Collagen crosslinking using riboflavin and UV light has been successfully used clinically to strengthen the cornea in several ectatic diseases, including keratoconus and post-LASIK ectasia.1 The stiffening of the cornea is believed to result from intrafibrillar photocrosslinking forming covalent bonds between collagen molecules and between collagen and other stromal proteins.2 The crosslinking mechanism with riboflavin as the photosensitizer requires the presence of oxygen.3

Another approach to protein-protein photocrosslinking using rose bengal (RB) with green light irradiation has been demonstrated in the cornea and several other tissues.4–7 RB, unlike riboflavin, associates strongly with tissue collagen8 and has limited penetration into tissue (less than 100 μm in corneal stroma), making it a promising candidate for certain photocrosslinking applications in the eye.9 It has been proposed for sealing corneal lacerations,10 crosslinking thin cornea,11 interface bonding of LASIK-flaps,5 and attaching IOL haptics to the capsular bag.12

RB photochemistry in aqueous solution follows two classical photochemical pathways from the triplet excited state (Fig. 1).3 The first involves the acceptance of an electron by the RB excited triplet state from a donor molecule, for example an amine, by the electron transfer pathway. In the second, energy is transferred to oxygen generating singlet oxygen, an excited state of oxygen. Both pathways, after further reaction steps, can lead to covalent bond formation (i.e., crosslinks) within and between macromolecules, potentially including collagen. A significant difference between these two pathways is that the electron transfer pathway does not require oxygen. The clinical applications of photocrosslinking involve solid tissues that are highly complex compared to simple aqueous solutions. For example, tissues contain a variety of electron donors and oxygen diffusion is reduced compared to solutions. These differences are likely to influence the balance between the two pathways or alter the overall efficiency of photocrosslinking.

The overall goal of this study was to increase the effectiveness and potential range of clinical applications for protein-protein crosslinking in tissue by using RB as the photosensitizer. To attain these goals, experiments were designed and carried out to gain a better understanding of the photochemical mechanisms that initiate crosslinking in tissue and to determine whether an oxygen-independent pathway via the electron transfer pathway efficiently crosslinks and stiffens cornea.
METHODS

Tissue Preparation

Mature New Zealand White freshly frozen rabbit eyes (Pel-Freez Biologicals, Rogers, AR, USA) were defrosted in a moist chamber. The epithelium was removed using a hockey knife (Katena, Denville, NJ, USA), and the surface was polished with a small swab. The nasal-temporal orientation of the cornea was identified by the two horizontal scleral vortex vessels and marked on the limbal region. A corneoscleral disc (~17 mm diameter) was obtained using keratoplasty scissors and fine forceps to remove the iridociliary complex. The resulting corneoscleral disc was placed in phosphate-buffered saline (PBS; Fisher Bioreagents, Fair Lawn, NJ, USA) containing 15% wt/vol dextran (molecular weight: 450,000–600,000; Sigma, St. Louis, MO, USA) for at least 30 minutes until a pachymetry (OQ Labscope OCT; Lumedica, Durham, NC, USA) below 400 µm thick, 90% of the RB fluorescence was measured as a function of distance from the stroma surface (Supplementary Fig. S1). RB remains near the slit because it has limited diffusion into stroma, presumably because it binds strongly to collagen.8 In corneas prepared for this study (~320 µm thick), 90% of the RB fluorescence was detected within ~90 µm of the stromal surface (Supplementary Fig. S2).

The corneoscleral discs were mounted into a Barron artificial anterior chamber (Katena) to guarantee natural curvature and pressure as well as to limit RB staining only to the outer surface. For staining, cornea was submerged in 1 mM RB (Sigma) in PBS.

Light Source

A continuous wave-potassium titanyl phosphate (CW-KTP) doubled laser (Oculight OR; IRIDEX, Mountain View, CA, USA) emitting 532 nm (green light) was used. The laser light was expanded to a 1.2-cm-diameter beam by coupling the optical fiber to a microscope objective (Edmund Optics, Barrington, NJ, USA) and then passing the light through a 30-degree diffusor (Luminit, St. Torrance, CA, USA) and refocusing into the incision with a 28G insulin syringe until the solution appeared to be evenly distributed. The portion of the cornea with the stained incision (~2.85 mm), 90% of the RB fluorescence was detected within ~90 µm of the stromal surface (Supplementary Fig. S2).

The corneoscleral discs were mounted into a Barron artificial anterior chamber (Katena) and a long manual incision was created using a 2.85-mm angled clear corneal knife (Katena). This long incision formed a slowly descending channel toward the endothelium. RB (1 mM) was injected into the incision with a 28G insulin syringe until the solution appeared to be evenly distributed. The portion of the cornea with the stained incision (~2.85 × 3–5 mm) was placed onto a microscope slide for horizontal optical coherence tomography (OCT; Telesto; Thorlabs, Newton, NJ, USA) B-scan parallel to and in the center of the incision. From the OCT scan, the depth of the incision could be determined. Absorption spectrum measurements were presented as percentage of the OD at 560 nm before irradiation.

Linear Tensile Strength Testing

RB (1 mM)-stained corneas were irradiated with 100 or 200 J/cm2 or served as unirradiated controls (n = 9–16/group; Table 1). Corneas were irradiated in air on the anterior chamber or, to provide oxygen-free conditions, were placed between two glass slides and surrounded by dextran solution. After irradiation, the tissue was kept in a moist chamber for at least 30 minutes before pachymetry measurement. Afterward, a 5-mm-wide nasaltemporal corneal strip was prepared using fixed forceps and mounted onto the stage of an optical microscope (Olympus IMT-2, Tokyo, Japan) equipped with an integrating sphere. Corneal disks (7 mm; n = 24) were prepared using a Barron donor cornea punch (Katena) and placed on a microscope slide for irradiation using different fluences without repositioning with RB in the same cornea to determine the influence of fluence on RB photobleaching. Irradiation without oxygen was achieved by covering the corneal disk with a second glass slide and surrounding it with dextran. All measurements were presented as percentage of the OD at 560 nm before irradiation.

Depth of RB Photobleaching

To test whether oxygen-independent crosslinking might occur at varying depths in the stroma, RB photobleaching was measured as a function of distance from the stroma surface of corneas penetrated by an inclined slit into which RB was inserted (Supplementary Fig. S1). RB remains near the slit because it has limited diffusion into stroma, presumably because it binds strongly to collagen.8 In corneas prepared for this study (~320 µm thick), 90% of the RB fluorescence was detected within ~90 µm of the stromal surface (Supplementary Fig. S2).

The corneoscleral discs were mounted into a Barron artificial anterior chamber (Katena) and a long manual incision was created using a 2.85-mm angled clear corneal knife (Katena). This long incision formed a slowly descending channel toward the endothelium. RB (1 mM) was injected into the incision with a 28G insulin syringe until the solution appeared to be evenly distributed. The portion of the cornea with the stained incision (~2.85 × 3–5 mm) was placed onto a microscope slide for horizontal optical coherence tomography (OCT; Telesto; Thorlabs, Newton, NJ, USA) B-scan parallel to and in the center of the incision. From the OCT scan, the depth of the incision could be determined. Absorption spectrum
Enhancement of Rose Bengal Corneal Crosslinking

RESULTS

Photobleaching of RB in Cornea

The light-induced decomposition of RB in cornea, as monitored by the loss and alteration of its absorption spectrum, was used to report whether the presence of oxygen or different additives changed the RB photochemistry and to suggest mechanisms that might be involved in collagen crosslinking (Fig. 2; Supplementary Fig. S3). Staining cornea with 1 mM RB produced an absorption maximum at 560 nm compared to 550 nm, the value in aqueous solution. The shoulder maximizing at 526 nm was about one-half (47% ± 3%) the height of the 560-nm peak (Fig. 2A) compared to the value of approximately one-third found for dilute RB solutions, suggesting that in cornea RB was aggregated and strongly interacted with collagen and possibly other proteins.

Influence of Oxygen.

Irradiation of RB-stained cornea with 50 and 100 J/cm² of green light produced a statistically significant decrease in the RB absorption, that is photobleaching, compared with unirradiated corneas (P < 0.001). Irradiation in air with 100 J/cm² reduced the absorption maximum to 25% ± 4% of the nonirradiated control without shifting the maximum wavelength from 560 nm. Photodecomposition of RB was altered two ways by an O₂-free environment. First, less bleaching occurred (47% ± 6% OD of control) compared to irradiation in air (25% ± 4% OD of control) (P < 0.001). Second, the spectrum shifted to shorter wavelengths, showing a maximum at 549 ± 3 nm after 100 J/cm² (Fig. 2A).

After further irradiation (500 J/cm²), the maximum had shifted to 530 ± 5 nm, indicating that some of the RB was converted to products absorbing at shorter wavelengths (Supplementary Fig. S3). Thus, the presence of oxygen strongly affected the RB photochemical pathways potentially available to initiate protein crosslinking.

Involvement of an Electron Transfer Mechanism.

To test whether an RB photosensitization mechanism involving electron transfer could occur in cornea, corneas were treated with two biomolecules previously established to transfer electrons to the RB excited triplet state (Fig. 2B). Arginine (200 mM) and ascorbate (200 mM) were included in the RB staining solution. After irradiation in air with 100 J/cm², arginine-treated corneas showed a decrease in OD at 560 nm to 18% ± 6% of control, a nonsignificant change compared to irradiation in air alone (25% ± 4% of control) (P = 0.13). However, a spectral shift to shorter wavelength (555 ± 3 nm) was observed. When arginine was tested in an O₂-free environment, rapid photobleaching was observed, including a loss of the 560-nm peak that was greater than the photobleaching produced by irradiation in air with arginine (P < 0.001) and comparable to the loss found by irradiation in air alone (P = 0.5). Irradiation in the presence of arginine in an O₂-free environment induced the greatest shift in the peak (510 ± 3 nm), indicating that products are formed from RB reactions that absorb at this shorter wavelength. The response to ascorbate differed from that found for arginine. Ascorbate caused a comparable increase of photobleaching in air (15% ±
5% of control) ($P = 0.016$) and under an O$_2$-free environment (16% $\pm$ 3% of control) ($P = 0.03$) when compared to RB photobleaching without ascorbate. Also, the shift of the maximum to shorter wavelength was observed both in O$_2$-free conditions (526 $\pm$ 4 nm) and in air (551 $\pm$ 5 nm).

**Involvement of Singlet Oxygen.** To test whether singlet oxygen generated by irradiation of RB in cornea is involved in RB photobleaching, the irradiation was carried out in the presence of deuterium oxide (D$_2$O), which extends the lifetime of singlet oxygen, thus allowing it to destroy more RB. Singlet oxygen-mediated RB photobleaching decreased the absorption without a shift in the absorption maximum. When corneas were treated with an RB solution prepared in D$_2$O and irradiated in air, the 560 nm peak was reduced to 12% $\pm$ 1% of the original peak, which is greater than the decrease observed in air alone ($P = 0.005$) (Fig. 2A). In addition, sodium azide (NaN$_3$; 200 mM), which quenches singlet oxygen and certain other reactive species, partially inhibited RB photobleaching (61% $\pm$ 7% OD of control) compared to irradiation alone (25% $\pm$ 4%) ($P < 0.001$). These results indicate that singlet oxygen is generated by irradiation in corneas stained with RB and participates in the destruction of RB.

**RB-Photosensitized Increase in Cornea Stiffness**

A single irradiation (100 or 200 J/cm$^2$) of RB-stained cornea almost completely bleached the RB absorption but did not induce sufficient crosslinking for accurate tensile strength measurements. Consequently, we established treatment conditions under which RB photobleaching occurred as a result of the irradiation, and RB staining was alternated to maintain the OD at 532 nm between 0.6 and 1.2 OD (transmittance, 25% to 6%) (Supplementary Fig. 5A). The treatment conditions established were retesting each 40 J/cm$^2$. Corneal thickness was constant in all experimental groups (Table 1) (ANOVA, $P = 0.08$).

An evaluation of the statistical differences in Young’s modulus between groups was carried out at 0.1% intervals between 0.1% and 10% strain. Because corneal stroma is not a uniform ideal material, Young’s modulus was not constant in this range of % strain but rather increased with increasing % strain (Figs. 3D–F). The range of % strain in which two groups differed significantly ($P < 0.05$) is shown in Table 2, and the P values as a function of % strain are shown in Figure S5.

**Influence of Oxygen.** Nonirradiated control samples did not differ in Young’s ($P > 0.05$, between 0.1%–10% strain). modulus. Treatment with RB and 532 nm irradiation in air stiffened the cornea significantly. In comparison to controls not treated with RB or irradiation, 100 J/cm$^2$ ($P < 0.05$, between 0.1%–7.9% strain) and 200 J/cm$^2$ ($P < 0.05$, between 0.1%–10% strain) increased Young’s modulus (Figs. 3A, 3D; Table 2). Nonirradiated control samples did not differ in Young’s ($P > 0.05$, between 0.1%–10% strain). modulus. Treatment with RB and 532 nm irradiation in air stiffened the cornea significantly. In comparison to controls not treated with RB or irradiation, 100 J/cm$^2$ ($P < 0.05$, between 0.1%–7.9% strain) and 200 J/cm$^2$ ($P < 0.05$, between 0.1%–10% strain) increased Young’s modulus (Figs. 3A, 3D; Table 2). In an O$_2$-free environment, irradiation with 200 J/cm$^2$ was not as effective as in the presence of O$_2$ ($P > 0.05$, between 0.1%–10% strain) (Figs. 3B, 3E).

**Involvement of an Electron Transfer Mechanism.** Because arginine and ascorbate enhanced RB photobleaching, indicating an electron transfer mechanism, their effects on RB-photosensitized cornea stiffening were measured (Fig. 3C, 3F; Table 2). Most interestingly, 200 mM arginine counteracted the inhibition of RB-photosensitized stiffness increase produced in O$_2$-free corneas irradiated with 200 J/cm$^2$ ($P < 0.05$ between 0.1%–8.4% and 9.1%–9.4% strain). In fact, the same increase in Young’s modulus was attained in arginine-treated samples in O$_2$-free environment as for those irradiated with 200 J/cm$^2$ in air ($P > 0.05$ between 0.1%–10% strain). However, the increase in stiffness of corneas irradiated in air (100 J/cm$^2$) was not enhanced by the presence of arginine ($P > 0.05$, between 0.1%–10% strain). In contrast, ascorbate did not counteract the effect of removing O$_2$ during the irradiation ($P > 0.05$, between 0.1%–10% strain).

**Involvement of Singlet Oxygen.** Because singlet oxygen was at least partially responsible for RB photobleaching, we evaluated whether replacing H$_2$O with D$_2$O increased corneal crosslinking. Irradiation (100 J/cm$^2$) in H$_2$O-based RB staining solution increased the tensile strength compared to unirradiated control ($P < 0.05$, between 0.1%–7.9% strain; Figs. 3B, 3E; Table 2). Irradiated D$_2$O-treated corneas showed an even greater increase in Young’s modulus compared to irradiation in the H$_2$O-based solution ($P < 0.05$, between 0.1%–0.4 and 3.0%–10% strain). In contrast, including NaN$_3$ (200 mM) in the RB staining solution entirely inhibited the RB-photosensitized increase in cornea stiffness compared to without NaN$_3$ ($P > 0.05$, between 0.1%–10% strain). These results are consistent with singlet oxygen as a required component of the photo-crosslinking mechanism.

**Depth of RB Photobleaching and Potential Photocrosslinking in Cornea**

Our results indicate that RB initiates photobleaching and photocrosslinking in cornea by an oxygen-dependent mechanism and, with arginine present, by an oxygen-independent mechanism. Oxygen-dependent crosslinking is limited by the rate of oxygen diffusion into the cornea and, thus, is expected to occur most efficiently near the anterior surface. To test this hypothesis, inclined slits stained with RB were irradiated and RB photobleaching was measured as a function of distance from the stroma surface (Supplementary Fig. S1).

Each incision received eight consecutive 50 J/cm$^2$ irradiations. Before and after each irradiation, a photographic en face image was taken (Supplementary Figs. S1D–F). Digitally, the OCT scan image was placed on top of photographic image to correlate photobleaching with tissue depth of the band of RB (Supplementary Fig. S1G). Photobleaching of RB in the incision was compared to photobleaching of corneas stained only on the surface in pairs of corneas with the same initial OD at 532 nm. Inside the incision, RB photobleaching was not apparent after irradiation with 50 J/cm$^2$, distinctly observable after 100 J/cm$^2$, and distinctly observable after 400 J/cm$^2$ in the example shown in Figure 4 and Supplementary Figure S6. In contrast, corneas that were only stained on the surface showed distinct photobleaching at the lowest fluence (50 J/cm$^2$) and virtually complete bleaching at 100 J/cm$^2$.

When the RB staining solution contained arginine (200 mM), substantial photobleaching occurred at much lower fluences than in incisions without arginine (Fig. 4; Supplementary Fig. S6). Photobleaching was even apparent after 50 J/cm$^2$ with arginine present, consistent with an oxygen-independent mechanism and suggesting that photocrosslinking could occur throughout the cornea. In addition, the color changed toward yellow from pink especially at the highest fluence, consistent with the shift of the absorption maximum to 510 nm observed in the absence of oxygen when arginine is present during the irradiation (Fig. 2B).

**Discussion**

The results of this study indicate that RB-photosensitized crosslinking stiffens the cornea efficiently both when oxygen is available as well as in anoxic tissue. In the absence of oxygen, electron transfer to photoexcited RB from an added biomolecule produces reactive intermediates that initiate crosslinking. In the presence of oxygen, energy transfer from photoexcited RB initiates a singlet oxygen-mediated mechanism for protein-protein crosslinks. Importantly, photosensi-
tized crosslinking that does not require oxygen may expand the range of clinical applications in cornea and other tissues.

RB photosensitization is often used to generate singlet oxygen in cells to study responses to reactive oxygen species. Our results now firmly demonstrate that it is also involved in the mechanism for tissue protein crosslinking. Both RB photobleaching and corneal stiffening were enhanced when the irradiations were carried out in D2O (Figs. 2A, 3B, 3E; Table 2), a signature of singlet oxygen involvement in the mechanism. In addition, sodium azide inhibited both of these RB-photosensitized effects in cornea. Although azide is not an entirely specific quencher, our previous study indicated that it

**FIGURE 3.** Stress-strain curves and Young’s modulus from tensile strength testing of RB-stained corneas before and after green light irradiation in the presence selected agents. (A, D) Comparison of two fluences, 100 and 200 J/cm², on cornea stiffness. (B, E) Influence of oxygen, of a singlet oxygen enhancer (D₂O) and of a singlet oxygen quencher (NaN₃) on cornea stiffness. (C, F) Effect of potential electron donors, arginine and ascorbate, on cornea stiffness. Lines represent mean values, and error bars indicate one standard deviation from the mean.
quenches singlet oxygen but not the RB triplet state. Our results are consistent with an earlier study showing that a riboflavin-photosensitized increase in cornea breaking strength involved singlet oxygen. Efficient RB-photosensitized crosslinking in cornea, in the absence of added enhancers, requires oxygen, as previously reported for riboflavin/UVA photo crosslinking. Both dyes generate singlet oxygen as well as produce reactive species by electron transfer processes upon irradiation in solution. However, their photochemistry in cornea may diverge because RB associates tightly to collagen, whereas riboflavin freely diffuses. An oxygen-dependent mechanism limits the rate and depth of photocrosslinking because oxygen is consumed during the reactions (so called “oxygen depletion”) and must be resupplied by diffusion from the surface. The results of our “incision experiment” in which RB was irradiated at different depths in the cornea in air demonstrate this limitation because photobleaching occurred near the stroma surface at a much lower fluence than at depths in the stroma (Fig. 4).

To overcome the limitations of oxygen-dependent photo-crosslinking, we evaluated an oxygen-independent electron transfer mechanism that is based on known photochemistry of RB. Certain molecules quench the excited triplet state of RB and form an anion radical of RB and cation radical of the donor molecule (Fig. 1). Subsequent deprotonation of the donor cation radical and rearrangement steps produce radical intermediates that could initiate protein crosslinking. Additional radicals formed by protonation of the RB anion radical also could lead to covalent crosslinks.

Arginine and ascorbate were selected as electron donors based on our previous study of RB in solution and in collagen gels containing RB showed that oxygen is depleted during irradiation with green light and, thus, singlet oxygen is not efficiently produced if the irradiance is greater than about 10 mW/cm². Similarly, riboflavin crosslinking using 3 mW/cm² was shown to deplete oxygen within 5 seconds at 100 l/min from the stromal surface, and after cessation of the irradiation, O₂ diffusion into the corneal stroma required 3 to 4 minutes to restore the preirradiation level. To explain the apparent contradiction between an oxygen-dependent mechanism and oxygen depletion, it was postulated that photocrosslinking occurs initially only near the O₂-rich stromal outer surface. Then, as all the potential crosslinkable sites on collagen have reacted, O₂ diffuses deeper and crosslinking occurs at a greater depth in the tissue.

Previous studies of the influence of oxygen depletion on photosensitization support this interpretation. A study in collagen gels containing RB showed that oxygen is depleted during irradiation with green light and, thus, singlet oxygen is not efficiently produced if the irradiance is greater than about 10 mW/cm². Similarly, riboflavin crosslinking using 3 mW/cm² was shown to deplete oxygen within 5 seconds at 100 l/min from the stromal surface, and after cessation of the irradiation, O₂ diffusion into the corneal stroma required 3 to 4 minutes to restore the preirradiation level. To explain the apparent contradiction between an oxygen-dependent mechanism and oxygen depletion, it was postulated that photocrosslinking occurs initially only near the O₂-rich stromal outer surface. Then, as all the potential crosslinkable sites on collagen have reacted, O₂ diffuses deeper and crosslinking occurs at a greater depth in the tissue.

Table 2. Comparisons of the Increase in Cornea Tensile Moduli Produced by RB Photosensitization in Studies to Test the Effects of Oxygen, Singlet Oxygen, and Electron Transfer Species

<table>
<thead>
<tr>
<th>Test</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Between Groups for Young’s Modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluence dependence in air</td>
<td>No RB, unirradiated</td>
<td>RB, unirradiated</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>100 J/cm², Air</td>
<td>200 J/cm², Air</td>
<td>0.1–7.9</td>
</tr>
<tr>
<td>Effect of O₂</td>
<td>RB, unirradiated</td>
<td>200 J/cm², Air</td>
<td>0.1–10</td>
</tr>
<tr>
<td>Enhancing singlet-oxygen half-life using D₂O</td>
<td>100 J/cm², Air</td>
<td>100 J/cm², D₂O, Air</td>
<td>0.1–0.4, 3.0–10</td>
</tr>
<tr>
<td>Quenching singlet oxygen using NaN₃</td>
<td>200 J/cm², Air</td>
<td>200 J/cm², NaN₃, Air</td>
<td>0.1–10</td>
</tr>
<tr>
<td>Arginine as an electron donor, O₂-free</td>
<td>200 J/cm², Arg, O₂-free</td>
<td>200 J/cm², O₂-free</td>
<td>0.1–8.4, 9.1–9.4</td>
</tr>
<tr>
<td>Ascorbic acid as an electron donor, O₂-free</td>
<td>200 J/cm², Asc, O₂-free</td>
<td>200 J/cm², O₂-free</td>
<td>None</td>
</tr>
</tbody>
</table>

* Statistically significant differences were evaluated between 0.1% and 10% strain.
gels in which RB anion radicals were detected. Electron transfer to the RB excited triplet state, in the absence of oxygen, leads to the loss of iodine from the RB structure and a final fluorescein product absorbing at 510 nm. Detection of the same 510-nm absorbing product in irradiated corneas treated with RB and arginine supports an electron transfer mechanism (Fig. 3B; Supplementary Fig. S3). Significantly, oxygen-independent crosslinking with arginine as a donor was as efficient as oxygen-dependent crosslinking (Fig. 3C, 3F; Table 2). In addition, this mechanism provides a pathway to efficient crosslinking at depth in tissues or at other locations with a low oxygen level.

Arginine appears to be a feasible choice for potential clinical applications. As a ubiquitous amino acid, it is well characterized and present in virtually all food and is part of many Food and Drug Administration and European Medicines Agency-approved formulations. It has been used in intravenous formulations in humans in high concentrations and been applied as an intravitreal injection to minipigs for retinal vein occlusions. Importantly, arginine has been used in concentrations comparable to those in this study as eye drops (2%) in mice and diffused through the cornea to reach the lens.

A light-initiated, oxygen-independent protein crosslinking technique may have clinical applications in poorly oxygenated tissues. For example, photo-bonding of an IOL haptic to the capsular bag by using RB and green light was enhanced by exogenously supplied oxygen. In practice, supplying oxygen may not be feasible and an oxygen-independent process would be essential. Another example is scleral crosslinking that has been proposed for the prevention of severe myopia in adolescents. Photo-crosslinking was applied within the orbit with a cerclage-like optical waveguide around the eye, where the oxygen availability between the waveguide and the sclera is limited. The addition of arginine could reduce the energy needed in this low oxygen setting. Apart from ocular tissues, photosensitized crosslinking has been applied to other situations with low oxygen availability, including skin incisions, vascular anastomosis, and nerve anastomosis.

In summary, RB-photosensitized crosslinking of cornea in the absence of oxygen can be enhanced by arginine to produce the same level of stiffness exhibited in the presence of oxygen, finding that fuels several additional potential clinical applications. Also, when oxygen is available, RB-photosensitized collagen crosslinking in cornea is oxygen-dependent and mediated by singlet oxygen despite low oxygen availability.

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References


