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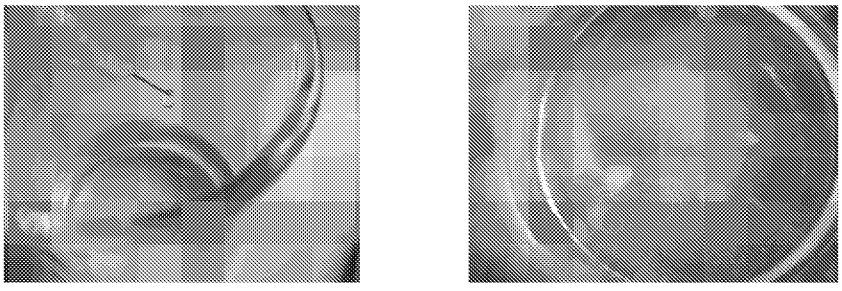

Figure 1A Figure 1B

Figure 1

(57) Abstract: The present invention describes neutral pH soluble collagen-glycosaminoglycan compositions and methods for augmenting soft tissue defects using the compositions. Soft tissue defects include dermal wrinkles and dermal folds, dermal contour unevenness and laxity and subdermal volume deficiencies. The compositions may also be used for and promoting cellular growth and stimulating tissue regeneration.

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SOFT TISSUE AUGMENTATION USING INJECTABLE, NEUTRAL PH SOLUBLE COLLAGEN-GLYCOSAMINOGLYCAN COMPOSITIONS

FIELD OF INVENTION

[0001] The present invention describes methods for augmenting soft tissue using injectable, soluble collagen in neutral pH and natural glycosaminoglycan compositions. This invention was inspired by natural extracellular matrix compositions of collagen and macromolecular glycosaminoglycan (such as hyaluronic acid). Collagen provides excellent biocompatibility for cell adhesion and tissue integration. And macromolecular glycosaminoglycan (like hyaluronic acid or heparosan, especially cross-linked hyaluronic acid or heparosan) with good water retaining property and mechanical properties contributed to the longevity of soft tissue argumentation effect.

[0002] The compositions may also be used for stimulating tissue regeneration. The compositions are chemically treated to produce temperature stable viscous solutions at neutral pH. Upon injection into tissues, the solutions rapidly undergo gelation and polymerization to form fibrous collagen matrices containing cross-linked or uncross-linked macromolecular glycosaminoglycan.

BACKGROUND

[0003] Collagen compositions have been utilized for more than 30 years to augment or smooth out soft tissue defects such as dermal wrinkles and dermal folds, to volumize furrows, or to correct dermal contour unevenness and laxity.

[0004] The collagen compositions utilized for soft tissue augmentation have been comprised of either reconstituted collagen fibrils prepared from solubilized collagen extracted from animal hides, reconstituted collagen fibrils prepared from soluble recombinant human collagen or intact collagen fibrils or fibers processed from human skin. In all cases the collagen composition has been composed of collagen fibrils/fibers or crosslinked collagen fibrils/fibers.

[0005] There are many references describing the application of collagen for soft tissue augmentation or for use as a dermal filler. Several key references are attached to this application. In addition there are many issued and pending patents referencing collagen for soft tissue augmentation. A list of these patents is also attached to this application.

[0006] Since soft tissues are primarily composed of collagen-based matrices, it makes sense to correct soft tissue defects with collagens or collagen-based compositions. There have been at least twelve FDA approved collagen products available for soft tissue augmentation in the U.S. since 1981. These products are generally called dermal fillers. However, at this time, most of the collagen-based fillers are no longer available in the U.S. market. They have been replaced by compositions that provide more durability including hyaluronic acid products, and products containing hydroxyapatite microbeads, poly-L-lactic acid particles, and polymethylmethacrylate microspheres.

[0007] There is still interest in having improved collagen-based compositions available for soft tissue augmentation because collagen serves as a scaffold capable of supporting cell attachment and cell proliferation, tissue integration *in vivo* through bioactive adhesion sites. The weakness of collagen-based composition of soft tissue augmentation is that collagen-based soft tissue filler generally undergo degradation and lost its augmentation effect in 3 to 6 months. Therefore, the compositions must exhibit increased durability.

[0008] Crosslinked macromolecular glycosaminoglycans like crosslinked hyaluronic acid are widely used for soft tissue augmentation because of its longevity and excellent safety profile. However, because macromolecular glycosaminoglycans lack cell adhesion, they are usually 'inert' to cell or tissue integration. (Figure 9). Combining collagen and macromolecular glycosaminoglycan was a strategy to develop soft tissue scaffold with cellular growth promoting properties and long duration in tissue space reducing lines, folds, fine lines, wrinkles, or scars, or a combination thereof. Dr. Oded Shoseyov and his colleagues invented photoinitiated dermal fillers, hyaluronic acid-collagen double crosslinked dermal fillers (US Patent No. 17/052216 assigned to Collagen Ltd). Light was applied to the surface of the epidermis superficial to induce polymerization of the combination including photoinitiator described in the patent.

[0009] Collagen is sensitive to temperature and ionic strength which drives spontaneous gel formation at proper temperature, under physiological conditions. The present invention describes methods for augmenting soft tissue using collagen- glycosaminoglycan compositions in the form of a viscous, biocompatible gel that can be easily injected through small needles (e.g., 27 gauge) and upon injection into tissues, rapidly undergoes gelation and fibril formation. The formed collagen- glycosaminoglycan matrix exhibits unique properties that prolong durability beyond that of any of the current injectable collagen fillers, and promoting cell ingrowth, tissue integration, healing or replacement due to degradation or injury of a collagen-comprising tissue beyond any of the current injectable hyaluronic acid products, and products containing hydroxyapatite microbeads, poly-L-lactic acid particles, and polymethylmethacrylate microspheres.

SUMMARY OF INVENTION

[0010] The disclosure herein relates to an injectable soft tissue filler comprising derivatized collagen or *in situ* polymerizing collagen and glycosaminoglycan, form a cellular growth promoting scaffolds, as well as methods of using the soft tissue fillers in some instances, for soft tissue augmentation.

[0011] In one aspect of the present application, provided herein is a composition for soft tissue augmentation comprising: (i) neutral pH soluble collagen; and (ii) glycosaminoglycan; and (iii) optionally, other active ingredients, wherein the neutral pH soluble collagen was mixed with glycosaminoglycan.

[0012] In some embodiments, the neutral pH soluble collagen is selected from the group consisting of derivatized collagen or in situ polymerizing collagen, or a combination thereof. In some embodiments, the glycosaminoglycan is selected from the group consisting of crosslinked and/or non-crosslinked glycosaminoglycan.

[0013] In some embodiments, said other active ingredients is selected from the group consisting of:

(a) a plasma or a platelet-rich plasma or at least one growth factor comprises plasma or platelet-rich plasma, preferably in a concentration of 1%~50% by weight;

(b) cell free fat extract or at least one growth factor comprises cell free fat extract, preferably in a concentration of 0.1%~5% by weight;

(c) cell free stem cell extract or at least one growth factor comprises cell free stem cell extract, preferably in a concentration of 0.1%~5% by weight;

(d) Extracellular Vehicles (EVs), secreted by stem cells, preferably in a concentration of 0.1%~5% by weight;

(e) essential ammio acids or at least one essential amino acid, preferably in a concentration of 0.1%~5% by weight;

(f) polynucleotide(PN) and/or polydeoxyribonucleotide (PDRN) extracted from the sperm cells of Oncorhynchus mykiss (Salmon trout) or Oncorhynchus keta (Chum Salmon) with a molecular weight ranging from 50 to 1500 kDa, preferably in a concentration of from 0.1~2% by weight;

(g) local anesthesia drugs such as lidocaine, procaine, preferably in a concentration of from 0.1% to 0.5% by weight;

(h) stabilizer or dissolution promotor, such as Methyl sulfonyl methane (MSM), preferably in a concentration of from 0.1% to 5% by weight; and

(i) any combinations thereof.

[0014] In some embodiments, the ratio of glycosaminoglycan to the neutral pH soluble collagen is between 10:1 to 1:10. In some embodiments, the concentration of glycosaminoglycan is in a range between 5 to 50 mg/ml.

[0015] In some embodiments, the source of collagen is selected from allogenetic tissue, mammal tissue (usually porcine, bovine, equine hides OR tendon) or marine species or axolotl hides derived matrix.

[0016] In some embodiments, the collagen is selected from full collagen or atelocollagen, or recombinant collagen or recombinant collagen peptides from microorganism, plants, insect cells or animal cells, or collagen mimic peptides.

[0017] In some embodiments, the derivatized collagen is derivatized with acetylation agents that alter the pKa of collagen and has one or more of the following features: (a) soluble at neutral pH (such as 6.5-7.5); (b) does not undergo fibrillogenesis at physiological pH; and/or (c) precipitates at acidic pH (such as 3.5-5.5, preferred 4.0~5.0).

[0018] In some embodiments, the derivatized collagen is derivatized with one or more agents selected from the group consisting of glutaric anhydride, succinic anhydride, maleic anhydride, citric acid anhydride, oxalic acid anhydride and ethylenediamine tetraacetic anhydride.

[0019] In some embodiments, the neutral pH soluble collagen forms rapidly polymerizing collagen gels as described in US10,111,981B2.

[0020] In some embodiments, the rapidly polymerizing collagen gels comprises a neutralized solution comprising an acid soluble collagen, EDTA/EGTA and a polyol, and wherein the acid soluble collagen comprises collagen selected from the group consisting of Type I collagen, Type II collagen, Type III collagen and combinations thereof.

[0021] In some embodiments, the acid soluble collagen in a concentration between 5 and 70 mg/ml. In some embodiments, said EDTA is disodium EDTA; and/or said EGTA is disodium EGTA. In some embodiments, EDTA or EGTA is in a concentration between 10 and 50 mM. In some embodiments, said polyol is a sugar alcohol, such as D-mannitol. In some embodiments, polyol is in a concentration between 2.5% and 4% (w/v). In some embodiments, said rapidly polymerizing collagen gels further comprises a disaccharide, fructose, or combinations thereof. In some embodiments, said rapidly polymerizing collagen gel has an osmolality of 280-360 mmol/kg.

[0022] In some embodiments, the glycosaminoglycan is one or more selected from the group consisting of hyaluronic acid, heparosan, heparin, chondroitin sulfate, dermatan sulfate, keratan sulfate, and any combinations thereof.

[0023] In some embodiments, the glycosaminoglycan is derived from allogenetic tissue, mammal tissue or marine species; and/or is produced through microbial fermentation.

[0024] In some embodiments, the molecular weight of glycosaminoglycan before crosslinking is from 1000Da~10000000Da.

[0025] In some embodiments, the crosslinker crosslinking Glycosaminoglycan are independently selected from 1,4-butanediol diglycidyl ether (BDDE), 1-[3- (Dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDC), polyethyleneglycol diglycidyl ether (PEGDE), N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC), Diepoxyoctane (DEO), Divinyl Sulfone (DVS) , glutaraldehyde, or p-phenylene biscarbodiimide or 1,2,7,8-diepoxyoctane, or Polyethylene glycol (PEG), or oligomers rich in amino groups (such as poly-lysine or poly-arginine or γ-polyglutamic acid) or combinations thereof.

[0026] In some embodiments, hyaluronic acid is selected from oligo-hyaluronan, hyaluronic acid produced by microbial fermentation using Streptococcus species or Bacillus species, or allogenetic or animal tissues (including rooster combs, human umbilical cord, bovine synovial fluid or vitreous humor) derived hyaluronic acid.

[0027] In some aspect of the present application, provided herein is a method of preparing a composition comprising (i) neutral pH soluble collagen; (ii) glycosaminoglycan; and (iii) optionally, other active ingredients, said method comprises one or more step selected from:

(a) combining part (i) with part (ii), for example by adding part (ii) to part (i) by utilizing vacuum planetary mixer, to form an injectable homogeneous gel, preferably with a revolution speed of 200 rpm~ 1,400 rpm and an autorotation speed of 100 rpm~ 700 rpm, preferably with a mixing time of 10~30 minutes with vacuum under sterile condition; or

(b) adding ethanol precipitated part (ii) to a salt or pH precipitate of part (i) and adding part (iii) (if present) and re-solubilizing the combination by dialysis or diafiltration or ultrafiltration process to form a homogeneous injectable gel; or

(c) combining part (i) ,part (ii) and part (iii) (if present), for example by sterile freeze-drying part (i) and part (ii) and part (iii) , and re-solubilizing the mixture of lyophilized part (i) ,part (ii) and part (iii) and dialyzing the combination to neutral pH form a homogeneous injectable gel .

[0028] In some aspect of the present application, provided herein is a method for augmenting soft tissue or inducing a cellular growth promoting scaffold in a tissue space under an epidermis in a subject in need thereof, comprising administering the composition of claim 1 to a site in need of the augment or induction..

[0029] In some embodiments, the composition is injected into soft tissue to correct soft tissue deficiencies. In some embodiments, the composition is injected into dermis to correct soft tissue deficiencies including wrinkles, dermal folds, dermal laxity, unevenness, facial emaciation, fat atrophy, cheek depression, eye socket depression, or a combination thereof. In some embodiments, the composition is injected into tissues other than dermis, including cartilage, to correct tissue deficiencies.

[0030] In some embodiments, the composition is injectable through a 25~30 gauge needle or cannula, such as a 25, 27 or 30 gauge needle or cannula.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0031] In the following, aspects of the invention will be elucidated by means of examples, with reference to the drawings. The drawings are diagrammatic and may not be drawn to scale. The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0032] Figure 1 shows *In situ* collagen polymerization in saline solution. Figure 1A: Injection of in situ polymerizable collagen into saline; Figure 1B: 60 seconds after injection of in situ polymerizable collagen in saline solution.

[0033] Figure 2 shows a photograph of Collagen-HA composition (80% In situ polymerizing collagen + 20% HA gel).

[0034] Figure 3 shows a photograph of Collagen-HA composition (80% In situ polymerizing collagen + 20% HA gel) polymerized in 2 minutes after injected into 37° buffer solution.

[0035] Figure 4 shows a photograph of Collagen - crosslinked-HA composition (50% In situ polymerizing collagen + 50% CXL-HA gel) polymerized in 2 minutes after injected into 37° buffer solution.

[0036] Figure 5 shows a TEM image of polymerized collagen-crosslinked-HA composition.

[0037] Figure 6 shows a TEM image of collagen fibrils of polymerized collagen-crosslinked-HA composition.

[0038] Figure 7 shows H&E stain of collagen-crosslinked-HA composition implant in rabbit ear (10X magnification).

[0039] Figure 8 shows H&E stain of collagen-crosslinked-HA composition implant in rabbit ear. Arrows showed cell ingrowth induced by the presence of collagen (20X magnification).

[0040] Figure 9 shows H&E stain of Crosslinked-HA composition implant in rabbit ear. Very few cell ingrowth in the implantation (20X magnification).

[0041] Figure 10 shows Injection force of derivatized collagen- heparosan-PRP composition measured by UTM.

DETAILED DESCRIPTION OF THE INVENTION

[0042] Described herein is a method for augmenting soft tissues using a combination of a soluble collagen and glycosaminoglycan especially hyaluronic acid capable of rapid polymerization when in contact with or mixed with tissue fluids. This method of using this collagen-glycosaminoglycan composition may augmenting soft tissues, such as correcting skin contour defects, or for enhancing soft tissue regeneration.

[0043] The *in situ* polymerizing collagen is a clear, viscous, soluble collagen at neutral pH that upon interacting with tissue fluids, instantly forms a cohesive clear gel that rapidly undergoes fibril formation to form an opaque collagen matrix which was described in US10111981B2 and US11235089B2. Upon injection into tissue, such as dermal tissue, the *in situ* polymerizing collagen forms a fibrous mass that has been shown to retain volume for time periods beyond 6 months.

[0044] The base collagen used to prepare the *in situ* polymerizing collagen may be extracted from animal hides, such as bovine hide or porcine hide, or may be cell derived human collagen, or recombinant human collagen. It is preferred that the base collagen be available in acid solution. Any acid soluble, fibril forming collagen type may be used. However, Type I, Type II, Type III collagen or their combination are preferred to prepare the *in situ* polymerizing collagen.

[0045] Particularly preferred collagen compositions for use in the invention are described in DeVore & Eiferman (US Patent No. 5,492,135 assigned to Euclid Systems Corporation). These

collagen compositions are initially soluble in form and, upon exposure to physiological fluids *in vivo*, undergo rapid polymerization. Such collagen solutions have been prepared at concentrations ranging from 10 mg/ml to over 70 mg/ml and at a pH ranging from 6.0-8.0.

[0046] In some embodiments of the invention, a neutralized, acid solubilized collagen, which remains in solution at physiological temperatures, is used to prepare *in situ* polymerizing collagen for soft tissue augmentation. These solutions must be extensively dialyzed against EDTA solutions and/or deionized water to reduce available cations and to prevent premature collagen fibrillogenesis. As the cations are removed, the pH of the collagen solution is increased to between about 6.8 and about 7.5 by adjusting the pH of the EDTA solution using 1N sodium hydroxide. The collagen preparation does not undergo typical fibrillogenesis in the absence of added unbound or free cations

[0047] In preferred embodiments, upon administration of the soluble collagen, the solution is converted to a gel or polymerized into a collagen fibrillar mass within 180 seconds, more preferably, within 120 seconds, most preferably, within 90 seconds. Preferably, the collagen-based solution is at a concentration of between 0.1-10%, more preferably, 0.5-7%, and most preferably between 2-5% collagen solids (w/v).

[0048] Glycosaminoglycan, also known as mucopolysaccharide, is a class of negatively-charged polysaccharide compounds. They are composed of repeating disaccharide units that are present in every mammalian tissue. Glycosaminoglycan is highly biological compatible. Glycosaminoglycan, such as hyaluronic acid and heparosan now can be produced from microbial fermentation and are widely used as soft tissue augmentation and intraarticular viscosupplement. And the addition of glycosaminoglycan or crosslinked Glycosaminoglycan does not affect *in situ* polymerizing collagen polymerization property. Accordingly, it is an object of the invention to provide a method for using a neutralized, acid solubilized collagen-glycosaminoglycan solution suitable for use in soft tissue augmentation. When such compositions are injected into tissues, they quickly undergo gel formation and subsequent rapid fibrillogenesis when contacted with tissue fluids containing cationic constituents such as sodium chloride.

[0049] The composition has been injected into rabbit ear and examined histologically for biocompatibility. Results demonstrated the collagen-glycosaminoglycan composition implant has improved durability comparing to collagen based implant with little to no reduction in original injection volume.

[0050] Another neutral pH soluble collagen solution is derivatized collagen in which the isoelectric point of collagen was altered from around 7 to 4 by the acylation of collagen.

[0051] Acylation reactions have been used to derivatize soluble and insoluble collagen and have been described by DeVore, et. al. in a series of patents (U.S. Pat. Nos. 4,713,446; 4,851,513; 4,969,912; 5,067,961; 5,104,957; 5,201,764; 5,219,895; 5,332,809; 5,354,336; 5,476,515; 5,480,427; 5,631,243; 6,161,544 and 17,744,428). However, none of these patents describe the use of chemically derivatized collagen combined with glycosaminoglycan substances, such as to treat soft tissue deficiencies or defects.

[0052] In the present invention, the chemically modified collagen- glycosaminoglycan compositions can be injected into superficial dermis, mid-dermis, or deep dermis to correct contour defects in facial skin or such compositions can be injected into the loose connective tissue surrounding lip muscle or into the body of the lip to enhance lip appearance. The collagen compositions are injectable through a 30 gauge needle. The material remains colorless and provides a long-lasting clinical effect. The collagen compositions can be prepackaged in ready-to-use syringes containing materials exhibiting several different degrees of durability

Definitions:

[0053] By "collagen" is meant all forms of collagen including those which have been processed or modified. The collagen may be of human or animal origin or may be produced using recombinant techniques. The present invention can use these and other typed of collagen including natural collagen and various collagen derivatives.

[0054] By "tissue" is meant an aggregation of similarly specialized cells in an organism, preferably, mammalian, and, most preferably, human, where the cells are exposed to the organism's extracellular fluid, and are united in performance of a function within an organism.

[0055] By "*in situ* polymerization" is meant formation of a collagen gel and subsequently a collagen fibrous mass, upon injection of soluble collagen into tissue.

[0056] The present invention provides a number of advantages. For example, the collagen compositions described herein are biocompatible, biodegradable, and stable in solution at neutral pH. The ability to chemically manipulate the collagen to form a neutral stable solution allows for injectable administration through a fine gauge needle (e.g., a 30 or 31 gauge needle). In addition to the ease of application, injectable delivery of the collagen solution allows access to the administration site while minimizing invasive injury to surrounding tissues. The density of the collagen solution is sufficient to fill a soft tissue defect or other specific delivery site and remain in place until gelation and fibril formation occurs, and maintenance of soft tissue augmentation for at least 6 months.

EXAMPLES

[0057] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1. Preparation of *in situ* polymerizing collagen solution

[0058] The *in situ* polymerizing collagen was prepared using methods described previously by DeVore and Eiferman (US Patent 5,492,135; assigned to Euclid Systems Corporation). Pure soluble Type I collagen was purchased from Advanced BioMatrix, Inc. Sodium chloride was added to the soluble, pepsin-digested collagen solution (3mg/mL) to a concentration of 0.8M to precipitate collagen. The white, opaque precipitate was recovered by centrifugation for 30 minutes at 3500 RPM and concentrated to approximately 50mg/mL by placement on filter paper. The concentrated collagen precipitate was placed in dialysis tubing with a MW cut off of 100,000 and dialyzed against 0.1N HCl for 16-18 hours. The resulting clear, viscous, redissolved collagen concentrate was then dialyzed against 0.035M EDTA (ethylenediaminetetraacetic acid, disodium salt dihydate, SigmaUltra ~99%). Dialysis was continued for 5 days with daily adjustment of pH from the starting pH of 4.5 to a final pH of 7.5. The final clear and viscous collagen concentrate was collected and centrifuged to remove air bubbles. The final clear, viscous collagen exhibited a pH of 7.4 and did not undergo fibril formation at room temperature. Collagen fibrillogenesis was not triggered by pH or temperature.

[0059] Evaluation of gelation and fibril formation.

Aliquots of the *in situ* polymerizing collagen were injected into 0.8M sodium chloride at 37°C and observed for the appearance of gel and fibrous collagen. As shown in Figure 1 the clear viscous collagen solution formed a white, opaque collagen matrix in less than 60 seconds.

Example 2. Evaluation of gelation and fibril formation of *in situ* polymerizing collagen-hyaluronic acid composition

[0060] The *in situ* polymerizing collagen-hyaluronic acid composition was made by directly mixing 24mg/mL *in situ* polymerizing collagen with 12mg/mL hyaluronic acid PBS solution at 80:20 by weight and centrifuging at 6000rpm to remove bubbles (Figure 2). And the *in situ* polymerizing collagen-hyaluronic acid composition was injected into 0.8M sodium chloride at 37°C and observed for the appearance of gel. As shown in Figure 3 the clear viscous solution formed a white, opaque matrix in less than 120 seconds.

Example 3. Evaluation of gelation and fibril formation of *in situ* polymerizing collagen-crosslinked hyaluronic acid composition

[0061] The *in situ* polymerizing collagen-hyaluronic acid composition was made by directly mixing 24mg/mL *in situ* polymerizing collagen with commercial crosslinked hyaluronic acid (Restylane®) at 50:50 by weight and centrifuging at 6000rpm to remove bubbles. And the *in situ* polymerizing collagen-crosslinked hyaluronic acid composition was injected into 0.8M sodium chloride at 37°C and observed for the appearance of gel. As shown in Figure 4 the clear viscous solution formed a slightly opaque gel. Transmission electron microscopy images (Figures 5 & 6) were taken for the gel, and collagen fibril structures were observed.

Example 4. Evaluation of biological compatibility and cell/tissue integration of *in situ* polymerizing collagen-crosslinked hyaluronic acid composition in rabbit ear

[0062] Five New Zealand rabbits were housed following the protocols from the guidelines for the use of laboratory animals. Up to 0.25 ml of *in situ* polymerizing collagen-crosslinked hyaluronic acid composition was injected via 27 or 25 gauge needle. After 4 weeks, two rabbits were euthanized followed by harvesting of the entire ear. Each ear was placed in formalin for histology. The implants were cut in the cross-section of maximum height and tissue block. Hematoxylin and eosin (H&E) stained slides at 10x (Figure 7) and 20x magnification (Figure 8) were examined to evaluate biological compatibility of *in situ* polymerizing collagen-crosslinked hyaluronic acid composition. Cellular infiltration induced by the collagen composition was observed by injection of the *in situ* polymerizing collagen-crosslinked hyaluronic acid composition, whereas the injection of Crosslinked-HA composition shows very few cell ingrowth in the implantation (Figure 10).

[0063] The rabbit ear thickness, total thickness, implantation length and width in the rest three rabbits were measured three times by the same person with a vernier caliper right after implantation, 1 week, 4 weeks, 8 weeks and 12 weeks after implantation. The mean of the three measurements were used. The height of the implantation was calculated through total thickness of the implantation and rabbit ear minus rabbit ear thickness. And the volume was calculated using the ellipsoid volume formula. With cell ingrowth induced by collagen, the implantation of collagen-crosslinked hyaluronic acid showed a better augmentation effect and long duration comparing to crosslinked hyaluronic acid alone.

Table 1. Estimate height and volume of *in situ* polymerizing collagen-crosslinked hyaluronic acid and crosslinked hyaluronic acid implantation in rabbit ears.

	Estimated Height of the implantation (mm)					Estimated Volume of the implantation (mm^3)				
	0	1 week	4 weeks	8 weeks	12 weeks	0	1 week	4 weeks	8 weeks	12 weeks
	rabbit 1									
Collagen +HA	4.16	2.45	2.68	1.96	2.73	347.20	157.86	252.36	203.06	217.89
HA	1.67	1.32	1.43	1.27	0.87	125.60	96.27	119.34	96.75	66.65
	rabbit 2									
Collagen +HA	3.98	2.48	3.63	4.75	3.19	258.59	182.82	381.71	624.39	307.72
HA	2.88	2.36	2.18	1.43	1.98	200.07	231.03	221.15	151.61	222.69
	rabbit 3									
Collagen +HA	3.37	2.41	3.57	2.88	4.09	187.71	148.67	239.93	257.56	746.17
HA	2.06	2.35	2.24	1.82	2.12	114.17	177.18	162.06	118.77	196.99

Example 5. Preparation of derivatized collagen-heparosan-Platelet rich plasma (PRP) composition

[0064] 200 mL of 3 mg/mL purified, soluble collagen (Porcogen, Lot #531131080) was filtered through 0.45 µm and 0.2 µm cartridge filters. The filtered collagen was place in a 500 mL beaker and adjusted to a pH of 9.0 using 10N and 1N NaOH. After stirring for 5 minutes at room temperature, pulverized glutaric anhydride powder (Sigma, >95%) was slowly added to the stirring collagen solution at a concentration equal to 10% of the collagen (60 mg). The pH of the collagen solution was maintained at pH 9.0 by addition of drops of 10N NaOH. The glutaric anhydride reaction continued for 15 minutes at which point drops of 6N HCl and 1N HCl were added to reduce the pH to approximately 4.5 to precipitate the derivatized collagen. The derivatized collagen was then placed in 50 mL centrifuge tubes and centrifuged at 3,500-5,000 rpm to precipitate the derivatized collagen. The recovered precipitate was then solubilized by adjusting the pH to 7.2 by adding drops of 10 N NaOH and 1N NaOH. The pH was monitored as NaOH was mixed with the derivatized collagen pellet. The neutralized, clear and transparent collagen gel was then placed in 50 mL centrifuge tubes and centrifuged to remove air bubbles.

[0065] The derivatized collagen was diluted to 2mg/mL and lyophilized under 0 °C for 48 hours. 0.1 grams of sodium heparosan powder (HTL Biotechnology, MW: 1800kDa~2400kDa) was added to 0.7 grams of lyophilized collagen sponge. And 15mL sterile PBS was used to re-solubilize collagen- heparosan mixture and the mixture was shaken at 50rpm under 10 °C for 72 hours. The neutralized, clear and transparent collagen- heparosan gel was placed in the tube and centrifuged to remove air bubbles. Derivatized collagen- heparosan-PRP composition was produced by adding 5ml PRP to the gel and homogeneously mixing by shaker at 50 rpm under 10 °C for 2 hours. PRP was prepared using human peripheral blood with Regenlab kit. Derivatized collagen- heparosan-PRP gel was loaded into 1mL BD glass syringes and centrifuged at 3000rpm for 5 min to remove bubbles.

[0066] 27 gauge needle was attached to the syringe and the injection force of the composite through 27gauge needle was evaluated by measuring compression force applied to syringe plug by universal testing machine (UTM). The injection force of derivatized collagen- heparosan-PRP gel was lower than 10N (Figure 10).

[0067] Although the present invention has been described with reference to exemplary embodiments, one skilled in the art can easily ascertain its essential characteristics and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention herein. Such equivalents are intended to be encompassed in the scope of the present invention.

[0068] All references, including patents, publications, and patent applications, mentioned in this specification are herein incorporated by reference in the same extent as if each independent publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

REFERENCES

Relevant technical publications

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Patents Referencing Collagens for Soft Tissue Augmentation

Citing Patent	Filing date	Issue date	Original Assignee	Title
US4291013	Oct 9, 1979	Sep 22, 1981	Merck Patent Gesellschaft Mit Beschrankter Haftung	Medicinally useful, shaped mass of collagen resorbable in the body
US4347234	Jun 15, 1981	Aug 31, 1982	Merck Patent Gesellschaft mit beschrankter Haftung	Medicinally useful, shaped mass of collagen resorbable in the body
US4424208	Jan 11, 1982	Jan 3, 1984	Collagen Corporation	Collagen implant material and method for augmenting soft tissue
US4488911	Sep 15, 1983	Dec 18, 1984		Non-antigenic collagen and articles of manufacture
US4582640	Oct 22, 1984	Apr 15, 1986	Collagen Corporation	Injectable cross-linked collagen implant material
US4592864	Jul 25, 1984	Jun 3, 1986	Koken Co., Ltd.	Aqueous atelocollagen solution and method of preparing same
US4642117	Mar 22, 1985	Feb 10, 1987	Collagen Corporation	Mechanically sheared collagen implant material and method

US4713446	Aug 6, 1986	Dec 15, 1987	Minnesota Mining and Manufacturing Company	Viscoelastic collagen solution for ophthalmic use and method of preparation
US4803075	Jun 25, 1986	Feb 7, 1989	Collagen Corporation	Injectable implant composition having improved intrudability
US4851513	Oct 5, 1987	Jul 25, 1989	Minnesota Mining and Manufacturing Company	Viscoelastic collagen solution for opthalmic use and method of preparation
US4883864	Oct 28, 1988	Nov 28, 1989	Minnesota Mining and Manufacturing Company	Modified collagen compound and method of preparation
US4969912	Feb 18, 1988	Nov 13, 1990	Autogenesis Technologies	Human collagen processing and autoimplant use
US4992226	Sep 16, 1988	Feb 12, 1991	Collagen Corporation	Method of making molds with xenogeneic collagen/mineral preparations for bone repair
US5103840	May 7, 1990	Apr 14, 1992		Viscoelastic collagen gel for ophthalmic surgery
US5104957	Feb 28, 1990	Apr 14, 1992	Autogenesis Technologies, Inc.	Biologically compatible collagenous reaction product and articles useful as medical implants produced therefrom
US5162430	Nov 14, 1989	Nov 10, 1992	Collagen Corporation	Collagen-polymer conjugates
US5201764	Jan 22, 1992	Apr 13, 1993	Autogenesis Technologies, Inc.	Biologically compatible collagenous reaction product and articles useful as medical implants produced therefrom
US5292802	Dec 2, 1992	Mar 8, 1994	Collagen Corporation	Collagen-polymer tubes for use in vascular surgery
US5304147	Nov 6, 1992	Apr 19, 1994	Johnson Medical Development Corp.	Injection syringe

US5306500	Aug 23, 1993	Apr 26, 1994	Collagen Corporation	Method of augmenting tissue with collagen-polymer conjugates
US5324519	Oct 28, 1991	Jun 28, 1994	Atrix Laboratories, Inc.	Biodegradable polymer composition
US5324775	Jul 2, 1992	Jun 28, 1994	Collagen Corporation	Biologically inert, biocompatible-polymer conjugates
US5328955	Jul 30, 1992	Jul 12, 1994	Collagen Corporation	Collagen-polymer conjugates
US5366498	Feb 10, 1993	Nov 22, 1994	Collagen Corporation	Device for treating fine superficial facial lines
US5376375	Jan 5, 1994	Dec 27, 1994	Collagen Corporation	Method of augmenting tissue using collagen-polymer conjugates
US5383930	Nov 2, 1992	Jan 24, 1995	Collagen Corporation	Method for treating fine superficial facial lines
US5413791	Feb 17, 1994	May 9, 1995	Collagen Corporation	Collagen-polymer conjugates
US5428024	Apr 19, 1994	Jun 27, 1995	Collagen Corporation	High concentration homogenized collagen compositions
US5436135	Jan 7, 1991	Jul 25, 1995	Pasteur Merieux Serums et Vaccins Imedex	New preparation of placenta collagen, their extraction method and their applications
US5446091	Jan 5, 1995	Aug 29, 1995	Collagen Corporation	Collagen-polymer conjugates containing an ether linkage
US5475052	May 2, 1994	Dec 12, 1995	Collagen Corporation	Collagen-synthetic polymer matrices prepared using a multiple step reaction
US5476515	Nov 24, 1993	Dec 19, 1995	Autogenesis Technologies, Inc.	Method of making intraocular lenses with injectable collagen-based compositions
US5480427	Jul 19, 1994	Jan 2, 1996	Autogenesus Technologies, Inc.	Biologically compatible collagenous reaction product and articles useful as medical implants produced

				therefrom
US5492135	Sep 9, 1992	Feb 20, 1996	Collagenesis, Inc.	Collagen modulators for use in photoablation excimer laser keratectomy
US5510418	Nov 3, 1993	Apr 23, 1996	Collagen Corporation	Glycosaminoglycan-synthetic polymer conjugates
US5550188	Jun 7, 1995	Aug 27, 1996	Collagen Corporation	Polymer conjugates ophthalmic devices comprising collagen-polymer conjugates
US5565519	Nov 3, 1993	Oct 15, 1996	Collagen Corporation	Clear, chemically modified collagen-synthetic polymer conjugates for ophthalmic applications
US5591444	Jul 28, 1995	Jan 7, 1997	Isolagen Technologies, Inc.	Use of autologous dermal fibroblasts for the repair of skin and soft tissue defects
US5660850	Jun 6, 1996	Aug 26, 1997	Isolagen Technologies, Inc.	Use of autologous dermal fibroblasts for the repair of skin and soft tissue defects
US5665372	Jun 6, 1996	Sep 9, 1997	Isolagen Technologies, Inc.	Autologous dermal fibroblasts for the repair of skin and soft tissue defects
US5800541	Jan 8, 1997	Sep 1, 1998	Collagen Corporation	Collagen-synthetic polymer matrices prepared using a multiple step reaction
US5807581	Sep 29, 1995	Sep 15, 1998	Collagen Corporation	Collagen-based injectable drug delivery system and its use
US5823671	Nov 8, 1995	Oct 20, 1998	Collagen Corporation	Apparatus and method of mixing materials in a sterile environment
US5830708	Jun 6, 1995	Nov 3, 1998	Advanced Tissue Sciences, Inc.	Methods for production of a naturally secreted extracellular matrix
US5840848	Jan 3, 1997	Nov 24, 1998	Autoimmune, Inc.	Method for preparation of type II

				collagen
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US5861486	Feb 16, 1996	Jan 19, 1999	Collagenesis, Inc.	Collagen modulators for use in photoablation eximer laser keratectomy
US5874500	Dec 18, 1996	Feb 23, 1999	Cohesion Technologies, Inc.	Crosslinked polymer compositions and methods for their use
US6051648	Jan 13, 1999	Apr 18, 2000	Cohesion Technologies, Inc.	Crosslinked polymer compositions and methods for their use
US6071530	Jun 26, 1997	Jun 6, 2000	Atrix Laboratories, Inc.	Method and composition for treating a bone tissue defect
US6129761	Jun 7, 1995	Oct 10, 2000	Reprogenesis, Inc.	Injectable hydrogel compositions
US6166130	Apr 30, 1999	Dec 26, 2000	Cohesion Technologies, Inc.	Method of using crosslinked polymer compositions in tissue treatment applications
US6284284	Oct 29, 1998	Sep 4, 2001	Advanced Tissue Sciences, Inc.	Compositions and methods for production and use of an injectable naturally secreted extracellular matrix
US6323278	Dec 8, 2000	Nov 27, 2001	Cohesion Technologies, Inc.	Method of making crosslinked polymer matrices in tissue treatment applications
US6337389	Oct 28, 1997	Jan 8, 2002	BioScience Consultants, L.L.C.	Method and process for the production of collagen preparations from invertebrate marine animals and compositions thereof
US6458889	Jun 15, 2001	Oct 1, 2002	Cohesion Technologies, Inc.	Compositions and systems for forming crosslinked biomaterials and associated methods of preparation and use

US6534591	Aug 17, 2001	Mar 18, 2003	Cohesion Technologies, Inc.	Cross-linked polymer compositions and methods for their use
US6682760	Apr 9, 2001	Jan 27, 2004	Colbar R&D Ltd.	Cross-linked collagen matrices and methods for their preparation
US6833408	Sep 30, 2002	Dec 21, 2004	Cohesion Technologies, Inc.	Methods for tissue repair using adhesive materials
US6911496	Jan 27, 2004	Jun 28, 2005	Cohesion Technologies, Inc.	Composition for administration of a biologically active compound
US6916910	Nov 28, 2001	Jul 12, 2005	Bioscience Consultants	Method and process for the production of collagen preparations from invertebrate marine animals and compositions thereof
US7025916	May 28, 2003	Apr 11, 2006	Organogenesis, Inc.	Process of making bioengineered collagen fibrils
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US7244270	Sep 16, 2004	Jul 17, 2007	Evera Medical	Systems and devices for soft tissue augmentation
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US7595377	Apr 28, 2006	Sep 29, 2009	Crosscart, Inc.	Substantially non-immunogenic injectable collagen
US7807150	Mar 8, 2004	Oct 5, 2010	Massachusetts Institute of Technology Children's Medical Center Corporation	Injectable composition containing crosslinkable material and cells for forming animal tissue
US7883693	Jan 31, 2006	Feb 8, 2011	AngioDevice International GmbH	Compositions and systems for forming crosslinked biomaterials and

				methods of preparation of use
US7932354	Mar 6, 2009	Apr 26, 2011	aap Biomaterials GmbH	Process for the production of collagen material
US8067031	Apr 28, 2005	Nov 29, 2011	AngioDevice International GmbH	Compositions and systems for forming crosslinked biomaterials and associated methods of preparation and use
US8084055	Sep 21, 2007	Dec 27, 2011	Purdue Research Foundation	Collagen preparation and method of isolation
US8124120	Dec 22, 2003	Feb 28, 2012	Anika Therapeutics, Inc.	Crosslinked hyaluronic acid compositions for tissue augmentation

CLAIMS

1. A composition for soft tissue augmentation comprising:
i) neutral pH soluble collagen; and
ii) glycosaminoglycan; and
iii) optionally, other active ingredients,
wherein the neutral pH soluble collagen was mixed with glycosaminoglycan.

2. The composition of claim 1, wherein
i) the neutral pH soluble collagen is selected from the group consisting of derivatized collagen or *in situ* polymerizing collagen, or a combination thereof; and/or

ii) the glycosaminoglycan is selected from the group consisting of crosslinked and/or non-crosslinked glycosaminoglycan; and/or

iii) said other active ingredients is selected from the group consisting of

(a) a plasma or a platelet-rich plasma or at least one growth factor comprises plasma or platelet-rich plasma;

(b) cell free fat extract or at least one growth factor comprises cell free fat extract;

(c) cell free stem cell extract or at least one growth factor comprises cell free stem cell extract;

(d) Extracellular Vehicles (EVs), secreted by stem cells;

(e) one or more essential amino acids;

(f) polynucleotide(PN) and/or polydeoxyribonucleotide (PDRN) extracted from the sperm cells of *Oncorhynchus mykiss* (Salmon trout) or *Oncorhynchus keta* (Chum Salmon) with a molecular weight ranging from 50 to 1500 kDa;

(g) local anesthesia drugs;

(h) stabilizer or dissolution promotor; and

(i) any combinations thereof.

3. The composition of claim 2, wherein

the concentration of active ingredient (a) is 1%~50% by weight; and/or

the concentration of active ingredient (b) is 1%~5% by weight; and/or

the concentration of active ingredient (c) is 0.1%~5% by weight; and/or

the concentration of active ingredient (d) is 0.1%~5% by weight; and/or

the concentration of active ingredient (e) is 0.1%~5% by weight; and/or

the concentration of active ingredient (f) is 0.1~2% by weight; and/or

the concentration of active ingredient (g) is 0.1% to 0.5% by weight; and/or

the concentration of active ingredient (h) is 0.1% to 5% by weight; and/or

active ingredient (g) is lidocaine or procaine; and/or

active ingredient (h) is Methyl sulfonyl methane (MSM).

4. The composition of claim 1, wherein

(a) the ratio of glycosaminoglycan to the neutral pH soluble collagen is between 10:1 to 1:10; or

(b) the concentration of glycosaminoglycan is in a range between 5 to 50 mg/ml.

5. The composition of claim 1, wherein the source of collagen is selected from allogeneic tissue, mammal tissue or marine species or axolotl hides derived matrix; and/or

the collagen is selected from full collagen or atelocollagen, or recombinant collagen or recombinant collagen peptides from microorganism, plants, insect cells or animal cells, or collagen mimic peptides.

6. The composition of claim 2, wherein the derivatized collagen is derivatized with acetylation agents that alter the pKa of collagen and has one or more of the following features:

(a) soluble at neutral pH;

(b) does not undergo fibrillogenesis at physiological pH; and/or

(c) precipitates at acidic pH.

7. The composition of claim 6, wherein the pH in feature (a) is pH 6.5-7.5; and/or the pH in feature (c) is pH 3.5-5.5.

8. The composition of claim 6, wherein the pH in feature (c) is pH 4.0~5.0.

9. The composition of claim 2, wherein the derivatized collagen is derivatized with one or more agents selected from the group consisting of glutaric anhydride, succinic anhydride, maleic anhydride, citric acid anhydride, oxalic acid anhydride and ethylenediamine tetraacetic anhydride.

10. The composition of claim 2, wherein the neutral pH soluble collagen forms rapidly polymerizing collagen gels; and

the rapidly polymerizing collagen gels comprises a neutralized solution comprising an acid soluble collagen, EDTA/EGTA and a polyol, and wherein the acid soluble collagen comprises collagen selected from the group consisting of Type I collagen, Type II collagen, Type III collagen and combinations thereof.

11. The composition of claim 10, wherein the acid soluble collagen in a concentration between 5 and 70 mg/ml; and/or

wherein said EDTA is disodium EDTA; and/or

wherein said EGTA is disodium EGTA; and/or

wherein said EDTA or EGTA is in a concentration between 10 and 50 mM; and/or

wherein said polyol is a sugar alcohol; and/or

wherein said polyol is in a concentration between 2.5% and 4% (w/v); and/or

wherein said rapidly polymerizing collagen gels further comprises a disaccharide, fructose, or combinations thereof; and/or

wherein said rapidly polymerizing collagen gel has an osmolality of 280-360 mmol/kg.

12. The composition of claim 11, wherein the sugar alcohol is D-mannitol.

13. The composition of claim 1, wherein the glycosaminoglycan is one or more selected from the group consisting of hyaluronic acid, heparosan, heparin, chondroitin sulfate, dermatan sulfate, keratan sulfate, and any combinations thereof.

14. The composition of claim 1, wherein the glycosaminoglycan is derived from allogenetic tissue, mammal tissue or marine species; and/or is produced through microbial fermentation.

15. The composition of claim 1, wherein the molecular weight of glycosaminoglycan before crosslinking is from 1000Da~10000000Da.

16. The composition of claim 2, wherein the crosslinker crosslinking glycosaminoglycan are independently selected from 1,4-butanediol diglycidyl ether (BDDE), 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDC), polyethyleneglycol diglycidyl ether (PEGDE), N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC), Diepoxyoctane (DEO), Divinyl Sulfone (DVS) , glutaraldehyde, or p-phenylene biscarbodiimide or 1,2,7,8-diepoxyoctane, or Polyethylene glycol (PEG), or oligomers rich in amino groups or combinations thereof.

17. The composition of claim 16, wherein the oligomers rich in amino groups is selected from lysine, poly-lysine, poly-arginine or γ-polyglutamic acid.

18. The composition of claim 13, wherein hyaluronic acid is selected from oligo-hyaluronan, hyaluronic acid produced by microbial fermentation using *Streptococcus* species or *Bacillus* species, or allogeneic or animal tissues derived hyaluronic acid.

19. A method of preparing a composition comprising (i) neutral pH soluble collagen; (ii) glycosaminoglycan; and (iii) optionally, other active ingredients, said method comprises one or more step selected from:

(a) combining part (i) with part (ii) to form an injectable homogeneous gel; or

(b) adding ethanol precipitated part (ii) to a salt or pH precipitate of part (i) and adding part (iii) (if present) and re-solubilizing the combination by dialysis or diafiltration or ultrafiltration process to form a homogeneous injectable gel; or

(c) combining part (i), part (ii) and part (iii) (if present), and re-solubilizing the mixture of lyophilized part (i), part (ii) and part (iii) and dialyzing the combination to neutral pH form a homogeneous injectable gel.

20. The method of claim 19, wherein in step (a), part (i) is combined with part (ii) by adding part (ii) to part (i) by utilizing vacuum planetary mixer to form an injectable homogeneous gel; and/or

wherein in step (a), part (i) is combined with part (ii) with a revolution speed of 200 rpm~ 1,400 rpm and an autorotation speed of 100 rpm~ 700 rpm, and with a mixing time of 10~30 minutes with vacuum under sterile condition; and/or

wherein in step (c), part (i), part (ii) and part (iii) (if present) are combined by sterile freeze-drying part (i) and part (ii) and part (iii).

21. A method for augmenting soft tissue or inducing a cellular growth promoting scaffold in a tissue space under an epidermis in a subject in need thereof, comprising administering the composition of claim 1 to a site in need of the augment or induction..

22. The method of claim 21, wherein the composition is injected into soft tissue to correct soft tissue deficiencies; and/or

wherein the composition is injected into dermis to correct soft tissue deficiencies including wrinkles, dermal folds, dermal laxity, unevenness, facial emaciation, fat atrophy, cheek depression, eye socket depression, or a combination thereof; and/or

wherein the composition is injected into tissues other than dermis, including cartilage, to correct tissue deficiencies; and/or

wherein the composition is injectable through a 25, 26, 27, 28, 29, 30 gauge needle or cannula.

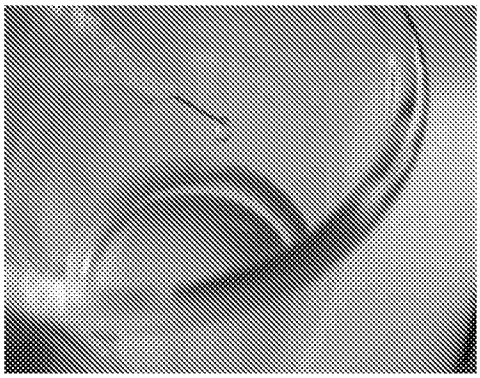

Figure 1A

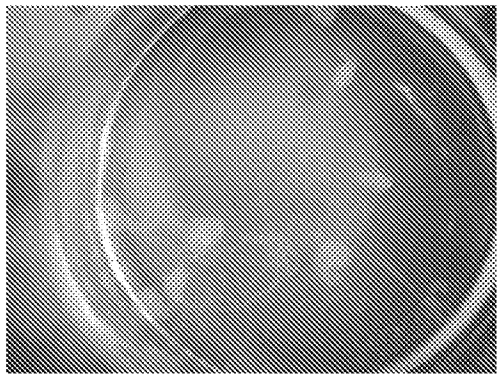

Figure 1B

Figure 1

Figure 2

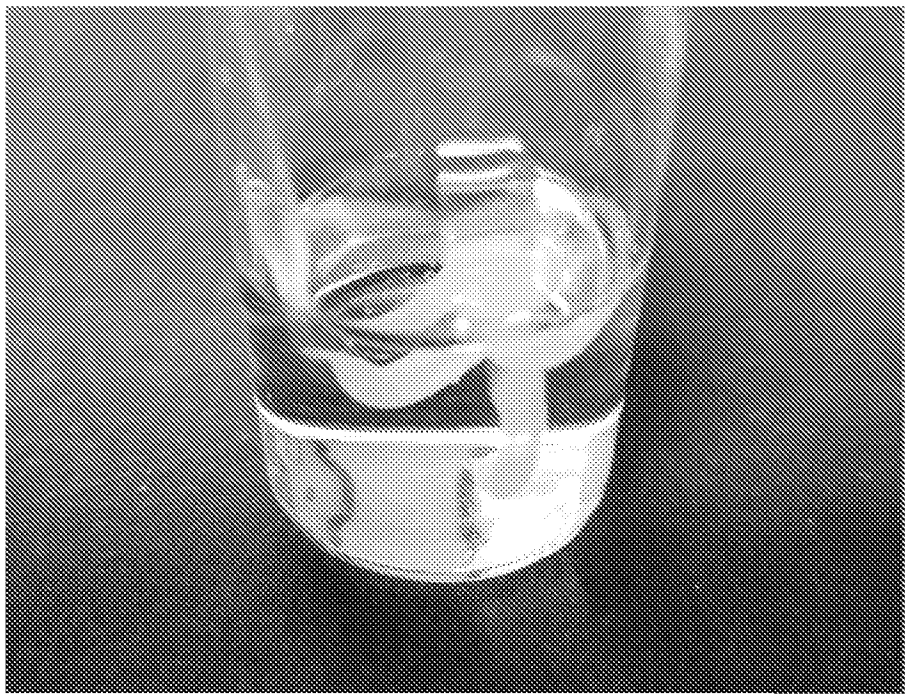

Figure 3

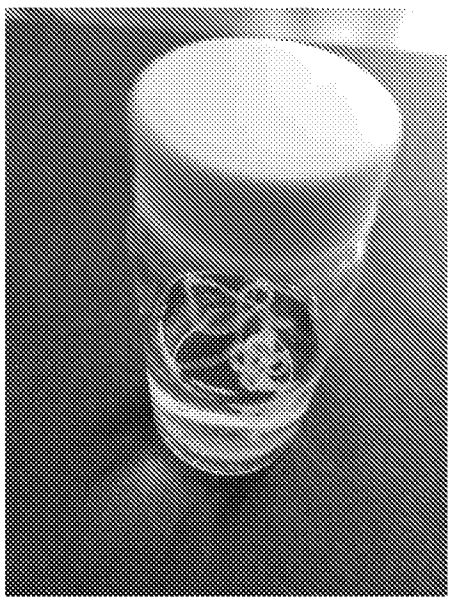

Figure 4

Figure 5

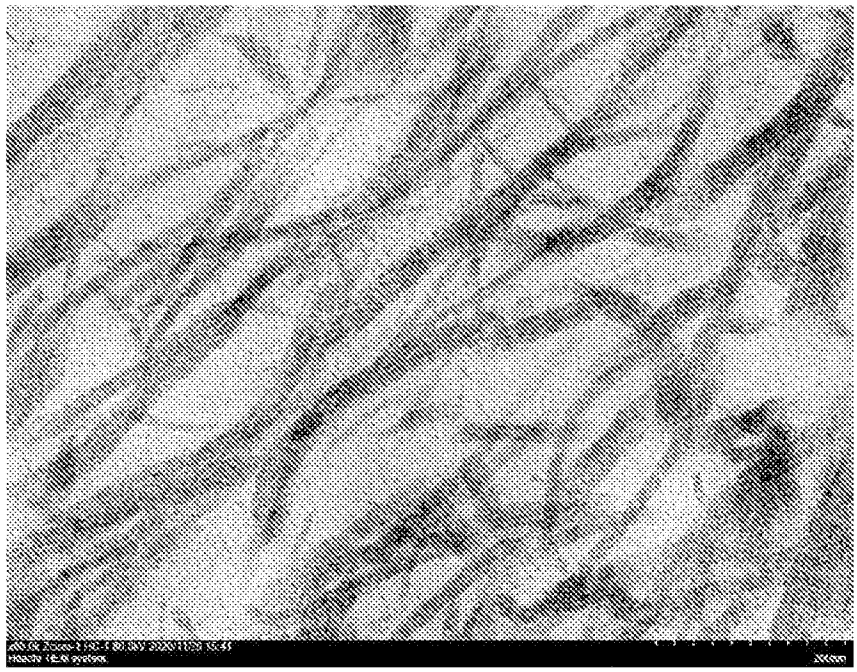

Figure 6

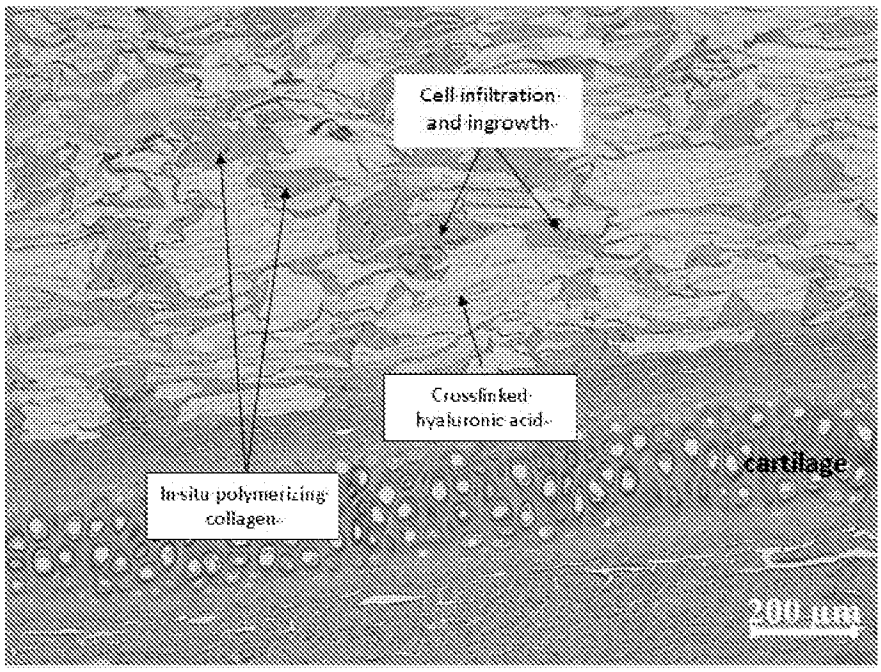

Figure 7

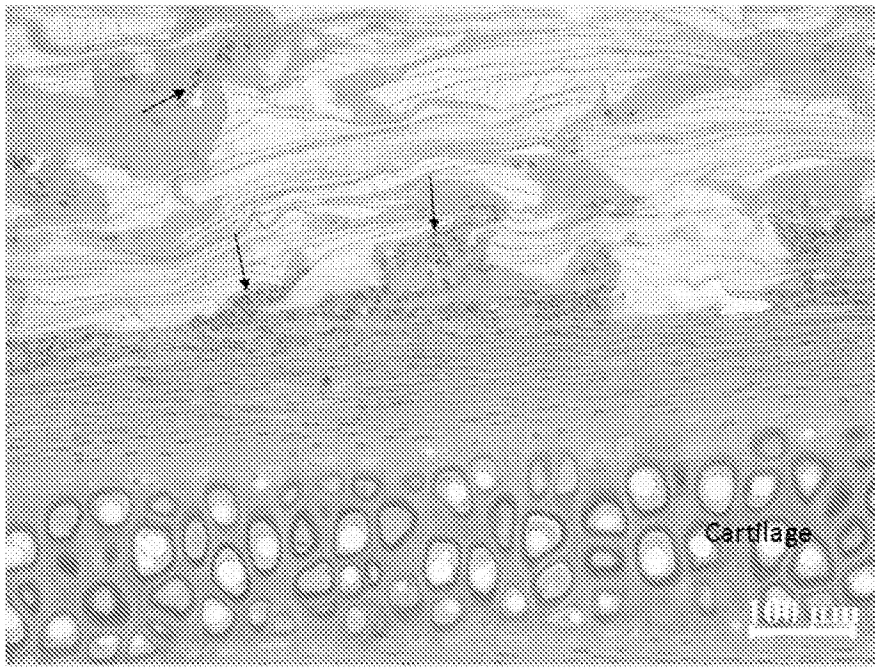

Figure 8

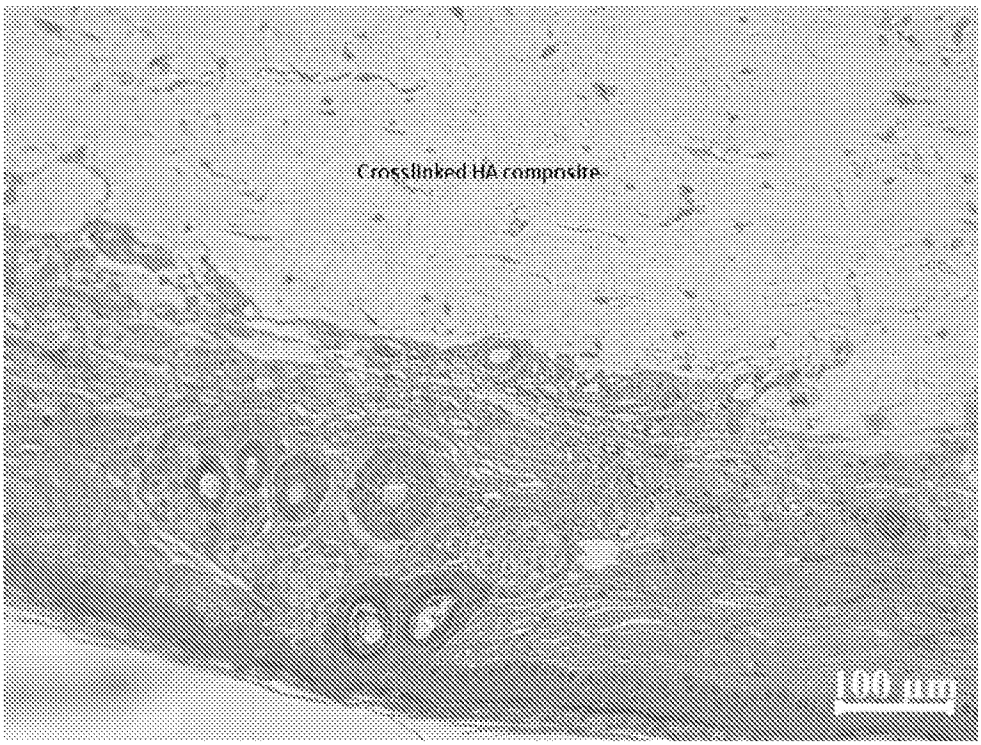

Figure 9

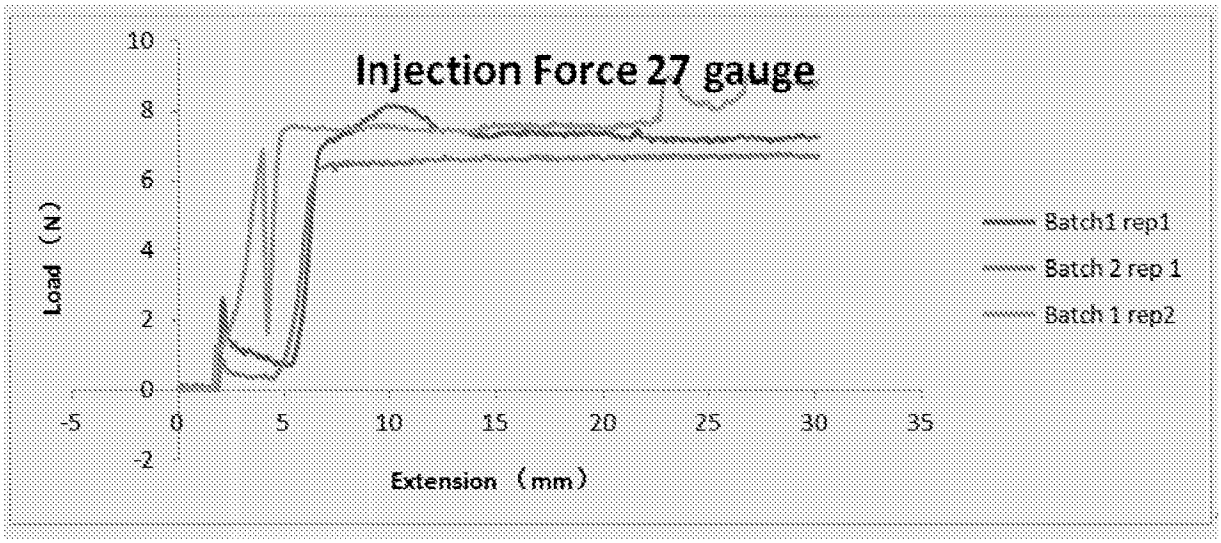

Figure 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/142904

A. CLASSIFICATION OF SUBJECT MATTER

A61L27/08(2006.01)i; A61L27/24(2006.01)i; A61K8/65(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61L, A61K

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)

CNTXT, EPTXT, TWTXT, USTXT, VEN, WOTXT, Elsevier, ISI Web of Science: augmentation, filling, collagen, Col, glycosaminoglycan, polysaccharide,methyl sulfonyl methane, MSM, heparin, hyaluronic acid, chitosan, alginate, heparosan, chondroitin sulfate, dermatan sulfate, keratan sulfate, nuetral, pH, soluble, soft tissue

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	CN 107213028 A (SHAA-N) SHAANXI HUIKANG BIOTECHNOLOGY CO LTD29 September 2017 (2017-09-29) paragraphs 6-24	1-20
X	CN 103834053 A (SHAA-N) SHAANXI BAIAO REGENERATIVE MEDICINE CO04 June 2014 (2014-06-04) claims 1-6	1-20
X	CN 103333349 A (SHAA-N) SHAANXI JUZI BIOTECHNOLOGY CO LTD02 October 2013 (2013-10-02) claims 1-8	1-20
X	WO 2019211854 A1 (COLL-N) COLLPLANT HOLDINGS LTD et al.07 November 2019 (2019-11-07) abstract, claims 1-5	1-20

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 2023	**20 May 2023**
Name and mailing address of the ISA/CN **CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	Authorized officer **GAO,Xin** Telephone No. **(+86) 010-62084785**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	CN 105860151 A (UYXB) UNIV NORTHWEST17 August 2016 (2016-08-17) claims 1-7	1-20
A	EP 1633275 A2 (BIOMERIX CORP) 15 March 2006 (2006-03-15) full text	1-20
A	EP 3744336 A1 (MIMEDX GROUP INC) 02 December 2020 (2020-12-02) full text	1-20
A	US 2011293669 A1 (BENNETT STEVEN et al.) 01 December 2011 (2011-12-01) full text	1-20
A	US 2013323128 A1 (UVCLEANING SYSTEMS INC) 05 December 2013 (2013-12-05) full text	1-20
A	US 2019231615 A1 (COVIDIEN LP) 01 August 2019 (2019-08-01) full text	1-20
A	US 5709991 A (CERUS CORP) 20 January 1998 (1998-01-20) full text	1-20
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/142904

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

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

1. ☑ Claims Nos.: **21-22**
because they relate to subject matter not required to be searched by this Authority, namely:

The subject-matter of claims 21-22 is method for treatment of the human body by surgery. Therefore, claims 21-22 are unsearchable.

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

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/142904

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