Original Research Article

Efficacy analysis of corneal crosslinking (CXL): comparing epi-on and epi-off procedures

ABSTRACT

Purpose: To analyze the factors influencing the corneal crosslinking (CXL) efficacy and comparison of epi-on and epi-off procedures.
Study Design: modeling the efficacy of epi-off and epi-on CXL.
Place and Duration of Study: New Taipei City, Taiwan, between November, 2021 and December, 2021.
Methodology: Solving the rate equations for the CXL efficacy which includes the roles of concentration of the photosensitizer, riboflavin (RF), RF depletion effects, dynamic of light intensity, and the non-uniform distribution of RF in the stroma, or the diffusion depth of RF. Both steady-state and transient state features are explored for the efficacy, crosslink depth (CD) and the effects of epithelium layer for the epi-on situation.

Results: The steady-state efficacy is proportional to the square-root of [RF-concentration] /[light-intensity], The competing factors of reduced RF, F(z), and reduced light intensity in the stroma determine the relative efficacy of epi-on and epi-off. For example, for F(z)<0.5, epi-on is more efficient than epi-off. In contrast, in the transient state (with efficacy <0.6), the efficacy is proportional to the light dose, and therefore epi-on is always less efficient than epi-off. The crosslink depth (CD) has an inverse trend, such that higher light intensity and lower RF concentration lead to deeper CD. The analytic formulas are developed under simplified conditions, in which numerical simulation is required for non-uniform distribution, and when RF depletion are included. Various strategies for improved steady-state efficacy efficacy and crosslink depth for epi-on CXL are explored including the use of higher RF concentration and lower light intensity; enhancing the RF diffusion by an electrode device, or diffusion enhancing medicine. The analytic formulas are compared with measured data.

Conclusion: For the steady-state epi-on is more efficient than epi-off, when the RF reduction factor is less than the light intensity gain factor. In contrast, in the transient state (with efficacy <0.6), the efficacy is proportional to the light dose, and therefore epi-on is always less efficient than epi-off.

Keywords: corneal crosslinking; efficacy; photosensitizer; riboflavin; UV light.

1. Introduction

In 1998, Spoerl et al [1,2] proposed the use of UVA light (at 365 nm) and a photosensitizer, riboflavin, for corneal collagen cross-linking (CXL) to increase the corneal biomechanical strength and stabilize the ectatic cornea. The standard Dresden (SD) protocol was proposed (in 2003) by Wollensak et al [3], in which a UVA light intensity of 3.0 mW/cm^2 was applied to the cornea for an irradiation time of 30 minutes, such that a light fluence (dose) of 5.4 J/cm^2 was delivered to the cornea. To shorten the irradiation time of the SD protocol, accelerated CXL was also developed [4,5], based on Bunsen and Roscoe law [6], leading to the AC protocol given by light intensity of I= (3,9,18,30,45) mW/cm^2, with the associated irradiation time is inverse proportional to the light intensity given by t= (30,10,5,3,2) minutes, such that the total dose applied to the cornea is fixed at 5.4 J/cm^2 [1].

The basic kinetics and modeling of CXL were published by various groups [6-10] and, more recently, by Lin et al [11-25]. The review articles by Lin et al [26,27] discussed the critical and controversial issues of CXL, including: (i) validation of SD protocol for the minimum (safety) corneal thickness of 400 m; (ii) validation of the Bunsen and Roscoe law (BRL) of reciprocity for accelerated CXL protocol; (iii) the role of oxygen in type-I and type-II CXL; (iv) Improved efficacy by pulsed light and by higher riboflavin concentration; (v) the new efficacy scaling law developed by Lin, a nonlinear law replacing the linear law based on BRL; (vi) New criteria for minimum corneal thickness, in which sub400μm thin corneas (214 - 398 um) was reported by Hafez et al [28].

The present article will further explore the features of CXL with an emphasis on the role of riboflavin concentration on the crosslink depth and the demarcation line depth, the critical parameters defining the outcome efficacy of CXL. We will also compare our formulas with the measured data [29,30]. Epi-on offers many clinical advantages of less post-operation pains, faster epithelium recovery and less haze. Therefore, strategy for improved the efficacy and crosslink depth for epi-on CXL is clinically important. The efficacy CXL of steady-state and transient-state are compared for epi-on and epi-off. Our formulas show that epi-on has lower transient-state efficacy than epi-off, as conventionally believed. However, opposite trend is found for the steady-state efficacy, a new finding to be explored experimentally.

2. Materials and Methods

2.1 Epi-off versus epi-on

As shown in Fig. 1, a cornea under a UV light (at 365 nm), with epi-on surface defined at z=0, and stroma thickness about 400 μm. The riboflavin concentration distribution for epi-off (Curve-1) and epi-on (Curve-2), noting that ep--off stroma surface defined by z=100 μm. Effect of RF distribution is approximated by a distribution function F(z)=1–0.5z/D, or C(z,t=0)=C_0F(z), with a

diffusion depth D in the stroma. We note that D is proportional to the waiting time of the pre-operation RF drops applying to the cornea. For a typical waiting time of 15 minutes (for epi-off), and 25 minutes (for epi-on), D is about 150 μm [12]. For example, for C(z=0)=0.2%, at z=300 μm, C(z)= 0.12% and 0.08% for epi-off and epi-off, respectively, in which C(z, epi-on) is about 65% of epi-off. We will show later that lower C(z) leads to lower efficacy.

The light intensity is given by $I(z,t)=I_0 \exp(-q'z)$, with the effective absorption constant given by [22] $q'=2.3(a'C_0+Q)(1-Bt)$, with $B=1.15C_0(a'-b')d'$, d' is the correction for a depleted $C(t)=C_0 \exp(-d't)$; and $a'=204$ (1/5 cm), $b'=120$ (1/% cm), and $Q=13.9$ cm^{-1}; are the extinction coefficients of RF and the photolysis product, and stroma, respectively. Given a value of $q'=0.0094$ (1/ μm), with neglected $B=0$, we calculate the light intensity reduction factor (R') due the epi-layer (having a thickness of $Z_0=70$ to 100 μm), $R'=\exp(-q'Z_0)=0.4$ to 0.56. Therefore, the reduced light intensity leads to a higher steady-state efficacy (to be shown later). Our formulas are consistent with the clinical studies of Lang et al [5] showing that accelerated CXL (ACX) had less efficacy than standard low intensity ($3mW/cm^2$) CXL for the same fluence (dose).

Combining the difference of D-value at z=300um, D(epi-on)=0.65 D(epi-off), we obtain the effective-ratio (of epi-on and epi-off), $ER=C_0/I_0=1.16$ to 1.63. This ER is a critical parameter defining efficacy and crosslinking depth (to be discussed later). We note that (to be shown later) due to the special feature of CXL steady-state efficacy is governed (proportional to) $ER^{0.5}=[F(z)C_0/I_0]^{0.5}\exp[0.5q'(z+Z_0)]$. Therefore, the competing factors of F(z) and $\exp(0.5q'Z_0)$ determines the relative efficacy of epi-on and epi-off. Our theory will predict that, under certain conditions, epi-on has a higher steady-state efficacy than that of epi-off, a feature in contrary to conventional concept, which is valid only for transient efficacy which is proportional to the light dose, or intensity x irradiation time (t), $E_0= tI_0$.

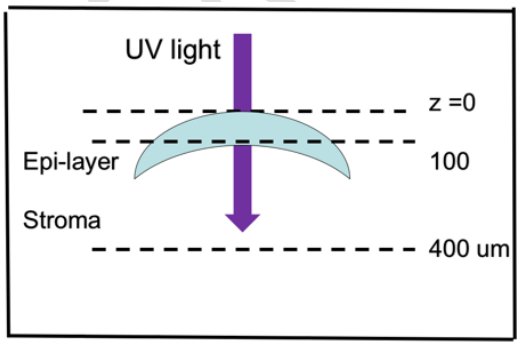

Fig. 1. Cornea under a UV light crosslinking, with (epi-on) surface defined at z=0, and stroma thickness about 400 um.

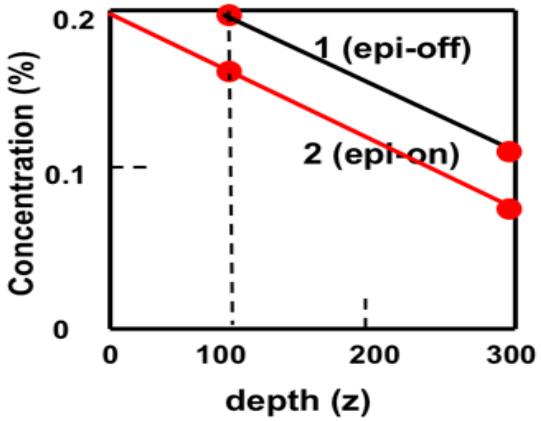

Fig. 2. Riboflavin concentration distribution for epi-off (Curve-1) and epi-on (Curve-2), noting that ep--off stroma surface defined by z=100 um.

2.2 Crosslink Rate Equation

Using the shorthand concentration notations: C and T for RF ground and excited triplet state; R for the active radical, S for the singlet oxygen; and [A] for the available extracellular matrix substrate, the crosslinking rate equation is given by [22, 23, 26]

$$\frac{d[A]}{dt} = -(K_3 T + K_1 R + K_2 S)[A] \qquad (1)$$

Eq. (1) includes three crosslink pathways: (i) the type-I direct coupling of T and the substrate [A]; (ii) and the coupling of the radical (R) and [A]; and (iii) the oxygen-mediated, type-II term due to the singlet oxygen coupling with [A]. Both type-I and type-II pathway can occur simultaneously, and the ratio between these processes depends on the type of photosensitizers (PS) used, the concentrations of PS, substrate and oxygen, the kinetic rates involved in the process, and the light intensity, dose, RF depletion rate etc.

In the type I mechanism, by receiving energy from UV light, RF becomes the excited RF triplet (T*), which interacts directly with the substrate (i.e., stromal proteins). In the type II mechanism, T* reacts with oxygen dissolved in stroma to produce reactive oxygen species (ROS), including singlet oxygen, hydroxyl radical and hydrogen peroxide, which generate photo-oxidation and photo-polymerization of the substrate. Overall, the dominant pathway of type I or type II depends on the type and concentration of photosensitizer, concentrations of substrate and the amount of oxygen [27].

2.3 The Efficacy formulas

Factors influencing the CXL efficacy include: UV light intensity, dose, exposure time, mode of exposure (pulsed or CW), riboflavin concentration, diffusion and drops pre-operation and interoperation administration, the concentration of oxygen in the stromal tissue (pre-op and inter-op), and environmental conditions. The length of the riboflavin presoaking time and viscosity of the riboflavin film also affect the crosslink depth. We will first consider the uniform RF distribution in the stroma, given by a distribution function F(z)=1–0.5z/D, with D>>500 μm, or F(z)=1. Therefore, the z-dependence of C(z,t) is mainly due to light absorption. The effects of D (with D about 150 to 200 μm) will be discussed later, in section 2.4.

For type-I dominant case, from Eq. (1), with $K_2S=0$, and for bimolecular termination dominant, we obtain, $R=(K_3[A]T/k')^{0.5}$, with $T= bI(z)gC(z,t)$. For g is approximated as $g=1/(K_3[A])$, R becomes $R=(T'/k')^{0.5}$, with $T'=bI(z)C(t)$, we obtain [26] (at a given z)

$$\frac{d[A]}{dt} = -T' + K_1\sqrt{T/k'}\,[A] \qquad (2)$$

Considering a non-perfect RF regeneration case with $C(t)=C_0 \exp(-d't)$, with $d'=bIg''$, and g'' defining the degree of regeneration (with g''=0, for the perfect case). the approximated analytic solution of Eq. (2) leads to the crosslink efficacy (CE) defined by $CE=1-[A]/[A_0]$,

$$CE = 1 - (1 + k\,H')\exp(-H) \qquad (3)$$

where $k=bI(z)C_0(z)/A_0$ is the contribution from the K_3T term; $H(t)=2d[1-\exp(-0.5d't)]/d'$, $H'(t) = [1-\exp(-0.5d''t)]/d''$, with $d=K_1(bIC_0/k')^{0.5}$, $d'=bIg''$ and $d''=d'-0.5d$. Eq. (3) has transient state, $H(t)=dt$, $H'=0.5d''t$. The steady state $H=2d/d'= K_1[C_0/(bgk'I)]^{0.5}$, and $H'(t)=1/d''$, which is proportional to $(C_0/I)^{0.5}$, an increasing function of C_0, but decreasing function of the light intensity, $I(z)=I_0 \exp(-A'z)$. Therefore, higher light intensity leads to lower steady-state efficacy. In contrast, the transient state H=dt, leading to efficacy proportional to $t(I_0C_0)^{0.5}$ or $(tE_0C_0)^{0.5}$, which is proportional to the light dose and irradiation time (t). These scaling laws having opposite power dependence of light intensity are also demonstrated numerically [15]. The special feature of steady-state efficacy is due to the RF concentration depletion which is proportional to light intensity, or $C(t)=C_0 \exp(-dt)$, with $d=K_1(bIC_0/k')^{0.5}$, and the CXL radical is proportional to $(T'/k')^{0.5}$, with $T'=bI(z,t)C(t)$, in which the time integral of R(z,t) defines the CXL efficacy, as shown the rate eq. (2).

2.4 Crosslink-Depth (CD) and Demarcation line depth (DLD)

CD and DLD are proportionally related, because both are defined by when CE is larger than an efficacy threshold value (E') for collagen tissue to be

effectively crosslinked, where E' may be different for CD and DLD. For type-I of Eq. (3), the steady state H=2d/d'= $2K_1[C_0/(bgk'I)]^{0.5}$, with $I(z)=I_0 \exp(-q'z)$; and let CE=E' (for H'=0), or H=ln[1/(1-E')]=lnE", we obtain (for type-I) DC (and DLD) are given by, assuming a uniform distribution, with F(z)=1, or diffusion depth D>>500 um, and redefining Z' =0 at stroma surface (for epi-off case)

$$Z' = (2/q')\ln(\ln E''/G) \qquad (4.a)$$

with $G= K_1[C_0/(bgk'I_0)]^{0.5}$; $q'=2.3(a'C_0+Q)(1-Bt)$, with $B=1.15C_0(a'-b')d'$, $K=2K'I_0^{-0.5}$, and E"=1/(1-E'); d' is the correction for a depleted $C(t)=C_0 \exp(-d't)$. Given a value of q'=0.01 (1/ μm), with neglected B=0, and let $G'= \ln E''(bgk']^{0.5}/K_1$, Eq. (4.a) becomes

$$Z' = 200 \ln(G'\sqrt{I_0/C_0}) \qquad (4.b)$$

for I_0 in mW/cm^2 and Z' in μm. We note that Z' is an increasing function of $[C_0/I_0]^{0.5}$ and ln(E").

2.5 Effect of diffusion depth (D)

In the above formulas, we assume a uniform RF distribution in the stroma. A more realistic modeling was also developed by Lin et al [15] to include the effect of RF distribution. given by a distribution function F(z)=1–0.5z/D, or $C(z,t=0)=C_0F(z)$, with a diffusion depth D in the stroma. Another factor is the light absorption due the epithelium layer (of epi-on case). Comparing to epi-off, the revised light intensity for epi-on is reduced by a factor of exp(-q'z"), with z" being the epi-layer (about 70 to 100 μm, mean of 80 μm, or 0.008 cm). Thus the effective absorption is revised to $q'=0.008+2.3(a'C_0+Q)(1-Bt)$, with $B=1.15C_0(a'-b')d'$ and d' is about 150 (1/sec), for I_0=30 mW/cm^2. For RF (and with C_0=0.2%, B=0), a'=204 (%.cm)$^{-1}$, b'=120 (%.cm)$^{-1}$ and Q=13.9 (1/cm) [15], q'=0.008+0.0094= 0.017 (for epi-on); and q'=0.0094 (for epi-off). To include the light intensity dynamics, we revise $I(z,t)=I_0\exp(+Bzt)$, thus Eq. (4.a) becomes,

$$Z' = \ln(G'\sqrt{I_0/C_0})/[q'(1-0.5/(q'D)] \qquad (5)$$

where q'=0.0094 (for epi-off), and 0.017 (for epi-on) which is 1.8 times larger. Therefore, to compensate the reduced CD in epi-on, we propose: (i) increase of light intensity (I_0) by a factor of 36, because ln6=1.8, to achieve the same Z'; (ii) using a 36 times lower C_0; (iii) using 10 times higher I_0 and 3.6 lower C_0; and (iv) enhancing the RF diffusion (or larger D value) by an electrode device, or diffusion enhancing medicine. We note that the above means for increased CD is opposite to that of efficacy, to be discussed later.

1. Results and Discussion

3.1 The role of RF concentration

Lombardo et al [30] disclosed an apparatus to measure the intrastromal riboflavin concentration of the cornea and provides biomarkers of treatment efficacy in real time. The differences between samples could be primarily caused by: (i) frequency application of riboflavin drops pre-op and intra-op, (ii) non-uniform distribution of riboflavin film over the stromal surface, and (iii) distribution of riboflavin in the stroma. The exponential decrease of intrastromal riboflavin concentration during UV illumination is due to the consumption of riboflavin in the stroma, which is also temporally monotonic decreasing. Their results were fit to an exponential law, $C(t) = C_0 \exp(-t/\tau)$, with C_0 being the initial value and intrastromal riboflavin consumption rate $1/\tau$, with τ=12.8 minutes, fro light intensity of 3 mW/cm^2, in which the mean consumption of riboflavin was 80±4%. During UV-A irradiation, the stromal riboflavin concentration decreased non-linearly. O'Brart et al [29] reported that efficacy is an increasing function of RF concentration (in the stroma).

Clinical studies of Lang et al [5] showed that accelerated CXL (ACX) had less efficacy than standard low intensity (3mW/cm^2) CXL for the same fluence (dose) based on BRL. To overcome this intrinsic drawback of ACX, Lin [20] recently proposed a new protocol called the riboflavin concentration controlled method (CCM) to improve the efficacy of ACX by supplemental RF during the UV exposure to compensate for fast depletion of RF by UV light. However, RF concentration, C(t), may be partially self-compensated due to the regeneration effect (RGE), in which $C(t) = C(z,t)=C_0 \exp(-blg''t)=C_0$, for a perfect RGE case with g''=0, which also depends on the type of photoinitiator [26]. The optimal concentration was also theoretically proposed [23], specially for the efficacy in the stroma (with z>0. From Eq. (4), the type-I steady-state efficacy given by $H = 2K_1[C_0/(bgk'I)]^{0.5}$, which is an increasing function of $[C_0/I_0]^{0.5}$.

As discussed in our previous paper [22], C(t) is a constant if there is a continuing resupply of RF, or for the case of a perfect REG of RF (or a catalytic cycle). To demonstrate the RF depletion, we conducted a measurement of RF (mixed with pure water) under UV irradiation at various RF concentrations, in which the increase of light intensity passing through the RF solution (path of 10 mm) was recorded indicating the decrease of RF which also has color changes. This feature can be explored by $I(z,t)=I_0\exp[-q'+Bt]z$, a revised time-dependent Beer-Lambert law (BLL) [11,17}, with $B=2.3(a'-b')C_0d'$, proportional to the depletion rate of RF (d') and its initial concentration C_0. If d'=0, then I(t,z) is time-independent.

Fig. 3 shows the calculated efficacy, based on Eq. (3), versus RF concentration (C_0) at various light intensity; also shown are the measured data of O'Brart et al [41], in which efficacy is an increasing function of C_0; however, it is a decreasing function of the light intensity (for a given light dose). For examples, (shown by the red lots of Fig.1), efficacy CE=(0.8, 0.7, 0.6), for I= (9,18,30) mW/cm^2, at a given C_0=0.25%. The measured data of O'Brart et al [41] are fit to our calculated curve.

Fig. 4 shows the calculated crosslink depth (CD) versus light intensity, based on Eq. (4.b), for various G'. We note that CD is an increasing function of

$(I_0/C_0]^{0.5}$ as shown by Eq. (4.b), which is proportional to the light intensity, but decreasing function of RF concentration, for steady-state efficacy. That is high concentration leads to a smaller CD. However, higher light intensity leads to deeper crosslink. These steady-state features are opposite to the transient state, in which CD is proportional to the light dose, $E_0=tI_0$ and C_0.

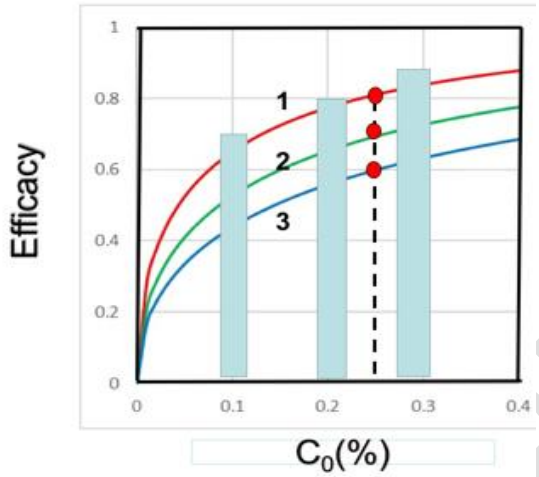

Fig. 3. Calculated efficacy versus RF concentration (C_0) at various light intensity I= (9,18,30) mW/cm^2, for curves (1,2,3); also shown are the measured data of O'Brart et al [29], shown by blue bars.

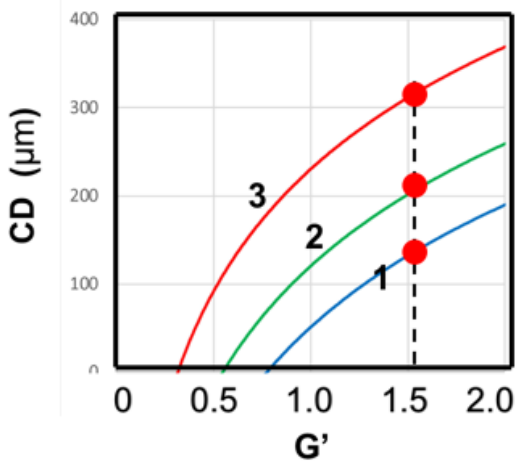

Fig. 4. Calculated crosslink depth (CD) versus G' for various light intensity, based on Eq. (4.b), for C_0= 0.25%, and various I_0=(3, 48, 60) mW/cm^2, for curves (1,2,3).

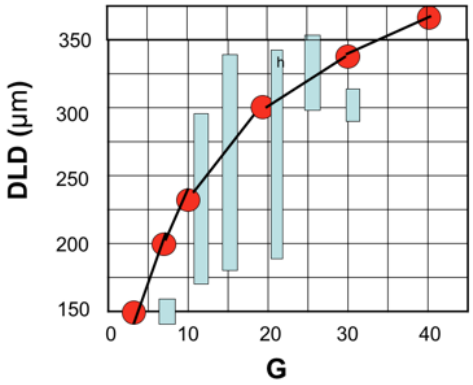

Fig. 5. Demarcation line depth (DLD) versus threshold ratio factor G, for intensity I= 10 mW/cm^2, where bars are measured data of Hafez et al [29], and red dots are calculated, based on Eq. (4.b)

3.2 The competing factors in epi-on and epi-off

A more realistic modeling was also developed by Lin et al [15] to include the effect of RF distribution, given by a distribution function F(z)=1–0.5z/D, or C(z,t=0)=C_0F(z), with a diffusion depth D in the stroma. We note that D is proportional to the waiting time of the pre-operation RF drops applying to the cornea. In this case CXL efficacy requires numerical simulation [15], with results shown in Fig. 6, in which the optimal efficacy is due to the competing effects of F(z), a decreasing function of z, and the steady-state efficacy is proportional to $[F(z)C_0/I_0]^{-0.5}\exp(0.5q'z)$, having an in creasing function of z, exp(0.5q'z). Mathematically this can be calculated by dG(z)/dz=0, to find the optimal z*, G(z)= F(z) exp(0.5q'z), and F(z)=1-0.5z/D.

The competing factors of F(z) and exp(q'Z_0) determines the relative efficacy of epi-on and epi-off. Given the example of (at a given z), F(z) of epi-on is about 0.65 of epi-off. On the other hand, the intensity reduction factor of epi-on, exp(-q'Z_0)=0.5, the net gain of epi-on efficacy is $[0.65/0.5]^{0.5}$=1.19, or about 20% higher than epi-off, for the same C_0 and I_0. However, if the epi-on RF reduction, F(z), (at a given z) is lower than the gain from reduced light intensity, exp(q'Z_0), we expect a lower efficacy of epi-on. The break-even point is when F(z) exp(q'Z_0)=1. A more rigorous analysis depends on the z-integral of F(z) , with z=0 to 300 μm, comparing to exp(q'Z_0)=1.16 to 1.63, for Z_0=70 to 100 μm. We also note that three factors influencing the role of RF concentration on the CXL efficacy: the RF depletion, dynamic of light intensity, and the non-uniform distribution of RF in the stroma. The above discussed features are for the steady-state efficacy. In contrast, in the transient state (with efficacy <0.6), the efficacy is proportional to the light dose, and therefore, epi-on is always less efficient than epi-off.

We note that most of the currently reported epi-on efficacy is lower than that of epi-off, consistent with our transient-state efficacy. The possible factors influencing the outcomes of the reported epi-on procedure include: poor diffusion, or F(z)<0.5, and lack of optimal resupply of RF drops during UV

irradiation. Strategies for improved efficacy and crosslink depth for epi-on CXL include: the use of higher RF concentration and lower light intensity (for a given light dose); enhancing the RF diffusion by an electrode device, or diffusion enhancing medicine.

The comparing efficacy features of steady-state and transient state are shown in Fig. 7, based on numerical simulations [18]. Greater details for the scaling laws for steady-state and transient state efficacy (for both type-I and type-II CXL) were reported by Lin et al [18, 26]. Clinical studies of Lang et al [5] showed consistent features as our theory that accelerated CXL (ACX) had less efficacy than standard low intensity for the same fluence (dose). Moreover, most of the measured epi-on efficacy is lower than that of epi-off, consistent with our transient state prediction, but in contrary to our predicted steady-state efficacy. Therefore, further clinical studies under controlled conditions are required in order to resolve the controversial issues. My group is currently conducting CXL surgeries at Shin Kong Wu Ho-Su Memorial Hospital. A recent review comparing epi-on and epi-off was reported [31]. However, there is no direct comparing of the CXL efficacy under the same protocols.

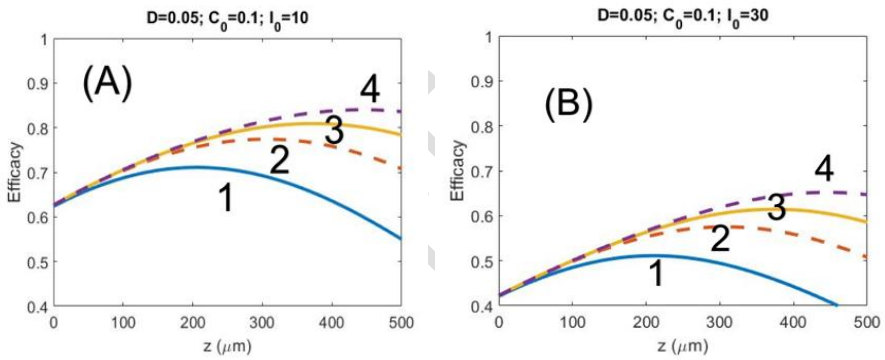

Fig. 6. Calculated CXL efficacy [15] versus corneal thickness (z) for diffusion depth D=500 um, C_0=0.1%, and for: (A) UV light intensity I_0=10 mW/cm^2 for various exposure time t=(3,5,7,10) sec, or dose of (0.03, 0.05, 0.07, 0.1) J/cm^2, shown by curves (1, 2, 3, 4), respectively; and (B) for I_0=30 mW/cm^2 with the same dose of (A).

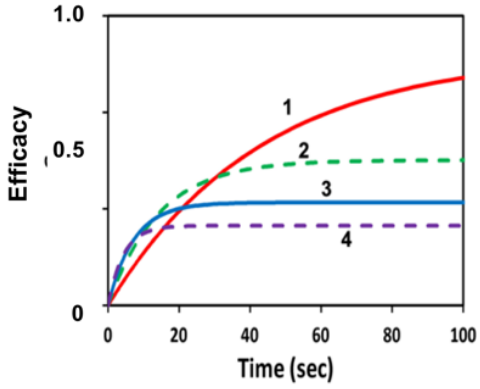

Fig. 7. The calculated efficacy [18] for light intensity I_0= (3,9,18,30) mW/cm^2 (curves 1,2,3,4), and C_0=0.1%, based on analytic formula Eq.(3).

4. *Conclusion*

Factors influencing the role of RF concentration on the CXL efficacy: the RF depletion effects, dynamic of light intensity, and the non-uniform distribution of RF in the stroma. The steady-state efficacy is proportional to $[F(z)C_0/I(z)]^{0.5.}$, and therefore epi-on is more efficient than epi-off, when the reduction factor F(z) >0.5. In contrast, in the transient state (with efficacy <0.6), the efficacy is proportional to the light dose, and therefore epi-on is always less efficient than epi-off. The CD has an inverse relation, such that higher light intensity and lower RF concentration lead to deeper CD. The analytic formulas of Eq. (3) and (4) need revisions based on numerical simulation.

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Conflicts of Interest

JT Lin is the CEO of Medical Photon, Inc.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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