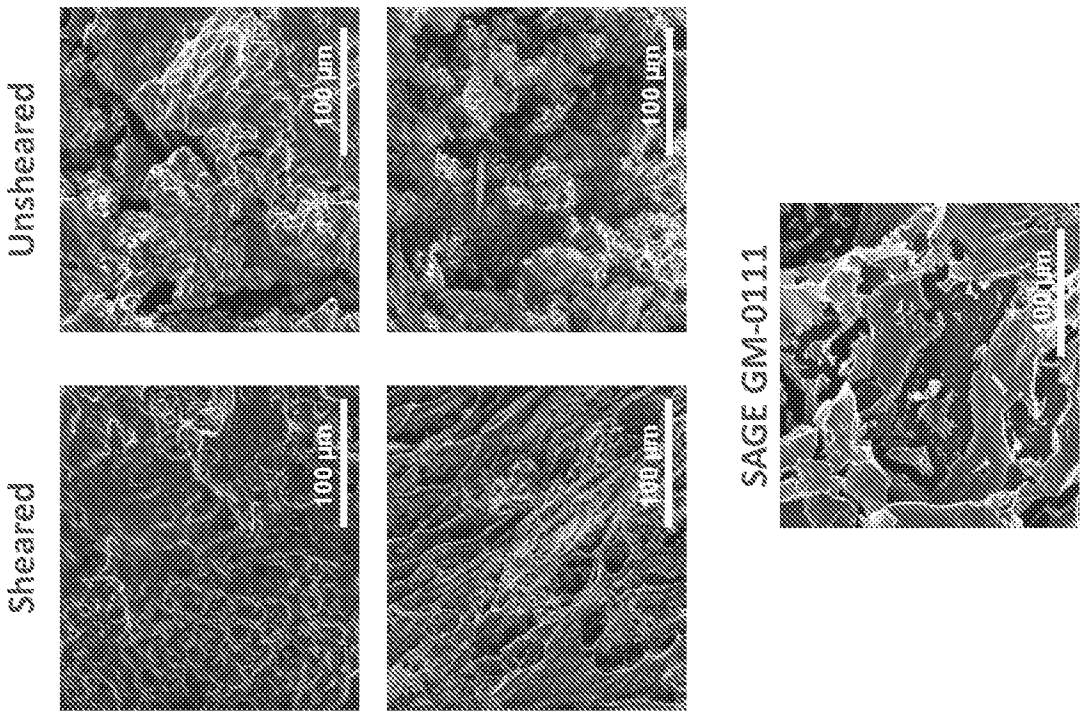

Sheared
Unsheared
SELP 815K 11% with 100 mg/ml SAGE GM-0111
SELP 815K 4% with 100 mg/ml SAGE GM-0111
SAGE GM-0111
100 μm

FIGURE 3

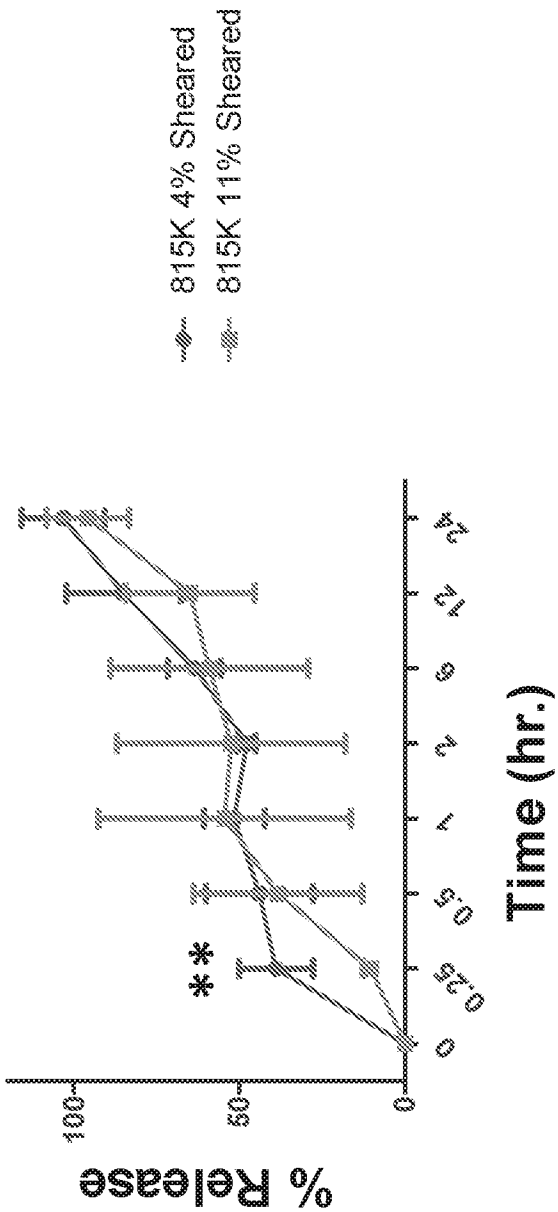
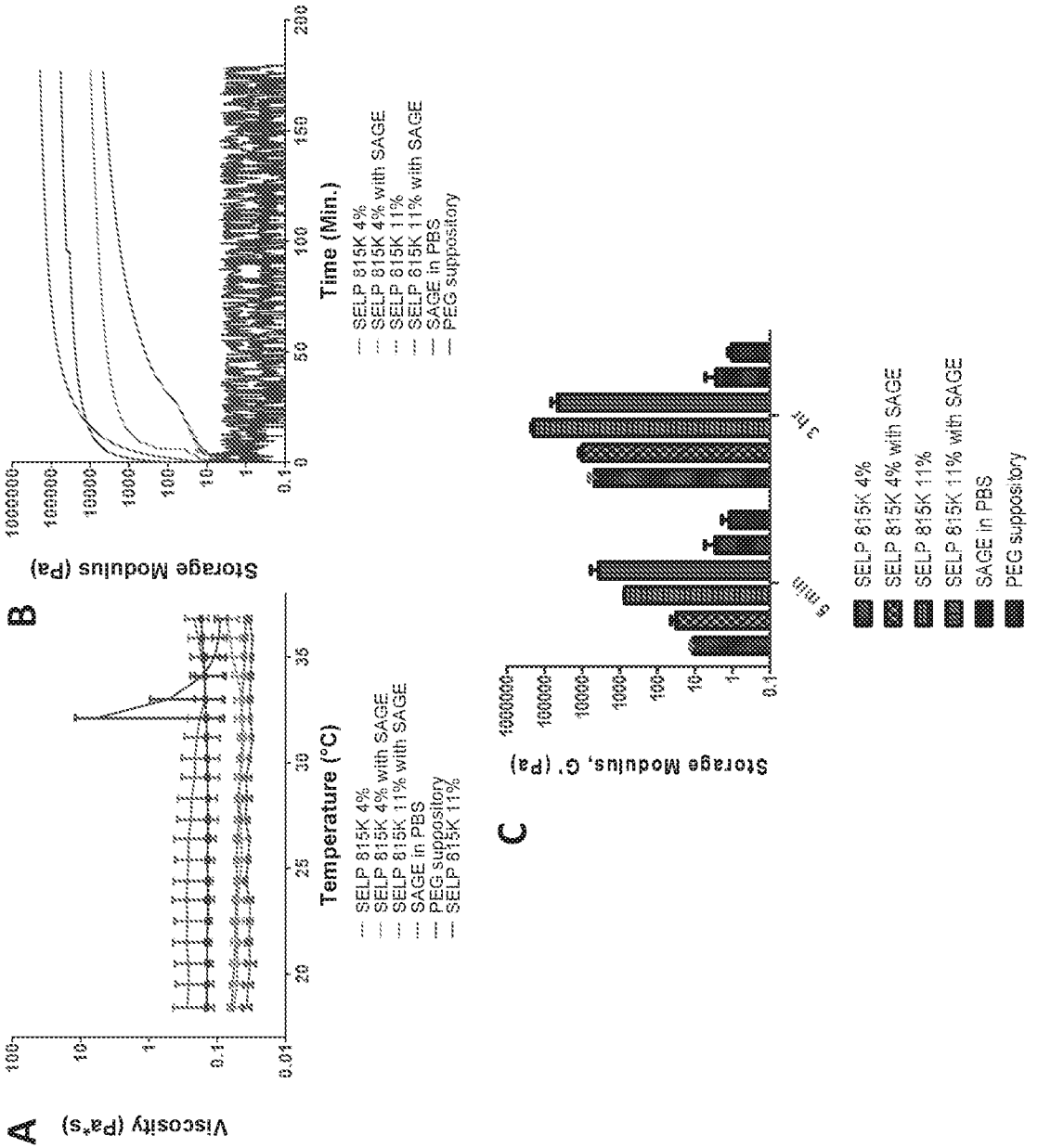

815K 4% Sheared
815K 11% Sheared
% Release
100
50
0
**
0
0.25
0.5
1
3
6
12
24
Time (hr.)

FIGURE 4



FIGURES 5A-5C

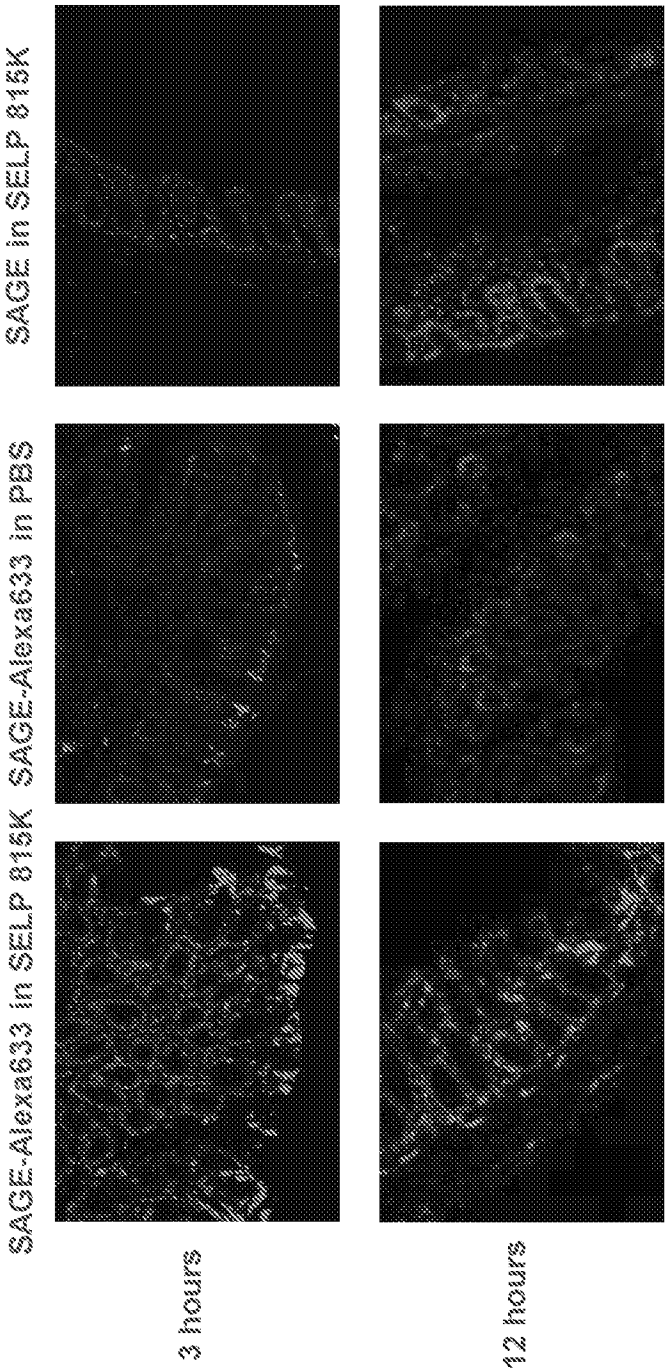
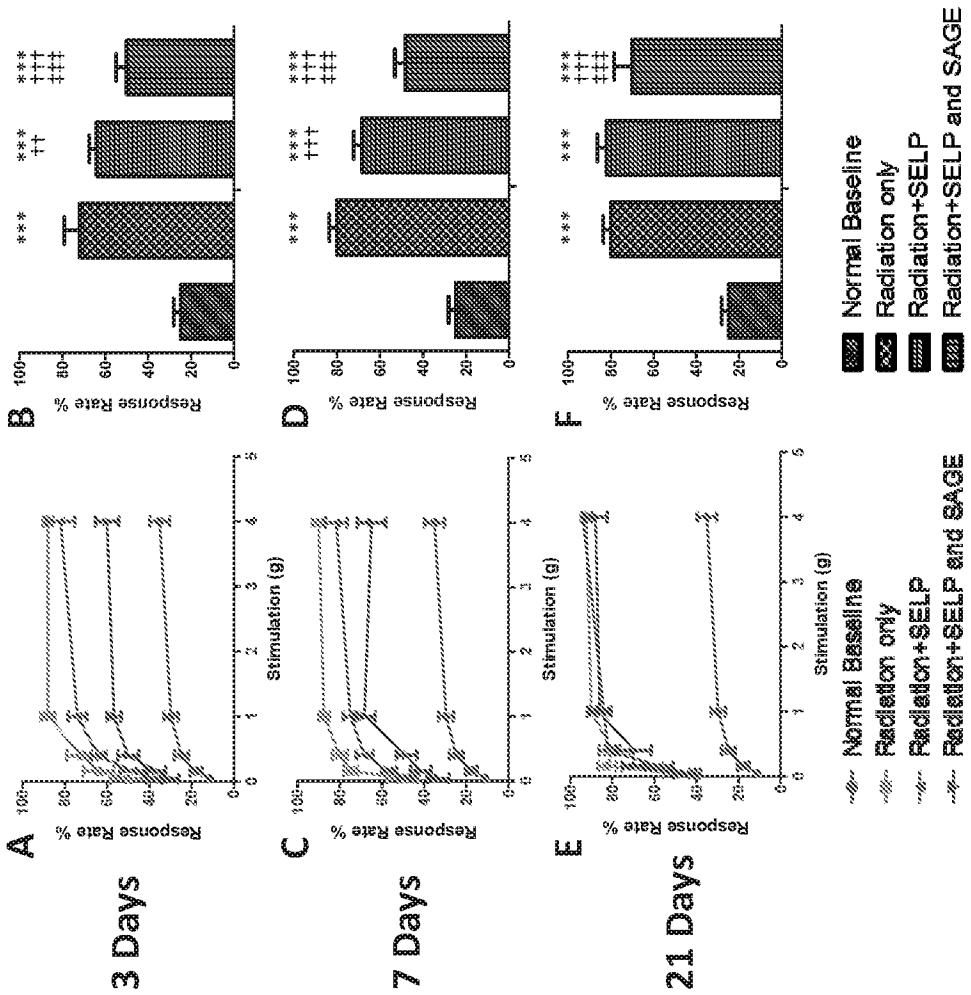

SAGE-Alexa633 in SELP 815K
SAGE-Alexa633 in PBS
SAGE in SELP 815K
3 hours
12 hours

FIGURE 6



FIGURES 7A-7E

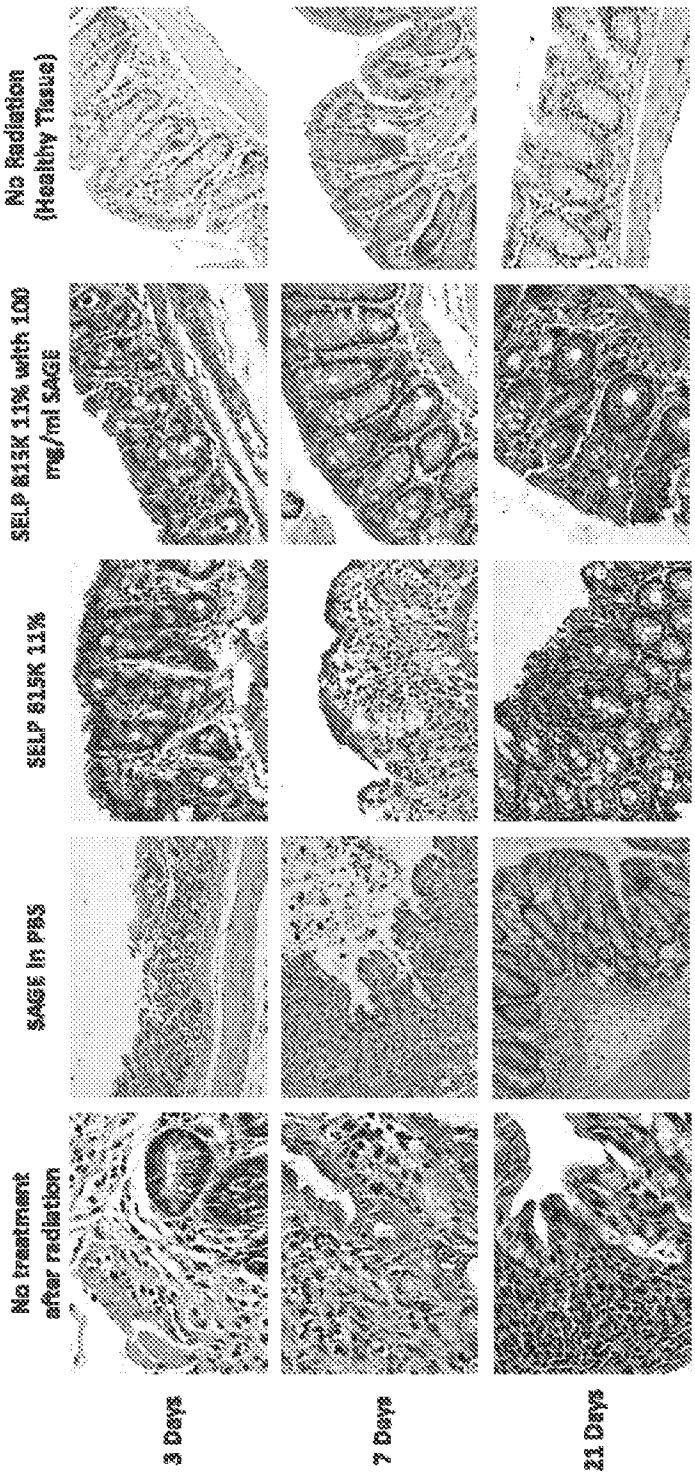

No Radiation (Healthy Tissue)
SELP 815K 11% with 100 mg/ml SAGE
SELP 815K 11%
SAGE in PBS
No treatment after radiation
3 Days
7 Days
21 Days

FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/51538

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/39, A61K 45/06, C07K 14/78, A61K 31/737, A61K 31/728, A61Q 11/00 (2017.01)
CPC - A61K 38/39, A61K 38/1767, A61K 45/06, A61K 9/0019, A61K 9/0024, A61K 47/42, A61K 31/737, A61K 31/728, A61K 2300/00, A61K 8/735, C07K 14/78, C07K 14/43586, A61Q 11/00, A61Q 19/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	Dubbini, et al. Injectable hyaluronic acid/PEG-p(HPMAm-lac)-based hydrogels dually cross-linked by thermal gelling and Michael addition. European Polymer Journal 2015, 72:423-437; Abstract, pg 427, Scheme 2; pg 425, Scheme 1	1, 2, 38-43 ---------- 3-37
A	Wirostko, et al. Ophthalmic Uses of a Thiol-Modified Hyaluronan-Based Hydrogel. Adv Wound Care (New Rochelle) 2014, 3(11):708-716); pg 710, col 2	1
Y	US 2007/0054878 A1 (Venbrocks, et al.) 08 March 2007 (08.03.2007) para [0030]-[0034]	3
Y	US 2014/0343011 A1 (Prestwich, et al.) 20 November 2014 (20.11.2014) claims 17-21; para [0004], [0005], [0070], [0083]-[0085], [0095], [0096]	3-10
Y	US 2015/0209385 A1 (University of Utah Research Foundation) 30 July 2015 (30.07.2015) claims 2-13, 31; Fig 2; para [0051], [0199]	3, 11-25, 37
Y	Poursaid. Design and development of silk-elastin like protein polymer liquid Embolics for treatment of hepatocellular carcinoma. Ph.D. Thesis August 2016 [Retrieved from the Internet 29 December 2017: <https://cdmbuntu.lib.utah.edu/utils/getfile/collection/etd3/id/4322/filename/4309.pdf>]; pg 5, 70-72, 89, 97, 99, 169	26-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 December 2017	06 FEB 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/51538

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/51538

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-43, directed to a composition comprising an anti-inflammatory polysaccharide and a gelling polymer. The composition will be searched to the extent that the gelling polymer encompasses silk-elastin like protein. It is believed that claims 1-43 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a composition comprising an anti-inflammatory polysaccharide and a silk-elastin like protein. Additional gelling polymer(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected gelling polymer(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a composition comprising an anti-inflammatory polysaccharide and a poloxomer, i.e., claims 1-26, 37-43.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-43, restricted to silk-elastin like protein

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/51538

In Continuation of Box III. Observations where unity of invention is lacking:

Group II: claims 44-78, directed to a method for using a composition of claim 1.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of each invention of Group I+ is a gelling agent recited therein.

The inventions of Group I+ do not include the technical feature of a method of using a composition of claim 1, as required by Group II.

Common Technical Features
The inventions of Groups I+ and II share the technical feature of a composition of claim 1. However, this shared technical feature does not represent a contribution over prior art as being anticipated by a paper titled "Injectable hyaluronic acid/PEG-p(HPMAm-lac)-based hydrogels dually cross-linked by thermal gelling and Michael addition" by Dubbini, et al. (European Polymer Journal 2015, 72:423-437 (hereinafter "Dubbini").

Dubbini discloses a composition (Abstract, "Fast in situ forming thermosensitive hydrogels consisted of vinyl sulfone bearing p(HPMAm-lac1-2)-PEG-p(HPMAm-lac1-2) triblock copolymers and thiol modified hyaluronic acid were prepared via a dual cross-linking strategy based on thermal gelation at 37C and simultaneous Michael addition cross-linking between vinyl sulfone and thiol moieties") comprising
- thiolated hyaluronic acid (pg 427, "Scheme 2. Synthesis route of thiolated hyaluronic acid"; pg 429, "Thiolated hyaluronic acid of a molecular weight of 37.9 kDa was used as a cross-linker") and
- a gelling polymer (pg 425, "Scheme 1. Synthesis route of vinyl sulfonated thermosensitive triblock copolymer VinylSulTC_n"),
- wherein the composition is a liquid prior to administration to a subject but converts to a gel upon administration to the subject (pg 427, "Immediately after mixing, the final solution containing VinylSulTC_n and HA-SH_n0 was placed in a preheated oven at 37C to allow thermal gelation and simultaneous Michael Addition cross-linking between thiol and vinyl sulfone groups").

Dubbini does not specifically disclose that thiol modified hyaluronic is an anti-inflammatory polysaccharide. However said limitation is met by/inherently present in Dubbini because thiol modified hyaluronic is an anti-inflammatory polysaccharide (please see a paper titled "Ophthalmic Uses of a Thiol-Modified Hyaluronan-Based Hydrogel" by Wirostko, et al. (Adv Wound Care (New Rochelle) 2014, 3(11):708-716); pg 710, col 2, "Crosslinked CMHA-S [thiol-modified carboxymethyl hyaluronic acid] hydrogels ... offer intrinsic ocular wound healing with anti-inflammatory..."). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions.

The inventions of Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.