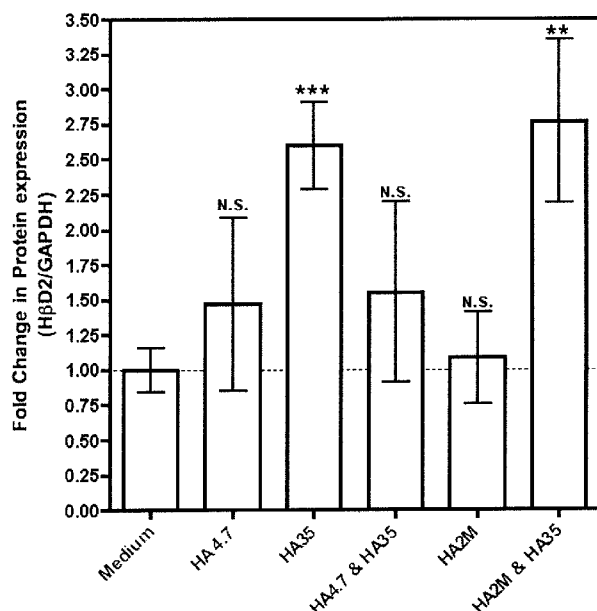




- (51) International Patent Classification:
A61K 47/36 (2006.01)
- (21) International Application Number:
PCT/US2012/036936
- (22) International Filing Date:
8 May 2012 (08.05.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/484,044 9 May 2011 (09.05.2011) US
61/609,657 12 March 2012 (12.03.2012) US
- (71) Applicant (for all designated States except US): **THE CLEVELAND CLINIC FOUNDATION** [US/US]; 9500 Euclid Ave., Cleveland, OH 44195 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **DE LA MOTTE, Carol** [US/US]; 8761 Avery Road, Broadview Heights, OH 44147 (US). **KESSLER, Sean** [US/US]; 3700 Archwood Drive, Rocky River, OH 44116 (US).
- (74) Agent: **BREEN, John, P.**; Barnes & Thornburg LLP, 11 South Meridian Street, Indianapolis, IN 46204 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

- (54) Title: COMPOSITION AND METHOD TO IMPROVE INTESTINAL HEALTH



N=12. Statistical significance evaluated by comparison of experimental groups to Medium treatment alone using one-tailed Student's T-test. 'N.S.' indicates no significant increase in expression relative to control, while '***' and '**' indicate $P < 0.01$ or $P < 0.001$ respectively.

Fig. 6A

(57) Abstract: Compositions and methods are provided for treating patients suffering from compromised intestinal function, including inflammatory bowel disease. The method comprises orally administering a composition comprising hyaluronan, where said hyaluronan has a molecular weight within the range of about 35 kDa.

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

COMPOSITION AND METHOD TO IMPROVE INTESTINAL HEALTH

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Nos.
5 61/484,044 and 61/609,657 filed on May 9, 2011 and March 12, 2012, respectively,
the disclosures of which are hereby expressly incorporated by reference in their
entirety.

BACKGROUND

10 Inflammatory bowel disease (IBD) is a collective term used to describe related
inflammatory disorders of the gastrointestinal tract whose etiology is not completely
understood. The two most common forms of IBD are ulcerative colitis (UC) and
Crohn's disease (CD). For most patients, IBD is a chronic condition with symptoms
lasting for months to years. The course of IBD varies widely, with intermittent
15 periods of remission (i.e., inactive disease) followed by periods of acute illness (i.e.,
active disease). Onset of IBD is predominant in young adulthood but can occur at any
age.

IBD has no cure. Current therapies for IBD are directed at reducing the
inflammatory process and at reducing the detrimental effects of the inflammatory
20 process associated with the disease, and include administration of anti-inflammatory
drugs (e.g., mesalamine, sulfasalazine, infliximab, adalimumab, prednisone,
budesonide) and of immunosuppressive drugs (e.g., 6-mercaptopurine, azathioprine,
cyclosporine). Such therapies are often associated with adverse side effects.

Hyaluronan, also referred to as hyaluronic acid or hyaluronate, is a non-
25 sulphated glycosaminoglycan comprised of repeating disaccharide units of N-acetyl-
glucosamine and B-glucuronic acid. Hyaluronan is distributed widely throughout
connective tissue of all organs, and is important for maintaining tissue hydration,
cushioning joints, preserving cell free space within specific tissues or regulation of
cell behavior such as migration or proliferation via activation of cell signaling
30 pathways. Hyaluronan is, under normal circumstances, a high molecular weight
(HMW) glycosaminoglycan, which is produced mainly by fibroblasts. As disclosed
herein compositions comprising hyaluronan of specific sizes (about 10 to about 35
kDa) is used to treat intestinal conditions and improve intestinal health in patients.

SUMMARY

Applicants have found that oral administration of specific sized hyaluronan fragments (approximately 35 kDa) reduces the severity of bacterially-driven colitis in mouse models. Accordingly, as disclosed herein compositions comprising low molecular weight hyaluronan are provided for oral administration to improve intestinal health in individuals. More particular, hyaluronan fragments of 10-75 kDa, and more typically about 35 kDa, have been discovered to induce anti-microbial protein production by epithelial cells that line the intestine. Additionally, applicants have found that hyaluronan fragments of molecular weight less than 10 kDa, more particularly at about 4.7 kDa, inhibits the anti-microbial protein production of epithelial cells induced by 35 kDa hyaluronan. One of the anti-microbial protein proteins induced by 35 kDa hyaluronan is human beta defensin 2, which is known to play an important role in protecting against intracellular and extracellular pathogens. Human β -defensin 2 is a small protein (7 kDa) with broad spectrum microbicidal activity. The protein is not constitutively expressed, but is regulated by TLR signaling. Accordingly, in one embodiment compositions comprising hyaluronan fragments of 10-75 kDa, and exclude hyaluronan fragments less than 10 kDa, are used to treat patients suffering from intestinal inflammation and/or intestinal bacterial infection.

In one embodiment, hyaluronan fragments of about 35 kDa are combined with known probiotics components and orally administered (either alone or in combination with other active agents) to individuals susceptible to intestinal infections or other intestinal distress. In one embodiment the low molecular weight hyaluronan is combined with lactoferrin. Lactoferrin is a multifunctional globular protein of the transferrin family with a molecular mass of about 80 kDa that is widely represented in various secretory fluids, such as milk, saliva, tears, and nasal secretions. In one embodiment a composition comprising hyaluronan fragments of 10-75 kDa, and more typically about 35 kDa, and lactoferrin is provided. More particularly, the composition is formulated for oral delivery using an oral pharmaceutically acceptable carrier. Such a composition provides enhanced protection to epithelial surfaces, especially those in contact with the external environment, including for example the

intestine, skin and lung cells, wherein the protective effect is greater than that achieved by either component by itself.

In one embodiment the hyaluronan comprising compositions, formulated for oral delivery, are used to treat patients suffering from a medical condition (e.g., Crohn's disease) or a medical treatment (e.g., antibiotics or radiation treatments) that disrupts normal intestinal flora and intestinal function. The composition can be administered prophylactically, or can be administered after the onset of the symptoms.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. provides a comparison of a cross-section of human colonic mucosa, demonstrating the changes that occur during an Inflammatory Bowel Disease flare in humans, including edema, epithelial/ crypt loss, expansion of smooth muscle layer and leukocyte infiltration.

Fig. 2 shows the changes in weight of mice treated with 2.5 % dextran sodium sulfate (DSS) in their drinking water in the presence or absence of 35 kDa hyaluronan. Treatment with 2.5 % dextran sodium sulfate (DSS) reproducibly initiates colitis and such treated mice are used as a model of colitis. The data shows that co-administration of 35 kDa hyaluronan provides a protective effect from DSS induced colitis.

Fig. 3 is a graph showing the induction of Human β -defensin by hyaluronan in HT29 colon epithelial cells. Human β -defensin is induced by hyaluronan in a size dependent manner.

Fig. 4A & 4B are graphs showing that the induction of Human β -defensin by hyaluronan in HT29 colon epithelial cells is both time (See Fig. 4A) and concentration (Fig. 4B) dependent.

Fig. 5 is a graph presenting data showing the efficacy of 35 kDa hyaluronan and lactoferrin in treating DSS induced colitis. Mice were administered 2.5 % dextran sodium sulfate (DSS) in their drinking water for three days. Starting on day 4 and proceeding through day 12, the mice were divided into four groups and received one of four treatments daily by gavage: 1) 35 kDa hyaluronan (—■—); 2) lactoferrin (--▼---); 3) a combination of 35 kDa hyaluronan and lactoferrin (--◆---); or 4) water (—▲—), as the control. In addition a fifth group of mice were not administered 2.5 %

-4-

dextran sodium sulfate (DSS) and served as a healthy control (—●—). Only the HA 35-lactoferrin treatment group recovered weight to the non-colitis control levels.

Fig. 6A is a bar graph presenting data from an experiment wherein HT29 colonic epithelial cells were contacted with 4.7 kDa, 35 kDa, 2000 kDa HA fragment preparations, individually or in mixed preparations, for 8 hours. More particularly, cells were contacted with HA4.7 (10 μ M, i.e., 0.047mg/ml); HA35 (10 μ M, i.e., 0.35mg/ml), HA2M (0.18 μ M, i.e., 0.35mg/ml) or mixtures thereof as indicated. Human beta defensin 2 (HBD2) levels were determined relative to the housekeeping protein Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Relative HBD2/GAPDH protein expression levels were determined by Western Blot analysis. The results demonstrate that 35 kDa hyaluronan sized fragments induced the greatest accumulation of HBD2, but that 4.7 kDa hyaluronan fragments inhibited the ability of HA35 to induce accumulations of HBD2. Fig. 6B is a reverse transcriptase PCR analysis of RNA isolated from human colon epithelium cells (HT29 cells) treated with various hyaluronan containing compositions, demonstrating the enhanced transcription induced by HA35 and milk protein fragments. Lane 1 is a base pair size standard, lane 2 is a control (no protein), lane 3 is HA 4.7 kDa fragments, lane 4 is HA 35 kDa fragments, lane 5 is human milk fragments and lane 6 is human milk fragments pretreated with hyaluronidase prior to contact with the cells. Only the HA 35 kDa fragments and the human milk fragments produced a substantial increase in HBD2 RNAs, whereby beta actin served as a loading control. Pretreating the human milk fragments with hyaluronidase eliminated that compositions ability to stimulate HBD2 RNA production.

DETAILED DESCRIPTION

DEFINITIONS

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent, but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

As used herein, the term “pharmaceutically acceptable carrier” includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein the term "pharmaceutically acceptable salt" refers to salts of compounds that retain the biological activity of the parent compound, and which are not biologically or otherwise undesirable.

As used herein, the term "treating" includes alleviation of the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms.

As used herein the term "patient" without further designation is intended to encompass any warm blooded vertebrate domesticated animal (including for example, but not limited to livestock, horses, cats, dogs and other pets) and humans.

The term "co-administered" as used herein refers to the administration of two compounds or compositions as part of a single therapeutic regiment. The first and second compounds/composition may be administered together as a mixture as a single dosage form or as separate, multiple dosage forms. Alternatively, the first and second compounds/composition may be administered consecutively to one another as two separate and distinct dosage forms. However, when two or more therapeutic agents are co-administered, each of the separate therapeutic agents are administered within a timeframe wherein the first therapeutic agent is still active *in vivo* upon administration of the second therapeutic agent or treatment. The co-administration of two or more therapeutic agents to a patient does not preclude the separate administration of any of those same therapeutic agents or any other active compound to the patient at another time during a course of treatment.

As used herein the term “colitis” refers to an inflammation of the large intestine (colon, caecum and rectum).

EMBODIMENTS

Hyaluronan is a carbohydrate polymer that is normally deposited in connective tissues around cells of vertebrate animals. Applicants have discovered that oral

-6-

administration of low molecular weight hyaluronan fragments ranging from about 10 to about 75 kDa, and more particularly ranging from about 15 to about 50 kDa induce antimicrobial protein production by epithelial cells that line the intestine. One such induced protein is human beta defensin 2, a protein important in protecting against intracellular pathogens.

Surprisingly, applicants have discovered that hyaluaronan fragments of less than 10 kDa, and more particularly of about 4.7 kDa, inhibit the antimicrobial protein production of epithelial cells that is induced by contacting the cells with hyaluronan fragments ranging from about 10 to about 75 kDa. In accordance with one embodiment a composition of low molecular weight hyaluronan fragments ranging from about 10 to about 75 kDa, is provided wherein said composition is substantially free of haluaronan fragments having a molecular weight of less than 10 kDa. In accordance with one embodiment hyaluronan fragments of less than 10 kDa fail to be detected in the compositions based on polyacrylamide gel electrophoresis analysis or by absorbance detection methods. In one embodiment NaCl elution of specific fragment size ranges is used to alter the HA fragment MW distribution of HA35, removing inhibitory small fragments. In accordance with one embodiment elution of the hyaluaronan fragments is conducted at 1.00 M NaCl or higher to eliminate HA4.7 from the recovered hyaluronan fragments. In accordance with one embodiment a composition comprising hyaluronan fragments ranging from about 10 to about 75 kDa is provided wherein haluaronan fragments having a molecular weight of less than 10 kDa comprise less than 1, 0.5, 0.1, 0.05, or 0.001 % of the hyaluronan content of the composition. In accordance with one embodiment a composition comprising hyaluronan fragments is provided wherein the hyaluronan component of said composition consists essentially of hyaluronan fragments having an mean distribution of about 25 to about 45 kDa, and in a further embodiment the composition comprises less than 1, 0.5, 0.1, 0.05, or 0.001 % of haluaronan fragments having a molecular weight of less than 10 kDa relative to the total detectable hyaluronan content. In one embodiment the hyaluronan compositions disclosed herein comprise less than 1% or less than 0.5% of haluaronan fragments having a molecular weight of 4.7 kDa or less relative to the total hyaluronan content of the composition.

In one embodiment a composition for increasing human beta defensin 2 production is provided wherein the composition comprises hyaluronan fragments

-7-

having a molecular weight of about 35 kDa, and a pharmaceutically acceptable carrier suitable for oral administration, with the proviso that the composition is substantially free of hyaluronan fragments of less than 10 kDa. In one embodiment the composition comprises hyaluronan fragments within the range of about 15 kDa to about 75 kDa, 25 to about 50 kDa or about 30 to about 45 kDa. In one embodiment the composition is substantially free of hyaluronan fragments of about 4.7 kDa. In one embodiment a composition is provided comprising low molecular weight hyaluronan and a pharmaceutically acceptable carrier, wherein the hyaluronan component of the composition consists essentially of hyaluronan fragments within the range of about 25 to about 50 kDa, or having an average molecular weight of about 35 kDa.

Applicants have found that compositions comprising hyaluronan fragments having a molecular weight of about 35 kDa, but are substantially free of hyaluronan fragments of less than 10 kDa, have efficacy in protecting and treating intestinal disorders including for example Crohn's Disease (CD). As shown in Table 1 patients suffering from active Crohn's Disease experience a large drop in human beta defensin 2 (HBD2) that is recovered in some degree during remission.

Table 1

Patient Group	N	Median Concentration of HD-5, ng/ml (IQR)	N	Median Concentration of HBD2, pg/ml (IQR)
Healthy individual	33	12.0 (7.0, 21.9)*	13	280 (170, 555)†
Active CD	32	18.4 (11.6, 49.6)*	12	20 (0, 360) †
CD in remission	17	9.0 (6.2, 18.0)	11	90 (40, 370)

Accordingly, one embodiment of the present disclosure comprises administering a composition comprising hyaluronan fragments having a molecular weight of about 35 kDa, with the proviso that the composition is substantially free of hyaluronan fragments of less than 10 kDa, to a patient suffering an intestinal disease or condition. In one embodiment the administered composition comprises hyaluronan fragments within the range of about 15 kDa to about 75 kDa, 25 to about 50 kDa or about 30 to about 45 kDa. In a further embodiment the administered composition comprises less than 1, 0.5, 0.1, 0.05, or 0.001 % of hyaluronan fragments having a molecular weight of less than 10 kDa, relative to the total hyaluronan content. In one

-8-

embodiment the administered hyaluronan composition comprises less than 1%, or less than 0.5%, of haluaronan fragments having a molecular weight of 4.7 kDa or less, relative to the total hyaluronan content of the composition. In one embodiment the composition is substantially free of hyaluronan fragments of about 4.7 kDa. In one
5 embodiment the hyaluronan compositions disclosed herein are used to treat Crohn's Disease or colitis. In particular, applicants observed that the oral administration of low molecular weight hyaluronan reduces the severity of bacterially-driven colitis in mouse models.

Accordingly, in one embodiment the hyaluaronan compositions disclosed
10 herein are used to treat any patient susceptible to intestinal bacterial infections or subject to intestinal inflammatory conditions caused by chronic disease. In accordance with one embodiment compositions are provided to enhance the intestinal health of the patient or treat any patient susceptible or suffering from compromised intestinal functioning. A patient with compromised intestinal functioning is intended
15 to include any patient suffering from inflammation and/or bacterial infection, or other condition that negatively impacts the functioning of the intestines.

The compositions disclosed herein can be used to prophalactically treat a chronic condition, and thus prevent symptoms associated with a medical condition (e.g., Crohn's disease), or a medical treatment (e.g., antibiotics or radiation
20 treatments), that disrupts normal intestinal flora and intestinal function. Alternatively, the composition can be administered after the onset of the symptoms or after exposure to agents that disrupt normal intestinal flora and intestinal function. In one embodiment the compositions disclosed herein are administered to patients suffering from colitis, including inflammatory bowel diseases such as ileitis, ulcerative colitis,
25 Barrett's syndrome, and Crohn's disease.

In accordance with one embodiment a composition comprising low molecular weight hyaluronan is provided for enhancing the intestinal health of the patient, wherein the composition is substantially free of hyaluronan fragments of less than 10 kDa, and more particularly, substantially free of hyaluaronan fragments of about 4.7
30 kDa. As used herein, low molecular weight hyaluronan is intended to encompass hyaluronan fragments ranging in size anywhere between 10 kDa to 75 kDa, 10 kDa to 50 kDa, 20 kDa to 45 kDa and 30 kDa to 40 kDa. In one embodiment the hyaluronan is about 35 kDa in size. In one embodiment the compositions are substantially free of

hyaluronan fragments having a molecular weight of less than 10 kDa or having a molecular weight of about 4.7 kDa or less. The hyaluronan compositions are formulated for oral delivery using a pharmaceutically acceptable carrier suitable for oral administration.

5 Formulations suitable for oral administration of the hyaluronan compositions disclosed herein can consist of (a) liquid solutions, such as an effective amount of hyaluronan dissolved in diluents, such as water, saline, or juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate
10 aqueous liquid or non-aqueous liquid; packed in liposome's; or as a bolus, etc.; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for
15 example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and cornstarch. Tablet forms can include one or more of lactose, sucrose, manifold, corn starch, potato starch, agonic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants,
20 diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise hyaluronan in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising hyaluronan in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such
25 excipients as are known in the art.

 Hyaluronan fragments having a size of about 10 kDa to about 75 kDa have also been found to stimulate interferon-mediated pathways. Accordingly, in one embodiment compositions comprising 10 kDa to about 75 kDa hyaluronan, but optionally substantially free of hyaluronans having a molecular weight of less than 10
30 kDa, are administered to patients as a defense against pathogens, including intestinal pathogens. In one embodiment the compositions are administered prophylactically, and in another embodiment the compositions are administered after known or suspected contact with an intestinal pathogen as a means of minimizing or preventing

compromised intestinal function. In accordance with one embodiment a method of stimulating interferon-mediated pathways is provided wherein a patient is orally administering a pharmaceutical composition comprising a purified hyaluronan, wherein the hyaluronan has a molecular weight within the range of about 5 to about 50 kDa. Optionally the composition is substantially free of hyaluronan fragments of 4.7 kDa or less. In one embodiment the composition comprises hyaluronan fragments averaging about 35 kDa in size. In one embodiment a composition comprising hyaluronan having a molecular weight of about 5 to about 25 kDa is administered to patients as a defense against rotavirus infection.

Composition comprising hyaluronan having a molecular weight of about 5 kDa have been found to induce defensin expression in monkey cells. In accordance with one embodiment compositions and methods are provided to induce anti-microbial protein production by epithelial cells that line the intestine and thus improve intestinal health in individuals. The method comprises administering orally to mammalian species a composition comprising relatively small molecular weight hyaluronan. More particularly, hyaluronan fragments of 10-50 kDa, and more typically about 35 KDa, have been discovered to enhance production of human beta defensin 2, which is known to play an important role in protecting against intracellular pathogens. In one embodiment, oral administration of hyaluronan fragments of about 10 to about 50 KDa is formulated as a probiotic (either alone or in combination with other active agents) to individuals susceptible to intestinal infections or other intestinal distress. In another embodiment the oral composition is formulated for treating patients suffering from a medical condition (e.g., Crohn's disease) or a medical treatment (e.g., antibiotics or radiation treatments) that disrupts normal intestinal flora and intestinal function.

In accordance with one embodiment a composition is provided comprising low molecular weight hyaluronan and a probiotic. More particularly, in one embodiment about 10 to about 50 kDa or about 10 to about 35 kDa sized hyaluronan is co-administered orally with a standard probiotic as a therapy to 1) aid infants susceptible to intestinal infection or infants not being breast fed; 2) aid patients whose medical condition (e.g., Crohn's disease) or medical treatment (e.g., antibiotics and radiation therapy) removes much of the beneficial intestinal flora that normally protects the intestine. In one embodiment compositions comprising about 10 to about

35 kDa sized hyaluronan are used as an adjunct to currently available bacterial probiotic treatments. Hyaluronan fragments of about 10 to about 50 kDa or about 10 to about 35 kDa in size can be co-administered either as a separate composition relative to the bacterial probiotic formulation or alternatively can be mixed with
5 current bacterial probiotic formulations and administered as a single composition.

Suitable probiotics include a plurality of beneficial microorganisms (such as lactobacilli, acidophilus, and other yogurt cultures), enzymes, or combinations thereof. Suitable probiotics also include any substance that promotes the growth of beneficial microorganisms in the composition or subject to which the composition is
10 administered, either alone or in combination with other probiotics.

The low molecular weight hyaluronan compositions disclosed herein can be further combined with other known agents that enhance intestinal health, including for example glucosamine. The compositions can also comprise at least one electrolyte, vitamin, mineral or trace element. Suitable electrolytes include sodium, potassium
15 and calcium, and can be present in the composition in a concentration of between about 0.1% and about 50%, including any fractional percentage in intervals of about 0.01%. Suitable vitamins and minerals include typical adult daily dosages, for example: Vitamin A (about 1000 to about 10,000 IU; Vitamin B1 or thiamine (about 50 mg); Vitamin B2 or riboflavin (about 50 mg); Vitamin B3 as niacin or niacinamide
20 (about 50 to about 500 mg); Vitamin B5 or pantothenic acid (about 50 to about 100 mg); Vitamin B6 or pyridoxine (about 50 mg); Vitamin B12 (about 300 to about 1000 mcg); Biotin (about 300 mcg); Choline (about 100 mg); Folic acid (about 800 mcg); Inositol (about 100 mg); Para-aminobenzoic acid (about 50 mg); Vitamin C (about 50 mg to about 3000 mg or more, in multiple daily doses); Bioflavonoids (mixed--about
25 500 mg); Hesperidin (about 100 mg); Rutin (about 25 mg); Vitamin D (about 400 IU); Vitamin E (about 200 to about 600 IU); Vitamin K (about 100 mcg); Apatite (for example micocrystalline hydroxyapatite--about 4762 mcg; Chromium (about 150 mcg); Copper (about 3 mg); Iodine (about 225 mcg); Iron (about 18 mg); Magnesium (about 750 to about 1,000 mg); Manganese (about 10 mg); Molybdenum (about 30
30 mcg); and Selenium (about 200 mg); and Zinc (about 50 mg) can also be included in the compositions of the present disclosure. Greater or lesser amounts of vitamins, minerals or trace elements for use in the composition are also contemplated.

-12-

Applicants have discovered that human milk contains hyaluronan.

Importantly, the size of the hyaluronan purified from milk includes hyaluronan fragments of the same size as the active, purified fragments that induce anti-microbial activity. Furthermore, the levels of hyaluronan in the mother's milk are highest immediately after delivery and decrease over approximately 10 weeks to a steady lower level. The infant formula products derived from cow's milk do not have levels of hyaluronan as high as in human milk during early lactation, and soy based infant formula has none. Accordingly, one aspect of the present invention is to supplement current standard infant formula with hyaluronan, having a molecular weight of about 10 to about 50 kDa or about 10 to about 35 kDa, to match the hyaluronan concentrations typically found in human milk during the first few months of lactation. Such hyaluronan supplemented infant formulas can be further supplemented with the addition of an external source of lactoferrin.

Lactoferrin (a protein) is one component contained in milk, that forms complexes with hyaluronan and is secreted by many types of epithelial lining cells. Other investigators have shown that lactoferrin has direct anti-microbial properties and inhibits certain inflammatory responses at epithelial surfaces in the intestine and on the skin. Lactoferrin is a multifunctional globular protein of the transferrin family with a molecular mass of about 80 kDa that is widely represented in various secretory fluids, such as milk, saliva, tears, and nasal secretions. Lactoferrin is one of the components of the immune system of the body, has antimicrobial activity (bacteriocide, fungicide) and is part of the innate defense, mainly at mucoses. In particular, lactoferrin provides antibacterial activity to human infants.

Accordingly, in one embodiment the low molecular weight hyaluronan compositions disclosed herein may be further combined with, or co-administered with, lactoferrin to provide an enhanced protection to epithelial surfaces wherein the protective effect is greater than that achieved by either component by itself, especially for epithelial surfaces in contact with the external environment, for example the intestine, skin and lung. This unique combination is particularly beneficial to infants and individuals susceptible to intestinal infections or other intestinal distress including premature infants or infants that are formula fed; patients with intestinal inflammatory conditions caused by chronic disease; patients who are immunocompromised; patients undergoing chemotherapy; or radiation therapy for cancer; patients susceptible to

nosocomial intestinal infection due to prolonged hospital or nursing home stays; patients suffering from a medical condition (e.g., Crohn's disease) or a medical treatment (e.g., antibiotics or radiation treatments) that disrupts normal intestinal flora and intestinal function. Such compositions are anticipated to have enhanced
5 protective effects on epithelial surfaces especially those in contact with the external environment, including for example the intestine, skin and lung cells.

In accordance with one embodiment a pharmaceutical composition for inhalation is provided for enhancing the health of lung tissues and prevent or remove/diminish bacterial infections of the lung. Any standard device can be used to
10 administer the hyaluronan compositions, including the hyaluronan/lactoferrin compositions, to the lung to treat pulmonary infections. In accordance with one embodiment the hyaluronan compositions disclosed herein are administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in
15 saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, e.g.: Rabinowitz J D and Zaffaroni A C, U.S. Pat. No. 6,803,031.

In accordance with one embodiment a pharmaceutical composition for oral
20 administration is provided for enhancing intestinal health. In one embodiment the composition comprises hyaluronan having a molecular weight within the range of about 15 to about 50 KDa, lactoferrin, and a pharmaceutically acceptable carrier suitable for oral administration. Optionally the compositions are substantially free of hyaluronan fragments having a molecular weight of 10 kDa or less, and in one
25 embodiment are substantially free of hyaluronan fragments having a molecular weight of 4.7 kDa or less. In one embodiment the combination of low molecular weight hyaluronan and lactoferrin is also used as an adjunct to current probiotic (beneficial bacteria) treatments or as an adjunct to current infant formula compositions.

In accordance with one embodiment a composition is provided comprising
30 hyaluronan having a molecular weight within the range of about 5 to about 75 kDa, 10 to about 50 kDa, 25 to about 35 kDa or about 35 kDa, lactoferrin, and a pharmaceutically acceptable carrier suitable for oral administration. In one embodiment the lactoferrin is selected from mammalian species, including for

-14-

example bovine, mouse or human lactoferrin proteins. In one embodiment the lactoferrin is human lactoferrin. In one embodiment the lactoferrin protein comprises the sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or a fragment or derivative of any of those sequences. In a further embodiment the
5 lactoferrin protein comprises an amino acid sequence that shares 70%, 80%, 85%, 90%, 95% or 99% sequence identity with SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4. In one embodiment the lactoferrin protein comprises the sequence of SEQ ID NO: 2.

In accordance with one embodiment, in compositions comprising lactoferrin
10 and hyaluronan, the lactoferrin and hyaluronan are conjugated to one another. Conjugates are formed by linking lactoferrin and hyaluronan, either directly, or indirectly through a linker. Such linkages can be accomplished by covalent chemical bonds, physical forces such electrostatic, hydrogen, ionic, van der Waals, or hydrophobic or hydrophilic interactions. A variety of non-covalent coupling systems
15 may be used, including biotin-avidin, ligand/receptor, enzyme/substrate, nucleic acid/nucleic acid binding protein, lipid/lipid binding protein, cellular adhesion molecule partners; or any binding partners or fragments thereof which have affinity for each other. In one embodiment the lactoferrin protein can be linked to hyaluronan via direct covalent linkage by reacting targeted amino acid residues of the peptide
20 with an organic derivatizing agent that is capable of reacting with reactive groups present on hyaluronan.

In one embodiment the hyaluronan and lactoferrin are covalently linked to one another via a bifunctional linker. In another embodiment the hyaluronan and lactoferrin are linked to a common lipid vesicle, such as a liposome or a micelle.
25 More particularly, in one embodiment the hyaluronan and lactoferrin are linked by covalent coupling to the exterior surface of the liposome or alternatively, one or both compounds can be linked by entrapping the compound within the liposome interior space.

In accordance with one embodiment a method of treating patients in need of
30 improved intestinal function is provided. The method comprises the steps of identifying patients suffering from compromised intestinal function, and orally administering a low weight hyaluronan composition as disclosed herein, where said hyaluronan has a molecular weight within the range of about 5 to about 75 kDa, about

25 to about 50 kDa or about 35 kDa. Optionally the composition is substantially free of hyaluronan having a molecular weight of about 4.7 kDa or less. In one embodiment the patients in need of improved intestinal function include infants and individuals susceptible to intestinal infections or other intestinal distress including premature infants or infants that are formula fed; patients with intestinal inflammatory conditions caused by chronic disease; patients who are immunocompromised; patients undergoing chemotherapy or radiation therapy for cancer; patients susceptible to nosocomial intestinal infection due to prolonged hospital or nursing home stays, patients suffering from a medical condition (e.g., Crohn's disease) or a medical treatment (e.g., antibiotics or radiation treatments) that disrupts normal intestinal flora and intestinal function. In one embodiment the low molecular weight hyaluronan composition further comprises lactoferrin.

In accordance with one embodiment a method of treating active colitis is provided. The method comprises the steps of identifying patients suffering from compromised intestinal function, and orally administering a low weight hyaluronan composition as disclosed herein, where said hyaluronan has a molecular weight within the range of about 10 to about 75 kDa, about 25 to about 50 kDa or about 35 kDa. In one embodiment the low molecular weight hyaluronan composition further comprises lactoferrin. In one embodiment the patient is suffering from an inflammatory bowel disease, including for example Crohn's disease.

In one embodiment a kit is provided for administering the low molecular weight hyaluronan compositions to a patient. In one embodiment the kit comprises the low molecular weight hyaluronan composition and a device for administering the composition to a patient. Depending on the route of administration, the kit may include an inhaler if said composition is an inhalable composition; a spoon, pump, or a vessel with or without volume markings if said composition is an oral liquid composition; or any other measuring or delivery device appropriate to the dosage formulation of the composition present in the kit.

The kit may alternatively, or in addition, include one or more containers, e.g., vials, tubes, bottles, optionally containing the low molecular weight hyaluronan composition in a lyophilized form or in an aqueous solution. Preferably, the kits will also include instructions for use. In one embodiment, the kits of this invention may comprise in a separate container a pharmaceutical composition comprising a second

-16-

therapeutic agent, for co-administration with hyaluronan. In one embodiment the second therapeutic composition comprises lactoferrin.

EXAMPLE 1.

- 5 Combination of HA-35 and lactoferrin promotes recovery from DSS- induced colitis in mice.

Colitis Model: Age matched male C57Bl/6 mice were treated with 2.5 % dextran sodium sulfate (DSS) in their drinking water for five days, a treatment which
10 reproducibly initiates colitis. DSS treatment was discontinued and normal drinking water was supplied immediately after the first treatment (see below).

Treatment: Subsequently, groups of mice were given one of four treatments delivered once per day by oral gavage (single dose delivered into the stomach) for up
15 to 12 days. The four treatments were:

- 1) water (250 µl);
- 2) 300 µg HA-35 (in 250 µl water);
- 3) 600 µg lactoferrin (in 250 µl water);
- 4) 300 µg hyaluronan and 600 µg lactoferrin (in 250 µl water). Treatment 4 is
20 a 1:1 molar ratio of hyaluronan 35 to lactoferrin.

Animals were weighed at regular intervals throughout the treatment, and harvested in groups of 5 at days 1, 4, 8 and 11 after beginning treatment, for histological examination of the colon tissue. The results obtained from groups of mice receiving treatments were compared with the results of a group of age and sex
25 matched control animals that did not receive DSS and therefore did not get colitis. Additionally a group of animals that had colitis induced, but never received any treatment was harvested on Day 5, and used as a positive control.

Results: Mice undergoing intestinal inflammation lose weight, a reflection of organ
30 dysfunction. Consistent with this colitis model, groups of mice that were treated with DSS lost weight for five days after discontinuing treatment. Mice treated with water, HA-35, or lactoferrin alone partially recovered, all three treatment groups rising to the same level, over the next week. However the group receiving HA-35 plus lactoferrin

-17-

recovered weight more rapidly and completely returned to the non-DSS control levels one week later. The average level of histological damage of each group at each time point overall paralleled the weight loss and subsequent gain. Again, the hyaluronan plus lactoferrin group was most like the untreated control at the end of the experiment. These results indicate a synergistic effect of the combined HA-35/lactoferrin treatment and not merely an additive effect of the two active components.

Example 2

10 In vitro cell response of endothelial cells to hyaluronan

Cell Culture - HT29 epithelial cells, a line initially derived from a human intestinal tumor, were washed and plated at a 1:15 area:area ratio in 12-well plates (Becton Dickinson, Franklin Lakes, NJ) and cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS) and incubated at 37° C in a humidified environment containing 5% CO₂ until 70-80% confluent (3 days). Purified, lyophilized HA was purchased from Lifecore Biomedical, LLC, Chaska, MN. The HA size designations were made on the basis of average molecular weight: 4.7 kDa (HA-4.7), 35 kDa (HA-35), 2000kDa (HA-2M). The final concentrations of HA fragment-supplemented medium treatments are as follows: HA4.7 alone at 0.047mg/ml (10 μM), HA35 alone at 0.35mg/ml (10 μM), HA2M alone at 0.35 mg/ml (0.18 μM), HA4.7 with HA35 at 0.047 mg/ml and 0.35mg/ml respectively, and HA35 with HA2M at 0.35mg/ml and 0.35mg/ml respectively. Fragment-supplemented medium treatments containing HA35 and either HA4.7 or HA2M were prepared by adding the HA4.7 or HA2M to an original purified HA35 preparation.

25 On the day of the experiment, growth medium was removed, and the HT29 cells were treated with fresh RPMI containing 10% FBS without or with specific or mixed molecular weight range HA preparations listed above for a total of 8 hours before the harvest of whole cell lysates and subsequent Western blot analysis. A total of 12 replicate cell culture wells per group were treated in this manner.

30

Detection of HβD2 by Immunoblot (Western) Analysis - Whole cell lysates from HT29 cells were isolated for Western blotting in the following lysis buffer: 300 mM NaCl, 50 mM Tris pH 8.0, 0.5% NP-40, 1 mM EDTA, 10% glycerol, protease

-18-

inhibitor for mammalian tissue P8340 (Sigma-Aldrich Inc., St. Louis, MO). Cell lysate proteins were separated by SDS-PAGE using pre-cast Tricine based 4-20% gradient gels (Invitrogen, Carlsbad, CA). Separated proteins were transferred at 4° C to PVDF membrane by electroblotting apparatus (Bio-Rad Laboratories, Hercules, CA) at 100 V for 45 m. PVDF membranes were air-dried at room temperature for 60 m prior to blocking with Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, NE) diluted to 50% concentration in Tris-buffered saline. The membrane was incubated with rabbit polyclonal antibody against H β D2 at 1:750 (Abcam, Cambridge, MA), and the primary antibody was followed by biotin conjugated anti-rabbit IgG at 1:25,000 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), and finally horseradish peroxidase (HRP)-conjugated streptavidin was added at 1:100,000 (GE Healthcare, Piscataway, NJ). Membrane-bound GAPDH protein expression was detected by incubation with rabbit polyclonal antibody against GAPDH at 1:5,000 (Abcam, Cambridge, MA) followed by HRP-conjugated donkey polyclonal anti-rabbit IgG (1:20,000, GE Healthcare, Piscataway, NJ). All washing steps were conducted in Tris-buffered saline with 0.1% Tween-20. Protein bands were visualized using ECL plus chemiluminescent development (GE Healthcare, Piscataway, NJ) and detection by BioMax XAR scientific imaging film (Carestream Health Inc., Rochester, NY). Differences in chemiluminescent signal intensity were quantified using the NCBI ImageJ software package .

Statistical analysis - Statistical difference between treatment groups was evaluated where appropriate by unpaired one-tailed Student's *t*-test and all error bars drawn to indicate the standard error of the means (S.E.M.). Differences were considered significant when $P < 0.05$. Statistical analysis and graphing was completed using GraphPad Prism version 4.0c.

Results

Cell contacted with fragment-supplemented medium treatments containing HA35 showed the greatest production of human beta defensin 2 production (See fig. 6). Cells contacted with the fragment-supplemented medium treatments containing both HA35 and HA4.7 produced less human beta defensin 2, indicating that the addition of 4.7 kDa HA and inhibited the HA-35kDa response. However, adding

-19-

2000 kDa HA to the fragment-supplemented medium treatments containing both HA35 did not inhibit the HA-35 kDa response. This suggests that the HA-35 kDa response is mediated through a receptor that recognizes the smaller molecular weight HA (in animals the receptor is believed to be the Toll like receptor-4) and the 4.7 size is a competitor for the HA35.

5

- 20 -

WE CLAIM:

1. A composition comprising
hyaluronan fragments having a molecular weight of about 35 kDa; and
a pharmaceutically acceptable carrier suitable for oral administration, with the proviso that said composition is substantially free of hyaluronan fragments having a molecular weight of less than 10 kDa.
2. The composition of claim 1 wherein the composition comprises
hyaluronan fragments having a molecular weight within the range of about 15 kDa to about 75 kDa.
3. The composition of claim 2 wherein the composition comprises
hyaluronan fragments having a molecular weight of about 25 to about 50 kDa.
4. The composition of claim 1 wherein the composition is substantially
free of hyaluronan fragments having a molecular weight of about 4.7 kDa.
5. The composition of claim 1 further comprising lactoferrin.
6. The composition of claim 5 wherein the lactoferrin and hyaluronan are
present in a 1:1 molar ratio.
7. The composition of claim 5 wherein the lactoferrin is conjugated to
hyaluronan.
8. The composition of claim 7, wherein the composition further
comprises liposomes wherein said lactoferrin and hyaluronan are linked to said
liposomes.

- 21 -

9. The composition of any one of claims 6-8 wherein said composition is combined with a probiotic.

10. The composition of any one of claims 6-8 wherein said composition is combined with a standard infant formula.

11. A method of treating patients in need of improved intestinal function, said method comprising the steps of
identifying patients suffering from compromised intestinal function; and
orally administering a composition comprising hyaluronan, where said hyaluronan has a molecular weight within the range of about 25 to about 50 kDa, with the proviso that the composition is substantially free of hyaluronan fragments having a molecular weight of less than 10 kDa.

12. The method of claim 11 where in the patient is suffering from an inflammatory bowel disease.

13. The method of claim 11 where in the patient is suffering from Crohn's disease.

14. The method of claim 11 wherein the composition further comprises lactoferrin.

15. A pharmaceutical composition for oral administration, said composition comprising
hyaluronan, having a molecular weight within the range of about 10 to about 50 KDa;
lactoferrin; and
a pharmaceutically acceptable carrier suitable for oral administration.

- 22 -

16. A method of stimulating interferon mediated pathways, said method comprising the steps of
orally administering to said patient a pharmaceutical composition comprising a purified hyaluronan, wherein the hyaluronan has a molecular weight within the range of about 10 to about 50 kDa.
17. The method of claim 16 wherein the composition further comprises lactoferrin.

Fig. 1

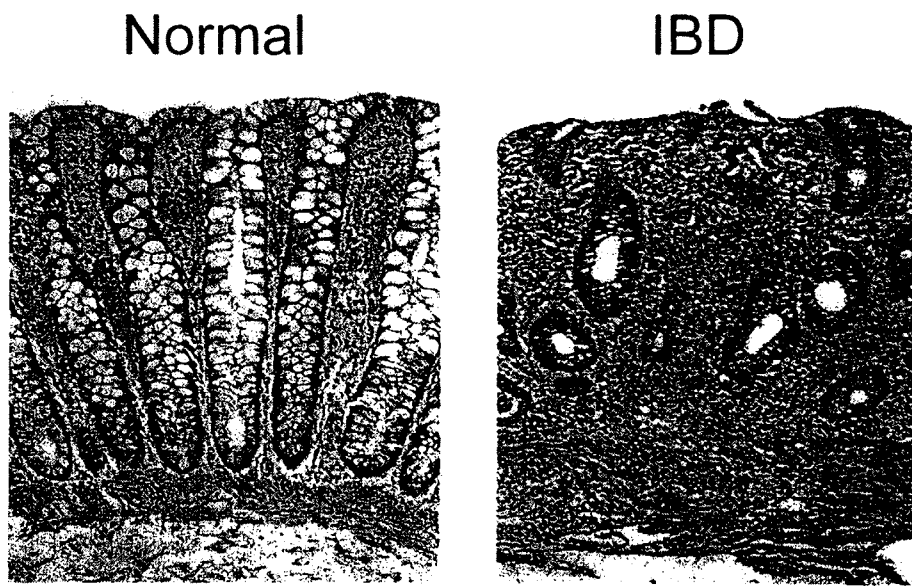
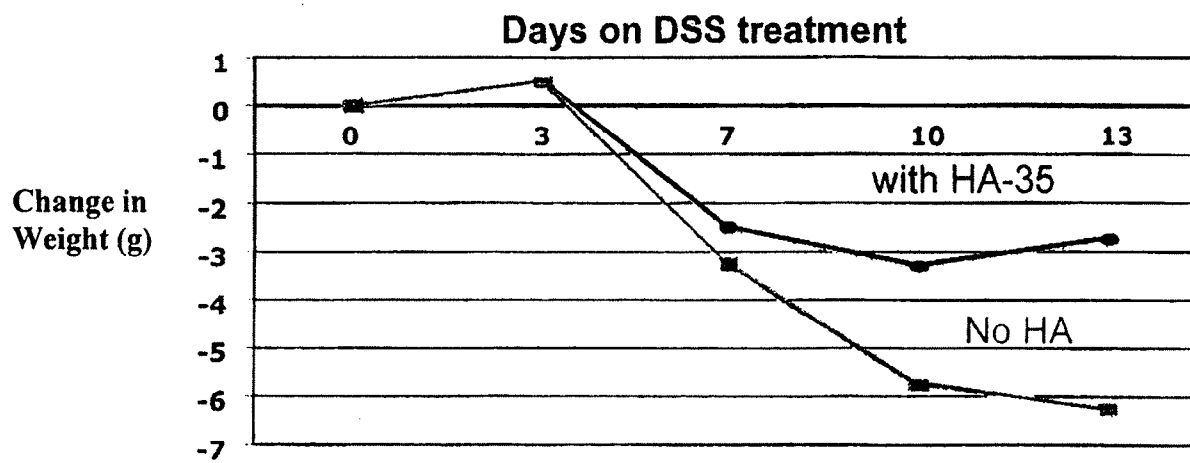


Fig. 2



2/6

Fig. 3

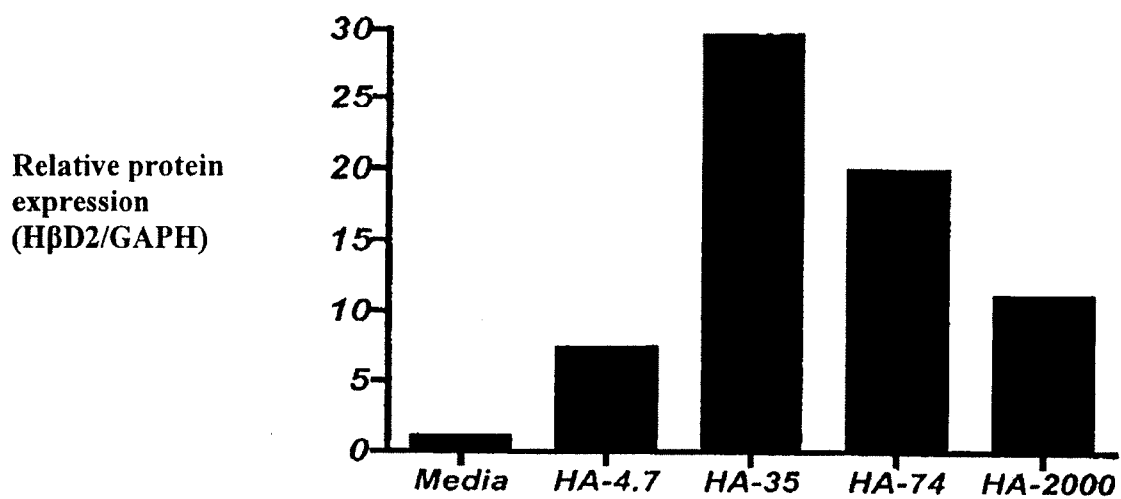
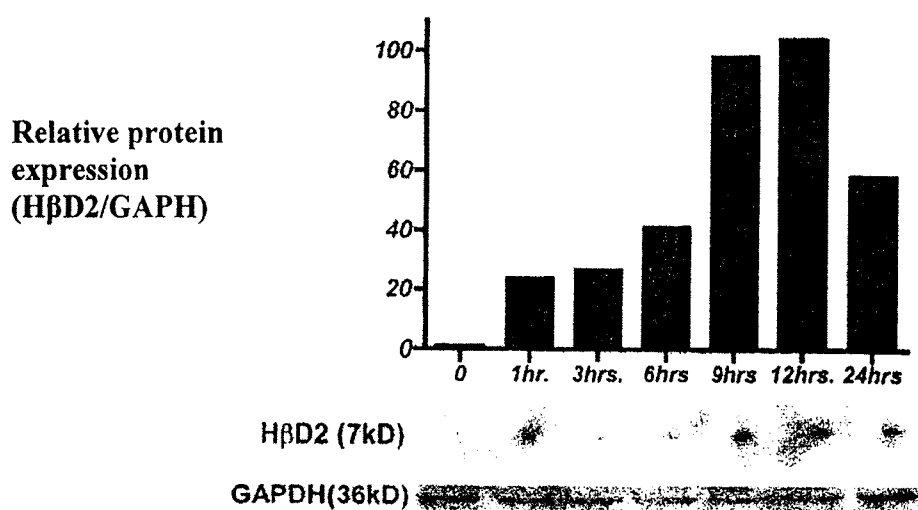


Fig. 4A



3/6

Fig. 4B

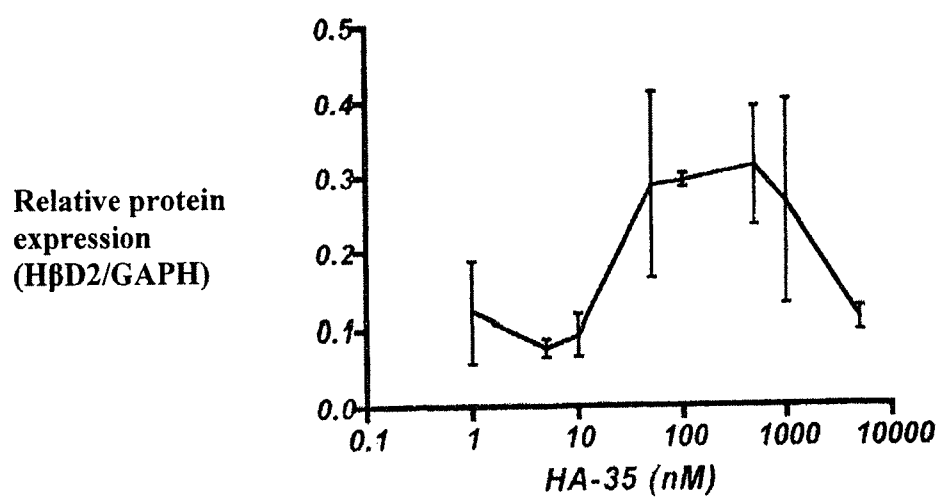


Fig. 5

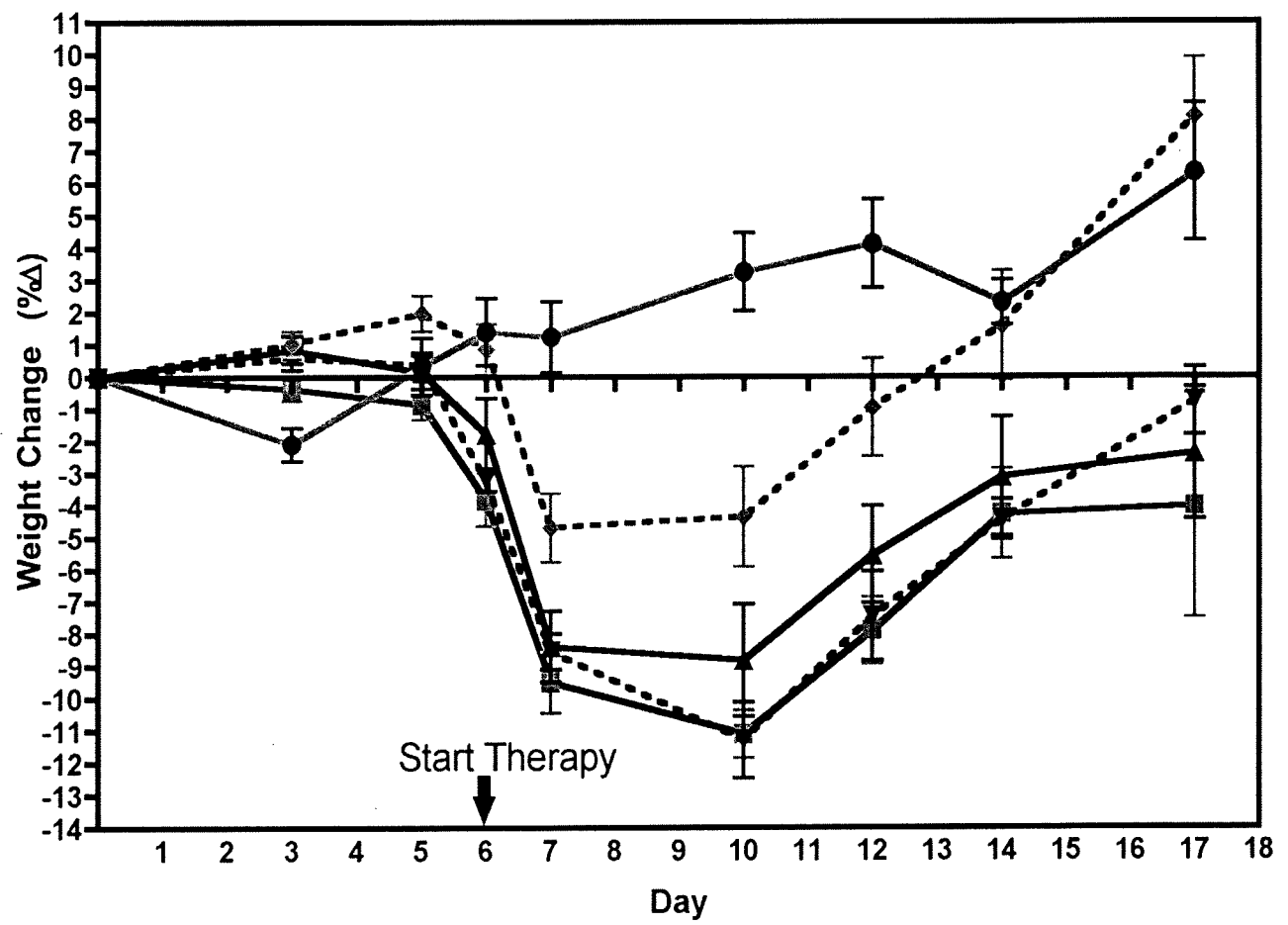
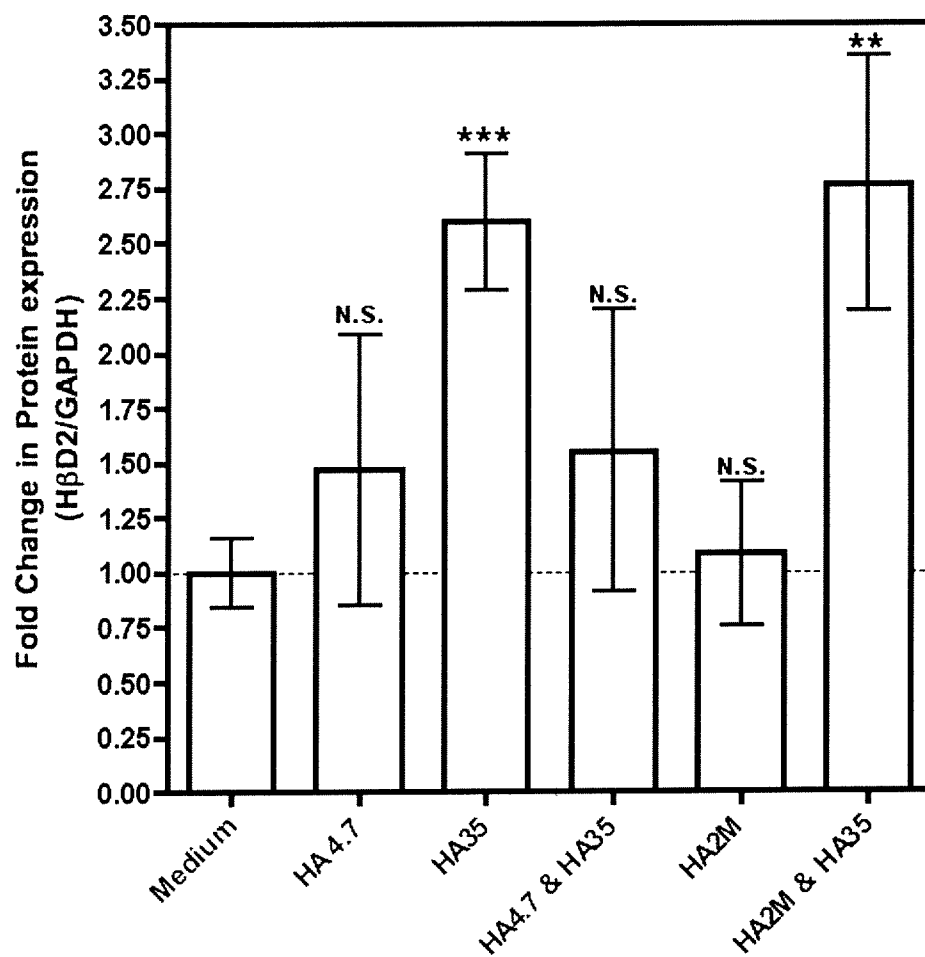
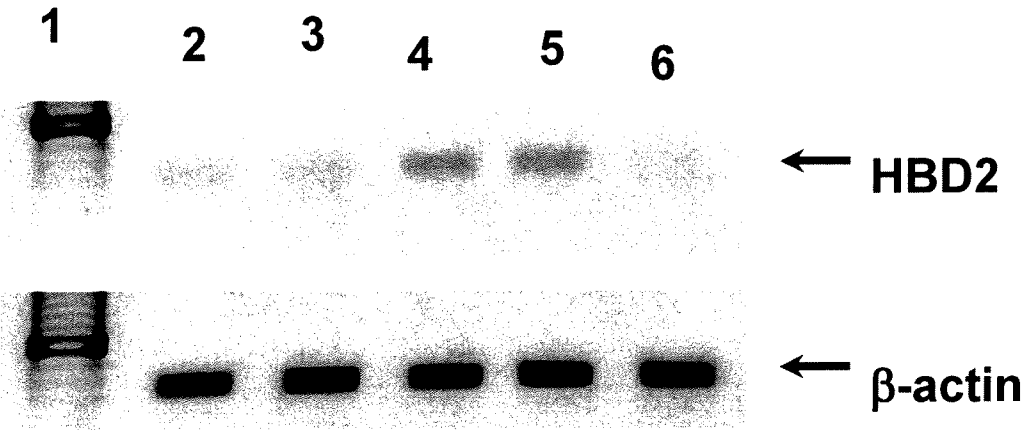


Fig. 6A



N=12. Statistical significance evaluated by comparison of experimental groups to Medium treatment alone using one-tailed Student's T-test. 'N.S.' indicates no significant increase in expression relative to control, while '***' and '***' indicate $P < 0.01$ or $P < 0.001$ respectively.

Fig. 6B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/036936

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 47/36 (2012.01)

USPC - 514/12.2

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)

IPC(8) - A23L 1/30; A61K38/17, 45/00, 47/36; A61P 1/00, 31/04; C07K14/47 (2012.01)

USPC - 424/93.4; 426/61; 514/12.2, 13.3

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

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

MicroPatent, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JING et al. Synchronized Chemoenzymatic Synthesis of Monodisperse Hyaluronan Polymers THE JOURNAL OF BIOLOGICAL CHEMISTRY. Vol. 279, No. 40, Issue of October 1, pp. 42345-42349, 2004 entire document	1-17
Y	ALAUZUN et al. Biocompatible, hyaluronic acid modified silicone elastomers. Biomaterials 31 (2010) 3471-3478 entire document	1-10
Y	US 2007/0142292 A1 (VARADHACHARY et al) 21 June 2007 (21.06.2007) entire document	5-17
Y	US 2010/0074870 A1 (RUSSELL et al) 25 March 2010 (25.03.2010) entire document	9, 10
Y	DE LA MOTTE et al. Platelet-Derived Hyaluronidase 2 Cleaves Hyaluronan into Fragments that Trigger Monocyte-Mediated Production of Proinflammatory Cytokines Am J Pathol 2009, 174:2254-2264. entire document	11-14

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 June 2012

Date of mailing of the international search report

06 JUL 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774