The Role of Riboflavin Concentration and Oxygen in the Efficacy and Depth of Corneal Crosslinking

The recent article of O'Brart et al.[1] discussed the roles of riboflavin concentration (in the stroma) on the efficacy of corneal collagen crosslinking (CXL). Clinical studies of Ng et al.[2] showed that accelerated CXL (ACXL) had less efficacy than standard CXL (SCXL) for the same fluence (dose) based on Bunsen–Roscoe reciprocal law (BRL). To overcome this intrinsic drawback of ACXL, Lin[3] recently proposed a new protocol called riboflavin (Rf) concentration-controlled method (CCM) to improve the efficacy of ACXL by supplemental Rf during the UV exposure to compensate the fast depletion of Rf by UV light.

This letter analyzes the role of Rf concentration and its limitation by a CXL depth formula. A new criterion of CXL efficacy based on crosslinking [strength] × [depth] is introduced for optimal protocol. In addition, the role of oxygen in both type-I and type-II CXL is briefly summarized.

CXL efficacy[4,5] defined by Eff = 1 − exp(−S), where the S-function for type-I and type-II CXL is shown in the Table. Our numerical calculations[5] showed that S2 follows BRL and is proportional to the light dose (E_0) and C[O_2]. In contrast, non-BRL feature occurs in type-I CXL (or S1) to be analyzed late. In contrast to the conventional belief that oxygen-mediated type-II plays the critical role of CXL, the kinetic model of Kamaev et al.[6] showed that CXL is predominated by type-I, whereas oxygen (or type-II) plays only a limited and transient role. Lin's 3-pathway model[5,7] showed mathematical details of the role of oxygen, supporting the claim of Kamaev et al.[6] Moreover, a recent clinical study of Lombardo et al.[8] showed a simple-exponential temporal profile of Rf concentration that implied that, in ambient environment, non–oxygen-mediated type-I mechanism is predominant.

For type-I CXL, the S-function (S1) is shown in the Table, where F(z)C_0 is the initial (at t = 0) Rf concentration (in the stroma) having a depth-profile defined by a diffusion depth (D), F(z) = 1–0.5z/D. In contrast to type-II (S2), in which oxygen plays a transient but critical role, type-I (S1) does not require oxygen and it is the predominant pathway of CXL efficacy.[4–8]

At steady-state (with btX>>1), S1 follows a nonlinear scaling law[4] that S1 is proportional to $(FC_0/I_0)^{0.5}$ exp(0.5Az), or $S1\alpha[C_0]^{0.5}$ (for z = 0) and stronger dependence of $S1\alpha[C_0\exp(Az)]^{0.5}$ (for z > 0), because A is also proportional to C_0. For example, on corneal surface (or z = 0), when C_0 is doubled (from 0.1% to 0.2%), S1 increases by a factor of 1.43. The Figure shows the theoretical Rf dose-curve (or S1 versus C_0) comparing to the data of O'Brart et al.[1] (their Fig. 3A, normalized, and fit at 0.1%); both show the nonlinear feature of $S1\alpha C_0^{0.5}$.

CXL depth (defined by when S1 is maximal) is given by[7] $z^* = \ln(NE_0)/A$, with N being a numerically fit constant given by N = 0.16 (for D >>1 cm) and N = 0.224 (for D = 500 μm). For example, when C_0 is doubled (from 0.1% to 0.2%), A increases and z^* is reduced by 1.48 times. The z^*-formula shows that higher Rf concentration results in an increased (or larger S1), but more superficial (or small z^*) crosslinking effect, as also indicated by O'Brart et al.[1] Our formulas lead to a new criterion of CXL efficacy based on the product of CXL [strength] (or S1) and [depth] (or z^*), that is, the [volume] of stroma being crosslinked. For a given C_0, deeper CXL may be achieved by larger fluence (E_0). However, to achieve clinically acceptable CXL efficacy by a minimal E_0, one requires an optimal range of C_0. For example, C_0 = 0.15% to 0.3%, and E_0 = 3.5 to 4.5 J/cm², such that [depth], z^* = 200 to 300 μm, and [strength], S1 = 1.5 to 2.0, or CXL efficacy Eff = 1 − exp(−S1) = 0.78 to 0.86. Our formulas also demonstrate that epi-on CXL (having a smaller D and C_0) is less efficient than epi-off CXL, as clinically reported. To conclude, the author would like to see further basic, clinical investigations to support the presented formulas, as suggested by the reviewers.

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Table. Abbreviations and Key Parameters[4,5]

CXL	Corneal Collagen Crosslinking
SCXL	Standard CXL (intensity 3 mW/cm²)
ACXL	Accelerated CXL (intensity 9 to 45 mW/cm²)
BRL	Bunsen–Roscoe reciprocal law
CCM	Riboflavin (Rf) concentration-controlled method
CXL efficacy S-functions	$S1 = K\sqrt{F(z)Co/(bX)}\ [1-\exp(-0.5btX)]$ $S2 = \int_0^t bC[O2]/([O2]+k)dt$ $X = \exp(-Az)$; $b = 0.62pkI_0$
C_0	Initial Rf concentration (at z = 0)
[O2]	Concentration of oxygen
F(z)	Depth-profile of Rf, with a diffusion depth (D)
$E_0 = tI_0$	UV light fluence (dose)
I_0	UV light intensity
t	Exposure time
p	Quantum yield of Rf triplet state
KI	A rate constant
A	Effective absorption, A = 290 m(1 - 0.25 z/D)C_0 + 32
m	Fit parameter = 0.4 to 0.6.

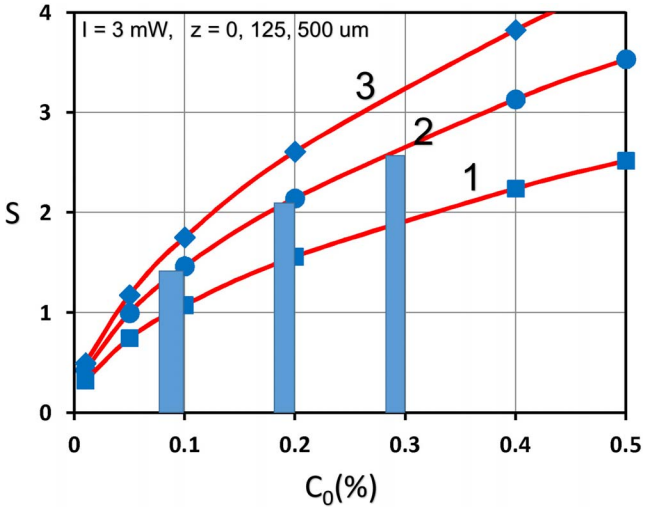

Figure. CXL efficacy versus Rf concentration shows the nonlinear feature, where theoretical curve (*red curve*) is compared with the clinical data (*bars*) of O'Brart et al.[1]

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