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(54) Title: INJECTABLE SCAFFOLDS AND USES THEREOF

(57) Abstract: The present invention provides a liquid composition comprising two water-soluble biodegradable natural polymers and having a particular viscosity that forms, upon cross-linking of said polymers, a highly biocompatible injectable hydrogel having particular physical properties, which can be used in various medical applications, e.g., for reducing lung volume in an individual suffering from emphysema. The invention further provides a kit comprising said liquid composition, and methods of use.

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INJECTABLE SCAFFOLDS AND USES THEREOF

TECHNICAL FIELD

[0001] The present invention provides a liquid composition comprising two water-soluble biodegradable natural polymers and having a particular viscosity that forms, upon cross-linking of said polymers, a highly biocompatible injectable hydrogel; a kit comprising said liquid composition; and methods of use.

BACKGROUND ART

Emphysema and its current, insufficient solutions

[0002] Emphysema is a type of chronic obstructive pulmonary disease (COPD) caused by damage to the alveoli, the small air sacs in the lung, where the exchange of oxygen and carbon dioxide takes place. The inner walls of the alveoli weaken, which causes them to expand and burst, creating one larger air sac as opposed to many tiny ones. This phenomenon results in a smaller surface area of the lungs, poor gas exchange, and consequently reduced oxygen levels and increased carbon dioxide levels in the blood.

[0003] According to the American Lung Association, COPD is the third leading cause of death in the United States, wherein about 80% of these deaths are due to smoking, holds an expensive burden on the governmental authorities, and results in a high rate of hospitalizations for those over 65 years old (American Lung Association, 2013; World Health Organization, 2016).

[0004] Currently, there is no treatment for emphysema that reverses the damage, but only slows the disease's progression. The main treatment for COPD includes medications such as bronchodilators, steroids, and antibiotics to treat infections that may result from the disease. However, this solution is better suited for patients with chronic bronchitis and asthma rather than those with emphysema, whose primary abnormality is lung hyperinflation due to destruction of elastic tissue. Medications are also costly and may include undesirable side effects. Depending on the severity of the disease, lung transplant or lung volume reduction surgery could be done to remove damaged sections of lung tissue. These methods are expensive, have limited organ donor availability, and are invasive, which always brings the risks of complications and mortality. A more efficient method of treatment for emphysema includes endoscopic lung volume reduction therapy using injectable hydrogels, which aims to collapse damaged areas of the lung and reduce

hyperinflation without the risk of invasive surgery (Díaz-Jimenez and Rodriguez, 2013). The AeriSeal lung sealant is an example of a product that works this way, in which the liquid-foam hydrogel is injected through a catheter to the lung periphery, where it polymerizes *in situ* and functions as a bioadhesive, forming a film of material on the lung surface that seals the damaged area and causes absorption atelectasis (Herth *et al.*, 2011). Clinical trials done by this company showed great safety and efficacy in patients with advanced upper lobe as well as homogeneous emphysema.

Injectable hydrogels in tissue engineering

[0005] Hydrogels are water-based semisolids comprised of networks of cross-linked hydrophilic molecules that maintain their state of hydration by trapping water through hydrogen bonding. They play a major role in tissue engineering and research, and have been used in many biomedical applications including, but not limited to, scaffolds, bioadhesives and sealants, and drug-delivery systems.

[0006] Scaffolds are made to fulfil several functions such as: (1) promoting cell-biomaterial interactions, cell adhesion, and extracellular matrix deposition; (2) allowing the sufficient transport of gases, nutrients, and regulatory factors to promote cell survival, proliferation, and differentiation; (3) being biodegradable at a manageable rate that approximates the rate of tissue regeneration under the culture conditions of interest; and (4) completing these tasks with a minimal degree of inflammation or toxicity (Dhandayuthapani *et al.*, 2011).

[0007] For some applications there is a need for injectable scaffolds that are injected via long and narrow catheters. Unlike tissue engineering approaches that use prefabricated scaffolds, injectable systems are a unique therapeutic method for areas that are difficult to reach in the body, as they have the ability to conform to any desired shape. Another advantage of such systems is that cells and bioactive molecules can be easily incorporated in the scaffold solution by mixing them in prior to injection, or by simultaneously injecting them together with the scaffold, making a homogeneous distribution within the scaffold matrix. Moreover, the minimally invasive procedure of injection may reduce patient discomfort, risk of infection, scar formation, treatment cost, and hospitalization time.

[0008] An example demonstrating the benefits of injectable hydrogels is that of Pape *et al.* (2015), in which a drug-loaded ureido-pyrimidinone-modified poly(ethylene glycol) hydrogel was injected (non-invasively and side-specific) into a pig's heart through a long,

flexible catheter in order to promote regeneration of lost or damaged myocardium. This research team used materials that allowed easy transitioning from a gel to a solution using environmental factors. At normal pH, the supramolecular hydrogel acts as a liquid (easily injectable) and at physiological pH, it forms a gel. These physical transitions allow the incorporation of bioactive drugs and/or other desired molecules within the hydrogel.

[0009] Another example of an injectable hydrogel is the AeriSeal technology. This company used aminated polyvinyl alcohol for their polymeric component, and 1,5-pentanedial for the cross-linker. By forming a hydrogel-based polymer formulation prior to injection via catheter, this technology allows the delivery of therapeutically active materials to the small airways and alveoli, which is an important characteristic for achieving consistent, effective lung volume reduction therapy. Due to their make-up being mostly water-based, hydrogels are soft and thus do not generally cause mechanical harm to the soft tissues in the lung, making them attractive candidates for therapeutic technologies (Díaz-Jimenez and Rodriguez, 2013).

[0010] The requirements from polymeric hydrogels for scaffold applications, in addition to biocompatibility, include certain mechanical and physical properties such as gelation time, tensile strength, young's modulus, viscosity, porosity, and degradation rate.

[0011] US 9,198,365 discloses a hydrogel comprising a non-natural polymer comprising a plurality of pendant nucleophilic or electrophilic groups, and a cross-linker comprising at least two pendant electrophilic or nucleophilic groups, respectively; and a method for reducing lung volume, e.g., so as to treat emphysema, by administration of said hydrogel.

[0012] US 20030181356 discloses a method for treating emphysema by reducing the amount of force the fibers in the lung must bear; and a composition comprising a lipid that, upon applying to an enlarged alveolus, exerts a surface tension within the alveolus that reduces the stress on fibers within the alveolus when inflated by a normal inspiration.

[0013] US 20040047855 discloses a method for performing non-surgical lung volume reduction in a patient suffering from, e.g., emphysema by administering, through the trachea, a composition comprising an enzyme, e.g., a protease; and collapsing a region of the lung, at least a portion of which was contacted by the composition administered.

[0014] EP 2609940 discloses a respiratory region volume inhibitor containing a coating film-forming component as a main component and capable of forming a coating film in a respiratory region, for administration to an emphysema-suffering pulmonary alveolar parenchyma in a human-respiratory region. As described in this publication, the coating

film-forming component preferably includes polymers such as inter alia gelatin and sodium alginate, or film-forming polymer precursors, and is configured such that a balloon-shaped closed pouch made of the coating film is formed in intimate contact with an inner surface of the respiratory region along an inner peripheral surface of the respiratory region in response to an external stimulation, and the balloon-shaped closed pouch is shrunk by reducing a pressure inside the balloon-shaped closed pouch from outside of the respiratory region.

[0015] US 20160206301 discloses a method for sealing a lung region by delivering a fluid containing a sealing agent into the target lung compartment, such that the target compartment is pressurized and the fluid flows through the collateral flow channel, and the sealing agent seals said collateral flow channel.

[0016] US 9,877,926 discloses a bioadhesive matrix formed by preparing a bioadhesive watery formulation and allowing a curing time to lapse, wherein said formulation comprises gelatin; alginate; and a coupling agent, wherein the concentrations of said gelatin, alginate and coupling agent in the formulation are 50-400 mg/ml, 10-60 mg/ml, and 10-30 mg/ml, respectively, and are selected such that, prior to curing, the formulation has a room temperature viscosity that ranges from 1-50 Pa-s, and the curing time for forming said matrix ranges from 5 seconds to 30 minutes.

[0017] Mehta *et al.* (2015) describe an endobronchial application of a synthetic hydrogel composed of two synthetic polyethylene glycols, a dilute hydrogen chloride solution and a sodium phosphate/sodium carbonate solution, for the closure of an alveolar-pleural fistula. As described, the components are mixed and the hydrogel thus obtained is injected into the airways through a flexible polyurethane catheter, deployed through the working channel of a bronchoscope, and forms a plug that seals the fistula.

SUMMARY OF INVENTION

[0018] It has now been found, in accordance with the present invention, that an aqueous composition comprising two biodegradable natural polymers, more specifically gelatin and alginate, and having a viscosity lower than 1 Pa-s, more particularly of about 0.1 to about 0.4 Pa-s, at ambient temperature, upon cross-linking of said two polymers with a carbodiimide such as N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), forms a hydrogel having a gelation time of 10-90 seconds and particular tensile strength, young's modulus, porosity and non-enzymatic degradation rate. The hydrogel

formed demonstrates very good *ex vivo* bonding strength and high biocompatibility, and may therefore be highly attractive for tissue adherence. Moreover, said hydrogel can be injected through long (e.g., 40- and 100-cm) catheters, and may thus be delivered into the body of a subject where it turns into a three-dimensional (3D) scaffold. Such scaffolds may be used in tissue engineering, e.g., for repairing or enhancing regeneration of a damaged/injured tissue of an internal organ, and in treating certain medical conditions, e.g., for reducing lung volume in a subject suffering from emphysema.

[0019] In one aspect, the present invention thus provides a liquid composition comprising a first water-soluble biodegradable natural polymer and a second water-soluble biodegradable natural polymer, wherein said first water-soluble biodegradable natural polymer is gelatin, optionally partially hydrolyzed; said second water-soluble biodegradable natural polymer is alginate or a salt thereof such as sodium alginate; and said composition has a viscosity of at least 0.02 pascal-second (Pa-s) and lower than 1 Pa-s at ambient temperature.

[0020] As shown herein, upon cross-linking of the first water-soluble biodegradable natural polymer and the second water-soluble biodegradable natural polymer, the liquid composition disclosed forms a hydrogel having particular gelation time as well as mechanical and physical properties. More specifically, the invention thus provides a liquid composition as defined above which, upon mixing with a solution comprising a water-soluble cross-linking agent ("cross-linker") capable of cross linking said polymers, forms an injectable hydrogel having a gelation time in the range of 10 to 90 seconds; and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air.

[0021] In another aspect, the present invention relates to a method for repairing or enhancing regeneration of a damaged/injured tissue of an internal organ in a subject in need thereof, said method comprising the steps of:

- (i) providing a liquid composition as defined above and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers; and
- either:

- (ii) mixing said liquid composition with said solution to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately
- (iii) administering to said damaged/injured tissue of said subject a therapeutically effective amount of said hydrogel which turns into a three-dimensional scaffold *in situ*,

or

- (iv) concomitantly administering to said damaged/injured tissue of said subject an effective amount of each one of said liquid composition and said solution, which are then mix and form a therapeutically effective amount of said hydrogel that turns into said three-dimensional scaffold *in situ*.

[0022] In still another aspect, the present invention relates to a method for reducing lung volume in a subject in need thereof, said method comprising the steps of:

- (i) providing a liquid composition as defined above, and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers; and

either:

- (ii) mixing said liquid composition with said solution to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately

- (iii) administering to the lung of said subject a therapeutically effective amount of said hydrogel which turns into a three-dimensional scaffold *in situ*,
or
- (iv) concomitantly administering to the lung of said subject an effective amount of each one of said liquid composition and said solution, which are then mix and form a therapeutically effective amount of said hydrogel that turns into said three-dimensional scaffold *in situ*.

[0023] The subject treated by any one of the methods disclosed herein may be a mammal, e.g., a human, non-human primate, horse, ferret, dog, cat, cow, or goat, but it is preferably a human, i.e., an individual.

[0024] In another aspect, the present invention relates to a liquid composition as defined above and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers, for use as a combination in repairing or enhancing regeneration of a damaged/injured tissue of an internal organ, wherein:

- (i) said liquid composition and said solution are first mixed to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air, and said hydrogel is then immediately administered to the damaged/injured tissue of the internal organ where it turns into a three-dimensional scaffold *in situ*, or
- (ii) said liquid composition and said solution are concomitantly administered to the damaged/injured tissue of the internal organ, where they mix and form said hydrogel that turns into said three-dimensional scaffold *in situ*.

[0025] In yet another aspect, the present invention relates to a liquid composition as defined above and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers, for use as a combination in reducing lung volume, wherein:

- (i) said liquid composition and said solution are first mixed to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90

seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air, and said hydrogel is then immediately administered to the lung where it turns into a three-dimensional scaffold *in situ*, or

- (ii) said liquid composition and said solution are concomitantly administered to the lung, where they mix and form said hydrogel that turns into said three-dimensional scaffold *in situ*.

[0026] In a further aspect, the present invention provides a kit comprising: (i) a liquid composition as defined above; (ii) a water-soluble cross-linking agent capable of cross linking said polymers, wherein said cross-linking agent is in the form of either a powder or a solution; and (iii) instructions for dissolving said cross-linking agent, when formulated as a powder, in a predetermined volume of an aqueous liquid so as to form a solution of said cross-linking agent; and either (a) mixing said liquid composition with said solution thereby forming an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent and having a gelation time as well as mechanical and physical properties as defined above; and immediately administering said hydrogel to a damaged/injured tissue of an internal organ, or to the lung, where it turns into a three-dimensional scaffold; or (b) concomitantly administering said liquid composition and said solution, separately, to a damaged/injured tissue of an internal organ, or to the lung, where they mix and form said hydrogel that turns into a three-dimensional scaffold.

BRIEF DESCRIPTION OF DRAWINGS

[0027] **Fig. 1** shows gelation time of varying concentrations of gelatin, with constant concentrations of alginate (10mg/mL) and EDC (20mg/mL), via no catheter, 40cm catheter, and 100cm catheter.

[0028] **Fig. 2** shows gelation time of varying polymer-to-air ratios for varying concentrations of gelatin, with constant concentrations of alginate (10mg/mL) and EDC (20mg/mL), via no catheter.

[0029] **Fig. 3** shows the viscosity of varying concentrations of gelatin, with constant alginate concentrations (10mg/mL).

[0030] **Figs. 4A-4B** show the effect of gelatin concentration on the tensile strength (**4A**) and young's modulus (**4B**) of Gel-Alg-EDC hydrogel with constant alginate (10mg/mL) and EDC (20mg/mL) concentrations.

[0031] **Figs. 5A-5B** show the influence of the foaming ratio (polymer:air ratio) on the tensile strength (**5A**) and young's modulus (**5B**) of both 200-10-20 and 200-10-10 Gel-Alg-EDC hydrogels.

[0032] **Figs. 6A-6B** show the influence of injection through 40- and 100-cm catheters on the tensile strength (**6A**) and young's modulus (**6B**) of both 200-10-10 and 200-10-20 Gel-Alg-EDC hydrogels, with a foaming ratio of 1.5.

[0033] **Figs. 7A-7B** show the influence of the foaming ratio (polymer:air ratio) on the tensile strength (**7A**) and young's modulus (**7B**) of 200-10-30 gel-al-EDC hydrogels.

[0034] **Fig. 8** shows enzymatic degradation of 200-10-10 (Gel-Alg-EDC) unfoamed plugs in varying amounts of collagenase dissolved in 100mL DDW (NED - no enzymatic degradation).

[0035] **Figs. 9A-9B** show non-enzymatic degradation (**9A**) and enzymatic degradation (0.2 mg collagenase in 100mL DDW) (**9B**) of unfoamed plugs with formulations 200-10-10 and 200-10-20 (Gel-Alg-EDC).

[0036] **Figs. 10A-10B** show non-enzymatic degradation (**10A**) and enzymatic degradation (**10B**) of 1:1 and 1.5:1 (polymer:air) foamed plugs with 200-10-10 (Gel-Alg-EDC) formulation.

[0037] **Figs 11A-11B** show non-enzymatic degradation (**11A**) and enzymatic degradation (**11B**) of 1:1 and 1.5:1 (polymer:air) foamed plugs with 200-10-20 (Gel-Alg-EDC) formulation.

[0038] **Fig. 12** shows weight loss in humid chamber without aqueous environment of plugs with 200-10-10 and 200-10-20 (Gel-Alg-EDC) formulations.

[0039] **Figs. 13A-13B** show the cell viability of two selected scaffold formulations after 24 and 48 hours: (**13A**) human neonatal foreskin fibroblast; (**13B**) human mesenchymal stem cells.

[0040] **Figs. 14A-14B** show human mesenchymal stem cells adhesion to hydrogel as affected by the foaming ratio (polymer:air) and polymer formulation (Gel-Alg-EDC) after 24 and 48 hours: (**14A**) The cells seeded on top of the hydrogel; (**14B**) The cells seeded inside the hydrogel.

DETAILED DESCRIPTION

[0041] In one aspect, the present invention provides a liquid composition comprising a first water-soluble biodegradable natural polymer and a second water-soluble biodegradable natural polymer, wherein said first water-soluble biodegradable natural polymer is gelatin, optionally partially hydrolyzed, and said liquid composition has a viscosity of at least 0.02 Pa-s and lower than 1 Pa-s at ambient temperature. Particular such liquid compositions are those wherein the second water-soluble biodegradable natural polymer is alginate, or a salt thereof such as sodium alginate, potassium alginate, calcium alginate, and magnesium alginate, but preferably sodium alginate.

[0042] The term "water-soluble biodegradable natural polymer" as used herein refers to any water-soluble, biodegradable, polymer occurring in nature, such as a polysaccharide, polypeptide, or protein. Non-limiting examples of such natural polymers, in addition to gelatin and alginate, include cellulose (a polysaccharide consisting of a linear chain of $\beta(1\rightarrow4)$ linked D-glucose units); starch (a polysaccharide consisting of glucose units linked by glycosidic bonds); chitosan (a linear polysaccharide of randomly distributed $\beta(1\rightarrow4)$ -linked D-glucoseamine (deacetylated unit) and N-acetyl-D-glucoseamine (acetylated unit)); guar gum (a polysaccharide consisting of a mannose backbone with galactose side groups); hyaluronic acid (also called hyaluronan, an anionic nonsulfated glycosaminoglycan); gellan gum (an anionic polysaccharide); chitin (polymer of N-acetylglucosamine); collagen; or albumin.

[0043] Gelatin is a mixture of peptides and proteins produced by partial hydrolysis, more particularly acid-, alkali-, or enzymatic hydrolysis, of collagen. Type A gelatin is obtained by acidic process and has a high density of amino groups causing a positive charge; and type B gelatin is obtained by alkaline process and has high density of carboxyl groups causing negative charge. There are different sources for collagen such as animal skin, bones, and connective tissues, which afford a variety of gelatin forms with a range of physical and chemical properties. Typically, gelatin contains eighteen amino acids that are linked in partially ordered fashion, wherein glycine or alanine is about a third to half of the residues, proline or hydroxyproline are about one fourth of the residues, and the remaining forth include acidic or basic amino acid residues. In order to dissolve gelatin in water it is necessary to reach a temperature of at least 35°C, by heating or stirring and adding hot water. Moderate heating enhances solubility and severe heating may cause aggregation or partial hydrolysis of gelatin. The viscosity of gelatin varies with type, concentration, time

and temperature. Acid processed gelatin has slightly greater intrinsic viscosity compared to alkali processed gelatin. Based on its molecular mass, gelatin may be referred to as "low-Bloom gelatin" (having Bloom number of 50-125 and an average molecular mass of 20000-25000 Da), "medium Bloom gelatin" (having Bloom number of 175-225 and an average molecular mass of 40000-50000 Da), and "high-Bloom gelatin" (having Bloom number of 225-325 and an average molecular mass of 50000-100000 Da). Yet, it should be understood that for the liquid composition of the present invention, any gelatin regardless of its molecular weight or Bloom number may be used.

[0044] Alginate, also called alginic acid or algin, is an anionic polysaccharide widely distributed in the cell walls of brown algae, more specifically a linear copolymer with homopolymeric blocks of (1-4)-linked β -D-mannuronate (M) and its C-5 epimer α -L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), or alternating M and G-residues (MG-blocks). Alginates may have different mannuronic and guluronic acid residues ratio (M/G ratio), and different molecular weight (from about 10,000 Da or less, to about 600,000 Da) based on which they may be referred to as "low-molecular weight alginate" (10,000-80,000 Da), "medium-molecular weight alginate" (80,000-200,000 Da), and "high-molecular weight alginate" (200,000-600,000 Da). Yet, it should be understood that for the liquid composition of the present invention, any alginate regardless of its molecular weight or M/G ratio, or salt thereof, may be used.

[0045] As stated above, the liquid composition disclosed herein may comprise any gelatin regardless of its molecular weight, as well as any alginate regardless of its molecular weight or M/G ratio, or salt thereof. Yet, it should be understood that the molecular weight of each one of the polymers may affect the properties of the liquid composition, e.g., its viscosity at ambient temperature, and consequently the properties of the injectable hydrogel formed upon cross-linking of said polymers, i.e., the gelation time thereof as well as one or more of the bonding strength, tensile strength, young's modulus, porosity, and non-enzymatic degradation rate thereof. Therefore, in order to get particular desired properties of the liquid composition and consequently the injectable hydrogel, specific molecular weights of each one of the polymers should be chosen; and when changing the source of either one or both of the gelatin and alginate, a totally different

molecular weight of one or both of said polymers might be needed in order to maintain said desired properties.

[0046] According to the present invention, the viscosity of the liquid composition at ambient temperature is at least 0.02 Pa-s and lower than 1 Pa-s, e.g., in a range of 0.02 to 0.95 Pa-s, 0.02 to 0.90 Pa-s, 0.02 to 0.80 Pa-s, 0.02 to 0.70 Pa-s, 0.02 to 0.60 Pa-s, 0.02 to 0.50 Pa-s, 0.04 to 0.50 Pa-s, 0.06 to 0.50 Pa-s, 0.08 to 0.50 Pa-s, 0.10 to 0.50 Pa-s, or 0.10 to 0.40 Pa-s.

[0047] In certain embodiments, the concentrations of said first water-soluble biodegradable natural polymer and said second water-soluble biodegradable natural polymer in said liquid composition are about 50 to about 400 mg/ml, and about 3 to about 40 mg/ml, respectively. In certain particular such compositions, the concentration of the first polymer is about 60 to about 380 mg/ml, about 80 to about 360 mg/ml, about 100 to about 340 mg/ml, about 120 to about 320 mg/ml, about 140 to about 300 mg/ml, about 160 to about 280 mg/ml, about 180 to about 260 mg/ml, or about 200 to about 240 mg/ml, e.g., about 180, 190, 200, 210, 220, 230 or 240 mg/ml. In other particular such compositions, the concentration of the second polymer is about 4 to about 38 mg/ml, about 5 to about 36 mg/ml, about 6 to about 34 mg/ml, about 7 to about 32 mg/ml, about 8 to about 30 mg/ml, about 9 to about 28 mg/ml, about 10 to about 26 mg/ml, about 11 to about 24 mg/ml, or about 12 to about 22 mg/ml. e.g., about 8, 9, 10, 11, or 12 mg/ml.

[0048] In certain embodiments, the liquid composition of the present invention, as defined in any one of the embodiments above, is foamed, i.e., in the form of a foam. Such compositions may be prepared by foaming with a gas, e.g., air, so as to reduce their density. The foamed liquid composition, upon cross-linking of the polymers comprised within, forms a foamed hydrogel that turns into a porous scaffold, wherein a higher ratio between the gas (air) and the polymers in the composition leads to a higher amount of gas in the hydrogel formed and results in higher porosity of the scaffold obtained. A porous scaffold obtained in the body of a subject, upon gelation of a foamed hydrogel, occupies a volume higher than that occupied by the same quantity of a non-foamed hydrogel. Furthermore, a porous scaffold would facilitate infiltration of cells, e.g., epithelial cells, fibroblasts or stem cells, and may thus enhance (promote) treatment progression. Particular foamed liquid composition according to the present invention are those wherein the ratio between said first- and second-water-soluble biodegradable natural polymers and the gas (air) in said composition is in a range of about 0.2:1 to about 5:1, e.g., in the range of about

0.4:1, about 0.6:1, about 0.8:1, about 1:1, about 1.2:1, about 1.4:1, about 1.6:1, about 1.8:1, about 2:1, about 2.2:1, about 2.4:1, about 2.6:1, about 2.8:1, about 3:1, about 3.2:1, about 3.4:1, about 3.6:1, about 3.8:1, about 4:1, about 4.2:1, about 4.4:1, about 4.6:1, or about 4.8:1, by volume (polymers:gas).

[0049] In certain embodiments, the liquid composition of the present invention, as defined in any one of the embodiments above, and particularly when it is foamed, further comprises a growth factor. Such a growth factor may enhance differentiation and/or proliferation of cells such as epithelial cells, fibroblasts or stem cells, upon infiltration into the scaffold formed in the body of the subject treated upon gelation of the hydrogel obtained by cross-linking said polymers. Examples of growth factors that may be comprised within the liquid composition include, without being limited to, epithelial growth factor (EGF) such as human EGF, as well as fibroblast growth factors (FGF) both acidic and basic.

[0050] In certain embodiments, the present invention provides a liquid composition as defined above, wherein the first polymer is gelatin, optionally partially hydrolyzed; the second polymer is alginate, or a salt thereof such as sodium alginate; and the concentrations of said first and second polymers in said composition are about 50 to about 400 mg/ml, and about 3 to about 40 mg/ml, respectively. In particular such embodiments, the concentration of the gelatin is about 180, 190, 200, 210, or 220 mg/ml, and the concentration of the alginate or salt thereof is about 8, 9, 10, 11, or 12 mg/ml. More particular such compositions comprise said polymers in concentrations of about 180 mg/ml and about 8 mg/ml, about 180 mg/ml and about 9 mg/ml, about 180 mg/ml and about 10 mg/ml, about 180 mg/ml and about 11 mg/ml, about 180 mg/ml and about 12 mg/ml, about 190 mg/ml and about 8 mg/ml, about 190 mg/ml and about 9 mg/ml, about 190 mg/ml and about 10 mg/ml, about 190 mg/ml and about 11 mg/ml, about 190 mg/ml and about 12 mg/ml, about 200 mg/ml and about 8 mg/ml, about 200 mg/ml and about 9 mg/ml, about 200 mg/ml and about 10 mg/ml, about 200 mg/ml and about 11 mg/ml, about 200 mg/ml and about 12 mg/ml, about 210 mg/ml and about 8 mg/ml, about 210 mg/ml and about 9 mg/ml, about 210 mg/ml and about 10 mg/ml, about 210 mg/ml and about 11 mg/ml, about 210 mg/ml and about 12 mg/ml, about 220 mg/ml and about 8 mg/ml, about 220 mg/ml and about 9 mg/ml, about 220 mg/ml and about 10 mg/ml, about 220 mg/ml and about 11 mg/ml, or about 220 mg/ml and about 12 mg/ml, respectively. Such liquid compositions may be foamed, e.g., wherein the ratio between the first and second polymers, and the gas

(air), in said composition is in a range of about 0.2:1 to about 5.0:1, preferably about 1.8:1 to about 2.2:1, by volume (polymers:air), and/or may further comprise a growth factor as defined above, e.g., EGF, or an acidic- or basic-FGF.

[0051] As shown herein, upon cross-linking of the two polymers, the liquid composition of the present invention forms a hydrogel having particular gelation time as well as mechanical and physical properties. More specifically, the invention thus provides a liquid composition as defined in any one of the embodiments above, which upon mixing with a solution comprising a water-soluble cross-linking agent capable of cross linking said first- and second polymers, forms an injectable hydrogel having a gelation time in the range of 10 to 90 seconds; and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air.

[0052] In particular embodiments, the injectable hydrogel formed upon mixing said liquid composition with said cross-linking agent solution has a tensile strength and young's modulus as defined above; tensile strength and porosity as defined above; tensile strength and non-enzymatic degradation rate as defined above; young's modulus and porosity as defined above; young's modulus and non-enzymatic degradation rate as defined above; porosity and non-enzymatic degradation rate as defined above; tensile strength, young's modulus and porosity as defined above; tensile strength, young's modulus and non-enzymatic degradation rate as defined above; young's modulus, porosity and non-enzymatic degradation rate as defined above; or tensile strength, young's modulus, porosity and non-enzymatic degradation rate as defined above. In more particular embodiments, the injectable hydrogel formed upon mixing said liquid composition with said cross-linking agent solution has a gelation time in the range of 10 to 90 seconds; a tensile strength in a range of about 0.5 to about 50 kPa; a young's modulus in a range of about 0.5 to about 50 kPa; a porosity in a range of about 10 to about 95%; and a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air.

[0053] In certain embodiments, the cross-linking agent capable of cross linking said polymers is a carbodiimide, formaldehyde, glutaraldehyde, glyceraldehyde, genipin, a polyepoxide, an isocyanate, or an acyl azide. In particular embodiments, said cross-linking

agent is EDC, exemplified herein. The concentration of the cross-linking agent in said cross-linking agent solution may be, without limiting, about 1 to about 50 mg/ml, e.g., about 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30 mg/ml.

[0054] In another aspect, the present invention relates to a method for repairing or enhancing regeneration of a damaged/injured tissue of an internal organ in a subject in need thereof, said method comprising the steps of:

- (i) providing a liquid composition as defined in any one of the embodiments above and a solution comprising a water-soluble cross-linking agent capable of cross linking said first polymer and said second polymer; and

either:

- (ii) mixing said liquid composition with said solution to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately after that, i.e., without any delay,
- (iii) administering to said damaged/injured tissue of said subject a therapeutically effective amount of said hydrogel which turns into a three-dimensional scaffold *in situ* and consequently repairs or enhances regeneration of said damaged/injured tissue;

or

- (iv) concomitantly administering to said damaged/injured tissue of said subject an effective amount of each one of said liquid composition and said solution, which are then mix and form a therapeutically effective amount of said hydrogel that turns into said three-dimensional scaffold *in situ* and consequently repairs or enhances regeneration of said damaged/injured tissue.

[0055] In certain embodiments, the method for repairing or enhancing regeneration of a damaged/injured tissue of an internal organ is used in a thoracic surgery, i.e., in a surgical treatment of an organ inside the thorax, e.g., so as to close a broncho-pleural fistula, i.e., a

fistula between the lung and the pleural space that may develop following pneumonectomy, post traumatically, or with certain types of infections; or a trachea-esophageal fistula, i.e., an abnormal connection between the trachea and the esophagus. In other embodiments, the method is used in gastroenterology, e.g., so as to close tears in the digestive mucosa, i.e., tears of the mucosal lining of the digestive tract, by an endoscopic procedure. In further embodiments, the method is used in ear-nose and throat (ENT) medicine, e.g., so as to close a pharyngeal- or laryngeal tear.

[0056] In still another aspect, the present invention relates to a method for reducing lung volume in a subject in need thereof, said method comprising the steps of:

- (i) providing a liquid composition as defined in any one of the embodiments above and a solution comprising a water-soluble cross-linking agent capable of cross linking said first polymer and said second polymer; and

either:

- (ii) mixing said liquid composition with said solution to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately after that, i.e., without any delay,
- (iii) administering to the lung of said subject a therapeutically effective amount of said hydrogel which turns into a three-dimensional scaffold *in situ* and consequently reduces said lung volume;

or

- (iv) concomitantly administering to the lung of said subject an effective amount of each one of said liquid composition and said solution, which are then mix and form a therapeutically effective amount of said hydrogel that turns into said three-dimensional scaffold *in situ* and consequently reduces said lung volume.

[0057] The injectable hydrogel or, alternatively, each one of the liquid composition and the cross-linking agent solution can be administered to the lung using any suitable

technique. In particular embodiments, administration of the hydrogel is carried out via a catheter; or administration of the liquid composition and the cross-linking agent solution is carried out, concomitantly, via two identical or different catheters, wherein each one of said catheters is capable of passing through the working channel of a flexible bronchoscope.

[0058] In certain embodiments, the subject treated by the method of the present invention suffers from COPD, e.g., from emphysema.

[0059] In certain embodiments, the liquid composition used in any one of the methods disclosed herein comprises gelatin, optionally partially hydrolyzed, as the first polymer, and alginate or a salt thereof as the second polymer; the concentrations of said gelatin and said alginate or salt thereof in said composition are about 50 to about 400 mg/ml, and about 3 to about 40 mg/ml, respectively; and said water-soluble cross-linking agent is a carbodiimide such as EDC. In particular such embodiments, the concentration of said gelatin is about 180, 190, 200, 210, or 220 mg/ml, and the concentration of said alginate or salt thereof is about 8, 9, 10, 11, or 12 mg/ml. More particular such compositions comprise said first and second polymers in concentrations of about 180 mg/ml and about 8 mg/ml, about 180 mg/ml and about 9 mg/ml, about 180 mg/ml and about 10 mg/ml, about 180 mg/ml and about 11 mg/ml, about 180 mg/ml and about 12 mg/ml, about 190 mg/ml and about 8 mg/ml, about 190 mg/ml and about 9 mg/ml, about 190 mg/ml and about 10 mg/ml, about 190 mg/ml and about 11 mg/ml, about 190 mg/ml and about 12 mg/ml, about 200 mg/ml and about 8 mg/ml, about 200 mg/ml and about 9 mg/ml, about 200 mg/ml and about 10 mg/ml, about 200 mg/ml and about 11 mg/ml, about 200 mg/ml and about 12 mg/ml, about 210 mg/ml and about 8 mg/ml, about 210 mg/ml and about 9 mg/ml, about 210 mg/ml and about 10 mg/ml, about 210 mg/ml and about 11 mg/ml, about 210 mg/ml and about 12 mg/ml, about 220 mg/ml and about 8 mg/ml, about 220 mg/ml and about 9 mg/ml, about 220 mg/ml and about 10 mg/ml, about 220 mg/ml and about 11 mg/ml, or about 220 mg/ml and about 12 mg/ml, respectively. Such liquid compositions may be foamed, e.g., wherein the ratio between the first and second polymers, and the gas (air), in said composition is in a range of about 0.2:1 to about 5.0:1, preferably about 1.8:1 to about 2.2:1, by volume (polymers:air), and/or may further comprise a growth factor as defined above, e.g., EGF, or an acidic- or basic-FGF.

[0060] According to the present invention, upon mixing of the liquid composition and the cross-linking agent-containing solution, either prior to administration into the body of the subject treated or *in situ* following concomitant administration of said composition and said

solution, said first and second polymers are cross-linked by said cross-linking agent, and a hydrogel comprising said polymers cross-linked is formed. The hydrogel formed has a gelation time in the range of 10 to 90 seconds; and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kPa; (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air. In particular embodiments, the hydrogel formed has a gelation time, tensile strength, young's modulus, porosity, and non-enzymatic degradation rate, each in the particular range defined above.

[0061] In certain embodiments, the liquid composition and the cross-linking agent solution are first mixed according to step (ii) of the method, and a therapeutically effective amount of the injectable hydrogel thus formed is then immediately delivered according to step (iii) to a damaged/injured tissue of an internal organ of the subject treated, or to the lung of said subject, where it turns into a three-dimensional scaffold.

[0062] In other embodiments, effective amounts of the liquid composition and the cross-linking agent solution are separately and concomitantly delivered according to step (iv) of the method to a damaged/injured tissue of an internal organ of the subject treated, or to the lung of said subject, where they mix to form a therapeutically effective amount of the hydrogel that then turns into a three-dimensional scaffold.

[0063] The term "therapeutically effective amount" as used herein with respect to the injectable hydrogel formed upon mixing the liquid composition and the cross-linker solution in step (ii) of the method, refers to an amount of said hydrogel that after administration to a damaged/injured tissue of an internal organ, or to a lung of a subject suffering from COPD, turns *in situ* into a three-dimensional scaffold with a volume sufficient for repairing or enhancing regeneration of said damaged/injured tissue, e.g., closing a broncho-pleural- or trachea-esophageal fistula, a tear in the digestive mucosa, or a pharyngeal- or laryngeal tear; or for occupying a damaged part/section of said lung thereby reducing the volume of said lung.

[0064] The term "effective amount" as used herein with respect to the liquid composition and the cross-linker solution concomitantly administered in step (iv) of the method to a damaged/injured tissue of an internal organ, or to a lung of a subject suffering from COPD, refers to an amount of said liquid composition or cross-linker solution, which upon mixing *in situ* with an effective amount of said cross-linker solution or liquid composition,

respectively, forms a therapeutically effective amount of an injectable hydrogel that turns into a three-dimensional scaffold with a volume sufficient for repairing or enhancing regeneration of said damaged/injured tissue, or for occupying a damaged part/section of said lung thereby reducing the volume of said lung.

[0065] In another aspect, the present invention relates to a liquid composition as defined in any one of the embodiments above and a solution comprising a water-soluble cross-linking agent capable of cross linking said first polymer and said second polymer, for use as a combination in repairing or enhancing regeneration of a damaged/injured tissue of an internal organ, wherein:

- (i) said liquid composition and said solution are first mixed to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air, and said hydrogel is then immediately administered to the damaged/injured tissue of the internal organ where it turns into a three-dimensional scaffold *in situ*, or
- (ii) said liquid composition and said solution are concomitantly administered to the damaged/injured tissue of the internal organ, where they mix and form said hydrogel that turns into said three-dimensional scaffold *in situ*.

[0066] In certain embodiments, the liquid composition and the cross-linking agent solution are used as defined above in a thoracic surgery, e.g., so as to close a broncho-pleural fistula or a trachea-esophageal fistula; in gastroenterology, e.g., so as to close tears in the digestive mucosa by an endoscopic procedure; or in ear-nose and throat medicine, e.g., so as to close a pharyngeal- or laryngeal tear.

[0067] In yet another aspect, the present invention relates to a liquid composition as defined in any one of the embodiments above and a solution comprising a water-soluble cross-linking agent capable of cross linking said first polymer and said second polymer, for use as a combination in reducing lung volume, wherein:

- (i) said liquid composition and said solution are first mixed to form an injectable hydrogel comprising said polymers cross-linked by said cross-

linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air, and said hydrogel is then immediately administered to the lung where it turns into a three-dimensional scaffold *in situ*, or

- (ii) said liquid composition and said solution are concomitantly administered to the lung, where they mix and form said hydrogel that turns into said three-dimensional scaffold *in situ*.

[0068] The injectable hydrogel or, alternatively, each one of the liquid composition and the cross-linking agent solution can be administered to the lung using any suitable technique. For example, administration of the hydrogel may be carried out via a catheter; or administration of the liquid composition and the cross-linking agent solution may be carried out, concomitantly, via two identical or different catheters, wherein each one of said catheters is capable of passing through the working channel of a flexible bronchoscope.

[0069] In a further aspect, the present invention provides a kit comprising: (i) a liquid composition as defined in any one of the embodiments above; (ii) a water-soluble cross-linking agent capable of cross linking said first polymer and said second polymer, wherein said cross-linking agent is in the form of either a powder or a solution; and (iii) instructions for dissolving said cross-linking agent, when formulated as a powder, in a predetermined volume of an aqueous liquid so as to form a solution of said cross-linking agent; and either (a) mixing said liquid composition with said solution thereby forming an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent and having a gelation time as well as mechanical and physical properties as defined above; and immediately administering said hydrogel to a damaged/injured tissue of an internal organ, or to the lung, where it turns into a three-dimensional scaffold; or (b) concomitantly administering said liquid composition and said solution, separately, to a damaged/injured tissue of an internal organ, or to the lung, where they mix and form said hydrogel that turns into a three-dimensional scaffold.

[0070] In certain embodiments, the cross-linking agent comprised within the kit disclosed herein is formulated as a solution, more specifically, as an aqueous solution, e.g., a watery

solution, and it is therefore ready for mixing with said liquid composition. The cross-linking agent solution may be contained within any suitable container, e.g., in one or more bottles or vials. In particular such embodiments, the liquid composition is contained within at least one syringe, and it is thus ready for administration into the body of the subject treated via, e.g., a catheter. In other embodiments, the cross-linking agent comprised within the kit of the present invention is formulated as a powder, and is therefore first dissolved in a predetermined volume of an aqueous liquid so as to form an aqueous solution. In particular such embodiments, said aqueous liquid is water.

[0071] The liquid composition comprised within the kit of the present invention may be contained within any suitable container, e.g., in one or more bottles or vials. In certain embodiments, the liquid composition is contained within at least one syringe, and it is thus ready for administration into the body of the subject treated via, e.g., a catheter.

[0072] The kit of the present invention, according to any one of the embodiments defined above, may further comprise a delivery mean for administering said hydrogel, or said liquid composition and said solution, to the body of the subject treated. Particular such delivery means may be, e.g., a single-lumen catheter for administering said hydrogel, or a double-lumen catheter for administering said liquid composition and said solution. Specific such single-lumen catheters or double-lumen catheters are capable of passing through the working channel of a flexible bronchoscope, and may thus enable delivering said hydrogel, or said liquid composition and said solution, to the lung of said subject.

[0073] Unless otherwise indicated, all numbers referring, e.g., to the concentrations of the components of the liquid composition disclosed, the ratios between said components, or the mechanical and physical properties of either the liquid composition or the injectable hydrogel disclosed, used in the present specification are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in this description and claims are approximations that may vary by up to plus or minus 10% depending upon the desired properties sought to be obtained by the invention.

[0074] The invention will now be illustrated by the following non-limiting Examples.

EXAMPLES

Materials and Methods

[0075] Coldwater fish skin "type A" gelatin (G7041), alginic acid sodium salt from brown algae (A1112), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and collagenase type 1A (from *Clostridium histolyticum*, Catalog Number C9891) were purchased from Sigma-Aldrich, Rehovot, Israel.

Preparation of gelatin-alginate polymer and EDC cross-linker

[0076] Preparation of the polymer is based on dissolving varying amounts of gelatin and alginate (Gel-Alg) in double-distilled water (DDW), under heating up to 60°C. For the cytotoxicity studies, Rhodamine B was used for non-covalent labelling (two drops of a 0.02% solution were added to 10 ml of sample at 60°C). Gelatin and alginate were characterized at concentrations of 150-300 and 10 mg/mL, respectively. The formulations are presented in the form of Gel-Al-EDC, where Gel is the concentration of gelatin, Al is the concentration of alginate, and EDC is the concentration of the carbodiimide crosslinking agent (all in mg/mL). The polymer solution containing fish gelatin was placed at room temperature, 25±2°C, for approximately ten minutes, thus allowing it to reach room temperature prior to application.

[0077] In all experiments, the dual-component scaffold solution was applied using a double-syringe with a static mixer at a 4:1 volume ratio (Mixpac L-System, Sulzer, Switzerland), which provides consistent mixing of the polymer and cross-linker solutions. The polymeric solution containing Gel-Al was loaded in the large chamber of the double syringe, while the EDC (cross-linker) solution was loaded in the small chamber. In some experiments, the mixed Gel-Al-EDC solutions were injected through 6Fr-diameter (2.00mm) catheters of lengths 40 cm and 100 cm.

Preparation of foamed polymers

[0078] Different ratios of polymer-to-air were used in creating foamed solutions in order to formulate more porous scaffolds. A 2.5mL (or 5mL) syringe of the Gel-Al polymer solution and a 2.5mL (or 5mL) syringe of air were inserted into a three-way stopcock, and a homogeneous foam mixture was created by alternating pushes of each syringe, for each ratio of polymer-to-air. The ratios that were studied varied between 0.5:1 and 2.5:1 (polymer:air).

Gelation time and fluidity through catheter

[0079] Gelation time indicates the time required for the polymer to reach its final desired state. Gelation time was determined as the time required for a magnetic bar to stop moving after submerging it in some of the Gel-Alg-EDC solution. About 1.5mL of the mixed solution was injected into a single well of a 12-well plate, containing a small magnetic stir bar, under mixing at 300 rpm at room temperature. The gelation time of the selected formulations injected via the two types of catheters were compared to gelation time of formulations injected without a catheter. Additionally, gelation time was compared between several formulations of non-foamed polymer and the same formulations with polymer-to-air ratios of 2.5:1, 1.5:1, and 1:1, without the use of catheters.

Viscosity measurements

[0080] The initial viscosity of the polymeric (Gel-Al) solution at the moment of application on the tissue is mainly affected by the viscosity of the aqueous Gel-Al solution. Viscosity measurements of polymer solutions were performed using a controlled stress rheometer (model DHR3, TA Instruments Ltd.) fitted with a cone-and-plate geometry (1° cone angle, 40 mm diameter), at a constant temperature of 25°C and a constant shear rate of 10 Hz, in order to investigate the polymer's initial viscosity.

Evaluation of mechanical properties

[0081] Cylindrical samples (8.5 mm diameter, 30 mm height) were prepared in a custom-made Teflon mold, with parchment paper lined on the inside, and analyzed 24 h after casting, in order to measure young's modulus and tensile strength. These parameters were measured using the 5500 Instron Universal Testing Machine (Instron Engineering Corp.). Cylindrical samples were subjected to tensile displacement at a rate of 5 mm/min until failure. Three specimens were tested for each formulation. Samples were compared at different hydrogel formulations as well as different ratios of polymer-to-air. This method was adopted from Ingenito *et al.* (2010).

Weight loss and biodegradation

[0082] Gel-Alg-EDC formulations having gelatin and alginate concentrations of 200 mg/mL and 10 mg/mL, respectively, were injected into 7.0×7.0×3.5 mm³ silicon molds, and after gelation were carefully removed and transferred into 24-well plates, and dried for ~48 hours. Subsequently, they were weighed (W_i), immersed in 2 mL DDW (for non-enzymatic degradation) or in 2 mL of 0.002 mg/mL collagenase solution (for enzymatic

degradation), and placed in a static incubator at 37°C and 100% relative humidity for 1, 2, 4, 6, and 24 hours. At each time point, the plugs were removed from the liquids, dried for 24 hours, and weighed again (W_f). The rate of degradation (both enzymatically and non-enzymatically) was measured by following the weight loss percentage of the samples, calculated using the following equation:

$$(1) \text{ Weight Loss \%} = (W_i - W_f) / W_i \times 100\%$$

3 repetitions were done for each time point.

[0083] In order to choose the final enzymatic concentration that would be used in the biodegradation experiments, an initial test was done by varying amounts of collagenase in 100 mL of DDW, including 5mg, 1mg, 0.2mg, and 0.05mg.

[0084] Another test was done in order to examine weight loss in a humid chamber over a longer amount of time, without having the plugs be submerged in an aqueous medium. The method was similar to the one described above, but for this test, each time point was every other week (as opposed to the hourly time points of the previous test). Plugs were removed (in triplicate) from the humid chamber at their respective time points, dried for 24 hours in a static incubator at 37°C with no humidity, and weighed to get their final weight. Weight loss percentage was calculated according to equation 1.

Statistical analysis

[0085] All data were processed using the Excel software. Statistical comparison between more than two groups was performed using the ANOVA (with Tukey Kramer post hoc) method via the XLSTAT software. A value of $p < 0.05$ was considered significant. Error bars indicate the standard deviation.

Study 1.

[0086] The aim of the current study was to investigate the effect of varying formulations of Gel-Alg-EDC hydrogels, as well as foaming ratios, on their mechanical and physical properties. Another goal was to examine if injection via catheters affects these properties.

Gelation time

[0087] The gelation time of hydrogels containing 10 mg/mL alginate and crosslinked with 20 mg/mL EDC were found to vary between about 20-40 seconds in different gelatin concentrations (**Fig. 1**). Moreover, the hydrogel's injection through catheters did not significantly affect the gelation time. This is most likely due to the fact that the hydrogel

remains liquid the entire time of injection via the catheters, which is a necessary factor in medical injection procedures. Subsequently, the next step was to determine gelation time of varying foaming ratios, without the use of a catheter (**Fig. 2**).

[0088] The longer timed results (i.e., 35-40 seconds) from the 150mg/mL gelatin concentration in **Fig. 1** most likely occurred due to their lower gelatin concentration, and thus, lower viscosity and weaker bonding strength. Considering the fact that the gelation time of only 1.5mL of the hydrogel was tested in this experiment, all results are shorter than the desired time for actual injection procedures, where a much greater quantity of the hydrogel is used. For example, the AeriSeal manufacturers used about 20 mL of liquid foam that polymerized within 2-3 minutes (Díaz-Jimenez and Rodriguez, 2013). Therefore, our material is suitable for therapeutic applications when used in greater quantities.

Viscosity measurements

[0089] Rheological tests were performed in order to elucidate the effect of the hydrogel's components on the initial viscosity, i.e., before the crosslinking reaction. In this experiment the hydrogel contained 10 mg/mL alginate. The viscosity of the hydrogel used for this project, which entailed a maximal gelatin concentration of 300 mg/mL, was compared to the 400-10 Gel-Al formulation used in previous studies done by our laboratory.

[0090] The viscosity characteristics of gelatin are primarily related to the molecular weight distribution of the gelatin molecules; as gelatin concentration increases, the viscosity should increase as well, which is supported by the results presented in **Fig. 3**.

Evaluation of the polymer's mechanical properties

[0091] The mechanical properties of the polymers being studied for emphysema treatment were evaluated in tension. Samples were made by the method described previously, in custom-made Teflon molds, and were taken out after 5-10 min. This method was found very easy and effective for producing ideal cylindrical samples. Using this method, Gel-Alg-EDC samples with different gelatin and EDC concentrations were produced, as well as those with varying polymer-to-air ratios.

[0092] As can be seen in **Figs. 4A-4B**, the young's modulus and the tensile strength of the cured hydrogels were determined. A significant increase in these parameters was found when the gelatin concentration reached 250 mg/mL. Therefore, all following experiments were done using Gel-Alg concentrations of 200-10 mg/mL.

[0093] These results are supported by the fact that the molecular weight (viscosity) and concentration of the polymer strongly affect the mechanical interlocking through mobility and penetration ability, entanglement of the 3D structure, and crosslinking density, all of which affect the mechanical properties (i.e., higher concentrations lead to stronger properties).

Foamed hydrogels

[0094] The effect of the foaming ratio (i.e., the polymer to air ratio) on the mechanical properties of the cylindrical hydrogels was examined on polymer formulations of 200-10 mg/mL (Gel-Alg) crosslinked with 10 and 20 mg/mL EDC. For the most part, it was seen that the tensile strength (**Fig. 5A**) and the young's modulus (**Fig. 5B**) of the hydrogels were higher at lower amounts of air incorporated in the hydrogel. This could be due to the fact that as the amount of polymer decreases, its bonding strength decreases as well, and its mechanical properties are weakened. According to Dunphy *et al.* (2014), the theoretical value of the young's modulus for a single alveolar wall in a normal lung is about 5kPa. In our results, both Gel-Al-EDC formulations with a foaming ratio of 1:1, and the 200-10-20 Gel-Al-EDC formulation with a foaming ratio of 1.25:1, have similar young's modulus values.

[0095] Furthermore, it was observed that as the EDC concentration increases, the bonding strength of the hydrogel increases as well. This phenomenon can be attributed to the role of EDC as a cross-linking agent, wherein increasing the concentration of EDC results in a denser crosslinked network throughout the polymer, and this, in turn, results in stronger mechanical properties.

Injection through catheters

[0096] Hydrogel cylindrical samples were casted in the Teflon mold by injection through both 40- and 100-cm catheters. This was evaluated using a foaming ratio of 1.5 on a hydrogel formulation of 200-10 (mg/mL) Gel-Alg crosslinked with 10 or 20 mg/mL EDC. The results presented in **Fig. 6** show that the injection does not affect the hydrogel's mechanical properties. Due to the quickness of the injection, the hydrogel does not reach its final polymerized phase prior to evacuation from the catheter; it remains liquid throughout the procedure. This phenomenon is necessary in catheter-based procedures. This further supports the notion that our hydrogel is a great candidate for injectable scaffold applications.

Foamed hydrogels with higher EDC concentration

[0097] Despite our previous studies showing increased cytotoxicity of hydrogels with higher EDC concentrations (data not shown), we wanted to observe their influence on the mechanical properties and compare the results to the previous formulations. As shown in **Fig. 7**, the results show a similar trend to that of the other two formulations. That is, as the amount of polymer increases, the mechanical properties become stronger. As can be further seen, the mechanical properties of the hydrogel become stronger also by increasing the EDC concentration, most likely because there is more of the cross-linker available to bond the polymeric chains and subsequently strengthen the network.

Conclusions

[0098] Current treatments for emphysema are either life-threatening, such as lung transplants, or are not adequate enough for improving quality of life for patients with this disease. As of now, the best method to fulfil this goal is lung volume reduction therapy, done by catheter-based injection of foamed polymeric solutions used as bioadhesives. However, bioadhesives are mostly 2-dimensional platforms whereas scaffolds can take a 3-dimensional shape and thus can better mimic natural physiological environments (Li *et al.*, 2015). Scaffolds are more suitable in allowing cell-cell and cell-matrix interactions *in vivo*, and are therefore better candidates for use in the therapeutic procedures.

[0099] The main goal of this study was to develop and study a hydrogel scaffold that can be injected through long catheters for emphysematous lung therapy. To fulfil this, we investigated the effect of the formulation parameters on the physical and mechanical properties of gelatin-alginate hydrogels crosslinked with EDC; and determined whether injection of the hydrogels via catheters affect these properties.

[00100] The results presented herein show that higher concentrations of gelatin result in higher viscosities and thus shorter gelation times; and that the hydrogel's mechanical strength in tension and Young's modulus are increased with the polymer to air ratio. As further shown, the Young's modulus of several of the formulations tested is similar to the theoretical value of the young's modulus of alveoli, verifying the hydrogel's potential for being applied in emphysematous lung treatment procedures. Another feature that highlights the benefit in using the hydrogel for these procedures is the fact that its properties are not affected by injection via catheters.

Study 2.

[00101] The aim of the current study was to investigate the effect of enzymes on the weight loss rate of both foamed and unfoamed gelatin-alginate-EDC polymer plugs. Selected formulations included 200-10-10 and 200-10-20 (mg/mL) (Gel-Alg-EDC), and selected foaming ratios included 1:1 and 1.5:1 (polymer:air).

[00102] The initial test was done in order to choose the optimal concentration of collagenase for the following experiments. In theory, the more enzymes present in the surrounding environment of the (natural) polymer, the faster the polymer will degrade. Likewise, the longer the polymer is submerged in enzymatic solution, the more it will degrade, and there will be a higher percentage of weight loss. As shown herein, for the most part, our results supported these theories.

[00103] The amount of enzymes used in an experiment should depend on the specific application. In the present case, we assumed a minimal amount of enzyme present in the surrounding environment, and we thus decided to use the concentration of 0.2 mg collagenase in 100mL DDW, i.e., 0.002 mg/mL collagenase solution. The reason not to use the 0.05 mg concentration was the difficulty in handling low quantities of the powder. This may be observed in the fact that the graph of 0.05 mg overlaps and exceeds the graph of 0.2 mg, which is unusual and probably caused by error in weighing the enzyme.

[00104] In theory, polymers that are more crosslinked have more inter-chain bonding, and should thus take a longer amount of time to break apart and degrade. Surprisingly, **Fig. 9** does not reflect this hypothesis, as it shows that the polymer with 20 mg/mL EDC (i.e., more crosslinked) lost more weight, in the same amount of time, than the polymer with 10 mg/mL EDC, by both non-enzymatic and enzymatic surrounding. Although neither graph has a significant difference between the two formulations, these results are unusual and unexpected. It should further be noted that both formulations completely degraded (100% weight loss) after 24 hours submerged in enzymatic medium. This could be useful for applications of injectable scaffolds that must degrade in a short period of time, such as drug-delivery systems and others.

[00105] In general, a material with a higher concentration of polymer should have stronger mechanical properties due to the greater amount of opportunities for inter-chain bonding. Likewise, if a material is more porous, it has a weaker inter-chain bonding and thus degrades faster and loses more weight compared to its less-porous counterpart during the same period of time. The results shown herein generally support this hypothesis. In

particular, **Figs. 10** (excluding **Fig. 10A**) and **11** show that the formulation with a 1.5:1 foaming ratio (i.e. more polymer) has a slightly lower weight loss percentage; and **Figs. 10A** and **11A** show that after 24 hours the foamed plugs with 20 mg/mL EDC lost slightly less weight than those of 10 mg/mL EDC. Notably, none of the results were significantly different, probably due to the small amount of material used for the preparation of the plugs.

[00106] Unlike the experiments described in Example 1, the experiment described herein tested the weight loss of the hydrogel (plugs) in a humid chamber, without having the plugs submerged in an aqueous environment. **Fig. 12** represents the weight loss of both formulations' plugs over the course of 10 weeks, and shows that there is no uniform pattern in the weight loss of either formulation, and there is also no significant difference between the formulations tested. The fact that neither formulation exceeds a weight loss of 15% proves the potential of long-term use of this material.

Conclusions

[00107] Many tissue-engineering applications involve bioadhesives and/or scaffolds based on natural or synthetic polymers. Bioadhesives are mostly 2-dimensional platforms (used greatly for wound-healing applications) whereas scaffolds can take a 3-dimensional shape and can thus better mimic natural physiological environments (Li *et al.*, 2015). Scaffolds, and more specifically injectable scaffolds, are more suitable in allowing cell-cell and cell-matrix interactions *in vivo*, and are therefore better candidates for use in therapeutic procedures.

[00108] The main goal of this study was to develop and study an injectable hydrogel scaffold that may be used in a variety of medical applications. To fulfil this, we investigated the effect of the formulation parameters on the physical properties (i.e., weight loss and enzymatic degradation rate) of gelatin-alginate hydrogels crosslinked with EDC.

[00109] The results presented herein do not support the theory that a more crosslinked hydrogel degrades during a longer time; however, this may be due to the minuscule amount of polymer used for the preparation of the plugs for the experiments. Additionally, it can be seen that polymer plugs with a higher polymer-to-air ratio took a slightly longer time to degrade, which supports the theory that a greater quantity of material (i.e., less porous scaffold) takes longer to break apart its inter-chain bonds. Finally, our material has proven to be relevant and beneficial for long-term applications.

Study 3.

[00110] The aim of this study was to investigate the adhesion of cells such as fibroblast and mesenchymal cells to the hydrogel formulations, either foamed or unfoamed, and whether the hydrogel formulations have cytotoxic effect on those cells.

Hydrogel morphology

[00111] ***Preparation of the confocal samples.*** Samples were made, in triplicate, of each polymer formulation and at varying polymer-to-air ratios, as described in the Experimental. Each sample consisted of 450µl of the hydrogel, which was injected to individual wells of a 24-well plate with a glass bottom. The samples were tested after 30 minutes.

[00112] ***Confocal Scanning Light Microscope (CSLM) instrumentation and observation conditions.*** Observations were made using a Zeiss LSM 510 META CSLM. A laser beam with a wavelength of 561nm was used for Rhodamine B. EC Plan-Neofluar 10×/0.3 M27 Zeiss objective lens was used. Serial optical sections of the samples were recorded and saved as .mdb format in 512×512 pixel resolution. The images were processed using SigmaScan Pro 5.

Cytotoxicity

[00113] ***Preparations of bioadhesive extract.*** After preparing the gelatin-alginate and crosslinking agent solutions, 0.1mL of the gelatin-alginate solution and 0.04mL of the crosslinking agent solution were poured into a 6.2×6.2×3.5 mm³ silicon mold in biosafety cabinet. After 24 hours the cubes were moved to 24-well plate. Bioadhesive extract medium was performed by immersing the samples in 1mL culture medium and incubation for 24h in the incubator (37°+1°C, 5% CO₂).

[00114] ***Cell culture.*** Primary human fibroblast cultures were obtained from neonatal foreskins, and bone marrow humane mesenchymal stem cells (hMSC-bm) were purchased from PromoCell. The cells were thawed and cultured in 75mm² flasks with Minimum Essential Medium (MEM; for fibroblasts) and Dulbecco's Modified Eagle's Medium (DMEM; for hMSC-bm) supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin-nystatin. The cells were kept in a humidified 37°C and 5% CO₂ environment.

[00115] **Alamar Blue assay for cell viability.** After reaching a confluence of at least 70%, the cells were separated from the bottom of the flasks using a "trypsin A" solution and were seeded into 96-well plates at concentrations of 5,000 cells per well with 0.2mL of fresh culture medium and incubated for 24 hours. After 24 hours the medium was removed and replaced with 0.2mL per well of bioadhesive extract medium, in triplicate. Cells cultured without bioadhesive extract medium served as negative control. The cells were cultured further for 24 and 48 hours.

[00116] An Alamar Blue (AB) assay was performed 24 and 48 hours after the addition of the bioadhesive extract to the wells, and was used to evaluate cell growth and viability in the presence of adhesive extracts. The procedure included replacing the original medium with 0.2mL of fresh medium containing 10% (v/v) AB and incubating the cells for 4 hours. Subsequently, duplicates of 0.1mL from each well were transferred into a 96-well plate for spectrophotometer analysis (Spectra max 340 PC384, Molecular Devices) at 570nm and 600nm. The percent reduction of the AB was calculated according to the manufacturer's protocol. The %AB reduction after the exposure of the bioadhesive extract for different periods was compared to the %AB reduction in the control cells' environment (cells that were not exposed to the extracts), to evaluate the cytotoxicity of the bioadhesive.

$$(2): \% \text{reduced Alamar blue} = \frac{\epsilon_{\lambda_2}^{ox} A_{\lambda_1}^t - \epsilon_{\lambda_1}^{ox} A_{\lambda_2}^t}{\epsilon_{\lambda_1}^{red} A_{\lambda_2}^c - \epsilon_{\lambda_2}^{red} A_{\lambda_1}^c}$$

where $\lambda_1=570$ nm, $\lambda_2=600$ nm; ϵ^{ox} and ϵ^{red} represent the molar extinction coefficient of the oxidized and reduced Alamar Blue respectively, at 570nm and 600nm; A^c and A^t represent the absorbance of control wells containing culture medium with Alamar Blue but without cells and test wells, respectively, at 570nm and 600nm.

Cell adhesion quantification

[00117] **Cell cultures.** MSC were cultured as described in "Cell culture" above and seeded into 96-well plate either (i) on top of the Gel-Al-EDC hydrogel (24 hours before seeding, 96-well plates were loaded with 100 μ L of Gel-Al-EDC hydrogel. The plates remained uncovered until cell seeding - 5,000 cells per well with 0.2 mL of fresh culture medium); or (ii) inside the Gel-Al-EDC hydrogel (100 μ L portions of hydrogel containing 5,000 cells were injected in 96-well plates. After 5 min, 0.2 mL of fresh culture medium was added to each well). Cells cultured directly onto uncoated wells represented "100% adherence". Another positive control included cells cultured onto wells with 2% gelatin coating. The cells were cultured further for 24 and 48 hours.

[00118] *Alamar Blue assay for cell adhesion and proliferation.* The Alamar Blue (AB) assay was used to evaluate cell adhesion and proliferation, and was performed 24 and 48 hours after seeding. The procedure included replacing the original medium with 0.2 mL of fresh medium containing 10% (v/v) AB and incubating the cells for 4 hours. Subsequently, duplicates of 0.1mL from each well were transferred into a 96-well plate for spectrophotometer analysis (Multiskan GO, Thermo Scientific) at 570nm and 600nm. The percent reduction of the AB was calculated according to the manufacturer's protocol. The %AB reduction after the exposure of cells seeded inside/on top of the hydrogel for different periods was compared to the %AB reduction in the control cells, in order to evaluate the adhesion and proliferation of the cells. Calculation of %AB reduction was done as described in "*Alamar Blue assay for cell viability*" above.

Statistical analysis

[00119] All data was processed using the Excel software. Statistical comparison between more than two groups was performed using the ANOVA (with Fisher) method via the XLSTAT software. A value of $p < 0.05$ was considered statistically significant.

Results

[00120] *Cytotoxicity.* The tested hydrogel formulations did not have any cytotoxic effect on the cells. Both fibroblast and mesenchymal cells showed over 70% viability for 24 and 48 hours tests (**Fig. 13**).

[00121] *Cell adhesion.* For all the tested formulations, MSC showed at least 50% adhesion to the hydrogel after 48 hours. After 48 hours, the foamed hydrogel showed better results than the unfoamed one. After 48 hours, the adhesion of cells injected with the hydrogel was slightly lower than that of the cells seeded independently on top of the hydrogel. Furthermore, neither the foaming nor the injection of the cells seems to cause any damage to the cells (**Fig. 14**).

[00122] *Microstructure.* A structure with 3 layers was observed: (a) a dense bottom layer close to the glass; (b) a porous middle layer; and (c) a highly porous layer (more than 40% porosity) on top. The formulation's layers are described in **Table 1**, and the pore size and porosity results are summarized in **Table 2**. Both the mean feret diameter and porosity are increased with the distance from the glass. For the 200-10-10 formulation, the porosity is increased with the polymer:air ratio, for the same distance from the glass. The mean feret

diameter for 200-10-20 formulations is more consistent, probably because the polymer gelation time is higher than that of the 200-10-10 formulation, and therefore coalescence of air bubbles does not occur. Practically, the layer with less porosity, near the glass, gives the hydrogel its strength. The mean diameter of our cells is 10-30 μ m, and the pore size of our scaffold structures is therefore suitable for cells' growth and proliferation.

Table 1: Layers thickness for each formulation and polymer:air ratio

Formulation	Polymer:air	Layer a [μ M]	Layer b [μ M]	Layer c [μ M]
200-10-10	0.5:1	0-200	200-450	450-810
	1:1	0-100	100-570	570-830
	1.5:1	0-100	100-450	450-780
	2.5:1	0-80	80-290	290-550
200-10-20	0.5:1	0-50	50-200	200-530
	1:1	0-100	100-200	200-650
	1.5:1	0-50	50-150	150-500
	2.5:1	0-70	70-200	200-540

Table 2: Structure summary

Formulation	Polymer:Air	Pore mean size	Mean Porosity
200-10-10	0.5:1	34.19 $\pm$ 8.52	15.37 $\pm$ 10.19
	1:1	41.93 $\pm$ 7.79	25.2 $\pm$ 11.12
	1.5:1	39.74 $\pm$ 6.15	26.32 $\pm$ 11.65
	2.5:1	36.94 $\pm$ 9.37	24.91 $\pm$ 13.42
200-10-20	0.5:1	37.02 $\pm$ 5.98	21.2 $\pm$ 7.18
	1:1	27.82 $\pm$ 2.26	13.73 $\pm$ 7.38
	1.5:1	31.48 $\pm$ 6.08	20.82 $\pm$ 9.83
	2.5:1	30.78 $\pm$ 5.2	16.05 $\pm$ 7.13

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CLAIMS

1. A liquid composition comprising a first water-soluble biodegradable natural polymer and a second water-soluble biodegradable natural polymer, wherein said first polymer is gelatin, optionally partially hydrolyzed; said second polymer is alginate or a salt thereof; and said composition has a viscosity of at least 0.02 pascal-second (Pa-s) and lower than 1 Pa-s at ambient temperature.
2. The liquid composition of claim 1, wherein said alginate salt is sodium alginate.
3. The liquid composition of claim 1, wherein the concentration of said gelatin in said composition is about 50 to about 400 mg/ml, preferably about 180 to about 220 mg/ml; and the concentration of said alginate or salt thereof in said composition is about 3 to about 40 mg/ml, preferably about 8 to about 12 mg/ml.
4. The liquid composition of any one of claims 1 to 3, wherein said composition is foamed.
5. The liquid composition of claim 4, wherein the ratio between said polymers and the air in said foamed composition is in a range of about 0.2:1 to about 5:1, preferably about 2:1, by volume (polymers:air).
6. The liquid composition of any one of claims 1 to 5, further comprising a growth factor such as epithelial growth factor (EGF), or an acidic- or basic-fibroblast growth factor (FGF).
7. The liquid composition of any one of claims 1 to 6, wherein upon mixing with a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers, forms an injectable hydrogel having a gelation time in the range of 10 to 90 seconds; and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air.
8. The liquid composition of claim 7, wherein said hydrogel has a tensile strength in a range of about 0.5 to about 50 kPa; a young's modulus in a range of about 0.5 to about 50

kPa; a porosity in a range of about 10 to about 95%; and a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air.

9. The liquid composition of claim 7 or 8, wherein said water-soluble cross-linking agent is a carbodiimide, formaldehyde, glutaraldehyde, glyceraldehyde, genipin, a polyepoxide, an isocyanate, or an acyl azide.

10. The liquid composition of claim 9, wherein said carbodiimide is N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

11. The liquid composition of any one of claims 7 to 10, wherein the concentration of said cross-linking agent in said solution is about 1 to about 50 mg/ml, preferably about 8 to about 12 mg/ml.

12. A method for repairing or enhancing regeneration of a damaged/injured tissue of an internal organ in an individual in need thereof, said method comprising the steps of:

- (i) providing a liquid composition according to any one of claims 7 to 11, and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers; and

either:

- (ii) mixing said liquid composition with said solution to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately
- (iii) administering to said damaged/injured tissue of said individual a therapeutically effective amount of said hydrogel which turns into a three-dimensional scaffold *in situ*,

or

- (iv) concomitantly administering to said damaged/injured tissue of said individual an effective amount of each one of said liquid composition and said solution,

which are then mix and form a therapeutically effective amount of said hydrogel that turns into said three-dimensional scaffold *in situ*.

13. The method of claim 12, for closure of a broncho-pleural fistula or a trachea-esophageal fistula.

14. The method of claim 12, for closure of tears in the digestive mucosa.

15. The method of claim 12, for closure of a pharyngeal- or laryngeal tear.

16. A method for reducing lung volume in an individual in need thereof, said method comprising the steps of:

- (i) providing a liquid composition according to any one of claims 7 to 11, and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers; and

either:

- (ii) mixing said liquid composition with said solution to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately
- (iii) administering to the lung of said individual a therapeutically effective amount of said hydrogel which turns into a three-dimensional scaffold *in situ*,

or

- (iv) concomitantly administering to the lung of said individual an effective amount of each one of said liquid composition and said solution, which are then mix and form a therapeutically effective amount of said hydrogel that turns into said three-dimensional scaffold *in situ*.

17. The method of claim 16, wherein said administering is carried out using a catheter capable of passing through the working channel of a bronchoscope.

18. The method of claim 16, wherein said individual suffers from emphysema.
19. The method of any one of claims 12 to 18, wherein the concentration of said gelatin in said composition is about 50 to about 400 mg/ml, preferably about 180 to about 220 mg/ml; the concentration of said alginate or salt thereof in said composition is about 3 to about 40 mg/ml, preferably about 8 to about 12 mg/ml; and said water-soluble cross-linking agent is a carbodiimide such as EDC.
20. The method of claim 19, wherein said liquid composition is foamed, and the ratio between said polymers and the air in said foamed composition is in a range of about 0.2:1 to about 5:1, preferably about 2:1, by volume (polymers:air).
21. The method of claim 19 or 20, wherein said liquid composition further comprises a growth factor such as epithelial growth factor (EGF), or an acidic- or basic-fibroblast growth factor (FGF).
22. A liquid composition according to any one of claims 7 to 11, and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers, for use as a combination in repairing or enhancing regeneration of a damaged/injured tissue of an internal organ, wherein:
- (i) said liquid composition and said solution are first mixed to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and said hydrogel is then immediately administered to the damaged/injured tissue of the internal organ where it turns into a three-dimensional scaffold *in situ*, or
 - (ii) said liquid composition and said solution are concomitantly administered to the damaged/injured tissue of the internal organ, where they mix and form said hydrogel that turns into said three-dimensional scaffold *in situ*.

23. The liquid composition and the solution for use according to claim 22, wherein said repairing or enhancing regeneration of a damaged/injured tissue is (i) closure of a broncho-pleural fistula or a trachea-esophageal fistula; (ii) closure of tears in the digestive mucosa; or (iii) closure of a pharyngeal- or laryngeal tear.

24. A liquid composition according to any one of claims 7 to 11, and a solution comprising a water-soluble cross-linking agent capable of cross linking said polymers, for use as a combination in reducing lung volume, wherein:

- (i) said liquid composition and said solution are first mixed to form an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and said hydrogel is then immediately administered to the lung where it turns into a three-dimensional scaffold *in situ*, or
- (ii) said liquid composition and said solution are concomitantly administered to the lung, where they mix and form said hydrogel that turns into said three-dimensional scaffold *in situ*.

25. The liquid composition and the solution for use according to claim 24, wherein said administering is carried out using a catheter capable of passing through the working channel of a bronchoscope.

26. The liquid composition and the solution for use according to any one of claims 22 to 25, wherein the concentration of said gelatin in said composition is about 50 to about 400 mg/ml, preferably about 180 to about 220 mg/ml; the concentration of said alginate or salt thereof in said composition is about 3 to about 40 mg/ml, preferably about 8 to about 12 mg/ml; and said water-soluble cross-linking agent is a carbodiimide such as EDC.

27. The liquid composition and the solution for use according to claim 26, wherein said liquid composition is foamed, and the ratio between said polymers and the air in said

foamed composition is in a range of about 0.2:1 to about 5:1, preferably about 2:1, by volume (polymers:air).

28. The liquid composition and the solution for use according to any one of claims 26 or 27, wherein said liquid composition further comprises a growth factor such as epithelial growth factor (EGF), or an acidic- or basic-fibroblast growth factor (FGF).

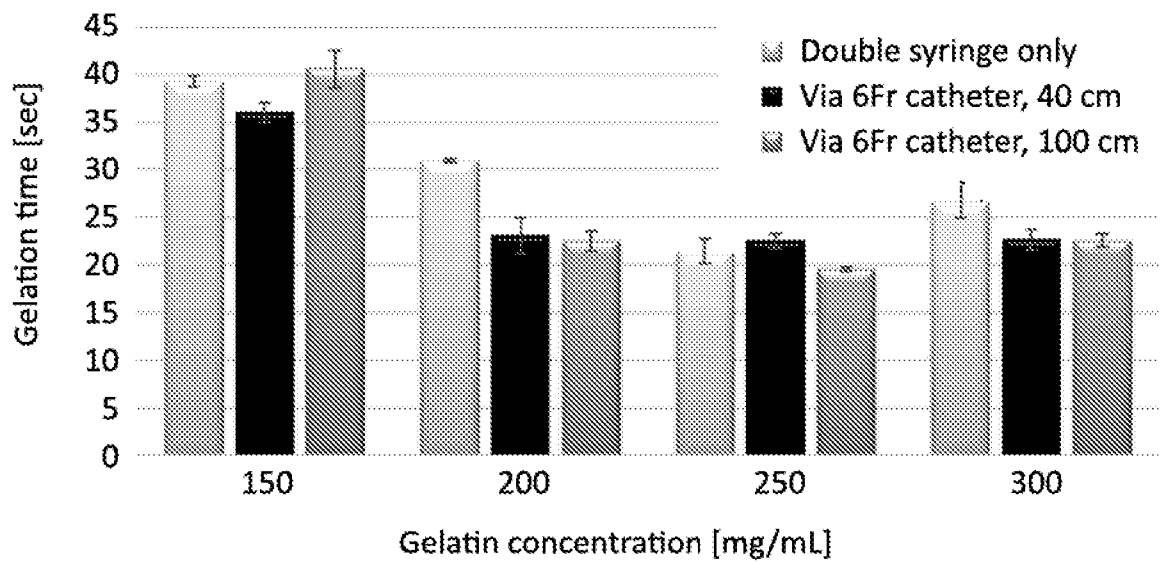
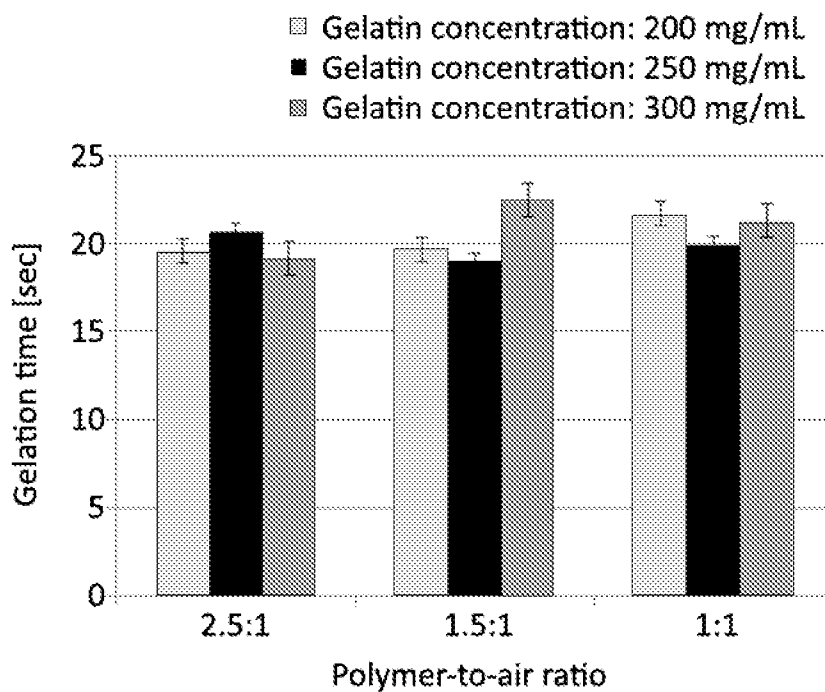
29. A kit comprising:

- (i) a liquid composition according to any one of claims 7 to 11;
- (ii) a water-soluble cross-linking agent capable of cross linking said polymers, wherein said cross-linking agent is in the form of either a powder or a solution; and
- (iii) instructions for dissolving said cross-linking agent, when formulated as a powder, in a predetermined volume of an aqueous liquid so as to form a solution of said cross-linking agent; and either
 - (a) mixing said liquid composition with said solution thereby forming an injectable hydrogel comprising said polymers cross-linked by said cross-linking agent, said hydrogel having a gelation time in the range of 10 to 90 seconds, and at least one of (a) a tensile strength in a range of about 0.5 to about 50 kilopascal (kPa); (b) a young's modulus in a range of about 0.5 to about 50 kPa; (c) a porosity in a range of about 10 to about 95%; and (d) a non-enzymatic degradation rate in a range of about 0.1% to about 50% per hour in water or about 0.005% to about 0.1% per hour in humid air; and immediately administering said hydrogel to a damaged/injured tissue of an internal organ, or to the lung, where it turns into a three-dimensional scaffold; or
 - (b) concomitantly administering said liquid composition and said solution, separately, to a damaged/injured tissue of an internal organ, or to the lung, where they mix and form said hydrogel that turns into a three-dimensional scaffold.

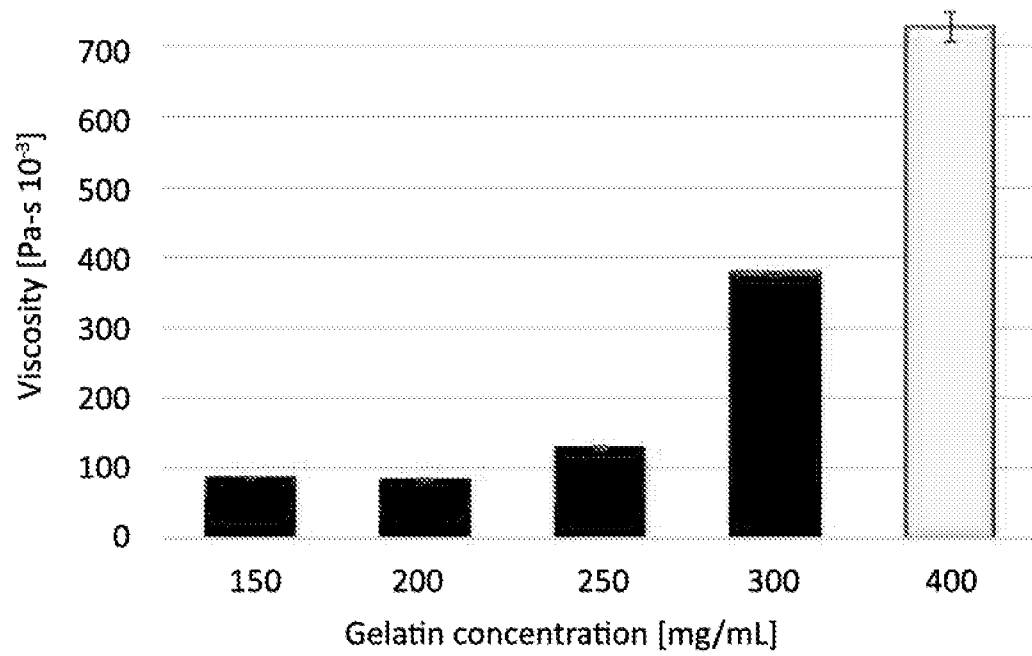
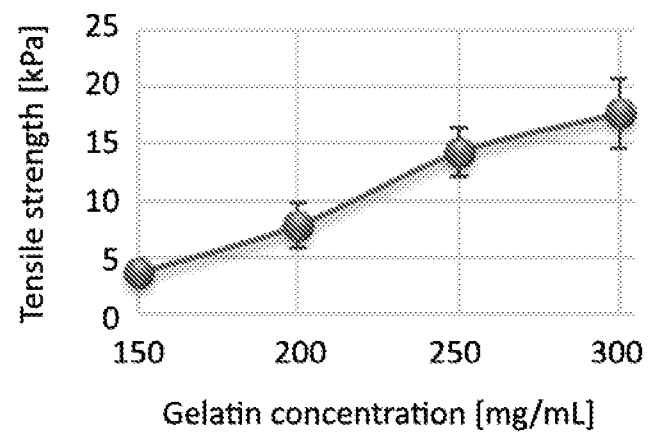
30. The kit of claim 29, wherein said liquid composition is contained in a syringe; or said cross-linking agent is in the form of a solution optionally contained in a syringe.

31. The kit of claim 29 or 30, further comprising a delivery mean for administering said hydrogel, or said liquid composition and said solution.
32. The kit of claim 31, wherein said delivery mean is a single-lumen catheter for administering said hydrogel, or a double-lumen catheter for administering said liquid composition and said solution.
33. The kit of claim 32, wherein said single-lumen catheter or double-lumen catheter is capable of passing through the working channel of a bronchoscope.

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Fig. 1**Fig. 2**

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Fig. 3**Fig. 4A**

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Fig. 4B

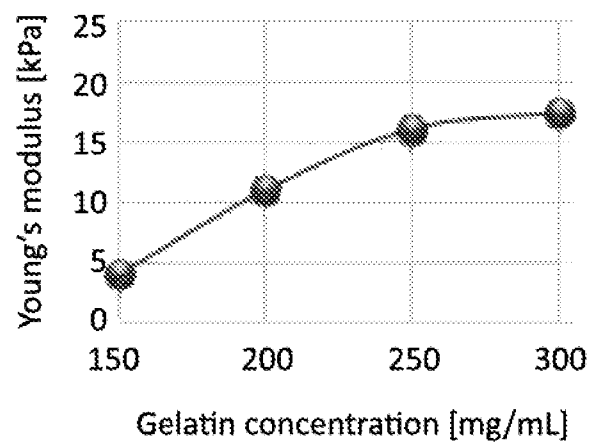
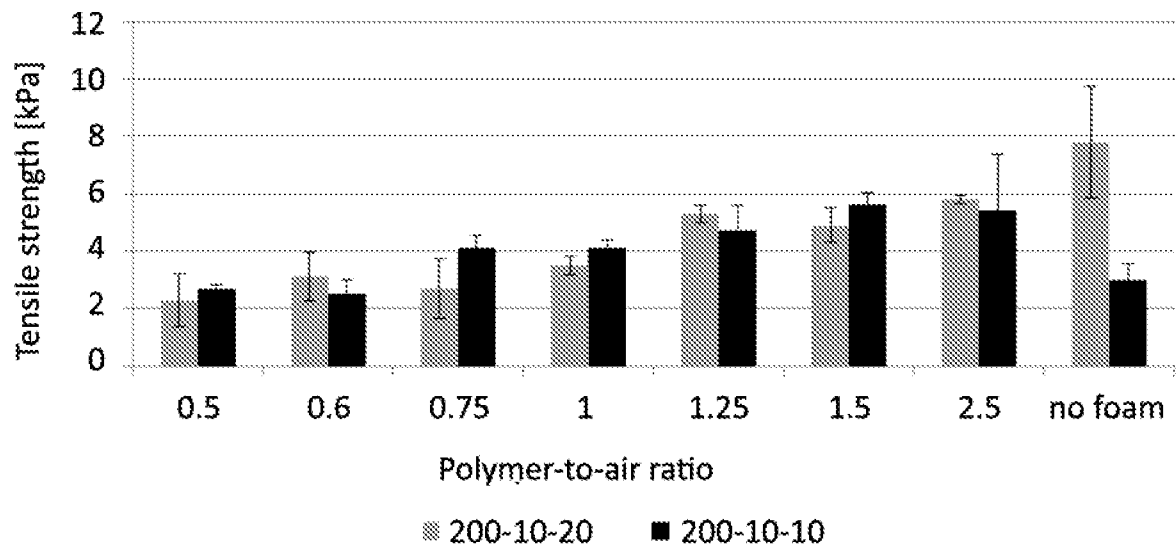
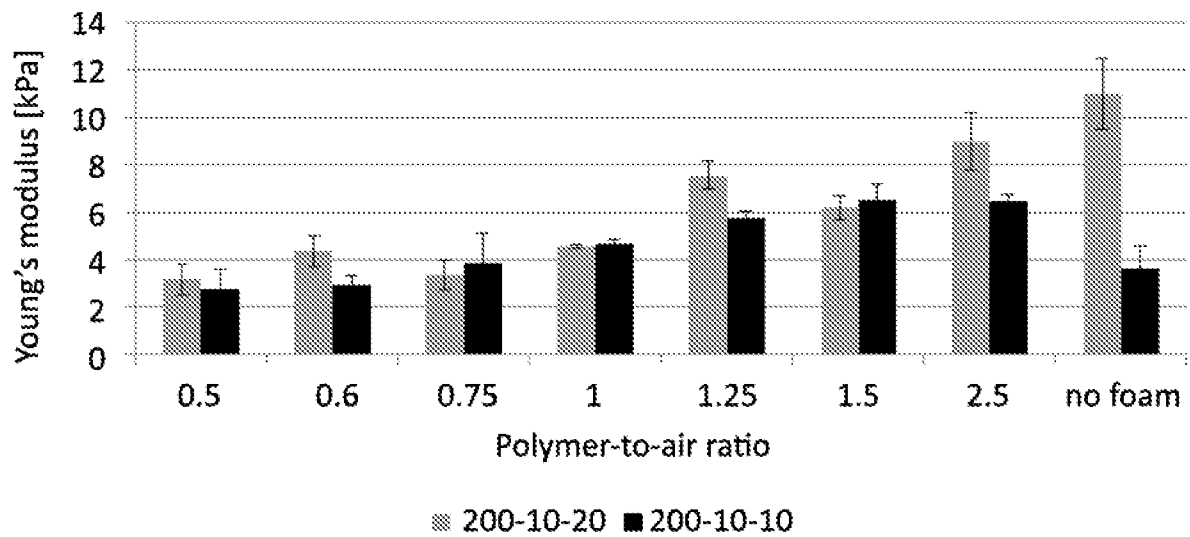
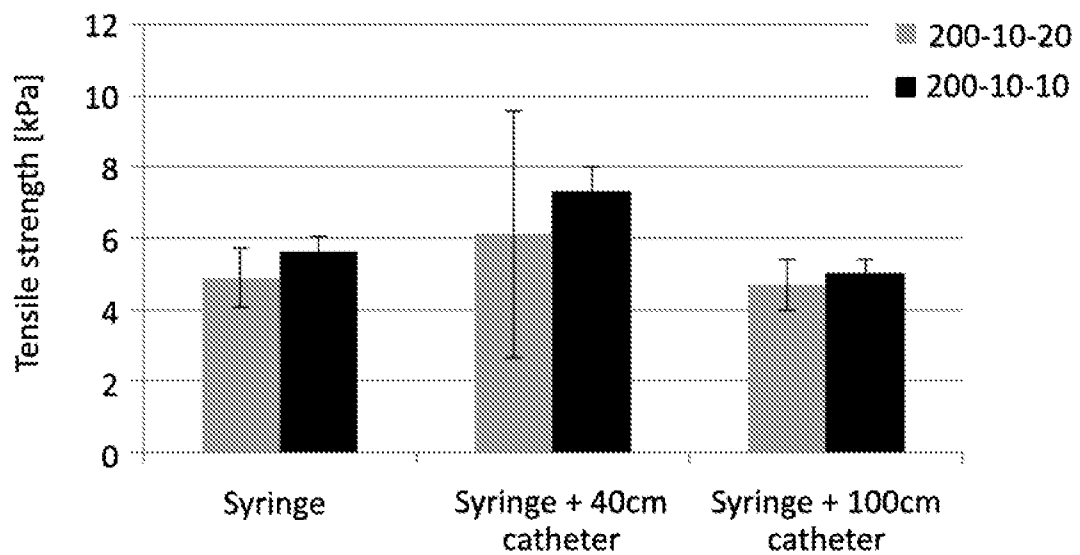


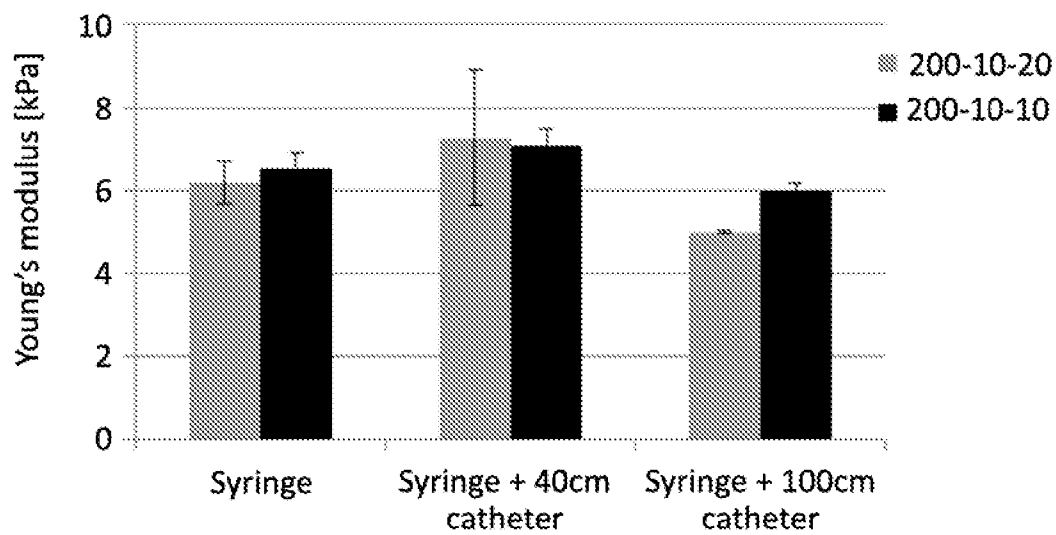
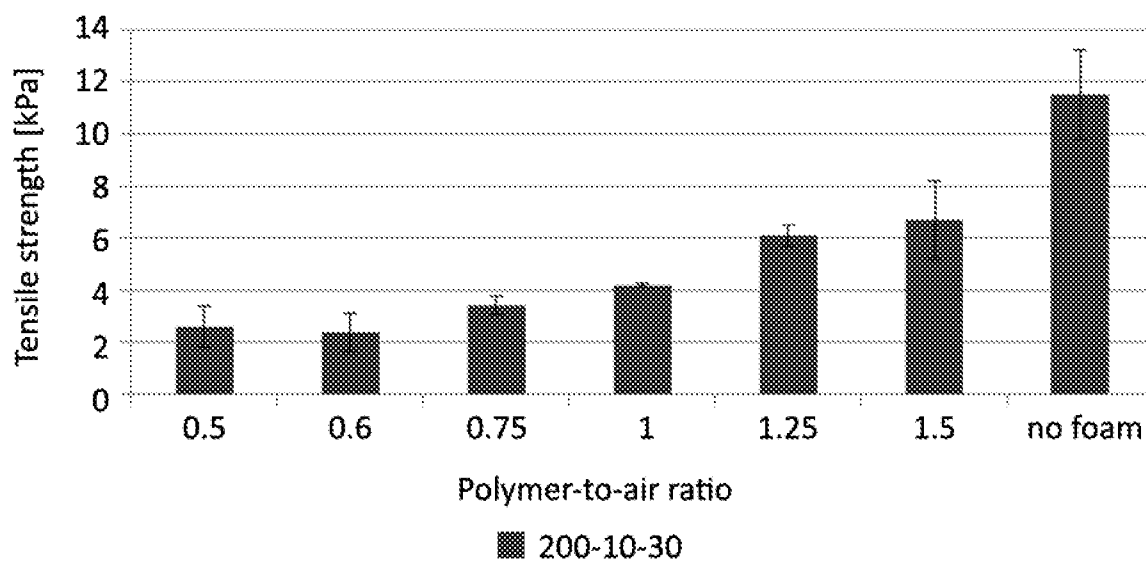
Fig. 5A



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Fig. 5B**Fig. 6A**

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Fig. 6B**Fig. 7A**

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Fig. 7B

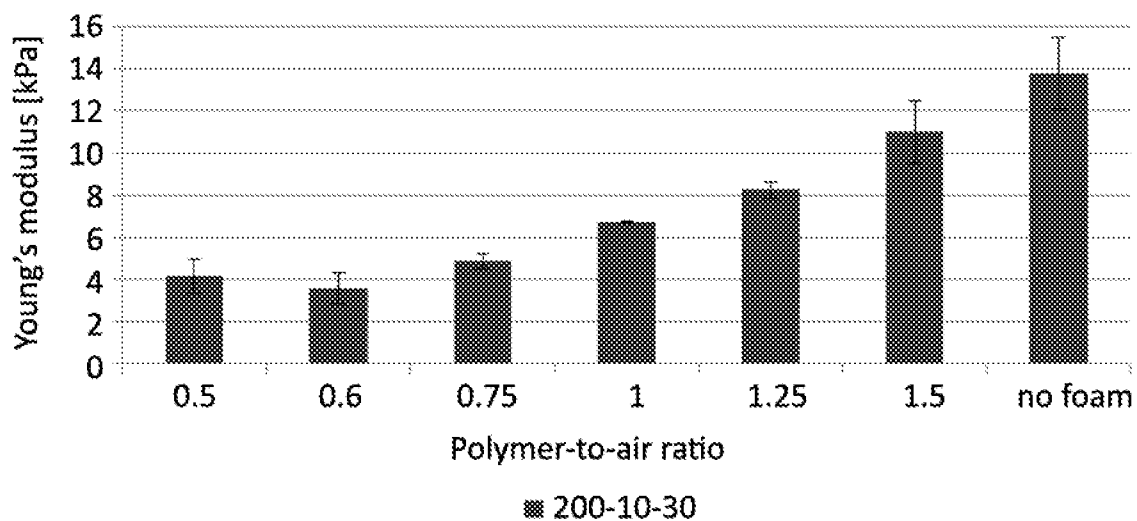
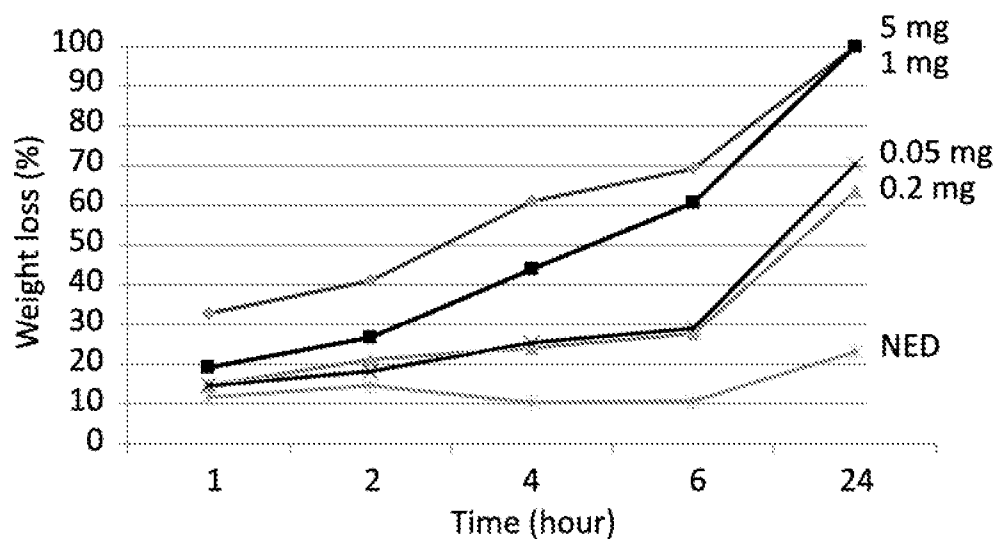


Fig. 8



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Fig. 9A

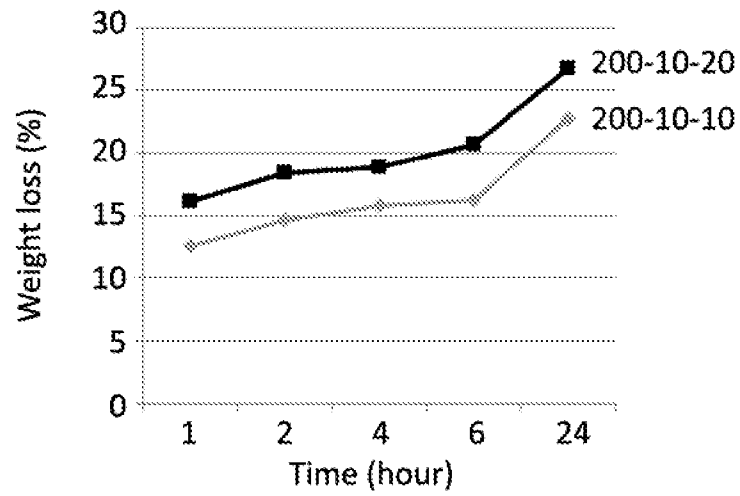
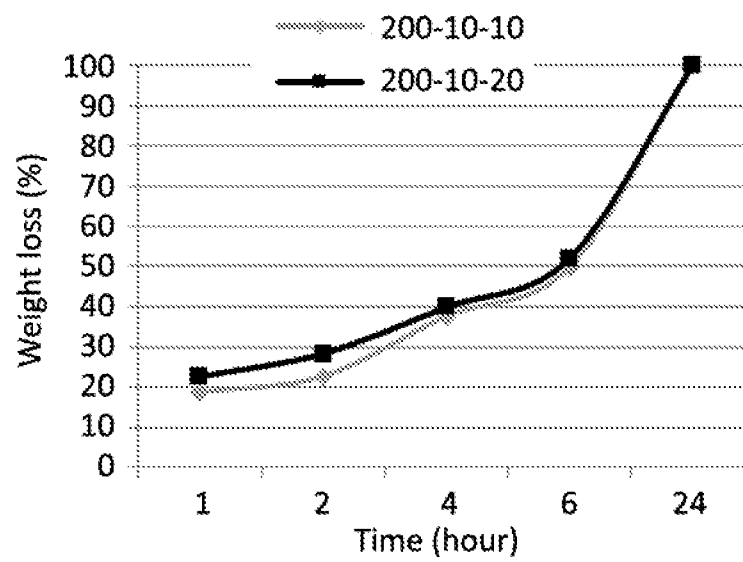


Fig. 9B



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Fig. 10A

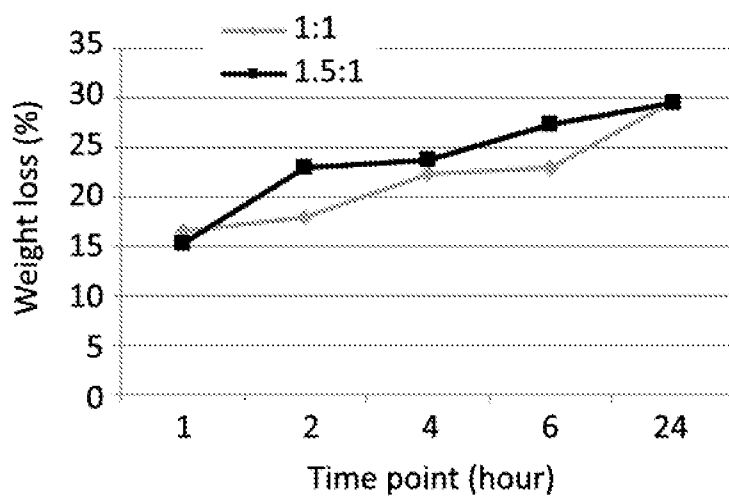
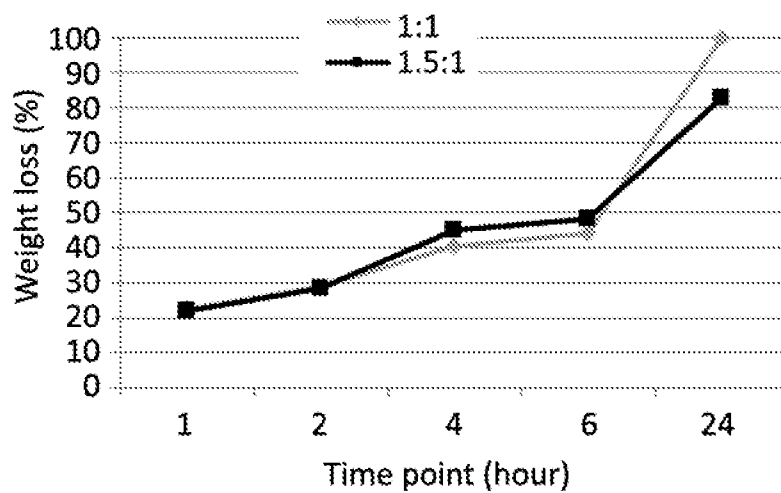


Fig. 10B



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Fig. 11A

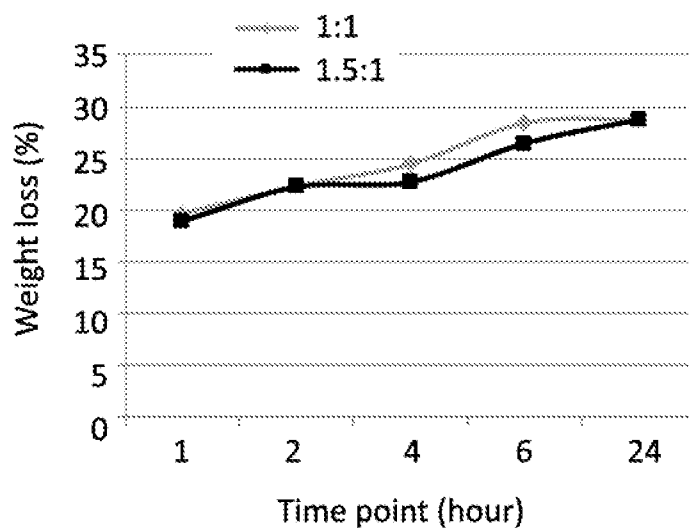
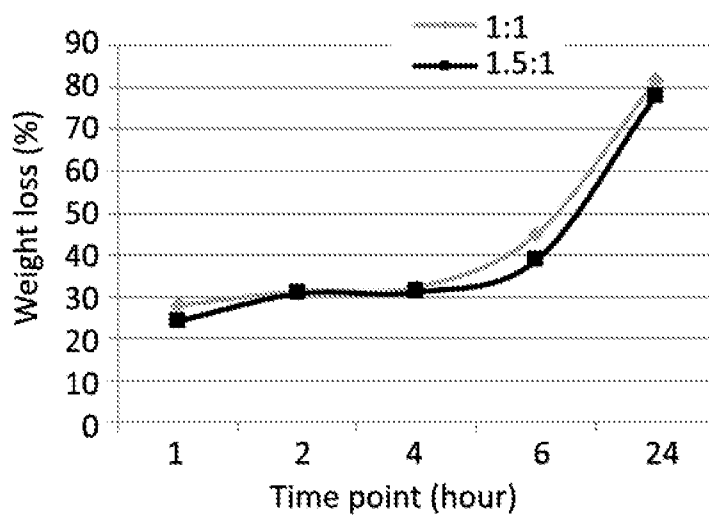


Fig. 11B



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Fig. 12

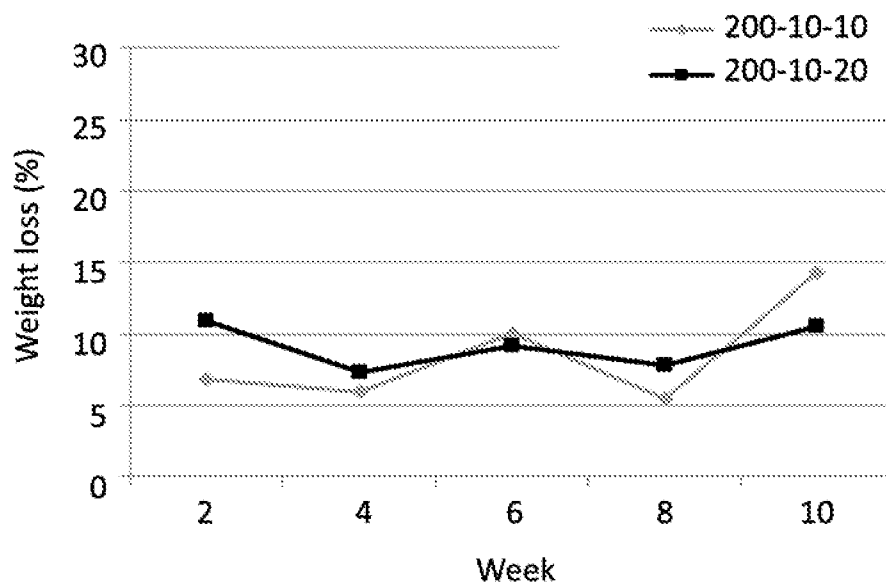
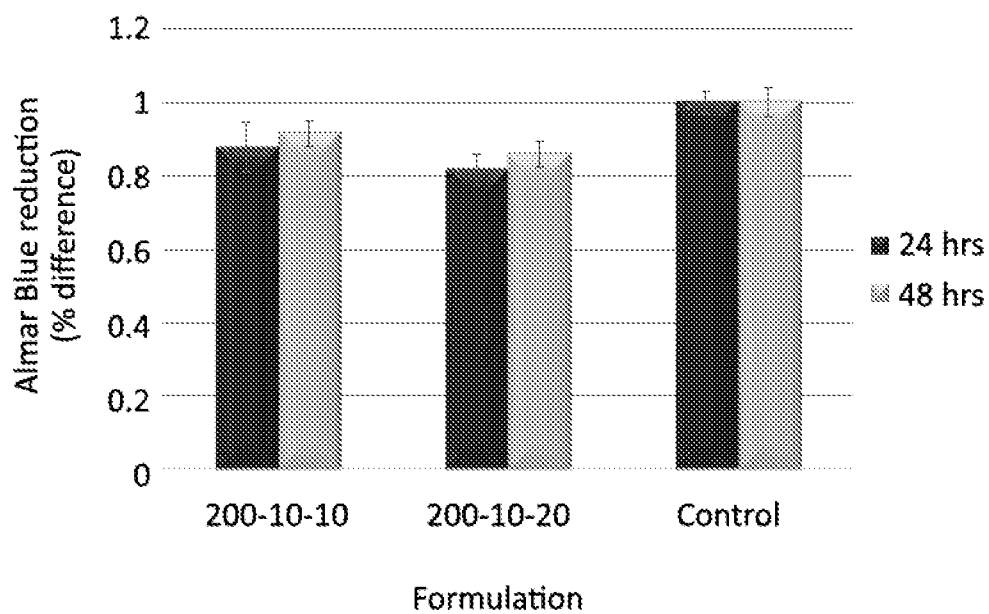
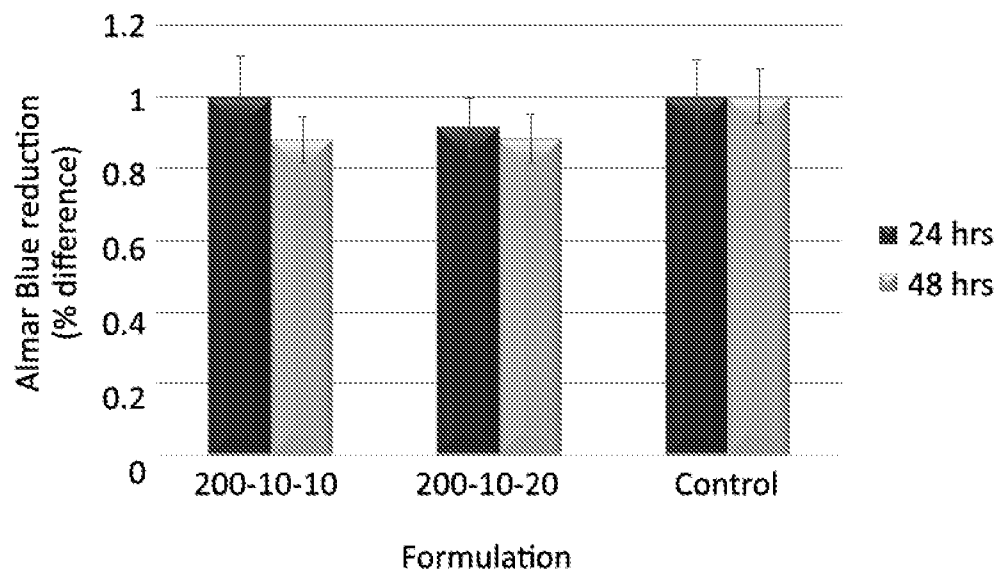
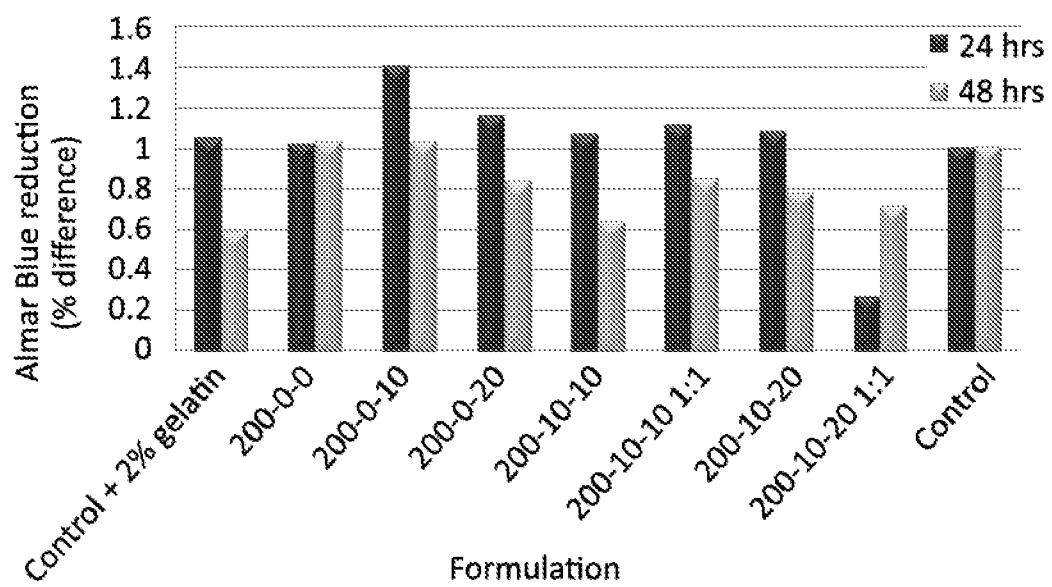


Fig. 13A

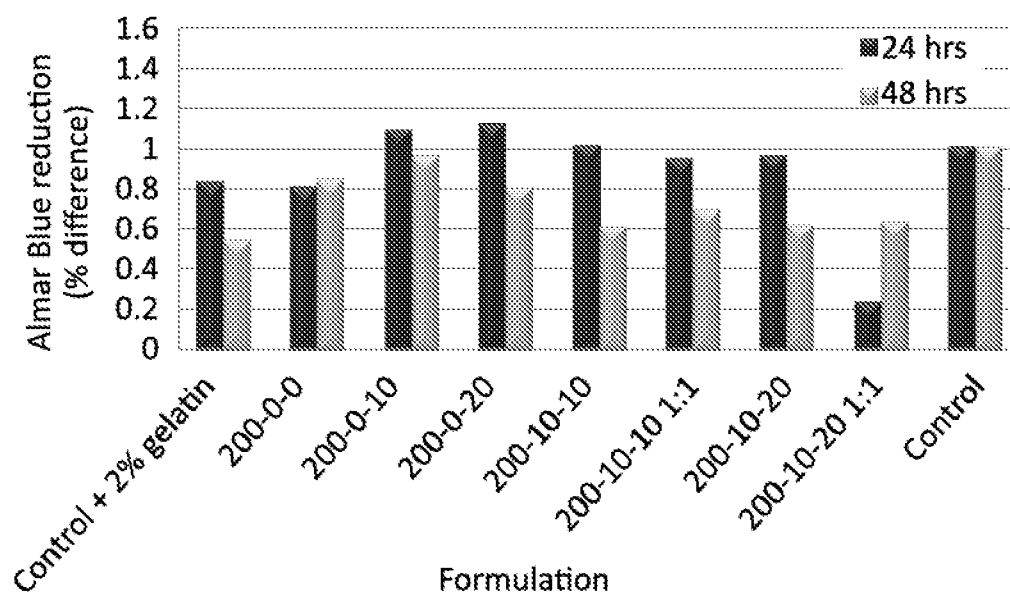


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Fig. 13B**Fig. 14A**

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Fig. 14B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2019/050130

A. CLASSIFICATION OF SUBJECT MATTER IPC (2019.01) A61L 24/10, A61B 17/12, A61K 9/00, A61L 31/14, A61M 16/04 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC (2019.01) A61B, A61K, A61L, A61M Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases consulted: Esp@cenet, Google Patents, Google Scholar, Orbit Search terms used: gelatin alginate low viscosity injection delivery emphysema		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2018197946 A1 ZILBERMAN MEITAL 01 Nov 2018 (2018/11/01) The whole document, in particular para. [0011], [0088], [0184]	1-3,6,9-11
X	EP 2609940 A1 TERUMO CORP 03 Jul 2013 (2013/07/03) Para.[0012], [0015]-[0016], [0019], [0028], [0053], [0095]	1-33
X	WO 2017165490 A1 HARVARD COLLEGE 28 Sep 2017 (2017/09/28) The whole document	1-33
A	Kretlow JD, Klouda L, Mikos AG. Injectable matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007; 59: 263-273. 30 May 2017 (2017/05/30) The whole document	1-33
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
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Date of the actual completion of the international search 10 Apr 2019		Date of mailing of the international search report 16 Apr 2019
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616		Authorized officer WIDLAN Fany Telephone No. 972-73-3927105

INTERNATIONAL SEARCH REPORT

International application No.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	CN 107050510 A UNIV NATIONAL DONG HWA 18 Aug 2017 (2017/08/18) The whole document	1-33
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