

Letters

Analytic Formulas on Factors Determining the Safety and Efficacy in UV-Light-Sensitized Corneal Cross-Linking

In a recent article by Laggner et al.,¹ they evaluated the applicability of second harmonic generation (SHG) imaging in the assessment of morphological and functional changes after riboflavin (RF) and ultraviolet (UV) irradiation-sensitized human corneal collagen cross-linking (CXL). To increase the efficacy of CXL through better penetration of RF into the corneal stroma, delivery of RF with iontophoresis was also proposed by Arboleda et al.²

While a large amount of clinical evidence demonstrates the safety and efficacy of CXL,³ there is no analytic formula available for predicting or analyzing the clinical outcomes. This letter will provide formulas to analyze certain important aspects of the results shown by Laggner et al.,¹ such as the roles of RF distribution profiles and the increased biochemical strength by higher RF doses.

It was known that the efficacy of the CXL process is related to the photoinitiation rate⁴ of RF defined by the product of the UV light intensity (I) and the RF concentration (C), $R(z,t) = 2a\phi I(z,t)C(z,t)$, where $z=0$ defines the corneal surface and t is the UV light exposure time; a is the extinction coefficients of RF at the UVA wavelength (approximately 370 nm), and ϕ is the quantum yield. As shown in the Figure, the numerical solution of the normalized RF concentration profile at various exposure time t for an initial RF concentration profile given by $C(z,t=0) = C_0F(z)$, $F(z) = 1 - 0.5z/D$, with D being the penetration depth of RF inside the corneal stroma, or the RF dosage defined by Laggner et al.¹ It should be mentioned that $t=0$ is defined as the time right before the UV light exposure and after approximately 20 minutes of RF diffusion time into the stroma.

The Figure shows that depletion of RF starts from the surface layer (at $z=0$) and gradually goes into the stroma volume. It should be noted that the central regime of 150 to 250 μm is the last portion cross-linked; that is, it absorbs more energy dose than other portions and is the major CXL regime, consistent with the measured feature of Laggner et al.¹ The efficacy of CXL may be also defined by a crosslinking time (T^*) in which RF concentration at a given stroma depth (z) is reduced to $C(z,t=T^*) = C_0F(z)\exp(-M)$. Our numerical simulation fit to an analytic formula: $T^* = (T_0/S)\exp(0.9Az)$, where $S = 0.5[1 + \exp(A - B)z]$; $A = 32 + 2.3bC_0(1 - 0.25z^2/D)$, $B = 32 + 2.3aC_0(1 - 0.25z^2/D)$, with a and b the extinction coefficients of the RF (initiator) and the photolysis product, respectively. The surface cross-linking time $T_0 = T^*(z=0) = 16.1M/(\phi I_0)$, with I_0 in mW/cm^2 , which shows the inverse proportional of T_0 and I_0 , or the basis of so-called accelerated CXL using high light intensity. For example, to deplete the surface layer of RF to 0.0182 of its initial value (or when $M=4$), $T_0 = (64.4, 21.5)$ seconds for $I_0 = (10, 30) \text{ mW}/\text{cm}^2$ and $\phi = 0.1$.

The Figure, for the case of $I_0 = 30 \text{ mW}/\text{cm}^2$, shows that it takes approximately 10 and 20 seconds to deplete the surface layer (at $z=0$) to a level of 0.135 and 0.0182, respectively, of the RF initial concentration. However, it takes a much longer time to deplete the volume depth (with $z > 0$) of the stroma, given by an exponential factor $\exp(0.9Az)$. For example, for the case of $I_0 = 30 \text{ mW}/\text{cm}^2$, the volume cross-linking times are $T^* = (25, 65)$ seconds, for $z = (200, 300) \mu\text{m}$, with $C_0 = 0.1\%$ and $D = 200 \mu\text{m}$, increasing to $T^* = (34, 78)$ seconds for a larger $D = 300 \mu\text{m}$. For lower light intensity of $I_0 = 10 \text{ mW}/\text{cm}^2$, it takes approximately 2 to 4 minutes to cross-link a corneal depth of 200 to 300 μm . The above calculated examples well predict the parameters currently used in most clinical protocols.

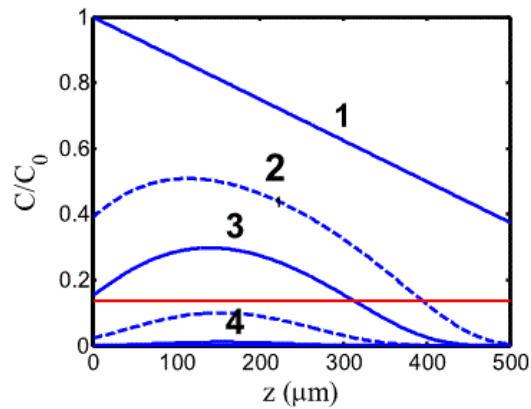


FIGURE. The normalized RF concentration profile at exposure time $t = (0, 5, 10, 20)$ seconds (for curves 1, 2, 3, 4) for an initial RF concentration $C_0 = 0.1\%$ and UV light intensity $I_0 = 30 \text{ mW}/\text{cm}^2$, where red indicates RF depletion level of 0.135.

The efficacy may be also related to the total energy dose during the exposure time of T^* , defined by $E = T^*I_0$, which is an exponential increasing function of zC_0 and the penetration depth (D). Therefore, the factors determining the efficacy of CXL include the initial concentration and profile of RF inside the corneal stroma and the intensity and exposure time of the UV irradiation. The above described features are consistent with the clinical outcome of Laggner et al.¹

My formulas show that high CXL efficacy may be achieved by larger intensity, longer UV exposure time, or higher concentration dose. However, CXL must be performed within the safety dose defined by $E^* = (E'/S)\exp(0.9Az)$, where E' is the cytotoxic energy threshold of endothelial cells, reported to be 0.32 and 0.65 J/cm^2 , for rabbit and human corneas.⁵ Therefore, the safety condition is given by $M < 4$ for $\phi = 0.1$ in human corneas. For a typical penetration depth $D = 300 \mu\text{m}$ and corneal thickness $z = (350, 400, 450) \mu\text{m}$, the safety dose $E^* = (3.8, 4.7, 5.6) \text{ J}/\text{cm}^2$ for $C = 0.1\%$, which increases to $E^* = (7.4, 9.3, 11.5) \text{ J}/\text{cm}^2$ for a higher $C = 0.2\%$. Finally, it should be noted that the commonly accepted criteria of corneal thickness larger than 400 μm under a fixed dose of $5.4 \text{ J}/\text{cm}^2$ is just one of my safety conditions, and requires high RF concentration ($C > 0.1\%$) and large penetration depth $D > 300 \mu\text{m}$. Greater detail will be shown elsewhere.⁵

To conclude, I have presented analytic formulas showing the roles of RF concentration (C) and its penetration depth (D), UV light intensity (I) and energy (or dose, E) for the efficacy and safety of CXL. The formulas, presented for the first time, may be used as guidance in predicting the clinical outcomes of CXL.

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Letters

Author Response: Analytic Formulas on Factors Determining the Safety and Efficacy in UV-Light-Sensitized Corneal Cross-Linking

We thank Jui-Teng Lin¹ for his interest in our recent work studying riboflavin (RF)-UVA-assisted corneal collagen cross-linking (CXL).² His comments on our data with a mathematic equation bring new perspectives to the corneal CXL biology.

Since RF-UVA CXL emerged as a medical alternative to treat corneal ectatic disorders, efficacy and safety have been the central topics for laboratory and clinical research.^{3,4} In photo-assisted CXL, the efficacy depends on both the RF tissue dose and the irradiation of UVA. How the depth-resolved profiles of RF and UVA affect CXL efficacy remains unclear. In our recent work,² we profiled the tissue distribution of various RF doses by confocal fluorescence microscopy. The corneal absorption of RF was positively related to the administered RF dosage. Interestingly, the saturation zone (RF penetration depth) was found to be localized in a stromal depth from 150 to 300 μm . Consistent with our findings, Mastropasqua et al.⁵ used HPLC to demonstrate that 80% of RF was absorbed by the first 300 μm corneal stroma. In parallel, an increase in UVA irradiation dose leads to a higher CXL efficacy, as demonstrated by Chai et al.⁶ Lin's mathematical formula, $R(z,t) = 2a\phi I(z,t)C(z,t)$, successfully models such RF-UVA-mediated CXL photokinetics.¹ The efficacy of CXL indicated by Lin's "normalized RF concentration" profile was positively correlated to UVA dosage.

Using second harmonic generation (SHG) microscopy, we further reported that the most effective zone of RF-UVA CXL was confined to 150 to 250 μm irrespective of RF dosage.² In comparison, Chai et al.⁶ indicated that the effective CXL region was restricted to the anterior 300 μm and not correlated to the UVA irradiation dosage. Such a depth threshold of CXL is well explained by Lin's formula. Analytically, he shows that the dose effect of UVA irradiation for CXL mainly takes place in a stromal depth of 150 to 250 μm .

Clinically, the safety range between therapeutic and phototoxic UVA irradiation doses is largely reduced when CXL treatment is applied to thin corneas with a limited UVA penetration depth. As commonly accepted, the safety threshold for corneal thickness in CXL therapy is set to be 400 μm . The effective CXL zone up to 300 μm , collectively supported by our

SHG image data, Mastropasqua's HPLC results, and Lin's mathematical model, justifies such a case selection criterion. Future laboratory studies will merit the clinical ramification of Lin's mathematical CXL model.

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