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(54) **CROSS-LINKED COLLAGEN AND USES THEREOF**

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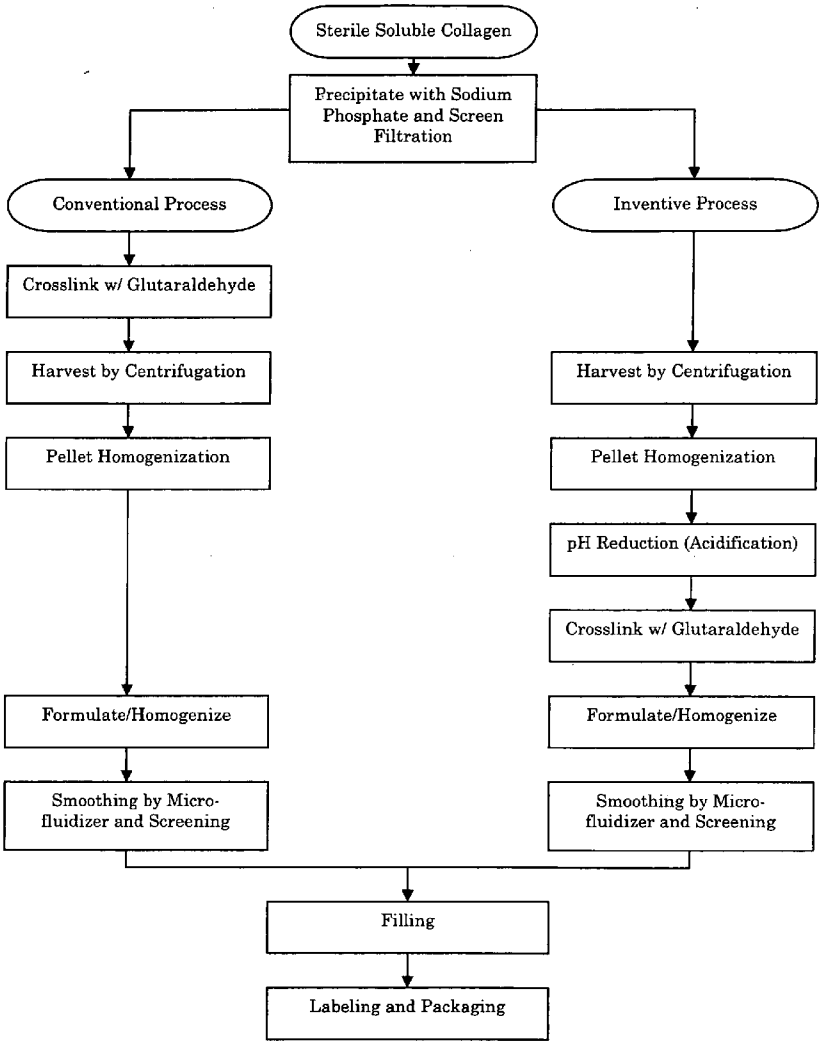
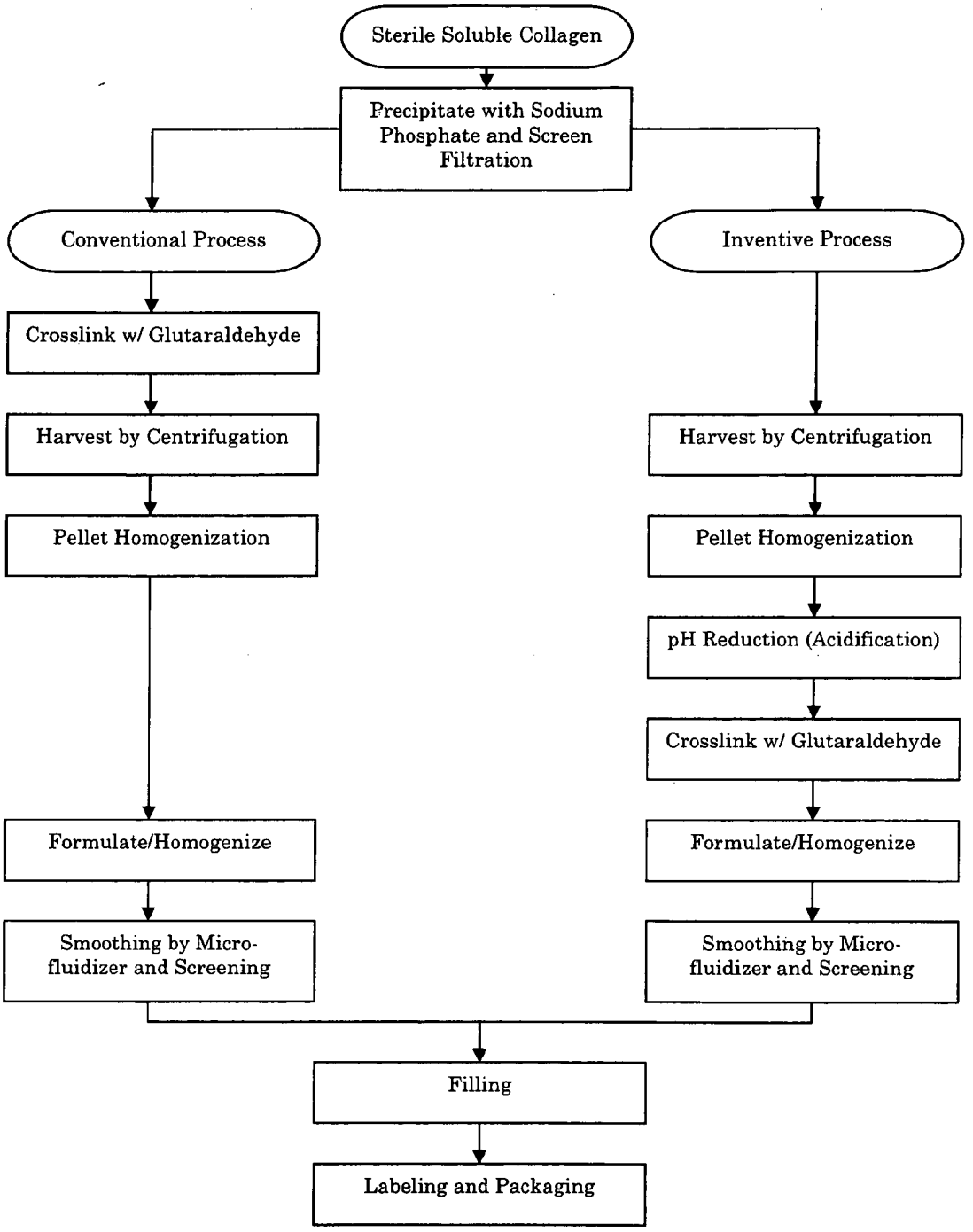
(57) **ABSTRACT**

The present invention discloses collagen cross-linked in a micro to non-fibrillar form and at a high concentration. The cross-linked collagen gel has improved volume stability or persistence than collagen cross-linked at a neutral pH. Also disclosed are methods for preparing the inventive cross-linked collagen and using such for augmenting soft tissues in mammals.

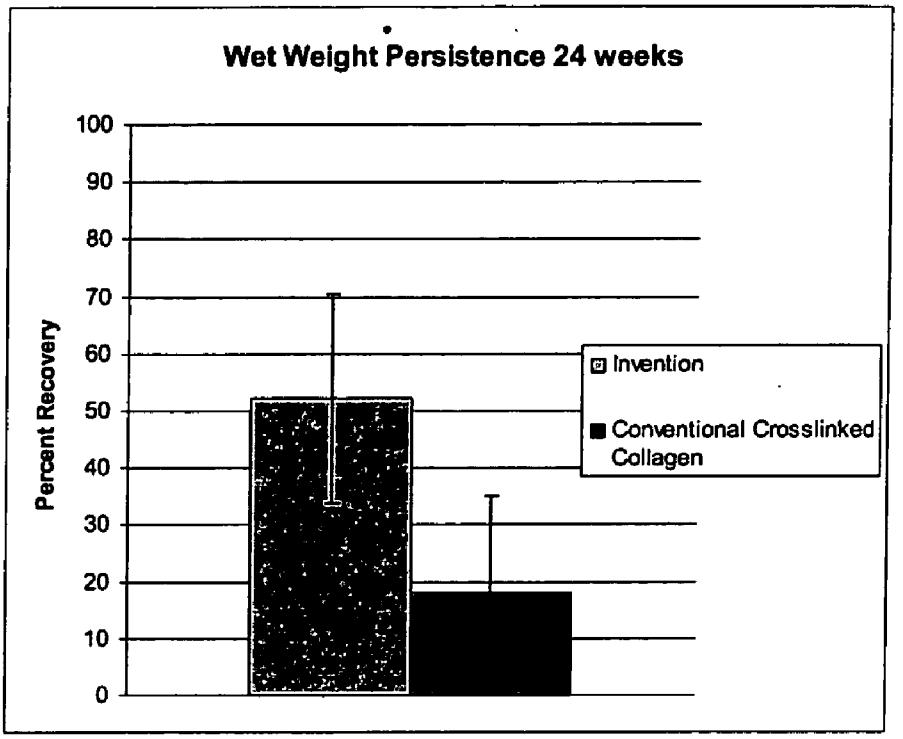


Comparison of Conventional and Inventive Processes



Comparison of Conventional and Inventive Processes

Figure 1


Wet Weight Persistence 24 weeks
Percent Recovery
100
90
80
70
60
50
40
30
20
10
0
Invention
Conventional Crosslinked Collagen

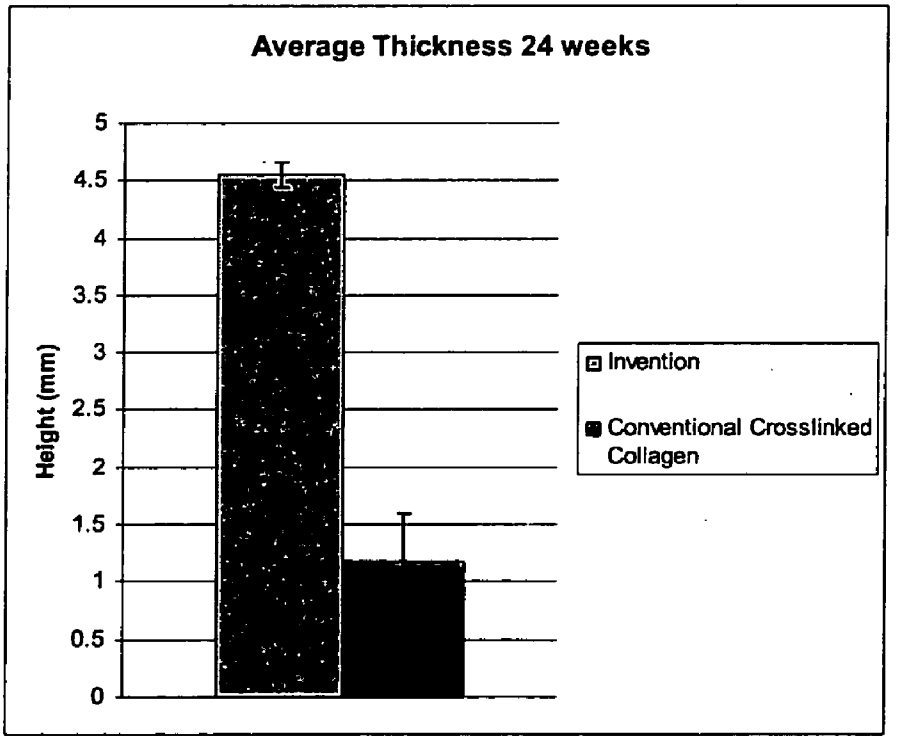

Average Thickness 24 weeks
Height (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Invention
Conventional Crosslinked Collagen

Figure 2

CROSS-LINKED COLLAGEN AND USES THEREOF

RELATED APPLICATION

[0001] This application is based, and claims priority under 35 U.S.C. § 120 to U.S. Provisional Patent Application No. 60/939,664 filed on May 23, 2007 and which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates in general to body treating compositions and methods. More specifically, the invention provides a cross-linked collagen implant of improved volume stability ("persistence") for augmenting soft tissue in mammals.

BACKGROUND OF THE INVENTION

[0003] Collagen has been used as a pharmaceutical carrier, as a surgical prosthesis (sutures and wound dressings), and as an implant material. In many instances, the collagen is cross-linked with chemical agents, radiation, or other means to improve its mechanical properties, decrease its immunogenicity, and/or increase its resistance to resorption. For example, U.S. Pat. No. 4,424,208 describes a collagen composition including cross-linked collagen and reconstituted collagen fibers having enhanced persistence. While these materials are remarkably effective, they shrink in volume after implantation due primarily to absorption of their fluid component by the body, although the shrinkage (syneresis) is less than non-cross-linked collagen. Since a constant volume or persistence is desirable, an additional injection or injections of supplemental implant material is required. It would thus be advantageous to provide collagen compositions having enhanced persistence after being introduced in vivo to a soft tissue treatment site.

SUMMARY OF THE INVENTION

[0004] The present invention relates to collagen cross-linked in a micro to non-fibrillar form and at a high concentration for augmenting soft tissue in mammals. The cross-linked collagen of the present invention has improved volume stability or persistence compared with collagen cross-linked at a neutral pH.

[0005] In one aspect, the invention features a method for preparing cross-linked collagen. The method involves the steps of obtaining micro to non-fibrillar collagen, treating the micro to non-fibrillar collagen with a cross-linking agent, and isolating cross-linked collagen. The micro to non-fibrillar collagen may be obtained by incubating fibrillar collagen in a suspension or solution of pH 2-5 or pH 9-12. In a preferred embodiment, the pH of the suspension or solution is between 4.2 and 5.0. The concentration of the micro to non-fibrillar collagen at the time of cross-linking is in the range of 3-150 mg/mL, and preferably, 38-52 mg/mL. Typically, the treating step includes treating the micro to non-fibrillar collagen with the cross-linking agent at pH 2-5 or pH 9-12, followed by treating the micro to non-fibrillar collagen with the cross-linking agent at pH 6-8 to encourage completion of the cross-linking. The method of the invention optionally may further include a step of admixing a local anesthetic agent (e.g., lidocaine) with the cross-linked collagen. Cross-linked collagen so prepared is within the invention.

[0006] Also provided in the present invention is cross-linked collagen comprising micro to non-fibrillar collagen and a cross-linking agent. The micro to non-fibrillar collagen is cross-linked by the cross-linking agent. Such cross-linked collagen may be prepared according to the method described above.

[0007] The cross-linked collagen may derive from type I, II, III, IV, or V collagen, or a combination thereof. It may also derive from telo-containing collagen, atelo-collagen, or derivatized collagen, or a combination thereof.

[0008] Preferably, the cross-linking agent is capable of forming covalent bonds between amino acid residues in the micro to non-fibrillar collagen. Examples of suitable cross-linking agents include, but are not limited to, carbodiimides, polyaldehydes, polysulfones, activated PEGs, epoxides, imidazoles, and diisocyanates. In one embodiment, the cross-linking agent is glutaraldehyde.

[0009] In some embodiments of the cross-linked type I collagen, the number of free hydroxy lysine and lysine residues per 1000 amino acid residues is in the range of 22-32, and more typically, in the range of 24-29.

[0010] The cross-linked collagen of this invention is in a gel but not fibrous state. It locks water in the gel and does not disperse like a fibrous collagen suspension. The cross-linked collagen in a gel state maintains its shape in vivo better than cross-linked collagen in a fibrous state. The fibers of the cross-linked collagen are smaller than those of fibrous collagen cross-linked at a neutral pH.

[0011] The invention further provides a composition containing the cross-linked collagen of the invention and a local anesthetic agent such as lidocaine. The local anesthetic agent is admixed with the cross-linked collagen. In addition, the invention provides a packaged product containing a syringe fitted with a needle, wherein the syringe is loaded with the cross-linked collagen of the invention.

[0012] In another aspect, the invention features a method for filling voids and defects and increasing tissue volume in a mammal. The method involves administering to a mammal the cross-linked collagen of the invention. Preferably, the cross-linked collagen is administered by intradermal or subcutaneous injection.

[0013] As used herein, "micro to non-fibrillar collagen" refers to collagen with a diameter of 5-70 nm; "fibrillar collagen" refers to collagen with a diameter of >70 nm; "fibrous collagen" refers to fibrillar collagen and larger fibers. "Telo-containing collagen" refers to collagen with intact telo peptide; "atelo-collagen" refers to collagen wherein the telo portions are removed partially or totally; "derivatized collagen" refers to chemically modified collagen. Examples of derivatized collagen include, but are not limited to, deamidated, methylated, succinylated, and phosphorylated collagen. Cross-linked collagen in a "gel state" contains micro to non-fibrillar collagen with a fiber diameter range of 5-70 nm; cross-linked collagen in a "fibrous state" contains fibrillar collagen with a fiber diameter of greater than 70 nm.

[0014] As used herein, "free hydroxyl-lysine and lysine" in collagen refers to unmodified hydroxyl-lysine and lysine; "neutral pH" refers to pH 6-8.

[0015] The present invention provides a cross-linked collagen filler for augmenting and filling soft tissue defects and voids with a material that plumps and bulks the soft tissue. The cross-linking collagen of the invention is particularly useful for deep dermal correction and sculpting. The superior

shape retention makes it ideal for areas that are hard to correct and where a biocompatible bolus can provide mechanical strength to the body.

[0016] The above-mentioned and other features of this invention and the manner of obtaining and using them will become more apparent, and will be best understood, by reference to the following description, taken in conjunction with the accompanying drawings. The drawings depict only typical embodiments of the invention and do not therefore limit its scope.

BRIEF DESCRIPTION OF THE FIGURES

[0017] FIG. **1** is a flow chart illustrating representative conventional and inventive processes for preparation of cross-linked collagen.

[0018] FIG. **2** shows the persistence of the inventive cross-linked collagen relative to the conventional cross-linked collagen.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention is based, at least in part, on the unexpected discovery that collagen cross-linked in a micro to non-fibrillar state and preferably at a high concentration has improved persistence compared to collagen cross-linked at a neutral pH.

[0020] More specifically, fibrillar collagen is fairly porous and allows the transport of cells and large molecules. By cross-linking smaller fiber (microfibrillar) collagen, more rigidity within the network was created to further increase the diffusion hindrance (Rosenblatt and Shenoy, 1993, Chain Rigidity and Diffusional Release in Biopolymer Gels, Proceed Intern Symp Control Rel Bioact Mater **20**, Controlled Release Society, Inc.). While fibrillar collagen matrices are capable of moderating the diffusion of large molecules, smaller fiber and non-fibrillar collagen can modulate the diffusion of smaller molecules (Rosenblatt et al., 1989, The Effect of Collagen Fiber Size Distribution on the Release Rate of Protein from Collagen Matrices by Diffusion, *J Controlled Release* 9:195-203). Cross-linked micro-fibrillar collagen has a tighter network or mesh, which creates a collagen matrix more resistant or persistent to biological degradation. In the present invention, as described in the Example below, type I collagen matrices were modified to reduce the penetration of cells and proteins into the matrices.

[0021] The present invention is particularly directed to the filling of voids and defects and increasing tissue volume in mammals with injectable cross-linked Type I/III collagen implants. Accordingly, the invention provides a method for preparing cross-linked collagen by cross-linking collagen in a micro to non-fibrillar state and preferably at a high concentration.

[0022] The cross-linked collagen of the present invention primarily derives from mammalian source materials, such as bovine or porcine corium, although human placenta material, collagen produced from human fibroblast cell culture, or recombinantly produced collagen expressed from a cell line may also be used. The donor need not be genetically similar to the host into which the material is ultimately implanted.

[0023] Referring to FIG. **1**, in a conventional process, purified, type I, pepsin digested human collagen from Allergan Medical Biomaterials (48490 Milmont Drive, Fremont, Calif. 94538) is reconstituted from a solution by neutralizing the solution at reduced temperatures and ionic strengths hypotonic relative to physiological conditions. The pH of the solution is raised to a level at which the collagen in solution reaggregates into fibrils. The reconstituted fibrous collagen is cross-linked with a cross-linking agent at a neutral pH. The cross-linked collagen is then harvested by centrifugation, formulated/homogenized, smoothed, and screened.

[0024] In contrast, the process of the present invention involves concentrating the collagen suspension by centrifugation. The pellet is homogenized, and the pH is adjusted to a non-neutral level prior to cross-linking. The collagen concentration at the time of cross-linking is usually in the range of 3-150 mg/mL, more typically 30-60 mg/mL, 35-55 mg/mL, or 38-52 mg/mL. The pH is adjusted to a non-neutral level appropriate for the cross-linking agent to be used. For example, the pH may be adjusted to about 4.5 for cross-linking by glutaraldehyde or to about 10 for cross-linking by divinylsulfone. A dilute solution of HCl or the like is typically added to adjust the pH to a desired acidic level, while NaOH or the like is used for adjusting the pH to a desired alkaline level.

[0025] The cross-linking begins at pH 2-5 or pH 9-12, more preferably at pH 2-3, 2-4, 3-4, 3-5, or 4-5, or pH 9-10, 9-11, 10-11, 10-12, or 11-12. At these pH ranges, the collagen fiber is unraveled to a micro to non-fibrillar state. More lysine groups are exposed and available for cross-linking. In addition, a high collagen concentration increases the reaction rate. The collagen is stabilized while being cross-linked into small fiber structures. Preferably, after the initial cross-linking, the pH is further adjusted to a neutral pH, e.g., pH 6-8, using 0.5 M phosphate, pH 11.2, or the like, to encourage complete cross-linking. The pH can then be adjusted back to neutral without causing the cross-linked collagen gel to collapse or to spontaneously form fibers.

[0026] Usually a cross-linking agent is polyfunctional, and more usually bifunctional. The cross-linking conditions of the present invention are such as to produce covalently cross-linked collagen that has improved persistence relative to an implant of a comparable formulation prepared according to the conventional process. When this degree of cross-linking has been reached, the cross-linking reaction is optionally quenched by adding a quenching agent. The quenching agent forms a water soluble adduct with the cross-linking agent. The concentration of the collagen in the suspension at the time of cross-linking, the concentration of the cross-linking agent, and the duration of the cross-linking reaction are important process conditions for obtaining the kind and degree of cross-linking that provides a product having enhanced persistence.

[0027] The collagen can be cross-linked by any of a number of conventional chemical cross-linking agents, including, but not limited to, glutaraldehyde, divinylsulfone, epoxides, carbodiimides, imidazole, N-hydroxy-succinimide (NHS), thiol derivatized polyethylene glycol (PEG), and the like.

[0028] Aldehydes are preferred cross-linking agents. Examples of aldehydes that may be used to cross-link collagen are formaldehyde, acetaldehyde, glyoxal pyruvic aldehyde, and dialdehyde starch. Glutaraldehyde is particularly preferred. Compounds that have functional groups that react with the functional groups of the cross-linking agent (e.g., aldehyde group) to form water soluble adducts may be used to quench the cross-linking reaction. Quenching agents that have free amino groups such as amino acids are preferred. Glycine is particularly preferred. The concentration of glutaraldehyde in the reaction mixture is typically about 0.001%

to about 0.05% by weight. The glutaraldehyde reacts with hydroxy lysine and lysine residues of the collagen fibers, thereby reducing the number of free hydroxy lysine and lysine in the collagen. At the glutaraldehyde concentrations mentioned above, the number of free hydroxy lysine and lysine residues per 1000 amino acid residues after cross-linking is about 22-32, more typically about 24-29. Hydroxy lysine and lysine content may be measured by reducing the cross-linked collagen with borohydride and hydrolyzing the reduced material under vacuum in 5.7 N HCl for 24 hours at 1000C. Amino acid analysis may be performed with available analyzers (e.g., a Durrum Model D-500 analyzer) and the hydroxy lysine and lysine residues quantified by comparing the hydroxy lysine and lysine/alanine ratio to those observed in non-cross-linked controls.

[0029] The duration of the cross-linking reaction is usually in the range of one-half hour to about one week. The reaction is normally carried out at about 10° C. to about 35° C. The quenching agent is added in at least stoichiometric proportions relative to the cross-linking agent.

[0030] A particularly preferred cross-linking protocol is about 38 to about 52 mg/mL collagen concentration, pH about 4.2 to about 5.0, and about 0.01% by weight glutaraldehyde for about 16 hours at approximately 22° C.

[0031] After the cross-linking reaction has been terminated, the cross-linked collagen product may be washed with an aqueous buffer solution to remove unreacted aldehyde, aldehyde polymers, and, if quenching was employed, unreacted quenching agent, and aldehyde-quenching agent adducts. A sodium phosphate-sodium chloride buffer solution, pH 6.9 to 7.4, is preferred. The washed product may be concentrated, such as by filtration or centrifugation, to a suitable protein concentration range, typically about 20 to about 65 mg/mL, more usually about 25 to about 40 mg/mL. Protein concentration may be adjusted to this range by addition of buffer or further concentration, as the case may be. The washed product has a free aldehyde content below about 20 ppm.

[0032] Formulation of the cross-linked collagen typically involves adjusting the ionic strength to isotonicity (i.e., about 0.15 to about 0.2) and adding a local anesthetic, such as lidocaine, to a concentration of about 0.3% by weight to reduce local pain upon injection. A particularly preferred cross-linking product has a collagen concentration of 30.0-37.0 mg/mL, a lidocaine concentration of 2.7-3.3 mg/mL, and a pH of 7.0-7.6. The cross-linked product is further homogenized, smoothed by microfluidization, and screened by forcing the collagen fibers through a screen of defined pore size.

[0033] The cross-linked collagen is then loaded into syringes fitted with a #25 gauge or larger gauge needle for injection. In the case of formulations used for dermal augmentation, the term "injectable" means that the formulation can be dispensed from syringes having a gauge as low as #25 under normal manual pressure without substantial spiking.

[0034] The above described steps for preparing the inventive cross-linked collagen are preferably carried out in sterile conditions using sterile materials.

[0035] The cross-linked collagen of the present invention may be injected intradermally or subcutaneously to augment soft tissue, to repair or correct congenital anomalies, acquired defects or cosmetic defects. Examples of such conditions are congenital anomalies such as hemifacial microsomia, malar and zygomatic hypoplasia, unilateral mammary hypoplasia, pectus excavatum, pectoralis agenesis (Poland's anomaly), and velopharyngeal incompetence secondary to cleft palate repair or submucous cleft palate (as a retropharyngeal implant); acquired defects (post traumatic, post surgical, or post infectious) such as depressed scars, subcutaneous atrophy (e.g., secondary to discoid lupis erythematosis), keratotic lesions, enopthalmos in the unucleated eye (also superior sulcus syndrome), acne pitting of the face, linear scleroderma with subcutaneous atrophy, saddle-nose deformity, Romberg's disease, and unilateral vocal cord paralysis; and cosmetic defects such as glabellar frown lines, deep nasolabial creases, circum-oral geographical wrinkles, sunken cheeks, and mammary hypoplasia.

[0036] In particular, the invention provides a soft tissue augmentation injectable that fills the space with a durable strong biocompatible bulking agent. Compared to the conventional cross-linked collagen, the collagen fiber of the present invention is reduced in size and forms a network that takes up more space. The inventive cross-linked collagen is elastic and resilient. It keeps its shape over time and resists breakdown and cellular infiltration.

[0037] The following example is intended to illustrate, but not to limit, the scope of the invention. While such example is typical of those that might be used, other procedures known to those skilled in the art may alternatively be utilized. Indeed, those of ordinary skill in the art can readily envision and produce further embodiments, based on the teachings herein, without undue experimentation.

EXAMPLE

Preparation of Inventive Cross-Linked Collagen

[0038] Purified, type I, pepsin digested human collagen from Allergan Medical Biomaterials (48490 Milmont Drive, Fremont, Calif. 94538) was precipitated by raising the pH to 7.0-7.6 and then centrifuging at 17000×g for 5-7 minutes. The supernatant was aseptically decanted from the centrifuge bottle, and the collagen pellet aseptically suctioned into a homogenization vessel. The precipitated collagen was aseptically homogenized. The protein concentration was 91.6 mg/mL.

[0039] 0.05 M HCl buffer and sterile filtered WFI (water for injection) were mixed with the homogenate. The protein concentration was 44.6 mg/mL; the pH was 4.8.

[0040] 3000 ppm glutaraldehyde buffer was mixed with the acid homogenate. The mixture was allowed to incubate for 1.5 hours and remixed, further incubated for 23 hours and remixed, and incubated again for 72 hours and remixed. The protein concentration was 37.1 mg/mL; the pH was 4.6.

[0041] The cross-linked homogenate was mixed with 0.5 M sodium phosphate buffer, pH 11.2, followed by 0.04 M sodium phosphate/2.6 M sodium chloride/60 mg/mL lidocaine buffer. The homogenate was allowed to incubate for 24 hours and remixed. The protein concentration was 31.9 mg/mL. The formulated homogenate was then passed through a microfluidizer and screened.

Comparison of Conventional and Inventive Cross-Linked Collagen

[0042] The conventional cross-linked collagen was obtained by precipitating and cross-linking purified, type I, pepsin digested human collagen from Allergan Medical Bio-

materials (48490 Milmont Drive, Fremont, Calif. 94538) at about 3 mg/mL and a neutral pH. The conventional cross-linked collagen was harvested by centrifugation, and then homogenized, formulated, smoothed using microfluidization and screened. The inventive cross-linked collagen was obtained by precipitating purified, type I, pepsin digested human collagen from Inamed Biomaterials (48490 Milmont Drive, Fremont, Calif. 94538) at about 3 mg/mL at a neutral pH and harvesting the collagen by centrifugation. The pH was reduced to 4.4-4.8, and the collagen was cross-linked. The inventive cross-linked collagen was then homogenized, formulated, smoothed using microfluidization, and screened.

[0043] The biocompatibility of the inventive cross-linked collagen was tested and compared to that of the conventional cross-linked collagen. Safety was assessed through a cytotoxicity study and multiple rabbit subcutaneous implantation studies. The data demonstrates that the inventive cross-linked collagen, like the conventional cross-linked collagen, was biocompatible.

[0044] More specifically, the cytotoxicity study was performed using the ISO Elution Method. The inventive cross-linked collagen implants caused no cell lysis or toxicity (Table I).

TABLE I

Cytotoxicity Study of the Conventional and Inventive Cross-linked Collagen Implants

	Confluent Monolayer	Percent Rounding	Percent Cells without Intrascyto-plasmic Granules	Percent Lysis	Grade	Reactivity
Conventional	0	0	0	0	0	None
Inventive	0	0	0	0	0	None

[0045] The rabbit subcutaneous implantation assay was used to compare tissue responses to the conventional and inventive cross-linked collagen implants at several different time-points. The tissue response to the inventive implant was similar to that seen with the conventional implant (Table II). The microscopic scores ranging from non-irritant to slight irritant are within the range of acceptable variability and considered to be satisfactory.

TABLE II

Rabbit Implantation Evaluation

Time Points	Conventional	Inventive
1 week	Non-irritant	Non-irritant
4 weeks	Non-irritant	Slight irritant
9 weeks	Not tested	Not tested
12 weeks	Non-irritant	Slight irritant

[0046] A rat subcutaneous implantation study was performed to compare the persistence of the inventive cross-linked collagen implant relative to the conventional cross-linked collagen implant. As part of the rat implantation study, a macroscopic evaluation of the implant site was performed. There was no capsule formation or adverse reaction for either implant for all time points studied (Table III).

TABLE III

Rat Subcutaneous Macroscopic Evaluation

Time Points	Conventional	Inventive
4 weeks	No capsule formation or adverse reaction	No capsule formation or adverse reaction
9 weeks	No capsule formation or adverse reaction	No capsule formation or adverse reaction
13 weeks	No capsule formation or adverse reaction	No capsule formation or adverse reaction
24 weeks	No capsule formation or adverse reaction	No capsule formation or adverse reaction

[0047] To assess effectiveness, the persistence of the inventive relative to the conventional cross-linked collagen was evaluated using wet weight recovery in conjunction with shape retention in the rat subcutaneous model (McPherson et al., 1988, Development and Biochemical Characterization of Injectable Collagen, *J Dermatol Surg Oncol* 14, Suppl 1). Shape retention is considered to be good measurement of the collagen implant's ability to maintain wrinkle correction. If the implant cannot maintain its shape, it may not correct a wrinkle effectively.

[0048] The data from the rat subcutaneous studies, summarized in FIG. **2**, indicates that the inventive implant, on average, had greater wet weight recovery and maintained its height better than the conventional implant after 24 weeks of implantation.

[0049] All patents and articles cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method for preparing cross-linked collagen, comprising:

obtaining micro to non-fibrillar collagen;

treating the micro to non-fibrillar collagen with a cross-linking agent; and

isolating cross-linked collagen.

2. The method of claim **1**, wherein the micro to non-fibrillar collagen is obtained by incubating fibrillar collagen in a suspension or solution of pH 2-5 or pH 9-12.

3. The method of claim **2**, wherein the micro to non-fibrillar collagen is obtained by incubating the fibrillar collagen in a suspension or solution of pH 4.2-5.0.

4. The method of claim **1**, wherein the concentration of the micro to non-fibrillar collagen is in the range of 3-150 mg/mL.

5. The method of claim **4**, wherein the concentration of the micro to non-fibrillar collagen is in the range of 38-52 mg/mL.

6. The method of claim **1**, wherein the treating step includes treating the micro to non-fibrillar collagen with the cross-linking agent at pH 2-5 or pH 9-12, followed by treating the micro to non-fibrillar collagen with the cross-linking agent at pH 6-8.

7. The method of claim **1**, wherein the cross-linked collagen derives from type I, II, III, IV or V collagen, or a combination thereof.

8. The method of claim **7**, wherein the cross-linked collagen derives from telo-containing collagen, atelo-collagen or derivatized collagen, or a combination thereof.

9. The method of claim **1**, wherein the cross-linking agent is capable of forming covalent bonds between amino acid residues in the micro to non-fibrillar collagen.

10. The method of claim **9**, wherein the cross-linking agent is selected from the group consisting of carbodiimides, polyaldehydes, polysulfones, activated PEGs, epoxides, imidazoles and diisocyanates.

11. The method of claim **10**, wherein the cross-linking agent is glutaraldehyde.

12. The method of claim **1**, further comprising admixing a local anesthetic agent with the cross-linked collagen.

13. The method of claim **12**, wherein the local anesthetic agent is lidocaine.

14. A method for filling voids and defects and increasing tissue volume in a mammal, comprising administering to a mammal the cross-linked collagen prepared according to the method of claim **1**.

15. The method of claim **14**, wherein the cross-linked collagen is administered by intradermal or subcutaneous injection.

16. Cross-linked collagen prepared according to the method of claim **1**.

17. The cross-linked collagen of claim **16**, wherein the number of free hydroxy lysine and lysine residues per 1000 amino acid residues in the cross-linked collagen is in the range of 22-32.

18. The cross-linked collagen of claim **17**, wherein the number of free hydroxy lysine and lysine residues per 1000 amino acid residues in the cross-linked collagen is in the range of 24-29.

19. The cross-linked collagen of claim **16**, wherein the cross-linked collagen is in a gel but not fibrous state.

20. The cross-linked collagen of claim **19**, wherein the cross-linked collagen locks water in the gel and does not disperse like a fibrous collagen suspension.

21. The cross-linked collagen of claim **19**, wherein the cross-linked collagen in the gel state maintains its shape in vivo better than cross-linked collagen in a fibrous state.

22. The cross-linked collagen of claim **19**, wherein the fibers of the cross-linked collagen are smaller than those of fibrous collagen cross-linked at a neutral pH.

23. A composition comprising the cross-linked collagen of claim **16** and a local anesthetic agent admixed with the cross-linked collagen.

24. The composition of claim **23**, wherein the local anesthetic agent is lidocaine.

25. A packaged product, comprising a syringe and a needle, wherein the syringe is loaded with the cross-linked collagen of claim **16**.

26. Cross-linked collagen, comprising:
micro to non-fibrillar collagen; and
a cross-linking agent,
wherein the micro to non-fibrillar collagen is cross-linked by the cross-linking agent.

27. The cross-linked collagen of claim **26**, wherein the cross-linked collagen derives from type I, II, III, IV or V collagen, or a combination thereof.

28. The cross-linked collagen of claim **27**, wherein the cross-linked collagen derives from telo-containing collagen, atelo-collagen or derivatized collagen, or a combination thereof.

29. The cross-linked collagen of claim **26**, wherein the cross-linking agent is capable of forming covalent bonds between amino acid residues in the micro to non-fibrillar collagen.

30. The cross-linked collagen of claim **29**, wherein the cross-linking agent is selected from the group consisting of carbodiimides, polyaldehydes, polysulfones, activated PEGs, epoxides, imidazoles and diisocyanates.

31. The cross-linked collagen of claim **30**, wherein the cross-linking agent is glutaraldehyde.

32. The cross-linked collagen of claim **26**, wherein the number of free hydroxy lysine and lysine residues per 1000 amino acid residues in the cross-linked collagen is in the range of 22-32.

33. The cross-linked collagen of claim **32**, wherein the number of free hydroxy lysine and lysine residues per 1000 amino acid residues in the cross-linked collagen is in the range of 24-29.

34. The cross-linked collagen of claim **26**, wherein the cross-linked collagen is in a gel but not fibrous state.

35. The cross-linked collagen of claim **34**, wherein the cross-linked collagen locks water in the gel and does not disperse like a fibrous collagen suspension.

36. The cross-linked collagen of claim **34**, wherein the cross-linked collagen in the gel state maintains its shape in vivo better than cross-linked collagen in the fibrous state.

37. The cross-linked collagen of claim **34**, wherein the fibers of the cross-linked collagen are smaller than those of fibrous collagen cross-linked at a neutral pH.

38. A composition comprising the cross-linked collagen of claim **26** and a local anesthetic agent admixed with the cross-linked collagen.

39. The composition of claim **38**, wherein the local anesthetic agent is lidocaine.

40. A packaged product, comprising a syringe and a needle, wherein the syringe is loaded with the cross-linked collagen of claim **26**.

41. A method for filling voids and defects and increasing tissue volume in a mammal, comprising administering to a mammal the cross-linked collagen of claim **26**.

42. The method of claim **41**, wherein the cross-linked collagen is administered by intradermal or subcutaneous injection.

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