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(54) Title: USE OF A URINARY BLADDER ECM HYDROGEL AS AN ESOPHAGEAL SUBMUCOSAL FLUID CUSHION

(57) Abstract: Methods are disclosed for dissecting a mucosa and a submucosa from a muscularis propria from a region of the esophagus of a subject. These methods include injecting submucosally into the region of the esophagus of the subject a pharmaceutical composition comprising a urinary bladder extracellular matrix (ECM) hydrogel, to form a cushion between the submucosa and the underlying muscularis propria at the region of the esophagus, wherein the ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 400 Pascal (Pa).

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**USE OF A URINARY BLADDER ECM HYDROGEL AS AN ESOPHAGEAL  
SUBMUCOSAL FLUID CUSHION**

**CROSS REFERENCE TO RELATED APPLICATION**

5           This application claims the benefit of U.S. Provisional Application No. 62/688,191, filed June 21, 2018, which is incorporated by reference herein in its entirety.

**FIELD OF THE DISCLOSURE**

10           This relates to endoscopic resection, specifically to the use of a urinary bladder matrix extracellular matrix (ECM) hydrogel as a submucosal cushion for dissecting a mucosa and a submucosa from a muscularis propria in the esophagus.

**BACKGROUND**

15           Endoscopy is a procedure that allows examination of the interior of a hollow organ or cavity of the body by means of an instrument called an endoscope, without employing invasive surgery. Endoscopy can be used for surgical procedures such as cauterization of a bleeding vessel, widening a narrow esophagus, removing polyps, adenomas and small tumors, performing biopsies or removing a foreign object. Endoscopic procedures can be performed in the gastrointestinal tract, the respiratory tract, the ear, the urinary tract, the female reproductive system and, through small  
20           incisions, in normally closed body cavities such as the abdominal or pelvic cavity (laparoscopy), the interior of a joint (arthroscopy) and organs of the chest (thoracoscopy and mediastinoscopy). The endoscope is an illuminated, usually fiber optic, flexible or rigid tubular instrument for visualizing the interior of a hollow organ or part (such as the esophagus) for diagnostic or therapeutic purposes, that typically has one or more working channels to enable passage of instruments (such as forceps,  
25           electrosurgical knife, endoscopic injection needles or scissors) or to facilitate the removal of bioptic samples. It includes a suitable lamp and imaging device at its distal portion, and it can be inserted through natural occurring openings of the body, such as the mouth, the anus, the ear, the nose or through small surgical incisions.

30           Endoscopic procedures are widely applied in the gastrointestinal tract. For example, endoscopic procedures can be used to examine the mucosa that covers the gastrointestinal cavities, and to detect small and large pathological lesions, such as inflammatory tissue, polyps, pseudo-polyps, serrated lesions, adenomas, ulcerations, dysplasias, pre-neoplastic and neoplastic formations, and tumors. Endoscopic procedures can be used for biopsies and removal of pathologic

lesions (polyps, adenomas, dysplasias, Barrett's dysplasia, pre-neoplastic and neoplastic formations, tumors). Surgical interventions include two types of endoscopic resection procedures commonly used in gastrointestinal endoscopy to remove pathological lesions: endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). These two techniques allow for minimally  
5 invasive treatment of gastrointestinal polyps, adenomas, dysplasias, Barrett's dysplasia and early-stage cancers that involve a minimum risk of lymph-node metastasis. A need remains for agents of use in endoscopic procedures in the esophagus.

### SUMMARY OF THE DISCLOSURE

10 The foregoing and other features and advantages of the invention will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

Methods are disclosed herein for dissecting a mucosa and a submucosa from a muscularis  
15 propria from a region of an esophagus of a subject. These methods include injecting submucosally into the region of the esophagus of the subject a pharmaceutical composition comprising a urinary bladder extracellular matrix (ECM) hydrogel to form a cushion between the submucosa and the underlying muscularis propria in the esophagus, wherein the ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b)  
20 a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 400 Pascal (Pa). The method dissects the mucosa and the submucosa from the underlying muscularis propria at the region of the esophagus. The method can also inhibit inflammation in the region of the esophagus in the subject.

Methods are also disclosed herein for dissecting a mucosa and a submucosa from a  
25 muscularis propria from a region of an esophagus of a subject. These methods include injecting submucosally into the region of the esophagus of the subject a pharmaceutical composition comprising a urinary bladder extracellular matrix (ECM) hydrogel to form a cushion between the submucosa and the underlying muscularis propria in the esophagus. The method dissects the mucosa and the submucosa from the underlying muscularis propria at the region of the esophagus.  
30 The method can also inhibit inflammation in the region of the esophagus in the subject.

In some embodiments, the method of dissecting comprises endoscopic mucosal resection or endoscopic mucosal dissection.

## BRIEF DESCRIPTION OF THE FIGURES

**FIGS. 1A-1C. In-vivo use of ECM as submucosal fluid cushion.** Use of urinary bladder extracellular matrix (UBM-ECM) hydrogel as a submucosal fluid cushion with visible areas where EMR has already been performed (A). Esophageal tissue removed with submucosal fluid cushion (SFC) shows a gel present at the site of the removed esophageal mucosa (B). Result of full circumferential EMR with full circumference of mucosa excised (C).

**FIGS. 2A and 2B.** are cross-sectional views of the urinary bladder. Urinary bladder submucosa (UBS) ECM hydrogels are prepared from urinary bladder submucosa (A), which includes the D- tunica submucosa (including muscularis mucosa and tunica submucosa) of the urinary bladder. Urinary bladder matrix (UBM) ECM hydrogels are prepared from UBM (B), which includes B-epithelial basement membrane and C-tunica propria of the urinary bladder. L indicates the location of the lumen. These figures are adapted from U.S. Patent No. 6,576,265, incorporated herein by reference.

**FIGS. 3A-3C.** Rheology data, including UBM flow viscosity (A), UBM time sweep (B), and UBM time to 50% gelation (C).

## DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

Disclosed herein is the use of a urinary bladder extracellular matrix (ECM) hydrogel as a submucosal cushion for dissecting a mucosa and a submucosa from a muscularis propria in the esophagus, such as in any endoscopic resection procedure commonly used in esophageal endoscopy to remove pathological lesions, for example EMR and ESD. The urinary bladder ECM hydrogel can be a UBM ECM hydrogel or a UBS ECM hydrogel.

### *Terms*

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

**Acid Protease:** An enzyme that cleaves peptide bonds, wherein the enzyme has increased

activity of cleaving peptide bonds in an acidic pH. For example and without limitation, acid proteases can include pepsin and trypsin.

**Barrett's Esophagus:** An abnormal change (metaplasia or dysplasia) in the cells of the esophagus, typically the lower (distal) portion of the esophagus. Barrett's esophagus is the diagnosis when portions of the normal stratified squamous epithelium lining of the esophagus are replaced by simple columnar epithelium with goblet cells. Barrett's esophagus is found in 5–15% of patients who seek medical care for gastroesophageal reflux disease (GERD), although a large subgroup of patients with Barrett esophagus do not have symptoms. Barrett's esophagus is strongly associated with esophageal adenocarcinoma, and is considered to be a premalignant condition. The main cause of Barrett's esophagus is thought to be an adaptation to chronic acid exposure from gastric reflux. The cells of Barrett's esophagus, after biopsy, are classified into four general categories: non-dysplastic, low-grade dysplasia, high-grade dysplasia, and frank carcinoma.

**Base:** A compound or a solution of a compound with a pH greater than 7. For example and without limitation, the base is an alkaline hydroxide or an aqueous solution of an alkaline hydroxide. In certain embodiments, the base is NaOH or NaOH in PBS.

**Comminute** (comminution and comminuting): The process of reducing larger particles into smaller particles, including, without limitation, by grinding, blending, shredding, slicing, milling, cutting, shredding. ECM can be comminuted while in any form, including, but not limited to, hydrated forms, frozen, air-dried, lyophilized, powdered, sheet-form.

**Diagnosis:** The process of identifying a disease by its signs, symptoms and results of various tests. The conclusion reached through that process is also called "a diagnosis." Forms of testing commonly performed include blood tests, medical imaging, and biopsy.

**Dissection:** The process of separating or cutting apart tissue, for example, during a surgical procedure.

**Endoscopic injection needles or endoscopic injection needle catheters:** Devices which are generally long (for example, up to about 230 cm) and which include a long catheter within which an inner injection tube having a distal injection needle that is slideably disposed. Generally, a proximal actuating handle is coupled to the catheter and the injection tube for moving one relative to the other. The needle can be retractable. Fluid access to the injection tube is typically provided via a luer connector on the handle.

Endoscopic injection needles are typically delivered to the injection site through the catheter of the endoscope. To protect the lumen from damage, the handle of the infusion needle device is manipulated to withdraw the distal injection needle into the lumen of the catheter before inserting

the device into the endoscope. When the distal end of the endoscopic injection needle device is located at the injection site, its handle is manipulated to move the injection needle distally out of the lumen of the catheter. In some embodiments, when advanced to the most distal position, the exposed portion of the injection needle can be about 4-6 mm in length.

5           **Endoscopic Mucosal resection (EMR):** An endoscopic technique developed for removal of sessile or flat neoplasms confined to the superficial layers (mucosa and submucosa) of the gastrointestinal (GI) tract. The mucosa and submucosa are resected from the underlying muscularis propria. An **endoscopic mucosal dissection (ESD)** refers to an endoscopic technique developed specifically for removing larger lesions, such as from the gastrointestinal tract, wherein the mucosa and submucosa are dissected from the other layers of the gastrointestinal tract. Both EMR and  
10           EMD typically involve injection of a substance under the targeted lesion, between the submucosa and underlying muscularis propria, to act as a cushion and elevate the submucosa and overlying mucosa. With EMR, the elevated lesion is then removed with a snare, mobilized into a small cup by suction. With ESD, the submucosa under the lesion is dissected with a specialized knife,  
15           causing separation of the submucosa and overlying mucosa. ESD enables removal of larger and potentially deeper lesions than possible with EMR with a curative intent. Both EMR and ESD are facilitated by injection of a substance into the submucosal plane of the esophagus which effectively separates the overlying mucosa from the underlying muscularis propria, and simultaneously elevates the mucosa above the adjacent esophageal mucosa. This separation of layers and elevation  
20           of affected tissue helps the surgeon isolate, grasp, and remove the tissue of interest.

**Extracellular Matrix (ECM):** The non-cellular component of tissues and organs. Natural ECM (ECM found in multicellular organisms, such as mammals and humans) is a complex mixture of structural and non-structural biomolecules, including, but not limited to, collagens, elastins, laminins, glycosaminoglycans, proteoglycans, antimicrobials, chemoattractants, cytokines, and  
25           growth factors, and typically differs in the specific composition between different tissues and organs. In mammals, ECM often comprises about 90% collagen by dry weight mass, in its various forms. Biologic scaffolds composed of ECM can be created by removing the cells from a given tissue or organ leaving behind the ECM. The composition and structure of ECM varies depending on the anatomic source of the tissue. For example, small intestinal submucosa (SIS), urinary  
30           bladder matrix (UBM), urinary bladder submucosa (UBS), esophagus (E) and liver stroma ECM each differ in their overall structure and composition due to the unique cellular niche needed for each tissue. An intact “extracellular matrix” and “intact ECM” bioscaffold consists of extracellular matrix that has not been solubilized, retains its’ 3-dimensional ultrastructure, and ideally retains

activity of its structural and non-structural biomolecules, including, but not limited to, collagens, elastins, laminins, glycosaminoglycans, proteoglycans, antimicrobials, chemoattractants, cytokines, and growth factors, such as, without limitation comminuted ECM as described herein.

The activity of the biomolecules within the ECM can be altered chemically or mechanically, for example, by chemical or enzymatic cross-linking and/or by dialyzing the ECM. Intact ECM essentially has not been enzymatically digested, cross-linked and/or dialyzed, meaning that the ECM has not been subjected to a digestion, dialysis and/or a cross-linking process, or conditions other than processes that occur naturally during storage and handling of ECM prior to solubilization. Thus, ECM that is dialyzed (in anything but a trivial manner which does not substantially affect the gelation and functional characteristics of the ECM in its uses described herein) is not considered to be "intact."

**Esophagogastroduodenoscopy (EGD) or Upper Gastrointestinal Endoscopy:** A diagnostic endoscopic procedure that visualizes any upper part of the gastrointestinal tract up to the duodenum. An "esophageal endoscopy" is any endoscopic procedure that visualizes the esophagus. An esophageal endoscopy may sometimes be performed as part of an EGD or upper gastrointestinal endoscopy. The terms are not mutually exclusive unless expressly stated to be so.

**Gelation:** The formation of a gel from a sol.

**Gastroesophageal Reflux Disease (GERD):** A chronic symptom of mucosal damage caused by stomach acid contents refluxing 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 (proximal portion) of the stomach closed, impaired expulsion of gastric reflux from the esophagus, or a hiatal hernia. These changes may be permanent or temporary.

**Flow Viscosity:** A measure of the resistance of a fluid to gradual deformation by shear stress or tensile stress. Viscosity is a property of a fluid which opposes the relative motion between the two surfaces of the fluid in a fluid that are moving at different velocities. When a fluid is forced through a tube, particles that compose the fluid generally move more quickly near the tube's axis and more slowly near its walls. Stress (such as a pressure difference between the two ends of the tube) is needed to overcome the friction between particle layers to keep the fluid moving. For a given velocity pattern, the stress required is proportional to the fluid's viscosity. Viscosity is measured with viscometers and rheometers. Viscosity can be measured as pascal second (Pa\*s). Water at 20°C has a viscosity of 1.002 mPa\*s.

**Hydrogel:** 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 natural or synthetic polymeric networks. Hydrogels also possess a degree of flexibility similar to natural tissue. The term “urinary bladder ECM hydrogel” includes UBM and UBS hydrogels.

**Inflammation:** A localized response elicited by injury to tissue. Inflammation is characterized by the appearance in or migration into any tissue space, unit or region of any class of leukocyte in numbers that exceed the number of such cells found within such region of tissue under normal (healthy) circumstances. Inflammation is orchestrated by a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.

**Isotonic Buffered Solution:** A solution that is buffered to a pH between 7.2 and 7.8 and that has a balanced concentration of salts to promote an isotonic environment.

**Low Grade Dysplasia and High Grade Dysplasia and Metaplasia (of the Esophagus):** Pathological conditions of the esophagus that are characterized by an abnormal cell morphology, but the cell type is still recognizable as squamous epithelium. Generally, in esophageal dysplasia there is an absence of apical mucin in the internal lining cells of the esophagus. At low power, these areas may appear more hyperchromatic as compared to uninvolved areas.

For high grade dysplasia, the changes in cell morphology become more pronounced, but the cells are still technically a type of squamous epithelium.

When the cells change from squamous epithelial cells to another cell type, such as a glandular cell that often is cuboidal or columnar in shape, the process is referred to as metaplasia. With metaplasia, the distortion of glandular architecture of the esophagus is usually present and may be marked; it is composed of branching and lateral budding of crypts, a villiform configuration of the mucosal surface, or intraglandular bridging of epithelium to form a cribriform pattern of "back-to-back" glands. There is abnormal epithelium on the mucosal surface with loss of nuclear polarity, characterized by "rounding up" of the nuclei, and absence of a consistent relationship of nuclei to each other.

**Preventing or treating:** Inhibiting a disease refers to inhibiting the partial or full development of a disease, for example in a person who is at risk for a disease such as one caused by inflammation. An example of a person at risk for esophageal adenocarcinoma is someone with Barrett's esophagus or GERD. Inhibiting a disease process includes preventing the development of the disease. “Treatment” refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition, such as after it has begun to develop.

**Shear Stress:** The component of stress coplanar with a material cross section. Shear stress arises from the force vector component parallel to the cross section. The formula to calculate

average shear stress is force per unit area

$$\tau = \frac{F}{A}$$

where  $\tau$  = the shear stress,  $F$  = the force applied,  $A$  = the cross-sectional area of material with area parallel to the applied force vector.

5           **Stricture:** A narrowing or tightening of the esophagus that causes swallowing difficulties. Symptoms of esophageal strictures include heartburn, bitter or acid taste in the mouth, choking, coughing, shortness of breath, frequent burping or hiccups, pain or trouble swallowing, vomiting blood and/or weight loss. Stricture of the esophagus can be caused by gastroesophageal reflux disease, esophagitis, a dysfunctional lower esophageal sphincter, disordered motility, lye ingestion, 10 or a hiatal hernia. Strictures can form after esophageal surgery and other treatments such as laser therapy or photodynamic therapy. While the area heals, a scar forms, causing the tissue to pull and tighten, leading to difficulty in swallowing. Stricture can be a result of inflammation. A barium swallow test or an upper gastrointestinal endoscopy can be used to diagnose esophageal stricture.

**Stiffness:** The rigidity of an object or fluid. The stiffness of the extracellular matrix is 15 important for guiding the migration of cells in durotaxis. Stiffness can be measure in Pascal (Pa), which are one newton per square meter.

**Therapeutic agent:** Used in a generic sense, it includes treating agents, prophylactic agents, and replacement agents. “Treatment” or “treating” means providing a substance, such as a urinary bladder ECM hydrogel, such as a UBM or UBS hydrogel, to a patient in an amount 20 sufficient to measurably reduce, inhibit, or mitigate any disease symptom, slow disease progression, or cause disease regression. In certain embodiments treatment of the disease may be commenced before the patient presents symptoms of the disease. The disclosed methods inhibit esophageal inflammation and/or mitigate the effects of esophageal inflammation.

**Therapeutically effective amount:** A “therapeutically effective amount” of a composition, 25 such as a urinary bladder ECM hydrogel, means an amount effective, when administered to a patient, to provide a therapeutic benefit such as an amelioration of symptoms, reduced decrease progression, or cause disease regression. A quantity of a urinary bladder ECM hydrogel is therapeutically effective if it is sufficient to achieve a desired effect in a subject being treated, such as to form a gel when injected into the submucosal tissue of the esophagus and dissect the overlying 30 mucosa from the underlying muscularis propria. The effective amount to form a submucosal cushion will be dependent on the preparation applied, the subject being treated, the severity and type of the affliction, and the manner of administration.

The urinary bladder ECM hydrogels of use in the methods disclosed herein have applications in both medical and veterinary settings. Therefore, the general term “subject” or “patient” is understood to include all animals, including, but not limited to, humans or veterinary subjects, such as other primates, dogs, cats, horses, and cows.

5           **Urinary Bladder ECM:** An extracellular matrix derived from urinary bladder of any mammal. This term includes urinary bladder matrix (UBM) ECM, and urinary bladder submucosa (UBS) ECM. The wall of the urinary bladder is composed of the following layers: the tunica mucosa (including a transitional epithelium layer and the tunica propria), a submucosa layer, up to three layers of muscle and the adventitia (a loose connective tissue layer)- listed in thickness cross-  
10           section from luminal to abluminal sides. UBS is prepared from a tissue composition comprising bladder submucosal tissue delaminated from abluminal muscle layers and at least the luminal portion of the tunica mucosa of a segment of vertebrate urinary bladder, see U.S. Patent No. 5,554,389, incorporated herein by reference). UBM ECM is prepared from urinary bladder epithelial basement membrane and the tunica propria that is immediately subjacent to the basement  
15           membrane, see U.S. Patent No. 6,576,265, incorporated herein by reference.

Unless otherwise explained, 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. The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly  
20           indicates otherwise. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term “comprises” means “includes.” The term  
25           “about” indicates within 5 percent. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

### 30                                    *Urinary Bladder Extracellular Matrix (ECM) Hydrogels*

Methods of preparing urinary bladder ECM hydrogels, are disclosed for example, in U.S. Patent No. 8,361,503, U.S. Patent No. 6,576,265 and U.S. Patent No. 5,554,389. Any method of producing a hydrogel can be used to produce the urinary bladder ECM hydrogels of use in the

methods disclosed herein. Additional methods are disclosed, for example, in U.S. Patent Nos. 4,902,508; 4,956,178; 5,281,422; 5,352,463; 5,372,821; 5,554,389; 5,573,784; 5,645,860; 5,771,969; 5,753,267; 5,762,966; 5,866,414; 6,099,567; 6,485,723; 6,576,265; 6,579,538; 6,696,270; 6,783,776; 6,793,939; 6,849,273; 6,852,339; 6,861,074; 6,887,495; 6,890,562; 5 6,890,563; 6,890,564; and 6,893,666 related to ECM. The ECM is derived from the urinary bladder. In certain embodiments, the urinary bladder ECM is isolated from a vertebrate animal, for example and without limitation, from a mammalian animal including, but not limited to, humans, monkeys, horses, pigs, cows and sheep. The mammal can be a veterinary animal. In specific non-limiting examples, the urinary bladder ECM is porcine or human. In some embodiments, the ECM 10 includes the basement membrane portion of the urinary bladder ECM. In certain embodiments, the urinary bladder ECM includes at least a portion of the basement membrane. In other embodiments, the ECM is harvested from a cell culture. Source tissue used for preparation of urinary bladder ECM can be harvested in a large variety of ways and once harvested, a variety of portion of the harvested tissue may be used. The urinary bladder ECM hydrogel can be a UBM or a UBS ECM 15 hydrogel, see FIGS. 2A and 2B.

As disclosed in U.S. Patent No. 8,361,503 (incorporated herein by reference), a urinary bladder ECM, such as porcine bladder ECM is prepared by abrading bladder tissue to remove the outer (abluminal) layers including both the tunica serosa, the tunica muscularis, and the tunica submucosa using a longitudinal wiping motion with a scalpel handle and moistened gauze. 20 Following eversion of the tissue segment, the luminal portion of the tunica mucosa is delaminated from the underlying tissue using the same wiping motion. After these tissues are removed, the resulting ECM consists of the tunica lamina propria and the overlying basement membrane. U.S. Patent No. 6,893,666, incorporated herein by reference, also discloses production of ECM from urinary bladder, skin, esophagus and small intestine.

25 In one embodiment, the ECM is isolated from harvested porcine urinary bladder to prepare urinary bladder matrix (UBM). Excess connective tissue and residual urine are removed from the urinary bladder. The tunica serosa, tunica muscularis externa, tunica submucosa and most of the muscularis mucosa can be removed by mechanical abrasion (see above) or by a combination of enzymatic treatment, hydration, and abrasion. Mechanical removal of these tissues can be 30 accomplished by abrasion using a longitudinal wiping motion to remove the outer layers (particularly the abluminal smooth muscle layers) and even the luminal portions of the tunica mucosa (epithelial layers). Mechanical removal of these tissues is accomplished by removal of mesenteric tissues with, for example, Adson-Brown forceps and Metzenbaum scissors and wiping

away the tunica muscularis and tunica submucosa using a longitudinal wiping motion with a scalpel handle or other rigid object wrapped in moistened gauze. The epithelial cells of the tunica mucosa can also be dissociated by soaking the tissue in a de-epithelializing solution, for example and without limitation, hypertonic saline. The resulting UBM comprises basement membrane of the tunica mucosa and the adjacent tunica propria, which is further treated with peracetic acid,  
5 lyophilized and powdered, see U.S. Patent No. 8,361,503.

In some embodiments, the epithelial cells can be delaminated by first soaking the tissue in a de-epithelializing solution such as hypertonic saline, for example and without limitation, 1.0 N saline, for periods of time ranging from 10 minutes to 4 hours. Exposure to hypertonic saline  
10 solution effectively removes the epithelial cells from the underlying basement membrane. The tissue remaining after the initial delamination procedure includes epithelial basement membrane and the tissue layers abluminal to the epithelial basement membrane. This tissue is next subjected to further treatment to remove the majority of abluminal tissues but not the epithelial basement membrane. The outer serosal, adventitial, smooth muscle tissues, tunica submucosa and most of  
15 the muscularis mucosa are removed from the remaining de-epithelialized tissue by mechanical abrasion or by a combination of enzymatic treatment, hydration, and abrasion.

Commercially available urinary bladder ECM preparations can also be used in the methods, devices and compositions described herein. Commercially available preparations include, but are not limited to MATRISTEM UBM™ (Acell Corporation; Jessup, Md.).

Urinary bladder ECM can be disinfected by any number of standard techniques, including,  
20 but not limited to, exposure to peracetic acid. Urinary bladder ECM can be sterilized by low dose gamma radiation, gas plasma sterilization, ethylene oxide treatment, supercritical CO<sub>2</sub>, or electron beam treatment. More typically, disinfection of ECM is obtained by soaking in 0.1% (v/v) peracetic acid, 4% (v/v) ethanol, and 95.9% (v/v) sterile water for two hours. The peracetic acid residue is  
25 removed by washing twice for 15 minutes with PBS (pH=7.4) and twice for 15 minutes with sterile water. ECM material can then be sterilized by propylene oxide or ethylene oxide treatment, gamma irradiation treatment (0.05 to 4 mRad), gas plasma sterilization, supercritical CO<sub>2</sub>, or electron beam treatment. The ECM can also be sterilized by treatment with glutaraldehyde, which causes cross  
30 linking of the protein material, but this treatment substantially alters the material such that it is slowly resorbed or not resorbed at all and incites a different type of host remodeling which more closely resembles scar tissue formation or encapsulation rather than constructive remodeling. Cross-linking of the protein material can also be induced with carbodiimide or dehydrothermal or photooxidation methods. As disclosed in U.S. Patent No. 8,361,503, ECM is disinfected by

immersion in 0.1% (v/v) peracetic acid (a), 4% (v/v) ethanol, and 96% (v/v) sterile water for 2 h. The ECM material is then washed twice for 15 min with PBS (pH=7.4) and twice for 15 min with deionized water.

Following isolation of the tissue of interest, decellularization is performed by various methods, for example and without limitation, exposure to hypertonic saline, peracetic acid, TRITON-X® or other detergents. Disinfection and decellularization can be simultaneous. For example, and without limitation, disinfection with peracetic acid, described above, also can serve to decellularize the ECM. Decellularized ECM can then be dried, either lyophilized (freeze-dried) or air dried. Dried ECM can be comminuted by methods including, but not limited to, tearing, milling, cutting, grinding, and shearing. The comminuted ECM can also be further processed into a powdered form by methods, for example and without limitation, such as grinding or milling in a frozen or freeze-dried state.

In order to prepare solubilized ECM tissue for use in preparing an ECM hydrogel, comminuted ECM is digested with an acid protease in an acidic solution to form a digest solution. The digest solution of ECM typically is kept at a constant stir for a certain amount of time at room temperature. The ECM digest can be used immediately or be stored at  $-20^{\circ}\text{C}$  or frozen at, for example and without limitation,  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .

Once the ECM is solubilized (typically substantially completely) the pH of the solution is raised to between 7.2 and 7.8, and according to one embodiment, to pH 7.4. Bases, such as bases containing hydroxyl ions, including NaOH, can be used to raise the pH of the solution. Likewise buffers, such as an isotonic buffer, including, without limitation, Phosphate Buffered Saline (PBS), can be used to bring the solution to a target pH, or to aid in maintaining the pH and ionic strength of the gel to target levels, such as physiological pH and ionic conditions. This forms a “pre-gel” solution. This “pre-gel” is in liquid form as a viscous solution at room temperature. The neutralized digest solution (pre-gel) can be gelled at temperatures approaching  $37^{\circ}\text{C}$ , wherein the temperature approaches physiological temperature. The method typically does not include a dialysis step prior to gelation, yielding a more-complete ECM-like matrix that typically gels at  $37^{\circ}\text{C}$  at specific rates (see below).

Thus, the ECM is derived from urinary bladder. The urinary bladder ECM can be produced from one subject, or from more than one subject, such as 2, 3, or 4 subjects, or from a urinary bladder cell line. The ECM can be commercially available urinary bladder ECM. In one non-limiting embodiment, the ECM is lyophilized and comminuted. The ECM is then solubilized with an acid protease in an acidic solution to produce digested ECM. The acid protease may be, without

limitation, pepsin or trypsin, or a combination thereof. The ECM can then be solubilized at an acid pH suitable or optimal for the protease, such as greater than about pH 2, or between pH and 4, for example in a 0.01M HCl solution. The solution typically is solubilized for about 12 to about 48 hours, depending upon the tissue type (e.g., see examples below), with mixing (stirring, agitation, admixing, blending, rotating, tilting, etc.). ECM hydrogel is prepared by (i) comminuting an extracellular matrix, (ii) solubilizing intact, non-dialyzed or non-cross-linked extracellular matrix by digestion with an acid protease in an acidic solution to produce a digest solution, (iii) raising the pH of the digest solution to a pH between 7.2 and 7.8 to produce a neutralized digest solution (pre-gel solution), and (iv) gelling the solution at a temperature of approximately 37°C within the esophagus of a subject of interest. When an acid protease is used to digest the ECM, the pre-gel solution and the resulting hydrogel may contain inactivated protease. Any of these urinary bladder ECM hydrogels are of use in the methods disclosed herein.

The urinary bladder ECM hydrogel, when exposed to temperatures of about 37°C, forms the gel. The urinary bladder ECM hydrogel in the “pre-gel” form can be frozen and stored at, for example and without limitation, -20°C or -80°C. The urinary bladder ECM hydrogel in the “pre-gel” form can be stored at room temperature, such about 25°C. Thus, the urinary bladder ECM hydrogel is in the pre-gel form at below 37°C, such as at 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4°C. The urinary bladder ECM hydrogel can be frozen for storage, and thus, can be stored at below 0°C. As used herein, the term “pre-gel form” or “pre-gel” refers to the urinary bladder ECM hydrogel wherein the pH is increased, but has not gelled. For example and without limitation, a urinary bladder ECM hydrogel in the pre-gel form has a pH between 7.2 and 7.8. The urinary bladder ECM hydrogel can be delivered in a pre-gel form to a subject with esophageal inflammation, such as orally, via a catheter, or endoscopically.

The urinary bladder ECM hydrogel in the pre-gel form is amenable to introduction into the esophagus of a patient. Once introduced submucosally into the esophagus, which is approximately 37°C, the urinary bladder ECM hydrogel gels and creates a cushion of ECM hydrogel between the muscularis propria and the submucosa of the esophagus, lifting the submucosa for surgical resection.

In some embodiments, the urinary bladder ECM hydrogel has the following characteristics:

- a time to 50% gelation of less than 20 minutes at a temperature of about 37°C;
- a flow viscosity suitable for infusion into the esophagus; and
- a stiffness of
  - about 10 to about 400 Pascal (Pa),
  - about 10 to about 450 Pa;
  - about 10 to about 600 Pa,
  - about 5 to about 1,000 Pa,
  - about 10 to 1,000 Pa, or
  - about 10 to about 70 Pa.

In embodiments, the urinary bladder ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 30 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 400 Pascal (Pa). In other embodiments, the ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 450 Pascal (Pa). In other embodiments, the urinary bladder ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 600 Pascal (Pa).

In other embodiments, the urinary bladder ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 5 to about 1,000 Pascal (Pa). In other embodiments, the urinary bladder ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 1,000 Pascal (Pa). In more embodiments, the urinary bladder ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of 10-70 Pascal (Pa).

In additional specific non-limiting examples, the urinary bladder ECM hydrogel is produced by (a) solubilizing acellular extracellular matrix by digestion of urinary bladder with an acid protease in an acidic solution to produce digested ECM; (b) raising the pH of the digested ECM to a pH between 7.2 and 7.8 to produce a neutralized digest solution; (c) diluting the digested ECM to a concentration of about 2 mg/ml to about 16 mg/ml, such as about 8 mg/ml to about 12 mg/ml of the urinary bladder ECM hydrogel. This urinary bladder ECM hydrogel is then introduced into the esophagus of the subject, where it gels.

The urinary bladder ECM hydrogel of use in the methods disclosed herein have a time to 50% gelation of less than 20 minutes at a temperature of about 37°C, such as less than 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 minutes. In some embodiments, the urinary bladder ECM hydrogel has a time to 50% gelation of less than 10 minutes at a temperature of about 37°C. In other embodiments, the time to 50% gelation is about 2 to about 20 minutes at a temperature of about 37°C. In further embodiments, the time to 50% gelation is about 2 to about 10 minutes at a temperature of about 37°C. In yet other embodiments the time to 50% gelation is about 3 to about 8 minutes at a temperature of about 37°C.

The disclosed urinary bladder ECM hydrogel can have a flow viscosity suitable for infusion/injection into the esophageal submucosal space. In some embodiments, the urinary bladder ECM hydrogels has a flow viscosity of about 0.1 to about 100 Pa\*s at a shear rate of 0.1/s, such as about 0.2, 1, 10, 20, 30, 40, 50, 60, 70, 80, or 90 Pa\*s at a sheer rate of 0.1/s. In further  
5      embodiments, the urinary bladder ECM hydrogel hydrogel has a flow viscosity of about 1 to about 40 Pa\*s at a shear rate of 0.1/s, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 Pa\*s at a shear rate of 0.1/s.

In other embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 0.01 to about 0.20 Pa\*s at a shear rate of 1000/s, or of about 0.01 to about 0.10 Pa\*s at a shear rate of  
10     1000/s, such as about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.19 or 0.2 at a shear rate of 1000/s.

In more embodiment, the urinary bladder ECM hydrogel has about 0.02 to about 0.8 Pa\*s at a shear rate of 100/s, or of about 0.1 to about 0.8 Pa\*s at a shear rate of 100/s, such as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, or 0.8 Pa\*s.

In further embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 10  
15     to about 100 Pa\*s at a shear rate of 0.2/s and a flow viscosity of about 0.01 to about 0.10 Pa\*s at a shear rate of 1000/s. In more embodiments, the ECM hydrogel has a flow viscosity of 1 to 40 Pa\*s at a shear rate of 0.1/s and 0.01 to 0.2 Pa\*s at a shear rate of 1000/s.

In other embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 0.1  
20     to about 25 Pa\*s, such as 1 to about 25 Pa\*s, or 1 to about 20 Pa\*s, or 1 to about 10 Pa\*s at a shear rate of 1/s, for example, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25 Pa\*s at a shear rate of 1/s. The shear rate can be, for example, 5, 10, 15 or 20 Pa\*s at a shear rate of 1/s. In other embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 0.025 to about 0.6 at a shear rate of 100/s, such as about 0.025, 0.030, 0.035,  
25     0.040, 0.045, 0.050, 0.055, or 0.060 at a shear rate of 100/s. The flow viscosity is about 0.1 to about 25 Pa\*s at a shear rate of 1/s, and is about 0.02 to about 0.8 Pa\*s at a shear rate of 100/s. In additional embodiments, the flow viscosity is about 1 to about 10 Pa\*s at a shear rate of 1/s, and is about 0.1 to about 0.3 at a shear rate of 100/s.

In further embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 10  
30     to about 100 Pa\*s at a shear rate of 0.2/s. In other embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 0.01 to about 0.10 Pa\*s at a shear rate of 1000/s. In other embodiments, the urinary bladder ECM hydrogel has a flow viscosity of about 1 to about 40 Pa\*s at a shear rate of 0.1/s and is 0.01 to 0.2 Pa\*s at a shear rate of 1000/s.

The disclosed urinary bladder ECM hydrogels have a stiffness i) about 10 to about 400 Pascal (Pa), ii) about 10 to about 600 Pa, iii) about 5 to about 1,000 Pa, iv) about 10 to 1,000 Pa, or v) about 10 to about 70 Pa. The urinary bladder ECM hydrogel can have a stiffness of about 10 to about 400 Pascal (Pa), such as about 10 to about 70 Pa, about 10 to about 100 Pascal (Pa), or about 10 to about 150 Pa, about 10 to about 200 Pa, or about 10 to about 250 Pa. In some embodiments, the disclosed urinary bladder ECM hydrogels have a stiffness of about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 Pa. In other embodiments, the disclosed urinary bladder ECM hydrogels have a stiffness of about 10 to about 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, or 400 Pa. In further embodiments, the disclosed urinary bladder ECM hydrogel can have a stiffness of about 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400Pa.

In some embodiments, the urinary bladder ECM concentration in the hydrogel is about 2 mg/ml to about 20 mg/ml, such as about 8 mg/ml to about 12 mg/ml or about 2 mg/ml to about 16 mg/ml. In other embodiments, the urinary bladder ECM concentration in the hydrogel is about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 mg/ml. Exemplary concentrations of use include, but are not limited to, about 9 mg/ml to about 11 mg/ml, and about 10 mg/ml to about 12 mg/ml. Additional exemplary concentrations include about 8 mg/ml to about 10 mg/ml, about 8 mg/ml to about 11 mg/ml, about 8 mg/ml to about 13 mg/ml, about 8 mg/ml to about 14 mg/ml, about 8 mg/ml to about 15 mg/ml, and about 8 mg/ml to about 16 mg/ml. Further exemplary concentrations of use also include about 6 mg/ml to about 12 mg/ml, about 13 mg/ml, about 14 mg/ml, about 15 mg/ml or about 16 mg/ml.

### *Methods of Treatment*

Methods are disclosed herein for dissecting a mucosa and a submucosa from a muscularis propria in the esophagus of a subject, these methods include injecting submucosally into the esophagus a pharmaceutical composition comprising a urinary bladder extracellular matrix (ECM) hydrogel, such as a UBS or a UBM ECM hydrogel, to form a cushion between the submucosa and the underlying muscularis propria at the region of the organ. The method can be an EMR or an EMD.

EMR is an endoscopic technique developed for removal of sessile or flat neoplasms confined to the superficial layers (mucosa and submucosa) of the GI tract. EMR is typically used for removal of lesions smaller than 2 cm or piecemeal removal of larger lesions. EMR also plays an

important role in the assessment of resected specimens for accurate pathological staging. In contrast to polypectomy, EMR involves the lifting up of a lesion from the muscular layer by injecting a fluid agent, commonly normal saline (NS) solution, into the submucosal layer. EMR is also useful for obtaining specimens for accurate histopathological staging to determine the risk of lymph-node metastasis. EMR facilitates the complete removal of the affected mucosa by excising through the middle or deeper portion of the gut wall submucosa. Various EMR techniques have been described and four methods involving snare resection are commonly used: (1) the inject and cut method; (2) the inject, lift, and cut method; (3) cap-assisted EMR (EMRC); and (4) EMR with ligation (EMRL). In the inject and cut technique, the diseased mucosa is lifted up from the muscular layer by creating a submucosal fluid cushion, captured, strangulated using an electrosurgical snare, and then resected. However, injection into the thin submucosal layer is a delicate process, the injected solution tends to dissipate quickly, flat and depressed lesions are hard to capture with the snare compared with protruded lesions, and large or awkwardly located lesions can be difficult to remove (Uraoka et al., *Drug Design, Development and Therapy* 2008:2 131-138). Injection-assisted EMR is frequently used for large flat colon polyps.

Endoscopic submucosal dissection (ESD) was specifically developed for removing larger lesions. Lesions are dissected directly along the submucosal layer using an electrosurgical knife, resulting in an en-bloc resection of even large lesions. ESD has been predicted to replace conventional surgery in treating certain cancerous stages, but since it has a higher rate of perforation and bleeding complications than conventional EMR, a greater degree of endoscopic skill and experience is required than for EMR. ESD can use numerous electrosurgical knives, such as an insulation-tipped diathermic knife, a needle knife, a hook knife, a flex knife, a triangle tipped knife, a flush knife, splash needle, and a small-caliber tip transparent hood. These knives can be used with a high frequency electrosurgical current (HFEC) generator. ESD is characterized by three steps: (1) injecting a fluid to form a submucosal cushion to elevate the lesion from the muscle layer; (2) circumferential cutting of the surrounding mucosa of the lesion; and (3) dissection of the connective tissue of the submucosa beneath the lesion (see Kakushima et al., *World J. Gastroenterol.* 14(9): 2962-2967, 2008, incorporated herein by reference. Various submucosal injection solutions had previously been developed and shown to be satisfactory for use during EMR, but introduction of the lengthier ESD procedure required a longer-lasting solution to help identifying the cutting line during dissection of the submucosal layer (Uraoka et al., *Drug Design, Development and Therapy* 2008:2 131-138). The presently disclosed methods meet this need.

A submucosal injection is used in EMR, as injection of fluid into the submucosa cushions

facilitates the isolation of the tissue to be removed just before capture of the target lesion, such as with a snare, thereby reducing thermal injury and the risk of perforation and hemorrhage while also facilitating resection. Submucosal injection plays an important role in the EMR procedure, as the solution must be retained in place for sufficient duration and needs to form a hemispheric shape to facilitate snaring. In addition, providing a sufficiently high submucosal elevation results in safe submucosal cutting during the ESD procedure (Uraoka et al., *Drug Design, Development and Therapy* 2008:2 131-138). Furthermore, as inflammation results from the procedure, any cushion retained at the procedure site should have anti-inflammatory properties. The urinary bladder ECM hydrogel will mitigate stricture and promote re-epithelialization. The presently disclosed methods also meets this need.

In some embodiments, the disclosed methods utilize an ECM hydrogel that has anti-inflammatory properties, and is inexpensive, non-toxic, easy to inject and provides a high, long-lasting submucosal cushion. The ECM hydrogel is administered in the pre-gel form, and then gels at the site of injection to form a cushion. The cushion can be dissected during the procedure so that some hydrogel remains on the underlying muscularis propria, thereby aiding healing. The disclosed ECM hydrogels facilitate closure of the wound created by removal of the resected mucosa/submucosa. In some embodiments, the procedure is an ESD. In other embodiments, the procedure is an EMR.

Normal saline solution (NS) and thinner solutions (*e.g.*, ELEVIEW™, see U.S. Patent No. 9,226,996, incorporated herein by reference) have been used as submucosal cushions for endoscopic resection, but the inherent characteristics of these solutions make it difficult to produce the proper submucosal fluid cushion, maintain the desired height, and retain the cushion at the desired location, because of the rapid dispersion of the solution. Furthermore, in ESD, once the mucosa/submucosa are removed, these agents will not be retained on the underlying muscularis propria. Furthermore, these agents do not aid the healing process, such as by reducing inflammation. The use of a urinary bladder ECM hydrogel meets these needs.

The urinary bladder ECM hydrogels disclosed herein can be used as in any ESD or ESR. As disclosed in U.S. Patent No. 9,364,580, incorporated herein by reference, endoscopic injection needles are devices which can be long (up to about 230) cm and which include a relatively long catheter within which an inner injection tube having a distal injection needle is slideably disposed. A proximal actuating handle is coupled to the catheter and the injection tube for moving one relative to the other when necessary. Fluid access to the injection tube is typically provided via a luer connector on the handle. Endoscopic injection needle devices are typically delivered to the

injection site through the working channel of the endoscope. In order to protect the lumen of the endoscope working channel from damage, the handle of the infusion needle device is manipulated to withdraw the distal injection needle into the lumen of the catheter before inserting the device into the endoscope. This prevents exposure of the sharp point of the injection needle as the device is moved through the lumen of the endoscope. When the distal end of the endoscopic injection needle device is located at the injection site, its handle is again manipulated to move the injection needle distally out of the lumen of the catheter. When advanced to the most distal position, the exposed portion of the injection needle is approximately 4-6 mm in length.

After the injection site has been pierced, the ECM in pre-gel form, usually contained in a 5 mL to 10 mL syringe provided with a luer-lock fitting connected to the handle of the injection needle, can be delivered through the injection tube and the needle into the injection site, such as between the submucosa and the underlying muscularis propria.

The injection needle and other accessories commonly used during endoscopic procedures, such as snares for polypectomy, clipping devices, biopsy forceps and similar, are passed through one or more specific channels of the endoscope, usually called working channels or operating channels. Depending upon the type of endoscope used the inner diameter of the working channel may vary. However, the most common endoscopes used in GI endoscopy have working channels with inner diameter in the range from about 2 mm to about 5 mm. Generally, the manufacturers of endoscopic accessories produce accessories having outer diameters which allow them to fit all the working channels. In some embodiments, the endoscopic injection needles, the outer diameter of catheter ranges from 1.9 mm to 2.3 mm, such as about 1.9, 2.0, 2.1, 2.2 or 2.3 cm. Thus, considering that the inner injection tube is contained in the outer catheter, its internal diameter is usually 1 mm or less. The disclosed urinary bladder ECM hydrogels, in the pre-gel form, can readily pass through these catheters.

The urinary bladder ECM hydrogel, in pre-gel form, can be used in an endoscopic resection procedure by sucking a volume of emulsion from its primary container by means of a syringe, injecting a suitable volume of said emulsion by means of an endoscopic injection needle inserted in the working channel of the endoscope immediately under the superficial mucosal layer, to deposit a liquid volume into the submucosal layer that becomes a cushion when in place: the elevation of the mucosal surface allow the endoscopist to perform an easy resection of the mucosal lesion found during the execution of the endoscopic procedure even if the lesion is flat and thus not protruding into a lumen, such as an esophageal lumen.

The presence of at least one dye into the cushion can aid an endoscopist to visualize the

structures beneath the mucosa (e.g. the submucosal layer and the external muscular wall), thereby lowering the risk that the endoscopist, performing the resection procedure, may cause damages to said structures. The use of the dye can allow visualization of the cushion cavity and the mucosal basement. The removal of the lesion from the mucosal surface generates a mucosal wound. The persistence of the cushion generated by the injected volume of the pharmaceutical composition allows the endoscopic resection procedure to be performed without the need to re-inject. The urinary bladder ECM hydrogel in pre-gel form is injected submucosally into the organ of the subject, such as at the region of a lesion or tumor, to form a cushion between the submucosa and the underlying muscularis propria at the region of the organ. The cushion can be dissected, such that a portion of the urinary bladder ECM hydrogel is maintained on the underlying muscularis propria and aid in the healing process.

The disclosed methods are of use in the esophagus. In a non-limiting example, the organ is the esophagus, and the method comprises a method of dissecting an esophageal carcinoma or adenocarcinoma from the esophagus. In another non-limiting example, the method comprises dissecting the mucosa and the submucosa from the esophagus of a subject who has Barrett's esophagus.

A urinary bladder ECM hydrogel, as disclosed herein, is maintained at a temperature at or below which it gels, such as at or below room temperature (e.g., about 25°C). The urinary bladder ECM hydrogel can be maintained, for example, at 25°C or 4°C prior to administration. An effective amount of the urinary bladder ECM hydrogel, in the pre-gel form, is then administered to the esophagus of the subject. The urinary bladder ECM hydrogel can be provided in in a lyophilized or frozen form, and reconstituted just prior to administration to the subject.

The disclosed methods are of use in any subject, including human and veterinary subjects. The subject can be any age. In one embodiment, a composition including a urinary bladder ECM hydrogel, in pre-gel form, is injected in a target tissue of a human to form a cushion which is then optionally subjected to an endoscopic surgical procedure, such as a resection procedure. The urinary bladder ECM can be from the same species as the subject being treated, or can be from a different species. In some embodiments, the subject is human, and the urinary bladder ECM hydrogel is derived from human and/or porcine urinary bladder. In other embodiments, the urinary bladder ECM hydrogel is derived from a non-human primates, dog, cat, horse, or cow. The urinary bladder ECM can also be from a commercial source.

The disclosed methods are invasive, as they require an injection that dissects a mucosa and a submucosa from a muscularis propria from a region of an organ of an intestinal tract of a subject.

Any of the methods disclosed herein can include injecting submucosally into the esophagus of the subject a pharmaceutical composition including a urinary bladder ECM hydrogel to form a cushion between the submucosa and the underlying muscularis propria at the region of the organ. The urinary bladder ECM hydrogel has the following characteristics: a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C; b) a flow viscosity suitable for infusion into the esophagus; and c) a stiffness of about 10 to about 400 Pascal (Pa). The urinary bladder ECM hydrogel gels and dissects the mucosa and the submucosa from the underlying muscularis propria and inhibits inflammation in the region of the organ in the subject. The urinary bladder ECM hydrogel, in pre-gel form, can be administered endoscopically or via a catheter.

In some embodiments, the resection procedure is an endoscopic mucosal resection or an esophageal endoscopic submucosal dissection, and the method comprises a method of dissecting an esophageal carcinoma or adenocarcinoma from the esophagus. In more embodiments, the method includes dissecting the mucosa and the submucosa from the esophagus of a patient who has dysplasia. In more embodiments, the method includes dissecting the mucosa and the submucosa from the esophagus of a subject who has Barrett's esophagus.

The methods can also include performing an endoscopic resection procedure on the cushion. In some embodiments, the methods include dividing the cushion such that hydrogel is retained on the underlying muscularis propria of the organ and the mucosa and the submucosa are removed from the region of the organ. In some non-limiting examples, the portion of the hydrogel cushion that is retained on the underlying muscularis propria downregulates pro-inflammatory macrophage activation in this tissue.

In some embodiments, the time to 50% gelation of the hydrogel is than 20 minutes at a temperature of about 37°C. In some specific non-limiting example, the time to 50% gelation is about 2 to about 20 minutes at about 37°C. In other specific non-limiting examples, the time to 50% gelation is about 2 to about 10 minutes at about 37°C. In further non-limiting examples, the time to 50% gelation is about 3 to about 8 minutes.

In additional embodiments, the flow viscosity in pre-gel form is sufficient for injection into the esophagus. In some embodiments, the flow viscosity of the urinary bladder ECM hydrogel is about 0.1 to about 100 Pa\*s at a shear rate of about 0.1/s and is about 0.01 to about 0.2 Pa\*s at a shear rate of 1000/s. In some non-limiting examples, the flow viscosity is about 0.1 to about 25 Pa\*s at a shear rate of 1/s, and is about 0.02 to about 0.8 Pa\*s at a shear rate of about 100/s.

In further embodiments, the urinary bladder ECM hydrogel has stiffness when introduced into the tissue of about 10 to about 400 Pascal (Pa); wherein the ECM hydrogel has a stiffness of

10-70 Pa. In further embodiments, the ECM concentration in the hydrogel is 2 mg/ml to about 16 mg/ml.

The urinary bladder ECM hydrogel can be produced by any method disclosed herein. In some embodiments, the ECM hydrogel is produced by (a) solubilizing decellularized extracellular matrix (ECM) by digestion of tissue with an acid protease in an acidic solution to produce digested ECM; and (b) raising the pH of the digested esophageal ECM to a pH between 7.2 and 7.8 to produce a neutralized digest solution. In further embodiments, step (b) raising the pH of the digested ECM includes adding a base or an isotonic buffer to raise the pH of the digested ECM. In further embodiments, an acid protease is used, such as pepsin, trypsin or a combination thereof.

In some embodiments, the urinary bladder ECM hydrogel is maintained at or below 25°C prior to administration to the subject. In some embodiments, the ECM hydrogel is maintained at about 4°C to about 28°C, such as about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28°C. The ECM hydrogel can be maintained at about 4°C, and used at about 4°C to about 25°C, or warmed to just about 25°C, just prior to use. In some embodiments, controlling temperature ensure that the ECM hydrogel is maintained in its pre-gel form, and thus is suitable for injection between the submucosa and the underlying muscularis propria. In further embodiments, the hydrogel gels upon administration to the subject, such as when reaching a temperature of, for example, 37°C.

## EXAMPLES

ELEVIEW™ and urinary bladder ECM hydrogels are different biomaterials. ELEVIEW™ (Aries Pharmaceuticals, Inc, Dublin, Ireland) is a commercially available low viscosity emulsion of Poloxamer 188 clinically used to provide submucosal lift for EMR and ESD procedures. ELEVIEW™ does not form a stably formed hydrogel at 37°C, but rather remains a liquid over an hour at 37°C. In contrast, urinary bladder ECM (prepared according to the method disclosed herein, i.e., by protease digestion of ECM) forms a hydrogel that is stably formed at 37°C (body temperature). ELEVIEW™ and urinary bladder ECM hydrogels, including UBM and UBS ECM hydrogels, can be injected underneath the mucosa. An ideal biomaterial would adhere to both layers. Urinary bladder ECM hydrogels have strong mucoadhesive properties, and beneficial biological properties. A urinary bladder ECM hydrogel can be effectively used as a submucosal cushion *in vivo*, and thus can be used to dissect the submucosa from the underlying muscularis propria.

## Example 1

### Materials and Methods

*Preparation of urinary bladder ECM:* Surfaces were sterilized with 70% ethanol and 25% Pyroclean. Porcine bladders (Animal Biotech Industries) stored at -20°C were thawed and washed thoroughly with Type I water. Bladders were split from apex to the dome and laid flat with the luminal side facing down. A beveled acrylic scraper and forceps were used to mechanically remove the smooth muscle tissue on the abluminal side (tunica serosa, tunica muscularis externa, tunica submucosa and tunica muscularis mucosa) leaving behind the tunica (lamina) propria and basement membrane (the tissue layers comprising UBM). The UBM was stored in Type I water at 4°C overnight and decellularized using 0.1% peracetic acid (PAA) in 4% ethanol for 4h, and 15 min washes of PBS, Type 1 water, PBS, and Type 1 water to create UBM. All decellularization steps were performed at room temperature agitated on a shaker plate at 300 rpm.

*Urinary bladder ECM hydrogel formation:* UBM was lyophilized and powdered using a Wiley mill, and 10 mg/mL of powdered ECM was digested by pepsin (1 mg/mL) in 0.01 M HCl for 48 hours at room temperature. ECM digests and pepsin control were stored at -20°C until use. On the day of the experiment, ECM digests and pepsin control were neutralized with 0.1 M NaOH (1/10 volume of pre-gel solution), 10x PBS at pH 7.4 (1/9 volume of the pre-gel solution) at 4°C and diluted to 5 mg/mL ECM concentration using ice-cold 1x PBS at 4°C. See Freytes et al.. Biomaterials **29**(11): p. 1630-7. 2008, incorporated herein by reference.

*Rheology:* The viscoelastic properties of urinary bladder ECM were determined with a temperature-controlled, 40 mm parallel plate rheometer (AR2000). The samples were kept at 4°C and loaded onto the rheometer with a parallel plate geometry pre-cooled to 10 °C. Mineral oil was used to seal the sample-plate interface and to minimize evaporation during the testing. A series of rheological tests were conducted for each sample in sequence. A steady state flow curve at 10°C was performed to determine the viscosity profile of the samples at a range of shear rates (0.1-1000 s<sup>-1</sup>). Plate temperature was rapidly raised from 10°C to 37°C, and an oscillatory time sweep was performed at 37°C, by applying a small, 0.5% oscillatory strain at a frequency of 1 rad/s to measure the maximum storage modulus (G'), maximum loss modulus (G'') and gelation kinetics. Data was extracted and analyzed in Prism (Version 6, GraphPad) for statistical analysis (n=3).

*In-vivo use of ECM as submucosal fluid cushion for EMR:* Anesthesia was induced with Acepromazine (0.01 mg/kg, SC) and ketamine (5-11 mg/kg), and surgical plane anesthesia maintained with 1-5% Isoflurane via endotracheal tube. Throughout the procedure and immediate post-operative period, animals were administered 2 ml/kg/h of lactated Ringer's solution I.V.

5 Temperature was controlled through warm water recirculating heating pads placed under the animal. Physiologic parameters such as heart, respiration rate, body temperature, and responsiveness were monitored during the procedure. Antibiotic prophylaxis with 25mg/kg of Cefazolin was administered before starting the procedure.

The animal was placed in supine position with and a Pentax EG3430K endoscope was used to evaluate the esophagus. Distance from the mouth to the GE junction was measured. After identifying reference points in the esophagus the mucosa and submucosa at the site of excision were separated by with injection blue-dyed Urinary Bladder Matrix - hydrogel at 8mg/ml using a Olympus Injectorforce 4mm 23G needle. at 4°C. This temperature was maintained at all times to prevent gelation and potential plugging of the needle. Approximately 2-5 ml of blue gel were injected per site. The full circumference of the mucosa (100%) for a length of 5 cm was removed using band-ligation EMR technique. For EMR a Cook Duette Kit with a ligation band was used. The mucosa was then excised with the use of a snare.

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### Example 2

#### **In-vivo use of a urinary bladder ECM hydrogel as submucosal fluid cushion for EMR**

The urinary bladder ECM-hydrogel was deliverable through a long endoscopic needle without any resistance. Elevation of the mucosa was successfully achieved and maintained to facilitate the EMR procedure and the blue dye was visible indicating the places where the dissection had been created for removal (**FIG. 1A**). Tissue was easily removed with the use of the snare.

25 Upon macroscopic observation, the removed esophageal mucosal tissue included part of the gel (**FIG. 1B**). The blue dye in the hydrogel appeared to diffuse across the circumference of the esophagus after removing the mucosa and a full-circumferential was achieved with the use of the hydrogel (**FIG. 1C**).

### Example 3

#### **Rheology Data**

Viscoelastic properties of UBM are shown in FIG. 3. A steady state flow curve similarly performed as described for UBM (FIG. 3A) show a concentration-dependent increase in viscosity with

increasing ECM concentration and shear-thinning profiles of the UBM hydrogel i.e., viscosity decreases with increasing shear rate for each ECM concentration.

UBM ECM (FIG. 3B) showed increasing storage modulus (stiffness) with increasing ECM. UBM showed a storage modulus ( $G'$ ) that is ~ an order of magnitude greater than the loss modulus ( $G''$ ) at each ECM concentration, following the definition of a stably formed ECM hydrogel (Freytes et al., *Biomaterials*, 2008. 29(11): p. 1630-7). UBM showed concentration-independent gelation times, i.e. gelation time remained constant for all ECM concentrations (FIG. 3C).

In view of the many possible embodiments to which the principles of our invention may be applied, it should be recognized that illustrated embodiments are only examples of the invention and should not be considered a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method for dissecting a mucosa and a submucosa from a muscularis propria from a region of an esophagus of a subject, comprising:

5 injecting submucosally into the region of the esophagus of the subject a pharmaceutical composition comprising a urinary bladder extracellular matrix (ECM) hydrogel to form a cushion between the submucosa and the underlying muscularis propria in the esophagus, wherein the ECM hydrogel has the following characteristics:

a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C;

b) a flow viscosity suitable for infusion into the esophagus; and

10 c) a stiffness of about 10 to about 400 Pascal (Pa);

thereby dissecting the mucosa and the submucosa from the underlying muscularis propria at the region of the esophagus and inhibiting inflammation in the region of the esophagus in the subject.

15 2. The method of claim 1, wherein the time to 50% gelation is about 2 to about 20 minutes at about 37°C.

3. The method of claim 1, wherein the time to 50% gelation is about 2 to about 10 minutes at about 37°C.

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4. The method of claim 2, wherein the time to 50% gelation is about 3 to about 8 minutes.

5. The method of any one of claims 1-4, wherein the flow viscosity is about 0.1 to about 100 Pa\*s at a shear rate of about 0.1/s and is about 0.01 to about 0.2 Pa\*s at a shear rate of 1000/s.

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6. The method of any one of claims 1-4, wherein the flow viscosity is about 0.1 to about 25 Pa\*s at a shear rate of 1/s, and is about 0.02 to about 0.8 Pa\*s at a shear rate of about 100/s.

7. The method of any one of claims 1-6, wherein the ECM hydrogel has a stiffness of 10-30 300 Pa.

8. The method of any one of claims 1-7, wherein the ECM concentration in the hydrogel is 2 mg/ml to about 16 mg/ml.

9. The method of any one of claims 1-8, wherein the ECM hydrogel is administered endoscopically or via a catheter.

10. The method of any one of claims 1-9, wherein the ECM hydrogel is produced by

5 (a) solubilizing decellularized extracellular matrix (ECM) by digestion of tissue with an acid protease in an acidic solution to produce digested ECM; and

(b) raising the pH of the digested ECM to a pH between 7.2 and 7.8 to produce a neutralized digest solution.

10 11. The method of claim 10, wherein (b) raising the pH of the digested ECM comprises adding a base or an isotonic buffer to raise the pH of the digested ECM.

12. The method of claim 10 or claim 11, wherein the acid protease is pepsin, trypsin or a combination thereof.

15 13. The method of any one of claims 1-12, wherein the ECM hydrogel is maintained at or below 25 ° C prior to administration to the subject.

20 14. The method of any one of claims 1-13, wherein the ECM hydrogel is injected endoscopically or via a catheter.

25 16. The method of any one of claims 1-14, wherein the organ is the esophagus, and the method comprises a method of dissecting an esophageal dysplasia, adenocarcinoma or carcinoma from the esophagus.

17. The method of claim 16, wherein the method comprises dissecting the mucosa and the submucosa from the esophagus of a subject who has Barrett's esophagus.

30 18. The method of any one of claim 1-17, further comprising performing an endoscopic resection procedure on the cushion.

19. The method of claim 18, wherein the resection procedure is an endoscopic mucosal resection or an endoscopic submucosal dissection to remove the dissected mucosa and submucosa.

20. The method of claim 19, wherein the method comprises:  
dividing the cushion such that hydrogel is retained on the underlying muscularis propria of  
the esophagus and the mucosa and the submucosa are removed from the region of the esophagus.

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21. The method of any one of claim 1-20, wherein the subject is human.

22. The method of any one of claims 1-21, wherein the method of dissecting comprises  
endoscopic mucosal resection or endoscopic mucosal dissection.

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23. The method of any one of claims 1-22, wherein the urinary bladder ECM hydrogel is a  
urinary bladder matrix (UBM) ECM hydrogel.

24. The method of any one of claims 1-22, wherein the urinary bladder ECM hydrogel is a  
urinary bladder submucosa (UBS) ECM hydrogel.

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25. A composition comprising a urinary bladder ECM hydrogel, wherein the ECM hydrogel  
has the following characteristics:

a) a time to 50% gelation of less than 20 minutes at a temperature of about 37°C;

20 b) a flow viscosity suitable for infusion into the esophagus; and

c) a stiffness of about 10 to about 400 Pascal (Pa);

for use in the method of any one of claims 1-25.

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FIG. 1A



FIG. 1B

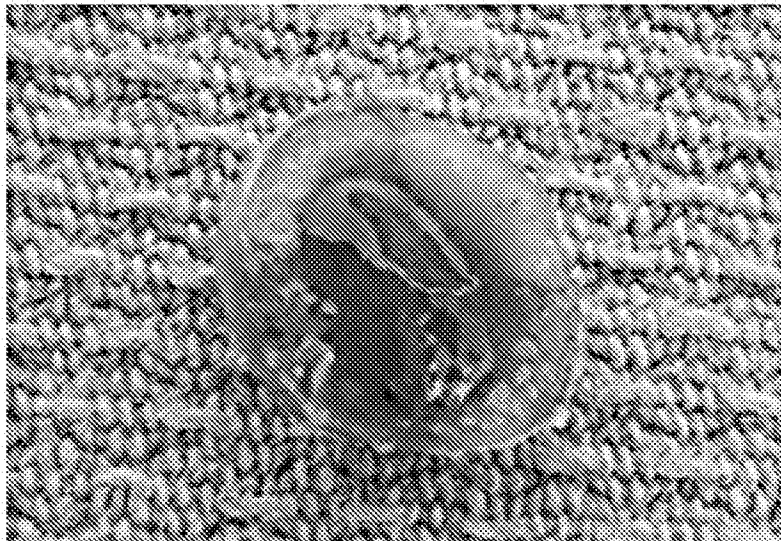


FIG. 1C



FIG. 2A

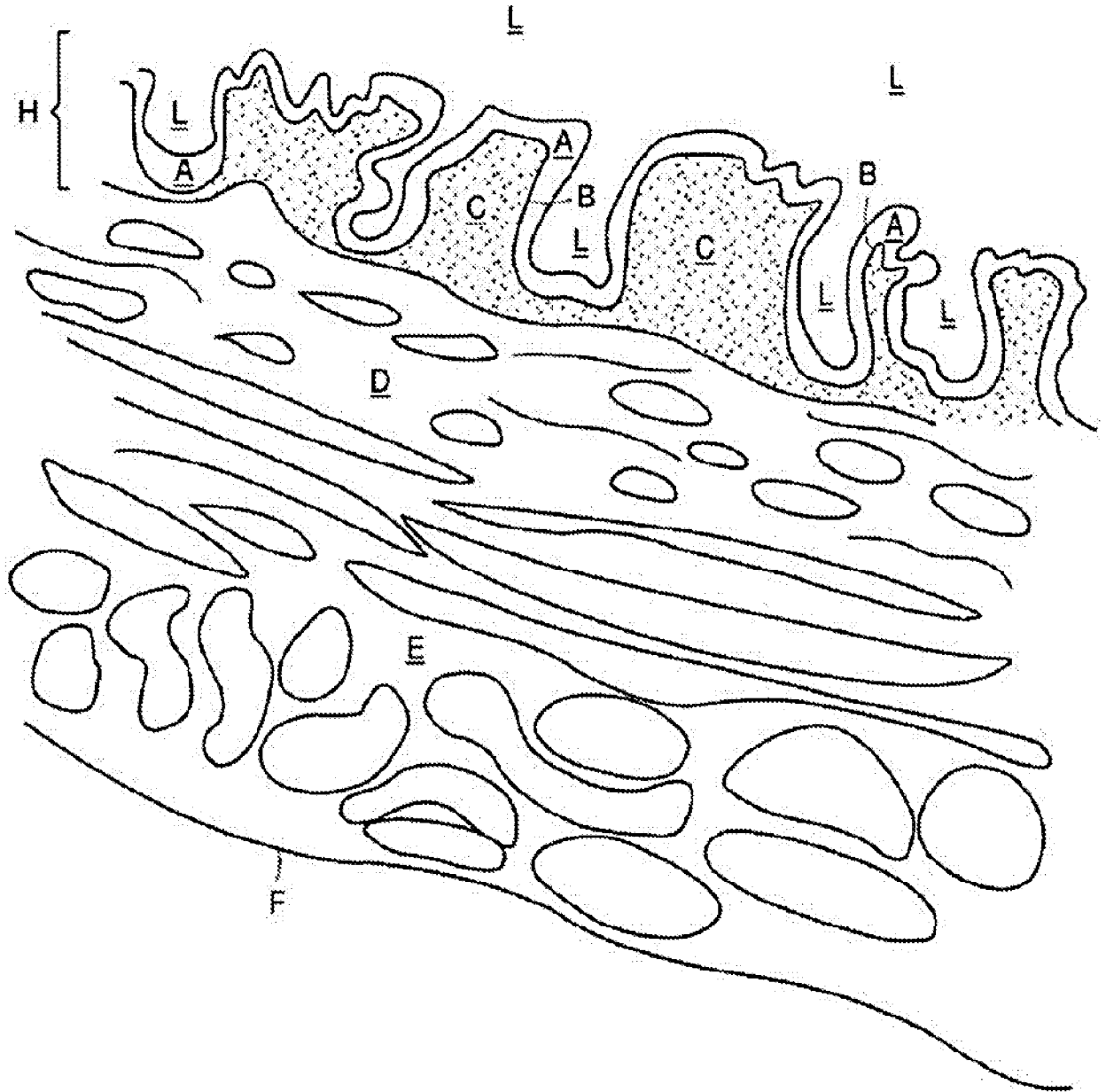
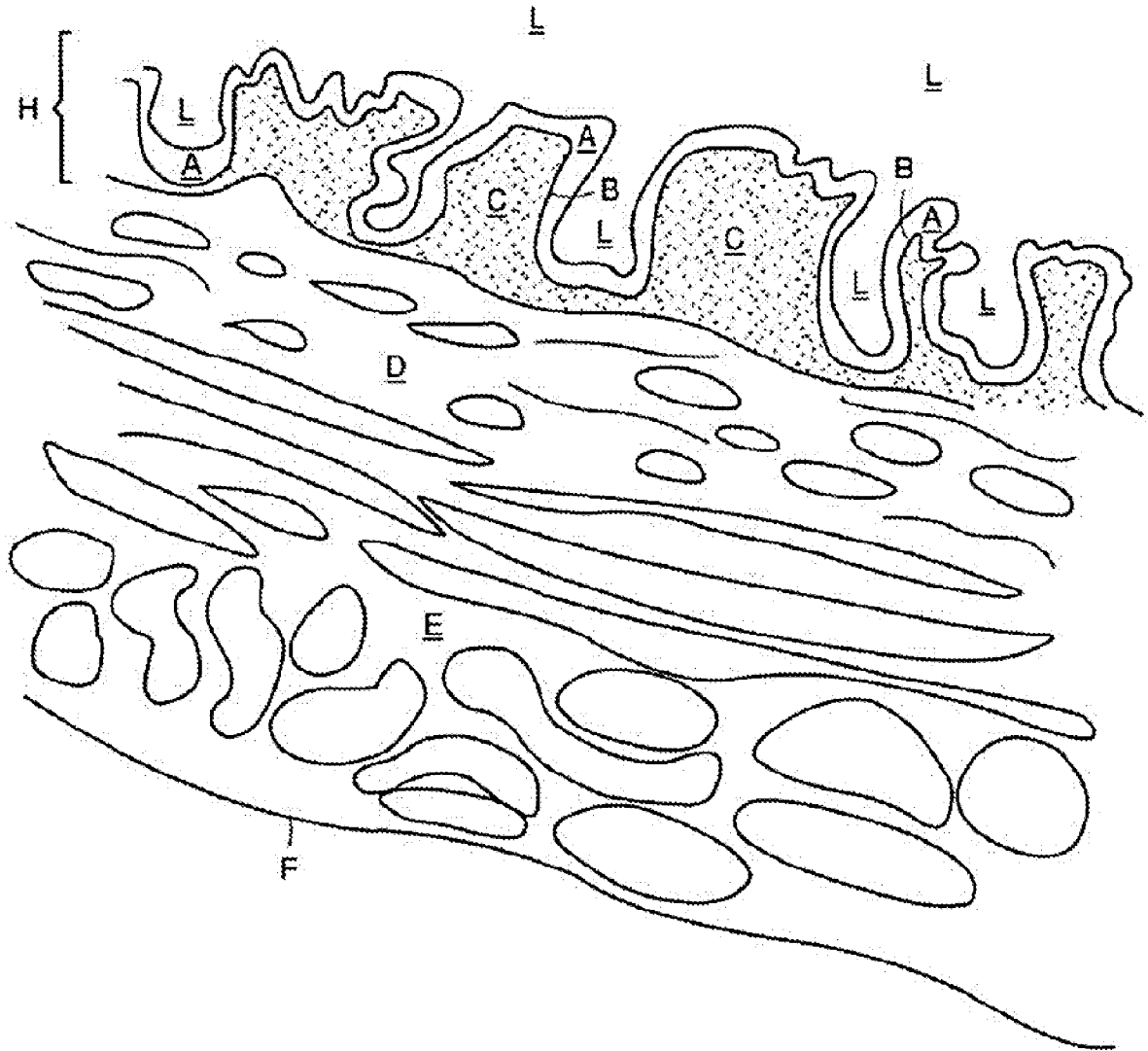


FIG. 2B



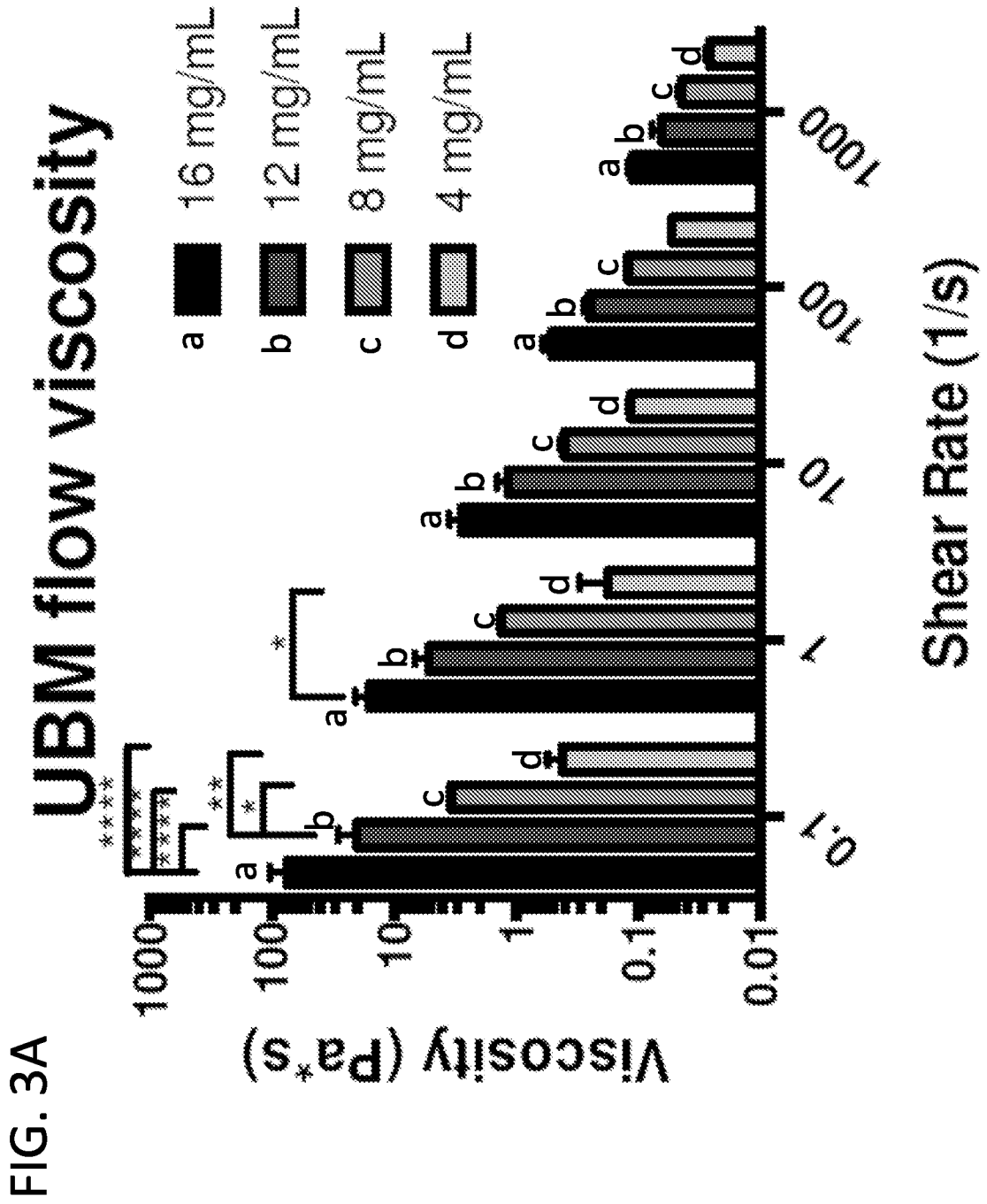


FIG. 3A

FIG. 3B

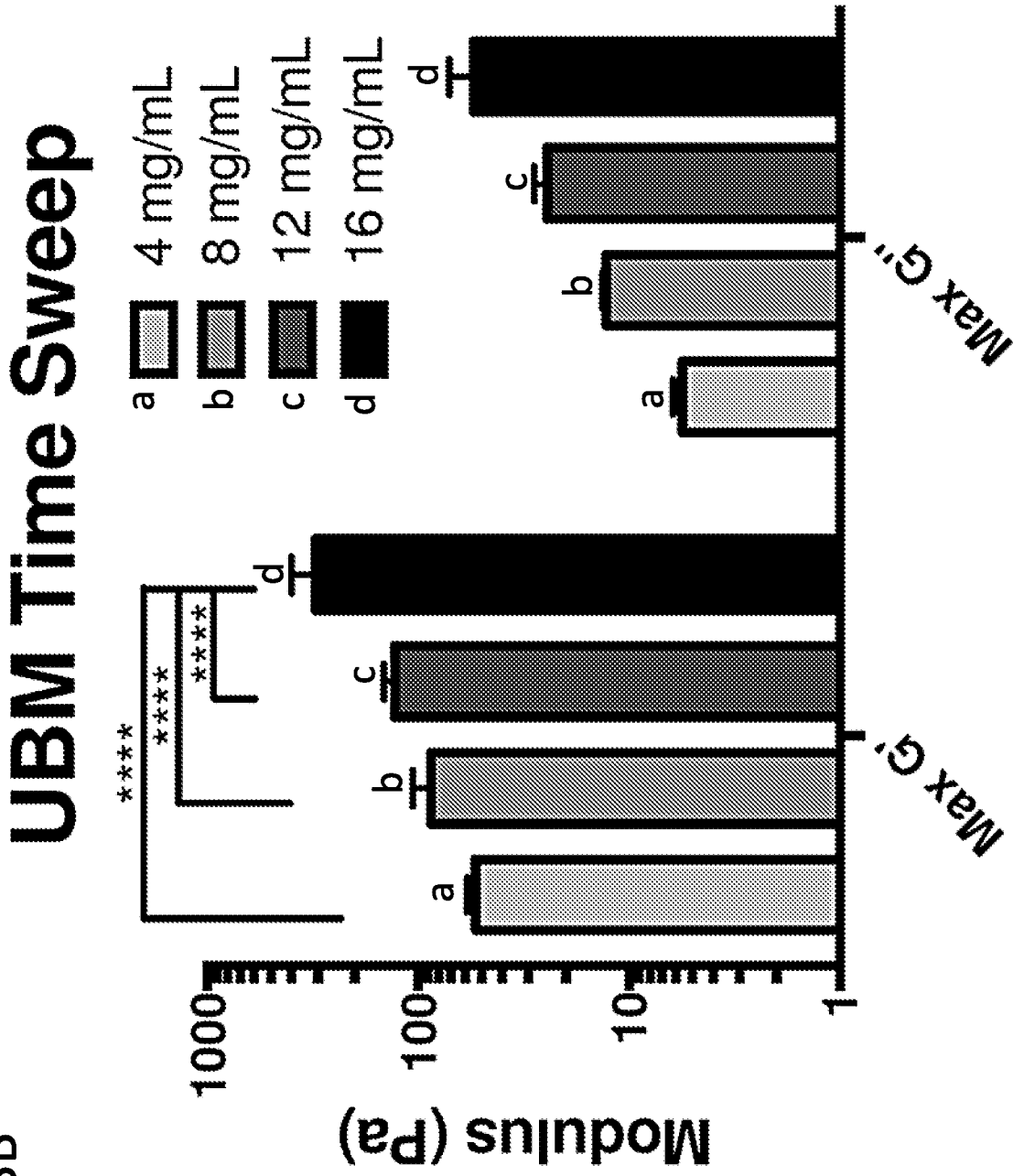
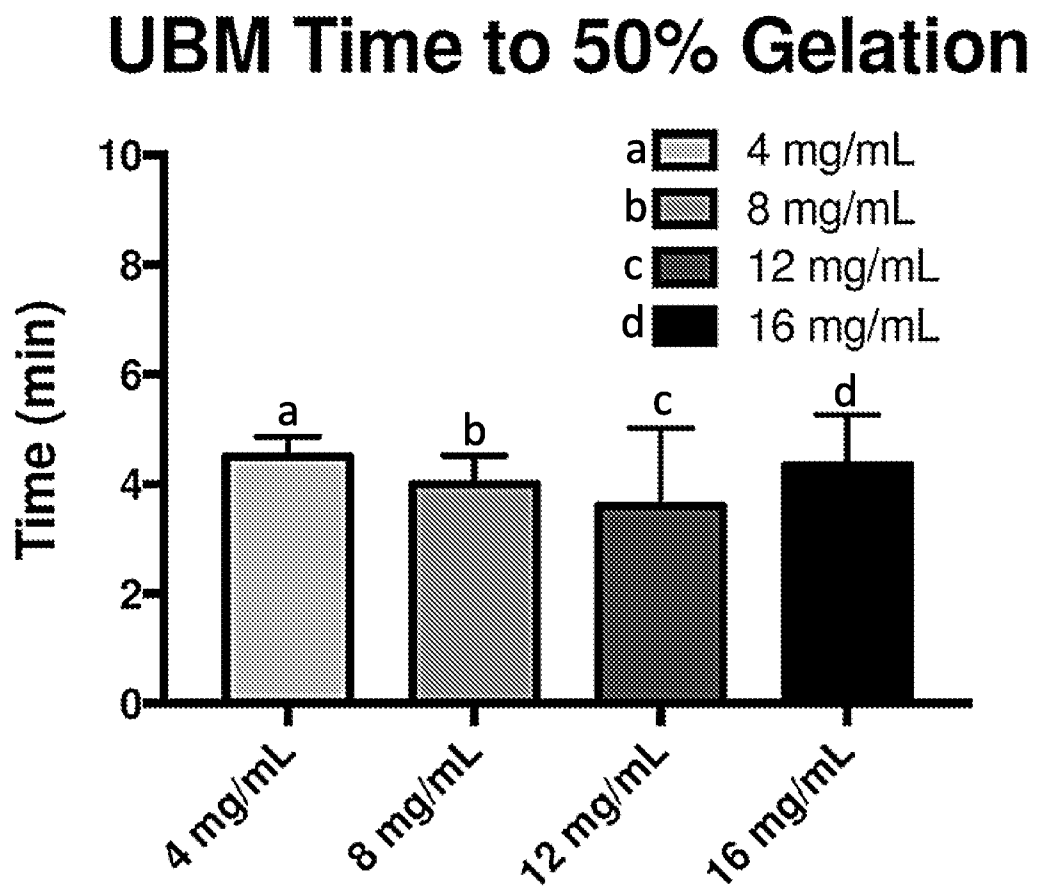


FIG. 3C



**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US2019/038320

<p><b>A. CLASSIFICATION OF SUBJECT MATTER</b>                  IPC (20190101) A61L 24/00, A61L 24/04, A61L 24/10, A61L 31/04, A61L 31/14, A61K 35/12                  CPC (20130101) A61L 24/0031, A61L 24/00, A61L 24/04, A61L 24/102, A61L 31/044, A61L 31/145, A61K 35/12                  According to International Patent Classification (IPC) or to both national classification and IPC</p>											
<p><b>B. FIELDS SEARCHED</b></p> <p>Minimum documentation searched (classification system followed by classification symbols)                  IPC (20190101) A61L 24/00, A61K 35/12                  CPC (20130101) A61L 24/0031, A61K 35/12</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)                  Databases consulted: CAPLUS, BIOSIS, EMBASE, MEDLINE, Google Scholar, Derwent Innovation                  Search terms used: extracellular matrix, ECM, hydrogel, Endoscopic mucosal resection, endoscopic submucosal dissection, submucosal, injection</p>											
<p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p> <table border="1"> <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>A</td> <td>WO 2015075024 A1 COSMO TECHNOLOGIES LTD[IE] 28 May 2015 (2015/05/28) examples, claims</td> <td>1-25</td> </tr> <tr> <td>A</td> <td>Uraoka, Toshio, et al. "Submucosal injection solution for gastrointestinal tract endoscopic mucosal resection and endoscopic submucosal dissection." Drug design, development and therapy 2 (2008): 131. Retrieved from the internet: &lt; <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2761197/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2761197/</a>&gt; 06 Feb 2009 (2009/02/06) the whole document</td> <td>1-25</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	A	WO 2015075024 A1 COSMO TECHNOLOGIES LTD[IE] 28 May 2015 (2015/05/28) examples, claims	1-25	A	Uraoka, Toshio, et al. "Submucosal injection solution for gastrointestinal tract endoscopic mucosal resection and endoscopic submucosal dissection." Drug design, development and therapy 2 (2008): 131. Retrieved from the internet: < <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2761197/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2761197/</a> > 06 Feb 2009 (2009/02/06) the whole document	1-25
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A	Uraoka, Toshio, et al. "Submucosal injection solution for gastrointestinal tract endoscopic mucosal resection and endoscopic submucosal dissection." Drug design, development and therapy 2 (2008): 131. Retrieved from the internet: < <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2761197/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2761197/</a> > 06 Feb 2009 (2009/02/06) the whole document	1-25									
<p><input type="checkbox"/> Further documents are listed in the continuation of Box C.      <input checked="" type="checkbox"/> See patent family annex.</p>											
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<p>Date of the actual completion of the international search</p> <p>19 Aug 2019</p>		<p>Date of mailing of the international search report</p> <p>05 Sep 2019</p>									
<p>Name and mailing address of the ISA:</p> <p>Israel Patent Office                  Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel                  Facsimile No. pctoffice@justice.gov.il</p>		<p>Authorized officer                  HOROWITZ Anat</p> <p>Telephone No. 972-73-3927172</p>									

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