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(54) Title: SYSTEMS AND METHODS FOR CROSS-LINKING TREATMENTS OF AN EYE

(57) **Abstract**: A system for corneal treatment includes a light source that activates cross-linking in at least one selected region of a cornea treated with a cross-linking agent. The light source delivers photoactivating light to the at least one selected region of the cornea according to a set of parameters. The system includes a controller that receives input relating to the cross-linking agent and the set of parameters. The controller includes computer-readable storage media storing: (A) program instructions for determining cross-linking resulting from reactions involving ROS including at least peroxides, superoxides, and hydroxyl radicals, and (B) program instructions for determining cross-linking from reactions not involving oxygen. The controller executes the program instructions to output a calculated amount of cross-linking in the at least one selected region of the cornea. In response to the calculated amount of cross-linking, the light source adjusts at least one value in the set of parameters.

SYSTEMS AND METHODS FOR CROSS-LINKING TREATMENTS OF AN EYE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/069,094, filed October 27, 2014, the contents of which are incorporated entirely herein by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present disclosure pertains to systems and methods for treating disorders of the eye, and more particularly, to systems and methods for cross-linking treatments of the eye.

Description of Related Art

[0003] Cross-linking treatments may be employed to treat eyes suffering from disorders, such as keratoconus. In particular, keratoconus is a degenerative disorder of the eye in which structural changes within the cornea cause it to weaken and change to an abnormal conical shape. Cross-linking treatments can strengthen and stabilize areas weakened by keratoconus and prevent undesired shape changes.

[0004] Cross-linking treatments may also be employed after surgical procedures, such as Laser-Assisted in situ Keratomileusis (LASIK) surgery. For instance, a complication known as post-LASIK ectasia may occur due to the thinning and weakening of the cornea caused by LASIK surgery. In post-LASIK ectasia, the cornea experiences progressive steepening (bulging). Accordingly, cross-linking treatments can strengthen and stabilize the structure of the cornea after LASIK surgery and prevent post-LASIK ectasia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1 illustrates an example system that delivers a cross-linking agent and photoactivating light to a cornea of an eye in order to generate cross-linking of corneal collagen, according to aspects of the present disclosure.

[0006] FIGS. 2A-B illustrate a diagram for photochemical kinetic reactions involving riboflavin and photoactivating light (e.g., ultraviolet A (UVA) light) applied during a corneal cross-linking treatment, according to aspects of the present disclosure.

[0007] FIGS. 3A-C illustrate graphs showing the correlation between model values and experimental data for oxygen depletion experiments, where the model values are based on a model of photochemical kinetic reactions according to aspects of the present disclosure.

[0008] FIG. 4 illustrates a graph showing the correlation between model values and experimental data for non-linear optical microscopy fluorescence experiments, where the model values are based on a model of photochemical kinetic reactions according to aspects of the present disclosure.

[0009] FIGS. 5A-D illustrate graphs showing the correlation between model values and experimental data for fluorescence data based on papain digestion method experiments, where the model values are based on a model of photochemical kinetic reactions according to aspects of the present disclosure.

[0010] FIGS. 6A-B illustrate graphs showing the correlation between model values and experimental data for corneal stromal demarcation line experiments, where the model values are based on a model of photochemical kinetic reactions according to aspects of the present disclosure.

[0011] FIGS. 7A-C illustrate graphs of cross-link profiles for treatments using different protocols, as generated by a model of photochemical kinetic reactions according to aspects of the present disclosure.

[0012] FIGS. 8A-C illustrate graphs of cross-link profiles for treatments using different protocols, as generated by a model of photochemical kinetic reactions, where the cross-link profiles are evaluated to determine the depth for a demarcation line for each protocol according to aspects of the present disclosure.

[0013] FIGS. 9A-B illustrate graphs of demarcation depth versus dose of photoactivating light based on cross-link profiles for treatments using different protocols, as generated by a model of photochemical kinetic reactions according to aspects of the present disclosure.

[0014] FIG. 10 illustrates a graph of cross-link profiles for treatments using different protocols as generated by a model of photochemical kinetic reactions, where the cross-link profiles are evaluated to determine the depth for a demarcation line for each protocol according to aspects of the present disclosure.

[0015] FIG. 11 illustrates the measurement of maximum keratometry (K_{max}) at six and twelve months relative to a baseline for corneas that were experimentally treated according to the protocols employed for FIG. 10.

[0016] FIG. 12A illustrates a graph that plots, for the biomechanical stiffness depth determined for each protocol in FIG. 10, the experimental change of K_{max} for months six and twelve corresponding to the respective protocol, according to aspects of the present disclosure.

[0017] FIG. 12B illustrates a graph that plots, for the area above the demarcation line for each protocol in FIG. 10, the experimental change of K_{max} for months six and twelve corresponding to the respective protocol, according to aspects of the present disclosure.

[0018] FIG. 13 illustrates an example system employing a model of photochemical kinetic reactions according to aspects of the present disclosure.

SUMMARY

[0019] According to aspects of the present disclosure, a system for corneal treatment includes a light source configured to activate cross-linking in at least one selected region of a cornea treated with a cross-linking agent. The light source is configured to deliver photoactivating light to the at least one selected region of the cornea according to a set of parameters. The system also includes a controller configured to receive input relating to the cross-linking agent and the set of parameters for the delivery of the photoactivating light. The controller includes computer-readable storage media storing: (A) a first set of program instructions for determining, from the input, cross-linking resulting from reactions involving reactive oxygen species (ROS) including at least peroxides, superoxides, and hydroxyl radicals, and (B) a second set of program instructions for determining, from the input, cross-linking from reactions not involving oxygen. The controller is configured to execute the first and second sets of program instructions to output a calculated amount of cross-linking in the at least one selected region of the cornea. In response to the calculated amount of cross-linking output by the controller, the light source is configured to adjust at least one value in the set of parameters for the delivery of the photoactivating light.

[0020] According to further aspects of the present disclosure,a system for corneal treatment includes a light source configured to activate cross-linking in at least one selected region of a cornea treated with a cross-linking agent. The light source is configured to deliver photoactivating light to the at least one selected region of the cornea according to a set

of parameters. The system also includes an oxygen source and an oxygen delivery device configured to provide a concentration of oxygen from the oxygen source to the at least one selected region of the cornea. The system also includes a controller configured to receive input relating to the cross-linking agent, the set of parameters for the delivery of the photoactivating light, and the concentration of oxygen. The controller includes computer-readable storage media storing: (A) a first set of program instructions for determining, from the input, cross-linking resulting from reactions involving reactive oxygen species (ROS) including at least peroxides, superoxides, and hydroxyl radicals, and (B) a second set of program instructions for determining, from the input, cross-linking from reactions not involving oxygen. The controller is configured to execute the first and second sets of program instructions to output a calculated amount of cross-linking in the at least one selected region of the cornea, the calculated amount of cross-linking indicating a three-dimensional distribution of cross-links. In response to the calculated amount of the cross-linking activity output by the controller, at least one of: (i) the light source is configured to adjust at least one value in the set of parameters for the delivery of the photoactivating light, or (ii) the oxygen delivery device is configured to adjust a value of the concentration of oxygen delivered to the at least one selected region of the cornea.

DESCRIPTION

[0021] FIG. 1 illustrates an example treatment system 100 for generating cross-linking of collagen in a cornea 2 of an eye 1. The treatment system 100 includes an applicator 132 for applying a cross-linking agent 130 to the cornea 2. In example embodiments, the applicator 132 may be an eye dropper, syringe, or the like that applies the photosensitizer 130 as drops to the cornea 2. The cross-linking agent 130 may be provided in a formulation that allows the cross-linking agent 130 to pass through the corneal epithelium 2a and to underlying regions in the corneal stroma 2b. Alternatively, the corneal epithelium 2a may be removed or otherwise incised to allow the cross-linking agent 130 to be applied more directly to the underlying tissue.

[0022] The treatment system 100 includes a light source 110 and optical elements 112 for directing light to the cornea 2. The light causes photoactivation of the cross-linking agent 130 to generate cross-linking activity in the cornea 2. For example, the cross-linking agent may include riboflavin and the photoactivating light may be ultraviolet A (UVA) (e.g., 365 nm) light. Alternatively, the photoactivating light may have another wavelength, such as a visible wavelength (e.g., 452 nm). As described further below, corneal cross-linking improves corneal strength by creating chemical bonds within the corneal tissue according to a system of photochemical kinetic reactions. For instance, riboflavin and the photoactivating light are applied to stabilize and/or strengthen corneal tissue to address diseases such as keratoconus or post-LASIK ectasia.

[0023] The treatment system 100 includes one or more controllers 120 that control aspects of the system 100, including the light source 110 and/or the optical elements 112. In an implementation, the cornea 2 can be more broadly treated with the cross-linking agent 130 (e.g., with an eye dropper, syringe, etc.), and the photoactivating light from the light source 110 can be selectively directed to regions of the treated cornea 2 according to a particular pattern.

[0024] The optical elements 112 may include one or more mirrors or lenses for directing and focusing the photoactivating light emitted by the light source 110 to a particular pattern on the cornea 2. The optical elements 112 may further include filters for partially blocking wavelengths of light emitted by the light source 110 and for selecting particular wavelengths of light to be directed to the cornea 2 for activating the cross-linking agent 130. In addition, the optical elements 112 may include one or more beam splitters for dividing a beam of light emitted by the light source 110, and may include one or more heat sinks for absorbing light emitted by the light source 110. The optical elements 112 may also accurately and precisely focus the photo-activating light to particular focal planes within the cornea 2, e.g., at a particular depths in the underlying region 2b where cross-linking activity is desired.

[0025] Moreover, specific regimes of the photoactivating light can be modulated to achieve a desired degree of cross-linking in the selected regions of the cornea 2. The one or more controllers 120 may be used to control the operation of the light source 110 and/or the optical elements 112 to precisely deliver the photoactivating light according to any combination of: wavelength, bandwidth, intensity, power, location, depth of penetration, and/or duration of treatment (the duration of the exposure cycle, the dark cycle, and the ratio of the exposure cycle to the dark cycle duration).

[0026] The parameters for photoactivation of the cross-linking agent 130 can be adjusted, for example, to reduce the amount of time required to achieve the desired cross-linking. In an example implementation, the time can be reduced from minutes to seconds. While some configurations may apply the photoactivating light at an irradiance of 5 mW/cm^2, larger irradiance of the photoactivating light, e.g., multiples of 5 mW/cm^2, can be applied to reduce the time required to achieve the desired cross-linking. The total dose of energy absorbed in the cornea 2 can be described as an effective dose, which is an amount of energy absorbed through an area of the corneal epithelium 2a. For example the effective dose for a region of the corneal surface 2A can be, for example, 5 J/cm^2, or as high as 20 J/cm^2 or 30 J/cm^2. The effective dose described can be delivered from a single application of energy, or from repeated applications of energy.

[0027] The optical elements 112 of the treatment system 100 may include a digital micro-mirror device (DMD) to modulate the application of photoactivating light spatially and temporally. Using DMD technology, the photoactivating light from the light source 110 is projected in a precise spatial pattern that is created by microscopically small mirrors laid out in a matrix on a semiconductor chip. Each mirror represents one or more pixels in the pattern of projected light. With the DMD one can perform topography guided cross-linking. The control of the DMD according to topography may employ several different spatial and temporal irradiance and dose profiles. These spatial and temporal dose profiles may be created using continuous wave illumination but may also be modulated via pulsed illumination by pulsing the illumination source under varying frequency and duty cycle regimes as described above. Alternatively, the DMD can modulate different frequencies and duty cycles on a pixel by pixel basis to give ultimate flexibility using continuous wave illumination. Or alternatively, both pulsed illumination and modulated DMD frequency and duty cycle combinations may be combined. This allows for specific amounts of spatially determined corneal cross-linking. This spatially determined cross-linking may be combined with dosimetry, interferometry, optical coherence tomography (OCT), corneal topography, etc., for pre-treatment planning and/or real-time monitoring and modulation of corneal cross-linking during treatment. Additionally, pre-clinical patient information may be combined with finite element biomechanical computer modeling to create patient specific pre-treatment plans.

[0028] To control aspects of the delivery of the photoactivating light, embodiments may also employ aspects of multiphoton excitation microscopy. In particular, rather than delivering a single photon of a particular wavelength to the cornea 2, the treatment system 100 may deliver multiple photons of longer wavelengths, i.e., lower energy, that combine to initiate the cross-linking. Advantageously, longer wavelengths are scattered within the cornea 2 to a lesser degree than shorter wavelengths, which allows longer wavelengths of light to penetrate the cornea 2 more efficiently than shorter wavelength light. Shielding effects of incident irradiation at deeper depths within the cornea are also reduced over conventional short wavelength illumination since the absorption of the light by the photosensitizer is much less at the longer wavelengths. This allows for enhanced control over depth specific cross-linking. For example, in some embodiments, two photons may be employed, where each photon carries approximately half the energy necessary to excite the molecules in the cross-linking agent 130 to generate the photochemical kinetic reactions described further below. When a cross-linking agent molecule simultaneously absorbs both photons, it absorbs enough energy to release reactive radicals in the corneal tissue. Embodiments may also utilize lower energy photons such that a cross-linking agent molecule must simultaneously absorb, for example, three, four, or five, photons to release a reactive radical. The probability of the near-simultaneous absorption of multiple photons is low, so a high flux of excitation photons may be required, and the high flux may be delivered through a femtosecond laser.

[0029] A large number of conditions and parameters affect the cross-linking of corneal collagen with the cross-linking agent 130. For example, when the cross-linking agent 130 is riboflavin and the photoactivating light is UVA light, the irradiance and the dose both affect the amount and the rate of cross-linking. The UVA light may be applied continuously (continuous wave (CW)) or as pulsed light, and this selection has an effect on the amount, the rate, and the extent of cross-linking.

[0030] If the UVA light is applied as pulsed light, the duration of the exposure cycle, the dark cycle, and the ratio of the exposure cycle to the dark cycle duration have an effect on the resulting corneal stiffening. Pulsed light illumination can be used to create greater or lesser stiffening of corneal tissue than may be achieved with continuous wave illumination for the same amount or dose of energy delivered. Light pulses of suitable length and frequency may be used to achieve more optimal chemical amplification. For pulsed light treatment, the on/off duty cycle may be between approximately 1000/1 to approximately 1/1000; the irradiance may be between approximately 1 mW/cm^2 to approximately 1000 mW/cm^2 average irradiance, and the pulse rate may be between approximately 0.01 HZ to approximately 1000 Hz or between approximately 1000 Hz to approximately 100,000 Hz.

[0031] The treatment system 100 may generate pulsed light by employing a DMD, electronically turning the light source 110 on and off, and/or using a mechanical or opto-electronic (e.g., Pockels cells) shutter or mechanical chopper or rotating aperture. Because of the pixel specific modulation capabilities of the DMD and the subsequent stiffness impartment based on the modulated frequency, duty cycle, irradiance and dose delivered to the cornea, complex biomechanical stiffness patterns may be imparted to the cornea to allow for various amounts of refractive correction. These refractive corrections, for example, may involve combinations of myopia, hyperopia, astigmatism, irregular astigmatism, presbyopia and complex corneal refractive surface corrections because of ophthalmic conditions such as keratoconus, pellucid marginal disease, post-lasik ectasia, and other conditions of corneal biomechanical alteration/degeneration, etc. A specific advantage of the DMD system and method is that it allows for randomized asynchronous pulsed topographic patterning, creating a non-periodic and uniformly appearing illumination which eliminates the possibility for triggering photosensitive epileptic seizures or flicker vertigo for pulsed frequencies between 2 Hz and 84 Hz.

[0032] Although example embodiments may employ stepwise on/off pulsed light functions, it is understood that other functions for applying light to the cornea may be employed to achieve similar effects. For example, light may be applied to the cornea according to a sinusoidal function, sawtooth function, or other complex functions or curves, or any combination of functions or curves. Indeed, it is understood that the function may be substantially stepwise where there may be more gradual transitions between on/off values. In addition, it is understood that irradiance does not have to decrease down to a value of zero during the off cycle, and may be above zero during the off cycle. Desired effects may be achieved by applying light to the cornea according to a curve varying irradiance between two or more values.

[0033] Examples of systems and methods for delivering photoactivating light are described, for example, in U.S. Patent Application Publication No. 2011/0237999, filed March 18, 2011 and titled "Systems and Methods for Applying and Monitoring Eye Therapy," U.S. Patent Application Publication No. 2012/0215155, filed April 3, 2012 and titled "Systems and Methods for Applying and Monitoring Eye Therapy," and U.S. Patent Application Publication No. 2013/0245536, filed March 15, 2013 and titled "Systems and Methods for Corneal Cross-Linking with Pulsed Light," the contents of these applications being incorporated entirely herein by reference.

[0002] The addition of oxygen also affects the amount of corneal stiffening. In human tissue, O_2 content is very low compared to the atmosphere. The rate of cross-linking in the cornea, however, is related to the concentration of O_2 when it is irradiated with photoactivating light. Therefore, it may be advantageous to increase or decrease the concentration of O_2 actively during irradiation to control the rate of cross-linking until a desired amount of cross-linking is achieved. Oxygen may be applied during the cross-linking treatments in a number of different ways. One approach involves supersaturating the riboflavin with O_2. Thus, when the riboflavin is applied to the eye, a higher concentration of

O_2 is delivered directly into the cornea with the riboflavin and affects the reactions involving O_2 when the riboflavin is exposed to the photoactivating light. According to another approach, a steady state of O_2 (at a selected concentration) may be maintained at the surface of the cornea to expose the cornea to a selected amount of O_2 and cause O_2 to enter the cornea. As shown in FIG. 1, for instance, the treatment system 100 also includes an oxygen source 140 and an oxygen delivery device 142 that optionally delivers oxygen at a selected concentration to the cornea 2. Example systems and methods for applying oxygen during cross-linking treatments are described, for example, in U.S. Patent No. 8,574,277, filed October 21, 2010 and titled "Eye Therapy," U.S. Patent Application Publication No. 2013/0060187, filed October 31, 2012 and titled "Systems and Methods for Corneal Cross-Linking with Pulsed Light," the contents of these applications being incorporated entirely herein by reference.

[0034] When riboflavin absorbs radiant energy, especially light, it undergoes photoactivation. There are two photochemical kinetic pathways for riboflavin photoactivation, Type I and Type II. Some of the reactions involved in both the Type I and Type II mechanisms are as follows:

Common reactions:

$Rf \rightarrow Rf_1^*,$ $\quad I;$ $\quad$ (r1)

$Rf_1^* \rightarrow Rf,$ $\quad \kappa 1;$ $\quad$ (r2)

$Rf_1^* \rightarrow Rf_3^*,$ $\quad \kappa 2;$ $\quad$ (r3)

Type I reactions:

$Rf_3^* + DH \rightarrow RfH^{\cdot} + D^{\cdot},$ $\quad \kappa 3;$ $\quad$ (r4)

$2RfH^{\cdot} \rightarrow Rf + RfH_2,$ $\quad \kappa 4;$ $\quad$ (r5)

Type II reactions:

$Rf_3^* + O_2 \rightarrow Rf + O_2^1,$ $\quad \kappa 5;$ $\quad$ (r6)

$DH + O_2^1 \rightarrow D_{ox},$ $\quad \kappa 6;$ $\quad$ (r7)

$D_{ox} + DH \rightarrow D - D,$ $\quad \kappa 7;$ $\quad$ **CXL** $\quad$ (r8)

[0035] In the reactions described herein, Rf represents riboflavin in the ground state. Rf^*_1 represents riboflavin in the excited singlet state. Rf^*_3 represents riboflavin in a triplet excited state. $Rf^{\cdot -}$ is the reduced radical anion form of riboflavin. $RfH^{\cdot}$ is the radical form of riboflavin. RfH_2 is the reduced form of riboflavin. DH is the substrate. $DH^{\bullet+}$ is the intermediate radical cation. $D^{\bullet}$ is the radical. D_{ox} is the oxidized form of the substrate.

[0036] Riboflavin is excited into its triplet excited state Rf^{*}_{3} as shown in reactions (r1) to (r3). From the triplet excited state Rf^{*}_{3}, the riboflavin reacts further, generally according to Type I or Type II mechanisms. In the Type I mechanism, the substrate reacts with the excited state riboflavin to generate radicals or radical ions, respectively, by hydrogen atoms or electron transfer. In Type II mechanism, the excited state riboflavin reacts with oxygen to form singlet molecular oxygen. The singlet molecular oxygen then acts on tissue to produce additional cross-linked bonds.

[0037] Oxygen concentration in the cornea is modulated by UVA irradiance and temperature and quickly decreases at the beginning of UVA exposure. Utilizing pulsed light of a specific duty cycle, frequency, and irradiance, input from both Type I and Type II photochemical kinetic mechanisms can be employed to achieve a greater amount of photochemical efficiency. Moreover, utilizing pulsed light allows regulating the rate of reactions involving riboflavin. The rate of reactions may either be increased or decreased, as needed, by regulating, one of the parameters such as the irradiance, the dose, the on/off duty cycle, riboflavin concentration, soak time, and others. Moreover, additional ingredients that affect the reaction and cross-linking rates may be added to the cornea.

[0038] If UVA radiation is stopped shortly after oxygen depletion, oxygen concentrations start to increase (replenish). Excess oxygen may be detrimental in the corneal cross-linking process because oxygen is able to inhibit free radical photopolymerization reactions by interacting with radical species to form chain-terminating peroxide molecules. The pulse rate, irradiance, dose, and other parameters can be adjusted to achieve a more optimal oxygen regeneration rate. Calculating and adjusting the oxygen regeneration rate is another example of adjusting the reaction parameters to achieve a desired amount of corneal stiffening.

[0039] Oxygen content may be depleted throughout the cornea, by various chemical reactions, except for the very thin corneal layer where oxygen diffusion is able to keep up with the kinetics of the reactions. This diffusion-controlled zone will gradually move deeper into the cornea as the reaction ability of the substrate to uptake oxygen decreases.

[0040] Riboflavin is reduced (deactivated) reversibly or irreversibly and/or photo-degraded to a greater extent as irradiance increases. Photon optimization can be achieved by allowing reduced riboflavin to return to ground state riboflavin in Type I reactions. The rate of return of reduced riboflavin to ground state in Type I reactions is determined by a number of factors. These factors include, but are not limited to, on/off duty cycle of pulsed light treatment, pulse rate frequency, irradiance, and dose. Moreover, the riboflavin concentration, soak time, and addition of other agents, including oxidizers, affect the rate of oxygen uptake. These and other parameters, including duty cycle, pulse rate frequency, irradiance, and dose can be selected to achieve more optimal photon efficiency and make efficient use of both Type I as well as Type II photochemical kinetic mechanisms for riboflavin photosensitization. Moreover, these parameters can be selected in such a way as to achieve a more optimal chemical amplification effect.

[0041] In addition to the photochemical kinetic reactions (r1)-(r8) above, however, the present inventors have identified the following photochemical kinetic reactions (r9)-(r26) that also occur during riboflavin photoactivation:

$Rf_3^* \to Rf,$ $\kappa 8;$ (r9)

$Rf_3^* + Rf \to 2RfH^{\cdot},$ $\kappa 9;$ (r10)

$RfH_2 + O_2 \to RfH^{\cdot} + H^+ + O_2^{-},$ $\kappa 10;$ (r11)

$RfH^{\cdot} + O_2 \to Rf + H^+ + O_2^{-},$ $\kappa 11;$ (r12)

$2\, RfH_2 + O_2^{-} \to 2\, RfH^{\cdot} + H_2O_2,$ $\kappa 12;$ (r13)

$2\, RfH^{\cdot} + O_2^{-} \to 2\, Rf + H_2O_2,$ $\kappa 13;$ (r14)

$RfH^{\cdot} + H_2O_2 \to OH^{\cdot} + Rf + H_2O,$ $\kappa 14;$ (r15)

$OH^{\cdot} + DH \to D^{\cdot} + H_2O,$ $\kappa 15;$ (r16)

$D^{\cdot} + D^{\cdot} \to D - D,$ $\kappa 16;$ **CXL** (r17)

$O_2^1 \to O_2,$ $k18;$ (r18)

$D^{\cdot} + RfH_2 \to RfH^{\cdot} + DH,$ $\kappa 19;$ (r19)

$Rf + Rf \underset{\kappa_a^-}{\overset{\kappa_a^+}{\rightleftarrows}} A_1,$ $\kappa_a = \kappa_a^+ / \kappa_a^-$ (r20)

$RfH_2 + RfH_2 \underset{\kappa_a^-}{\overset{\kappa_a^+}{\rightleftarrows}} A_2,$ $\kappa_a = \kappa_a^+ / \kappa_a^-$ (r21)

$Rf + RfH_2 \underset{\kappa_b^-}{\overset{\kappa_b^+}{\rightleftarrows}} A_3,$ $\kappa_b = \kappa_b^+ / \kappa_b^-$ (r22)

$$Rf_1^* + A \rightarrow Rf + A, \qquad \kappa_{1a} \qquad (r23)$$

$$Rf_3^* + A \rightarrow Rf + A, \qquad \kappa_{3a} \qquad (r24)$$

$$2\,O_2^- \rightarrow O_2 + H_2O_2, \qquad \kappa_{12} \qquad (r25)$$

$$OH^\circ + \text{CXL} \rightarrow inert\ products, \qquad \kappa_{OH} \qquad (r26)$$

[0042] FIG. 2A illustrates a diagram for the photochemical kinetic reactions provided in reactions (r1) through (r26) above. The diagram summarizes photochemical transformations of riboflavin (Rf) under UVA photoactivating light and its interactions with various donors (DH) via electron transfer. As shown, cross-linking activity occurs: (A) through the presence of singlet oxygen in reactions (r6) through (r8) (Type II mechanism); (B) without using oxygen in reactions (r4) and (r17) (Type I mechanism); and (C) through the presence of peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radicals ($^\cdot OH$) in reactions (r13) through (r17).

[0043] As shown in FIG. 2A, the present inventors have also determined that the cross-linking activity is generated to a greater degree from reactions involving peroxide, superoxide, and hydroxyl radicals. Cross-linking activity is generated to a lesser degree from reactions involving singlet oxygen and from non-oxygen reactions. Some models based on the reactions (r1)-(r26) may account for the level of cross-linking activity generated by the respective reactions. For instance, where singlet oxygen plays a smaller role in generating cross-linking activity, models may be simplified by treating the cross-linking activity resulting from singlet oxygen as a constant.

[0044] All the reactions start from Rf_3^* as provided in reactions (r1)-(r3). The quenching of Rf_3^* occurs through chemical reaction with ground state Rf in reaction (r10), and through deactivation by the interaction with water in reaction (r9).

[0045] As described above, excess oxygen may be detrimental in corneal cross-linking process. As shown in FIG. 2A, when the system becomes photon-limited and oxygen-abundant, cross-links can be broken from further reactions involving superoxide, peroxide, and hydroxyl radicals. Indeed, in some cases, excess oxygen may result in net destruction of cross-links versus generation of cross-links.

[0046] As described above, a large variety of factors affect the rate of the cross-linking reaction and the amount of biomechanical stiffness achieved due to cross-linking. A number of these factors are interrelated, such that changing one factor may have an unexpected effect on another factor. However, a more comprehensive model for understanding the relationship between different factors for cross-linking treatment is provided by the photochemical kinetic reactions (r1)-(r26) identified above. Accordingly, systems and methods can adjust various parameters for cross-linking treatment according to this photochemical kinetic cross-linking model, which provides a unified description of oxygen dynamics and cross-linking activity. The model can be employed to evaluate expected outcomes based on different combinations of treatment parameters and to identify the combination of treatment parameters that provides the desired result. The parameters, for example, may include, but is not limited to: the concentration(s) and/or soak times of the applied cross-linking agent; the dose(s), wavelength(s), irradiance(s), duration(s), and/or on/off duty cycle(s) of the photoactivating light; the oxygenation conditions in the tissue; and/or presence of additional agents and solutions.

[0047] A model based on the reactions (r1)-(r19) has been validated by at least four different methods of evaluating cross-linking activity:

- Oxygen depletion experiments
- Non-linear optical microscopy fluorescence experiments
- Fluorescence data based on papain digestion method experiments
- Corneal stromal demarcation line correlation experiments

[0048] For the oxygen depletion experiments, O_2 concentrations were measured and calculated at a depth of approximately 100 μm to approximately 200 μm for corneas treated with riboflavin. FIG. 3A illustrates a graph of data showing the correlation between the theoretical values based on the model and experimental data for corneas exposed to continuous wave UVA photoactivating light at an irradiance of 3mW/cm^2. FIGS. 3B-C illustrate graphs of data showing the correlation between model values and experimental data for corneas exposed to long term pulses and short term pulses, respectively, at an irradiance of 3 mW/cm^2.

[0049] For the non-linear optical microscopy fluorescence experiments, the cross-linking profiles based on corneal depth were determined for corneas treated with riboflavin and exposed to UVA photoactivating light at an irradiance of 3mW/cm^2. FIG. 4 illustrates a graph of data showing the correlation between model and experimental data for corneas exposed for 15 minutes and 30 minutes. The third party experimental data was published in Dongyul Chai et al. "Quantitative Assessment of UVA-riboflavin Corneal Cross-Linking Using Nonlinear Optical Microscopy," Investigative Ophthalmology & Visual Science. June 2011, Vol. 52, No. 7, pp. 4231-4238, the contents of which are incorporated entirely herein by reference.

[0050] For the fluorescence data based on papain digestion method experiments, cross-linking concentrations were evaluated based on fluorescent light intensity. FIG. 5A illustrates a graph of data showing the correlation of model values and experimental data for corneal flaps (taken from 0 to approximately 100 μm deep) exposed to combinations of riboflavin concentrations (0.1%, 0.25%, and 0.5%) and 5.4 J/cm^2 doses of UVA photoactivating light at irradiances of 3 mW/cm^2 and 30 mW/cm^2 for 3 minutes and 30 minutes. Similarly, FIG. 5B illustrates a graph of data showing the correlation of model values and experimental data for corneal flaps (taken from approximately 100 μm to approximately 200 μm deep) exposed to combinations of riboflavin concentrations (0.1%, 0.25%, and 0.5%) and 5.4 J/cm^2 doses of UVA photoactivating light at irradiances of 3 mW/cm^2 and 30 mW/cm^2 for 3 minutes and 30 minutes. FIG. 5C illustrates a graph of data showing the correlation of model values and experimental data for corneal flaps treated with a concentration of riboflavin and exposed to full oxygen concentration and 5.4 J/cm^2 and 7.2 J/cm^2 doses of continuous wave UVA photoactivating light at irradiances of 3 mW/cm^2, 10 mW/cm^2, 15 mW/cm^2, 30 mW/cm^2, 45 mW/cm^2, 60 mW/cm^2, and 100 mW/cm^2. FIG. 5D illustrates a graph of data showing the correlation of model values and experimental data for corneal flaps (taken from approximately 0 μm to approximately 200 μm deep) treated with a concentration of 0.1% riboflavin and exposed to air or full oxygen concentration and a 5.4 J/cm^2 doses of continuous wave UVA photoactivating light at irradiances of 3 mW/cm^2, 10 mW/cm^2, 15 mW/cm^2, 30 mW/cm^2, 45 mW/cm^2, 60 mW/cm^2, and 100 mW/cm^2.

[0051] For the corneal stromal demarcation correlation experiments, corneal stromal demarcation lines were evaluated for treated corneas. Corneal stromal demarcation lines indicate the transition zone between cross-linked anterior corneal stroma and untreated posterior corneal stroma. Aspects of the method for these experiments are described further by Theo Seiler and Farhad Hafezi. "Corneal Cross-Linking-Induced Stromal Demarcation Line." Cornea, Oct. 2006; 25:1057–59, the contents of which are incorporated entirely herein by reference. The corneal FIG. 6A illustrates a graph of data showing the correlation of model values and experimental data for the depths of corneal stromal demarcation lines for the protocols described in FIG. 6B.

[0052] The four evaluations described above show a strong correlation between the experimental data and the calculations generated by a model based on the photochemical kinetic reactions identified above. The model is extremely effective and accurate in predicting the results of riboflavin cross-linking treatments applied according to various combinations of parameters. Accordingly, using such a model, systems and methods can more efficiently and predictably achieve a desired profile of cross-linking activity throughout the cornea. The model allows the systems and methods to identify a more optimal combination of parameters for cross-linking treatment. Therefore, the model can be used to determine the set up for different aspects of cross-linking treatment systems as described above.

[0053] As shown in FIG. 2B, aspects of the system of reactions can be affected by different parameters. For instance, the irradiance at which photoactivating light is delivered to the system affects the photons available in the system to generate Rf_3^* for subsequent reactions. Additionally, delivering greater oxygen into the system drives the oxygen-based reactions. Meanwhile, pulsing the photoactivating light affects the ability of the reduced riboflavin to return to ground state riboflavin by allowing additional time for oxygen diffusion. Of course, other parameters can be varied to control the system of reactions.

[0054] A model based on the photochemical kinetic reactions (r1)-(r26) can generate cross-link profiles for treatments using different protocols as shown in FIGS. 7A-C. In particular, each protocol determines the dose of the photoactivating UVA light, the irradiance for the UVA photoactivating light, the treatment time, and the concentration of oxygen delivered to the corneal surface. The cornea has been treated with a formulation including 0.1% concentration riboflavin. FIG. 7A illustrates cross-link profiles for treatments that deliver a dose of 7.2 J/cm^2 of UVA light under normal (ambient) oxygen according to different irradiances and different treatment times. FIG. 7B illustrates cross-link profiles for treatments that employ different irradiances of continuous or modulated (pulsed) UVA light and different treatment times under normal or 100% oxygen concentration. FIG. 7C illustrates cross-link profiles for treatments that deliver an irradiance of 3 mW of UVA light for 30 minutes with different oxygen conditions (normal, 100%, or 0.01x) at the corneal surface.

[0055] The cross-link profiles in FIGS. 7A-C provide the cross-link concentration as a function of corneal depth. In general, the three-dimensional distribution of cross-links in the cornea as indicated by each cross-link profile depends on the combination of different treatment parameters. Protocols employing different sets of treatment parameters can be provided as input into the model and the model can output the resulting three-dimensional distribution of cross-links in the cornea. Accordingly, the model can be used to select treatment parameters to achieve the desired distribution of cross-links in the cornea.

[0056] As described above, corneal stromal demarcation lines indicate the transition zone between cross-linked anterior corneal stroma and untreated posterior corneal stroma. As also shown in FIG. 8A-C, cross-link profiles generated by the model can be evaluated to determine the depth at which the demarcation line may appear at a cross-link concentration of approximately 5 mol/m^3. Here, the demarcation line may be understood as the threshold at which a healing response occurs in response to the distribution of cross-links as well as the effect of reactive oxygen species on the corneal tissue. The cornea has been treated with a formulation including 0.1% concentration riboflavin. FIG. 8A illustrates a cross-link profile for a treatment that delivers a dose of 5.4 J/cm^2 of photoactivating UVA light under normal oxygen according to an irradiance of 3 mW/cm^2 and a treatment time of 30 minutes. FIG. 8A shows that a cross-link concentration of approximately 5 mol/m^3 (demarcation line) occurs at a depth of approximately 290 μm in the resulting cross-link profile. FIG. 8B illustrates cross-link profiles for treatments that deliver different doses of photoactivating UVA light according to different irradiances and different treatment times under normal oxygen. FIG. 8C illustrates cross-link profiles for treatments that deliver different doses of photoactivating UVA light according to different irradiances and different treatment times under normal or 100% oxygen concentration.

[0057] FIGS. 8B-C shows that the depths for the demarcation line vary with the different cross-link profiles generated by the different sets of treatment parameters. The depths of the demarcation line indicated by the different cross-link profiles may be employed to select treatment parameters. For instance, treatment parameters may be selected to ensure that the cross-links do not occur at a depth where undesired damage may result to the endothelium. This analysis allows the treatment system to accommodate different corneal thicknesses, particularly thin corneas.

[0058] Correspondingly, FIGS. 9A-B illustrate graphs of demarcation depth (cross-link concentration of approximately 5 mol/m^3) as a function of dose of UVA photoactivating light. The determination of the demarcation depths are based on cross-link profiles generated by the model for treatments using different protocols. The cornea has been treated with a formulation including 0.1% concentration riboflavin. FIG. 9A illustrates graphs for treatments that deliver continuous or pulsed UVA photoactivating light according to different irradiances under normal oxygen. FIG. PB illustrates graphs for treatments that deliver continuous or pulsed UVA photoactivating light according to different irradiances under a greater concentration of oxygen.

[0059] FIG. 10 illustrates the cross-link profiles for treatments employing different protocols as generated by the model. FIG. 10 also shows a demarcation line that corresponds to biomechanical stiffness threshold at a cross-link concentration of 10 mol/m^3. The demarcation line intersects the cross-link profiles at varying depths (biomechanical stiffness depth) based on the different treatment parameters of the protocols. FIG. 11 illustrates the measurement of maximum keratometry (K_{max}) (diopters) at three, six, and twelve months relative to a baseline for corneas that were experimentally treated according to the protocols employed for FIG. 10.

[0060] FIGS. 12A-B illustrate the correlation between the experimental data of FIG. 11 and the cross-link profiles generated for FIG. 10 by the model. For the biomechanical stiffness depth determined for each protocol in FIG. 10, FIG. 12A plots the experimental change of K_{max} for months six and twelve corresponding to the respective protocol. FIG. 12A also shows a quadratic fit of the plotted data for each month six and twelve. The quadratic fit is consistent with the quadratic nature of shear forces (in the x-y plane) resulting from a force placed on a disk (along the z-axis) according to thin shell theory.

[0061] Meanwhile, for the area above the demarcation line for the cross-link profile for each protocol in FIG. 10, FIG. 12B plots the experimental change of K_{max} for months six and twelve corresponding to the respective protocol. FIG. 12B also shows a linear fit of the plotted data for each month six and twelve.

[0062] The quadratic fit for the two curves in FIG. 12A are substantially similar. Similarly, the linear fit for the two curves in FIG. 12B are substantially similar. The correlations shown in FIGS. 12A-B indicate that there is a predictable biomechanical/healing response over time for a given set of treatment parameters. In view of the verification of the experimental data points, the model, as well as thin shell analysis, one can predictably determine refractive change according to the radius and depth of the disk corresponding to the myopic correction. In general, the distribution of cross-links effects refractive change. By accurately determining the distribution of cross-links, the model can be employed to determine this refractive change.

[0063] FIG. 13 illustrates the example system 100 employing a model based on the photochemical kinetic reactions (r1)-(r26) identified above. The controller 120 includes a processor 122 and computer-readable storage media 124. The storage media 124 stores program instructions for determining an amount of cross-linking when the photoactivating light from the light source 110 is delivered to a selected region of a cornea treated with a cross-linking agent. In particular, a photochemical kinetic model 126 based on the reactions (r1)-(r26) may include a first set of program instructions A for determining cross-linking resulting from reactions involving reactive oxygen species (ROS) including combinations of peroxides, superoxides, hydroxyl radicals, and/or singlet oxygen and a second set of program instructions B for determining cross-linking from reactions not involving oxygen. The controller 120 receives input relating to treatment parameters and/or other related information. The controller 120 can then execute the program instructions A and B to output information relating to three-dimensional cross-link distribution(s) for the selected region of the cornea based on the input. The three-dimensional cross-link distribution(s) may then be employed to determine how to control aspects of the light source 110, the optical elements 112, the cross-linking agent 130, the applicator 132, the oxygen source 140, and/or oxygen delivery device 142 in order to achieve a desired treatment in selected region of the cornea. (Of course, the system 100 shown in FIG. 13 and this process can be used for treatment of more than one selected region of the same cornea.)

[0064] According to one implementation, the three-dimensional cross-link distribution(s) may be evaluated to calculate a threshold depth corresponding to a healing response due to the cross-links and an effect of the reactive-oxygen species in the selected region of the cornea. Additionally or alternatively, the three-dimensional cross-link distribution(s) may be evaluated to calculate a biomechanical tissue stiffness threshold depth corresponding to a biomechanical tissue response in the selected region of the cornea. The information on the depth of the healing response and/or the biomechanical tissue stiffness in the cornea can be employed to determine how to control aspects of the light source 110, the optical elements 112, the cross-linking agent 130, the applicator 132, the oxygen source 140, and/or oxygen delivery device 142. Certain healing response and/or biomechanical tissue stiffness may be desired or not desired at certain depths of the cornea.

[0065] As described above, according to some aspects of the present disclosure, some or all of the steps of the above-described and illustrated procedures can be automated or guided under the control of a controller (e.g., the controller 120). Generally, the controllers may be implemented as a combination of hardware and software elements. The hardware aspects may include combinations of operatively coupled hardware components including microprocessors, logical circuitry, communication/networking ports, digital filters, memory, or logical circuitry. The controller may be adapted to perform operations specified by a computer-executable code, which may be stored on a computer readable medium.

[0066] As described above, the controller may be a programmable processing device, such as an external conventional computer or an on-board field programmable gate array (FPGA) or digital signal processor (DSP), that executes software, or stored instructions. In general, physical processors and/or machines employed by embodiments of the present disclosure for any processing or evaluation may include one or more networked or non-networked general purpose computer systems, microprocessors, field programmable gate arrays (FPGA's), digital signal processors (DSP's), micro-controllers, and the like, programmed according to the teachings of the example embodiments of the present disclosure, as is appreciated by those skilled in the computer and software arts. The physical processors and/or machines may be externally networked with the image capture device(s), or may be integrated to reside within the image capture device. Appropriate software can be readily prepared by programmers of ordinary skill based on the teachings of the example embodiments, as is appreciated by those skilled in the software art. In addition, the devices and subsystems of the example embodiments can be implemented by the preparation of application-specific integrated circuits or by interconnecting an appropriate network of conventional component circuits, as is appreciated by those skilled in the electrical art(s). Thus, the example embodiments are not limited to any specific combination of hardware circuitry and/or software.

[0067] Stored on any one or on a combination of computer readable media, the example embodiments of the present disclosure may include software for controlling the devices and subsystems of the example embodiments, for driving the devices and subsystems of the example embodiments, for enabling the devices and subsystems of the example embodiments to interact with a human user, and the like. Such software can include, but is not limited to, device drivers, firmware, operating systems, development tools, applications software, and the like. Such computer readable media further can include the computer program product of an embodiment of the present disclosure for performing all or a portion (if processing is distributed) of the processing performed in implementations. Computer code devices of the example embodiments of the present disclosure can include any suitable interpretable or executable code mechanism, including but not limited to scripts, interpretable programs, dynamic link libraries (DLLs), Java classes and applets, complete executable programs, and the like. Moreover, parts of the processing of the example embodiments of the present disclosure can be distributed for better performance, reliability, cost, and the like.

[0068] Common forms of computer-readable media may include, for example, a floppy disk, a flexible disk, hard disk, magnetic tape, any other suitable magnetic medium, a CD-

ROM, CDRW, DVD, any other suitable optical medium, punch cards, paper tape, optical mark sheets, any other suitable physical medium with patterns of holes or other optically recognizable indicia, a RAM, a PROM, an EPROM, a FLASH-EPROM, any other suitable memory chip or cartridge, a carrier wave or any other suitable medium from which a computer can read.

[0069] While the present disclosure has been described with reference to one or more particular embodiments, those skilled in the art will recognize that many changes may be made thereto without departing from the spirit and scope of the present disclosure. Each of these embodiments and obvious variations thereof is contemplated as falling within the spirit and scope of the invention. It is also contemplated that additional embodiments according to aspects of the present disclosure may combine any number of features from any of the embodiments described herein.

WHAT IS CLAIMED IS:

1. A system for corneal treatment, comprising:

a light source configured to activate cross-linking in at least one selected region of a cornea treated with a cross-linking agent, the light source being configured to deliver photoactivating light to the at least one selected region of the cornea according to a set of parameters; and

a controller configured to receive input relating to the cross-linking agent and the set of parameters for the delivery of the photoactivating light, the controller including computer-readable storage media storing:

(A) a first set of program instructions for determining, from the input, cross-linking resulting from reactions involving reactive oxygen species (ROS) including at least peroxides, superoxides, and hydroxyl radicals, and

(B) a second set of program instructions for determining, from the input, cross-linking from reactions not involving oxygen,

the controller being configured to execute the first and second sets of program instructions to output a calculated amount of cross-linking in the at least one selected region of the cornea,

wherein, in response to the calculated amount of cross-linking output by the controller, the light source is configured to adjust at least one value in the set of parameters for the delivery of the photoactivating light.

2. The system of claim 1, further comprising an oxygen source and an oxygen delivery device configured to provide a concentration of oxygen from the oxygen source to the at least one selected region of the cornea,

wherein the input received by the controller further relates to the concentration of oxygen and the controller is configured to execute the first and second sets of program instructions to output the calculated amount of cross-linking based additionally on the concentration of oxygen.

3. The system of claim 1, wherein the calculated amount of the cross-linking indicates a three-dimensional distribution of cross-links in the at least one selected region of the cornea.

4. The system of claim 3, wherein the controller is further configured to calculate a threshold depth corresponding to a healing response due to the three-dimensional distribution

of cross-links and an effect of the reactive-oxygen species in the at least one selected region of the cornea.

5. The system of claim 3, wherein the controller is further configured to calculate a biomechanical tissue stiffness threshold depth corresponding to a biomechanical tissue response due to the three-dimensional distribution of cross-links in the at least one selected region of the cornea.

6. The system of claim 3, further comprising an oxygen source and a delivery device configured to provide a concentration of oxygen from the oxygen source to the at least one selected region of the cornea, wherein the concentration of the oxygen further determines the three-dimensional distribution of cross-links.

7. The system of claim 1, wherein the set of parameters for the delivery of the photoactivating light includes at least one of a wavelength, an irradiance, a dose, pulsing, or continuous wave.

8. The system of claim 1, wherein the controller is coupled to the light source, and the controller is configured to control the light source to deliver the photoactivating light in response to the calculated amount of the cross-linking activity output by the controller.

9. The system of claim 1, wherein the cross-linking agent is riboflavin and the light source delivers ultraviolet photoactivating light.

10. The system of claim 1, wherein the input relating to the cross-linking agent indicates a specified concentration of the cross-linking agent and a soak time for the treatment of the cross-linking agent.

11. The system of claim 1, wherein the first set of program instructions further determines cross-linking resulting from reactions involving reactive oxygen species (ROS) including singlet oxygen.

12. A system for corneal treatment, comprising:

a light source configured to activate cross-linking in at least one selected region of a cornea treated with a cross-linking agent, the light source being configured to deliver photoactivating light to the at least one selected region of the cornea according to a set of parameters;

an oxygen source and an oxygen delivery device configured to provide a concentration of oxygen from the oxygen source to the at least one selected region of the cornea; and

a controller configured to receive input relating to the cross-linking agent, the set of parameters for the delivery of the photoactivating light, and the concentration of oxygen, the controller including computer-readable storage media storing:

(A) a first set of program instructions for determining, from the input, cross-linking resulting from reactions involving reactive oxygen species (ROS) including at least peroxides, superoxides, and hydroxyl radicals, and

(B) a second set of program instructions for determining, from the input, cross-linking from reactions not involving oxygen,

the controller being configured to execute the first and second sets of program instructions to output a calculated amount of cross-linking in the at least one selected region of the cornea, the calculated amount of cross-linking indicating a three-dimensional distribution of cross-links,

wherein, in response to the calculated amount of the cross-linking activity output by the controller, at least one of: (i) the light source is configured to adjust at least one value in the set of parameters for the delivery of the photoactivating light, or (ii) the oxygen delivery device is configured to adjust a value of the concentration of oxygen delivered to the at least one selected region of the cornea.

13. The system of claim 12, wherein the controller is further configured to calculate a threshold depth corresponding to a healing response due to the three-dimensional distribution of cross-links and an effect of the reactive-oxygen species in the at least one selected region of the cornea.

14. The system of claim 12, wherein the controller is further configured to calculate a biomechanical tissue stiffness threshold depth corresponding to a biomechanical tissue response due to the three-dimensional distribution of cross-links in the at least one selected region of the cornea.

15. The system of claim 12, wherein the set of parameters for the delivery of the photoactivating light includes at least one of a wavelength, an irradiance, a dose, pulsing, or continuous wave.

16. The system of claim 12, wherein the controller is coupled to the light source, and the controller is configured to control the light source to deliver the photoactivating light in response to the calculated amount of the cross-linking activity output by the controller.

17. The system of claim 12, wherein the cross-linking agent is riboflavin and the light source delivers ultraviolet photoactivating light.

18. The system of claim 12, wherein the input relating to the cross-linking agent indicates a specified concentration of the cross-linking agent and a soak time for the treatment of the cross-linking agent.

19. The system of claim 12, wherein the first set of program instructions further determines cross-linking resulting from reactions involving reactive oxygen species (ROS) including singlet oxygen.

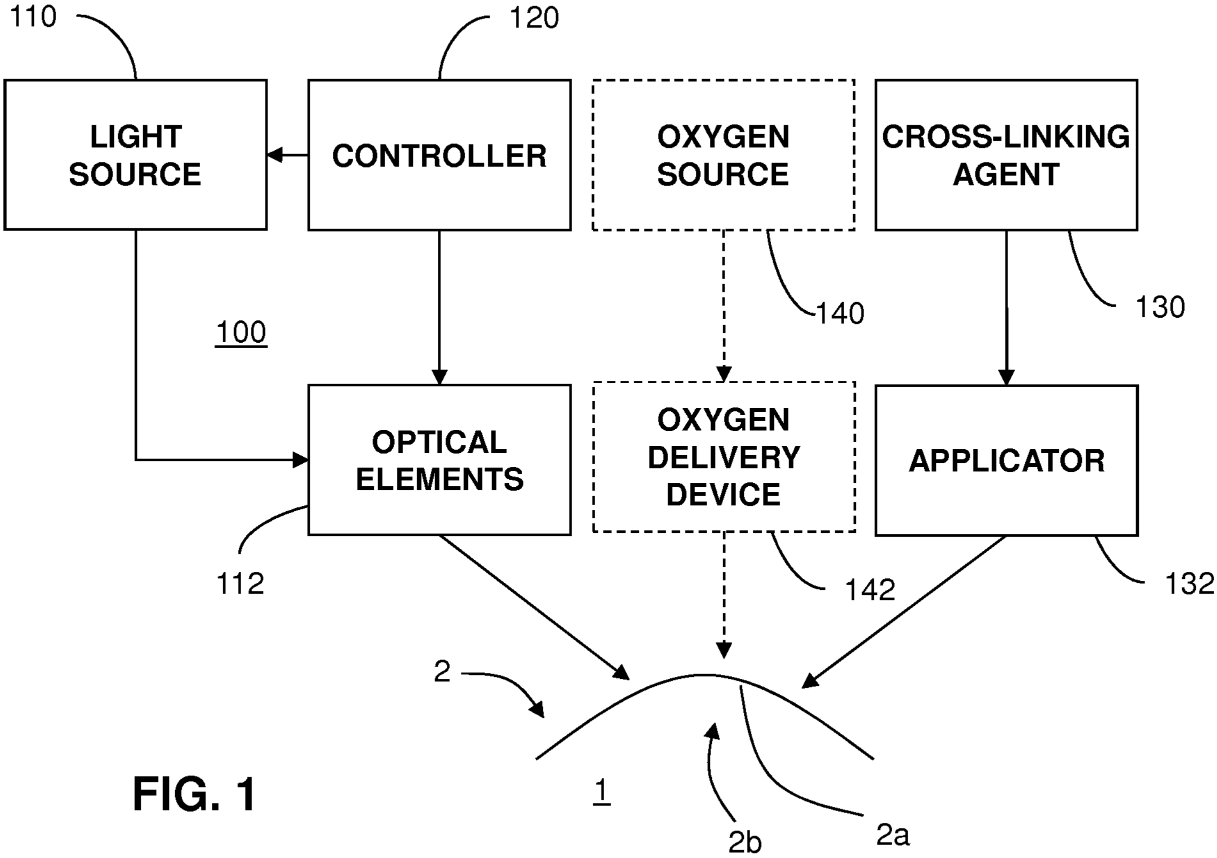

110
120
LIGHT SOURCE
CONTROLLER
OXYGEN SOURCE
CROSS-LINKING AGENT
140
130
100
OPTICAL ELEMENTS
OXYGEN DELIVERY DEVICE
APPLICATOR
112
142
132
2
1
2b
2a

FIG. 1

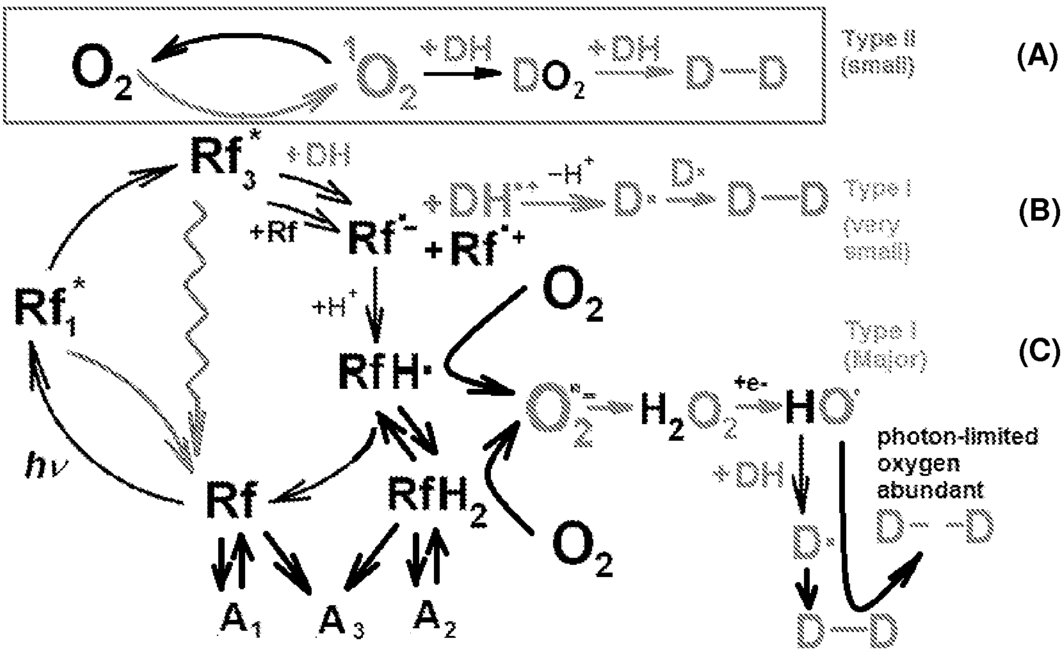

Type II (small)
(A)
Type I (very small)
(B)
Type I (Major)
(C)
photon-limited
oxygen abundant
hν

FIG. 2A

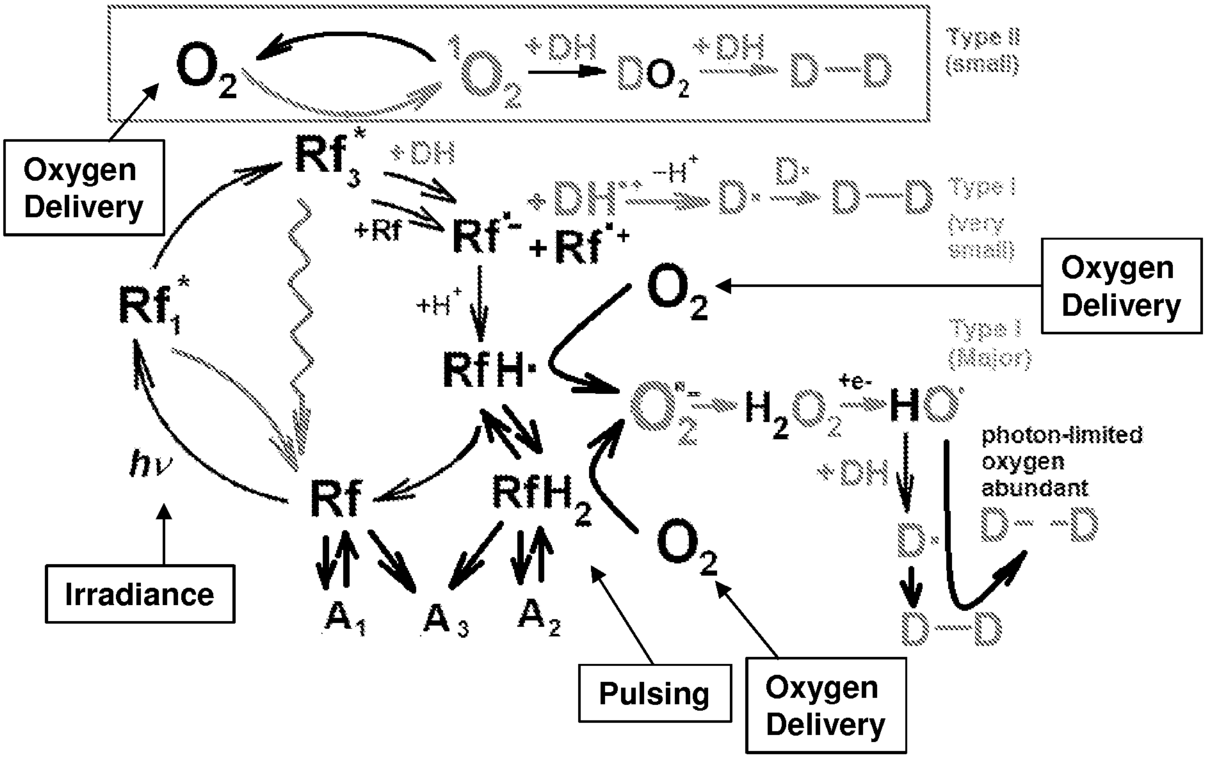

Type II
(small)
Oxygen Delivery
Type I
(very small)
Oxygen Delivery
Type I
(Major)
photon-limited
oxygen
abundant
hν
Irradiance
Pulsing
Oxygen Delivery

FIG. 2B

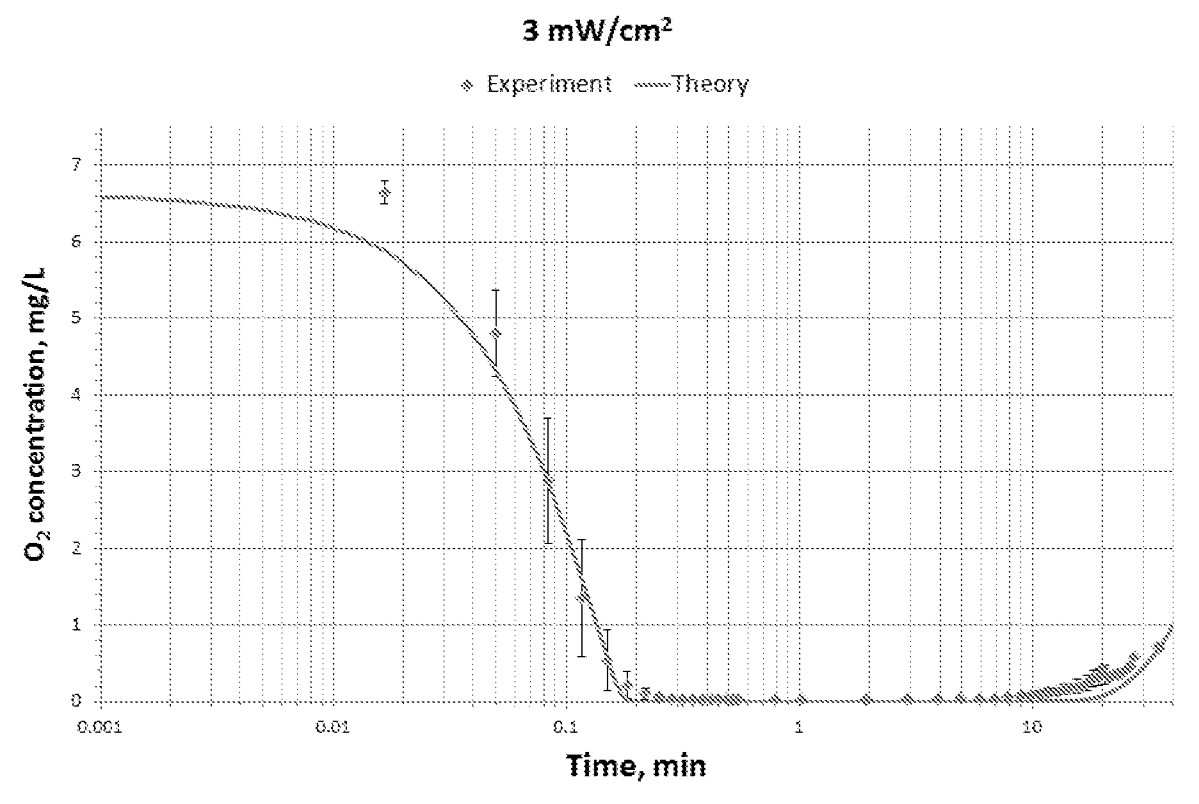

3 mW/cm²
Experiment
Theory
O₂ concentration, mg/L
Time, min

FIG. 3A

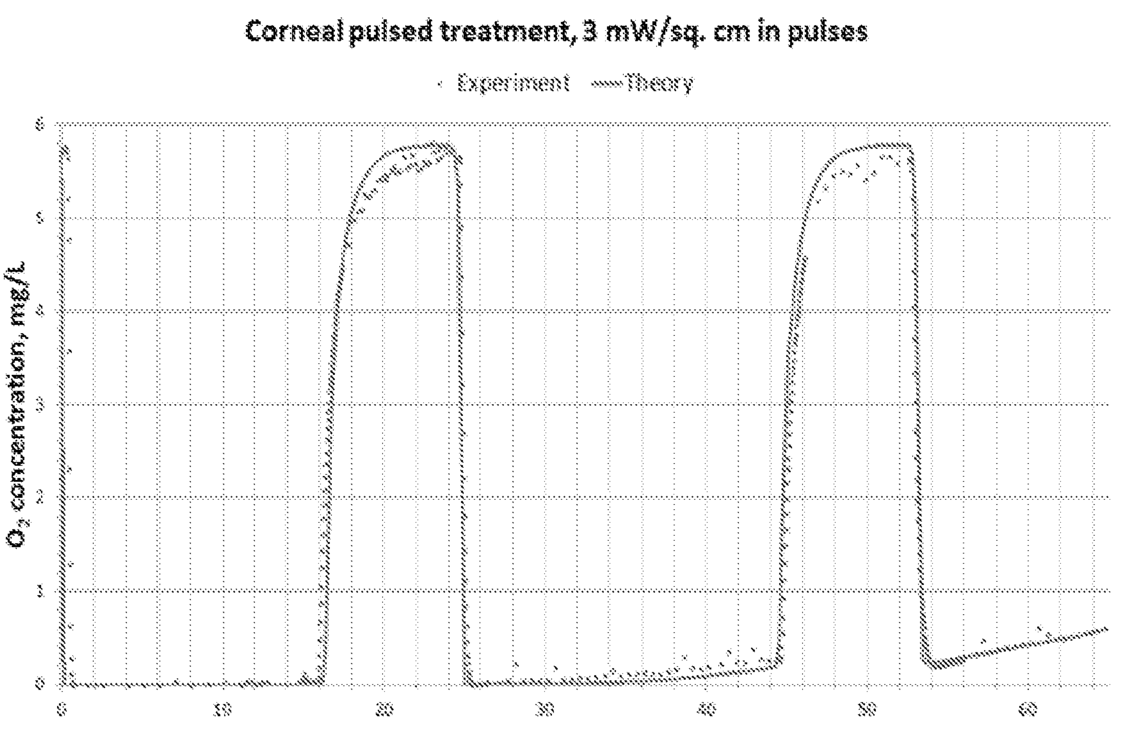

Corneal pulsed treatment, 3 mW/sq. cm in pulses
Experiment
Theory
O2 concentration, mg/L
0
1
2
3
4
5
6
0
10
20
30
40
50
60
Time, min

FIG. 3B

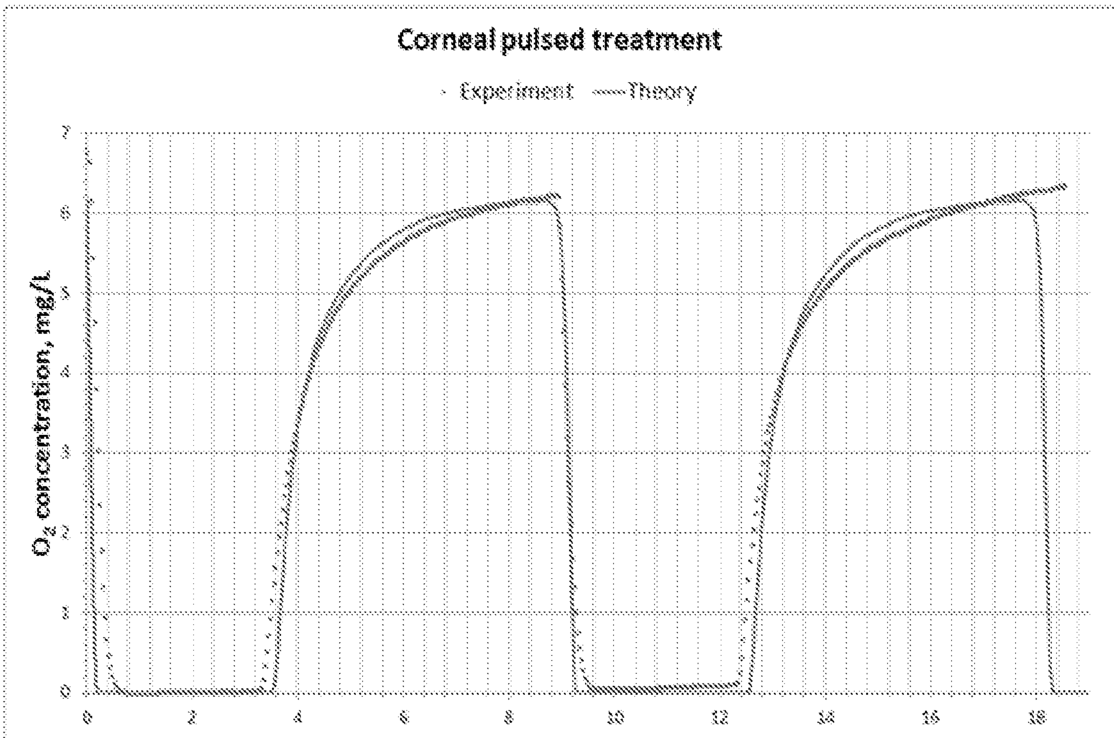

Corneal pulsed treatment
Experiment
Theory
O2 concentration, mg/L
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
18
Time, min

FIG. 3C

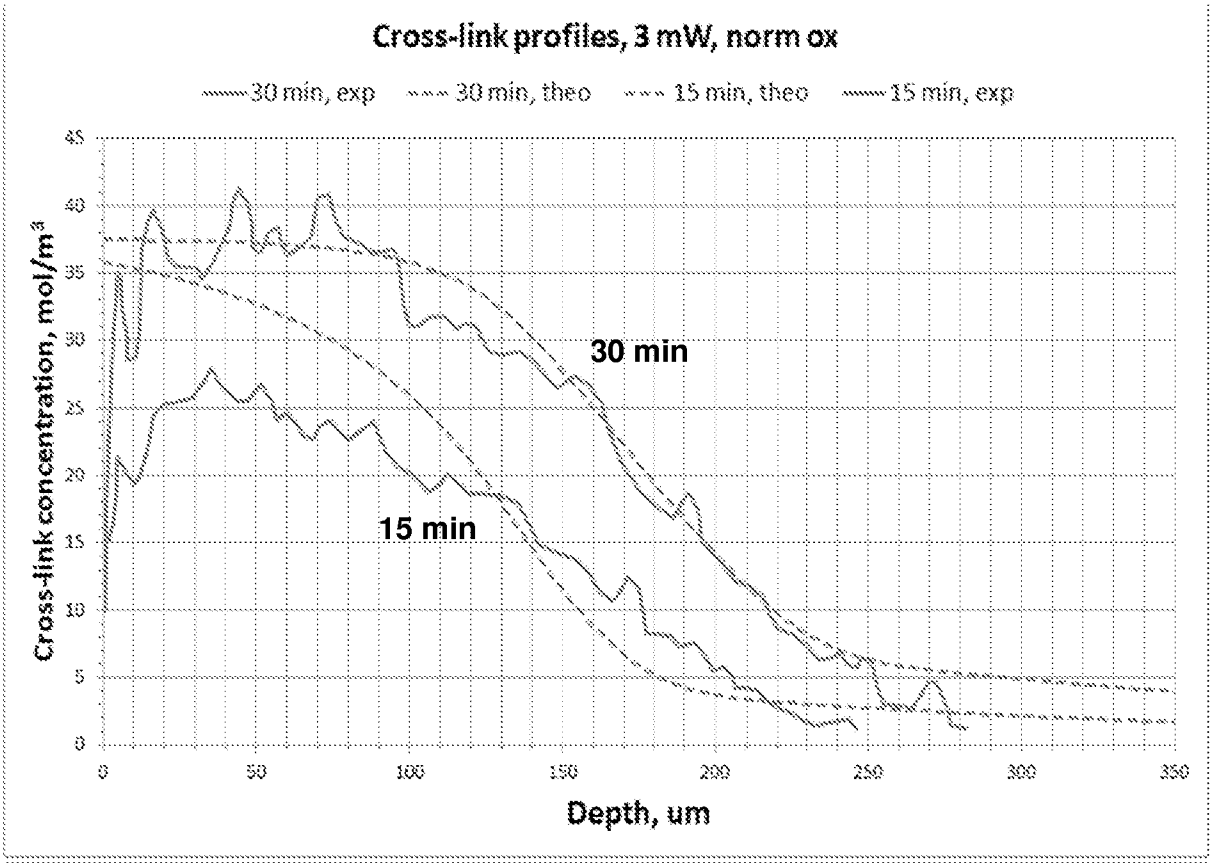

Cross-link profiles, 3 mW, norm ox
30 min, exp
30 min, theo
15 min, theo
15 min, exp
Cross-link concentration, mol/m³
Depth, um
30 min
15 min
0
5
10
15
20
25
30
35
40
45
50
100
150
200
250
300
350

FIG. 4

Upper 100 um of flap

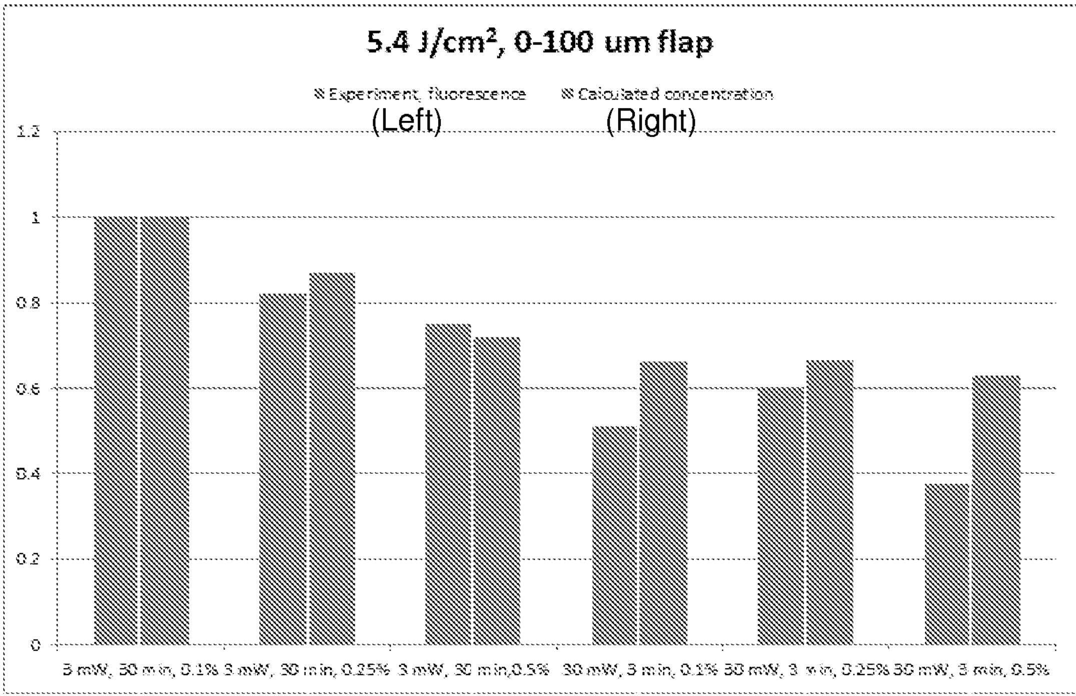


FIG. 5A

Flap slice from 100 to 200 um

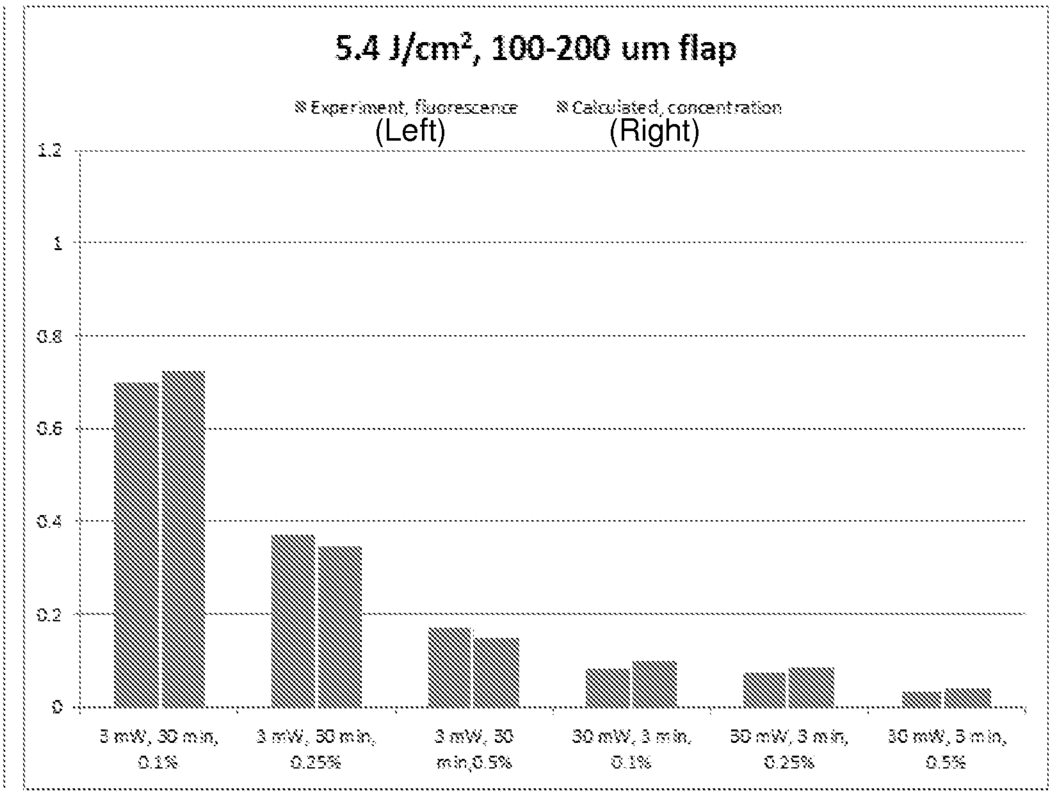


FIG. 5B

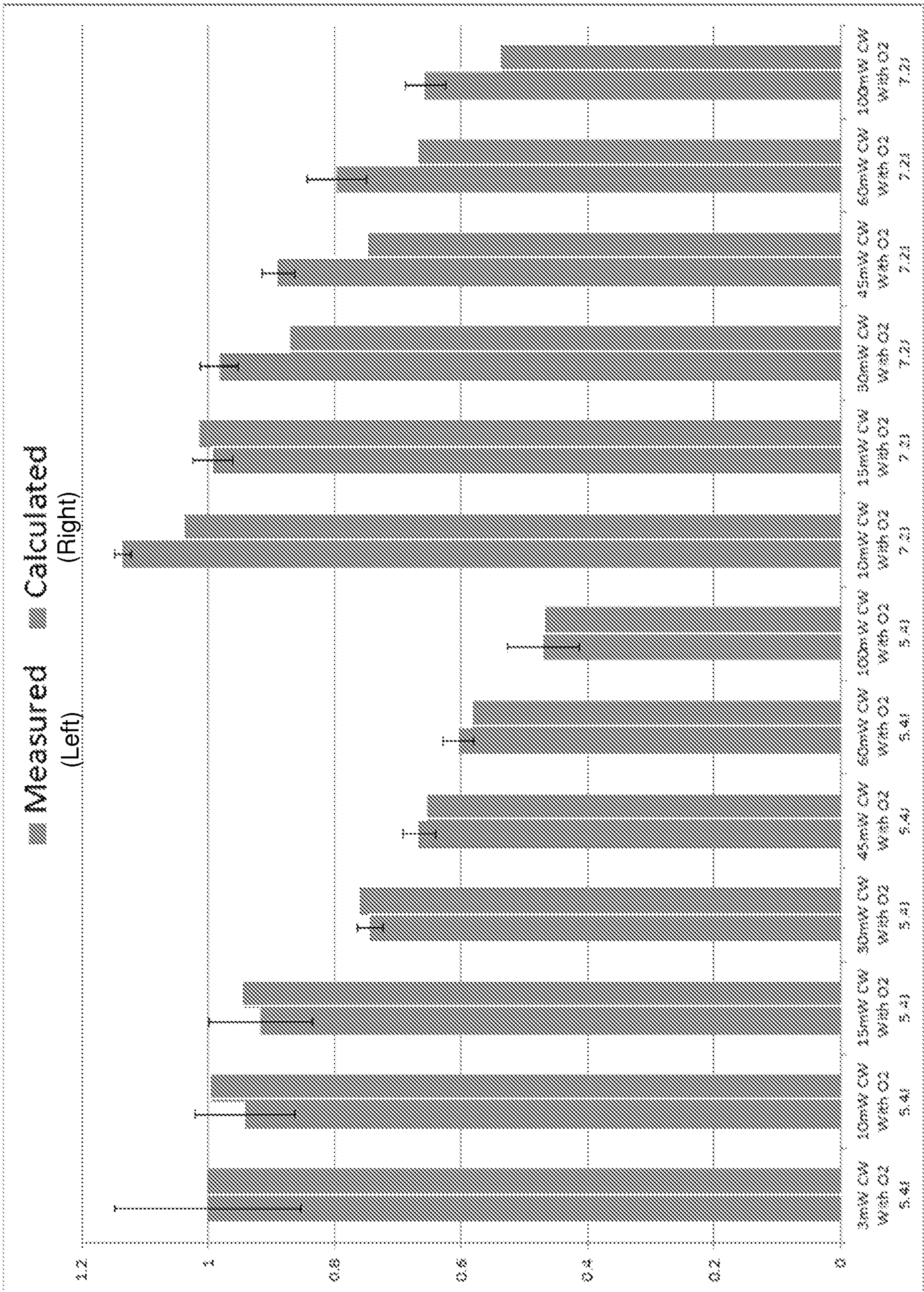


FIG. 5C

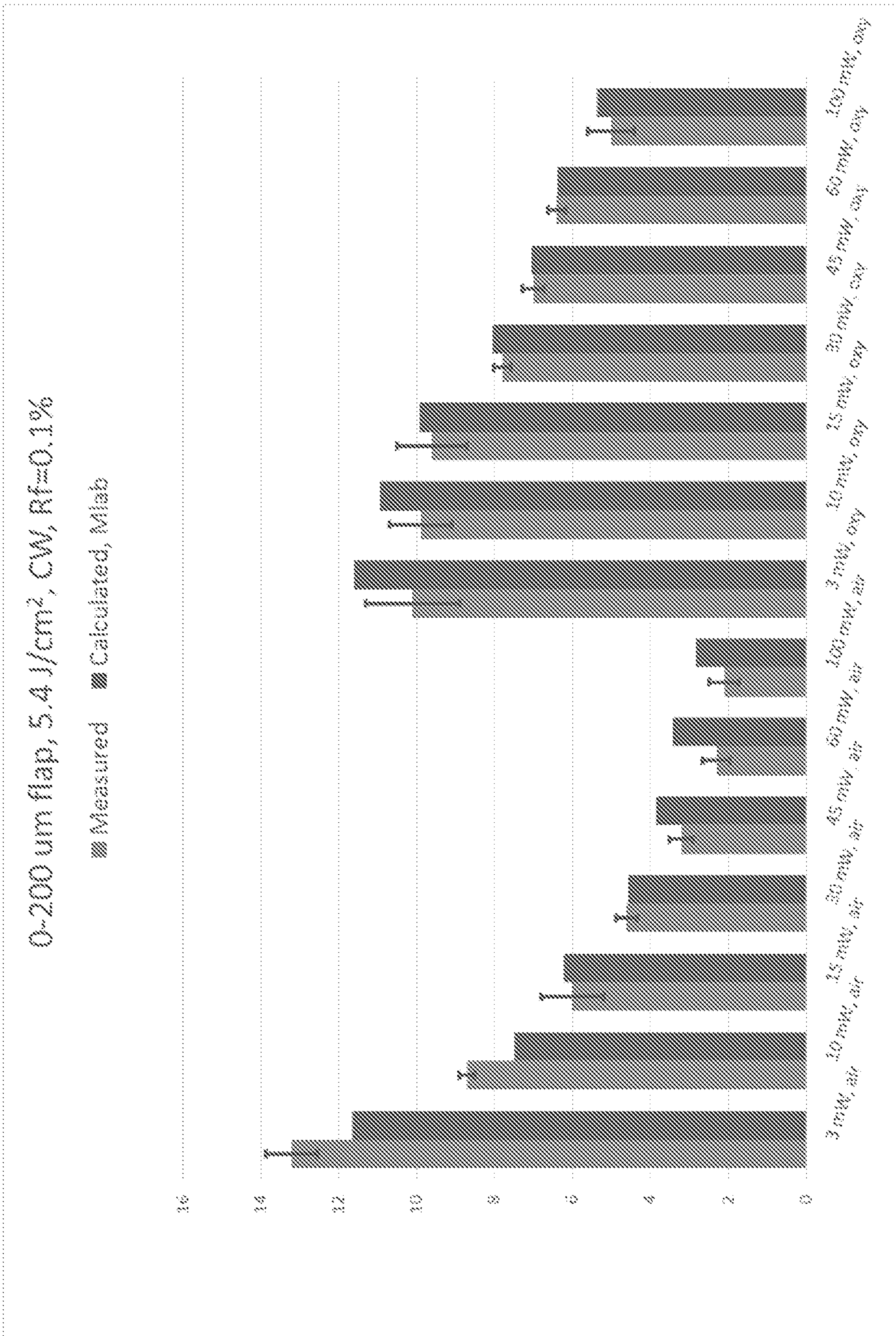

0-200 um flap, 5.4 J/cm², CW, Rf=0.1%
Measured
Calculated, Mlab
0
2
4
6
8
10
12
14
16
3 mW, air
10 mW, air
15 mW, air
30 mW, air
45 mW, air
60 mW, air
100 mW, air
3 mW, oxy
10 mW, oxy
15 mW, oxy
30 mW, oxy
45 mW, oxy
60 mW, oxy
100 mW, oxy

FIG. 5D

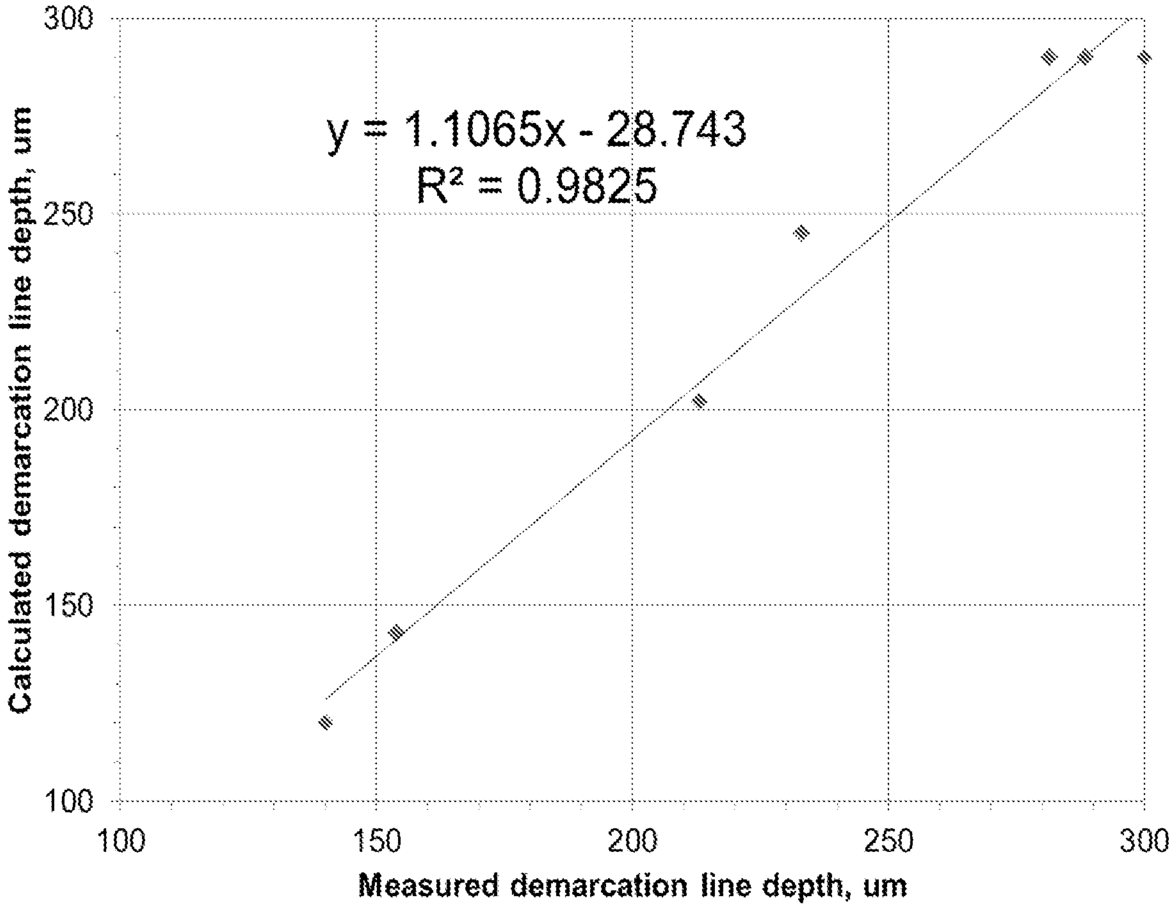

y = 1.1065x - 28.743
R² = 0.9825
300
250
200
150
100
100
150
200
250
300
Calculated demarcation line depth, um
Measured demarcation line depth, um

FIG. 6A

Protocol	Literature source	Measured depth, um	Calculated depth, um
30 min presoak (for calculations) 3 mW/cm², 30 min treatment (Dresden, 5.4 J/cm²)	[1] [3] [2] [7]	261.4±53.3 ~300 319.87 ± 31.84 μm (range, 264-364 μm) centrally, 212.87 ± 24.5 μm (range, 176-279 μm) nasally, and 218.84 ± 21.91 μm (range, 191-281 μm) temporally. 358.78 μm ± 49.34 (SD) (range 258.5 to 410 μm)	290
15 min presoak 30 mW/cm², 3 min treatment (Accelerated, 5.4 J/cm²)	[4]	140.4±39.1	120
15 min presoak 30 mW/cm², 4 min treatment (7.2 J/cm²)	[4]	153.85±33.11	143
10 min presoak 15 mW/cm² (average), 8 min treatment (7.2 J/cm²) 1 sec on and 1 sec off"	[4]	213±47	202
10 min presoak 10 mW/cm² (average), 12 min treatment (7.2 J/cm²) 2 sec on and 1 sec off"	[4]	233±92	245
15 min presoak 12 mW/cm² (average), 10 min treatment (7.2 J/cm²)	[5,6]	150-200	223
20 min presoak 30 mW/cm² (average), 4 min treatment (7.2 J/cm²)	[5,6]	140-180	143
10 minutes with 9 mW/cm² of UVA (5.4 J/cm²)	[7]	288.46 ± 42.37 μm (range 238.5 to 353.5 μm)	200

1. Yam JC, *et al.*, *Corneal collagen cross-linking demarcation line depth assessed by Visante OCT after CXL for keratoconus and corneal ectasia.* J Refract Surg. (Jul. 2012), 28(7):475-81.
2. Kymionis GD, *et al.*, *Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography*, J Cataract Refract Surg. (May 2014), 40(5):736-40.
3. Theo Seiler, MD, PhD and Farhad Hafezi, MD., *Corneal Cross-Linking–Induced Stromal Demarcation line*, Cornea (2006), 25:1057–1059.
4. Luigi Fontana and Antonello Moramarco. *Esperienze personali con CXL accelerato*, UOC Oculistica ASMN-IRCCS Reggio Emilia. Roma, 20 Sept. 2014.
5. C. Mazzotta. *In Vivo Corneal Micro-Structural Analysis in Accelerated Corneal Collagen X-Linking*. UOC Oculistica ASMN-IRCCS Reggio Emilia. Roma, 20 Sept. 2014.
6. Cosimo Mazzotta, *et al.*, *Qualitative Investigation of Corneal Changes after Accelerated Corneal Collagen Cross-linking (A-CXL) by In vivo Confocal Microscopy and Corneal OCT*, J Clin Exp Ophthalmol (2013), 4:6.
7. George D Kymionis, *et al.*, *Evaluation of the corneal collagen cross-linking demarcation line profile using anterior segment optical coherence tomography*, Cornea (2013), 32: 907-10.

FIG. 6B

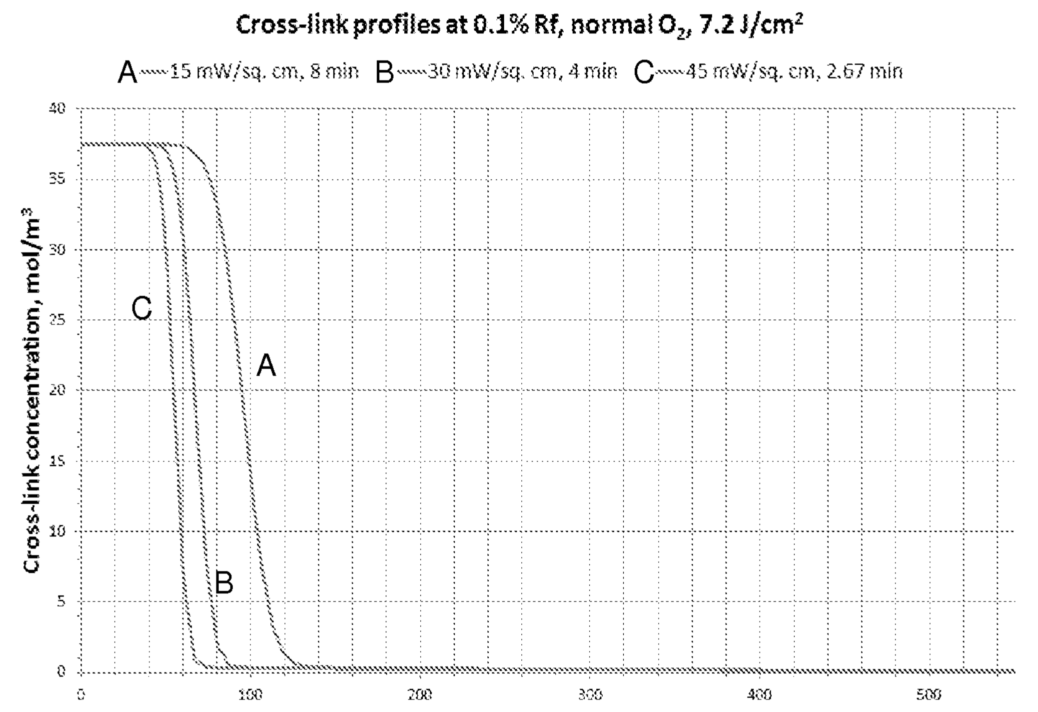

Cross-link profiles at 0.1% Rf, normal O_2, 7.2 J/cm^2
A 15 mW/sq. cm, 8 min
B 30 mW/sq. cm, 4 min
C 45 mW/sq. cm, 2.67 min
Cross-link concentration, mol/m^3
Depth, um
A
B
C

FIG. 7A

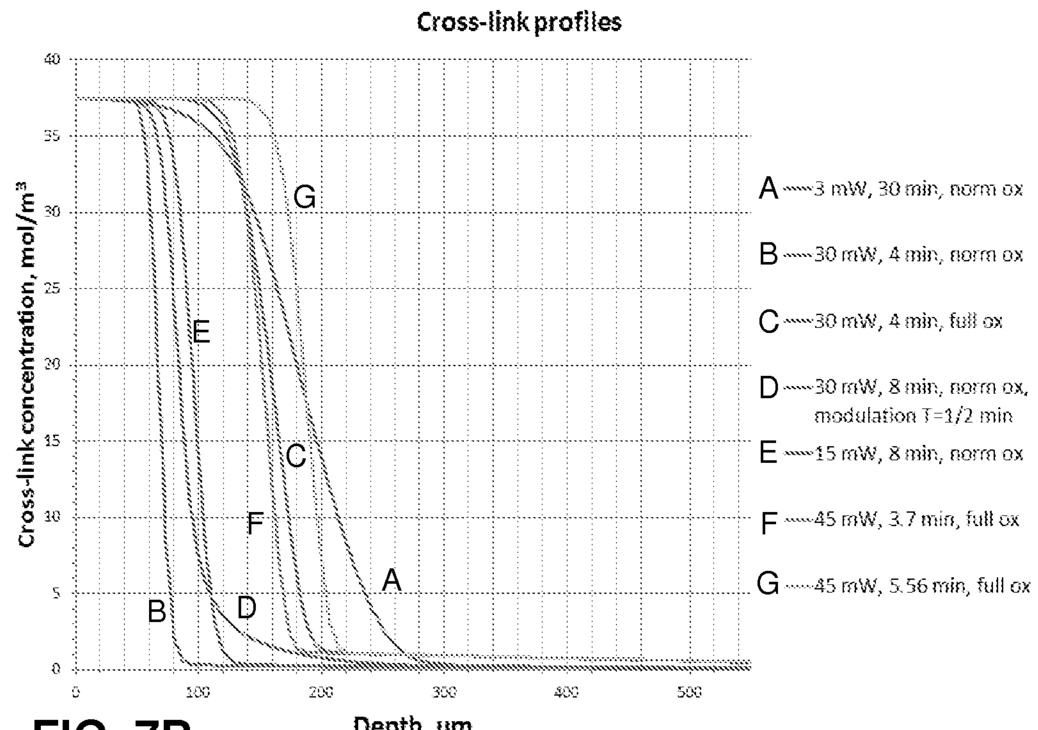

Cross-link profiles
Cross-link concentration, mol/m^3
Depth, um
A 3 mW, 30 min, norm ox
B 30 mW, 4 min, norm ox
C 30 mW, 4 min, full ox
D 30 mW, 8 min, norm ox, modulation T=1/2 min
E 15 mW, 8 min, norm ox
F 45 mW, 3.7 min, full ox
G 45 mW, 5.56 min, full ox

FIG. 7B

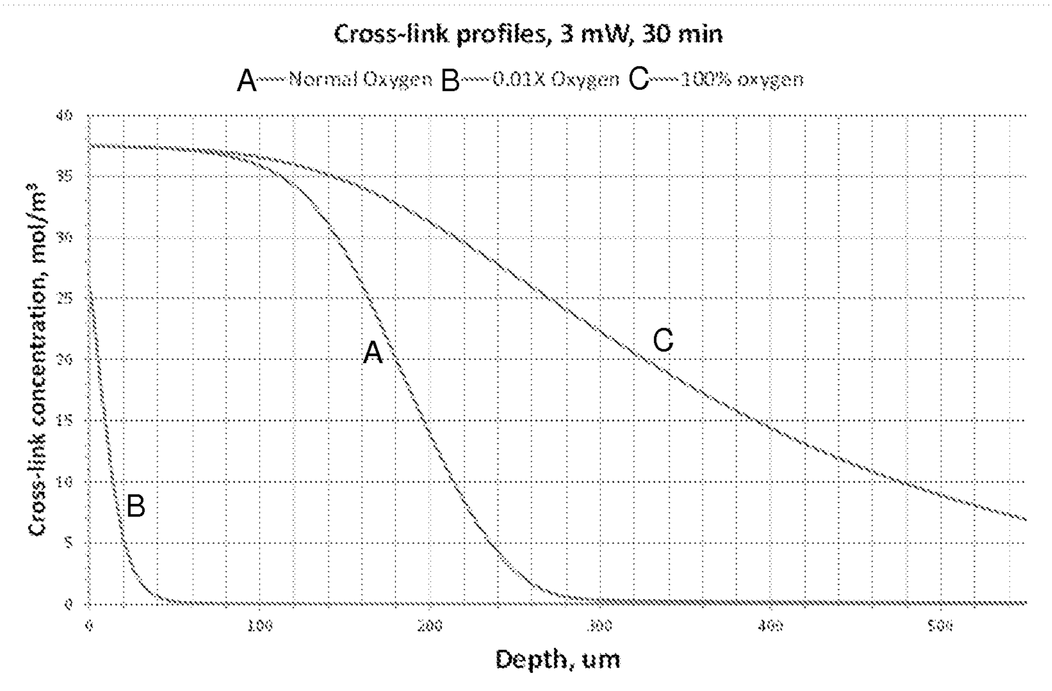

Cross-link profiles, 3 mW, 30 min
A Normal Oxygen B 0.01X Oxygen C 100% oxygen
Cross-link concentration, mol/m³
Depth, um
A
B
C
0
5
10
15
20
25
30
35
40
0
100
200
300
400
500

FIG. 7C

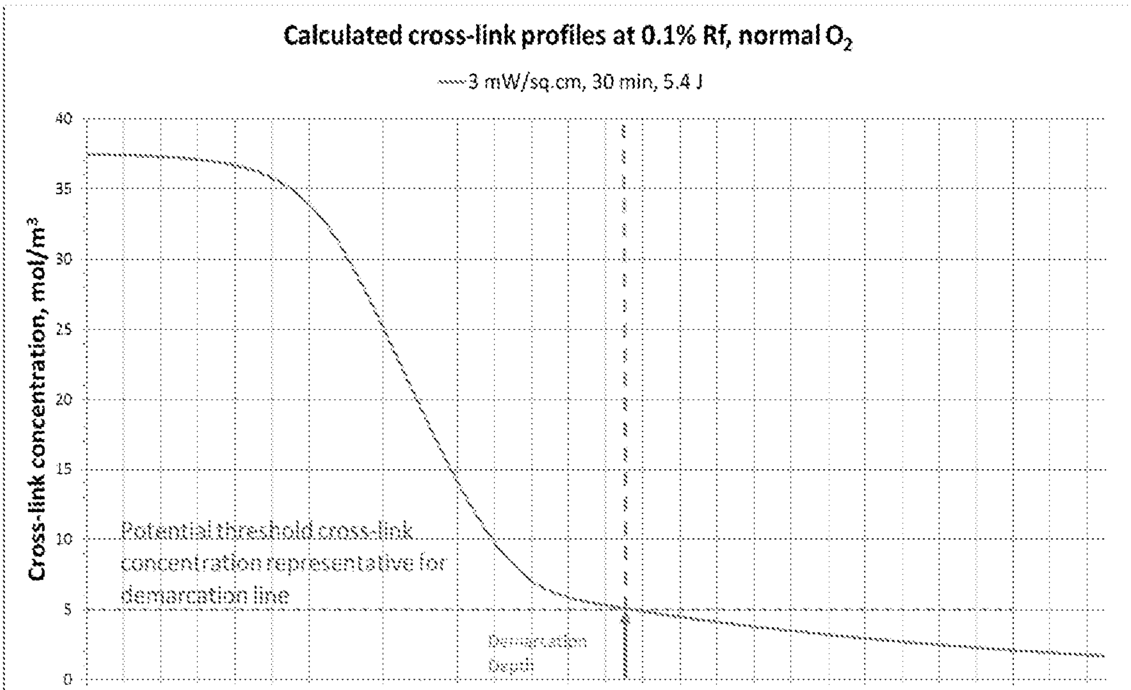

Calculated cross-link profiles at 0.1% Rf, normal O2
3 mW/sq.cm, 30 min, 5.4 J
Cross-link concentration, mol/m³
Depth, um
Potential threshold cross-link concentration representative for demarcation line
Demarcation Depth
0
5
10
15
20
25
30
35
40
0
100
200
300
400
500

FIG. 8A

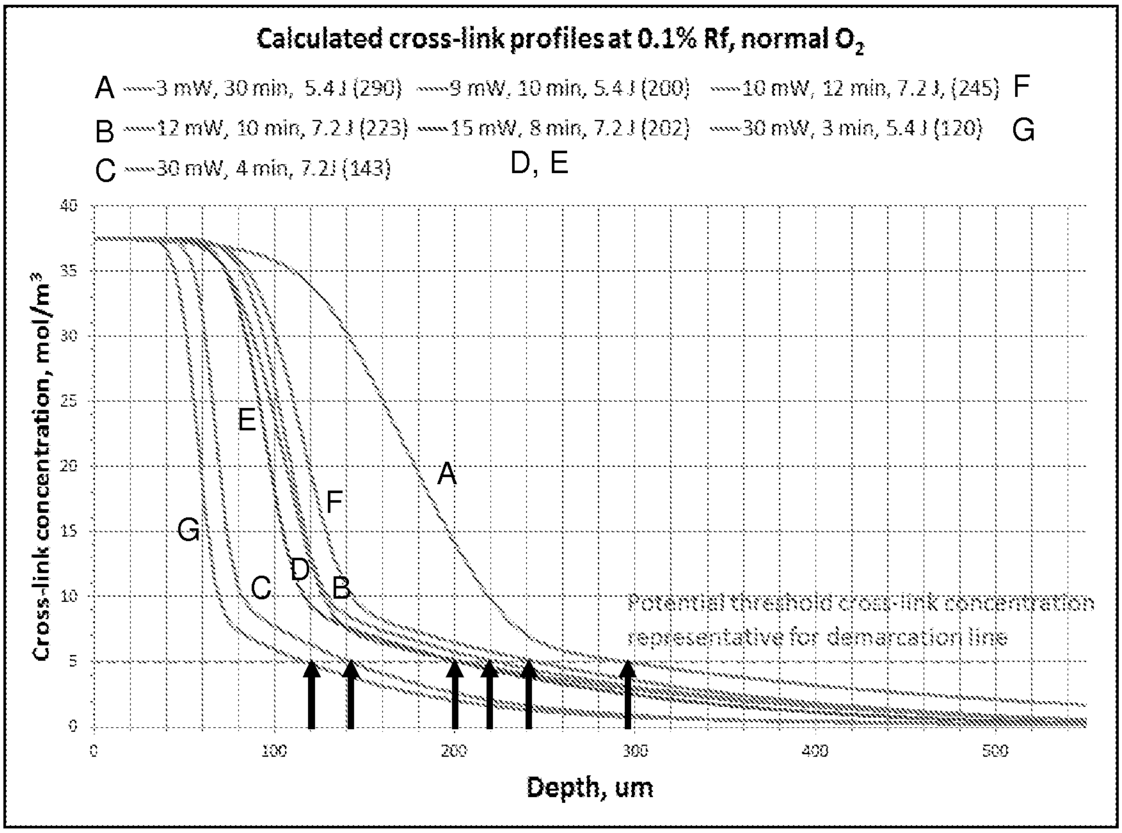

Calculated cross-link profiles at 0.1% Rf, normal O_2
A 3 mW, 30 min, 5.4 J (290)
9 mW, 10 min, 5.4 J (200)
10 mW, 12 min, 7.2 J, (245) F
B 12 mW, 10 min, 7.2 J (223)
15 mW, 8 min, 7.2 J (202)
30 mW, 3 min, 5.4 J (120) G
C 30 mW, 4 min, 7.2J (143)
D, E
Cross-link concentration, mol/m^3
Depth, um
Potential threshold cross-link concentration representative for demarcation line

FIG. 8B

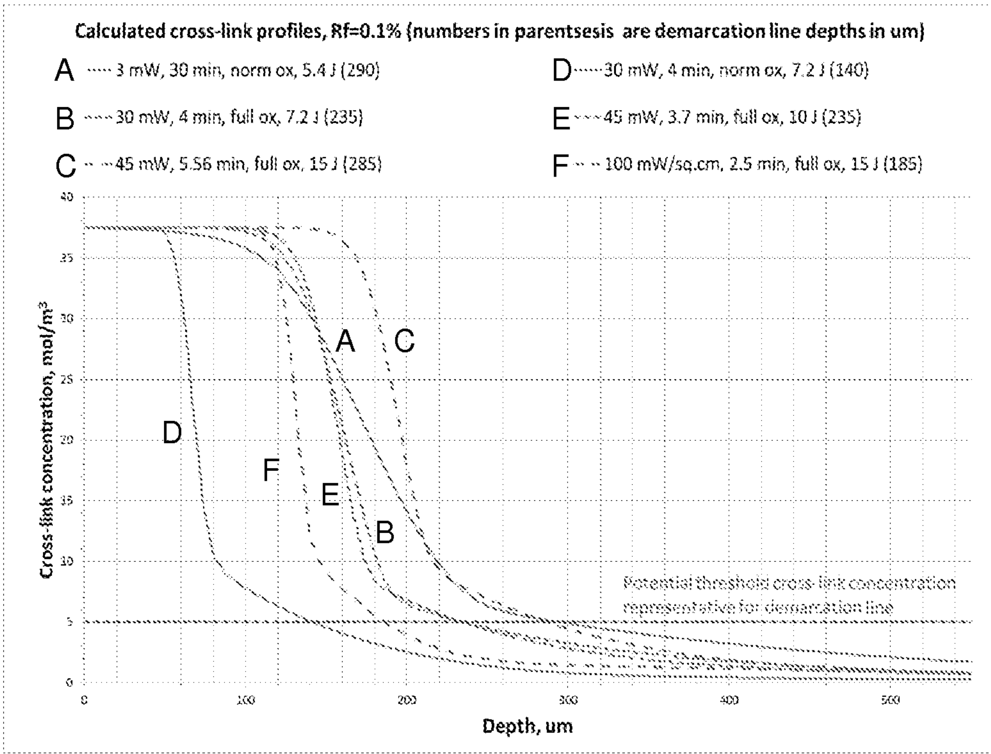

Calculated cross-link profiles, Rf=0.1% (numbers in parentsesis are demarcation line depths in um)
A 3 mW, 30 min, norm ox, 5.4 J (290)
D 30 mW, 4 min, norm ox, 7.2 J (140)
B 30 mW, 4 min, full ox, 7.2 J (235)
E 45 mW, 3.7 min, full ox, 10 J (235)
C 45 mW, 5.56 min, full ox, 15 J (285)
F 100 mW/sq.cm, 2.5 min, full ox, 15 J (185)
Cross-link concentration, mol/m^3
Depth, um
Potential threshold cross-link concentration representative for demarcation line

FIG. 8C

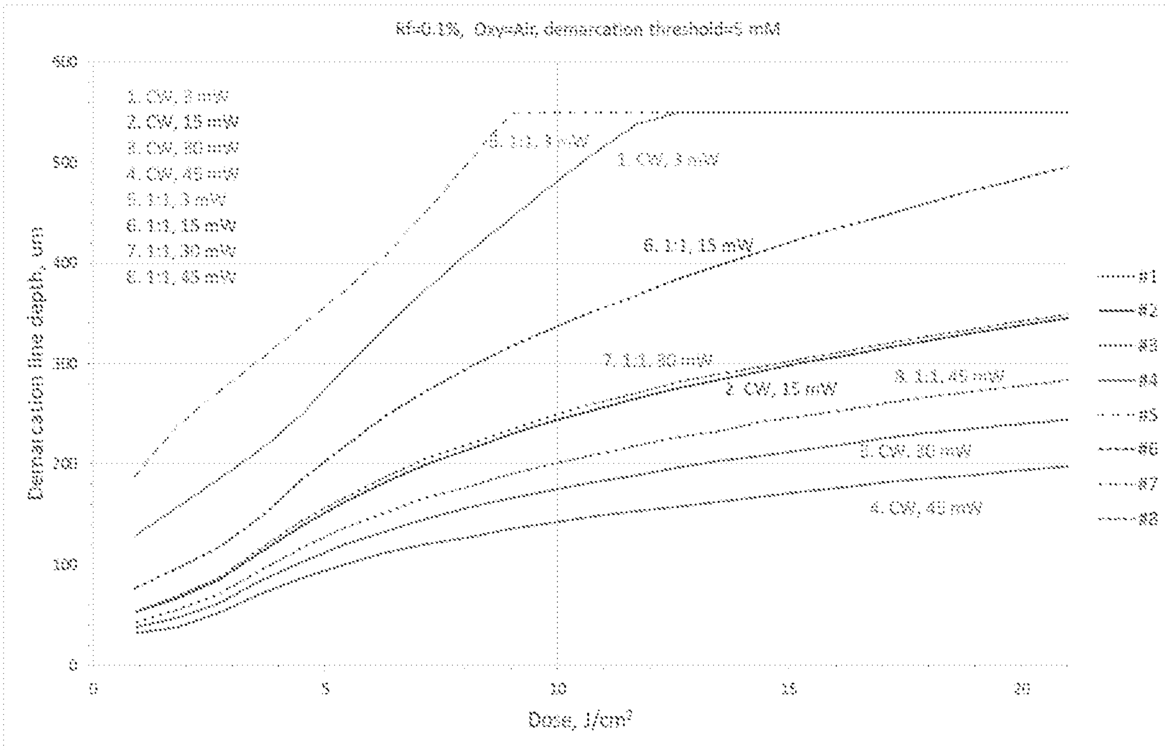

Rf=0.1%, Oxy=Air, demarcation threshold=5 mM
Demarcation line depth, um
Dose, J/cm²
0
100
200
300
400
500
600
0
5
10
15
20
1. CW, 3 mW
2. CW, 15 mW
3. CW, 30 mW
4. CW, 45 mW
5. 1:1, 3 mW
6. 1:1, 15 mW
7. 1:1, 30 mW
8. 1:1, 45 mW
5. 1:1, 3 mW
1. CW, 3 mW
6. 1:1, 15 mW
7. 1:1, 30 mW
2. CW, 15 mW
8. 1:1, 45 mW
3. CW, 30 mW
4. CW, 45 mW
#1
#2
#3
#4
#5
#6
#7
#8

FIG. 9A

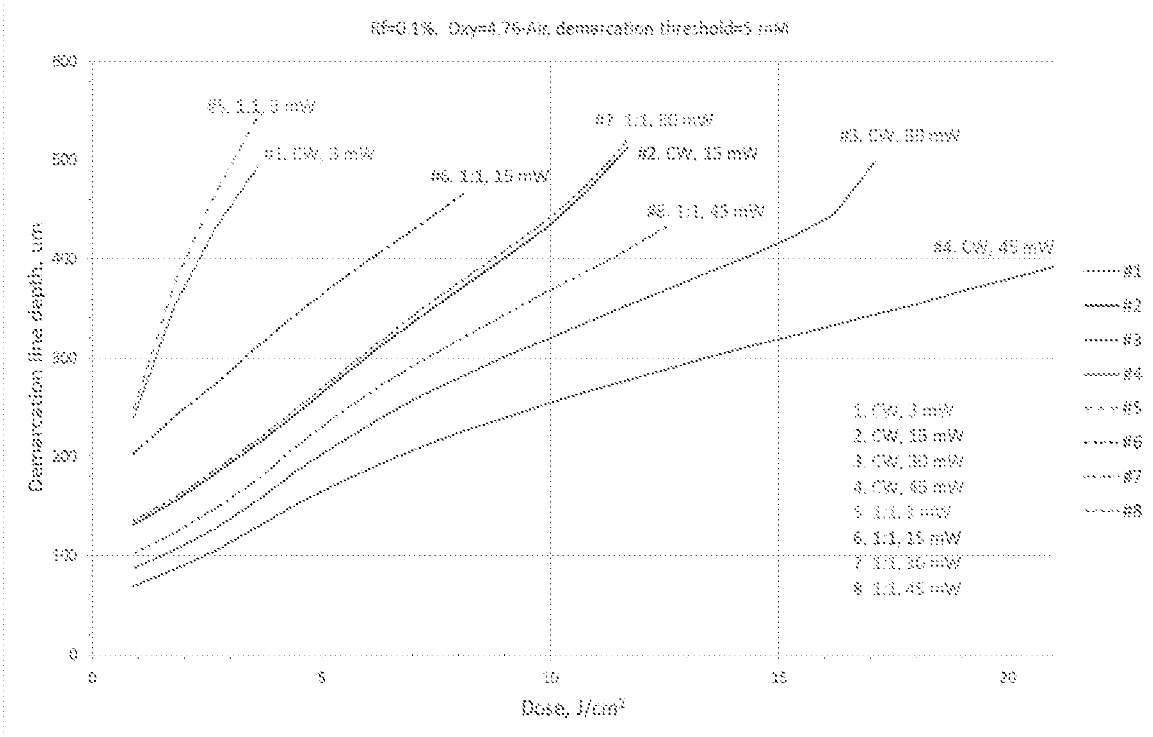
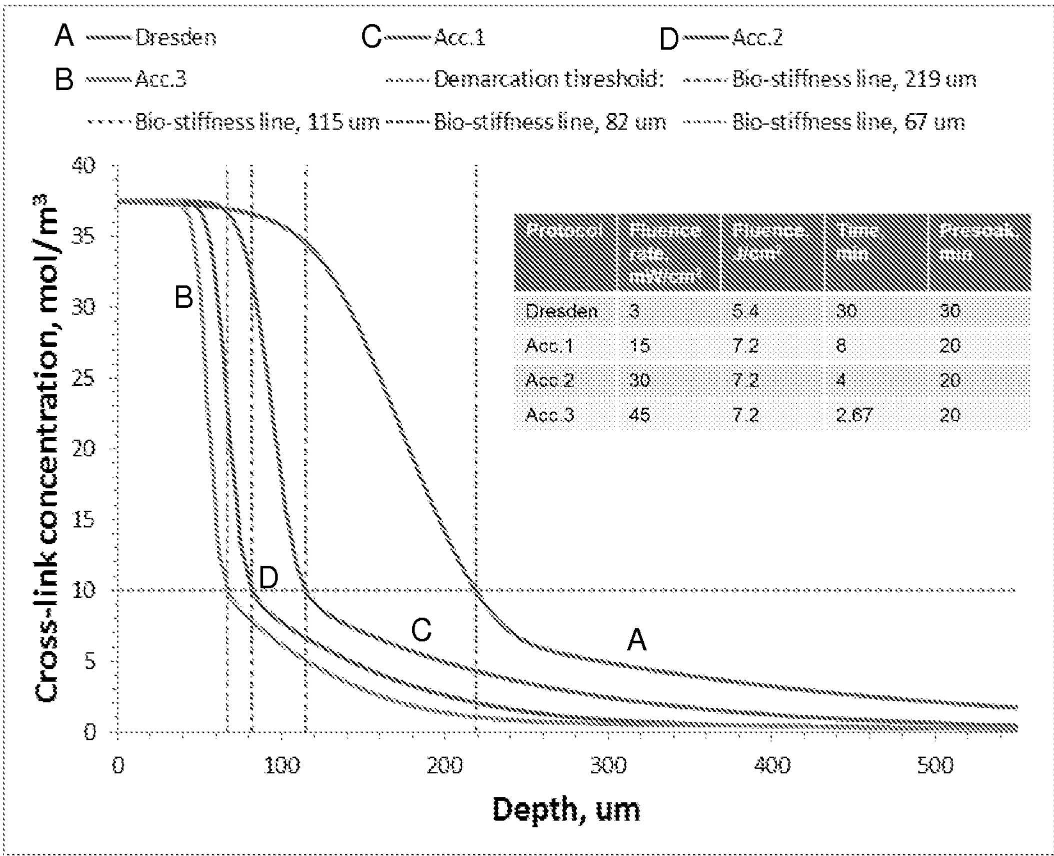

Rf=0.1%, Oxy=4.76·Air, demarcation threshold=5 mM
Demarcation line depth, um
Dose, J/cm²
0
100
200
300
400
500
600
0
5
10
15
20
#5, 1:1, 3 mW
#1. CW, 3 mW
#6. 1:1, 15 mW
#7 1:1, 30 mW
#2. CW, 15 mW
#8. 1:1, 45 mW
#3. CW, 30 mW
#4. CW, 45 mW
1. CW, 3 mW
2. CW, 15 mW
3. CW, 30 mW
4. CW, 45 mW
5 1:1, 3 mW
6. 1:1, 15 mW
7 1:1, 30 mW
8 1:1, 45 mW
#1
#2
#3
#4
#5
#6
#7
#8

FIG. 9B



Protocol	Fluence rate mW/cm²	Fluence J/cm²	Time min	Presoak min
Dresden	3	5.4	30	30
Acc.1	15	7.2	8	20
Acc.2	30	7.2	4	20
Acc.3	45	7.2	2.67	20

FIG. 10

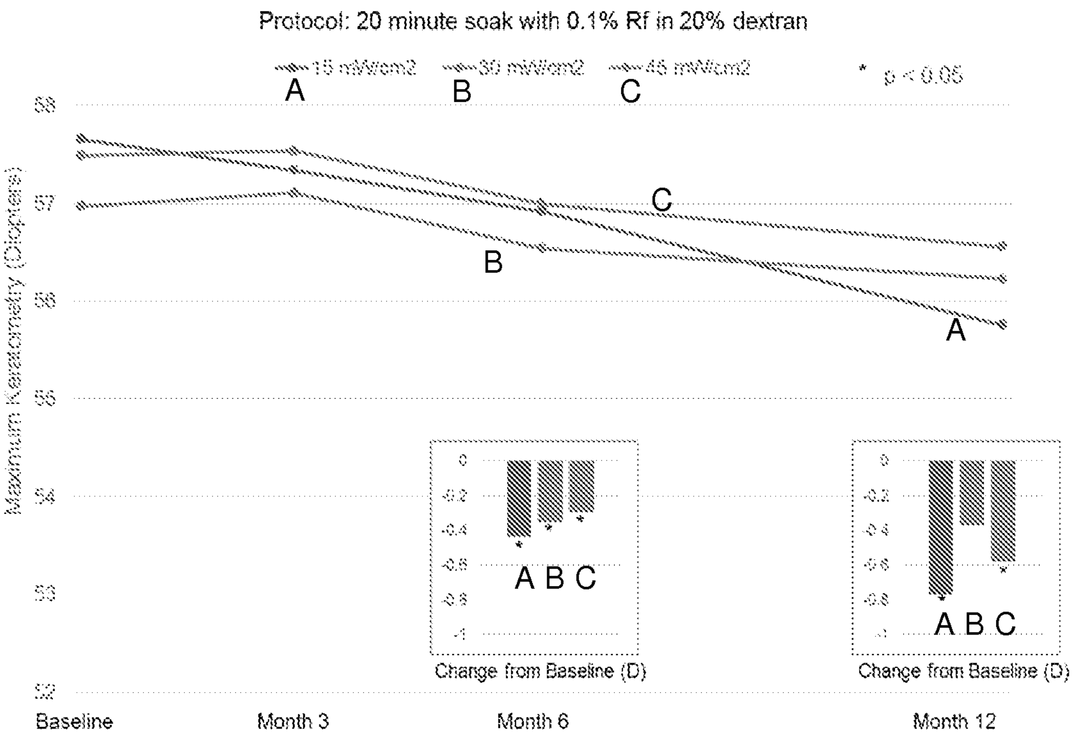


FIG. 11

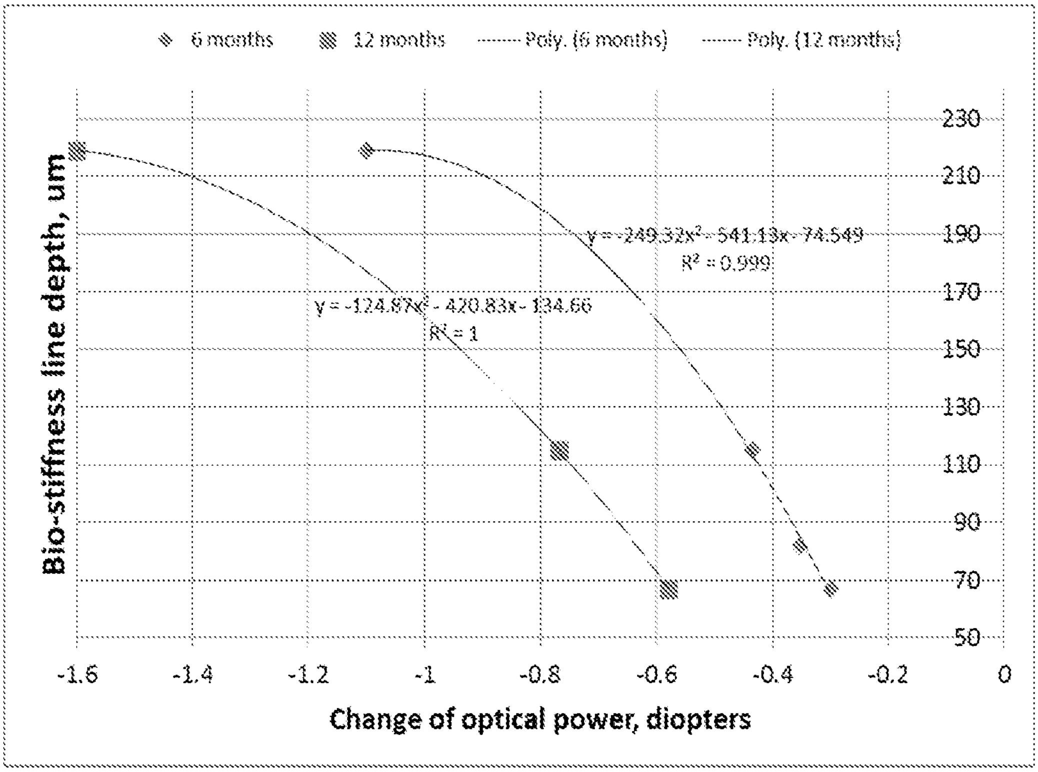


FIG. 12A

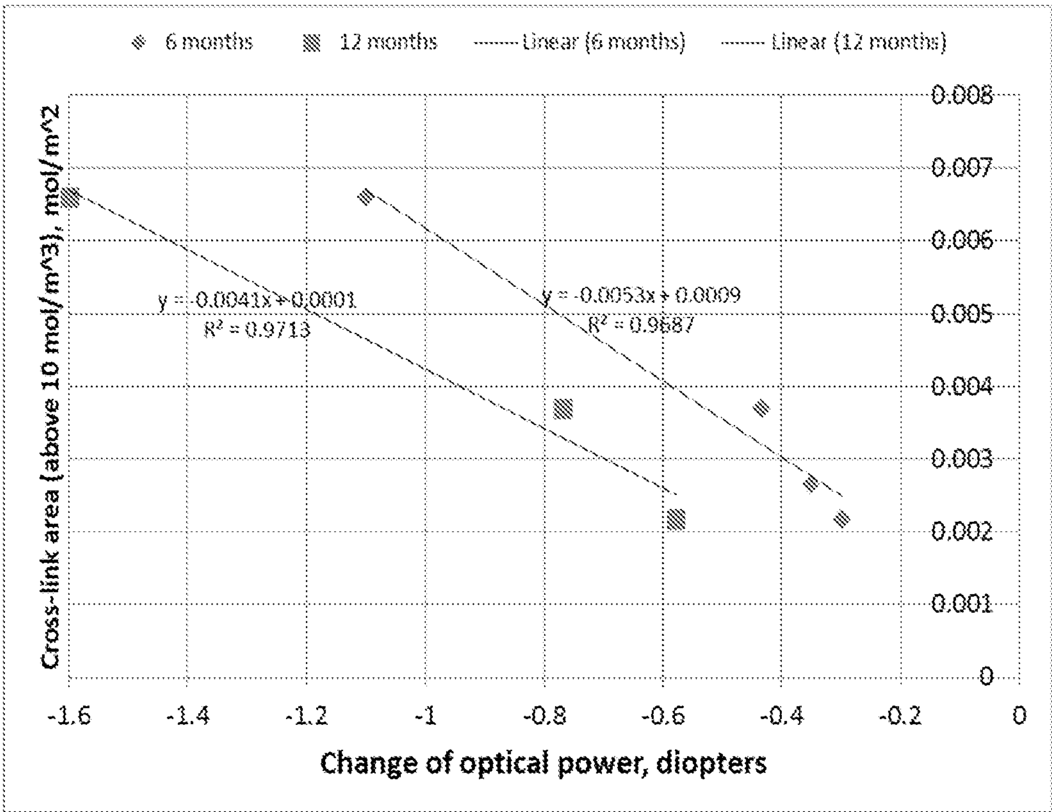


FIG. 12B

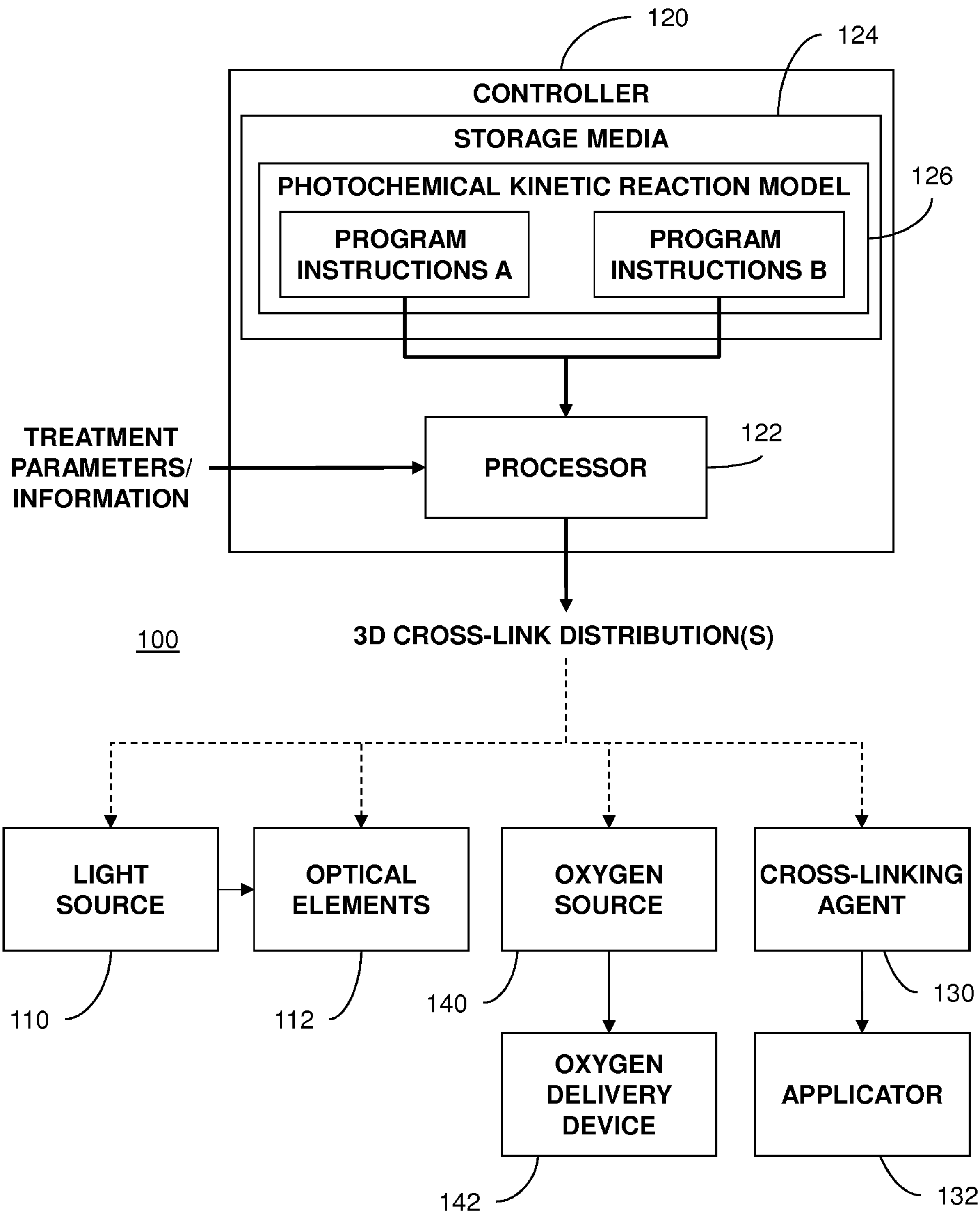

120
124
CONTROLLER
STORAGE MEDIA
PHOTOCHEMICAL KINETIC REACTION MODEL
126
PROGRAM INSTRUCTIONS A
PROGRAM INSTRUCTIONS B
TREATMENT PARAMETERS/ INFORMATION
PROCESSOR
122
100
3D CROSS-LINK DISTRIBUTION(S)
LIGHT SOURCE
OPTICAL ELEMENTS
OXYGEN SOURCE
CROSS-LINKING AGENT
110
112
140
130
OXYGEN DELIVERY DEVICE
APPLICATOR
142
132

FIG. 13

INTERNATIONAL SEARCH REPORT	International application No. PCT/US 2015/057628

A. CLASSIFICATION OF SUBJECT MATTER

A61F 9/007 *(2006.01)*
A61N 5/06 *(2006.01)*

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)

A61F 9/007, A61N 5/06

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)

PatSearch (RUPTO internal), PubMed, NCBI, Rambler, Yandex

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 8574277 B2 (AVEDRO INC.) 05.11.2013	1-19
A	RU 2456971 C1 (FEDERALNOE GOSUDARSTVENNOE UCHREZHDENIE "MEZHOTRASLEVOY NAUCHNO-TEKHNICHESKIY KOMPLEKS "MIKROKHIRURGIYA GLAZA" IMENI AKADEMIKA S.N. FEDOROVA FEDERALNOGO AGENTSTVA PO VYSOKOTEKHNOLOGICHNOY MEDITSINSKOY POMOSCHI") 27.07.2012	1-19
A	HAMMER Arthur et al. Corneal Biomechanical Properties at Different Corneal Cross-Linking (CXL) Irradiances. IOVS, May 2014, Vol. 55, No. 5, pp.2881-2884	1-19

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2016 (11.01.2016)	21 January 2016 (21.01.2016)

Name and mailing address of the ISA/RU:	Authorized officer
Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	A. Shitov Telephone No. (495)531-64-81