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(54) Title: EXTRACELLULAR MATRIX-BINDING SYNTHETIC PEPTIDOGLYCANS

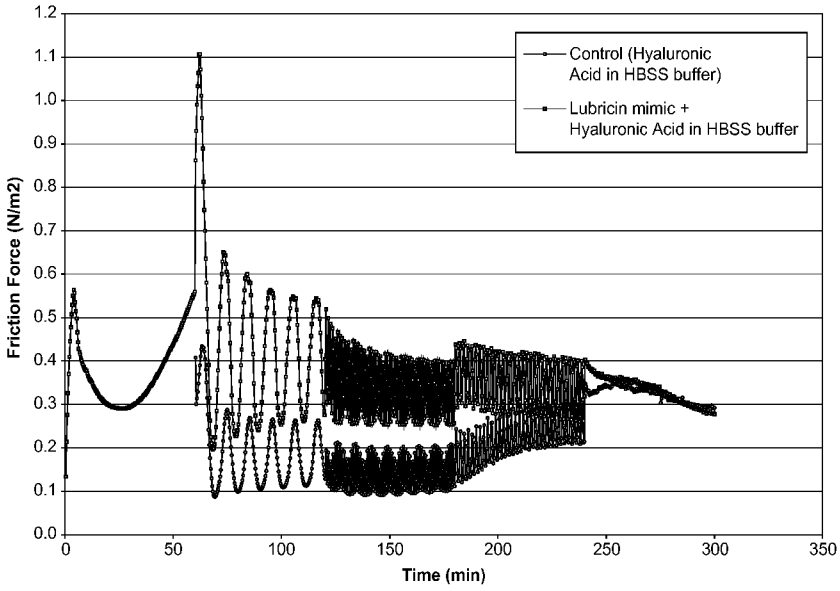


FIG. 1

(57) Abstract: This disclosure provides extracellular matrix-binding synthetic peptidoglycans comprised of one or more synthetic peptides conjugated to a glycan and methods of their use.

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EXTRACELLULAR MATRIX-BINDING SYNTHETIC PEPTIDOGLYCANS

Field of Invention

This disclosure provides extracellular matrix-binding synthetic peptidoglycans comprised of one or more synthetic peptides conjugated to a glycan and methods of their use.

Background

In most tissues, cells are surrounded by an extracellular matrix (ECM) containing proteins such as collagen, laminin, and fibronectin. As mammals age and in some disease states, the extracellular matrix in certain areas of the body (e.g., in synovial joints, the vitreous humor, the spinal discs, the skin, etc.) can degrade, causing undesirable symptoms, such as various forms of arthritis, loss of vision, and the like.

Lubricin, also known as superficial zone protein (SZP) or PRG4, is a mucinous glycoprotein secreted by tissues lining the interior surfaces of animal joints (see Schumacher, B.L., et al., Arch Biochem Biophys, 1994, 311(1): 144-52). Lubricin acts as a chondroprotective barrier against direct solid-to solid contact in joints when the kinematic conditions are conducive to surface sliding in the boundary lubrication regime, characterized by the formation of an adsorbed molecular layer conformal with the articular tissue surface topography (see Neu, C.P., K. Komvopoulos, and A.H. Reddi, Tissue Engineering, Part B: Reviews, 2008). In the absence of a strongly adsorbing, continuous, self-replenishing boundary lubricant layer, intermittent asperity-asperity interactions lead to rapid deterioration of the join surface by various mechanical wear processes, such as adhesion, abrasion, surface fatigue, and delamination. Lubricin tribosupplementation has been shown to reduce cartilage degeneration (see Jay, G.D., et al., Arthritis and rheumatism, 2012, 64(4): 1162-71, and Teeple, E., et al., The American Journal of Sports Medicine, 2011, 39(1): 164-72). Reducing friction at the articular cartilage interface will suppress cartilage wear and surface damage.

Another extracellular matrix is the vitreous humor, a complex gel-like network which fills the posterior cavity of the eye, is composed of approximately 99 wt% water, 0.9 wt% salts, less than 0.1 wt% heterotypic collagen fibrils (type II, V/XI and IX), and a hyaluronan network. It serves several purposes (including developmental, optical, protective) and its degradation has

been implicated in several ocular pathologies, such as retinal tear, retinal detachment, retinal edema, choroidal detachment, vitreous hemorrhage, and glaucoma.

In addition, degeneration of the nucleus pulposus, a gel-like substance is the inner core of the spinal disc, results in reduced ability of the spinal disc to transmit loads evenly and efficiently between vertebral bodies, and leads to damage in the annular region of the disc, known as the annulus fibrosis. The nucleus pulposus functions to distribute hydraulic pressure in all directions within each disc under compressive loads and is comprised of chondrocyte-like cells, collagen fibrils, and proteoglycan aggrecans that aggregate through hyaluronic chains. Fissures or tears in the annulus can translate into a disc that herniates or ruptures, resulting in impingement of the nerves in the region of the disc and finally lower back or leg pain.

This disclosure provides extracellular matrix-binding synthetic peptidoglycans for use in supplementing and/or replacing extracellular matrix fluids in the body, thus treating and/or preventing diseases or disorders resulting from the degradation thereof.

Summary

Provided herein is an extracellular matrix-binding synthetic peptidoglycan, wherein the extracellular matrix-binding synthetic peptidoglycan comprises a glycan; from about 1 to 60 collagen binding peptide(s); and from about 1 to 60 hyaluronic acid binding peptide(s). In the extracellular matrix-binding synthetic peptidoglycan, the collagen binding peptide(s) and the hyaluronic acid binding peptide(s) are covalently bonded to the glycan. The synthetic peptidoglycan can comprise any glycan, including, but not limited to, dextran, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparan, heparin, keratin, keratan sulfate, or hyaluronic acid.

Also provided is a pharmaceutical composition comprising an extracellular matrix-binding synthetic peptidoglycan comprising a glycan; from about 1 to 80 collagen binding peptide(s); and from about 1 to 80 hyaluronic acid binding peptide(s), wherein the collagen binding peptide(s) and the hyaluronic acid binding peptide(s) are covalently bonded to the glycan.

Synthetic peptidoglycans as described herein may be useful in supplementing and/or protecting tissues that have both collagen and hyaluronic acid, such as cartilage, the nucleus

pulposus, and the vitreous humor of the eye. Accordingly, provided is a method of treating and/or preventing cartilage degeneration in a patient comprising administering to a patient in need thereof a pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein. In addition, provided is a method of treating and/or preventing vitreous humor degeneration in a patient comprising administering to a patient in need thereof the pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein. Also provided is a method of treating and/or preventing nucleus pulposus degeneration in a patient comprising administering to a patient in need thereof the pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein.

It is further contemplated that the extracellular matrix-binding synthetic peptidoglycans and pharmaceutical compositions thereof can be used in vascular intervention procedures including, for example, to prevent any one or a combination of platelet binding to exposed collagen of the denuded endothelium, platelet activation, thrombosis, inflammation resulting from denuding the endothelium, intimal hyperplasia, and vasospasm. The extracellular matrix-binding synthetic peptidoglycans described herein can also be used to stimulate endothelial cell proliferation and can bind to collagen in a denuded vessel.

Brief Description of the Drawings

Certain aspects are best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

Figure 1 shows the frictional force in the case of undamaged cartilage (with no aggrecan depletion) and shows that with a synthetic peptidoglycan according to according to the disclosure, the friction increases between the cartilage surfaces.

Figure 2 shows the frictional force in the case of damaged cartilage (with aggrecan depletion to simulate osteoarthritis) and shows that when the synthetic peptidoglycan according to Example 1 is added to the cartilage, friction is lowered between the cartilage surfaces.

Figure 3 shows the rheological measurements on bovine vitreous with no treatment (control), PBS buffer and trypsin (Figure 3A), the rheological measurements on bovine vitreous treated with trypsin, and trypsin in combination with three different peptidoglycans (Figure 3B), and the rheological measurements on bovine vitreous with no treatment (control), PBS buffer, and three different peptidoglycans without trypsin (Figure 3C).

Detailed Description

It is to be understood that this disclosure is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

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

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. As used herein the following terms have the following meanings.

As used herein, the term "comprising" or "comprises" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude other materials or steps that do not materially affect the basic and novel characteristic(s) claimed. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this disclosure.

The term "about" when used before a numerical designation, e.g., temperature, time, amount, and concentration, including range, indicates approximations which may vary by (+) or (−) 10%, 5% or 1%.

As used herein, the term "synovial cavity" refers to the space between the bones of a synovial joint that is filled with synovial fluid.

As used herein, the term "treating and/or preventing" refers to preventing, curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease state, disease progression or other abnormal condition.

As used herein, the term "patient" refers to a subject (i.e., human) at risk for or suffering from a disease state, disease progression or other abnormal or deleterious condition.

As used herein, the term "synthetic peptidoglycan" refers to a synthetic conjugate that comprises a glycan and one or more synthetic peptides covalently bonded thereto. The glycan portion can be made synthetically or derived from animal sources. The synthetic peptides can be covalently bonded directly to the glycan or via a linker. For methods of conjugating hyaluronic acid binding peptides to glycans, see, e.g., WO 2012/162534. For methods of conjugating collagen binding peptides to glycans, see, e.g., US 2013/0190246, US 2012/0100106, and US 2011/0020298, the disclosures of which are incorporated herein by reference in their entirety.

As used herein, the term "covalently bonded" refers to a bond in which one or more pairs of electrons are shared by two atoms.

As used herein, the term "glycan" refers a compounds consisting of a large number of monosaccharides linked glycosidically. In certain embodiments, the glycan is a glycosaminoglycans, which comprise 2-aminosugars linked in an alternating fashion with uronic acids, and include polymers such as heparin, heparan sulfate, chondroitin, keratin, and dermatan. Accordingly, non-limiting examples of glycans which can be used in the embodiments described herein include alginate, agarose, dextran, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparan, heparin, keratin, keratan sulfate, and hyaluronic acid.

As used herein, "hyaluronic acid binding peptide" refers to a synthetic peptide comprising a hyaluronic acid binding sequence. In the various embodiments described herein, the peptide component of the synthetic peptidoglycan can comprise an amino acid sequence selected from the group consisting of: GAHWQFNALTVR, GAHWQFNALTVRGG, GDRRRRRMWHRQ, GKHLGGKHRRSR, RGTHHAQKRRS, RRHKSGHIQGSK, SRMHGRVRGRHE, RRRAGLTAGRPR, RYGGHRTSRKWV, RSARYGHRRGVG,

GLRGNRRVFARP, SRGQRGRLGKTR, DRRGRSSLPKLAGPVEFPDRKIKGRR, RMRRKGRVKHWG, RGGARGRHKTGR, TGARQRGLQGGWGPRHLRGKDQPPGR, RQRRRDLTRVEG, STKDHNRGRRNVGPVSRSTLRDPIRR, RRIGHQVGGRRN, RLESRAAGQRRA, GGPRRHLGRRGH, VSKRGHRRTAHE, RGTRSGSTR, RRRKKIQGRSKR, RKSYGKYQGR, KGRYSISR, RRRCGQKK, KQKIKHVVKLK, KLKSQLVKRK, RYPISRPRKR, KVGKSPPVR, KTFGKMKPR, RIKWSRVSK or KRTMRPTRR, or any peptide sequence comprising a sequence with at least about 80% sequence identity to the amino acid sequence. Additional peptides that can be included as the peptide component of the hyaluronic acid binding synthetic peptidoglycans include peptides which have an Arg-Arg (R-R) motif, such as one or more peptides selected from the group consisting of RRASRSRGQVGL, GRGTHHAQKRRS, QPVRRLGTPVVG, ARRAEGKTRMLQ, PKVRGRRHQASG, SDRHRRRREADG, NQRVRRVKHPPG, RERRERHAVARHGPGLERDARNLARR, TRPGGKRGGQVGPPAGVLHGRRARS, NVRSRRGHRMNS, DRRRGRTRNIGN, KTAGHGRRWSRN, AKRGEGRREWPR, GGDRRKAHKLQA, RRGGRKWGSFEG and RQRRRDLTRVEG (see, e.g., Amemiya et al, Biochem. Biophys. Acta, 2005, 1724, 94-99, incorporated herein by reference). In another embodiment, the peptide is selected from the group consisting of RDGTRYVQKGEYR, HREARSGKYK, PDKKHKLYGV, and WDKERSRYDV (see, e.g., Yang et al, EMBO Journal, 1994, 13, 286-296, and Goetinck et al, J. Cell. Biol, 1987, 105, 2403-2408, both of which are incorporated herein by reference).

As used herein, the term "collagen binding peptide" refers to a synthetic peptide comprising a collagen binding sequence. The "collagen binding peptide" can have amino acid homology with a portion of a protein normally or not normally involved in collagen fibrillogenesis. In one embodiment, the collagen binding peptide comprises from about 5 to about 40 amino acids, or from about 5 to about 20 amino acids, or from about 5 to about 10 amino acids. In some embodiments, these peptides have homology or sequence identity to the amino acid sequence of a small leucine-rich proteoglycan, a platelet receptor sequence, or a protein that regulates collagen fibrillogenesis. In various embodiments, the collagen binding peptide comprises an amino acid sequence selected from the group consisting of RRANAALKAGELYKSILY, RLDGNEIKR, AHEEISTTNEGVM, GELYKSILY, NGVFKYRPRYFLYKHAYFYPPLKRFPVQ, CQDSETRTFY, TKKTLRT,

GLRSKSKKFRRPDIQYPDATDEDITSHM, SQNPVQP, SYIRIADTNIT, KELNLVYT, GSITTIDVPWNVGC, and GSITTIDVPWNV any peptide sequence comprising a sequence with at least about 80% sequence identity to the amino acid sequence. In certain embodiments, the collagen binding peptide comprises an amino acid sequence that has about 80%, about 85%, about 90%, about 95%, about 98%, or about 100% sequence identity with the collagen binding domain(s) of the von Willebrand factor or a platelet collagen receptor as described in Chiang, et al. J. Biol. Chem. 277: 34896-34901 (2002), Huizing a, et al., Structure 5: 1147-1156 (1997), Romijn, et al., J. Biol. Chem. 278: 15035-15039 (2003), and Chiang, et al., Cardio. & Haemato. Disorders-Drug Targets 7: 71-75 (2007), each incorporated herein by reference.

In any of the embodiments described herein, any one or more of the synthetic peptides (e.g., the hyaluronic acid binding peptide and/or the collagen binding peptide) may have a glycine-cysteine (GC) attached to the C-terminus of the peptide, and/or a glycine-cysteine-glycine (GCG) attached to the N-terminus of the peptide. For example, the collagen binding peptide may comprise an amino acid sequence selected from the group consisting of RRANAALKAGELYKSILYGC, RLDGNEIKRGC, AHEEISTTNEGVMGC , GCGGELYKSILY, NGVFKYRPRYFLYKHAYFYPPLKRFPVQGC, CQDSETRTFYGC, TKKTLRTGC, GLRSKSKKFRRPDIQYPDATDEDITSHMGC, SQNPVQPGC, SYIRIADTNITGC, KELNLVYTGC, GSITTIDVPWNVGC, GCGGELYKSILYGC and GELYKSILYGC.

As used herein, the term "amino acid" refers to either a natural and/or unnatural or synthetic amino acid, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics. Single letter and three letter abbreviations of the naturally occurring amino acids are listed below in Table 1. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

Table 1

1-Letter	3-Letter	Amino Acid
Y	Tyr	L-tyrosine
G	Gly	L-glycine
F	Phe	L-phenylalanine
M	Met	L-methionine

1-Letter	3-Letter	Amino Acid
A	Ala	L-alanine
S	Ser	L-serine
I	Ile	L-isoleucine
L	Leu	L-leucine
T	Thr	L-threonine
V	Val	L-valine
P	Pro	L-proline
K	Lys	L-lysine
H	His	L-histidine
Q	Gln	L-glutamine
E	Glu	L-glutamic acid
W	Trp	L-tryptohan
R	Arg	L-arginine
D	Asp	L-aspartic acid
N	Asn	L-asparagine
C	Cys	L-cysteine

In any of the embodiments described herein, a synthetic peptide (e.g., a hyaluronic acid binding peptide and/or a collagen binding peptide) comprises any amino acid sequence described in the preceding paragraph or an amino acid sequence with 80%, 85%, 90%, 95%, 98%, or 100% homology to any of these amino acid sequences. In various embodiments, the peptide components of the synthetic peptidoglycan described herein can be modified by the inclusion of one or more conservative amino acid substitutions. As is well-known to those skilled in the art, altering any non-critical amino acid of a peptide by conservative substitution should not significantly alter the activity of that peptide because the side-chain of the replacement amino acid should be able to form similar bonds and contacts to the side chain of the amino acid which has been replaced. Non-conservative substitutions are possible provided that these do not excessively affect the hyaluronic acid binding activity of the peptide.

As used herein, the term "homology" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. A peptide (or a polypeptide or peptide region) has a certain percentage (for example, 60%, 65%, 70%, 75%,

80%, 85%, 90%, 95%, 98% or 99%) of "homology" or "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art (e.g., BLAST), and for example, those described in Ausubel et al. eds. (2007) Current Protocols in Molecular Biology.

As is well-known in the art, a "conservative substitution" of an amino acid or a "conservative substitution variant" of a peptide refers to an amino acid substitution which maintains: 1) the secondary structure of the peptide; 2) the charge or hydrophobicity of the amino acid; and 3) the bulkiness of the side chain or any one or more of these characteristics. Illustratively, the well-known terminologies "hydrophilic residues" relate to serine or threonine. "Hydrophobic residues" refer to leucine, isoleucine, phenylalanine, valine or alanine, or the like. "Positively charged residues" relate to lysine, arginine, ornithine, or histidine. "Negatively charged residues" refer to aspartic acid or glutamic acid. Residues having "bulky side chains" refer to phenylalanine, tryptophan or tyrosine, or the like. A list of illustrative conservative amino acid substitutions is given in Table 2.

Table 2

For Amino Acid	Replace With
Alanine	D-Ala, Gly, Aib, β-Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp

For Amino Acid	Replace With
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

In one embodiment, the synthetic peptidoglycans of the disclosure bind, either directly or indirectly to collagen and/or hyaluronic acid. The terms "binding" or "bind" as used herein are meant to include interactions between molecules that may be detected using, for example, a hybridization assay, surface plasmon resonance, ELISA, competitive binding assays, isothermal titration calorimetry, phage display, affinity chromatography, rheology or immunohistochemistry. The terms are also meant to include "binding" interactions between molecules. Binding may be "direct" or "indirect". "Direct" binding comprises direct physical contact between molecules. "Indirect" binding between molecules comprises the molecules having direct physical contact with one or more molecules simultaneously. For example, it is contemplated that synthetic peptidoglycans of the disclosure directly bind and interact with both hyaluronic acid and type II collagen, and can be used to restore the low friction properties of articular cartilage, thus protect the surface from mechanical wear. This binding can result in the formation of a "complex" comprising the interacting molecules. A "complex" refers to the binding of two or more molecules held together by covalent or non-covalent bonds, interactions or forces.

Synthetic Peptidoglycans

The present disclosure provides an extracellular matrix-binding synthetic peptidoglycan comprising: a) a glycan; b) from about 1 to about 80 collagen binding peptide(s); and c) from about 1 to about 80 hyaluronic acid binding peptide(s); wherein the peptides of b) and c) are covalently bonded to the glycan.

In the synthetic peptidoglycan disclosed herein, the glycan can be any glycan, or polysaccharide, including but not limited to, dextran, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparan, heparin, keratin, keratan sulfate, and hyaluronic acid. In some

embodiments, the glycan is chondroitin sulfate. In some embodiments, the glycan is dermatan sulfate. In some embodiments, the glycan is hyaluronic acid.

The peptides can be bonded to the glycan directly or via a linker. In certain embodiments, the peptides are covalently bonded to the glycan via a linker. The linker can be any suitable bifunctional linker, e.g., 3-(2-pyridyldithio)propionyl hydrazide (PDPH), N-[β-maleimidopropionic acid]hydrazide (BMPH), and the like. In any of the various embodiments described herein, the sequence of the peptide may be modified to include a glycine-cysteine segment to provide an attachment point for a glycan. In certain embodiments, the linker is N-[β-maleimidopropionic acid]hydrazide (BMPH).

Depending on the desired properties of the synthetic peptidoglycan, the total number of peptides bound to the glycan can be varied. In certain embodiments, the total number of peptides present in the synthetic peptidoglycan is from about 2 to about 160, from about 10 to about 160, from about 20 to about 160, from about 30 to about 160, from about 40 to about 160, from about 40 to about 150, from about 40 to about 140, from about 10 to about 120, or from about 20 to about 110, or from about 20 to about 100, or from about 20 to about 90, or from about 30 to about 90, or from about 40 to about 90, or from about 50 to about 90, or from about 50 to about 80, or from about 60 to about 80, or about 10, or about 20, or about 30, or about 40, or about 50, or about 60, or about 70, or about 80, or about 90, or about 100, or about 110, or about 120. In certain embodiments, the total number of peptides present in the synthetic peptidoglycan is less than about 50. In certain embodiments, the total number of peptides present in the synthetic peptidoglycan is from about 10 to about 40. In certain embodiments, the total number of peptides present in the synthetic peptidoglycan is about 22. In certain embodiments, the total number of collagen binding peptides is from about 5 to about 20, or about 10, or about 11. In certain embodiments, the total number of hyaluronic acid binding peptides is from about 5 to about 20, or about 10, or about 11.

In one aspect, the collagen binding peptides present in the extracellular matrix-binding synthetic peptidoglycans described herein comprise have binding affinity to one or more of collagen types I, II, III, or IV. One or more collagen binding peptide having a specified binding affinity can be used in the extracellular matrix-binding synthetic peptidoglycans described herein. For example, the extracellular matrix-binding synthetic peptidoglycans can comprise at

least one collagen binding peptide which has binding affinity to type I collagen and at least one collagen binding peptide which has binding affinity to type II collagen. In another aspect, the collagen binding peptides have binding affinity to type I collagen. In certain aspects, the collagen binding peptides have binding affinity to type II collagen.

Suitable collagen binding peptides are known in the art (see, e.g., US 2013/0190246, US 2012/0100106, and US 2011/0020298, the disclosures of which are incorporated herein by reference in their entirety). In one embodiment, the collagen binding peptide comprises from about 5 to about 40 amino acids. In some embodiments, these peptides have homology to the amino acid sequence of a small leucine-rich proteoglycan, a platelet receptor sequence, or a protein that regulates collagen fibrillogenesis.

In various embodiments, the collagen binding peptide comprises an amino acid sequence selected from the group consisting of WYRGRLGC, RRANAALKAGELYKSILYGC, RLDGNEIKRGC, AHEEISTTNEGVMGC , GCGGELYKSILY, NGVFKYRPRYFLYKHAYFYPPLKRFPVQGC, CQDSETRTFY, TKKTLRTGC, GLRSKSKKFRRPDIQYPDATDEDITSHMGC, SQNPVQPGC, SYIRIADTNITGC, SYIRIADTNIT, KELNLVYT, KELNLVYTGC, GSITTIDVPWNV, GELYKSILYGC and GSITTIDVPWNVGC, or any peptide sequence comprising a sequence with at least about 80% sequence identity, or at least about 80% sequence identity, or at least about 85% sequence identity, or at least about 90% sequence identity, or at least about 95% sequence identity, or at least about 98% sequence identity. In certain embodiments, the collagen binding peptide is WYRGRLGC, or any peptide that has at least about 80% sequence identity, or at least about 85% sequence identity, or at least about 90% sequence identity, or at least about 95% sequence identity, or at least about 98% sequence identity. In certain embodiments, the collagen binding peptide is about 80%, about 85%, about 90%, about 95%, about 98%, or about 100% homologous with the collagen binding domain(s) of the von Willebrand factor or a platelet collagen receptor as described in Chiang, et al. J. Biol. Chem. 277: 34896-34901 (2002), Huizing a, et al., Structure 5: 1147-1156 (1997), Romijn, et al., J. Biol. Chem. 278: 15035-15039 (2003), and Chiang, et al., Cardio. & Haemato. Disorders-Drug Targets 7: 71-75 (2007), each incorporated herein by reference.

Suitable hyaluronic acid binding peptides are known in the art (see, e.g., WO 2012/162534). In the various embodiments described herein, the peptide component of the synthetic peptidoglycan can comprise an amino acid sequence selected from the group consisting of GAHWQFNALTVRGG, GDRRRRRMWHRQ, GKHLGGKHRRSR, RGTHHAQKRRS, RRHKSGHIQGSK, SRMHGRVRGRHE, RRRAGLTAGRPR, RYGGHRTSRKWV, RSARYGHRRGVG, GLRGNRRVFARP, SRGQRGRLGKTR, DRRGRSSLPKLAGPVEFPDRKIKGRR, RMRRKGRVKHWG, RGGARGRHKTGR, TGARQRGLQGGWGPRHLRGKDQPPGR, RQRRRDLTRVEG, STKDHNRGRRNVGPVSRSTLRDPIRR, RRIGHQVGGRRN, RLESRAAGQRRA, GGPRRHLGRRGH, VSKRGHRRTAHE, RGTRSGSTR, RRRKKIQGRSKR, RKSYGKYQGR, KGRYSISR, RRRCGQKK, KQKIKHVVKLK, KLKSQLVKRK, RYPISRPRKR, KVGKSPPVR, KTFGKMKPR, RIKWSRVSK and KRTMRPTRR, or any peptide sequence comprising a sequence with at least about 80% sequence identity, or at least about 80% sequence identity, or at least about 85% sequence identity, or at least about 90% sequence identity, or at least about 95% sequence identity, or at least about 98% sequence identity.

Additional peptides that can be included as the peptide component of the hyaluronic acid binding synthetic peptidoglycans include peptides which have an Arg-Arg (R-R) motif, such as one or more peptides selected from the group consisting of RRASRSRGQVGL, GRGTHHAQKRRS, QPVRRLGTPVVG, ARRAEGKTRMLQ, PKVRGRRHQASG, SDRHRRRREADG, NQRVRRVKHPPG, RERRERHAVARHGPGLERDARNLARR, TRPGGKRGGQVGPPAGVLHGRRARS, NVRSRRGHRMNS, DRRRGRTRNIGN, KTAGHGRRWSRN, AKRGEGRREWPR, GGDRRKAHKLQA, RRGGRKWGSFEG and RQRRRDLTRVEG, or any peptide sequence comprising a sequence with at least about 80% sequence identity, or at least about 80% sequence identity, or at least about 85% sequence identity, or at least about 90% sequence identity, or at least about 95% sequence identity, or at least about 98% sequence identity (see, e.g., Amemiya et al, Biochem. Biophys. Acta, 2005, 1724, 94-99, incorporated herein by reference). In certain embodiments, the collagen binding peptide is GAHWQFNALTVRGG, or any peptide sequence comprising a sequence with at least about 80% sequence identity, or at least about 80% sequence identity, or at least about 85% sequence identity, or at least about 90% sequence identity, or at least about 95% sequence

identity, or at least about 98% sequence identity. In another embodiment, the peptide is selected from the group consisting of RDGTRYVQKGEYR, HREARSGKYK, PDKKHKLYGV, and WDKERSRYDV (see, e.g., Yang et al, EMBO Journal, 1994, 13, 286-296, and Goetinck et al, J. Cell. Biol., 1987, 105, 2403-2408, both of which are incorporated herein by reference).

In another embodiment, the hyaluronic acid binding peptide may be selected from a group consisting of the B-X7-B homology, in which B is either lysine or arginine and X is any non-acidic amino acid residue (i.e., any amino acid other than aspartic acid or glutamic acid), where at least one of the 7 X residues is a basic residue (i.e., arginine, lysine, or histidine). Peptides may also be selected by phage display, utilizing positive selection for binding to hyaluronic acid. An hyaluronic acid binding peptide may be determined by its interaction with hyaluronic acid, and measured by any of the techniques used to evaluate molecular interactions. For example, surface plasmon resonance, ELISA, competitive binding assays, isothermal titration calorimetry, affinity chromatography, rheology or immunohistochemistry. Peptides that are considered "hyaluronic acid binding" may interact with hyaluronic acid or hyaluronic acid-containing tissues such that the interaction is not attributed to known chemically reactive groups. The interaction may be due to hydrophobic and charge interactions resulting from the amino acid residues in the peptide. The interaction may be measured by immobilizing hyaluronic acid on a microplate and incubating with hyaluronic acid binding peptides followed by detection techniques such as biotin-avidin with the use of a chromophore. The interaction may also be measured by mechanical influence on hyaluronic acid-containing fluids, gels, or tissues that have been incubated with the hyaluronic acid binding peptide, or with a peptidoglycan containing an hyaluronic acid binding peptide or peptides.

For identifying a peptide, a peptide selected from phage display, or one that is identified from a hyaluronic acid binding motif in a protein, can be synthesized and evaluated for its interaction with hyaluronic acid. For example, a B-X7-B sequence could be synthesized with a biotin modification at the N-terminus and incubated on a hyaluronic acid coated microplate. A dose response binding curve can be generated to determine the ability of the peptide to bind to hyaluronic acid.

Similarly for a collagen binding peptide, a synthetic peptide derived from a phage display library selected for collagen binding can be generated. The peptide can be synthesized and

evaluated for binding to collagen by any of the techniques such as SPR, ELISA, ITC, affinity chromatography, or others known in the art. An example could be a biotin modified peptide sequence that is incubated on a microplate containing immobilized collagen. A dose response binding curve can be generated using a streptavidin-chromophore to determine the ability of the peptide to bind to collagen.

In one embodiment, provided herein is an extracellular matrix-binding synthetic peptidoglycan comprising:

a) chondroitin sulfate;

b) from about 1 to about 80 collagen binding peptide(s); and

c) from about 1 to about 80 hyaluronic acid binding peptide(s);

wherein the peptides of b) and c) are covalently bonded to the glycan; and further wherein the collagen binding peptide(s) are WYRGRLGC and the hyaluronic acid binding peptide(s) are GAHWQFNALTVRGGGC. In certain embodiments, the peptides are covalently bonded to the glycan via a linker, such as N-[β-maleimidopropionic acid]hydrazide (BMPH).

In another embodiment, provided herein is an extracellular matrix-binding synthetic peptidoglycan comprising:

a) chondroitin sulfate;

b) from about 5 to about 20 collagen binding peptide(s); and

c) from about 5 to about 20 hyaluronic acid binding peptide(s);

wherein the peptides of b) and c) are covalently bonded to the glycan via N-[β-maleimidopropionic acid]hydrazide (BMPH); and further wherein the collagen binding peptide(s) are WYRGRLGC and the hyaluronic acid binding peptide(s) are GAHWQFNALTVRGGGC.

In various embodiments described herein, the peptides described herein can be modified by the inclusion of one or more conservative amino acid substitutions. As is well known to those skilled in the art, altering any non-critical amino acid of a peptide by conservative substitution should not significantly alter the activity of that peptide because the side-chain of the replacement amino acid should be able to form similar bonds and contacts to the side chain of

the amino acid which has been replaced. Non-conservative substitutions may too be possible, provided that they do not substantially affect the binding activity of the peptide (i.e., hyaluronic acid or collagen binding affinity).

In various embodiments described herein, the synthetic peptidoglycan is resistant to aggrecanase. An aggrecanase is characterized in the art as any enzyme known to cleave aggrecan. In one embodiment, the synthetic peptidoglycan does not contain a polymerizable group, such as methacrylates, ethacrylates, itaconates, acrylamides, thiols, peptides and aldehydes.

Synthesis of Peptidoglycans

The peptides used in the synthetic peptidoglycans described herein (i.e., the hyaluronic acid binding peptide and the collagen peptide) may be purchased from a commercial source or partially or fully synthesized using methods well known in the art (e.g., chemical and/or biotechnological methods). In certain embodiments, the peptides are synthesized according to solid phase peptide synthesis protocols that are well known in the art. In another embodiment, the peptide is synthesized on a solid support according to the well-known Fmoc protocol, cleaved from the support with trifluoroacetic acid and purified by chromatography according to methods known to persons skilled in the art. In other embodiments, the peptide is synthesized utilizing the methods of biotechnology that are well known to persons skilled in the art. In one embodiment, a DNA sequence that encodes the amino acid sequence information for the desired peptide is ligated by recombinant DNA techniques known to persons skilled in the art into an expression plasmid (for example, a plasmid that incorporates an affinity tag for affinity purification of the peptide), the plasmid is transfected into a host organism for expression, and the peptide is then isolated from the host organism or the growth medium, e.g., by affinity purification. Recombinant DNA technology methods are described in Sambrook et al., "Molecular Cloning: A Laboratory Manual", 3rd Edition, Cold Spring Harbor Laboratory Press, (2001), incorporated herein by reference, and are well-known to the skilled artisan.

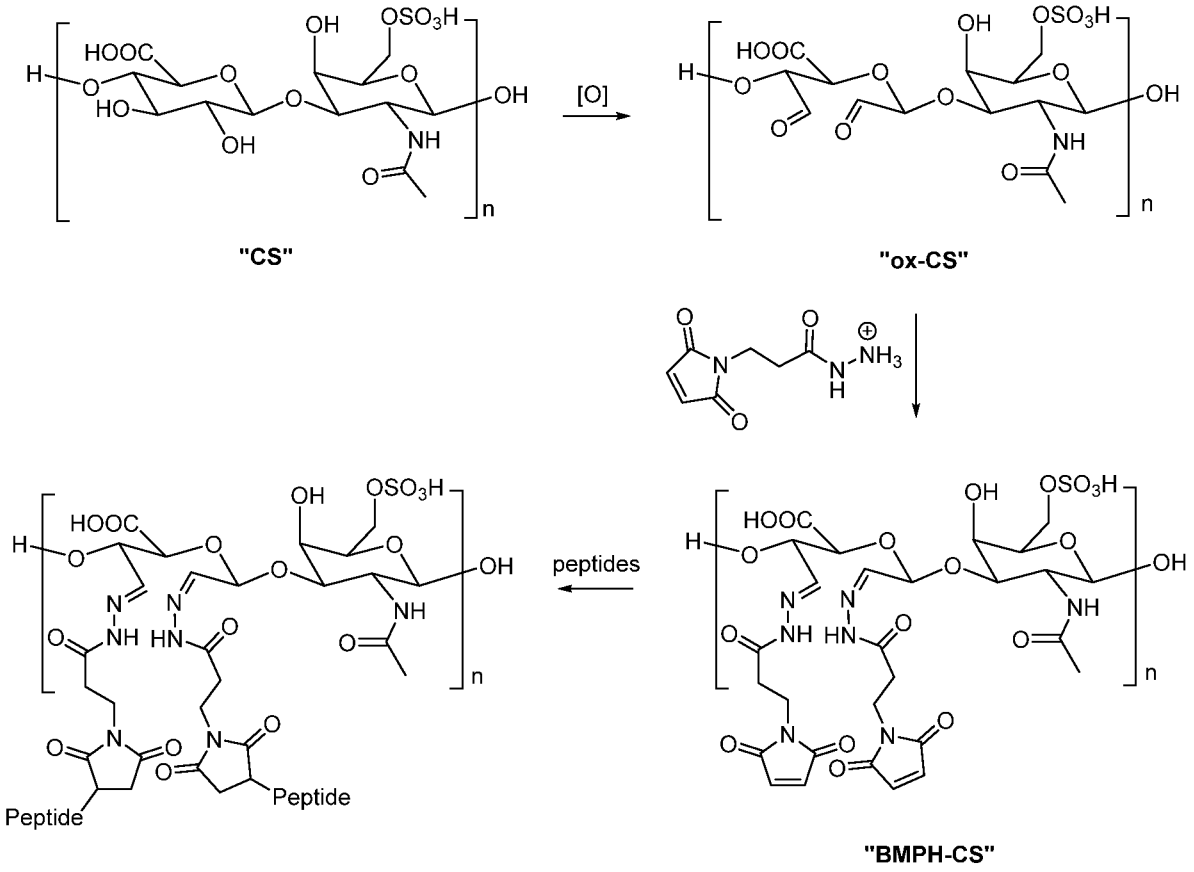
In certain embodiments, the peptides are covalently bonded to the glycan directly (i.e., without a linker). In such embodiments, the synthetic peptidoglycan may be formed by covalently bonding the peptides to the glycan through the formation of one or more amide, ester or imino bonds between an acid, aldehyde, hydroxy, amino, or hydrazo group on the glycan. All

of these methods are known in the art or are further described in the Examples section of this application or in Hermanson G.T., Bioconjugate Techniques, Academic Press, pp. 169-186 (1996), incorporated herein by reference. As shown in Scheme 1, the glycan (e.g., "CS") can be oxidized using a periodate reagent, such as sodium periodate, to provide aldehyde functional groups on the glycan (e.g., "ox-CS") for covalently bonding the peptides to the glycan. In such embodiments, the peptides may be covalently bonded to a glycan by reacting a free amino group of the peptide with an aldehyde functional groups of the oxidized glycan, e.g., in the presence of a reducing agent, utilizing methods known in the art.

In embodiments where the peptides are covalently bonded to the glycan via a linker, the oxidized glycan (e.g., "ox-CS") can be reacted with a linker (e.g., any suitable bifunctional liker, such as 3-(2-pyridyldithio)propionyl hydrazide (PDPH) or N-[β-maleimidopropionic acid]hydrazide (BMPH)) prior to contacting with the peptides. The linker typically comprises about 1 to about 30 carbon atoms, or about 2 to about 20 carbon atoms. Lower molecular weight linkers (i.e., those having an approximate molecular weight of about 20 to about 500) are typically employed. In addition, structural modifications of the linker are contemplated. For example, amino acids may be included in the linker, including but not limited to, naturally occurring amino acids as well as those available from conventional synthetic methods, such as beta, gamma, and longer chain amino acids.

As shown in Scheme 1, in one embodiment, the peptides are covalently bonded to the glycan (e.g., "CS") by reacting an aldehyde function of the oxidized glycan (e.g., "ox-CS") with 3-(2-pyridyldithio)propionyl hydrazide (PDPH) or N-[β-maleimidopropionic acid]hydrazide (BMPH) to form an glycan intermediate (e.g., "BMPH-CS") and further reacting the glycan intermediate with peptides containing at least one free thiol group (i.e., -SH group) to yield the synthetic peptidoglycan. In yet another embodiment, the sequence of the peptides may be modified to include an amino acid residue or residues that act as a spacer between the HA- or Collagen- binding peptide sequence and a terminating cysteine (C). For example a glycine-cysteine (GC) or a glycine-glycine-glycine-cysteine (GGGC) or glycine-serine-glycine-cysteine (GSGC) segment may be added to provide an attachment point for the glycan intermediate.

Scheme 1



Accordingly, in one embodiment, the synthetic peptidoglycans described herein are provided by a) oxidizing at least one vicinal diol group of a glycan to provide a glycan having at least two aldehyde functional groups; b) optionally reacting the glycan with a linker; and reacting the glycan with from about 1 to about 80 collagen binding peptide(s); and from about 1 to about 80 hyaluronic acid binding peptide(s), such that the peptides are covalently bonded to the glycan.

The synthetic peptidoglycan can be synthesized by sequentially adding the peptides having different binding affinities to the glycan (i.e., oxidized glycan or glycan intermediate), or alternatively, adding all peptides simultaneously. The synthetic peptidoglycans can be isolated and/or purified using known methods, such as size exclusion chromatography, at any point in the synthesis.

Pharmaceutical Compositions and Administration

Disclosed herein is a pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan, wherein the synthetic peptidoglycan comprises a glycan having from about 1 to about 80 collagen binding peptide(s) and from about 1 to about 80 hyaluronic acid binding peptide(s) covalently bonded to the glycan.

As is known in the art, the components as well as their relative amounts are determined by the intended use and method of delivery. The pharmaceutical compositions may be for oral, topical or parenteral delivery, including intra-articular, intervertebral or intraocular delivery. In any of the embodiments described herein, the synthetic peptidoglycan can be administered alone or in combination with suitable pharmaceutical carriers or diluents. Diluent or carriers used in the pharmaceutical compositions can be selected so that they do not diminish the desired effects of the synthetic peptidoglycan. The pharmaceutical composition may be in any suitable form. Examples of suitable dosage forms include aqueous solutions, for example, a solution in isotonic saline, 5% glucose or other well-known pharmaceutically acceptable liquid carriers such as alcohols, glycols, esters and amides. In certain embodiments, the pharmaceutical composition further comprises one or more pH buffering agent, one or more ionic strength modifying agent, and/or one or more viscosity modulating agent.

In certain embodiments, the pharmaceutical composition further comprises a collagen binding peptidoglycan. Suitable "collagen binding peptidoglycans" are described in the art (see, e.g., US 2013/0190246, US 2012/0100106, and US 2011/0020298). In certain embodiments, the collagen binding peptidoglycan comprises chondroitin sulfate conjugated to from about 1 to about 20 collagen binding peptide(s) as described herein. In certain embodiments, the collagen binding peptide(s) of the collagen binding peptidoglycan is WYRGRLGC.

In certain embodiments, the pharmaceutical composition further comprises a hyaluronic acid binding peptidoglycan. Suitable "hyaluronic acid binding peptidoglycans" are described in the art (see, e.g., WO 2012/162534). In certain embodiments, the hyaluronic acid binding peptidoglycan comprises chondroitin sulfate conjugated to from about 1 to about 20 hyaluronic acid binding peptide(s) as described herein. In certain embodiments, the hyaluronic acid binding peptide(s) of the hyaluronic acid binding peptidoglycan is GAHWQFNALTVRGGGC.

Suitable pH buffering agents for use in the pharmaceutical compositions herein described include, for example, acetate, borate, carbonate, citrate, and phosphate buffers, as well as hydrochloric acid, sodium hydroxide, magnesium oxide, monopotassium phosphate, bicarbonate, ammonia, carbonic acid, hydrochloric acid, sodium citrate, citric acid, acetic acid, disodium hydrogen phosphate, borax, boric acid, sodium hydroxide, diethyl barbituric acid, and proteins, as well as various biological buffers, for example, TAPS, Bicine, Tris, Tricine, HEPES, TES, MOPS, PIPES, cacodylate, or MES.

Suitable ionic strength modifying agents include, for example, glycerin, propylene glycol, mannitol, glucose, dextrose, sorbitol, sodium chloride, potassium chloride, and other electrolytes.

Suitable viscosity modulating agents include but are not limited to, ionic and non-ionic water soluble polymers; crosslinked acrylic acid polymers such as the "carbomer" family of polymers, e.g., carboxypolyalkylenes that may be obtained commercially under the Carbopol™ trademark; hydrophilic polymers such as polyethylene oxides, polyoxyethylene-polyoxypropylene copolymers, and polyvinylalcohol; cellulosic polymers and cellulosic polymer derivatives such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methyl cellulose, carboxymethyl cellulose, and etherified cellulose; gums such as tragacanth and xanthan gum; sodium alginate; gelatin, hyaluronic acid and salts thereof, chitosans, gellans or any combination thereof. Typically, non-acidic viscosity enhancing agents, such as a neutral or basic agent are employed in order to facilitate achieving the desired pH of the formulation.

In some embodiments, the synthetic peptidoglycans can be combined with minerals, amino acids, sugars, peptides, proteins, vitamins (such as ascorbic acid), or laminin, collagen, fibronectin, hyaluronic acid, fibrin, elastin, or aggrecan, or growth factors such as epidermal growth factor, platelet-derived growth factor, transforming growth factor beta, or fibroblast growth factor, and glucocorticoids such as dexamethasone or viscoelastic altering agents, such as ionic and non-ionic water soluble polymers; acrylic acid polymers; hydrophilic polymers such as polyethylene oxides, polyoxyethylene-polyoxypropylene copolymers, and polyvinylalcohol; cellulosic polymers and cellulosic polymer derivatives such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methyl cellulose, carboxymethyl cellulose, and etherified cellulose; poly(lactic acid),

poly(glycolic acid), copolymers of lactic and glycolic acids, or other polymeric agents both natural and synthetic.

Parenteral formulations may be suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. The preparation of parenteral formulations under sterile conditions, for example, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well known in the art.

In certain embodiments, the solubility of the synthetic peptidoglycan may need to be enhanced. In such cases, the solubility may be increased by the use of appropriate formulation techniques, such as the incorporation of solubility-enhancing compositions such as mannitol, ethanol, glycerin, polyethylene glycols, propylene glycol, poloxomers, and others known in the art.

The synthetic peptidoglycan may be sterilized to remove unwanted contaminants including, but not limited to, endotoxins and infectious agents. Sterilization techniques which do not adversely affect the structure and biotropic properties of the synthetic peptidoglycan can be used. In certain embodiments, the synthetic peptidoglycan can be disinfected and/or sterilized using conventional sterilization techniques including propylene oxide or ethylene oxide treatment, sterile filtration, gas plasma sterilization, gamma radiation, electron beam, and/or sterilization with a peracid, such as peracetic acid. In one embodiment, the synthetic peptidoglycan can be subjected to one or more sterilization processes. Alternatively, the synthetic peptidoglycan may be wrapped in any type of container including a plastic wrap or a foil wrap, and may be further sterilized.

In various embodiments, the synthetic peptidoglycan can be administered intravenously or into muscle, for example. Suitable routes for parenteral administration include intravascular, intravenous, intraarterial, intramuscular, cutaneous, subcutaneous, percutaneous, intradermal, and intraepidermal delivery. Suitable means for parenteral administration include needle (including microneedle) injectors, infusion techniques, and catheter-based delivery.

In various embodiments described herein, formulations for parenteral administration may be formulated to be for immediate and/or modified release. Modified release formulations include delayed, sustained, pulsed, controlled, targeted and programmed release formulations.

Thus, a synthetic peptidoglycan may be formulated as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing modified release of the active compound. Illustrative examples of such formulations include drug-coated stents and copolymeric (dl-lactic, glycolic)acid (PGLA) microspheres. In another embodiment, the synthetic peptidoglycan or composition comprising the synthetic peptidoglycan may be continuously administered, where appropriate.

In any of the embodiments described herein, the synthetic peptidoglycan or composition comprising the synthetic peptidoglycan can be administered intravascularly into the patient (e.g., into an artery or vein) in any suitable way. In various embodiments described herein, the synthetic peptidoglycan or composition comprising the synthetic peptidoglycan can be administered into a vessel of a patient prior to, during, or after vascular intervention. In various embodiments, vascular interventions, such as percutaneous coronary intervention (PCI), can include, for example, stenting, atherectomy, grafting, and angioplasty, such as balloon angioplasty. Illustratively, the vascular intervention can be one which involves temporarily occluding an artery, such as a coronary artery or a vein (e.g., balloon angioplasty), or it can be one which does not involve temporarily occluding an artery or a vein (e.g., non-balloon angioplasty procedures, stenting procedures that do not involve balloon angioplasty, etc.). Illustrative modes of delivery can include a catheter, parenteral administration, a coating on a balloon, through a porous balloon, a coated stent, and any combinations thereof or any other known methods of delivery of drugs during a vascular intervention procedure. In one illustrative embodiment, the target vessel can include a coronary artery, e.g., any blood vessel which supplies blood to the heart tissue of a patient, including native coronary arteries as well as those which have been grafted into the patient, for example, in an earlier coronary artery bypass procedure.

Exemplary pharmaceutical compositions for use with the synthetic peptidoglycans for parenteral administration or catheter-based delivery may comprise: a) a synthetic peptidoglycan as described herein; b) a pharmaceutically acceptable pH buffering agent to provide a pH in the range of about pH 4.5 to about pH 9; c) an ionic strength modifying agent in the concentration range of about 0 to about 300 millimolar; and d) water soluble viscosity modifying agent in the concentration range of about 0.25% to about 10% total formula weight or any individual component a), b), c), or d) or any combinations of a), b), c) and d).

Suitable dosages of the synthetic peptidoglycan can be determined by standard methods, for example by establishing dose-response curves in laboratory animal models or in clinical trials and can vary significantly depending on the patient condition, the disease state being treated, the route of administration and tissue distribution, and the possibility of co-usage of other therapeutic treatments. The effective amount to be administered to a patient is based on body surface area, patient weight or mass, and physician assessment of patient condition. In various exemplary embodiments, an effective dose ranges from about 1 ng/kg to about 10 mg/kg, 100 ng/kg to about 1 mg/kg, from about 1 μg/kg to about 500 μg/kg, or from about 100 μg/kg to about 400 μg/kg. In each of these embodiments, dose/kg refers to the dose per kilogram of patient mass or body weight. In other illustrative aspects, effective doses ranges from about 0.01 μg to about 1000 mg per dose, 1 μg to about 100 mg per dose, or from about 100 μg to about 50 mg per dose, or from about 500 μg to about 10 mg per dose or from about 1 mg to 10 mg per dose, or from about 1 to about 100 mg per dose, or from about 1 mg to 5000 mg per dose, or from about 1 mg to 3000 mg per dose, or from about 100 mg to 3000 mg per dose, or from about 1000 mg to 3000 mg per dose. In any of the various embodiments described herein, effective doses ranges from about 0.01 μg to about 1000 mg per dose, 1 μg to about 100 mg per dose, about 100 μg to about 1.0 mg, about 50 μg to about 600 μg, about 50 μg to about 700 μg, about 100 μg to about 200 μg, about 100 μg to about 600 μg, about 100 μg to about 500 μg, about 200 μg to about 600 μg, or from about 100 μg to about 50 mg per dose, or from about 500 μg to about 10 mg per dose or from about 1 mg to 10 mg per dose. In other illustrative embodiments, effective doses can be 1 μg, 10 μg, 25 μg, 50 μg, 75 μg, 100 μg, 125 μg, 150 μg, 200 μg, 250 μg, 275 μg, 300 μg, 350 μg, 400 μg, 450 μg, 500 μg, 550 μg, 575 μg, 600 μg, 625 μg, 650 μg, 675 μg, 700 μg, 800 μg, 900 μg, 1.0 mg, 1.5 mg, 2.0 mg, 10 mg, 100 mg, or 100 mg to 30 grams.

Any effective regimen for administering the synthetic peptidoglycan can be used. For example, the synthetic peptidoglycan can be administered as a single dose, or as a multiple-dose daily regimen. Further, a staggered regimen, for example, one to five days per week can be used as an alternative to daily treatment.

In various embodiments described herein, the patient is treated with multiple injections of the synthetic peptidoglycan. In one embodiment, the patient is injected multiple times (e.g., about 2 up to about 50 times) with the synthetic peptidoglycan, for example, at 12-72 hour

intervals or at 48-72 hour intervals. Additional injections of the synthetic peptidoglycan can be administered to the patient at an interval of days or months after the initial injections(s).

Methods

The synthetic peptidoglycans described herein may be useful in replacing, rejuvenating, or supplementing tissues that have both collagen and hyaluronic acid, such as cartilage, synovial fluid, and the vitreous humor.

Cartilage degeneration

A well-lubricated surface on articular cartilage leads to optimal functionality of synovial joints. As occurs in osteoarthritis, however, a reduced lubrication results in cartilage degradation and fibrillation; which in turn contribute to joint dysfunction and pain. Reduced lubrication also leads to joint dysfunction and pain in other forms of arthritis, including rheumatoid arthritis.

As shown in Example 2, the synthetic peptidoglycans provided herein can be used to mimic some of the functions of lubricin, a mucinous glycoprotein secreted by tissues lining the interior surfaces of animal joints. The synthetic peptidoglycan thus has the potential to enhance lubrication at an articular cartilage surface, thereby reducing wear-induced erosion of the cartilage. The synthetic peptidoglycan also has the potential to protect macromolecules, like hyaluronic acid and type II collagen, from enzyme-induced degradation.

Accordingly, provided is a method of treating and/or preventing cartilage degeneration in a patient comprising administering to a patient in need thereof a pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein. In one embodiment, the patient is treated by injecting the pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan into a synovial cavity.

It is also contemplated that the synthetic peptidoglycans can be used to treat and/or prevent articular cartilage disease by protecting the articular cartilage matrix from traumatic and cytokine-induced enzymatic degradation.

Vitreous humor degeneration

The vitreous humor is a viscoelastic, gel-like substance that fills the posterior cavity of the eye. Vitreous replacements have been used to replace a dysfunctional vitreous humor, for example in cases where opacification or the physical collapse and liquefaction of the vitreous has

occurred, and as a temporary or permanent vitreous replacement during retinal surgery. A suitable vitreous replacement should be transparent, biocompatible, and they should have a density and refractive index close to the natural vitreous.

Accordingly, provided is a method of treating and/or preventing vitreous humor degeneration in a patient comprising administering to a patient in need thereof a pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein.

Nucleus pulposus degeneration

The nucleus pulposus is a gel-like substance present in spinal discs, and functions to distribute hydraulic pressure in all directions within each disc under compressive loads and is comprised of chondrocyte-like cells, collagen fibrils, and proteoglycan aggrecans that aggregate through hyaluronic chains. Degeneration of the nucleus pulposus results in reduced ability of the disc to transmit loads evenly and efficiently between vertebral bodies, and leads to damage in the annular region of the disc, known as the annulus fibrosis. Fissures or tears in the annulus can translate into a disc that herniates or ruptures, resulting in impingement of the nerves in the region of the disc and finally lower back or leg pain.

Attempts have also been made to replace only the nucleus pulposus. Replacement of the nucleus pulposus is expected to arrest the initial dehydration of the degenerated nucleus and return the disc to a fully hydrated state so that the degenerative process, including the associated pain, is postponed or prevented and the mechanical function is restored to the vertebral segment.

It is contemplated that the synthetic peptidoglycans described herein will bind to and protect the annulus fibrosis. Accordingly, provided is a method of treating and/or preventing annulus fibrosis degeneration in a patient comprising administering to a patient in need thereof a pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein. Also provided is a method of treating and/or preventing nucleus pulposus degeneration in a patient comprising administering to a patient in need thereof a pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan described herein.

Table of abbreviations

The following abbreviations used herein have the following meanings.

µg	Microgram
µm	Micrometer
BMPH	N-[β-maleimidopropionic acid]hydrazide
CS	Chondroitin sulfate
DNA	Deoxyribonucleic acid
HBSS	Hank's Balanced Salt Solution
HEPES	hydroxyethyl piperazineethanesulfonic acid
hr	Hour
Hz	Hertz
kg	Kilogram
M	Molar
M	Molar
m	Meter
MES	2-ethanesulfonic acid
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
MOPS	3-(N-morpholino)propanesulfonic acid
N	Newton
Pa	pascal
PBS	Phosphate buffered saline
PDPH	3-(2-pyridyldithio)propionyl hydrazide
PIPES	piperazine-N,N′-bis(2-ethanesulfonic acid)
rad/s	radian per second
TAPS	3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]propane-1-sulfonic acid
TES	2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid
wt%	Weight percent

Examples

Example 1: Synthesis of peptidoglycans

Reagents

The peptides GAHWQFNALTVRGGGC (GAH) and WYRGRLGC (WYRGRL) were purchased from Genscript (Piscataway, NJ). *N*-[ß-maleimidopropionic acid] hydrazide, trifluoroacetic acid salt (BMPH) was purchased from Pierce (Rockford, IL).

Methods

The synthesis of collagen type I and hyaluronic acid binding peptidoglycans has been previously described (see, e.g., Bernhard J.C., Panitch A. Acta Biomater 2012; 8: 1543-1550, and Paderi J.E., Panitch A. Biomacromolecules 2008; 9: 2562-2566). The present synthetic peptidoglycan was prepared according to the methods described therein and modified as follows.

Chondroitin sulfate (10 mg/mL) was oxidized with sodium meta-periodate in 0.1 M sodium acetate buffer pH 5.5. The degree of oxidation was controlled by the concentration of sodium meta-periodate. Heterobifunctional crosslinker BMPH was conjugated to functionalized chondroitin sulfate by reacting about 40-fold molar excess BMPH to chondroitin sulfate in 1xPBS pH 7.2 buffer at room temperature for 2 hours. The degree of functionalization was determined by measuring the consumption of BMPH during purification of CS-BMPH from excess BMPH. In the final step of conjugation, peptides WYRGRLGC and GAHWQFNALTVRGGGC were dissolved in water and each was added at one-half molar equivalent to the number of BMPH reactive groups on chondroitin. For example, chondroitin sulfate with an average of about 22 BMPH functional groups was conjugated with about 11 WYRGRLGC peptides and about 11 GAHWQFNALTVRGGGC peptides. The mixture containing both peptides was reacted for 2 hours at room temperature followed by purification in ultrapure water and lyophilization.

All intermediates were purified by size-exclusion chromatography using an ÄKTA Purifier FPLC (GE Healthcare) and a column packed with polyacrylamide beads (Bio-Rad Labs). The final product was similarly purified using a column packed with sephadex G-25 beads. After synthesis, the synthetic peptidoglycan can be lyophilized and stored for extended periods of time at -80 °C.

Example 2: Lubricin Mimetic

The ability of the lubrican mimetic peptidoglycan to reduce the frictional force between cartilage surfaces was tested as follows. A cartilage plug was glued to the plate of a rheometer, and a second cartilage plug was glued to the rotating fixture of the rheometer. The two cartilage surfaces were put together with a 5N force, and then a shear was applied. The torque was measured and used to calculate the frictional force between the surfaces.

Rheometer

1. Set the conditioning step:
    a. Temperature: 37 °C
    b. Normal Force: 5 N
    c. Normal Force Tolerance: 0.1 N
    d. Duration: 1 hr
2. Set the peak hold steps:
    a. Angular Velocity range: 0.001 – 100.0 rad/s
    b. Sample Points: 180
    c. Temperature: 37°C
    d. Duration: 1hr
    e. Gap limit down: 10000 μm
    f. Gap limit up: 100 μm
3. Weigh the cartilage plugs.
4. Glue the larger cartilage plug to a strip of tape placed inside a large petri dish. Place the petri dish on the rheometer plate and hold it in place with two clamps.
5. Glue the smaller cartilage plug to a strip of tape and place it on the geometry head (20 mm).
6. Add 10 μL of hyaluronic acid to the larger plug and lower the geometry head until both plugs are touching. Add 20 mL of HBSS to the petri dish to maintain hydration.

7. Run the steps and name the file when prompted.
8. Check once in a while if the HBSS has evaporated. If it has, add more.
9. Once the steps are done, open Data Analysis and open the file.
10. Right click on the file name and select Table. A table with the sample points will show up. For the different steps for each sample, a pull down tab appears and you can select one step at a time and the values will show up on the screen.
11. Copy and paste in Excel.
12. Calculate the time in minutes and the torque in N/m.
13. Calculate the total frictional force (F):

$$F = \frac{(3+\alpha)(r_o^3 - r_i^3)}{(2+\alpha)(r_o^4 - r_i^4)} M$$

, where α=1, M=torque, r_o=outside radius of plug, and r_i=inside radius of plug.

14. Calculate the average frictional force per unit area (f) of the cartilage plugs:

$$f = \frac{F}{\pi(r_o^2 - r_i^2)}$$

, where r_o=outside radius of plug, r_i=inside radius of plug, and F =total frictional force.

15. Calculate the friction coefficient using: $\mu = F/W$ where F=total frictional force and W=weight.

Data

Figure 1 shows the frictional force in the case of undamaged cartilage (with no aggrecan depletion) and shows that with the lubricin mimetic peptidoglycan according to the disclosure, the friction increases between the cartilage surfaces. Figure 2 shows the frictional force in the case of damaged cartilage (with aggrecan depletion to simulate osteoarthritis) and shows that when the lubricin mimetic peptidoglycan according to the disclosure is added to the cartilage, friction is lowered between the cartilage surfaces. Accordingly, when the lubrican mimetic synthetic peptidoglycan according to the disclosure was applied between the two cartilage

surfaces, the frictional force was decreased at higher oscillatory shear, suggesting that it can protect the cartilage during rapid movements.

Example 3: Vitreous humor mimetic

Methods

The synthetic peptidoglycans according to the present disclosure can be tested as follows. Viscoelastic properties can be measured using a rheometer, for example the TAInstruments ARG2 rheometer. A cone and plate geometry or a parallel plate geometry can be used. Vitreous humor (e.g., bovine) can be placed within the geometry. The temperature can be controlled and frequency sweeps can be performed from ranges of about 0.1 Hz to about 100 Hz (e.g., 1 Hz) and storage, loss, and complex modulus can be measured at an oscillatory shear stress between about 0.1 Pa and about 10 Pa (e.g., 10 Pa). Temperature: 37 °C

Data

Figure 3A shows the rheological measurements on bovine vitreous with no treatment (control), PBS buffer and trypsin, where the trypsin treated bovine vitreous exhibited a lower viscosity, thus mimicking the disease state.

In Figure 3B and Figure 3C, GAH is a synthetic peptidoglycan comprising chondroitin sulfate with hyaluronic acid binding peptide (i.e., GAHWQFNALTVRGGGC) covalently bonded thereto (via BMPH), WYR is a synthetic peptidoglycan comprising chondroitin sulfate with collagen II binding peptide (i.e., WYRGRLGC) covalently bonded thereto (via BMPH), and WYRGAH is a mixture of the two synthetic peptidoglycans WYR and GAH just described.

Figure 3B shows the restoration of the vitreous humor mechanical strength after treatment with the synthetic peptidoglycans.

Figure 3C shows that the mechanical strength of the vitreous humor is sufficiently maintained in the presence of the synthetic peptidoglycans.

Based on the data shown in Figures 3A, 3B and 3C, it is contemplated that the present synthetic peptidoglycans can be used to treat diseases and disorders affecting the vitreous humor.

What is Claimed is:

1. An extracellular matrix-binding synthetic peptidoglycan comprising:

a) a glycan;

b) from about 1 to about 80 collagen binding peptide(s); and

c) from about 1 to about 80 hyaluronic acid binding peptide(s);

wherein the peptides of b) and c) are covalently bonded to the glycan.

2. The synthetic peptidoglycan of claim 1, wherein the glycan is dextran, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparan, heparin, keratin, keratan sulfate, or hyaluronic acid.

3. The synthetic peptidoglycan of claim 1, wherein the glycan is dermatan sulfate.

4. The synthetic peptidoglycan of claim 1, wherein the glycan is chondroitin sulfate.

5. The synthetic peptidoglycan of claim 1, wherein the glycan is hyaluronic acid.

6. The synthetic peptidoglycan of claim 1, wherein the peptides are covalently bonded to the glycan via a linker.

7. The synthetic peptidoglycan of claim 6, wherein the linker is N-[β-maleimidopropionic acid]hydrazide (BMPH).

8. The synthetic peptidoglycan of claim 1, wherein the synthetic peptidoglycan comprises a total of less than about 50 peptides.

9. The synthetic peptidoglycan of claim 1, wherein the synthetic peptidoglycan comprises a total of from about 10 to about 40 peptides.

10. The synthetic peptidoglycan of claim 1, wherein the synthetic peptidoglycan comprises a total of about 22 peptides.

11. The synthetic peptidoglycan of claim 1, wherein the collagen binding peptide has binding affinity to one or more of collagen types I, II, III, or IV.

12. The synthetic peptidoglycan of claim 1, wherein the collagen binding peptide comprises at least one collagen binding peptide that binds to type I collagen and at least one collagen binding peptide that binds to type II collagen.

13. The synthetic peptidoglycan of claim 1, wherein the collagen binding peptide comprises an amino acid sequence selected from the group consisting of:

i) WYRGRLGC, RRANAALKAGELYKSILYGC, RLDGNEIKRGC, AHEEISTTNEGVMGC , GCGGELYKSILY, NGVFKYRPRYFLYKHAYFYPPLKRFPVQGC, CQDSETRTFY, TKKTLRTGC, GLRSKSKKFRRPDIQYPDATDEDITSHMGC, SQNPVQPGC, SYIRIADTNITGC, SYIRIADTNIT, KELNLVYT, KELNLVYTGC, GSITTIDVPWNV, GELYKSILYGC and GSITTIDVPWNVGC, or

ii) any peptide sequence comprising a sequence with at least about 80% sequence identity to the amino acid sequence of i).

14. The synthetic peptidoglycan of claim 1, wherein the collagen binding peptide is WYRGRLGC, RRANAALKAGELYKSILYGC, RLDGNEIKRGC, AHEEISTTNEGVMGC , GCGGELYKSILY, NGVFKYRPRYFLYKHAYFYPPLKRFPVQGC, CQDSETRTFY, TKKTLRTGC, GLRSKSKKFRRPDIQYPDATDEDITSHMGC, SQNPVQPGC, SYIRIADTNITGC, SYIRIADTNIT, KELNLVYT, KELNLVYTGC, GSITTIDVPWNV, GELYKSILYGC or GSITTIDVPWNVGC.

15. The synthetic peptidoglycan of claim 1, wherein the collagen binding peptide is WYRGRLGC.

16. The synthetic peptidoglycan of claim 1, wherein the hyaluronic acid binding peptide comprises an amino acid sequence selected from the group consisting of:

i) GAHWQFNALTVRGG, GDRRRRRMWHRQ, GKHLGGKHRRSR, RGTHHAQKRRS, RRHKSGHIQGSK, SRMHGRVRGRHE, RRRAGLTAGRPR, RYGGHRTSRKWV, RSARYGHRRGVG, GLRGNRRVFARP, SRGQRGRLGKTR, DRRGRSSLPKLAGPVEFPDRKIKGRR, RMRRKGRVKHWG, RGGARGRHKTGR, TGARQRGLQGGWGPRHLRGKDQPPGR, RQRRRDLTRVEG, STKDHNRGRRNVGPVSRSTLRDPIRR, RRIGHQVGGRRN, RLESRAAGQRRA, GGPRRHLGRRGH, VSKRGHRRTAHE, RGTRSGSTR, RRRKKIQGRSKR, RKSYGKYQGR, KGRYSISR, RRRCGQKK, KQKIKHVVKLK, KLKSQLVKRK, RYPISRPRKR, KVGKSPPVR, KTFGKMKPR, RIKWSRVSK and KRTMRPTRR; or

ii) any peptide sequence comprising a sequence with at least about 80% sequence identity to the amino acid sequence of i).

17. The synthetic peptidoglycan of claim 1, wherein the hyaluronic acid binding peptide is GAHWQFNALTVRGG, GDRRRRRMWHRQ, GKHLGGKHRRSR, RGTHHAQKRRS, RRHKSGHIQGSK, SRMHGRVRGRHE, RRRAGLTAGRPR, RYGGHRTSRKWV, RSARYGHRRGVG, GLRGNRRVFARP, SRGQRGRLGKTR, DRRGRSSLPKLAGPVEFPDRKIKGRR, RMRRKGRVKHWG, RGGARGRHKTGR, TGARQRGLQGGWGPRHLRGKDQPPGR, RQRRRDLTRVEG, STKDHNRGRRNVGPVSRSTLRDPIRR, RRIGHQVGGRRN, RLESRAAGQRRA, GGPRRHLGRRGH, VSKRGHRRTAHE, RGTRSGSTR, RRRKKIQGRSKR, RKSYGKYQGR, KGRYSISR, RRRCGQKK, KQKIKHVVKLK, KLKSQLVKRK, RYPISRPRKR, KVGKSPPVR, KTFGKMKPR, RIKWSRVSK or KRTMRPTRR.

18. The synthetic peptidoglycan of claim 1, wherein the hyaluronic acid binding peptide is GAHWQFNALTVRGGGC.

19. An extracellular matrix-binding synthetic peptidoglycan comprising:

a) chondroitin sulfate;

b) from about 1 to about 80 collagen binding peptide(s); and

c) from about 1 to about 80 hyaluronic acid binding peptide(s);

wherein the peptides of b) and c) are covalently bonded to the glycan; and further wherein the collagen binding peptide(s) is WYRGRLGC and the hyaluronic acid binding peptide(s) is GAHWQFNALTVRGGGC.

20. The synthetic peptidoglycan of claim 19, wherein the peptides are covalently bonded to the glycan via a linker.

21. The synthetic peptidoglycan of claim 20, wherein the linker is N-[β-maleimidopropionic acid]hydrazide (BMPH).

22. An extracellular matrix-binding synthetic peptidoglycan comprising:

a) chondroitin sulfate;

b) from about 5 to about 20 collagen binding peptide(s); and

c) from about 5 to about 20 hyaluronic acid binding peptide(s);

wherein the peptides of b) and c) are covalently bonded to the glycan via N-[β-maleimidopropionic acid]hydrazide (BMPH); and further wherein the collagen binding peptide(s) are WYRGRLGC and the hyaluronic acid binding peptide(s) are GAHWQFNALTVRGGGC.

23. A pharmaceutical composition comprising the extracellular matrix-binding synthetic peptidoglycan of any one of claims 1-22.

24. A method of treating and/or preventing degradation of a hyaluronic acid rich tissue in a patient comprising administering to a patient in need thereof the pharmaceutical composition of claim 23.

25. The method of claim 24, wherein the hyaluronic acid rich tissue is the skin.

26. A method of treating and/or preventing cartilage degeneration in a patient comprising administering to a patient in need thereof the pharmaceutical composition of claim 23.

27. A method of treating and/or preventing cartilage degeneration in a patient comprising injecting the pharmaceutical composition of claim 23 into a synovial cavity of a patient in need thereof.

28. A method of treating and/or preventing vitreous humor degeneration in a patient comprising administering to a patient in need thereof the pharmaceutical composition of claim 23.

29. A method of treating and/or preventing nucleus pulposus degeneration in a patient comprising administering to a patient in need thereof the pharmaceutical composition of claim 23.

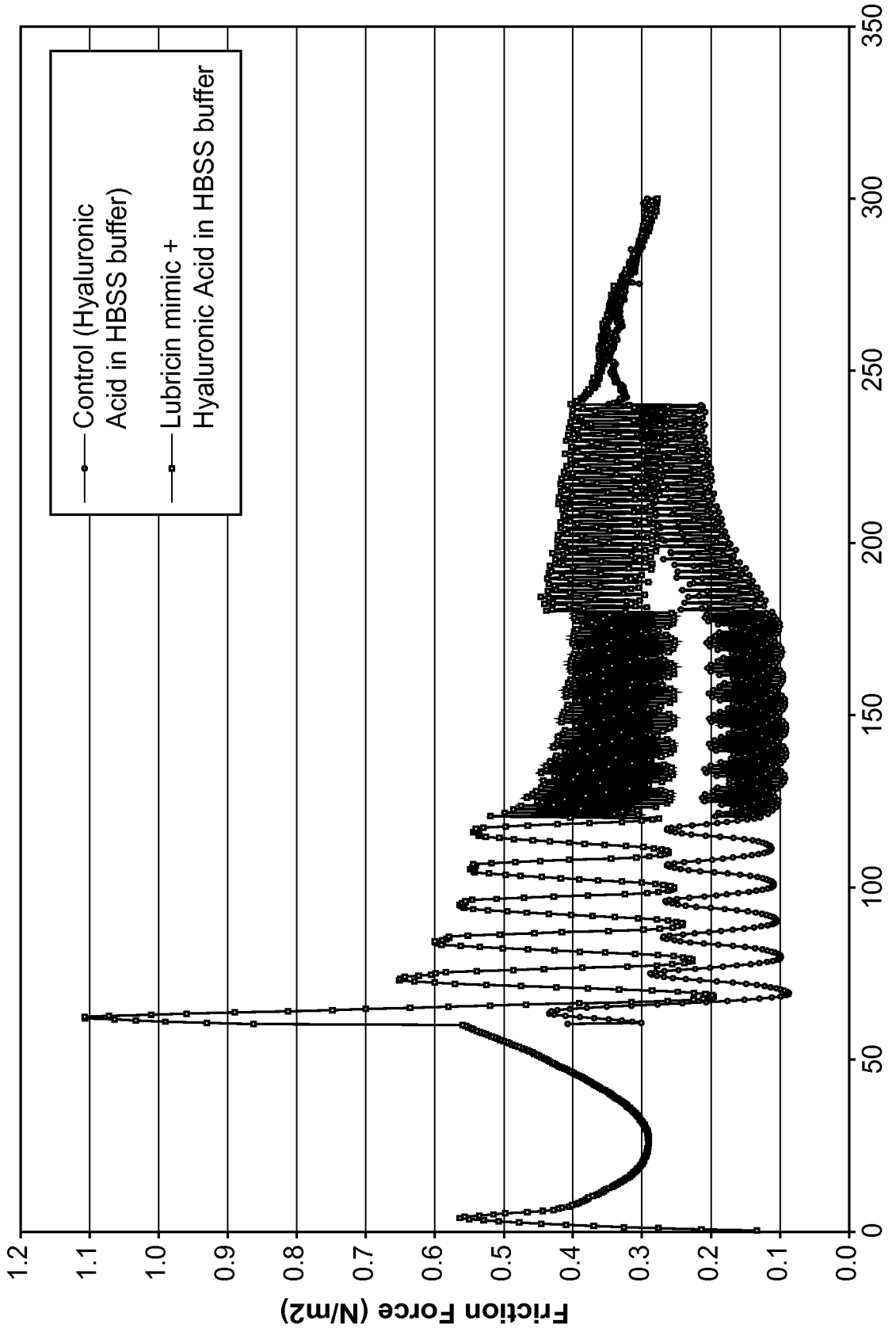

Control (Hyaluronic Acid in HBSS buffer)
Lubricin mimic + Hyaluronic Acid in HBSS buffer
Friction Force (N/m2)
Time (min)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
0
50
100
150
200
250
300
350

FIG. 1

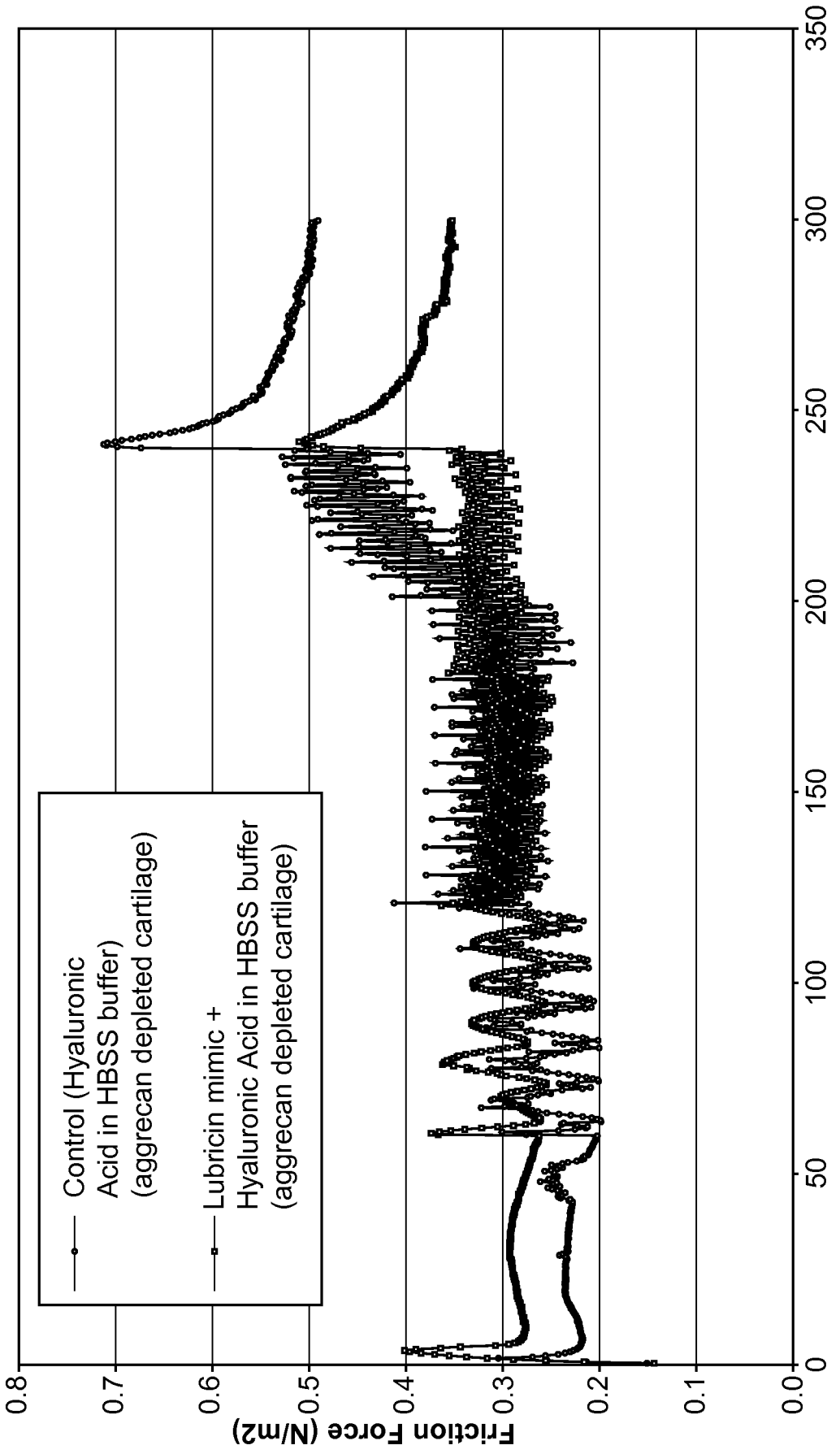

Friction Force (N/m2)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Time (min)
0
50
100
150
200
250
300
350
Control (Hyaluronic Acid in HBSS buffer) (aggrecan depleted cartilage)
Lubricin mimic + Hyaluronic Acid in HBSS buffer (aggrecan depleted cartilage)

FIG. 2

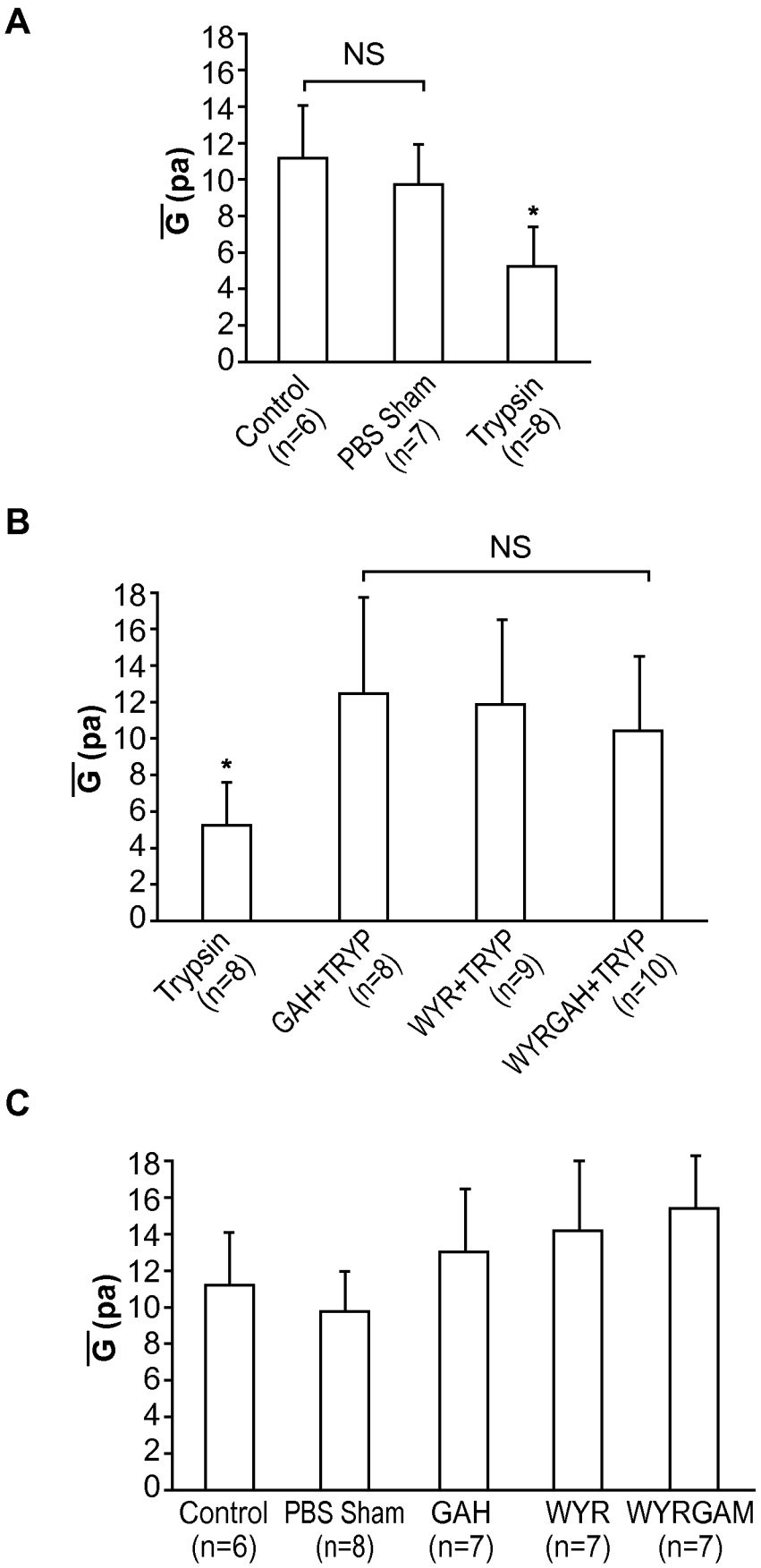

A
NS
$\overline{G}$ (pa)
18
16
14
12
10
8
6
4
2
0
*
Control (n=6)
PBS Sham (n=7)
Trypsin (n=8)
B
NS
$\overline{G}$ (pa)
*
Trypsin (n=8)
GAH+TRYP (n=8)
WYR+TRYP (n=9)
WYRGAH+TRYP (n=10)
C
$\overline{G}$ (pa)
Control (n=6)
PBS Sham (n=8)
GAH (n=7)
WYR (n=7)
WYRGAM (n=7)

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/029596

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00(2006.01)i, A61K 38/00(2006.01)i

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)
C07K 19/00; C07K 7/08; C07K 7/06; A61K 38/00; C07K 14/78; A61K 39/00; C07K 17/14; A61K 38/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: synthetic peptidoglycan, collagen binding peptide, hyaluronic acid binding peptide, glycan, BMPH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012-162534 A2 (PURDUE RESEARCH FOUNDATION) 29 November 2012. See abstract; claims 1-4; and pages 8, 26, 35 and 37.	1-23
A	WO 2009-120995 A2 (PURDUE RESEARCH FOUNDATION) 01 October 2009. See the whole document.	1-23
A	US 7592009 B2 (HUBBELL, et al.) 22 September 2009. See the whole document.	1-23
A	BERNHARD, et al., 'Synthesis and characterization of an aggrecan mimic.' Acta Biomaterialia, 2012, Vol.8, No.4, pp.1543-1550. See the whole document.	1-23
A	WO 2012-112767 A2 (PURDUE RESEARCH FOUNDATION) 23 August 2012. See the whole document.	1-23

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2014 (25.07.2014)	**28 July 2014 (28.07.2014)**

Name and mailing address of the ISA/KR
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/029596

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

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

1. ☒ Claims Nos.: 24-29
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 24-29 pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv) to search.

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/029596

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012-162534 A2	29/11/2012	AU 2012-258706 A1	19/12/2013
		CA 2837096 A1	29/11/2012
		EP 2714718 A2	09/04/2014
		KR 10-2014-0041579 A	04/04/2014
		WO 2012-162534 A3	11/07/2013
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		EP 2280720 A2	09/02/2011
		JP 2011-515499 A	19/05/2011
		US 2011-0020298 A1	27/01/2011
		WO 2009-120995 A3	28/01/2010
US 7592009 B2	22/09/2009	AU 2007-322346 A1	29/05/2008
		CA 2665839 A1	29/05/2008
		EP 2084275 A2	05/08/2009
		JP 2010-505444 A	25/02/2010
		US 2008-0118523 A1	22/05/2008
		US 2010-0080850 A1	01/04/2010
		WO 2008-063291 A2	29/05/2008
		WO 2008-063291 A3	02/10/2008
WO 2012-112767 A2	23/08/2012	US 2013-0323311 A1	05/12/2013
		WO 2012-112767 A3	26/10/2012

摘要

本发明提供了包括偶联到多糖上的一种或多种合成肽的细胞外基质结合的合成肽聚糖及其使用方法。