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Kinetics of Enhancement for Corneal Cross-linking: Proposed Model for a Two-initiator System

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aims: To derive kinetic equations and analytic formulas for efficacy enhancement of corneal collagen crosslinking (CXL) in a 2-initiator system.
Study Design: Modeling the kinetics of CXL.
Place and Duration of Study: Taipei, Taiwan, between between January 2019 to June, 2019.
Methodology: Coupled rate equations are derived for two initiators system for a type-II process, consisting of a primary initiator (PA), and a co-initiator (PB) as an enhancer, having 3 cross linking pathways: Two radical-mediated (or electron transfer) pathways, and one oxygen-mediated (or energy transfer) pathway. For a type-II process, the triplet state T* interacts with the co-initiator, PB, to form the primary radicals R', and an active intermediates radical, R, which could interact with the substrate [M] for crosslink, or be inhibited by oxygen [O_2], or bimolecular termination. Rate equations, based on lifetime of triplet-state and oxygen singlet-state, are used to analyze the measured results in a rose-Bengal system with an enhanced initiator.
Results: Additive enhancer-monomer of arginine added to a rose Bengal photosensitizer may enhance the production of free radicals under a green-light CXL. D_2O may extends the lifetime of oxygen singlet state and thus improve the efficacy. Our formulas predicted features are consistent with the measured results.
Conclusion: Efficacy may be improved by enhancer-monomer or extended lifetime of photosensitizer triplet-state or oxygen singlet state.

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Keywords: Corneal crosslinking; corneal keratoconus; efficacy; kinetic modeling; oxygen; riboflavin; rose Bengal, ultraviolet light.

1. INTRODUCTION

Photochemical kinetics of corneal collagen crosslinking (CXL) has been extensively studied using UVA light and riboflavin (RF) as the photosensitizer [1-6]. Much less efforts have been reported using green-light and rose Bengal (RB) as the photosensitizer [7-9]. Comparison of animal studies of corneal biomechanical properties after *in vivo* and *ex vivo* CXL using rose-Bengal–green light (RGX) or riboflavin-UVA (UVX) was reported by Bekesi et al. [8] that the CXL anterior part was 100 and 143 to 188 um for RGX and UVX, respectively. We have previously considered a single-initiator system for the UVX, in which type-I is the predominant process [6,10,11]. In contrast, RGX is predominated by an oxygen-mediated type-II process, where the single-initiator radical induced type-I conversion is not very efficient [7]. Therefore, additive enhancer-initiator was proposed by Wertheimer et al. [7] for improved overall (type-I plus type-II) efficacy. For type-I predominant UVX, single-initiator has sufficient efficacy and there is no need to add another enhancer-monomer. A 2-initiator system offers an enhanced efficacy, however, it is limited by the available enhancers, and more difficult to control the precise concentrations and might take longer preoperative time for sufficient diffusion of the two initiators. In comparison, a single-initiator system is simpler and clinically easier to administrate its concentration.

This article will present a 2-initiator model system, for the first time, to analyze the measured results of Wertheimer et al. [7], where efficacy may be enhanced by an enhancer-initiator or increase the lifetime of photoinitiator (PA) triplet-state and oxygen singlet-state.

2. MATERIALS AND METHODS

As shown by Fig. 1, a two-initiator system is proposed: [M] for the corneal collagen substrate monomer. This system involves 3 crosslinking pathways: two radical-mediated (or electron transfer) pathways (1 and 2), and one oxygen-mediated (or energy transfer) pathway (3). The ground state photoinitiator PA is excited to its first-excited state (PS*) and triplet excited state T* by a quantum yield (q). In a type-I process, [T] interacts directly with [M] for a crosslink. For a type-II process, T* interacts with the co-initiator, PB, to form the primary radicals R', then produces the reactive intermediates radical, R, which could interact with [M] for crosslink, or be inhibited by oxygen [O_2], or bimolecular termination. For a type-II (or oxygen-mediated) process, T* interacts with [O_2] to form a singlet oxygen [O_1] which could interact with [M] for crosslink, or relaxed to [O_2].

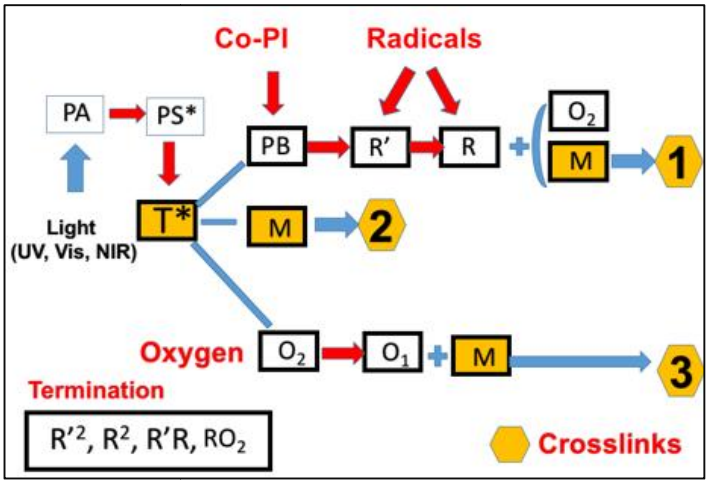

Fig. 1. Schematics of 3 photochemical pathways in a two-initiator system, PA and PB, in the presence of oxygen O_2, for radical-mediated pathways (1 and 2), and oxygen-mediated pathway (3)

2.1 Photochemical Kinetics

The kinetic equations for a previous 1-initiator system [6] are revised for a 2-initiator system as follows; where short-hand notations for the concentrations are used: C_1 and C_2 for initiator PA and PB ground state, T for PA triplet state, $[O_2]$ and X for oxygen ground and singlet state; R' and R the radicals.

$$\frac{\partial C_1}{\partial t} = -bI(z,t)\,C_1 + (k_5 + k_3[O_2])T \quad (1.a)$$

$$\frac{\partial C_2}{\partial t} = -k_{72}TC_2 \quad (1.b)$$

$$\frac{\partial T}{\partial t} = bI(z,t)C_1 - (k_5 + k_3[O_2] + k_{71}[M] + k_{72}C_2)T \quad (1.c)$$

$$\frac{\partial R'}{\partial t} = k_{72}TC_2 - k_{12}RR' - 2k_{t1}R'^2 \quad (1.d)$$

$$\frac{\partial R}{\partial t} = 2k_{t1}R'^2 - 2k_{t1}R^2 - (k_{12}R' + k_9[O_2] + k'[M])R \quad (1.e)$$

$$\frac{\partial X}{\partial t} = k_3[O_2]T - (k_6 + k_{81}C_1 + k_{82}C_2)X \quad (1.f)$$

$$\frac{\partial [O_2]}{\partial t} = P - (k_3T + k_9R)[O_2] + k_8X \quad (1.g)$$

$$\frac{\partial [M]}{\partial t} = -(k_{71}[T] + k_8X + k'R)[M] \quad (1.h)$$

where b=83.6a'wq, with w being the UV light wavelength (in cm) and *q* is the triplet state [T] quantum yield, a' in (1/mM/%) and I(z,t) in mW/cm^2, b=0.62q, for UV light at 365 nm (with a'=204 (1/mM/%). For RGX with green light, the b value is approximately 4 time larger and having a smaller penetration depth of 100 um [8]. Eq. (1.g) also includes an oxygen source term given by $P=(1-X/X_0)\,P_0$, with a maximum rate constant P_0. All the reaction rate constants are defined by the associated coupling terms. For examples, in Eq. (1.b), k_{72} is for the reaction of PB and T, which has a ground state relaxation rate k_5; in Eq. (1.c), k_{41} is for the reaction of [M] and R, which is coupled with R' by k_{12} and a bimolecular termination rate of k_{t1}; In Eq. (1.f), k' is for the reaction of [M] and R, which is coupled with oxygen by k_9 and a bimolecular termination rate of k_{t1}.

The dynamic light intensity is given by [3,4]

$$\frac{\partial I(z,t)}{\partial z} = -A'(z,t)I(z,t) \quad (2.a)$$

$$A'(z,t) = 2.3[(a' - b')C(z,t) + b'C_0F' + Q'] \quad (2.b)$$

a' and b' are the extinction coefficients of PA and PB and the photolysis product, respectively; Q' is the absorption coefficient of the stroma at UV (365 nm) or green (532 nm) wavelength.

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 [2,3] described as follows. The life time of triplet states of PS, the radicals (R and R'), 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, one may set the time dependences, dT/dt=dX*/dt=dR/dt=dR'/dt=dS/dt=dS'/dt=0, or the so called quasi-steady-state conditions; defining $k_{9=}k$, $k_{81}= k_{82} =k_8$, $k_{71}= k_{72} =k_7$, $k_4 = k_5 =k'$, $k_{t1}= k_{t2} =k_T$; $k_{37}= k_3/k_7$, $k_{18} = k_8/k_{11}$, $k_{57}= k_5/k_7$, $k_{61}= k_6/k_{11}$, $T=bIgC_1$, $X=bIg'[O_2]K_{12}$, $g=1/(k_{37}[O_2]+[M]+C_2+k_{57})$, $g'=k_{37}g/(k_{61}+ k_{81}C_1+ k_{81}C_2)$, Eq. (2) becomes [4,5]

$$\frac{\partial C_1}{\partial t} = -([M] + kC_2)gB_1C_1 \quad (3.a)$$

$$\frac{\partial C_2}{\partial t} = -g'B_1C_2 \quad (3.b)$$

$$\frac{\partial [O_2]}{\partial t} = -(bIgK_{22}C_1 + k_9R)[O_2] + P \quad (3.c)$$

$$\frac{\partial [M]}{\partial t} = -R_T[M] = -[bIC_1(g + g'[O_2]) + k'R]\,[M] \quad (3.d)$$

The radicals, R' and R, are given by the solution of the following steady-state of Eq. (1.c) and (1.d): For $k_{12}R'<<2k_TR'$, $k_{22}S'<<2k_TS'$ Eq. (1.c) and (1.d) give steady-state $R=[bIgC[A]/k_T]^{0.5}$, and $S=[bIgC[B]/k_T]^{0.5}$. Using these R and S, we may find the steady-state solution of Eq. (1.d) and (1.e) given by

$$bIgC_2 - k_{12}RR' - 2k_TR'^2 = 0 \quad (4.a)$$

$$2k_TR'^2 - 2k_TR^2 - (k_{12}R' + k_9[O_2] + k'[M])R = 0 \quad (4.b)$$

Eq. (4.a) has solution given by, for $2k_TR'>>k_{12}R$, $R' =[0.5bIgC_2/k_T]^{0.5}$; which gives solution of Eq. (4.b)

$$R = \left(\frac{1}{4k_T}\right)[-G + \sqrt{G^2 + 8k_TbIgC_2} \quad (4.c)$$

where where $G=k_{12}R'+k_9[O_2]+k'[M]$. For $2bIgC_2 >> G^2$, we obtain an approximate

$$R = \frac{0.5bIgC_2}{\sqrt{2k_T}} - G \quad (4.d)$$

2.2 Conversion Efficacy

The conversion efficacy of monomer [A] is defined by $C_A=1- [A]/[A]_0 = 1- \exp(-S)$, where the S function is given by the time integral of the total rate function, R_T.

Using the total rate function given by Eq. (3.c) and R given by Eq. (4.c), we obtain

$$R_T = bI(gC_1 + K_{13}C_2 + g'C_1[O_2]) - k'G \quad (5)$$

where $K_{13}=0.5/k_T^{0.5}$, We note that R_T consists of four parts: the coupling of [M] with the PA triplet-state (the first term $bIgC_1$), with the radical (K_{13} term via co-initiator, PB), with the singlet oxygen (K_{12} term), has an inhibition term given by k'G.

To discuss the role of the lifetimes of the triplet-state (T_1) and singlet-oxygen (T_2), Eq. (3.c) may be expressed by as follows:

$$\frac{\partial[M]}{\partial t} = -bI(k_7T_1)(C_1+K_{13}C_2 + KT_2C_1[O_2])\,[M] - k'G[M] \quad (6)$$

where $g= k_7T_1$, $K=k_8/k_7$), $g=1/(k_{37}[O_2]+[M]+C_2 +k_{57})$, and

$$T_1 = 1/(k_5 + k_3[O_2] + k_{71}[M] + k_{72}C_2]) \quad (7.a)$$

$$T_2 = 1/(k_6 + k_{81}C_1 + k_{82}C_2) \quad (7.b)$$

3. RESULTS AND DISCUSSION

Numerical results of Eq. (8) will be presented elsewhere, the following important features are available just based on Eq. (8) and (9) without solving them numerically.

Analysis of UVX and RGX

Eq. (5) defines the conversion rate (R_T) of monomers, which has three parts contributed by the the coupling of [A] or [B] with the triplet-state ([T]), the singlet oxygen [O-], and the radicals R' and S' (the K_{13} term). As shown by Eq. (5.e) and (6), R_T proportional to the lifetimes T_1 (for triplet state), and T_2 (for singlet-oxygen), oxygen concentration, $[O_2]$, and the rate constants (kj). Base on Eq. (6) and (7), we are able to analyze the measured results of Wertheimer et al. [7] as follows:

(a) For UVX system, with $k'R>>bIgC_1$, $bIg'C_1[O_2]$, as proposed by Kamaev et al. [8] Semchishen et al. [9] and Lin [4,5] that CXL is predominated by type-I, which does not require oxygen, in contrast to Kling et al. [12] proposing type-II oxygen=mediated system. The type-I conversion efficacy, given by $C_A=1-\exp(-S)$, with S is he time integral of R_T, and has a state-state value proportional to the $[C_1/(bI)]^{0.5}$, for a bimolecular termination process [5]. In this UVX system, the oxygen inhibition effect reduces the lifetime, as shown by Eq. (6) with $T_1=g/k_7$, which has a reduction factor ($k_{37}[O_2]+[M]+C_2 +k_{57}$), and thus reduces the conversion efficacy. The oxygen inhibition effect also defines a so called "induction time" for the efficacy function, when oxygen is totally depleted, and thus type-I efficacy starts to increase faster.

(b) In contrast to UVX, RGX is predominated by an oxygen-mediated type-II process, where the single-monomer radical (R') induced type-I conversion is not very efficient. Therefore, additive enhanced co-initiator (PB) was proposed by Wertheimer et al. [7] for improved efficacy.

(c) For RGX with an additive enhancer (arginine), the conversion could be enhanced due to the extra radical term in Eq. (6), $K_{13}C_2$, with in which the total rate function given by Eq. (5.e) has an enhance factor of $k'R+K_{13}C_2/(gC_1)$; which is proportional to the initial concentration ratio $[C_{20}]/C_{10}$ and the PB-induced radical (R). Therefore, in the absence of oxygen and without the co-initiator, PB, (or $C_2=0$), the conversion is contributed from $k_{71}T_1$, which might not sufficient for effective crosslinking, which is enhanced by the and its radical R. These features are measured by Wertheimer et al [7] that in an O_2-free environment, arginine was required for an increase in tensile strength.

(d) In the presence of deuterium oxide (D_2O), which extends the lifetime of singlet oxygen, i.e., a smaller relaxation rate (k_6), thus a larger T_2, based on our Eq. (7.b). In contrast, sodium azide which quenches singlet oxygen and other reactive radicals (R' and R), i.e., increasing of k_5 and k_6, thus shortening T_1 and T_2, and partially inhibited RB photobleaching, shown by Eq. (6) with a reduced conversion as measured by

Wertheimer et al. [7]. This feature is also predicted by our quenching factors shown in Eq. (2.b), Q, which reduce the lifetimes.

(e) As measured by Wertheimer et al. [7], the increase in stiffness of corneas irradiated in air was not enhanced by arginine at low dose of 100 J/cm^2. This may be analyzed by our formula that the arginine-enhancement factor is proportional to $E_F=(btE_0k_{72}C_{20}/C_{10})$, with E_0 being the light dose, thus there is a threshold light dose (or intensity x time) and threshold arginine initial concentration C_{20} to achieve stiffness increase in cornea.

(f) Higher dose (E_0) is needed for a deeper crosslink depth. Thus, for a given depth, the enhanced dose of arginine will reduce the required light dose (E_0). Our formula predicts the measured feature of Wertheimer et al. that RB staining solution contained arginine substantial photo-bleaching occurred at much lower fluence than in incisions without arginine. It also predicts that there is a threshold light dose and enhancement-threshold initial concentration of arginine.

(g) For RGX, type-II is predominant, as proposed by Wertheimer et al. [7], whereas type-I is predominant in UVX [4,5,10,11]. This controversial issue of the role of oxygen in RF-system was explained by Wertheimer et al. [7] that O_2 diffused deeper and crosslinking occurs at a greater depth in the tissue, after the crosslinkable sites was reacted. However, our formulas support the kinetic proposed by Kamaev et al. [10] and Semchishen et al. [11] that type-I and type-II co-exist, initially, and then predominated by type-I after oxygen is depleted.

Greater details of the roles of oxygen in CXL [12-16], anticancer [17-18] and 3D bioprinting [19-20] have been reported. Numerical results of this study to investigate the role of oxygen, comparing to the clinical data of Wertheimer et al [7] will be published elsewhere.

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4. CONCLUSION

Efficacy may be improved by additive enhancer-monomer in a 2-monomer system or extended lifetime of photosensitizer triplet-state or oxygen singlet. Our theory predicts the measured results.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

The author is the CEO of New Vision Inc. and has financial interest.

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