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(54) Title: HIGH-MOLECULAR-WEIGHT FUCANS FOR TREATING FIBROUS ADHESIONS AND OTHER DISEASES AND CONDITIONS

(57) Abstract: High-molecular-weight fucan compositions comprising a therapeutically effective, medically acceptable fucan in a composition comprising wherein the fucan, for example, has a molecular weight distribution in which more than 60 % w/w of the composition has a molecular weight above 100 kDa.

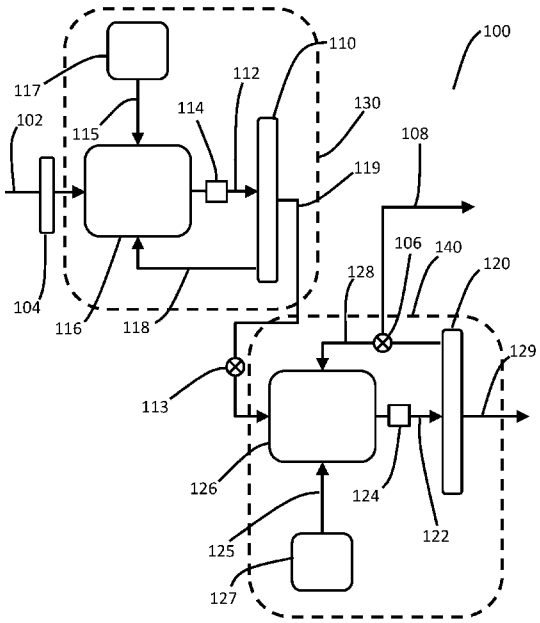

FIG. 1

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HIGH-MOLECULAR-WEIGHT FUCANS FOR TREATING FIBROUS ADHESIONS AND OTHER DISEASES AND CONDITIONS

CLAIM FOR PRIORITY

[0001] The present application claims the benefit of co-pending United States provisional patent application no. 62,711,364, filed July 27, 2018; United States provisional patent application no. 62,711,372, filed July 27, 2018; United States provisional patent application no. 62/711,335, filed July 27, 2018; United States Provisional Patent Application Serial No. 62/713,399, filed August 1, 2018; United States provisional patent application No. 62/722,135, filed August 23, 2018; United States provisional patent application No. 62/755,311, filed November 2, 2018; United States provisional patent application No. 62/793,514, filed on January 17, 2019; United States provisional patent application No. 62/861,223, filed June 13,2019; co-pending United States Provisional Patent Application Serial No. 62/713,392, filed August 1, 2018; United States provisional patent application No. 62/713,413, filed August 1, 2018; United States provisional patent application No. 62/722,137, filed August 23, 2018; United States provisional patent application No. 62/755,318, filed on November 2, 2018; United States provisional patent application No. 62/861,228, filed June 13, 2019; co-pending United States Provisional Patent Application Serial No. 62/755,328, filed November 2, 2018; United States provisional patent application No. 62/793,654, filed January 17, 2019; and, United States provisional patent application No. 62/861,235, filed June 13, 2019, all of which applications are incorporated herein by reference in their entirety.

BACKGROUND

[0002] Fucans (including fucoidan) are sulfated polysaccharides. In general terms, this means that they are molecules made up of a number of sugar groups, and also have sulfur atoms attached to the sugar groups. The main sugar group is called "fucose", which is sugar that has 6 carbon atoms and has the chemical formula $C_6H_{12}O_5$. "Fucoidan" (or fucoidin) indicates fucans derived from brown algae (seaweed). Fucans can exist alone, or in a mixture of other sugars, for example in a mixture of sugars such as xylose, galactose, glucose, glucuronic acid and/or mannose. These other

sugars may be extracted from the seaweed or other source with the fucan. Although fucans are currently derived from natural sources such as the brown algae (seaweeds), sea cucumbers, etc., mentioned herein, "fucan" includes polymer molecules having the chemical and structural motifs of the fucans as discussed herein regardless of the ultimate source(s) of the fucans.

[0003] Fucoidan can be obtained from a variety of species of brown algae including but not limited to: *Adenocystis utricularis*, *Ascophyllum nodosum*, *Chorda filum*, *Cystoseirabies marina*, *Durvillaea antarctica, Ecklonia kurome*, *Ecklonia maxima, Eisenia bicyclis, Fucus evanescens*, *Fucus vesiculosis*, *Hizikia fusiforme*, *Himanthalia Elongata, Kjellmaniella crassifolia*, *Laminaria brasiliensis*, *Laminaria cichorioides*, *Laminaria hyperborea*, *Laminaria japonica*, *Laminaria saccharina*, *Lessonia trabeculata, Macrocystis pyrifera, Pelvetia fastigiata*, *Pelvetia Canaliculata, Saccharina japonica, Saccharina latissima, Sargassum stenophylum*, *Sargassum thunbergii*, *Sargassum confusum, Sargassum fusiforme* and *Undaria pinnatifida*. These exemplary species are all from the taxonomic class *Phaeophyceae* and the majority of these species fall into the families of *Fucales* and *Laminariaceae*.

[0004] Fucans including fucoidan have been shown to be efficacious in serving to inhibit, prevent, remove, reduce, or otherwise treat the formation of fibrous adhesions. They have also found use in the treatment of other related diseases and conditions.

[0005] Thus, there has gone unmet a need for compositions comprising fucans having desired high-molecular-weights, including in some embodiments such compositions being modified to have desired sulfation levels and/or medically viable, low endotoxin levels. The present compositions, systems and methods, etc., provide these and/or other advantages.

SUMMARY

[0006] The present compositions, systems, devices, materials and methods, etc., provide high-molecular-weight fucans. Such high-molecular-weight fucans can be obtained from feedstock fucan compositions or other starting or initial fucan compositions that have fucans with a broad molecular weight distribution comprising a desired high-molecular-weight segment/portion (i.e., broad molecular weight fucan compositions from which the high-molecular weight fucans can be derived; such starting fucan compositions may or may not be crude or have been previously processed or purified). The desired high-molecular-weight fucan has a molecular weight

distribution consisting essentially of the desired high-molecular-weight segment/portion of the starting fucan broad molecular weight distribution wherein a substantial quantity of the broad molecular weight distribution at the low molecular weight end has been eliminated, suppressed, or otherwise attenuated such that any remaining amounts are inconsequential.

[0007] In some aspects, the compositions, systems, methods, etc., herein comprise high-molecular-weight fucans such as fucoidans can comprise, consist essentially of, or consist of. a molecular weight distribution wherein at least 60% w/w of the distribution can be greater than 100 kDa when measured using an aqueous gel permeation chromatography set up consisting essentially of:

one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of can be between about 50 kDa and about 5,000 kDa, one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of can be between about 1 kDa and about 6,000 kDa and one 40 mm guard column with a 6 mm inner diameter packed with hydroxylated polymethacrylate-based gel, the two analytical gel permeation chromatography columns and the one guard column contained in a column compartment at about 30 °C;

a refractive index detector at about 30 °C;

0.1M sodium nitrate mobile phase run at 0.6 mL/min; and

quantification against a peak molecular weight standard curve consisting essentially of a first dextran standard with a peak molecular weight of about 2,200 kDa, a second dextran standard with a peak molecular weight of can be between about 720 kDa and about 760 kDa, a third dextran standard with a peak molecular weight can be between about 470 kDa and about 510 kDa, a fourth dextran standard with a peak molecular weight can be between about 370 kDa and about 410 kDa, a fifth dextran standard with a peak molecular weight can be between about 180 kDa and about 220 kDa, and a sixth dextran standard with a peak molecular weight can be between about 40 kDa and 55 kDa.

[0008] In some embodiments, at least about 70% w/w, 80% w/w, 90% w/w, 93% w/w, 94% w/w, 95% w/w, 97% w/w, 98% w/w, or 99% w/w of the distribution can be greater than 100 kDa. The weight average molecular weight can be between about 100 kDa and 10,000 kDa; between about 140 kDa and 8,100 kDa; between about 370 kDa and 8100 kDa; between about 370 kDa and 5300 kDa; between about 370 kDa and 8100 kDa; between about 370 kDa and 5300 kDa; between about 370 kDa and 1900 kDa; between about 590 kDa and 1600 kDa; between about 590 kDa and 1600 kDa; or between about 860 kDa and 1600 kDa. In some embodiments, the weight average molecular weight can be about 1,100 kDa, about 1,200 kDa, or about 1,300 kDa. The number average molecular weight can be between about 50 kDa and 3,000 kDa; between about 60 kDa and 2,000 kDa; between about 140 kDa and 2,000 kDa; between about 140 kDa and 520 kDa; or between about 230 kDa and 450 kDa. At least 55% w/w, 71% w/w, or 91% w/w of the distribution can be greater than about 200 kDa. At least 22%, 54% w/w, or 90% w/w of the distribution can be greater than about 500 kDa.

[0009] In some embodiments, the high-molecular-weight fucans can consist essentially of, comprise, or consist of, a molecular weight distribution wherein can be between about 61% w/w and 80% w/w of the distribution can be between about 200 kDa and 1600 kDa when measured using an aqueous gel permeation chromatography set up as set forth above and elsewhere herein. The high-molecular-weight fucans can consist essentially of, comprise, or consist of, a molecular weight distribution wherein at least 60% w/w of the distribution can be greater than about 1600 kDa when measured using an aqueous gel permeation chromatography set up as set forth above and elsewhere herein.

[00010] The sulfate content can be between about 20% w/w and 60% w/w, about 30% w/w and 55% w/w, or about 32% w/w and 52% w/w. The total carbohydrate content can be between about 27% w/w and 80% w/w. The total fucose content as a percentage of the total carbohydrate content can be at least about 30% w/w, 50% w/w, 70% w/w, 80% w/w, 90% w/w or 95% w/w. The total galactose content as a percentage of the total carbohydrate content can be below about 60% w/w, or can be between about 2% w/w and 20% w/w, or can be below about 10% w/w. The total of glucuronic acid, mannose, rhamnose and xylose content as a percentage of the total carbohydrate content can be below about 30% w/w.

[00011] The high-molecular-weight fucans when dissolved in water at a concentration of 50 mg/mL has a viscosity of can be between about 4 cP and 50 cP; between about 10 cP and 40 cP; or between about 15 cP and 30 cP. The high-molecular-weight fucans can be a white solid, and when dissolved in water at a concentration from 1 mg/mL through 100 mg/mL forms a solution that can be one of clear-colorless. The fucan can comprise less than about 5% w/w or 2% w/w acetyl content. The fucan can comprise an acetyl content of substantially 0% w/w when measured by 2D ^{1}H-^{13}C heteronuclear multiple quantum coherence at 70 °C with solvent signal suppression on a 600 MHz spectrometer equipped with 5-mm cold probe, in the range from 10-30 ppm in the carbon dimension, in 8 increments of 256-512 scans each.

[00012] Also included herein are methods, including methods that can comprise making or using the high-molecular-weight fucans herein, including for treating fibrous adhesions. Further included herein are medically acceptable fucan compositions that can comprise a therapeutically effective amount of the high-molecular-weight fucans in a medically acceptable buffer or diluent. Methods also include treating a condition or disease in an animal that can comprise selecting the medically acceptable fucan compositions herein to treat the condition or disease and administering a therapeutically effective amount comprising between about 0.5 mg/kg and 50 mg/kg; 0.04 mg/kg and 25 mg/kg; 0.2 mg/kg and 10 mg/kg; 1 mg/kg and 5 mg/kg; 1.5 mg/kg and 3 mg/kg; 5 mg/kg and 10 mg/kg.

[00013] The condition or disease can be a fibrous adhesion at a target site in the animal, and the administering can comprise administering the therapeutically effective amount to the target site.

[00014] The medical compositions can be between about 0.02 mg/mL and 100 mg/mL of the high-molecular-weight fucans, wherein the medical compositions is configured and composed to treat a disease or condition in an animal. The medical compositions can also be between about 0.5 mg/mL and 5 mg/mL, or about 2.5 mg/mL, of the high-molecular-weight fucans.

[00015] The medical compositions can be a medical device including a liquid medical device. The medical compositions can be pharmaceutical compositions, which can be liquid pharmaceutical compositions.

[00016] The methods herein also include use of a dosage range comprising between about 0.01 mL/kg and 15 mL/kg; about 0.03 mL/kg and 4 mL/kg; about 0.06 mL/kg and 2 mL/kg; or, about 2 mL/kg and 4 mL/kg of the medical compositions to treat a disease or condition in an animal.

[00017] The methods for treating fibrous adhesions in a patient can comprise administering the medical compositions to a target site in the patient. The target site can be a surgical site and the administering can be performed at least one of a) after opening a surgical wound at the surgical site, b) during surgery, and c) after closing the surgical wound. The administering can be performed after surgery and before closing the surgical wound. The administering can take less than 3 minutes, 2 minutes or 1 minute. The target site can be at least one of a lesion, abrasion and injury site. The target site can be at least one of a pelvic cavity, an abdominal cavity, a dorsal cavity, a cranial cavity, a spinal cavity, a ventral cavity, a thoracic cavity, a pleural cavity, a pericardial cavity, skin, a joint, a muscle, a tendon and a ligament.

[00018] In further embodiments, the methods herein include methods for obtaining a high-molecular-weight fucans. Such methods can comprise:

providing in a starting solution a starting fucan compositions having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment;

subjecting the starting solution to a first tangential flow filtration across a first higher molecular weight cutoff tangential flow filtration filter to produce a first permeate fucan compositions; and

subjecting the first permeate fucan compositions to a second tangential flow filtration across a second lower molecular weight cutoff tangential flow filtration filter to produce a second retentate fucan compositions consisting essentially of the desired high-molecular-weight fucans.

[00019] The methods further can comprise collecting the second retentate fucan compositions consisting essentially of the desired high-molecular-weight fucans, and the first higher molecular weight cutoff tangential flow filtration filter has a higher molecular weight cutoff of can be between about 50 kDa and about 1000 kDa and the second lower molecular weight cutoff tangential flow filtration filter has a lower molecular weight cutoff of can be between about 30 kDa and about 100 kDa. The higher molecular weight cutoff can be about 300 kDa and the lower molecular weight cutoff can be about 100 kDa.

[00020] Methods for obtaining a high-molecular-weight fucans can comprise:

providing a starting fucan compositions having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment in a starting solution;

subjecting the starting solution to tangential flow filtration across a first lower molecular weight cutoff tangential flow filtration filter to produce a first retentate fucan compositions; and

subjecting the first retentate fucan compositions to tangential flow filtration across a second higher molecular weight cutoff tangential flow filtration filter to produce a second permeate fucan compositions consisting essentially of the desired high-molecular-weight fucans.

[00021] The methods further can comprise collecting the second permeate fucan compositions consisting essentially of the desired high-molecular-weight fucans. The first tangential flow filtration can comprise diafiltering the starting solution across the first lower molecular weight cutoff tangential flow filtration filter. The second tangential flow filtration can comprise diafiltering the first retentate fucan compositions across the second higher molecular weight cutoff tangential flow filtration filter. The first lower molecular weight cutoff tangential flow filtration filter has a lower molecular weight cutoff of can be between about 30 kDa and about 100 kDa and the second higher molecular weight cutoff tangential flow filtration filter has a higher molecular weight cutoff of can be between about 50 kDa and about 1000 kDa. The lower molecular weight cutoff can be about 100 kDa and the higher molecular weight cutoff can be about 300 kDa.

[00022] Methods for obtaining a high-molecular-weight fucans can comprise:

providing a starting fucan compositions having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment in a starting solution, the starting fucan compositions can comprise low atomic weight cations ionically bound to the sulfate groups on fucan in the compositions; and

subjecting the starting solution to tangential flow filtration against a cationic additive solution can comprise a cationic additive having a greater molecular weight than the low atomic weight cations to produce a retentate fucan compositions consisting essentially of the desired high-molecular-weight fucans.

[00023] The methods further can comprise collecting the retentate fucan compositions consisting essentially of the desired high-molecular-weight fucans. The low atomic weight cations comprise at least one of an alkali metal, an alkaline earth metal, aluminum and ammonium. The cationic additive can comprise at least one of choline, polyvinylpyrrolidone, taurine, polyamine, chitosan, histone, and collagen. The methods further can comprise adding to the starting solution the

cationic additive before subjecting the starting solution to tangential flow filtration. The tangential flow filtration can comprise diafiltering the starting solution against the cationic additive solution. The methods still further can comprise removing the cationic additive by diafiltering the retentate fucan compositions against a salt solution over a second tangential flow filtration filter having a molecular weight cutoff that can be lower than a molecular weight cutoff of the first tangential flow filtration filter.

[00024] The salt solution can comprise a chloride, bromide, iodide, fluoride, sulfate, sulfite, carbonate, bicarbonate, phosphate, nitrate, nitrite, acetate, citrate, silicate and/or cyanide of an alkali metal, alkaline earth metal, aluminum and/or ammonium. The methods can also comprise removing salt by diafiltering the retentate fucan compositions against a low-ionic strength solution.

[00025] Methods for obtaining a high-molecular-weight fucans can comprise:

providing a centrifuge container can comprise a bottom end and a top end and a permeable barrier therebetween, the permeable barrier can comprise a gradient material therebetween;

placing a starting fucan compositions having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment in the centrifuge container and above the permeable barrier; and

centrifuging the centrifuge container for a period of time sufficient to produce a precipitate consisting essentially of the desired high-molecular-weight fucans.

[00026] The methods further can comprise collecting the desired high-molecular-weight fucans from the centrifuge container. The permeable barrier can comprise a single segment of gradient material. The permeable barrier can comprise a plurality of segments of gradient material. The gradient material can comprise at least one of sucrose, polysucrose, glycerol, sorbitol, CsCl, Cs_2SO_4, KBr, diatrizoate, Nycodenz® and iodixanol. The centrifugal force can be between about 10,000 gravities to about 1,000,000 gravities. The centrifugal force can be between 60,000 gravities to about 500,000 gravities.

[00027] Methods for obtaining a high-molecular-weight fucans can comprise:

providing a centrifuge container can comprise a bottom end and a top end;

placing a starting fucan compositions in a starting solution, having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment in the centrifuge container; and

centrifuging the centrifuge container for a period of time sufficient to produce a precipitate consisting essentially of the desired high-molecular-weight fucans.

[00028] The methods further can comprise collecting the desired high-molecular-weight fucans as a precipitate from the centrifuge container. The centrifugal force can be between about 60,000 gravities to about 1,000,000 gravities. The centrifugal force can be between 200,000 gravities to about 500,000 gravities.

[00029] Methods for obtaining a high-molecular-weight fucans can comprise:

subjecting a starting fucan compositions having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment to gel electrophoresis wherein the starting fucan compositions can be displaced according to mass-to-charge ratio across an electrophoresis gel;

selecting a portion of the electrophoresis gel consisting essentially of the desired high-molecular-weight fucans; and

extracting the desired high-molecular-weight fucans from the selected portion of the electrophoresis gel.

[00030] The subjecting the starting fucan compositions to gel electrophoresis can comprise applying a potential difference across the electrophoresis gel can be between about 10 Volt/cm and 200 Volt/cm. The electrophoresis gel can comprise at least one of agarose, polyacrylamide, polydimethylacrylamide and starch. The electrophoresis gel further can comprise at least one of tris-acetate EDTA, tris-borate EDTA and phosphate buffered saline. Extracting the desired high-molecular-weight fucans from the selected portion of the electrophoresis gel can comprise agitating the selected portion of the electrophoresis gel in a solvent. The solvent can comprise at least one of water, methanol, ethanol and isopropanol.

[00031] Methods for obtaining a high-molecular-weight fucans can comprise:

providing a starting fucan compositions having a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment, and an ion exchange macroporous resin; and

subjecting the starting fucan compositions to ion exchange with the ion exchange macroporous resin to obtain an ion exchange treated fucan compositions consisting essentially of the desired high-molecular-weight fucans.

[00032] The methods further can comprise collecting the desired high-molecular-weight fucans from the ion exchange treated fucan compositions. Providing the starting fucan compositions further can comprise desalting the starting fucan compositions before subjecting the starting fucan compositions to ion exchange. A mass ratio of the starting fucan composition:ion exchange macroporous resin can be between about 1:100 and about 10:1. The mass ratio can be between about 1:10 and about 5:1. The starting fucan compositions can be subjected to ion exchange for a period of can be between about 5 minutes and about 100 hours. The ion exchange macroporous resin can comprise at least one of an anion exchange macroporous resin and a mixed charge macroporous resin. The anion exchange macroporous resin can be a strong base macroporous resin. The strong base macroporous resin can comprise quaternary amine groups. The anion exchange macroporous resin can be a weak base macroporous resin. The weak base macroporous resin can comprise at least one of primary, secondary or tertiary amine groups. The ion exchange macroporous resin can comprise at least one of styrene, agarose, dextran, acrylate, methacrylate, methyl methacrylate, butyl methacrylate, divinylbenzene, cellulose, silica, and ceramic. The ion exchange macroporous resin has a pore size of can be between about 5 nm and about 1000 nm, about 10 nm and about 100 nm, or about 15 nm and about 50 nm. The ion exchange macroporous resin can have an exclusion limit of can be between about 50 kDa and about 50,000 kDa, about 1,000 kDa and about 9,000 kDa, or about 100 kDa and about 1,000 kDa. The starting fucan compositions can be subjected to anion-exchange for a period of can be between about 5 minutes and about 100 hours or between about 1 hour and about 30 hours.

[00033] Methods for obtaining a high-molecular-weight fucans can comprise:

providing a starting fucan compositions with a broad molecular weight distribution comprising a desired high-molecular-weight fucans segment in a starting solution, and a gel media;

subjecting the starting solution to preparative gel permeation chromatography, wherein the starting fucan compositions can be displaced from a first input end to a second output end across the gel media according to molecular weight; and

collecting from the second output end at least one aliquot consisting essentially of the desired high-molecular-weight fucans segment.

[00034] The methods further can comprise collecting multiple aliquots and combining the aliquots. The gel media can be contained in a column. The gel media can comprise at least one of polyhydroxymethacrylate, sulfonated styrene-divinylbenzene, silica, a hydrophilic bonded phase or polymer, polystyrene, divinylbenzene, methacrylate, methyl methacrylate, butyl methacrylate, cellulose, ceramic, agarose and dextran. The gel media has a pore size of can be between about 3 nm and about 3000 nm, 3 nm and about 3000 nm, about 5 nm and about 10,000 nm, about 10 nm and about 100 nm, about 50 nm and about 500 nm, about 200 nm and about 2,000 nm, or about 500 nm and about 5,000 nm. The gel media has an exclusion limit of can be between about 100 Da and about 100,000 kDa, about 100 kDa and about 30,000 kDa, about 1,000 kDa and about 100,000 kDa, about 1,000 kDa and about 10,000 kDa, or about 5,000 kDa and about 50,000 kDa.

[00035] These and other aspects, features and embodiments are set forth within this application, including the following Detailed Description and attached drawings. Unless expressly stated otherwise, all embodiments, aspects, features, etc., can be mixed and matched, combined and permuted in any desired manner.

BRIEF DESCRIPTION OF THE DRAWINGS

[00036] **FIG. 1** schematically depicts an exemplary two-filter system for the segmentation of a starting fucan composition on the basis of molecular weight using sequential tangential flow filtration, the starting fucan having a broad molecular weight distribution.

[00037] **FIG. 2** schematically depicts an exemplary further embodiment of a two-filter system for the segmentation of a starting fucan composition on the basis of molecular weight using sequential tangential flow filtration, the starting fucan having a broad molecular weight distribution.

[00038] **FIG. 3** schematically depicts an exemplary system for obtaining a desired high-molecular-weight fucan from a starting fucan composition using cation-augmented tangential flow filtration, the starting fucan having a broad molecular weight distribution.

[00039] **FIG. 4** schematically depicts an exemplary system for centrifugally precipitating a high-molecular-weight fucan from a starting fucan composition using a multi-segment barrier of gradient material, the starting fucan having a broad molecular weight distribution.

[00040] **FIG. 5** schematically depicts an exemplary system for centrifugally precipitating a high-molecular-weight fucan from a starting fucan composition using a single segment barrier, the starting fucan having a broad molecular weight distribution.

[00041] **FIG. 6** schematically depicts an exemplary system for obtaining a high-molecular-weight fucan from a starting fucan composition by gel electrophoresis-extraction, the starting fucan having a broad molecular weight distribution.

[00042] **FIG. 7** schematically depicts an exemplary system for obtaining a high-molecular-weight fucan from a starting fucan composition by dialysis, the starting fucan having a broad molecular weight distribution.

[00043] **FIG. 8** schematically depicts an exemplary system for obtaining a desired high-molecular-weight fucan from a starting fucan composition using ion adsorption, the starting fucan having a broad molecular weight distribution.

[00044] **FIG 9A** depicts NMR results demonstrating that certain fucans treated according to methods herein undergo structural changes to the fucans.

[00045] **FIG. 9B** depicts 2-D NMR results demonstrating that certain fucans treated according to methods herein undergo chemical structural changes to the fucans.

[00046] **FIG. 10** shows an exemplary system for the centrifugal precipitation of a high-molecular-weight fucan from a starting fucan composition using a multi-segment sucrose barrier, the starting fucan having a broad molecular weight distribution.

[00047] The drawings present exemplary embodiments of the present disclosure. The drawings are not necessarily to scale and certain features may be exaggerated or otherwise represented in a manner to help illustrate and explain the present systems, methods, etc. Actual embodiments of the systems, methods, etc., herein may include further features or steps not shown in the drawings. The exemplifications set out herein illustrate embodiments of the systems, methods, etc., in one or more forms, and such exemplifications are not to be construed as limiting the scope of the disclosure in any manner. The embodiments herein are not exhaustive and do not limit the disclosure to the precise form disclosed, for example in the following detailed description.

DETAILED DESCRIPTION

[00048] The current compositions, systems, methods, etc., presented herein comprise high-molecular-weight fucans. The present compositions can be effective for medical treatments, post-surgical treatments, disease inhibition, etc. In some embodiments, the fucan is fucoidan. The present high-molecular-weight fucans can themselves be, or can be included on or in, medical devices, medical materials, combination products or in pharmaceutically acceptable, therapeutically and/or medically effective compositions.

[00049] The following paragraphs turn to a brief discussion of some of the methodologies that can be used to create the high-molecular-weight fucans and compositions herein from starting fucans and compositions via various methods that can be performed using any suitable reaction mixture such as solutions, suspensions, solids, gels or other modalities depending on the chosen method(s).

Compositions

[00050] The current compositions, systems, etc., presented herein provide, in certain embodiments, fucans and medically acceptable high-molecular-weight fucans and compositions comprising therapeutically effective amounts of high-molecular-weight fucans for the treatment of fibrous adhesions, such as surgical adhesions, arthritis, psoriasis or other diseases as desired.

[00051] The high-molecular-weight fucans presented herein may be used for a plurality of applications, including the inhibition, prevention, removal, reduction, or other treatment of fibrous adhesions and other targets and other diseases and/or conditions. Treatment includes that the high-molecular-weight fucans reduce or prevent the development of a target disease or other condition, such as reducing or preventing the formation of fibrous adhesions at a target site, formation of fibrous adhesions at a target site, which is typically a selected target site identified by a surgeon or other practitioner as comprising or reasonably susceptible to having fibrous adhesions (or other diseases or conditions), and also includes elimination of existing diseases or other conditions, including for example the elimination of already-existing fibrous adhesions. For such inhibition, prevention, removal, reduction, or other treatment, the high-molecular-weight fucan is typically provided in a medically acceptable medical device, combination product, or pharmaceutically effective composition that contains additional components such as binders, adjuvants, excipients, etc., as well as, if desired, additional medically active substances such as secondary drugs that are

contained within the composition but not attached to the fucan, and/or that can be attached to the fucan.

[00052] The molecular weight distribution of the high-molecular-weight fucans may be measured using any desired, appropriate measurement system. Different systems can yield different readings or results from different compositions having essentially the same make-up, or even from the same batch when measured differently. One suitable measurement system is an aqueous gel permeation chromatography set up consisting essentially of one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 50 kDa and about 5,000 kDa, one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 1 kDa and about 6,000 kDa and one 40 mm guard column with a 6 mm inner diameter packed with hydroxylated polymethacrylate-based gel, the two analytical gel permeation chromatography columns and the one guard column contained in a column compartment at about 30 °C, a refractive index detector at about 30 °C, 0.1M sodium nitrate mobile phase run at 0.6 mL/min, and quantification against a peak molecular weight standard curve consisting essentially of a first dextran standard with a peak molecular weight of about 2,200 kDa, a second dextran standard with a peak molecular weight of between about 720 kDa and about 760 kDa, a third dextran standard with a peak molecular weight between about 470 kDa and about 510 kDa, a fourth dextran standard with a peak molecular weight between about 370 kDa and about 410 kDa, a fifth dextran standard with a peak molecular weight between about 180 kDa and about 220 kDa, and a sixth dextran standard with a peak molecular weight between about 40 kDa and 55 kDa. The peak molecular weight standard curve may further comprise a dextran standard with a peak molecular weight between 3 kDa and 5 kDa.

[00053] The high-molecular-weight fucans herein can have a weight average molecular weight over 100kDa and comprise about 50% w/w or more of their molecular weight distribution above 100kDa. Such high-molecular-weight fucans show greater efficacy in the inhibition, prevention, removal, reduction, and/or other treatment of fibrous adhesions than fucans with weight average molecular weight below 100kDa and comprising less than about 50% of their molecular weight distribution above 100kDa at the same dose. High-molecular-weight fucans with weight average

molecular weight above 300 kDa, comprising about 70% or more of their molecular weight distribution above 100kDa show even greater efficacy in the inhibition, prevention, removal, reduction, and/or other treatment of fibrous adhesions at the same dose.

[00054] In some embodiments, high-molecular-weight fucans herein are configured for use in inhibition, prevention, removal, reduction, or other treatment of fibrous adhesions that result in greater than about 65%, 70%, 80%, 90%, 95%, or 99% efficacious prevention, inhibition or other treatment of post-surgical adhesions. Such high-molecular-weight fucans can also be configured for such treatment of other targets.

[00055] The high-molecular-weight fucans herein may comprise a molecular weight distribution in which more than about 60%, 70%, 75%, 80%, 90%, 95 or 99% w/w of the fucan has a molecular weight above 100kDa.

[00056] In other embodiments, the high-molecular-weight fucans herein may comprise a weight average molecular weight between about 100 kDa and 10,000 kDa, between about 140 kDa or 200 kDa and 9,000 kDa, between about 350 kDa or 370 kDa and 8,000 kDa, between about 450 kDa and 7,000 kDa, between about 580 kDa and 5,300 kDa or 6,000 kDa, between about 580 kDa or 590 kDa and 5,500 kDa, between about 400 kDa and 2,800 kDa or between about 800 kDa or 860 kDa and about 2,000 kDa for example about 850 kDa, about 930 kDa, about 1,100 kDa, about 1,200 kDa, about 1,300 kDa, about 1,600 kDa and about 1,800 kDa.

[00057] In yet other embodiments, the high-molecular-weight fucans herein may comprise a peak molecular weight between about 60 kDa or 70 kDa and 7,000 kDa, between about 100 kDa or 140 kDa and 6000 kDa, between about 200 kDa or 230 kDa and 5000 kDa, between about 250 kDa and 4000 kDa, between about 350 kDa and 3000 kDa, between about 500 kDa and 2000 kDa, or between about 400 kDa and about 1000 kDa, for example, about 450 kDa, 500 kDa, 550 kDa, 600 kDa, 650 kDa, 700 kDa and 750 kDa.

[00058] In yet other embodiments, the high-molecular-weight fucans herein may comprise a number average molecular weight between about 50 kDa and 3,000 kDa, between about 100 kDa and 2,000 kDa, between about 200 kDa and 1,500 kDa, between about 300 kDa and 2,000 kDa, between about 400 kDa and 1,000 kDa, or between about 250 kDa and about 600 kDa, for example, about 300 kDa, 350 kDa, 400 kDa, 450 kDa, 500 kDa and 550 kDa.

[00059] In yet other embodiments, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which more than about 55% w/w or 60% w/w of the fucan may have a molecular weight above 200 kDa, or more than about 70% w/w or 71% w/w of the fucan may have a molecular weight above 200 kDa. In yet other embodiments, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which more than 22% w/w or 30% w/w of the fucan may have a molecular weight above 500 kDa, or more than 50% w/w or 54% w/w of the fucan may have a molecular weight above 500 kDa.

[00060] In yet other embodiments, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which less than about 10% w/w of the fucan has a molecular weight below 50 kDa, or less than about 5% w/w of the fucan has a molecular weight below 50 kDa, or less than about 2% w/w of the fucan has a molecular weight below 50 kDa.

[00061] In yet other embodiments, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which less than about 5% w/w of the fucan has a molecular weight below 10 kDa, or less than about 2% w/w of the fucan has a molecular weight below 10 kDa.

[00062] In yet other embodiments, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which less than about 5% w/w of the fucan has a molecular weight below 5 kDa, or less than about 2% w/w of the fucan has a molecular weight below 5 kDa.

[00063] In yet another aspect, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which between 61% w/w and 80% w/w or 85% w/w of the fucan has a molecular weight between 200 kDa and 1600 kDa. More particularly, more than 70% w/w of the fucan may have a molecular weight above 200 kDa, and more than 30% of the fucan may have a molecular weight above 500 kDa.

[00064] In yet another aspect, the high-molecular-weight fucans herein may comprise a molecular weight distribution in which more than about 20% w/w, 40% w/w or 60% w/w of the fucan has a molecular weight above 1600 kDa. More particularly, more than about 70% w/w of the fucan may have a molecular weight above 200 kDa, or more than about 80% w/w of the fucan may have a molecular weight above 200 kDa.

[00065] The high-molecular-weight fucans herein may have a sulfation level of between about 14% w/w and 70% w/w, between about 20% w/w and 60% w/w, between about 30% w/w and 55% w/w, or between about 32% w/w or 35% w/w and 52% w/w.

[00066] The high-molecular-weight fucans herein may have a molar ratio of total fucose:total sulfate of between 1:0.5 and 1:4, between about 1:0.8 and 1:3.5, between about 1:1 and 1:2.5, between about 1:1.2 and 1:2.0, or between about 1:1.5 and 1:3.

[00067] The high-molecular-weight fucans herein may have a molar ratio of total fucose and galactose:total sulfate of between about 1:0.5 and 1:4, between about 1:0.8 and 1:3.5, between about 1:1 and 1:2.5, between about 1:1.2 and 1:2.0, or between about 1:1.5 and 1:3.

[00068] The high-molecular-weight fucans herein may have a total carbohydrate content of between 27% w/w and 80% w/w, between about 30% w/w and 70% w/w, between about 40% w/w and 90% w/w, or between about 50% w/w and 96% w/w.

[00069] The high-molecular-weight fucans herein may have a fucose content as a percentage of total carbohydrate of between about 30% w/w and 100% w/w, between about 40% w/w and 95% w/w, between about 50% w/w and 90% w/w, between about 80% w/w and 100% w/w, or between about 90% w/w and 100% w/w.

[00070] The high-molecular-weight fucans herein may have a galactose content as a percentage of total carbohydrate of between about 0% w/w and 60% w/w, between about 3% w/w and 30% w/w, between about 2% w/w and 20% w/w or between about 5% w/w and 10% w/w.

[00071] The high-molecular-weight fucans herein may have a glucuronic acid content as a percentage of total carbohydrate content between about 0% w/w and 10% w/w, a mannose content as a percentage of total carbohydrate content between about 0% w/w and 7% w/w, a rhamnose content as a percentage of total carbohydrate content between 0% w/w and 4% w/w, and a xylose content as a percentage of total carbohydrate content between 0% w/w and 20% w/w. The high-molecular-weight fucans herein may have a total of glucuronic acid, mannose, rhamnose, glucose and xylose content as a percentage of the total carbohydrate content below about 30% w/w or below about 12% w/w.

[00072] In some embodiments, the high-molecular-weight fucans herein, when dissolved at a concentration of about 50 mg/mL in water, have a viscosity of between about 4 cP and about 50 cP, between about 5 cP and about 40 cP, between about 10 cP and about 30 cP, about 15 cP, about 20 cP and about 25 cP. In certain embodiments, the high-molecular-weight fucans herein, when dissolved in water at 1 mg/mL through 100 mg/mL form a solution that is one of clear and colorless, clear and light yellow or clear and light brown.

[00073] In certain embodiments, the high-molecular-weight fucans herein can have an acetyl content of less than about 5% w/w, less than about 2% w/w, and about 0% w/w. In some embodiments, the high-molecular-weight fucans herein comprise substantially 0% w/w acetyl content when measured by 2D ^{1}H-^{13}C heteronuclear multiple quantum coherence at 70 °C with solvent signal suppression on a 600 MHz spectrometer equipped with 5-mm cold probe, in the range from 10-30 ppm in the carbon dimension, in 8 increments of 256-512 scans each.

Methods

[00074] Methods, systems, etc., are presented for obtaining high-molecular-weight fucans obtained from a starting fucan composition, such as a feedstock fucan composition, having a broad molecular weight distribution (a broad molecular weight distribution starting fucan) that encompasses and extends beyond the desired high-molecular-weight segment, the desired high-molecular-weight segment being a portion of the broad molecular weight distribution wherein a quantity of the broad molecular weight distribution at the low molecular weight end has been eliminated, suppressed or otherwise attenuated. At least one of these methods may be used in the preparation of high-molecular-weight fucans, for example, comprising more than about 60%, 70%, 80%, 90% or 95% w/w of their molecular weight distribution above 100kDa. In some embodiments, the current disclosure presents high-molecular-weight fucans that are suitable for medical and surgical applications, for example, the prevention of surgical adhesions.

Tangential flow filtration

[00075] Some of the methods discussed herein utilize tangential flow filtration (TFF). Consistent with typical identification of tangential flow filtration (TFF) filters, the nominal molecular weight cut-off (MWCO) value for a given TFF filter will selectively retain on its retentate side a solution containing molecules that did not cross the filter barrier and thus generally have molecular weights and/or sizes greater than the molecular weight of molecules that do cross/permeate the barrier to the permeate side. Thus, molecular weight cut-off values for TFF filters are typically not absolute for any given polymer or nominal cut-off value: a given TFF filter will pass or retain some molecules both above and below the nominal molecular weight cut-off. The actual cut-

off/selectively values and effects of a nominal TFF filter for a particular polymer can be routinely determined for the particular polymer.

[00076] A number of factors can affect the permeation behavior of the TFF filters. These factors may be dependent on the TFF filters themselves or dependent on an attribute of the target polymers, for example the folding behavior and folded structure of the target polymer can affect the behavior of the target polymer in crossing/not-crossing the TFF filter's MWCO barrier. Regarding the TFF filters themselves, as is known, a number of factors can affect the permeation behavior of the TFF filters. For example, manufacturing methods can cause a variety of hole sizes within the specific TFF filter, which variety can include holes both larger and smaller than the nominal MWCO. Thus, a TFF filter having a nominal molecular weight cut-off value will substantially pass/retain molecules at the nominal molecular weight cut-off value, but can also pass/retain some molecules below and/or above such value.

Gel permeation chromatography

[00077] Gel permeation chromatography was employed to evaluate the molecular weight distributions obtained for the experimental examples. There are a large number of different parameters, columns and standards available for use in gel permeation chromatography, resulting in a variety of instrumentation set-ups available for the analysis of molecular weight. For molecular weight determinations herein, the GPC were conducted using the following parameters: The mobile phase was 0.1M sodium nitrate run at 0.6 mL/min. The column compartment and detector were at 30 °C. A Waters 2414 refractive index detector was used for detection.

[00078] Suitable GPC columns include GPC columns compatible with aqueous solvents, for example columns packed with at least one of sulfonated styrene-divinylbenzene, NH-functionalized acrylate copolymer network, modified silica and hydroxylated polymethacrylate-based gel. For the analyses herein, three columns were used in series, comprising one 40 mm long guard column with an inner diameter (ID) of 6 mm packed with 6 μm particle size hydroxylated polymethacrylate-based gel, followed by a first 300 mm analytical GPC column with a 7.8 mm ID packed with 12 μm particle size hydroxylated polymethacrylate-based gel that has an exclusion limit of about 7,000 kDa and an effective molecular weight range of between about 50 kDa and about 5,000 kDa, followed by a second 300 mm analytical GPC column with a 7.8 mm ID packed

with 10 μm particle size hydroxylated polymethacrylate-based gel that has an exclusion limit of about 7,000 kDa and an effective molecular weight range of between about 1 kDa and about 6,000 kDa. The total effective molecular weight range of the column set up was between about 1 kDa and about 6,000 kDa. An example of this column set up can be Ultrahydrogel® guard-Ultrahydrogel® 2000-Ultrahydrogel® Linear columns connected in series.

[00079] Samples run were quantified against a standard curve comprising of traceable standards from the American Polymer Standards Corporation: DXT3755K (peak molecular weight=2164 kDa), DXT820K (peak molecular weight=745 kDa), DXT760K (peak molecular weight=621 kDa), DXT670K (peak molecular weight=401 kDa), DXT530K (peak molecular weight=490 kDa), DXT500K (peak molecular weight=390 kDa), DXT270K (peak molecular weight=196 kDa), DXT225K (peak molecular weight=213 kDa), DXT150K (peak molecular weight=124 kDa), DXT55K (peak molecular weight=50 kDa), DXT50K (peak molecular weight=44 kDa) and DXT5K (peak molecular weight=4 kDa), the peak molecular weights of these standards being between about 4 kDa and about 2,200 kDa. The standard curve used may, for example, include Dextran 3755 kDa, at least one of Dextran 50 kDa and Dextran 55 kDa, and between 3 to 6 additional traceable standards discussed herein, the calibration points being the peak molecular weights of the calibrants used. An example calibration curve may consist of DXT3755K, DXT 820K, DXT530K, DXT500K, DXT225K and DXT55K. The columns used herein had a total effective molecular weight range that encompassed and extended beyond the peak molecular weight range of the standards used for quantification of the fucans.

[00080] A molecular weight stated for a fucan/fucoidan polymer herein is a value of molecular weight about which there will always be a distribution of molecules of higher and lower molecular weights, increasing or decreasing in amount or percentage as the molecular weight increases or decreases away from the specified molecular weight. The distribution may, but is not required to, have a generally Gaussian or distorted Gaussian shape.

[00081] Results in the tables herein contain abbreviations used for certain characteristics of a molecular weight distribution. Gel permeation chromatography is denoted by GPC, peak retention time is denoted by PRT, peak molecular weight is denoted by PMW, weight average molecular weight is denoted by WAMW, number average molecular weight is denoted by NAMW,

percentage distribution is denoted by % dist., molecular weight is denoted by MW, polydispersity index is denoted by PDI and molecular weight cutoff is denoted by MWCO.

[00082] The following paragraphs turn to a brief general discussion of some methodologies that can be used to create the high-molecular-weight fucans herein.

Sequential tangential flow filtration segmentation

[00083] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan composition by a sequential TFF segmentation method. The methods can comprise: providing a starting fucan composition comprising the desired molecular weight segment, for example a high-molecular-weight segment, the starting fucan composition having a starting broad molecular weight distribution; subjecting the starting fucan composition to tangential flow filtration across a first, higher MWCO tangential flow filtration filter having an average molecular weight cutoff within the starting molecular weight distribution; collecting from the first TFF filter a first permeate fucan composition comprising a reduced proportion of high-molecular-weight fucans compared with the starting fucan composition; subjecting the first permeate fucan composition to tangential flow filtration across a second, lower MWCO tangential flow filtration filter having a lower average molecular weight cutoff within the starting molecular weight distribution than the first TFF filter; and, collecting from the second TFF filter a fucan with the desired molecular weight segment in the retentate fucan composition.

[00084] The methods can comprise further steps as desired, for example pre-filtering the starting fucan composition through a pre-filter capable of filtering out particulates or moieties greater than a desired size, or other unwanted materials. Passing the starting fucan composition over the first TFF filter may comprise passing the starting fucan composition over the TFF filter while applying pressure to the starting fucan composition. Passing the permeate fucan composition of the first TFF filter over the second TFF filter may comprise passing the permeate fucan composition of the first TFF filter over the second TFF filter while applying pressure to the permeate fucan composition of the first TFF filter.

[00085] Passing the starting fucan composition over the first TFF filter may comprise recirculating the retentate fucan composition of the first TFF filter over the first TFF filter. Recirculating the retentate fucan composition of the first TFF filter over the first TFF filter may comprise diafiltering

the retentate fucan composition over the first TFF filter. Recirculating the retentate fucan composition of the first TFF filter over the first TFF filter may comprise determining a weight average molecular weight of the permeate fucan composition of the first TFF filter. Recirculating the retentate fucan composition of the first TFF filter over the first TFF filter may comprise recirculating the retentate fucan composition of the first TFF filter over the first TFF filter until the weight average molecular weight of fucan in the permeate fucan composition of the first TFF filter has a predetermined desired value.

[00086] Passing a permeate fucan composition from the first TFF filter over the second TFF filter may comprise recirculating the permeate fucan composition over the second TFF filter. Recirculating the permeate fucan composition over the second TFF filter may comprise diafiltering the permeate fucan composition over the second TFF filter. Recirculating the permeate fucan composition over the second TFF filter may comprise determining a weight average molecular weight of a retentate fucan composition of the second TFF filter. Recirculating the permeate fucan composition over the second TFF filter may comprise recirculating the fucan over the second TFF filter until the weight average molecular weight of the retentate fucan composition of the second TFF filter has a predetermined desired value.

[00087] **FIG. 1** shows schematically an exemplary molecular weight-based segmentation system (higher-to-lower) **100** comprising two different, higher and lower, molecular weight cut-off (MWCO) TFF filters, which in the embodiment shown are provided as higher molecular weight cut-off TFF filter **110** and lower molecular weight cut-off TFF filter **120**; the TFF filters can be provided in any acceptable format, the current examples use cassettes. Higher molecular weight cut-off TFF filter **110** has a MWCO that is greater than the MWCO of lower molecular weight cut-off TFF filter **120**. By way of example, higher molecular weight cut-off TFF filter **110** may have a MWCO of 30 kiloDalton (kDa), 50 kDa, 70 kDa, 100 kDa, 300 kDa and 1000 kDa, while the MWCO of lower molecular weight cut-off TFF filter **120** may be, for example, 5 kDa, 10 kDa, 30 kDa, 50 kDa and 100 kDa. By way of example, selecting a combination of a higher molecular weight cut-off TFF filter and a lower molecular weight cut-off TFF filter, molecular weight based segmentation system (higher-to-lower) **100** can be used to obtain a molecular weight segment between molecular weight cut-off TFF filters of 5-30 kDa, 10-30 kDa, 5-50 kDa, 10-50 kDa, 30-50 kDa, 10-70 kDa, 30-70 kDa, 50-70 kDa, 5-100 kDa, 10-100 kDa, 30-100 kDa, 50-100 kDa, 70-

100 kDa, 5-300 kDa, 10-300 kDa, 30-300 kDa, 50-300 kDa, 70-300 kDa and 100-300 kDa. In some embodiments, the molecular weight segment can be a high-molecular-weight segment.

[00088] A starting fucan composition is supplied as a solution via input supply line **102** to higher MWCO subsystem fucan container **116**. The starting fucan may be present in a suitable solvent at a concentration between 0.1% w/v and 30% w/v, such as between 1% w/v and 10% w/v, for example, at 5% w/v. The starting fucan in a suitable solvent may be pre-filtered through pre-filter **104** to remove undesired particulate matter. The solution containing the starting fucan composition may comprise further non-fucan components such as desired buffers, diluents, etc., as desired, for example for other fucan processing steps or downstream uses of the fucan. The gauge (effective hole size) of the pre-filter will typically be greater than the largest polymer molecules to be isolated by means of the molecular weight based segmentation system (higher-to-lower) **100**.

[00089] Higher MWCO subsystem pump **114** pumps a solution containing the starting fucan composition to higher molecular weight cut-off TFF filter **110** of higher MWCO TFF subsystem **130** via higher MWCO TFF filter supply line **112**. Higher molecular weight cut-off TFF filter **110** is typically supplied as a cassette designed to allow an input fluid to pass over its filter on its retentate side. The format of the molecular weight cutoff filter may be without limitation a plate and frame system; a spiral wound cartridge system; a hollow fiber system; a flow cell system; and centrifugal filter system. The permeate exits via higher MWCO subsystem permeate output line **119** and the treated input fluid, i.e., retentate fluid, leaves as retentate via higher MWCO subsystem retentate return line **118**. Higher MWCO subsystem pump **114** provides a level of pressure over higher molecular weight cut-off TFF filter **110** between its retentate and permeate sides. In **FIG. 1**, the retentate fluid from higher molecular weight cut-off TFF filter **110** is returned to higher MWCO subsystem fucan container **116** via higher MWCO subsystem retentate return line **118**, while permeate fluid is produced via higher MWCO subsystem permeate output line **119** for use outside of the higher MWCO TFF subsystem **130**. While higher MWCO subsystem pump **114** recirculates the prefiltered fucan and retentate over higher molecular weight cut-off TFF filter **110**, solvent may be supplied from higher MWCO subsystem solvent container **117** via higher MWCO subsystem solvent supply line **115**, for example to replenish solvent lost via the permeate and/or to ensure that a predetermined number of diavolumes of input starting fucan and solvent are circulated over the higher molecular weight cut-off TFF filter **110**.

[00090] Higher-to-lower MWCO inter-subsystem valve **113** may be shut off (closed) during the above processing, and permeate fluid from higher molecular weight cut-off TFF filter **110** of higher MWCO TFF subsystem **130** can be collected into a container (not shown) for storage or other use before being supplied to lower MWCO subsystem fucan container **126** of lower MWCO TFF subsystem **140**. The starting fucan composition can be cycled as many times as desired through higher MWCO TFF subsystem **130.**

[00091] The collected permeate from higher MWCO TFF subsystem **130** may then be supplied to lower MWCO subsystem fucan container **126** of lower MWCO TFF subsystem **140** via a higher MWCO subsystem permeate output line **119**. In other embodiments, the collected permeate may be transferred in a container (not shown) to lower MWCO subsystem fucan container **126**. In yet other embodiments of the system, the higher-to-lower MWCO inter-subsystem valve **113** may be maintained open and the permeate of higher molecular weight cut-off TFF filter **110** may be supplied via higher MWCO subsystem permeate output line **119** on a continuous basis to lower MWCO subsystem fucan container **126**. The distribution of higher molecular weight molecules in the permeate of higher molecular weight cut-off TFF filter **110** is attenuated or suppressed compared with the distribution of higher molecular weight molecules in the starting fucan composition.

[00092] The permeate supplied to lower MWCO TFF subsystem **140** is filtered in a similar way over lower molecular weight cut-off TFF filter **120** as discussed above for higher molecular weight cut-off TFF filter **110**. That is, after the permeate from higher MWCO TFF subsystem **130** is supplied to lower MWCO subsystem fucan container **126,** lower MWCO subsystem pump **124** pumps it to lower molecular weight cut-off TFF filter **120** of lower MWCO TFF subsystem **140** via lower MWCO TFF filter supply line **122**. Lower MWCO subsystem pump **124** maintains a level of pressure over lower molecular weight cut-off TFF filter **120** between its retentate and permeate sides. In **FIG. 1**, the retentate of lower molecular weight cut-off TFF filter **120** is returned to lower MWCO subsystem fucan container **126** via lower MWCO subsystem retentate return line **128**, while a permeate is produced via lower MWCO subsystem permeate output line **129** for further use or discarding outside lower MWCO TFF subsystem **140**. If the lower MWCO subsystem pump **124** recirculates the permeate from higher molecular weight cut-off TFF filter **110** and retentate from lower molecular weight cut-off TFF filter **120** to pass again over lower

molecular weight cut-off TFF filter **120** (as with the higher molecular weight cut-off filtration filter, this recirculation can be repeated as often as desired), solvent may be supplied from lower MWCO subsystem solvent container **127** via lower MWCO subsystem solvent supply line **125** and lower MWCO subsystem fucan container **126** to replenish solvent lost via the lower MWCO subsystem permeate output line **129** and/or to ensure that a predetermined number of diavolumes of retentate of lower molecular weight cut-off TFF filter **120** and solvent are circulated over the lower molecular weight cut-off TFF filter **120**.

[00093] During the tangential flow filtration operation of lower MWCO TFF subsystem **140**, lower MWCO subsystem retentate-line valve **106** may be closed. When the permeate supplied to lower MWCO TFF subsystem **140** from higher MWCO TFF subsystem **130** has been filtered to a desired degree, lower MWCO subsystem retentate-line valve **106** is opened and the retentate of lower molecular weight cut-off TFF filter **120** is supplied via lower MWCO subsystem retentate output line **108**. This provides a fucan with the desired molecular weight segment from a starting fucan composition, for example a high-molecular-weight fucan.

[00094] The output fucan has a desired molecular weight segment with a molecular weight distribution typically predominantly between the average molecular weight cut-off of the higher molecular weight cut-off TFF filter **110** and the average molecular weight cut-off of the lower molecular weight cut-off TFF filter **120**. However, considering the width and complexity of the starting fucan molecular weight distribution and the variability of polymer behavior and TFF filters, the output polymer molecular weight distribution may not peak between the average molecular weight cut-off values of the two TFF filters. For example, excessively high or low folding of the fucan can result in selection of appropriately sized but unusually dense (or not) fucans in the desired molecular weight segment. Thus, in terms of the fucans present after the sequential TFF discussed herein, the output desired molecular weight segment consists essentially of a desired molecular weight segment derived from the original starting fucan composition that was supplied to molecular weight based isolation system (higher-to-lower) **100**.

[00095] Further embodiments can comprise: providing a starting fucan composition comprising the desired molecular weight segment, for example a high-molecular-weight segment, the starting fucan composition having a starting molecular weight distribution; subjecting the starting fucan composition to tangential flow filtration across a first tangential flow filtration filter having an

average molecular weight cutoff within the starting molecular weight distribution; collecting from the first TFF filter a first retentate fucan composition comprising a reduced proportion of low molecular weight fucans compared with the starting fucan composition; subjecting the first retentate fucan composition to tangential flow filtration across a second tangential flow filtration filter having a higher average molecular weight cutoff within the starting molecular weight distribution than the first TFF filter; and collecting from the second TFF filter a fucan with the desired molecular weight segment in the permeate fucan composition.

[00096] The methods may further comprise pre-filtering the starting fucan composition through a pre-filter capable of filtering out moieties greater than a desired size. Passing the starting fucan composition over the first TFF filter may comprise passing the starting fucan composition over the first TFF filter while applying pressure to the starting fucan composition. Passing the retentate fucan composition of the first MCWO filter over the second TFF filter may comprise passing the retentate fucan composition of the first TFF filter over the second TFF filter while applying pressure to the retentate fucan composition of the first TFF filter in the second TFF filter.

[00097] Passing the starting fucan composition over the first TFF filter may comprise recirculating the retentate fucan composition of the first TFF filter over the first TFF filter. Recirculating the retentate fucan composition of the first TFF filter over the first TFF filter may comprise diafiltering the retentate fucan composition over the first TFF filter. Recirculating the retentate fucan composition of the first TFF filter over the first TFF filter may comprise determining a weight average molecular weight of the retentate fucan composition of the first TFF filter. Recirculating the retentate fucan composition of the first TFF filter over the first TFF filter may comprise recirculating the retentate fucan composition of the first TFF filter over the first TFF filter until the weight average molecular weight of fucan in the retentate fucan composition of the first TFF filter has a predetermined desired value.

[00098] Passing a retentate fucan composition from the first TFF filter over the second TFF filter may comprise recirculating the retentate fucan composition over the second TFF filter. Recirculating the retentate fucan composition over the second TFF filter may comprise diafiltering the retentate fucan composition over the second TFF filter. Recirculating the retentate fucan composition over the second TFF filter may comprise determining a weight average molecular weight of a permeate fucan composition of the second TFF filter. Recirculating the retentate fucan

composition over the second TFF filter may comprise recirculating the retentate fucan composition over the second TFF filter until the weight average molecular weight of the permeate fucan composition of the second TFF filter has a predetermined desired value.

[00099] **FIG. 2** shows a further embodiment of the methods, systems, etc., herein. In **FIG. 2**, subsystems **130** and **140** of **FIG. 1** are reversed in terms of process order to form molecular weight-based segmentation system (lower-to-higher) **100'**. As in the method discussed in **FIG. 1**, the starting fucan enters the system through input supply line**102** and is pre-filtered by pre-filter **104**. However, in contrast to the method above in **FIG. 1**, the pre-filtered starting fucan is processed first in lower MWCO TFF subsystem **140** then in higher MWCO TFF subsystem **130**. In lower MWCO TFF subsystem **140** the starting fucan composition is passed over lower molecular weight cut-off TFF filter **120**, which is the TFF filter with the lower average MWCO value. In this embodiment, it is the retentate and not the permeate of lower molecular weight cut-off TFF filter **120** that exits lower MWCO TFF subsystem **140** on lower MWCO subsystem retentate output line **121**. Such retentate exits through lower-to-higher MWCO inter-subsystem valve **123** to be supplied to higher MWCO subsystem fucan container **116** of higher MWCO TFF subsystem **130**. The retentate is then pumped by higher MWCO subsystem pump **114** via higher MWCO TFF filter supply line **112** to pass over higher molecular weight cut-off TFF filter **110**, which is the TFF filter with the higher MWCO.

[000100] Within lower MWCO TFF subsystem **140**, lower MWCO subsystem pump **124** pumps the permeate from lower MWCO subsystem fucan container **126** to lower molecular weight cut-off TFF filter **120** via lower MWCO TFF filter supply line **122**. In **FIG. 2**, the retentate of lower molecular weight cut-off TFF filter **120** is returned to lower MWCO subsystem fucan container **126** via lower MWCO subsystem retentate return line **128**, while a permeate is produced via lower MWCO subsystem permeate output line **129** for further use or discarding outside lower MWCO TFF subsystem **140**. If the retentate is recirculated to pass again over lower molecular weight cut-off TFF filter **120**, solvent may be supplied from lower MWCO subsystem solvent container **127** via lower MWCO subsystem solvent supply line **125** and lower MWCO subsystem fucan container **126** to replenish solvent lost via the lower MWCO subsystem permeate output line **129** and/or to ensure that a predetermined number of diavolumes of retentate of lower molecular weight cut-off TFF filter **120** and solvent are circulated over the lower molecular weight cut-off TFF filter 120.

[000101] Lower-to-higher MWCO inter-subsystem valve **123** may be shut during the above processing, and the retentate of lower molecular weight cut-off TFF filter **120** of lower MWCO TFF subsystem **140** can be collected into a container (not shown) before being supplied to higher MWCO subsystem fucan container **116** of higher MWCO TFF subsystem **130**. The collected retentate is supplied to higher MWCO subsystem fucan container **116** of higher MWCO TFF subsystem **130** via a physical lower MWCO subsystem retentate output line **121**. In other embodiments, the collected retentate may be transferred in a container (not shown) to higher MWCO subsystem fucan container **116**. In yet other embodiments, the lower-to-higher MWCO inter-subsystem valve **123** may be maintained open and the retentate of lower molecular weight cut-off TFF filter **120** supplied via lower MWCO subsystem retentate output line **121** on a continuous basis to higher MWCO subsystem fucan container **116**. The distribution of lower molecular weight molecules in the retentate from lower molecular weight cut-off TFF filter **120** is attenuated or suppressed compared with the distribution of lower molecular weight molecules in the starting fucan.

[000102] As higher MWCO TFF subsystem **130** processes the retentate from lower molecular weight cut-off TFF filter **120** of lower MWCO TFF subsystem **140**, the permeate of higher molecular weight cut-off TFF filter **110** is produced on higher MWCO subsystem permeate output line **119**. While higher MWCO subsystem pump **114** recirculates the retentate fucan of lower MWCO TFF subsystem **140** over higher molecular weight cut-off TFF filter **110**, solvent may be supplied from higher MWCO subsystem solvent container **117** via higher MWCO subsystem solvent supply line **115** to replenish solvent lost via the permeate and/or to ensure that a predetermined number of diavolumes of retentate fucan of lower MWCO TFF subsystem **140** and solvent are circulated over the higher molecular weight cut-off TFF filter **110**.

[000103] In **FIG. 2**, the retentate fluid from higher molecular weight cut-off TFF filter **110** is returned to higher MWCO subsystem fucan container **116** via higher MWCO subsystem retentate return line **118**, while permeate fluid is produced via higher MWCO subsystem permeate output line **119** for use outside of the higher MWCO TFF subsystem 130. In **FIG. 2**, the output fucan with the desired molecular weight segment produced through higher MWCO subsystem permeate output line **119** has a molecular weight distribution predominantly between the average molecular weight cut-off of the first higher molecular weight cut-off TFF filter **110** and the average molecular

weight cut-off of the second lower molecular weight cut-off TFF filter **120**. However, considering the width and complexity of the starting fucan molecular weight distribution and the variability of polymer behavior and TFF filters, the output polymer molecular weight distribution may not peak between the average molecular weight cut-off values of the two TFF filters. For example, excessively high or low folding of the fucan can result in selection of appropriately sized but unusually dense (or not) fucans in the desired molecular weight segment. Thus, in terms of the fucans present after the sequential TFF discussed herein, the output fucan consists essentially of a desired molecular weight segment of fucan derived from the original starting fucan composition that was supplied to molecular weight based segmentation system (lower-to-higher) **100'**. This output fucan with a desired molecular weight segment can also be derived from the pre-filtered starting fucan composition created after prefiltering by pre-filter **104** and then supplied to lower MWCO TFF subsystem **140**.

Cation augmented tangential flow filtration

[000104] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan by cation augmented TFF, the methods comprising: providing the starting fucan composition having low atomic weight cations and a molecular weight distribution comprising a desired high-molecular-weight segment; cation treating the starting fucan composition with a cationic additive having cations of greater molecular weight than the low atomic weight cations to replace the low atomic weight cations with additive cations; subjecting the cation-treated fucan composition to tangential flow filtration across a first tangential flow filtration filter having an average molecular weight cutoff based on a molecular weight distribution of the desired high-molecular-weight fucan segment to generate a first retentate fucan composition; subjecting the first retentate fucan composition to tangential flow filtration across a second lower MWCO tangential flow filtration filter having an average molecular weight cutoff based on a molecular weight distribution of the cationic additive to generate a second retentate fucan composition; subjecting the second retentate fucan composition to diafiltration with a salt solution to generate a third retentate fucan composition; subjecting the third fucan retentate composition to diafiltration across the same second tangential flow filtration filter with a low

conductivity diafiltration solution to produce a fourth retentate fucan composition; and collecting the fourth retentate solution comprising the desired high-molecular-weight fucan.

[000105] The methods can comprise further steps as desired, for example pre-filtering the starting fucan composition through a pre-filter capable of filtering out particulates or moieties greater than a desired size, or other unwanted materials. Passing the starting fucan composition over the first TFF filter may comprise passing the starting fucan composition over the TFF filter while applying pressure to the starting fucan composition. Passing the retentate fucan composition of the first TFF filter over the second TFF filter may comprise passing the retentate fucan composition of the first TFF filter over the second TFF filter while applying pressure to the retentate fucan composition of the first TFF filter.

[000106] Subjecting the first retentate fucan composition to tangential flow filtration across the second tangential flow filtration filter and treating the second retentate fucan composition with a salt solution may be done simultaneously. Treating the second retentate fucan composition with a salt may comprise treating the second retentate fucan composition with a chloride, bromide, iodide, fluoride, sulfate, sulfite, carbonate, bicarbonate, phosphate, nitrate, nitrite, acetate, citrate, silicate and/or cyanide of an alkali metal, alkaline earth metal, aluminum and/or ammonium. Treating the first retentate fucan composition with a sodium salt may comprise treating the first retentate with sodium chloride.

[000107] Cation treating the starting fucan composition with a cationic additive may comprise treating the starting fucan with a cationic additive having cations of greater molecular weight than the low atomic weight cations within the starting fucan. The cationic additive may be a polycationic additive. Cation treating the starting fucan composition with a cationic additive may comprise treating the starting fucan with a zwitterionic additive having zwitterions of greater molecular weight than the low atomic weight cations within the starting fucan.

[000108] Subjecting the cation-treated fucan composition to tangential flow filtration across a first tangential flow filtration filter may comprise recirculating the cation-treated fucan composition over the first TFF filter. Recirculating the cation-treated fucan composition over the first TFF filter may comprise diafiltering the cation-treated fucan composition over the first TFF filter with a solution of the cationic additive. Recirculating the cation-treated fucan composition over the first TFF filter may comprise determining a weight average molecular weight of fucan in the cation-

treated fucan composition. Recirculating the cation-treated fucan composition over the first TFF filter may comprise recirculating the cation-treated fucan composition over the first TFF filter until the weight average molecular weight of cation-treated fucan in the cation-treated fucan composition has a predetermined desired value, producing the first retentate fucan composition.

[000109] Subjecting the first retentate fucan composition to tangential flow filtration across a second lower MWCO tangential flow filtration filter may comprise recirculating the first retentate fucan composition over the second TFF filter. Recirculating the first retentate fucan composition over the second TFF filter may comprise diafiltering the first retentate fucan composition of the second TFF filter with a salt solution. Recirculating the first retentate fucan composition over the second TFF filter may comprise determining a weight average molecular weight of fucan in the first retentate fucan composition. Recirculating the first retentate fucan composition over the second TFF filter may comprise recirculating the first retentate fucan composition over the second TFF filter until the weight average molecular weight of fucan from the first retentate fucan composition has a predetermined desired value, producing the second retentate fucan composition.

[000110] Subjecting the second retentate fucan composition to diafiltration with a salt solution may comprise recirculating the second retentate fucan composition over the second TFF filter. Recirculating the second retentate fucan composition over the second TFF filter may comprise diafiltering the second retentate fucan composition of the first TFF filter with a salt solution comprising at least one of a chloride, bromide, iodide, fluoride, sulfate, sulfite, carbonate, bicarbonate, phosphate and nitrate of an alkali metal, alkaline earth metal, aluminum and ammonium, for example sodium chloride. Subjecting the third retentate fucan composition to tangential flow filtration across the second MWCO tangential flow filtration filter may comprise recirculating the third retentate fucan composition over the second TFF filter. Recirculating the third retentate fucan composition over the second TFF filter may comprise diafiltering the third retentate fucan composition of the second TFF filter with a low conductivity solution. The low conductivity solution may be deionized water.

[000111] Cation treating the starting fucan composition with a cationic additive may comprise treating the input fucan with at least one of choline, polyvinylpyrrolidone, taurine, polyamine, chitosan, histone, and collagen.

[000112] **FIG. 3** shows a schematic diagram of a cation-augmented TFF system (CATS) **100″** for the separation of a fucan on the basis of molecular weight. CATS **100″** employs a number of elements already discussed at the hand of **FIG. 1** and **FIG. 2**. A solution containing the starting fucan composition is supplied via input supply line **102** to higher MWCO subsystem fucan container **116**. The starting fucan composition in a suitable solvent may be pre-filtered through pre-filter **104** to remove undesired particulate matter. The solution containing the starting fucan composition may comprise further non-fucan components such as desired buffers, diluents, etc., as desired, for example for other fucan processing steps or downstream uses of the fucan. The gauge of the pre-filter will typically be greater than the largest polymer molecules to be separated by means of the CATS **100″**.

[000113] Cationic additive, for example choline, polyvinylpyrrolidone, polyaniline, may be added to the pre-filtered starting fucan composition in higher MWCO subsystem fucan container **116**. Higher MWCO subsystem pump **114** pumps fucan to higher MWCO TFF filter **150** of higher MWCO TFF subsystem **130′** via higher MWCO TFF filter supply line **112**. Higher MWCO TFF filter **150** is typically supplied as a cassette designed to allow an input fluid supplied to it to pass over its filter on its retentate side, while allowing a permeate to exit via one output line and treated input fluid to leave as retentate via another output line. The format of the molecular weight cutoff filter may be without limitation a plate and frame system; a spiral wound cartridge system; a hollow fiber system; a flow cell system; and centrifugal filter system. For this embodiment, the cut off molecular weight of higher MWCO TFF filter **150** is chosen to separate a desired portion of the high-molecular-weight end of the cation-treated fucan obtained by treating the pre-filtered starting fucan with the cationic additive.

[000114] Higher MWCO subsystem pump **114** provides a level of pressure over higher MWCO TFF filter **150** between its retentate and permeate sides. In **FIG. 3**, the retentate of higher MWCO TFF filter **150** is returned to higher MWCO subsystem fucan container **116** via higher MWCO subsystem retentate return line **118**, while permeate is produced via higher MWCO subsystem permeate output line **119** for use outside higher MWCO TFF subsystem **130′** or to be discarded. While higher MWCO subsystem pump **114** recirculates the prefiltered starting fucan composition and retentate over higher MWCO TFF filter **150**, cationic additive flush solution from cationic additive flush solution container **137** may be supplied via cationic additive flush solution supply

line **135**, for example to replenish solution lost via the permeate on higher MWCO subsystem permeate output line **119** and/or to ensure that a predetermined number of diavolumes of input starting fucan and cationic additive flush solution are circulated over the higher MWCO TFF filter **150**. By controlling cationic additive flush solution valve **136**, the cationic additive flush solution may be added in a pulse process. In other embodiments, the cationic additive flush solution may be added in a continuous mode. In other embodiments, the cationic additive flush solution may be added all at once. If choline has been chosen as cationic additive for the input starting fucan, then the cationic additive flush solution employed is a choline solution, for example a choline chloride solution. The number of diavolumes of retentate and choline flush solution to process over higher MWCO TFF filter **150** may be predetermined, four diavolumes being a generally useful number.

[000115] Higher-to-lower MWCO inter-subsystem valve **113** may be shut (closed) during the above processing, and retentate of higher MWCO TFF filter **150** of higher MWCO TFF subsystem **130′** collected into a container (not shown) before being supplied to lower MWCO subsystem fucan container **126** of lower MWCO TFF subsystem **140′**. The collected retentate may then be supplied to lower MWCO subsystem fucan container **116** of lower MWCO TFF subsystem **140′** via higher MWCO subsystem retentate output line **111**. In other embodiments, the collected retentate may be transferred in a container (not shown) to lower MWCO subsystem fucan container **126**. In yet other embodiments of the system, the higher-to-lower MWCO inter-subsystem valve **113** may be maintained open and the retentate of higher MWCO TFF filter **150** may be supplied via higher MWCO subsystem retentate output line **111** on a continuous basis to lower MWCO subsystem fucan container **126**. The distribution of lower molecular weight molecules in the retentate of higher MWCO TFF filter **150** is attenuated or suppressed compared with the distribution of lower molecular weight molecules in the starting fucan composition.

[000116] The lower MWCO TFF subsystem **140′** removes the choline cations from the cation-treated fucan and restores sodium cations to the fucan, thereby returning the cation-treated fucan to about its original ionic components, but with a different desired high-molecular-weight distribution. During the processing of fucan solutions by lower MWCO TFF subsystem **140′,** lower MWCO subsystem output valve **106′** controlling the lower MWCO subsystem retentate output line **108** from lower MWCO subsystem fucan container **126** may be closed. As lower MWCO TFF subsystem **140′** processes the retentate from higher MWCO TFF filter **150** of higher MWCO TFF

subsystem **130′**, the permeate of lower MWCO TFF filter **160** is produced on lower MWCO subsystem permeate output line **129** via which is employed elsewhere or is discarded.

[000117] While lower MWCO subsystem pump **114** recirculates the retentate of lower MWCO TFF subsystem **140′** over lower MWCO TFF filter **160**, a sodium salt solution, for example 2M NaCl solution, may be supplied from sodium salt solution container **142** via sodium salt solution supply line **146** by appropriate control of sodium salt solution control valve **144**. For this method, the cut off molecular weight of lower MWCO TFF filter **160** is chosen to separate cationic additive released from the fucan by the sodium salt treatment. As the process of lower MWCO TFF subsystem **140′** proceeds, the free choline chloride resulting from the replacement of the choline cations on the fucan with sodium cations from the NaCl transfers to the permeate of lower MWCO TFF filter **160** and leaves CATS **100″** via lower MWCO subsystem permeate output line **129**. The sodium salt solution may be used, for example to replenish solution lost via the permeate on lower MWCO subsystem permeate output line **129** and/or to ensure that a predetermined number of diavolumes of sodium salt solution and retentate from higher MWCO TFF subsystem **130′** are circulated over the lower MWCO TFF filter **160**. By controlling sodium salt solution control valve **144**, the sodium salt solution may be added in a pulse process. In other embodiments, the sodium salt solution may be added in a continuous mode. When a suitable number of diavolumes of sodium salt solution and retentate have been circulated over lower MWCO TFF filter **160**, sodium salt solution control valve **144** may be closed and low conductivity diafiltration solution valve **145** opened. The number of diavolumes of sodium salt solution to process over lower MWCO TFF filter **160** may be predetermined. Lower-MWCO-subsystem pump **124** provides a level of pressure over lower-MWCO TFF filter **160** between its retentate and permeate sides. In **FIG. 3**, the retentate of lower MWCO TFF filter **160** is returned to lower MWCO subsystem fucan container **126** via lower MWCO subsystem retentate return line **128**, while permeate is produced via lower MWCO subsystem permeate output line **129** for use outside lower MWCO TFF subsystem **140'** or to be discarded.

[000118] Low conductivity diafiltration solution valve **145** may be opened to allow low conductivity diafiltration solution from low conductivity diafiltration solution container **143** to enter lower MWCO subsystem fucan container **126** via low conductivity diafiltration solution supply line **147**, the retentate and low conductivity diafiltration solution may be processed over

lower MWCO TFF filter **160** to remove the free sodium salt generated during the sodium salt treatment of the retentate of lower MWCO TFF filter **160**. The low conductivity diafiltration solution may be, for example, deionized water. To this end, the conductivity of permeate on lower MWCO subsystem permeate output line **129** may be measured to ensure it drops to a desired level, this serving as indication that the sodium salt has been removed to a suitable degree. The number of diavolumes of low conductivity diafiltration solution to process over lower MWCO TFF filter **160** may be predetermined. When the sodium salt has been suitably removed from the retentate of lower MWCO TFF filter **160**, low conductivity diafiltration solution valve **145** maybe shut and lower MWCO subsystem retentate output line **108** opened to deliver the product of CATS **100''** on lower MWCO subsystem retentate output line **108**.

Centrifugal precipitation

[000119] A high-molecular-weight fucan may be obtained from a broad distribution starting fucan by centrifugal precipitation.

[000120] Turning to **FIG. 4**, a centrifugal precipitation system **600** for centrifugal precipitating a high-molecular-weight fucan from a starting fucan composition is shown. The system **600** comprises a centrifuge container **610** comprising a gradated permeable barrier **620**. The permeable barrier may be gradated on the basis of density, with density decreasing from a first-bottom end **630** toward a second-top end **640** of the centrifuge container **610**. The gradated permeable barrier **620** may be comprised of different materials of different densities. The gradated permeable barrier **620** may be comprised of solutions of different concentrations of one solute dissolved in a suitable solvent. Suitable solvents may be, for example without limitation, one of water and a water-alcohol solution. The solute, also known as "gradient material" may be for example without limitation one or more of glycerol, sorbitol, CsCl, Cs_2SO_4, KBr, diatrizoate, Nycodenz®, iodixanol and suitable saccharides, including (poly) sucrose. The gradated permeable barrier **620** may comprise a continuous gradient of decreasing gradient material concentration from the first-bottom end **630** to the second-top end **640** of the centrifuge container **610**. In other embodiments, the gradated permeable barrier **620** may comprise a plurality of distinct gradations in density, for example gradated permeable barrier segments **620a**, **620b**, and **620c**, as shown in **FIG. 4**. A solution containing the starting fucan composition, suitably pre-filtered through a pre-filter to remove

particulate matter, is disposed to be the starting fucan composition **650** proximate the second-top end **640** of the centrifuge container **610** and in contact with the gradated permeable barrier **620**. The pre-filter may be, for example without limitation, a 0.22 μm particulate filter.

[000121] In operation the centrifuge container is subjected to centrifugal force having a force component directed from the second-top end **640** to the first-bottom end **630** of the container as indicated by centrifugal force arrow **660** in **FIG. 4**. This may be achieved in a suitable centrifuge, schematically shown as centrifuge box **670** in **FIG. 4** and adapted to accommodate the centrifuge container **610**. Suitable centrifuges are well known in the art and will not be further discussed herein. The centrifugal force may be between about 1,000 gravities to about 1,000,000 gravities, for example between about 10,000 gravities to about 200,000 gravities, between about 60,000 gravities to about 500,000 gravities and between about 190,000 gravities to about 800,000 gravities.

[000122] Associated with the system of **FIG. 4**, the method for centrifugally precipitating a high-molecular-weight fucan from a starting fucan composition comprises establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material having a first-bottom gradated permeable barrier material end **622** in contact with a first-bottom end **630** of the centrifuge container **610**; disposing in contact with an opposing second-top gradated permeable barrier material end **624** of the gradated permeable barrier **620** proximate a second-top end **640** of the centrifuge container **610** the starting fucan composition comprising a desired high-molecular-weight segment; subjecting the centrifuge container **610** to a centrifugal force **660** directed from the second-top end **640** to the first-bottom end **630** of the centrifuge container **610**; and collecting precipitated high-molecular-weight fucan at the first-bottom end **630** of the centrifuge container **610**. Disposing the starting fucan composition **650** in contact with the lowest density gradient material may comprise pre-filtering the starting fucan composition through a suitable pre-filter.

[000123] Establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material may comprise establishing a plurality of segments of gradient material, the density of the gradient material segments decreasing from the first-bottom end **630** of the centrifuge container **610** toward the second-top end **640** of the centrifuge container **610**. Establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material may comprise establishing within the centrifuge container **610** a gradated permeable

barrier **620** of a saccharide. Establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material may comprise establishing within the centrifuge container **610** a gradated permeable barrier **620** of sucrose**.** Establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material may comprise establishing within the centrifuge container **610** a gradated permeable barrier **620** of at least one of glycerol, sorbitol, CsCl, Cs_2SO_4, KBr, diatrizoate, Nycodenz® and iodixanol. Establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material may comprise establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material dissolved in a solvent. Establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material may comprise establishing within the centrifuge container **610** a gradated permeable barrier **620** of a gradient material dissolved in one of water and a water-alcohol solution.

[000124] **FIG. 5** shows another embodiment of a centrifugal precipitation system **600′** for centrifugally precipitating a high-molecular-weight fucan from a starting fucan composition. Employing similar numbering as in **FIG. 4**, this embodiment uses a permeable barrier **620′** having a single barrier segment **620c′** of gradient material of which a first-bottom permeable barrier material end **622′** is in contact with a first-bottom end **630** of the centrifuge container **610**. In this embodiment, the starting fucan composition is directly in contact with an opposing second-top permeable barrier material end **624′** of the permeable barrier **620'**. In this embodiment the method comprises subjecting the centrifuge container **610** to a centrifugal force **660** directed from the second-top end **640** to the first-bottom end **630** of the centrifuge container **610** and collecting precipitated high-molecular-weight fucan at the first-bottom end **630** of the centrifuge container **610**. Disposing the starting fucan composition **650** in contact with the lowest density gradient material may comprise pre-filtering the starting fucan composition through a suitable pre-filter.

[000125] Other embodiments require no barrier to be employed and the container with starting fucan composition is centrifuged to subject the centrifuge container **610** to a centrifugal force **660** directed from the second-top end **640** to the first-bottom end **630** of the centrifuge container **610** and collecting precipitated high-molecular-weight fucan at the first-bottom end **630** of the centrifuge container **610**.

Gel electrophoresis-extraction

[000126] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan by gel electrophoresis-extraction. The methods can comprise: subjecting the starting fucan composition comprising a desired high-molecular-weight-segment to gel electrophoresis wherein the starting fucan composition is displaced according to mass to charge ratio by the action of an applied electric potential difference; selecting a portion of the electrophoresis gel on the basis of the potential difference and the desired high-molecular-weight fucan; and extracting the desired high-molecular-weight fucan from the selected gel portion.

[000127] Subjecting the starting fucan composition to gel electrophoresis may comprise first pre-filtering the starting fucan composition in solution through a pre-filter to remove undesired particulate matter. Subjecting the starting fucan composition to gel electrophoresis may comprise preparing the starting fucan composition in a solution at a concentration of between 0.1% w/v and 30% w/v. Extracting the desired high-molecular-weight fucan may comprise extracting the desired high-molecular-weight fucan from a gel portion that extends along the a direction of the potential difference for a distance of between 0.1 mm and 1000 mm. Extracting the desired high-molecular-weight fucan may comprise extracting the gel portion using one of water, methanol, ethanol, isopropanol, a water/alcohol mix and a salt solution.

[000128] Subjecting the starting fucan composition to gel electrophoresis may comprise displacing the starting fucan composition in solution for a predetermined amount of time. Subjecting the starting fucan composition to gel electrophoresis across the electrophoresis gel may comprise displacing the starting fucan composition across the electrophoresis gel while the gel is immersed in a buffer solution. Subjecting the starting fucan composition to gel electrophoresis across the electrophoresis gel may comprise preparing the gel from a gel material and the buffer solution. Preparing the gel from the gel material and the buffer solution may comprise preparing the gel from the buffer and one of agarose, polyacrylamide, polydimethylacrylamide and starch. Preparing the gel from the gel material and the buffer solution may comprise preparing the gel from one of tris-acetate EDTA, tris-borate EDTA and phosphate buffered saline together with a gel material. Displacing the starting fucan composition under the action of an applied electric potential difference may comprise displacing the starting fucan composition under the action of an applied electric field strength of between about 1 Volt/cm and about 500 Volt/cm, for example

between about 5 Volt/cm to about 50 Volt/cm, between about 10 Volt/cm to about 200 Volt/cm and between about 50 Volt/cm to about 300 Volt/cm.

[000129] An electrophoresis-extraction system **900** for obtaining a desired high-molecular-weight fucan from a starting fucan composition is shown in **FIG. 6**. Electrophoresis-extraction system **900** comprises an electrophoresis chamber **910**, shown as transparent and containing electrophoresis gel **916**, and an electrophoresis buffer **918**. The electrophoresis gel **916** material may be, for example without limitation, one of agarose, polyacrylamide and a starch. The an electrophoresis buffer **918** may be for example without limitation one of tris-acetate EDTA, tris-borate EDTA and phosphate buffered saline. Proximate and parallel to a first side of electrophoresis gel **916** is fashioned within electrophoresis gel **916** a well **912** in which the starting fucan composition in solution is placed.

[000130] Direct current power supply **920** applies a potential difference across electrophoresis buffer **918** in electrophoresis chamber **910** by means of cathode **922** and anode **924.** The electric potential difference between the cathode **922** and the anode **924** induces the fucan anions in the starting fucan composition to migrate along the gel away from the cathode **922** and toward the anode **924** along a direction given by migration direction arrow **926** so that, if the potential difference is maintained for a given period of time, different molecular weight molecules of the starting fucan composition will have been displaced from the well **912** by different distances toward the anode **924**. The rate of displacement is determined by the mass to charge ratio of the fucan molecule. The lower molecular weight fucans will displace more rapidly and will, after a fixed period of time under the action of the electric potential difference, be displaced further than the higher molecular weight fucans. Theoretical displacement distances **914** indicate different theoretical distances of displacement of different molecular weight fucan molecules, the lower molecular weight fucan molecules being displaced further from the cathode **922** at any given period of time.

[000131] To obtain a desired high-molecular-weight fucan from the starting fucan composition post-electrophoresis, the corresponding portion of the electrophoresis gel **916** is selected and the high-molecular-weight fucan extracted from that portion of the gel. One non-limiting method of doing that is to submerge the portion of the electrophoresis gel **916** in an extractant solution and

agitate the gel-solution mixture. In one embodiment, the agitation may be accomplished by shaking. In another embodiment, the agitation may be accomplished by high-shear mixing.

Membrane dialysis

[000132] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan by membrane dialysis. Consistent with typical identification of dialysis membranes, the nominal MWCO value for a given dialysis membrane will selectively allow passage of a solution containing molecules generally having molecular weights less than the molecular weight of molecules that do not cross/permeate the dialysis membrane. Molecular weight cut-off values for dialysis membranes are typically not absolute for any given polymer or nominal cut-off value: a given dialysis membrane will pass or retain some molecules both above and below the nominal molecular weight cut-off. The actual cut-off/selectively values and effects of a nominal MWCO dialysis membrane for a particular polymer can be routinely determined for the particular polymer.

[000133] A number of factors can affect the permeation behavior of the dialysis membranes. These factors may be dependent on the dialysis membranes themselves or dependent on an attribute of the target polymers, for example the folding behavior and folded structure of the target polymer can affect the behavior of the target polymer in crossing/not-crossing the dialysis membrane's MWCO barrier. Regarding the dialysis membrane themselves, for example, manufacturing methods can cause a variety of hole sizes within the specific dialysis membrane, which variety can include holes both larger and smaller than the nominal MWCO cut-off. Thus, a dialysis membrane having a nominal molecular weight cut-off value will substantially allow passage of molecules below the nominal molecular weight cut-off value, but can also pass/retain some molecules below and/or above such value.

[000134] The methods can comprise subjecting the starting fucan composition comprising a desired high-molecular-weight segment to dialysis against a dialysate through a membrane with a molecular weight cut-off greater than 100kDa to produce a dialyzed fucan composition comprising the high-molecular-weight fucan; and collecting the dialyzed fucan composition comprising the high-molecular-weight fucan.

[000135] Turning to **FIG. 7**, a membrane dialysis system **800** for obtaining a high-molecular-weight fucan from a starting fucan composition is shown. System **800** comprises a dialysis cell **820** having a dialysis membrane **825** that allows low molecular weight fucan molecules to pass through it. The starting fucan composition in a suitable solvent enters membrane dialysis system **800** and passes into fucan container **810** via input supply line **801** and through pre-filter **802**. The pre-filter may be, for example a 0.22 μm pre-filter to remove unwanted particulate matter.

[000136] The pre-filtered starting fucan composition is circulated through the dialysis cell **820** on a first side of the dialysis membrane **825** by way of dialysis system supply line **812** and dialyzed fluid return line **816** by dialysis system pump **814**. A dialysate fluid is circulated from dialysate container **830** through the dialysis cell **820** on a second side of the dialysis membrane **825** by way of dialysate supply line **832** and dialysate fluid return line **836** by dialysate pump **834**. The dialysate fluid is selected to flow freely through the dialysis membrane **825**. Suitable dialysate fluids include but are not limited to deionized water and solutions of sodium chloride, phosphate buffer, sodium phosphate, phosphate buffered saline, tris-HCl buffer, sodium citrate, citrate buffer, sodium ascorbate, ascorbic acid, sodium sulfite and ethylenediamine-tetraacetic acid (EDTA). Suitable dialysis membranes have pore sizes chosen to preferentially stop passage of fucan molecules of molecular weight greater than 200 kDa. Further suitable dialysis membranes have pore sizes that preferentially prevent the passage of molecules of molecular weight greater than 300 kDa, 500 kDa, and 1000 kDa. Each of these membranes may be employed to obtain a corresponding high-molecular-weight fucan from a starting fucan composition comprising fewer fucan molecules with molecular weights smaller than the dialysis membrane pore size or cut-off molecular weight relative to the broad starting molecular weight distribution. The dialysis membrane may be, without limitation, one of a cellulose ester and a regenerated cellulose membrane. The concentration of the solution containing the starting fucan composition may be between 0.1% w/v and 30% w/v.

[000137] As fucan molecules pass through dialysis membrane **825** their concentration builds up in the dialysate fluid and this starts to oppose the dialysis process. At a desired point in time dialysate supply valve **845** may be opened to allow fresh dialysate fluid into dialysate container **830** from dialysate supply container **840** via dialysate supply line **842**.

[000138] After a suitable dialysis period, dialyzed fluid output valve **815** may be opened to allow the dialyzed fucan composition to be drawn from dialysis system **800** via dialyzed fluid output line **818**. Dialysate fluid output valve **835** may be opened to allow the dialysis fluid containing low molecular weight fucan molecules to be drawn on dialysate fluid output line **838**.

Selective precipitation

[000139] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan by selective precipitation. The methods can comprise: providing the starting fucan composition comprising a desired high-molecular-weight segment as a solution of the starting fucan composition in water; adding to the solution containing the starting fucan composition a fucan-precipitant to obtain a supersaturated fucan-solvent mix; triggering precipitation of a portion of the broad molecular weight distribution starting fucan by adding an ionic-precipitation triggering compound to the supersaturated fucan-solvent mix to produce a precipitated high-molecular-weight fucan from the starting fucan composition and a solution containing remaining fucans; and extracting the precipitated high-molecular-weight fucan from the mix. Suitable fucan-precipitants include solvents with a relative polarity of less than 0.765, for example, ethanol, isopropanol, propanol, acetone, methanol, dimethyl sulfoxide, dimethyl formamide, ethylene glycol, tetrahydrofuran, acetonitrile, glyme, diglyme and dioxane, the solubility of the fucan decreasing as the polarity of the precipitating fluid decreases. The values for relative polarity can be normalized from measurements of solvent shifts of absorption spectra. See for example Christian Reichardt, Solvents and Solvent Effects in Organic Chemistry, Wiley-VCH Publishers, 3rd ed., 2003. Suitable ionic-precipitation triggering compounds include but are not limited to salts and bases of monovalent, divalent and trivalent cations, for example, chlorides, bromides, iodides, fluorides, sulfates, sulfites, carbonates, bicarbonates, phosphates, nitrates, nitrites, acetates, citrates, silicates, hydroxides, oxides and/or cyanides of an alkali metal, alkaline earth metal, aluminum and/or ammonium. In some embodiments, the ionic precipitation triggering compound comprises at least one of NaCl, KCl, NaOH, $MgCl_2$ and $CaCl_2$. Suitable concentrations of the starting fucan composition in water are between 0.01% w/v and 30% w/v. Particular fucans lending themselves to the above method include but are not limited to fucoidan.

[000140] The methods may further comprise desalting the starting fucan composition before adding the fucan-precipitant. The desalting may comprise diafiltrating the starting fucan composition across a molecular weight cutoff filter. The diafiltrating may comprise diafiltrating the starting fucan composition with distilled water. The diafiltrating may comprise diafiltrating the starting fucan composition across a molecular weight cutoff filter having a molecular weight cutoff smaller than a desired molecular weight in the desired high-molecular-weight fucan, for example, a 5 kDa, 10 kDa, 30 kDa, 50 kDa, 70 kDa, 100 kDa, 200 kDa or 300 kDa molecular weight cut-off. The methods may further comprise pre-filtering a solution containing the starting fucan composition through a suitable pre-filter to remove undesired particulate matter.

[000141] Extracting the precipitated high-molecular-weight fucan from the mix may comprise at least one of centrifugation, sedimentation, filtration and hydrodynamic flow separation.

Anionic adsorption

[000142] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan by anionic adsorption. The methods can comprise: providing dissolved in a starting solution, the starting fucan composition having a broad starting molecular weight distribution comprising a desired high-molecular-weight segment; subjecting the starting fucan composition in the starting solution to ion exchange with an ion-exchange macroporous resin having a pore size based on a desired separation molecular weight within the starting fucan molecular weight distribution to convert the starting fucan composition into a first ion exchange-treated fucan composition; collecting the first ion exchange-treated fucan composition comprising the desired high-molecular-weight fucan; after the ion exchange with the starting fucan composition subjecting the macroporous resin to a salt solution to extract fucan molecules from the resin into the salt solution, producing a low molecular weight fucan-rich salt solution; desalting the low molecular weight fucan-rich salt solution to form a second ion exchange-treated fucan composition; and collecting the second ion exchange-treated fucan composition comprising a low-molecular-weight fucan.

[000143] The methods may further comprise desalting the starting fucan composition before the subjecting to ion exchange. The desalting may comprise diafiltrating the starting fucan composition across a molecular weight cutoff TFF filter. The diafiltrating may comprise

diafiltrating the starting fucan composition across a molecular weight cutoff TFF filter having a molecular weight cutoff smaller than a desired molecular weight in the high-molecular-weight fucan, for example a 5 kDa, 10 kDa, 30 kDa, 50 kDa, 70 kDa, 100 kDa and/or a 300 kDa molecular weight cutoff TFF filter.

[000144] In another embodiment, a method for producing from a starting fucan composition a desired high-molecular-weight fucan composition, can comprise: providing dissolved in a starting solution a starting fucan composition having a broad starting molecular weight distribution comprising a desired high-molecular-weight segment; subjecting the dissolved starting fucan composition to ion exchange with an ion-exchange macroporous resin having a pore size based on a desired separation molecular weight within the starting fucan molecular weight distribution to convert the starting fucan composition into a first ion exchange-treated fucan composition; and collecting the first ion exchange-treated fucan composition comprising the desired high-molecular-weight fucan. The further embodiments may further comprise desalting the starting fucan composition before the subjecting to ion exchange. The desalting may comprise diafiltrating the starting fucan composition across a molecular weight cutoff TFF filter. The diafiltrating may comprise diafiltrating the starting fucan composition across a molecular weight cutoff TFF filter having a molecular weight cutoff smaller than a desired molecular weight in a molecular weight distribution of the desired high-molecular-weight fucan for example a 5 kDa, 10 kDa, 30 kDa, 50 kDa, 70 kDa, 100 kDa and/or a 300 kDa molecular weight cutoff TFF filter.

[000145] Subjecting the macroporous resin to a salt solution may comprise subjecting the macroporous resin to a sodium salt solution, for example a solution comprising at least one of a chloride, bromide, iodide, fluoride, sulfate, sulfite, carbonate, bicarbonate, phosphate, nitrate, nitrite, acetate, citrate, silicate and/or cyanide of an alkali metal, alkaline earth metal, aluminum and/or ammonium. Subjecting the macroporous resin to a sodium salt solution may comprise subjecting the macroporous resin to a sodium chloride solution. Desalting the low molecular weight fucan-rich salt solution may comprise diafiltrating the low molecular weight fucan-rich salt solution across a molecular weight cutoff TFF filter. The diafiltrating may comprise diafiltrating the low molecular weight fucan-rich salt solution across a molecular weight cutoff TFF filter having a molecular weight cutoff smaller than a desired molecular weight in a molecular weight

distribution of the desired low molecular weight fucan-rich salt solution for example a 5 kDa, 10 kDa, 30 kDa, 50 kDa, 70 kDa and/or 100 kDa molecular weight cutoff TFF filter.

[000146] Subjecting the dissolved starting fucan composition to ion exchange with an ion-exchange macroporous resin may comprise adjusting a ratio of the starting fucan to resin to a predetermined mass ratio. The predetermined mass ratio may be between about 1:100 fucan:resin and about 10:1 fucan:resin, 5:1 fucan:resin, or 2:1 fucan:resin. In other embodiments, the predetermined mass ratio may be between about 1:100 fucan:resin and about 1:1 fucan:resin. In yet other embodiments, the predetermined mass ratio may be between about 1:100 fucan:resin and about 1:2 fucan:resin. In yet further embodiments, the predetermined mass ratio may be between about 1:50 fucan:resin and about 1:5 fucan:resin. In yet further embodiments, the predetermined mass ratio may be between about 1:20 fucan:resin and about 1:1 fucan:resin, for example, about 1:2 fucan:resin, 1:4 fucan:resin, 1:6 fucan:resin, 1:8 fucan:resin and 1:10 fucan:resin.

[000147] Subjecting the dissolved starting fucan composition to ion exchange with an ion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with the resin for a predetermined period of time. The predetermined period of time may be between zero and 300 hours. In other embodiments, the predetermined period of time may be between zero and 100 hours. In further embodiments, the predetermined period of time may be between 5 minutes and 30 hours, for example between about 8 hours and about 24 hours. In yet further embodiments, the predetermined period of time may be between 1 and 10 hours, for example between about 4 hours and about 10 hours. In yet further embodiments, the predetermined period of time may be between about 2 and about 5 hours.

[000148] Subjecting the dissolved starting fucan composition to ion exchange with an ion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with an anion-exchange macroporous resin. Subjecting the dissolved starting fucan composition to ion exchange with an anion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with a strong base anion-exchange macroporous resin. Subjecting the dissolved starting fucan composition to ion exchange with an anion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with a weak base anion-exchange macroporous resin. "Strong base" and "weak base" are used according to their ordinary meanings, for example a "strong base" being

a resin that does not lose charge under any typical ion-exchange circumstances, for example a quaternary amine functionalized resin , and a weak base being a resin that does lose charge under high pH conditions, for example, a primary, secondary or tertiary amine functionalized resin. Subjecting the dissolved starting fucan composition to ion exchange may comprise subjecting the dissolved starting fucan composition to ion exchange with a mixed charge macroporous resin.

[000149] Subjecting the dissolved starting fucan composition to ion exchange with an anion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with a macroporous resin comprising at least one of primary, secondary, tertiary and quaternary amine groups. The primary amine groups may be NH_2 groups. The secondary amine groups may be at least one of, for example without limitation, benzylamine groups and dimethyl amine groups. The tertiary amine groups may be at least one of, for example without limitation, diethylaminoethyl groups and dimethylaminoethyl groups. The quaternary amine groups may be for example without limitation trimethyl ammonium and triethyl ammonium groups. The resin may comprise, but is not limited to, one or more of styrene, agarose, dextran, acrylate, methacrylate, methyl methacrylate, butyl methacrylate, divinylbenzene, cellulose, silica, and ceramic.

[000150] Subjecting the dissolved starting fucan composition to ion exchange with an ion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with an ion exchange resin having a pore size between 5 nm and 1000 nm, for example between 5 nm and 100 nm, between 10 nm or 15 nm and 50 nm, between 20 nm and 80 nm, between 5 nm and 30nm, between 100 nm and 500 nm, between 300 nm and 900 nm or between 200 nm and 400 nm. Subjecting the dissolved starting fucan composition to ion exchange with an ion-exchange macroporous resin may comprise subjecting the dissolved starting fucan composition to ion exchange with an ion exchange resin has an exclusion limit of between 50 kDa and 50,000 kDa, for example between 50 kDa and 10,000 kDa, between 100 kDa and 5,000 kDa, between 10,000 kDa and 40,000 kDa, between 1,000 kDa and 9,000 kDa, between 2,000 kDa and 7,000 kDa or between 500 kDa and 2,000 kDa. The exclusion limit can be based on the exclusion limit for globular proteins.

[000151] **FIG. 8** shows a schematic diagram of an exemplary ion adsorption system **300** for the segmentation of a fucan on the basis of molecular weight. A solution containing the starting fucan

composition is supplied via input supply line **301** and pre-filter **306** to TFF subsystem fucan container **176**. In a desalting process, tangential flow filtration (TFF) subsystem pump **174** pumps the starting fucan composition to TFF filter **171** of TFF subsystem **170** via TFF subsystem filter supply line **172**. The format of the TFF filter **171** may be without limitation any one of a plate and frame system; a spiral wound cartridge system; a hollow fiber system; a flow cell system; and centrifugal filter system.

[000152] In the system of **FIG. 8**, TFF subsystem **170** serves as a desalination subsystem. TFF filter **171** is typically supplied as a cassette designed to allow an input fluid supplied to it to pass over its filter on its retentate side, while allowing a permeate to exit via one output line and treated input fluid to leave as retentate via another output line. For the present method, the cut off molecular weight of TFF filter **171** is chosen to allow permeation of salt components in the starting fucan solution while retaining the fucan in the retentate for subsequent ion adsorption treatment in ion exchange subsystem **180**. TFF subsystem pump **174** maintains a level of pressure over TFF filter **171** between its retentate and permeate sides. In **FIG. 8**, the retentate of TFF filter **171** is returned to TFF subsystem fucan container **176** via TFF subsystem retentate line **178**, while permeate containing the unwanted non-fucan salt components is produced via TFF subsystem permeate output line **179** for use outside TFF subsystem **170** or to be discarded.

[000153] While TFF subsystem pump **174** recirculates the starting fucan composition and retentate over TFF filter **171**, water or a low conductivity flush solution from TFF subsystem solvent container **177** may be supplied via TFF subsystem solvent supply line **175**. The flush solution is used to replenish retentate solution lost via the permeate on TFF subsystem permeate output line **179** and/or to ensure that a predetermined number of diavolumes of input starting fucan and solvent are circulated over the TFF filter **171**. By controlling TFF subsystem solvent supply valve **173**, flush solution may be added in a pulse process. In other embodiments, the solvent may be added in a continuous mode. The continuous mode of adding the solvent has efficiency benefits. The number of diavolumes of solvent to process over TFF filter **171** may be predetermined. In some embodiments, the solvent may be deionized water.

[000154] Inter-subsystem valve **302** may be shut during the above processing, and retentate of TFF filter **171** of TFF subsystem **170** collected into a container (not shown) before being supplied to ion exchange subsystem fucan container **186** of ion exchange subsystem **180**. The collected

retentate may then be supplied to ion exchange subsystem fucan container **186** of ion exchange subsystem **180** via TFF subsystem retentate output line **303**. In other embodiments, the collected retentate may be transferred in a container (not shown) to ion exchange subsystem fucan container **186**. In yet other embodiments of the system, the inter-subsystem valve **302** may be maintained open and the retentate of TFF filter **171** may be supplied via TFF subsystem retentate output line **303** on a continuous basis to ion exchange subsystem fucan container **186**. The retentate supplied to ion exchange subsystem **180** may be anticipated to have a lower salt content remaining that may interfere with the processing of fucan in ion exchange subsystem **180** and is a desalinated fucan composition.

[000155] Ion exchange container **181** of ion exchange subsystem **180** contains a volume of macroporous ion exchange resin **189**. In some embodiments, the macroporous ion exchange resin is an anion exchange resin. In some embodiments, the macroporous ion exchange resin is a mixed charge resin. The pore size of the macroporous ion exchange resin **189** is chosen to preferentially adsorb fucan molecules of molecular weight below a predetermined value from a solution containing a broad molecular weight distribution starting fucan, preferentially leaving behind in the solution fucan molecules that have a greater molecular weight than the predetermined value. One form of this category of resin is based on substantially spherical particles of styrene crosslinked with divinylbenzene and having pores containing quaternary ammonium groups. In some embodiments, the pore size may be between 10nm and 100nm. The fucan molecules may or may not be preferentially adsorbed into the pores of the resin based on the hydrodynamic size of the fucan molecules.

[000156] During the processing of the desalinated fucan composition from TFF subsystem **170** in ion exchange container **181,** ion exchange subsystem output valve **304** controlling the ion exchange subsystem output line **305** from ion exchange subsystem fucan container **186** may be closed. Ion exchange subsystem salt solution supply valve **183b** and ion exchange subsystem salt solution return valve **183c** may similarly be closed and ion exchange subsystem fucan return valve **183a** opened. While ion exchange subsystem fucan pump **184a** recirculates a solution containing the desalinated fucan composition through ion exchange container **181** via ion exchange subsystem fucan supply line **182a** and ion exchange subsystem fucan pump **184a**, macroporous ion exchange resin **189** adsorbs the lower molecular weight fucan molecules, thereby causing the solution in ion

exchange subsystem fucan return line **188a** to contain the desired high-molecular-weight fucan. After flowing through the ion exchange container **181**, the solution containing the desired high-molecular-weight fucan is returned to ion exchange subsystem fucan container **186** via ion exchange subsystem fucan return line **188a**.

[000157] The average molecular weight of the fucans in ion exchange subsystem fucan container **186** may be measured or monitored. When the solution in ion exchange subsystem fucan container **186** has been circulated for a suitable period of time, or when the fucans in the solution have attained a predetermined desired average molecular weight value, ion exchange subsystem output valve **304** may be opened to produce a first ion exchange treated fucan composition as the first output product of ion adsorption system **300** via ion exchange subsystem output line **305**. This first output product comprises, for example, a high-molecular-weight fucan with a molecular weight distribution wherein the quantity of the input starting fucan broad molecular weight distribution at the low molecular weight end has been suppressed or attenuated such that the resulting molecular weight distribution is displaced towards the higher end of the molecular weight distribution of the input starting fucan composition supplied to ion adsorption system **300** on input supply line **301**.

[000158] Ion exchange subsystem output valve **304** may be closed again, as may ion exchange subsystem fucan return valve **183a**, and ion exchange subsystem salt solution supply valve **183b** and ion exchange subsystem salt solution return valve **183c** opened to allow salt solution from ion exchange subsystem salt solution container **187** to enter the circulation in ion exchange subsystem **180** via ion exchange subsystem salt solution supply line **182b**. Ion exchange subsystem salt solution pump **184b** now circulates salt solution via ion exchange subsystem salt solution supply line **182b** through the macroporous ion exchange resin **189** in ion exchange container **181** and back to ion exchange subsystem salt solution container **187** via ion exchange subsystem salt solution return line **188b** and ion exchange subsystem salt solution return valve **183c**. In this process, the salt displaces the fucan adsorbed within the pores of the macroporous ion exchange resin and releases the freed fucan into the salt solution in circulation in ion exchange subsystem **180**. The salt solution may be circulated for a predetermined time. In other embodiments, the average molecular weight of the fucan in the salt solution in ion exchange subsystem **180** may be measured and the recirculation of the salt solution terminated when the average molecular weight of the fucan in salt solution reaches a predetermined desired value.

[000159] In some embodiments, a predetermined amount of a low ionic content solution may be used to wash the resin prior to initiating the circulation of salt solution from ion exchange subsystem salt solution container **187**. In some embodiments, this low ionic content solution may be deionized water.

[000160] At this point ion exchange subsystem output valve **304** may be opened again and the pumps and valves of ion exchange subsystem **180** suitably operated to allow the second product of ion adsorption system **300** drawn from ion exchange subsystem output line **305** in the form of a low molecular weight fucan-rich salt solution. The second product may be filtered, for example without limitation in a centrifuge over a suitable centrifugal filter or tangential flow filtration filter, to separate the low-molecular-weight fucan from the unwanted salt. This produces a second output low-molecular-weight fucan. This second output low-molecular-weight fucan, in contrast with the first output high-molecular-weight fucan discussed above, has a fucan molecular weight distribution wherein a portion of the input starting fucan broad molecular weight distribution at the high-molecular-weight end has been suppressed or attenuated such that the resulting molecular weight distribution is displaced towards the lower end of the molecular weight distribution of the input starting fucan composition supplied to ion adsorption system **300** on input supply line **301**.

[000161] Given the width and complexity of the starting fucan molecular weight distribution and the vagaries of polymer behavior and ion exchange resins, the two output fucan molecular weight distributions may not peak where anticipated from a consideration of the pore size of the macroporous ion exchange resin. If that occurs, however, the two output fucan molecular weight distributions will still be displaced with respect to each other, representing the segmentation of the starting fucan composition into a comparatively higher molecular weight fucan corresponding to the first product, and a comparatively lower molecular weight fucan corresponding to the second product. The first product corresponds to large and heavy fucan molecules preferentially not adsorbed by the resin, while the second product conversely corresponds to fucan molecules preferentially adsorbed by the resin and are on average smaller and lighter than those not adsorbed.

Preparative gel permeation chromatography

[000162] A high-molecular-weight fucan may be obtained from a broad molecular weight distribution starting fucan by preparative gel permeation chromatography. The methods can

comprise providing packed in a column format a gel media specified for gel permeation chromatography (GPC) of polymers in an aqueous solution; providing a starting fucan composition comprising a desired high-molecular-weight segment dissolved in an aqueous solvent suitable for gel permeation chromatography on the gel media; subjecting the solution containing the starting fucan composition to preparative gel permeation chromatography, wherein the fucan is displaced according to molecular weight across the gel media in the column at a predetermined flow rate between a first input end of the column and a second output end of the column; collecting eluent from the second output end of the column in pre-determined aliquots based on a desired segmentation of the starting fucan composition, each aliquot comprising a segmented fucan composition; pooling the desired aliquots based on the desired segmentation of the starting fucan composition to obtain a pooled GPC aliquot composition comprising the desired high-molecular-weight fucan.

[000163] Subjecting the solution containing the starting fucan composition to preparative gel permeation chromatography may comprise first pre-filtering the starting fucan composition in solution through a pre-filter to remove undesired particulate matter. Subjecting the solution containing the starting fucan composition to preparative gel permeation chromatography may comprise preparing the starting fucan composition in a solution at a concentration of between 0.1% w/v and 20% w/v. Subjecting the solution containing the starting fucan composition to preparative gel permeation chromatography may comprise using at least one of a peristaltic pump, isocratic pump, binary pump, quaternary pump and gradient pump to accomplish the displacement across the column containing gel media. Subjecting the solution containing the starting fucan composition to preparative gel permeation chromatography may comprise displacing the solution across the column containing the gel media at a predetermined flow rate of between 0.0005 milliliters per minute per gel media surface area (mL/min/cm^2) to 5 mL/min/cm^2, between 0.005 mL/min/cm^2 to 0.5 mL/min/cm^2, between 0.01 mL/min/cm^2 to 0.25 mL/min/cm^2, 0.05 mL/min/cm^2, 0.1 mL/min/cm^2, 0.15 mL/min/cm^2 and 0.2 mL/min/cm^2.

[000164] Collecting eluent from the second output end of the column may comprise collecting aliquots of eluent between about 0.1 mL and 1000 mL, between about 1 mL and 100 mL, between about 5 mL and 50 mL, about 10 mL, about 20 mL, about 30 mL and about 40 mL. Collecting the aliquots from the second output end of the column may comprise measuring the molecular weight

distributions of the aliquots by analytical GPC. Measuring the aliquots by analytical GPC may be done simultaneously with the collecting of the column eluent.

[000165] Pooling the desired aliquots may involve measuring the molecular weight distributions of the aliquots by analytical GPC and pooling only aliquots with desired molecular weight distributions. Pooling the desired aliquots may be done simultaneously with the collecting of the column eluent.

[000166] The gel media used may comprise at least one of polyhydroxymethacrylate, sulfonated styrene-divinylbenzene, silica, a hydrophilic bonded phase or polymer, polystyrene, divinylbenzene, methacrylate, methyl methacrylate, butyl methacrylate, cellulose, ceramic, agarose and dextran. The gel media used may have pores with diameters of at least one of about 3 nm, 5 nm, 10 nm, 20 nm, 50 nm, 100 nm, 200 nm, 500 nm, 1,000 nm, 2,000 nm, 3,000 nm, 5,000 nm and 10,000 nm. The gel media used may have pores with exclusion limits of at least one of about 100 Da, 100 kDa 1,000 kDa, 5,000 kDa, 10,000 kDa, 30,000 kDa, 50,000 kDa and 100,000 kDa. The exclusion limits may be based on the exclusion limit for globular proteins, or a polysaccharide, for example, dextran and/or pullulan.

[000167] The solvent used to dissolve the starting fucan composition may comprise at least one of water, sodium nitrate, lithium nitrate, monosodium phosphate, disodium phosphate, trisodium phosphate, lithium chloride, lithium bromide, lithium iodide sodium chloride, sodium bromide, sodium iodide, potassium chloride, potassium bromide, potassium iodide, sodium hydroxide, lithium hydroxide, potassium hydroxide, sodium sulfate, sodium sulfite, methanol, ethanol and acetonitrile.

Chemical Structural modification

[000168] The methods, systems etc. discussed herein can comprise chemical structural modification of the fucan composition, particularly the fucans in the fucan composition. The chemical structural modification may involve removal of functional groups from the fucan, for example, O-acetyl, N-acetyl, methoxy, hydroxyl, carboxylic and/or sulfate functional groups from the fucan structure. The chemical structural modification may involve the use of a wide variety of chemical reagents, for example, acids, bases, detergents and/or oxidizing agents.

Diseases and conditions

Fibrous adhesions

[000169] A fibrous adhesion is a type of scar that forms between two parts of the body, usually after surgery (surgical adhesion). Fibrous adhesions can cause severe problems. For example, fibrous adhesions involving the female reproductive organs (ovaries, Fallopian tubes) can cause infertility, dyspareunia and severe pelvic pain. Fibrous adhesions that occur in the bowel can cause bowel obstruction or blockage, and fibrous adhesions can also form in other places such as around the heart, spine and in the hand. In addition to surgery, fibrous adhesions can be caused for example by endometriosis, infection, chemotherapy, radiation, trauma and cancer.

[000170] A variety of fibrous adhesions are discussed in this document. Terms such as surgical adhesions, post-surgical adhesions, postoperative adhesions, adhesions due to pelvic inflammatory disease, adhesions due to mechanical injury, adhesions due to radiation, adhesions due to radiation treatment, adhesions due to trauma, and adhesions due to presence of foreign material all refer to adherence of tissues to each other due to a similar mechanism and are all included in the term fibrous adhesions.

[000171] Fibrous adhesion formation is a complex process in which tissues that are normally separated in the body grow into each other. Surgical adhesions (also known as post-surgical adhesions) develop from the otherwise normal wound healing response of the tissues to trauma and have been reported to occur in over two-thirds of all abdominal surgical patients (Ellis, H., *Surg. Gynecol. Obstet.* 133: 497 (1971)). The consequences of these fibrous adhesions are varied and depend upon the surgical site or other site, such as a disease site, involved. Problems may include chronic pain, obstruction of the intestines and even an increased risk of death after cardiac surgery (diZerega, G. S., *Prog. Clin. Biol. Res.* 381: 1-18 (1993); diZerega, G. S., *Fertil. Steril.* 61:219-235 (1994); Dobell, A. R., Jain, A. K., Ann. *Thorac. Surg.* 37: 273-278 (1984)). In women of reproductive age, fibrous adhesions involving the uterus, fallopian tubes or ovaries are estimated to account for approximately 20% of all infertility cases (Holtz, G., Fertil. *Steril.* 41: 497-507 (1984); Weibel, M.A. and Majno, G. *Am. J. Surg.* 126: 345-353 (1973)).

[000172] The process of fibrous adhesion formation initially involves the establishment of a fibrin framework and normal tissue repair. The normal repair process allows for fibrinolysis

alongside mesothelial repair. However, in fibrous adhesion formation the fibrin matrix matures as fibroblasts proliferate into the network and angiogenesis occurs resulting in the establishment of an organized fibrous adhesion within about 3 to 5 days (Buckman, R. F., et al., *J. Surg. Res.* 21: 67-76 (1976); Raferty, A. T., *J. Anat.* 129: 659-664 (1979)). Inflammatory processes include neutrophil activation in the traumatized tissues, fibrin deposition and bonding of adjacent tissues, macrophage invasion, fibroblast proliferation into the area, collagen deposition, angiogenesis and the establishment of permanent fibrous adhesion tissues.

[000173] Various attempts have been made to prevent surgical adhesions. These involve pharmacological approaches targeted at influencing the biochemical and cellular events that accompany surgical traumas well as barrier methods for the separation of affected tissues. For example, the use of peritoneal lavage, heparinized solutions, procoagulants, modification of surgical techniques such as the use of microscopic or laparoscopic surgical techniques, the elimination of talc from surgical gloves, the use of smaller sutures and the use of physical barriers (films, gels or solutions) aiming to minimize apposition of serosal surfaces, have all been attempted. Currently, preventive therapies also include prevention of fibrin deposition, reduction of inflammation (steroidal and non-steroidal anti-inflammatory drugs) and removal of fibrin deposits.

[000174] Interventional attempts to prevent the formation of post-surgical adhesions have included the use of hydroflotation techniques or barrier devices. Hydroflotation involves the instillation of large volumes of polymer solutions such as dextran (Adhesion Study Group, *Fertil. Steril.* 40:612-619 (1983)), or carboxymethyl cellulose (Elkins, T. E., et al., *Fertil. Steril.* 41:926-928 (1984)), into the surgical space in an attempt to keep the organs apart. Synthetic barrier membranes made from oxidized regenerated cellulose (*e.g.,* Interceed™), polytetrafluoroethylene (Gore-tex surgical membrane) and fully resorbable membranes made from a modified hyaluronic acid/carboxymethylcellulose (HA/CMC) combination (Seprafilm™) have also been used to reduce post-surgical adhesion formation in both animals and humans (Burns, J. W., et al., *Eur. J. Surg.* Suppl. 577: 40-48 (1997); Burns, J. W., et al., *Fertil. Steril.* 66:814-821 (1996); Becker, J. M., et al., *J. Am. Coll. Surg.* 183:297-306 (1996)). The success of these HA/CMC membranes may derive from their ability to provide tissue separation during the peritoneal wound repair process when fibrous adhesions form. The membranes were observed to form a clear viscous coating on

the injured tissue for 3-5 days after application, a time period that is compatible with the time course of post-surgical adhesion formation (Ellis, H., *Br. J. Surg.* 50: 10-16 (1963)). Unfortunately, limited success has been seen with these methods.

[000175] Peritonitis involves inflammation of the peritoneum. Peritonitis can cause severe problems. For example, abdominal pain, abdominal tenderness and abdominal guarding. Peritonitis may involve spontaneous, anatomic and/or peritoneal dialysis related inflammation. Peritonitis may involve an infection, for example, perforation of a hollow viscus, disruption of the peritoneum, spontaneous bacterial peritonitis, and systemic infections may result in infection and peritonitis. Peritonitis may also not involve an infection, for example, leakage of sterile body fluids into the peritoneum, and sterile abdominal surgery may result in peritonitis. Various attempts have been made to prevent and/or treat peritonitis. For example, general supportive measures such as intravenous rehydration, antibiotics, and surgery. There is an unmet need for compounds, compositions, methods and the like (including delivery approaches) to inhibit, or otherwise treat and/or prevent, peritonitis, preferably more effectively with few side effects.

[000176] The high-molecular-weight fucans discussed herein can be used to treat fibrous adhesions in a patient and can be included as a component of, or be, a high-molecular-weight fucan medical device, combination or pharmaceutical product configured and composed to treat fibrous adhesions. For example, a high-molecular-weight fucan medical device comprising between about 0.02 mg/mL to about 100 mg/mL, for example 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 0.9 mg/mL, 1 mg/mL, 2.5 mg/mL, 5 mg/mL 7.5 mg/mL, of a high-molecular-weight fucan herein dissolved in a physiological salt solution. The physiological salt solution can be, for example, Lactated Ringer's Injection USP (LRS), normal saline and physiological Dextran solution.

[000177] The high-molecular-weight fucan medical devices, which can be liquid medical devices, herein can contain pharmaceutically acceptable excipients such as buffers, stabilizers, preservatives, adjuvants, etc. Such high-molecular-weight fucan medical devices can be used to treat fibrous adhesions pre-, during, or post-surgery by administering between about 0.01 mL/kg (per kilogram bodyweight of the patient or target) to about 10 mL/kg or 15 mL/kg of the fucan medical devices in the preceding paragraph. Doses include, for example, about 0.03 mL/kg, 0.1 mL/kg, 0.2 mL/kg, 0.4 mL/kg, 0.5 mL/kg, 0.6 mL/kg, 1 mL/kg, 1.2 mL/kg, 2 mL/kg, 3 mL/kg, 4 mL/kg, 5 mL/kg, 8 mL/kg, 10 mL/kg and 15 mL/kg of the high-molecular-weight fucan medical

device to the surgical site of the patient. In further embodiments, such high-molecular-weight fucan medical devices can be used to treat fibrous adhesions at any selected target site, for example lesions, abrasions, injury sites, surgical sites and post-surgical sites by administering between about 0.04 mg/kg or 0.1 mg/kg to about 25 mg/kg or 50 mg/kg. Some examples of such doses include, for example, about 0.04 mg/kg, 0.075 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.3 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 7.5 mg/kg, 8 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg and 50 mg/kg of the fucans herein, including for example the high-molecular-weight fucans herein, to the surgical site of the patient. The administering can be accomplished, for example, by instilling a liquid medical device generally throughout the target area; directing the liquid medical device at a specific location(s) within the target area; spraying the liquid medical device generally or at a specific location(s) within the target area; or, spraying or otherwise delivering the liquid medical device via an applicator, which can be a spray applicator through a trocar, catheter, endoscope or other minimally invasive device, onto a specific location(s) that a surgeon or other practitioner has identified as particularly susceptible to or concerning for development of fibrous adhesions. In another aspect, the administering can be done after opening of the surgical wound but before the surgical procedure; during the surgical procedure, or after the surgical procedure but before the surgical wound has been closed. If desired, the liquid medical device can also be administered after the surgery is completed (for example through a syringe and needle) and can be administered to non-surgical target sites as well. The surgical site of the patient can be, for example, at least one of the pelvic cavity, abdominal cavity, dorsal cavity, cranial cavity, spinal cavity, ventral cavity, thoracic cavity, pleural cavity, pericardial cavity, skin, joints or muscles. The administering of the high-molecular-weight fucan medical device into the surgical site of the patient can be accomplished in less than about 15 minutes, 10 minutes, 8 minutes, 6 minutes, 5 minutes, 4 minutes, 3 minutes, 2 minutes, 1 minute, 45 seconds, 30 seconds, 20 seconds, 15 seconds, 10 seconds and 5 seconds.

[000178] Examples of administering the high-molecular-weight fucan medical device to a surgical site include without limitation administering the high-molecular-weight fucan medical device at the surgical site of a Cesarean section surgical procedure; a microvascular free flap reconstruction surgical procedure, a full thickness skin graft surgical procedure, a V-Y advancement flap surgical procedure, a fasciocutaneous rotation flap surgical procedure, an

arthroplasty surgical procedure, a mastectomy surgical procedure, a sequestrectomy surgical procedure, a saucerization surgical procedure, an osteotomy surgical procedure, an osteoplasty surgical procedure, a patellectomy surgical procedure, a synovectomy surgical procedure, a capsulectomy surgical procedure, a tendon or ligament repair surgical procedure, a tenolysis surgical procedure, a tenotomy surgical, a fasciotomy surgical procedure, a meniscal repair surgical procedure, a vertebrectomy surgical procedure, a ethmoidectomy surgical procedure, a Caldwell Luc's operation surgical procedure, a dacryocystorhinostomy surgical procedure, a lysis nasal synechia surgical procedure, a thymectomy surgical procedure, a pneumonolysis surgical procedure, a pneumonectomy surgical procedure, thoracoplasty surgical procedure, a bilobectomy surgical procedure, a portal hypertension surgery surgical procedure, a splenectomy surgical procedure, a esophagectomy surgical procedure, a peritonitis surgery surgical procedure, a gastrectomy surgery surgical procedure, a jejunojejunostomy surgery surgical procedure, a laparoscopic cholecystectomy surgery surgical procedure, a laparoscopic common bile duct exploration surgical procedure, a gastroenterostomy surgical procedure, a bariatric surgery surgical procedure, a bowel resection & anastomosis surgical procedure, a segemental hepatectomy surgical procedure, a lobectomy surgical procedure, a pancreatomy surgical procedure, a pancreaticoduodenectomy surgical procedure, a tumor resection surgical procedure, a laparoscopic nephrectomy surgical procedure, a cystectomy surgical procedure, an abdominal or pelvic adhesion lysis surgical procedure, a hysterosalpingostomy surgical procedure, a salpingoplasty surgical procedure, an ectopic pregnancy laparoscopic surgery surgical procedure, a joint replacement surgery surgical procedure, a broken bone repair surgical procedure, a hysterectomy surgical procedure, a gallbladder removal surgical procedure, a heart bypass surgical procedure, an angioplasty surgical procedure, an atherectomy surgical procedure, a breast biopsy surgical procedure, a carotid endarterectomy surgical procedure, a cataract surgery surgical procedure, a coronary artery bypass surgical procedure, a dilation and curettage surgical procedure, a hernia repair surgical procedure, a lower back pain surgery surgical procedure, a partial colectomy surgical procedure, prostatectomy surgical procedure and a tonsillectomy surgical procedure, after opening the surgical wound, during surgery, before closing the surgical wound and/or after closing the surgical wound.

Cancers Generally

[000179] Cancer has been the second leading cause of death in the U.S. and accounts for over 20% of all mortalities. Cancer is a proliferative disease and is characterized by the uncontrolled division of certain cells, which may lead to the formation of one or more tumors. A number of methods are used to treat cancer, including surgery, radiation, chemotherapy and combinations thereof. Although surgery is a relatively common method used for some localized tumors, there is still a significant chance of tumor recurrence after tumor excision.

[000180] Treating cancers and other proliferative diseases has been limited by the potential for damage or toxicity to non-cancerous, healthy tissues. In radiation and surgical treatments, the procedure has been generally confined to and proximal to the tumor sites. However, there can be significant risk to patients undergoing surgical removal of cancerous tissues (*e.g.,* in removal of prostate or brain tumors there can be a significant risk of non-repairable damage to surrounding vital tissues, for example via potential reduced need for resection of non-tumor tissues. Furthermore, in focused radiation treatment, which has been given as a first line treatment for prostate cancer, there are similar risks. In the chemotherapeutic treatment of cancer, the drug has been administered systemically, so that the whole body is exposed to the drug. These drugs are designed to be toxic to cancer cells, but they are also (generally) toxic to non-cancerous cells so that patients become quite ill when undergoing drug treatments for cancer. Through experience, oncologists are able to give doses of these drugs that may be tolerated by some patients. However, these doses are often not successful in treating cancers.

[000181] One problem with any method of treating cancer has been the local recurrence of the disease. For example, approximately 700,000 Americans are diagnosed with localized cancer annually (approximately 64% of all cancer patients) and almost half a million are treated using surgical methods. Unfortunately, 32% of patients treated with surgery relapse after the initial treatment (approximately 21% relapse at the initial surgical site and 11% at distant metastatic sites). Almost 100,000 patients die annually due to localized recurrence of cancer. This has been especially true in breast cancer where 39% of patients undergoing lumpectomy will experience local recurrence of the disease.

[000182] Staging is a method of judging the progress of the cancer (solid tumor) in a patient. A simplified approach puts patients into three groups or stages based on how far the cancer has advanced:

[000183] *Stage 1:* The cancer can be treated by surgically removing part of the organ. This is also known as the resectable stage.

[000184] *Stage 2:* The cancer has advanced past the point of being resectable but is still confined to the organ itself.

[000185] *Stage 3:* The tumor has spread to other organs.

[000186] Many cancers are treated with anti-proliferative agents including, for example, 5-fluorouracil (Efudex®), vinca alkaloids (for example, vincristine (Oncovin®)), anthracyclines (for example, doxorubicin (Adriamycin®)), cisplatin (Platinol-AQ®), gemcitabine hydrochloride (Gemzar®), methotrexate and paclitaxel. Some examples of the toxicities associated with the anti-proliferative agents, methotrexate and paclitaxel, are discussed elsewhere herein. Methotrexate has been used to treat several cancers including, for example, bladder, breast, cervical, head and neck, hepatic, lung, and testicular cancers. Paclitaxel has been used to treat several cancers including, for example, ovarian, breast, and non-small cell lung cancers (*Compendium of Pharmaceutical and Specialties Thirty-fifth Edition, 2000).*

[000187] Toxicities due to 5-fluorouracil can include cardiovascular toxicity such as myocardial ischemia; central nervous system toxicities such as euphoria, acute cerebellar syndrome and ataxia; dermatologic toxicities such as alopecia and dermatitis; gastrointestinal toxicities such as nausea, vomiting and oral or gastrointestinal ulceration; hematologic toxicities such as leukopenia, thrombocytopenia and anemia; hypersensitivity toxicities such as anaphylaxis and contact hypersensitivity; ocular toxicities such as increased lacrimation, photophobia and conjunctivitis; and, other toxicities such as fever. 5-fluorouracil has been used to treat many cancers including, for example, breast, colorectal, gastric, hepatic, bladder, head and neck, non-small cell lung, ovarian, pancreatic, and prostate cancers (*Compendium of Pharmaceutical and Specialties Thirty-fifth Edition, 2000).*

[000188] Toxicities due to vincristine include central nervous system toxicities such as seizures in children and hallucinations; dermatologic toxicity such as alopecia; extravasation toxicity such as vesicant; gastrointestinal toxicities such as nausea, vomiting, constipation and stomatitis;

hematologic toxicity such as myelosuppression; neurologic toxicities such as peripheral neuropathy and autonomic neuropathy; ocular toxicities such as double vision, transient blindness and optic atrophy; renal/metabolic toxicities such as urinary retention, hyperuricemia and bladder atony; respiratory toxicity such as shortness of breath; and, other toxicity such as fever in children. This anti-proliferative agent has been used to treat several cancers including, for example, Hodgkin's disease, small cell lung, Wilm's tumor, and testicular cancers (*Compendium of Pharmaceutical and Specialties Thirty-fifth Edition, 2000).*

[000189] Toxicities due to doxorubicin include cardiovascular toxicities such as electrocardiographic abnormalities and cardiomyopathy; dermatologic toxicities such as alopecia and nail changes; extravasation hazard toxicity such as vesicant; gastrointestinal toxicities such and nausea, vomiting and stomatitis; genitourinary toxicity such as red coloration of urine; hematologic toxicity such as myelosuppression; hypersensitivity toxicities such as anaphylaxis and skin rash; ocular toxicity such as conjunctivitis; reproductive toxicity such as infertility; and, other toxicity such as hyperuricemia. This anti-proliferative agent has been used to treat several cancers including, for example, breast, small cell lung, and ovarian cancers (*Compendium of Pharmaceutical and Specialties Thirty-fifth Edition, 2000).*

[000190] Toxicities due to cisplatin include cardiovascular toxicity such as electrocardiographic changes; dermatologic toxicity such as hyperpigmentation; extravasation hazard toxicity such as irritant; gastrointestinal toxicities such as nausea and vomiting; hematologic toxicities such as myelosuppression and hemolytic anemia; hypersensitivity toxicity such as anaphylactic; neuromuscular toxicity such as peripheral neuropathy and acute encephalopathy; ocular toxicity such as retrobulbar neuritis; otologic toxicities such as hearing loss and tinnitus; renal/metabolic toxicities such as toxic nephropathy and hypokalemia; and, other toxicity such as infertility. This anti-proliferative agent has been used to treat several cancers including, for example, bladder, small cell lung, ovarian, testicular, brain, breast, cervical, head and neck, hepatoblastoma, and thyroid cancers (*Compendium of Pharmaceutical and Specialties Thirty-fifth Edition, 2000).* Toxicities due to gemcitabine hydrochloride include, for example, hematologic toxicities such as myelosuppression; gastrointestinal toxicities such as nausea, vomiting and stomatitis; hepatic toxicities such as transient elevations of serum transaminases; renal toxicities such as proteinuria, hematuria, hemolytic uremic syndrome and renal failure; dermatologic toxicity such as rash and

alopecia; edema toxicities such as edema and peripheral edema; and, other toxicity such as fever. This anti-proliferative agent has been used to treat pancreatic and non-small cell lung cancers (*Compendium of Pharmaceutical and Specialties Thirty-fifth Edition, 2000).*

[000191] The present discussion comprises prevention or treatment of localized cancers or solid tumors that can be treated include those of the prostate, breast, pancreas, liver, kidney, genitourinary system, brain, gastrointestinal system, respiratory system, and head and neck. The compositions, etc., herein may prevent or treat cancers, including metastases, by allowing controlled release of high-molecular-weight fucan at a site somewhat distant from the target tumors by allowing effective concentrations of the high-molecular-weight fucan to reach the tumors and/or metastases by diffusion or even systemic transport. Some of these cancers are discussed further in the following paragraphs.

Prostate Cancer

[000192] Prostate cancer is a malignant tumor that arises in the cells lining the prostate gland. In the U.S., an estimated 200,000 patients will develop prostate cancer this year, and more than 30,000 will die of the disease. Prostate cancer has a death to new cases ratio of ~15%. The cancer may remain within the prostate, or it may spread to surrounding tissues or to distant sites (most often lymph nodes and bone). Usually prostate cancer spreads silently, producing symptoms only when it has progressed beyond the prostate. If prostate cancer has been diagnosed and treated during early stages, in some studies patients have had a 5-year survival rate of 94%.

[000193] Prostate cancer is often discussed as a disease of men over age 50. In fact, 80% of men with prostate cancer are 60 years of age and older. A man's chances of being diagnosed with prostate cancer during his lifetime are about 1 in 10, roughly the same as a woman's chances of having breast cancer. The number of reported new cases has risen dramatically in recent years as a result of improved tests that can detect the disease early in its development, often long before symptoms appear. The likelihood of developing prostate cancer in any given year increases with age but rises dramatically after age 50.

[000194] Current treatment options for prostate cancer depend upon the extent of disease progression, the patient's age and overall health. Elderly patients, who have only early stage cancer or who suffer from additional, more serious diseases, may be treated conservatively, whereas those

whose cancer is advanced may undergo more aggressive treatment. Prostate cancer has been treated by various methods, including radiation therapy (external beam radiation or brachytherapy), hormone withdrawal or castration (surgical or chemical), anti-proliferative agents, surgery, and expectant therapy (that is, "watchful waiting"). No treatment guarantees an absolute cure, and some have considerable side effects.

[000195] Early stage prostate cancer (that is, the tumor is localized to the prostate) may be treated with "watchful waiting". Surgery for prostate cancer has been recommended for patients whose overall health has been otherwise good and the tumor is confined to the prostate gland. A common treatment for localized cancer of the prostate in men under the age of 70 has been radical prostatectomy (that is, surgical removal of the prostate).

[000196] Patients whose cancer is localized in the prostate area are commonly treated with external beam radiation (EBR). The radiation kills cancer cells and shrinks tumors. EBR accounts for less than 20% of localized prostate cancer treatment, with approximately 50% of these patients experiencing post radiation recurrences of the disease. Combined with early stage prostate cancer detection and increased demand from patients, brachytherapy (*i.e.*, local radiation therapy) use has been expected to grow. In 1995, only 2.5% of newly diagnosed patients were treated using brachytherapy. Brachytherapy involves the implantation of radioactive metal "seeds" in the prostate tumor.

[000197] Treatment for prostate cancer that has spread involves removal of the testicles or hormone therapy. Both are used to inhibit or stop the production of the testosterone that has been driving the cancer growth. Approximately 20% of all prostate cancer patients undergo hormone withdrawal therapy. Hormone therapies include goserelin acetate (Zoladex®) or leuprolide acetate (Lupron®). Anti-proliferative agents used to treat prostate cancer have included 5-fluorouracil.

Breast Cancer

[000198] In the U.S., breast cancer has been the most common cancer among women, with about 180,000 new cases diagnosed every year (male breast cancer accounts for about 5% of all diagnosed breast cancers). It has been surpassed only by lung cancer as a cause of death in women, and it has been responsible for approximately 50,000 deaths annually. An American woman has a one in eight (or about 13%) chance of developing breast cancer during her lifetime. Over the

past decade, most reported breast cancers were small, primary (arising independently; not caused by a metastasis) tumors. Roughly 70% to 80% of newly diagnosed patients exhibited early-stage disease (Stage 1 or 2), and a majority had no involvement of the axillary (underarm) lymph nodes.

[000199] Most breast cancers are carcinomas (that is, malignant tumors that grow out of epithelial tissues). Less than 1% of breast cancers are sarcomas, or tumors arising from connective tissue, bone, muscle or fat. In addition, most breast cancers (about 75%) are ductal carcinomas, arising in the tissues that line the milk ducts. A much smaller number of cancers (about 7%) are found within the breast lobules and are called lobular carcinomas. Paget's disease (cancer of the areola and nipple) and inflammatory carcinoma account for nearly all other forms of breast cancer.

[000200] Breast cancer treatment has been complicated and depends on many factors. Two important factors are the type of tumor and the stage of progression. Tumor characteristics, in particular, help to separate individuals into two groups: (1) those who are at low risk of cancer recurrence and (2) those who are at high risk of cancer recurrence. Specific prognostic factors place patients in either of these groups. These factors include tumor size; presence of female sex hormone estrogen and progesterone (ER/PR) receptors; cellular growth cycle phase (whether tumor cells are actively dividing or are in "S-phase"); presence of a protein known as "her-2-neu protein"; tumor grade, an indicator of tumor cell differentiation or change; and, tumor ploidy, the number of sets of genetic material within tumor cells.

[000201] Treatment of primary disease without significant lymph node involvement has been by lumpectomy and radiotherapy. More significant lymph node involvement may warrant mastectomy and removal of auxiliary lymph nodes. At this stage the chance of metastasis and local recurrence has been high. Treatment of metastatic disease has been palliative, involving radiation therapy and chemotherapy, which are immunosuppressive, cytotoxic and leukopenia. Anti-proliferative agents including, for example, 5-fluorouracil, doxorubicin, methotrexate, and paclitaxel, have been approved for use against breast cancer.

Pancreatic Cancer

[000202] The pancreas is an organ of the digestive system located near the stomach and small intestine. It has two major functions: the production of enzymes and hormones. Cancers of the

pancreas can occur in the exocrine (*i.e.*, enzymes) pancreas (*e.g.*, classic pancreatic adenocarcinomas) or can occur in the endocrine (*i.e.*, hormones) pancreas.

[000203] Cancers of the exocrine pancreas are a very serious health issue. In the U.S., approximately 28,000 patients are diagnosed with pancreatic cancer, while about the same number die annually from this disease. Pancreatic cancer occurs equally in males and females. Due to difficulties in diagnosis, the intrinsic aggressive nature of pancreatic cancers, and the sparse systemic treatment options available, only approximately 4% of patients diagnosed with pancreatic adenocarcinoma live for 5 years after diagnosis. Pancreatic cancer has been the 5th leading cause of cancer death, following breast, lung, colon, and prostate cancer.

[000204] The choice of treatment for pancreatic cancer depends largely on the stage of the tumor. Possible treatments include surgery, anti-proliferative agents, radiation, and biological therapy. Surgery has been usually reserved for Stage 1 patients whose cancer is deemed resectable. Sometimes a combination of therapies, such as radiation and anti-proliferative agent given before or after surgery, can increase a patient's chances of survival. Pancreatic cancer that is deemed unresectable (usually Stage II or later) may be treated using anti-proliferative agents in clinical trials. Anti-proliferative agents, such as, for example, gemcitabine or 5-fluorouracil have had some effect against pancreatic cancer and gemcitabine has been used as a palliative agent. Toxicities due to these anti-proliferative agents are discussed elsewhere herein. Radiation therapy has some effect against pancreatic cancer when used in combination with chemotherapy. Radiation therapy alone may subdue symptoms. This form of treatment has also been used in Stage II or later pancreatic cancers.

Bladder Cancer

[000205] In 1998, it was estimated that over 54,000 new cases of bladder cancer would be diagnosed in the U.S. and about 15,000 deaths would be attributed to the disease. Bladder cancer has been the fourth most common cancer among American men and the ninth most common cancer among American women. It occurs three times more frequently in men than in women. Primarily a disease of older men, bladder cancer has been a significant cause of illness and death. The risk of bladder cancer increases steeply with age (80% of cases occur in people older than 50 years), with over half of all bladder cancer deaths occurring after age 70. In white men over 65, the annual

disease rate of bladder cancer has been approximately 2 cases per 1,000 persons; this contrasts with a rate of 0.1 cases per 1,000 persons under 65. During one's lifetime, the probability of developing bladder cancer has been greater than 3%; however, the probability of dying, from bladder cancer has been small (<1%). Bladder cancer rarely occurs in people who are younger than 40 years of age.

[000206] Recent studies suggest that certain genes and inherited metabolic abilities may play a role in bladder cancer. Transitional cell carcinoma (TCC) has been the most common form of bladder cancer. TCC usually occurs as a superficial (surface), papillary (wart-like), exophytic (outward-growing) mass upon a stalk-like base. In some cases, though, TCC may be attached on a broad base or it may appear ulcerated (within an indented lesion). Papillary TCCs often start out as areas of hyperplasia that later dedifferentiate or lose individual cell characteristics. Only about 10% to 30% of papillary TCCs develop into invasive cancers. By contrast, nonpapillary forms of TCC are more likely to become invasive. As noted, such TCCs may appear ulcerated or flat. Flat, nonpapillary TCC that has been made up of anaplastic epithelium has been classified as carcinoma *in situ* (CIS or TIS). The tissue of CIS contains cells that are large, have noticeable nucleoli (round body within a cell; involved in protein synthesis), and lack normal polarity.

[000207] The treatment of bladder cancer depends upon many factors. The most important of these factors are the type of tumor that is present and its stage. Common treatments include transurethral resection (TUR), electrosurgery, laser surgery, intravesical therapy, anti-proliferative agents, surgical therapy, cystectomy, and radiation therapy. Examples of anti-proliferative agents used to treat bladder cancer include, for example, 5-fluorouracil, cisplatin and methotrexate. Toxicities due to the anti-proliferative agents, 5-fluorouracil, cisplatin, and methotrexate, are discussed elsewhere herein.

Brain Cancer

[000208] Brain tumors are often inoperable and more than 80% of patients die within 12 months of diagnosis. Approximately 18,000 new cases of primary intracranial (brain) cancer are diagnosed each year in the U.S. This represents about 2 percent of all adult cancers. More than 50 percent of these are high-grade gliomas (*i.e.,* glioblastoma multiform and anaplastic

astrocytoma tumors). Patients with these tumors often suffer from severe disabilities such as motor dysfunction, seizures, and vision abnormalities.

[000209] Tumors that begin in brain tissue are known as primary brain tumors. Primary brain tumors are classified by the type of tissue in which they begin. The most common brain tumors are gliomas, which begin in the glial (supportive) tissue. Others include astrocytomas, brain stem gliomas, ependymomas and oligodendrogliomas.

[000210] Surgical removal of brain tumors has been recommended for most types and in most locations and should be as complete as possible within the constraints of preservation of neurologic function. An exception to this rule has been for deep-seated tumors, such as pontine gliomas, which are diagnosed on clinical evidence and are treated without initial surgery approximately 50% of the time. In many cases, however, diagnosis by biopsy is performed. Stereotaxic biopsy can be used for lesions that are difficult to reach and resect. Patients who have brain tumors that are either infrequently curable or unresectable should be considered candidates for clinical trials that evaluate radiosensitizers, hyperthermia, or interstitial brachytherapy used in conjunction with external-beam radiation therapy to improve local control of the tumor or for studies that evaluate new drugs and biological response modifiers.

[000211] Radiation therapy has a major role in the treatment of most tumor types and can increase the cure rate or prolong disease-free survival. Radiation therapy may also be useful in the treatment of recurrences in patients treated initially with surgery alone. Chemotherapy may be used before, during, or after surgery and radiation therapy. Recurrent tumors are treated with chemotherapy as well. Anti-proliferative agents used in the treatment of brain cancers include cisplatin. Examples of the toxicities associated with this anti-proliferative agent are discussed elsewhere herein.

Restenosis

[000212] Restenosis is a form of chronic vascular injury leading to vessel wall thickening and loss of blood flow to the tissue supplied by the blood vessel. This inflammatory disease can occur in response to vascular reconstructive procedures including any manipulation that relieves vessel obstruction. Thus, restenosis has been a major restrictive factor limiting the effectiveness of these procedures.

[000213] The present discussion comprises prevention or treatment of restenosis, for example by administering to a blood vessel a therapeutically effective amount of the combination of an oligonucleotide therapeutic and an anti-inflammatory agent. Suitable compositions include a polymeric carrier that can be surgically implanted at a restenosis site, or potential restenosis site, or can be injected via a catheter as a polymeric paste or gel. Suitable compositions may comprise high-molecular-weight fucans discussed herein.

Arthritis

[000214] Rheumatoid arthritis (RA) is a debilitating chronic inflammatory disease characterized by pain, swelling, synovial cell proliferation (pannus formation) and destruction of joint tissue. In the advanced stage, the disease often damages critical organs and may be fatal. The disease involves multiple members of the immune system (macrophages/monocytes, neutrophils, B cells and T cells) complex cytokine interactions and synovial cell malfunction and proliferation. Early aggressive treatment has been recommended with disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate, which drug is discussed elsewhere herein.

[000215] Crystal induced arthritis has been characterized by crystal induced activation of macrophages and neutrophils in the joints and is followed by excruciating pain for many days. The disease progresses so that the intervals between episodes gets shorter and morbidity for the patient increases. This disease has been generally treated symptomatically with non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac sodium (Voltaren®). This anti-inflammatory agent has toxicities which include central nervous system toxicities such as dizziness and headache; dermatologic toxicities such as rash and pruritus; gastrointestinal toxicities such as exacerbated ulcerative colitis and Crohn's disease; genitourinary toxicities such as acute renal failure and renal papillary necrosis; hematologic toxicities such as agranulocytosis, leukopenia and thrombocytopenia; hepatic toxicities such as elevated liver transaminases and hepatitis; and, other toxicities such as asthma and anaphylaxis.

[000216] The present discussion comprises prevention or treatment of rheumatoid arthritis, for example via administering to a patient a therapeutically effective amount of an oligonucleotide therapeutic and optionally an anti-inflammatory agent. Suitable compositions include a polymeric carrier that can be injected into a joint as a controlled release carrier of the anti-inflammatory agent

and microparticulates as controlled release carriers of the oligonucleotide therapeutic (which in turn has been incorporated in the polymeric carrier). Suitable compositions may comprise high-molecular-weight fucans discussed herein. Such polymeric carriers may take the form of polymeric microspheres, pastes or gels.

Inflammatory conditions

[000217] The compositions, etc., herein may optionally inhibit or treat inflammatory conditions involving neutrophils for example comprising administering to a patient compositions containing an oligonucleotide therapeutic and an anti-inflammatory agent. Examples of such conditions include crystal-induced arthritis; osteoarthritis; non-rheumatoid inflammatory arthritis; mixed connective tissue disease; Sjögren's syndrome; ankylosing spondylitis; Behçet's syndrome; sarcoidosis; psoriasis; eczema; inflammatory bowel disease; chronic inflammatory lung disease; neurological disorders; and, multiple sclerosis. Some of these diseases are discussed further in the following paragraphs.

Chronic inflammatory skin diseases (including psoriasis and eczema)

[000218] Psoriasis is a common, chronic inflammatory skin disease characterized by raised, thickened and scaly lesions which itch, burn, sting and bleed easily. While these diseases have cellular proliferation and angiogenic components in later stages of the disease, patients often have accompanying arthritic conditions. Symptoms may be treated with steroidal anti-inflammatory agents such as prednisone or anti-proliferative agents such as methotrexate, which agents are discussed elsewhere herein. The compositions herein may also be used to inhibit or otherwise treat and/or prevent chronic inflammatory skin diseases, for example psoriasis and/or eczema.

[000219] The following provides some additional representative examples of inflammatory diseases that can be treated with compositions discussed herein, include, for example, arterial embolization in arteriovenous malformations (vascular malformations); menorrhagia; acute bleeding; central nervous system disorders; and, hypersplenism; inflammatory skin diseases such as psoriasis; eczematous disease (atopic dermatitis, contact dermatitis, eczema); immunobullous disease; and, inflammatory arthritis which includes a variety of conditions including rheumatoid arthritis, mixed connective tissue disease, Sjögren's syndrome, ankylosing spondylitis, Behçet's

syndrome, sarcoidosis, crystal induced arthritis and osteoarthritis (all of which feature inflamed, painful joints as a prominent symptom).

Ischemia

[000220] Ischemia or ischaemia involves a restriction in blood supply, which may include a shortage of supply of oxygen, glucose and other components required for proper tissue function, resulting in damage and/or dysfunction of tissue. Ischemia can cause severe problems. For example, tissues can become anoxic, necrotic, and clots can form. Various attempts have been made to prevent and/or treat ischemia. For example, restoration of blood flow, or reperfusion. Restoration of blood, however, involves the reintroduction of oxygen, which can cause additional damage due to the production of free radicals, resulting in reperfusion injury. Reperfusion injury can cause severe problems. The compositions herein may be used to inhibit or otherwise treat and/or prevent, ischemia, and/or reperfusion injury.

Endotoxemia

[000221] Endotoxemia is the presence of endotoxins in the blood. Endotoxemia can cause severe problems. For example, endotoxemia can lead to septic shock. The compositions herein may be used to inhibit, or otherwise treat and/or prevent, endotoxemia.

Keloid scarring

[000222] Keloid trait causes wounds to heal with raised scars. Keloid traits' raised scars involve abnormal fibrous scarring. Keloid trait causes severe problems, for example, pain and disfigurement. The compositions herein may be used to inhibit, or otherwise treat and/or prevent, keloid trait and its resulting raised scars.

[000223] Keloid (keloid scar) is a type of scar that expands in growths over normal skin. Keloids involve abnormal collagen growth, including type I and type III collage abnormal growth. Keloids cause severe problems, for example, pain, itchiness, and if infected may ulcerate. Attempts have been made to treat or prevent keloids including the use of surgery, dressings, steroid injections and laser therapy. The compositions herein may be used to inhibit, or otherwise treat and/or prevent, keloids.

Dermatitis

[000224] Dermatitis includes inflammation of the skin including atopic dermatitis and contact dermatitis. For example, contact dermatitis involves localized rash and/or irritation of the skin following contact of the skin with a foreign substance. For example, atopic dermatitis is a chronically relapsing, pruritic skin disease. Atopic dermatitis is sometimes called prurigo Besnier, neurodermitis, endogenous eczema, flexural eczema, infantile eczema, childhood eczema and prurigo diathsique. Eczema is a disease in a form of dermatitis. Other types of dermatitis include spongiotic dermatitis, seborrhoeic dermatitis (dandruff), dyshidrotic dermatitis (pompholyx), urticaria, vesicular dermatitis (bullous dermatitis), and popular urticaria. Dermatitis can cause severe problems. For example, dry skin, skin rashes, skin edema, skin redness, skin itchiness, skin crusting, cracking, blistering, oozing and bleeding. Attempts have been made to treat or prevent dermatitis including the use of corticosteroids and coal tars. The compositions herein may be used to inhibit, or otherwise treat and/or prevent, dermatitis including atopic dermatitis, eczema, contact dermatitis, spongiotic dermatitis, seborrhoeic dermatitis, dyshidrotic dermatitis, urticaria, vesicular dermatitis, and popular urticaria.

Rosacea

[000225] Rosacea is a chronic disease or condition typically characterized by facial erythema. Rosacea can cause severe problems. For example, rosacea typically begins as redness on the forehead, nose or cheeks and can also cause redness on the neck, ears, scalp and chest. For example, rosacea can cause additional symptoms including telangiectasia, papules, pustules, painful sensations, and in advanced cases rhinophyma (red lobulated nose) may develop. Rosacea subtypes include erythematotelangiectatic rosacea, papulopustular rosacea, phymatous rosacea, and ocular rosacea. Attempts have been made to treat or prevent rosacea including the use of anti-inflammatories and antibiotics. The compositions herein may be used to inhibit, or otherwise treat and/or prevent, rosacea including its erythematotelangiectatic, papulopustular, rosacea and ocular subtypes.

Medical Device, Medical Material, Combination, and Pharmaceutical Products

[000226] The discussion herein also provides medical devices, medical materials, combination, and pharmaceutical products, comprising compositions as discussed herein in a medical device, medical material, combination product or pharmaceutically acceptable container. The products can also include a notice associated with the container, typically in a form prescribed by a governing agency regulating the manufacture, use, or sale of medical devices, medical materials, combination, and pharmaceuticals or biopharmaceuticals, whereby the notice is reflective of approval by the agency of the compositions, such as a notice that a high-molecular-weight fucan has been approved as an anti-proliferative agent or anti-inflammatory agent, e.g., for human or veterinary administration to treat proliferative diseases or inflammatory diseases (such as, for example, inflammatory arthritis, restenosis, surgical adhesions, psoriasis and peritonitis). Instructions for the use of the high-molecular-weight fucan herein may also be included. Such instructions may include information relating to the dosing of a patient and the mode of administration.

[000227] The present application is further directed to methods of making the various elements of the high-molecular-weight fucan, systems etc., discussed herein, including making the compositions themselves, as well as to methods of using the same, including for example treatment of the conditions, diseases, etc., herein.

[000228] The present application further comprises medical devices, medical materials, medical combination products, and pharmaceutical products for treatment of fibrous adhesions, arthritis, psoriasis or other diseases as desired comprising high-molecular-weight fucans presented herein. The materials, etc., can be used in a medicament for treating fibrous adhesions, such as a surgical adhesions, arthritis, psoriasis or other diseases as desired. Also provided are methods of manufacturing and using such medicaments able to reduce symptoms associated with at least one of fibrous adhesions, arthritis, and psoriasis in a patient including a human patient, comprising combining a pharmaceutically effective amount of a fucan such as fucoidan as discussed herein with a pharmaceutically acceptable excipient or buffer.

[000229] The following Examples provide exemplary discussions of certain embodiments herein but the disclosure and claims are not limited thereto.

Example 1: Chemical structural modification

[000230] An exudate-extract was obtained from *Laminaria Hyperborea.* The exudate-extract was filtered and small molecules were removed by tangential flow filtration (TFF) over a 100 kDa filter. A sample of the resulting retentate was lyophilized to obtain otherwise unmodified sample A. The resulting retentate was brought to 0.25 M NaOH by addition of 10 M NaOH solution and left at room temperature for 16 hours. The resulting sample was then centrifugally filtered over a 50 kDa filter and the resulting retentate collected and lyophilized to obtain base-treated sample B. Both unmodified sample A and base-treated sample B were analyzed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and the resulting ^{1}H-NMR spectrum are shown in **FIG. 9A.**

[000231] **FIG. 9A** demonstrates the chemical structural modification of the fucan accomplished, the broad peak with a chemical shift about 2.0 ppm that is present in the unmodified sample A is not present in the base-treated sample B.

[000232] Unmodified sample A and base-treated/modified sample B were further analyzed by 2D ^{1}H-^{13}C heteronuclear multiple quantum coherence (HMQC). The HMQC spectra, shown in **FIG. 9B**, were acquired at 70 °C with solvent signal suppression on a 600 MHz spectrometer equipped with 5-mm cold probe. A high number of scans of the HMQC spectra were acquired in the range from 10-30 ppm in the carbon dimension in 8 increments of 256-512 scans each; such scans were combined to create the spectra in **FIG. 9B**.

[000233] The HMQC spectra for unmodified sample A has a cross-peak corresponding to O-acetyl groups, indicated by the signal circled in **FIG. 9B**. This cross-peak is not present in the spectra for base-treated sample B. This demonstrates the removal of acetyl groups from the fucan, and thus chemical structural modification of the fucan in base-treated sample B by the NaOH treatment.

Example 2: Tangential flow filtration

[000234] A high-molecular-weight fucan may be obtained by tangential flow filtration. A broad distribution starting fucan is dissolved in distilled water at 50mg/mL. In this example, the broad distribution fucan is diafiltered against distilled water over a 100kDa molecular weight cut-off (MWCO) tangential flow filter (TFF) cassette for 4 diavolumes to remove unwanted lower molecular weight components and the retentate of the TFF process is collected comprising the high-molecular-weight fucan. The diafiltration may be accomplished with any desired MWCO

TFF filter, for example a 50kDa, 70kDa, 100kDa, 300kDa, 500kDa and 1000kDa MWCO TFF cassette. The resulting high-molecular-weight fucan has a higher average molecular weight than the broad molecular weight distribution starting fucan.

Example 3: Sequential tangential flow filtration segmentation

[000235] An input broad molecular weight distribution starting fucoidan having a weight average molecular weight of 365.6 kDa and Polydispersity index (PDI)=3.58 that had been pre-filtered through a 0.22 micron filter was provided. A TFF filter cassette of 100 kDa MWCO supplied by Pall of Port of Washington was employed as the higher MWCO TFF cassette and a 50 kDa TFF cassette supplied by Pall of Port of Washington employed as the lower MWCO TFF cassette. The process was repeated for the following TFF cassette pairs: a TFF filter of MWCO 300 kDa supplied by Millipore of Burlington, MA and a TFF filter of MWCO 100 kDa supplied by Pall of Port of Washington, a TFF filter of MWCO 50 kDa supplied by Pall of Port of Washington and a filter of 30 kDa supplied by Pall of Port of Washington, a TFF filter of 30 kDa supplied by Pall of Port of Washington and a TFF filter of 10 kDa supplied by Pall of Port of Washington. The cassettes were all of the Polyethersulfone (PES) type.

[000236] After sequential tangential low filtration as discussed above, the various obtained fucans, including high-molecular-weight fucans comprising high-molecular-weight segments of the starting fucan molecular weight distribution, were analyzed using gel permeation chromatography (GPC). The results are shown in Table 1 below.

	GPC PRT (Mins)	**PMW (kDa)**	**WAMW (kDa)**	**NAMW (kDa)**	**% dist. MW> 100kDa**	**% dist. MW> 200kDa**	**% dist. MW> 500kDa**	**PDI**
Input	25.51	299.6	365.6	102.2	76.3	57.2	23.1	3.58
MWCO of TFF filter pairs (kDa)								
300-100	25.62	278.3	394.2	151.9	83.5	63.3	24.9	2.60
100-50	27.96	59.8	125.1	42.6	37.3	17.4	3.3	2.94
50-30	30.33	12.6	20.6	9.8	1.6	0.3	0.0	2.11
30-10	34.22	1.0	2.1	1.2	--	--	--	1.66

Table 1. TFF segmentation of fucoidan

Example 4: Cation augmented tangential flow filtration

[000237] A broad molecular weight distribution input starting fucoidan composition in a starting solution having a weight average molecular weight of 436.4 kDa with a polydispersity index (PDI) of 3.24 that had been pre-filtered through a 0.22 micron filter was provided. Choline, a biocompatible water-soluble quaternary ammonium salt, was selected as the chemical additive. Choline was added in a 1:2 choline:fucoidan mass ratio to the pre-filtered starting solution and the resulting mixture stirred until the choline was dissolved. The choline may or may not bind to the sulfate sites on the fucoidan molecules. In a first TFF process, the choline treated-fucoidan solution was then subjected to tangential flow filtration over a 300 kDa filter cassette to obtain a first retentate comprising a choline bound high-molecular-weight fucoidan, being a choline-treated retentate. During this first choline-augmented TFF process, the choline treated-fucoidan solution was diafiltered with four diavolumes of 1% w/v choline flush solution. The choline-treated retentate of the first TFF process was collected and subjected to a second TFF process to replace the choline cations with sodium cations.

[000238] The second TFF process, being a decholinating TFF process, comprised diafiltering the choline-treated retentate of the first TFF process over a 50 kDa filter cassette while treating the retentate with NaCl to replace the choline cations with sodium cations. In this example, the choline-treated retentate was diafiltered with 4 volumes of 2 M NaCl to remove the choline additive from the high-molecular-weight fucoidan. The decholinated retentate of this second TFF process was then diafiltered with deionized water until the conductivity of the permeate had dropped to below 5 mS/cm to indicate the removal of excess NaCl. After cation augmented TFF as discussed above, samples of the various retentates in the process comprising the high-molecular-weight fucan were analyzed using gel permeation chromatography (GPC). The results are shown in Table 2 below.

	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
Input	24.67	436.4	490.7	151.3	82.3	66.1	34.8	3.24
MWCO of TFF filter retentate (kDa)								

300	24.71	423.6	525.5	206.7	88.5	72.6	37.3	2.54
100	24.10	639.8	740.7	411.0	97.9	90.6	59.1	1.80

Table 2. Cation-augmented TFF segmentation of fucoidan

Example 5: Centrifugal precipitation

[000239] A starting solution containing 0.5% w/v starting fucoidan composition that had been pre-filtered through a 0.22µm pre-filter was provided. A step gradient of 20%, 10%, and 5% w/v sucrose in water was created in a centrifuge tube, with 5% layer being the topmost layer and the 20% layer being in the bottom of the centrifuge tube. The 0.5% starting solution containing the starting fucoidan composition was then layered on top of the 5% w/v sucrose layer. The resulting layer structure is shown in **FIG. 10**. The tube with these four layers was then centrifuged at 190,000 gravities (g) for 6 hours. The supernatant solution was decanted and the precipitate remaining in the centrifuge tube, containing the desired high-molecular-weight fucan, was re-dissolved in water. The re-dissolved high-molecular-weight fucan was then analyzed by gel permeation chromatography (GPC). The results are shown in Table 3 below.

	GPC PRT (Mins)	**PMW (kDa)**	**WAMW (kDa)**	**NAMW (kDa)**	**% dist. MW> 100kDa**	**% dist. MW> 200kDa**	**% dist. MW> 500kDa**	**PDI**
Input	24.57	471.7	590.1	200.6	87.4	72.3	40.3	2.94
Re-dissolved fucoidan precipitate	22.95	1472.9	1113.0	492.3	98.2	91.0	69.1	2.26

Table 3. Centrifugal precipitation of fucoidan using a 5%-10%-20% sucrose barrier

Example 6: Gel electrophoresis-extraction

[000240] A starting fucoidan composition with a broad molecular weight distribution was provided. The starting fucoidan composition was dissolved at 50 mg/mL, pre-filtered through a 0.22 micron filter and loaded onto a 0.5% agarose gel cast from 380 mL of agarose. The loaded gel was submerged in a running buffer of 40 mM tris-acetate 1 mM EDTA, also known as TAE

buffer. A voltage of 90 V was applied across the buffer for 50 minutes with the anode proximate the starting fucoidan composition well. This allowed the fucoidan to separate by mass to charge ratio through the gel. For visualization purposes, the gel was stained with methylene blue, a dye known to stain fucoidan. The agarose gel was then cut in 1 cm wide segments parallel to the well, starting 1 cm from the well. The segments of the gel were agitated in distilled water to extract the fucoidan segments from the gel by shaking the mixture.

[000241] The electrophoresis-extracted fucoidan segments, potentially comprising high-molecular-weight fucoidans, were analyzed by gel permeation chromatography (GPC). The results are shown in Table 4 below.

	GPC PRT (Mins)	**PMW (kDa)**	**WAMW (kDa)**	**NAMW (kDa)**	**% dist. MW> 100kDa**	**% dist. MW> 200kDa**	**% dist. MW> 500kDa**	**PDI**
Input	24.67	462.6	581.3	170.9	86.7	73.0	40.8	3.40
Distance of gel segment from well								
1-2 cm	25.07	349.4	619.9	81.33	71.3	55.9	30.7	7.62
2-3 cm	25.16	327.8	362.0	118.2	76.2	56.7	23.9	3.06
3-4 cm	25.47	263.6	288.4	103.7	70.9	48.8	16.7	2.78
4-5 cm	25.58	242.7	279.1	66.0	62.8	42.2	14.9	4.23

Table 4. GPC results of the electrophoresis-based separation of pre-filtered starting fucoidan across agarose gel with TAE buffer.

Example 7: Membrane dialysis

[000242] A 5% w/v starting solution containing a starting fucoidan composition that had been pre-filtered through a 0.22 micron filter was provided. The starting solution was placed in a cellulose acetate dialysis tubing of nominal molecular weight cutoff 300kDa. The dialysis tubing was sealed

and placed in a container with 20 liters of deionized water. The deionized water was replaced with fresh deionized water every 12 hours to ensure continuous diffusion across the membrane pores. The dialysis process was allowed to continue for about 5 days.

[000243] The pre-filtered starting fucoidan composition and the post-dialysis, high-molecular-weight fucoidan in the dialysis tube were both analyzed using gel permeation chromatography (GPC). The results are shown in Table 5 below.

	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
Input	24.57	471.7	590.1	200.6	87.4	72.4	40.3	2.94
Dialyzed Fucoidan	24.29	599.1	777.0	374.5	96.8	88.6	57.0	2.07

Table 5. GPC results of the dialysis of fucoidan against deionized water across a 300kDa membrane.

Example 8: Selective precipitation

[000244] A starting fucoidan composition that had been pre-filtered through a 0.22µm pre-filter and desalted via diafiltering with deionized water over a 100kDa TFF cassette to remove unwanted low molecular weight salts that may interfere with the precipitation process was provided. A series of identical starting solutions of the prefiltered and desalted starting fucoidan in distilled water were prepared. The solvent compositions were brought up to different pre-determined concentrations of ethanol. This prepared the different solvent environments for the precipitation of the fucoidan from the solution compositions identified in Table 6 below. A minimal amount of an ionic agent in the form of NaCl was added to each solution composition to initiate the precipitation of fucoidan from the solution. The mixes of precipitate and solution composition were centrifuged at 2300 gravities for 10 minutes. The liquid supernatant was decanted in each case and the solid fucoidan collected.

The solid fucoidan were re-dissolved in distilled water and analyzed by gel permeation chromatography. The results are shown in Table 6 below.

% Ethanol in the fucoidan solution	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
40	24.85	394.1	447.0	133.5	76.6	62.5	31.3	3.35
50	25.96	182.1	347.9	149.2	80.8	53.9	20.3	2.33
60	25.43	263.1	335.5	119.1	73.9	51.9	20.0	2.82
70	24.91	376.1	382.6	117.2	75.7	56.8	25.4	3.26

Table 6. Selective precipitation of fucoidan using ethanol as precipitating solvent

Example 9: Anionic adsorption

[000245] A starting solution containing about 500 mg of a broad molecular weight distribution desalted starting fucoidan was recirculated on about 14 mL of DEAE-Sepharose® resin for about 16 hours to bind the low molecular weight fucoidan to the active sites on the resin. After about 16 hours the recirculating solution was collected. This separated the high-molecular-weight fucoidan from the low molecular weight fucoidan that had bonded to the resin. 10% w/v NaCl was then recirculated on the resin for 4 hours to displace the low molecular weight fucoidan from the resin. The fucoidan rich-salt solution was then collected and desalted over a 5 kDa centrifugal filter to separate the collected low molecular weight fucoidan from the unwanted salt. GPC was performed on the desalted starting fucoidan, the high-molecular-weight fucoidan not adsorbed during the ion exchange process, and the low molecular weight fucoidan extracted from the resin. The results are shown in Table 7 below.

	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
Input	24.57	462.4	576.6	198.0	87.1	71.9	39.6	2.91
Fucoidan not adsorbed	24.20	601.3	844.5	391.4	96.8	90.9	60.5	2.16
Fucoidan adsorbed	25.82	193.6	245.8	119.2	73.0	43.8	10.7	2.06

Table 7. Anion exchange segmentation of fucoidan: DEAE-Sepharose as resin

Example 10: Anionic adsorption

[000246] A starting solution containing about 1 g of a broad molecular weight distribution desalted fucoidan was mixed with about 10 g Amberlyst® A26 resin for about 16 hours to bind the low molecular weight fucoidan to the active sites on the resin. The solution containing the high-molecular-weight fucoidan was subsequently separated from the resin by decanting. 20% w/v NaCl was then mixed with the resin for about 4 hours to displace the low molecular weight fucoidan from the resin. The fucoidan rich salt solution was then separated from the resin and desalted over a 5 kDa centrifugal filter to separate the collected low molecular weight fucoidan from the unwanted salt. GPC was performed on the desalted starting fucoidan, the high-molecular-weight fucoidan not adsorbed during the ion exchange process, and the low molecular weight fucoidan extracted from the resin. The results are shown in Table 8 below.

	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
Input	25.02	517.7	536.9	148.2	82.7	67.7	38.1	3.62
Fucoidan not adsorbed	24.73	625.8	867.4	463.1	98.7	93.0	62.6	1.87
Fucoidan adsorbed	27.30	112.4	172.3	86.4	58.0	25.4	4.7	2.00

Table 8. Anion exchange separation of fucoidan: Amberlyst ™ A26 OH

Example 11: Anionic adsorption

[000247] A starting solution containing about 1 g of a broad molecular weight distribution desalted fucoidan was mixed with about 10 g of three different resins, being Amberlyst® A26 OH^-, Ambersep® 900 OH^-, and Lewatit® VPOC 1065 in three separate containers. The solution-resin mixtures were incubated for about 16 h to bind the low molecular weight fucoidan to the active sites on the resin. The solution containing the high-molecular-weight fucoidan was subsequently separated from the resin by decanting. The pores of the Amberlyst® and Ambersep® product had quaternary amine groups while the pores of the Lewatit product had primary benzylamine groups. The first two products were strongly basic anion exchange resins, while the third was a weakly basic anion exchange resin. The fucoidan not adsorbed during the ion-exchange process were then analyzed by GPC. The results are shown in Table 9 below.

	GPC PRT	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW>	% dist. MW>	% dist. MW>	PDI

	(Mins)				100kDa	200kDa	500kDa	
Input	24.66	461.9	521.5	151.8	83.0	67.3	36.5	3.44
Resin used								
Ambersep® 900 OH^-	24.51	513.0	609.1	323.6	96.1	85.5	47.3	1.88
Amberlyst® A26 OH^-	24.58	489.8	591.0	309.5	95.6	83.5	45.1	1.91
Lewatit® VPOC 1065	24.54	501.2	585.6	219.6	89.5	75.3	42.4	2.67

Table 9. Anion exchange separation of fucoidan: comparison of fucoidans prepared by recirculation on 3 resins.

Example 12: Anionic adsorption

[000248] A starting solution containing about 1g of a broad molecular weight distribution desalted fucoidan was mixed with about 10g Ambersep® 900 OH^- for up to 53 hours. The fucoidan in the mixture not adsorbed during the ion-exchange process were then analyzed by GPC at various time points during the anion adsorption process. The results are shown in Table 10 below.

	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
Input	26.71	624.2	955.9	339.9	95.2	84.6	56.3	2.81
Ion exchange time (hours)								
1	26.66	642.4	1049.2	386.0	96.6	87.3	59.5	2.72
4	26.42	756.4	1151.7	470.9	98.2	91.7	65.5	2.45
24	26.33	801.9	1205.2	589.9	99.5	95.9	72.1	2.04
53	26.25	843.6	1257.9	656.9	99.8	97.5	75.6	1.91

Table 10. Anion exchange separation of fucoidan: comparison of anion exchange times

Example 13: Anionic adsorption

[000249] A starting solution containing about 1g of the broad molecular weight distribution desalted fucoidan was mixed with various amounts of Ambersep® 900 OH^- for about 16 hours to bind the low molecular weight fucoidan to the active sites on the resin. The solution containing

the high-molecular-weight fucoidan was subsequently separated from the resin by decanting. The fucoidan not adsorbed during the ion-exchange process were then analyzed by GPC. The results are shown in Table 11 below.

	GPC PRT (Mins)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% dist. MW> 100kDa	% dist. MW> 200kDa	% dist. MW> 500kDa	PDI
Input	24.66	442.4	498.5	146.3	82.4	66.1	34.8	3.41
Mass ratio of Fucoidan: resin								
1:1	24.51	491.1	548.1	203.2	87.6	72.0	39.0	2.70
1:5	24.48	500.9	633.9	306.6	95.3	82.9	46.6	2.07
1:10	24.41	523.8	688.1	376.4	98.0	88.8	51.9	1.83

Table 11. Anion exchange separation of fucoidan: comparison of different fucoidan to resin ratios

Example 14: Preparative gel permeation chromatography

[000250] A starting fucoidan composition with a broad molecular weight distribution is provided. The starting fucoidan composition is dissolved at 10 mg/mL in 60 mL 0.1 M sodium nitrate. 20 mL of the starting solution containing the starting fucoidan composition is pumped at 40 mL/min through each of a 50 mm inner diameter, 250 mm length column containing Sepax® SRT-10/10C SEC-1000, Agilent® PL Aquagel®-OH MIXED-H and TSKGel® G4000SW respectively, all of which contain modified silica-hydrophilic bonded phase gel media. Elution is carried out at the same flow rate using 0.1 M sodium nitrate. After 5 minutes of elution, 40 mL aliquots are collected until a total of 1000 mL, or 25 aliquots, have been collected. The molecular weight distribution of a sample of each aliquot is measured by analytical GPC. Aliquots containing weight average molecular weights between 200 kDa and 600 kDa are pooled. Aliquots containing weight average molecular weights between 600 kDa and 1000 kDa are pooled. Aliquots containing weight average molecular weights between 1000 kDa and 1400 kDa are pooled. Aliquots containing weight average molecular weights between 1400 kDa and 1800 kDa are pooled. The rest of the aliquots are discarded. Each pooled preparative GPC aliquot composition contains a desired high-molecular-weight fucan.

Example 15: Preparative gel permeation chromatography

[000251] A starting fucoidan composition with a broad molecular weight distribution is provided. The starting fucoidan composition is dissolved at 10 mg/mL in 60 mL 0.1M sodium nitrate. 20 mL of the starting solution containing the starting fucoidan composition is pumped at 40 mL/min through each of a 50 mm inner diameter, 250 mm length column containing Waters® HSPgel AQ MB-H, PSS® Suprema® Combination Ultrahigh and TSKGel® GMPWXL respectively, all of which contain hydroxylated polymethacrylate-based gel media. Elution is carried out at the same flow rate using 0.1M sodium nitrate. After 5 minutes of elution, 40 mL aliquots are collected until a total of 1000 mL, or 25 aliquots, have been collected. The molecular weight distribution of a sample of each aliquot is measured by analytical GPC. Aliquots containing at least 90% of their molecular weight distribution above 100 kDa, at least 80% of their molecular weight distribution above 200 kDa and/or at least 50% of their molecular weight distribution above 500 kDa are pooled. The rest of the aliquots are discarded. Each pooled preparative GPC aliquot composition contains a desired high-molecular-weight fucan.

Example 16: Preparation of low and high-molecular-weight fucans

[000252] The methods discussed herein may be used, combined, modified and permuted in any manner to obtain high-molecular-weight fucans.

[000253] Twenty fucans, some with high and some with low molecular weights, were prepared from feedstock/starting fucan compositions having broad molecular weight distributions to evaluate the efficacy of high and low molecular weight fucans in medical and surgical applications. The twenty fucans are hereafter referred to as fucan 1 to fucan 20. Fucan 1 to fucan 5 were light brown solids. Fucan 6, fucan 8 to fucan 15 and fucan 17 were white solids. The preparation of low-molecular weight fucans, being fucan 1 to fucan 6, involved numerous different methodologies. Fucan 3 was extracted from brown seaweed and found to be a low-molecular weight fucan. Fucan 2 was obtained from FMC BioPolymer® and found to be a low-molecular weight fucan. Fucan 1 and fucan 5 were obtained by methods discussed in example 3, using MWCO TFF filters under 100kDa. Fucan 4 was obtained by methods discussed in example 10. Fucan 6 was obtained by chemical degradation of a high-molecular-weight fucan with hydrogen peroxide.

[000254] The preparation of high-molecular-weight fucans, being fucan 7 to fucan 20, involved numerous different methodologies including treatment with sodium hydroxide, and in some cases other bases as well. The preparation of fucan 7, fucan 8, fucan 11, fucan 12 to fucan 17 and fucan 20 involved a combination of the method discussed in example 12 and tangential flow filtration against a low ionic strength solution. The preparation of fucan 10 involved a combination of cationic augmented tangential flow filtration and sequential tangential flow filtration methods discussed above. Fucan 9, fucan 18 and fucan 19 were extracted from brown seaweed, further processed by tangential flow filtration against a low ionic strength solution and found to be high-molecular-weight fucans.

Example 17: Molecular weight determination of crude fucans used to make fucan 7 to fucan 14

[000255]Gel permeation chromatography was used to evaluate the molecular weight distributions of crude fucans used to make fucans 7 to 14. Crude fucan 1 refers to the crude fucan used to make fucan 7 and fucan 8. Crude fucan 2 refers to the crude fucan used to make fucan 9, fucan 10, fucan 11 and fucan 13. Crude fucan 3 refers to the crude fucan used to make fucan 12. Crude fucan 4 refers to the crude fucan used to make fucan 14. The results of such analyses are shown in Table 12.

[000256] Results in the tables below contain abbreviations used for certain characteristics of a molecular weight distribution. Gel permeation chromatography is denoted by GPC, peak molecular weight is denoted by PMW, weight average molecular weight is denoted by WAMW, number average molecular weight is denoted by NAMW, percentage distribution is denoted by % dist., molecular weight is denoted by MW and polydispersity index is denoted by PDI.

	PMW (kDa)	**WAMW (kDa)**	**NAMW (kDa)**	**% dist. <10 kDa**	**% dist. <20 kDa**	**% dist. <50 kDa**	**% dist. >100 kDa**	**% dist. >200 kDa**	**% dist. >500 kDa**	**PDI**
Crude fucan 1	92.0	259.3	22.1	8.3	14.7	30.4	52.3	34.7	14.5	11.7
Crude fucan 2	512.3	535.3	128.5	0.5	2.1	8.9	80.4	65.1	36.6	4.2
Crude	594.6	493.4	4.4	22.0	27.3	35.7	57.4	49.3	31.3	113.3

fucan 3										
Crude fucan 4	662.5	790.6	245.4	0.1	0.5	3.4	90.9	80.2	52.0	3.2

Table 12

Example 18: Molecular weight determination of low and high-molecular-weight fucans

[000257]Gel permeation chromatography was used to evaluate the molecular weight distributions obtained for fucans 1 to 20.

[000258] Table 13 and Table 14 list the molecular weight distribution profiles obtained for twenty fucans. Table 14 provides molecular weight distribution profiles for the same twenty fucans shown in Table 13, providing the molecular weight distribution profiles in a different manner that shown in Table 13, providing thereby two different perspectives on the molecular weight distribution of the various fucans. As can be seen from the results, a broad range of different molecular weight distributions in fucans has been accomplished. Fucans with a weight average molecular weight between 28 kDa and 8250 kDa have been obtained with a plurality of distribution profiles.

[000259] Results in the tables below contain abbreviations used for certain characteristics of a molecular weight distribution. Gel permeation chromatography is denoted by GPC, peak molecular weight is denoted by PMW, weight average molecular weight is denoted by WAMW, number average molecular weight is denoted by NAMW, percentage distribution is denoted by % dist., molecular weight is denoted by MW and polydispersity index is denoted by PDI.

	PMW (kDa)	**WAMW (kDa)**	**NAMW (kDa)**	**% dist. <10 kDa**	**% dist. <20 kDa**	**% dist. <50 kDa**	**% dist. >100 kDa**	**% dist. >200 kDa**	**% dist. >500 kDa**	**PDI**
Fucan 1	17.5	28.3	14.2	16.6	51.7	87.9	3.0	0.6	0.0	1.99
Fucan 2	21.0	72.4	9.9	26.5	44.0	67.3	18.4	9.1	2.3	7.29
Fucan 3	70.4	105.9	52.4	0.6	5.8	33.1	35.3	12.1	1.3	2.02
Fucan 4	107.1	136.1	79.9	0.1	1.5	15.4	53.4	19.8	1.1	1.70
Fucan 5	80.2	171.9	60.4	0.8	5.3	26.5	47.9	25.4	6.6	2.84
Fucan 6	195.1	192.1	87.4	0.4	2.3	14.0	64.4	35.8	5.5	2.20
Fucan 7	242.5	366.5	137.2	0.0	0.5	7.0	77.7	54.6	21.9	2.67

Fucan 8	307.1	395.8	170.2	0.0	0.2	4.0	83.8	62.2	25.4	2.33
Fucan 9	459.3	514.0	198.5	0.1	0.4	3.4	87.8	71.4	37.2	2.62
Fucan 10	390.2	497.9	228.9	0.0	0.0	1.7	90.4	73.3	35.1	2.17
Fucan 11	457.3	592.8	300.9	0.0	0.0	0.7	95.4	82.9	43.8	1.97
Fucan 12	535.8	760.1	350.6	0.0	0.1	0.9	96.5	88.3	54.3	2.17
Fucan 13	612.3	857.0	448.7	0.0	0.0	0.2	98.6	92.4	61.4	1.91
Fucan 14	393.1	930.1	296.6	0.0	0.0	1.1	93.6	81.1	43.6	3.14
Fucan 15	409.4	772.0	291.8	0.0	0.0	1.1	94.0	81.5	43.6	2.65
Fucan 16	743.0	1618.0	387.5	0.0	0.1	1.4	92.9	86.6	68.2	4.18
Fucan 17	686.2	1876.7	524.9	0.0	0.0	0.3	98.4	93.0	69.9	3.58
Fucan 18	6238.6	3957.4	519.7	0.0	0.1	1.7	82.3	78.8	71.4	7.61
Fucan 19	4315.2	5336.8	2009.5	0.0	0.0	0.0	93.7	93.3	90.1	2.66
Fucan 20	6170.2	8101.9	846.3	0.0	0.0	0.3	94.7	91.1	83.6	9.57

Table 13: A first perspective on the molecular weight distribution of 20 fucans

	% dist. <5 kDa	**% dist. between 5-60 kDa**	**% dist. between 60-200 kDa**	**% dist. between 200-1600 kDa**	**% dist. >1600 kDa**
Fucan 1	3.5	87.9	8.1	0.6	0.0
Fucan 2	13.0	58.6	19.4	9.0	0.0
Fucan 3	0.0	41.3	46.6	12.1	0.0
Fucan 4	0.0	20.9	59.1	20.1	0.0
Fucan 5	0.1	32.8	41.7	25.0	0.4
Fucan 6	0.0	18.5	45.6	35.8	0.0
Fucan 7	0.0	10.0	35.4	52.3	2.4
Fucan 8	0.0	6.2	31.5	60.0	2.3
Fucan 9	0.0	5.0	23.6	67.4	4.0
Fucan 10	0.0	3.0	23.7	69.8	3.5
Fucan 11	0.0	1.3	15.8	78.0	4.9
Fucan 12	0.0	1.3	10.5	78.9	9.4
Fucan 13	0.0	0.3	7.2	80.4	12.0
Fucan 14	0.0	1.8	16.3	68.7	13.1

Fucan 15	0.0	1.7	16.1	72.4	9.8
Fucan 16	0.0	2.1	9.4	60.9	37.6
Fucan 17	0.0	0.5	6.5	62.4	30.6
Fucan 18	0.0	2.3	5.7	35.2	56.8
Fucan 19	0.0	0.0	0.3	24.9	74.8
Fucan 20	0.0	0.6	5.2	28.7	65.5

Table 14: A second perspective on the molecular weight distribution of 20 fucans

Example 19: Sulfate, total carbohydrate and monosaccharide content of high-molecular-weight fucans

[000260] High-molecular-weight fucans fucan 7 to fucan 18 and fucan 20 were dissolved in deionized water, hydrolyzed under acidic conditions and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for % w/w total sulfur content, performed by ALS Environmental laboratories in Burnaby, British Columbia. Sulfur content was converted to sulfate content by multiplying the sulfur content by the molar ratio of sulfate to sulfur to obtain % w/w sulfate content of the fucan. The sulfate contents of fucan 7 to 18 and fucan 20 are shown in table 15 below.

	Sulfate content (% w/w)
Fucan 7	23.93
Fucan 8	40.95
Fucan 9	40.32
Fucan 10	33.15
Fucan 11	44.87
Fucan 12	41.02
Fucan 13	36.18
Fucan 14	40.45
Fucan 15	39.79
Fucan 16	14.39
Fucan 17	51.30
Fucan 18	21.11
Fucan 20	25.60

Table 15 – Sulfate content of fucan 7 to fucan 18 and fucan 20

[000261] High-molecular-weight fucans fucan 7, fucan 11, fucan 16, fucan 18 and fucan 20 were analyzed for total carbohydrate and monosaccharide composition by gas spectrometry-mass spectroscopy (GC-MS) performed by the complex carbohydrate research center at the University

of Georgia. The high-molecular-weight fucans were derivatized by acidic methanolysis to produce O-trimethylsilyl (O-TMS) derivatives. After derivatization, the fucans are analyzed on an Agilent 7890A gas chromatography system interfaced to an Agilent 5975C mass spectrometry detector using a Supelco Equity-1 fused silica capillary column (30 m, 0.25 mm inner diameter). The results for the total carbohydrate content and the monosaccharide composition of the high-molecular-weight fucans are shown in table 16 below. Carbohydrate in the table below is abbreviated "carb.".

	Total carb. content (% w/w of the fucan)	**Fucose (% w/w of the total carb. content)**	**Galactose (% w/w of total carb. content)**	**Xylose (% w/w of total carb. content)**	**Mannose (% w/w of total carb. content)**	**Rhamnose (% w/w of total carb. content)**
Fucan 7	32.7	44.4	52.9	0.5	0.4	0.3
Fucan 11	59.5	91.9	8.1	0.0	0.0	0.0
Fucan 16	25.9	48.3	9.9	15.5	5.9	0.3
Fucan 18	41.2	92.0	4.7	2.1	0.4	0.2
Fucan 20	30.1	84.7	10.6	3.3	0.9	0.0

Table 16 – Total carbohydrate and monosaccharide composition of five fucans

Example 20: Rat epidural adhesion treatment

[000262] Fucoidan solutions using the twenty fucans identified in the example 18 were prepared in Lactated Ringers Injection USP (LRS). Fucan 1 to fucan 16, fucan 18 and fucan 20 were prepared at 100 mg/mL in LRS. Fucan 19 was prepared at 50 mg/mL in LRS. Fucan 17 was prepared at 500 mg/mL in LRS. Laminectomy surgery was performed on Sprague Dawley rats, the average weights of the rats and the dose in milligram per kilogram shown in table 17 below. A line block along the lumbar spine was created with bupivacaine solution. The back of the rat was cleaned and then covered with sterile drapes. The lumbar fascia was opened through a midline skin incision, lumbosacral fascia was incised and the paralumbar muscles was dissected to expose the underlying vertebral laminae. Bone at the centre of the vertebrae was removed. Throughout the procedure, haemostasis was maintained by irrigation with Lactated Ringer's Injection USP (LRS) and pressure with cotton swabs. The exposed dura was treated directly with 15 microlitres of LRS (control) or fucoidan solution. The muscle and skin layers were closed with sutures and the rats were allowed to recover for one week before sacrifice for adhesion quantification. The presence and size of adhesions on the dura were noted. The dimensions of the adhesions and the exposed

dura were recorded and used to calculate an adhesion coverage percentage, being the adhesion area as a percentage of the total exposed dura area.

Equation 1: Adhesion coverage (%) = 100 x epidural adhesion area ÷total exposed dura area

[000263] The control group receiving LRS was determined to have a 65% adhesion coverage using equation 1. The adhesion coverage for the twenty fucans disclosed in Table 13 to Table 16 are shown in Table 17 below as the reduction in adhesion coverage relative to the control group. A negative value denoted where an increase in adhesion coverage was seen relative to the control group.

	Average Rat Weight (kg)	Dose (mg)	Dose per animal weight (mg/kg)	Number of rats scored	% Reduction in epidural adhesion coverage vs. control
Fucan 1	0.41	1.5	3.7	4	-40% (i.e., 40% increase in fibrous adhesions compared to control)
Fucan 2	0.59	1.5	2.5	3	9%
Fucan 3	0.39	1.5	3.8	4	-10%
Fucan 4	0.65	1.5	2.3	4	83%
Fucan 5	0.53	1.5	2.9	4	46% (i.e., 46% decrease in fibrous adhesions compared to control)
Fucan 6	0.46	1.5	3.3	4	44%
Fucan 7	0.47	1.5	3.2	3	100%
Fucan 8	0.36	1.5	4.2	3	100%
Fucan 9	0.39	1.5	3.8	2	100%
Fucan 10	0.40	1.5	3.8	4	100%
Fucan 11	0.58	1.5	2.6	2	100%
Fucan 12	0.44	1.5	3.4	2	100%

Fucan 13	0.64	1.5	2.3	3	100%
Fucan 14	0.37	1.5	4.0	4	100%
Fucan 15	0.50	1.5	3.0	3	100%
Fucan 16	0.45	1.5	3.3	3	100%
Fucan 17	0.59	7.5	12.8	3	100%
Fucan 18	0.59	1.5	2.5	2	100%
Fucan 19	0.39	0.8	1.9	3	100%
Fucan 20	0.56	1.5	2.7	2	100%

Table 17: Reduction in rat epidural adhesion relative to control LRS using 20 different fucans

[000264] As may be seen by comparing the results of Table 17 with the molecular weight of the fucans given in Tables 13 and Table 14, fucans with a weight average molecular weight over 130 kDa and containing about 60% or more of their molecular weight distribution above 100 kDa show greater efficacy in the inhibition, prevention, removal, reduction, or other treatment of rat epidural adhesions than fucans with weight average molecular weight below 100 kDa containing about 60% or less of their molecular weight distribution above 100 kDa at the same dose. There is also a further indication that fucans with weight average molecular weight above 300 kDa, containing about 70% or more of their molecular weight distribution above 100 kDa show even greater efficacy in the inhibition, prevention, removal, reduction, or other treatment of rat epidural adhesions at the same dose.

Example 21: Rabbit uterine horn adhesion treatment with fucan 1 and fucan 10

[000265] Uterine horn surgery was performed on both uterine horns in each rabbit. Prior to surgery, the rabbits were weighed and then prepared for surgery by premedication with ketamine and xylazine.

[000266] Fucoidan solution was prepared at 0.07 mg/mL in Lactated Ringers Injection USP, sterilizing by filtration. All instruments were sterile, and a sterile field was maintained throughout the surgeries. The abdomen was cleaned and entered via a midline abdominal incision. The uterine horns were located, exteriorized and scraped to induce damage. The abdominal wall near the scraped uterine horns was also scraped. The damaged uterine horns and abdominal wall were placed next to each other and stabilized with sutures. 15 mL/kg fucoidan solution per rabbit weight

was applied to the abdominal cavity before the incision was closed. Adhesion was evaluated two weeks after the surgery. Length of the uterine horn adhesion was measured with a ruler. The uterine horn adhesion coverage percentage, being the length of the adhesion as a percentage of the total damaged uterine horn length was calculated as:

Equation 2: Adhesion coverage (%) = 100 x uterine horn adhesion length ÷ total damaged uterine horn length

[000267] The same surgical method was applied to 3 New Zealand White rabbits, receiving 15 mL/kg of control Lactated Ringer's Injection USP (LRS) instead of fucoidan solution.

[000268] The control group receiving LRS was determined to have a 41% adhesion coverage using equation 2. Table 18 shows the results obtained using the method discussed above for fucans fucan 1 and fucan 10, being representative examples of respectively a fucan with the majority of its molecular weight distribution below 100 kDa and even below 50 kDa and a fucan with the majority of its molecular weight distribution above 100 kDa and even above 200 kDa. The results in the table below are shown as the reduction in adhesion coverage relative to the control group.

	Dose per animal weight (mg/kg)	**Number of Uterine Horns**	**% Reduction in uterine horn adhesion coverage vs. control**
Fucan 1 - low molecular weight	1	6	21% (i.e., 21% decrease in fibrous adhesions compared to control)
Fucan 10 - high-molecular-weight	1	8	100%

Table 18: Reduction in rabbit uterine horn adhesion using two different fucans relative to control LRS

[000269] As may be seen from the results in Table 18, fucans having the majority of their distribution above 100 kDa, or even above 200 kDa, have a higher efficacy in the inhibition, prevention, removal, reduction, or other treatment of rabbit uterine horn adhesion as compared with fucans having a majority of their distribution under 100 kDa or even under 50 kDa at the same dose.

Example 22: Rabbit uterine horn adhesion with fucan 17

[000270] To determine the efficacy of the high-molecular-weight fucan 17 in inhibiting surgical adhesions, the following double uterine horn (DUH) surgeries were performed on both horns of a total of three New Zealand White rabbits. Prior to surgery, the rabbits were weighed and then prepared for surgery by premedication with ketamine and xylazine.

[000271] Fucoidan solution was prepared at 5 mg/mL in Lactated Ringers Injection USP (LRS), sterilizing by filtration. All instruments were sterile, and a sterile field was maintained throughout the surgeries. The abdomen was cleaned and entered via a midline abdominal incision. The uterine horns were located, exteriorized and scraped to induce damage. The abdominal wall near the scraped uterine horns was also scraped. The damaged uterine horns and abdominal wall were placed next to each other and stabilized with sutures. The top third and the bottom third of the muscle incision was closed and 5 mL/kg fucoidan solution per rabbit weight was applied to the abdominal cavity. The muscle incision was temporarily closed and the fucoidan solution left in the abdominal cavity for 30 minutes. The muscle incision was reopened and the abdominal cavity was flushed with 10 mL/kg LRS. The majority of the fluid in the abdominal cavity was suctioned out before the incision was closed. Adhesion formation was evaluated two weeks after the surgery. Length of the uterine horn adhesion was measured with a ruler. The uterine horn adhesion coverage percentage, being the length of the adhesion as a percentage of the total damaged uterine horn length was calculated using equation 2.

[000272] Table 19 shows the results obtained using the method discussed above for fucan 17, being a representative example of a high-molecular-weight fucan. The results in the table below are shown as the mean adhesion length across the 6 uterine horns scored.

[000273] Table 19 provides the results of treating six uterine horns with fucan 17.

	Dose (mg/kg)	**Number of Uterine Horns**	**Mean % adhesion length**
Fucan 17	25	6	0% (i.e., no adhesions were found)

Table 19: Adhesion length using fucan 17

[000274] As may be seen from the results of Table 19, high-molecular-weight fucans may be used to successfully inhibit, prevent, remove, reduce, or otherwise treat post-surgical uterine horn adhesions.

Example 23: Uterine horn fibrous adhesion treated with a high-molecular-weight fucan composition

[000275] To determine the efficacy of a high-molecular-weight fucan composition comprising a number average molecular weight of about 228 kDa, a weight average molecular weight of about 1210 kDa, a peak molecular weight of about 575 kDa and having a molecular weight distribution wherein about 89% of the distribution is above 100 kDa and wherein about 30% of the distribution is above 1000 kDa, in inhibiting surgical adhesions, the following double uterine horn (DUH) surgeries were performed on both horns of a total of twenty New Zealand White rabbits. Prior to surgery, the rabbits were weighed and then prepared for surgery by premedication with midazolam and dexmeditomidine.

[000276] Fucoidan solution was prepared at each concentration of 0.02 mg/mL, 0.1 mg/mL, 0.5 mg/mL, or 2.5 mg/mL in Lactated Ringers Injection USP (LRS), sterilizing by filtration. All instruments were sterile, and a sterile field was maintained throughout the surgeries. The abdomen was cleaned and entered via a midline abdominal incision. The uterine horns were located, exteriorized and scraped to induce damage. The abdominal wall near the scraped uterine horns was also scraped. The damaged uterine horns and abdominal wall were placed next to each other and stabilized with sutures. About 2 mL/kg fucoidan solution per rabbit weight was applied to the abdominal cavity before the incision was closed. Adhesion was evaluated two weeks after the surgery. Five rabbits were treated and evaluated for each fucoidan concentration prepared. Length of the uterine horn adhesion was measured with a ruler. The uterine horn adhesion length was calculated using equation 2.

[000277] The same surgical method was applied to 5 additional New Zealand White rabbits for control, each receiving about 2 mL/kg of control Lactated Ringer's Injection USP (LRS) instead of fucoidan solution. The control group receiving LRS was determined to have a 100% adhesion coverage using equation 2. Table 20 shows the results obtained using the method discussed above for the high-molecular-weight fucan composition at different concentrations and dosages (in total forty uterine horns were treated, 10 each for each concentration of the high-molecular-weight fucan composition); the results are shown as the reduction in adhesion coverage relative to the control group.

Concentration (mg/mL)	Dose (mg/kg)	Number of Uterine Horns	% Reduction in uterine horn adhesion coverage vs. control
0.02	0.04	10	10% (i.e., 10% decrease in fibrous adhesions compared to control)
0.1	0.2	10	30% (i.e., 30% decrease in fibrous adhesions compared to control)
0.5	1	10	71% (i.e., 71% decrease in fibrous adhesions compared to control)
2.5	5	10	95% (i.e., 95% decrease in fibrous adhesions compared to control)

Table 20: Decrease in rabbit uterine horn adhesion using a high-molecular-weight fucan composition relative to control LRS

[000278] As can be seen from the results of Table 20, high-molecular-weight fucan compositions can be used to successfully inhibit, prevent, remove, reduce, or otherwise treat post-surgical uterine horn adhesions.

Reference Numeral List:

100 Molecular weight based segmentation system (higher-to-lower)
100′ Molecular weight based segmentation system (lower-to-higher)
100" Cation-augmented TFF system (CATS)
102 Input supply line
104 Pre-filter
106 Lower MWCO subsystem retentate-line valve
106' Lower MWCO subsystem output valve
108 lower MWCO subsystem retentate output line
110 Higher molecular weight cut-off TFF filter
111 Higher MWCO subsystem retentate output line
112 Higher MWCO TFF filter supply line
113 Higher-to-lower MWCO inter-subsystem valve
114 Higher MWCO subsystem pump
115 Higher MWCO subsystem solvent supply line
116 Higher MWCO subsystem fucan container
117 Higher MWCO subsystem solvent container
118 Higher MWCO subsystem retentate return line
119 Higher MWCO subsystem permeate output line
120 Lower molecular weight cut-off TFF filter
121 Lower MWCO subsystem retentate output line 108
122 Lower MWCO TFF filter supply line

123 Lower-to-higher MWCO inter-subsystem valve
124 Lower MWCO subsystem pump
125 Lower MWCO subsystem solvent supply line
126 Lower MWCO subsystem fucan container
127 Lower MWCO subsystem solvent container
128 Lower MWCO subsystem retentate return line
129 Lower MWCO subsystem permeate output line
130 Higher MWCO TFF subsystem
130' Higher MWCO TFF subsystem (FIG. 3)
135 Cationic additive flush solution supply line
136 Cationic additive flush solution valve
137 Cationic additive flush solution container
140 Lower MWCO subsystem
140' Lower MWCO TFF subsystem (FIG. 3)
142 Sodium salt solution container
143 Low conductivity diafiltration solution container
144 Sodium salt solution control valve
145 Low conductivity diafiltration solution valve
146 Sodium salt solution supply line
147 Low conductivity diafiltration solution supply line
150 Higher MWCO TFF filter
160 Lower MWCO TFF filter
600 Centrifugal precipitation system for obtaining a high-molecular-weight fucan from a starting fucan composition
600′ Centrifugal precipitation system for obtaining a high-molecular-weight fucan from a starting fucan composition
610 Centrifuge container
620 Gradated permeable barrier
620′ Permeable barrier
620a Barrier segment (first – highest density)
620b Barrier segment (second – intermediate density)
620c Barrier segment (third – lowest density)
620c′ Single barrier segment
622 First-bottom gradated permeable barrier material end
622′ First-bottom permeable barrier material end
624 Second-top gradated permeable barrier material end
624′ Second-top permeable barrier material end
630 First-bottom end of centrifuge container 610
640 Second-top end of centrifuge container 610

650 Starting fucan composition
660 Arrow indicating direction of centrifugal force on container 610
670 Centrifuge box
900 Electrophoresis-extraction system
910 Electrophoresis chamber
912 Well
914 Theoretical Displacement distances
916 Electrophoresis gel
918 Electrophoresis buffer
920 Direct current power supply
922 Cathode
924 Anode
926 Migration direction arrow (depicting displacement direction of anions)
800 Membrane dialysis system for obtaining a high-molecular-weight fucan from a starting fucan composition
801 Input supply line
802 Pre-filter
810 Fucan container
812 Dialysis system supply line
814 Dialysis system pump
815 Dialyzed fluid output valve
816 Dialyzed fluid return line
818 Dialyzed fluid output line
820 Dialysis cell
825 Dialysis membrane
830 Dialysis container
832 Dialysate supply line
834 Dialysate pump
835 Dialysate fluid output valve
836 Dialysate fluid return line
838 Dialysate fluid output line
840 Dialysate container
842 Dialysate supply line
845 Dialysate supply valve
170 Tangential flow filtration (TFF) subsystem
171 TFF filter
172 TFF subsystem filter supply line
173 TFF subsystem solvent supply valve
174 TFF subsystem pump

175 TFF subsystem solvent supply line
176 TFF subsystem fucan container
177 TFF subsystem solvent container
178 TFF subsystem retentate line
179 TFF subsystem permeate output line
180 Ion exchange subsystem
181 Ion exchange container
182a Ion exchange subsystem fucan supply line
182b Ion exchange subsystem salt solution supply line
183a Ion exchange subsystem fucan return valve
183b Ion exchange subsystem salt solution supply valve
183c Ion exchange subsystem salt solution return valve
184a Ion exchange subsystem fucan pump
184b Ion exchange subsystem salt solution pump
186 Ion exchange subsystem fucan container
187 Ion exchange subsystem salt solution container
188a Ion exchange subsystem fucan return line
188b Ion exchange subsystem salt solution return line
189 Macroporous ion exchange resin
300 Ion adsorption system
301 Input supply line
302 Inter-subsystem valve
303 TFF subsystem retentate output line
304 Ion exchange subsystem output valve
305 Ion exchange subsystem output line
306 Pre-filter

[000279] All terms used herein are used in accordance with their ordinary meanings unless the context or definition clearly indicates otherwise. Also unless expressly indicated otherwise, in this disclosure the use of "or" includes "and" and vice-versa. Non-limiting terms are not to be construed as limiting unless expressly stated, or the context clearly indicates, otherwise (for example, "including," "having," and "comprising" typically indicate "including without limitation"). Singular forms, including in the claims, such as "a," "an," and "the" include the plural reference unless expressly stated, or the context clearly indicates otherwise.

[000280] Unless otherwise indicated, adjectives herein such as “substantially” and “about” that modify a condition or relationship characteristic of a feature or features of an embodiment, indicate

that the condition or characteristic is defined to within tolerances that are acceptable for operation of the embodiment for an application for which it is intended.

[000281] The scope of the present methods, compositions, systems, etc., includes both means plus function and step plus function concepts. However, the claims are not to be interpreted as indicating a "means plus function" relationship unless the word "means" is specifically recited in a claim, and are to be interpreted as indicating a "means plus function" relationship where the word "means" is specifically recited in a claim. Similarly, the claims are not to be interpreted as indicating a "step plus function" relationship unless the word "step" is specifically recited in a claim, and are to be interpreted as indicating a "step plus function" relationship where the word "step" is specifically recited in a claim.

[000282] From the foregoing, it will be appreciated that, although specific embodiments have been discussed herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the discussion herein. Accordingly, the systems and methods, etc., include such modifications as well as all permutations and combinations of the subject matter set forth herein and are not limited except as by the appended claims or other claim having adequate support in the discussion and figures herein.

What is claimed is:

1. A high-molecular-weight fucan consisting essentially of a molecular weight distribution wherein at least 92% w/w of the distribution is greater than 100 kDa when measured using an aqueous gel permeation chromatography set up consisting essentially of:

one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 50 kDa and about 5,000 kDa, one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 1 kDa and about 6,000 kDa and one 40 mm guard column with a 6 mm inner diameter packed with hydroxylated polymethacrylate-based gel, the two analytical gel permeation chromatography columns and the one guard column contained in a column compartment at about 30 ^{o}C;

a refractive index detector at about 30 ^{o}C;

0.1M sodium nitrate mobile phase run at 0.6 mL/min; and

quantification against a peak molecular weight standard curve consisting essentially of a first dextran standard with a peak molecular weight of about 2,200 kDa, a second dextran standard with a peak molecular weight of between about 720 kDa and about 760 kDa, a third dextran standard with a peak molecular weight between about 470 kDa and about 510 kDa, a fourth dextran standard with a peak molecular weight between about 370 kDa and about 410 kDa, a fifth dextran standard with a peak molecular weight between about 180 kDa and about 220 kDa, and a sixth dextran standard with a peak molecular weight between about 40 kDa and 55 kDa.

2. The high-molecular-weight fucan of claim 1, wherein at least 93% w/w of the distribution is greater than 100 kDa.
3. The high-molecular-weight fucan of claim 1, wherein at least 94% w/w of the distribution is greater than 100 kDa.
4. The high-molecular-weight fucan of claim 1, wherein at least 95% w/w of the distribution is greater than 100 kDa.

5. The high-molecular-weight fucan of claim 1, wherein at least 97% w/w of the distribution is greater than 100 kDa.
6. The high-molecular-weight fucan of claim 1, wherein at least 98% w/w of the distribution is greater than 100 kDa.
7. The high-molecular-weight fucan of claim 1, wherein at least 99% w/w of the distribution is greater than 100 kDa.
8. The high-molecular-weight fucan of any one of claims 1 to 7, comprising a weight average molecular weight between about 100 kDa and 10,000 kDa.
9. The high-molecular-weight fucan of claim 8, comprising a weight average molecular weight between about 140 kDa and 8,100 kDa.
10. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is between about 370 kDa and 8100 kDa.
11. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is between about 370 kDa and 5300 kDa.
12. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is between about 370 kDa and 1900 kDa.
13. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is between about 590 kDa and 1600 kDa.
14. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is between about 860 kDa and 1600 kDa.
15. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is about 1,100 kDa.
16. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is about 1,200 kDa.
17. The high-molecular-weight fucan of claim 8, wherein the weight average molecular weight is about 1,300 kDa.
18. The high-molecular-weight fucan of any one of claims 1 to 17, comprising a number average molecular weight between about 50 kDa and 3,000 kDa.
19. The high-molecular-weight fucan of claim 18, wherein the number average molecular weight is between about 60 kDa and 2,000 kDa.

20. The high-molecular-weight fucan of claim 18, wherein the number average molecular weight is between about 140 kDa and 2,000 kDa.
21. The high-molecular-weight fucan of claim 18, wherein the number average molecular weight is between about 140 kDa and 520 kDa.
22. The high-molecular-weight fucan of claim 18, wherein the number average molecular weight is between about 230 kDa and 450 kDa.
23. The high-molecular-weight fucan of any one of claims 1 to 22, wherein at least 55% w/w of the distribution is greater than about 200 kDa.
24. The high-molecular-weight fucan of claim 23, wherein at least 71% w/w of the distribution is greater than about 200 kDa.
25. The high-molecular-weight fucan of claim 23, wherein at least 91% w/w of the distribution is greater than about 200 kDa.
26. The high-molecular-weight fucan of any one of claims 1 to 25, wherein at least 22% w/w of the distribution is greater than about 500 kDa.
27. The high-molecular-weight fucan of claim 26, wherein at least 54% w/w of the distribution is greater than about 500 kDa.
28. The high-molecular-weight fucan of claim 26, wherein at least 90% w/w of the distribution is greater than about 500 kDa.
29. A high-molecular-weight fucan consisting essentially of a molecular weight distribution wherein between about 61% w/w and 80% w/w of the distribution is between about 200 kDa and 1600 kDa when measured using an aqueous gel permeation chromatography set up consisting essentially of:

    one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 50 kDa and about 5,000 kDa, one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 1 kDa and about 6,000 kDa and one 40 mm guard column with a 6 mm inner diameter packed with hydroxylated polymethacrylate-based gel, the two analytical gel permeation

chromatography columns and the one guard column contained in a column compartment at about 30 °C;

a refractive index detector at about 30 °C;

0.1M sodium nitrate mobile phase run at 0.6 mL/min; and

quantification against a peak molecular weight standard curve consisting essentially of a first dextran standard with a peak molecular weight of about 2,200 kDa, a second dextran standard with a peak molecular weight of between about 720 kDa and about 760 kDa, a third dextran standard with a peak molecular weight between about 470 kDa and about 510 kDa, a fourth dextran standard with a peak molecular weight between about 370 kDa and about 410 kDa, a fifth dextran standard with a peak molecular weight between about 180 kDa and about 220 kDa, and a sixth dextran standard with a peak molecular weight between about 40 kDa and 55 kDa.

30. A high-molecular-weight fucan consisting essentially of a molecular weight distribution wherein at least 60% w/w of the distribution is greater than about 1600 kDa when measured using an aqueous gel permeation chromatography set up consisting essentially of:

one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 50 kDa and about 5,000 kDa, one 300 mm analytical gel permeation chromatography column with a 7.8 mm inner diameter packed with hydroxylated polymethacrylate-based gel, having an effective molecular weight range of between about 1 kDa and about 6,000 kDa and one 40 mm guard column with a 6 mm inner diameter packed with hydroxylated polymethacrylate-based gel, the two analytical gel permeation chromatography columns and the one guard column contained in a column compartment at about 30 °C;

a refractive index detector at about 30 °C;

0.1M sodium nitrate mobile phase run at 0.6 mL/min; and

quantification against a peak molecular weight standard curve consisting essentially of a first dextran standard with a peak molecular weight of about 2,200 kDa, a second dextran standard with a peak molecular weight of between about 720 kDa and about 760 kDa, a third dextran standard with a peak molecular weight between about 470 kDa and about 510 kDa, a fourth

dextran standard with a peak molecular weight between about 370 kDa and about 410 kDa, a fifth dextran standard with a peak molecular weight between about 180 kDa and about 220 kDa, and a sixth dextran standard with a peak molecular weight between about 40 kDa and 55 kDa.

31. The high-molecular-weight fucan of any of claim 1 to 30, wherein the sulfate content is between about 20% w/w and 60% w/w.
32. The high-molecular-weight fucan of claim 31, wherein the sulfate content is between about 30% w/w and 55% w/w.
33. The high-molecular-weight fucan of claim 31, wherein the sulfate content is between about 32% w/w and 52% w/w.
34. The high-molecular-weight fucan of any one of claims 1 to 33, wherein the total carbohydrate content is between about 27% w/w and 80% w/w.
35. The high-molecular-weight fucan of claim 34, wherein the total fucose content as a percentage of the total carbohydrate content is at least about 30% w/w.
36. The high-molecular-weight fucan of claim 34, wherein the total fucose content as a percentage of the total carbohydrate content is at least about 50% w/w.
37. The high-molecular-weight fucan of claim 34, wherein the total fucose content as a percentage of the total carbohydrate content is at least about 70% w/w.
38. The high-molecular-weight fucan of claim 34, wherein the total fucose content as a percentage of the total carbohydrate content is at least about 80% w/w.
39. The high-molecular-weight fucan of claim 34, wherein the total fucose content as a percentage of the total carbohydrate content is at least about 90% w/w.
40. The high-molecular-weight fucan of claim 34, wherein the total fucose content as a percentage of the total carbohydrate content is at least about 95% w/w.
41. The high-molecular-weight fucan of claim 34, wherein the total galactose content as a percentage of the total carbohydrate content is below about 60% w/w.
42. The high-molecular-weight fucan of claim 34, wherein the total galactose content as a percentage of the total carbohydrate content is between about 2% w/w and 20% w/w.
43. The high-molecular-weight fucan of claim 34, wherein the total galactose content as a percentage of the total carbohydrate content is below about 10% w/w.

44. The high-molecular-weight fucan of claim 34, wherein the total of glucuronic acid, mannose, rhamnose, glucose and xylose content as a percentage of the total carbohydrate content is below about 30% w/w.
45. The high-molecular-weight fucan of any one of claims 1 to 44 wherein the high-molecular-weight fucan when dissolved in water at a concentration of 50 mg/mL has a viscosity of between about 4 cP and 50 cP.
46. The high-molecular-weight fucan of any one of claims 1 to 44 wherein the high-molecular-weight fucan when dissolved in water at a concentration of 50 mg/mL has a viscosity of between about 10 cP and 40 cP.
47. The high-molecular-weight fucan of any one of claims 1 to 44 wherein the high-molecular-weight fucan when dissolved in water at a concentration of 50 mg/mL has a viscosity of between about 15 cP and 30 cP.
48. The high-molecular-weight fucan of any one of claims 1 to 47 wherein the high-molecular-weight fucan is a white solid.
49. The high-molecular-weight fucan of any one of claims 1 to 48 wherein the high-molecular-weight fucan when dissolved in water at a concentration from 1 mg/mL through 100 mg/mL forms a solution that is one of clear-colorless.
50. The high-molecular-weight fucan of any one of claims 1 to 49 wherein the fucan comprises less than 5% w/w acetyl content.
51. The high-molecular-weight fucan of any one of claims 1 to 49 wherein the fucan comprises less than 2% w/w acetyl content.
52. The high-molecular-weight fucan of any one of claims 1 to 49 wherein the fucan comprises an acetyl content of substantially 0% w/w when measured by 2D ^{1}H-^{13}C heteronuclear multiple quantum coherence at 70 °C with solvent signal suppression on a 600 MHz spectrometer equipped with 5-mm cold probe, in the range from 10-30 ppm in the carbon dimension, in 8 increments of 256-512 scans each.
53. A method comprising making the high-molecular-weight fucan of any one of claims 1 to 52.
54. A method comprising using the high-molecular-weight fucan of any one of claims 1 to 52.
55. The method of claim 54 wherein the using comprises treating fibrous adhesions.

56. A medically acceptable fucan composition comprising a therapeutically effective amount of the high-molecular-weight fucan of any one of claims 1 to 52 in a medically acceptable buffer or diluent.
57. A method of treating a condition or disease in an animal comprising selecting the medically acceptable fucan composition of claim 56 to treat the condition or disease and administering a therapeutically effective amount comprising between about 0.5 mg/kg and 50 mg/kg of the high-molecular-weight fucan to the animal.
58. A method of treating a condition or disease in an animal comprising selecting the medically acceptable fucan composition of claim 56 to treat the condition or disease and administering a therapeutically effective amount between about 0.04 mg/kg and 25 mg/kg of the high-molecular-weight fucan to the animal.
59. The method of claim 57 or 58 wherein the therapeutically effective amount is between about 0.2 mg/kg and 10 mg/kg.
60. The method of claim 57 or 58 wherein the therapeutically effective amount is between about 1 mg/kg and 5 mg/kg.
61. The method of claim 57 or 58 wherein the therapeutically effective amount is between about 1.5 mg/kg and 3 mg/kg.
62. The method of claim 57 or 58 wherein the therapeutically effective amount is between about 5 mg/kg and 10 mg/kg.
63. The method of any of claims 57 to 62 wherein the condition or disease is a fibrous adhesion at a target site in the animal, and wherein the administering comprises administering the therapeutically effective amount to the target site.
64. A medical composition comprising between about 0.02 mg/mL and 100 mg/mL of the high-molecular-weight fucan of any one of claims 1 to 52, wherein the medical composition is configured and composed to treat a disease or condition in an animal.
65. The medical composition of claim 64 comprising between about 0.5 mg/mL and 5 mg/mL of the high-molecular-weight fucan.
66. The medical composition of claim 64 comprising about 2.5 mg/mL of the high-molecular-weight fucan.

67. The medical composition of any one of claims 64 to 66 wherein the medical composition is a medical device.
68. The medical composition of any one of claims 64 to 66 wherein the medical composition is a liquid medical device.
69. The medical composition of any one of claims 64 to 66 wherein the medical composition is a pharmaceutical composition.
70. The medical composition of any one of claims 64 to 66 wherein the medical composition is a liquid pharmaceutical composition.
71. The medical composition of any one of claims 64 to 70 wherein the disease or condition is a fibrous adhesion.
72. The use of a dosage range comprising between about 0.01 mL/kg and 15 mL/kg of the medical composition of any one of claims 64 to 71 to treat a disease or condition in an animal.
73. The use of a dosage range comprising between about 0.03 mL/kg and 4 mL/kg of the medical composition of any one of claims 64 to 71 to treat a disease or condition in an animal.
74. The use of a dosage range comprising between about 0.06 mL/kg and 2 mL/kg of the medical composition of any one of claims 64 to 71 to treat a disease or condition in an animal.
75. The use of a dosage range comprising between about 2 mL/kg and 4 mL/kg of the medical composition of any one of claims 64 to 71 to treat a disease or condition in an animal.
76. A method for treating a selected disease or condition in a patient comprising identifying a selected target site in a patient comprising or reasonably susceptible to having the selected disease or condition and then administering the medical composition of any one of claims 64 to 71 to a target site in the patient.
77. The method of claim 76 wherein the disease or condition is fibrous adhesions.
78. The method of claim 76 or 77 wherein the target site is a surgical site and the administering is performed at least one of a) after opening a surgical wound at the surgical site, b) during surgery, and c) after closing the surgical wound.
79. The method of claim 76 or 77 wherein the administering is performed after surgery but before closing the surgical wound.
80. The method of claim 76 or 77 wherein the administering takes less than 3 minutes.
81. The method of claim 76 or 77 wherein the administering takes less than 2 minutes.

82. The method of claim 76 or 77 wherein the administering takes less than 1 minute.

83. The method of claim 76 or 77 wherein the target site is at least one of a lesion, abrasion and injury site.

84. The method of claim 76 or 77 wherein the target site is at least one of a pelvic cavity, an abdominal cavity, a dorsal cavity, a cranial cavity, a spinal cavity, a ventral cavity, a thoracic cavity, a pleural cavity, a pericardial cavity, skin, a joint, a muscle, a tendon and a ligament.

85. A method for obtaining a high-molecular-weight fucan comprising:

providing in a starting solution a starting fucan composition having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment;

subjecting the starting solution to a first tangential flow filtration across a first higher molecular weight cutoff tangential flow filtration filter to produce a first permeate fucan composition; and

subjecting the first permeate fucan composition to a second tangential flow filtration across a second lower molecular weight cutoff tangential flow filtration filter to produce a second retentate fucan composition consisting essentially of the desired high-molecular-weight fucan.

86. The method of claim 85 wherein the method further comprises collecting the second retentate fucan composition consisting essentially of the desired high-molecular-weight fucan.

87. The method of claim 85 wherein the first higher molecular weight cutoff tangential flow filtration filter has a higher molecular weight cutoff of between about 50 kDa and about 1000 kDa and the second lower molecular weight cutoff tangential flow filtration filter has a lower molecular weight cutoff of between about 30 kDa and about 100 kDa.

88. The method of claim 87 wherein the higher molecular weight cutoff is about 300 kDa and the lower molecular weight cutoff is about 100 kDa.

89. A method for obtaining a high-molecular-weight fucan comprising:

providing a starting fucan composition having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment in a starting solution;

subjecting the starting solution to tangential flow filtration across a first lower molecular weight cutoff tangential flow filtration filter to produce a first retentate fucan composition; and

subjecting the first retentate fucan composition to tangential flow filtration across a second higher molecular weight cutoff tangential flow filtration filter to produce a second permeate fucan composition consisting essentially of the desired high-molecular-weight fucan.

90. The method of claim 89 wherein the method further comprises collecting the second permeate fucan composition consisting essentially of the desired high-molecular-weight fucan.

91. The method of claim 89 wherein the first tangential flow filtration comprises diafiltering the starting solution across the first lower molecular weight cutoff tangential flow filtration filter.

92. The method of claim 89 wherein the second tangential flow filtration comprises diafiltering the first retentate fucan composition across the second higher molecular weight cutoff tangential flow filtration filter.

93. The method of claim 89 wherein the first lower molecular weight cutoff tangential flow filtration filter has a lower molecular weight cutoff of between about 30 kDa and about 100 kDa and the second higher molecular weight cutoff tangential flow filtration filter has a higher molecular weight cutoff of between about 50 kDa and about 1000 kDa.

94. The method of claim 92 wherein the lower molecular weight cutoff is about 100 kDa and the higher molecular weight cutoff is about 300 kDa.

95. A method for obtaining a high-molecular-weight fucan comprising:

providing a starting fucan composition having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment in a starting solution, the starting fucan composition comprising low atomic weight cations ionically bound to the sulfate groups on fucan in the composition; and

subjecting the starting solution to tangential flow filtration against a cationic additive solution comprising a cationic additive having a greater molecular weight than the low atomic weight cations to produce a retentate fucan composition consisting essentially of the desired high-molecular-weight fucan.

96. The method of claim 95 wherein the method further comprises collecting the retentate fucan composition consisting essentially of the desired high-molecular-weight fucan.

97. The method of claim 95 wherein the low atomic weight cations comprise at least one of an alkali metal, an alkaline earth metal, aluminum and ammonium.

98. The method of claim 95 wherein the cationic additive comprises at least one of choline, polyvinylpyrrolidone, taurine, polyamine, chitosan, histone, and collagen.
99. The method of claim 95 further comprising adding to the starting solution the cationic additive before subjecting the starting solution to tangential flow filtration.
100. The method of claim 95 wherein the tangential flow filtration comprises diafiltering the starting solution against the cationic additive solution.
101. The method of claim 95 further comprising removing the cationic additive by diafiltering the retentate fucan composition against a salt solution over a second tangential flow filtration filter having a molecular weight cutoff that is lower than a molecular weight cutoff of the first tangential flow filtration filter.
102. The method of claim 101 wherein the salt solution comprises a chloride, bromide, iodide, fluoride, sulfate, sulfite, carbonate, bicarbonate, phosphate, nitrate, nitrite, acetate, citrate, silicate and/or cyanide of an alkali metal, alkaline earth metal, aluminum and/or ammonium.
103. The method of claim 101 further comprising removing salt by diafiltering the retentate fucan composition against a low-ionic strength solution.
104. A method for obtaining a high-molecular-weight fucan comprising:

providing a centrifuge container comprising a bottom end and a top end and a permeable barrier therebetween, the permeable barrier comprising a gradient material therebetween;

placing a starting fucan composition having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment in the centrifuge container and above the permeable barrier; and

centrifuging the centrifuge container for a period of time sufficient to produce a precipitate consisting essentially of the desired high-molecular-weight fucan.
105. The method of claim 104 wherein the method further comprises collecting the desired high-molecular-weight fucan from the centrifuge container.
106. The method of claim 104 wherein the permeable barrier comprises a single segment of gradient material.
107. The method of claim 104 wherein the permeable barrier comprises a plurality of segments of gradient material.

108. The method of any of claims 106 and 107 wherein the gradient material comprises at least one of sucrose, polysucrose, glycerol, sorbitol, CsCl, Cs_2SO_4, KBr, diatrizoate, Nycodenz® and iodixanol.

109. The method of claim 104 wherein the centrifugal force is between about 10,000 gravities to about 1,000,000 gravities.

110. The method of claim 104 wherein the centrifugal force is between 60,000 gravities to about 500,000 gravities.

111. A method for obtaining a high-molecular-weight fucan comprising:

providing a centrifuge container comprising a bottom end and a top end;

placing a starting fucan composition in a starting solution, having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment in the centrifuge container; and

centrifuging the centrifuge container for a period of time sufficient to produce a precipitate consisting essentially of the desired high-molecular-weight fucan.

112. The method of claim 111 further comprising collecting the desired high-molecular-weight fucan as a precipitate from the centrifuge container.

113. The method of claim 111 wherein the centrifugal force is between about 60,000 gravities to about 1,000,000 gravities.

114. The method of claim 111 wherein the centrifugal force is between 200,000 gravities to about 500,000 gravities.

115. A method for obtaining a high-molecular-weight fucan comprising:

subjecting a starting fucan composition having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment to gel electrophoresis wherein the starting fucan composition is displaced according to mass-to-charge ratio across an electrophoresis gel;

selecting a portion of the electrophoresis gel consisting essentially of the desired high-molecular-weight fucan; and

extracting the desired high-molecular-weight fucan from the selected portion of the electrophoresis gel.

116. The method of claim 115 wherein subjecting the starting fucan composition to gel electrophoresis comprises applying a potential difference across the electrophoresis gel between about 10 Volt/cm and 200 Volt/cm.
117. The method of claim 115 wherein the electrophoresis gel comprises at least one of agarose, polyacrylamide, polydimethylacrylamide and starch.
118. The method of claim 117 wherein the electrophoresis gel further comprises at least one of tris-acetate EDTA, tris-borate EDTA and phosphate buffered saline.
119. The method of claim 115 wherein extracting the desired high-molecular-weight fucan from the selected portion of the electrophoresis gel comprises agitating the selected portion of the electrophoresis gel in a solvent.
120. The method of claim 119 wherein the solvent comprises at least one of water, methanol, ethanol and isopropanol.
121. A method for obtaining a high-molecular-weight fucan comprising:

providing a starting fucan composition having a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment, and an ion exchange macroporous resin; and

subjecting the starting fucan composition to ion exchange with the ion exchange macroporous resin to obtain an ion exchange treated fucan composition consisting essentially of the desired high-molecular-weight fucan.

122. The method of claim 121 wherein the method further comprises collecting the desired high-molecular-weight fucan from the ion exchange treated fucan composition.
123. The method of claim 121 wherein providing the starting fucan composition further comprises desalting the starting fucan composition before subjecting the starting fucan composition to ion exchange.
124. The method of claim 121 wherein a mass ratio of the starting fucan composition:ion exchange macroporous resin is between about 1:100 and about 10:1.
125. The method of claim 124 wherein the mass ratio is between about 1:10 and about 5:1.
126. The method of claim 121 wherein the starting fucan composition is subjected to ion exchange for a period of between about 5 minutes and about 100 hours.
127. The method of claim 121 wherein the ion exchange macroporous resin comprises at least one of an anion exchange macroporous resin and a mixed charge macroporous resin.

128. The method of claim 127 wherein the anion exchange macroporous resin is a strong base macroporous resin.
129. The method of claim 128 wherein the strong base macroporous resin comprises quaternary amine groups.
130. The method of claim 127 wherein the anion exchange macroporous resin is a weak base macroporous resin.
131. The method of claim 130 wherein the weak base macroporous resin comprises at least one of primary, secondary or tertiary amine groups.
132. The method of claim 121 wherein the ion exchange macroporous resin comprises at least one of styrene, agarose, dextran, acrylate, methacrylate, methyl methacrylate, butyl methacrylate, divinylbenzene, cellulose, silica, and ceramic.
133. The method of claim 121 wherein the ion exchange macroporous resin has a pore size of between about 5 nm and about 1000 nm.
134. The method of claim 133 wherein the pore size is between about 10 nm and about 100 nm.
135. The method of claim 133 wherein the pore size is between about 15 nm and about 50 nm.
136. The method of claim 121 wherein the ion exchange macroporous resin has an exclusion limit of between about 50 kDa and about 50,000 kDa.
137. The method of claim 136 wherein the exclusion limit is between about 1,000 kDa and about 9,000 kDa.
138. The method of claim 136 wherein the exclusion limit is between about 100 kDa and about 1,000 kDa.
139. A method for obtaining a high-molecular-weight fucan comprising:

providing a starting fucan composition with a broad molecular weight distribution comprising a desired high-molecular-weight fucan segment in a starting solution, and a gel media;

subjecting the starting solution to preparative gel permeation chromatography, wherein the starting fucan composition is displaced from a first input end to a second output end across the gel media according to molecular weight; and

collecting from the second output end at least one aliquot consisting essentially of the desired high-molecular-weight fucan segment.

140. The method of claim 139 wherein the method further comprises collecting multiple aliquots and combining the aliquots.
141. The method of claim 139 wherein the gel media is contained in a column.
142. The method of claim 139 wherein the gel media comprises at least one of polyhydroxymethacrylate, sulfonated styrene-divinylbenzene, silica, a hydrophilic bonded phase or polymer, polystyrene, divinylbenzene, methacrylate, methyl methacrylate, butyl methacrylate, cellulose, ceramic, agarose and dextran.
143. The method of claim 139 wherein the gel media has a pore size of between about 3 nm and about 10,000 nm.
144. The method of claim 143 wherein the pore size is between about 3 nm and about 3000 nm.
145. The method of claim 139 wherein the gel media has an exclusion limit of between about 100 Da and about 100,000 kDa.
146. The method of claim 145 wherein the gel media has an exclusion limit of between about 100 kDa and about 30,000 kDa.

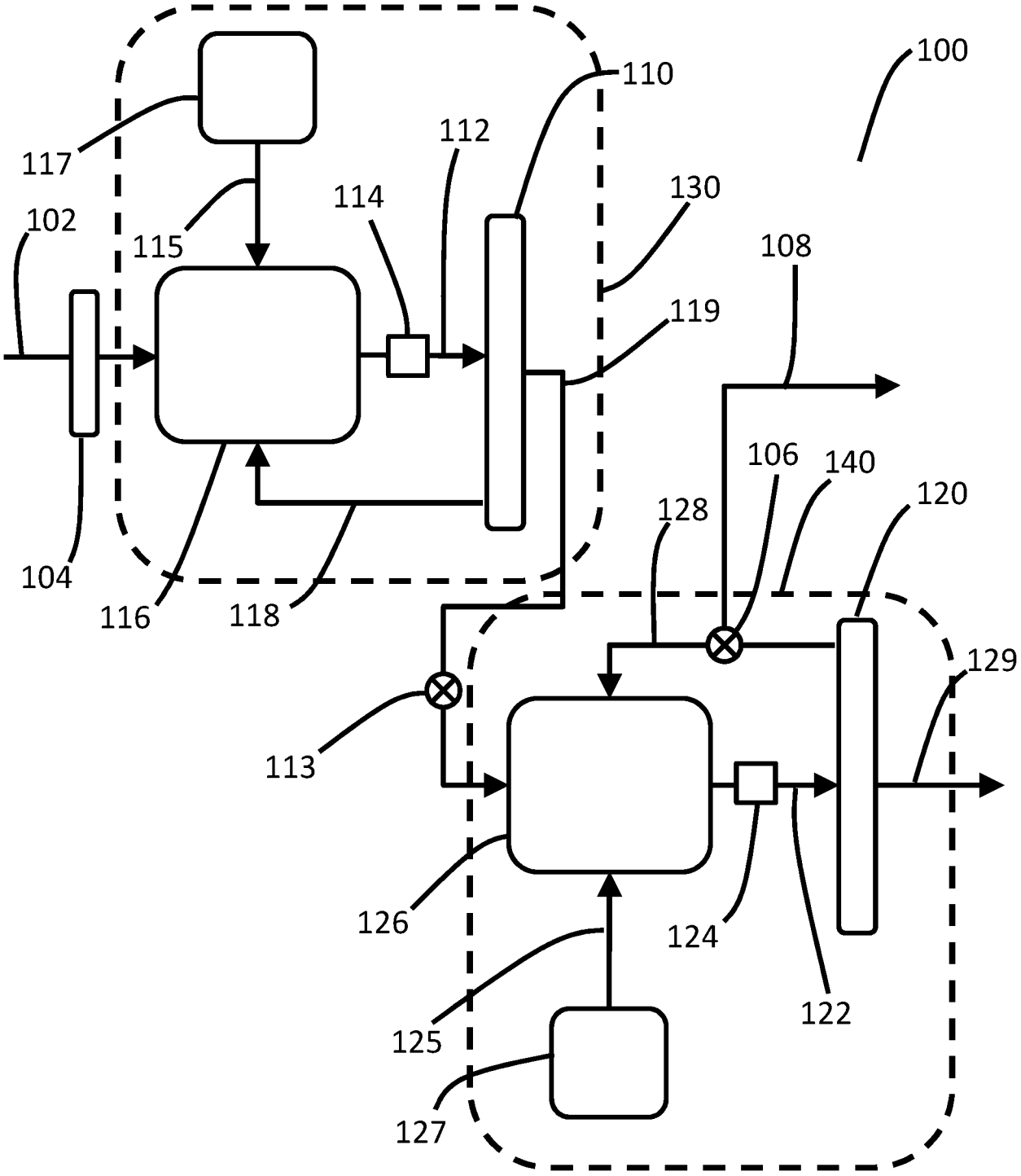
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FIG. 1

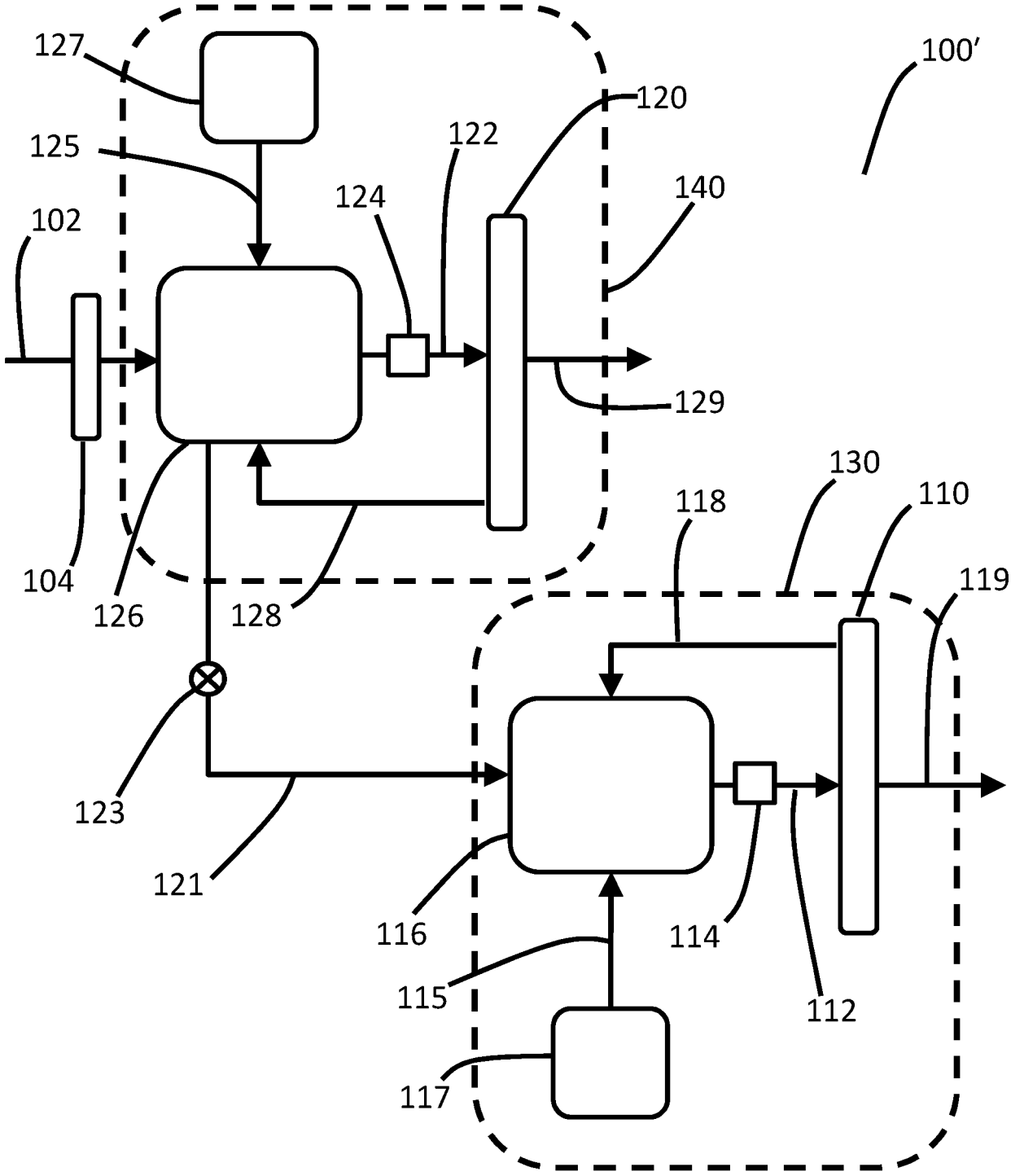
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FIG. 2

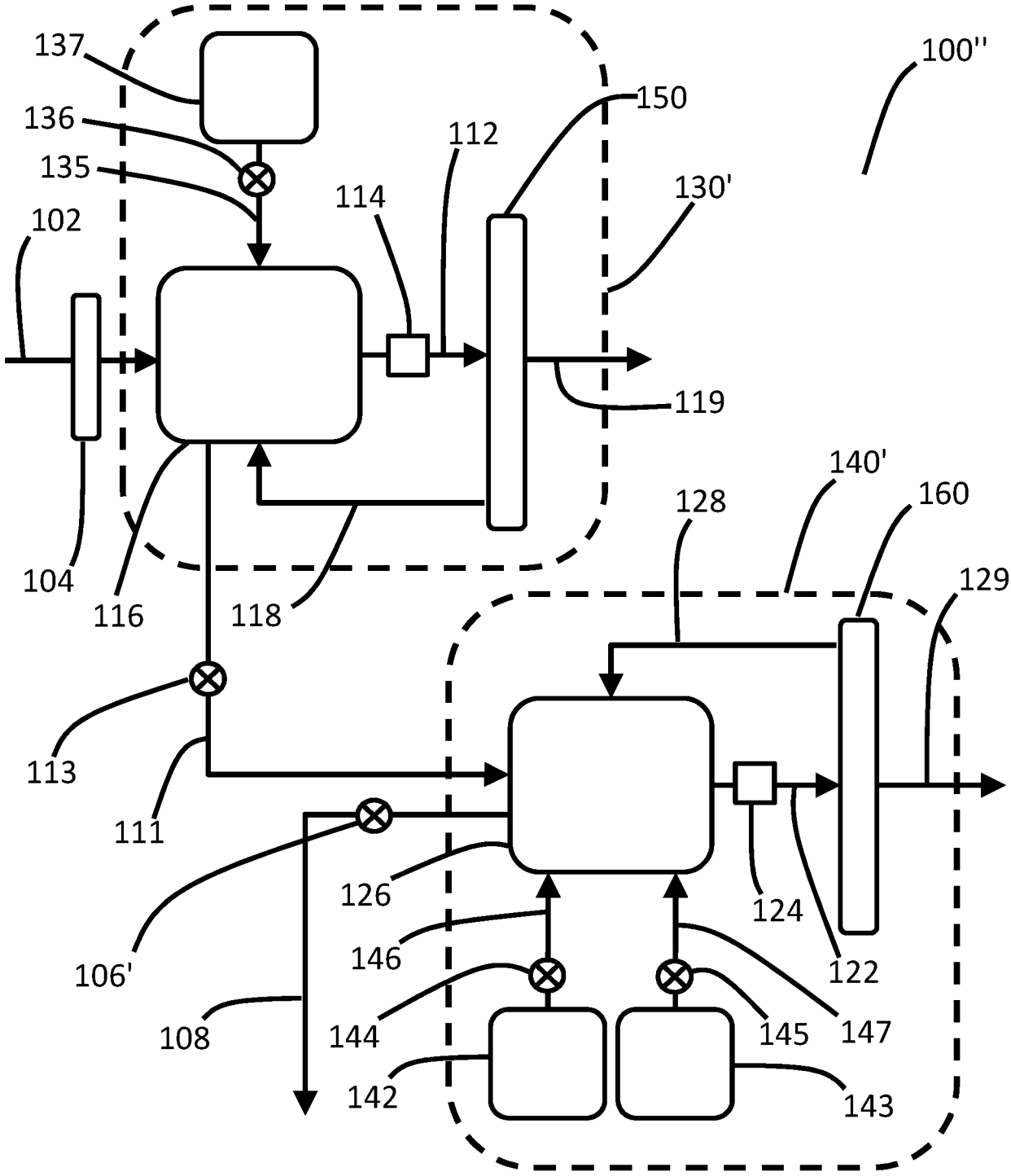
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FIG. 3

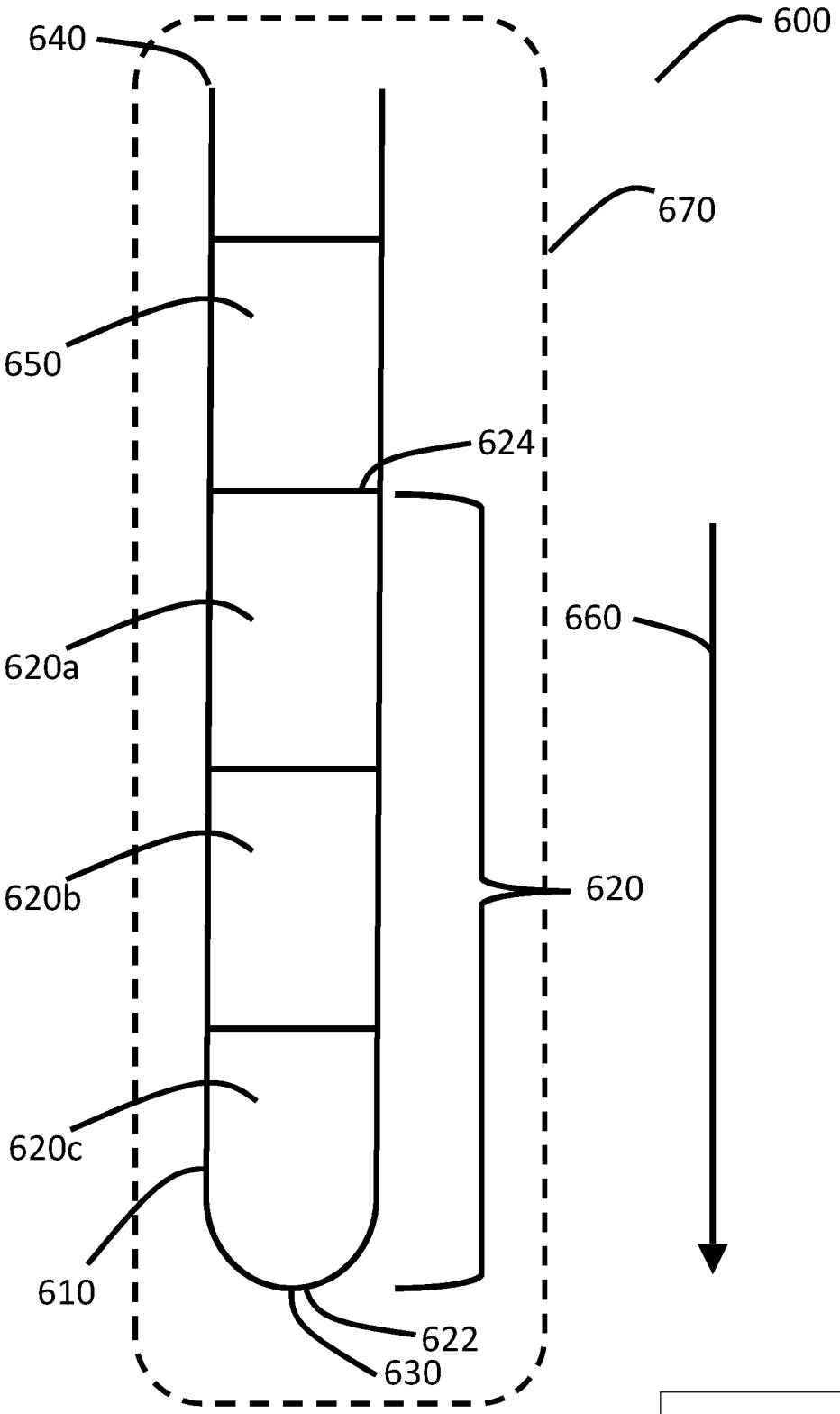

FIG. 4

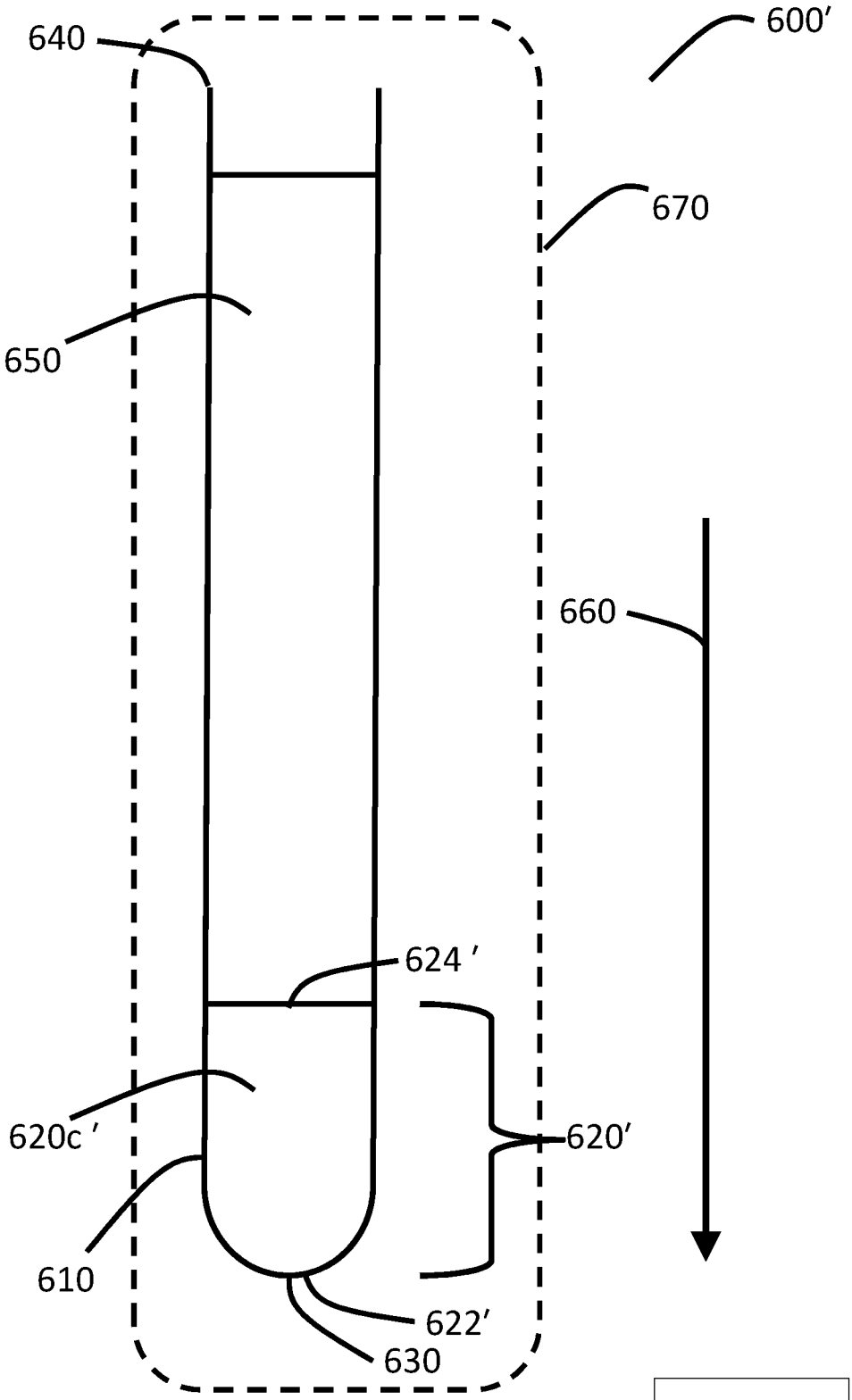

FIG. 5

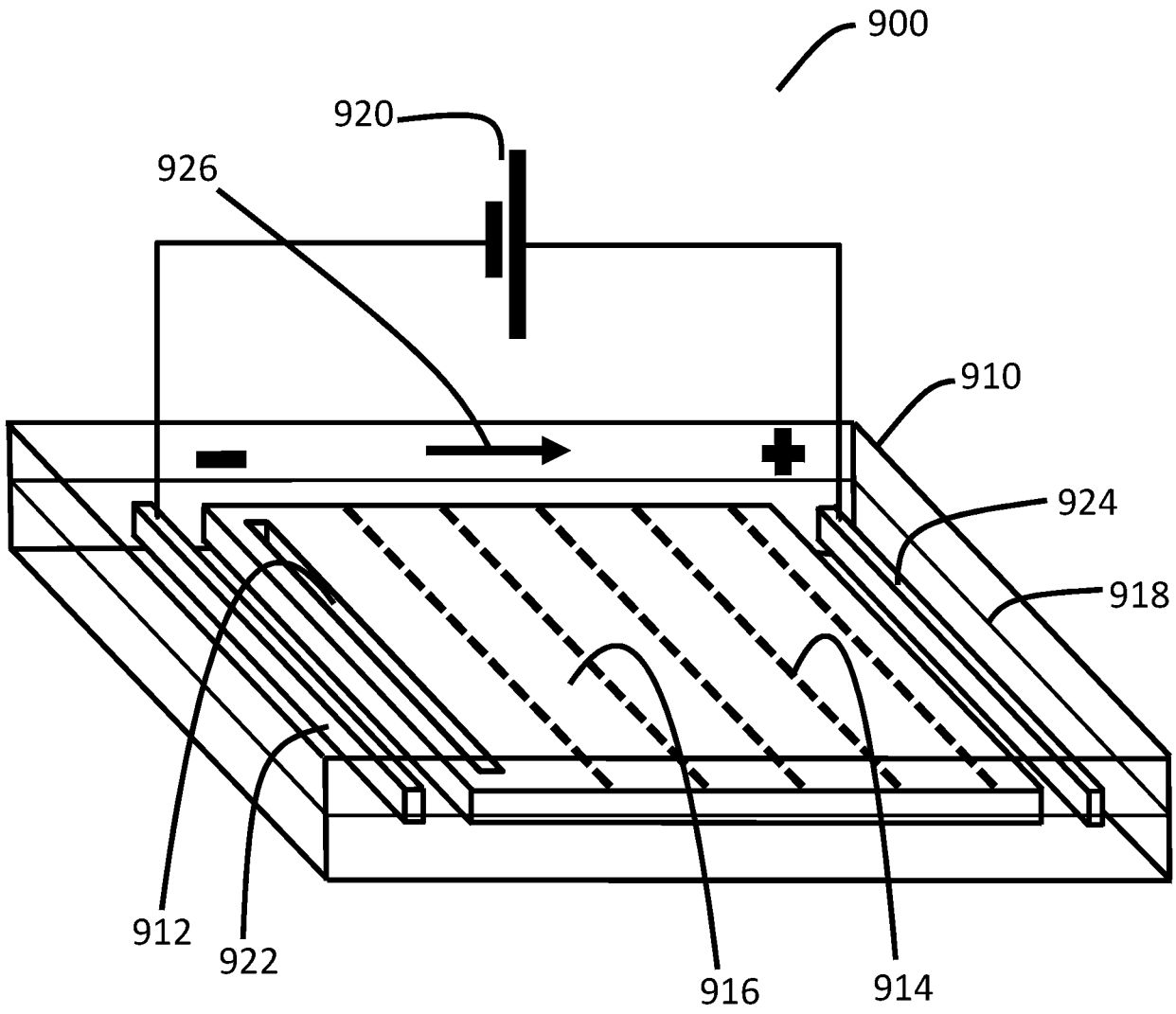

FIG. 6

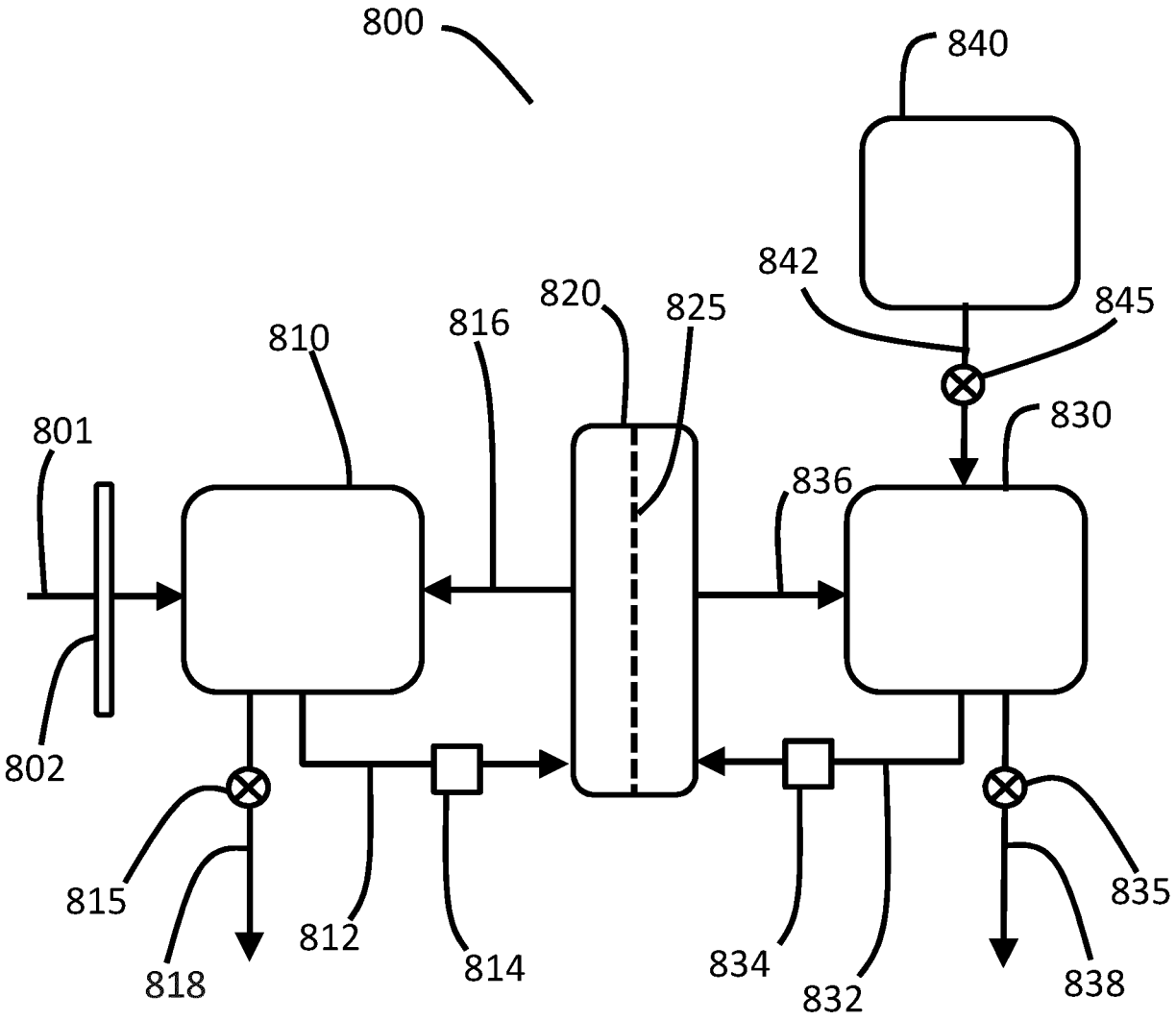
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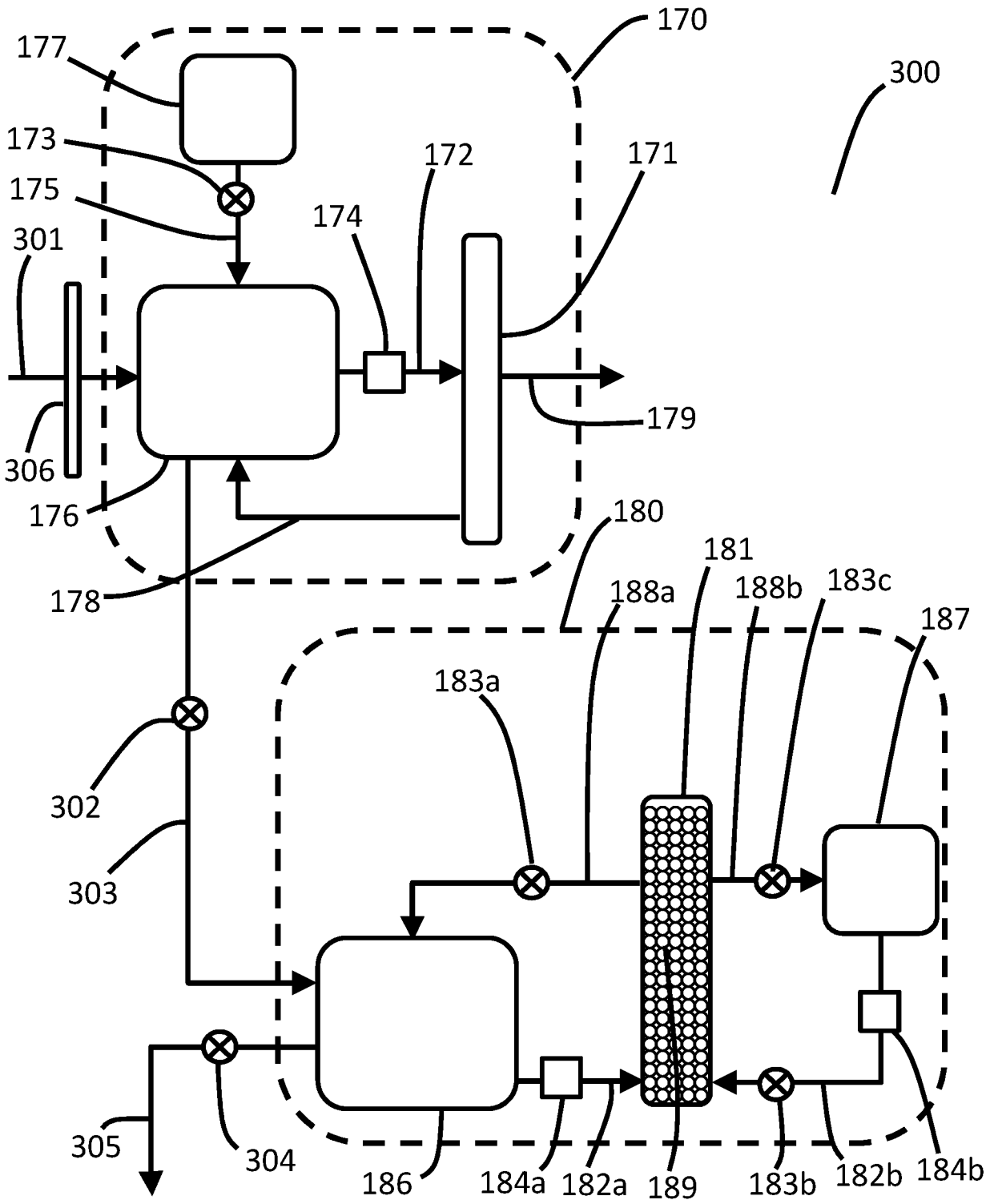
FIG. 7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178
181
188a
188b
183c
187
183a
302
303
305
304
186
184a
182a
189
183b
182b
184b

FIG. 8

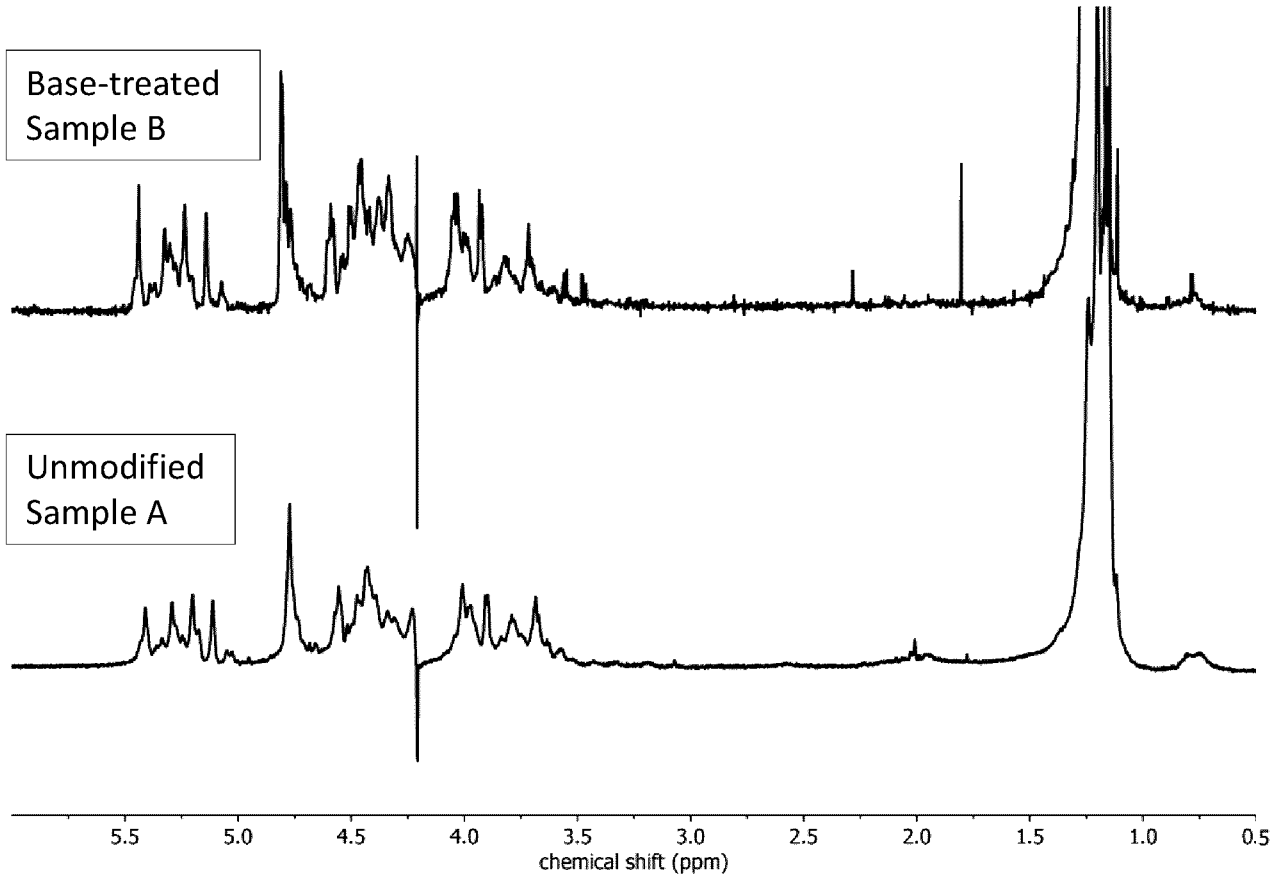
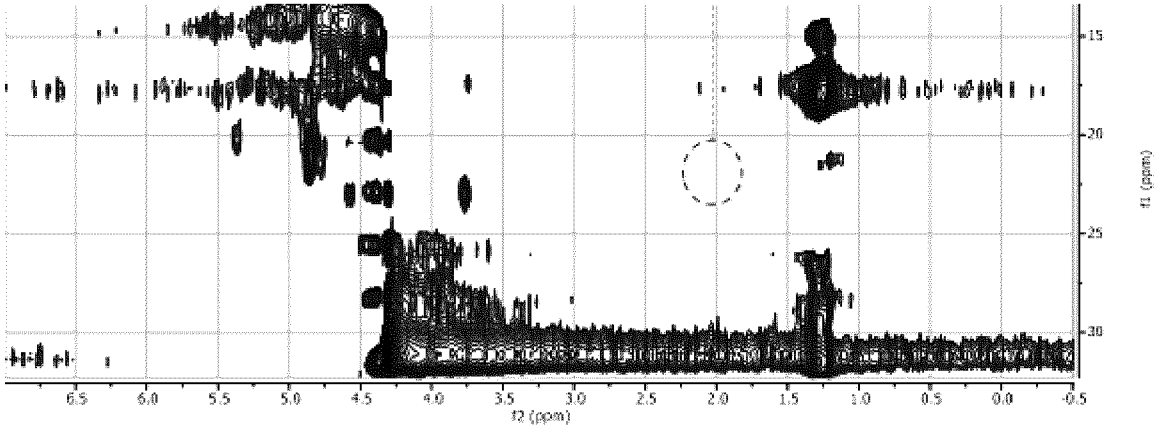

Base-treated
Sample B
Unmodified
Sample A
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
chemical shift (ppm)

FIG. 9A

Base-treated
Sample B



Unmodified
Sample A

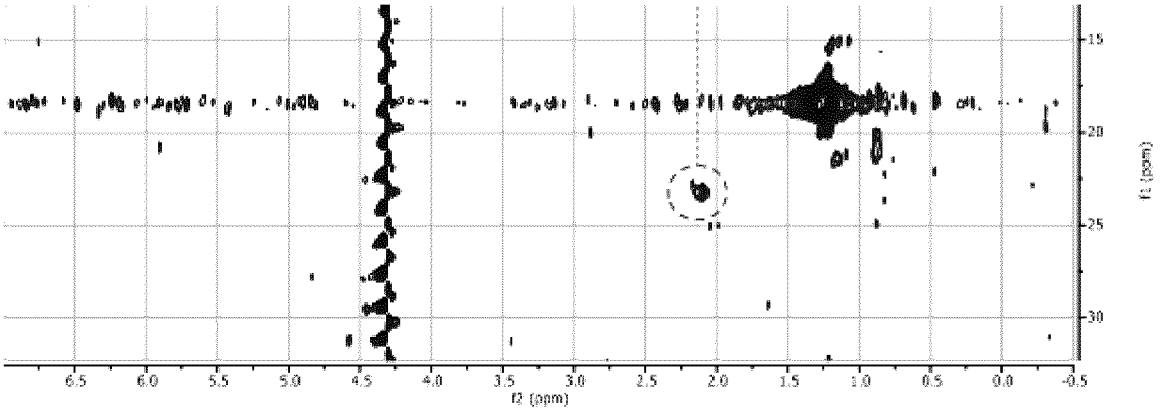


FIG. 9B

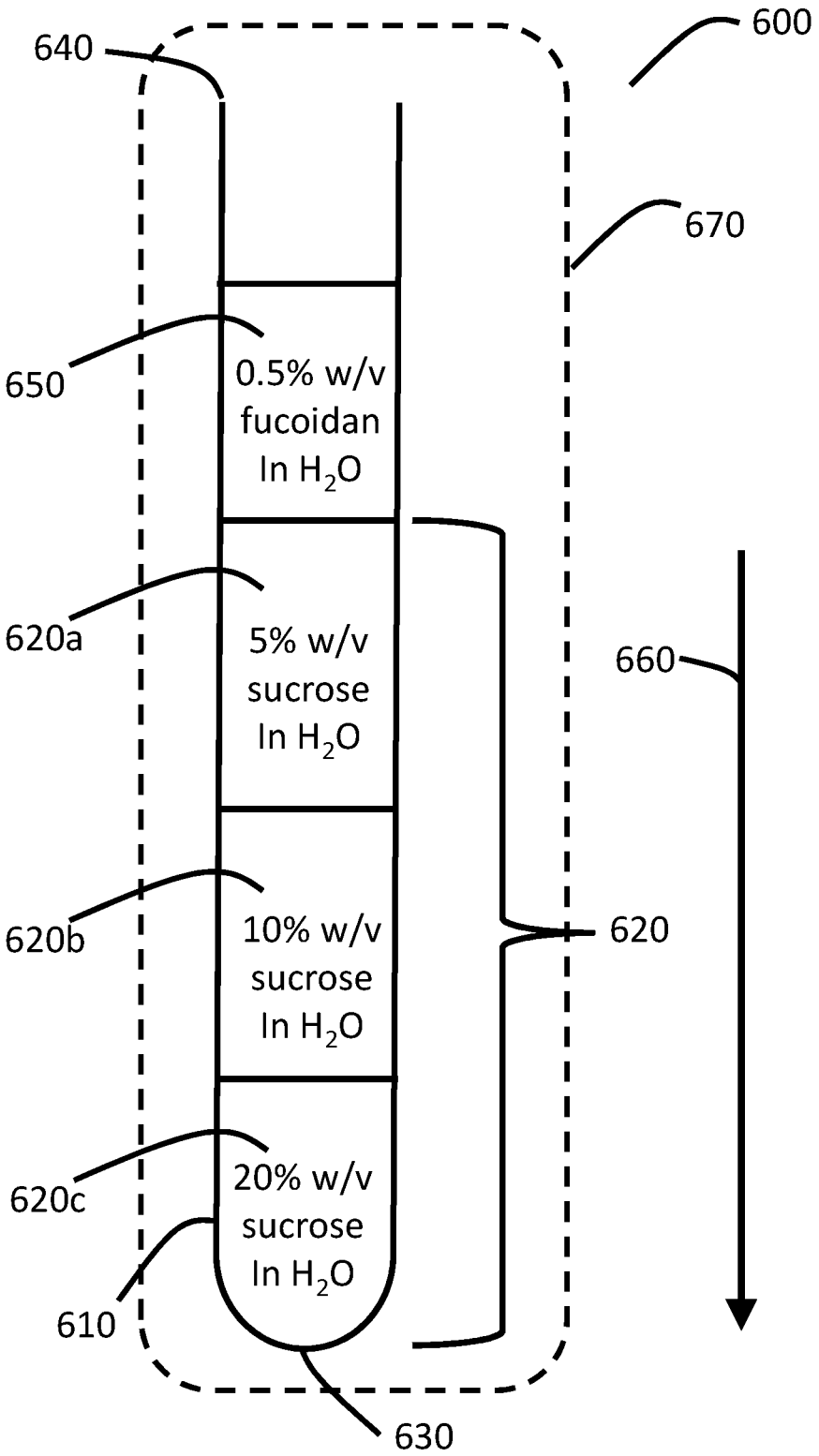

640
600
670
650
0.5% w/v fucoidan In H_2O
620a
5% w/v sucrose In H_2O
660
620b
10% w/v sucrose In H_2O
620
620c
20% w/v sucrose In H_2O
610
630

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/051027

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C08L 5/00*** (2006.01) , ***A61K 31/737*** (2006.01) , ***A61P 41/00*** (2006.01) , ***C08B 37/00*** (2006.01)

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)
C08L 5/00 (2006.01) , ***A61K 31/737*** (2006.01) , ***A61P 41/00*** (2006.01) , ***C08B 37/00*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Google patent, Questel Orbit FAMPAT,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D1: CHIZHOV et al., "A study of fucoidan from the brown seaweed Chorda filum" Carbohydrate Research, 20 July 1999 (20-07-1999), Vol 320, pp108-119. (whole document)	1-84, 121-138
X	D2: CN105399848B 16 March 2016 (16-03-2016) google translation.	1-84, 121-138
X	D3: US2018/0051097 SPRINGATE, CHRISTOPHER 22 February 2018 (22-02-2018)	1-84
X	D4: CN106832022 13 June 2017 (13-06-2917) abstract google patents	85-94, 111-114
X	D5: US8426381B Thibodeau, Alain et al. 23 April 2013 (23.04.2013)	104-111, 115-120

☑ 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
"D"	document cited by the applicant in the international application
"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 19 November 2019 (19-11-2019)	Date of mailing of the international search report 20 November 2019 (20-11-2019)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Rebecca Gardner (819) 639-8460

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/051027

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim 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. ☐ Claim 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:

Group A: Claims 1-84 are directed to high molecular weight fucans and their use to treat fibrous adhesions;
Group B: Claims 85-103 are directed to methods to make high molecular weight fucans via tangential flow filtration;
Group C: Claims 104-114 are directed to methods to make high molecular weight fucans via centrifuge;
Group D: Claims 115-120 are directed to methods to make high molecular weight fucans via gel electrophoresis;
Group E: Claims 121-138 are directed to methods to make high molecular weight fucans via ion exchange macroporous resin; and
Group F: Claims 139-146 are directed to methods to make high molecular weight fucans via gel permeation chromatography.

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 claim 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 claim Nos.:

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.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D6:WU et al., "Structural Analysis and Anticoagulant Activities of the Novel Sulfated Fucan Possessing a Regular Well-Defined Repeating Unit from Sea Cucumber", Marine Drugs, 13 April 2015 (13-04-2015) Vol 13, p 2063-2084.	139-146
X	D7:PEREIRA, M.S. et al., "Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans" Glycobiology, 1 October 2002 (01-10-2002) Vol 12(10), pp573-580. **Retrieved from the internet:<** **https://doi.org/10.1093/glycob/cwf077>**	139-146

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		EP2056890A2	13 May 2009 (13-05-2009)
		EP2056890B1	18 November 2015 (18-11-2015)
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		US2011021457A1	27 January 2011 (27-01-2011)
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		WO2011011881A1	03 February 2011 (03-02-2011)
		ZA201200999B	29 May 2013 (29-05-2013)
CN106832022A	13 June 2017 (13-06-2017)	None	
US8426381B2	23 April 2013 (23-04-2013)	US2009215720A1	27 August 2009 (27-08-2009)
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		WO2007028256A2	15 March 2007 (15-03-2007)
		WO2007028256A3	26 April 2007 (26-04-2007)

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2021.03.16

(21)申请号 201980050281.2

(22)申请日 2019.07.24

(30)优先权数据
62/711,335 2018.07.27 US
62/711,364 2018.07.27 US
62/711,372 2018.07.27 US
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62/713,413 2018.08.01 US
62/722,135 2018.08.23 US
62/722,137 2018.08.23 US
62/755,311 2018.11.02 US
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62/793,514 2019.01.17 US
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62/861,223 2019.06.13 US
62/861,228 2019.06.13 US
62/861,235 2019.06.13 US

(85)PCT国际申请进入国家阶段日
2021.01.27

(86)PCT国际申请的申请数据
PCT/CA2019/051027 2019.07.24

(87)PCT国际申请的公布数据
WO2020/019078 EN 2020.01.30

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(74)专利代理机构 北京市柳沈律师事务所 11105
代理人 涂滔

(51)Int.Cl.
C08L 5/00(2006.01)
A61K 31/737(2006.01)
A61P 41/00(2006.01)
C08B 37/00(2006.01)

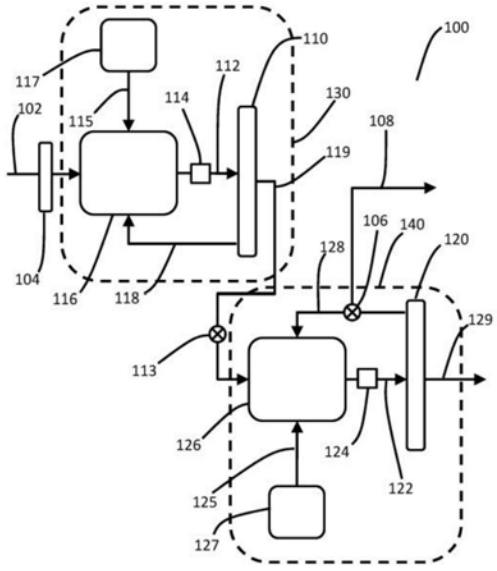
权利要求书10页 说明书73页 附图11页

(54)发明名称

用于治疗纤维性粘连和其他疾病和病症的高分子量褐藻糖胶

(57)摘要

高分子量褐藻糖胶组合物包括治疗有效的、医疗上可接受的褐藻糖胶，在组合物中包括，其中，例如，所述褐藻糖胶具有分子量分布，其中，大于60%w/w的所述组合物具有大于100kDa的分子量。



CN 112513165 A

1.一种基本上由分子量分布组成的高分子量褐藻糖胶,其中,当使用水性凝胶渗透色谱法装置测量时,所述分布的至少92%w/w大于100kDa,所述水性凝胶渗透色谱法装置基本上由以下组成:

具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱,装填有羟基化聚甲基丙烯酸酯基凝胶,具有约50kDa至约5,000kDa的有效分子量范围;具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱,装填有羟基化聚甲基丙烯酸酯基凝胶,具有约1kDa至约6,000kDa的有效分子量范围;以及具有6mm内径的一个40mm保护柱,装填有羟基化聚甲基丙烯酸酯基凝胶,所述两个分析型凝胶渗透色谱柱及所述一个保护柱被容纳在处于约30℃的柱室中;

折射率检测器,处于约30℃;

0.1M硝酸钠流动相,以0.6mL/min运行;以及

相对于峰分子量标准曲线进行量化,所述峰分子量标准曲线基本上由以下组成:第一右旋糖酐标准物,具有的峰分子量为约2,200kDa;第二右旋糖酐标准物,具有的峰分子量为约720kDa至约760kDa;第三右旋糖酐标准物,具有的峰分子量为约470kDa至约510kDa;第四右旋糖酐标准物,具有的峰分子量为约370kDa至约410kDa;第五右旋糖酐标准物,具有的峰分子量为约180kDa至约220kDa;以及第六右旋糖酐标准物,具有的峰分子量为约40kDa至55kDa。

2.根据权利要求1所述的高分子量褐藻糖胶,其中,所述分布的至少93%w/w大于100kDa。

3.根据权利要求1所述的高分子量褐藻糖胶,其中,所述分布的至少94%w/w大于100kDa。

4.根据权利要求1所述的高分子量褐藻糖胶,其中,所述分布的至少95%w/w大于100kDa。

5.根据权利要求1所述的高分子量褐藻糖胶,其中,所述分布的至少97%w/w大于100kDa。

6.根据权利要求1所述的高分子量褐藻糖胶,其中,所述分布的至少98%w/w大于100kDa。

7.根据权利要求1所述的高分子量褐藻糖胶,其中,所述分布的至少99%w/w大于100kDa。

8.根据权利要求1至7中任一项所述的高分子量褐藻糖胶,包括的重均分子量为约100kDa至10,000kDa。

9.根据权利要求8所述的高分子量褐藻糖胶,包括的重均分子量为约140kDa至8,100kDa。

10.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约370kDa至8100kDa。

11.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约370kDa至5300kDa。

12.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约370kDa至1900kDa。

13.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约590kDa至

1600kDa。

14.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约860kDa至1600kDa。

15.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约1,100kDa。

16.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约1,200kDa。

17.根据权利要求8所述的高分子量褐藻糖胶,其中,所述重均分子量为约1,300kDa。

18.根据权利要求1至17中任一项所述的高分子量褐藻糖胶,包括的数均分子量为约50kDa至3,000kDa。

19.根据权利要求18所述的高分子量褐藻糖胶,其中,所述数均分子量为约60kDa至2,000kDa。

20.根据权利要求18所述的高分子量褐藻糖胶,其中,所述数均分子量为约140kDa至2,000kDa。

21.根据权利要求18所述的高分子量褐藻糖胶,其中,所述数均分子量为约140kDa至520kDa。

22.根据权利要求18所述的高分子量褐藻糖胶,其中,所述数均分子量为约230kDa至450kDa。

23.根据权利要求1至22中任一项所述的高分子量褐藻糖胶,其中,所述分布的至少55%w/w大于约200kDa。

24.根据权利要求23所述的高分子量褐藻糖胶,其中,所述分布的至少71%w/w大于约200kDa。

25.根据权利要求23所述的高分子量褐藻糖胶,其中,所述分布的至少91%w/w大于约200kDa。

26.根据权利要求1至25中任一项所述的高分子量褐藻糖胶,其中,所述分布的至少22%w/w大于约500kDa。

27.根据权利要求26所述的高分子量褐藻糖胶,其中,所述分布的至少54%w/w大于约500kDa。

28.根据权利要求26所述的高分子量褐藻糖胶,其中,所述分布的至少90%w/w大于约500kDa。

29.一种基本上由分子量分布组成的高分子量褐藻糖胶,其中,当使用水性凝胶渗透色谱法装置测量时,所述分布的约61%w/w至80%w/w为约200kDa至1600kDa,所述水性凝胶渗透色谱法装置基本上由以下组成:

具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱,装填有羟基化聚甲基丙烯酸酯基凝胶,具有约50kDa至约5,000kDa的有效分子量范围;具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱,装填有羟基化聚甲基丙烯酸酯基凝胶,具有约1kDa至约6,000kDa的有效分子量范围;以及具有6mm内径的一个40mm保护柱,装填有羟基化聚甲基丙烯酸酯基凝胶,所述两个分析型凝胶渗透色谱柱及所述一个保护柱被容纳在处于约30℃的柱室中;

折射率检测器,处于约30℃;

0.1M硝酸钠流动相,以0.6mL/min运行;以及

相对于峰分子量标准曲线进行量化,所述峰分子量标准曲线基本上由以下组成:第一

右旋糖酐标准物，具有的峰分子量为约2,200kDa；第二右旋糖酐标准物，具有的峰分子量为约720kDa至约760kDa；第三右旋糖酐标准物，具有的峰分子量为约470kDa至约510kDa；第四右旋糖酐标准物，具有的峰分子量为约370kDa至约410kDa；第五右旋糖酐标准物，具有的峰分子量为约180kDa至约220kDa；以及第六右旋糖酐标准物，具有的峰分子量为约40kDa至55kDa。

30.一种基本上由分子量分布组成的高分子量褐藻糖胶，其中，当使用水性凝胶渗透色谱法装置测量时，所述分布的至少60％w/w大于约1600kDa，所述水性凝胶渗透色谱法装置基本上由以下组成：

具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱，装填有羟基化聚甲基丙烯酸酯基凝胶，具有约50kDa至约5,000kDa的有效分子量范围；具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱，装填有羟基化聚甲基丙烯酸酯基凝胶，具有约1kDa至约6,000kDa的有效分子量范围；以及具有6mm内径的一个40mm保护柱，装填有羟基化聚甲基丙烯酸酯基凝胶，所述两个分析型凝胶渗透色谱柱及所述一个保护柱被容纳在处于约30℃的柱室中；

折射率检测器，处于约30℃；

0.1M硝酸钠流动相，以0.6mL/min运行；以及

相对于峰分子量标准曲线进行量化，所述峰分子量标准曲线基本上由以下组成：第一右旋糖酐标准物，具有的峰分子量为约2,200kDa；第二右旋糖酐标准物，具有的峰分子量为约720kDa至约760kDa；第三右旋糖酐标准物，具有的峰分子量为约470kDa至约510kDa；第四右旋糖酐标准物，具有的峰分子量为约370kDa至约410kDa；第五右旋糖酐标准物，具有的峰分子量为约180kDa至约220kDa；以及第六右旋糖酐标准物，具有的峰分子量为约40kDa至55kDa。

31.根据权利要求1至30中任一项所述的高分子量褐藻糖胶，其中，所述硫酸酯含量为约20％w/w至60％w/w。

32.根据权利要求31所述的高分子量褐藻糖胶，其中，所述硫酸酯含量为约30％w/w至55％w/w。

33.根据权利要求31所述的高分子量褐藻糖胶，其中，所述硫酸酯含量为约32％w/w至52％w/w。

34.根据权利要求1至33中任一项所述的高分子量褐藻糖胶，其中，所述总碳水化合物含量为约27％w/w至80％w/w。

35.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总岩藻糖含量占所述总碳水化合物含量的百分比为至少约30％w/w。

36.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总岩藻糖含量占所述总碳水化合物含量的百分比为至少约50％w/w。

37.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总岩藻糖含量占所述总碳水化合物含量的百分比为至少约70％w/w。

38.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总岩藻糖含量占所述总碳水化合物含量的百分比为至少约80％w/w。

39.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总岩藻糖含量占所述总碳水化合物含量的百分比为至少约90％w/w。

40.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总岩藻糖含量占所述总碳水化合物含量的百分比为至少约95％w/w。

41.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总半乳糖含量占所述总碳水化合物含量的百分比低于约60％w/w。

42.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总半乳糖含量占所述总碳水化合物含量的百分比为约2％w/w至20％w/w。

43.根据权利要求34所述的高分子量褐藻糖胶，其中，所述总半乳糖含量占所述总碳水化合物含量的百分比低于约10％w/w。

44.根据权利要求34所述的高分子量褐藻糖胶，其中，葡糖醛酸、甘露糖、鼠李糖、葡萄糖和木糖的总含量占所述总碳水化合物含量的百分比低于约30％w/w。

45.根据权利要求1至44中任一项所述的高分子量褐藻糖胶，其中，当以50mg/mL的浓度溶解在水中时，所述高分子量褐藻糖胶的粘度为约4cP至50cP。

46.根据权利要求1至44中任一项所述的高分子量褐藻糖胶，其中，当以50mg/mL的浓度溶解在水中时，所述高分子量褐藻糖胶的粘度为约10cP至40cP。

47.根据权利要求1至44中任一项所述的高分子量褐藻糖胶，其中，当以50mg/mL的浓度溶解在水中时，所述高分子量褐藻糖胶的粘度为约15cP至30cP。

48.根据权利要求1至47中任一项所述的高分子量褐藻糖胶，其中，所述高分子量褐藻糖胶为白色固体。

49.根据权利要求1至48中任一项所述的高分子量褐藻糖胶，其中，当以1mg/mL至100mg/mL的浓度溶解在水中时，所述高分子量褐藻糖胶形成一种澄清无色的溶液。

50.根据权利要求1至49中任一项所述的高分子量褐藻糖胶，其中，所述褐藻糖胶包括小于5％w/w的乙酰基含量。

51.根据权利要求1至49中任一项所述的高分子量褐藻糖胶，其中，所述褐藻糖胶包括小于2％w/w的乙酰基含量。

52.根据权利要求1至49中任一项所述的高分子量褐藻糖胶，其中，当在70℃下伴随溶剂信号抑制在配备有5-mm冷探针的600MHz光谱仪上在碳尺寸10-30ppm范围内以256-512的8次增量扫描每次通过2D ^{1}H-^{13}C异核多量子相干测量时，所述褐藻糖胶包括的乙酰基含量基本为0％w/w。

53.一种方法，包括制备根据权利要求1至52中任一项所述的高分子量褐藻糖胶。

54.一种方法，包括使用根据权利要求1至52中任一项所述的高分子量褐藻糖胶。

55.根据权利要求54所述的方法，其中，所述使用包括治疗纤维性粘连。

56.一种医疗上可接受的褐藻糖胶组合物，包括医疗上可接受的缓冲剂或稀释剂中的治疗有效量的权利要求1至52中任一项所述的高分子量褐藻糖胶。

57.一种治疗动物的病症或疾病的方法，包括：选择权利要求56所述的医疗上可接受的褐藻糖胶组合物以治疗所述病症或疾病，以及向所述动物给药包括约0.5mg/kg至50mg/kg的治疗有效量的所述高分子量褐藻糖胶。

58.一种治疗动物的病症或疾病的方法，包括：选择权利要求56所述的医疗上可接受的褐藻糖胶组合物以治疗所述病症或疾病，以及向所述动物给药约0.04mg/kg至25mg/kg的治疗有效量的所述高分子量褐藻糖胶。

59.根据权利要求57或58所述的方法，其中，所述治疗有效量为约0.2mg/kg至10mg/kg。

60.根据权利要求57或58所述的方法，其中，所述治疗有效量为约1mg/kg至5mg/kg。

61.根据权利要求57或58所述的方法，其中，所述治疗有效量为约1.5mg/kg至3mg/kg。

62.根据权利要求57或58所述的方法，其中，所述治疗有效量为约5mg/kg至10mg/kg。

63.根据权利要求57至62中任一项所述的方法，其中，所述病症或疾病是所述动物的目标部位处的纤维性粘连，并且其中，所述给药包括向所述目标部位给药所述治疗有效量。

64.一种医疗组合物，包括约0.02mg/mL至100mg/mL的权利要求1至52中任一项所述的高分子量褐藻糖胶，其中，所述医疗组合物被配置和构成为治疗动物的疾病或病症。

65.根据权利要求64所述的医疗组合物，包括约0.5mg/mL至5mg/mL的所述高分子量褐藻糖胶。

66.根据权利要求64所述的医疗组合物，包括约2.5mg/mL的所述高分子量褐藻糖胶。

67.根据权利要求64至66中任一项所述的医疗组合物，其中，所述医疗组合物是医疗器械。

68.根据权利要求64至66中任一项所述的医疗组合物，其中，所述医疗组合物是液体医疗器械。

69.根据权利要求64至66中任一项所述的医疗组合物，其中，所述医疗组合物是药物组合物。

70.根据权利要求64至66中任一项所述的医疗组合物，其中，所述医疗组合物是液体药物组合物。

71.根据权利要求64至70中任一项所述的医疗组合物，其中，所述疾病或病症是纤维性粘连。

72.包括约0.01mL/kg至15mL/kg的剂量范围的权利要求64至71中任一项所述的医疗组合物用于治疗动物的疾病或病症的用途。

73.包括约0.03mL/kg至4mL/kg的剂量范围的权利要求64至71中任一项所述的医疗组合物用于治疗动物的疾病或病症的用途。

74.包括约0.06mL/kg至2mL/kg的剂量范围的权利要求64至71中任一项所述的医疗组合物用于治疗动物的疾病或病症的用途。

75.包括约2mL/kg至4mL/kg的剂量范围的权利要求64至71中任一项所述的医疗组合物用于治疗动物的疾病或病症的用途。

76.一种用于治疗患者的选择的疾病或病症的方法，包括：确认患者的包括或相当容易患有所述选择的疾病或病症的选择的目标部位，然后向所述患者的目标部位给药权利要求64至71中任一项所述的医疗组合物。

77.根据权利要求76所述的方法，其中，所述疾病或病症是纤维性粘连。

78.根据权利要求76或77所述的方法，其中，所述目标部位是外科手术部位，并且所述给药是在以下情况中的至少一种情况下进行的：a)在所述外科手术部位处打开外科手术伤口之后，b)在外科手术期间，以及c)在闭合所述外科手术伤口之后。

79.根据权利要求76或77所述的方法，其中，所述给药在外科手术之后但在闭合所述外科手术伤口之前进行。

80.根据权利要求76或77所述的方法，其中，所述给药耗时少于3分钟。

81.根据权利要求76或77所述的方法，其中，所述给药耗时少于2分钟。

82.根据权利要求76或77所述的方法，其中，所述给药耗时少于1分钟。

83.根据权利要求76或77所述的方法，其中，所述目标部位是病变、擦伤和损伤部位中的至少一种。

84.根据权利要求76或77所述的方法，其中，所述目标部位是以下中的至少一种：骨盆腔、腹腔、背侧腔、颅腔、脊髓腔、腹侧腔、胸腔、胸膜腔、心包腔、皮肤、关节、肌肉、肌腱和韧带。

85.一种用于获得高分子量褐藻糖胶的方法，包括：

以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物，所述宽分子量分布包括所需高分子量褐藻糖胶区段；

使所述起始溶液通过第一较高截留分子量切向流过滤过滤器进行第一切向流过滤，以产生第一渗透褐藻糖胶组合物；以及

使所述第一渗透褐藻糖胶组合物通过第二较低截留分子量切向流过滤过滤器进行第二切向流过滤，以产生基本上由所需高分子量褐藻糖胶组成的第二渗余褐藻糖胶组合物。

86.根据权利要求85所述的方法，其中，所述方法还包括：收集基本上由所需高分子量褐藻糖胶组成的所述第二渗余褐藻糖胶组合物。

87.根据权利要求85所述的方法，其中，所述第一较高截留分子量切向流过滤过滤器具有约50kDa至约1000kDa的较高截留分子量，并且所述第二截留较低分子量切向流过滤过滤器具有约30kDa至约100kDa的较低截留分子量。

88.根据权利要求87所述的方法，其中，所述较高截留分子量为约300kDa，并且所述较低截留分子量为约100kDa。

89.一种用于获得高分子量褐藻糖胶的方法，包括：

以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物，所述宽分子量分布包括所需高分子量褐藻糖胶区段；

使所述起始溶液通过第一较低截留分子量切向流过滤过滤器进行切向流过滤，以产生第一渗余褐藻糖胶组合物；以及

使所述第一渗余褐藻糖胶组合物通过第二较高截留分子量切向流过滤过滤器进行切向流过滤，以产生基本上由所需高分子量褐藻糖胶组成的第二渗透褐藻糖胶组合物。

90.根据权利要求89所述的方法，其中，所述方法还包括：收集基本上由所需高分子量褐藻糖胶组成的所述第二渗透褐藻糖胶组合物。

91.根据权利要求89所述的方法，其中，所述第一切向流过滤包括：通过所述第一较低截留分子量切向流过滤过滤器对所述起始溶液进行渗滤。

92.根据权利要求89所述的方法，其中，所述第二切向流过滤包括：通过所述第二较高截留分子量切向流过滤过滤器对所述第一渗余褐藻糖胶组合物进行渗滤。

93.根据权利要求89所述的方法，其中，所述第一较低截留分子量切向流过滤过滤器具有约30kDa至约100kDa的较低截留分子量，并且所述第二较高截留分子量切向流过滤过滤器具有约50kDa至约1000kDa的较高截留分子量。

94.根据权利要求92所述的方法，其中，所述较低截留分子量为约100kDa，并且所述较高截留分子量为约300kDa。

95.一种用于获得高分子量褐藻糖胶的方法,包括:

以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物,所述宽分子量分布包括所需高分子量褐藻糖胶区段,所述起始褐藻糖胶组合物包括与所述组合物中的褐藻糖胶上的硫酸酯基团离子键合的低原子量阳离子;以及

使所述起始溶液相对于阳离子添加剂溶液进行切向流过滤,所述阳离子添加剂溶液包括具有比所述低原子量阳离子大的分子量的阳离子添加剂,以产生基本上由所需高分子量褐藻糖胶组成的渗余褐藻糖胶组合物。

96.根据权利要求95所述的方法,其中,所述方法还包括:收集基本上由所需高分子量褐藻糖胶组成的所述渗余褐藻糖胶组合物。

97.根据权利要求95所述的方法,其中,所述低原子量阳离子包括碱金属、碱土金属、铝和铵中的至少一种。

98.根据权利要求95所述的方法,其中,所述阳离子添加剂包括胆碱、聚乙烯吡咯烷酮、牛磺酸、多胺、壳聚糖、组蛋白和胶原蛋白中的至少一种。

99.根据权利要求95所述的方法,还包括:在使所述起始溶液进行切向流过滤之前将所述阳离子添加剂添加到所述起始溶液中。

100.根据权利要求95所述的方法,其中,所述切向流过滤包括:相对于所述阳离子添加剂溶液对所述起始溶液进行渗滤。

101.根据权利要求95所述的方法,还包括:通过在具有比所述第一切向流过滤过滤器的截留分子量小的截留分子量的第二切向流过滤过滤器上相对于盐溶液对所述渗余褐藻糖胶组合物进行渗滤来去除所述阳离子添加剂。

102.根据权利要求101所述的方法,其中,所述盐溶液包括碱金属、碱土金属、铝和/或铵的氯化物、溴化物、碘化物、氟化物、硫酸盐、亚硫酸盐、碳酸盐、碳酸氢盐、磷酸盐、硝酸盐、亚硝酸盐、乙酸盐、柠檬酸盐、硅酸盐和/或氰化物。

103.根据权利要求101所述的方法,还包括:通过相对于低离子强度溶液渗滤所述渗余褐藻糖胶组合物来去除盐。

104.一种用于获得高分子量褐藻糖胶的方法,包括:

提供离心容器,所述离心容器包括底端和顶端以及在其间的可渗透屏障,所述可渗透屏障在其间包括梯度材料;

将具有宽分子量分布的起始褐藻糖胶组合物置于所述离心容器中和所述可渗透屏障上方,所述宽分子量分布包括所需高分子量褐藻糖胶区段;以及

将所述离心容器离心足够长的时间段以产生基本上由所需高分子量褐藻糖胶组成的沉淀物。

105.根据权利要求104所述的方法,其中,所述方法还包括:从所述离心容器收集所需高分子量褐藻糖胶。

106.根据权利要求104所述的方法,其中,所述可渗透屏障包括梯度材料的单个区段。

107.根据权利要求104所述的方法,其中,所述可渗透屏障包括梯度材料的多个区段。

108.根据权利要求106和107中任一项所述的方法,其中,所述梯度材料包括蔗糖、聚蔗糖、甘油、山梨糖醇、CsCl、Cs_2SO_4、KBr、泛影酸盐、Nycodenz®和碘克沙醇中的至少一种。

109.根据权利要求104所述的方法,其中,离心力为约10,000重力至约1,000,000重力。

110.根据权利要求104所述的方法,其中,离心力为60,000重力至约500,000重力。

111.一种用于获得高分子量褐藻糖胶的方法,包括:

提供包括底端和顶端的离心容器;

将起始溶液形式的具有宽分子量分布的起始褐藻糖胶组合物置于所述离心容器中,所述宽分子量分布包括所需高分子量褐藻糖胶区段;以及

将所述离心容器离心足够长的时间段以产生基本上由所需高分子量褐藻糖胶组成的沉淀物。

112.根据权利要求111所述的方法,还包括:从所述离心容器收集作为沉淀物的所需高分子量褐藻糖胶。

113.根据权利要求111所述的方法,其中,离心力为约60,000重力至约1,000,000重力。

114.根据权利要求111所述的方法,其中,离心力为200,000重力至约500,000重力。

115.一种用于获得高分子量褐藻糖胶的方法,包括:

使具有宽分子量分布的起始褐藻糖胶组合物进行凝胶电泳,所述宽分子量分布包括所需高分子量褐藻糖胶区段,其中,所述起始褐藻糖胶组合物根据电泳凝胶上的质荷比进行置换;

选择所述电泳凝胶的一部分,其基本上由所需高分子量褐藻糖胶组成;以及

从所述电泳凝胶的选择的部分提取所需高分子褐藻糖胶。

116.根据权利要求115所述的方法,其中,使所述起始褐藻糖胶组合物进行凝胶电泳包括:在所述电泳凝胶上施加约10伏/cm至200伏/cm的电势差。

117.根据权利要求115所述的方法,其中,所述电泳凝胶包括琼脂糖、聚丙烯酰胺、聚二甲基丙烯酰胺和淀粉中的至少一种。

118.根据权利要求117所述的方法,其中,所述电泳凝胶还包括三乙酸酯EDTA、三硼酸EDTA和磷酸盐缓冲盐水中的至少一种。

119.根据权利要求115所述的方法,其中,从所述电泳凝胶的选择的部分提取所需高分子量褐藻糖胶包括:在溶剂中搅拌所述电泳凝胶的选择的部分。

120.根据权利要求119所述的方法,其中,所述溶剂包括水、甲醇、乙醇和异丙醇中的至少一种。

121.一种用于获得高分子量褐藻糖胶的方法,包括:

提供具有宽分子量分布的起始褐藻糖胶组合物和离子交换大孔树脂,所述宽分子量分布包括所需高分子量褐藻糖胶区段;以及

使所述起始褐藻糖胶组合物与所述离子交换大孔树脂进行离子交换,以获得经离子交换处理的基本上由所需高分子量褐藻糖胶组成的褐藻糖胶组合物。

122.根据权利要求121所述的方法,其中,所述方法还包括:从所述经离子交换处理的褐藻糖胶组合物收集所需高分子量褐藻糖胶。

123.根据权利要求121所述的方法,其中,提供所述起始褐藻糖胶组合物还包括:在使所述起始褐藻糖胶组合物进行离子交换之前对所述起始褐藻糖胶组合物进行脱盐。

124.根据权利要求121所述的方法,其中,所述起始褐藻糖胶组合物:离子交换大孔树脂的质量比为约1:100至约10:1。

125.根据权利要求124所述的方法,其中,所述质量比为约1:10至约5:1。

126.根据权利要求121所述的方法，其中，使所述起始褐藻糖胶组合物进行离子交换的时间段为约5分钟至约100小时。

127.根据权利要求121所述的方法，其中，所述离子交换大孔树脂包括阴离子交换大孔树脂和混合电荷大孔树脂中的至少一种。

128.根据权利要求127所述的方法，其中，所述阴离子交换大孔树脂是强碱大孔树脂。

129.根据权利要求128所述的方法，其中，所述强碱大孔树脂包括季胺基。

130.根据权利要求127所述的方法，其中，所述阴离子交换大孔树脂是弱碱大孔树脂。

131.根据权利要求130所述的方法，其中，所述弱碱大孔树脂包括伯、仲或叔胺基中的至少一种。

132.根据权利要求121所述的方法，其中，所述离子交换大孔树脂包括苯乙烯、琼脂糖、右旋糖酐、丙烯酸酯、甲基丙烯酸酯、甲基丙烯酸甲酯、甲基丙烯酸丁酯、二乙烯基苯、纤维素、二氧化硅和陶瓷中的至少一种。

133.根据权利要求121所述的方法，其中，所述离子交换大孔树脂的孔径为约5nm至约1000nm。

134.根据权利要求133所述的方法，其中，所述孔径为约10nm至约100nm。

135.根据权利要求133所述的方法，其中，所述孔径为约15nm至约50nm。

136.根据权利要求121所述的方法，其中，所述离子交换大孔树脂的排阻极限为约50kDa至约50,000kDa。

137.根据权利要求136所述的方法，其中，所述排阻极限为约1,000kDa至约9,000kDa。

138.根据权利要求136所述的方法，其中，所述排阻极限为约100kDa至约1,000kDa。

139.一种用于获得高分子量褐藻糖胶的方法，包括：

提供起始溶液形式的具有宽分子量分布的起始褐藻糖胶组合物和凝胶介质，所述宽分子量分布包括所需高分子量褐藻糖胶区段；

使所述起始溶液进行制备型凝胶渗透色谱法，其中，所述起始褐藻糖胶组合物根据分子量在所述凝胶介质上从第一输入端到第二输出端进行置换；以及

从所述第二输出端收集至少一个等分部分，所述等分部分基本上由所需高分子量褐藻糖胶区段组成。

140.根据权利要求139所述的方法，其中，所述方法还包括：收集多个等分部分并且合并所述等分部分。

141.根据权利要求139所述的方法，其中，所述凝胶介质被容纳在色谱柱中。

142.根据权利要求139所述的方法，其中，所述凝胶介质包括聚羟基甲基丙烯酸酯、磺化的苯乙烯-二乙烯基苯、二氧化硅、亲水性键合相或聚合物、聚苯乙烯、二乙烯基苯、甲基丙烯酸酯、甲基丙烯酸甲酯、甲基丙烯酸丁酯、纤维素、陶瓷、琼脂糖和右旋糖酐中的至少一种。

143.根据权利要求139所述的方法，其中，所述凝胶介质的孔径为约3nm至约10,000nm。

144.根据权利要求143所述的方法，其中，所述孔径为约3nm至约3000nm。

145.根据权利要求139所述的方法，其中，所述凝胶介质的排阻极限为约100Da至约100,000kDa。

146.根据权利要求145所述的方法，其中，所述凝胶介质的排阻极限为约100kDa至约

30,000kDa。

用于治疗纤维性粘连和其他疾病和病症的高分子量褐藻糖胶

[0001] 要求优先权

[0002] 本申请要求共同审查中的于2018年7月27日提交的美国临时专利申请号62,711,364;于2018年7月27日提交的美国临时专利申请号62,711,372;于2018年7月27日提交的美国临时专利申请号62/711,335;于2018年8月1日提交的美国临时专利申请序列号62/713,399;于2018年8月23日提交的美国临时专利申请号62/722,135;于2018年11月2日提交的美国临时专利申请号62/755,311;于2019年1月17日提交的美国临时专利申请号62/793,514;于2019年6月13日提交的美国临时专利申请号62/861,223;共同审查中的于2018年8月1日提交的美国临时专利申请序列号62/713,392;于2018年8月1日提交的美国临时专利申请号62/713,413;于2018年8月23日提交的美国临时专利申请号62/722,137;于2018年11月2日提交的美国临时专利申请号62/755,318;于2019年6月13日提交的美国临时专利申请号62/861,228;共同审查中的于2018年11月2日提交的美国临时专利申请序列号62/755,328;于2019年1月17日提交的美国临时专利申请号62/793,654;以及于2019年6月13日提交的美国临时专利申请号62/861,235,上述专利申请的内容通过引用整体并入本文。

背景技术

[0003] 褐藻糖胶(包括岩藻多糖)是硫酸酯化的多糖。一般而言,这意味着褐藻糖胶是由许多糖基团组成的分子,并且还具有附接到糖基团上的硫原子。主要的糖基团称为“岩藻糖”,它是具有6个碳原子并且具有化学式$C_6H_{12}O_5$的糖。“岩藻多糖”(或墨角藻多糖)指示衍生自褐藻(海藻)的褐藻糖胶。褐藻糖胶可以单独存在,或者存在于其他糖的混合物中,例如存在于诸如木糖、半乳糖、葡萄糖、葡糖醛酸和/或甘露糖之类的糖的混合物中。这些其他糖可以与褐藻糖胶一起提取自海藻或其他来源。尽管褐藻糖胶目前衍生自天然来源诸如本文提及的褐藻(海藻)、海参等,但“褐藻糖胶”包括具有如本文所讨论的褐藻糖胶的化学和结构基序的聚合物分子,而与褐藻糖胶的一种或多种最终来源无关。

[0004] 岩藻多糖可获自多种褐藻物种,包括但不限于:小腺囊藻(Adenocystisutricularis)、泡叶藻(Ascophyllum nodosum)、绳藻(Chorda filum)、Cystoseirabies marina、南极公牛藻(Durvillaea antarctica)、褐藻门昆布(Eckloniakurome)、极大昆布(Ecklonia maxima)、爱森藻(Eisenia bicyclis)、岩藻(Fucus evanescens)、墨角藻(Fucus vesiculosis)、羊栖菜(Hizikiafusiforme)、伸长海条藻(HimanthaliaElongata)、笼目海带(Kjellmaniellacrassifolia)、Laminaria brasiliensis、拟菊苣海带(Laminaria cichorioides)、极北海带(Laminaria hyperborea)、日本真海带(Laminaria japonica)、糖海带(Laminaria saccharina)、Lessonia trabeculata、巨藻(Macrocystispyrifera)、Pelvetiafastigiata、沟鹿角菜(PelvetiaCanaliculata)、Saccharina japonica、糖海带(Saccharina latissima)、Sargassum stenophylum、鼠尾藻(Sargassum thunbergii)、海嵩子(Sargassum confusum)、马尾藻科植物羊栖菜(Sargassum fusiforme)及裙带菜(Undaria pinnatifida)。这些示例性物种均来自分类纲褐藻纲(Phaeophyceae)且这些物种中的大部

分属于以下科:墨角藻目(Fucales)及海带科(Laminariaceae)。

[0005] 包括岩藻多糖的褐藻糖胶已示出有效用于抑制、预防、去除、减少或以其他方式治疗纤维性粘连的形成。还发现其用于治疗其他相关疾病及病症。

[0006] 因此,对包含具有所需高分子量的褐藻糖胶的组合物的需求尚未得到满足,在一些实施方式中,包括将此类组合物修饰为具有所需的硫酸酯化水平和/或医疗上可行的低内毒素水平。本发明的组合物、系统和方法等提供了这些和/或其他优点。

发明内容

[0007] 本发明的组合物、系统、器械、材料和方法等提供了高分子量褐藻糖胶。此类高分子量褐藻糖胶可以从这样的原料褐藻糖胶组合物或其他起始或初始褐藻糖胶组合物获得:其具有的褐藻糖胶具有宽分子量分布,包括所需的高分子量区段/部分(即,可以衍生出高分子量褐藻糖胶的宽分子量褐藻糖胶组合物;此类起始褐藻糖胶组合物可以是或可以不是粗制的,或者已被预先处理或纯化)。所需的高分子量褐藻胶具有的分子量分布基本上由起始褐藻糖胶宽分子量分布的所需的高分子量区段/部分组成,其中,在低分子量末端处的大量的宽分子量分布已被消除、抑制或减弱,以使任何剩余量都无关紧要。

[0008] 在一些方面,本文的组合物、系统、方法等包括高分子量褐藻糖胶,例如岩藻多糖,其可以包括分子量分布、基本上由分子量分布组成、或者由分子量分布组成,其中,当使用水性凝胶渗透色谱法装置测量时,所述分布的至少60%w/w大于100kDa,所述水性凝胶渗透色谱法装置基本上由以下各项组成:

[0009] 一根内径为7.8mm的300mm分析型凝胶渗透色谱柱,其装填有羟基化聚甲基丙烯酸酯基凝胶,有效分子量范围为约50kDa至约5,000kDa;一根内径为7.8mm的300mm分析型凝胶渗透色谱柱,其装填有羟基化聚甲基丙烯酸酯基凝胶,有效分子量范围为约1kDa至约6,000kDa;以及一根内径为6mm的40mm保护柱,其装填有羟基化聚甲基丙烯酸酯基凝胶,所述两根分析型凝胶渗透色谱柱和所述一根保护柱被容纳在处于约30℃的柱室中;

[0010] 处于约30℃的折射率检测器;

[0011] 以0.6mL/min运行的0.1M硝酸钠流动相;以及

[0012] 相对于峰分子量标准曲线进行量化,所述峰分子量标准曲线基本上由以下各项组成:第一右旋糖酐标准物,具有的峰分子量为约2,200kDa;第二右旋糖酐标准物,具有的峰分子量为约720kDa至约760kDa;第三右旋糖酐标准物,具有的峰分子量为约470kDa至约510kDa;第四右旋糖酐标准物,具有的峰分子量为约370kDa至约410kDa;第五右旋糖酐标准物,具有的峰分子量为约180kDa至约220kDa;以及第六右旋糖酐标准物,具有的峰分子量为约40kDa至55kDa。

[0013] 在一些实施方式中,所述分布的至少约70%w/w、80%w/w、90%w/w、93%w/w、94%w/w、95%w/w、97%w/w、98%w/w或99%w/w可以大于100kDa。所述重均分子量可以为约100kDa至10,000kDa;约140kDa至8,100kDa;约370kDa至8100kDa;约370kDa至5300kDa;约370kDa至8100kDa;约370kDa至5300kDa;约370kDa至1900kDa;约590kDa至1600kDa;约590kDa至1600kDa;或约860kDa至1600kDa。在一些实施方式中,所述重均分子量可以为约1,100kDa、约1,200kDa、或约1,300kDa。所述数均分子量可以为约50kDa至3,000kDa;约60kDa至2,000kDa;约140kDa至2,000kDa;约140kDa至520kDa;或约230kDa至450kDa。所述分布的

至少55%w/w、71%w/w或91%w/w可以大于约200kDa。所述分布的至少22%、54%w/w或90%w/w可以大于约500kDa.

[0014] 在一些实施方式中，所述高分子量褐藻糖胶可以基本上由分子量分布组成、包括分子量分布或由分子量分布组成，当使用上文和本文其他地方阐述的水性凝胶渗透色谱法装置测量时，所述分布的约61%w/w至80%w/w为约200kDa至1600kDa。所述高分子量褐藻糖胶可以基本上由分子量分布组成、包括分子量分布或由分子量分布组成，其中，当使用上文和本文其他地方阐述的水性凝胶渗透色谱法装置测量时，所述分布的至少60%w/w可以大于约1600kDa。

[0015] 所述硫酸酯含量可以为约20%w/w至60%w/w、约30%w/w至55%w/w、或约32%w/w至52%w/w。所述总碳水化合物含量可以为约27%w/w至80%w/w。所述总岩藻糖含量占所述总碳水化合物含量的百分比可以为至少约30%w/w、50%w/w、70%w/w、80%w/w、90%w/w或95%w/w。所述总半乳糖含量占所述总碳水化合物含量的百分比可以低于约60%w/w、或可以为约2%w/w至20%w/w、或可以低于约10%w/w。葡糖醛酸、甘露糖、鼠李糖和木糖的总含量占所述总碳水化合物含量的百分比可以低于约30%w/w。

[0016] 当以50mg/mL的浓度溶于水中时，所述高分子量褐藻糖胶的粘度可为约4cP至50cP；约10cP至40cP；或约15cP至30cP。所述高分子量褐藻糖胶可为白色固体，并且当以1mg/mL至100mg/mL的浓度溶于水中时，所述高分子量褐藻糖胶可形成一种澄清无色的溶液。所述褐藻糖胶可包含少于5%或2%w/w的乙酰基含量。另外，当在70℃下伴随溶剂信号抑制在配备有5-mm冷探针的600MHz光谱仪上在碳尺寸10-30ppm范围内以256-512的8次增量扫描每次通过2D ^{1}H-^{13}C异核多量子相干测量时，所述褐藻糖胶包含的乙酰基含量基本为0%w/w。

[0017] 本文还包括这样的方法，所述方法可以包括：制备或使用本文的高分子量褐藻糖胶，包括用于治疗纤维性粘连。本文还包括医疗上可接受的褐藻糖胶组合物，其可以包括医疗上可接受的缓冲剂或稀释剂中的治疗有效量的高分子量褐藻糖胶。方法还包括治疗动物的病症或疾病，其可以包括：选择本文的医疗上可接受的褐藻糖胶组合物以治疗所述病症或疾病，以及给药包括约0.5mg/kg至50mg/kg；0.04mg/kg至25mg/kg；0.2mg/kg至10mg/kg；1mg/kg至5mg/kg；1.5mg/kg至3mg/kg；5mg/kg至10mg/kg的治疗有效量。

[0018] 所述病症或疾病可以是所述动物的目标部位处的纤维性粘连，并且所述给药可以包括：向所述目标部位给药所述治疗有效量。

[0019] 所述医疗组合物可以是约0.02mg/mL至100mg/mL的高分子量褐藻糖胶，其中，所述医疗组合物被配置和构成为治疗动物的疾病或病症。所述医疗组合物还可为约0.5mg/mL至5mg/mL、或约2.5mg/mL的高分子量褐藻糖胶。

[0020] 所述医疗组合物可以是包括液体医疗器械的医疗器械。所述医疗组合物可以是药物组合物，其可以是液体药物组合物。

[0021] 本文的方法还包括：包括约0.01mL/kg至15mL/kg；约0.03mL/kg至4mL/kg；约0.06mL/kg至2mL/kg；或约2mL/kg至4mL/kg的剂量范围的医疗组合物用于治疗动物的疾病或病症的用途。

[0022] 用于治疗患者的纤维性粘连的方法可以包括：将医疗组合物给药于患者的目标部位。所述目标部位可以是外科手术部位，并且所述给药可以在以下情况中的至少一种情况

下进行:a)在所述外科手术部位处打开外科手术伤口后,b)在手术期间,以及c)在闭合所述外科手术伤口之后。所述给药可以在外科手术之后但在闭合所述外科手术伤口之前进行。所述给药可以耗时少于3分钟、2分钟或1分钟。所述目标部位可以是病变部位、擦伤部位和损伤部位中的至少一种。所述目标部位可以是骨盆腔、腹腔、背侧腔、颅腔、脊髓腔、腹侧腔、胸腔、胸膜腔、心包腔、皮肤、关节、肌肉、肌腱和韧带。

[0023] 在进一步的实施方式中,本文的方法包括用于获得高分子量褐藻糖胶的方法。这些方法可以包括:

[0024] 以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物,所述宽分子量分布包括所需高分子量褐藻糖胶区段;

[0025] 使所述起始溶液通过第一较高截留分子量切向流过滤过滤器进行第一切向流过滤,以产生第一渗透褐藻糖胶组合物;以及

[0026] 使所述第一渗透褐藻糖胶组合物通过第二较低截留分子量切向流过滤过滤器进行第二切向流过滤,以产生基本上由所述所需高分子量褐藻糖胶组成的第二渗余褐藻糖胶组合物。

[0027] 所述方法还可以包括:收集基本上由所述所需高分子量褐藻糖胶组成的所述第二渗余褐藻糖胶组合物,并且所述第一较高截留分子量切向流过滤过滤器的较高截留分子量可以为约50kDa至约1000kDa,并且所述第二较低截留分子量切向流过滤过滤器的较低截留分子量可以为约30kDa至约100kDa。所述较高截留分子量可以为约300kDa,并且所述较低截留分子量可以为约100kDa。

[0028] 用于获得高分子量褐藻糖胶的方法,可以包括:

[0029] 以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物,所述宽分子量分布包括所需高分子量褐藻糖胶区段;

[0030] 使所述起始溶液通过第一较低截留分子量切向流过滤过滤器进行切向流过滤,以产生第一渗余褐藻糖胶组合物;以及

[0031] 使所述第一渗余褐藻糖胶组合物通过第二较高截留分子量切向流过滤过滤器进行切向流过滤,以产生基本上由所述所需高分子量褐藻糖胶组成的第二渗透褐藻糖胶组合物。

[0032] 所述方法还可以包括:收集基本上由所述所需高分子量褐藻糖胶组成的所述第二渗透褐藻糖胶组合物。所述第一切向流过滤可包括:使所述起始溶液通过所述第一较低截留分子量切向流过滤过滤器进行渗滤。所述第二切向流过滤可包括:使所述第一渗余褐藻糖胶组合物通过所述第二较高截留分子量切向流过滤过滤器进行渗滤。所述第一较低截留分子量切向流过滤过滤器的较低截留分子量可以为约30kDa至约100kDa,并且所述第二较高截留分子量切向流过滤过滤器的较高截留分子量可以为约50kDa至约1000kDa。所述较低截留分子量可以为约100kDa,并且所述较高截留分子量可以为约300kDa。

[0033] 用于获得高分子量褐藻糖胶的方法,可以包括:

[0034] 以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物,所述宽分子量分布包括所需高分子量褐藻糖胶区段,所述起始褐藻糖胶组合物可以包括与所述组合物中的褐藻糖胶上的硫酸酯基团离子键合的低原子量阳离子;以及

[0035] 使所述起始溶液相对于阳离子添加剂溶液进行切向流过滤,所述阳离子添加剂溶

液可以包括具有比所述低原子量阳离子大的分子量的阳离子添加剂，以产生基本上由所需高分子量褐藻糖胶组成的渗余褐藻糖胶组合物。

[0036] 所述方法还可以包括：收集基本上由所述所需高分子量褐藻糖胶组成的所述渗余褐藻糖胶组合物。所述低原子量阳离子包括碱金属、碱土金属、铝和铵中的至少一种。所述阳离子添加剂可以包括胆碱、聚乙烯吡咯烷酮、牛磺酸、多胺、壳聚糖、组蛋白和胶原蛋白中的至少一种。所述方法还可以包括：在使所述起始溶液进行切向流过滤之前，将所述阳离子添加剂添加到所述起始溶液中。所述切向流过滤可以包括：将所述起始溶液相对于所述阳离子添加剂溶液进行渗滤。所述方法还可以包括：通过在第二切向流过滤过滤器上将所述渗余褐藻糖胶组合物相对于盐溶液进行渗滤来去除所述阳离子添加剂，所述第二切向流过滤过滤器的截留分子量可以低于所述第一切向流过滤过滤器的截留分子量。

[0037] 所述盐溶液可以包括碱金属、碱土金属、铝和/或铵的氯化物、溴化物、碘化物、氟化物、硫酸盐、亚硫酸盐、碳酸盐、碳酸氢盐、磷酸盐、硝酸盐、亚硝酸盐、乙酸盐、柠檬酸盐、硅酸盐和/或氰化物。所述方法还可以包括：通过将所述渗余褐藻糖胶组合物相对于低离子强度溶液进行渗滤来去除盐。

[0038] 用于获得高分子量褐藻糖胶的方法可以包括：

[0039] 提供离心容器，所述离心容器可以包括底端和顶端以及在其间的可渗透屏障，所述可渗透屏障可以在其间包括梯度材料；

[0040] 将具有宽分子量分布的起始褐藻糖胶组合物置于所述离心容器中和所述可渗透屏障上方，所述宽分子量分布包括所需高分子量褐藻糖胶区段；以及

[0041] 将所述离心容器离心足够长的时间段以产生基本上由所需高分子量褐藻糖胶组成的沉淀物。

[0042] 所述方法还可以包括：从所述离心容器中收集所需高分子量褐藻糖胶。所述可渗透屏障可以包括梯度材料的单个区段。所述可渗透屏障可以包括梯度材料的多个区段。所述梯度材料可以包括蔗糖、聚蔗糖、甘油、山梨糖醇、CsCl、Cs_2SO_4、KBr、泛影酸盐、Nycodenz®和碘克沙醇中的至少一种。离心力可以为约10,000重力至约1,000,000重力。离心力可以为60,000重力至约500,000重力。

[0043] 用于获得高分子量褐藻糖胶的方法可以包括：

[0044] 提供包括底端和顶端的离心容器；

[0045] 将起始溶液形式的具有宽分子量分布的起始褐藻糖胶组合物置于所述离心容器中，所述宽分子量分布包括所需高分子量褐藻糖胶区段；以及

[0046] 将所述离心容器离心足够长的时间段以产生基本上由所需高分子量褐藻糖胶组成的沉淀物。

[0047] 所述方法还可以包括：从所述离心容器中收集作为沉淀物的所需高分子量褐藻糖胶。离心力可以为约60,000重力至约1,000,000重力。离心力可以为200,000重力至约500,000重力。

[0048] 用于获得高分子量褐藻糖胶的方法可以包括：

[0049] 使具有宽分子量分布的起始褐藻糖胶组合物进行凝胶电泳，所述宽分子量分布包括所需高分子量褐藻糖胶区段，其中，所述起始褐藻糖胶组合物可以根据电泳凝胶上的质荷比进行置换；

[0050] 选择所述电泳凝胶的一部分，其基本上由所需高分子量褐藻糖胶组成；以及

[0051] 从所述电泳凝胶的选择的部分提取所需高分子褐藻糖胶。

[0052] 所述使所述起始褐藻糖胶组合物进行凝胶电泳可包括：在所述电泳凝胶上施加电势差，所述电势差可以为约10伏/cm至200伏/cm。所述电泳凝胶可以包括琼脂糖、聚丙烯酰胺、聚二甲基丙烯酰胺和淀粉中的至少一种。所述电泳凝胶还可以包括三乙酸酯EDTA、三硼酸EDTA和磷酸盐缓冲盐水中的至少一种。从所述电泳凝胶的选择的部分中提取所需高分子量褐藻糖胶可以包括：在溶剂中搅动所述电泳凝胶的选择的部分。所述溶剂可以包括水、甲醇、乙醇和异丙醇中的至少一种。

[0053] 用于获得高分子量褐藻糖胶的方法可以包括：

[0054] 提供具有宽分子量分布的起始褐藻糖胶组合物和离子交换大孔树脂，所述宽分子量分布包括所需高分子量褐藻糖胶区段；以及

[0055] 使所述起始褐藻糖胶组合物与所述离子交换大孔树脂进行离子交换，以获得经离子交换处理的基本上由所需高分子量褐藻糖胶组成的褐藻糖胶组合物。

[0056] 所述方法还可以包括：从经离子交换处理的褐藻糖胶组合物中收集所需高分子量褐藻糖胶。提供所述初始褐藻糖胶组合物还可以包括：在使所述起始褐藻糖胶组合物进行离子交换之前使所述起始褐藻糖胶组合物脱盐。所述起始褐藻糖胶组合物:离子交换大孔树脂的质量比可以为约1:100至约10:1。所述质量比可以为约1:10至约5:1。可以使所述起始褐藻糖胶组合物进行离子交换的时间段为约5分钟至约100小时。所述离子交换大孔树脂可以包括阴离子交换大孔树脂和混合电荷大孔树脂中的至少一种。所述阴离子交换大孔树脂可以是强碱大孔树脂。所述强碱大孔树脂可以包括季胺基。所述阴离子交换大孔树脂可以是弱碱大孔树脂。所述弱碱大孔树脂可以包括伯、仲或叔胺基中的至少一种。所述离子交换大孔树脂可以包括苯乙烯、琼脂糖、右旋糖酐、丙烯酸酯、甲基丙烯酸酯、甲基丙烯酸甲酯、甲基丙烯酸丁酯、二乙烯基苯、纤维素、二氧化硅和陶瓷中的至少一种。所述离子交换大孔树脂的孔径可以为约5nm至约1000nm、约10nm至约100nm、或约15nm至约50nm。所述离子交换大孔树脂的排阻极限可以为约50kDa至约50,000kDa、约1,000kDa至约9,000kDa、或约100kDa至约1,000kDa。可以使所述起始褐藻糖胶组合物进行阴离子交换的时间段为约5分钟至约100小时或约1小时至约30小时。

[0057] 用于获得高分子量褐藻糖胶的方法可以包括：

[0058] 以起始溶液的形式提供具有宽分子量分布的起始褐藻糖胶组合物和凝胶介质，所述宽分子量分布包括所需高分子量褐藻糖胶区段；

[0059] 使所述起始溶液进行制备型凝胶渗透色谱法，其中，所述起始褐藻糖胶组合物可以根据分子量在所述凝胶介质上从第一输入端到第二输出端进行置换；以及

[0060] 从所述第二输出端收集至少一个等分部分，所述等分部分基本上由所需高分子量褐藻糖胶区段组成。

[0061] 该方法还可以包括：收集多个等分部分并将这些等分部分合并。所述凝胶介质可以被容纳在色谱柱中。所述凝胶介质可以包括聚甲基丙烯酸甲酯、磺化的苯乙烯-二乙烯基苯、二氧化硅、亲水性键合相或聚合物、聚苯乙烯、二乙烯基苯、甲基丙烯酸酯、甲基丙烯酸甲酯、甲基丙烯酸丁酯、纤维素、陶瓷、琼脂糖和右旋糖酐中的至少一种。所述凝胶介质的孔径可以为约3nm至约3000nm、3nm至约3000nm、约5nm至约10,000nm、约10nm至约100nm、约

50nm至约500nm、约200nm至约2,000nm、或约500nm至约5,000nm。所述凝胶介质的排阻极限可以为约100Da至约100,000kDa、约100kDa至约30,000kDa、约1,000kDa至约100,000kDa、约1,000kDa至约10,000kDa、或约5,000kDa至约50,000kDa。

[0062] 在本申请中阐述了这些和其他方面、特征和实施方式，包括以下具体实施方式和附图。除非另有明确说明，否则所有实施方式、方面、特征等都可以以任何期望的方式混合和匹配、组合和置换。

附图说明

[0063] 图1示意性地描绘了示例性的两过滤器系统，其用于使用顺序切向流过滤基于分子量将起始褐藻糖胶组合物进行分段，所述起始褐藻糖胶具有宽分子量分布。

[0064] 图2示意性地描绘了两过滤器系统的另一示例性实施方式，其用于使用顺序切向流过滤基于分子量对起始褐藻糖胶组合物进行分段，所述起始褐藻糖胶具有宽分子量分布。

[0065] 图3示意性地描绘了示例性系统，其用于使用阳离子增强的切向流过滤从起始褐藻糖胶组合物中获得所需高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0066] 图4示意性地描绘了示例性系统，其用于使用梯度材料的多区段屏障从起始褐藻糖胶组合物中离心沉淀高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0067] 图5示意性地描绘了示例性系统，其用于使用单区段屏障从起始褐藻糖胶组合物中离心沉淀高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0068] 图6示意性地描绘了示例性系统，其用于通过凝胶电泳提取从起始褐藻糖胶组合物中获得高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0069] 图7示意性地描绘了示例性系统，其用于通过透析从起始褐藻糖胶组合物中获得高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0070] 图8示意性地描绘了示例性系统，其用于使用离子吸附从起始褐藻糖胶组合物中获得所需高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0071] 图9A示出了NMR结果，表明根据本文的方法处理的某些褐藻糖胶经历了褐藻糖胶的结构变化。

[0072] 图9B描绘了2-D NMR结果，表明根据本文的方法处理的某些褐藻糖胶经历了褐藻糖胶的化学结构变化。

[0073] 图10示出了示例性系统，其用于使用多区段蔗糖屏障从起始褐藻糖胶组合物中离心沉淀高分子量褐藻糖胶，所述起始褐藻糖胶具有宽分子量分布。

[0074] 附图呈现了本公开的示例性实施方式。附图不一定按比例，并且某些特征可以以有助于例示和解释本发明系统、方法等的方式被放大或以其他方式表示。本文的系统、方法等的实际实施方式可以包括附图中未示出的另外的特征或步骤。本文陈述的范例以一种或多种形式例示了系统、方法等的实施方式，并且这样的范例不应被解释为以任何方式限制本公开的范围。本文的实施方式不是穷举性的，并且不将本公开限制为例如在以下详细描述中公开的精确形式。

具体实施方式

[0075] 本文呈现的当前组合物、系统、方法等包括高分子量褐藻糖胶。本发明组合物可对医疗治疗、术后治疗、疾病抑制等有效。在一些实施方式中，褐藻糖胶为岩藻多糖。本发明的高分子量褐藻糖胶可自身为医疗器械、医疗材料、组合产品，或可包括于医疗器械、医疗材料、组合产品之上或之中，或可包括于药物学上可接受的、治疗上和/或医疗上有效的组合物中。

[0076] 以下段落转向对一些方法学的简要讨论，这些方法学可用于通过各种方法从起始褐藻糖胶和组合物产生本文的高分子量褐藻糖胶和组合物，所述各种方法可以使用任何合适的反应混合物(例如溶液、悬浮液、固体、凝胶或其他形态，具体取决于所选择的方法)来执行。

[0077] 组合物

[0078] 在某些实施方式中，本文呈现的当前组合物、系统等提供褐藻糖胶及医疗上可接受的高分子量褐藻糖胶和组合物，其包括治疗有效量的高分子量褐藻糖胶，以用于治疗纤维性粘连(诸如外科手术粘连)、关节炎、牛皮癣或视需要的其他疾病。

[0079] 本文呈现的高分子量褐藻糖胶可用于多种应用，包括抑制、预防、去除、减少或以其他方式治疗纤维性粘连及其他目标、疾病和/或病症。治疗包括所述高分子量褐藻糖胶减小或预防目标疾病或其他病症的发展，诸如减小或预防目标部位处形成纤维性粘连，该目标部位通常为由外科医生或其他从业者确认为包括或相当易患纤维性粘连(或者其他疾病或病症)的选择的目标部位，并且还包括消除现有疾病或其他病症，包括(例如)消除已存在的纤维性粘连。对于这样的抑制、预防、去除、减少或其他方式的处理，高分子量褐藻糖胶通常以医疗上可接受的医疗器械、组合产品或药物学上有效的组合物来提供，其包含附加组分，诸如粘结剂、佐剂、赋形剂等，以及(视需要)附加的医疗上的活性物质，诸如包含于该组合物中但不附接至褐藻糖胶和/或可附接至褐藻糖胶的次要药品。

[0080] 高分子量褐藻糖胶的分子量分布可使用任何所需的、适当的测量系统来测量。不同系统当以不同方式测量时可由具有基本上相同构成的不同组合物或甚至由同一批产生不同读数或结果。一种合适的测量系统为水性凝胶渗透色谱法装置，其基本上由以下组成：具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱，装填有羟基化聚甲基丙烯酸酯基凝胶，具有约50kDa至约5,000kDa的有效分子量范围；具有7.8mm内径的一个300mm分析型凝胶渗透色谱柱，装填有羟基化聚甲基丙烯酸酯基凝胶，具有约1kDa至约6,000kDa的有效分子量范围；以及具有6mm内径的一个40mm保护柱，装填有羟基化聚甲基丙烯酸酯基凝胶，所述两个分析型凝胶渗透色谱柱和所述一个保护柱被容纳在约30℃下的柱室中；折射率检测器，在约30℃下；0.1M硝酸钠流动相，以0.6mL/min运行；以及相对于峰分子量标准曲线进行量化，该峰分子量标准曲线基本上由以下组成：第一右旋糖酐标准物，峰分子量为约2,200kDa；第二右旋糖酐标准物，峰分子量为约720kDa至约760kDa；第三右旋糖酐标准物，峰分子量为约470kDa至约510kDa；第四右旋糖酐标准物，峰分子量为约370kDa至约410kDa；第五右旋糖酐标准物，峰分子量为约180kDa至约220kDa；以及第六右旋糖酐标准物，峰分子量为约40kDa至55kDa。峰分子量标准曲线还可以包括峰分子量为3kDa至5kDa的右旋糖酐标准物。

[0081] 本文的高分子量褐藻糖胶可具有超过100kDa的重均分子量，并且其分子量分布的

约50％w/w或更多高于100kDa。与在相同剂量下重均分子量低于100kDa且其分子量分布的小于约50％高于100kDa的的褐藻糖胶相比，此类高分子量褐藻糖胶在抑制、预防、去除、减少和/或以其他方式治疗纤维性粘连方面显示出更大的功效。在相同剂量下，重均分子量高于300kDa、其分子量分布的约70％或更多高于100kDa的高分子量褐藻糖胶在抑制、预防、去除、减少和/或以其他方式治疗纤维性粘连方面显示出更高的功效。

[0082] 在一些实施方式中，本文的高分子量褐藻糖胶被配置用于抑制、预防、去除、减少或以其他方式治疗纤维性粘连，其引起大于约65％、70％、80％、90％、95％或99％的手术后粘连的预防、抑制或其他治疗有效。此类高分子量褐藻糖胶也可以被配置用于其他目标的这种治疗。

[0083] 本文中的高分子量褐藻糖胶可以包括分子量分布，其中，大于约60％、70％、75％、80％、90％、95或99％w/w的褐藻糖胶具有高于100kDa的分子量。

[0084] 在其他实施方式中，本文的高分子量褐藻糖胶可以包括的重均分子量为约100kDa至10,000kDa、约140kDa或200kDa至9,000kDa、约350kDa或370kDa至8,000kDa、约450kDa至7,000kDa、约580kDa至5,300kDa或6,000kDa、约580kDa或590kDa至5,500kDa、约400kDa至2,800kDa、或约800kDa或860kDa至约2,000kDa，例如约850kDa、约930kDa、约1100kDa、约1200kDa、约1300kDa、约1600kDa和约1800kDa。

[0085] 在其他实施方式中，本文的高分子量褐藻糖胶可以包括的峰分子量为约60kDa或70kDa至7,000kDa、约100kDa或140kDa至6000kDa、约200kDa或230kDa至5000kDa、约250kDa至4000kDa、约350kDa至3000kDa、约500kDa至2000kDa、或约400kDa至约1000kDa，例如约450kDa、500kDa、550kDa、600kDa、650kDa、700kDa和750kDa。

[0086] 在其他实施方式中，本文的高分子量褐藻糖胶可以包括的数均分子量为约50kDa至3,000kDa、约100kDa至2,000kDa、约200kDa至1,500kDa、约300kDa至2,000kDa、约400kDa和1,000kDa、或约250kDa至约600kDa，例如，约300kDa、350kDa、400kDa、450kDa、500kDa和550kDa。

[0087] 在其他实施方式中，本文的高分子量褐藻糖胶可以包括分子量分布，其中，大于约55％w/w或60％w/w的褐藻糖胶可具有高于200kDa的分子量，大于约70％w/w或71％w/w的褐藻糖胶可具有高于200kDa的分子量。在其他实施方式中，本文的高分子量褐藻糖胶可以包括分子量分布，其中，大于22％w/w或30％w/w的褐藻糖胶可具有大于500kDa的分子量，或大于50％w/w或54％w/w的褐藻糖胶可具有大于500kDa的分子量。

[0088] 在其他实施方式中，本文的高分子量褐藻糖胶可以包括分子量分布，其中，小于约10％w/w的褐藻糖胶具有小于50kDa的分子量，或小于约5％w/w的褐藻糖胶具有小于50kDa的分子量，或小于约2％w/w的褐藻糖胶具有小于50kDa的分子量。

[0089] 在其他实施方式中，本文的高分子量褐藻糖胶可以包括分子量分布，其中，小于约5％w/w的褐藻糖胶的分子量低于10kDa，或小于约2％w/w的褐藻糖胶的分子量低于10kDa。

[0090] 在其他实施方式中，本文的高分子量褐藻糖胶可包含分子量分布，其中小于约5％w/w的褐藻糖胶的分子量低于5kDa，或小于约2％w/w的褐藻糖胶的分子量低于5kDa。

[0091] 在另一方面，本文的高分子量褐藻糖胶可包含分子量分布，其中，61％w/w至80％w/w或85％w/w的褐藻糖胶的分子量为200kDa至1600kDa。更特别地，大于70％w/w的褐藻糖胶可具有高于200kDa的分子量，并且大于30％的褐藻糖胶可具有高于500kDa的分子量。

[0092] 在另一方面，本文中的高分子量褐藻糖胶可包含分子量分布，其中大于约20％w/w、40％w/w或60％w/w的褐藻糖胶具有的分子量大于1600kDa。更特别地，大于约70％w/w的褐藻糖胶可具有高于200kDa的分子量，或大于约80％w/w的褐藻糖胶可具有高于200kDa的分子量。

[0093] 本文中的高分子量褐藻糖胶可具有的硫酸酯化水平为约14％w/w至70％w/w、约20％w/w至60％w/w、约30％w/w至55％w/w、或约32％w/w或35％w/w至52％w/w。

[0094] 本文的高分子量褐藻糖胶可具有的总岩藻糖:总硫酸酯的摩尔比为1:0.5至1:4、约1:0.8至1:3.5、约1:1至1:2.5、约1:1.2至1:2.0、或约1:1.5至1:3。

[0095] 本文中的高分子量褐藻糖胶可具有的总岩藻糖和半乳糖:总硫酸酯的摩尔比为约1:0.5至1:4、约1:0.8至1:3.5、约1:1至1:2.5、约1:1.2至1:2.0、或约1:1.5至1:3。

[0096] 本文中的高分子量褐藻糖胶可具有的总碳水化合物含量为27％w/w至80％w/w、约30％w/w至70％w/w、约40％w/w至90％w/w、或约50％w/w至96％w/w。

[0097] 本文中的高分子量褐藻糖胶可具有的岩藻糖含量占总碳水化合物的百分比为约30％w/w至100％w/w、约40％w/w至95％w/w、约50％w/w至90％w/w、约80％w/w至100％w/w、或约90％w/w至100％w/w。

[0098] 本文中的高分子量褐藻糖胶可具有的半乳糖含量占总碳水化合物的百分比为约0％w/w至60％w/w、约3％w/w至30％w/w、约2％w/w至20％w/w、或约5％w/w至10％w/w。

[0099] 本文的高分子量褐藻糖胶可具有的葡糖醛酸含量占总碳水化合物含量的百分比为约0％w/w至10％w/w，甘露糖含量占总碳水化合物含量的百分比为约0％w/w至7％w/w，鼠李糖含量占总碳水化合物含量的百分比为0％w/w至4％w/w，并且木糖含量占总碳水化合物含量的百分比为0％w/w至20％w/w。本文的高分子量褐藻糖胶可具有的葡糖醛酸、甘露糖、鼠李糖、葡萄糖和木糖的总含量占总碳水化合物含量的百分比低于约30％w/w或低于约12％w/w。

[0100] 在一些实施方式中，当以50mg/mL浓度溶解于水中时，本文的高分子量褐藻糖胶具有以下粘度:约4cP至约50cP、约5cP至约40cP、约10cP至约30cP、约15cP、约20cP及约25cP。在某些实施方式中，当以1mg/mL至100mg/mL溶解于水中时，本文的高分子量褐藻糖胶形成为一种澄清且无色、澄清且淡黄色、或澄清且浅棕色的溶液。

[0101] 在某些实施方式中，本文的高分子量褐藻糖胶可具有小于约5％w/w、小于约2％w/w和约0％w/w的乙酰基含量。在一些实施方式中，当在70℃下伴随溶剂信号抑制在配备有5-mm冷探针的600MHz光谱仪上在碳尺寸10-30ppm范围内以256-512的8次增量扫描每次通过2D ^{1}H-^{13}C异核多量子相干测量时，本文的高分子量褐藻糖胶包含的乙酰基含量基本为0％w/w。

[0102] 方法

[0103] 提出了用于从起始褐藻糖胶组合物(例如原料褐藻糖胶组合物)获得高分子量褐藻糖胶的方法、系统等，其具有宽分子量分布(宽分子量分布起始褐藻糖胶)，其涵盖并延伸超过所需高分子量区段，所需高分子量区段是宽分子量分布的一部分，其中，在低分子量末端处的宽分子量分布的量已被消除、抑制或减弱。这些方法中的至少一种可用于制备高分子量褐藻糖胶，例如，其分子量分布的约60％、70％、80％、90％或95％w/w高于100kDa。在一些实施方式中，本公开提出了适用于医疗和外科手术应用(例如，预防外科手术粘连)的高

分子量褐藻糖胶。

[0104] 切向流过滤

[0105] 本文所论述的方法中的一些利用切向流过滤(TFF)。与切向流过滤(TFF)过滤器的典型识别一致,给定TFF过滤器的标称截留分子量(MWCO)值将在其截留物侧上选择性地保留包含未穿过过滤器屏障的分子的溶液,且因此通常具有大于穿过/渗透屏障至渗透物侧的分子的分子量的分子量和/或大小。因此,TFF过滤器的截留分子量值典型地对于任何给定聚合物或标称截留值并不绝对:给定TFF过滤器将通过或保留高于及低于标称截留分子量两者的一些分子。用于具体聚合物的标称TFF过滤器的实际截留/选择性值及影响可针对具体聚合物来常规地决定。

[0106] 大量因素可影响TFF过滤器的渗透行为。这些因素可取决于TFF过滤器自身或取决于目标聚合物的属性,例如目标聚合物的折叠行为及折叠结构可影响目标聚合物在穿过/不穿过TFF过滤器的MWCO屏障中的行为。如已知的,关于TFF过滤器自身,大量因素可影响TFF过滤器的渗透行为。例如,制造方法可导致特定TFF过滤器内的各种孔大小,其变体可包括大于及小于标称MWCO的两种孔。因此,具有标称截留分子量值的TFF过滤器将基本上通过/保留处于标称截留分子量值的分子,但还可通过/保留低于和/或高于这样的值的一些分子。

[0107] 凝胶渗透色谱法

[0108] 凝胶渗透色谱法用于评估实验实施例获得的分子量分布。存在大量可用于凝胶渗透色谱法的不同参数、色谱柱及标准物,引起多种仪器装置可用于分子量的分析。对于本文的分子量测定,使用以下参数进行GPC:流动相为以0.6mL/min运行的0.1M硝酸钠。柱室及检测器处于30℃。Waters 2414折射率检测器用于检测。

[0109] 合适的GPC柱包括与水性溶剂兼容的GPC柱,例如装填有以下中的至少一种的色谱柱:磺化苯乙烯-二乙烯基苯、NH官能化的丙烯酸酯共聚物网络、修饰的二氧化硅及羟基化聚甲基丙烯酸酯基凝胶。对于本文的分析,串联使用三个色谱柱,包括具有6mm内径(ID)的一个40mm长的保护柱,装填有6μm粒度的羟基化聚甲基丙烯酸酯基凝胶;接着是具有7.8mm ID的第一300mm分析型GPC柱,装填有12μm粒度的羟基化聚甲基丙烯酸酯基凝胶,其具有约7,000kDa的排阻极限以及约50kDa至约5,000kDa的有效分子量范围;接着是具有7.8mm ID的第二300mm分析型GPC柱,装填有10μm粒度的羟基化聚甲基丙烯酸酯基凝胶,其具有约7,000kDa的排阻极限以及约1kDa至约6,000kDa的有效分子量范围。色谱柱装置的总有效分子量范围为约1kDa至约6,000kDa。此色谱柱装置的实例可为串联连接的Ultrahydrogel®保护柱-Ultrahydrogel®2000-Ultrahydrogel®线性柱。

[0110] 相对于包括来自American Polymer Standards Corporation的可追踪标准物的标准曲线来量化样品运行:DXT3755K(峰分子量=2164kDa)、DXT820K(峰分子量=745kDa)、DXT760K(峰分子量=621kDa)、DXT670K(峰分子量=401kDa)、DXT530K(峰分子量=490kDa)、DXT500K(峰分子量=390kDa)、DXT270K(峰分子量=196kDa)、DXT225K(峰分子量=213kDa)、DXT150K(峰分子量=124kDa)、DXT55K(峰分子量=50kDa)、DXT50K(峰分子量=44kDa)和DXT5K(峰分子量=4kDa),这些标准物的峰分子量为约4kDa至约2,200kDa。所使用标准曲线可例如包括Dextran 3755kDa,Dextran 50kDa及Dextran 55kDa中的至少一种及

在3至6个之间的本文所论述的附加可追踪标准物，校正点为所使用校正物的峰分子量。示例性校正曲线可由以下组成:DXT3755K、DXT820K、DXT530K、DXT500K、DXT225K及DXT55K。本文中所使用的色谱柱具有涵盖且延伸超出用于褐藻糖胶的量化的标准物的峰分子量范围的总有效分子量范围。

[0111] 规定用于本文的褐藻糖胶/岩藻多糖聚合物的分子量为这样的分子量值，在该分子量值附近将始终存在较高及较低分子量的分子分布，随着分子量远离指定分子量增大或减小而在数量或百分比上增大或减小。分布可以(但并非必需)具有通常的高斯或失真(distorted)高斯形状。

[0112] 本文的表格中的结果包含用于分子量分布的某些特征的缩写。凝胶渗透色谱法由GPC表示，峰截留时间由PRT表示，峰分子量由PMW表示，重均分子量由WAMW表示，数均分子量由NAMW表示，分布百分比由％分布(％dist.)表示，分子量由MW表示，多分散性指数由PDI表示，并且截留分子量由MWCO表示。

[0113] 以下段落转向对可用于产生本文中的高分子量褐藻糖胶的一些方法学的简要一般性讨论。

[0114] 顺序切向流过滤分段

[0115] 可以通过顺序TFF分段方法从宽分子量分布起始褐藻糖胶组合物获得高分子量褐藻糖胶。所述方法可以包括:提供包含所需分子量区段(例如高分子量区段)的起始褐藻糖胶组合物，该起始褐藻糖胶组合物具有起始宽分子量分布;使起始褐藻糖胶组合物通过第一较高MWCO切向流过滤过滤器进行切向流过滤，该过滤器具有在起始分子量分布内的平均截留分子量;从第一TFF过滤器中收集第一渗透褐藻糖胶组合物，其与起始褐藻糖胶组合物相比包含降低比例的高分子量褐藻糖胶;使第一渗透褐藻糖胶组合物通过第二较低MWCO切向流过滤过滤器进行切向流过滤，该过滤器具有比第一TFF过滤器低的在起始分子量分布内的平均截留分子量;以及从第二TFF过滤器中收集在渗余褐藻糖胶组合物中具有所需分子量区段的褐藻糖胶。

[0116] 所述方法可以根据需要包括其他步骤，例如通过能够过滤掉大于所需尺寸的颗粒或部分或其他不需要的材料的预过滤器来预过滤起始褐藻糖胶组合物。使起始褐藻糖胶组合物通过第一TFF过滤器可包括:在将压力施加至起始褐藻糖胶组合物的同时，使起始褐藻糖胶组合物通过TFF过滤器。使第一TFF过滤器的渗透褐藻糖胶组合物通过第二TFF过滤器可以包括:在将压力施加至第一TFF过滤器的渗透褐藻糖胶组合物的同时，使第一TFF过滤器的渗透褐藻糖胶组合物通过第二TFF过滤器。

[0117] 使起始褐藻糖胶组合物通过第一TFF过滤器可包括:使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环。使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环可以包括:在第一TFF过滤器上对渗余褐藻糖胶组合物进行渗滤。使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环可以包括:确定第一TFF过滤器的渗透褐藻糖胶组合物的重均分子量。使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环可以包括:使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环，直到第一TFF过滤器的渗透褐藻糖胶组合物中的褐藻糖胶的重均分子量具有预定的期望值。

[0118] 使来自第一TFF过滤器的渗透褐藻糖胶组合物通过第二TFF过滤器可包括:使渗透

褐藻糖胶组合物在第二TFF过滤器上再循环。使渗透褐藻糖胶组合物在第二TFF过滤器上再循环可以包括:在第二TFF过滤器上对渗透褐藻糖胶组合物进行渗滤。使渗透褐藻糖胶组合物在第二TFF过滤器上再循环可以包括:确定第二TFF过滤器的渗余褐藻糖胶组合物的重均分子量。使渗透褐藻糖胶组合物在第二TFF过滤器上再循环可以包括:使褐藻糖胶在第二TFF过滤器上再循环,直到第二TFF过滤器的渗余褐藻糖胶组合物的重均分子量具有预定的期望值。

[0119] 图1示意性地示出了示例性的基于分子量的分段系统(较高至较低)100,其包括两个不同的(较高和较低的)截留分子量(MWCO)TFF过滤器,其在所示的实施方式中被提供为较高截留分子量TFF过滤器110和较低截留分子量TFF过滤器120;TFF过滤器可以任何可接受的形式提供,当前示例使用盒件。较高截留分子量TFF过滤器110的MWCO大于较低截留分子量TFF过滤器120的MWCO。举例来说,较高截留分子量TFF过滤器110的MWCO可以为30千道尔顿(kDa)、50kDa、70kDa、100kDa、300kDa和1000kDa,而较低截留分子量TFF过滤器120的MWCO可以为,例如5kDa、10kDa、30kDa、50kDa和100kDa。举例来说,选择较高截留分子量TFF过滤器和较低截留分子量TFF过滤器的组合,可以使用基于分子量的分段系统(较高至较低)100来获得截留分子量TFF过滤器之间的分子量区段:5-30kDa、10-30kDa、5-50kDa、10-50kDa、30-50kDa、10-70kDa、30-70kDa、50-70kDa、5-100kDa、10-100kDa、30-100kDa、50-100kDa、70-100kDa、5-300kDa、10-300kDa、30-300kDa、50-300kDa、70-300kDa和100-300kDa。在一些实施方式中,分子量区段可以是高分子量区段。

[0120] 起始褐藻糖胶组合物作为溶液经由输入供应管线102供应至较高MWCO子系统褐藻糖胶容器116。起始褐藻糖胶可以以以下浓度存在于合适的溶剂中:0.1%w/v至30%w/v的浓度,例如1%w/v至10%w/v,例如5%w/v。可以通过预过滤器104对在合适溶剂中的起始褐藻糖胶进行预过滤以去除不希望有的颗粒物质。含有起始褐藻糖胶组合物的溶液可根据需要包含其他非褐藻糖胶组分,例如所需的缓冲剂、稀释剂等,例如用于褐藻糖胶的其他褐藻糖胶处理步骤或下游用途。预过滤器的规格(有效孔径)通常大于要通过基于分子量的分段系统(较高至较低)100分离的最大聚合物分子。

[0121] 较高MWCO子系统泵114通过较高MWCO TFF过滤器供应管线112将包含起始褐藻糖胶组合物的溶液泵送到较高MWCO TFF子系统130的较高截留分子量TFF过滤器110。通常以盒件形式提供较高截留分子量TFF过滤器110,该盒件被设计为允许输入流体通过其截留侧的过滤器。截留分子量过滤器的形式可以是但不限于:板框系统;螺旋盘绕药筒系统;中空纤维系统;流通池系统;和离心过滤系统。渗透物通过较高MWCO子系统渗透物输出管线119离开,并且经处理的输入流体(即截留流体)通过较高MWCO子系统截留物返回管线118离开。较高MWCO子系统泵114在其截留侧和渗透侧之间在较高截留分子量TFF过滤器110上提供一定的压力水平。在图1中,来自较高截留分子量TFF过滤器110的截留流体通过较高MWCO子系统截留物返回管线118返回到较高MWCO子系统褐藻糖胶容器116,而通过较高MWCO子系统渗透物输出管线119产生渗透流体以在较高MWCO TFF子系统130外部使用。虽然较高MWCO子系统泵114在较高截留分子量TFF过滤器110上再循环预过滤的褐藻糖胶和截留物,但溶剂可以从较高MWCO子系统溶剂容器117经由较高MWCO子系统溶剂供应管线115供应,例如以补充通过渗透物损失的溶剂和/或确保预定透析体积(diavolumes)量的输入起始褐藻糖胶和溶剂在较高截留分子量TFF过滤器110上循环。

[0122] 在以上处理期间，较高至较低MWCO子系统间阀113可以被切断(关闭)，并且来自较高MWCO TFF子系统130的较高截留分子量TFF过滤器110的渗透流体可以被收集到容器(未示出)中用于储存或其他用途，然后再供应给较低MWCO TFF子系统140的较低MWCO子系统褐藻糖胶容器126。可以根据需要通过较高MWCO TFF子系统130将起始褐藻糖胶组合物循环多次。

[0123] 然后，可以将来自较高MWCO TFF子系统130的收集的渗透物通过较高MWCO子系统渗透物输出管线119供应到较低MWCO TFF子系统140的较低MWCO子系统褐藻糖胶容器126中。在其他实施方式中，收集的渗透物可以在容器(未示出)中被转移到较低MWCO子系统褐藻糖胶容器126。在系统的其他实施方式中，较高至较低MWCO子系统间阀113可保持打开，并且较高截留分子量TFF过滤器110的渗透物可通过较高MWCO子系统渗透物输出管线119被连续地供应给较低MWCO子系统褐藻糖胶容器126。与起始褐藻糖胶组合物中较高分子量的分布相比，较高截留分子量TFF过滤器110的渗透物中较高分子量的分子的分布被减弱或抑制。

[0124] 供应到较低MWCO TFF子系统140的渗透物以与以上针对较高截留分子量TFF过滤器110所讨论的类似的方式在较低截留分子量TFF过滤器120上过滤。也就是说，在将来自较高MWCO TFF子系统130的渗透物供应至较低MWCO子系统褐藻糖胶容器126之后，较低MWCO子系统泵124将其通过较低MWCO TFF过滤器供应管线122泵送到较低MWCO TFF子系统140的较低截留分子量TFF过滤器120。较低MWCO子系统泵124在其截留侧和渗透侧之间在较低截留分子量TFF过滤器120上维持一定的压力水平。在图1中，较低截留分子量TFF过滤器120的截留物通过较低MWCO子系统截留物返回管线128返回到较低MWCO子系统褐藻糖胶容器126，同时通过较低MWCO子系统渗透物输出管线129产生渗透物以用于在较低MWCO TFF子系统140之外进一步使用或丢弃。如果较低MWCO子系统泵124使来自较高截留分子量TFF过滤器110的渗透物和来自较低截留分子量TFF过滤器120的截留物再循环，以再次通过较低截留分子量TFF过滤器120(利用较高截留分子量过滤过滤器，可以根据需要多次重复这种再循环)，则可以从较低MWCO子系统溶剂容器127通过较低MWCO子系统溶剂供应管线125和较低MWCO子系统褐藻糖胶容器126供应溶剂，以补充通过较低MWCO子系统渗透物输出管线129损失的溶剂和/或确保较低截留分子量TFF过滤器120的预定透析体积量的截留物和溶剂在较低截留分子量TFF过滤器120上循环。

[0125] 在较低MWCO TFF子系统140的切向流过滤操作期间，较低MWCO子系统截留物管线阀106可以关闭。当从较高MWCO TFF子系统130供应至较低MWCO TFF子系统140的渗透物已过滤至所需程度时，较低MWCO子系统截留物管线阀106被打开，并且较低截留分子量TFF过滤器120的截留物通过较低MWCO子系统截留物输出管线108供应。这从起始褐藻糖胶组合物(例如高分子量褐藻糖胶)提供具有所需分子量区段的褐藻糖胶。

[0126] 输出褐藻糖胶具有所需分子量区段，其分子量分布通常主要在较高截留分子量TFF过滤器110的平均截留分子量与较低截留分子量TFF过滤器120的平均截留分子量的之间。但是，考虑到起始褐藻糖胶分子量分布的宽度和复杂性以及聚合物行为和TFF过滤器的可变性，输出聚合物分子量分布可能不会在这两个TFF过滤器的平均截留分子量之间达到峰值。例如，褐藻糖胶的过高或过低的折叠会导致在所需分子量区段中选择适当大小但异常稠密(或否)的褐藻糖胶。因此，就本文所述的顺序TFF之后存在的褐藻糖胶而言，输出所

需分子量区段基本上由衍生自原始起始褐藻糖胶组合物的所需分子量区段组成，其被供应至基于分子量的分离系统(较高至较低)100。

[0127] 进一步的实施方式可包括：提供包含所需分子量区段(例如高分子量区段)的起始褐藻糖胶组合物，所述起始褐藻糖胶组合物具有起始分子量分布；使所述起始褐藻糖胶组合物通过第一切向流过滤过滤器进行切向流过滤，该过滤器具有在起始分子量分布内的平均截留分子量；从第一TFF过滤器中收集第一渗余褐藻糖胶组合物，与起始褐藻糖胶组合物相比，该组合物包含降低比例的低分子量褐藻糖胶；使第一渗余褐藻糖胶组合物通过第二切向流过滤过滤器进行切向流过滤，该过滤器具有比第一TFF过滤器高的在起始分子量分布内的平均截留分子量；以及从第二TFF过滤器收集在渗透褐藻糖胶组合物中具有所需分子量区段的褐藻糖胶。

[0128] 该方法可以进一步包括：通过能够过滤出大于期望尺寸的部分的预过滤器来预过滤起始褐藻糖胶组合物。使起始褐藻糖胶组合物通过第一TFF过滤器可以包括：在将压力施加至起始褐藻糖胶组合物的同时，使起始褐藻糖胶组合物通过第一TFF过滤器。使第一MCWO过滤器的渗余褐藻糖胶组合物通过第二TFF过滤器可以包括：在第二TFF过滤器中向第一TFF过滤器的渗余褐藻糖胶组合物施加压力的同时，使第一TFF过滤器的渗余褐藻糖胶组合物通过第二TFF过滤器。

[0129] 使起始褐藻糖胶组合物通过第一TFF过滤器可包括：使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环。使第一TFF过滤器的渗余褐藻糖胶组合物在第一TFF过滤器上再循环可以包括：在第一TFF过滤器上对渗余褐藻糖胶组合物进行渗滤。在第一TFF过滤器上再循环第一TFF过滤器的渗余褐藻糖胶组合物可以包括：确定第一TFF过滤器的渗余褐藻糖胶组合物的重均分子量。在第一TFF过滤器上再循环第一TFF过滤器的渗余褐藻糖胶组合物可以包括：在第一TFF过滤器上再循环第一TFF过滤器的渗余褐藻糖胶组合物，直到第一TFF过滤器的渗余褐藻糖胶组合物中的褐藻糖胶的重均分子量具有预定的期望值。

[0130] 使来自第一TFF过滤器的渗余褐藻糖胶组合物通过第二TFF过滤器，可以包括：使渗余褐藻糖胶组合物在第二TFF过滤器上再循环。在第二TFF过滤器上再循环渗余褐藻糖胶组合物可以包括：在第二TFF过滤器上对渗余褐藻糖胶组合物进行渗滤。在第二TFF过滤器上再循环渗余褐藻糖胶组合物可以包括：确定第二TFF过滤器的渗透褐藻糖胶组合物的重均分子量。在第二TFF过滤器上再循环渗余褐藻糖胶组合物可以包括：在第二TFF过滤器上再循环渗余褐藻糖胶组合物，直到第二TFF过滤器的渗透褐藻糖胶组合物的渗透褐藻糖胶的重均分子量具有预定的期望值。

[0131] 图2示出了本文的方法、系统等的另一实施方式。在图2中，图1的子系统130和140的处理顺序相反，以形成基于分子量的分段系统(较高至较低)100'。如在图1中讨论的方法中，起始褐藻糖胶通过输入供应管线102进入系统，并被预过滤器104预过滤。然而，与上文图1的方法相反，首先在较低MWCO TFF子系统140中处理预过滤的起始褐藻糖胶，然后在较高MWCO TFF子系统130中对其进行处理。在较低MWCO TFF子系统140中，起始褐藻糖胶组合物通过较低截留分子量TFF过滤器120，其是具有较低平均MWCO值的TFF过滤器。在该实施方式中，是较低截留分子量TFF过滤器120的截留物而不是渗透物离开较低MWCO子系统截留物输出管线121上的较低MWCO TFF子系统140。这种截留物通过较低至较高MWCO子系统间阀

123离开，其将被供应到较高MWCO TFF子系统130的较高MWCO子系统褐藻糖胶容器116。截留物然后由较高MWCO子系统泵114经由较高MWCO TFF过滤器供应管线112泵送通过较高截留分子量TFF过滤器110，其是较高MWCO的TFF过滤器。

[0132] 在较低MWCO TFF子系统140中，较低MWCO子系统泵124通过较低MWCO TFF过滤器供应管线122将渗透物从较低MWCO子系统褐藻糖胶容器126泵送到较低截留分子量TFF过滤器120。在图2中，较低截留分子量TFF过滤器120的截留物通过较低MWCO子系统截留物返回管线128返回到较低MWCO子系统褐藻糖胶容器126，而渗透物通过较低MWCO子系统渗透物输出管线129产生，以用于在较低MWCO TFF子系统140外部进一步使用或丢弃。如果截留物再次循环通过较低截留分子量TFF过滤器120，则可以通过较低MWCO子系统溶剂供应管线125和较低MWCO子系统褐藻糖胶容器126从较低MWCO子系统溶剂容器127供应溶剂，以补充通过较低MWCO子系统渗透物输出管线129损失的溶剂和/或确保较低截留分子量TFF过滤器120的预定透析体积量的截留物和溶剂在较低截留分子量TFF过滤器120上循环。

[0133] 在上述处理期间，可以关闭较高至较低MWCO子系统间阀123，并且可以将较低MWCO TFF子系统140的较低截留分子量TFF过滤器120的截留物收集到容器(未示出)中，然后供应给较高MWCO TFF子系统130的较高MWCO子系统褐藻糖胶容器116。将收集的截留物通过物理较低MWCO子系统截留物输出管线121供应至较高MWCO TFF子系统130的较高MWCO子系统褐藻糖胶容器116。在其他实施方式中，收集的截留物可以在容器(未示出)中转移到较高MWCO子系统褐藻糖胶容器116。在又一些实施方式中，较低至较高MWCO子系统间阀123可以保持打开，并且较低截留分子量TFF过滤器120的截留物通过较低MWCO子系统截留物输出管线121连续地供应到较高MWCO子系统褐藻糖胶容器116。与起始褐藻糖胶中的较低分子量的分布相比，来自较低截留分子量TFF过滤器120的截留物中的较低分子量的分布被减弱或抑制。

[0134] 当较高MWCO TFF子系统130处理来自较低MWCO TFF子系统140的较低截留分子量TFF过滤器120的截留物时，较高截留分子量TFF过滤器110的渗透物在较高MWCO子系统渗透物输出管线119上产生。当较高MWCO子系统泵114在较高截留分子量TFF过滤器110上循环较低MWCO TFF子系统140的渗余褐藻糖胶时，可以通过较高MWCO子系统溶剂供应管线115从较高MWCO子系统溶剂容器117供应溶剂，以补充经由渗透物损失的溶剂和/或确保较低MWCO TFF子系统140的预定透析体积量的渗余褐藻糖胶和溶剂在较高截留分子量TFF过滤器110上循环。

[0135] 在图2中，来自较高截留分子量TFF过滤器110的截留流体通过较高MWCO子系统截留物返回管线118返回至较高MWCO子系统褐藻糖胶容器116，而渗透流体则通过较高MWCO子系统渗透物输出管线119产生以用于在较高MWCO TFF子系统130的外部使用。在图2中，通过较高MWCO子系统渗透物输出管线119产生的具有所需分子量区段的输出褐藻糖胶，其分子量分布主要在第一较高截留分子量TFF过滤器110的平均截留分子量与第二较低截留分子量TFF过滤器120的平均截留分子量之间。但是，考虑到起始褐藻糖胶分子量分布的宽度和复杂性以及聚合物行为和TFF过滤器的可变性，输出聚合物分子量分布可能不会在这两个TFF过滤器的平均截留分子量之间达到峰值。例如，褐藻糖胶的过高或过低的折叠会导致在所需分子量区段中选择适当大小但异常稠密(或否)的褐藻糖胶。因此，就本文所述的连续TFF之后存在的褐藻糖胶而言，输出褐藻糖胶基本上由衍生自原始起始褐藻糖胶组合物的

褐藻糖胶的所需分子量区段组成，其被供应至基于分子量的分段系统(较低至较高)100'。具有所需分子量区段的该输出褐藻糖胶也可以从由预过滤器104进行预过滤之后产生的经预过滤的起始褐藻糖胶组合物中获得，然后供应给较低MWCO TFF子系统140。

[0136] 阳离子增强的切向流过滤

[0137] 可以通过阳离子增强的TFF从宽分子量分布起始褐藻糖胶获得高分子量褐藻糖胶，所述方法包括：提供起始褐藻糖胶组合物，其具有低分子量阳离子和分子量分布，所述分子量分布包括所需高分子量区段；用具有比低原子量阳离子更大分子量的阳离子的阳离子添加剂对起始褐藻糖胶组合物进行阳离子处理，以用添加剂阳离子代替低原子量阳离子；使经阳离子处理的褐藻糖胶组合物通过第一切向流过滤过滤器进行切向流过滤，该过滤器具有基于所需高分子量褐藻糖胶区段的分子量分布的平均截留分子量，以产生第一渗余褐藻糖胶组合物；使第一渗余褐藻糖胶组合物通过第二较低MWCO切向流过滤过滤器进行切向流过滤，该过滤器具有基于阳离子添加剂的分子量分布的平均截留分子量，以产生第二渗余褐藻糖胶组合物；用盐溶液使第二渗余褐藻糖胶组合物渗滤以产生第三渗余褐藻糖胶组合物；用低电导率渗滤溶液使所述第三褐藻糖胶截留组合物在相同的第二切向流过滤器中进行渗滤，以产生第四渗余褐藻糖胶组合物；以及收集包含所需高分子量褐藻糖胶的第四截留溶液。

[0138] 所述方法可以根据需要包括其他步骤，例如通过能够滤出大于所需尺寸的颗粒或部分的预滤器或其他不需要的材料对起始褐藻糖胶组合物进行预过滤。使起始褐藻糖胶组合物通过第一TFF过滤器可包括：在将压力施加至起始褐藻糖胶组合物的同时，使起始褐藻糖胶组合物通过TFF过滤器。使第一TFF过滤器的渗余褐藻糖胶组合物通过第二TFF过滤器可以包括：在向第一TFF过滤器的渗余褐藻糖胶组合物施加压力的同时，使第一TFF过滤器的渗余褐藻糖胶组合物通过第二TFF过滤器。

[0139] 可以使第一渗余褐藻糖胶组合物通过第二切向流过滤器进行切向流过滤并用盐溶液处理第二渗余褐藻糖胶组合物。用盐处理第二渗余褐藻糖胶组合物可以包括：用碱金属、碱土金属、铝和/或铵的氯化物、溴化物、碘化物、氟化物、硫酸盐、亚硫酸盐、碳酸盐、碳酸氢盐、磷酸盐、硝酸盐、亚硝酸盐、乙酸盐、柠檬酸盐、硅酸盐和/或氰化物处理第二渗余褐藻糖胶组合物。用钠盐处理第一渗余褐藻糖胶组合物可以包括：用氯化钠处理第一渗余褐藻糖胶组合物。

[0140] 用阳离子添加剂对起始褐藻糖胶组合物进行阳离子处理可包括：用阳离子添加剂处理起始褐藻糖胶，所述阳离子添加剂的分子量大于起始褐藻糖胶内的低原子量阳离子。阳离子添加剂可以是聚阳离子添加剂。用阳离子添加剂处理起始褐藻糖胶组合物的阳离子可包括：用两性离子添加剂处理起始褐藻糖胶，该两性离子的分子量大于起始褐藻糖胶内的低原子量阳离子的分子量。

[0141] 使经阳离子处理的褐藻糖胶组合物通过第一切向流过滤器进行切向流过滤可以包括：使经阳离子处理的褐藻糖胶组合物在第一TFF过滤器上再循环。在第一TFF过滤器上再循环经阳离子处理的褐藻糖胶组合物可以包括：用阳离子添加剂的溶液在第一TFF过滤器上对经阳离子处理的褐藻糖胶组合物进行渗滤。在第一TFF过滤器上再循环经阳离子处理的褐藻糖胶组合物可以包括：确定经阳离子处理的褐藻糖胶组合物中的褐藻糖胶的重均分子量。在第一TFF过滤器上再循环经阳离子处理的褐藻糖胶组合物可以包括：在第一TFF

过滤器上再循环经阳离子处理的褐藻糖胶组合物，直到经阳离子处理的褐藻糖胶组合物中的经阳离子处理的褐藻糖胶的重均分子量具有预定的期望值，从而产生第一渗余褐藻糖胶组合物。

[0142] 使第一渗余褐藻糖胶组合物通过第二较低MWCO切向流过滤过滤器进行切向流过滤可包括：使第一渗余褐藻糖胶组合物在第二TFF过滤器上再循环。在第二TFF过滤器上再循环第一渗余褐藻糖胶组合物可以包括：用盐溶液渗滤第二TFF过滤器的第一渗余褐藻糖胶组合物。在第二TFF过滤器上再循环第一渗余褐藻糖胶组合物可以包括：确定第一渗余褐藻糖胶组合物中褐藻糖胶的重均分子量。在第二TFF过滤器上再循环第一渗余褐藻糖胶组合物可以包括：在第二TFF过滤器上再循环第一渗余褐藻糖胶组合物，直到来自第一渗余褐藻糖胶组合物的褐藻糖胶的重均分子量具有预定的期望值，从而产生第二渗余褐藻糖胶组合物。

[0143] 用盐溶液对第二渗余褐藻糖胶组合物进行渗滤可包括：使第二渗余褐藻糖胶组合物在第二TFF过滤器上再循环。在第二TFF过滤器上再循环第二渗余褐藻糖胶组合物可以包括：用盐溶液渗滤第一TFF过滤器的第二渗余褐藻糖胶组合物，所述盐溶液包括碱金属、碱土金属、铝和铵的氯化物、溴化物、碘化物、氟化物、硫酸盐、亚硫酸盐、碳酸盐、碳酸氢盐、磷酸盐和硝酸盐中的至少一种，例如氯化钠。使第三渗余褐藻糖胶组合物通过第二MWCO切向流过滤过滤器进行切向流过滤可包括：使第三渗余褐藻糖胶组合物在第二TFF过滤器上再循环。在第二TFF过滤器上再循环第三渗余褐藻糖胶组合物可以包括：用低电导率溶液渗滤第二TFF过滤器的第三渗余褐藻糖胶组合物。低电导率溶液可以是去离子水。

[0144] 用阳离子添加剂对起始褐藻糖胶组合物进行阳离子处理可包括：用胆碱、聚乙烯吡咯烷酮、牛磺酸、多胺、壳聚糖、组蛋白和胶原中的至少一种处理输入褐藻糖胶。

[0145] 图3示出了用于基于分子量分离褐藻糖胶的阳离子增强的TFF系统(CATS)100”的示意图。CATS 100”采用了许多已经在图1和图2的前面讨论过的要素。将包含起始褐藻糖胶组合物的溶液通过输入供应管线102供应至较高MWCO子系统褐藻糖胶容器116。可以通过预过滤器104将合适溶剂中的起始褐藻糖胶组合物进行预过滤，以去除不希望有的颗粒物质。含有起始褐藻糖胶组合物的溶液可根据需要包含其他非褐藻糖胶组分，例如所需的缓冲剂、稀释剂等，例如用于褐藻糖胶的其他褐藻糖胶处理步骤或下游用途。预过滤器的规格通常大于要通过CATS 100”分离的最大聚合物分子。

[0146] 可以将阳离子添加剂，例如胆碱、聚乙烯吡咯烷酮、聚苯胺，添加到较高MWCO子系统褐藻糖胶容器116中的预过滤的起始褐藻糖胶组合物中。较高MWCO子系统泵114通过较高MWCO TFF过滤器供应管线112将褐藻糖胶泵至较高MWCO TFF子系统130’的较高MWCO TFF过滤器150。较高MWCO TFF过滤器150通常作为盒体供应，其被设计为允许供应给它的输入流体通过其截留侧的过滤器，同时允许渗透物通过一个输出管线排出，并且经处理的输入流体通过另一个输出管线作为截留物离开。截留分子量过滤器的形式可以是但不限于板框系统；螺旋盘绕药筒系统；中空纤维系统；流通池系统；和离心过滤系统。对于该实施方式，选择较高MWCO TFF过滤器150的截留分子量以分离通过用阳离子添加剂处理预过滤的起始褐藻糖胶而获得的经阳离子处理的褐藻糖胶的高分子量末端的所需部分。

[0147] 较高MWCO子系统泵114在其截留侧和渗透侧之间提供较高MWCO TFF过滤器150上的压力水平。在图3中，较高MWCO TFF过滤器150的截留物通过较高MWCO子系统截留物返回

管线118返回到较高MWCO子系统褐藻糖胶容器116，而渗透物通过较高MWCO子系统渗透物输出管线119产生，以在较高MWCO TFF子系统130'之外使用或被丢弃。当较高MWCO子系统泵114在较高MWCO TFF过滤器150上再循环预过滤的起始褐藻糖胶组合物和截留物时，可通过阳离子添加剂冲洗溶液供应管线135供应来自阳离子添加剂冲洗溶液容器137的阳离子添加剂冲洗溶液，例如以补充经由较高MWCO子系统渗透物输出管线119损失的溶剂和/或确保在较高MWCO TFF过滤器150上循环预定透析体积量的输入起始褐藻糖胶和阳离子添加剂冲洗溶液。通过控制阳离子添加剂冲洗溶液阀136，可以在脉冲过程中添加阳离子添加剂冲洗溶液。在其他实施方式中，阳离子添加剂冲洗溶液可以以连续模式添加。在其他实施方式中，阳离子添加剂冲洗溶液可以一次全部添加。如果已经选择胆碱作为输入起始褐藻糖胶的阳离子添加剂，则所用的阳离子添加剂冲洗溶液是胆碱溶液，例如氯化胆碱溶液。可以预先确定要在较高MWCO TFF过滤器150上处理的截留物和胆碱冲洗溶液的透析体积量，四透析体积通常是有用的。

[0148] 较高至较低MWCO子系统间阀113可在以上处理期间切断(关闭)，并且将较高MWCO TFF子系统130'的较高MWCO TFF过滤器150的截留物收集到容器(未示出)中，然后供应至较低MWCO TFF子系统140'的较低MWCO子系统褐藻糖胶容器126。然后可以通过较高MWCO子系统截留物输出管线111将收集的截留物供应至较低MWCO TFF子系统140'的较低MWCO子系统褐藻糖胶容器116。在其他实施方式中，可以将收集的截留物在容器(未示出)中转移至较低MWCO子系统褐藻糖胶容器126。在该系统的其他实施方式中，较高至较低MWCO子系统间阀113可以保持打开，并且较高MWCO TFF过滤器150的截留物可以通过较高MWCO子系统截留物输出管线111连续地供应给较低MWCO子系统褐藻糖胶容器126。与起始褐藻糖胶组合物中较低分子量的分布相比，较高MWCO TFF过滤器150的截留物中较低分子量的分布被减弱或抑制。

[0149] 较低MWCO TFF子系统140'从经阳离子处理的褐藻糖胶中去除胆碱阳离子，并将钠阳离子恢复到褐藻糖胶中，从而使经阳离子处理的褐藻糖胶返回至大约其原始离子组分，但是具有不同的所需高分子重量分布。在较低MWCO TFF子系统140'处理褐藻糖胶溶液期间，可以关闭较低MWCO子系统输出阀106'，其从较低MWCO子系统褐藻糖胶容器126控制较低MWCO子系统截留物输出管线108。当较低MWCO TFF子系统140'处理来自较高MWCO TFF子系统130'的较高MWCO TFF过滤器150的截留物时，较低MWCO TFF过滤器160的渗透物在较低MWCO子系统渗透物输出管线129上产生，通过该管线在其他地方使用或丢弃。

[0150] 当较低MWCO子系统泵114在较低MWCO TFF过滤器160上循环较低MWCO TFF子系统140'的截留物时，可以通过适当地控制钠盐溶液控制阀144通过钠盐溶液供应管线146从钠盐溶液容器142供应钠盐溶液，例如2M NaCl溶液。对于该方法，选择较低MWCO TFF过滤器160的截留分子量，以分离通过钠盐处理从褐藻糖胶中释放的阳离子添加剂。随着较低MWCO TFF子系统140'的过程的进行，由来自NaCl的钠阳离子取代在褐藻糖胶上的胆碱阳离子而产生的游离胆碱氯化物转移至较低MWCO TFF过滤器160的渗透物，并通过较低MWCO子系统渗透物输出管线129离开CATS 100"。可以使用钠盐溶液，例如以补充通过较低MWCO子系统渗透物输出管线129上的渗透液损失的溶液，和/或确保来自较高MWCO TFF子系统130'的预定透析体积量的钠盐溶液和截留物在较低MWCO TFF过滤器160上循环。通过控制钠盐溶液控制阀144，可以以脉冲过程添加钠盐溶液。在其他实施方式中，可以以连续模式添加钠盐

溶液。当适当透析体积量的钠盐溶液和截留物已经在较低MWCO TFF过滤器160上循环时，可以关闭钠盐溶液控制阀144并打开低电导率渗滤溶液阀145。可以预先确定要在较低MWCO TFF过滤器160上处理的钠盐溶液的透析体积量。较低MWCO子系统泵124在较低MWCO TFF过滤器160的截留侧和渗透侧之间提供一定水平的压力。在图3中，较低MWCO TFF过滤器160的截留物通过较低MWCO子系统截留物返回管线128返回到较低MWCO子系统褐藻糖胶容器126，而渗透物通过较低MWCO子系统渗透物输出管线129产生，以在较低MWCO TFF子系统140'外部使用或被丢弃。

[0151] 可以打开低电导率渗滤溶液阀145以允许来自低电导率渗滤溶液容器143的低电导率渗滤溶液通过低电导率渗滤溶液供应管线147进入较低MWCO子系统褐藻糖胶容器126，截留物和低电导率渗滤溶液可以在较低MWCO TFF过滤器160上进行处理以去除在较低MWCO TFF过滤器160的截留物的钠盐处理期间产生的游离钠盐。低电导率渗滤溶液可以是例如去离子水。为此，可以测量较低MWCO子系统渗透物输出管线129上的渗透物的电导率，以确保其下降到所需水平，这用于表明钠盐已经被去除到合适的程度。可以预先确定在较低MWCO TFF过滤器160上处理的低电导率渗滤溶液的透析体积量。当已经从较低MWCO TFF过滤器160的截留物中适当去除钠盐时，低电导率渗滤溶液阀145可以关闭并且较低MWCO子系统截留物输出管线108打开以将CATS 100'的产物输送到较低MWCO子系统截留物输出管线108上。

[0152] 离心沉淀

[0153] 可以通过离心沉淀从宽分布的起始褐藻糖胶中获得高分子量褐藻糖胶。

[0154] 转向图4，示出了用于从起始褐藻糖胶组合物中离心沉淀高分子量褐藻糖胶的离心沉淀系统600。系统600包括具有分级的可渗透屏障620的离心容器610。可渗透屏障可基于密度来分级，密度从离心容器610的第一底端630朝向第二顶端640减小。分级的可渗透屏障620可以由不同密度的不同材料组成。分级的可渗透屏障620可以由溶解在合适溶剂中的不同浓度的一种溶质的溶液组成。合适的溶剂可以是例如但不限于水和水-乙醇溶液中的一种。溶质，也称为"梯度材料"，例如但不限于可以是甘油、山梨糖醇、CsCl、Cs_2SO_4、KBr、泛影酸盐、Nycodenz®、碘克沙醇和合适的糖类(包括(聚)蔗糖)中的一种或多种。分级的可渗透屏障620可包括从离心容器610的第一底端630到第二顶端640的梯度材料浓度递减的连续梯度。在其他实施方式中，分级的可渗透屏障620可包括多个不同的密度的等级，例如分级的可渗透屏障区段620a、620b和620c，如图4所示。将包含起始褐藻糖胶组合物的溶液适当地通过预过滤器进行预过滤以去除颗粒物质，将其设置为紧邻离心容器610的第二顶端640并与分级的可渗透屏障620接触的起始褐藻糖胶组合物650。预过滤器可以是例如但不限于0.22μm的颗粒过滤器。

[0155] 在操作中，离心容器受到离心力，该离心力具有从容器的第二顶端640指向容器的第一底端630的力分量，如图4中的离心力箭头660所示。这可以在合适的离心机中实现，该离心机在图4中示意性地示出为离心机箱670，并适于容纳离心容器610。合适的离心机在本领域中是众所周知的，因此在此不再赘述。离心力可以为约1,000重力至约1,000,000重力，例如约10,000重力至约200,000重力、大约60,000重力至约500,000重力、以及约190,000重力至约800,000重力。

[0156] 与图4的系统相关联，用于从起始褐藻糖胶组合物离心沉淀高分子量褐藻糖胶的

方法包括:在离心容器610内建立梯度材料的分级的可渗透屏障620,其具有与离心容器610的第一底端630接触的第一底部分级可渗透屏障材料端622;将包含所需高分子量区段的起始褐藻糖胶组合物设置为与邻近离心容器610的第二顶端640的分级的可渗透屏障620的相对的第二顶部分级的可渗透屏障材料端624接触;使离心容器610受到从离心容器610的第二顶端640指向第一底端630的离心力660;以及在离心容器610的第一底端630处收集沉淀的高分子量褐藻糖胶。将起始褐藻糖胶组合物650设置为与最低密度梯度材料接触可以包括:通过合适的预过滤器对起始褐藻糖胶组合物进行预过滤。

[0157] 在离心容器610内建立梯度材料的分级的可渗透屏障620可包括:建立多个梯度材料区段,梯度材料区段的密度从离心容器610的第一底端630朝向离心容器610的第二顶端640减小。在离心容器610中建立梯度材料的分级的可渗透屏障620可以包括:在离心容器610中建立糖的分级的可渗透屏障620。在离心容器610中建立梯度材料的分级的可渗透屏障620可包括:在离心容器610内建立蔗糖的分级的可渗透屏障620。在离心容器610内建立梯度材料的分级的可渗透屏障620可包括:在离心容器610内建立甘油、山梨糖醇、CsCl、Cs_2SO_4、KBr、泛铝酸盐、Nycodenz®和碘克沙石中的至少一种的分级的可渗透屏障620。在离心容器610内建立梯度材料的分级的可渗透屏障620可以包括:在离心容器610内建立由溶剂溶解的梯度材料的分级的可渗透屏障620。在离心容器610内建立梯度材料的分级的可渗透屏障620可包括:在离心容器610内建立由溶解在水和水-乙醇溶液之一中的梯度材料的分级的可渗透屏障620。

[0158] 图5示出了离心沉淀系统600'的另一个实施方式,该离心沉淀系统用于从起始褐藻糖胶组合物中离心沉淀高分子量褐藻糖胶。使用与图4中类似的编号,该实施方式使用具有梯度材料的单个屏障区段620c'的可渗透屏障620',其第一底部可渗透屏障材料端622'与离心容器610的第一底端630接触。在一个实施方式中,起始褐藻糖胶组合物直接与可渗透屏障620'的相对的第二顶部可渗透屏障材料末端624'接触。在该实施方式中,该方法包括:使离心容器610承受被引导为从离心容器610的第二顶端640到第一底端630的离心力660,并在离心容器610的第一底端630收集沉淀的高分子量褐藻糖胶。将起始褐藻糖胶组合物650设置为与最低密度梯度材料接触可以包括:通过合适的预过滤器预过滤起始褐藻糖胶组合物。

[0159] 其他实施方式不需要采用屏障,并且将具有起始褐藻糖胶组合物的容器离心以使离心容器610受到被引导为从离心容器610的第二顶端640到第一底端630的离心力660,并在离心容器610的第一底端630收集沉淀的高分子量褐藻糖胶。

[0160] 凝胶电泳提取

[0161] 可以通过凝胶电泳提取从宽分子量分布的起始褐藻糖胶获得高分子量褐藻糖胶。所述方法可以包括:使包含所需高分子量区段的起始褐藻糖胶组合物进行凝胶电泳,其中,所述起始褐藻糖胶组合物通过施加的电势差的作用根据质荷比进行置换;根据电势差和所需高分子量褐藻糖胶选择电泳凝胶的一部分;以及从选择的凝胶部分中提取所需高分子量褐藻糖胶。

[0162] 使起始褐藻糖胶组合物进行凝胶电泳可包括:首先通过预过滤器将溶液形式的起始褐藻糖胶组合物进行预过滤以去除不希望有的颗粒物质。使起始褐藻糖胶组合物进行凝胶电泳可包括:以浓度在0.1%w/v至30%w/v之间的溶液制备起始褐藻糖胶组合物。提取所

需高分子量褐藻糖胶可以包括:从沿着电势差的方向延伸0.1mm至1000mm的距离的凝胶部分提取所需高分子量褐藻糖胶。提取所需高分子量褐藻糖胶可包括:使用水、甲醇、乙醇、异丙醇、水/醇混合物和盐溶液中的一种提取凝胶部分。

[0163] 使起始褐藻糖胶组合物进行凝胶电泳可包括:将起始褐藻糖胶组合物在溶液中置换预定的时间。使起始褐藻糖胶组合物在整个电泳凝胶上进行凝胶电泳可包括:在凝胶浸入缓冲溶液中的同时在整个电泳凝胶上置换起始褐藻糖胶组合物。使起始褐藻糖胶组合物在整个电泳凝胶上进行凝胶电泳可包括:由凝胶材料和缓冲溶液制备凝胶。由凝胶材料和缓冲溶液制备凝胶可包括:由缓冲剂和琼脂糖、聚丙烯酰胺、聚二甲基丙烯酰胺和淀粉中的一种制备凝胶。由凝胶材料和缓冲溶液制备凝胶可包括:由三乙酸酯EDTA、三硼酸EDTA和磷酸盐缓冲盐水中的一种与凝胶材料一起制备凝胶。在施加的电势差的作用下置换起始褐藻糖胶组合物可包括在这样的电场强度的作用下置换起始褐藻糖胶组合物:约1伏/cm至约500伏/cm,例如约5伏/cm至约50伏/cm、约10伏/cm至约200伏/cm、以及约50伏/cm至约300伏/cm。

[0164] 在图6中示出了用于从起始褐藻糖胶组合物获得所需的高分子量褐藻糖胶的电泳提取系统900。电泳提取系统900包括显示为透明且包含电泳凝胶916的电泳室910和电泳缓冲液918。电泳凝胶916材料可以是例如但不限于琼脂糖、聚丙烯酰胺和淀粉中的一种。电泳缓冲液918可以是例如但不限于三乙酸酯EDTA、三硼酸EDTA和磷酸盐缓冲盐水中的一种。在电泳凝胶916内接近并平行于电泳凝胶916的第一侧形成孔912,在该孔中放置溶液形式的起始褐藻糖胶组合物。

[0165] 直流电源920通过阴极922和阳极924在电泳室910中的电泳缓冲液918上施加电势差。阴极922和阳极924之间的电势差引发了起始褐藻糖胶组合物中的褐藻糖胶阴离子沿迁移方向箭头926给出的方向沿着凝胶迁移远离阴极922并朝阳极924迁移,这样,如果在给定的时间段内保持电势差,则起始褐藻糖胶组合物的不同分子量分子将以朝向阳极924的不同距离从孔912被置换。置换速率由褐藻糖胶分子的质荷比确定。较低分子量的褐藻糖胶将更迅速地置换,并且在电势差的作用下经过固定的时间段之后,其将比较高分子量的褐藻糖胶更多地置换。理论位移距离914表示不同分子量的褐藻糖胶分子的置换的不同理论距离,在任何给定的时间段,较低分子量的褐藻糖胶分子从阴极922进一步置换。

[0166] 为了从电泳后的起始褐藻糖胶组合物获得所需的高分子量褐藻糖胶,选择电泳凝胶916的相应部分,并从该凝胶的该部分中提取高分子量褐藻糖胶。一种非限制性的方法是将电泳凝胶916的一部分浸没在萃取剂溶液中并搅动凝胶溶液混合物。在一个实施方式中,搅拌可通过摇动完成。在另一个实施方式中,可以通过高剪切混合来完成搅拌。

[0167] 膜透析

[0168] 可以通过膜透析从宽分子量分布的起始褐藻糖胶获得高分子量褐藻糖胶。与典型的透析膜鉴定相一致,给定透析膜的标称MWCO值将选择性地允许溶液通过,该溶液所含分子的分子量通常小于不穿过/渗透透析膜的分子的分子量。对于任何给定的聚合物或标称截留值,透析膜的分子量截留值通常不是绝对的:给定的透析膜将通过或保留高于或低于标称分子量截留的一些分子。对于特定聚合物,可以常规地确定特定聚合物的标称MWCO透析膜的实际截留/选择性值和效果。

[0169] 许多因素可以影响透析膜的渗透行为。这些因素可能取决于透析膜本身或取决于

目标聚合物的属性，例如目标聚合物的折叠行为和折叠结构会影响目标聚合物在穿越/不穿越透析膜的MWCO屏障方面的行为。关于透析膜本身，例如，制造方法会在特定的透析膜内引起各种尺寸的孔，该各种孔可包括比标称MWCO截留大和小的孔。因此，具有标称分子量截留的透析膜将大体上允许低于标称分子量截留的分子通过，但是也可以使低于和/或高于该值的一些分子通过/保留。

[0170] 该方法可以包括使包含所需高分子量区段的起始褐藻糖胶组合物通过截留分子量大于100kDa的膜针对透析液进行透析，以产生包含该高分子量褐藻糖胶的透析褐藻糖胶组合物；以及收集包含高分子量的褐藻糖胶的透析的褐藻糖胶组合物。

[0171] 转向图7，示出了用于从起始褐藻糖胶组合物获得高分子量褐藻糖胶的膜透析系统800。系统800包括具有透析膜825的透析单元820，其允许低分子量褐藻糖胶分子通过。合适溶剂中的起始褐藻糖胶组合物进入膜透析系统800，并通过输入供应管线801并通过预过滤器802进入褐藻糖胶容器810。该预过滤器可以是例如0.22μm的预过滤器，以去除不希望有的颗粒物。

[0172] 通过透析系统泵814，通过透析系统供应管线812和透析液返回管线816，将预过滤的起始褐藻糖胶组合物循环通过透析膜825的第一侧上的透析单元820。通过透析液供应管线832通过透析液供应管线832从透析液容器830通过透析膜825的第二侧上的透析单元820循环透析液。透析液泵834选择透析液流体自由地流过透析膜825。合适的透析液包括但不限于去离子水和氯化钠、磷酸盐缓冲液、磷酸钠、磷酸盐缓冲盐水、tris-HCl缓冲液、柠檬酸钠、柠檬酸盐缓冲液、抗坏血酸钠、抗坏血酸、亚硫酸钠和乙二胺四乙酸(EDTA)。合适的透析膜的孔径被选择为优先阻止分子量大于200kDa的褐藻糖胶分子通过。其他合适的透析膜具有优先阻止分子量大于300kDa、500kDa和1000kDa的分子通过的孔径。这些膜中的每一种都可以用于从起始的褐藻糖胶组合物获得相应的高分子量褐藻糖胶，所述起始褐藻糖胶组合物包含较少的褐藻糖胶分子，其分子量小于透析膜的孔径或相对于宽的起始分子量分布的截留分子量。透析膜可以是但不限于纤维素酯和再生纤维素膜中的一种。包含起始褐藻糖胶组合物的溶液的浓度可以在0.1％w/v与30％w/v之间。

[0173] 当褐藻糖胶分子通过透析膜825时，其浓度在透析液中积累，并且这开始与透析过程相反。在期望的时间点，透析液供应阀845可以被打开以允许新鲜的透析液经由透析液供应管线842从透析液供应容器840进入透析液容器830。

[0174] 在适当的透析时间段之后，可以打开透析液输出阀815以允许通过透析液输出管线818从透析系统800中抽取透析的褐藻糖胶组合物。可以打开透析液输出阀835以允许含有低分子量褐藻糖胶分子的透析液被抽取到透析液输出管线838上。

[0175] 选择性沉淀

[0176] 可以通过选择性沉淀从宽分子量分布的起始褐藻糖胶获得高分子量褐藻糖胶。所述方法可以包括：以起始褐藻糖胶组合物在水中的溶液的形式提供包含所需的高分子量区段的起始褐藻糖胶组合物；向包含起始褐藻糖胶组合物的溶液中添加褐藻糖胶沉淀剂以获得过饱和的褐藻糖胶-溶剂混合物；通过将离子沉淀触发化合物添加到过饱和的褐藻糖胶-溶剂混合物中来触发一部分宽分子量分布的起始褐藻糖胶的沉淀，以从起始褐藻糖胶组合物和含有剩余褐藻糖胶的溶液中产生沉淀的高分子量褐藻糖胶；以及从混合物中提取沉淀出的高分子量褐藻糖胶。合适的褐藻糖胶沉淀剂包括相对极性小于0.765的溶剂，例如乙

醇、异丙醇、丙醇、丙酮、甲醇、二甲基亚砜、二甲基甲酰胺、乙二醇、四氢呋喃、乙腈、甘醇二甲醚、二甘醇二甲醚和二恶烷，褐藻糖胶的溶解度随着沉淀液极性的降低而降低。相对极性的值可以通过吸收光谱的溶剂位移的测量来归一化。参见例如Christian Reichardt, Solvents and Solvent Effects in Organic Chemistry(《有机化学中的溶剂和溶剂作用》),Wiley-VCH Publishers,第3版,2003年。合适的离子沉淀触发化合物包括但不限于单价、二价和三价阳离子的盐和碱，例如碱金属、碱土金属、铝和/或铵的氯化物、溴化物、碘化物、氟化物、硫酸盐、亚硫酸盐、碳酸盐、碳酸氢盐、磷酸盐、硝酸盐、亚硝酸盐、乙酸盐、柠檬酸盐、硅酸盐、氢氧化物、氧化物和/或氰化物。在一些实施方式中，离子沉淀触发化合物包含NaCl、KCl、NaOH、$MgCl_2$和$CaCl_2$中的至少一种。水中的起始褐藻糖胶组合物的合适浓度为0.01％w/v至30％w/v。适用于上述方法的特定褐藻糖胶包括但不限于岩藻多糖。

[0177] 所述方法可以进一步包括：在添加褐藻糖胶沉淀剂之前使起始褐藻糖胶组合物脱盐。脱盐可包括：使起始褐藻糖胶组合物通过截留分子量过滤器进行渗滤。渗滤可包括：用蒸馏水渗滤起始褐藻糖胶组合物。渗滤可包括：使起始褐藻糖胶组合物通过截留分子量过滤器进行渗滤，所述过滤器的截留分子量小于所需的高分子量褐藻糖胶中的所需分子量，例如5kDa、10kDa、30kDa、50kDa、70kDa、100kDa、200kDa或300kDa的截留分子量。所述方法可以进一步包括：通过合适的预过滤器预过滤包含起始褐藻糖胶组合物的溶液，以去除不希望有的颗粒物质。

[0178] 从混合物中提取沉淀的高分子量褐藻糖胶可包括离心、沉降、过滤和流体动力流分离中的至少一种。

[0179] 阴离子吸附

[0180] 可以通过阴离子吸附从宽分子量分布的起始褐藻糖胶中获得高分子量褐藻糖胶。所述方法可以包括：提供溶解在起始溶液中的具有宽起始分子量分布的起始褐藻糖胶组合物，所述起始分子量分布包含所需的高分子量区段；使起始溶液中的褐藻糖胶组合物与离子交换大孔树脂进行离子交换，该多孔树脂的孔径基于起始褐藻糖胶分子量分布内的所需分离分子量，以将褐藻糖胶组合物转化为经第一离子交换处理的褐藻糖胶组合物；收集包含所需的高分子量褐藻糖胶的第一经离子交换处理的褐藻糖胶组合物；与起始褐藻糖胶组合物进行离子交换后，将大孔树脂置于盐溶液中，从树脂中提取褐藻糖胶分子到盐溶液中，制得富含低分子量褐藻糖胶的盐溶液；使富含低分子量褐藻糖胶的褐藻糖胶盐溶液脱盐以形成第二经离子交换处理的褐藻糖胶组合物；以及收集包含低分子量褐藻糖胶的第二经离子交换处理的褐藻糖胶组合物。

[0181] 该方法可以进一步包括：在进行离子交换之前使起始褐藻糖胶组合物脱盐。脱盐可包括：使起始褐藻糖胶组合物通过截留分子量TFF过滤器进行渗滤。渗滤可以包括：在截留分子量TFF过滤器上渗滤起始褐藻糖胶组合物，所述过滤器的截留分子量小于高分子量褐藻糖胶中的所需分子量，例如5kDa、10kDa、30kDa、50kDa、70kDa、100kDa和/或300kDa的截留分子量TFF过滤器。

[0182] 在另一个实施方式中，从起始褐藻糖胶组合物产生所需的高分子量褐藻糖胶组合物的方法可以包括：提供溶解在起始溶液中的起始褐藻糖胶组合物，其具有宽起始分子量分布，所述宽起始分子量分布包括所需的高分子量区段；使溶解的起始褐藻糖胶组合物与在起始褐藻糖胶分子量分布内具有基于所需分离分子量的孔径的离子交换大孔树脂进行

离子交换，以将起始褐藻糖胶组合物转化为经离子交换处理的第一褐藻糖胶组合物；收集包含所需的高分子量褐藻糖胶的第一经离子交换处理的褐藻糖胶组合物。进一步的实施方式可以进一步包括：在进行离子交换之前使起始褐藻糖胶组合物脱盐。脱盐可包括：使起始褐藻糖胶组合物通过截留分子量TFF过滤器进行渗滤。渗滤可包括：在截留分子量TFF过滤器上渗滤起始褐藻糖胶组合物，所述过滤器的截留分子量小于所需的高分子量褐藻糖胶的分子量分布中的所需分子量，例如5kDa、10kDa、30kDa、50kDa、70kDa、100kDa和/或300kDa截留分子量TFF过滤器。

[0183] 使大孔树脂经受盐溶液可包括：使大孔树脂经受钠盐溶液，例如包含碱金属、碱土金属、铝和/或铵的氯化物、溴化物、碘化物、氟化物、硫酸盐、亚硫酸盐、碳酸盐、碳酸氢盐、磷酸盐、硝酸盐、亚硝酸盐、乙酸盐、柠檬酸盐、硅酸盐和/或氰化物中的至少一种的溶液。使大孔树脂经受钠盐溶液可包括：使大孔树脂经受氯化钠溶液。使富含低分子量褐藻糖胶的盐溶液脱盐可包括：使富含低分子量褐藻糖胶的盐溶液通过截留分子量TFF过滤器进行渗滤。渗滤可包括：在截留分子量TFF过滤器上渗滤富含低分子量褐藻糖胶的盐溶液，所述过滤器的截留分子量小于所需的富含低分子量褐藻糖胶的盐溶液的分子量分布中的所需分子量，例如5kDa、10kDa、30kDa、50kDa、70kDa和/或100kDa截留分子量TFF过滤器。

[0184] 使溶解的起始褐藻糖胶组合物与离子交换大孔树脂进行离子交换可包括：将起始褐藻糖胶与树脂的比例调节至预定的质量比。预定的质量比可以为约1:100的褐藻糖胶:树脂至约10:1的褐藻糖胶:树脂、5:1的褐藻糖胶:树脂、或2:1的褐藻糖胶:树脂。在其他实施方式中，预定质量比可以为约1:100的褐藻糖胶:树脂至约1:1的褐藻糖胶:树脂。在其他实施方式中，预定质量比可以为约1:100的褐藻糖胶:树脂至约1:2的褐藻糖胶:树脂。在另外的实施方式中，预定的质量比可以为约1:50的褐藻糖胶:树脂至约1:5的褐藻糖胶:树脂。在另外的实施方式中，预定的质量比可以为约1:20的褐藻糖胶:树脂至约1:1的褐藻糖胶:树脂，例如，约1:2的褐藻糖胶:树脂、1:4的褐藻糖胶:树脂、1:6的褐藻糖胶:树脂、1:8的褐藻糖胶:树脂和1:10的褐藻糖胶:树脂。

[0185] 使溶解的起始褐藻糖胶组合物与离子交换大孔树脂进行离子交换可包括：使溶解的起始褐藻糖胶组合物与树脂进行离子交换预定的时间段。预定时间段可以为零至300小时。在其他实施方式中，预定时间段可以为零至100小时。在其他实施方式中，预定时间段可以为5分钟至30小时，例如为约8小时至大约24小时。在另外的实施方式中，预定时间段可以为1至10小时，例如为约4小时至约10小时。在其他实施方式中，预定时间段可以为约2小时至约5小时。

[0186] 使溶解的起始褐藻糖胶组合物与离子交换大孔树脂进行离子交换可包括：使溶解的起始褐藻糖胶组合物与阴离子交换大孔树脂进行离子交换。使溶解的起始褐藻糖胶组合物与阴离子交换大孔树脂进行离子交换可包括：使溶解的起始褐藻糖胶组合物与强碱阴离子交换大孔树脂进行离子交换。使溶解的起始褐藻糖胶组合物与阴离子交换大孔树脂进行离子交换可包括：使溶解的起始褐藻糖胶组合物与弱碱阴离子交换大孔树脂进行离子交换。“强碱”和“弱碱”根据其通常含义使用，例如“强碱”是在任何典型的离子交换环境下都不会失去电荷的树脂，例如季胺官能化树脂，而“弱碱”是在高pH条件下会失去电荷的树脂，例如伯、仲或叔胺官能化树脂。使溶解的起始褐藻糖胶组合物进行离子交换可包括：使溶解的起始褐藻糖胶组合物与混合电荷大孔树脂进行离子交换。

[0187] 使溶解的起始褐藻糖胶组合物与阴离子交换大孔树脂进行离子交换可包括:使溶解的起始褐藻糖胶组合物与包含伯、仲、叔和季胺基中的至少一种的大孔树脂进行离子交换。伯胺基可以是NH_2基团。仲胺基可以是例如但不限于苄胺基和二甲基胺基中的至少一种。叔胺基可以是例如但不限于二乙基氨基乙基和二甲基氨基乙基中的至少一种。季胺基可以例如是但不限于三甲基铵和三乙基铵基。该树脂可以包括但不限于苯乙烯、琼脂糖、右旋糖酐、丙烯酸酯、甲基丙烯酸酯、甲基丙烯酸甲酯、甲基丙烯酸丁酯、二乙烯基苯、纤维素、二氧化硅和陶瓷中的一种或多种。

[0188] 使溶解的起始褐藻糖胶组合物与离子交换大孔树脂进行离子交换可包括:使溶解的起始褐藻糖胶组合物与孔径为5nm至1000nm的离子交换树脂进行离子交换,例如5nm至100nm、10nm或15nm至50nm、20nm至80nm、5nm至30nm、100nm至500nm、300nm至900nm、或200nm至400nm。使溶解的起始褐藻糖胶组合物与离子交换大孔树脂进行离子交换可包括:使溶解的起始褐藻糖胶组合物与离子交换树脂进行离子交换,所述离子交换树脂的排阻极限为50kDa至50,000kDa,例如50kDa至10,000kDa、100kDa至5,000kDa、10,000kDa至40,000kDa、1,000kDa至9,000kDa、2,000kDa至7,000kDa或500kDa至2,000kDa。排阻极限可以基于球状蛋白质的排阻极限。

[0189] 图8示出了用于基于分子量的褐藻糖胶的分段的示例性离子吸附系统300的示意图。包含起始褐藻糖胶组合物的溶液通过输入供应管线301和预过滤器306供应到TFF子系统褐藻糖胶容器176。在脱盐过程中,切向流过滤(TFF)子系统泵174通过TFF子系统过滤器供应管线172将起始褐藻糖胶组合物泵送到TFF子系统170的TFF过滤器171。TFF过滤器171的形式可以是但不限于以下各项中的任何一种:板框系统;螺旋盘绕药筒系统;中空纤维系统;流通池系统;和离心过滤系统。

[0190] 在图8的系统中,TFF子系统170用作脱盐子系统。TFF过滤器171通常被提供为盒体,该盒体被设计成允许供应给它的输入流体在其截留物侧通过其过滤器,同时允许渗透物通过一个输出管线排出,并且经处理的输入流体通过另一个输出管线作为截留物离开。对于本方法,选择TFF过滤器171的截留分子量以允许盐组分渗透到起始褐藻糖胶溶液中,同时将褐藻糖胶保留在截留物中,用于随后在离子交换子系统180中的离子吸附处理。TFF子系统泵174保持TFF过滤器171在其截留侧和渗透侧之间的压力水平。在图8中,TFF过滤器171的截留物通过TFF子系统截留物管线178返回到TFF子系统褐藻糖胶容器176,而含有不希望有的非褐藻糖胶组分的渗透物通过TFF子系统渗透物输出管线179产生,供在TFF子系统170外部使用或丢弃。

[0191] 当TFF子系统泵174在TFF过滤器171上再循环起始褐藻糖胶组合物和截留物时,可以通过TFF子系统溶剂供应管线175供应来自TFF子系统溶剂容器177的水或低电导率冲洗溶液。冲洗溶液用于补充通过TFF子系统渗透物输出管线179上的渗透物损失的截留物溶液,和/或确保在TFF过滤器171上循环预定透析体积量的输入起始褐藻糖胶和溶剂。通过控制TFF子系统溶剂供应阀173,冲洗溶液可以在脉冲过程中添加。在其他实施方式中,可以以连续模式添加溶剂。连续添加溶剂的方式具有效率优势。可以预先确定要在TFF过滤器171上处理的溶剂的透析体积量。在一些实施方式中,溶剂可以是去离子水。

[0192] 子系统间阀302可以在上述处理过程中关闭,并且在将TFF子系统170的TFF过滤器171的截留物收集到容器(未示出)中,然后再供应给离子交换子系统180的离子褐藻糖胶容

器186。然后可以将收集的截留物通过TFF子系统截留物输出管线303提供给离子交换子系统180的离子交换子系统褐藻糖胶容器186。在其他实施方式中，可以将收集的截留物在容器(未示出)中转移到离子交换子系统褐藻糖胶容器186中。在系统的其他实施方式中，子系统间阀302可以保持打开，并且TFF过滤器171的截留物可以连续地经由TFF子系统截留物输出管线303供应到离子交换子系统褐藻糖胶容器186。可以预期供应给离子交换子系统180的截留物具有较低的盐含量，这可能会干扰在离子交换子系统180中处理褐藻糖胶，并且其是脱盐的褐藻糖胶组合物。

[0193] 离子交换子系统180的离子交换容器181容纳一定体积的大孔离子交换树脂189。在一些实施方式中，大孔离子交换树脂是阴离子交换树脂。在一些实施方式中，大孔离子交换树脂是混合电荷树脂。选择大孔离子交换树脂189的孔径以优先从包含宽分子量分布的起始褐藻糖胶的溶液中吸附分子量低于预定值的褐藻糖胶分子，优先留在溶液中具有较大分子量的褐藻糖胶分子比预定值大。这类树脂的一种形式是基于基本上球形的苯乙烯颗粒，该颗粒与二乙烯基苯交联并且具有包含季铵基团的孔。在一些实施方式中，孔径可以为10nm至100nm。基于所述褐藻糖胶分子的流体动力尺寸，所述褐藻糖胶分子可以或可以不被优先吸附到树脂的孔中。

[0194] 在处理来自离子交换容器181中的TFF子系统170的脱盐褐藻糖胶组合物的过程中，控制来自离子交换子系统褐藻糖胶容器186的离子交换子系统输出管线305的离子交换子系统输出阀304可以关闭。离子交换子系统盐溶液供应阀183b和离子交换子系统盐溶液返回阀183c可以类似地关闭，而离子交换子系统褐藻糖胶返回阀183a可以打开。在离子交换子系统褐藻糖胶泵184a经由离子交换子系统褐藻糖胶供应管线182a和离子交换子系统褐藻糖胶泵184a通过离子交换容器181使包含脱盐的褐藻糖胶组合物的溶液再循环的同时，大孔离子交换树脂189吸附较低分子量的褐藻糖胶分子，从而使离子交换子系统褐藻糖胶返回管线188a中的溶液包含所需的高分子量褐藻糖胶。在流过离子交换容器181之后，包含所需的高分子量褐藻糖胶的溶液通过离子交换子系统褐藻糖胶返回管线188a返回到离子交换子系统褐藻糖胶容器186。

[0195] 可以测量或监测离子交换子系统褐藻糖胶容器186中褐藻糖胶的平均分子量。当使离子交换子系统褐藻糖胶容器186中的溶液循环一段合适的时间时，或者当溶液中的褐藻糖胶达到预定的所需平均分子量值时，可以打开离子交换子系统输出阀304以产生第一经由离子交换子系统输出管线305进行离子交换处理的褐藻糖胶组合物作为离子吸附系统300的第一输出产物。该第一输出产物包括例如具有分子量分布的高分子量褐藻糖胶，其中在低分子量末端处的输入起始褐藻糖胶的宽分子量分布的量已被抑制或减弱，使得所得分子量分布向输入供应管线301时供应至离子吸附系统300的输入起始褐藻糖胶组合物的分子量分布的较高端发生位移。

[0196] 离子交换子系统输出阀304可以再次关闭，离子交换子系统褐藻糖胶返回阀183a也可以关闭，离子交换子系统盐溶液供应阀183b和离子交换子系统盐溶液返回阀183c打开以允许盐溶液进行离子交换子系统盐溶液容器187通过离子交换子系统盐溶液供应管线182b进入离子交换子系统180中的循环。离子交换子系统盐溶液泵184b现在使盐溶液通过离子交换子系统盐溶液供应管线182b通过离子交换容器181中的大孔离子交换树脂189循环，并通过离子交换子系统盐溶液返回管线188b和离子交换子系统盐溶液返回阀183c返回

离子交换子系统盐溶液容器187。在该过程中，盐置换了吸附在大孔离子交换树脂的孔内的褐藻糖胶，并在离子交换子系统180中循环地将游离的褐藻糖胶释放到盐溶液中。盐溶液可以循环预定时间。在其他实施方式中，可以测量离子交换子系统180中的盐溶液中的褐藻糖胶的平均分子量，并且当盐溶液中的褐藻糖胶的平均分子量达到预定的期望值时，盐溶液的再循环终止。

[0197] 在一些实施方式中，在开始从离子交换子系统盐溶液容器187开始盐溶液的循环之前，可以使用预定量的低离子含量溶液来洗涤树脂。在一些实施方式中，该低离子含量溶液可以是去离子水。

[0198] 此时，离子交换子系统输出阀304可再次被打开，并且离子交换子系统180的泵和阀适当地操作以允许从离子交换子系统输出管线305抽取的离子吸附系统300的第二产物为以下形式：富含低分子量褐藻糖胶的盐溶液。可以将第二产物过滤，例如但不限于在合适的离心过滤器或切向流过滤器上的离心分离中，以将低分子量褐藻糖胶与不需要的盐分离。这产生了第二输出低分子量褐藻糖胶。与上面讨论的第一输出高分子量褐藻糖胶相比，该第二输出低分子量褐藻糖胶具有褐藻糖胶分子量分布，其中在高分子量下一部分输入起始褐藻糖胶分子量分布较宽。末端分子量已被抑制或减弱，使得所得的分子量分布朝着在输入供应管线301上供应至离子吸附系统300的输入起始褐藻糖胶组合物的分子量分布的下端移动。

[0199] 考虑到起始褐藻糖胶分子量分布的宽度和复杂性以及聚合物行为和离子交换树脂的变化，在考虑大孔离子交换树脂的孔径的情况下，两种输出褐藻糖胶分子量分布可能不会达到峰值。但是，如果发生这种情况，则两种输出的褐藻糖胶分子量分布仍将相对于彼此发生位移，这表示将起始褐藻糖胶组合物区段为与第一产物相对应的相对较高分子量的褐藻糖胶和相对较低的分子量。褐藻糖胶对应于第二产物。第一产物对应于优先未被树脂吸附的大而重的褐藻糖胶分子，而第二产物相反对应于优选被树脂吸附的褐藻糖胶分子，并且平均比未吸附的分子更小、更轻。

[0200] 制备型凝胶渗透色谱

[0201] 可以通过制备性凝胶渗透色谱法从宽分子量分布的起始褐藻糖胶获得高分子量褐藻糖胶。该方法可以包括：以柱形式装填用于水溶液中聚合物的凝胶渗透色谱法(GPC)所指定的凝胶介质；提供起始褐藻糖胶组合物，其包含溶解在适合于凝胶介质上的凝胶渗透色谱法的水性溶剂中的所需的高分子量区段；使包含起始褐藻糖胶组合物的溶液经受制备型凝胶渗透色谱，其中，所述褐藻糖胶根据分子量在色谱柱的第一输入端和第二输出端之间以预定的流速在色谱柱中的凝胶介质上移位；基于所述起始褐藻糖胶组合物的期望区段，以预定的等分部分从所述色谱柱的第二输出端收集洗脱液，每个等分部分包括分段的褐藻糖胶组合物；基于起始褐藻糖胶组合物的期望分段，合并期望的等分部分，以获得包含期望的高分子量褐藻糖胶的合并的GPC等分部分组合物。

[0202] 使包含起始褐藻糖胶组合物的溶液经受制备型凝胶渗透色谱法可包括：首先通过预过滤器将溶液中的起始褐藻糖胶组合物预过滤以去除不希望有的颗粒物质。使包含起始褐藻糖胶组合物的溶液经受制备型凝胶渗透色谱法可以包括：以浓度在0.1％w/v至20％w/v之间的溶液制备起始褐藻糖胶组合物。使包含起始褐藻糖胶组合物的溶液经历制备型凝胶渗透色谱法可包括使用蠕动泵、等度泵、二元泵、四元泵和梯度泵中的至少一种来完成在

包含凝胶介质的色谱柱上的置换。从褐藻糖胶组合物开始进行制备型凝胶渗透色谱分析，可以包括将溶液以预定流速通过含有凝胶介质的色谱柱：每分钟每凝胶介质表面积0.0005毫升(mL/min/cm^2)至5mL/min/cm^2、0.005mL/min/cm^2至0.5mL/min/cm^2、0.01mL/min/cm^2至0.25mL/min/cm^2、0.05mL/min/cm^2、0.1mL/min/cm^2、0.15mL/min/cm^2和0.2mL/min/cm^2。

[0203] 从色谱柱的第二输出端收集洗脱液可包括收集约0.1mL至1000mL、约1mL至100mL、约5mL至50mL、约10mL、约20mL、约30mL和约40mL的洗脱液的等分部分。从色谱柱的第二输出端收集等分部分可以包括通过分析GPC测量等分部分的分子量分布。通过分析型GPC测量等分部分可与收集柱洗脱液同时进行。

[0204] 合并所需的等分部分可包括通过分析GPC测量等分部分的分子量分布，并仅合并具有所需分子量分布的等分部分。合并所需的等分部分可以与收集柱洗脱液同时进行。

[0205] 所使用的凝胶介质可以包括聚羟基甲基丙烯酸酯、磺化的苯乙烯-二乙烯基苯、二氧化硅、亲水键合相或聚合物、聚苯乙烯、二乙烯基苯、甲基丙烯酸酯、甲基丙烯酸甲酯、甲基丙烯酸丁酯、纤维素、陶瓷、琼脂糖和右旋糖酐中的至少一种。所使用的凝胶介质可以具有直径为约3nm、5nm、10nm、20nm、50nm、100nm、200nm、500nm、1,000nm、2,000nm、3,000nm、5,000nm和10,000nm中的至少之一的孔。所使用的凝胶介质可以具有排阻极限为约100Da、100kDa、1,000kDa、5,000kDa、10,000kDa、30,000kDa、50,000kDa和100,000kDa中至少之一的孔。排阻极限可以基于球状蛋白质或多糖例如右旋糖酐和/或普鲁兰多糖的排阻极限。

[0206] 用于溶解起始褐藻糖胶组合物的溶剂可包含水、硝酸钠、硝酸锂、磷酸二氢钠、磷酸二钠、磷酸三钠、氯化锂、溴化锂、碘化锂氯化钠、溴化钠、碘化钠、氯化钾、溴化钾、碘化钾、氢氧化钠、氢氧化锂、氢氧化钾、硫酸钠、亚硫酸钠、甲醇、乙醇和乙腈中的至少一种。

[0207] 化学结构修饰

[0208] 本文讨论的方法、系统等可包括对褐藻糖胶组合物，特别是褐藻糖胶组合物中的褐藻糖胶进行化学结构修饰。化学结构修饰可涉及从褐藻糖胶结构中去除官能团，例如，从褐藻糖胶结构中去除O-乙酰基、N-乙酰基、甲氧基、羟基、羧基和/或硫酸酯官能团。化学结构修饰可涉及多种化学试剂的使用，例如酸、碱、去污剂和/或氧化剂。

[0209] 疾病和病症

[0210] 纤维性粘连

[0211] 纤维性粘连通常是在外科手术后在身体的两个部位之间形成的一种疤痕类型(外科手术粘连)。纤维性粘连可能导致严重问题。例如，涉及女性生殖器官(卵巢、输卵管)的纤维性粘连可能导致不育、性交困难及严重的骨盆疼痛。在肠中出现的纤维性粘连可能导致肠梗阻或阻塞，且纤维性粘连还可能在其他位置，诸如心脏周围、脊椎及手部形成。除外科手术之外，纤维性粘连可能例如由子宫内膜异位症、感染、化疗、放射、创伤及癌症引起。

[0212] 在本文档中，论述了各种纤维性粘连。诸如外科手术粘连、外科手术后粘连(post-surgical adhesion)、术后粘连(postoperative adhesion)、因盆腔炎所致的粘连、因机械性损伤所致的粘连、因放射所致的粘连、因放射治疗所致的粘连、因创伤所致的粘连以及因外来材料的存在所致的粘连等术语均是指因类似机制所致的组织间彼此粘连并且均包括在术语纤维性粘连中。

[0213] 纤维性粘连形成是复杂的过程，其中体内正常分离的组织生长至彼此中。外科手术粘连(也称为外科手术后粘连)是由组织对创伤的正常伤口愈合反应以外的反应发展而

来的并且已被报导在超过三分的二的全部腹部外科手术患者中出现(Ellis,H.,Surg.Gynecol.Obstet.133:497(1971))。这些纤维性粘连的后果是变化的且取决于所涉及的外科手术部位或其他部位,诸如疾病部位。面临的问题可能包括慢性疼痛、肠梗阻及甚至心脏外科手术后的死亡风险增大(diZerega,G.S.,Prog.Clin.Biol.Res.381:1-18(1993);diZerega,G.S.,Fertil.Steril.61:219-235(1994);Dobell,A.R.,Jain,A.K.,Ann.Thorac.Surg.37:273-278(1984))。据估计,在生殖期女性中,涉及子宫、输卵管或卵巢的纤维性粘连占全部不育情况的大约20%(Holtz,G.,Fertil.Steril.41:497-507(1984);Weibel,M.A.和Majno,G.Am.J.Surg.126:345-353(1973))。

[0214] 纤维性粘连形成过程最初涉及建立纤维蛋白框架及正常组织修复。正常修复过程允许沿间皮修复的纤维蛋白溶解。然而,在纤维性粘连形成中,纤维蛋白基质随着成纤维细胞增殖成熟为网络且发生血管新生,使得在约3至5天内建立经组织化的纤维性粘连(Buckman,R.F.,等人,J.Surg.Res.21:67-76(1976);Raferty,A.T.,J.Anat.129:659-664(1979))。炎性过程包括创伤组织中的中性粒细胞活化、纤维蛋白沉淀及邻接组织结合、巨噬细胞侵袭、成纤维细胞增殖至区域中、胶原沉淀、血管新生及建立永久纤维性粘连组织。

[0215] 已作出各种尝试以防止外科手术粘连。这些尝试涉及针对影响伴随外科手术创伤的生物化学及细胞事件的药理学方法以及用于分离受影响的组织的屏障方法。例如,使用腹腔灌洗、肝素化溶液、促凝剂、诸如使用显微术或腹腔术外科手术技术的修饰外科技术、消除来自外科手术用手套的滑石、使用更小的缝合线以及使用旨在最小化浆膜表面的并置的物理屏障(膜、凝胶或溶液)均已尝试。当前,预防性疗法还包括预防纤维蛋白沉淀、减少炎性(类固醇及非类固醇抗炎药)及去除纤维蛋白沉淀物。

[0216] 防止形成外科手术后粘连的介入尝试已包括使用水浮选(hydroflotation)技术或屏障器械。水浮选涉及将较大体积的聚合物溶液,诸如右旋糖酐(Adhesion Study Group,Fertil.Steril.40:612-619(1983))或羧甲基纤维素(Elkins,T.E.,等人,Fertil.Steril.41:926-928(1984))灌注至外科手术间隙中以尝试保持器官分开。由经氧化再生纤维素制成的合成屏障膜(例如,Interceed™)、聚四氟乙烯(戈尔-特克斯手术膜)及由经修饰的透明质酸/羧甲基纤维素(HA/CMC)组合制成的可完全再吸收的膜(Seprafilm™)也已用于减少动物及人类两者体内的外科手术后粘连形成(Burns,J.W.,等人,Eur.J.Surg.Suppl.577:40-48(1997);Burns,J.W.,等人,Fertil.Steril.66:814-821(1996);Becker,J.M.,等人,J.Am.Coll.Surg.183:297-306(1996))。这些HA/CMC膜的成功可能源自其能够在纤维性粘连形成时在腹膜伤口修复过程期间提供组织分离。据观察,在应用后3-5天(与外科手术后粘连形成的时程兼容的时间段),膜在受伤组织上形成清楚的粘性涂层(Ellis,H.,Br.J.Surg.50:10-16(1963))。不幸地,利用这些方法取得的成功有限。

[0217] 腹膜炎涉及腹膜的炎症。腹膜炎可能导致严重的问题。例如,腹痛、腹部压痛及腹部防护。腹膜炎可能涉及自发性、解剖和/或腹膜透析相关的炎症。腹膜炎可能涉及感染,例如,空腔脏器穿孔、腹膜破裂、自发性细菌腹膜炎以及全身性感染可能引起感染及腹膜炎。腹膜炎还可能不涉及感染,例如,无菌体液渗漏至腹膜中,以及无菌腹部外科手术可能引起腹膜炎。已作出各种尝试以预防和/或治疗腹膜炎。例如,通用支持性测量诸如静脉内补液、抗生素及外科手术。未能满足对抑制或以其他方式治疗和/或预防腹膜炎,优选更有效的且

副作用小的化合物、组合物和方法等(包括递送方法)的需求。

[0218] 本文讨论的高分子量褐藻糖胶可用于治疗患者中的纤维性粘连,并且可以作为高分子量褐藻糖胶医疗器械、组合物或药物产品的组成部分或被配置和构成为用于治疗纤维性粘连。例如,一种高分子量褐藻糖胶医疗器械,其包含约0.02mg/mL至约100mg/mL,例如0.1mg/mL、0.2mg/mL、0.3mg/mL、0.5mg/mL、0.9mg/mL、1mg/mL、2.5mg/mL、5mg/mL、7.5mg/mL的本文中溶解在生理盐溶液中的高分子量褐藻糖胶。生理盐溶液可以是例如乳酸钠林格注射液USP(LRS)、生理盐水和生理右旋糖酐溶液。

[0219] 高分子量褐藻糖胶医疗器械,其可以是液体医疗器械,在本文中可以包含药学上可接受的赋形剂,例如缓冲剂、稳定剂、防腐剂、佐剂等。此类高分子量褐藻糖胶医疗器械可以用于在手术前、手术中或手术后,通过在先前段落中给药约0.01mL/kg(每公斤患者或目标体重)至约10mL/kg或15mL/kg的褐藻糖胶医疗器械来治疗纤维性粘连。剂量包括例如约0.03mL/kg、0.1mL/kg、0.2mL/kg、0.4mL/kg、0.5mL/kg、0.6mL/kg、1mL/kg、1.2mL/kg、2mL/kg、3mL/kg、4mL/kg、5mL/kg、8mL/kg、10mL/kg和15mL/kg的高分子量褐藻糖胶医疗器械到达患者的手术部位。在一些实施方式中,这样的高分子量褐藻糖胶医疗器械可以用于通过给药约0.04mg/kg或0.1mg/kg至约25mg/kg或50mg/kg来治疗任何选择的目标部位的纤维性粘连,例如损伤、擦伤、损伤部位、手术部位和手术后部位。这样的剂量的一些实例包括例如约0.04mg/kg、0.075mg/kg、0.1mg/kg、0.2mg/kg、0.5mg/kg、1mg/kg、1.3mg/kg、2mg/kg、3mg/kg、4mg/kg、5mg/kg、7.5mg/kg、8mg/kg、10mg/kg、15mg/kg、20mg/kg、25mg/kg和50mg/kg,可以例如通过在整个目标区域中一般注入液体医疗器械来完成给药;例如,将本文所述的褐藻糖胶,包括例如本文的高分子量褐藻糖胶,给药至患者的手术部位。将液体医疗器械引导到目标区域内的特定位置;一般或在目标区域内的特定位置喷涂液体医疗器械;或者,通过施料器将液体医疗器械喷雾或以其他方式递送,该施料器可以是通过套管针、导管、内窥镜或其他微创器械通过喷雾器施加到外科医生或其他从业者特别确定的特定位置容易产生纤维性粘连或与之相关。在另一方面,可以在手术伤口张开之后但在手术程序之前进行给药。在手术过程中,或在手术过程之后但在手术伤口闭合之前。如果需要,也可以在手术完成后(例如通过注射器和针头)给药液体医疗器械,也可以将其给药到非手术目标部位。患者的手术部位可以是例如骨盆腔、腹腔、背侧腔、颅腔、脊髓腔、腹侧腔、胸腔、胸膜腔、心包腔、皮肤、关节或肌肉中的至少之一。可以在不到约15分钟、10分钟、8分钟、6分钟、5分钟、4分钟、3分钟、2分钟、1分钟、45秒、30秒、20秒、15秒、10秒和5秒的时间内完成将高分子量褐藻糖胶医疗器械给药于患者手术部位的操作。

[0220] 将高分子量褐藻糖胶医疗器械给药于手术部位的实例包括但不限于在剖宫产手术方法的外科手术部位施予高分子量褐藻糖胶医疗器械;无血管皮瓣再造外科手术、全层皮瓣移植手术、VY推进皮瓣外科手术、筋膜皮肤旋转皮瓣外科手术、人工关节置换外科手术、乳腺切除术外科手术、死骨切除术外科手术、椎间盘摘除术外科手术、截骨术外科手术、整形外科手术、髌骨切除术手术、滑膜切除术外科手术、囊膜切开术外科手术、腱或韧带修复外科手术、腱切术外科手术、腱切断术、筋膜切开术外科手术、半月板修复外科手术、椎骨切除外科手术、筛窦切除外科手术、Caldwell Luc氏手术外科手术、泪囊鼻腔吻合术、溶胞鼻粘连外科手术、胸腺切除外科手术、肺气溶解手术、肺切除术、胸腔镜整形外科手术、双叶切除外科手术、门静脉高压手术外科手术、脾切除外科手术、食管切除外科手术、腹膜炎外

科手术、胃切除外科手术、空肠空肠造口外科手术、腹腔镜胆囊切除外科总手术、腹腔镜手术外科手术、胃肠造口外科手术、减肥手术外科手术、肠切除和吻合外科手术手术、节段性肝切除外科手术手术、肺叶切除外科手术手术、胰腺外科手术、胰十二指肠切除外科手术手术、肿瘤切除外科手术、腹腔镜肾手术外科手术、腹部或骨盆粘连溶解手术、子宫输卵管造口术、输卵管成形术、异位妊娠腹腔镜外科手术、关节置换外科手术、断骨修复外科手术、子宫切除术外科手术、胆囊摘除外科手术、心脏搭桥外科手术、血管成形术外科手术、旋磨术外科手术、乳腺穿刺活检外科手术、颈动脉内膜切除术手术、白内障手术、冠状动脉搭桥手术、扩张刮宫手术、疝气修复手术、下背痛手术、局部结肠切除术、前列腺切除术和扁桃体切除术手术过程:在打开手术伤口后,在手术过程中,在关闭手术伤口之前和/或在关闭手术伤口之后。

[0221] 一般癌症

[0222] 癌症已成为美国死亡的第二主要原因且占全部死亡率的20%以上。癌症为增殖性疾病且其特征为某些细胞的不可控分裂,其可导致形成一种或多种肿瘤。大量方法用于治疗癌症,包括外科手术、放射、化疗及其组合。尽管外科手术是用于一些局部肿瘤的相对普遍的方法,但在肿瘤切除后仍存在明显肿瘤复发机率。

[0223] 治疗癌症及其他增殖性疾病受对非癌性、健康组织的潜在损伤或毒性限制。在放射及外科手术治疗中,手术总体上受限于肿瘤部位且靠近肿瘤部位。然而,对于经历手术去除癌性组织的患者来说,可能存在明显的风险(例如,在去除前列腺或脑瘤中,可能存在对于周围重要组织的明显的不可修复损伤风险,例如经由潜在地减小对切除非肿瘤组织的需求。另外,在作为前列腺癌的一线治疗的集中放射治疗中,存在类似风险。在癌症的化学治疗性治疗中,药物全身性给药,使得整个身体暴露于药物。这些药物经设计对癌细胞是有毒的,但其对非癌细胞(通常)也是有毒的,使得患者在经历用于癌症的药物治疗时变得非常虚弱。经由试验,肿瘤学家能够给予对一些患者耐受的这些药物的剂量。然而,这些剂量通常不能成功治疗癌症。

[0224] 任何治疗癌症的方法都存在的一个问题是疾病的局部复发。例如,每年大约700,000名美国人经诊断患有局部癌症(大约64%的全部癌症患者)且近似五十万患者使用外科手术方法进行治疗。不幸地,经手术治疗的32%患者在初始治疗后复发(大约21%在初始手术部位处复发及11%在远处转移性部位处复发)。每年接近100,000名患者死于癌症的局部复发。这在乳腺癌中更是如此,其中经历乳房肿瘤切除术的39%的患者将经受疾病的局部复发。

[0225] 分期为判定患者体内的癌症(实体肿瘤)的进展的方法。简化方法基于癌症已进展程度将患者分成三组或三期:

[0226] 1期:可以通过手术去除器官的部分来治疗癌症。这还称为可切除期。

[0227] 2期:癌症已进展超过可切除点但仍受限于器官本身。

[0228] 3期:肿瘤已扩散至其他器官。

[0229] 许多癌症采用抗增殖剂,包括例如,5-氟尿嘧啶(Efudex®)、长春花生物碱(vinca alkaloid)(例如,长春新碱(vincristine)(Oncovin®))、蒽环素(anthracycline)(例如,阿霉素(Adriamycin®))、顺铂(Platinol-AQ®)、盐酸吉西他滨(gemcitabine

hydrochloride)(Gemzar®)、甲胺喋呤及紫杉醇治疗。与抗增殖剂、甲胺喋呤及紫杉醇相关联的毒性的一些实例在本文中的其他地方论述。甲胺喋呤已用于治疗几种癌症,包括例如,膀胱癌、乳腺癌、宫颈癌、头部及颈部癌、肝癌、肺癌及睾丸癌。紫杉醇已用于治疗几种癌症,包括例如,卵巢癌、乳腺癌及非小细胞肺癌(Compendium of Pharmaceutical and Specialties Thirty-fifth Edition,2000)。

[0230] 因5-氟尿嘧啶所致的毒性可以包括心脏血管毒性,诸如心肌缺血;中枢神经系统毒性,诸如欣快症、急性小脑综合征及共济失调;皮肤病学毒性,诸如秃发症及皮炎;胃肠毒性,诸如恶心、呕吐及口腔或胃肠溃烂;血液毒性,诸如白细胞减少症、血小板减少及贫血症;超敏感性毒性,诸如全身性过敏反应及接触过敏;眼部毒性,诸如增加流泪、畏光及结膜炎;及其他毒性,诸如发热。5-氟尿嘧啶已用于治疗多种癌症,包括例如,乳腺癌、结肠直肠癌、胃癌、肝脏癌、膀胱癌、头部及颈部癌、非小细胞肺癌、卵巢癌、胰腺癌及前列腺癌(Compendium of Pharmaceutical and Specialties Thirty-fifth Edition,2000)。

[0231] 因长春新碱所致的毒性包括中枢神经系统毒性,诸如儿童癫痫及幻觉;皮肤病学毒性,诸如秃发症;外渗毒性,诸如水疱;胃肠毒性,诸如恶心、呕吐、便秘及口炎;血液毒性,诸如骨髓抑制;神经毒性,诸如周围神经病变及自主神经病;眼部毒性,诸如复视、瞬时目盲及视神经萎缩;肾/代谢毒性,诸如尿潴留、高尿酸血症及膀胱乏力;呼吸毒性,诸如呼吸短促;及其他毒性,诸如儿童发热。这种抗增殖剂已用于治疗几种癌症,包括例如,霍奇金病、小细胞肺癌、威尔姆肿瘤及睾丸癌(Compendium of Pharmaceutical and Specialties Thirty-fifth Edition,2000)。

[0232] 因阿霉素所致的毒性包括心脏血管毒性,诸如心电图异常及心肌病;皮肤病学毒性,诸如秃发症及指甲变化;外渗危害毒性,诸如水疱;胃肠毒性,诸如恶心、呕吐及口炎;泌尿生殖系统毒性,诸如尿液红色;血液毒性,诸如骨髓抑制;超敏感性毒性,诸如过敏反应及皮疹;眼部毒性,诸如结膜炎;生殖毒性,诸如不孕症;及其他毒性,诸如高尿酸血症。这种抗增殖剂已用于治疗几种癌症,包括例如,乳腺癌、小细胞肺癌及卵巢癌(Compendium of Pharmaceutical and Specialties Thirty-fifth Edition,2000)。

[0233] 因顺铂所致的毒性包括心脏血管毒性,诸如心电图变化;皮肤病学毒性,诸如色素沉着;外渗危害毒性,诸如刺激性;胃肠毒性,诸如恶心及呕吐;血液毒性,诸如骨髓抑制及溶血性贫血;超敏感性毒性,诸如过敏反应;神经肌肉毒性,诸如周围神经病及急性脑病;眼部毒性,诸如球后神经炎;耳科毒性,诸如听觉损失及耳鸣;肾/代谢毒性,诸如中毒性肾病及低钾血症;及其他毒性,诸如不育。这种抗增殖剂已用于治疗几种癌症,包括例如,膀胱癌、小细胞肺癌、卵巢癌、睾丸癌、脑癌、乳腺癌、宫颈癌、头部及颈部癌、肝母细胞瘤癌及甲状腺癌(Compendium of Pharmaceutical and Specialties Thirty-fifth Edition,2000)。因盐酸吉西他滨所致的毒性包括例如血液毒性,诸如骨髓抑制;胃肠毒性,诸如恶心、呕吐及口炎;肝毒性,诸如血清转胺酶的瞬时升高;肾毒性,诸如蛋白尿、血尿、溶血性尿毒症综合征及肾衰竭;皮肤病学毒性,诸如皮疹及秃发症;水肿毒性,诸如水肿及周围水肿;及其他毒性,诸如发热。这种抗增殖剂已用于治疗胰腺癌及非小细胞肺癌(Compendium of Pharmaceutical and Specialties Thirty-fifth Edition,2000)。

[0234] 本发明论述包括预防或治疗可治疗的局部癌症或实体肿瘤,包括前列腺、乳腺、胰腺、肝、肾、泌尿生殖系统、脑、胃肠系统、呼吸系统及头部及颈部的局部癌症或实体肿瘤。本

文中的组合物等可以通过允许在距目标肿瘤远处的部位处控制释放高分子量褐藻糖胶，通过允许有效浓度的高分子量褐藻糖胶利用扩散或甚至全身性输送达至肿瘤和/或转移瘤来预防或治疗癌症，包括转移瘤。在以下段落中进一步论述这些癌症中的一些。

[0235] 前列腺癌

[0236] 前列腺癌为在衬在前列腺腺体的细胞中产生的恶性肿瘤。在美国，今年估计200,000名患者将罹患前列腺癌，且超过30,000名患者将死于该疾病。

[0237] 前列腺癌的新发病例死亡率为15％。癌症可能保持在前列腺内，或其可能扩散至周围组织或远部位(最经常淋巴结及骨骼)。通常前列腺癌无症状地扩散，仅在其已进展超出前列腺时才产生症状。在一些研究中，若前列腺癌已在早期期间经诊断且经治疗，患者的5年存活率为94％。

[0238] 前列腺癌经常论述为超过50岁的男性疾病。实际上，患有前列腺癌的80％男性为60岁且更年长。男性在其寿命期间诊断患有前列腺癌的机率为约1/10，与女性患乳腺癌的机率大致相同。近年来，由于可以在疾病出现早期(经常在症状呈现的前很久)检测到疾病的改良测试，经报导的新病例的数目显著地上升。任一给定年份中罹患前列腺癌的可能性随着年龄增加，但在50岁后显著地上升。

[0239] 用于前列腺癌的当前治疗选项取决于疾病进展程度、患者的年龄及总体健康状况。仅患有早期癌症或受额外更严重疾病影响的老年患者可以保守治疗，然而癌症为晚期的老年患者可能经历更具侵袭性的治疗。前列腺癌已通过各种方法治疗，包括放射疗法(外部辐射束放射或近距离放射疗法)、激素戒断或去势(外科手术或化学品)、抗增殖剂、外科手术及期望治疗(即，“观察等待”)。没有任何治疗保证绝对治愈，且一些具有相当大的副作用。

[0240] 早期前列腺癌(即，肿瘤在前列腺局部)可以“观察等待”治疗。前列腺癌的外科手术经推荐用于总体健康状况在其他方面良好且肿瘤受限于前列腺腺体的患者。针对70岁以下的男性前列腺的局部癌症的普遍治疗为根治性前列腺切除术(即，外科手术去除前列腺)。

[0241] 其癌症为前列腺区域局部的患者通常以外部辐射束放射(EBR)治疗。放射杀灭癌细胞且缩小肿瘤。EBR占局部前列腺癌治疗的小于20％，其中这些患者中大约50％经历放射后疾病复发。与早期前列腺癌检测及来自患者的增加的需求组合，近距离放射疗法(即，局部放射疗法)的使用预期将会增加。在1995年，仅2.5％的新诊断患者使用近距离放射疗法治疗。近距离放射疗法涉及将放射性金属“种”植入前列腺肿瘤中。

[0242] 用于已扩散的前列腺癌的治疗涉及去除睾或激素疗法。两种皆用于抑制或终止驱动癌症生长的睾固酮的产生。大约20％的全部前列腺癌患者经历激素戒断疗法。激素疗法包括醋酸戈舍瑞林乙(goserelin acetate)(Zoladex®)或醋酸亮丙瑞林(leuprolide acetate)(Lupron®)。用于治疗前列腺癌的抗增殖剂包括5-氟尿嘧啶。

[0243] 乳腺癌

[0244] 在美国，乳腺癌已成为女性当中最常见的癌症，其中每年诊断约180,000个新病例(男性乳腺癌占全部经诊断乳腺癌的约5％)。乳腺癌已成为女性的仅次于肺癌的致死原因，且其已引起每年大约50,000起死亡。美国女人在其寿命期间具有1/8(或约13％)的罹患乳

癌机率。在过去十年间，报导最多的乳腺癌为小、原发性(独立地产生；并非由转移瘤所引起)肿瘤。大致70％至80％的新诊断患者展现早期疾病(1期或2期)，且大部分尚未涉及腋下(手臂下方)淋巴结。

[0245] 大部分乳腺癌为癌(即，从上皮组织中生长出的恶性肿瘤)。小于1％的乳腺癌为肉瘤或由结缔组织、骨骼、肌肉或脂肪产生的肿瘤。此外，大部分乳腺癌(约75％)为导管癌，在与乳导管并排的组织中产生。更小量的癌症(约7％)在乳小叶内发现且被称作小叶癌。佩吉特病(Paget's disease)(乳晕及乳头癌)及炎性瘤占几乎全部其他形式的乳癌。

[0246] 乳腺癌治疗为复杂的且取决于多种因素。两个重要因素为肿瘤类型和进展阶段。尤其，肿瘤特征帮助将个体分为两个组：(1)处于癌症复发低风险的个体及(2)处于癌症复发高风险的个体。特定预后因素将患者置放在这些组中的任一者中。这些因素包括肿瘤大小；女性性激素雌激素及孕酮(ER/PR)受体的存在；细胞生长周期阶段(肿瘤细胞是否有效地分裂或处于“S阶段”)；被称为“her-2-neu”蛋白的蛋白的存在；肿瘤级别、肿瘤细胞分化或改变的指示物；及肿瘤倍性，肿瘤细胞内的基因物质的集合数目。

[0247] 已通过肿块切除术及放射疗法来治疗无明显淋巴结涉及的原发性疾病。更明显的淋巴结涉及可保证乳房切除术及去除辅助淋巴结。在此阶段，癌转移及局部复发的机率已经较高。涉及放射疗法及化疗的转移性疾病的治疗只是缓解性的，其为免疫抑止、细胞毒性及白血球减少。包括例如，5-氟尿嘧啶、阿霉素、甲胺喋呤及紫杉醇的抗增殖剂已被批准用于抗乳腺癌。

[0248] 胰腺癌

[0249] 胰腺为位于接近胃及小肠的消化系统的器官。其具有两个主要功能：产生酶和激素。胰腺癌可能出现在外分泌(即，酶)胰腺(例如，典型胰腺腺癌)中或可能出现在内分泌(即，激素)胰腺中。

[0250] 外分泌胰腺癌是极严重的健康问题。在美国，大约28,000名患者经诊断患有胰腺癌，同时每年约相同数目死于该疾病。胰腺癌同等地出现在男性及女性中。由于诊断困难、胰腺癌的固有侵袭性本质及稀少的可用全身性治疗选项，在诊断后，仅大约4％的经诊断患有胰腺癌的患者存活5年。胰腺癌已成为继乳腺癌、肺癌、结肠癌及前列腺癌之后的第5个癌症死亡的主要原因。

[0251] 对于胰腺癌的治疗选择很大程度上取决于肿瘤阶段。可能的治疗包括外科手术、抗增殖剂、放射及生物疗法。外科手术已通常保留用于其癌症视为可切除的1期患者。有时，在手术之前或之后给予疗法(诸如放射及抗增殖剂)的组合可以增大患者的存活机率。可以在临床试验中使用抗增殖剂治疗被视为不可切除的胰腺癌(通常II期或晚期)。诸如例如，吉西他滨或5-氟尿嘧啶的抗增殖剂对胰腺癌具有一些效果且吉西他滨已用作姑息性药剂。本文中其他地方论述因这些抗增殖剂所致的毒性。放射疗法在与化疗组合使用时对胰腺癌具有一些效果。仅放射疗法可以抑制症状。这种治疗形式也已经用于II期或晚期胰腺癌。

[0252] 膀胱癌

[0253] 在1998年，在美国估计将诊断出超过54,000个新的膀胱癌病例且约15,000例死亡将归因于该疾病。膀胱癌已成为美国男性中第四种最常见癌症且在美国女性中为第九种最常见癌症。膀胱癌在男性中出现的频率为女性的三倍。主要地，年长男性疾病中，膀胱癌已成为显著疾病及死亡原因。膀胱癌风险随着年龄增长而大幅度增加(80％病例在大于50岁

的人群中出现)，其中超过二分的一的全部膀胱癌死亡在70岁后出现。在超过65岁的白人男性中，膀胱癌的年疾病率已为每1,000个人中有大约2个病例；这与65岁以下的每1,000个人中有0.1个病例的比率形成对比。在个人寿命期间，罹患膀胱癌的机率已大于3％；然而，膀胱癌的死亡机率较小(＜1％)。膀胱癌罕见地出现在小于40岁的人群中。

[0254] 近期研究表明某些基因及遗传性代谢能力可能在膀胱癌中起作用。移行细胞癌(TCC)已为膀胱癌的最常见形式。TCC通常以浅表(表面)、乳头状(疣状)、茎状基底上的外生性(向外成长)块的形式出现。但在一些情况下，TCC可附接于宽广基底上或其可呈现溃疡(在凹痕式病变内)。乳头状TCC通常从增殖区域开始，随后去分化或失去个别细胞特征。仅约10％至30％的乳头状TCC发展成浸润性癌症。相比之下，非乳头状形式的TCC更可能变成浸润性的。如所述，此类TCC可能呈现溃疡或平整的。由退行性上皮组成的平整、非乳头状TCC已经被分类为原位癌(CIS或TIS)。CIS的组织包含大的具有明显核仁(细胞内的圆形体；涉及蛋白质合成)且缺少正常极性的细胞。

[0255] 膀胱癌的治疗取决于多种因素。这些因素中最重要的是呈现的肿瘤类型及其阶段。普遍治疗包括经尿道切除术(TUR)、电外科手术、激光手术、膀胱内治疗、抗增殖剂、外科手术疗法、膀胱切除术及放射疗法。用于治疗膀胱癌的抗增殖剂的实例包括例如5-氟尿嘧啶、顺铂及甲胺喋呤。因抗增殖剂、5-氟尿嘧啶、顺铂及甲胺喋呤所致的毒性在本文中其他地方论述。

[0256] 脑癌

[0257] 脑瘤经常为不可手术的且多于80％的患者在诊断后12个月内死亡。在美国，每年诊断出大约18,000例原发性颅内(脑)癌新病例。这相当于全部成年人癌症的2％。多于50％的这些癌症为高度神经胶质瘤(即，多形性胶质母细胞瘤及间变性星形细胞瘤肿瘤)。患有这些肿瘤的患者通常遭受严重残疾，诸如运动屏障、癫痫及视觉异常。

[0258] 在脑组织中开始的肿瘤被称为原发性脑瘤。原发性脑瘤通过其开始的组织类型进行分类。最常见脑瘤为开始于胶质(支持性)组织中的神经胶质瘤。其他的脑瘤包括星形细胞瘤、脑干神经胶质瘤、室管膜瘤及少突神经胶质瘤。

[0259] 已针对大部分类型及大部分位置建议外科手术去除脑瘤且应尽可能在保留神经功能的限制内完成。此规则的例外为深部肿瘤，诸如桥脑胶质瘤，其根据临床证据诊断且大约有50％的时间无需进行初次外科手术即可接受治疗。然而，在许多情况下，执行活组织检查诊断。立体定向的活组织检查可用于难以到达及切除的病变。患有脑瘤的罕见地可治愈或不可切除的患者应被视为用于评估辐射增敏剂、热疗或间质性近距离放射治疗结合外部辐射束放射疗法使用以改善肿瘤的局部控制的临床试验或用于评估新药物及生物反应调节剂的研究的候选者。

[0260] 放射疗法在治疗大部分肿瘤类型中具有主要作用且可增大治愈率或延长无病生存期。放射疗法还可以适用于治疗最初仅通过外科手术治疗的患者的复发。可以在外科手术及放射疗法之前、期间或之后使用化疗。还通过化疗来治疗复发性肿瘤。用于治疗脑癌的抗增殖剂包括顺铂。与这种抗增殖剂相关联的毒性的实例在本文中其他地方论述。

[0261] 再狭窄

[0262] 再狭窄为引起血管壁增厚和丧失由血管供应至组织的血流的慢性血管损伤形式。此炎性疾病可以响应于包括减轻血管梗阻的任何手术的血管重建手术而出现。因此，再狭

窄已成为限制这些手术的有效性的主要限定性因素。

[0263] 本发明论述包括例如通过向血管给药治疗有效量的寡核苷酸治疗剂与抗炎剂的组合来预防或治疗再狭窄。适合的组合物包括可以以外科手术方式植入再狭窄部位或潜在再狭窄部位处或可以以聚合性糊剂或凝胶形式经由导管注射的聚合性载体。适合的组合物可以包括本文中所论述的高分子量褐藻糖胶。

[0264] 关节炎

[0265] 类风湿性关节炎(RA)为衰弱的慢性炎性疾病,其特征为关节组织的疼痛、肿胀、滑膜细胞增殖(血管翳形成)及破坏。在晚期,该疾病经常损害关键器官且可致命。该疾病涉及免疫系统(巨噬细胞/单核细胞、中性粒细胞、B细胞及T细胞)复杂的细胞介素相互作用及滑膜细胞功能失常及增殖的多个成员。已推荐用疾病修饰抗风湿药物(DMARD),诸如甲胺喋呤用于早期侵袭性治疗,该药物在本文中其他地方论述。

[0266] 结晶诱发的关节炎的特征为关节中结晶诱发的巨噬细胞及中性粒细胞活化且之后许多天伴随剧烈疼痛。疾病进展使得发作间隔变得更短且患者的发病率增加。此疾病总体上已通过非类固醇抗炎药(NSAID),诸如双氯芬酸钠(Voltaren®)对症治疗。此抗炎剂具有毒性,包括中枢神经系统毒性,诸如眩晕及头痛;皮肤病学毒性,诸如皮疹及瘙痒;胃肠毒性,诸如加重的溃疡性结肠炎及克罗恩病;泌尿生殖毒性,诸如急性肾衰竭及肾乳头坏死;血液毒性,诸如粒细胞缺乏、白血球减少及血小板减少;肝毒性,诸如升高的肝转胺酶及肝炎;及其他毒性,诸如哮喘及过敏反应。

[0267] 本发明论述包括例如经由向患者给药治疗有效量的寡核苷酸治疗剂及可选的抗炎剂来预防或治疗类风湿性关节炎。适合的组合物包括聚合性载体,其可以以抗炎剂及微颗粒的受控释放载体形式以寡核苷酸治疗剂的受控释放载体形式(其反过来已经并入于聚合性载体中)注射至关节中。适合的组合物可以包括本文中所论述的高分子量褐藻糖胶。所述聚合性载体可以采取聚合物微球体、糊剂或凝胶的形式。

[0268] 炎症病症

[0269] 本文中的组合物等可选地抑制或治疗涉及嗜中性白细胞的炎性病症,例如包含向患者给药包括寡核苷酸治疗剂及抗炎剂的组合物。所述病症的实例包括结晶诱发的关节炎;骨关节炎;非类风湿性炎性关节炎;混合结缔组织疾病;干燥综合征;强直性脊柱炎;贝赫切特综合征;结节病;银屑病;湿疹;炎性肠病;慢性炎性肺病;神经屏障及多发性硬化。在以下段落中进一步论述这些疾病中的一些。

[0270] 慢性炎性皮肤病(包括牛皮癣和湿疹)

[0271] 牛皮癣为常见的慢性炎性皮肤病,其特征为发痒、灼热、蜇伤且易于出血的突起的、增厚的及鳞片状病变。当这些疾病在疾病的晚期具有细胞增殖及血管生成组分时,患者通常具有伴随的关节炎病症。症状可以通过诸如泼尼松的类固醇抗炎剂或诸如甲胺喋呤的抗增殖剂来治疗,这些药剂在本文的其他地方论述。本文中的组合物还可以用于抑制或以其他方式治疗和/或预防慢性炎性皮肤病,例如银屑病和/或湿疹。

[0272] 以下提供可以通过本文中所论述的组合物治疗的炎性疾病的一些额外代表性实例,包括例如动静脉畸形(血管畸形)的动脉栓塞;月经过多;急性出血;中枢神经系统屏障及脾功能亢进;炎性皮肤病,诸如银屑病;湿疹疾病(异位性皮炎、接触性皮炎、湿疹);免疫大疱疾病;及包括各种病症的炎性关节炎,所述病症包括类风湿性关节炎、混合结缔组织疾

病、干燥综合征、强直性脊柱炎、贝赫切特综合征、结节病、结晶诱发的关节炎及骨关节炎(以上所有特征以发炎的疼痛关节为重要症状)。

[0273] 缺血

[0274] 缺血或局部缺血涉及血液供应限制,可以包括恰当组织功能所需的氧、葡萄糖及其他组分供应不足,使得组织损坏和/或功能不全。缺血可能导致严重问题。例如,组织可能变得缺氧、坏死且可形成结块。已作出各种尝试以预防和/或治疗缺血。例如,复原血流或再灌注。然而,复原血液涉及再引入氧,其可能导致因产生自由基所致的额外损坏,进而导致再灌注损伤。再灌注损伤可能导致严重问题。本文的组合物可以用于抑制或以其他方式治疗和/或预防缺血和/或再灌注损伤。

[0275] 内毒素血症

[0276] 内毒素血症是在血液中存在内毒素。内毒素血症可能导致严重问题。例如,内毒素血症可能引起败血症休克。本文中的组合物可以用于抑制或以其他方式治疗和/或预防内毒素血症。

[0277] 瘢痕疙瘩瘢痕

[0278] 瘢痕疙瘩特质致使伤口通过突起的瘢痕愈合。瘢痕疙瘩特质的突起瘢痕涉及异常纤维性瘢痕。瘢痕疙瘩特质导致严重问题,例如疼痛及外形损伤。本文中的组合物可用于抑制或以其他方式治疗和/或预防瘢痕疙瘩特质及其引起的突起的瘢痕。

[0279] 瘢痕疙瘩(瘢痕疙瘩瘢痕)是生长扩增超过正常皮肤的瘢痕类型。瘢痕疙瘩涉及异常胶原生长,包括I型及III型胶原异常生长。瘢痕疙瘩导致严重问题,例如疼痛、瘙痒,并且若经感染,则可能溃烂。已作出尝试以治疗或预防瘢痕疙瘩,包括使用外科手术、敷料、类固醇注射及激光疗法。本文中的组合物可以用于抑制或以其他方式治疗和/或预防瘢痕疙瘩。

[0280] 皮炎

[0281] 皮炎包括皮肤炎症,所属皮肤炎症包括特应性皮炎及接触性皮炎。例如,接触性皮炎涉及在皮肤与外来物质接触的后皮肤的局部皮疹和/或刺激。例如,特应性皮炎为长期复发性、瘙痒皮肤病。特应皮炎有时被称作贝尼埃氏痒疹(prurigo Besnier)、神经性皮炎、内源性湿疹、曲湿疹、婴儿湿疹、儿童湿疹及痒疹病(prurigo diathsique)。湿疹为呈皮炎形式的疾病。其他类型的皮炎包括海绵性皮炎、脂溢性皮炎(皮屑)、出汗屏障性皮炎(汗疱疹)、荨麻疹、泡状皮炎(大疱性皮炎)及流行性风疹。皮炎可能导致严重问题。例如,干燥皮肤、皮肤皮疹、皮肤水肿、皮肤发红、皮肤瘙痒、皮肤结痂、开裂、起泡、渗泌及出血。已作出尝试以治疗或预防皮炎,包括使用皮质类固醇及煤焦油。本文中的组合物可以用于抑制或以其他方式治疗和/或预防皮炎,包括特应性皮炎、湿疹、接触性皮炎、海绵性皮炎、脂溢性皮炎、出汗屏障性皮炎、荨麻疹、泡状皮炎及流行性荨麻疹。

[0282] 红斑痤疮

[0283] 红斑痤疮为典型地特征化为面部红斑的慢性疾病或病症。红斑痤疮可能导致严重问题。例如,红斑痤疮通常从额头、鼻子或脸颊发红开始,还可能导致脖子、耳朵、头皮和胸部发红。红斑痤疮可能导致包括毛细管扩张、丘疹、脓包、疼痛感觉的额外症状,且在晚期病例中,可能产生肥大性酒渣鼻(红色分叶鼻)。红斑痤疮亚型包括红斑狼疮样红斑痤疮、脓包性丘疹样红斑痤疮、结块性红斑痤疮及眼部红斑痤疮。已作出尝试以治疗或预防红斑痤疮,包括使用非类固醇抗炎药及抗生素。本文中的组合物可以用于抑制或以其他方式治疗和/

或预防红斑痤疮，包括其红斑狼疮、脓包性丘疹、红斑痤疮及眼部亚型。

[0284] 医疗器械、医疗材料、组合和药物产品

[0285] 本文的论述还提供医疗器械、医疗材料、组合及药物产品，其包括在医疗器械、医疗材料、组合产品或药学上可接受的容器中的如本文所论述的组合物。该产品还可包括与容器相关联的注意事项，典型地以由监察医疗器械、医疗材料、组合及药剂或生物药剂的制造、使用或销售的管理机构规定的形式，从而该注意事项反映该组合物经该机构批准，诸如高分子量褐藻糖胶已经批准作为例如用于人类或兽医给药以治疗增殖性疾病或炎性疾病(例如炎性关节炎、再狭窄、外科手术粘连、银屑病及腹膜炎)的抗增殖剂或抗炎剂的注意事项。还可以包括使用本文中的高分子量褐藻糖胶的说明书。所述说明书可以包括关于患者的给药及投药模式的信息。

[0286] 本申请进一步涉及制备本文讨论的高分子量褐藻糖胶，系统等的各种元件的方法，包括制备组合物本身，以及使用该组合物的方法，包括本文中对状况，疾病等的示例性治疗。

[0287] 本申请进一步包括用于治疗纤维性粘连、关节炎、银屑病或视需要的其他疾病的医疗器械、医疗材料、药物组合产品及药物产品，其包括本文中呈现的高分子量褐藻糖胶及高分子量褐藻糖胶组合物。所述材料等可以用于治疗纤维性粘连，诸如外科手术粘连、关节炎、银屑病或视需要的其他疾病的药物品中。还提供制造且使用能够减少与患者，包括人类患者体内的纤维性粘连、关节炎及银屑病中的至少一种相关联的症状的所述药物的方法，所述方法包括将药学上有效量的褐藻糖胶，诸如如本文所论述的岩藻多糖与药学上可接受的赋形剂或缓冲剂组合。

[0288] 以下实施例提供了本文中某些实施方式的示例性讨论，但是本公开和权利要求书不限于此。

[0289] 实施例1：化学结构修饰

[0290] 渗出物-提取物获自极北海带(Laminaria Hyperborea)。渗出物-提取物通过切向流过滤(TFF)通过100kDa过滤器来过滤并去除小分子。将所得渗余物的样品冻干以获得以其他方式未修饰的样品A。通过添加10M NaOH溶液且在室温下静置16小时使所得渗余物达至0.25M NaOH。然后将所得样品通过50kDa过滤器离心过滤且收集所得渗余物并冻干以获得经碱处理的样品B。通过质子核磁共振光谱法(^{1}H-NMR)分析未修饰的样品A及经碱处理的样品B两者且图9A中示出所得^{1}H-NMR光谱。

[0291] 图9A证明了已完成的褐藻糖胶的化学结构修饰：存在于未修饰的样品A中的具有约2.0ppm化学位移的较宽峰并不存在于经碱处理的样品B中。

[0292] 通过2D ^{1}H-^{13}C异核多量子相干(HMQC)进一步分析未修饰的样品A及经碱处理/修饰的样品B。在70℃下伴随溶剂信号抑制在配备有5-mm冷探针的600MHz光谱仪上获得图9B中示出的HMQC谱。在碳尺寸10-30ppm范围内以256-512的8次增量的扫描每次获得HMQC谱的大量扫描；这样的扫描经组合以产生图9B中的光谱。

[0293] 未修饰的样品A的HMQC谱具有对应于O-乙酰基的交叉峰，由图9B中的带圆圈信号指示。此交叉峰不存在于经碱处理的样品B的光谱中。这证明了从褐藻糖胶中去除乙酰基，且因此通过NaOH处理来对经碱处理的样品B中的褐藻糖胶进行化学结构修饰。

[0294] 实施例2：切向流过滤

[0295] 可以通过切向流过滤获得高分子量的褐藻糖胶。将宽分布起始褐藻糖胶以50mg/mL的浓度溶于蒸馏水中。在此实施例中，在100kDa截留分子量(MWCO)切向流过滤器(TFF)盒上，对4透析体积的宽分布褐藻糖胶进行了蒸馏水渗滤，以去除不需要的较低分子量组分，并收集了TFF过程的截留物，其包含高分子量的褐藻糖胶。渗滤可以用任何所需的MWCO TFF滤器完成，例如50kDa、70kDa、100kDa、300kDa、500kDa和1000kDa MWCO TFF盒。所得的高分子量褐藻糖胶具有比宽分子量分布起始褐藻糖胶更高的平均分子量。

[0296] 实施例3:顺序切向流过滤分段

[0297] 提供了输入宽分子量分布起始岩藻多糖，其平均分子量为365.6kDa，并且多分散指数(PDI)＝3.58，其已通过0.22微米过滤器被预过滤。将华盛顿港的Pall提供的100kDa MWCO的TFF过滤器盒用作较高MWCO TFF盒，并将华盛顿港的Pall提供的50kDa的TFF盒用作较低MWCO TFF盒。对以下TFF盒对重复该过程:由马萨诸塞州伯灵顿的Millipore提供的MWCO 300kDa的TFF过滤器和由华盛顿港的Pall提供的MWCO 100kDa的TFF过滤器，由华盛顿港的Pall提供的MWCO 50kDa的TFF过滤器和由华盛顿港的Pall提供的30kDa的过滤器，由华盛顿港的Pall提供的30kDa的TFF过滤器和由华盛顿港的Pall提供的10kDa的TFF过滤器。盒件均为聚醚砜(PES)类型。

[0298] 在如上所述的顺序切向流过滤之后，使用凝胶渗透色谱法(GPC)分析了各种获得的褐藻糖胶，包括包含起始褐藻糖胶分子量分布的高分子量区段的高分子量褐藻糖胶。结果显示在下表1中。

[0299]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	25.51	299.6	365.6	102.2	76.3	57.2	23.1	3.58
TFF 过滤器对的 MWCO (kDa)								
300-100	25.62	278.3	394.2	151.9	83.5	63.3	24.9	2.60
100-50	27.96	59.8	125.1	42.6	37.3	17.4	3.3	2.94

[0300]

50-30	30.33	12.6	20.6	9.8	1.6	0.3	0.0	2.11
30-10	34.22	1.0	2.1	1.2	--	--	--	1.66

[0301] 表1.岩藻多糖的TFF分段

[0302] 实施例4:阳离子增强的切向流过滤

[0303] 提供了起始溶液形式的宽分子量分布输入起始岩藻多糖组合物,其已通过0.22微米过滤器预过滤,具有的重均分子量为436.4kDa且多分散指数(PDI)为3.24。生物相容性水溶性季铵盐胆碱被选作化学添加剂。将胆碱以1:2的胆碱:岩藻多糖质量比加入到预过滤的起始溶液中,并将所得混合物搅拌直至胆碱溶解。胆碱可以或可以不结合到岩藻多糖分子上的硫酸酯位点。在第一TFF过程中,然后将经胆碱处理的岩藻多糖溶液在300kDa的过滤器盒上进行切向流过滤,以获得包含胆碱结合的高分子量岩藻多糖的第一截留物,其为经胆碱处理的截留物。在该第一胆碱增强的TFF过程中,将经胆碱处理的岩藻多糖溶液用4透析体积的1%w/v胆碱冲洗溶液的进行渗滤。收集第一TFF过程的经胆碱处理的截留物,并进行第二TFF过程,以用钠阳离子代替胆碱阳离子。

[0304] 第二TFF过程是脱胆碱的TFF过程,包括在50kDa过滤器盒上渗滤第一TFF过程的经胆碱处理的截留物,同时用NaCl处理截留物以用钠阳离子代替胆碱阳离子。在该实施例中,用4体积的2M NaCl渗滤经胆碱处理的截留物,以从高分子量岩藻多糖中去除胆碱添加剂。然后,用去离子水对第二TFF过程的脱胆碱截留物进行渗滤,直到渗透物的电导率降至5mS/cm以下,以表明已去除过量的NaCl。在如上所述的阳离子增强的TFF之后,使用凝胶渗透色谱法(GPC)分析包括高分子量褐藻糖胶的方法中的各种截留物的样品。

[0305] 结果示于下表2。

[0306]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	24.67	436.4	490.7	151.3	82.3	66.1	34.8	3.24
TFF 过滤器截留物的 MWCO (kDa)								
300	24.71	423.6	525.5	206.7	88.5	72.6	37.3	2.54
100	24.10	639.8	740.7	411.0	97.9	90.6	59.1	1.80

[0307] 表2.岩藻多糖的阳离子增强的TFF分段

[0308] 实施例5:离心沉淀

[0309] 提供了包含0.5%w/v的起始褐藻糖胶组合物的起始溶液,该组合物已经通过0.22μm的预过滤器进行了预过滤。在离心管中在水中形成20%、10%和5%w/v蔗糖的逐步梯度,其中5%的层是最顶层,而20%的层在离心管的底部。然后将包含起始岩藻多糖组合物的0.5%起始溶液分层在5%w/v蔗糖层上。所得的层结构在图10中示出。然后将具有这四层的试管在190,000重力(g)下离心6小时。倒出上清液,将残留在离心管中的沉淀物重新溶解在水中,该沉淀物中含有所需的高分子量褐藻糖胶。然后通过凝胶渗透色谱法(GPC)分析重新溶解的高分子量褐藻糖胶。结果示于下表3。

[0310]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	24.57	471.7	590.1	200.6	87.4	72.3	40.3	2.94
重新溶解的岩藻多糖沉淀物	22.95	1472.9	1113.0	492.3	98.2	91.0	69.1	2.26

[0311] 表3.使用5%-10%-20%蔗糖屏障的岩藻多糖的离心沉淀物

[0312] 实施例6:凝胶电泳提取

[0313] 提供了具有宽分子量分布的起始岩藻多糖组合物。将起始岩藻多糖组合物以50mg/mL溶解,通过0.22微米过滤器预过滤,并加载到由380mL琼脂糖铸成的0.5%琼脂糖凝胶上。将加载的凝胶浸入40mM三乙酸1mM EDTA的运行缓冲液中,该缓冲液也称为TAE缓冲液。将90V电压施加在缓冲液上50分钟,使阳极靠近起始岩藻多糖组合物孔。这使得岩藻多糖通过凝胶按质荷比分离。为了可视化,用亚甲基蓝对凝胶进行染色,亚甲基蓝是一种已知可以对岩藻多糖染色的染料。然后从孔开始1cm将琼脂糖凝胶切成与孔平行的1cm宽区段。将凝胶的区段在蒸馏水中搅拌,以通过摇动混合物从凝胶中提取岩藻多糖区段。

[0314] 通过凝胶渗透色谱法(GPC)分析了电泳提取的岩藻多糖区段,其可能包含高分子量岩藻多糖。结果显示在下表4中。

[0315]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	24.67	462.6	581.3	170.9	86.7	73.0	40.8	3.40
凝胶区段距孔的距离								
1-2 cm	25.07	349.4	619.9	81.33	71.3	55.9	30.7	7.62
2-3 cm	25.16	327.8	362.0	118.2	76.2	56.7	23.9	3.06
3-4 cm	25.47	263.6	288.4	103.7	70.9	48.8	16.7	2.78
4-5 cm	25.58	242.7	279.1	66.0	62.8	42.2	14.9	4.23

[0316]

[0317] 表4.使用TAE缓冲液在琼脂糖凝胶上通过预过滤的起始岩藻多糖进行电泳分离的GPC结果。

[0318] 实施例7:膜透析

[0319] 提供了包含已通过0.22微米过滤器预过滤的起始岩藻多糖组合物的5%w/v的起始溶液。将起始溶液置于标称截留分子量为300kDa的乙酸纤维素透析管中。将透析管密封并放置在装有20升去离子水的容器中。每12小时用新鲜的去离子水替换去离子水,以确保在膜孔中连续扩散。允许透析过程持续约5天。

[0320] 使用凝胶渗透色谱法(GPC)分析透析管中的预过滤的起始岩藻多糖组合物和透析后的高分子量岩藻多糖。结果示于下表5。

[0321]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	24.57	471.7	590.1	200.6	87.4	72.4	40.3	2.94
透析的岩藻多糖	24.29	599.1	777.0	374.5	96.8	88.6	57.0	2.07

[0322]

[0323] 表5.岩藻多糖在300kDa膜上相对于去离子透析的GPC结果。

[0324] 实施例8:选择性沉淀

[0325] 提供了起始岩藻多糖组合物,其已经通过0.22μm的预过滤器进行了预过滤,并且通过在100kDa TFF盒上用去离子水进行渗滤以进行脱盐以去除可能干扰沉淀过程的不想要的低分子量盐。准备了一系列相同的起始褐藻糖胶在蒸馏水中的预过滤和脱盐起始溶液。使溶剂组合物达到不同的乙醇预定浓度。这为从下表6所示的溶液组合物中沉淀出岩藻多糖制备了不同的溶剂环境。将最少量的NaCl形式的离子剂添加到每种溶液组合物中,以引发岩藻多糖从溶液中的沉淀。将沉淀物和溶液组合物的混合物在2300重力下离心10分钟。分别倾析出液体上清液,并收集固体岩藻多糖。

[0326] 将固体岩藻多糖重新溶解于蒸馏水中,并通过凝胶渗透色谱法进行分析。结果显示在下表6中。

[0327]

%岩藻多	GPC	PMW	WAMW	NAMW	% 分	% 分	% 分	PDI

[0328]

糖溶液中的乙醇	PRT (分钟)	(kDa)	(kDa)	(kDa)	布 MW> 100 kDa	布 MW> 200 kDa	布 MW> 500 kDa	
40	24.85	394.1	447.0	133.5	76.6	62.5	31.3	3.35
50	25.96	182.1	347.9	149.2	80.8	53.9	20.3	2.33
60	25.43	263.1	335.5	119.1	73.9	51.9	20.0	2.82
70	24.91	376.1	382.6	117.2	75.7	56.8	25.4	3.26

[0329] 表6.使用乙醇作为沉淀溶剂对岩藻多糖的选择性沉淀

[0330] 实施例9:阴离子吸附

[0331] 将包含约500mg的宽分子量分布的脱盐的岩藻多糖的起始溶液在约14mL的DEAE-Sepharose®树脂上再循环约16小时,以使低分子量岩藻多糖与树脂上的活性位点结合。约16小时后,收集再循环溶液。这将高分子量岩藻多糖与已经与树脂键合的低分子量岩藻多糖分离。然后将10%w/v的NaCl在树脂上再循环4小时以从树脂上置换低分子量岩藻多糖。然后收集富含岩藻多糖的盐溶液,并通过5kDa离心过滤器脱盐,以将收集的低分子量岩藻多糖与不需要的盐分离。对脱盐的起始岩藻多糖、在离子交换过程中未吸附的高分子量岩藻多糖和从树脂中提取的低分子量岩藻多糖进行GPC。结果显示在下表7中。

[0332]

	GPC	PMW	WAMW	NAMW	% 分	% 分布	% 分布	PDI

[0333]

	PRT (分钟)	(kDa)	(kDa)	(kDa)	布 MW> 100 kDa	MW> 200 kDa	MW> 500 kDa	
输入	24.57	462.4	576.6	198.0	87.1	71.9	39.6	2.91
未吸附的岩藻多糖	24.20	601.3	844.5	391.4	96.8	90.9	60.5	2.16
吸附的岩藻多糖	25.82	193.6	245.8	119.2	73.0	43.8	10.7	2.06

[0334] 表7.岩藻多糖的阴离子交换区段:DEAE-Sepharose作为树脂

[0335] 实施例10:阴离子吸附

[0336] 将含有约1g的宽分子量分布的脱盐岩藻多糖的起始溶液与约10g的 Amberlyst® A26树脂混合约16小时,以使低分子量岩藻多糖与树脂上的活性位点结合。随后通过倾析将包含高分子量岩藻多糖的溶液与树脂分离。然后将20%w/v的NaCl与树脂混合约4小时以从树脂上置换低分子量岩藻多糖。然后将富含岩藻多糖的盐溶液与树脂分离,并通过5kDa离心过滤器脱盐,以将收集的低分子量岩藻多糖与不需要的盐分离。对脱盐的起始岩藻多糖,在离子交换过程中未吸附的高分子量岩藻多糖和从树脂中提取的低分子量岩藻多糖进行GPC。结果显示在下表8中。

[0337]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	25.02	517.7	536.9	148.2	82.7	67.7	38.1	3.62
未吸附的岩藻多糖	24.73	625.8	867.4	463.1	98.7	93.0	62.6	1.87
吸附的岩藻多糖	27.30	112.4	172.3	86.4	58.0	25.4	4.7	2.00

[0338] 表8.岩藻多糖的阴离子交换分离:Amberlyst™ A26 OH

[0339] 实施例11:阴离子吸附

[0340] 将含有约1g的宽分子量分布的脱盐岩藻多糖的起始溶液与约10g的三种不同的树脂在三个分开的容器中混合,分别是Amberlyst®A26OH⁻、Ambersep®900OH⁻和Lewatit® VPOC 1065。将溶液-树脂混合物温育约16小时,以使低分子量岩藻多糖与树脂上的活性位点结合。随后通过倾析将包含高分子量岩藻多糖的溶液与树脂分离。Amberlyst®和Ambersep®产品的孔具有季胺基,而Lewatit产品的孔具有伯苄胺基。前两种产品是强碱性阴离子交换树脂,而第三种是弱碱性阴离子交换树脂。然后通过GPC分析在离子交换过程中未吸附的岩藻多糖。结果显示在下表9中。

[0341]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	24.66	461.9	521.5	151.8	83.0	67.3	36.5	3.44
使用的树脂								
Ambersep® 900 OH^-	24.51	513.0	609.1	323.6	96.1	85.5	47.3	1.88
Amberlyst® A26 OH^-	24.58	489.8	591.0	309.5	95.6	83.5	45.1	1.91
Lewatit®VPOC 1065	24.54	501.2	585.6	219.6	89.5	75.3	42.4	2.67

[0342] 表9.岩藻多糖的阴离子交换分离:通过在3种树脂上再循环制备的岩藻多糖的比较。

[0343] 实施例12:阴离子吸附

[0344] 将包含约1g的宽分子量分布的脱盐岩藻多糖的起始溶液与约10g的 Ambersep® 900OH^-混合达53小时。然后,在阴离子吸附过程中的各个时间点,通过GPC对离子交换过程中未吸附的混合物中的岩藻多糖进行分析。结果显示在下表10中。

[0345]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	26.71	624.2	955.9	339.9	95.2	84.6	56.3	2.81
离子交换时间(小时)								
1	26.66	642.4	1049.2	386.0	96.6	87.3	59.5	2.72
4	26.42	756.4	1151.7	470.9	98.2	91.7	65.5	2.45
24	26.33	801.9	1205.2	589.9	99.5	95.9	72.1	2.04
53	26.25	843.6	1257.9	656.9	99.8	97.5	75.6	1.91

[0346] 表10.岩藻多糖的阴离子交换分离:阴离子交换时间的比较

[0347] 实施例13:阴离子吸附

[0348] 将包含约1g的宽分子量分布的脱盐岩藻多糖的起始溶液与各种量的Ambersep® 900OH⁻混合约16小时,以使低分子量岩藻多糖与树脂上的活性位点结合。随后通过倾析将包含高分子量岩藻多糖的溶液与树脂分离。然后通过GPC分析在离子交换过程中未吸附的岩藻多糖。结果示于下表11。

[0349]

	GPC PRT (分钟)	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 MW> 100 kDa	% 分布 MW> 200 kDa	% 分布 MW> 500 kDa	PDI
输入	24.66	442.4	498.5	146.3	82.4	66.1	34.8	3.41
岩藻多糖:树脂的质量比								
1:1	24.51	491.1	548.1	203.2	87.6	72.0	39.0	2.70
1:5	24.48	500.9	633.9	306.6	95.3	82.9	46.6	2.07
1:10	24.41	523.8	688.1	376.4	98.0	88.8	51.9	1.83

[0350] 表11.岩藻多糖的阴离子交换分离:不同的岩藻多糖与树脂比率的比较

[0351] 实施例14:制备型凝胶渗透色谱法

[0352] 提供具有宽分子量分布的起始岩藻多糖组合物。将起始岩藻多糖组合物以10mg/mL的浓度溶于60mL的0.1M硝酸钠中。将含有起始岩藻多糖组合物的20mL起始溶液以40mL/min的速度泵入内径分别为50mm、长度为250mm的含有Sepax®SRT-10/10C SEC-1000、Agilent®PL Aquagel®-OH MIXED-H和TSKGel® G4000SW的色谱柱中,其均包含修饰的硅胶亲水键合相凝胶介质。使用0.1M硝酸钠以相同的流速进行洗脱。洗脱5分钟后,收集40mL等分部分,直到总共收集了1000mL或25等分部分。通过分析型GPC测量每个等分部分的分子量分布。合并重均分子量在200kDa和600kDa之间的等分部分。合并重均分子量在600kDa和1000kDa之间的等分部分。合并重均分子量在1000kDa和1400kDa之间的等分部分。合并重均分子量在1400kDa和1800kDa之间的等分部分。其余的等分部分均被丢弃。每种合并的制备GPC等分部分组合物均包含所需的高分子量褐藻糖胶。

[0353] 实施例15:制备型凝胶渗透色谱

[0354] 提供了具有宽分子量分布的起始岩藻多糖组合物。将起始岩藻多糖组合物以10mg/mL的浓度溶于60mL 0.1M的硝酸钠中。将含有起始岩藻多糖组合物的20mL起始溶液以40mL/min的速度泵入内径分别为50mm、长度为250mm的含有Waters®HSPgel AQ MB-H、PSS® Suprema® Combination Ultrahigh和TSKGel® GMPWXL的色谱柱中,其均包含羟基

化聚甲基丙烯酸酯基凝胶介质。使用0.1M硝酸钠以相同的流速进行洗脱。洗脱5分钟后，收集40mL等分部分，直到总共收集了1000mL或25等分部分。通过分析型GPC测量每个等分部分的分子量分布。合并包含其分子量分布的至少90％大于100kDa、其分子量分布的至少80％大于200kDa和/或其分子量分布的至少50％大于500kDa的等分部分。其余的等分部分均被丢弃。每种合并的制备GPC等分部分组合物均包含所需的高分子量褐藻胶。

[0355] 实施例16：低和高分子量褐藻糖胶的制备

[0356] 本文所讨论的方法可以以任何方式使用、组合、修改和置换以获得高分子量的褐藻糖胶。

[0357] 从具有宽分子量分布的原料/起始褐藻糖胶组合物制备二十种具有高分子量和低分子量的褐藻糖胶，以评价高分子量和低分子量褐藻糖胶在医疗和外科手术应用中的功效。以下将这二十种褐藻糖胶称为褐藻糖胶1至褐藻糖胶20。褐藻糖胶1至褐藻糖胶5是浅棕色固体。褐藻糖胶6、褐藻糖胶8至褐藻糖胶15和褐藻糖胶17为白色固体。低分子量褐藻糖胶(即褐藻糖胶1制褐藻糖胶6)的制备涉及许多不同的方法学。从棕色海藻中提取了褐藻糖胶3，发现它是一种低分子量的褐藻糖胶。褐藻糖胶2获自FMC BioPolymer®，并发现是低分子量褐藻糖胶。通过实施例3中讨论的方法，使用MWCO TFF过滤器在100kDa下获得褐藻糖胶1和褐藻糖胶5。通过实施例10中讨论的方法获得褐藻糖胶4。通过用过氧化氢化学降解高分子量的褐藻糖胶获得褐藻糖胶6。

[0358] 高分子量褐藻糖胶(即褐藻糖胶7制褐藻糖胶20)的制备涉及许多不同的方法学，包括用氢氧化钠处理，在某些情况下还包括其他碱。褐藻糖胶7、褐藻糖胶8、褐藻糖胶11、褐藻糖胶12至褐藻糖胶17和褐藻糖胶20的制备涉及实施例12中讨论的方法与针对低离子强度溶液的切向流过滤的组合。褐藻糖胶10的制备涉及上述阳离子增强切向流过滤和顺序切向流过滤方法的组合。从棕色海藻中提取了褐藻糖胶9、褐藻糖胶18和褐藻糖胶19，然后通过对低离子强度溶液进行切向流过滤进行进一步处理，发现它们是高分子量褐藻糖胶。

[0359] 实施例17：用于制备褐藻糖胶7至褐藻糖胶14的粗制褐藻糖胶的分子量测定

[0360] 使用凝胶渗透色谱法来评估用于制备褐藻糖胶7至14的粗制褐藻糖胶的分子量分布。粗制褐藻糖胶1是指用于制备褐藻糖胶7和褐藻糖胶8的粗制褐藻糖胶。粗制褐藻糖胶2是指用来制造褐藻糖胶9、褐藻糖胶10、褐藻糖胶11和褐藻糖胶13的粗制褐藻糖胶。粗褐藻糖胶3是指用于制造褐藻糖胶12的粗褐藻糖胶。粗褐藻糖胶4是指用于制造褐藻糖胶14的粗褐藻糖胶。在表12中示出了这种分析的结果。

[0361] 下表中的结果包含用于分子量分布的某些特征的缩写。凝胶渗透色谱法用GPC表示，峰分子量用PMW表示，重均分子量用WAMW表示，数均分子量用NAMW表示，百分比分布用％分布表示，分子量用MW表示，多分散指数由PDI表示。

[0362]

	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	%分布<10 kDa	%分布<20 kDa	%分布<50 kDa	%分布>100 kDa	%分布>200 kDa	%分布>500 kDa	PDI
粗制褐藻糖胶1	92.0	259.3	22.1	8.3	14.7	30.4	52.3	34.7	14.5	11.7

[0363]

粗制褐藻糖胶2	512.3	535.3	128.5	0.5	2.1	8.9	80.4	65.1	36.6	4.2
粗制褐藻糖胶3	594.6	493.4	4.4	22.0	27.3	35.7	57.4	49.3	31.3	113.3
粗制褐藻糖胶4	662.5	790.6	245.4	0.1	0.5	3.4	90.9	80.2	52.0	3.2

[0364] 表12

[0365] 实施例18:低分子量和高分子量褐藻糖胶的分子量测定使用凝胶渗透色谱法来评估褐藻糖胶1至20的获得的分子量分布。

[0366] 表13和表14列出了针对二十种褐藻糖胶获得的分子量分布曲线。表14提供了与表13中所示相同的二十种褐藻糖胶的分子量分布曲线,并以与表13中所示方式不同的方式给出了分子量分布曲线,从而提供了各种褐藻糖胶分子量分布的两种不同观点。从结果可以看出,已经完成了在褐藻糖胶中的广泛范围的不同分子量分布。已经获得具有28kDa至8250kDa之间的重均分子量的具有多种分布曲线的褐藻糖胶。

[0367] 下表中的结果包含用于分子量分布的某些特征的缩写。凝胶渗透色谱法用GPC表示,峰分子量用PMW表示,重均分子量用WAMW表示,数均分子量用NAMW表示,百分比分布用%

分布表示,分子量用MW表示,多分散指数由PDI表示。

[0368]

	PMW (kDa)	WAMW (kDa)	NAMW (kDa)	% 分布 <10 kDa	% 分布 <20 kDa	% 分布 <50 kDa	%分布>100 kDa	%分布>200 kDa	%分布>500 kDa	PDI
褐藻糖胶1	17.5	28.3	14.2	16.6	51.7	87.9	3.0	0.6	0.0	1.99
褐	21.0	72.4	9.9	26.5	44.0	67.3	18.4	9.1	2.3	7.29

[0369]

藻糖胶2										
褐藻糖胶3	70.4	105.9	52.4	0.6	5.8	33.1	35.3	12.1	1.3	2.02
褐藻糖胶4	107.1	136.1	79.9	0.1	1.5	15.4	53.4	19.8	1.1	1.70
褐藻糖胶5	80.2	171.9	60.4	0.8	5.3	26.5	47.9	25.4	6.6	2.84
褐藻糖胶	195.1	192.1	87.4	0.4	2.3	14.0	64.4	35.8	5.5	2.20

[0370]

6										
褐藻糖胶7	242.5	366.5	137.2	0.0	0.5	7.0	77.7	54.6	21.9	2.67
褐藻糖胶8	307.1	395.8	170.2	0.0	0.2	4.0	83.8	62.2	25.4	2.33
褐藻糖胶9	459.3	514.0	198.5	0.1	0.4	3.4	87.8	71.4	37.2	2.62
褐藻糖胶10	390.2	497.9	228.9	0.0	0.0	1.7	90.4	73.3	35.1	2.17
褐藻	457.3	592.8	300.9	0.0	0.0	0.7	95.4	82.9	43.8	1.97

[0371]

糖胶11										
褐藻糖胶12	535.8	760.1	350.6	0.0	0.1	0.9	96.5	88.3	54.3	2.17
褐藻糖胶13	612.3	857.0	448.7	0.0	0.0	0.2	98.6	92.4	61.4	1.91
褐藻糖胶14	393.1	930.1	296.6	0.0	0.0	1.1	93.6	81.1	43.6	3.14
褐藻糖胶15	409.4	772.0	291.8	0.0	0.0	1.1	94.0	81.5	43.6	2.65

[0372]

褐藻糖胶16	743.0	1618.0	387.5	0.0	0.1	1.4	92.9	86.6	68.2	4.18
褐藻糖胶17	686.2	1876.7	524.9	0.0	0.0	0.3	98.4	93.0	69.9	3.58
褐藻糖胶18	6238.6	3957.4	519.7	0.0	0.1	1.7	82.3	78.8	71.4	7.61
褐藻糖胶19	4315.2	5336.8	2009.5	0.0	0.0	0.0	93.7	93.3	90.1	2.66
褐藻糖	6170.2	8101.9	846.3	0.0	0.0	0.3	94.7	91.1	83.6	9.57

[0373]

胶20										

[0374] 表13:20种褐藻糖胶的分子量分布的第一个观点

[0375]

	% 分布 <5 kDa	% 分布 5-60 kDa	% 分布 60-200 kDa	% 分布 200-1600 kDa	% 分布>1600 kDa
褐藻糖胶 1	3.5	87.9	8.1	0.6	0.0
褐藻糖胶 2	13.0	58.6	19.4	9.0	0.0
褐藻糖胶 3	0.0	41.3	46.6	12.1	0.0
褐藻糖胶 4	0.0	20.9	59.1	20.1	0.0
褐藻糖胶 5	0.1	32.8	41.7	25.0	0.4
褐藻糖胶 6	0.0	18.5	45.6	35.8	0.0
褐藻糖胶 7	0.0	10.0	35.4	52.3	2.4

[0376]

褐藻糖胶 8	0.0	6.2	31.5	60.0	2.3
褐藻糖胶 9	0.0	5.0	23.6	67.4	4.0
褐藻糖胶 10	0.0	3.0	23.7	69.8	3.5
褐藻糖胶 11	0.0	1.3	15.8	78.0	4.9
褐藻糖胶 12	0.0	1.3	10.5	78.9	9.4
褐藻糖胶 13	0.0	0.3	7.2	80.4	12.0
褐藻糖胶 14	0.0	1.8	16.3	68.7	13.1
褐藻糖胶 15	0.0	1.7	16.1	72.4	9.8
褐藻糖胶 16	0.0	2.1	9.4	60.9	37.6
褐藻糖胶 17	0.0	0.5	6.5	62.4	30.6
褐藻糖胶 18	0.0	2.3	5.7	35.2	56.8

[0377]

褐藻糖胶 19	0.0	0.0	0.3	24.9	74.8
褐藻糖胶 20	0.0	0.6	5.2	28.7	65.5

[0378] 表14:20种褐藻糖胶的分子量分布的第二个观点

[0379] 实施例19:高分子量褐藻糖胶的硫酸酯、总碳水化合物和单糖含量

[0380] 将高分子量褐藻糖胶褐藻糖胶7至褐藻糖胶18和褐藻糖胶20溶解在去离子水中，在酸性条件下水解，并通过电感耦合等离子体质谱法(ICP-MS)分析总硫含量%w/w，由不列颠哥伦比亚省本那比的ALS环境实验室执行。通过将硫含量乘以硫酸酯与硫的摩尔比，将硫含量转化为硫酸酯含量，以获得褐藻糖胶的%w/w硫酸酯含量。褐藻糖胶7至18和褐藻糖胶20的硫酸酯含量示于下表15中。

[0381]

	硫酸酯含量 (% w/w)
褐藻糖胶 7	23.93
褐藻糖胶 8	40.95
褐藻糖胶 9	40.32
褐藻糖胶 10	33.15

[0382]

褐藻糖胶11	44.87
褐藻糖胶12	41.02
褐藻糖胶13	36.18
褐藻糖胶14	40.45
褐藻糖胶15	39.79
褐藻糖胶16	14.39
褐藻糖胶17	51.30
褐藻糖胶18	21.11
褐藻糖胶20	25.60

[0383] 表15-褐藻糖胶7至褐藻糖胶18和褐藻糖胶20的硫酸酯含量

[0384] 由佐治亚大学的复杂碳水化合物研究中心通过气相色谱-质谱法(GC-MS)分析了高分子量的褐藻糖胶褐藻糖胶7、褐藻糖胶11、褐藻糖胶16、褐藻糖胶18和褐藻糖胶20的总碳水化合物和单糖组成。通过酸性甲醇分解将高分子量褐藻糖胶聚糖衍生化，以产生O-三甲基甲硅烷基(O-TMS)衍生物。衍生化后，使用Supelco Equity-1熔融石英毛细管色谱柱(30m，内径0.25mm)，在连接到Agilent 5975C质谱检测器的Agilent 7890A气相色谱系统上分析褐藻糖胶。高分子量褐藻糖胶的总碳水化合物含量和单糖组成的结果示于下表16中。下表中的碳水化合物缩写为“carb.”。

[0385]

	总碳水化合物含量(褐藻糖胶的% w/w)	岩藻糖(总碳水化合物含量的% w/w)	半乳糖(总碳水化合物含量的% w/w)	木糖(总碳水化合物含量的% w/w)	甘露糖(总碳水化合物含量的% w/w)	鼠李糖(总碳水化合物含量的% w/w)
褐藻糖胶7	32.7	44.4	52.9	0.5	0.4	0.3
褐藻糖胶11	59.5	91.9	8.1	0.0	0.0	0.0
褐藻糖胶16	25.9	48.3	9.9	15.5	5.9	0.3
褐藻糖胶18	41.2	92.0	4.7	2.1	0.4	0.2
褐藻糖胶20	30.1	84.7	10.6	3.3	0.9	0.0

[0386] 表16-五种褐藻糖胶的总碳水化合物和单糖组成

[0387] 实施例20:大鼠硬膜外粘连治疗

[0388] 使用实施例18中鉴定的二十种褐藻糖胶的岩藻多糖溶液在乳酸钠林格注射液USP(LRS)中制备。在LRS中以100mg/mL制备了褐藻糖胶1至褐藻糖胶16、褐藻糖胶18和褐藻糖胶20。在LRS中以50mg/mL制备褐藻糖胶19。在LRS中以500mg/mL制备褐藻糖胶17。对Sprague Dawley大鼠进行了椎板切除手术,大鼠的平均体重和以毫克每千克为单位的剂量在下表17中显示。用布比卡因溶液沿腰椎形成一个线阻滞。清洁大鼠的背部,然后用无菌盖布覆盖。通过中线皮肤切口打开腰筋膜,切开腰筋膜,并解剖腰旁肌,露出下面的椎板。去除椎骨中央的骨头。在整个过程中,通过使用乳酸钠林格注射液USP(LRS)冲洗并用棉签加压来维持止血。将裸露的硬脑膜直接用15微升LRS(对照)或岩藻多糖溶液处理。用缝线封闭肌肉和皮肤层,让大鼠恢复一周,然后处死以进行粘连量化。记录硬膜上粘连的存在和大小。记录粘连和暴露的硬膜的尺寸,并用于计算粘连覆盖率,即粘连面积占总暴露硬膜面积的百分比。

[0389] 等式1:粘连覆盖率(%)=100x硬膜外粘连面积÷总暴露硬膜面积

[0390] 使用等式1确定接受LRS的对照组具有65%的粘连覆盖率。表13至表16中公开的二十种褐藻糖胶的粘连覆盖率示于下表17中,显示相对于对照组的粘连覆盖率降低。负值表示相对于对照组观察到的粘连覆盖率增加。

[0391]

	平均大鼠重量 (kg)	剂量(mg)	每动物重量的剂量 (mg/kg)	评分大鼠的数量	与对照相比硬膜外粘连覆盖率降低%
褐藻糖胶 1	0.41	1.5	3.7	4	-40% (即，与对照相比，纤维性粘连增加 40%)
褐藻糖胶 2	0.59	1.5	2.5	3	9%
褐藻糖胶 3	0.39	1.5	3.8	4	-10%
褐藻糖胶 4	0.65	1.5	2.3	4	83%
褐藻糖胶 5	0.53	1.5	2.9	4	46% (即，与对照相比，纤维性粘连降低 46%)
褐藻糖胶 6	0.46	1.5	3.3	4	44%
褐藻糖胶 7	0.47	1.5	3.2	3	100%
褐藻糖胶 8	0.36	1.5	4.2	3	100%
褐藻糖胶 9	0.39	1.5	3.8	2	100%
褐藻糖胶 10	0.40	1.5	3.8	4	100%

[0392]

[0393]

褐藻糖胶11	0.58	1.5	2.6	2	100%
褐藻糖胶12	0.44	1.5	3.4	2	100%
褐藻糖胶13	0.64	1.5	2.3	3	100%
褐藻糖胶14	0.37	1.5	4.0	4	100%
褐藻糖胶15	0.50	1.5	3.0	3	100%
褐藻糖胶16	0.45	1.5	3.3	3	100%
褐藻糖胶17	0.59	7.5	12.8	3	100%
褐藻糖胶18	0.59	1.5	2.5	2	100%
褐藻糖胶19	0.39	0.8	1.9	3	100%
褐藻糖胶20	0.56	1.5	2.7	2	100%

[0394] 表17:使用20种不同的褐藻糖胶相对于对照LRS的大鼠硬膜外粘连降低

[0395] 通过将表17的结果与表13和表14中给出的褐藻糖胶的分子量进行比较可以看出,在相同剂量下,与包含约60%或更少的其分子量分布大于100kDa的重均分子量低于100kDa的褐藻糖胶相比,重均分子量大于130kDa和包含约60%或更多其分子量分布大于100kDa的褐藻糖胶在抑制、预防、去除、减少或以其他方式治疗大鼠硬膜外粘连方面显示出更大功效。还有进一步的迹象表明,在相同剂量下,重均分子量高于300kDa的褐藻糖胶,包含约70%或更多的其分子量分布高于100kDa,在抑制、预防、去除、减少或以其他方式治疗大鼠硬膜外粘连方面显示出更高的功效。

[0396] 实施例21:用褐藻糖胶1和褐藻糖胶10对兔子子宫角进行粘连治疗

[0397] 对每只兔子的两个子宫角进行子宫角手术。在手术之前，将兔子称重，然后通过氯胺酮和甲苯噻嗪的预用药准备进行手术。

[0398] 在乳酸钠林格注射液USP中以0.07mg/mL制备岩藻多糖溶液，通过过滤灭菌。所有仪器都是无菌的，并且在整个手术过程中都保持无菌区域。清洁腹部并通过中线腹部切口进入。子宫角被定位、外部化和刮擦以引起损伤。刮除子宫角附近的腹壁也被刮除。受损的子宫角和腹壁彼此相邻放置，并用缝合线固定。在切开切口之前，将每只兔子体重15mL/kg的岩藻多糖溶液给药于腹腔。术后两周评估粘连。用尺子测量子宫角粘连的长度。子宫角粘连覆盖率，即粘连长度占总受损子宫角长度的百分比，计算如下：

[0399] 等式2:粘连覆盖率(%)＝100x子宫角粘连长度÷总受损子宫角长度

[0400] 将相同的手术方法应用于3只新西兰白兔，接受15mL/kg的对照乳酸钠林格注射液USP(LRS)代替岩藻多糖溶液。

[0401] 使用等式2确定接受LRS的对照组具有41％的粘连覆盖率。表18显示了使用以上讨论的针对褐藻糖胶褐藻糖胶1和褐藻糖胶10的方法所获得的结果，分别是这样的褐藻糖胶的代表性实例:大部分分子量分布低于100kDa甚至低于50kDa的褐藻糖胶，以及大部分分子量分布高于100kDa甚至高于200kDa的褐藻糖胶。下表中的结果显示为相对于对照组的粘连覆盖率降低。

[0402]

	每动物体重的剂量 (mg/kg)	子宫角数	与对照相比子宫角粘连覆盖率降低%
褐藻糖胶 1 –低分子量	1	6	21% (即，与对照相比，纤维性粘连降低 21%)
褐藻糖胶 10 – 高分子量	1	8	100%

[0403] 表18:相对于对照LRS，使用两种不同的褐藻糖胶减少兔子子宫角粘连

[0404] 从表18的结果可以看出，与在相同剂量下其大部分分布在100kDa或甚至在50kDa以下的褐藻糖胶相比，具有大部分分布高于100kDa或甚至高于200kDa的褐藻糖胶在抑制、预防、去除、减少或以其他方式治疗兔子子宫角粘连方面具有更高的功效。

[0405] 实施例22:用褐藻糖胶17治疗兔子子宫角粘连

[0406] 为了确定高分子量褐藻糖胶17在抑制手术粘连中的功效，在总共三只新西兰白兔的两个角上进行了以下双子宫角(DUH)手术。在手术之前，将兔子称重，然后通过氯胺酮和甲苯噻嗪的预用药准备进行手术。

[0407] 在乳酸林格氏注射液USP(LRS)中以5mg/mL制备岩藻多糖溶液，通过过滤灭菌。所有仪器都是无菌的，并且在整个手术过程中都保持无菌区域。清洁腹部并通过中线腹部切口进入。子宫角被定位、外部化和刮擦以引起损伤。刮除子宫角附近的腹壁也被刮除。受损的子宫角和腹壁彼此相邻放置，并用缝合线固定。闭合肌肉切口的顶部三分之一和底部三

分之一，并将每只兔子体重5mL/kg岩藻多糖溶液给药于腹腔。暂时关闭肌肉切口，将岩藻多糖溶液留在腹腔中30分钟。重新打开肌肉切口，并用10mL/kg LRS冲洗腹腔。在关闭切口之前，将腹腔中的大部分液体吸出。术后两周评估粘连形成。用直尺测量子宫角粘连的长度。使用公式2计算子宫角粘连覆盖率，即粘连长度占受损子宫角总长度的百分比。

[0408] 表19显示了使用以上讨论的针对褐藻糖胶17的方法获得的结果，所述褐藻糖胶是高分子量褐藻糖胶的代表性实例。下表中的结果显示为在所评分的6个子宫角上的平均粘连长度。

[0409] 表19提供了用褐藻糖胶17治疗六个子宫角的结果。

[0410]

	剂 量 (mg/kg)	子宫角数	平均粘连长度%
褐藻糖胶 17	25	6	0% (即， 未发现粘连)

[0411] 表19:使用褐藻糖胶17的粘连长度

[0412] 从表19的结果可以看出，高分子量褐藻糖胶可用于成功地抑制、预防、去除、减少或以其他方式治疗手术后子宫角粘连。

[0413] 实施例23:用高分子量褐藻糖胶组合物治疗子宫角纤维性粘连

[0414] 为了确定包含约228kDa的数均分子量、约1210kDa的重均分子量、约575kDa的峰分子量和具有分子量分布(其中约89％的分布大于100kDa，其中约30％的分布大于1000kDa)的高分子量褐藻糖胶组合物在抑制手术粘连方面的功效，在总共二十只新西兰白兔的两个角上进行了以下双子宫角(DUH)手术。在手术之前，将兔子称重，然后通过用咪达唑仑和右美托咪定的处方药进行手术准备。

[0415] 在乳酸钠林格注射液USP(LRS)中以0.02mg/mL、0.1mg/mL、0.5mg/mL或2.5mg/mL的每种浓度制备岩藻多糖溶液，通过过滤灭菌。所有仪器都是无菌的，并且在整个手术过程中都保持无菌区域。清洁腹部并通过中线腹部切口进入。子宫角被定位、外部化和刮擦以引起损伤。刮除子宫角附近的腹壁也被刮除。受损的子宫角和腹壁彼此相邻放置，并用缝合线固定。在切开切口之前，将每只兔子体重约2mL/kg的岩藻多糖溶液给药于腹腔。术后两周评估粘连。治疗五只兔子并评估每个岩藻多糖的浓度。用直尺测量子宫角粘连的长度。使用等式2计算子宫角粘连长度。

[0416] 将相同的手术方法应用于另外5只新西兰白兔作为对照，每只兔子接受约2mL/kg的对照乳酸钠林格注射液USP(LRS)代替岩藻多糖溶液。使用等式2确定接受LRS的对照组具有100％的粘连覆盖率。表20显示了使用上述方法对不同浓度和剂量的高分子量褐藻糖胶组合物所获得的结果(总共治疗四十个子宫角，每种浓度的高分子量褐藻糖胶组合物各10个);结果显示为相对于对照组的粘连覆盖率降低。

[0417]

浓度(mg/mL)	剂量(mg/kg)	子宫角数	与对照相比子宫角粘连覆盖率降低%
0.02	0.04	10	10% (即，与对照相比纤维性粘连降低 10%)
0.1	0.2	10	30% (即，与对照相比纤维性粘连降低 30%)
0.5	1	10	71% (即，与对照相比纤维性粘连降低 71%)
2.5	5	10	95% (即，与对照相比纤维性粘连降低 95%)

[0418] 表20:相对于对照LRS,使用高分子量褐藻糖胶组合物降低了兔子宫角粘连

[0419] 从表20的结果可以看出,高分子量的褐藻糖胶组合物可以用于成功地抑制、预防、去除、减少或以其他方式治疗手术后子宫角粘连。

[0420] 附图标记列表:

[0421] 100 基于分子量的分段系统(较高至较低)

[0422] 100’ 基于分子量的分段系统(较低至较高)

[0423] 100” 阳离子增强的TFF系统(CATS)

[0424] 102 输入供应管线

[0425] 104 预过滤器

[0426] 106 较低MWCO子系统截留物管线阀

[0427] 106’ 较低MWCO子系统输出阀

[0428] 108 较低MWCO子系统截留物输出管线

[0429] 110 较高截留分子量TFF过滤器

[0430] 111 较高MWCO子系统截留物输出管线

[0431] 112 较高MWCO TFF过滤器供应管线

[0432] 113 较高至较低MWCO子系统间阀

[0433] 114 较高MWCO子系统泵

[0434] 115 较高MWCO子系统溶剂供应管线

[0435] 116 较高MWCO子系统褐藻糖胶容器

[0436] 117 较高MWCO子系统溶剂容器

[0437] 118 较高MWCO子系统截留物返回管线

[0438] 119 较高MWCO子系统渗透物输出管线

[0439] 120 较低截留分子量TFF过滤器

[0440] 121 较低MWCO子系统截留物输出管线108

[0441] 122 较低MWCO TFF过滤器供应管线

[0442] 123 较低至较高MWCO子系统间阀门

[0443] 124 较低MWCO子系统泵

[0444] 125 较低MWCO子系统溶剂供应管线

[0445] 126 较低MWCO子系统褐藻糖胶容器

[0446] 127 较低MWCO子系统溶剂容器

[0447] 128 较低MWCO子系统截留物返回管线

[0448] 129 较低MWCO子系统渗透物输出管线

[0449] 130 较高MWCO TFF子系统

[0450] 130’ 较高MWCO TFF子系统(图3)

[0451] 135 阳离子添加剂冲洗溶液供应管线

[0452] 136 阳离子添加剂冲洗溶液阀

[0453] 137 阳离子添加剂冲洗液容器

[0454] 140 较低MWCO子系统

[0455] 140’ 较低MWCO TFF子系统(图3)

[0456] 142 钠盐溶液容器

[0457] 143 低电导率渗滤溶液容器

[0458] 144 钠盐溶液控制阀

[0459] 145 低电导率渗滤溶液阀

[0460] 146 钠盐溶液供应管线

[0461] 147 低电导率渗滤溶液供应管线

[0462] 150 较高MWCO TFF过滤器

[0463] 160 较低MWCO TFF过滤器

[0464] 600 离心沉淀系统,用于从起始褐藻糖胶组合物获得高分子量褐藻糖胶

[0465] 600’ 离心沉淀系统,用于从起始褐藻糖胶组合物获得高分子量褐藻糖胶

[0466] 610 离心容器

[0467] 620 分级的可渗透屏障

[0468] 620′ 可渗透屏障

[0469] 620a 屏障区段(第一-最高密度)

[0470] 620b 屏障区段(第二-中等密度)

[0471] 620c 屏障区段(第三-最低密度)

[0472] 620c’ 单屏障区段

[0473] 622 第一底部分级的可渗透屏障材料端

[0474] 622’ 第一底部可渗透屏障材料端

[0475] 624 第二顶部分级的可渗透屏障材料端

[0476] 624’ 第二顶部可渗透屏障材料端

[0477] 630 离心容器610的第一底端

[0478] 640 离心容器610的第二顶端

[0479] 650 起始褐藻糖胶组合物

[0480] 660 箭头指示容器610上的离心力方向

[0481] 670 离心盒

[0482] 900 电泳提取系统

[0483] 910 电泳室

[0484] 912 孔

[0485] 914 理论位移距离

[0486] 916 电泳凝胶

[0487] 918 电泳缓冲液

[0488] 920 直流电源

[0489] 922 阴极

[0490] 924 阳极

[0491] 926 迁移方向箭头(表示阴离子的位移方向)

[0492] 800 膜透析系统,用于从起始褐藻糖胶组合物获得高分子量褐藻糖胶

[0493] 801 输入供应管线

[0494] 802 预过滤器

[0495] 810 褐藻糖胶容器

[0496] 812 透析系统供应管线

[0497] 814 透析系统泵

[0498] 815 透析液输出阀

[0499] 816 透析液返回管线

[0500] 818 透析液输出管线

[0501] 820 透析单元

[0502] 825 透析膜

[0503] 830 透析容器

[0504] 832 透析液供应管线

[0505] 834 透析泵

[0506] 835 透析液输出阀

[0507] 836 透析液返回管线

[0508] 838 透析液输出管线

[0509] 840 透析液容器

[0510] 842 透析液供应管线

[0511] 845 透析液供应阀

[0512] 170 切向流过滤(TFF)子系统

[0513] 171 TFF过滤器

[0514] 172 TFF子系统过滤器供应管线

[0515] 173 TFF子系统溶剂供应阀

[0516] 174 TFF子系统泵

[0517] 175 TFF子系统溶剂供应管线

[0518] 176 TFF子系统褐藻糖胶容器

[0519] 177 TFF子系统溶剂容器

[0520] 178 TFF子系统截留物管线

[0521] 179 TFF子系统渗透物输出管线

[0522] 180 离子交换子系统

[0523] 181 离子交换容器

[0524] 182a 离子交换子系统褐藻糖胶供应管线

[0525] 182b 离子交换子系统盐溶液供应管线

[0526] 183a 离子交换子系统褐藻糖胶返回阀

[0527] 183b 离子交换子系统盐溶液供应阀

[0528] 183c 离子交换子系统盐溶液返回阀

[0529] 184a 离子交换子系统褐藻糖胶泵

[0530] 184b 离子交换子系统盐溶液泵

[0531] 186 离子交换子系统褐藻糖胶容器

[0532] 187 离子交换子系统盐溶液容器

[0533] 188a 离子交换子系统褐藻糖胶返回管线

[0534] 188b 离子交换子系统盐溶液返回管线

[0535] 189 大孔离子交换树脂

[0536] 300 离子吸附系统

[0537] 301 输入供应管线

[0538] 302 子系统间阀

[0539] 303 TFF子系统截留物输出管线

[0540] 304 离子交换子系统输出阀

[0541] 305 离子交换子系统输出管线

[0542] 306 预过滤器

[0543] 除非上下文或定义另有清楚说明，否则本文中使用的所有术语均按照其普通含义使用。另外，除非另有明确说明，否则在说明书中使用的“或”包括“和”，反之亦然。除非另有明确指出或上下文另有清楚说明，否则非限制性术语不应解释为限制性的(例如，“包括(including)”、“具有”和“包含(comprising)”通常表示“包括但不限于”)。除非另有明确指出或上下文另有清楚说明，否则单数形式(包括在权利要求书中，诸如“一(a)”、“一(an)”和“该(the)”)包括复数引用。

[0544] 除非另有指出，否则本文中对实施方式的一个或多个特征的条件或关系特征进行修饰的形容词(诸如“基本上”和“约”)表示，该条件或特征被定义为，对于该实施方式针对预期应用的操作而言，处于可接受的允许范围内。

[0545] 本发明的方法、组合物、系统等的范围包括装置加功能以及步骤加功能这两种概念。然而，除非在权利要求中具体地陈述词语“装置(means)”，否则权利要求不应被解释为表示“装置加功能”的关系，而在权利要求中具体地陈述词语“装置”的情况下，权利要求应解释为表示“装置加功能”的关系。类似地，除非在权利要求中具体地陈述词语“步骤”，否则

权利要求不应解释为表示“步骤加功能”的关系，而在权利要求中具体地陈述词语“步骤”的情况下，权利要求应解释为表示“步骤加功能”的关系。

[0546] 根据以上所述，应当理解，尽管出于说明的目的已经在本文中讨论了具体的实施方式，但是可以在不背离本文所讨论的精神和范围的情况下进行各种修改。因此，系统和方法等包括对本文提出的主题所做的这样的修改以及所有排列和组合，并且仅受所附权利要求或在本文的讨论和附图中具有足够支持的其他权利要求的限制。

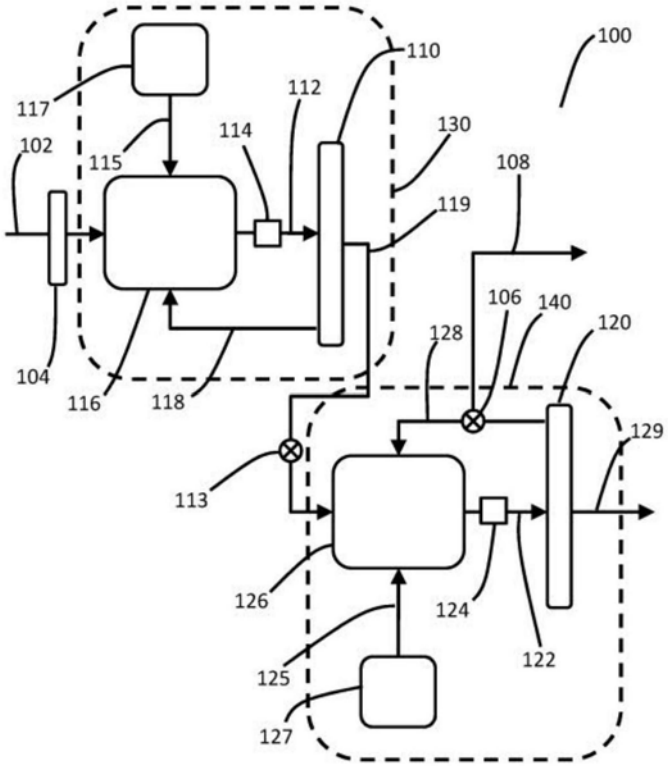

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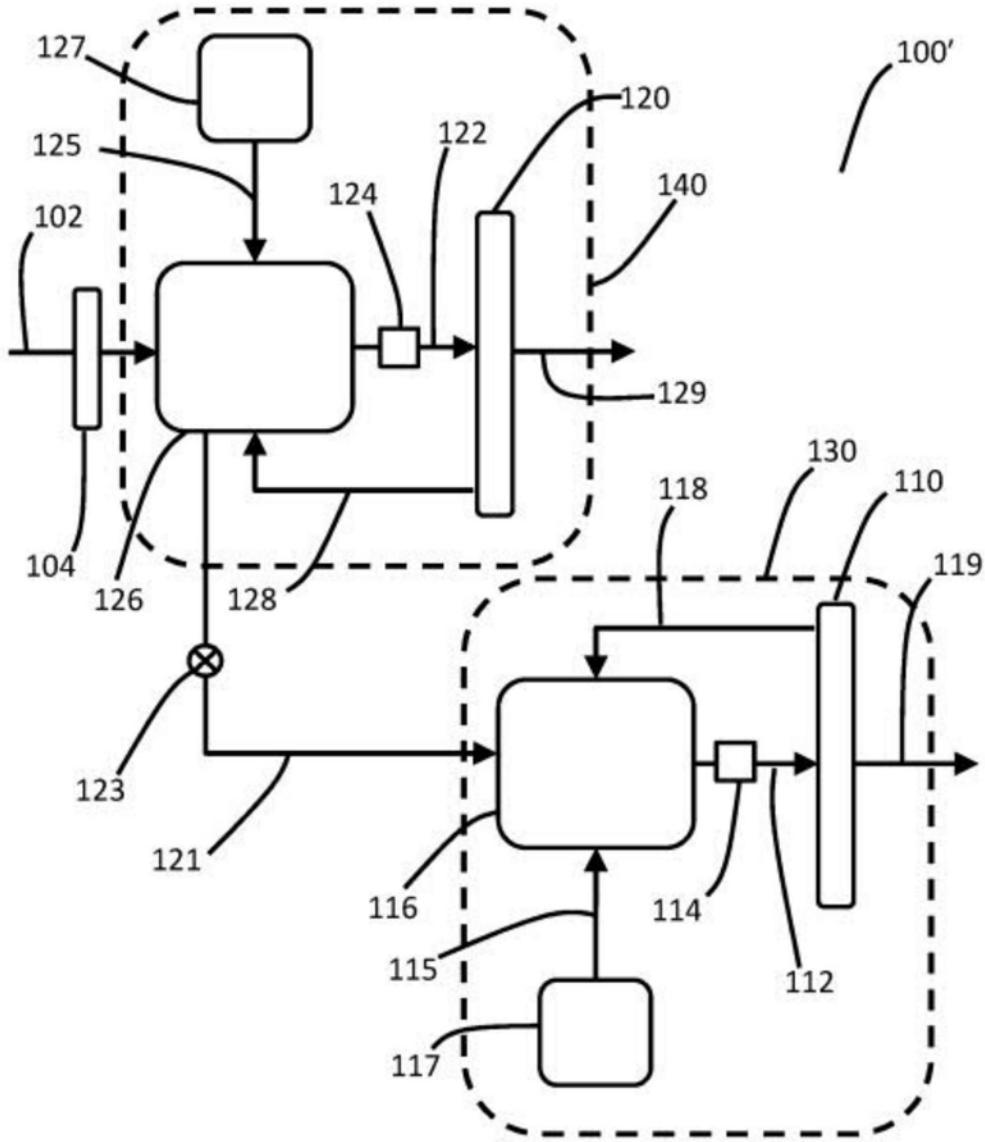
图1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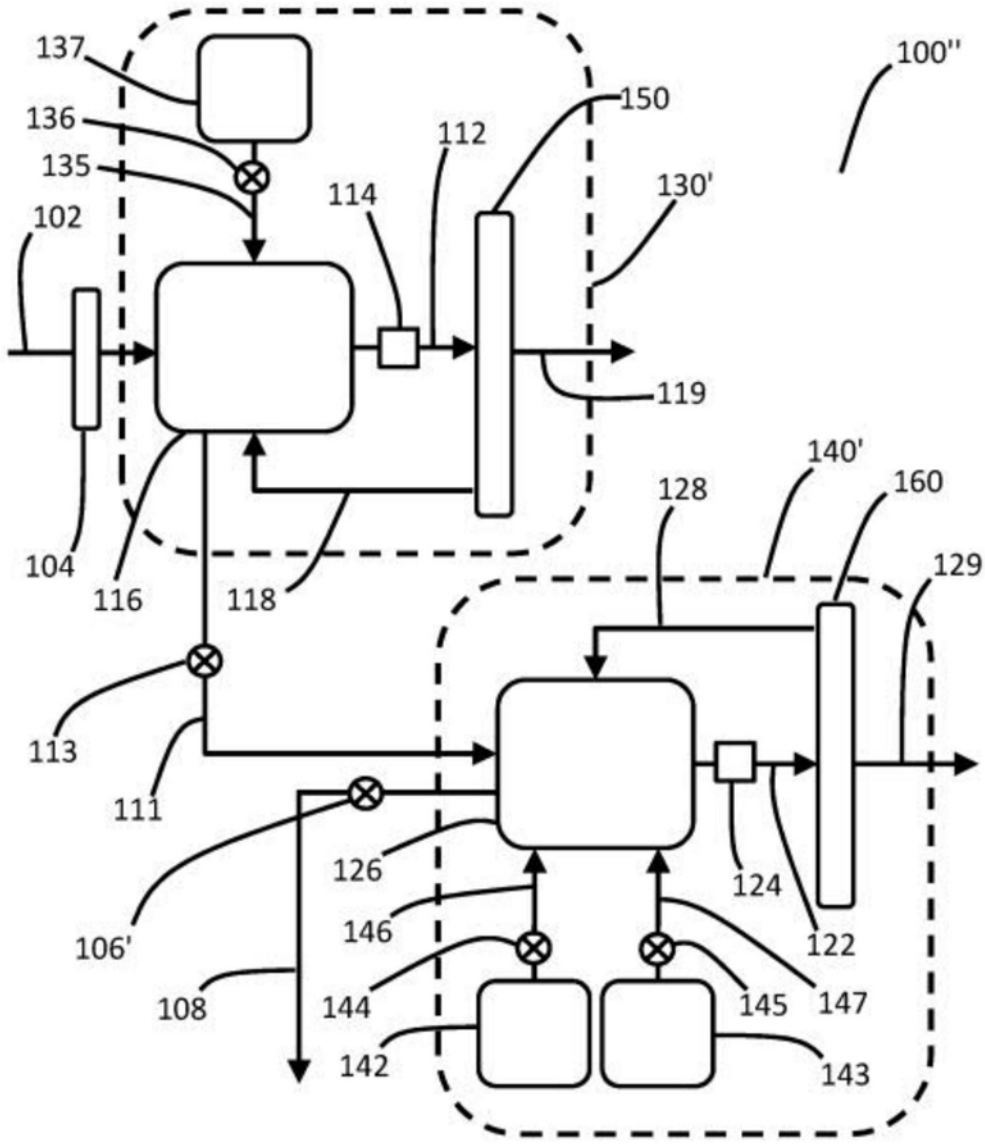
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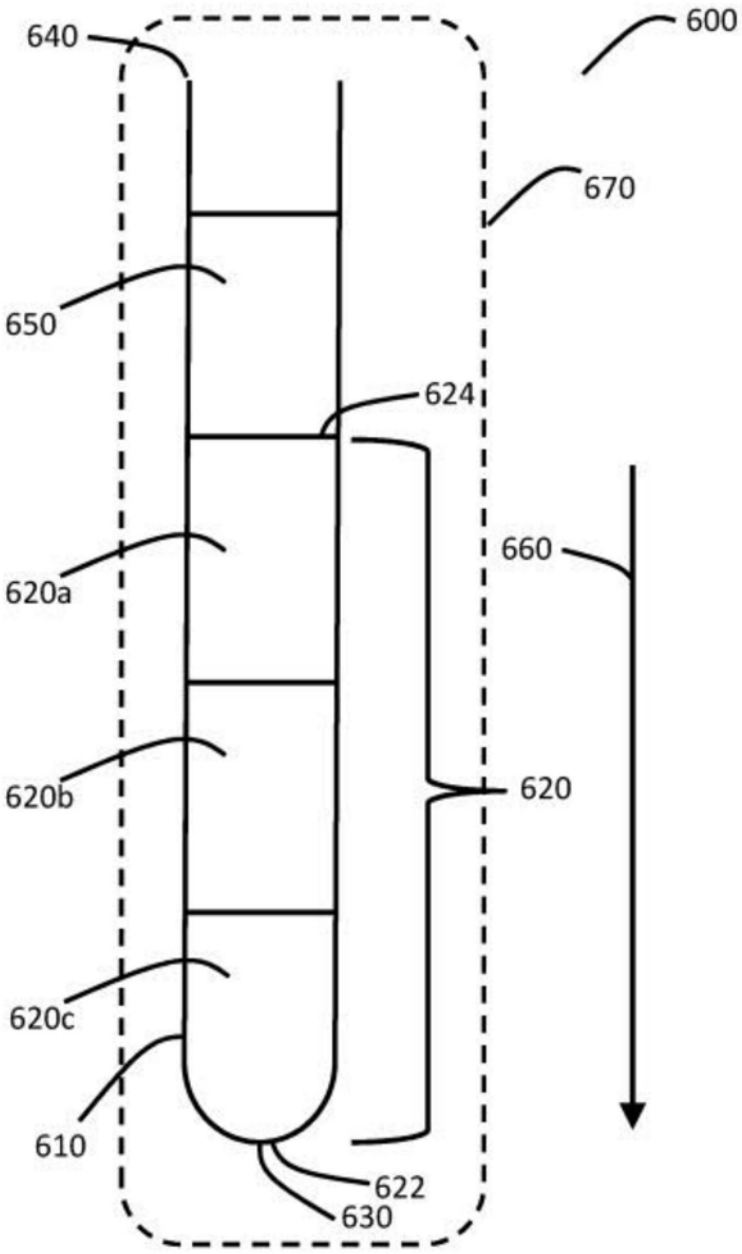
图3



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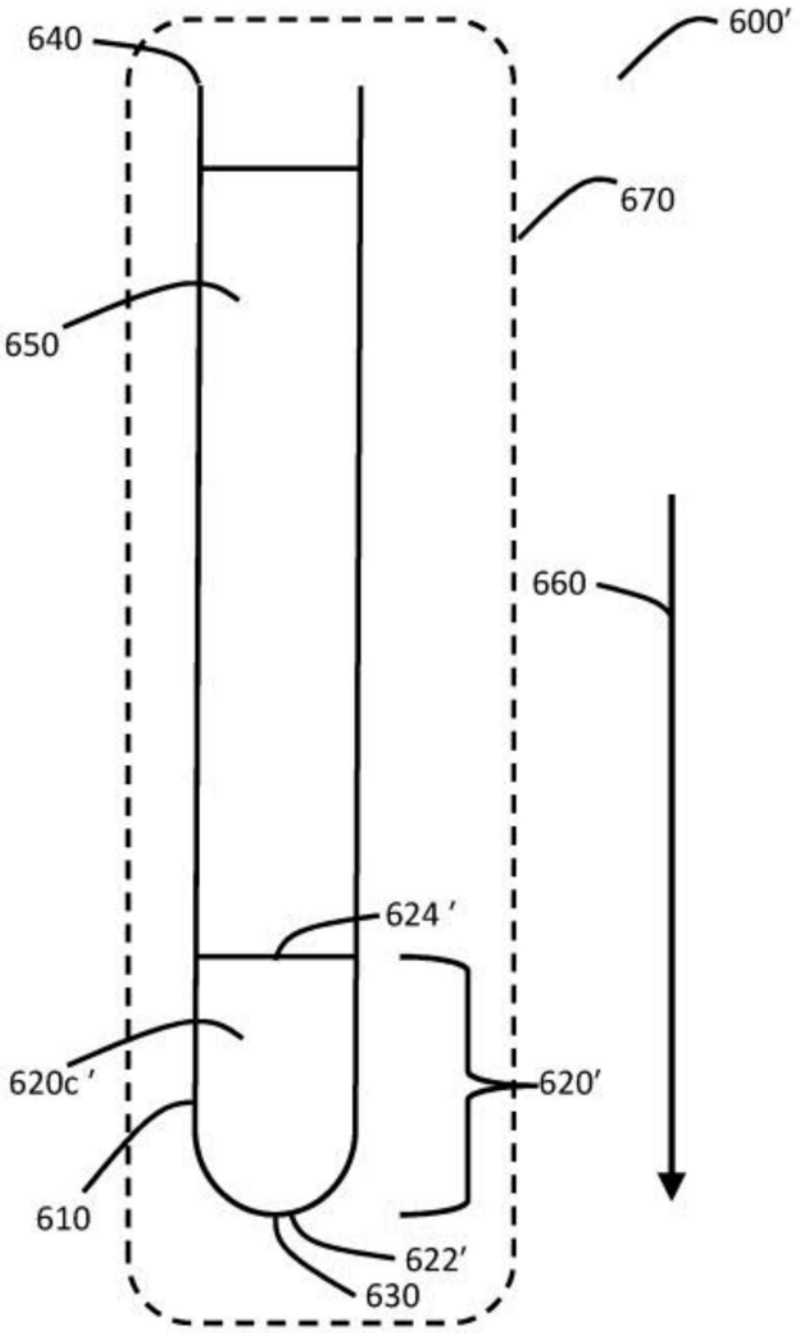

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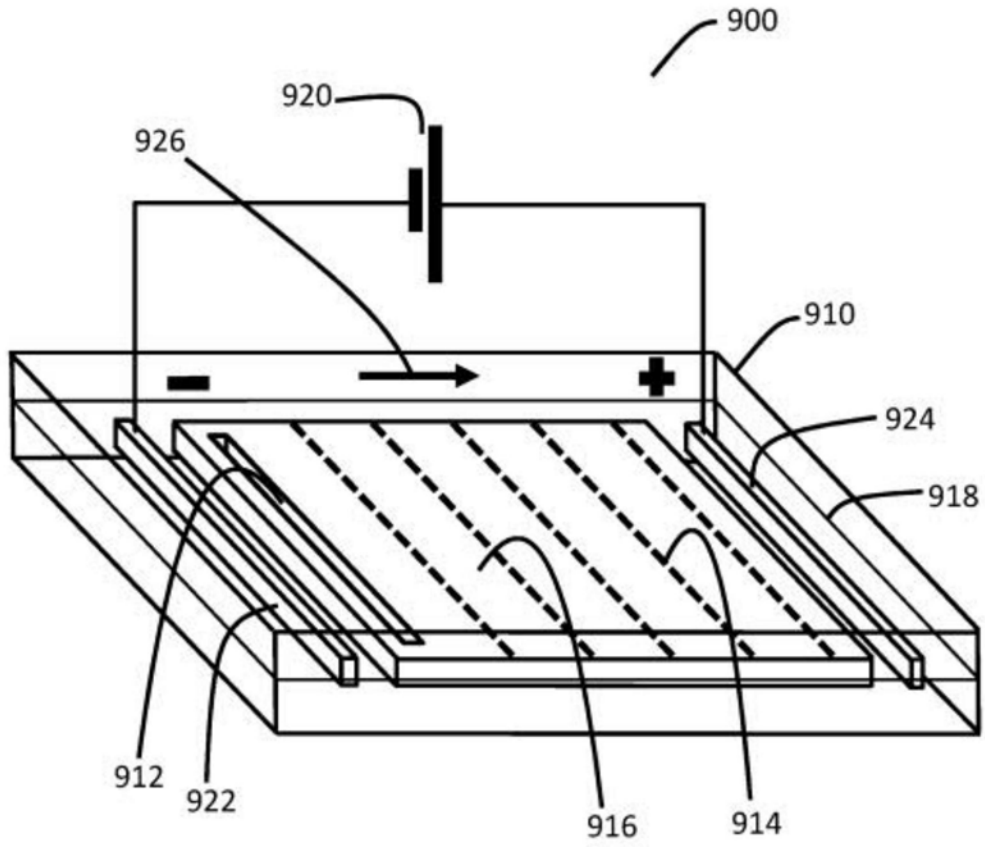

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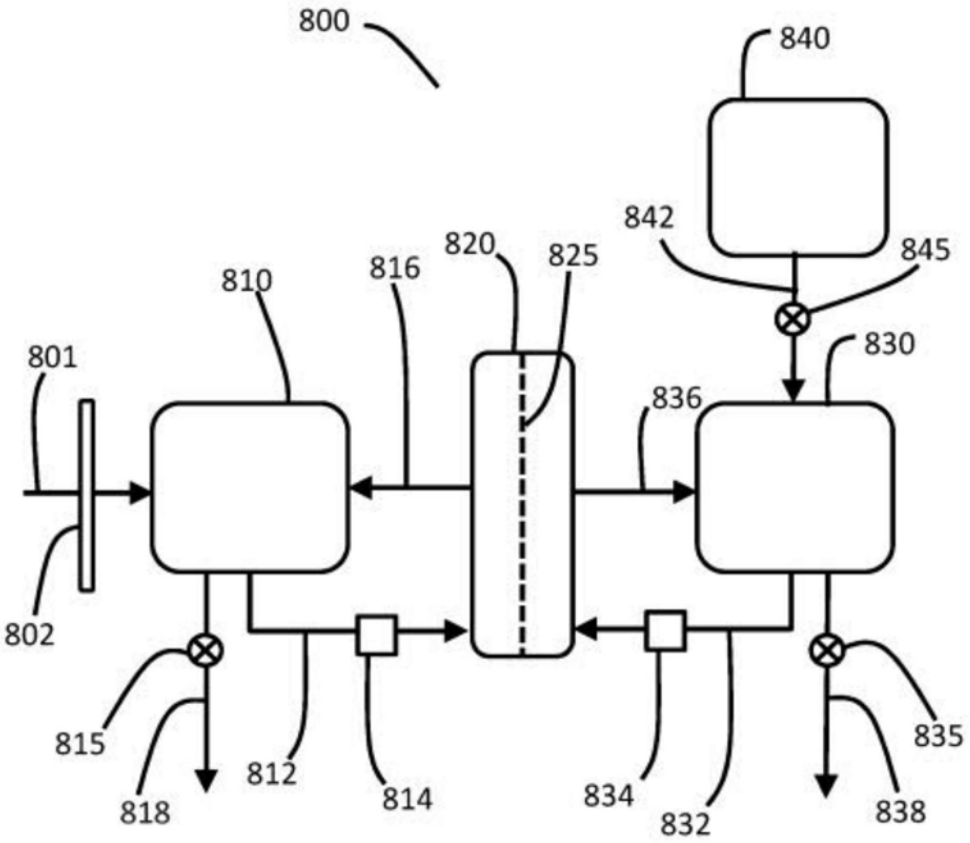
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图7

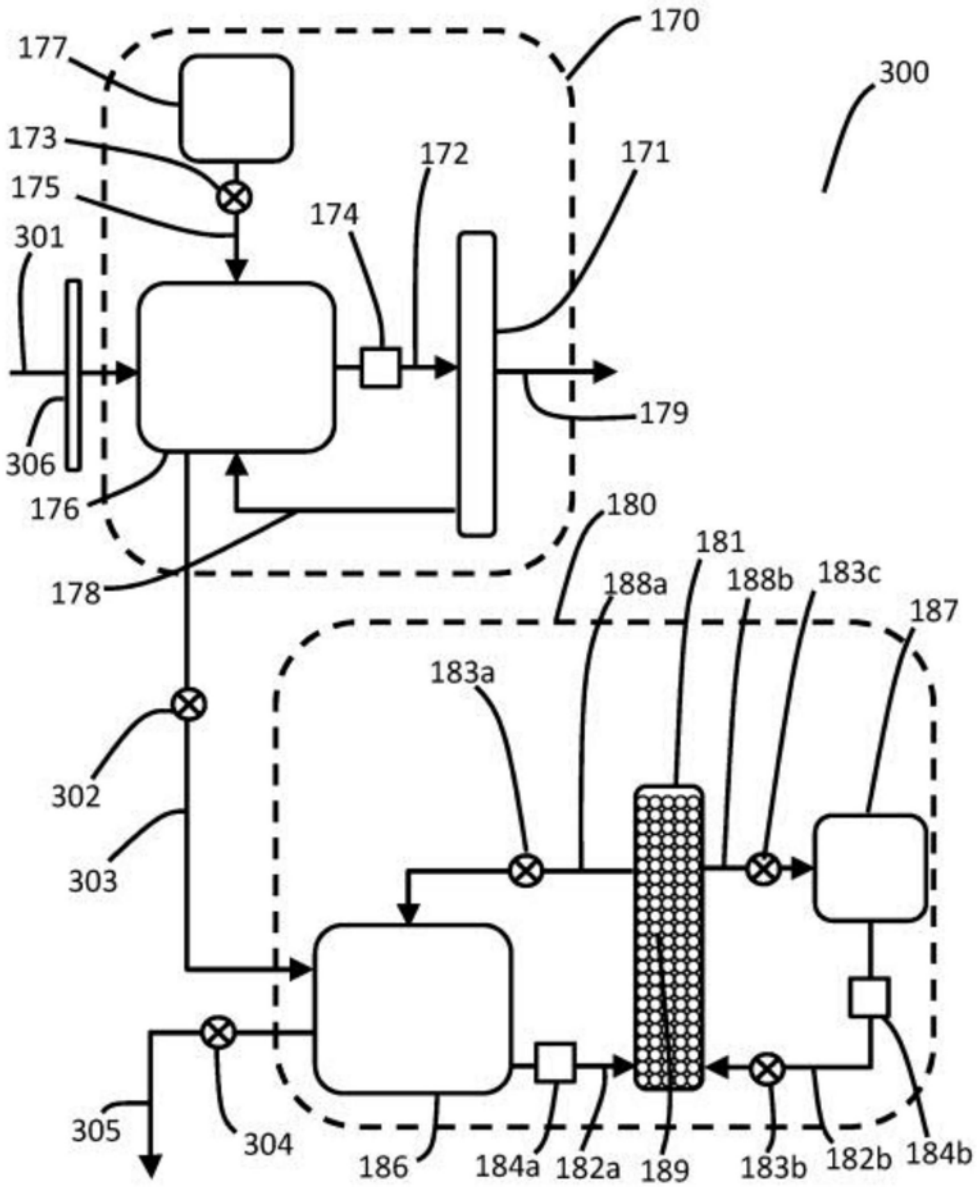
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图8

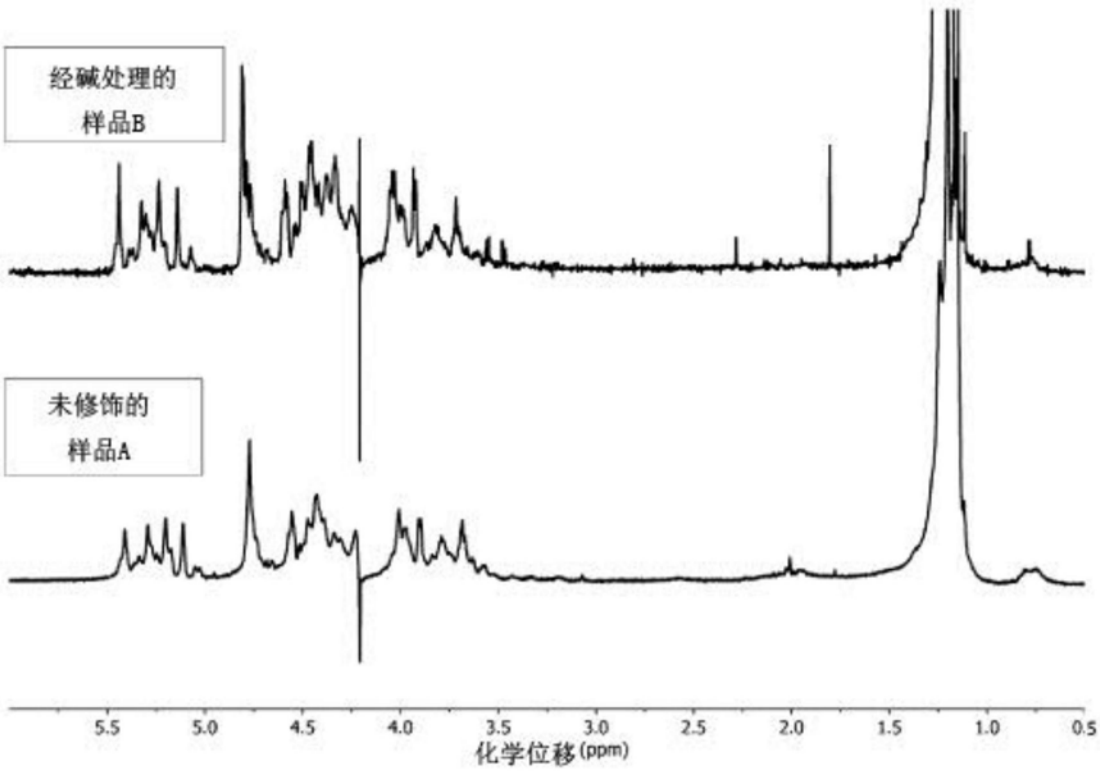
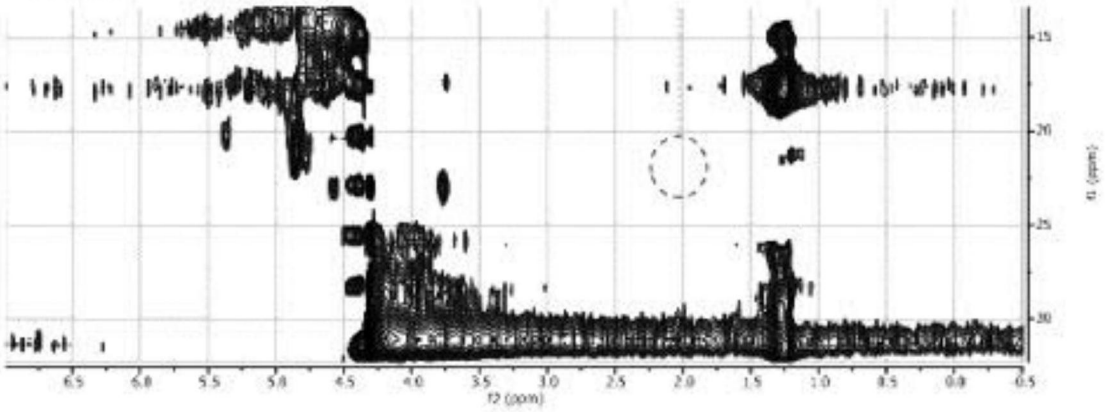


图9A

经碱处理的

样品B



未修饰的

样品A

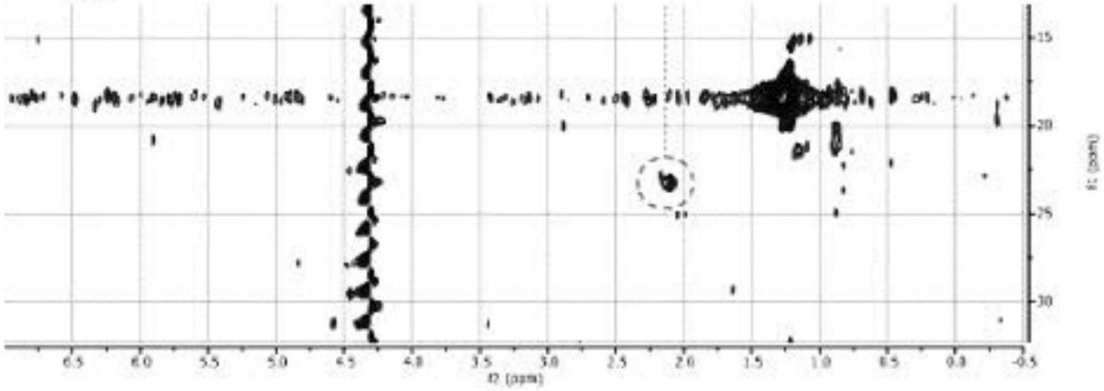
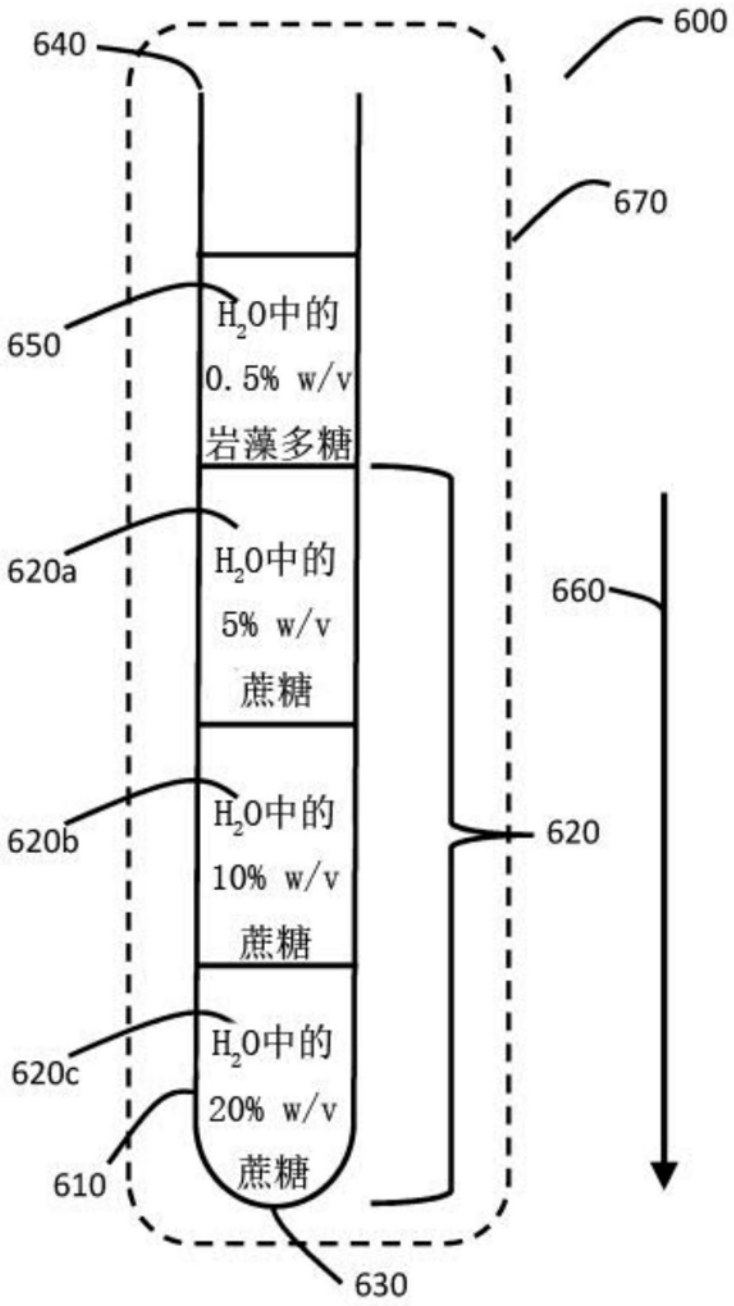


图9B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5% w/v
蔗糖
660
620b
H_2O中的
10% w/v
蔗糖
620
620c
H_2O中的
20% w/v
蔗糖
610
630

图10