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Corneal Collagen Cross-linking (CXL): Controversy and Fundamentals

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Abbreviations: CXL: Corneal Collagen Cross-Linking; SCXL: Standard CXL (intensity 3 mW/cm^2); ACXL: Accelerated CXL (intensity 9 to 45 mW/cm^2), BRL: Bunsen–Roscoe Reciprocal Law; CCM: Riboflavin (Rf) Concentration-Controlled Method

INTRODUCTION

Corneal collages cross-linking (CXL) is a technology using riboflavin solution as the photosensitizer activated by a UVA light (at 365 nm) to change the biomechanical properties of the corneal stroma. CXL has been used clinically for various corneal conditions such as keratoconus, keratitis, corneal ectasia and corneal ulcers. It has also been used to preventively treat thin corneas, which carry a higher risk of ectasia after LASIK vision correction. Other potential applications include the reduction of postoperative regression in vision correction and scleral treatment in malignant myopia, scleromalacia and low tension glaucoma. The first animal data was reported by Wollensak in 2003 for the treatment of keratoconus [1]. Extensive review of CXL has been covered in detail in a recent book edited by Hafezi and Randleman [2], This Editorial Review will first address the current controversial issues with comments and resolutions. Then it will summarize the principles/formulas of and define the key parameters influencing the efficacy of CXL.

The controversial issues to be discussed include:

- Safety criteria (and the minimum corneal thickness)
- Dynamic profiles and depletion of riboflavin
- Validation of Bunsen Roscoe law (BRL)
- Intensity cutoff maximum
- The role of oxygen and pulsed mode
- CXL efficacy (type-I and type-II)
- Dresden vs. Modern protocols

Controversial Issues

Safety criteria

Figure 1 shows z versus a normalized dose N=(E/E') that z is a nonlinear increasing function of N, but a decreasing function of RF concentration. Accurate z* depends on the measured Ed which needs further studies and value of A, which also needs measured parameters. The criteria [1,2] J/cm^2, 400 um] is just one of the special case, for Ed=0.35 mW/cm^2 (under the Dresden protocol) and cannot be the safety standard.

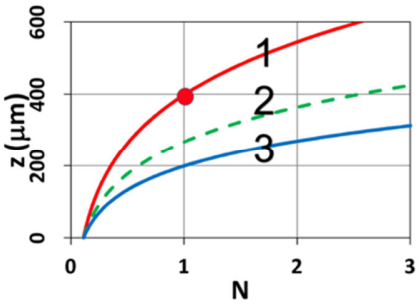

Figure 1. Minimum corneal thickness versus the normalized dose (fluence) N=(E/E'), for riboflavin concentration C_0= (0.1, 0.2, 0.3) %, for curves (1,2,3), for a diffusion depth D=500 um.

Table 1. CXL safety, crosslink depth (z) and time (T*)

Minimum corneal thickness
z*=(1/A) [2.2+ ln(E/E')]
A : effective absorption, A=290(1-0.25z/D)C_0+32.
E and E': UV dose in general and at threshold (at z=0).
C_0: initial RF concentration (at z=0),
Crosslink time (T*) and depth (z)
T*= T_0 exp(Az)
T_0=on surface (z=0)=258/I_0
z = ln(NE_0)/*A*,
N=0.16 (for D>>1 cm) and N=0.224(for D=500 um).

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Dynamic profiles and depletion of RF

Conventional modeling [4,5] assumed a constant RF concentration during the crosslink, which is true only under the so-called Dresden protocol [1,2], in which the RF is constantly re-supplied to compensate its depletion. However, it also reduced the available effective dose to approximately about 70% to 80% of the applied dose 5.4 J/cm^2. The constant-RF also underestimated the UV light intensity, which in general, is an increasing function of time (when RF depletion is accurately included), given by [6,7] $I(z,t)=I_0 \exp[-A(z,t)]$ with A(z,t) is a decreasing function of time when C(z,t) is depleted, given by $A(z,t)= 2.3[(a-b)C(z,t)G(z)+bC_0]+Q$, where a,b and Q are, respectively, the absorption constant of RF, photolysis product and stroma (without RF); G(z)=1-0.25z/D; and F(z)=1-0.5z/D is the initial RF distribution defined by a diffusion constant (D) [6].

The dynamic profiles of RF and concentration and UV light intensity are shown in **Figure 2,** where C(z,t) is a decreasing function (depletion) of time, whereas I(z,t) is an increasing function of time due to reduced absorption, A(z,t). Crosslink time, T*(z,t), defined by the when C(z,t) is depleted to 0.13, and is shown in Table 1., which also defines a crosslink depth when the CXL efficacy reaching it maximum. We note that z is an increasing function of the UV dose, but decreasing function of A and C_0.

The UV light intensity increases from its initial value $I(z,t)=I_0 \exp[-A_1 z]$ to steady-state vale given by $I(z,t)=I_0 \exp[-A_2 z]$, with $A_1= 2.3aC_0+Q$, $A_2=2.3bC_0+Q$. For C_0=0.1%, a=204 (1/cm/%), b=50(1/cm/%), and Q=32 (/cm), we obtain A_1 =79 (1/cm), and A_2 =43.5 (1/cm), with an averaged value of 61 (1/cm), which are much larger than the RF-constant model with a value of 42.5 (1/cm). If one assumes Q=b=0, then A=46.9 (1/cm), which is smaller than our averaged value of 61 (1/cm). Numerical simulation of Lin and Cheng [8], also showed another fit $A=2.3[mbC_0 + Q]$, with m=1.5 for b=50(1/%/cm), which is fit to the CXL efficacy (at steady state). In this fitting, (for D=500 um), A=49 and 66 (1/cm) for C_0=0.1% and 0.2%.

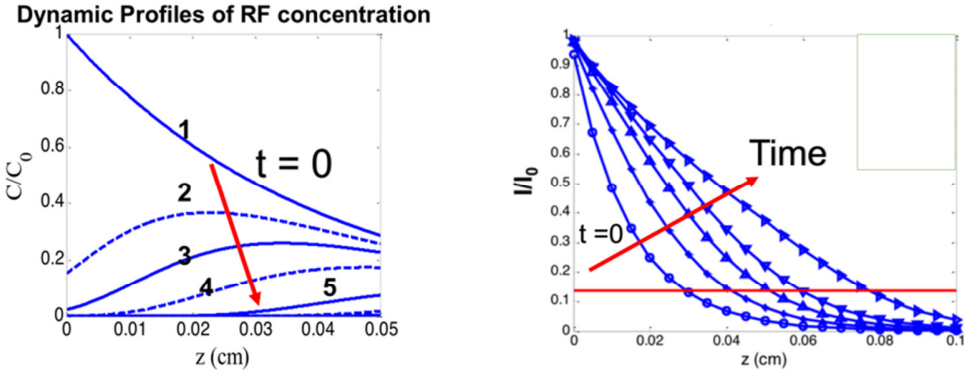

Figure 2. The normalized RF concentration and UV light intensity profiles for t=0 and t = (0, 25,50,100, 200) seconds [6].

Validation of Bunsen Roscoe law (BRL)

To shorten the CXL treatment duration while maintaining the similar CXL efficacy, various accelerated (AC) protocols to replace the SD protocol have been proposed based on the BRL of reciprocity [8] stating that the effect of a photo-biological reaction is proportional only to the total irradiation dose (E=It), or the product of intensity (I) and exposure time (t). To achieve the same efficacy, the required exposure time based on BRL is given by t=E/I, which gives the protocol for AC; for example, t= (30, 10, 5, 3, 2) minutes for I= (3,9,18,30,45) mW/cm^2. Validation of BRL has been challenged by Lin's non-linear law and the S-formulas for CXL efficacy [7,9]. Wernli, et al. [11] also pointed out the limitation of BRL due to the sudden drop of efficacy at UV intensity around 50 mW/cm^2. To improve the CXL efficacy, extended exposure time and/or dose, has been proposed to compensate the drawback of exposure time predicted by BRL [9]. Moreover, a concentration-controlled method (CCM) was proposed by Lin [10] to improve the CXL efficacy by resupply of RF during the UV exposure.

The role of oxygen and pulsed mode

CXL efficacy is governed by both oxygen-mediated (OM) and non-oxygen-mediated (NOM) 3-pathway processes, rather than the conventionally believed type-II only (oxygen-mediated) mechanism [12,13]. Both type-I and type-II reactions can occur simultaneously, and the ratio between these processes depends on the type of photosensitizers (PS) used, the concentrations of PS, substrate and oxygen, the kinetic rates involved in the process, and the light intensity, dose, PS depletion rate etc. The CXL 3-pathway kinetics maybe described as follows. For type-I, the riboflavin triplet state [T] may interact directly with the stroma collagen substrate [A] under NOM (with a rate constant k_8, pathway-1); or with the ground-state oxygen [$^3O_2$] to form reactive oxygen species [O-] under OM; and in type-II process, [T] interacts with [$^3O_2$] to form a singlet oxygen [$^1O_2$]. [T] may

also relax to riboflavin ground state (with a rate constant k_5). Both reactive oxygen species (ROS), [O-] and [$^1O_2$], can either relax to [$^3O_2$], or interact with [A] for crosslinking.

Schumacher, et al. [4] reported the NOM-type-I CXL, in contrast to Kling, et al. [5] claiming that oxygen-mediated type-II played the critical role of CXL efficacy. Furthermore, Kamaev, et al. [12] claimed that CXL is NOM-type-I dominant, while the OM-type-II only plays a limited and transient role, as shown by **Figure 3.** If Kling, et al. [5] were correct, then all the reported results of epi-on CXL would not be possible, since only limited and transient oxygen supply is available. Lin [13] proposed mathematically, model in supporting the claims of Kamaev, et al. Pulsed mode was claimed to have higher efficacy than CW mode [5]. This conclusion, I believe, is due to clinical measured errors and/or non-controlled comparison of RF concentration during the UV exposure, based in Lin and Kamaev studies [12-14] that OM-type-II only plays a limited and transient role. As shown by **Figure 4**, the role of oxygen resupply (and pulsed mode) take few minutes. Therefore, pulsing in few seconds would not help the Type-II efficacy.

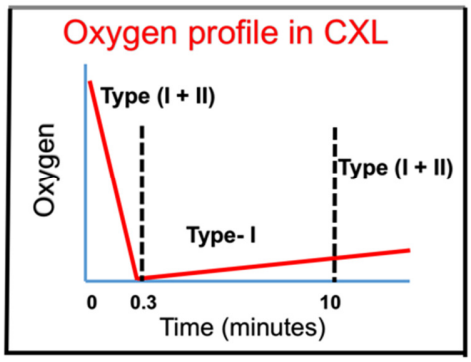

Figure 3. Schematics of the oxygen concentration profiles in CXL; in the transient stage, both type-I and type–II coexist until the oxygen is depleted; then type-I dominates before the oxygen is replenished [14].

CXL efficacy (type-I and type-II)

CXL efficacy defined by Eff=1-exp(-S), where the S-function for type-I and type-II CXL are shown in **Table 1.** Our numerical calculations[5] showed that S2 follows BRL and 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, Kamaev et al [12] kinectic model showed that CXL is predominated by type-I, while oxygen (or type-II) only plays a limited and transient role. Lin's 3-path-way model [14] showed mathematical details of the role of oxygen, supporting the claim of Kamaev et al.

For type-I CXL, the S-function (S1) is shown in Table 1, 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.

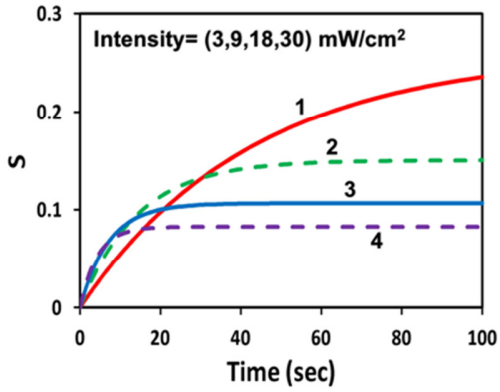

Figure 4. The S-profile (S1) for type-I CXL (at z=0), for intensity I_0= (3,9,18,30) mW/cm^2 (curves 1,2,3,4), for C_0=0.1% and D=500 um, showing that higher intensity has higher efficacy in the transient-state, but lower in the steady-state [9].

Dresden vs. Modern protocols

The standard Dresden (SD) protocol was proposed by Wollensak et al [2] in 2003, where a UVA light (at 365 nm) was used to treat cornea 9 mm zone at an intensity of 3.0 mW/cm^2 for 30 minutes, delivering a fluence (dose) of 5.4 J/cm^2. Modern protocols, named as CCM by Lin [10], used a limited resupply of RF to eliminate the extra blocking effect due to over resupplied RF in Dresden protocol.

CXL efficacy is influenced by multiple factors including, the UV light intensity, exposure period and dose, the initial concentration profiles of RF and oxygen, the quantum yield of the RF triplet state, the kinetic rate constants of RF (in type-I) and oxygen (in type-II). Besides, the protocol procedures defining how the RF drops are applied pre-operatively and during the UV exposure are also important, because they define the initial, and intra-procedure RF concentration profiles (or diffusion depth). For example, the frequency of RF drops (Fdrop) applied on the cornea after the UV is turned on, and the waiting period (Twait) for each RF drops instillation during the UV exposure. In the conventional Dresden protocol, Fdrop is about 5 to 10 times and Twait=0. In contrast, our proposed concentration-controlled method (CCM) uses Fdrop is about 1 to 3 times (for RF replenishment) and Twait is 1 or 2 minutes (for enough diffusion depth, with D>150 um).

Kling, et al. [15] recently reported the use of 1.5 mW/cm^2 intensity for 30 minutes exposure (or 2.7 J/cm^2 dose) has similar efficacy as that of 3 mW/cm^2 and 30 minutes

exposure (5.4 J/cm^2 dose). This feature may be easily realized by our S-function which has an optimal dose predicted to be about 3 to 4 J/cm^2, and the 5.4 J/cm^2 (for 3 mW/cm^2) is certainly higher than the optimal value [16].

Cut-off maximum intensity

Validation of BRL for accelerated CXL has been studied by Wernli, et al. [11] by the Cutoff maximum intensity about 50 mW/cm^2 and a minimum crosslinking time about 2 minutes. These criteria may be derived by our S-function as follow. Taking a threshold value of S_0 (the minimum S for efficient crosslinking as that of Dresden 3 mW/cm^2), or $4KC_0Fexp(Az)/(aqKI_0) > S_0^2$, from our S-formula, which leads to a cutoff maximum intensity (on the corneal surface, z=0) given by $I^*=4KC_0/(aqKS^{*2})$. For C_0=0.1%, q=0.5, K=7.8, K'=0.05, a=0.622, we obtain $I^*=201/S_0^2$, or I*= (50.3,22.3) mW/cm^2, for S_0= (2,3), i.e., Ceff=1.exp(-S_0) = (0.86,0.95). these values predict what was reported by Wernli et al [11]. We should note that the S-formula is valid for the situation of non-controlled RF concentration, i.e., no extra RF drops were applied during the UV exposure (or Fdrop=0). A concentration-controlled methods (with Fdrop=1 to 3) was proposed to overcome the limitation of maximum intensity [10].

New standard for CXL efficacy

At steady-state (with bt>>1), S1 follows a nonlinear scaling law [10,16] that S1 is promotional to $(C_0E_0/I_0)^{0.5}$ exp(0.5Az) showing that S1 is proportional to $C_0^{0.5}$ (for z=0) and stronger dependence of exp(0.5Az) $C_0^{0.5}$ (for z>0), noting that A is also proportional to C_0, A=290F(z)C_0+32 (in cm^{-1}). For example, at z=0, S1(for C_0=0.2%)=1.43 S1(for C_0=0.1%), i.e., S1 increases by a factor of 1.43 when the Rf concentration (in the stroma) is doubled. Our formulas show that higher Rf concentrations result in an increased but more superficial cross-linking effect, as also clinically indicated by O'Brart, et al. [17].

CXL depth (defined by a maximal S1) is given by (for simplified case of F=1), z*=ln (NE_0)/A, with A=[290C_0+32], N being a numerically fit constant. Therefore, when C_0 is doubled (from 01% to 0.2%), A increases by (58+32)/(29+32)=1.48, and z* is reduced by 1.48 times. Therefore, a more appropriate CXL efficacy [18] should be defined by the product of [strength] (or the maximal value of S1) and the [depth] (or z*), i.e., the volume of stroma being cross-linked. It should be noted that deeper CXL (or larger z*) may be achieved by larger fluence (E_0), i.e, more superficial CXL in higher C_0 may be compensated by larger light-dose. However, considering optimal CXL with minimal UV exposure time (or dose), one requires an optimal range of C_0-0.15% to 0.3% and and E_0 = 3.5 to 4.5 J/cm^2, such that [depth] z*=200 to 300 um, with [strength] S1=1.5 to 2.0 (or CXL efficacy 1-exp(-S1)=0.78 to 0.86), noting that high C_0 causes a competing of [strength] and [depth] which needs to be optimized. Greater detail with numerical simulation will be presented elsewhere.

CONCLUSION

We have presented the resolutions of controversial issues in CXL via factors influencing the CXL efficacy. To improve the efficacy of ACXL, a CCM was proposed. The key parameters and fundamentals are summarized in **Table 1 and 2.**

Table 2. CXL efficacy[4,5]

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)=1-0.5z/D: depth-profile of RF
$E_0 = tI_0$, UV light fluence (dose);
I_0 is UV light intensity; t is exposure time,
p : quantum yield of Rf triplet state;
K: effective rate; k: a rate constant;

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