Original research article

Photochemical Kinetic Modeling for Oxygen-enhanced UV-light-activated Corneal Collagen Crosslinking

ABSTRACT

Aims: To derive analytic formulas for the efficacy of type-II corneal collagen crosslinking (CXL) based on coupled macroscopic kinetic equations.

Study design: modeling and analysis of type-II CXL

Place and Duration of Study: New Vision Inc, Taipei, between Feb. 2017 and June 2017.

Methodology: Coupled macroscopic kinetic equations are derived under the quasi-steady state condition. The critical parameters influencing the efficacy of type –II CXL include: concentration and diffusion depth of the riboflavin, C(z,t), and the oxygen [O_2], the quantum yield, the UV light intensity (I_0), dose and irradiation duration. Second-order solutions of C(z,t) and [O_2] are derived to calculate the type-II efficacy proportional to the time integration of C(z,t) [O_2]/([O_2]+b). In the transient state with enough amount of oxygen, type-II process dominates over type-I During the CXL, the oxygen profile is a decreasing function of time, UV light intensity and the stroma depth, where strong oxygen depletion (for high intensity) results a lower type-II efficacy.

Conclusion: Oxygen is not required in type-I CXL, whereas it is a must element in type-II CXL which has an efficacy is a nonlinear increasing function of the UV light dose (or fluence tI_0), given by ln [1+ Bt], with B is proportional to C_0I_0 . Type-II efficacy has an optimal dose, whereas type-I steady state efficacy is a decreasing function I_0.

Keywords: corneal crosslinking, CXL, efficacy, type-II, oxygen, kinetic modeling, ultraviolet light, riboflavin, photodynamic therapy

1. INTRODUCTION

Photochemical kinetics of CXL and the biomechanical properties of corneal tissue after CXL are reported [1]. However, much less efforts have been invested in basic theoretical studies of photopolymerization [2-13], where Lin et al presented the first dynamic modeling for the safety of CXL [2,3]. The safety and efficacy issues of CXL have been reported theoretically [4-6]. The critical parameters influencing the efficacy of corneal collagen crosslinking (CXL) include: initial concentration and diffusion depth of the riboflavin (for type-I CXL) and the oxygen (for type-II CXL), the quantum yield, the UV light intensity, dose and irradiation duration.

It has been reported that oxygen concentration in the cornea is modulated by UV irradiance and temperature and quickly decreases at the beginning of UV light exposure [9,14]. The oxygen concentration tends to deplete within about 10-15 seconds for irradiance of 3

mW/cm^2 and within about 3-5 seconds for irradiance of 30 mW/cm^2 [9]. By using pulsed UV light of a specific duty cycle, frequency, and irradiance, input from both Type I and Type II photochemical kinetic mechanisms may be optimized to achieve the greatest amount of photochemical efficiency. The rate of reactions may either be increased or decreased by regulating one of the parameters such as the irradiance, the dose, the on/off duty cycle, riboflavin concentration, soak time, and others [1,9].

The prior works of Zhu et al [7,8], Schumacher et al [9,10], and Kling [13] assumed a constant UV light intensity and ignoring the RF depletion based on the conventional Beer-Lambert law, underestimated the UV light intensity in the stroma during the CXL. The prior work also assumed a flat RF concentration and ignored the absorption of the photolytic products. Our model will remove all the above described oversimplified assumptions for a much more realistic and accurate prediction of the key parameters influencing the CXL efficacy. A generalized, time-dependent Beer-Lambert law is employed to solve the dynamic UV light intensity [4,6]. The type-I efficacy (without oxygen) has been reported by Lin et al [4,6], this study will focus on the oxygen-enhanced type-II efficacy

2. MATERIAL AND METHODS

2.1 The Modeling System

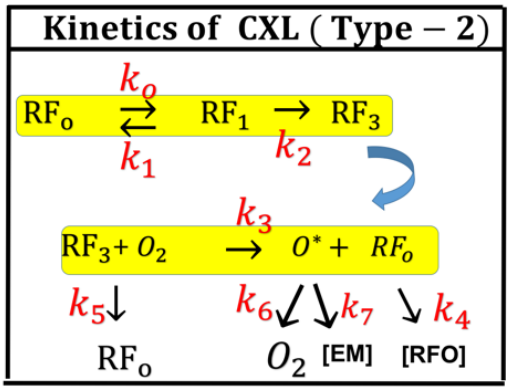

Fig. 1. The kinetics of type –II CXL. The ground state RF molecules [RF_0] is excited by the UV light to singlet excited state (RF_1), and then triplet excited state (RF_3). interacts with the ground oxygen (O_2) to form a reactive oxygen singlet (ROS), O*. The ROS could be relaxed to its ground state oxygen (O_2), or interacts with the extracellular matrix (EM) to form cross linking.

As shown in Fig. 1, the CXL type –II process is described as follows. The ground state RF molecules is excited by the UV light to its singlet excited state (RF_1), which could be relaxed to its ground state or to a triplet excited state (RF_3). In type-I process, (RF_3) further interact with the stroma collagen substrate for crosslinking. For type-II process, (RF_3) interacts with the ground oxygen (O_2) to form a reactive oxygen singlet (ROS), O*. The ROS could be

relaxed to its ground state oxygen (O_2), or interacts with the extracellular matrix (EM) to kill bacterial (to treat corneal ulcers) or to form cross linking.

The kinetic equations for the concentration of various components are shown by using short-hand notations: C(z,t) and C*(z,t) for the RF ground and singlet state [RF_0] and [RF_1]; X(z,t) and X*(z,t) for the ground state [O_2] and singlet oxygen [O^*_2], T(z,t) for the RF triplet state of [Rf3*], and [EM] for the available extracellular matrix (EM); given by [6-9]

$$\frac{\partial C(z,t)}{\partial t} = -k_0 C + k_1 C^* + k_3 XT - k_4 X^* C + k_5 T \quad (1.a)$$

$$\frac{\partial C^*(z,t)}{\partial t} = k_0 C - k_1 C^* - k_2 C^* \quad (1.b)$$

$$\frac{\partial T(z,t)}{\partial t} = k_2 C^* - k_3 XT - k_5 T \quad (1.c)$$

$$\frac{\partial X(z,t)}{\partial t} = -P_1 \quad (1.d)$$

$$\frac{\partial X^*(z,t)}{\partial t} = P_1 - k_4 X^* C - k_7 [EM] X^* \quad (1.e)$$

$$\frac{\partial [EM]}{\partial t} = -k_7 X^* [EM] \quad (1.f)$$

where

$$P_1 = a' I(z,t) k_3 XT - k_6 X^* \quad (1.g)$$

And the UV light intensity is given by

$$\frac{\partial I(z,t)}{\partial z} = -A(z,t) I(z,t) \quad (2.a)$$

$$A(z,t) = 2.3[(\varepsilon_1 - \varepsilon_2) X(z,t) + \varepsilon_2 X_0 F(z) + Q] \quad (2.b)$$

$a' = 83.6 p \varepsilon_1 \lambda$, with p being the type-I quantum yield and λ being the UV light wavelength.; ε_1 and ε_2 are the extinction coefficients of RF and the photolysis product, respectively; Q is the absorption coefficient of the stroma at the UV wavelength.

Time integration of the singlet oxygen concentration, or Eq. (1.f), efficacy of the type-II cross linking given by the time integration of the singlet oxygen concentration. The normalized efficacy of type-II cross linking defined by Ceff =1-[EM]/$[EM]_0$ = 1-exp(-S), with S-function given by [7,8]

$$S = k_7 \int_0^t X^* \, dt \quad (3)$$

In the above described CXL model, the UV light intensity in the corneal stroma is given by a time-dependent Beer-Lambert law [2,4,6]

$$I(z,t) = I_0 \exp\left[-\int_0^z A(z',t)dz'\right] \quad (4)$$

where the time-dependent extinction coefficient A(t) shows the dynamic feature of the UV light absorption due to the RF concentration depletion. Without the RF, A(t) becomes a constant given by the absorption coefficient of the corneal stroma tissue reported to be[27] A=2.3Q, with Q=13.9 (1/cm). With the RF in the stroma, the initial (at t=0) overall absorption has an extra absorption defined by the extinction coefficient and initial concentration of the RF, i.e., $A(z,t=0)=A_1=2.3\ (Q+\varepsilon_1 C_0)$, with the reported data [6,9] $\varepsilon_1 =$ 204 $(\%\cdot cm)^{-1}$. For t>0, A(t) is an increasing function due to the deletion of RF in time and defined by both the extinction coefficient of the RF(ε_1) and its photolysis product (ε_2), where ε_2 is not yet available for human, but was estimated to be about 50 to 120 $(\%\cdot cm)^{-1}$, based on measured data in RF solution under UV light irradiation[2,3]. The steady state light intensity is given by the steady state absorption of $A(z)=A_2=2.3\ (Q+\varepsilon_2 C_0)$. We have previously derived the effective intensity by its mean value suing $A(z,t) = 0.5\ (A_1 + A_2)$, or using a numerically fit $A(z,t)=2.3\ (Q+m\varepsilon_2 C_0)$, with fit parameter m=1.2 to 1.5 for ε_2 is 50 to 120 $(\%\cdot cm)^{-1}$. These methods provide us analytic formulas for the efficacy in type-I CXL [4,6]. Similar approaches maybe used for type-II CXL as follows.

The kinetic equations (1) and (2) may be numerically calculated to find the CXL efficacy, which however is too complex for us to analyze the roles of each of the parameters. For comprehensive modeling we will use the so-called quasi-steady state assumption described as follows. The life time of the singlet and triplet states of photosensitizer (C* and T) and the singlet oxygen (X*) are very short (ns to μs time scale) since they either decay or react with cellular matrix immediately after they are created. Thus, it is reasonable is to set the time dependences, dC*/dt=dT/dt=dX*/dt=0, or the quasi-steady-state conditions introduced by Zhu et al. [7,8] in a different medical system. These conditions lead to the macroscopic kinetic equation for the concentration of the ground state RF, C(z,t) and the ground state oxygen, X(z,t), as follows.

$$\frac{\partial C(z,t)}{\partial t} = -KI(z,t)[1+qH(z,t)]C(z,t) \quad (5.a)$$

$$\frac{\partial X}{\partial t} = -bqKI(z,t)H(z,t) \quad (5.b)$$

$$\frac{\partial I(z,t)}{\partial z} = -A(z,t)I(z,t) \quad (5.c)$$

$$H(z,t) = C(z,t)X(z,t)/[X(z,t)+k] \quad (5.d)$$

where $K = 83.6\varepsilon_1 \lambda p$; λ is the UV light wavelength; p and q are the type-I and type-II

quantum yield, respectively, given by p= $k_2/(k_1+k_2)$ and q=(k_3 k_4)/(k_6+k_7[EM]); k= k_5/k_3, b= k_7[EM]/(k_4 k_5) having a typical value 1000 to 1500. Eq. (3) has been generalized for the situation that both type-I and type-II CXL occur. It reduces to type-I only, when q=0, or dX/dt=0, or there is no oxygen supply in the process.

The initial concentration profiles (at t=0) of the RF and oxygen may be calculated or measured based on Fick's second law of diffusion [9,10,14]. For analytic solution, we have chosen the distribution profile given by [3,6]: F(z,D) = 1 – 0.5z/D for RF solution, or C(z,t=0)=C_0F(z), with a diffusion depth D in the stroma; and F'(D',z) =1 – 0.5z/D' for the oxygen, or X(z,0)= X_0F'(D',z), with a different diffusion depth D'. The typical diffusion depths are: D is 300 to 500 um and D' is 100 to 200 um.

The prior work of Zhu et al [7,8], Schumacher et al [9,10], and Kling [13] assumed a constant UV light intensity and ignoring the RF depletion, i.e., X(z,t) =X_0, is a constant in Eq. (2.b), based on the conventional Beer-Lambert law which overestimated the A(z,t) as its initial value when t>0. The prior work also assume a flat RF concentration, or F(z,t)=1 and used an oversimplified model to assume no absorption of the photolytic products, or ε_2 =0. Therefore, our model system based on Eq. (1) and (2), is much more realistic than the prior works using oversimplified assumptions.

2.2 Analytic Formulas

We will first derive the analytic formulas for the efficacy of type-II CXL (without the type-I), we approximate H(z,t)= C(1-k/X)/k_3, such that the first-order solution (with k/X<<1), of Eq. (5.b) is given by the solution of dX/dC=b/X, or

$$X(z,t) = X_0F'(z) - b\ln[C_0/C(z,t)] \qquad (6)$$

Using the first-order solution of C(z,t) in Eq. (6), we find the second-order solution of Eq. (5.a) and (5.b) for the oxygen, X(z,t), and RF concentration, C(z,t) given by

$$X(z,t) = X_0F'(z) - b\ln[1+Bt] \qquad (7.a)$$

$$C(z,t) = C_0F(z)/[1+Bt] \qquad (7.b)$$

$$B(z,t) = 1 - k'[1 - k'V(z,t)] \qquad (7.c)$$

$$V(z,t) = \ln[1+B't] \qquad (7.d)$$

with k'= k/X_0, B'(z,t)=(1-k')apqI'(z)C_0F(z). where we have used the mean intensity I'(z)=I_0exp(-A'z), with A' is the fit steady-state value of A (z,t), or its mean value, A'=0.5(A_1 + A_2) as defined earlier.

Therefore, the second-order quasi-steady state of the singlet oxygen, X*=(apq/k_4)I'(z)H(z,t), is approximated by the Eq. (7) and its time integration, from Eq. (3), gives the S function

$$S(z,t) = (k_7/k_4)b_1V(z,t)[1 - b_2V(z,t)] \qquad (8)$$

where b_1=1-k/X_0, b_2=0.5kb/[apqI'(z)X_0^2].

We note that S(z,t) has an optimal calculated by dS/dV=0, to obtain an optimal dose given by ln(1+B't)=1/b_2.

For more complex case that both type-I and type-II coexit in the process, analytic formulas are also available, when q/k_3 <<1, that is type-I is the dominant process. Using the similar approach in type-II only situation, we obtain the second-order solution

$$C(z,t) = C_0\, F(z)\exp\left[-B''(z)t\right] \quad (9.a)$$

$$B''(z) = KI'(z) + K'(q/t)C_0\, F(z)E' \quad (9.b)$$

$$E'(z,t) = 1 - \exp\left[-KI'(z)t\right] \quad (9.c)$$

where K'=(1-k')/k_3. For the special case when q=0, or only type-I rocess, Eq. (9.a) reduces to our previous formula. Uisng Eq. (9), we may solve for the oxgen concentration

$$X(z,t) = X_0 F'(z) - b\ln[(1+K'qC_0)/(1+K'qC(z,t)] \quad (10)$$

However, theer is no analytic solution for the overall efficacy when type-I and type-II coexist.

Comparing to the type-II S function in Eq. (8), the type-I efficacy Ceff (I)=1-exp(-S'), with S' function given by [4,6]

$$S'(z,t) = P(z,t)\sqrt{4KC_0F(z)/(aI'(z))} \quad (11.a)$$

$$P(z,t) = 1 - \exp[-0.5\alpha t I'(z)] \quad (11.b)$$

which is derived from the type-I rate equation of formation of polymers from the monomers, [M], given by [6,11]

$$\frac{\partial [M](z,t)}{\partial t} = -R(z,t)[M] \quad (12.a)$$

$$R(z,t) = \sqrt{a'KC(z,t)I(z,t)} \quad (12.b)$$

and S' is given by the time integration of R(z,t).

2. RESULTS

The following calculations are based on the numerical solutions of Eq. (5) with input parameters of: p=q=0.316, (or pq=0.1), b=1000, k=1.0; initial RF concentration C_0 =0.1, oxygen concentration X_0=10 mg/L. As shown by Fig. 1, the normalized oxygen profiles at z=0 and 100 um, for various UV light intensity of (3,5,10,30) mW/cm^2, and oxygen initial diffusion depth of D'= (100, 200) um. As predicted by Eq. (7.a), the oxygen concentration [O_2] is a decreasing function of time and depth (z), but it is an increasing function of the

oxygen concentration diffusion depth (D'). It is also a strong decreasing function of the UV light intensity, in consistent with the clinically measured data. [ ]. It should be noted that our modeling data has the similar trend as that of Kling [13], which, however, is not as accurate as ours due to their simplified assumption of constant RF concentration in the Beer-Lambert law, or A(z,t) in Eq. (2.b) is time-independent.

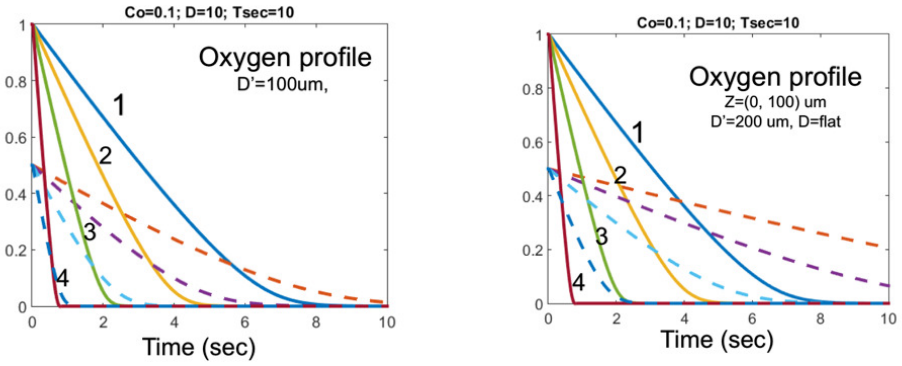

Fig. 2. The normalized oxygen profiles at z=0 (solid curves) and 100 um (dashed curves), UV light intensity of (3,5,10,30) mW/cm^2 (curves 1,2,3,4); and oxygen initial diffusion depth (D') of 100 um (left figure) and 200 um (right figure), for initial RF flat distribution (or F=1.0).

From Eq. (7) to Eq. (12), the key features of type-I and type-II are summarized and compared as follows:

(a) In both type-I and type-II, the RF concentration is depleted by the UV light dose, but they have different functional form, given by Eq. (6) and Eq. (9).

(b) In the transient stage with enough amount of oxygen, type-II process dominates over type-I, whereas type-I becomes the dominating process after oxygen is depleted and converted to the singlet oxygen. As shown by Eq. (8), the type-II efficacy is proportional to Bt, or the products of the quantum yield (pq), and the RF initial concentration, $C_0F(z)$.

(c) Both type-I and type-II efficacies are nonlinear increasing function of the UV light dose (or fluence) in the transient state.

(d) At steady-state, type-I efficacy is a decreasing function of UV light intensity and the corneal thickness, as shown by Eq. (11); whereas type-II has different dependence to the UV light dose, having an optimal dose with steady state efficacy being a decreasing function of light dose.

(e) The type-I efficacy is reduced by the type-II quantum yield (q) when type-II co-exits. Depletion of the RF concentration is much higher in type-I than type-II which as shown by Eq. (5.a) with is proportional to (pq) having a value about 0.1 to 0.2, whereas the depletion of type-I is proportional to *p* (about 0.3 to 0.5). The conventionally believed no depletion of RF in type-II process is not correct, specially when the type-II is mixed with

type-I.

(f) Oxygen is not required in type-I, whereas it is a must element in type-II, as shown by Eq. (5.b), (7.d) and Eq. (8), V(z)=0, when B(z)=0, or X_0=0.

(g) As shown by Fig. 2, the oxygen profile is a decreasing function of time, UV light intensity, and the stroma depth (z). Strong oxygen depletion (in high UV intensity) results a lower type-II efficacy.

4. CONCLUSION

We have present the analytic formulas for type-II CXL efficacy based on coupled macroscopic kinetic equations. In the transient stage with enough amount of oxygen, type-II process dominates over type-I. The oxygen profile is a decreasing function of time, UV light intensity, and the stroma depth (z). Strong oxygen depletion (in high UV intensity) results a lower type-II efficacy. Oxygen is not required in type-I, whereas it is a must element in type-II CXL which has an efficacy is a nonlinear increasing function of the UV light dose (or fluence tI_0), given by ln [1+ Bt], with B is proportional to C_0I_0 . Type-II efficacy has an optimal dose, whereas type-I steady state efficacy is a decreasing function I_0.

This article focuses on the analytic formulas and the features derived from them. Greater details of the roles of each of the parameters of [p,q,k,b,I, D.D'] on the type-II efficacy require numerical simulation of Eq. (5), which will be presented elesewhere. The formulas developed in this study provide guidance for further clinical studies. The features predicted in this study is based on a modeling system which may not represent a real system. Moreover, parameters used in the calculatuons would require further clnical measurement for more accurate values.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

REFERENCES

1. .Hafezi F and Randleman JB. editors. Corneal Collagen Cross-linking, second ed. Thorofare (NJ): SLACK; 2017.
2. Lin JT, Liu HW, Cheng DC. On the dynamic of UV-Light initiated corneal cross-linking. J. Med Biolog Eng. 2014;34:247-250; doi: 10.5405/jmbe.15332.
3. Lin JT, Cheng DC, Chang C, Yong Zhang. The new protocol and dynamic safety of UV-light activated corneal collagen cross-linking. Chinese J Optom Ophthalmol Vis Sci. 2015;17:140-147.

4. Lin JT. Combined analysis of safety and optimal efficacy in UV-light-activated corneal collagen crosslinking. Ophthalmology Research. 2016; 6(2):1-14.
5. Lin JT. Efficacy and Z* formula for minimum corneal thickness in UV-light crosslinking. Cornea. 2017 (in press).
6. Lin JT, Cheng DC. Modeling the efficacy profiles of UV-light activated corneal collagen crosslinking. PloS One. 2017;12:e0175002.
7. Zhu TC, Finlay JC, Zhou X, et al. Macroscopic Modeling of the singlet oxygen production during PDT. Proc SPIE. 2007; 6427:6427O81–6427O812.
8. Wang KKH, Finlay JC, Busch TM, et al. Explicit dosimetry for photodynamic therapy: macroscopic singlet oxygen modeling. Journal of Biophotonics. 2010; 3(5-6):304–318. [PubMed: 20222102].
9. Kamaev P, Friedman MD, Sherr E, Muller D. Cornea photochemical kinetics of corneal cross-linking with riboflavin. Vis. Sci. 2012;53:2360-2367.
10. Schumacher S, Mrochen M, Wernli J, Bueeler M, Seiler T. Optimization model for UV-riboflavin corneal cross-linking. Invest Opthamol Vis Sci. 2012;53:762-769.
11. Semchishen A, Mrochen A, Semchishen V. Model for optimization of the UV-A/Riboflavin strengthening (cross-linking) of the cornea: percolation threshold. Photochemistry and photobiology, 2015; 91:1403-1411.
12. Caruso C, Epstein RL, Ostaacolo C, et al. Customized cross-linking- A mathematical model. Cornea, 2017; 36:600-604.
13. Kling S, Hafezi F. An algorithm to predict the biomechanical stiffening effect in corneal cross-linking. J Refract Surg 2017; 32:128-136.
14. Kling S, Richoz O, Hammer A, et al. Increased biomechanical efficacy of corneal cross-linking in thin corneas due to higher oxygen availability. J Refract Surg. 2015;31:840–846.