Abstract:
This disclosure provides a method for treating a gastro-esophageal injury in a patient by topically applying a pharmaceutical composition to a lesion on a gastro-esophageal tissue, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 1 to about 80 collagen-binding peptide(s) bonded to the glycan.
Cross Reference To Related Application

This application claims the benefit under 35 U.S.C. 119(e) to U.S. Provisional Application Ser. No. 62/066,819, filed on October 21, 2014, the entirety of which is incorporated herein by reference.

Field

This disclosure provides compositions and methods for treating or preventing injuries of gastroesophageal tissues, in particular injuries caused by the gastroesophageal reflux disease (GERD). The compositions and methods are also useful for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, and ameliorating peptic ulcer disease (PUD) symptoms, for injured esophagus.

Background

The esophagus is relatively protected against injury but can be harmed gradually by backflow of acid from the stomach (gastroesophageal reflux or GERD). The esophagus may also be harmed suddenly by caustic or acidic chemicals (erosive esophagitis), irritating drugs, a sharp object, or extreme pressure. Extreme pressure can occur during violent vomiting, and violent vomiting can cause tears in the esophagus (esophageal laceration, or Mallory-Weiss Syndrome).

Gastroesophageal reflux disease (GERD), also referred to as gastroesophageal reflux disease (GORD), gastric reflux disease, or acid reflux disease, is a chronic symptom of mucosal damage caused by stomach acid coming up from the stomach into the esophagus. GERD is usually caused by changes in the barrier between the stomach and the esophagus, including abnormal relaxation of the lower esophageal sphincter, which normally holds the top of the stomach closed, impaired expulsion of gastric reflux from the esophagus, or a hiatal hernia. These changes may be permanent or temporary.

Treatment is typically via lifestyle changes and medications such as proton pump inhibitors, ¾ receptor blockers or antacids with or without alginic acid. Surgery may be an option in those who do not improve. In the Western world between 10 and 20% of the population is affected. The effectiveness of these treatments, however, is limited.
Summary
The present disclosure provides compositions and methods for treating or preventing injuries of gastroesophageal tissues, in particular injuries caused by the gastroesophageal reflux disease (GERD), maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms, for injured esophagus in a patient. In some embodiments, the method entails topically applying a pharmaceutical composition to a gastroesophageal tissue such as an exposed tissue or a lesion. In some embodiments, the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 1 to about 80 collagen-binding peptide(s) or peptides having one or more collagen-binding units bonded to the glycan.

In some aspects, the patient suffers from a gastroesophageal reflux disease (GERD). In some aspects, the lesion is caused by an iatrogenic intervention. In some aspects, the lesion has undergone an esophagogastroduodenoscopy (EGD) ablation.

In some aspects, the patient suffers from esophageal stricture. In some aspects, the patient is in need of EGD dilation.

In some aspects, the patient suffers from a peptic ulcer disease (PUD).

Without limitation, in any of the above embodiments, the pharmaceutical composition can be applied through esophagogastroduodenoscopy (EGD).

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.

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.
1. 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%.

The following abbreviations used herein have the following meanings.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>BID</td>
<td>Administered Twice Daily</td>
</tr>
<tr>
<td>BMPH</td>
<td>N-[β-maleimidopropionic acid]hydrazide</td>
</tr>
<tr>
<td>BMPH-CS</td>
<td>BMPH Linker-Chondroitin Sulfate Conjugate</td>
</tr>
<tr>
<td>CD44</td>
<td>Cell-Surface Glycoprotein CD44 Antigen</td>
</tr>
<tr>
<td>cps</td>
<td>Centipoise</td>
</tr>
<tr>
<td>CS</td>
<td>Chondroitin sulfate</td>
</tr>
<tr>
<td>Dex</td>
<td>Dextran</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DS</td>
<td>Dermatan Sulfate</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-Fluorenlymethoxycarbonyl</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast Growth Factor</td>
</tr>
</tbody>
</table>
As used herein, the term "treating and/or preventing" refers to preventing, curing, reversing, attenuating, alleviating, minimizing, suppressing or halting gastroesophageal injuries.

As used herein, the term "patient" refers to a subject suffering from or at risk of developing a gastroesophageal injury.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
</tr>
<tr>
<td>Hep</td>
<td>Heparin</td>
</tr>
<tr>
<td>HEPES</td>
<td>2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid</td>
</tr>
<tr>
<td>HLB</td>
<td>Hydrophil/Lipophil/Balance</td>
</tr>
<tr>
<td>HPC</td>
<td>Hydroxyl Propylcellulose</td>
</tr>
<tr>
<td>HPMC</td>
<td>Hydroxypropylmethyl Cellulose</td>
</tr>
<tr>
<td>ITC</td>
<td>Isothermal Titration Calorimeters</td>
</tr>
<tr>
<td>kDa</td>
<td>KiloDalton</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>MES</td>
<td>2-ethanesulfonic acid</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mOsm</td>
<td>Milliosmole</td>
</tr>
<tr>
<td>mV</td>
<td>Millivolt</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PDPH</td>
<td>3-(2-pyridyl)dithio)propionyl hydrazide</td>
</tr>
<tr>
<td>PIPES</td>
<td>piperazine-N,N'-bis(2-ethanesulfonic acid)</td>
</tr>
<tr>
<td>QD</td>
<td>Administered Once Daily</td>
</tr>
<tr>
<td>SILY</td>
<td>RRANAALKAGELYKSILY (SEQ ID NO: 1)</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface Plasmon Resonance</td>
</tr>
<tr>
<td>TAPS</td>
<td>3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]propane-1-sulfonic acid</td>
</tr>
<tr>
<td>TES</td>
<td>2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid</td>
</tr>
<tr>
<td>Tris</td>
<td>2-Amino-2-hydroxymethyl-propane-1,3-diol</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight/Weight</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight/Volume</td>
</tr>
</tbody>
</table>
As used herein, the term "administering" or "administration" refers to the delivery of one or more therapeutic agents to a patient.

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.

A "peptide" is a chain of two or more amino acid monomers linked by peptide (amide) bonds. 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. As used herein, the term "peptide" is intended to refer a linear or branched chain of amino acids linked by peptide (amid) bonds. In one embodiment, the peptide comprises from about 3 to about 120 amino acids, or from about 3 to about 110 amino acids, or from about 3 to about 100 amino acids, or from about 3 to about 90 amino acids, or from about 3 to about 80 amino acids, or from about 3 to about 70 amino acids, or from about 3 to about 60 amino acids, or from about 3 to about 50 amino acids, or from about 3 to about 40 amino acids, or from about 5 to about 120 amino acids, or from about 5 to about 100 amino acids, or from about 5 to about 90 amino acids, or from about 5 to about 80 amino acids, or from about 5 to about 70 amino acids, or from about 5 to about 60 amino acids, or from about 5 to about 50 amino acids, or from about 5 to about 40 amino acids, or from about 5 to about 30 amino acids, or from about 5 to about 20 amino acids, or from about 5 to about 10 amino acids.

As used herein, the terms "peptidoglycan," "proteoglycan," "proteoglycan mimic," or "synthetic peptidoglycan" are used interchangeably and refer 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 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. In some embodiments, the term synthetic proteoglycan includes proteoglycans. In one embodiment, the molecular weight range for the synthetic proteoglycan is from about 13 kDa to about 1.2 MDa, or from about 500 kDa to about 1 MDa, or from about 20 kDa to about 90 kDa, or from about 10 kDa to about 70 kDa.

As used herein, the term "glycan" refers to a compound having a large number of monosaccharides linked glycosidically. In certain embodiments, the glycan is a
glycosaminoglycan (GAG), 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 (Dex), chondroitin, chondroitin sulfate (CS), dermatan, dermatan sulfate (DS), heparan sulfate, heparin (Hep), keratin, keratan sulfate, and hyaluronic acid (HA). In one embodiment the molecular weight of the glycan is a key parameter in its biological function. In another embodiment the molecular weight of the glycan is varied to tailor the effects of the synthetic proteoglycan mimic (see e.g., Radek, K. A., et al, Wound Repair Regen., 2009, 17: 118-126; and Taylor, K. R., et al, J. Biol. Chem., 2005, 280:5300-5306). In another embodiment, the glycan molecular weight is about 46 kDa. In another embodiment, the glycan is degraded by oxidation and alkaline elimination (see e.g., Fransson, L. A., et al., Eur. J. Biochem., 1980, 106:59-69) to afford degraded glycan having a lower molecular weight (e.g., from about 10 kDa to about 50 kDa). In some embodiments, the glycan is unmodified.

In one embodiment, the GAG is heparin. Various molecular weights for the heparin can be used in the proteoglycans described herein, such as from a single disaccharide unit of about 650-700 Da, to a glycan of about 70 kDa. In some embodiments, the heparin is from about 10 kDa to about 70, or from about 10 to about 46 kDa, or from about 10 to about 20 kDa. In some embodiments, the heparin is up to about 15, or about 16, or about 17, or about 18, or about 19, or about 20 kDa.

In another embodiment, the GAG is dermatan sulfate (DS). The biological functions of DS are extensive, and include the binding and activation of growth factors FGF-2, FGF-7, and FGF-10, which promote endothelial cell and keratinocyte proliferation and migration. In one embodiment, the DS molecular weight is about 46 kDa. In another embodiment, the DS is degraded by oxidation and alkaline elimination (see e.g., Fransson, L. A., et al., Eur. J. Biochem., 1980, 106:59-69) to afford degraded DS having a low molecular weight (e.g., 10 kDa). In some embodiments, the weight range of the DS is from about 10 kDa to about 70 kDa.

As used herein, the terms "bonded" and "covalently bonded" can be used interchangeably, and refer to the sharing of one or more pairs of electrons by two atoms. In one embodiment, the peptide is bonded to the glycan. In one embodiment the peptide is covalently bonded to the glycan, with or without a linker. In one embodiment the peptide is
covalently bonded to the glycan via a linker. In one embodiment the peptide is directly bonded to the glycan.

In one embodiment, the synthetic proteoglycans of the disclosure bind, either directly or indirectly to collagen. 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. 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.

**Collagen-Binding Peptides**

"Collagen-binding peptides" are peptides comprising 1 to about 120 amino acids having one or more collagen-binding units (or sequences). As used herein, the term "collagen-binding unit" is intended to refer to an amino acid sequence within a peptide which binds to collagen. "Collagen-binding" indicates an interaction with collagen that could include hydrophobic, ionic (charge), and/or Van der Waals interactions, such that the compound binds or interacts favorably with collagen. This binding (or interaction) is intended to be differentiated from covalent bonds and nonspecific interactions with common functional groups, such that the peptide would interact with any species containing that functional group to which the peptide binds on the collagen. Peptides can be tested and assessed for binding to collagen using any method known in the art. See, e.g., Li, Y., et al, Current Opinion in Chemical Biology, 2013, 17: 968-975, Helmes, B.A., et al., J. Am. Chem. Soc. 2009, 131, 11683-1 1685, and Petsalaki, E., et al, PLoS Comput Biol, 2009, 5(3): e1000335. In one embodiment, the peptide, or the collagen-binding unit of the peptide, binds to collagen with a dissociation constant ($K_d$) of less than about 1 mM, or less than about 900 µM, or less than about 800 µM, or less than about 700 µM, or less than about 600 µM, or less than about 500 µM, or less than about 400 µM, or less than about 300 µM, or less than about 200 µM, or less than about 100 µM.
The peptide can have amino acid homology with a portion of a protein normally or not normally involved in collagen fibrillogenesis. 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 peptide comprises an amino acid sequence selected from

**RRANAALKAGELYKSILY** (SEQ ID NO: 1), **GELYKSILY** (SEQ ID NO: 2),
**RRANAALKAGELYKCILY** (SEQ ID NO: 3), **GELYKCILY** (SEQ ID NO: 4),
**RLDGNEIKR** (SEQ ID NO: 5), **AHEEISTTNEGVM** (SEQ ID NO: 6),
**NGVFKYRPRLYKHAYFYPPLKRFPVQ** (SEQ ID NO: 7), **CQDSETRTFY** (SEQ ID NO: 8), **TKKTLRT** (SEQ ID NO: 9), **GLRSKSKFRRPDQYPDATDEPITSMH** (SEQ ID NO: 10), **SQNPVQP** (SEQ ID NO: 11), **SYIRIADTNIT** (SEQ ID NO: 12), **KELNLYVT** (SEQ ID NO: 13), **GSIT** (SEQ ID NO: 14), **GSITTIDVPWNV** (SEQ ID NO: 15),
**GQLYKSILY** (SEQ ID NO: 16), **RRANAALKAGQLYKSILY** (SEQ ID NO: 17), or a sequence having at least about 80% sequence identity, or at least about 83% 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 thereto, provided the sequence is capable of binding to collagen.

In certain embodiments, the peptide comprises an amino acid sequence that has at least about 80%, or at least about 83%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98%, or at least about 100% sequence identity with the collagen-binding domain(s) of the von Willebrand factor (vWF) or a platelet collagen receptor as described in Chiang, T.M., et al. J. Biol. Chem., 2002, 277: 34896-34901, Huizinga, E.G. et al, Structure, 1997, 5: 1147-1156, Romijn, R.A., et al, J. Biol. Chem., 2003, 278: 15035-15039, and Chiang, et al, Cardio. & Haematol. Disorders-Drug Targets, 2007, 7: 71-75, each incorporated herein by reference. A non-limiting example is **WREPSFCALS** (SEQ ID NO: 18), derived from vWF.

Various methods for screening peptide sequences for collagen-binding affinity (or a collagen-binding domain/unit) are routine in the art. Other peptide sequences shown to have collagen-binding affinity (or a collagen-binding unit) which can be used in the proteoglycans and methods disclosed herein include but are not limited to, **pAWHCTTKFPHHYCLYBip** (SEQ ID NO: 19), **pAHKCPWHLYTTHYCFBip** (SEQ ID NO: 20), **PAHKCPWHLYTTHYCF** (SEQ ID NO: 21), etc., where Bip is biphenylalanine and βA is beta-alanine (see, Abd-Elgaliel, W.R., et al, Biopolymers, 2013, 100(2), 167-173),
GROGER (SEQ ID NO: 22), GMOGER (SEQ ID NO: 23), GLOGEN (SEQ ID NO: 24),
GLOGER (SEQ ID NO: 25), GLKGEN (SEQ ID NO: 26), GFOGERGVEGPOGPA (SEQ
ID NO: 27), etc., where O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006,
Chem. Soc. 2009, 131, 11683-1 1685), WREPSFCALS (SEQ ID NO: 18) (see, Takagi, J., et
al, Biochemistry, 1992, 31, 8530-8534), WYRGRL (SEQ ID NO:29 ), etc. (see, Rothenfluh
WTCVGDHKTWKC (SEQ ID NO: 31), QWHCTTRFPHHYCLYG (SEQ ID NO: 32), etc.
(see, U.S. 2007/0293656), STWTWNGSAWTWNEGGK (SEQ ID NO:33 ),
5
STWTWNGTNWTRNDGGK (SEQ ID NO: 34), etc. (see, WO/2014/059530),
CVWLWEQC (SEQ ID NO: 35) cyclic CVWLWENC (SEQ ID NO: 36), cyclic
CVWLWEQC (SEQ ID NO: 37), (see, Depraetere H., et al, Blood. 1998, 92, 4207-4211;
and Duncan R., Nat Rev Drug Discov, 2003, 2(5), 347-360), CMTSPWRC (SEQ ID NO:
10
CPGRVMHGLHLGDDEGPC ( SEQ ID NO: 39) (see, Muzzard, J., et al, PLoS one. 4 (e
5585) I- 10), KLWLLPK ( SEQ ID NO: 40) (see, Chan, J. M., et al, Proc Natl Acad Sci
U.S.A., 2010, 107, 2213- 2218), and CQDSETRTFY ( SEQ ID NO: 8), etc. (see, U.S.
131(33), 11683-1 1685), wherein each is hereby incorporated by reference in its entirety.
15

Additional peptide sequences shown to have collagen-binding affinity (or a collagen-
binding unit) which can be used in the proteoglycans and methods disclosed herein include
but are not limited to, LSELRLHEN (SEQ ID NO: 41), LTELHLDNN (SEQ ID NO: 42),
LSELRLHNN (SEQ ID NO: 43), LSELRLHAN (SEQ ID NO: 44), and LRELHLNNN (SEQ
ID NO: 45) (see, Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10984).
20

In certain embodiments, the peptides include one or more sequences selected from the
group consisting of RVMHGLHLGDDE (SEQ ID NO: 46), D-amino acid
EDDGLHLGHMVR (SEQ ID NO: 47), RVMHGLHLGNQ (SEQ ID NO: 48), D-amino
acid QNNGLHLGHMVR (SEQ ID NO: 49), RVMHGLHLGNQ (SEQ ID NO: 50),
GQLYKSLYGSG-4K2K (SEQ ID NO: 51) (a 4-branch peptide), GSGQLYKSLY (SEQ ID
30
NO: 52), GSGGQLYKSLY (SEQ ID NO: 53), KQLNLVYT (SEQ ID NO: 54 ),
CVWLWQQC (SEQ ID NO: 55), WREPSFSALS (SEQ ID NO: 56),
GHRPLDJKREEAPSRPAPPPISGG GYR ( SEQ ID NO: 57), and
GHRPLNKRQQ APSLRPAPPPISG GYR (SEQ ID NO: 58).
Similarly for a collagen-binding peptide, a peptide derived from a phage display library selected for collagen 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 (e.g., SILYbiotin) 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, the peptides comprise one or more collagen-binding units which binds any one or more of collagen type I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV. In one embodiment, the peptide binds to type I collagen with a dissociation constant ($K_d$) of less than about 1 mM, or less than about 900 μM, or less than about 800 μM, or less than about 700 μM, or less than about 600 μM, or less than about 500 μM, or less than about 400 μM, or less than about 300 μM, or less than about 200 μM, or less than about 100 μM. In one embodiment, the peptide binds to type II collagen with a dissociation constant ($K_d$) of less than about 1 mM, or less than about 900 μM, or less than about 800 μM, or less than about 700 μM, or less than about 600 μM, or less than about 500 μM, or less than about 400 μM, or less than about 300 μM, or less than about 200 μM, or less than about 100 μM. In one embodiment, the peptide binds to type III collagen with a dissociation constant ($K_d$) of less than about 1 mM, or less than about 900 μM, or less than about 800 μM, or less than about 700 μM, or less than about 600 μM, or less than about 500 μM, or less than about 400 μM, or less than about 200 μM, or less than about 100 μM. In one embodiment, the peptide binds to type IV collagen with a dissociation constant ($K_d$) of less than about 1 mM, or less than about 900 μM, or less than about 800 μM, or less than about 700 μM, or less than about 600 μM, or less than about 500 μM, or less than about 400 μM, or less than about 200 μM, or less than about 100 μM.

As used herein, the term "sequence identity" refers to a level of amino acid residue or nucleotide identity between two peptides or between two nucleic acid molecules. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are identical at that position. A peptide (or a polypeptide or peptide region) has a certain percentage (for example, at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 83%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98%, or at least about 99%) of "sequence identity" to another sequence means that, when aligned, that percentage
of bases (or amino acids) are the same in comparing the two sequences. It is noted that, for any sequence ("reference sequence") disclosed in this application, sequences having at least about 60\%, or at least about 65\%, or at least about 70\%, or at least about 75\%, or at least about 80\%, or at least about 83\%, or at least about 85\%, or at least about 90\%, or at least about 95\%, or at least about 98\% or at least about 99\% sequence identity to the reference sequence are also within the disclosure. Likewise, the present disclosure also includes sequences that have one, two, three, four, or five substitution, deletion or addition of amino acid residues or nucleotides as compared to the reference sequences.

In any of the embodiments described herein, any one or more of the synthetic peptides (e.g., the collagen-binding peptide) may have a spacer sequence comprising from one to about five amino acids. It is contemplated that any amino acid, natural or unnatural, can be used in the spacer sequence, provided that the spacer sequence does not significantly interfere with the intended binding of the peptide. Exemplary spacers include, but are not limited to, short sequences comprising from one to five glycine units (e.g., G, GG, GGG, GGGG (SEQ ID NO: 59), or GGGGG (SEQ ID NO: 60)), optionally comprising cysteine (e.g., GC, GCG, GSGC (SEQ ID NO: 61), or GGC) and/or serine (e.g., GSG, or GSGSG (SEQ ID NO: 62)), or from one to five arginine units (e.g., R, RR, RRR, etc.). The spacer can also comprise non-amino acid moieties, such as polyethylene glycol (PEG), 6-aminohexanoic acid, succinic acid or combinations thereof, with or without an amino acid spacer. The spacer can be attached to either the C-terminus or the N-terminus of the peptide to provide a point of attachment for a glycan or a glycan-linker conjugate. In certain embodiments, the spacer comprises more than one binding site (may be linear or branched) such that more than one peptide sequence can be bound thereto, thus creating a branched construct. The binding sites on the spacer can be the same or different, and can be any suitable binding site, such as an amine or carboxylic acid moiety, such that a desired peptide sequence can be bound thereto (e.g. via an amide bond). Thus in certain embodiments, the spacer contains one or more lysine, glutamic acid or aspartic acid residues. Such constructs can provide peptides having more than one collagen-binding unit of the formula \( P_n L \), where \( P \) is a collagen-binding unit, \( L \) is a spacer and \( n \) is an integer from 2 to about 10, or from 2 to 8, or from 2 to 6, or from 2 to 5, or from 2 to 4, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10. For example, the spacer \( L \) can be an amino acid sequence such as KGSG (SEQ ID NO: 63), KKGSG (SEQ ID NO: 64), or KKKGGSG (SEQ ID NO: 65), etc., providing 2, 3, or 4 binding sites, respectively.
In certain embodiments, spacers, or a combination thereof, can be used to create further branching. For example, the spacer may comprise one or more amino acids which contain a side chain capable of linking additional peptides or collagen-binding units. Exemplary amino acids for including in such spacers include, but are not limited to, lysine, glutamic acid, aspartic acid, etc. In certain embodiments, the spacer comprises from 2 to 6 amino acids, or 3 or 4 amino acids. In certain embodiments, the spacer comprises one or more amino acid sequences of the formula KXX, where each X is independently a natural or unnatural amino acid. Specific examples of spacers which can be used alone or in combination to make branched constructs include, but are not limited to, KRR, KKK, (K)_nGSG, and (KRR)_n-KGSG, where n is 0 to 5, or 1, 2, 3, 4, or 5. Exemplary collagen-binding constructs include, but are not limited to, (GELYKSIYLGSG)\_2KSG (SEQ ID NO: 66), (GELYKSIYLGSG)\_3KKGSG (SEQ ID NO: 67), (GELYKSIYLGSG)\_4KKKGSG (SEQ ID NO: 68), (GQLYKSIYLGSG)\_2KSG (SEQ ID NO: 69), (GQLYKSIYLGSG)\_3KKSG (SEQ ID NO: 70), (GQLYKSIYLGSG)\_4KKKGSG (SEQ ID NO: 71) and (GQLYKSIYLGSG)\_4(KRR)\_2-KGSG (SEQ ID NO: 72). Other collagen-binding constructs include amino acids containing functional groups comprising one or more binding sites such as lysine, glutamic acid, aspartic acid, etc.

Accordingly, in certain embodiments, the synthetic peptide is RYPISRPKRGSG (SEQ ID NO: 73), RRANAALKAGEYKSIYGC (SEQ ID NO: 74), or GELYKSIYGC (SEQ ID NO: 75).

In any of the embodiments described herein, a synthetic peptide (e.g., a collagen-binding peptide) comprises any amino acid sequence described in the preceding paragraph or an amino acid sequence having at least about 80%, or at least about 83%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98%, or at least about 100% homology to any of these amino acid sequences. In various embodiments, the peptide components of the synthetic proteoglycan 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.

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 1.

Table 1

<table>
<thead>
<tr>
<th>For Amino Acid</th>
<th>Replace With</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>D-Ala, Gly, Aib, β-Ala, L-Cys, D-Cys</td>
</tr>
<tr>
<td>Arginine</td>
<td>D-Arg, Lys, D-Lys, Orn D-Orn</td>
</tr>
<tr>
<td>Asparagine</td>
<td>D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln</td>
</tr>
<tr>
<td>Cysteine</td>
<td>D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-Fluorenylmethoxycarbonyl</td>
</tr>
<tr>
<td>Glutamine</td>
<td>D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln</td>
</tr>
<tr>
<td>Glycine</td>
<td>Ala, D-Ala, Pro, D-Pro, Aib, β-Ala</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met</td>
</tr>
<tr>
<td>Leucine</td>
<td>Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile</td>
</tr>
<tr>
<td>Lysine</td>
<td>D-Lys, Arg, D-Arg, Orn, D-Orn</td>
</tr>
<tr>
<td>Methionine</td>
<td>D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp</td>
</tr>
<tr>
<td>Proline</td>
<td>D-Pro</td>
</tr>
<tr>
<td>Serine</td>
<td>D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys</td>
</tr>
<tr>
<td>Threonine</td>
<td>D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp</td>
</tr>
<tr>
<td>Valine</td>
<td>D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met</td>
</tr>
</tbody>
</table>

As used herein, the term "extracellular matrix" refers to the extracellular part of tissue that provides structural and biochemical support to the surrounding cells.
As used herein, the term "linker" refers to chemical bond, atom, or group of atoms that connects two adjacent chains of atoms in a large molecule such as a peptide, synthetic proteoglycan, protein or polymer. In various embodiments, the linker comprises two or more chemically orthogonal functionalities on a rigid scaffold (e.g., any suitable bifunctional linker, such as N-[P-maleimidopropionic acid] hydrazide (BMPH), 3-(2-pyridyldithio)propionyl hydrazide (PDPH)), or the peptide GSG.

As used herein, the term "composition" refers to a preparation suitable for administration to an intended patient for therapeutic purposes that contains at least one pharmaceutically active ingredient, including any solid form thereof. The composition may include at least one pharmaceutically acceptable component to provide an improved formulation of the compound, such as a suitable carrier. In certain embodiments, the composition is formulated as a film, gel, patch, or liquid solution.

As used herein, the term "topically" refers to administering a composition non-systemically to the surface of a tissue and/or organ (internal or, in some cases, external) to be treated, for local effect.

As used herein, the term "pharmaceutically acceptable" indicates that the indicated material does not have properties that would cause a reasonably prudent medical practitioner to avoid administration of the material to a patient, taking into consideration the disease or conditions to be treated and the respective route of administration. For example, it is commonly required that such a material be essentially sterile.

As used herein, the term "pharmaceutically acceptable carrier" refers to pharmaceutically acceptable materials, compositions or vehicles, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any supplement or composition, or component thereof, from one organ, or portion of the body, to another organ, or portion of the body, or to deliver an agent to the internal surface of a vein.

As used herein, the term "formulated" or "formulation" refers to the process in which different chemical substances, including one or more pharmaceutically active ingredients, are combined to produce a dosage form. In certain embodiments, two or more pharmaceutically active ingredients can be co-formulated into a single dosage form or combined dosage unit, or formulated separately and subsequently combined into a combined dosage unit. A sustained release formulation is a formulation which is designed to slowly release a
therapeutic agent in the body over an extended period of time, whereas an immediate release formulation is a formulation which is designed to quickly release a therapeutic agent in the body over a shortened period of time.

As used herein, the term "delivery" refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical composition in the body as needed to safely achieve its desired therapeutic effect. In some embodiments, an effective amount of the composition is formulated for delivery onto a gastroesophageal tissue of a patient.

As used herein, the term "solution" refers to solutions, suspensions, emulsions, drops, ointments, liquid wash, sprays, liposomes which are well known in the art. In some embodiments, the liquid solution contains an aqueous pH buffering agent which resists changes in pH when small quantities of acid or base are added. In certain embodiments, the liquid solution contains a lubricity enhancing agent.

As used herein, the term "polymer matrix" or "polymeric agent" refers to a biocompatible polymeric materials. The polymeric material described herein may comprise, for example, sugars (such as mannitol), peptides, protein, laminin, collagen, hyaluronic acid, 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.

As used herein, the term "absorbable" refers to the ability of a material to be absorbed into the body. In certain embodiments, the polymeric matrix is absorbable, such as, for example collagen, polyglycolic acid, polylactic acid, polydioxanone, and caprolactone. In other embodiments, the polymer is non-absorbable, such as, for example polypropylene, polyester or nylon.

As used herein, the term "pH buffering agent" refers to an aqueous buffer solution which resists changes in pH when small quantities of acid or base are added to it. pH Buffering solutions typically comprise of a mixture of weak acid and its conjugate base, or vice versa. For example, pH buffering solutions may comprise phosphates such as sodium phosphate, sodium dihydrogen phosphate, sodium dihydrogen phosphate dihydrate, disodium
hydrogen phosphate, disodium hydrogen phosphate dodecahydrate, potassium phosphate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate; boric acid and borates such as, sodium borate and potassium borate; citric acid and citrates such as sodium citrate and disodium citrate; acetates such as sodium acetate and potassium acetate; carbonates such as sodium carbonate and sodium hydrogen carbonate, etc. pH Adjusting agents can include, for example, acids such as hydrochloric acid, lactic acid, citric acid, phosphoric acid and acetic acid, and alkaline bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and sodium hydrogen carbonate, etc. In some embodiments, the pH buffering agent is a phosphate buffered saline (PBS) solution (i.e., containing sodium phosphate, sodium chloride and in some formulations, potassium chloride and potassium phosphate).

As used herein, the term "concurrently" refers to simultaneous (i.e., in conjunction) administration. In one embodiment, the administration is coadministration such that two or more pharmaceutically active ingredients, including any solid form thereof, are delivered together at one time.

As used herein, the term "sequentially" refers to separate (i.e., at different times) administration. In one embodiment, the administration is staggered such that two or more pharmaceutically active ingredients, including any solid form thereof, are delivered separately at different times.

2. Methods

The present disclosure, in one embodiment, provides a new approach to address the unmet need of treating or preventing a gastroesophageal injury in a patient. In general, the new approach entails applying a pharmaceutical composition that includes a synthetic collagen-binding proteoglycan of the present disclosure on the injured gastroesophageal tissue or cell.

Such application of the composition can generate a coating of the synthetic collagen-binding proteoglycan. The synthetic collagen-binding proteoglycan can bind to collagen exposed on the esophageal tissue through physical peptide-collagen interactions. When bound to collagen, the proteoglycan has a number of functions including 1) acting as a barrier to platelet attachment/activation, 2) protecting collagen from degradation by inhibiting MMP access, and/or 3) sequestering growth factors FGF-2, FGF-7, and FGF-10, thus promoting endothelial and epithelial cell proliferation and migration, leading to tissue repair and recovery.
The collagen-binding proteoglycan, in one embodiment, includes a polysaccharide backbone with covalently attached collagen-binding peptides. The synthetic proteoglycans can compete for platelet binding sites on collagen and prevent platelet binding and activation. The glycan backbone can be negatively charged and bind water molecules, creating a hydrophilic barrier over the collagen surface that prevents platelet and protein adhesion. By masking the exposed collagen, rather than inhibiting normal platelet function, the proteoglycan can provide a local treatment that addresses the initial steps in the cascade to inflammation and intimal hyperplasia.

This new approach is contemplated to be useful for treating gastroesophageal injuries, including but not limited to those caused by GERD or iatrogenic interventions. It is further contemplated that patients of the following categories can benefit from this approach:

- GERD associated esophageal lesion requiring esophagogastroduodenoscopic (EGD) ablation
- Esophageal stricture requiring EGD dilation
- Peptic Ulcer Disease (PUD) requiring EGD treatment.

The pharmaceutical composition can be topically applied to one or more lesions of the injured gastroesophageal tissue. Given the limited accessibility of the tissue, it is contemplated that use of a delivery device is beneficial. For instance, the composition can be delivered during an esophagogastroduodenoscopy (EGD) procedure or using an esophagogastroduodenoscope.

Esophagogastroduodenoscope is an instrument used to get an image of the upper gastrointestinal tract, namely, the esophagus, the stomach and the duodenum. The esophagogastroduodenoscope is inserted through the mouth. Esophagogastroduodenoscopy can be used to identify ulcers, gastritis, esophagitis, varices, duodenitis, Barrett's esophagus, hiatal hernias, and tumors. An esophagogastroduodenoscope procedure is performed on patients with a variety of symptoms; that include: nausea, vomiting, abdominal bloating, abdominal pain, heartburn, reflux, family history of cancer, jaundice, weight loss, anemia, and gastrointestinal bleeding.

In some embodiments, the composition of the present disclosure can be applied to a cell or tissue of the esophagus or the stomach during an esophagogastroduodenoscope procedure. For instance, the images or live view from the esophagogastroduodenoscope can be used as a guide to applying the composition topically on the cell or tissue.
In one embodiment, the composition is loaded to esophagastroduodenoscope (such as at the tip close to the lens), optionally at an applicator attached to the esophagastroduodenoscope. In some aspects, the applicator further includes a release mechanism that can be controlled remotely by a medical professional. Therefore, when the esophagastroduodenoscope reaches at the target cell or tissue, the composition can be released and applied to the cell or tissue.

It is contemplated that the synthetic proteoglycans provided in the solution can be tailored with respect to the peptide identity, the number of peptides attached to the glycosaminoglycan (GAG) backbone, and the GAG backbone identity to promote recovery of an injured gastroesophageal tissue. Thus, a number of molecular design parameters can be engineered to optimize the target effect.

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan.

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin.

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin.

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin.
In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin and collagen-binding peptide(s) is (GQLYKSILY)_4-(KRR)_2-KGSG (SEQ ID NO: 72).

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin and collagen-binding peptide(s) is RRANAALKAGELYKSILY (SEQ ID NO: 1).

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin and collagen-binding peptide(s) is (GQLYKSILY)_4-(KRR)_2-KGSG (SEQ ID NO: 72).

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin and collagen-binding peptide(s) is RRANAALKAGELYKSILY (SEQ ID NO: 1).

In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an
injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is dermatan sulfate.

5 In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is dermatan sulfate and collagen-binding peptide(s) is RRANAALKAGELYKSYL (SEQ ID NO: 1).

10 In certain embodiments, the disclosure provides a method for maintaining esophageal patency, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is dermatan sulfate and collagen-binding peptide(s) is RRANAALKAGELYKSYL (SEQ ID NO: 1).

15 In certain embodiments, the disclosure provides a method for maintaining esophageal patency, reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is dermatan sulfate and collagen-binding peptide(s) is (GQLYKSYL)_4-(KRR)_2-KGSG (SEQ ID NO: 72).

20 In certain embodiments, the disclosure provides a method for maintaining esophageal patency, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to
about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is dermatan sulfate and collagen-binding peptide(s) is (GQLYKSILY)_{4-(KRR)_{2}-KGSG (SEQ ID NO: 72).

A method for treating a gastroesophageal injury in a patient, comprising topically applying a pharmaceutical composition to a lesion on a gastroesophageal tissue, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 1 to about 80 collagen-binding peptide(s) bonded to the glycan.

In certain embodiments, the disclosure provides a method for treating a gastroesophageal injury in a patient, comprising topically applying a pharmaceutical composition to a lesion on a gastroesophageal tissue, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan.

In certain embodiments, the disclosure provides a method for treating a gastroesophageal injury in a patient, comprising topically applying a pharmaceutical composition to a lesion on a gastroesophageal tissue, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin or dermatan sulfate.

In certain embodiments, the disclosure provides a method for treating a gastroesophageal injury in a patient, comprising topically applying a pharmaceutical composition to a lesion on a gastroesophageal tissue, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 5 to about 40 collagen-binding peptide(s) bonded to the glycan, wherein the glycan is heparin or dermatan sulfate and collagen-binding peptide(s) is RRANAALKAGELYKSILY (SEQ ID NO: 1).

In one embodiment, the molecule configuration consists of a heparin GAG backbone with attached collagen-binding peptide(s). In another embodiment, the molecule configuration consists of a dermatan sulfate (DS) GAG backbone with attached collagen-binding peptide(s). DS may be useful because of its ability to promote epithelial cell migration and proliferation.

It is contemplated that other variants of GAG-peptide provided herein are also capable of inhibiting platelet activation through binding to type I collagen. In one embodiment the
synthetic proteoglycans include a collagen-binding peptide such as
RRANAALKAGELYKSILY (SEQ ID NO: 1), referred to as "SILY". In another
embodiment, the synthetic proteoglycans include a collagen-binding peptide such as
(GQLYKSILY)_{4}(KRR)_{2}-KGSG (SEQ ID NO: 72).

In another embodiment, the synthetic proteoglycan comprises collagen-binding
peptide(s) (SILY) conjugated to GAG backbones comprising heparin (Hep-SILY), dermatan
sulfate (DS-SILY), or dextran (Dex-SILY) (see, e.g., US 2011/0020298 and 2013/0190246).
In another embodiment, the synthetic proteoglycan comprises collagen-binding peptide(s)
(GQLYKSILY)_{4}(KRR)_{2}-KGSG conjugated to GAG backbones selected from heparin,
dermatan sulfate or dextran.

In certain embodiments, the synthetic proteoglycan comprises (GQLYKSILY)_{4}(KRR)_{2}-
KGSG conjugated to heparin. In some embodiments, the synthetic proteoglycan
comprises about 1-15 peptides, or 1-10 peptides, or 1-5 peptides or 1-2 peptides conjugated
to heparin. In some embodiments, the synthetic proteoglycan comprises from about 1 to
about 75 percent (%) functionalization, or from about 5 to about 30 percent (%) functionalization,
wherein the percent (%) functionalization is determined by a percent of disaccharide units on the heparin which are functionalized with (GQLYKSILY)_{4}(KRR)_{2}-
KGSG (SEQ ID NO: 72).

Hep-SILY refers to the synthetic proteoglycan having about 5-20 SILY peptide(s)
conjugated to heparin. In some embodiments, the synthetic proteoglycan comprises 5-10
SILY peptide(s) or 10-15 SILY peptide(s) or 5-20 SILY peptide(S) conjugated to heparin.
In some embodiments, the synthetic proteoglycan comprises from about 1 to about 75
percent (%) functionalization, or from about 5 to about 30 percent (%) functionalization,
wherein the percent (%) functionalization is determined by a percent of disaccharide units on
heparin which are functionalized with SILY peptide(s). Hep-SILY optionally contains a
linker between the SILY peptide(s) and heparin.

DS-SILY refers to the synthetic proteoglycan having about 5-20 SILY peptide(s)
conjugated to dermatan sulfate (DS). In some embodiments, the synthetic proteoglycan
comprises 5-10 SILY peptide(s) or 10-15 SILY peptide(s) or 5-20 SILY peptide(S)
conjugated to dermatan sulfate. In some embodiments, the synthetic proteoglycan comprises
from about 1 to about 75 percent (%) functionalization, or from about 5 to about 30 percent
(%) functionalization, wherein the percent (%) functionalization is determined by a percent of
disaccharide units on the dermatan sulfate which are functionalized with SILY peptide(s). DS-SILY optionally contains a linker between the SILY peptide(s) and DS.

Palifermin is a keratocyte growth factor useful for oral mucositis treatment. The proteoglycan of present disclosure binds to collagen and also binds to endogenous or exogenous growth factors such as palifermin. Therefore, such a formulation provides targeted delivery of palifermin. In some embodiments, this disclosure provides a method for delivering palifermin. In certain embodiments, the method comprises applying a composition comprising proteoglycan and palifermin to a patient in need thereof. In other embodiments, this disclosure provides a method for treating oral mucositis in a patient wherein the method comprises applying a composition comprising a proteoglycan and palifermin to the patient in need thereof.

**Synthetic Proteoglycans**

In one embodiment, the synthetic proteoglycan comprises a glycan having from about 1 to about 80 collagen-binding peptide(s) bonded to the glycan.

In various embodiments of the methods disclosed herein, the glycan is dextran, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparan sulfate, heparin, keratin, keratan sulfate, or hyaluronic acid. In some embodiments the glycan can be any glycan (e.g., glycosaminoglycan or polysaccharide). In some embodiments, the glycan is dextran. In some embodiments, the glycan is chondroitin. In other embodiments, the glycan is chondroitin sulfate. In some embodiments, the glycan is dermatan. In some embodiments, the glycan is dermatan sulfate. In other embodiments, the glycan is heparan sulfate. In other embodiments, the glycan is heparin. In other embodiments, the glycan is keratin. In some embodiments, the glycan is keratan sulfate. In other embodiments, the glycan is hyaluronic acid. Various glycans may be employed including, a wide range of molecular weights, such as from about 1 kDa to about 2 MDa, or from about 10 kDa to about 2 MDa. In some embodiments, the glycan is from about 3 to about 5 MDa. In some embodiments, the glycan is up to about 3 MDa, or up to about 5 MDa, or up to about 60 MDa.

The peptide(s) can be bonded to the glycan directly or via a linker. In some embodiments, the linker can be any suitable bifunctional linker, e.g., N-[β-maleimidopropionic acid]hydrazide (BMPH), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), and the like. In any of the various embodiments described herein, the sequence of the peptide may be modified to include a glycine-cysteine (GC) attached to the C-terminus of
the peptide and/or a glycine-cysteine-glycine (GCG) attached to the N-terminus to provide an
attachment point for a glycan or a glycan-linker conjugate. In certain embodiments, the
linker is N-[P-maleimidopropionic acid] hydrazide (BMPH). In certain embodiments, the
linker is 3-(2-pyridyl)thiopropionyl hydrazide (PDPH). In some embodiments, the peptide
to linker ratio is from about 1:1 to about 5:1. In other embodiments, the peptide to linker
ratio is from about 1:1 to about 10:1. In other embodiments, the peptide to linker ratio is
from about 1:1 to about 2:1, or from about 1:1 to about 3:1, or from about 1:1 to about 4:1, or
from about 1:1 to about 5:1, or from about 1:1 to about 6:1, or from about 1:1 to about 7:1, or
from about 1:1 to about 8:1, or from about 1:1 to about 9:1. In one embodiment, the peptide
linker ratio is about 1:1. In one embodiment, the peptide linker ratio is about 2:1. In one
embodiment, the peptide linker ratio is about 3:1. In one embodiment, the peptide linker ratio
is about 4:1. In one embodiment, the peptide linker ratio is about 5:1. In one embodiment,
the peptide linker ratio is about 6:1. In one embodiment, the peptide linker ratio is about 7:1.
In one embodiment, the peptide linker ratio is about 8:1. In one embodiment, the peptide
linker ratio is about 9:1. In one embodiment, the peptide linker ratio is about 10:1.

Depending on the desired properties of the synthetic proteoglycan, the total number of
peptides bonded to the glycan can be varied. In certain embodiments, the total number of
peptides present in the synthetic proteoglycan is from about 1 or 2 to about 160, or from
about 10 to about 160, or from about 20 to about 160, or from about 30 to about 160, or from
about 40 to about 160, or from about 40 to about 150, or from about 40 to about 140, or 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 synthetic
proteoglycan comprises less than about 50 peptides. In various embodiments, the synthetic
proteoglycan comprises from about 5 to about 40 peptides. In some embodiments, the
synthetic proteoglycan comprises from about 10 to about 40 peptides. In other embodiments,
the synthetic proteoglycan comprises from about 5 to about 20 peptides. In various
embodiments, the synthetic proteoglycan comprises from about 4 to about 18 peptides. In
certain embodiments, the synthetic proteoglycan comprises less than about 20 peptides. In
certain embodiments, the synthetic proteoglycan comprises less than about 18 peptides. In
certain embodiments, the synthetic proteoglycan comprises less than about 15 peptides. In
certain embodiments, the synthetic proteoglycan comprises less than about 10 peptides. In certain embodiments, the synthetic proteoglycan comprises about 20 peptides. In certain embodiments, the synthetic proteoglycan comprises about 40 peptides. In certain embodiments, the synthetic proteoglycan comprises about 18 peptides. In certain embodiments, the synthetic proteoglycan comprises from about 5 to about 40, or from about 10 to about 40, or from about 5 to about 20, or from about 4 to about 18, or about 10, or about 11, or about 18, or about 20 peptides.

In any of the embodiments described herein, the number of peptides per glycan is an average, where certain synthetic proteoglycans in a composition may have more peptides per glycan and certain synthetic proteoglycans have less peptides per glycan. Accordingly, in certain embodiments, the number of peptides as described herein is an average in a composition of synthetic proteoglycans. For example, in certain embodiments, the synthetic proteoglycans are a composition where the average number of peptides per glycan is about 5. In other embodiments, the average number of peptides per glycan is about 6, or about 7, or about 8, or about 9, or about 10, or about 11, or about 12, or about 13, or about 14, or about 15, or about 16, or about 17, or about 18, or about 19, or about 20, or about 25, or about 30. In certain embodiments, the number of peptides per glycan may be described as a "percent (%) functionalization" based on the percent of disaccharide units which are functionalized with peptide on the glycan backbone. For example, the total number of available disaccharide units present on the glycan can be calculated by dividing the molecular weight (or the average molecular weight) of a single disaccharide unit (e.g., about 550-800 Da, or from about 650-750 Da) by the molecular weight of the glycan (e.g., about 25 kDa up to about 70 kDa, or even about 100 kDa). For example, in some embodiments, the number of available disaccharide units present on the glycan is from about 10 to about 80, or from about 10 to about 70, or from about 15 to about 70, or from about 20 to about 70, or from about 30 to about 70, or from about 35 to about 70, or from about 40 to about 70, or from about 10 to about 50, or from about 20 to about 50, or from about 25 to about 50, or from about 10 to about 30, or from about 15 to about 30, or from about 20 to about 30, or from about 15, or about 20, or about 25, or about 30, or about 35, or about 40, or about 45, or about 50, or about 55, or about 60, or about 65, or about 70.

Therefore, in certain embodiments, the glycan comprises from about 1 to about 50, or from about 5 to about 30% functionalization, or about 25% functionalization, wherein the percent (%) functionalization is determined by a percent of disaccharide units on the glycan.
which are functionalized with peptide. In some embodiments, the percent (\%) functionalization of the glycan is from about 1% to about 50%, or from about 3% to about 40%, or from about 5% to about 30\%, or from about 10\% to about 20\%, or about 1%, or about 2\%, or about 5%, or about 10\%, or about 15%, or about 20%, or about 25%, or about 30\%, or about 35%, or about 40\%, or about 45\%, or about 50\%, or about 55\%, or about 60\%, or about 65\%, or about 70\%, or about 75\%, or about 80\%, or about 85\%, or about 90\%, or about 95\%, or about 100\%.

In one aspect, the collagen-binding peptide has binding affinity to one or more of collagen types I, II, III, or IV. In some embodiments, the collagen-binding peptide binds to type I collagen. In other embodiments, the collagen-binding peptide binds to type IV collagen. In certain embodiments, one or more collagen-binding peptide having a specified binding affinity can be used in the synthetic proteoglycans described herein. For example, the synthetic proteoglycans 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 IV collagen. In another aspect, the collagen-binding peptides have binding affinity to type I collagen. In another aspect, the collagen-binding peptides have binding affinity to type IV collagen. In certain aspects, the collagen-binding peptides have binding affinity to type II collagen. In certain aspects, the collagen-binding peptides have binding affinity to type III collagen.

Suitable collagen-binding peptides are known (see, e.g., US 2013/0190246, US 2012/0100106, and US 201 1/0020298, the disclosures of which are incorporated herein by reference in their entirety) or can be found by methods known in the art. In certain embodiments, 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:

i) RRANAALKAGELYKSILY (SEQ ID NO: 1), GELYKSILY (SEQ ID NO: 2),
RRANAALKAGELYKCI Lyon (SEQ ID NO: 3), GELYKCI Lyon (SEQ ID NO: 4),
RLDGNEIKR (SEQ ID NO: 5), AHEEISTTNEGVM (SEQ ID NO: 6),
NGVFKYRPYFLYKHAYFYPPPLKRFPVQ (SEQ ID NO: 7), CQDSETRTFY (SEQ ID NO: 8), TKKTLRT (SEQ ID NO: 9), GLRSKSKKFRRPDIQYPDATDEDITSHM (SEQ ID NO: 9).
NO: 10), SQNPVQP (SEQ ID NO: 11), SYIRIADTNIT (SEQ ID NO: 12), KELNLVYT (SEQ ID NO: 13), GSIT (SEQ ID NO: 14), GSITTIDVPNV (SEQ ID NO: 15), GQLYKSILY (SEQ ID NO: 16), RRANAALKAGLYKSILY (SEQ ID NO: 17); or ii) a peptide comprising a sequence with at least about 80% sequence identity to the amino acid sequence of i) and capable of binding to collagen. In some embodiments, the collagen-binding peptide(s) is RRANAALKAGLYKSILY (SEQ ID NO: 1) or a peptide having at least about 80% sequence identity to RRANAALKAGLYKSILY (SEQ ID NO: 1) and capable of binding to collagen. In some embodiments, the peptide sequence comprises a sequence with at least about 80%, sequence identity, or at least about 83% 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 to the amino acid sequence of i) and capable of binding to collagen. In certain embodiments, the collagen-binding peptide is at least about 80%, or at least about 83%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98%, or at least about 100% homologous with the collagen-binding domain(s) of the von Willebrand factor or a platelet collagen receptor as described in Chiang, T. M. et al. J. Biol. Chem., 2002, 277: 34896-34901; Huizinga, E.G. et al, Structure, 1997, 5: 1147-1156; Romijn, R. A. et al, J. Biol. Chem., 2003, 278: 15035-15039; and Chiang, et al, Cardio. & Haemato. Disorders-Drug Targets, 2007, 7: 71-75, each incorporated herein by reference.

In various embodiments, the collagen-binding peptide comprises an amino acid spacer. Accordingly, in certain embodiments, the collagen-binding peptide comprises an amino acid sequence selected from:

i) RRANAALKAGELYKSILYGC (SEQ ID NO: 76), RLDGNEIKRGC (SEQ ID NO: 77), AHEEISTNENGMGC (SEQ ID NO: 78), GCGGELYKSILY (SEQ ID NO: 79), NGVFKYRPRYFLKHAYFYPPLKRFPVQGC (SEQ ID NO: 80), CQDSETRTFYGC (SEQ ID NO: 81), TKKTLRTGC (SEQ ID NO: 82), GLRSKSSKFRRPDQYPDATDEDITSMHGC (SEQ ID NO: 83), SQNPVQPFGC (SEQ ID NO: 84), SYIRIADTNITGC (SEQ ID NO: 85), KELNLVYTGC (SEQ ID NO: 86), GSITTIDVPNVGC (SEQ ID NO: 87), GCGGELYKSILYGC (SEQ ID NO: 88) and GELYKSILYGC (SEQ ID NO: 75); or

ii) a peptide comprising a sequence with at least about 80% sequence identity to the amino acid sequence of i) and capable of binding to collagen. In some embodiments, the
peptide sequence comprises a sequence with at least about 80% sequence identity, or at least about 83% 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 to the amino acid sequence of i) and capable of binding to collagen.

In one embodiment, the synthetic proteoglycan comprises heparin having from about 5 to about 40 collagen-binding peptide(s) bonded thereto and wherein the collagen-binding peptide(s) is RRANAALKAGELYKSILY (SEQ ID NO: 1) or a peptide having at least about 80% sequence identity to RRANAALKAGELYKSILY (SEQ ID NO: 1) and capable of binding to collagen.

In another embodiment, the synthetic proteoglycan comprises heparin having from about 5 to about 40 collagen-binding peptide(s) bonded thereto and wherein the collagen-binding peptide(s) is (GQLYKSILY)₄-(KRR)₂-KGSG (SEQ ID NO: 72) or a peptide having at least about 80% sequence identity to (GQLYKSILY)₄-(KRR)₂-KGSG (SEQ ID NO: 72) and capable of binding to collagen.

In another embodiment, the synthetic proteoglycan comprises dermatan sulfate having from about 5 to about 40 collagen-binding peptide(s) bonded thereto and wherein the collagen-binding peptide(s) is RRANAALKAGELYKSILY (SEQ ID NO: 1) or a peptide having at least about 80% sequence identity to RRANAALKAGELYKSILY (SEQ ID NO: 1) and capable of binding to collagen.

In another embodiment, the synthetic proteoglycan comprises dermatan sulfate having from about 5 to about 40 collagen-binding peptide(s) bonded thereto and wherein the collagen-binding peptide(s) is (GQLYKSILY)₄-(KRR)₂-KGSG (SEQ ID NO: 72) or a peptide having at least about 80% sequence identity to (GQLYKSILY)₄-(KRR)₂-KGSG (SEQ ID NO: 72) and capable of binding to collagen.

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 (e.g., \textbf{SILY} \textbf{I}_i \textbf{S}_n \textbf{I}_n) 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 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., collagen-binding affinity).

3. Synthesis of Synthetic Proteoglycans

The peptides used in the method described herein (i.e., the collagen-binding 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 proteoglycan 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. See, e.g., Hermanson G.T., Bioconjugate Techniques, Academic Press, pp. 169-186 (1996), incorporated herein by reference. As shown in Scheme 1, the glycan (e.g., chondroitin sulfate "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 linker, 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., chondroitin sulfate "CS") by reacting an aldehyde function of the oxidized glycan (e.g., "ox-CS") with N-[P-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 proteoglycan. Alternatively, an oxidized glycan can be purchased or prepared via alternative technology known by one of skill in the art. 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.
4. Compositions

In one embodiment, the synthetic proteoglycan is administered in a composition. The present disclosure provides compositions comprising a synthetic proteoglycan and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are known to one having ordinary skill in the art may be used, including water or saline. 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 compositions provided in accordance with the present disclosure can be formulated as a solution for delivery to a gastroesophageal tissue. Diluent or carriers employed in the compositions can be selected so that they do not diminish the desired effects of the synthetic proteoglycan. Examples of suitable compositions include aqueous solutions, for example, a solution in isotonic saline, 5% glucose. Other well-known pharmaceutically acceptable liquid carriers such as alcohols, glycols, esters and amides, may be employed. In certain embodiments, the composition further comprises one or more excipients, such as, but not limited to ionic strength modifying agents, solubility enhancing agents, sugars such as mannitol or sorbitol, pH buffering agent, surfactants, stabilizing polymer, preservatives, and/or co-solvents.
In certain embodiments, the composition is an aqueous solution. Aqueous solutions are suitable for use in composition formulations based on ease of formulation, as well as an ability to easily administer such compositions by means of instilling the solution in. In certain embodiments, the compositions are suspensions, viscous or semi-viscous gels, or other types of solid or semi-solid compositions. In some embodiments, the composition is in the form of foams, ointments, liquid wash, gels, sprays and liposomes, which are very well known in the art. Alternatively, the topical administration is an infusion of the provided synthetic proteoglycan to the gastroesophageal tissue via a device selected from a esophagogastroduodenoscopic device, a pump-catheter system, a continuous or selective release device. In certain embodiments, the composition is a solution that is directly applied to or contacts the injured gastroesophageal tissue. In some embodiments, the composition comprises a polymer matrix. In other embodiments, the composition is absorbable. In certain embodiments, the composition comprises a pH buffering agent. In some embodiments, the composition contains a lubricity enhancing agent.

In certain embodiments, a polymer matrix or polymeric material is employed as a pharmacetically acceptable carrier or support for the composition. The polymeric material described herein may comprise natural or unnatural polymers, for example, such as sugars, peptides, protein, laminin, collagen, hyaluronic acid, 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. In certain embodiments, the compositions provided herein is formulated as films, gels, foams, or and other dosage forms.

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

In certain embodiments, the solubility of the synthetic proteoglycan 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.

In certain embodiments, the composition contains a lubricity enhancing agent. As used herein, lubricity enhancing agents refer to one or more pharmaceutically acceptable polymeric materials capable of modifying the viscosity of the pharmaceutically acceptable carrier. Suitable polymeric materials include, but are not limited to: ionic and non-ionic water soluble polymers; hyaluronic acid and its salts, chondroitin sulfate and its salts, dextran, gelatin, chitosans, gellans, other proteoglycans or polysaccharides, or any combination thereof; cellulose polymers and cellulosic polymer derivatives such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methyl cellulose, carboxymethyl cellulose, and etherified cellulose; collagen and modified collagens; galactomannans, such as guar gum, locust bean gum and tara gum, as well as polysaccharides derived from the foregoing natural gums and similar natural or synthetic gums containing mannose and/or galactose moieties as the main structural components (e.g., hydroxypropyl guar); gums such as tragacanth and xanthan gum; gellan gums; alginate and sodium alginate; chitosans; vinyl polymers; hydrophilic polymers such as polyethylene oxides, polyoxyethylene-polyoxypropylene copolymers, and polyvinylalcohol; carboxyvinyl polymers or crosslinked acrylic acid polymers such as the "carbomer" family of polymers, e.g., carboxypolyalkylenes that may be obtained commercially under the Carbopol™ trademark; and various other viscous or viscoelastomeric substances. In one embodiment, a lubricity enhancing agent is selected from the group consisting of hyaluronic acid, dermatan, chondroitin, heparin, heparan, keratin, dextran, chitosan, alginate, agarose, gelatin, hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methyl cellulose, carboxymethyl cellulose, and etherified cellulose, polyvinyl alcohol, polyvinylpyrrolidinone, povidone, carbomer 941, carbomer 940, carbomer 971P, carbomer 974P, or a pharmaceutically acceptable salt thereof. In one embodiment, a lubricity enhancing agent is applied concurrently with the synthetic proteoglycan. Alternatively, in one embodiment, a lubricity enhancing agent is applied sequentially to the synthetic proteoglycan. In one embodiment, the lubricity enhancing agent is chondroitin sulfate. In one embodiment, the lubricity enhancing agent is hyaluronic acid. The lubricity enhancing agent can change the viscosity of the composition.
For further details pertaining to the structures, chemical properties and physical properties of the above lubricity enhancing agents, see e.g., U.S. 5,409,904, U.S. 4,861,760 (gellan gums), U.S. 4,255,415, U.S. 4,271,143 (carboxyvinyl polymers), WO 94/10976 (polyvinyl alcohol), WO 99/51273 (xanthan gum), and WO 99/06023 (galactomannans).

Typically, non-acidic lubricity 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 proteoglycans 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.

Suitable pH buffering agents for use in the compositions herein 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. In certain embodiments, an appropriate buffer system (e.g., sodium phosphate, sodium acetate, sodium citrate, sodium borate or boric acid) is added to the composition to prevent pH drift under storage conditions. In some embodiments, the buffer is a phosphate buffered saline (PBS) solution (i.e., containing sodium phosphate, sodium chloride and in some formulations, potassium chloride and potassium phosphate). The particular concentration will vary, depending on the agent employed. In certain embodiments, the pH buffer system (e.g., sodium phosphate, sodium acetate, sodium citrate, sodium borate or boric acid) is added to maintain a pH within the range of from about pH 4 to about pH 8, or about pH 5 to about pH 8, or about pH 6 to about pH 8, or about pH 7 to about
pH 8. In some embodiments, the buffer is chosen to maintain a pH within the range of from about pH 4 to about pH 8. In some embodiments, the pH is from about pH 5 to about pH 8. In some embodiments, the buffer is a saline buffer. In certain embodiments, the pH is from about pH 4 and about pH 8, from about pH 3 to about pH 8, or from about pH 4 to about pH 7. In some embodiments, the composition is in the form of a film, gel, patch, or liquid solution which comprises a polymeric matrix, pH buffering agent, a lubricity enhancing agent and a synthetic proteoglycan wherein the composition optionally contains a preservative; and wherein the pH of said composition is within the range of about pH 4 to about pH 8.

Surfactants are employed in the composition to deliver higher concentrations of synthetic peptidoglycan. The surfactants function to solubilize the inhibitor and stabilize colloid dispersion, such as micellar solution, microemulsion, emulsion and suspension. Suitable surfactants comprise c polysorbate, poloxamer, polyosyl 40 stearate, polyoxyl castor oil, tyloxapol, triton, and sorbitan monolaurate. In one embodiment, the surfactants have hydrophile/lipophile/balance (HLB) in the range of 12.4 to 13.2 and are acceptable for ophthalmic use, such as TritonX1 14 and tyloxapol.

In certain embodiments, stabilizing polymers, i.e., demulcients, are added to the composition. The stabilizing polymer should be an ionic/charged example, more specifically a polymer that carries negative charge on its surface that can exhibit a zeta-potential of (−)10-50 mV for physical stability and capable of making a dispersion in water (i.e. water soluble). In one embodiment, the stabilizing polymer comprises a polyelectrolyte or polyelectrolytes if more than one, from the family of cross-linked polycrylates, such as carbomers and Pemulen®, specifically Carbomer 974p (polyacrylic acid), at a range of about 0.1% to about 0.5% w/w.

In one embodiment, the composition comprises an agent which increases the permeability of the synthetic proteoglycan to the extracellular matrix of blood vessels. Preferably the agent which increases the permeability is selected from benzalkonium chloride, saponins, fatty acids, polyoxyethylene fatty ethers, alkyl esters of fatty acids, pyrrolidones, polyvinylpyrrolidone, pyruvic acids, pyroglutamic acids or mixtures thereof.

The synthetic proteoglycan 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 proteoglycan can be used. In certain embodiments, the synthetic proteoglycan 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 proteoglycan can be subjected to one or more sterilization processes. Alternatively, the synthetic proteoglycan may be wrapped in any type of container including a plastic wrap or a foil wrap, and may be further sterilized.

In some embodiments, preservatives are added to the composition to prevent microbial contamination during use. Suitable preservatives added to the compositions comprise benzalkonium chloride, benzoic acid, alkyl parabens, alkyl benzoates, chlorobutanol, chlorocresol, cetyl alcohols, fatty alcohols such as hexadecyl alcohol, organometallic compounds of mercury such as phenylmercury nitrate or borate, diazolidinyl urea, diisopropyl adipate, dimethyl polysiloxane, salts of EDTA, vitamin E and its mixtures. In certain embodiments, the preservative is selected from benzalkonium chloride, chlorobutanol, benzododecinium bromide, methyl paraben, propyl paraben, phenylethyl alcohol, edentate disodium, sorbic acid, or polyquaternium-1. In certain embodiments, the ophthalmic compositions contain a preservative. In some embodiments, the preservatives are employed at a level of from about 0.001% to about 1.0% w/v. In certain embodiments, the ophthalmic compositions do not contain a preservative and are referred to as "unpreserved". In some embodiments, the unit dose compositions are sterile, but unpreserved.

In some embodiments, separate or sequential administration of the synthetic proteoglycan and other agent is necessary to facilitate delivery of the composition into to the gastroesophageal tissue. In certain embodiments, the synthetic proteoglycan and the other agent can be administered at different dosing frequencies or intervals. For example, the synthetic proteoglycan can be administered daily, while the other agent can be administered less frequently. Additionally, as will be apparent to those skilled in the art, the synthetic proteoglycan and the other agent can be administered using the same route of administration or different routes of administration.

Any effective regimen for administering the synthetic proteoglycan can be used. For example, the synthetic proteoglycan 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, the synthetic proteoglycan can be administered topically, such as by film, gel, patch, or liquid solution. In some of the embodiments, the compositions
provided are in a buffered, sterile aqueous solution. In certain embodiments, the solutions have a viscosity of from about 1 to about 100 centipoises (cps), or from about 1 to about 200 cps, or from about 1 to about 300 cps, or from about 1 to about 400 cps. In some embodiments, the solutions have a viscosity of from about 1 to about 100 cps. In certain embodiments, the solutions have a viscosity of from about 1 to about 200 cps. In certain embodiments, the solutions have a viscosity of from about 1 to about 300 cps. In certain embodiments, the solutions have a viscosity of from about 1 to about 400 cps. In certain embodiments, the solution is in the form of an injectable liquid solution. In other embodiments, the compositions are formulated as viscous liquids, i.e., viscosities from several hundred to several thousand cps, gels or ointments. In these embodiments, the synthetic proteoglycan is dispersed or dissolved in an appropriate pharmaceutically acceptable carrier.

Exemplary compositions for use with the synthetic proteoglycans for catheter-based delivery may comprise: a) a synthetic proteoglycan as described herein; b) a pharmaceutically acceptable carrier; c) a polymer matrix; d) a pH buffering agent to provide a pH in the range of about pH 4 to about pH 8; and e) a water soluble lubricity enhancing agent in the concentration range of about 0.25% to about 10% total formula weight or any individual component a), b), c), d) or e), or any combinations of a), b), c), d) or e).

The proteoglycan of the present disclosure binds to collagen and also binds to endogenous or exogenous growth factors. Palifermin is a keratinocyte growth factor useful for oral mucositis treatment. In certain embodiments, the present disclosure provides a composition comprising a proteoglycan and palifermin. Such a composition is useful for the targeted delivery of the palifermin in oral mucositis patients.

5. Pharmaceutical Formulations

Formulations contemplated by the present disclosure may also be for administration by injection include aqueous or oil suspensions, or emulsions, with sesame oil, corn oil, cottonseed oil, or peanut oil, as well as elixirs, mannitol, dextrose, or a sterile aqueous solution, and similar pharmaceutical vehicles. Aqueous solutions in saline are also conventionally used for injection, but less preferred in the context of the present disclosure. Ethanol, glycerol, propylene glycol, liquid polyethylene glycol, and the like (and suitable mixtures thereof), cyclodextrin derivatives, and vegetable oils may also be employed. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of
surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersosal, and the like.

Sterile injectable solutions are prepared by incorporating the component in the required amount in the appropriate solvent with various other ingredients as enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In making pharmaceutical compositions that include synthetic proteoglycans described herein, the active ingredient is usually diluted by an excipient or carrier and/or enclosed within such a carrier that can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material (as above), which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of films, gels, patches, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compounds, soft and hard gelatin films, gels, patches, sterile injectable solutions, and sterile packaged powders.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents.

Films used for drug delivery are well known in the art and comprise non-toxic, non-irritant polymers devoid of leachable impurities, such as polysaccharides (e.g., cellulose, maltodextrin, etc.). In some embodiments, the polymers are hydrophilic. In other embodiments, the polymers are hydrophobic. The film adheres to tissues to which it is
applied, and is slowly absorbed into the body over a period of about a week. Polymers used in the thin-film dosage forms described herein are absorbable and exhibit sufficient peel, shear and tensile strengths as is well known in the art. In some embodiments, the film is injectable. In certain embodiments, the film is administered to the patient prior to, during or after surgical intervention.

Gels are used herein refer to a solid, jelly-like material that can have properties ranging from soft and weak to hard and tough. As is well known in the art, a gel is a non-fluid colloidal network or polymer network that is expanded throughout its whole volume by a fluid. A hydrogel is a type of gel which comprises a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent and can contain a high degree of water, such as, for example greater than 90% water. In some embodiments, the gel described herein comprises a natural or synthetic polymeric network. In some embodiments, the gel comprises a hydrophilic polymer matrix. In other embodiments, the gel comprises a hydrophobic polymer matrix. In some embodiments, the gel possesses a degree of flexibility very similar to natural tissue. In certain embodiments, the gel is biocompatible and absorbable. In certain embodiments, the gel is administered to the patient prior to, during or after surgical intervention.

Liquid solution as used herein refers to solutions, suspensions, emulsions, drops, ointments, liquid wash, sprays, liposomes which are well known in the art. In some embodiments, the liquid solution contains an aqueous pH buffer agent which resists changes in pH when small quantities of acid or base are added. In certain embodiments, the liquid solution is administered to the patient prior to, during or after surgical intervention.

Exemplary formulations may comprise: a) synthetic proteoglycan as described herein; b) pharmaceutically acceptable carrier; c) polymer matrix; and d) pH buffering agent to provide a pH in the range of about pH 4 to about pH 8, wherein said solution has a viscosity of from about 3 to about 30 cps for a liquid solution. In certain embodiments, the solutions have a viscosity of from about 1 to about 100 centipoises (cps), or from about 1 to about 200 cps, or from about 1 to about 300 cps, or from about 1 to about 400 cps. In some embodiments, the solutions have a viscosity of from about 1 to about 100 cps. In certain embodiments, the solutions have a viscosity of from about 1 to about 200 cps. In certain embodiments, the solutions have a viscosity of from about 1 to about 300 cps. In certain embodiments, the solutions have a viscosity of from about 1 to about 400 cps.
Alternatively, exemplary formulations may comprise: a) synthetic proteoglycan as described herein; b) pharmaceutically acceptable carrier; and c) hydrophilic polymer as matrix network, wherein said compositions are formulated as viscous liquids, i.e., viscosities from several hundred to several thousand cps, gels or ointments. In these embodiments, the synthetic proteoglycan is dispersed or dissolved in an appropriate pharmaceutically acceptable carrier.

In certain embodiments, the synthetic proteoglycan, or a composition comprising the same, is lyophilized prior to, during, or after, formulation. Accordingly, also provided herein is a lyophilized composition comprising a proteoglycan or composition comprising the same as described herein.

6. Dosing

Suitable dosages of the synthetic proteoglycan 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, a dose ranges from about 0.0001 mg to about 10 mg. 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 500 mg per dose, or from about 1 mg to 3000 mg per dose, or from about 100 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 about 10 mg per dose. In other illustrative embodiments, effective doses can be about 1 µg, about 10 µg, about 25 µg, about 50 µg, about 75 µg, about 100 µg, about 125 µg, about 150 µg, about 200 µg, about 250 µg, about 275 µg, about 300 µg, about 350 µg, about 400 µg, about 450 µg, about 500 µg, about 550 µg, about 575 µg, about 600 µg, about 625 µg, about 650 µg, about 675 µg, about 700 µg,
about 800 µg, about 900 µg, 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 100 mg, or about 100 mg to about 30 grams. In certain embodiments, the dose is from about 0.01 mL to about 10 mL.

In some embodiments, the compositions are packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. In certain embodiments, suitable preservatives as described above can be added to the compositions. In some embodiments, the composition contains a preservative. In certain embodiments the preservatives are employed at a level of from about 0.001% to about 1.0% w/v. In some embodiments, the unit dose compositions are sterile, but unpreserved.

In one embodiment, an effective amount of a composition comprising a synthetic proteoglycan and pharmaceutically acceptable carrier is administered to a patient in need to treat a gastroesophageal injury or ameliorate one or more symptoms of the injury, for instance, without limitation.

Examples

Example 1. Synthesis of DS-SILY

Dermatan sulfate (DS) was dissolved in 0.1 M sodium phosphate buffer at pH 5.5 to make a solution of a concentration of 20 mg/mL. The degree of functionalization was controlled by the concentration of the periodate. Periodate solutions of various concentrations were prepared by dissolving it in 0.1 M sodium phosphate buffer at pH 5.5 according to the following table.

<table>
<thead>
<tr>
<th>Target (SILY/DS)</th>
<th>Peridote Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The DS solution was mixed with the periodate solution in a ratio of 1:1 (V:V) for two hours at room temperature to provide the oxidized DS, which was purified using Biogel P6 column with phosphate buffer saline. SILY peptide having a terminal GSG-NHNH₂ bound thereto (i.e., RRANAALKAGELYKSILYGSG-NHNH₂ (SEQ ID NO: 77)) was dissolved in water to provide a concentration of 1 mg/mL using sonication if needed. The SILY peptide was slowly added to the oxidized DS at room temperature and stirred for about 2 hours protecting it from light. The pH of the reaction mixture was maintained above 6. Optionally, one mole of similarly functionalized SILYbiot₂ (biotin-labeled peptide) can be reacted with
one mole of DS and then unlabeled SILY peptide can be added up (molar equivalent - 1 ) to
the number of aldehydes expected. For example, for DS-SILY\textsubscript{20}, 1 mole of SILY\textsubscript{biotin} and 19
moles of SILY unlabeled were added. However, the addition of SILY\textsubscript{biotin} was optional. DS-
SILY\textsubscript{20} was also prepared by adding 20 moles of SILY-unlabeled to one mole of DS. The
product was purified with water to provide the desired DS-SILY.

Additional proteoglycans, including heparin-containing proteoglycans, are made via
similar synthesis or in accordance with Scheme 1 shown above.

**Example 2. Clinical Trial Protocol for Treating Gastroesophageal Injury**

This example proposes a clinical trial to test the ability of proteoglycans selected from
those described herein, such as Hep-SILY, other heparin-containing proteoglycans including
the branched peptides as discussed above, or DS-SILY in treating gastroesophageal injuries. The
text agent will be proteoglycans prepared in a solution or gel. Gastroesophageal injuries
constitute significant morbidity and costs to the patient and healthcare system. Maintaining
esophageal patency consumes significant resources.

The disease target is any gastroesophageal injury, either spontaneous from GERD or
iatrogenic from interventions. This is a randomized, multi-center, safety and effectiveness
study of topical proteoglycans administered via esophagogastroduodenoscopy (EGD) for
treatment of gastroesophageal injuries.

The objectives of this trial include, 1) to assess the overall safety profile of EGD-
delivered proteoglycan treatment dosed at the time of EGD intervention, and 2) to determine
the effectiveness of EGD-delivered proteoglycan in reducing restenosis rates in
gastroesophageal injuries.

Fifty (50) patients will be enrolled for the trial, 25 of which will receive proteoglycan
(EGD-delivered) and 25 vehicle (EGD-delivered).

The key inclusion criteria include (a) GERD associated esophageal lesion requiring
EGD ablation, (b) esophageal stricture requiring EGD dilation, and (c) peptic ulcer disease
(PUD) requiring EGD treatment. The key exclusion criteria include H/O severe allergic
diseases.

The visit schedule will be one, six, and 12 weeks. The safety assessment include (1)
ascertainment of adverse events (AEs) and Serious AEs (SAEs), (2) physical
examination/vitals: with special attention given to local findings, and (3) labs including
proteoglycan antibodies (baseline, week 1, 12 and 24). The EGD delivery can be repeated at 6 and 12 weeks.

To measure the patency of the esophagus, quantitative barium swallow will be conducted pre-treatment and at end of the study.

The primary endpoint of this trial is reduced rate of recurrent stricture, and the secondary endpoints are time to advancement of oral diet, time to recurrent stricture and improved PUD score or symptoms. It is contemplated that the trial will succeed on all of these endpoints.
What is Claimed is:

1. A method for maintaining esophageal patency in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 1 to about 80 collagen-binding peptide(s) bonded to the glycan.

2. A method for treating a gastroesophageal injury in a patient, comprising topically applying a pharmaceutical composition to a lesion on a gastroesophageal tissue, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 1 to about 80 collagen-binding peptide(s) bonded to the glycan.

3. The method of claim 1 or 2, wherein the patient suffers from a gastroesophageal reflux disease (GERD).

4. The method of claim 2, wherein the lesion is caused by an iatrogenic intervention.

5. The method of claim 2, wherein the lesion has undergone an esophagogastroduodenoscopy (EGD) ablation.

6. The method of claim 1 or 2, wherein the patient suffers from esophageal stricture.

7. The method of claim 6, wherein the patient is in need of EGD dilation.

8. The method of claim 1 or 2, wherein the patient suffers from a peptic ulcer disease (PUD).

9. The method of any preceding claim, wherein the pharmaceutical composition is applied through esophagogastroduodenoscopy (EGD).

10. The method of any preceding claim, wherein the glycan is dextran, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparan sulfate, heparin, keratin, keratan sulfate, or hyaluronic acid.

11. The method of any preceding claim, wherein the peptide(s) are covalently bonded to the glycan via a linker.

12. The method of claim 11, wherein the linker is N-[P-maleimidopropionic acid] hydrazide (BMPH), 3-(2-pyridylthio)propionyl hydrazide (PDPH) or the peptide GSG.
13. The method of any preceding claim, wherein the synthetic proteoglycan comprises from about 5 to about 40 peptides.

14. The method of any preceding claim, wherein the collagen-binding peptide comprises an amino acid sequence selected from:

   i) RRANAALKAGELYKSILY (SEQ ID NO: 1), RLDGNEIKR (SEQ ID NO: 2), AHEEI STTNEGVM (SEQ ID NO: 3), GELYKSILY (SEQ ID NO: 4), NGVFKYRPRYFLYKAYFYPPLKRPFPVQ (SEQ ID NO: 5), CQDSETRTFY (SEQ ID NO: 6), TKKTLRT (SEQ ID NO: 7), GLRSDKKKFRRPDIQYPDATDEDITSHM (SEQ ID NO: 8), SQNPVQP (SEQ ID NO: 9), SYRIADTNIT (SEQ ID NO: 10), KELNLVYT (SEQ ID NO: 11), GSITTDVPWNVC (SEQ ID NO: 12), GSITTDVPWNV (SEQ ID NO: 13), RRANAALKAGELYKCIL (SEQ ID NO: 14), GELYKCILY (SEQ ID NO: 15), GQLYKSILY (SEQ ID NO: 16), or RRANAALKAGQLYKSILY (SEQ ID NO: 17); or

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

15. The method of any preceding claim, wherein the pharmaceutical composition is formulated as a film, gel, patch, or liquid solution.

16. The method of claim 15, wherein the liquid solution further comprises a polymer matrix.

17. The method of any preceding claim, wherein the collagen-binding peptide(s) is RRANAALKAGELYKSILY (SEQ ID NO: 1).

18. The method of any of claims 1-16, wherein the collagen-binding peptide(s) is (GQLYKSILY)_4-(KRR)_2-KGSG (SEQ ID NO: 72).

19. The method of claim 17 or 18, wherein the glycan is heparin.

20. The method of claim 17 or 18, wherein the glycan is dermatan sulfate.

21. A method for reducing restenosis, reducing recurrent stricture, expediting advancement to oral diet, or ameliorating peptic ulcer disease (PUD) symptoms in a patient suffering from a gastroesophageal injury, comprising topically applying a pharmaceutical composition to an injured gastroesophageal tissue of the patient, wherein the pharmaceutical composition comprises an effective amount of a synthetic proteoglycan comprising a glycan having from about 1 to about 80 collagen-binding peptide(s) bonded to the glycan.
**INTERNATIONAL SEARCH REPORT**

**International application No**

PCT/US2015/056778

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K38/14 A61K47/48 A61K31/727 A61K31/737 C07K14/47 C07K9/00

**ADD.**

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)

A61K C07K A61P

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 2010/129547 AI (PURDUE RESEARCH FOUNDATION [US]; PANITCH ALYSSA [US]; PADERI JOHN E [U]) 11 November 2010 (2010-11-11) cited in the application on see claims, page 35; examples 3, 10, 11, 19, 35, 40, sequences -----</td>
<td>1-21</td>
</tr>
<tr>
<td>Y</td>
<td>WO 2011/163492 AI (PURDUE RESEARCH FOUNDATION [US]; PADERI JOHN E [US]; PANITCH ALYSSA [U]) 29 December 2011 (2011-12-29) cited in the application on see claims, page 1, examples 17, 20, 27, 41, sequences -----</td>
<td>1-21</td>
</tr>
</tbody>
</table>

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

* "Z" document member of the same patent family

**Date of the actual completion of the international search**

12 February 2016

**Date of mailing of the international search report**

11/03/2016

**Name and mailing address of the ISA/**

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

**Authorized officer**

Merckl i ng-Rui z, V
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>US 2015038427 Al</td>
<td>05-02-2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2010129547 Al</td>
<td>11-11-2010</td>
</tr>
<tr>
<td>WO 2011163492 Al</td>
<td>29-12-2011</td>
<td>A U 2011270862 Al</td>
<td>10-01-2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2803167 Al</td>
<td>29-12-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2585112 Al</td>
<td>01-05-2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2013190246 Al</td>
<td>25-07-2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2015038425 Al</td>
<td>05-02-2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2011163492 Al</td>
<td>29-12-2011</td>
</tr>
</tbody>
</table>