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(71) Applicant *(for all designated States except US)*: **PRIAVISION, INC.** [US/US]; 1455 Adams Drive, Menlo Park, CA 94025 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **HEREKAR, Satish** [US/US]; 3361 Emerson Street, Palo Alto, CA 94306 (US).

(74) Agents: **ALLEN, Kenneth, R.** et al.; Townsend And Townsend And Crew LLP, Two Embarcadero Center, Eighth Floor, San Francisco, CA 94111-3834 (US).

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(54) Title: METHOD AND MATERIAL FOR IN SITU CORNEAL STRUCTURAL AUGMENTATION

(57) Abstract: A method and material for augmenting the shape and thickness of the cornea in situ includes applying a clear liquid collagen mixed with a customized crosslinker onto the augmentation surface or in a cavity (with or without a mold) and exposing the mixture to UVA radiation in vivo. Application of UVA at varying dosages demonstrate progressive optically clear gelation and biomechanical adherence properties, and in vitro optical properties (RI), mechanical suture strength and rheometric parameters are comparable to native corneal stromal tissue. Photochemical corneal collagen augmentation according to the invention makes it suitable to reconstruct and strengthen diseased and damaged eyes, ulcerated corneas, as well as provide a substrate for refractive onlay/inlay procedures and lamellar transplantation.

METHOD AND MATERIAL FOR IN SITU CORNEAL STRUCTURAL AUGMENTATION

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] NOT APPLICABLE

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] NOT APPLICABLE

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK.

[0003] NOT APPLICABLE

BACKGROUND OF THE INVENTION

[0004] The present invention relates generally to biomedical techniques. More particularly, the invention relates to a method and materials for augmenting corneal, scleral, and retinal tissue and for treating, such as by repairing and reshaping, ocular tissues and eventually for refractive surgery.

[0005] Corneal and other ocular structural weakness, such as scleral structural weaknesses, can have several origins, including genetic, iatrogenic, accidents and shortcoming of desired surgical correction. Furthermore, ulcerations, melts, and the like, may require localized repair. Refractive corrections refer to either corneal reshaping surgery or addition of prosthetics (inlays/onlays/cavity augmentations) or some combination thereof. Localized repair is currently performed by lamellar surgery and requires precise in situ "fitting" of biocompatible host and donor tissues and maintaining smooth interfaces and biocompatibility thereafter, all of which are not insignificant issues. Laser-based surface shaving surgery complications are well

publicized and may be easily referenced in current literature. Suturing has its own set of difficulties and shortcomings, as does tissue gluing.

[0006] It is long known that collagen exposed to riboflavin, also known as vitamin B2, in the presence of ultraviolet light produces cross-linking, which is useful as a cell scaffold for rebuilding cartilaginous defects. It is also known that corneal tissue can be stiffened by cross-linking by UVA irradiation in the presence of riboflavin eyedrops. However, problems with riboflavin-mediated cross-linking in collagen include shrinkage and increased opacity. (Representative citations herein are not necessarily prior art.) In the ocular domain, as published since the filing of the priority application upon which this invention is based, work has been reported on techniques for corneal cross-linking by photopolymerization of stromal fibers in the presence of riboflavin by irradiation with ultraviolet light. See Cosimo Mazzotta PhD, Angelo Balestrazzi PhD, Stefano Baiocchi PhD, Claudio Traversi MD PhD, Aldo Caporossi MD (2007), "Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: *in vivo* confocal microscopic evaluation," *Clinical & Experimental Ophthalmology*, Volume 35 (6), pp. 580–582, (August 2007). (doi:10.1111/j.1442-9071.2007.01536.x). No separate application of augmentation materials or suggestions of mixtures of photoreactive augmentation materials was reported in that study. Moreover, the researcher reported negative results: The therapy caused stromal haze after the cross-linking treatment.

[0007] Others have reported on the results of collagen cross-linking induced by riboflavin exposed to UVA. Wollensak reported on collagen cross-linking induced by riboflavin UVA and involving injection of a polymeric composition forming a gel into the eye in *The Journal of Cataract & Refractive Surgery*, Vol. 30 (3), pp. 689-695 (March 2004). Augmentation by onlay in particular was not addressed.

[0008] Therefore, there remains in the art a need for a method for effective augmentation therapy, and well as the formulation of materials that can be used in effective augmentation therapy, for ocular applications.

SUMMARY OF THE INVENTION

[0009] According to the invention, a method and material for augmenting the shape and thickness of ocular tissues, in particular the cornea, in situ are disclosed. The

method includes applying a clear liquid collagen mixed with a customized cross-linker, either as a layer or as molding material, depending upon customized thickness/shape properties, and exposing the mixture to ultraviolet radiation, typically UVA, in vivo, for a period corresponding to the thickness of the stratum, and typically less than five minutes. Application of UVA at varying dosages yields progressive optically clear gelation and biomechanical adherence properties, and the in-vitro optical properties, mechanical suture strength and rheometric characteristics are comparable to native corneal stromal tissue. Photochemical-based corneal collagen augmentation according to the invention makes it suitable for clinical use to reconstruct and strengthen diseased and ulcerated corneas, as well as provide a substrate for refractive augmentation procedures and lamellar transplantation, in particular as a suitable therapy for corneal augmentation.

[0010] The invention will be better understood by reference to the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] NOT APPLICABLE

DETAILED DESCRIPTION OF THE INVENTION

[0012] A suitable photochemical-based ocular tissue and corneal augmentation material according to the invention comprises clear liquid collagen of bovine, porcine and recombinant human origin at concentration levels of about 0.5% to about 7% weight per volume, and preferably between 1% and 5% wpv, at a pH between 6.5 and 7.0, mixed with a customized, non-toxic, water soluble cross-linker (XL, see below) having the active ingredient riboflavin in dilutions of around at least 1:500 to about 50:500 and preferably between 1:100 and 5:100 (XL:Collagen). A method according to the invention involves applying the fluid mixture (a formable gel) to ocular tissue to a preselected profile and thickness and irradiating the formable gel with ultraviolet energy that is reactive with the cross-linker in the mixture, in particular by irradiating with directed or focused or coherent (laser) ultra-violent light and more particularly Ultraviolet Type A (UVA) radiation at varying dosages, such as ~370nm UVA light at 6mW/cm^2 to 30mW/cm^2 for varying periods, such as 1 minute

to about 5 minutes to 15 minutes and as long as about 30 minutes. As a result, the material polymerizes into an augmenting stratum that adheres to the treated host substrate. Tests of the material in representative trials resulted in establishing suitable parameters of concentration, wavelength and exposure intensity and exposure time for collagen/riboflavin mixtures for corneal augmentation over a range of thicknesses. The following is a table showing what are believed to be baseline parameters for corneal augmentation of various thicknesses:

Optimal Parameters for Corneal Augmentation

Thickness	Collagen Conc.(w/v)	XL:Coll Dilution	UVA fluence	Exp Time
100 μm	1%	1:500	12 mW/cm^2 (6-30)	3 min.
200 μm	3%	1:100	12 mW/cm^2 (6-30)	4 min.
300 μm	5%	5:100	12 mW/cm^2 (6-30)	5 min.

[0013] In ranges of collagen concentrations of .5% to 7% weight per volume collagen and cross-linker to collagen dilution of 1:500 to 1:100, there is a tradeoff between mechanical strength vs. optical clarity and transparency for thin augmentation layers. Thus, the table shows nominal effective values, subject to variances within a reasonable range. Examination of the boundaries of the variances demonstrate that the values are valid over a range of an estimated three standard deviations from the stated optimal parameters without loss of cross-linking effectiveness. Mechanical strength for example is increased by increasing collagen concentration. Increasing fluence time beyond 10 minutes does not lead to further improvement in properties. Procedures and results are listed in Appendix A, fully incorporated herein. The results were optically clear and progressive gelation reaching a stable state after the end of exposure. There is little further gelation after about one hour, since the riboflavin cross-linker no longer produces reactive species.

The resultant object binds with the underlying substrate, typically living tissue, and it can be subjected to other procedures, such as excimer laser-based ablation, femto-second pulsed ablation, or other conventional surgical procedures. The photochemical gelation procedure was tested for biomechanical adherence properties in vitro and on moldings of tissue or collagen in varying thicknesses (100□m, 200□m, 400 □m, and 100□m+100□m--a multilayer cross-linking laminate).

[0014] Preliminary in vitro lab results (following initial optimization of the cross-linking procedure) of optical properties (RI), mechanical suture strength and rheometric measurements compared to native corneal stromal tissue were found to be equivalent or better.

[0015] In situ collagen corneal molding using UVA and riboflavin combinations are able to produce a clear adherent layer of transparent collagen on top of corneas, as well as in Petri dishes and contact lenses.

[0016] The procedure of photochemical augmentation has biocompatibility, as well as optical, biomechanical and adhesive properties, suitable for human therapy with the potential to reconstruct and strengthen diseased and ulcerated corneas, scleral and other ocular tissues, to use in strabismus of ocular muscle or tendon repair, as well as provide a substrate for refractive onlay/inlay procedures, cavity augmentation and lamellar transplantation.

[0017] The procedure may be implemented with a mold formed for example by a contact lens, or it may be used without a mold, such as by spray application of photo-chemically cross-linkable collagen mixtures.

[0018] Various factors which represent tradeoffs may be manipulated to optimize for desired results. These include collagen concentration, which is correlated with mechanical and optical properties, acidity, which is correlated with translucency and biocompatibility, cross-linker concentration and UVA exposure time, related to setting of the shape of collagen, which is correlated with ease of manipulation and potential for radiation damage to the tissue, and thickness of applied layer per exposure, which is a material consideration. The procedure herein disclosed contemplates single or multi-regional photo-initiating exposure, as well as exposure of large contiguous areas.

[0019] The invention has been explained with reference to specific embodiments. Other embodiments will be evident to those of ordinary skill in the art. It is therefore not intended that the invention be limited, except as indicated by the appended claims.

APPENDIX A

	chill on ice for 10 mins water, collagen xl	30 mg/ml final	66mg/ml init
trial 1	syringe 1 ml collagen soln 1:100 XL syringe mix 500 uL water remove bubbles syringe mix add 400 uL water syringe mix remove bubbles syringe mix HCL 100 uL add 2 syringe mix to form clear soln ~ 2mL 20 uL XL soln mixed with 2ml ph measures 2 UVA 10 mw/cm2exposure 2 mins not still gelled UVA exposure 3 mins not gelled, 10 mW/cm2	BD 3mL luer lock tip syringe UVA Oven varies hi to lo 20mW/cm2 to 10 mW/cm2	
trial 2	syringe 1 ml collagen soln 1:10 XL syringe mix 500 uL water remove bubbles syringe mix add 400 uL water syringe mix remove bubbles syringe mix HCL 100 uL add 2 syringe mix to form clear soln ~ 2mL 200 uL XL soln mixed with 2ml ph measures 2 UVA 10mw/cm2 exposure 5 mins gelled but temp too high		
trial 3	syringe 1 ml collagen soln 5:100 XL syringe mix 500 uL water remove bubbles syringe mix add 400 uL water syringe mix remove bubbles syringe mix HCL 100 uL add 2 syringe mix to form clear soln ~ 2mL add 50 uL 1N NaOH, syringe mix 100 uL XL soln mixed with 2ml ph measures 3.6 UVA 10 mw/cm2exposure 3 mins gelled		
trial			

4

syringe 1 ml collagen soln
5:100 XL
syringe mix 500 uL water
remove bubbles syringe mix
add 400 uL water syringe mix
remove bubbles syringe mix
HCL 100 uL add
2 syringe mix to form clear soln ~ 2mL
add 50 uL 1N NaOH, syringe mix
100 uL XL soln mixed with 2ml
ph measures 5.2
UVA 11mw/cm2 exposure 3 mins gelled

trial
5

syringe 1 ml collagen soln
5:100 XL
syringe mix 500 uL water
remove bubbles syringe mix
add 400 uL water syringe mix
remove bubbles syringe mix
HCL 100 uL add
2 syringe mix to form clear soln ~ 2mL
add 50 uL 1N NaOH, syringe mix
100 uL XL soln mixed with 2ml
ph measures 5.5
paraffin wax paper and Petri dish collagen chip
UVA 11mw/cm2 exposure 3 mins gelled OPTICAL CLARITY GOOD

trial
6

syringe 1 ml collagen 50mg/ml soln
1:10 XL
syringe mix 30 uL water
remove bubbles centrifuge 5 mins 10k rpm
HCL 160 uL add
2 syringe mix to form clear soln ~ 1.13mL
add 60 uL 1N NaOH, syringe mix
130 uL XL soln mixed with 1.12ml
ph measures 6.1
paraffin wax paper and Petri dish collagen chip
UVA 11mw/cm2 exposure 3 mins no gel

trial
7 DOUBLE LAYERED XL PETRI DISH

syringe 1 ml collagen soln
5:100 XL
syringe mix 500 uL water

remove bubbles syringe mix
add 400 uL water syringe mix
remove bubbles syringe mix
HCL 100 uL add
2 syringe mix to form clear soln ~ 2mL
add 50 uL 1N NaOH, syringe mix
100 uL XL soln mixed with 2ml
ph measures 5.5
paraffin wax paper and Petri dish collagen chip
UVA 11mw/cm2 exposure 3 mins gelled

trial
8 DOUBLE LAYERED XL CL
syringe 1 ml collagen soln
5:100 XL
syringe mix 500 uL water
remove bubbles syringe mix
add 400 uL water syringe mix
remove bubbles syringe mix
HCL 100 uL add
2 syringe mix to form clear soln ~ 2mL
add 50 uL 1N NaOH, syringe mix
100 uL XL soln mixed with 2ml
ph measures 5.5
paraffin wax paper and Petri dish collagen chip
UVA 11mw/cm2 exposure 3 mins gelled

trial
9 initial 98 mg/ml, target 50 mg/ml
syringe 1 ml collagen soln
1:10 XL

syringe mix vigorously 500 uL water	vol=	1.96	ml	vol of water=	660	ul

remove bubbles syringe mix
add 160 uL water syringe mix
remove bubbles syringe mix
HCL 100 uL add
2 syringe mix to form clear soln ~ 2mL
200 uL XL soln mixed with 2ml
ph measures 5.5
paraffin wax paper and Petri dish collagen chip
UVA 11mw/cm2 exposure 3 mins gelled OPTICAL CLARITY GOOD

trial
10 initial 98 mg/ml, target 50 mg/ml
syringe 1 ml collagen soln
5:100 XL

syringe mix vigorously 500 uL water
remove bubbles syringe mix
add 160 uL water syringe mix
remove bubbles syringe mix
HCL 100 uL add
2 syringe mix to form clear soln ~ 2mL
add 50 uL 1N NaOH, syringe mix
100 uL XL soln mixed with 2ml
ph measures 5.5
add 50 uL water
paraffin wax paper and Petri dish collagen chip
UVA 11mw/cm2 exposure 2, 3, 5 mins gelled OPTICAL CLARITY GOOD

trial
11 initial 98 mg/ml, target 10 mg/ml

syringe 0.5 ml collagen soln	Vol=	4.9	ml	vol of water=	4150
5:100 XL						

syringe mix with 2000ul water
add 2150 ul water and HCL 50ul to get collagen solution
add 25 ul 1N NaOH, 200 ul XL soln mixed to form clear soln ~5mL
UVA 11mw/cm2 exposure 2, 3, 5 mins

WHAT IS CLAIMED IS:

1. A method for augmenting ocular tissue comprising:

mixing clear liquid collagen selected from one of bovine, porcine and recombinant human origin with a small percentage of cross-linker having as the active ingredient riboflavin to obtain a mixture;

applying the mixture to a living eye tissue; and

exposing the mixture in situ to Ultraviolet A (UVA) radiation at dosages between 6mW/cm^2 and 30mW/cm^2 for periods between 1 minute to 30 minutes to form a layer of tissue augmentation of selected thickness.

2. The method according to claim 1 wherein the living eye tissue is corneal tissue and said tissue augmentation is substantially clear.

3. The method of claim 1 wherein optimal and range parameters are substantially as follows:

Approx. Thickness	**Collagen Conc.(w/v)**	**XL:Coll. Dilution**	**UVA fluence**	**Exp. Time**
100 □m	5% (0.5-7)	1:500 (1-50:500)	12 mW/cm^2 (6-30)	3min. (1-5)
200 □m	5% (0.5-7)	1:100 (1-50:500)	12 mW/cm^2 (6-30)	4 min. (1-15)
300 □m	5% (0.5-7)	5:100 (5-50:500)	12 mW/cm^2 (6-30)	5 min. (1-30)

4. A material for promoting augmentation of ocular tissue comprising:

an ultraviolet radiation-reactive cross-linker mixed into liquid collagen.

5. The material according to claim 4 wherein said ultraviolet radiation-reactive cross-linker is reactive to UVA.

6. The material according to claim 5 wherein said reactive to UVA cross-linker is riboflavin.

7. The material according to claim 6 wherein said liquid collagen is selected from bovine, porcine and recombinant human collagen.

8. A method for augmenting corneal and ocular tissue comprising the steps of:

applying a fluid mixture of liquid collagen having an unreacted ultraviolet radiation-reactive cross-linker to stromal tissue; and

irradiating in vivo said fluid mixture upon said corneal tissue with optical radiation of an effective wavelength and in sufficient dosage and duration sufficient to cause binding to said stromal tissue and gelation of a preselected rigidity in said fluid mixture.

9. The method according to claim 8 wherein said fluid mixture is clear and said ocular tissue is corneal tissue.

10. A method for augmenting ocular tissue including corneal tissue comprising the steps of:

applying a fluid mixture of liquid collagen having therein an unreacted ultraviolet radiation-reactive cross-linker to a mold;

irradiating said fluid mixture in vitro upon said mold with optical radiation of an effective wavelength and in sufficient dosage and duration to cause gelation in a preselected rigidity in said fluid mixture; and thereafter

causing the binding said fluid mixture to ocular tissue.

11. The method according to claim 10 wherein said fluid mixture is clear and said ocular tissue is corneal tissue.