(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 3 572 087 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Sequence listing
Remarks
Sequence listing replaced or added

(48) Corrigendum issued on:
04.10.2023 Bulletin 2023/40

(45) Date of publication and mention of the grant of the patent:
07.06.2023 Bulletin 2023/23

(21) Application number: **17892942.8**

(22) Date of filing: **22.09.2017**

(51) International Patent Classification (IPC):
A61K 31/702 *(2006.01)* ***A23L 33/10*** *(2016.01)*
A61P 19/02 *(2006.01)* ***A61P 17/00*** *(2006.01)*
A23L 33/125 *(2016.01)*

(52) Cooperative Patent Classification (CPC):
(C-Sets available)
A61K 31/702; A23L 33/125; A61P 17/00; A61P 19/02; A23V 2002/00 (Cont.)

(86) International application number:
PCT/KR2017/010489

(87) International publication number:
WO 2018/135719 (26.07.2018 Gazette 2018/30)

(54) COMPOSITION FOR PREVENTING OR TREATING OSTEOARTHRITIS CONTAINING SIALYLLACTOSE OR SALT THEREOF AS ACTIVE INGREDIENT

ZUSAMMENSETZUNG ZUR PRÄVENTION ODER BEHANDLUNG VON OSTEOARTHRITIS MIT SIALYLLACTOSE ODER SALZ DAVON ALS WIRKSTOFF

COMPOSITION DESTINÉE À LA PRÉVENTION OU AU TRAITEMENT DE L'ARTHROSE CONTENANT COMME PRINCIPE ACTIF DU SIALYLLACTOSE OU UN SEL CORRESPONDANT

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **23.01.2017 KR 20170010540**
31.05.2017 KR 20170067915

(43) Date of publication of application:
27.11.2019 Bulletin 2019/48

(73) Proprietor: **Cupione Co., Ltd.**
Gangnam-gu, Seoul 06159 (KR)

(72) Inventors:
- **YANG,Siyoung**
  Suwon-si
  Gyeonggi-do 16224 (KR)
- **JEON, Jimin**
  Daegu 41164 (KR)
- **KANG, Li-Jung**
  Busan 46292 (KR)
- **CHO, Chanmi**
  Jindo-gun
  Jeollanam-do 58909 (KR)

(74) Representative: **Vossius & Partner**
Patentanwälte Rechtsanwälte mbB
Siebertstrasse 3
81675 München (DE)

(56) References cited:
WO-A1-98/48817
WO-A1-2005/011633
WO-A1-2009/095240
WO-A1-2010/116317
WO-A1-2011/100979
KR-A- 20140 088 805
US-A- 5 164 374
US-A1- 2016 199 424

- **ANANTHILA ANANDACOOMARASAMY ET AL: "Current evidence for osteoarthritis treatments", THERAPEUTIC ADVANCES IN MUSCULOSKELETAL DISEASE, vol. 2, no. 1, 1 February 2010 (2010-02-01), pages 17-28, XP055739730, ISSN: 1759-720X, DOI: 10.1177/1759720X09359889**

Processed by Luminess, 75001 PARIS (FR)

(Cont. next page)

- **SGAMBATO, A. ET AL.: 'Different Sialoside Epitopes on Collagen Film Surfaces Direct Mesenchymal Stem Cell Fate' ACS APPLIED MATERIALS & INTERFACES vol. 8, no. 24, 2015, pages 14952 - 14957, XP055504376**

Remarks:

The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

(52) Cooperative Patent Classification (CPC): (Cont.)

C-Sets
A23V 2002/00, A23V 2200/318, A23V 2250/612

Description

TECHNICAL FIELD

[0001] The present invention relates to a composition for preventing or treating osteoarthritis containing 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

BACKGROUND ART

[0002] Osteoarthritis (OA) is a degenerative joint disease primarily caused by inhibition of cartilage extracellular matrix (ECM) synthesis and promotion of cartilage tissue destruction. Many etiological risk factors and pathophysiological processes associated with aging contribute to the progression of osteoarthritis. Joint instability, mechanical stress including injury, and aging-related factors that predispose one to osteoarthritis are potential osteoarthritis-causing mechanisms. These factors activate biochemical pathways in chondrocytes which are a unique cell type that synthesizes various catabolic and anabolic factors, leading to degradation of the ECM by matrix metalloproteinase (Mmp) and cessation of ECM synthesis via dedifferentiation and apoptosis of chondrocytes (Pelletier JP et al., Arthritis Rheum., 44:1237-47, 2001). In particular, cartilage tissue that constitutes a joint is not normally regenerated *in vivo* once it is damaged. If cartilage tissue in a joint is damaged, the cartilage tissue damage impedes daily activities with severe pain. If the damage becomes chronic, it causes fatal osteoarthritis which interferes with normal life or professional activities.

[0003] Until now, therapeutic agents for arthritis have not been developed. Generally, non-steroidal anti-inflammatory drugs (NSAIDs) are used for the purpose of alleviating joint inflammation. However, since NSAID-based drugs are primarily intended to temporarily relieve joint inflammation, NSAID-based drugs do not provide adequate treatment for osteoarthritis which is a non-inflammatory arthritis that requires enhancement of cartilage formation and inhibition of cartilage destruction (Pritchard MH et al., Annals of the Rheumatic Diseases, 37:493-503, 1978). Such NSAIDs are suitable as a therapeutic agent for the prevention of inflammation in rheumatoid arthritis which is an inflammatory arthritis. However, it is pointed out that NSAIDs accelerate cartilage damage or have adverse effects on the cardiovascular system, gastrointestinal tract, kidney, liver, etc.

[0004] Further, an autologous osteochondral transplantation method which was developed for cartilage formation involves collecting cartilage and subchondral bone from a normal part of a patient, and transplanting them into a hole which is made in the damaged cartilage site by drilling, thereby generating hyaline cartilage. Although this method has been successful in some patients, it cannot be universally applied because the method can be performed only for autologous transplant-eligible patients with less cartilage damage (Peterson L et al., J Bone Joint Surg Am. 85-A Suppl:17-24, 2003).

[0005] Meanwhile, among breast milk oligosaccharides, 3'- or 6'-sialyllactose has anti-inflammatory properties that influence intestinal microflora activity, and there is a report that 3'- or 6'-sialyllactose enriches intestinal microflora (Izquierdo-Useros N et al., Plos Biol, 2012, 10). Since sialyllactose is present in breast milk, side effects of ingesting sialyllactose have already been verified, and thus various functions thereof are being studied. Administration of sialyllactose to a patient with rheumatoid arthritis was confirmed to have therapeutic effects on autoimmune diseases caused by change in IgG (US 5164374). However, there have been no reports about prophylactic and therapeutic effects of 3'- or 6'-sialyllactose on osteoarthritis.

[0006] Accordingly, the present inventors have made intensive efforts to find a novel substance capable of efficiently preventing or treating osteoarthritis, and as a result, have found that 3'- or 6'-sialyllactose may promote cartilage formation and inhibit cartilage destruction simultaneously, thereby completing the present disclosure.

SUMMARY OF INVENTION

[0007] An object of the present disclosure is to provide a pharmaceutical composition and a food for preventing, or treating osteoarthritis, the pharmaceutical composition and the food including 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

[0008] Disclosed herein is a method of treating osteoarthritis, the method including administering the composition including 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

[0009] Further disclosed herein is the use of the composition including 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient in the treatment of osteoarthritis.

[0010] In order to achieve the above object, the present disclosure provides a pharmaceutical composition for preventing or treating osteoarthritis, the pharmaceutical composition including 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

[0011] Further, the present disclosure provides a food for preventing or improving osteoarthritis, the food including 3'- or 6'-sialyllactose or a salt thereof acceptable for use as an active ingredient in food.

[0012] Further, the present disclosure provides a method of treating osteoarthritis, the method including administering the composition including 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

[0013] Further, the present disclosure provides use of the composition including 3'- or 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient in the treatment of osteoarthritis.

BRIEF DESCRIPTION OF DRAWINGS

[0014]

FIG. 1 illustrates a mechanism by which osteoarthritis is induced by various catabolic and anabolic factors.

FIG. 2 illustrates chemical structural formulae of (A) 3'-sialyllactose (3'-SL) and (B) 6'-sialyllactose (6'-SL).

FIG. 3 illustrates that 3'-sialyllactose and 6'-sialyllactose did not show cytotoxicity against chondrocytes when chondrocytes were treated with (A) 3'-sialyllactose or (B) 6'-sialyllactose at various concentrations.

FIG. 4 illustrates that type II collagen (Col2a1) expression was increased by treatment of chondrocytes with 0 μM, 50 μM, 100 μM, or 250 μM of 3'-sialyllactose (A and B), Col2a1 expression which was decreased by IL-1β was increased by treatment with 3'-sialyllactose (C and D), and Sox-9 activity which was decreased by IL-1β was increased by treatment with 3'-sialyllactose (E).

FIG. 5 illustrates that type II collagen (Col2a1) expression was increased by treatment of chondrocytes with 0 μM, 50 μM, 100 μM, or 250 μM of 6'-sialyllactose (A), Col2a1 expression which was decreased by IL-1β was increased by treatment with 6'-sialyllactose (B), and Sox-9 which is a transcription factor that regulates type II collagen expression was decreased by IL-1β but increased again by 6'-sialyllactose (C).

FIG. 6 illustrates that Mmp3 and Mmp13 expression inducing cartilage destruction was increased by IL-1β in chondrocytes (A and B), and Mmp3 and Mmp13 expression which was increased by IL-1β was decreased by 3'-sialyllactose (C and D).

FIG. 7 illustrates that Mmp3 and Mmp13 expression inducing cartilage destruction was increased by IL-1β in chondrocytes (A), and Mmp3 and Mmp13 expression which was increased by IL-1β was decreased by 6'-sialyllactose (B).

FIG. 8 illustrates that Erk phosphorylation that was increased by IL-1β in chondrocytes was inactivated by 3'-sialyllactose.

DETAILED DESCRIPTION OF INVENTION AND DESIRED EMBODIMENTS

[0015] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as those generally understood by one of ordinary skill in the art to which the present disclosure belongs. Generally, the nomenclature used herein is well known and commonly employed in the art.

[0016] Arthritis is largely classified into non-inflammatory arthritis and inflammatory arthritis, and non-inflammatory arthritis may be represented by osteoarthritis (OA) and inflammatory arthritis may be represented by rheumatoid arthritis (Yusuf E et al., Ann Rheum Dis, 70:60-67,2011; Berebaum F et al., Osteoarthritis Cartilage, 21:16-21, 2013).

[0017] Osteoarthritis is also called degenerative arthritis, and the etiology thereof is still obscure, but it is known that a variety of triggers such as heredity, trauma, obesity, aging, metabolic abnormalities, etc. are involved. These triggers lead to imbalance between attacking factors and defensive factors in chondrocytes, which promotes cartilage tissue destruction and cartilage wear, and as a result, patients feel pain and experience limitation in movement of the joint due to characteristic pathological changes of osteoarthritis (Pelletier JP et al., Arthritis Rheum., 44:1237-47, 2001).

[0018] In contrast, rheumatoid arthritis (RA) is known to be mainly caused by disease progression due to autoimmune reaction, unlike osteoarthritis caused by destruction of chondrocyte and cartilage tissue. Rheumatoid arthritis is an autoimmune diseases characterized by inflammation and proliferation of synoviocytes, and develops periarticular osteoporosis and bony erosion, unlike osteoarthritis. Rheumatoid arthritis is progressed by spreading of inflammation of synovial membrane to joint capsule, ligament, tendon, and invading to bone. Therefore, osteoarthritis and rheumatoid arthritis are completely different from each other in the etiology and progression, and treatment methods thereof are also different.

[0019] Therapeutic agents for rheumatoid arthritis known until now include non-steroidal anti-inflammatory drugs (NSAIDs), penicillamine, steroidal hormones, TNF inhibitors, interleukin inhibitors, JAK inhibitors, anti-CD related inhibitors, etc., which are suitable for blocking inflammation mechanism (Pritchard MH et al., Ann Rheum Dis, 37:493-503, 1978; 2014 Frost & Sullivan report: Product and pipeline analysis of the global rheumatoid arthritis therapeutics market). NSAIDs and steroidal hormone are used for osteoarthritis patients for the purpose of pain relief and anti-inflammation, but these drugs may not function as practical therapeutic agents for osteoarthritis because they aim at relieving symptoms rather than treating the disease itself (Abramson SB et al., Osteoarthritis Cartilage, 7:380-1, 1999). In addition, since osteoarthritis which is mainly caused by destruction of chondrocyte and cartilage tissue is quite different from rheumatoid arthritis which is inflammatory arthritis, in terms of the cause and symptoms, a method of treating osteoarthritis is also

different from that of rheumatoid arthritis.

[0020] For example, until 2014, development of most therapeutic agents for osteoarthritis proceeded in a direction that cartilage regeneration is promoted by transplantation of various scaffolds with Col2a1 and ECM secretion-promoted mesenchymal stem cells into cartilage defects. In contrast, development of therapeutic agents for rheumatoid arthritis proceeds in a direction that inflammatory cytokines are ultimately inhibited by developing TNF inhibitors, interleukin inhibitors, JAK inhibitors, anti-CD-related inhibitors, etc. (2014 Frost & Sullivan report: 1. A product and pipeline Analysis of the Global knee cartilage repair market, 2. Product and pipeline analysis of the global rheumatoid arthritis therapeutics market). That is, it can be seen that therapeutic targets of osteoarthritis having a non-inflammatory feature and rheumatoid arthritis having an inflammatory feature take different forms according to various types of arthritis.

[0021] Based on these results, it can be seen that osteoarthritis and rheumatoid arthritis have completely different causes of disease, and therapeutic agents which are currently under development are focused on cartilage regeneration for osteoarthritis and inflammation inhibition for rheumatoid arthritis. Accordingly, target strategy for the treatment of osteoarthritis should be different from target strategy for the treatment of inflammatory rheumatoid arthritis.

[0022] Furthermore, document WO 98/48817 discloses certain methods and compositions for inhibiting proliferation of endothelial cells by contacting the cells with a sialic acid or a sialyl glycoside. These methods are useful for treating conditions that are characterized by undesirable celllular proliferation.

[0023] As used herein, the terms "osteoarthritis (OA)" and "degenerative arthritis" may be used interchangeably with each other, and it should be understood that they have the same meanings.

[0024] In the present disclosure, it was confirmed that 3'- or 6'-sialyllactose promotes expression of type II collagen (Col2a1) that plays an important role in joint formation and inhibits expression of Mmp3 and Mmp13 that promote destruction of cartilage tissue at the same time, while having no cytotoxicity on chondrocytes. It was also confirmed that 3'- or 6'-sialyllactose is directly involved in the regulation of Sox9 which is a transcription factor involved in Col2a1 expression, and 3'-sialyllactose directly regulates the pErk signal transduction pathway involved in Mmp3 and Mmp13 expression.

[0025] Accordingly, an aspect of the present disclosure relates to a pharmaceutical composition for preventing or treating osteoarthritis, the pharmaceutical composition including sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

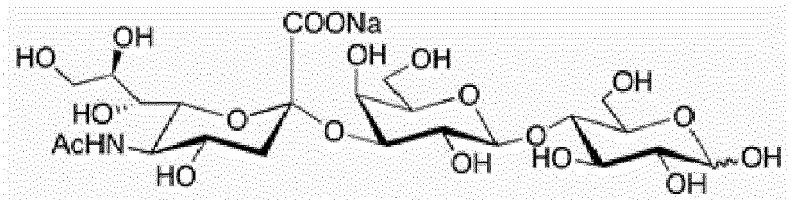
[0026] In the present disclosure, the sialyllactose may be 3'-sialyllactose or 6'-sialyllactose.

[0027] As used herein, the term "pharmaceutically acceptable salt" refers to a formulation of a compound that does not cause significant irritation to an organism to which the compound is administered and does not abrogate the biological activity and properties of the compound. The pharmaceutical salts may include acid addition salts which may form non-toxic acid addition salts containing pharmaceutically acceptable anions, for example, inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, hydrobromic acid, hydriodic acid, etc.; organic carbonic acids such as tartaric acid, formic acid, citric acid, acetic acid, trichloroacetic acid, trifluoroacetic acid, gluconic acid, benzoic acid, lactic acid, fumaric acid, maleic acid, salicylic acid, etc.; sulfonic acids such as methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, etc. For example, the pharmaceutically acceptable salt may also include metal salts or alkali earth metal salts formed by lithium, sodium, potassium, calcium, magnesium, etc.; amino acid salts such as lysine, arginine, guanidine, etc.; organic salts such as dicyclohexylamine, N-methyl-D-glucamine, tris(hydroxymethyl)methylamine, diethanolamine, choline, triethylamine, etc.

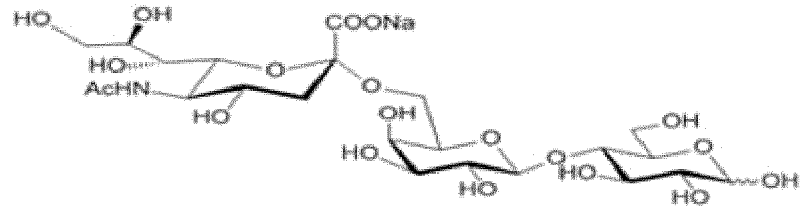
[0028] In the present disclosure, the pharmaceutically acceptable salt of 3'- or 6'-sialyllactose may be Na, but is not limited thereto.

[0029] The salt of 3'-sialyllactose may have a structure of the following Formula 1, and the salt of 6'-sialyllactose may have a structure of the following Formula 2, but are not limited thereto:

[Formula 1]



[Formula 2]



[0030] A test single compound used in the present disclosure is 3'- or 6'-sialyllactose having a structural formula of $C_{23}H_{38}NO_{19}Na$, which is a natural source-derived single compound abundant in breast milk (FIG. 2).

[0031] In the present disclosure but not encompassed by the present invention, the 3'- or 6'-sialyllactose may include a derivative thereof.

[0032] As used herein, the term "derivative" refers to a compound which is modified by introduction, substitution, oxidation, reduction, etc. of functional groups of 3'- or 6'-sialyllactose without significant changes in the structure and properties of a parent compound. There is no limitation in a kind of the functional groups, and for example, the functional groups may include each independently C1 to C20 bicyclic hydrocarbon groups substituted or unsubstituted with a hydroxyl group, a phenoxy group, a thienyl group, a furyl group, a pyridyl group, a cyclohexyl group, an alkyl alcohol group, an alkyl dialcohol group, or a substituted or unsubstituted phenyl group; C3 to C30 cyclic hydrocarbon groups substituted or unsubstituted with a hydroxyl group, a hydroxymethyl group, a methyl group, or an amino group; or sugar residues, but are not limited thereto.

[0033] As used herein, the term "sugar residue" refers to a group available on elimination of one hydrogen atom from a polysaccharide molecule, and therefore, the sugar residue may be, for example, a residue derived from a monosaccharide or an oligosaccharide.

[0034] As used herein, the term "substituted" means, unless otherwise specified, that at least one hydrogen atom among functional groups is substituted with a halogen atom (F, Cl, Br, or I), a hydroxyl group, a nitro group, a cyano group, an imino group (=NH, =NR, where R is a C1 to C10 alkyl group), an amino group (-NH_2,-NH(R'), -N(R")(R"'), where R',R",R"' are each independently a C1 to C10 alkyl group), an amidino group, a hydrazine group, a hydrazone group, a carboxyl group, a C1 to C20 alkyl group, C6 to C30 aryl group, a C3 to C30 cycloalkyl group, a C3 to C30 heteroaryl group, or a C2 to C30 heterocycloalkyl group.

[0035] In the present disclosure, a pH range at which the 3'- or 6'-sialyllactose or 3'- or 6'-sialyllactose derivative shows stability may be pH 4 to pH 10, but is not limited thereto.

[0036] In the present disclosure, the pharmaceutical composition for preventing or treating osteoarthritis including 3'- or 6'-sialyllactose as an active ingredient may have one or more of the following properties of:

1) increasing expression of type II collagen (Col2a1);
2) decreasing expression of matrix metalloproteinases (Mmp3) or matrix metalloproteinase13 (Mmp13);
3) increasing Sox-9 activity; and
4) increasing inactivation of p-ERK.

[0037] In the present disclosure, the pharmaceutical composition may further include a pharmaceutically acceptable carrier, excipient, or diluent. The "pharmaceutically acceptable carrier" refers to a substance that may be added to the active ingredient to aid preparation or stabilization of a formulation without causing a significant adverse toxicological effect on a patient.

[0038] The carrier refers to a carrier or diluent that does not cause irritation to a patient and does not abrogate the biological activity and properties of 3'- or 6'-sialyllactose of the present disclosure. When the composition is formulated into a liquid solution, the pharmaceutically acceptable carrier may be a mixture of one or more of saline, sterile water, Ringer's solution, buffered saline, an albumin injectable solution, a dextrose solution, a maltodextrin solution, glycerol, and ethanol, which are sterile and biocompatible. If necessary, other common additives, including an antioxidant, a buffer, a bacteriostatic agent, etc. may be added thereto. Further, a diluent, a dispersant, a surfactant, a binder, and a lubricant may be additionally added thereto to prepare the composition as a formulation for injection such as an aqueous solution, a suspension, and an emulsion, or as a pill, a capsule, a granule, or a tablet. Other carriers are described, for example, in a literature [Remington's Pharmaceutical Sciences (E. W. Martin)].

[0039] Pharmaceutically acceptable carriers may include sterile aqueous solutions or dispersions and sterile powders for extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. The composition may be formulated for parenteral injection. The

composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, etc.), and suitable mixtures thereof. In some cases, the composition may include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride. Sterile injectable solutions may be prepared by incorporating a required amount of 3'- or 6'-sialyllactose in an appropriate solvent with one or a combination of ingredients described above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those described above. In the case of sterile powders for the preparation of sterile injectable solutions, preparation methods are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient and any additional desired ingredient from a previously sterile-filtered solution thereof.

[0040] Further, the pharmaceutical composition according to the present disclosure may be administered orally or parenterally in an administration dose and frequency which may vary depending on severity of a patient suffering from pain. The composition may be administered to a patient in a bolus or continuous form, as needed.

[0041] The composition including sialyllactose of the present disclosure may inhibit cartilage destruction due to aging of the joint and may promote cartilage formation, thereby treating osteoarthritis.

[0042] Methods of treating osteoarthritis known until now may include replacement arthroplasty, arthroplasty, joint transplantation, and autologous chondrocyte implantation. However, since replacement arthroplasty requires joint incision, it impose pain and burden on a patient, and the procedure is complicated and difficult. In addition, replacement arthroplasty is performed only for autologous transplant-eligible patients, and thus there are many restrictions in the treatment (Peterson L et al., J Bone Joint Surg Am, 85:17-24, 2003). Autologous chondrocyte implantation is a method of obtaining chondrocytes from a cartilage tissue collected from a normal site of a patient, culturing and proliferating the desired number of the chondrocytes ex *vivo,* and then introducing the chondrocytes into a damaged site of cartilage. However, this procedure is also complicated and difficult, because donor tissues are limited, and a surgery is required for collection of a tissue for implantation (Yoon et al., Jorunal of Rheumatic Diseases, 19, 2012). In addition, there is a method of obtaining mesenchymal stem cells from a tissue such as autologous bone marrow, muscle, fat, etc., differentiating the cells ex *vivo,* and then injecting the cells into a damaged site of cartilage. However, there is a risk that mesenchymal stem cells may differentiate into hypertrophic chondrocytes when TGF-b is used to induce differentiation of mesenchymal stem cells into chondrocytes, and mesenchymal stem cells may differentiate into osteophytes when BMP is used to induce differentiation of mesenchymal stem cells into chondrocytes (1. Park et al., J of Korean Orthopaedic Research Society, 18:2, 2015; Mamidi MK et al., Osteoarthritis Cartilage, 24:1307-16, 2016). Substantially, most drugs or health foods for osteoarthritis which have been developed until now tend to focus on pain relief and anti-inflammation effects rather than focusing on chondrocyte activation and cartilage regeneration which are critical to osteoarthritis treatment.

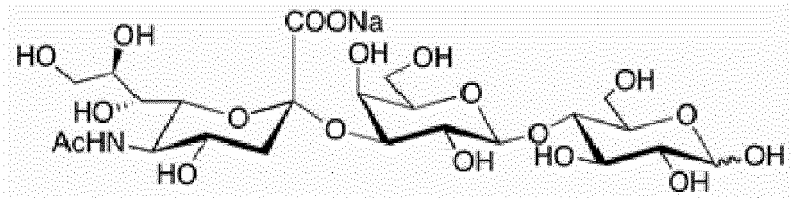
[0043] Therefore, 3'- or 6'-sialyllactose which is one of breast milk components having no adverse effect on human body is expected to be used as a raw material that may prevent, treat, or improve osteoarthritis and may solve problems of the known therapeutic drugs or health foods for osteoarthritis, including side effects, reduced cartilage regeneration effects, and safety.

[0044] Another aspect of the present disclosure relates to a method of treating osteoarthritis, the method including administering the composition including sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

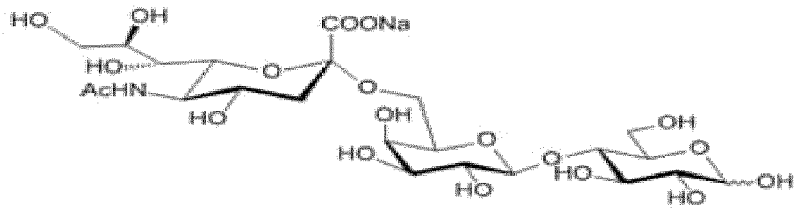
[0045] Still another aspect of the present disclosure relates to use of the composition including sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient in the treatment of osteoarthritis.

[0046] In the present disclosure, the sialyllactose may be 3'-sialyllactose or 6'-sialyllactose, and more preferably, the salt of 3'-sialyllactose may have a structure of the following Formula 1, and the salt of 6'-sialyllactose may have a structure of the following Formula 2, but are not limited thereto:

[Formula 1]



[Formula 2]



[0047] Still another aspect of the present disclosure relates to a food for preventing or improving osteoarthritis, the food including sialyllactose or a salt thereof acceptable for use as an active ingredient in food.

[0048] In the present disclosure, the sialyllactose may be 3'-sialyllactose or 6'-sialyllactose.

[0049] In the present disclosure, the salt of 3'-sialyllactose acceptable for food use may have the structure of Formula 1, and the salt of 6'-sialyllactose acceptable for food use may have the structure of Formula 2, but are not limited thereto.

[0050] In the present disclosure, the salt of 3'- or 6'-sialyllactose acceptable for food use may be Na, but is not limited thereto.

[0051] In the present disclosure, but as not encompassed by the present invention, the 3'- or 6'-sialyllactose may include a derivative thereof.

[0052] The food of the present disclosure may be prepared in any form of a functional food, a nutritional supplement, a health food, and a food additive. For example, as the health food, the 3'-sialyllactose of the present disclosure may be drunken after being prepared in a form of teas, juices, and drinks, or may be taken after granulation, encapsulation, and powdering. Further, the functional food may be prepared by adding 3'-sialyllactose of the present disclosure to beverages (including alcoholic beverages), fruits and their processed foods (e.g., canned fruits, bottled foods, jam, marmalade, etc.), fish, meat, and their processed food (e.g., ham, sausage, corn beef, etc.), breads and noodles (e.g., udon noodles, buckwheat noodles, ramen noodles, spaghetti, macaroni, etc.), fruit juices, various drinks, cookies, taffy, dairy products (e.g., butter, cheese, etc.), edible vegetable oils, margarine, vegetable proteins, retort foods, frozen foods, various seasonings (e.g., soybean paste, soy sauce, sauce, etc.), etc.

[0053] Further, the health functional food includes various forms, such as functional food, nutritional supplements, health food, and food additives, as a food composition, and may be provided in various forms according to a general method known in the art, for example, by preparing the 3'- or 6'-sialyllactose in a form of tea, juice, or drink, or by granulating, encapsulating, or powdering the 3'- or 6'-sialyllactose, or adding the compound or the extract to various foods including beverages, fruits and their processed foods, fish, meat and their processed foods, breads, noodles, seasonings, etc.

EXAMPLES

[0054] Hereinafter, the present disclosure will be described in more detail with reference to embodiments. However, it is apparent to those skilled in the art that these embodiments are for more detailed explanation, and the scope of the present disclosure is not intended to be limited by these embodiments.

Example 1: Measurement of Cytotoxicity of Sialyllactose on Chondrocytes

[0055] Chondrocytes were obtained from cartilage tissues derived from femoral heads, femoral condyles, and tibial plateaus of normal mouse at 5 days after birth. The obtained chondrocytes were cultured in DMEM medium (Gibco, USA) containing 10%(v/v) fetal bovine serum (Gibco, USA), 50 μg/ml of streptomycin (Sigma-Aldrich, USA) and 50 unit/ml of penicillin (Sigma-Aldrich, USA).

[0056] In order to confirm that 3'- or 6'-sialyllactose has no cytotoxicity on chondrocytes, chondrocytes were cultured in a 96-well culture plate at a density of 9×10^3 cells/well, and then treated with 3'- or 6'-sialyllactose (Genechem Inc., Daejeon, Korea) at a concentration of 0 μM, 10 μM, 50 μM, 100 μM, or 250 μM, followed by incubation in a 5% CO_2 incubator at 37°C for 24 hrs. Cytotoxicity of 3'- or 6'-sialyllactose on chondrocytes was confirmed by measuring absorbance at 450 nm using an EZ-Cytox Cell viability assay kit (DoGen, Korea).

[0057] As a result, 3'-sialyllactose and 6'-sialyllactose did not show cytotoxicity on chondrocytes at any concentration, suggesting that they do not adversely affect chondrocyte proliferation (FIG. 3).

Example 2: Examination of Effects of Sialyllactose on Cartilage Formation and Regeneration

2-1: Increase of Expression of Type II Collagen (Col2a1)

[0058] In order to examine effects of 3'- or 6'-sialyllactose on cartilage formation and regeneration, the chondrocytes obtained in Example 1 were incubated for 36 hrs and then treated with 3'- or 6'-sialyllactose at a concentration of 0 μM, 10 μM, 50 μM, 100 μM, or 250 μM, followed by further incubation for 36 hrs.

[0059] Next, in order to perform qRT-PCR, RNA was extracted from the chondrocytes using a TRI reagent (Molecular Research Center Inc.), and cDNA obtained by reverse transcription of RNA was amplified by PCR using primers of SEQ ID NOS: 1 and 2 under condition of annealing temperature of 55°C to examine expression of type II collagen (Col2a1, 173bp) which is essential for cartilage formation. As a control group, Gapdh (450 bp, annealing temperature of 58°C) was examined by using primers of SEQ ID NOS: 3 and 4.

SEQ ID NO: 1: 5'-CACACTGGTAAGTGGGGCAAGA-3' (Col2a1-S)
SEQ ID NO: 2: 5'-GGATTGTGTTGTTTCAGGGTTCG-3' (Col2a1-AS)
SEQ ID NO: 3: 5'-TCACTGCCACCCAGAAGAC-3' (Gapdh-S)
SEQ ID NO: 4: 5'-TGTAGGCCATGAGGTCCAC-3' (Gapdh-AS)

[0060] Further, a whole cell lysate was extracted from the chondrocytes using a lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris, 5 mM NaF) containing protease and phosphatase inhibitor cocktails (Roche), and Col2a1 expression in the cells was examined. Western blotting was performed using anti-Col2a1 antibody (Millipore) and anti-Erk antibody (Cell signaling), and thickness and concentration of Western blot bands were measured by a computer program and relative values thereof were determined by densitometry (FIGS. 4A and 4B).

[0061] As a result, it was confirmed that Col2a1 expression in chondrocytes was increased by 3'-sialyllactose or 6'-sialyllactose, indicating that 3'-sialyllactose and 6'-sialyllactose have the effect of promoting cartilage formation (FIGS. 4A and 4B and FIG. 5A).

2-2: Increase of Expression of Type II Collagen (Col2a1) Suppressed by IL-1β

[0062] IL-1β is a representative inflammatory cytokine inhibiting Col2a1 expression in chondrocytes. Chondrocytes were incubated for 36 hrs, and then treated with 5 ng/ml of IL-1β (GeneScript, USA) for 24 hrs to confirm that Col2a1 expression was decreased by IL-1β.

[0063] In order to examine whether the decreased Col2a1 expression is increased again in chondrocytes by 3'- or 6'-sialyllactose, qRT-PCR and Western blotting were performed in the same manner as in Example 2-1.

[0064] As a result, it was confirmed that Col2a1 expression suppressed by IL-1β in chondrocytes was gradually increased by 3'- or 6'-sialyllactose (FIGS. 4C and 4D and FIG. 5B), indicating that cartilage formation and regeneration may be promoted by 3'-sialyllactose or 6'-sialyllactose.

Example 3: Activation of Cartilage Formation and Regeneration Signaling Pathways by Sialyllactose

[0065] Col2a1 expression essential for cartilage formation and regeneration is regulated by a transcription factor Sox-9, and therefore, it was examined whether Sox-9 transcription factor is regulated by 3'-sialyllactose.

[0066] A Sox-9 reporter gene was prepared by inserting 48-bp Sox9 binding site in the first intron of human Col2a1 gene into the upstream of SV40 promoter in pGL3 vector (Zhou G et al., J Biol Chem 1998, 12, 14989-97).

[0067] 1 μg of the Sox-9 reporter gene was transfected into chondrocytes using lipofectamine 2000 (Invitrogen) for 3 hrs. The transfected cells were co-treated with 5 ng/ml interleukin 1 beta (IL-1β) and 0 μM, 10 μM, 50 μM, 100 μM, or 250 μM of 3'- or 6'-sialyllactose for 24 hrs, and then chondrocytes were recovered to examine Sox-9 activity by luciferase activity.

[0068] As a result, it was confirmed that Sox-9 activity decreased by IL-1β was restored by 3'- or 6'-sialyllactose (FIG. 4E and FIG. 5C), indicating that 3'- or 6'-sialyllactose directly regulates Sox-9 activity, leading to regulation of Col2a1 expression essential for cartilage formation. In other words, cartilage formation and regeneration are promoted by 3'-sialyllactose or 6'-sialyllactose.

Example 4: Examination of Inhibition of Articular Inflammation and Cartilage Destruction by Sialyllactose

[0069] IL-1β is a representative inflammatory cytokine that decreases Col2a1 essential for cartilage formation in chondrocytes and also promotes articular inflammation and cartilage tissue destruction. Chondrocytes were treated with 5 ng/ml of IL-1β by time, and then qRT-PCR was performed using conditions and primers of the following Table 1

according to the method of Example 2-1 to examine inhibition of Mmp3 and Mmp13 expression.

[Table 1]

SEQ ID NO.	Sequence (5'-3')	Sense/ Antisense	Gene	Size (bp)	Annealing temperature (AT, °C)
5	TCCTGATGTTGGTGGCTTCAG	S	Mmp3	102	58
6	TGTCTTGGCAAATCCGGTGTA	AS			
7	TGATGGACCTTCTGGTCTTCTGG	S	Mmp13	473	55
8	CATCCACATGGTTGGGAAGTTCT	AS			

[0070] Secretory proteins such as Mmp3 and Mmp13 were allowed to react at 0°C for 20 min after reacting 900 μl of serum-free medium (conditioned medium) with 100 μl of trichloroacetic acid (TCA). Next, a supernatant was discarded by centrifugation at 12,000 rpm and 4°C for 10 min, and then reacted with 500 μl of 100% cold acetone at 20°C for 1 hr. The sample reacting with 100% acetone was centrifuged to discard a supernatant, and proteins were finally precipitated and detected. Western blotting was performed using anti-Mmp3 antibody (Abcam) and anti-Mmp13 antibody (Abcam), and thickness and concentration of Western blot bands were measured by a computer program and relative values thereof were determined by densitometry.

[0071] As a result, it was confirmed that Mmp3 and Mmp13 expression which induces cartilage tissue destruction causing articular inflammation was increased in chondrocytes by IL-1β (FIGS. 6A, 6B, and 7A).

[0072] Accordingly, the chondrocytes were treated with 5 ng/ml of IL-1β and 0 μM, 10 μM, 50 μM, 100 μM, or 250 μM of 3'- or 6'-sialyllactose for 24 hrs to examine Mmp3 and Mmp13 expression levels. qRT-PCR was performed using the conditions and primers of Table 1, and Western blotting was performed to confirm that Mmp3 and Mmp13 expression increased by IL-1β in chondrocytes was decreased by 3'- or 6'-sialyllactose in a concentration-dependent manner (FIGS. 6C, 6D, and 7B), indicating that articular inflammation and cartilage tissue destruction may be alleviated and inhibited by 3'-sialyllactose or 6'-sialyllactose.

Example 5: Inhibition of Cartilage Destruction Signal Transduction Pathway by Sialyllactose

[0073] Mmp3 and Mmp13 which are cartilage-destroying factors and are increased by IL-1β are activated via various signal transduction pathways in chondrocytes. Accordingly, it was examined whether 3'-sialyllactose is able to block various signal transduction pathways which are regulated by IL-1β.

[0074] Chondrocytes of mouse knee joint were treated with 5 ng/ml of IL-1β for 10 min to examine activation of extracellular-signal regulated kinase (Erk) through Erk phosphorylation.

[0075] Chondrocytes were co-treated with 5 ng/ml of IL-1β and 0 μM, 50 μM, 100 μM, or 250 μM of 3'-sialyllactose, and Erk phosphorylation increased by IL-1β was confirmed to be decreased by 3'-sialyllactose (FIG. 8). That is, Western blotting and densitometry showed that among the signal transduction pathways capable of activating Mmp3 and Mmp13 by IL-1β, Erk signal transduction pathway may be inhibited by 3'-sialyllactose, thereby inhibiting Mmp3 and Mmp13.

[0076] In general, Erk activation or promotion is also found in tissues of osteoarthritis patients (Yang et al., Nat Med, 2010), suggesting that 3'-sialyllactose may strongly inhibit the cartilage destruction signaling pathway that is most involved in osteoarthritis patients.

Statistical analysis

[0077] All results of Examples of the present disclosure were analyzed by the nonparametric statistical method using data based on ordinal grading systems, such as Mankin scores. qRT-PCR data presented as the fold change were initially tested for conformation to a normal distribution using the Shapiro-Wilk test, then analyzed by Student's t-test and analysis of variance (ANOVA) with post hoc tests each for pair-wise comparisons and multi-comparisons as appropriate. Significance was accepted at the 0.05 level of probability ($P < 0.05$).

INDUSTRIAL APPLICABILITY

[0078] 3'- or 6'-sialyllactose of the present disclosure may promote cartilage formation and may effectively inhibit cartilage destruction at the same time, and therefore, it may be useful as a composition for preventing or treating osteoarthritis.

Sequence List Free Text

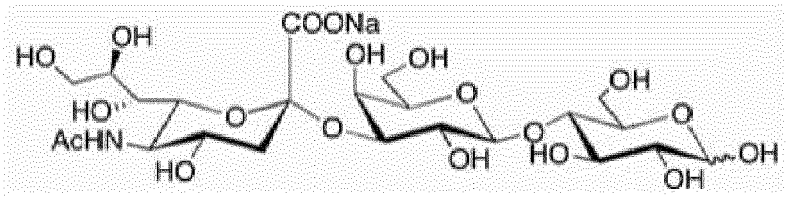
[0079] The electronic file was attached.

Claims

1. A pharmaceutical composition for use in preventing or treating osteoarthritis comprising 3'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

2. The pharmaceutical composition for use according to claim 1, wherein the salt of 3'-sialyllactose has a structure of the following Formula 1:

[Formula 1]



3. The pharmaceutical composition for use according to claim 1, wherein the composition has one or more of the following characteristics of:

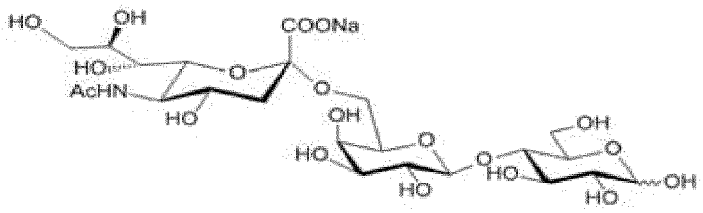
    1) increasing expression of type II collagen (Col2a1);
    2) decreasing expression of matrix metalloproteinases (Mmp3) or matrix metalloproteinase13 (Mmp13);
    3) increasing Sox-9 activity; and
    4) increasing inactivation of p-ERK.

4. The pharmaceutical composition for use according to claim 1, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

5. A pharmaceutical composition for use in preventing or treating osteoarthritis without angiogenesis in a cartilage comprising 6'-sialyllactose or a pharmaceutically acceptable salt thereof as an active ingredient.

6. The pharmaceutical composition for use according to claim 5, wherein the salt of 6'-sialyllactose has a structure of the following Formula 2:

[Formula 2]



7. The pharmaceutical composition for use according to claim 5, wherein the composition has one or more of the following characteristics of:

    1) increasing expression of type II collagen (Col2a1);
    2) decreasing expression of matrix metalloproteinases (Mmp3) or matrix metalloproteinase13 (Mmp13); and
    3) increasing Sox-9 activity.

8. The pharmaceutical composition for use according to claim 5, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

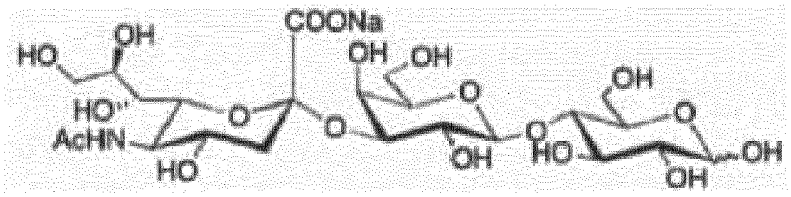
9. A food product comprising 3'-sialyllactose or a food acceptable salt thereof as an active ingredient and/or 6'-sialyllactose or a food acceptable salt thereof as an active ingredient as shown in Formula 1 or 2, respectively, for use in preventing or improving osteoarthritis.

Patentansprüche

1. Arzneimittel zur Verwendung bei der Vorbeugung oder Behandlung von Osteoarthritis, umfassend 3'-Sialyllactose oder ein pharmazeutisch verträgliches Salz davon als Wirkstoff.

2. Arzneimittel zur Verwendung nach Anspruch 1, wobei das Salz von 3'-Sialyllactose eine Struktur mit der folgenden Formel 1 aufweist:

[Formel 1]



3. Arzneimittel zur Verwendung nach Anspruch 1, wobei die Zusammensetzung eine oder mehrere der folgenden Eigenschaften aufweist:

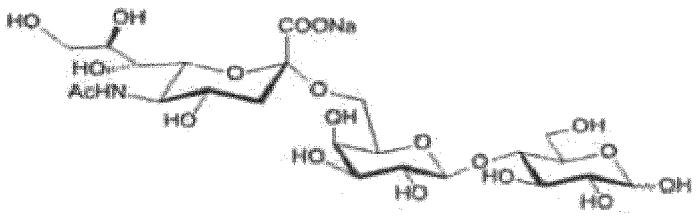
1) Erhöhen der Expression von Kollagen Typ 2 (Col2a1);
2) Verringern der Expression von Matrix-Metalloproteinase-3 (Mmp-3) oder Matrix-Metalloproteinase-13(Mmp-13);
3) Erhöhen der Sox-9-Aktivität; und
4) Erhöhen der Inaktivierung von p-ERK.

4. Arzneimittel zur Verwendung nach Anspruch 1, des Weiteren umfassend einen pharmazeutisch verträglichen Träger, einen pharmazeutisch verträglichen Exzipienten oder ein pharmazeutisch verträgliches Verdünnungsmittel.

5. Arzneimittel zur Verwendung bei der Vorbeugung oder Behandlung von Osteoarthritis ohne Angiogenese in einem Knorpel, umfassend 6'-Sialyllactose oder ein pharmazeutisch verträgliches Salz davon als Wirkstoff.

6. Arzneimittel zur Verwendung nach Anspruch 5, wobei das Salz von 6'-Sialyllactose eine Struktur mit der folgenden Formel 2 aufweist:

[Formel 2]



7. Arzneimittel zur Verwendung nach Anspruch 5, wobei die Zusammensetzung eine oder mehrere der folgenden Eigenschaften aufweist:

1) Erhöhen der Expression von Kollagen Typ 2 (Col2a1);
2) Verringern der Expression von Matrix-Metalloproteinase-3 (Mmp-3) oder Matrix-Metalloproteinase-13 (Mmp-13); und
3) Erhöhen der Sox-9-Aktivität;

8. Arzneimittel zur Verwendung nach Anspruch 5, des Weiteren umfassend einen pharmazeutisch verträglichen Träger, einen pharmazeutisch verträglichen Exzipienten oder ein pharmazeutisch verträgliches Verdünnungsmittel.

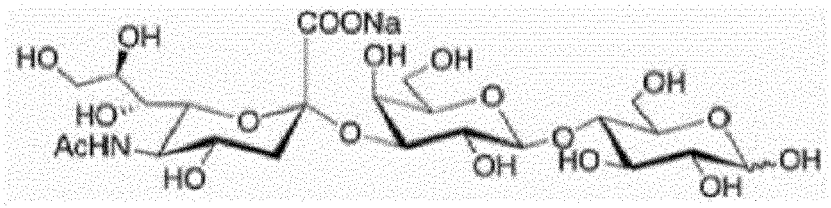
9. Nahrungsmittelprodukt, umfassend 3'-Sialyllactose oder ein Nahrungsmittel-verträgliches Salz davon als Wirkstoff und/oder 6'-Sialyllactose oder ein Nahrungsmittel-verträgliches Salz davon als Wirkstoff, wie in Formel 1 bzw. 2 gezeigt, zur Verwendung bei der Vorbeugung oder Verbesserung von Osteoarthritis.

Revendications

1. Composition pharmaceutique pour une utilisation dans la prévention ou le traitement de l'ostéoarthrite comprenant du 3'-sialyllactose ou un sel pharmaceutiquement acceptable correspondant en tant qu'un ingrédient actif.

2. Composition pharmaceutique pour une utilisation selon la revendication 1, le sel de 3'-sialyllactose ayant une structure de la formule 1 suivante :

[formule 1]



.

3. Composition pharmaceutique pour une utilisation selon la revendication 1, la composition ayant l'une ou plusieurs des caractéristiques suivantes de :

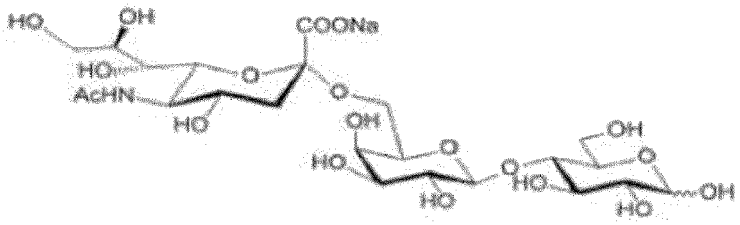
1) augmentation de l'expression de collagène de type II (Col2a1) ;
2) diminution de l'expression de la métalloprotéinase matricielle 3 (Mmp3) ou de la métalloprotéinase matricielle 13 (Mmp13) ;
3) augmentation de l'activité de Sox-9 ; et
4) augmentation de l'inactivation de p-ERK.

4. Composition pharmaceutique pour une utilisation selon la revendication 1, comprenant en outre un support, excipient ou diluant pharmaceutiquement acceptable.

5. Composition pharmaceutique pour une utilisation dans la prévention ou le traitement de l'ostéoarthrite sans angiogenèse dans un cartilage comprenant du 6'-sialyllactose ou un sel pharmaceutiquement acceptable correspondant en tant qu'un ingrédient actif.

6. Composition pharmaceutique pour une utilisation selon la revendication 5, le sel de 6'-sialyllactose ayant une structure de la formule 2 suivante :

[formule 2]



.

7. Composition pharmaceutique pour une utilisation selon la revendication 5, la composition ayant l'une ou plusieurs des caractéristiques suivantes de :

1) augmentation de l'expression de collagène de type II (Col2a1) ;
2) diminution de l'expression de la métalloprotéinase matricielle 3 (Mmp3) ou de la métalloprotéinase matricielle 13 (Mmp13) ; et
3) augmentation de l'activité de Sox-9.

8. Composition pharmaceutique pour une utilisation selon la revendication 5, comprenant en outre un support, excipient ou diluant pharmaceutiquement acceptable.

9. Produit alimentaire comprenant du 3'-sialyllactose ou un sel acceptable sur le plan alimentaire correspondant en tant qu'un ingrédient actif et/ou du 6'-sialyllactose ou un sel acceptable sur le plan alimentaire correspondant en tant qu'un ingrédient actif comme présenté respectivement dans la formule 1 ou 2, pour une utilisation dans la prévention ou l'amélioration de l'ostéoarthrite.

FIG. 1

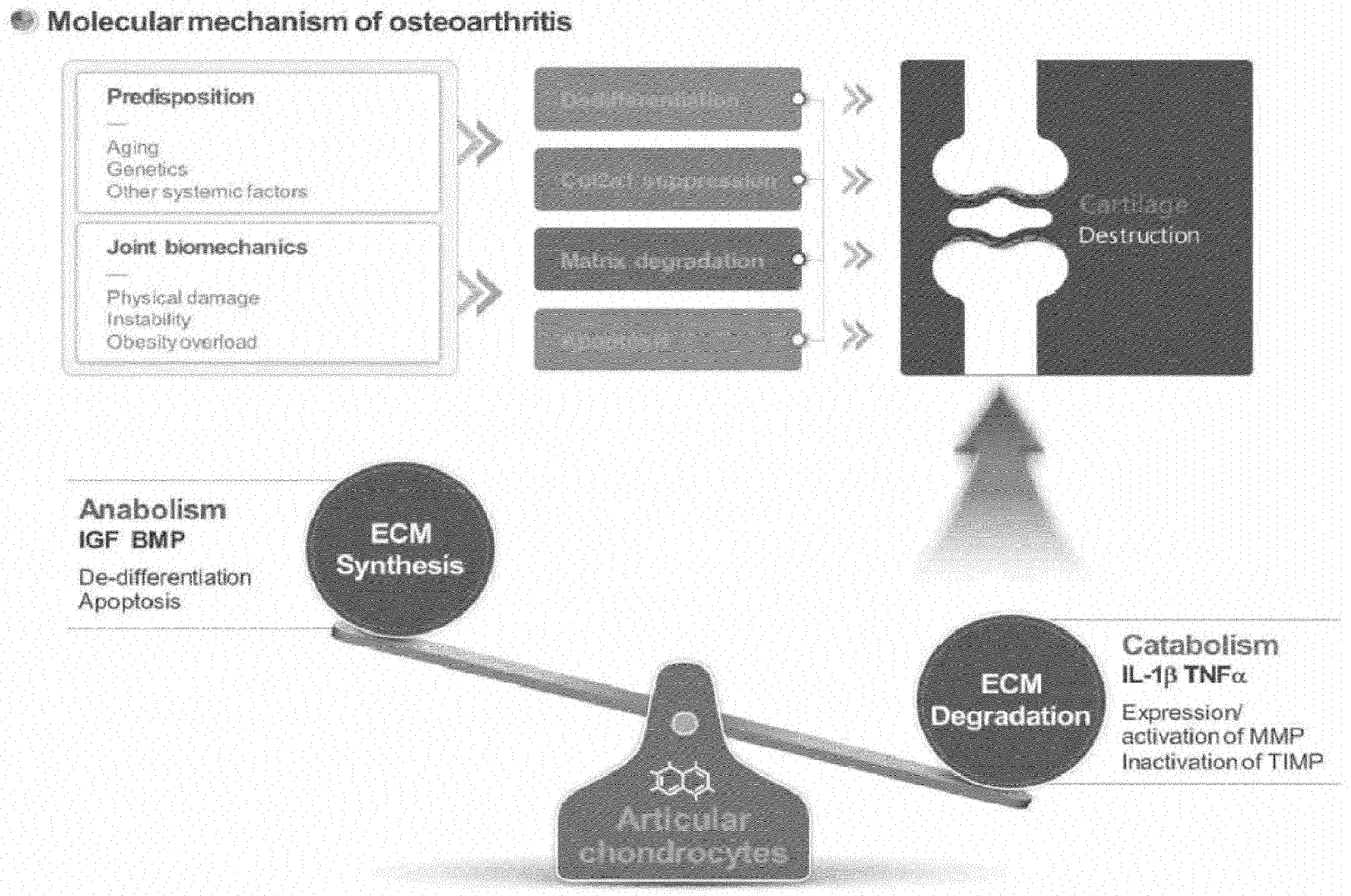

Molecular mechanism of osteoarthritis
Predisposition
Aging
Genetics
Other systemic factors
Joint biomechanics
Physical damage
Instability
Obesity overload
Matrix degradation
Cartilage
Destruction
Anabolism
IGF BMP
De-differentiation
Apoptosis
ECM
Synthesis
ECM
Degradation
Catabolism
IL-1β TNFα
Expression/
activation of MMP
Inactivation of TIMP
Articular
chondrocytes

FIG. 2A

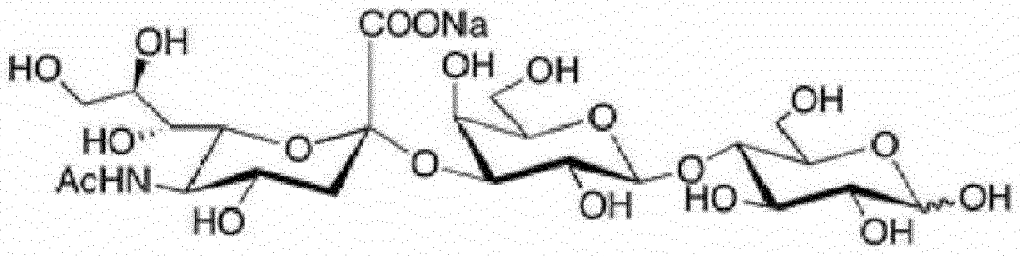

COONa
OH
HO
HO
AcHN
HO
O
O
OH
OH
O
OH
O
HO
OH
OH
O

FIG. 2B

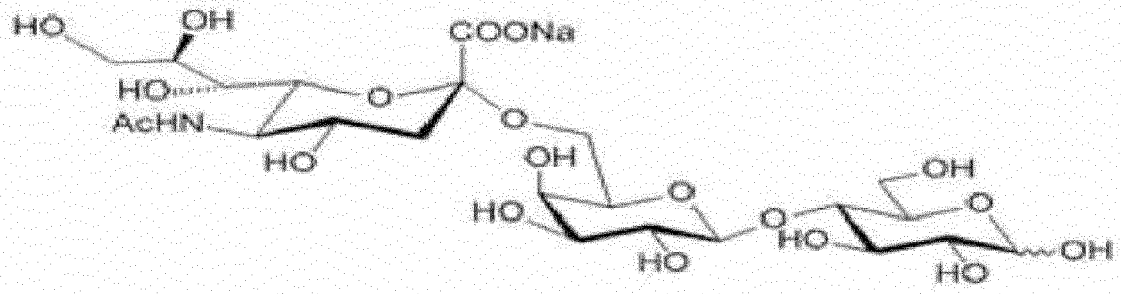

HO
OH
HO
AcHN
HO
O
COONa
O
OH
HO
HO
O
O
OH
HO
OH
HO
OH

FIG. 3A

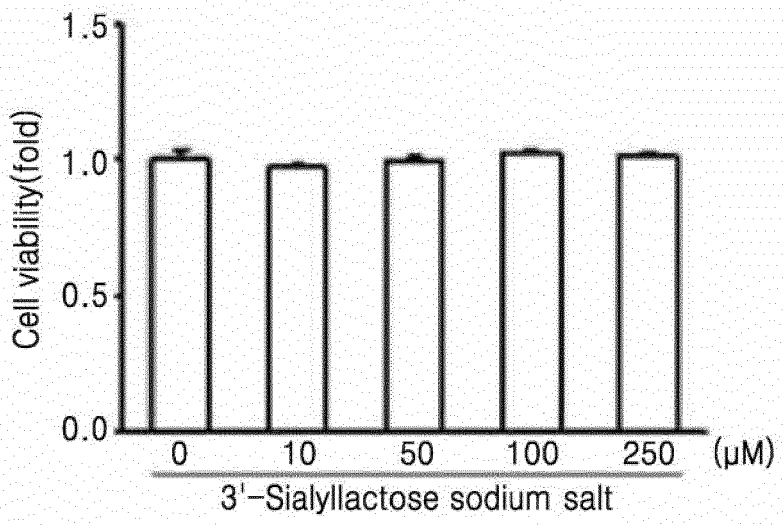

1.5
1.0
0.5
0.0
Cell viability(fold)
0
10
50
100
250
(μM)
3'-Sialyllactose sodium salt

FIG. 3B

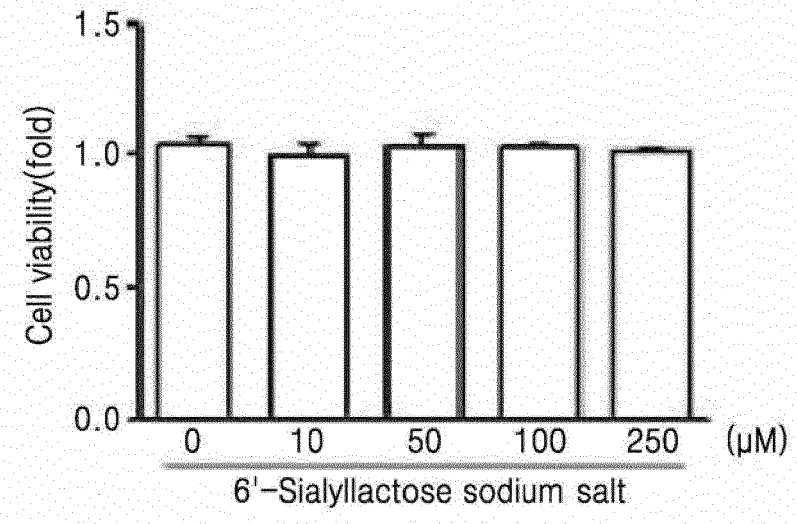

1.5
1.0
0.5
0.0
Cell viability(fold)
0
10
50
100
250
(μM)
6'-Sialyllactose sodium salt

FIG. 4

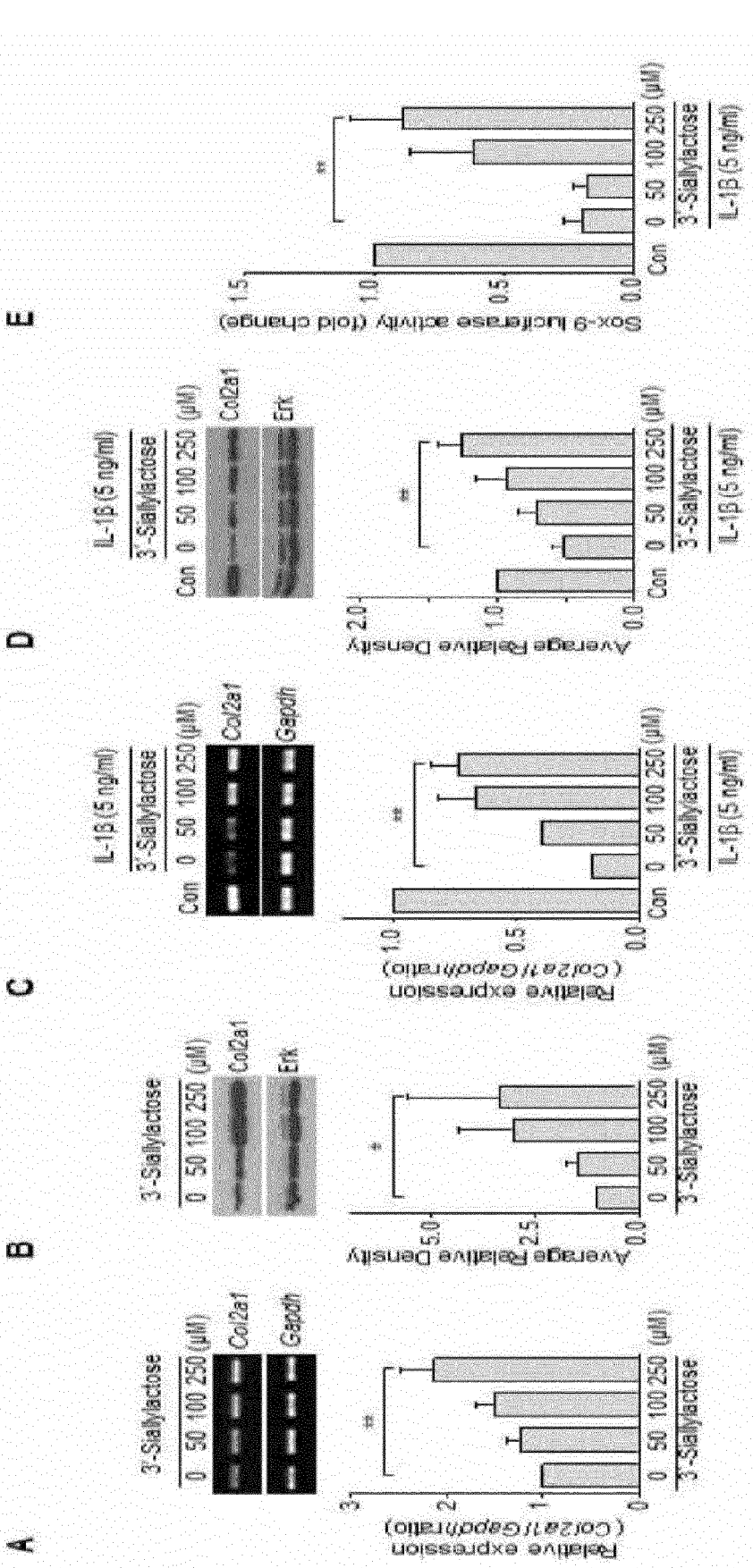

A
B
C
D
E
3'-Sialyllactose
0 50 100 250 (μM)
Col2a1
Gapdh
Erk
Relative expression
(Col2a1/Gapdh ratio)
Average Relative Density
IL-1β (5 ng/ml)
Con
Sox-9 luciferase activity (fold change)
*
**

FIG. 5

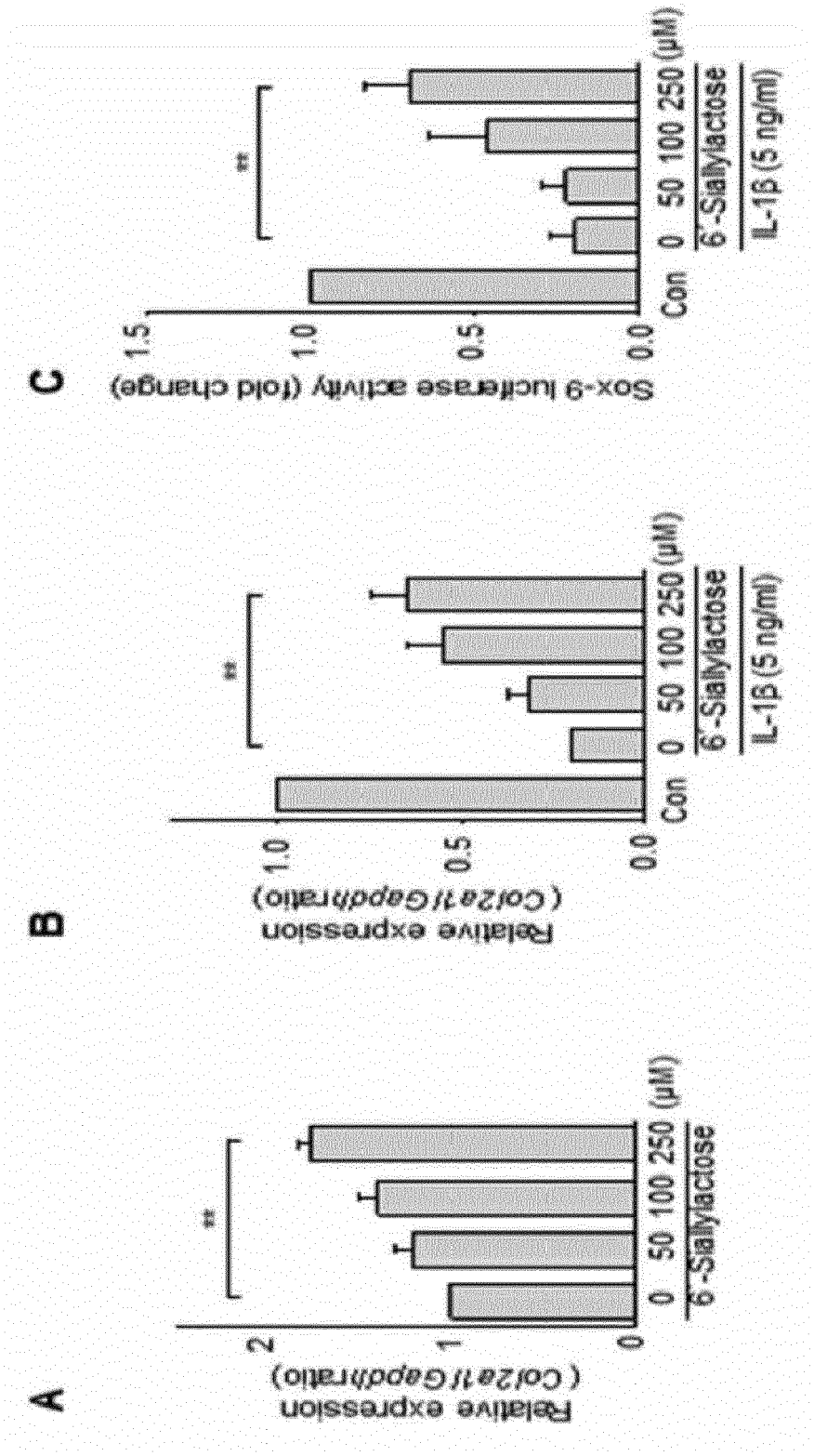

A
Relative expression
(Col2a1/Gapdh ratio)
2
1
0
**
0 50 100 250 (μM)
6'-Sialyllactose
B
Relative expression
(Col2a1/Gapdh ratio)
1.0
0.5
0.0
**
Con 0 50 100 250 (μM)
6'-Sialyllactose
IL-1β (5 ng/ml)
C
Sox-9 luciferase activity (fold change)
1.5
1.0
0.5
0.0
**
Con 0 50 100 250 (μM)
6'-Sialyllactose
IL-1β (5 ng/ml)

FIG. 6

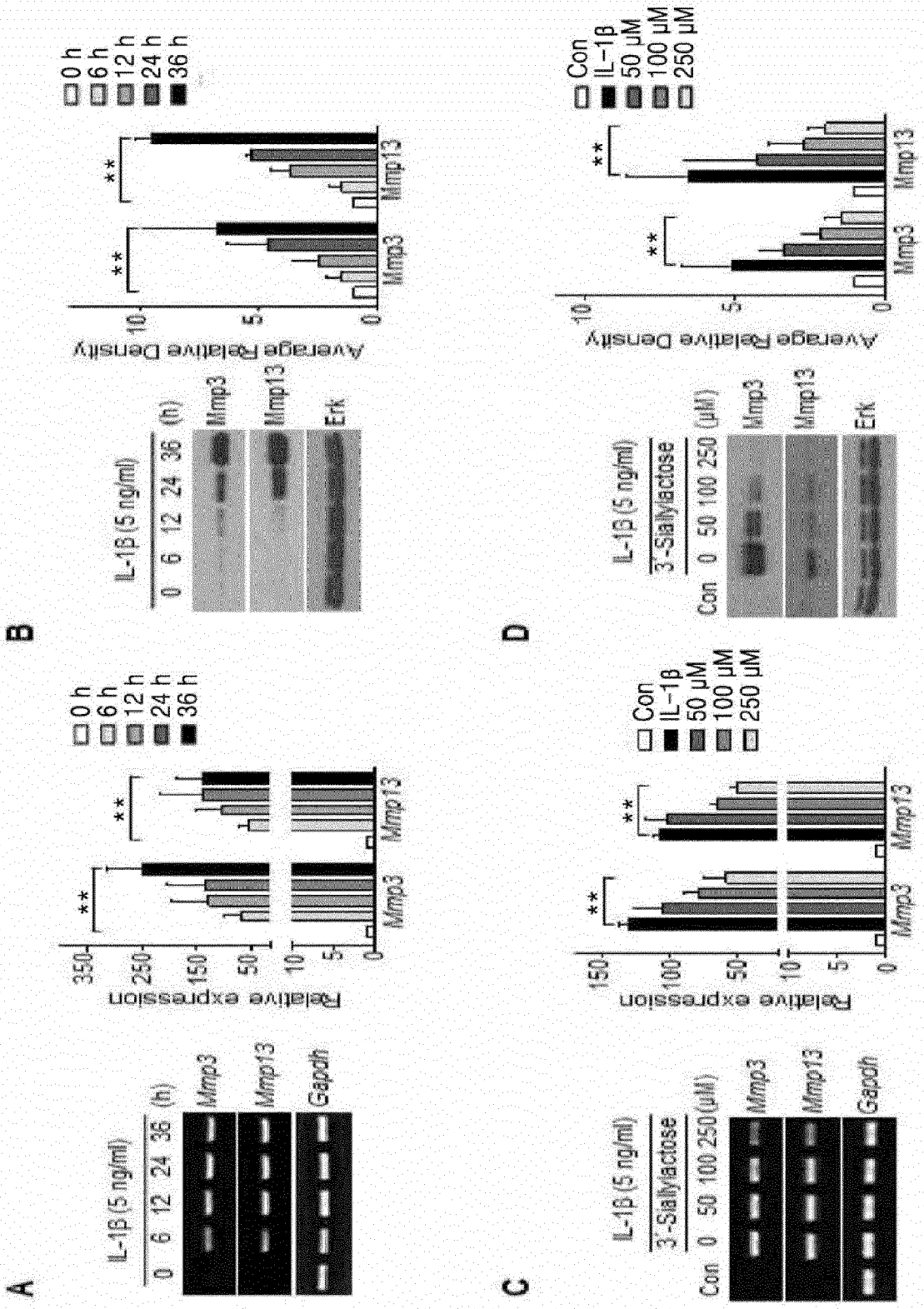

A
IL-1β (5 ng/ml)
0 6 12 24 36 (h)
Mmp3
Mmp13
Gapdh
Relative expression
0 h
6 h
12 h
24 h
36 h
B
Average Relative Density
C
Con
IL-1β
50 μM
100 μM
250 μM
3'-Sialyllactose
0 50 100 250 (μM)
D
Erk

FIG. 7

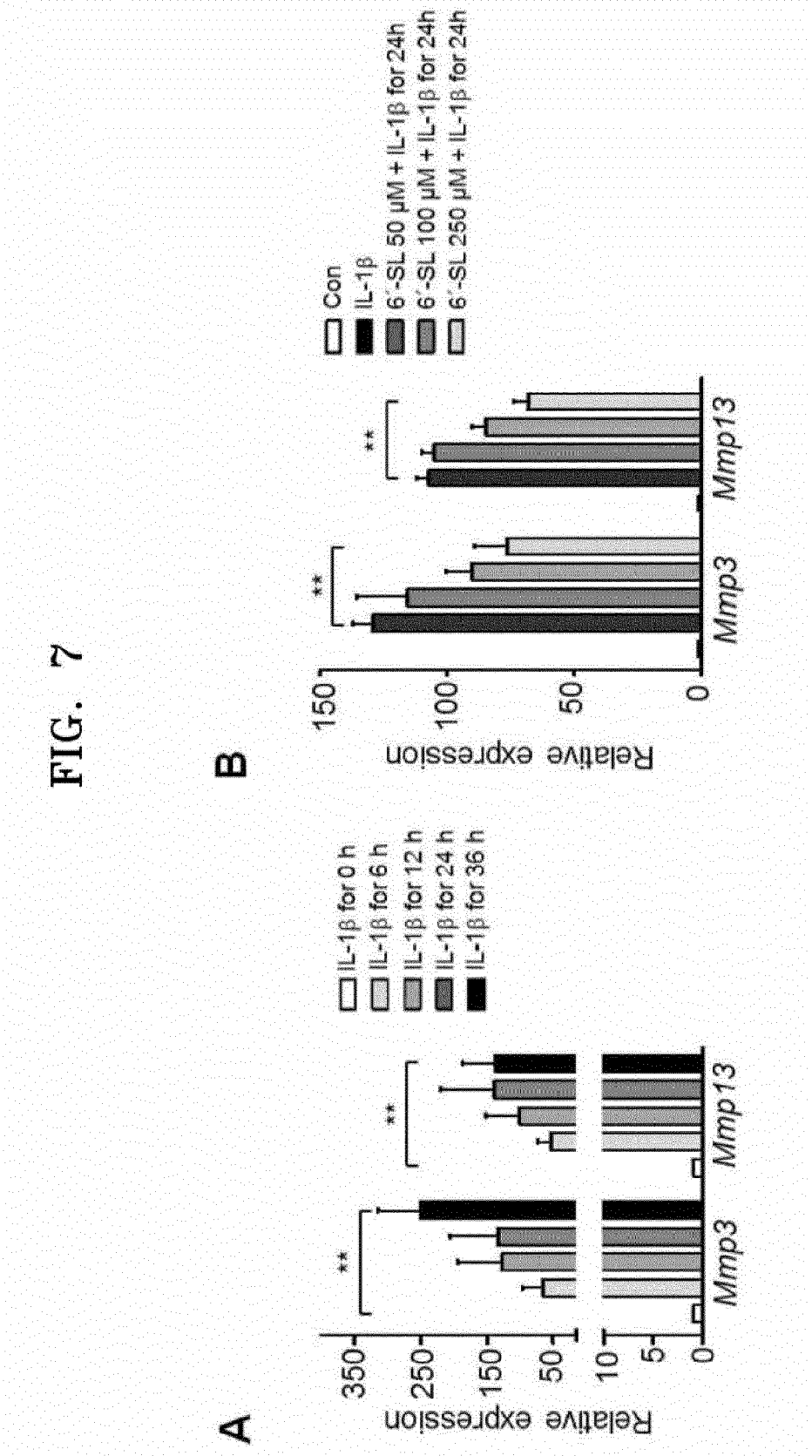
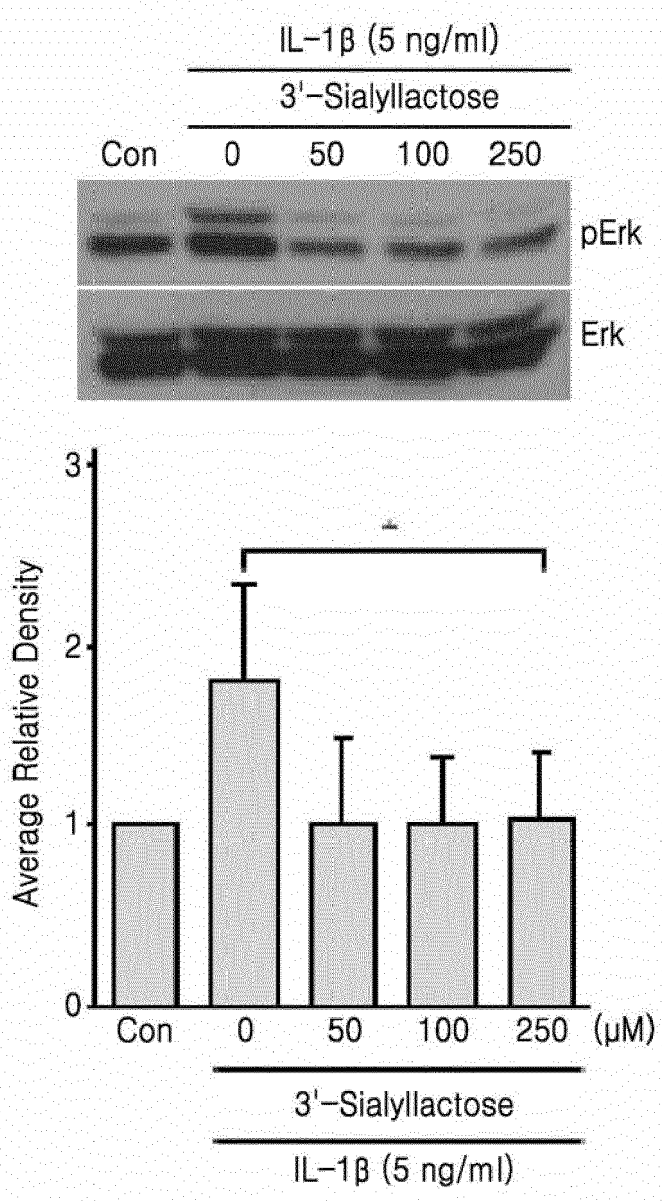

A
Relative expression
350
250
150
50
10
5
0
**
**
Mmp3
Mmp13
IL-1β for 0 h
IL-1β for 6 h
IL-1β for 12 h
IL-1β for 24 h
IL-1β for 36 h
B
Relative expression
150
100
50
0
**
**
Mmp3
Mmp13
Con
IL-1β
6´-SL 50 μM + IL-1β for 24h
6´-SL 100 μM + IL-1β for 24h
6´-SL 250 μM + IL-1β for 24h

FIG. 8


IL-1β (5 ng/ml)
3'-Sialyllactose
Con
0
50
100
250
pErk
Erk
Average Relative Density
3
2
1
0
Con
0
50
100
250
(μM)
3'-Sialyllactose
IL-1β (5 ng/ml)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5164374 A **[0005]**
- WO 9848817 A **[0022]**

Non-patent literature cited in the description

- **PELLETIER JP et al.** *Arthritis Rheum,* 2001, vol. 44, 1237-47 **[0002]**
- **PRITCHARD MH et al.** *Annals of the Rheumatic Diseases,* 1978, vol. 37, 493-503 **[0003]**
- **PETERSON L ; J BONE et al.** *Joint Surg Am.,* 2003, vol. 85, 17-24 **[0004]**
- **IZQUIERDO-USEROS N et al.** *Plos Biol,* 2012, vol. 10 **[0005]**
- **YUSUF E et al.** *Ann Rheum Dis,* 2011, vol. 70, 60-67 **[0016]**
- **BEREBAUM F et al.** *Osteoarthritis Cartilage,* 2013, vol. 21, 16-21 **[0016]**
- **PELLETIER JP et al.** *Arthritis Rheum.,* 2001, vol. 44, 1237-47 **[0017]**
- **PRITCHARD MH et al.** *Ann Rheum Dis,* 1978, vol. 37, 493-503 **[0019]**
- **ABRAMSON SB et al.** *Osteoarthritis Cartilage,* 1999, vol. 7, 380-1 **[0019]**
- **PETERSON L et al.** *J Bone Joint Surg Am,* 2003, vol. 85, 17-24 **[0042]**
- **YOON et al.** *Jorunal of Rheumatic Diseases,* 2012, vol. 19 **[0042]**
- **PARK et al.** *J of Korean Orthopaedic Research Society,* 2015, vol. 18, 2 **[0042]**
- **MAMIDI MK et al.** *Osteoarthritis Cartilage,* 2016, vol. 24, 1307-16 **[0042]**
- **ZHOU G et al.** *J Biol Chem,* 1998, vol. 12, 14989-97 **[0066]**
- **YANG et al.** *Nat Med,* 2010 **[0076]**