A Proposed Concentration-Controlled New Protocol for Optimal Corneal Crosslinking Efficacy in the Anterior Stroma

The recent article of Bekesi et al.1 made two important conclusions about corneal collagen crosslinking (CXL): Firstly, biomechanical properties determined after ex vivo CXL may not provide entirely accurate information about the responses to CXL in vivo; secondly, within the crosslinked regions, rose bengal crosslinking (RGX) stiffened the cornea to a greater degree than ultraviolet-A (UVA) crosslinking (UXV) at 1 and 2 months, suggesting a greater density of crosslinks produced by RGX. The intent of this letter is to provide mathematical formulas to analyze and comment on the above clinically measured features and also propose a new protocol called the riboflavin concentration-controlled method (CCM), which, as theoretically demonstrated, is more efficient than the conventional Dresden protocol (using a saturated concentration) or other noncontrolled concentration methods based on the Bunsen-Roscoe reciprocal law.

For type I, epi-off CXL, the efficacy is given by2-3 \[ \text{Ceff} = 1 - \exp(-S) \] with \( S \) being the crosslinking rate function given by \[ S = \sqrt{\frac{4kFCo}{aXl0}} \left[ 1 - \exp\left(-\frac{bI}{I_0}\right) \right] \], where \( k \) is an effective rate constant; \( X = \exp(-Az) \); \( z \) is the stromal thickness; \( A \) is an effective absorption coefficient given by \( A = 2.5 \text{ma}_c C_0 (1 - 0.25z/D) + Q \); \( b = 0.5a_0(I_0) \); \( a = 0.622p \); \( p \) being the quantum yield and \( m = 1.5 \) a fit parameter; \( a_0 \) and \( Q \) are the absorption coefficient of the photolysis and the stroma, respectively. \( C_0 \) is the initial (at \( t = 0 \)) riboflavin (RF) concentration having a mean value \( C_0 = 0.1\% \). \( D \), which has a nonlinear scaling law \( S^m \) is proportional to \( (E_0/I_0)^{0.5} \), or \( \exp[(tI_0)^{0.5}] \), in contrast to the Bunsen–Roscoe law (BRL) that \( S \) is proportional to \( E_0 \) such that the irradiation time in accelerated CXL is given by a reciprocal law \( t = E_0/I_0 \). Our \( S \)-formula shows that, for the same dose, high UV powers (9–40 mW) deplete the RF faster and reach a lower steady-state efficacy and shallower crosslink depth than the conventional lower power (1.5–3 mW). For a given efficacy threshold (\( S' \)), the \( S \)-formula may be used to calculate the maximum (or cutoff) intensity \( I^* = 1.7kFCo/(aXl0^2) \), which is approximately 45 to 60 mW/cm\(^2\), and the associated minimum crosslink time \( (t^* = E_0/I^*) \), approximately 2 to 5 minutes, which provide the valid range of BRL and have been demonstrated by the clinical data of Wernli et al.4

For example, for \( C_0 = 0.1\% \), \( a_2 = 50 (1/(cm\%)) \), \( k = 0.39(1/s) \), and quantum yield \( p = 0.5 \), we obtain steady-state \( S' = 14.1/F(Xl0)^{0.5} \) and the crosslink depth \( (z^*) \) is approximately 200 to 500 \( \mu \)m, depending on the UV light dose range of 1.0 to 3.0 J/cm\(^2\). The measured data of RGX (ex vivo) by Bekesi et al.1 with \( z^* = 100 \mu m \) are much smaller than UXV. However, the protocols and mechanisms in RGX (dominated by a type II CXL) and in UXV (dominated by a type I CXL) are different and cannot be fairly compared. Our \( S \)-formula shows the CXL strength (or density) in type I is proportional to \( \sqrt{4kFCo/(aXl0)} \), showing that the anterior stroma always has lower efficacy than the posterior, whereas in type II CXL strength is proportional to \( 3/2 \frac{4kFCo}{aXl0}\) which has a reverse dependence on \( z \), because \( X = \exp(-Az) \). However, both are decreasing function of the light intensity \( I_0 \) and increasing function of the diffusion depth \( D \), suggesting a greater density (strength) of crosslinks produced by RGX than UXV, as indicated clinically by Bekesi et al.1 Furthermore, the CXL efficacy may be strongly influenced by the frequency of RF instilled during the UV exposure (defined as \( F_{drop} \)) to be detailed as follows. The \( F_{drop} \) in RGX and UXV are different in the study of Bekesi et al.1

To overcome the drawback of low efficacy in accelerated CXL as predicted by the \( S \)-formula, a RF CCM is proposed as follows. In the conventional Dresden protocol, extra RF drops were instilled during the UV exposure (with a frequency \( F_{drop} = 5-10 \)), which reduced the effective dose from 5.4 J/cm\(^2\) to approximately 4.0 J/cm\(^2\), based on our calculations.2-3 For an optimal protocol (for fast and efficient CXL in the anterior stroma), I propose \( F_{drop} = 1 \) to 4 to compensate the fast RF depletion in the anterior stroma, especially in high intensity (>18 mW/cm\(^2\)). In contrast to the conventional Dresden protocol, which keeps the RF in a saturated condition during the UV exposure, CCM proposes to turn off the UV light after each of the extra RF drops applied to the stroma and waiting for a period approximately 1.0 to 2.0 minutes to allow enough RF diffusion (with a depth diffusion \( D > 150 \mu m \)) before it is turned on again. In the above proposed CCM, my theory predicts comparable efficacy (for the same dose) for intensity of 1.5 to 45 mW/cm\(^2\), based on a combined efficacy formula defined as \( c-Ceff = 1 - \exp\left[-(S1 + S2 + \ldots + Sj)\right] \), with \( j = F_{drop} \), and \( F_{drop} \) is given by the integer portion of square root of \( \lfloor I_0/z \rfloor \), that is, \( F_{drop} = (1, 1, 2, 3, 3, 4) \), for \( I_0 = (1.5, 3, 9, 18, 30, 45) \) mW/cm\(^2\) and exposure time \( t = (30, 30, 10, 5, 3, 2) \) minutes. The above CCM proposes that higher intensity requires larger \( F_{drop} \) (or more RF resupply) to compensate the faster bleaching effect in the anterior stroma (100–250 \( \mu m \)), which is re-treated by \( F_{drop} \) times, and the waiting period (with UV off) after each RF drops secures enough diffusing depth (\( D > 150 \mu m \)). Numerical simulation of \( c-Ceff \) (to be shown elsewhere) under the new CCM protocol shows a stronger correlation with the measured data of Wernli et al.3 than the simple protocol (with \( F_{drop} = 0 \) or Dresden protocol (with \( F_{drop} > 5 \)). The Figure shows an example of \( c-Ceff \) for various

**FIGURE.** The combined CXL efficacy shows \( c-Ceff > 80\% \) within the anterior stroma (0–200 \( \mu m \)) under the concentration-controlled protocol for UV intensity \( I_0 = (3, 9, 18, 30, 45) \) mW/cm\(^2\) (for curves from **top to low**). Also shown is the low efficacy in high intensity (>18 mW/cm\(^2\)) in posterior stroma (>200 \( \mu m \)).
UV intensities of 3 to 45 mW/cm², with $F_{\text{drop}} = (1, 2, 3, 4)$, where all cases have efficacy above the threshold value 80% within the anterior stroma crosslinked depth range of 0 to 200 µm. In comparison, curves in the range 400 to 500 µm are associated with the situation of $F_{\text{drop}} = 0$, which shows low efficacy $< 80\%$ for high intensity with $I_0 > 18$ mW/cm², where diffusion depth $D = 500, 200, 150, \text{and } 150$ µm were used for $F_{\text{drop}} = 1, 2, 3, \text{and } 4$. The cutoff (or maximum) intensity with $c$-$C_{\text{eff}} < 80\%$ (within 0–200 µm stroma) predicted by the $S$-formula is approximately 45 to 55 mW/cm², consistent with the clinical data of Wernli et al.¹ The above formulas also demonstrate that not only crosslink depth ($z^* > 150$ µm) but also crosslink strength ($S^* > 1.6$, or $c$-$C_{\text{eff}} > 0.8$) is required in order to achieve high crosslinked stroma volume which is proportional to $(z^*S^*)$, as also suggested by Bekesi et al.¹

To conclude, the theoretically proposed CCM using an accelerated CXL while keeping efficacy similar to that of the conventional CXL requires much more basic clinical study to validate the multiple factors influencing the CXL efficacy, including $C_0, F, I_0, D$, and $F_{\text{drop}}$, and the associated cutoff maximum intensity ($I^*$) and minimum exposure time ($T^*$), as shown by the $S$- and $z^*$ formulas for type I CXL. Greater details of photochemical kinetics of type II CXL and its influencing factors of efficacy, such as oxygen environment and the generation of reactive oxygen species (ROS), were shown elsewhere.² Many debating issues about the CXL efficacy, such as pulsing versus continuous wave operation, accelerated versus conventional CXL, the minimum corneal thickness, and the role of oxygen in types I and II CXL require further clinical studies, although they have been partially resolved theoretically.³