·Original Article/Corneal Collagen Cross-linking·

The new protocol and dynamic safety of UV-light activated corneal collagen cross-linking

Juiteng Lin, Da-chuan Cheng, Chaokai Chang, Zhang Yong

【Abstract】 **Objective** To discuss the critical issues of the dynamics of UV-light-photoinitiated cross-linking in corneal collagen (CXL) and to confirm the dynamics of riboflavin (vitamin-B2) absorption under UV light. **Methods** Coupled dynamic equations are numerically solved and analytic formulas are derived for three critical parameters: the safe depth (z*), the safe dose (E*) and the cross-linking time (t*). Time-dependent absorption of UV light due to the depletion of the initiator is measured and shown by a dynamic spectrum of riboflavin. The critical issues of CXL are explored by seven parameters: the extinction coefficient, concentration, the penetration depth of the riboflavin, the UV light intensity and dose, irradiation duration, and corneal thickness. **Results** The safe dose (E*) has a wide range from 2.3 to 8.2 (J/cm^2) for riboflavin concentrations of 0.1% to 0.2% and penetration depths of 0.02 to 0.04 cm. It is shown by mathematical modeling that a higher light intensity and extinction coefficient lead to shorter t* for a given cross-linking depth, while t* increases with corneal thickness (z*). The safety depth decreases as a function of the extinction coefficient and initiator concentration. **Conclusion** A new cross-linking protocol is suggested based on new findings, which include the safe depth (z*), the safe dose (E*), the cross-linking time (T*), and the safe riboflavin concentration.

【Key words】 Modeling; Keratoconus; Ophthalmic devices; UV light; Corneal collagen cross-linking

1 Introduction

The kinetics of photoinitiated cross-linking systems have been studied by many researchers analytically, numerically and experimentally[1-2]. However, most prior studies have been limited to photo-polymerization for chemical engineering applications, and far fewer attempts have been made for human corneas[3-4]. CXL systems have been commercialized for years for human clinical use[5-15]. However, much fewer efforts have been invested in basic theoretical studies[16-20]. Recently, Lin presented the first dynamic modeling of CXL confirmed by measurements[19-20].

Before 1998, the only treatment options for keratoconus were custom contact lenses, intracorneal ring-segment implantation and corneal transplantation. Corneal transplantation presents a lifelong risk of rejection of the corneal graft, as well as numerous other complications that can lead to permanent loss of vision and even loss of the eye. In 1998, the CXL procedure for treatment of keratoconus using the riboflavin vitamin and UV light was developed by Seiler, Spoerl and Wollensak[15]. Today, doctors are performing CXL and successfully treating patients since 2006 in over 400 centers outside the United States (including all 25 nations in the European Union). As of April 2014, the FDA has had 63 registered studies related to CXL in the USA. Through research studies coordinated by the CXLUSA Study Group and others, select participating centers are now able to provide this breakthrough treatment to qualified patients. FDA has cleared the CXL procedures in March, 2015.

CXL has been used clinically for various corneal conditions such as keratoconus, keratitis, corneal ectasia and corneal ulcers[15]. It has also been used to preventively treat thin corneas, which carry a higher risk of ectasia after LASIK vision correction. Other potential applications include the reduction of postoperative regression in vision correction and scleral treatment in malignant myopia, scleromalacia and low tension glaucoma. CXL has been covered in greater detail in a recent book edited by Hafezi and Randleman[15] and in a review article by Chunyu et al[21]. To increase the speed of CXL procedures, accelerated CXL using high UV power was proposed and various devices with different powers were introduced by Avedro (USA), up to 45 mW; MLase (Germany), 18 mW; Peschke (Switzerland), 30 mW; and New Vision Inc. (Taiwan), 90 mW. In addition, pulsed mode of the UV light was proposed for potential improvement on safety[22-23]. To enhance riboflavin diffusion, a femtosecond-laser-created pocket was proposed[24] as well as iontophoresis[25]. More recently, a corneal topography-guided CXL was commercialized by Avedro (USA) based on a pending US patent[26].

Many factors can affect the cross-linking reaction and the amount of biomechanical stiffness achieved. These factors include

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riboflavin concentration, condition of the cornea, temperature, presence of the oxidizing agent (riboflavin), the UV light intensity, its dose and the on-off duty cycle, as well as other factors[23]. Furthermore, changing one factor may have an unexpected effect on another factor.

In CXL, both type-I and type-II photochemcical reactions occur. Type-I reaction is favored at low oxygen concentrations, and type-II at high concentrations. In type-I reaction, the substrate reacts with the sensitizer in the excited state to generate radicals or radical ions, by hydrogen atoms or electron transfer, respectively. In the type-II mechanism, the excited sensitizer reacts with oxygen to form singlet molecular oxygen-species. The singlet molecular oxygen-species then acts on tissue to produce additional cross-linked bonds[15,27].

It has been reported that oxygen concentration in the cornea is modulated by UV irradiance and temperature and that it quickly decreases at the beginning of the UV light exposure[23]. The oxygen tends to be depleted within 10-15 seconds for an irradiance of 3 mW/cm^2 and within 3-5 seconds for an irradiance of 30 mW/cm^2. By using the pulsed UV light of a specific duty cycle, frequency, and irradiance, both type I and type II photochemical kinetic mechanisms may be optimized to achieve the greatest amount of photochemical efficiency. Moreover, the rate of reactions may either be increased or decreased as needed, by regulating parameters such as the irradiance, dose, the on/off duty cycle, riboflavin concentration and soak time, and others[23].

The critical issues of CXL to be explored in this paper will be characterized by the following seven key parameters: the extinction coefficient (A), concentration (C) and penetration depth (d) of riboflavin, then intensity (I), dose (E) and irradiation duration (t) of the UV light and finally, corneal thickness (z). The dose is further defined by the product of the light intensity and the exposure duration, i.e., E=It. The extinction coefficient is further defined by three parameters: the molar extinction coefficients of riboflavin, the photolysis product and corneal stroma.

Based on the above-described parameter sets (A, C, E, d, z), we will define and derive analytic formulas for the following critical parameters for safety:

(1)the safe depth (z*) (or the minimum corneal thickness) for a given set of A, C, E, d; (2)the safe dose (E*) (or the maximum light energy) for a given set of A, C, d, z; (3)the safe concentration (C*) (or the minimum riboflavin concentration) for a given set of A, E, d, z.

We will also introduce a cross-linking time (t*) defined by the duration of light exposure needed for riboflavin concentration depletion to exp (-M) of its initial value, with M=2 to 4. This paper intends to provide both analytic and numerical results relating to the clinically important issues listed above. The time-dependent absorption of UV light resulting from depletion of the initiator is theoretically predicted and then confirmed by measured data. A general formula will be derived to cover various situations including the UV light acting on various absorbing media of the corneal stroma (without riboflavin), on riboflavin solution only, and stroma with riboflavin solution in the case of uniform and non-uniform saturation. Numerical calculations for the dynamic profile of the riboflavin concentration and the UV light intensity are presented. We will demonstrate that the conventionally used fixed-light dose of 5.4 J/cm^2 may lead to a high risk, particularly in the case of low riboflavin concentration. Our theory will show that the safe dose cannot be set as a constant, instead, it is a function of the combined parameter set (A, C, E, d, z). Finally, a new protocol based on the new finding and theory of this paper is suggested.

2 Methods and Theory

2.1 *The Modeling System*

As shown in Fig.1, a simplified corneal model consists of its epithelial layer and the underlying stromal collagen, where z represents corneal thickness and z=0 defines the corneal surface. The UV light is incident-normal to the corneal surface, which is covered by a thin layer of riboflavin (B2) solution. A typical CXL protocol is to administer riboflavin solution (0.1% to 0.25%) on the corneal surface five times at five-minute intervals and wait until the B2 solution diffuses into the top layer at approximately 300 μm. The CXL procedures could be conducted (as shown by Fig.1) either with epithelium off (epi-off) with a 0.1% riboflavin-dextran solution or with epithelium on (epi-on) with a 0.25% riboflavin aqueous solution. The riboflavin penetration depth in the epi-on case is normally less than that for epi-off (as shown in Fig.1).

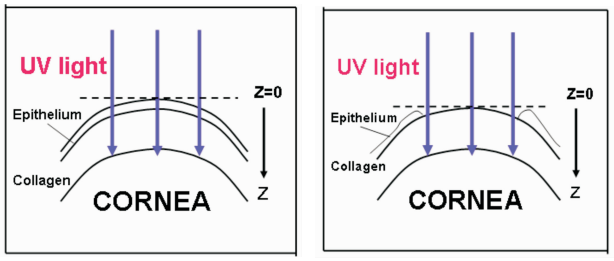

Fig.1 A corneal model system under UV light cross-linking for the epi-on (left) and the epi-off (right) cases, where z is along the corneal thickness direction and z=0 defines the corneal surface.

The epi-on case with two layers of different mediums (the epithelium and the stroma) is more complicated than the epi-off case. The theory developed in this paper can apply to both epi-on and epi-off cases with slight revisions. However, we will focus on the more efficient epi-off case as shown by Fig.2, where the stroma has a non-uniform riboflavin distribution covered by a uniform riboflavin layer (thickness d', and an initial concentration C_0). For a typical protocol where the extra riboflavin solution on the corneal surface is washed out before UV light is applied, only region 2, the non-uniform B2 diffusion in the stroma, is required, where the distribution is calculated by an exponential function $C_0(z)=\exp(-z/d)$ having a penetration depth (d).

2.2 *The Dynamic Equations*

In our modeling system, we will first consider the case of the uniform distribution of riboflavin and then the non-uniform case. The formulas for the uniform case are for the depth of z<d' describing the dynamics of the B2 top layer on the corneal surface as shown by Fig.2. Without the top layer of B2, the formulas developed will be based on the initial B2 concentration C_0 and z=d' is redefined as z=0 where both uniform and non-uniform cases will be presented.

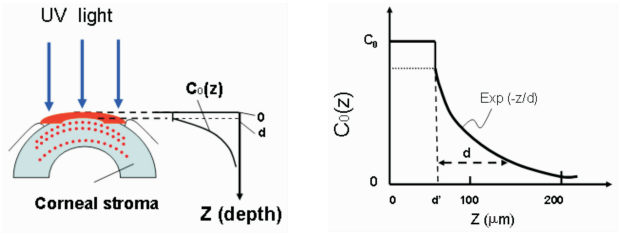

Fig.2 The initial (at t=0 before the UV light exposure) riboflavin concentration distribution on the corneal surface (uniform layer for z<d') and inside the stroma (for z>d') is calculated by an exponential function $C_0(z)=\exp(-z/d)$ with a penetration depth (d).

In the above-described corneal modeling system, the concentrations of the B2 photoinitiator C (z, t) and the UV light intensity I (z, t) inside the B2 top layer or inside the stroma may be described by coupled equations as follows[1,19-20].

$$\frac{\partial C(z,\ t)}{\partial t}=-\alpha I(z,\ t)C(z,\ t) \qquad (1)$$

$$\frac{\partial I(z,\ t)}{\partial z}=-2.3[(\varepsilon_1-\varepsilon_2)C(z,\ t)+\varepsilon_2 C_0(z)+\varepsilon_3]I(z,\ t) \qquad (2)$$

Where $\alpha=83.6\lambda\phi\varepsilon_1$, with ϕ being the quantum yield, λ being the UV light wavelength and ε_1 and ε_2 being the molar extinction coefficients of the riboflavin (initiator) and the photolysis product, respectively. $C_0(z)$ is the initial concentration in the stroma, which in general is z dependent[7]. The extinction coefficient of the riboflavin (B2) solution (at 365 nm) has been reported[13] as $\varepsilon_1=204$ $(\%\cdot cm)^{-1}$, or 8.16 $(mM\cdot cm)^{-1}$. In a corneal system, we have added ε_3, the absorption coefficient of the corneal stroma tissue reported to be ε_3= 7.4 (1/cm) and 13.9 (1/cm), respectively, with and without the epithelium[14]. In Eq. (2), the following units are used: C(z,t) in weight percent (%), I(z, t) in (mW/cm^2), λ in cm, and ε_j (for j=1, 2) in $(\%\cdot cm)^{-1}$. The above differential equations will be solved analytically and numerically under the initial and boundary conditions $C(z=0,\ t=0)=C_0$ and $I(z=0,\ t=0)=I_0$[19-20].

For uniform B2 surface layer on top of the stroma (with z< d', shown in Fig.2), there is no stroma absorption ($\varepsilon_3=0$) and only the extinction coefficients of the Riboflavin (initiator) (ε_1) and the photolysis product (ε_2) are needed in Eq. (1) and (2).

As shown by Eq. (1) and (2) that there are three major UV absorption components in the CXL process: the absorption of the stroma tissue (ε_3), which is independent of the B2 concentration, the absorption of the unreacted B2 solution ($\varepsilon_1 C_0$), and the photolysis product ($\varepsilon_2 C_0$), both being proportional to the initial B2 concentration C_0.

Analytic approximate solution of Eq.(1) and (2) leads to the light intensity given by a revised time-dependent Lambda-Beer law[20].

$$I(z,\ t)=I_0\exp[-A(t)z] \qquad (3)$$

Where the time-dependent extinction coefficient A (t) shows the dynamic feature of the UV light absorption due to the B2 concentration depletion. To be shown later that A(t) is given by an expression of A(t)=A-gt/z, where gt is a time-dependent factor.

2.3 *The Measurements*

As predicted by our theory and given by Eq.(3), the dynamic extinction coefficient A(t) is a decreasing function of time (t). To test our theory, we set up a simple laboratory cubic tube (10 mm wide) filled with riboflavin solution at 0.005% and 0.0075% concentrations under a UV light intensity of $I_0=100$ mW/cm^2. The dynamic absorption spectrum was taken initially (t=0) and at 2 and 8 minutes, as shown in Fig.3.

Our measured, transmitted UV light intensity I (at z=1.0 cm) versus time shows the increase in light transmission due to depletion of the photoinitiator concentration. Using the relation of $A(t)=\ln(I(z,t)/I_0)/z$, (at z=1.0 cm), we calculate A (t, at z=1.0 cm) versus t and plug the data in Fig.4 to show the nonlinear dependence of A (t) measured at z=10 mm for initial riboflavin concentrations of 0.005% (top curve) and 0.0075% (lower curve). These data are consistent with the features predicted by Eq.(9), in which A (t) is a deceasing function of time (t). In other words, the dynamic A(t) starts from its initial value A_1 and reduces to its steady state value A_2. We have also observed the color change (from dark to light) of riboflavin solution after a few minutes of UV light illumination. The riboflavin depletion (the cross-linking process) starts from the entrance surface (at z=0) and gradually reaches the exit surface (at z=10 mm). The data of Fig.4 also provide us the ratio of A_1/A_2, which (when $\varepsilon_3=0$), gives the ratio $\varepsilon_1/\varepsilon_2 \approx 1.43$, or ε_2 is about 0.7 of ε_1. Given $\varepsilon_1=204$ $(\%\cdot cm)^{-1}$, we find $\varepsilon_2=143$ $(\%\cdot cm)^{-1}$. In our calculations, we will use these parameters as the best available values.

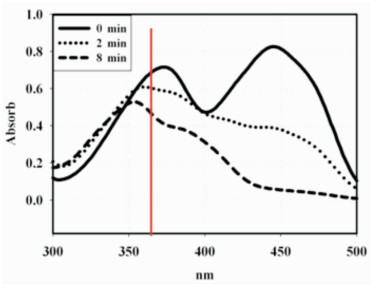

Fig.3 The dynamic absorption spectrum of a riboflavin solution (with 0.005% concentration, at 10 mm depth) under a UV light intensity of $I_0=100$ mW/cm^2, initially (solid curve) and at 2 and 8 minutes (dotted and dashed curves). Also shown is the vertical line indicating the wavelength at 365 nm[19].

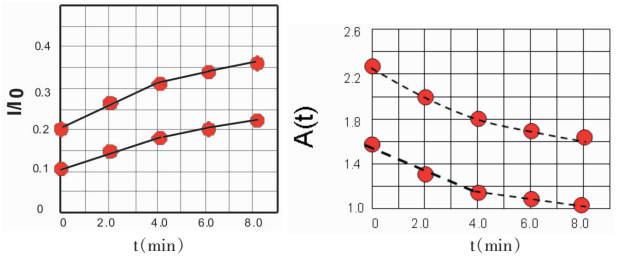

Fig.4 The measured UV light transmitted intensity (normalized by its initial value) and the extinction coefficient A(t), showing the nonlinear dependence on t, where the initial riboflavin concentration is 0.0075% (top curve) and 0.005% (lower curve)[20].

3 Results and Discussions

3.1 *The Dynamic Formulas*

As shown in Fig.4 for the measured data, the UV light intensity in the riboflavin (B2) solution is time-dependent due to B2 depletion. Therefore, a higher risk is involved when B2 is gradually depleted over time under the UV light irradiation,

particularly when a low B2 concentration (<0.1%) is applied. The modeling system (shown by Fig.2) defines z=0 as the surface when B2 is applied on the corneal surface (with a thickness d), whereas z=d is redefined as z=0 when there is no B2 layer on the surface. In addition, with the B2 surface layer, the light intensity at z=d becomes the boundary condition for the B2-diffused stroma. We will derive a general formula describing the situation of a corneal stroma with diffused B2 solution. This general formula may be used for various situations including: UV light absorbed by corneal stroma only (without B2), B2 solution only, and stroma with B2 solution for the uniform and non-uniform cases.

The following discussion is for the case without a B2 surface layer, and z=d is redefined as z=0 (Fig.2).

Absorption of UV light inside the stroma with B2, or when $\varepsilon_2\varepsilon_3\neq 0$, numerical solutions of Eq.(1) and (2) are required and only the initial state (at t=0) and the steady state with $C(z, \infty)=0$ can be solved analytically. We consider an initial B2 concentration distribution inside the stroma (as shown by Fig.2) given by $C(z, 0)=C_0(z)=C_0\exp(-z/d)$, having a penetration depth (d) defined by when its surface value (at z=0) is reduced to 1/e (or 0.367). Defining an absorption function A_j given by (with j=1 or 2)

$$A_j=2.3\varepsilon_3+\left(\frac{2.3d\varepsilon_jC_0}{z}\right)[1-\exp(-z/d)] \quad (4.a)$$

The initial light intensity (at t=0) is obtained by the integration of Eq.(2)

$$I(z, 0)=I_0\exp(-A_1z) \quad (4.b)$$

And the steady-state light intensity is derived by using $C(z, t=\infty)=0$ in Eq.(2)

$$I(z, \infty)=I_0\exp(-A_2z) \quad (4.c)$$

Where A_1 (for j=1) is the initial state (at t=0) absorption coefficient which is independent on ε_2; and A_2 (for j=2) is the steady-state absorption coefficient which is independent on ε_1 because of B2 concentration depletion, $C(z, t=\infty)=0$, as shown in Eq.(2) for the case of uniform B2 distribution (or when d>>corneal thickness, or z/d<<1). However, our numerical data (to be shown later) indicate that for the non-uniform case, the steady state $C(z, t=\infty)<<C_0$ only for z<200 μm. For z>400 μm, C (z, t) is about 0.6 to 0.8 of C_0 due to the exp (-z/d) term. Therefore Eq.(4.c) is only valid for the uniform case.

For a finite time, the time evolution of I (z, t) and C(z,t) require numerical simulations (to be shown later) and only the approximate solutions are analytically available. Using the initial conditions of Eq. (4.b), we solve Eq. (1) for the first-order approximation for the B2 concentration

$$C_{(1)}(z, t)=C_0\exp[-z/d-(\alpha I_0t)\exp(-A_1z)] \quad (5)$$

Eq.(5) shows that C (z,t) has a maximum value given by dC/dz(at z=z')=0. We obtain an approximate formula

$$z'=\frac{1n(2.3d\varepsilon_3\alpha I_0t)}{2.3(\varepsilon_3+\varepsilon_1C_0)} \quad (6)$$

The maximum value of C (z, t) only occurs for d<z and not for d>>z (the uniform case). It also requires $2.3\varepsilon_3(\alpha I_0t)>1/d$. Greater numerical detail is shown later.

Using Eq. (5) to solve Eq. (2), we obtain the first-order approximation of the light intensity,

$$I(z, t)=I_0\exp[-A_1z+gt] \quad (7.a)$$

$$g=\left(\frac{2.3(\varepsilon_1-\varepsilon_2)\alpha I_0C_0}{A'}\right)\exp[-2.3d\varepsilon_1C_0][1-\exp(-A'z)] \quad (7.b)$$

$$A'=2.3\varepsilon_3+1/d \quad (7.c)$$

Where we have used a Taylor expansion for the second exponential term in Eq.(5), and Eq.(7) is valid for gt<1. For gt>1, numerical method is required. We note that the time-dependent factor (gt) proportional to $(\varepsilon_1-\varepsilon_2)\alpha I_0$, also provides the rate of change (m) of the dynamic intensity given by the derivative of I (z, t), m=dI(z,t)/dt=gI(z,t).

Using Eq.(7), we solve for the second-order approximation for the B2 concentration by the time integration of Eq.(1),

$$C(z, t)=C_0F(t)\exp[-z/d-(\alpha I_0t)\exp(-A_1z)] \quad (8.a)$$

$$F(t)=[(\exp(gt)-1)/gt] \quad (8.b)$$

Above equations (7) and (8) will be used to find the safe depth, safe dose and the cross-linking time later. We will now discuss various situations for the UV light acting on various absorbing media: (1)corneal stroma (without B2), (2)B2 solution only, and (3) stroma with B2 solution for the uniform (with d>>z) and non-uniform (d<z) cases.

Case (1) stromal tissue (without B2)

We will first discuss the safety issues of UV light irradiation on the cornea without the protection of B2 solution. In this situation, the UV light intensity in the corneal stroma is given by the Lambert-Beer law[20]

$$I(z)=I_0\exp[-Az] \quad (9)$$

Where $A=2.3\varepsilon_3$, ε_3 is the molar extinction coefficient of the cornea reported to be ε_3=7.4 and 13.9 $(\%\cdot cm)^{-1}$, respectively, with and without the epithelium[14]. Eq.(9) may also be easily obtained from Eq.(7) for the special case that C_0=0, g=0, and A'=A.

Case (2): uniform surface layer of B2

Eq.(6), (7) and (8) may be used to describe the intensity dynamics of the uniform B2 surface layer on top of the stroma (with z<d, shown in Fig.2), where there is no stroma absorption (ε_3=0) and only the extinction coefficients of B2 solution (ε_1) and the photolysis product (ε_2) are needed in Eq.(7.b). Therefore, $A_1=2.3(\varepsilon_1C_0)$, $A_2=2.3(\varepsilon_2C_0)$. Using the reported[13] parameters of ε_1=204 $(\%\cdot cm)^{-1}$, we obtain, from Eq. (4), the initial light intensity

$$I(z, 0)=I_0\exp\{-0.047C_0z\} \quad (9.a)$$

Where z is in μm (or 0.0001 cm) and C_0 is in %. And the steady light intensity

$$I(z, \infty)=I_0\exp\{-0.00023\varepsilon_2C_0z\} \quad (9.b)$$

For example, for ε_2=143 (1/cm), ε_1=204 $(\%\cdot cm)^{-1}$ and C_0=0.1%, we obtain I (z, 0)=(0.79, 0.62) I_0, and I (z, ∞)=(0.85, 0.72)I_0, at z=50, 100)μm. Therefore, for a B2 layer thickness of 50 um, the UV light intensity decreases exponentially (at z=50 um) to 79% of its surface value (at z=0) initially (at t=0), but it increases to its steady-state value of 85% at the time B2 is completely depleted. The intensity at the interface, z=d, defines the surface UV intensity for the stroma in a two-layer system, where the stroma (with B2 inside) is covered with a thin layer of B2. For higher concentrations, C_0=0.2%, lower light intensity is expected at z=d=50 μm, with I(z, 0)=0.63 I_0, and I(z, ∞)=0.72 I_0.

Case (3) stromal layer with diffused B2

For the uniform case with 1/d=0, we obtain, from Eq.(6) and (7), $A_1=2.3(\varepsilon_1C_0+\varepsilon_3)$, $A_2=2.3(\varepsilon_2C_0+\varepsilon_3)$. For ε_1=204 $(\%\cdot cm)^{-1}$, ε_2=143 $(\%\cdot cm)^{-1}$ and ε_3=13.9 cm^{-1} we obtain the initial light intensity from Eq. (4.b)

$$I(z, 0)=I_0\exp[-(0.047C_0+0.0032)z] \quad (10.a)$$

Where z in um and C_0 in %. And the steady-light intensity from Eq.(4.c),

$$I(z, 0)=I_0 \exp[-(0.033C_0+0.0032)z] \quad (10.b)$$

For example, for C_0=0.1%, we obtain I (z, 0)=(0.45, 0.2, 0.09)I_0, and I(z, ∞)=(0.52, 0.27, 0.14)I_0, at z=(100, 200, 300)μm, respectively. That is, at z=200, the intensity increases from its initial value 0.2 I_0 to the steady-state value 0.27 I_0 in the case of C_0=0.1%, which is reduced to 0.08 I_0 and 0.14 I_0, respectively, for a higher C_0=0.2%.

Table.1 shows the initial (at t =0) UV light intensity normalized by its initial value, the normalized intensity, $I(z)/I_0$, at various depths (z) in the absorbing media of the corneal stroma, B2 solution only, and stroma with B2 solution for the uniform case (with d=0.1 cm), and non-uniform case (with d=0.01 cm) based on Eq.(4), (6), (8) and (10) for an initial B2 concentration C_0= 0.1%. We note that the case (3) uniform case has a higher light intensity than that of case (4), the non-uniform case, due to less absorption of the initial B2 solution, which has an exponential decaying function of z. That is, the non-uniform case has a higher risk than the uniform distribution case. We shall emphasize that the initial (at t=0) intensity of the UV light listed in Table 1, except for case (1) without B2, is smaller than the intensity when t>0 due to B2 depletion, as shown by our dynamic formula Eq.(7). The initial intensity is given by Eq.(7) when gt=0. Therefore, a higher risk occurs for longer UV exposure durations, where the risk factor is given by gt term in Eq.(7). Greater detail is discussed later.

Table 1 Normalized initial (at t=0) UV light intensity $I(z, 0)/I_0$ at various depths (z=0 to 400 μm.) in various absorbing media based on Eq.(4), (9) and (10).

Depth(um)/Media	0	100	200	300	400
(1)Corneal stroma	1.0	0.72	0.52	0.37	0.27
(2)B2 only(0.1%)	1.0	0.63	0.39	0.25	0.15
(3)Stroma+B2(uniform case)	1.0	0.45	0.20	0.09	0.04
(4)Stroma+B2(with d=0.01 cm)	1.0	0.56	0.34	0.23	0.16

3.2 *The safe dose*

The coupled dynamic equations Eq.(1) and (2) for the non-uniform distribution case can only be solved numerically and are shown later. A comprehensive modeling is shown by the light intensity (shown by Fig.5), in which the transient regime (for t<T*) given by the gt term in Eq.(7) and the steady-state regime when t>T*, where T* is the cross-linking time to be defined later, G (z) and H (z) are the initial and steady-state intensities at a given depth (z). The total energy absorbed (the dose) by the stroma is given by the area covered by the intensity curve.

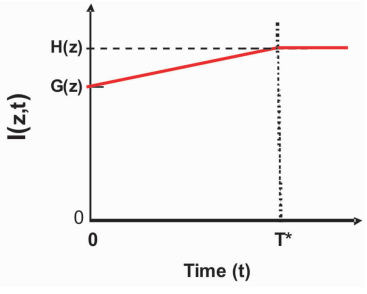

Fig.5 Schematic of the light intensity I (z,t) profiles at a given depth (z) defined by an linearly increasing function (for 0<t<T*) and steady state value H(z) (for t>T*).

The safe dose ($E^*=I_0T^*$) is defined when the total energy absorbed (or dose) by the stroma equals the damage threshold energy (E'), or

$$E'(z, t)=\int_0^t I(z, t')dt' \quad (11)$$

As shown by Fig.5, the area E'=0.5 $(I_2-I_1)T^*$. Using Eq.(4), we obtain

$$E^*(z, t)=\frac{2E'}{1+B}\exp(A_2z) \quad (12.a)$$

$$B=\exp[(A_2-A_1)z] \quad (12.b)$$

Where a=6.2 (cm^2/J) when E* and E' are in J/cm^2 and for a quantum yield ϕ=0.1; and A_j(with j=1, 2) are defined by Eq.(4).

For E'=0.32 J/cm^2, ε_3=13.9(1/cm), ε_2=143(1/cm) and ε_1=204 $(\%\cdot cm)^{-1}$, Eq.(12.a) becomes

$$E^*(z)=[0.64/(1+B)]\exp(A_2z) \quad (13.a)$$

$$A_2=0.0032+\left(\frac{0.0329dC_0}{z}\right)[1-\exp(-z/d)] \quad (13.b)$$

$$B=\exp[-140dC_0(1-\exp(-z/d))] \quad (13.c)$$

Where z and d in um, C_0 in % and E* in J/cm^2.

Eq.(12) shows that z* is an increasing function of the UV light exposure duration (t) due to the existence of B2 and its depletion. That is, a thicker cornea is required for a longer exposure duration (t) for a given safe energy (E*). The dynamic feature of A (t) of Eq.(7) indicates that the safe energy is an accumulated quantity and is proportional to the gt term in Eq.(7), or F (t) term in Eq.(11.c). Higher risk results from a longer exposure duration at a given UV light intensity; or a higher UV light intensity at a given exposure duration.

For the case of UV light in stroma tissue without a B2 solution, the safe depth for the epi-off case with A=32 cm^{-1}, C_0= 0, g'=0, and Eq.(13) reduces to z*=312.5ln[E*/E']. For example, for E'=0.32 J/cm^2 and a corneal safe depth of z*=400 um, we obtain E*=1.15 J/cm^2 (for E'=0.32 J/cm^2), and E*=2.3 J/cm^2 (for E'=0.64 J/cm^2). For the same safe energy (E*), a shorter exposure duration allows a higher light intensity. For example, for $E^*=I_0T^*$= 1.15 J/cm^2, I^0=(9.6, 19.2)mW/cm^2 for t=(2.0, 1.0) minutes.

With the presence of a B2 solution, the commonly accepted dose E=5.4 J/cm^2 [15] is much higher than the above stroma-only situation due to the extra absorption of the B2 solution and the photolysis product. Greater details are discussed as follows.

We will show the role of the endothelium damage threshold (E'), the penetration depth (d) and the B2 concentration (C_0), as shown in Fig.6, for E'=0.32(J/cm^2) and for depth of z=300 μm and 400 μm with various C_0 based on Eq.(13). Fig. 6 shows that the safety dose (E*) is an increasing function of the concentration (C_0) and the penetration depth (d) for a given corneal thickness of 400 μm. For C^0=0.1%, E*=(2.3, 3.1)J/cm^2 for d=(0.02, 0.04)cm, which increases to E*=(4.5, 8.2)J/cm^2 for a higher C_0=0.2%. The data show that a higher dose requires a thicker cornea (or larger z*). In addition, a lower concentration requires a larger corneal thickness for a given dose. Eq.(13) shows that the safe depth is a decreasing function of the product of ε_1dC_0.

Based on the above-presented examples, the conventional dose used in CXL 5.4 (J/cm^2) is higher than most of our safe doses (E*) and it meets our safety criteria only for the following situations for a corneal thickness of 400 um and: (i)d=0.02 cm and C_0>0.23%; (ii)d=0.04 cm, and C_0>0.16%, (iii)C_0=0.2% and

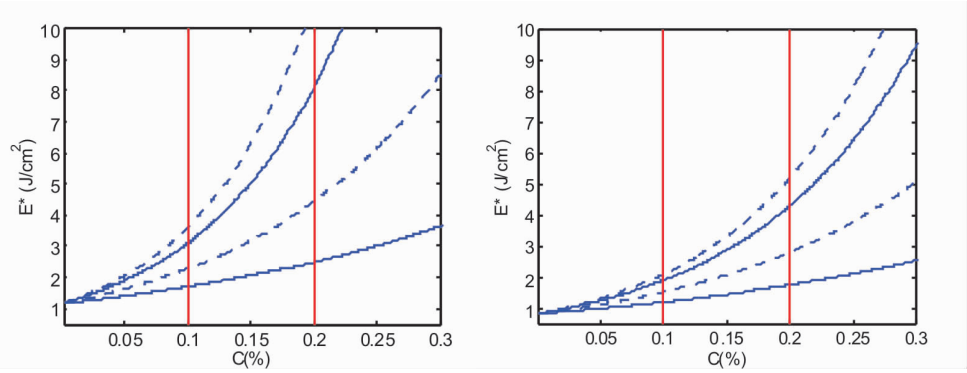

Fig.6 The energy (dose) E* versus concentration (C_0), for z=300 μm (right figure) and 400 μm (left figure), and d=(0.1, 0.2, 0.4, 0.6)cm, for low to high curves.

d>0.03 cm. We must emphasize that the safe dose (E*) depends on the parameter set (C_0, E*, E', d, z) and has a wide range from 2.3 to 8.2 J/cm² for B2 concentrations of C_0=(0.1%–0.2%) and penetration depths (d) of 0.02 to 0.04 cm. Therefore the fixed dose value of 5.4 J/cm² is over estimated, particularly for C_0<0.2% and d<0.03 cm. It should be mentioned that the conventional dose of 5.4 J/cm² and a concentration of 0.1% should have resulted endothelial damage, if no extra B2 is administrated during the UV exposure. The extra B2 administration protects the endothelium, however, it also reduces the CXL efficacy, where a majority of the UV energy was waisted in depleting the surface B2 layer on the cornea before it is transmitted to the stroma.

3.3 *The cross-linking time*

We will now consider the situation where there is no B2 solution on top of the corneal surface and the B2 concentration is non-uniformly distributed in the stroma. Cross-linking time may be defined in a variety of ways. Basically, it is used to define when the cross-linking procedure is mostly completed, or when the B2 initial concentration is mostly depleted by the UV light and the procedure reaches a steady state having a very low reaction rate. Based on the above-described concept, we define the cross-linking time (t*) as when the B2 concentration on the is reduced to C(z, t)=$C_0\exp(-M)$ at t=t*, where M has a value ranging from 2 to 7 depnding on the depletion level of the B2 concentration at a depth z.

The solution of Eq.(1) and (2) to solve for t=t* is highly nonlinear and cannot be solved analytically. Numerical results will be shown elsewhere. We will focus on a comprehensive method shown by Fig.5. Similar to Eq.(11), using a comprehensive method shown by Fig.5, the time integration of Eq.(1) leads to

$$C(z,\ t)=C_0\exp[-z/d]\exp[-\alpha F(z,\ t)] \tag{14.a}$$

$$F(z,\ t)=\int_0^t I(z,\ t')dt' \tag{14.b}$$

As shown by Fig.5, F(z, t) may be approximated by F(z, t) =(tI_2)[B+0.5 (1−B)(t/T*)], where t≤T*. Using the cross linking time (t*) defined by C(0, t=t*)=$C_0\exp(-M)$, we find the cross linking time (t*) is related to M, T* and E' as follows

$$M=\frac{2\alpha E'}{(1+B)}\left(\frac{t^*}{T^*}\right)\left[B+\frac{1-B}{2}\left(\frac{t^*}{T^*}\right)\right] \tag{15}$$

For t*=T*, M=αE' where α=83.6λϕε$_1$ and T*=E*/I_0, given by Eq.(12). We should note that aE' defines the degree of the B2 concentration depletion, or the CXL efficacy. For examples, for ε$_1$= 204 (%·cm)$^{-1}$, quantum yield [27] ϕ=0.38, a=23.6, E'=0.32 J/cm², aE'=7.5 and the initial B2 concentration depleted to exp (−7.5)= 0.3% at a given depth (z) and a UV light exposure duration of t=T*. For a shorter exposure duration of t*=0.5T*, M=0.25aE'(1+ 3B)/(1+B) which is a decreasing function of z while it is an increasing function of the quantum yield. For example, for t*= 0.5T*, M=(3.5, 3.3) for C_0=(0.1, 0.2)% and d=0.02 cm; and M reduces to M=(3.4, 3.0), for a larger d=0.04 cm. For t*=0.5T*, M=(3.5, 3.3, 3.4, 3.0), the corresponding B2 concentration is depleted to (2.4, 3.7, 3.3, 4.9)%, which are higher than 0.3% for M=7.5 (when t*=T*). Therefore, for a depleted concentration of 2.4% to 4.9%, the required dose is half of the safety dose (E* for t=T*) and the cross linking time is 0.5T*.

From Eq.(12), we obtain T*=E*/I_0, given by

$$T^*(z)=\frac{2E'}{(1+B)I_0}\exp(A_2z) \tag{16}$$

On the surface (at z=0), B=1, E'=0.32 J/cm², T_0=320/I_0 (for I0 in mW/cm²). Therefore, T^0=(32, 10.7) seconds, for I_0=(10, 30)mW/cm². Eq.(16) shows that T* (z) is a nonlinear increasing function of z, whereas it is a decreasing function of the product of the light intensity and extinction coefficient. For examples, at z= 400 um, T*=(7.1, 14.0, 9.6, 27)T_0, for the case of (d, C_0)= (0.2, 0.1%), (0.02, 0.2%), (0.04, 0.1%), and (0.04,0.2%), respectively. That is, using a 30 mW/cm² intensity, T_0 =10.7 seconds on the surface, and takes (76, 150, 103, 289) minutes, respectively for the above 4 cases. For M in the range of 3.0 to 3.5, the cross liking time (t*) equals half of T*, or t*=0.5T*= (3.6, 7.0, 4.8, 13.5)T_0, to cross link a stroma depth of z.

During the CXL procedure, applying a B2 layer on top of the treated surface will provide extra protection but also largely reduces the available light intensity in the stroma. As shown by Fig.2, for example (as shown by Table 1), a thin layer of 100 μm B2 will reduce the light intensity to 63% on the stroma surface (at z=d'). The above-described extra B2 surface layer protection also needs a longer cross-linking time due to the reduced intensity.

4 Clinical guidance and new protocol

Our theory presented above as well as the measured data are providing the basis for a clinical guidance for CXL, summarized as follows:

(a)Concerning the minimum corneal thickness: The safe depth (z*), or minimum corneal thickness, depends on the parameter set (C_0, E*, E', d) and can be calculated by Eq.(12) and it is a decreasing function of the product of dC_0. That is, for a given dose, thin (300–380 μm) corneas require a higher concentration and deeper penetration (or longer riboflavin soaking time before UV irradiation). On the contrary, when dC_0 is small (<0.004), a larger

corneal thickness (400–500 μm) is required. (b)For a given UV light intensity (I=10 to 90 mW/cm^2), light exposure duration (t) must be controlled to meet the safe dose (E*=IT*) condition shown by Eq.(12) as follows.

(1)For d=0.02 cm and C_0=0.1%, examples for the safe set include (z*, E*)=(350 μm, 1.9 J/cm^2), (400 μm, 2.3 J/cm^2) and (500 μm, 3.7 J/cm^2). (2)For d=0.02 cm and C_0=0.2%, examples for the safet set include (z*, E*)=(350 μm, 4.3 J/cm^2), (400 μm, 4.5 J/cm^2), (500 μm, 9.0 J/cm^2). (3)For d=0.04 cm and C_0=0.1%, examples for the safe set include (z*, E*)=(350 μm, 2.5 J/cm^2), (400 μm, 3.1 J/cm^2), (500 μm, 4.9 J/cm^2). (4)For d=0.04 cm and C_0=0.2%, examples for the safe set include (z*, E*)=(350 μm, 6.0 J/cm^2), (400 μm, 8.2 J/cm^2), (500 μm, 14.4 J/cm^2). (5)For a corneal thickness of z*=400 μm, the commercial light dose of 5.4 (J/cm^2) will result in endothelial damage for d<0.02, and C_0<0.2% (using the endothelial damage threshold E'=0.32 J/cm^2). Its safety sets include: d=0.02 cm and C_0>0.23% (for corneal thickness z*>400 μm; d=0.04 cm and C^0=0.23%, for corneal thickness z*>300 μm. (6)For a corneal thickness of z*=400 um, the safe set includes: (i)for d=0.02 cm, E*=(2.3, 4.5)J/cm^2, for C_0=(0.1, 0.2)%; (ii)for d=0.04 cm, E*=(3.1, 8.2) J/cm^2, for C_0=(0.1, 0.2)%.

An extra riboflavin surface layer on the cornea should be washed off prior to UV light irradiation. Extra exposure duration is expected to deplete the surface layer of riboflavin before the UV light can be efficiently transmitted into the stroma. The reduced UV light transmission due to the riboflavin layer may be calculated by Eq. (9). The conventional protocol proposes that during UV exposure, isotonic riboflavin solution instillation is continued every 2 min for 30 min. Our new protocol proposes that no riboflabin solution should be applied during UV exposure to maximize CXL efficiency.

5 The commercial CXL devices

6 Conclusions

We have presented a comprehensive model for the kinetics of UV light –initiated corneal collagen cross –linking. CXL is characterized by the key parameters: extinction coefficient (A), concentration (C) and the penetration depth (d) of the riboflavin initiator, the UV light intensity (I), dose (E), irradiation duration (t), and finally the corneal thickness (z), The safety dose (E*) depends on the parameter set (C_0, E*, E', d, z) and has a wide range from 2.3 to 8.2 (J/cm^2) for riboflavin concentrations of C_0=(0.1%–0.2%) and penetration depths (d) from 0.02 to 0.04 cm. Therefore, the fixed–dose value of 5.4 J/cm^2 may cause endothelial damage for C_0<0.2% and d<0.03 mm, if no riboflabin solution is applied during UV exposure.

We have shown that the safe depth (z*) is a decreasing function of the product of $\varepsilon_1 dC_0$. Higher light intensities and smaller initiator concentrations both require a shorter cross–linking time to achieve a given cross–linking depth. Based on our findings, both in theory and in measured data, we are able to suggest a new protocol for CXL that is fast and safe.

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References:

[1] Odian G. Principles of Polymerization[M]. New York: Wiley, 1991.
[2] Miller GA, Gou L, Narayanan V, et al. Modeling of photobleaching for the photoinitiation of thick polymerization systems[J]. J Polym Sci Pol Chem, 2002, 40(6): 793-808.
[3] Terrones G, Pearlstein AJ. Effects of optical attenuation and consumption of a photobleaching initiator on local initiation rates in photopolymerizations [J]. Macromolecules., 2001, 34(10): 3195-3204.
[4] Stephenson N, Kriks D, El-Maazawi M, et al. Spatial and temporal evolution of the photo initiation rate for thick polymer systems illuminated on both sides[J]. Polym Int, 2005, 54(10): 1429-1439.
[5] Meek KM, Hayes S. Corneal cross-linking—A review[J]. Ophthalmic Physiol Opt, 2013, 33(2): 78-93.
[6] Kamaev P, Friedman MD, Sherr E, et al. Photochemical kinetics of corneal cross-linking with riboflavin[J]. Invest Ophthalmol Vis Sci, 2012, 53(4): 2360-2367.
[7] Søndergaard AP, Hjortdal J, Breitenbach T, et al. Corneal distribution of riboflavin prior to collagen cross-linking[J]. Curr Eye Res, 2010, 35 (2): 116-121.
[8] Chai D, Gaster RN, Roizenblatt R, et al. Quantitative assessment of UVA-riboflavin corneal cross-linking using nonlinear optical microscopy [J]. Invest Ophthalmol Vis Sci, 2011, 52(7): 4231-4238.
[9] Spoerl E, Mrochen M, Sliney D, et al. Safety of UVA-riboflavin cross-linking of the cornea[J]. Cornea, 2007, 26(4): 385-389.
[10] Lamy R, Chan E, Zhang H, et al. Ultrasound-enhanced penetration of topical riboflavin into the corneal stroma[J]. Invest Ophthalmol Vis Sci, 2013, 54(8): 5908-5912.
[11] Wollensak G, Spoerl E, Wilsch M, et al. Endothelial cell damage after riboflavin-ultraviolet-A treatment in the rabbit[J]. J Cataract Refract Surg, 2003, 29(29): 1786-90.
[12] Wernli J, Schumacher S, Spoer E, et al. The efficacy of corneal cross-linking shows a sudden decrease with very high intensity UV light and short treatment time[J]. Invest Ophthalmol Vis Sci, 2013, 54(2): 1176-1180.
[13] Schumacher S, Mrochen M, Spoerl E. Absorption of UV-light by riboflavin

Table 2 Comparison of the commercial CXL devices (as of Feb. 2015)

Model name	Manufacturer(country)	Power(mW/cm^2)	Spot size(mm)	Output mode
VEGA	CSO(Italy)	3 or 10	7–12	CW only
Lightlink	Lightmed Corp(USA)	0,5 to 30	7–12	CW only
KXL	AVEDRO(USA)(note–2)	3 to 45	7–12	CW & pulse
PHOENIX	PESCHKE(Swaziland)	3 to 30	7–12	CW only
CCL–VARIO 365	MLase(Germany)	3–9–18–45	7–11	CW only
UV–360	New Vision Inc(Taiwan)(note–1)	3 to 120	7–12 & 2–5 DUAL mode	CW & pulse

Note–1: New Vision Inc offers dual mode with a small spot hand–held design for localized or irregular treatment for PACK–VET and localized (irregular) PACK. Note–2: Avedro offers topography–guided CXL.

solutions with different concentration [J]. J Refract Surg,2012,28(2):91-92.
[14] Koppen C, Gobin L, Tassignon MJ. The absorption characteristics of the human cornea in ultraviolet-a crosslinking[J]. Eye Contact Lens, 2010,36(2):77-80.
[15] Hafezi F, Randleman JB. Corneal collagen cross-linking[M]. Thorofare, New Jersey: Slack,2013.
[16] Schumacher S, Mrochen M, Wernli J, et al. Optimization model for UV-riboflavin corneal cross-linking[J]. Invest Opthalmol Vis Sci,2012,53(2):762-769.
[17] Lin JT, Liu HW, Cheng DC. Modeling the kinetics of enhanced photo-polymerization by a collimated and a reflecting focused UV laser[J]. Polymers,2014,6(5):1489-1501.
[18] Lin JT, Cheng DC. Optimal focusing and scaling law for uniform photo-polymerization in a thick medium using a focused UV laser[J]. Polymers, 2014,6(2):552-564.
[19] Lin JT. Analysis on the critical issues of UV light induced corneal cross linking[J]. Int J Latest Res Eng Comp,2013,1:104-110.
[20] Lin, JT, Liu HW, Cheng DC. On the dynamic of UV-light initiated corneal cross linking[J]. Med Biolog Eng,2014,34(3):247-250.
[21] Tian C, Peng X, Fan Z, et al. Corneal collagen cross-linking in keratoconus: A systematic review and meta-analysis[J]. Sci Rep,2014,4:5652.
[22] Mazzotta C, Traversi C, Caragiuli S, et al. Pulsed vs continuous light accelerated corneal collagen crosslinking: In vivo qualitative investigation by confocal microscopy and corneal OCT[J]. Eye,2014,28(10):1179-1183.
[23] Freidman MD, et al. Systems and methods for corneal cross-linking with pulsed light[P]. US Patent Publication number WO2014014521 A1(2014).
[24] Balidis M, Konidaris VE, Ioannidis G, et al. Femtosecond-assisted intrastromal corneal cross-linking for early and moderate keratoconus[J]. Eye,2014,28(10):1258-1260.
[25] Bikbova G1, Bikbov M. Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin[J]. Acta Ophthalmol,2014,92(1):e30-34.
[26] Muller D, Marshall J, Friedman MD, et al. Controlled cross-linking initiation and corneal topography feedback systems for directing cross-linking[P]. US Patent Publication number EP2712311A1(2014).
[27] Kamaev P, Friedman MD, Sherr E, et al. Photochemical Kinetics of corneal cross-linking with riboflavin[J]. Invest Ophthalmol Vis Sci,2012, 53(4):2360-2367.

紫外线激发的角膜胶原交联术的动态安全

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【摘要】 目的 探讨紫外线引发角膜交联光动力学的关键问题,并以实验数据确认紫外线在核黄素内的吸收动力学理论。**方法** 耦合动力学方程的数值求解和分析公式推导出 3 个重要的参数:安全深度(z*),安全剂量(E*) 和交联时间(T*)。经由对时间函数的核黄素吸收系数实验测量, 核黄素的动态频谱显示光敏剂随时间的耗竭。角膜交联光动力是由 7 个关键参数决定:核黄素(B2)的消光系数,浓度,光敏剂穿透深度,紫外线的强度和剂量,照射时间以及角膜厚度。**结果** 核黄素浓度范围为 0.1%~0.2%,穿透深度范围为 0.02~0.04 cm,安全剂量范围为 2.3~8.2 J/cm^2。我们的数学模式显示,较高的紫外线强度和消光系数导致较短的表面交联时间,而角膜厚度大的交联时间也增加。安全深度为消光系数和光敏剂浓度的反比函数。**结论** 基于我们的新的发现,角膜胶原交联术的动态安全参数包括安全深度,安全剂量,交联时间,和安全光敏剂浓度,我们提出一个新的交联临床规范。

【关键词】 模型; 圆锥角膜; 眼科设备; 紫外线; 角膜胶原交联

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