·Basic Research·

Analysis of the effective dose of ultraviolet light in corneal cross-linking

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Abstract

• AIM: To analyze the efficacy of ultraviolet (UV) light initiating corneal cross-linking (CXL).

• METHODS: The time-dependent absorption of UV light due to the depletion of the initiator (riboflavin) was calculated. The effective dose of CXL with corneal surface covered by a thin layer of riboflavin was derived analytically. The cross linking time was calculated by the depletion level of the riboflavin concentration. A comprehensive method was used to derive analytic formulas.

• RESULTS: The effective dose of CXL was reduced by a factor (R) which was proportional to the thickness (d) and concentrations (C_0) of the riboflavin surface layer. Our calculations showed that the conventional dose of 5.4 J/cm² had a reduced effective dose of 4.3 and 3.45 J/cm², for d was 100 and 200 μm, respectively, and C_0=0.1%. The surface cross linking time was calculated to be T*=10.75s, for a depletion level of 0.135 and UV initial intensity of 30 mW/cm². The volume T* was exponentially increasing and proportional to exp (bdC_0), with b being the steady state absorption coefficient.

• CONCLUSION: The effective dose of CXL is reduced by a factor proportional to the thickness and concentrations of the riboflavin surface layer. The wasted dose should be avoided by washing out the extra riboflavin surface layer prior to the UV light exposure.

• **KEYWORDS:** keratoconus; collagen corneal cross-linking; ultraviolet radiation; riboflavin; safety efficacy
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INTRODUCTION

Photo-polymerization has been used in chemical engineering applications [1-4] and more recently for corneal cross-linking (CXL) [5-14]. However, much fewer efforts have been invested in basic theoretical studies [15-19]. Many factors can affect the CXL reaction and the amount of biomechanical stiffness achieved. These factors include riboflavin concentration, condition of the cornea, temperature, presence of the oxidizing agent (riboflavin), the ultraviolet (UV) light intensity, its dose and the on-off duty cycle. The safety and efficacy of CXL have been reported clinically by various methods, including the accelerated CXL using high UV power, pulsed mode operation for improved oxygen supply, diffusion in the de-epithelialized stroma (standard method), diffusion through the epithelium into the stroma (transepithelial method), or direct introduction of riboflavin into the stroma (pocket technique, ring technique, needle technique), and enrichment of riboflavin in the stroma by iontophoresis[20-21].

The conventional CXL procedures are based on the Dresden protocol which requires a surface safety dose of 5.4 J/cm² and a minimum corneal thickness of 400 μm. During the UV exposure, riboflavin drops were applied every few minutes for saturated riboflavin concnetraion in the stroma and extra protection of the corneal endothelial cells. However, the effective dose of CXL is reduced by the extra absorption of this surface layer. For maximum efficacy, the wasted-dose can be avoided by washing out the extra surface riboflavin layer after its sufficient diffusion into the stroma and prior to the UV light exposure. The dynamic in the stroma has been studied elsewhere [18-19] and this paper will focus on the riboflavin surface layer and its influence on the efficacy of CXL. We shall note that previous study [15] assuming a time-independent riboflavin concentration, or without the depletion feature, would underestimate the steady-state intensity and hence overestimate the safety dose. The riboflavin depletion plays the major role of the dynamic feature of CXL.

We will also introduce a CXL time defined by the duration of light exposure needed for riboflavin concentration

depletion to exp (-M) of its initial value, with M is 2 to 4. The dynamic in the stroma has been studied elsewhere[16-19], this paper will focus on the role of the B2 surface layer.

METHODS AND MATERIALS

The Modeling System As shown in Figure 1, a simplified corneal model consists of its epithelial layer and the underlying stroma collagen, where z represents corneal thickness and z=d defines the corneal surface. The UV light is incident-normal to the corneal surface, which is covered by a thin layer of riboflavin (or B2) solution. The CXL procedures could be conducted 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 due to the less efficient diffusion (f) riboflavin in the epi-on case. This paper will discuss the epi-off case and the influence of the surface B2 layer (Figure 1). The dynamic in the stroma (z>d) has been studied elsewhere[16-19], this paper will focus on the role of the B2 surface layer, or z<d.

The Dynamic Equations 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 surface layer or inside the stroma may be described by coupled integral equations (Eq.) as follows[1-2,16]:

$$I(z,t)=I_0 \int_0^z exp[-(a-b)C(z't)-bC_0F(z')-c]dz' \quad (1a)$$

$$C(z,t)=C_0F(z)exp\left\{-g\int_0^t I(z,t')dt'\right\} \quad (1b)$$

where F(z) is the distribution profile of the initial B2 solution (Figure 1) with F (z)=1, for z<d; $g=83.6a\lambda\varphi$, with φ being the quantum yield, λ being the UV light wavelength; a and b being the molar extinction coefficients of the riboflavin (initiator) and the photolysis product, respectively. Q is the absorption coefficient of the corneal stroma tissue reported to be Q=32 cm^{-1} without the epithelium [13]. The extinction coefficient of the riboflavin (or B2) solution (at 365 nm) has been reported [12,15] as a=469 $(\%\cdot cm)^{-1}$ and extinction coefficients of the photolysis product [19] b is about 40% to 60% of a, or b=(188-263) $(\%\cdot cm)^{-1}$. The following units are used: C(z,t) in weight percent (%), I(z,t) in (mW/cm^2), λ in cm, a and b in $(\%\cdot cm)^{-1}$.

The above coupled 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]. We shall note that previous study [15] using Eq. (1a) for the UV intensity and assuming a time-independent B2 concentration, or without the second coupled Eq. (1b), would underestimate the steady-state intensity and also the safety dose. The B2 depletion defined by Eq. (1b) plays the major role of the dynamic feature of CXL.

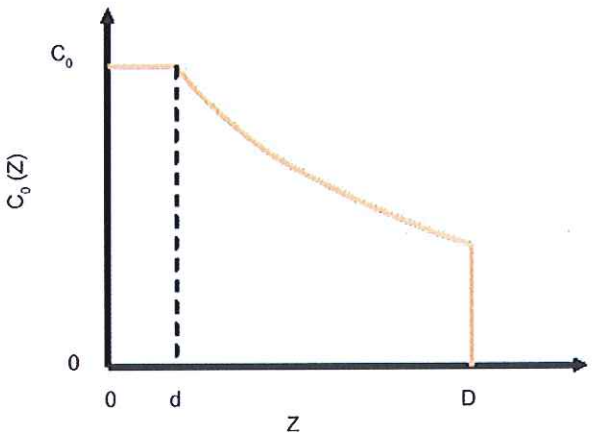

Figure 1 A corneal model system for the initial distribution of the riboflavin surface layer (z<d) and inside the stroma (z>d).

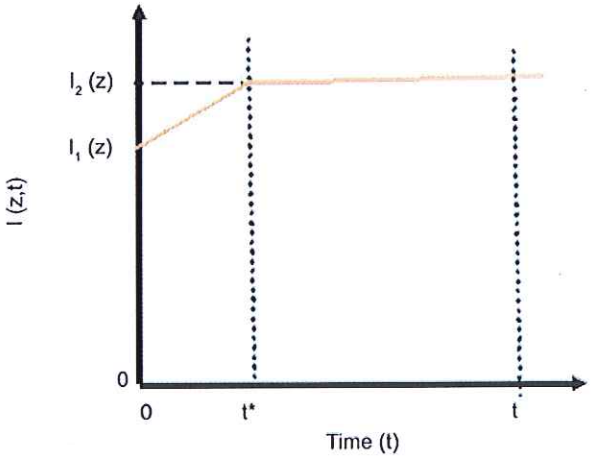

Figure 2 Schematic of the light intensity I (z,t) profiles at a given depth (z) in the transient (for t<t*) and steady state (for t>t*) defined by Eq.(3).

Our goal is to study the role of the B2 surface layer on the efficacy of CXL. For uniform B2 surface layer on top of the stroma (with z<d, shown in Figure 1), there is no stroma absorption (c=0) and only the extinction coefficients of the riboflavin (initiator) (a) and the photolysis product (b) are needed in Eq.(1). In addition, for the B2 surface layer, F (z)=1 for a uniform distribution.

Analytic approximate solution of Eq.(1) and (2) leads to the UV light intensity given by a revised time-dependent Lambda-Beer law[19]

$$I(z,t)=I_0 exp[-A(t)z] \quad (2)$$

Where the time-dependent extinction coefficient A (t) shows the dynamic feature of the UV light absorption due to the B2 concentration depletion. This feature will be shown both theoretically and experimentally later.

The exact UV light intensity profiles require numerical solution of Eq. (1). However, the initial and steady state solutions are analytically available as follows[16]:

$$I_j(z,t)=I_0 exp[-A_j z] \quad (3)$$

With $A_1=aC_0$ for the initial state and $A_2=bC_0$ for the steady state for Q=0 in the B2 surface layer.

For a comprehensive method, as shown by Figure 2, the time

integration function E (d,t), or the area covered by the red line of Figure 2, defines the dose absorbed by the B2 surface layer (with thickness d) for an exposure time (t). Therefore the effective dose is given by

$$E(d,t)=\int_0^t I(d,t')dt'=RE_0 \qquad (4a)$$

where the dose reduction factor (R) is approximated by the steady-state formula[16]

$$R=exp(-bC_0d) \qquad (4b)$$

and $E_0=tI_0$ is the dose applied on the corneal surface (at z=0) with a reduction factor defined by $R=Eeff/E_0$.

The Cross-linking Time CXL time may be defined in a variety of ways. Basically, it is used to define the level of depletion of the B2 initial concentration and the procedure reaches a steady state having a very low reaction rate. Based on the above-described concept, we define the CXL time (T*) as when the B2 concentration on the is reduced to C(z, t)=C_0exp (-M), at t=T*, where M has a value ranging from 2 to 4 depending 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. Using Eq. (4) for the integration of Eq.(1.b), we obtain

$$T^*(z,t)=T_0exp(bC_0z) \qquad (5)$$

where $T_0=M/(gI_0)$ is the surface CXL time (at z=0). For a quantum efficiency of 0.1, we have g=0.062, or $T_0=16.1M/(\emptyset I_0)$ with I_0 in mW/cm^2.

RESULTS

The Dynamic Intensity Profiles As shown in Figure 3, the initial intensity (solid curve) increases due to B2 depletion and reaches its steady-state (dashed red curve) defined by Eq.(3). The intensity has a faster exponential decay for z>d due to the addition absorption of the corneal stroma, whereas for z<d the absorption is due to B2 solution only. The dynamic in the stroma has been studied elsewhere [19]. This paper will focus on the role of the B2 surface layer, or z<d.

Figure 4 shows the calculated time-dependent UV light intensity at a given position z=200 μm for various B2 initial concentration C_0=(0.1, 0.15, 0.2)% (from top to low curves), where we have used a=469 (%•cm)$^{-1}$, Q=32 cm^{-1} and b=0.5a. The calculated time increasing feature of the UV light intensity is consistent with our measured data shown by Figure 5. The measured UV light transmitted intensity (normalized by its initial value) showing the time increasing feature, where the initial riboflavin concentration is 0.0075% (top curve) and 0.005% (lower curve)[18].

The Cross-linking Time As shown by Figure 6, the initial constant concentration (curve 1) is depleted to lower curves (2 to 7) at various time of t= (5, 10, 20, 40, 60, 80)s, for an initial concentration of C_0=0.2%. We may easily see that the B2 depletion starts from the surface. It takes longer exposure time to deplete the volume layer and the cross linking time (T*) is given by Eq.(5). Also shown in Figure 6 is the red line defined by a depletion level of C/C_0=0.135, or exp(-M) with M=2. The cross points of the red line and the

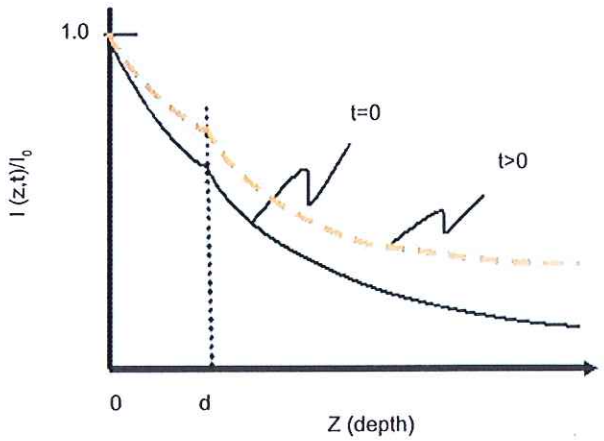

Figure 3 Schematics of the light intensity initially (solid curve) and at steady-state (dashed red curve).

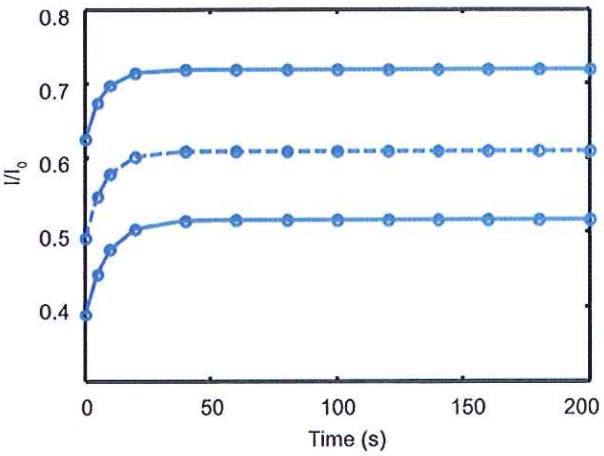

Figure 4 The calculated light intensity (normalized by its initial value) at a given depth z=200 μm for various riboflavin concentration C_0=(0.1, 0.15, 0.2)% (from top to low curves).

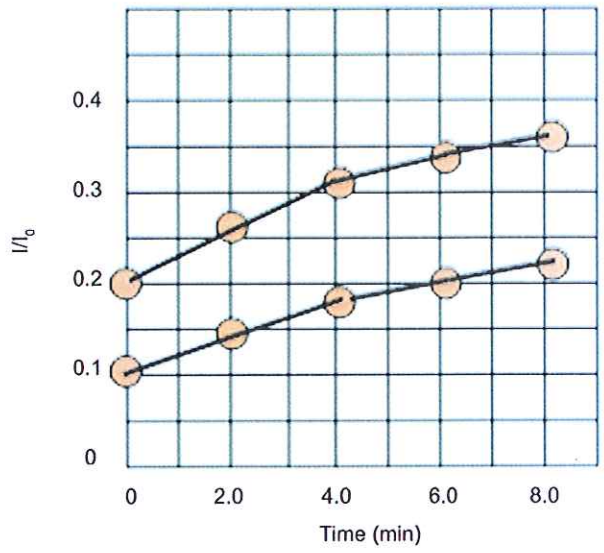

Figure 5 The measured UV light transmitted intensity for riboflavin concentration of 0.0075% (top curve) and 0.005% (lower curve).

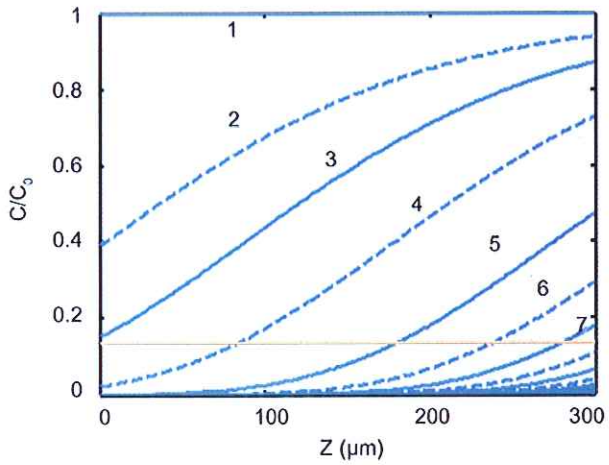

Figure 6 The normalized B2 concentration at various time t= (0, 5, 10, 20, 40, 60, 80)s (for curve 1 to 7), for an initial concentration C_0=0.2%.

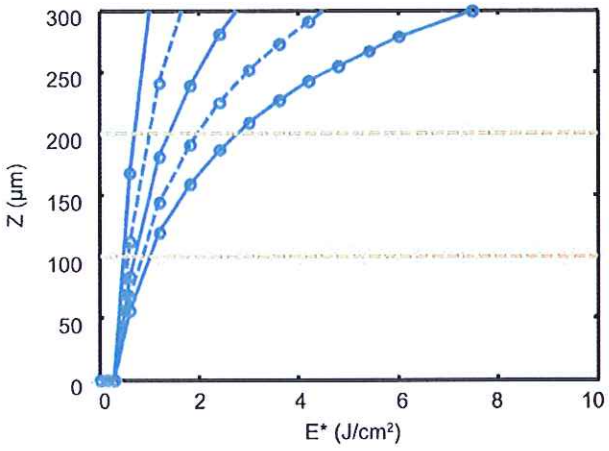

Figure 7 The required dose to deplete the B2 layer at various thickness (d), for various concentration of C_0=(0.1, 0.15, 0.2, 0.25, 0.3)%, (curves left to right), for a=469 (%·cm)$^{-1}$, b= 0.5a, Q=32 cm^{-1}.

concentration curves defines the cross linking time (T*) for various depth of the B2 layer. The corresponding dose, defined by $E^*=T^*I_0$ for various concentration of C_0= (0.1, 0.15, 0.2, 0.25 0.3)% for curves left to right (Figure 7).

The Effective Dose As shown by Eq.(4), the dose applied on the corneal surface (at z=0) $E_0=tI_0$ is reduced by a factor R due to the dose absorbed by the B2 surface layer (with thickness d), where $R=Eeff/E_0$ is given by Eq. (4b). For b=235 (%·cm)$^{-1}$ and Q=32 cm^{-1}, we calculate the reduction factor, for R=(0.8, 0.64), for d=(100, 200) μm and C_0=0.1%, which is further decrease to R=(0.64, 0.41), for C_0=0.2%.

The effective dose given by Eq.(4) defines the available dose at z=d after the absorption of the B2 layer. That is, less CXL efficacy in thicker B2 surface layer with high concentration. At steady state, or when the B2 layer is largely depleted, the UV light surface intensity (I_0) is reduced to $I_0\exp(-bC_0d)$, as shown by Eq.(4b). For example, the conventional dose 5.4 J/cm^2 is reduced to an effective dose of 5.4× 0.8=4.3 J/cm^2, that is 20% of the dose, or 1.08 J/cm^2 dose is wasted in the B2 surface layer having a thickness of 100 μm and concentration of 0.1%. The effective dose is further reduced to 5.4×0.64=3.45 J/cm^2 for a thicker B2 layer of 200 μm.

The cross linking time (T*) defined by the depletion of the riboflavin is given by Eq.(5). The surface CXL time (at z=0) given by $T_0=M/(gI_0)$ is calculated T*=10.75s, for M=2 and g=0.0062 (for a quantum efficiency of 0.1) and UV initial intensity of 30 mW/cm^2. While B2 surface layer provides extra protection of the corneal endothelial cells, the wasted dose (defined by 1-R) should be avoided by washing out the extra B2 surface layer after its sufficient diffusion into the stroma and prior to the UV light exposure. As shown earlier, a B2 layer of 100 and 200 μm causes the effective dose reduced to 69% and 49% , respectively, for 0.1% concentration; and 48% and 25%, for 0.2% concentration.

In conclusion, the effective dose of CXL is reduced by a factor (R) which is proportional to the thickness and concentrations of the riboflavin surface layer. For maximum efficacy, the wasted dose (defined by R) should be avoided by washing out the extra B2 surface layer.

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Conflicts of Interest: Zhang Y, None; **Wang KC,** None; **Chang CK,** None; **Lin JT,** None.

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