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Oxygen kinetics during corneal crosslinking with and without supplementary oxygen

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Purpose: To measure and simulate oxygen kinetics during corneal crosslinking (CXL) at different irradiances with and without supplementary oxygen.

Design: Experimental, laboratory study.

Methods: In de-epithelialized porcine eyes, a femtosecond-laser generated tunnel was used to place a fiber-probe in corneal depths of 100, 200 and 300µm to measure the local oxygen concentration. After riboflavin imbibition, the corneas were irradiated at 3, 9, 18 and 30mW/cm^2 while the oxygen concentration was measured. All experiments were performed under normoxic (21%) and hyperoxic (>95%) conditions. The obtained data were used to identify parameters of a numerical model for oxygen consumption and diffusion.

Results: The equilibrium stromal oxygen concentration under atmospheric oxygen at 3mW/cm^2 was 2.3% in 100µm decreasing to <1% in 300µm. With 9, 18 and 30mW/cm^2, no oxygen was available in 200µm respectively 100µm or deeper. Using a hyperoxic environment, the concentration was 50% using 3mW/cm^2 in 100µm, decreasing to 40% in 300µm. At 9mW/cm^2 the concentrations were 5%, 3% and 1% in 100, 200 and 300µm, respectively. Using 18 and 30mW/cm^2 all oxygen was depleted at 100µm, however, oxygen half-lives were longer at 18mW/cm^2 than at 30mW/cm^2. The oxygen model was able to reproduce the experiments and indicated an exponential decay with increasing distance to the anterior surface.

Conclusion: Supplementary oxygen increases the oxygen-availability during CXL. At higher irradiances, supplementary oxygen is beneficial and eliminates the bottleneck of oxygen allowing a potentially more efficient crosslinking. The calibrated numerical model can quantify the spatial oxygen concentration related to different scenarios such as irradiance or environmental oxygen concentration.

Oxygen kinetics during corneal crosslinking with and without supplementary oxygen

Running title: Oxygen kinetics during CXL

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Introduction

Corneal crosslinking (CXL) is an effective procedure to halt the progression of keratectasia such as keratoconus[1,2] or post-LASIK ectasia[3] with low rates of complication.[4,5] By means of riboflavin as the photosensitizer and the activation by UV-irradiation, reactive oxygen species (ROS) are created, inducing new bonds in the extracellular matrix of the cornea.[6] If one of the three "ingredients" does not contribute to the process, CXL is ineffective. The first factor, riboflavin, has been investigated extensively and corneal riboflavin concentrations with an epi-off application have been found to be sufficient[7], also in deeper corneal layers not restricting CXL. The second, UV-light, is not a restricting factor because even at an irradiance of 3mW/cm^2, CXL demonstrates the best biomechanical effect.[8] Therefore, irradiance cannot be the bottleneck for accelerated epi-off protocols. Richoz and coworkers[9] performed crosslinking in an oxygen-free environment and found no significant stiffening effect emphasizing the importance of oxygen in CXL. To date, only few reports have been published on the basics of corneal oxygen kinetics during crosslinking[10,11] or on the biomechanical effect of supplementary oxygen during CXL.[10,12]

The aim of this study was to provide experimental data on the oxygen consumption during CXL by determination of the oxygen profile at different depths using different irradiances and oxygen environments. Oxygen kinetics might be the missing piece to understand the invalidity of Bunsen-Roscoe's law in CXL.

Materials and Methods

Preparation of eyes

Freshly enucleated porcine eyes were obtained 2-3 hours postmortem from a local abattoir. The eyes were stored at 4^{o} C and used within 8 hours. Prior to the experiments, the corneas were examined for corneal opacities and epithelial defects. The eyes were divided in six different groups (n = 5 for each group). Three of the groups were subjected to CXL in an atmospheric environment (room air, 21% oxygen) while the rest of the groups underwent CXL in a hyperoxic environment (>95% oxygen). Before any treatment, the corneal epithelium was removed using a blunt hockey knife and by means of a femtosecond-laser (FEMTO LDV Z8, Ziemer Ophthalmic Systems, Port Switzerland), a small tunnel with a width of 0.6mm and length of 5mm (Figure 1) was created for each subgroup at a depth *D* of either 100, 200 or 300 microns (controlled by OCT). The tunnel was generated to guide the oxygen micro-sensor and to guarantee the proper depth of the sensor within the corneal stroma.

Oxygen micro-sensor

Prior to data acquisition, the optic oxygen micro-sensor (NTH-PSt1, PreSens, Regensburg, Germany) was connected to a microfiber optic oxygen meter (Micro TX3, PreSens, Regensburg, Germany) and calibrated at room temperature following the manufacturer's guidelines using two data points at 0% and 20.9% oxygen. To provide smoother measurements, dynamic signal averaging has been applied, meaning every yielded data point per second consists of an average of 4 measurement points. Since the sensor has a response time of <2 seconds, measurements during the first 2 seconds of each data set were excluded from evaluation. Although the oxygen microsensor has a theoretical limit of detection of 0.05% for gaseous and dissolved O_2, premeasurements in corneal tissue indicated a lower limit of detection of below 1%.[13]

Oxygen measurements and crosslinking

The eyes were kept in a custom-built chamber with 2 access paths for inserting the oxygen micro-sensor and for applying riboflavin drops and the UV-beam. In addition, an oxygen gas tank was connected via a tube to assure an oxygen concentration above 95% for groups with a hyperoxic environment. The oxygen content inside the chamber was controlled during the experiments by a separate oxygen sensor (Fibox 4, PreSens, Regensburg, Germany). After mounting the eye, the sensor was inserted into the channel under a microscope, so that the fiberoptic tip of the sensor reached the end of the tunnel to guarantee the proper depth (Figure 1). The oxygen concentration measurements were then initiated and meanwhile drops of 0.1% riboflavin (Vibex rapid, Avedro, Waltham, MA) were applied each 2 minutes for a total of 10 minutes. Then the UV-source (KXL, Avedro, Waltham, MA) was focused onto the surface of the cornea, centered next to the end of the tunnel delivering continuous irradiances ranging from 3 to 30mW/cm^2 with a diameter of 9mm. Each measurement was recorded until either an equilibrium of the oxygen concentration had been established or until the concentration decreased to a level of less than 1%.

Data analysis

Descriptive statistics were performed using Microsoft Excel. Every experimental data set (one eye, time course of oxygen concentration in 1 depth during CXL) was fit to reduce the measurement noise (MATLAB R2019b, The MathWorks, Natick, MA) as presented in Figure 2.

$$O_2 = b_1 e^{b_2 t} + b_3 \quad \text{(equ. 1)}$$

b_1 describes the speed of the oxygen consumption, b_2 represents the negative time constant of the exponential decay, while b_3 is the equilibrium oxygen concentration. To approximate the time constant of the decay, the half-life of each fit function (equ. 1) was determined and for the equilibrium oxygen concentration the fit-parameter b_3 (equ. 1) was used. For the initial oxygen depletion rate, the first derivate at $t = 1\,sec$ was taken.

To compare oxygen concentrations between different depths of the cornea Mann-Whitney-U-Tests were performed (Winstat for Excel, R. Finch, Germany). Significance was accepted if p<0.05.

Numerical model

The transport of oxygen in the cornea is governed by a diffusion equation (equ. 2);

$$D\frac{\partial^2 P_{O2}}{\partial x^2} - \frac{Q}{k} = \frac{\partial P_{O2}}{\partial t} \quad \text{(equ. 2)}$$

where *D* is the diffusivity ($\frac{mm^2}{s}$), *x* is the position (mm), P_{O2} is the partial pressure of oxygen ($mmHg$), *k* is the Henry's solubility constant ($\frac{ml(O2)}{ml(tissue)\times mmHg}$), *t* is time ($s$) and *Q* is the oxygen consumption due to the CXL induced by UV-light ($\frac{ml(O_2)}{ml(tissue)\times s}$). In this study, we assume that this consumption is linearly dependent on the UV-irradiance and follows an exponential decay with the distance from the anterior corneal surface. Moreover, a Michaelis-Menten–type relationship for oxygen uptake was used to account for oxygen availability (equ. 3);

$$Q = Q_{CXL}\frac{P_{O2}}{K+P_{O2}} + Q_{stroma}\frac{P_{O2}(20+155)}{155(20+P_{O2})},\ Q_{CXL} = Q_0 W e^{-x/\zeta} \quad \text{(equ. 3)}$$

where Q_0 ($\frac{ml(O_2)\times cm^2}{ml(tissue)\times s\times mW}$) and ζ (μm) are model parameters, *W* $\left(\frac{mW}{cm^2}\right)$ is the irradiance, and Q_{stroma} $\left(\frac{ml(O_2)}{ml(tissue)\times s}\right)$ is the consumption of oxygen by the stromal cells.[14]

The model was divided in three layers; the stroma (0.85 mm), the endothelium (0.005 mm), and a posterior boundary layer (0.1 mm) describing the diffusion kinetic related

to the aqueous humor. The diffusivity of the stroma (Dk_{str}) was a model parameter, but the diffusivity of the endothelium and of the boundary layer were constrained to a fixed ratio (Dk_{bc} = 0.1 Dk_{str} and Dk_{end} = 0.2 Dk_{str}).[14]
The partial differential equation solver *pdepe* in MATLAB (MATLAB R2019b, The MathWorks, Natick, MA) was used to solve the diffusion equation. The different oxygen concentration were simulated by changing the anterior and posterior boundary conditions; the 100% configuration was modeled with a partial pressure of oxygen of 775 mmHg on the anterior surface and 375 mmHg on the posterior side, while the 20% configuration was modeled with pressures of 155 mmHg and 50 mmHg on the anterior and posterior side. The values chosen for these boundary conditions were provided by direct measurements of the environmental and aqueous oxygen concentrations. An iterative least squares optimization process was used to determine the five model parameters Q_0, Q_{stroma}, ζ, K, and D_{str} that best reproduced the experimental data for all measurement depths and irradiances.

Results

Equilibrium oxygen concentration levels for different corneal depths and irradiations

Once the UV irradiation was initiated, oxygen levels quickly descended and stabilized at an equilibrium. Table 1 shows the equilibrium in percentage of oxygen and the half-life in a hyperoxic environment as well as for an atmospheric oxygen environment, respectively. The highest oxygen availability was found for both groups in the 3mW/cm^2 subgoup in 100 microns with 2.3±2.5% in the normoxic and 50.2±6.2% in the hyperoxic environment. In both subgroups decreasing oxygen levels were observed, when measured in deeper corneal layers (p=0.009) or higher irradiances (p=0.009) illustrated in Figure 3. After cessation of the UV-irradiation, oxygen concentrations recovered quickly and returned to initial levels (Figure 4a and 4b).

Averaged initial oxygen depletion rates for different corneal depths and irradiations

Based on the derivative of the corresponding fit function, the initial depletion rate (in combination with the re-diffused oxygen) for the O_2 concentration was calculated. The corresponding depletion rates are listed in Table 2. Depletion rates increase with higher applied irradiances and decrease with depth. An anomaly is observed at 100 microns depth in the hyperoxic subgroup for all irradiances with lower depletion rates than at 200 microns depth.

Diffusion model

The experimentally decays of the oxygen were fit to equ. (3). The optimized parameters for all 4 irradiances yielded the following model parameters:

$$Q_0 = 1.45\ 10^{-3}\ \frac{ml(O_2) \times cm^2}{ml(tissue) \times s \times mW}$$

$$Q_{stroma} = 9.91\ 10^{-6}\ \frac{ml(O_2)}{ml(tissue) \times s}$$

$$\zeta = 75.84\ \mu m$$

$$K = 7.57\ mmHg$$

$$Dk_{str} = 4.92\ 10^{-8}\ \frac{ml(O_2) \times mm^2}{ml\ (tissue) \times mmHg \times s}$$

With these parameters the model was able to reproduce the measured decay curves mostly within one standard deviation. The best fit between model and experiment was observed at a depth of 300 microns, while largest difference between the model and the experiments was observed at 100 microns for the different irradiances in the hyperoxic environment (Figure 5).

Discussion

The main findings of this study are:

(1) Using the conventional 3mW/cm^2 Dresden protocol, oxygen is available in the anterior 300 microns of the corneal stroma throughout the entire CXL-procedure. At irradiances of 9mW/cm^2 and higher, all oxygen is consumed within in the anterior 200 microns or 100 microns, respectively.
(2) A hyperoxic environment can substantially increase available stromal oxygen, allowing a sufficient CXL deeper than 300 microns with irradiances less than 18mW/cm^2.
(3) Higher oxygen depletion rates are found with higher irradiances and at higher oxygen concentrations.
(4) A set of parameters describing the oxygen consumption were identified enabling the calculation of the evolution of the oxygen concentration at any position within the cornea for different UV-irradiances.

Beside the well-examined and in surplus available factors riboflavin and UV-light, a third factor, oxygen, is crucial to achieve sufficient CXL. The mechanism of photosensitized production of reactive oxygen species (ROS) such as singlet oxygen has been described before[15,16] and McCall[17] confirmed this pathway for CXL by ROS inhibitors (azide) or stimulators (D_2O) altering the biomechanical effect of CXL. Kamaev and coworkers[10] proposed type I (low oxygen availability) and type II (sufficient oxygen availability) reactions and demonstrated experimentally the depletion and thus scarcity of oxygen during CXL. In contrast to our results, they found no residual oxygen later than 15 seconds at 3mW/cm^2 and 5 seconds at 30mW/cm^2-irradiation in 100 microns depth. However, they have applied the 0.1% riboflavin in distilled water each 30 seconds for up to 50 minutes leading to massive corneal swelling. With an anticipated flap-thickness of >200 microns their results are in agreement to ours. Recently, also Hill et al.[11] found no oxygen at 200 microns depth using 3mW/cm^2 in an atmospheric environment, however, with similar consumption times of around 30 seconds. On the other hand, our finding of stromal oxygen down to 300 microns at 3mw/cm^2 and down to 200 microns at 9mW/cm^2 at atmospheric oxygen conditions matches to clinically determined demarcation line depth.[18] To date there are no further publications reporting oxygen levels and consumption times for epi-off CXL at atmospheric oxygen levels. Based on our data for atmospheric oxygen conditions, the maximum CXL effect has to be expected at 3mW/cm^2 and less, which is also supported by theoretical and experimental data.[8,19] For hyperoxic conditions, the optimal irradiance lays somewhere between 9 to 18mW/cm^2 since the oxygen concentration is above 1% until a depth of 300 microns and deeper for 9mW/cm^2. It is obvious that with a continuous irradiance of 30mW/cm^2 even a hyperoxic atmosphere over the cornea does not provide enough oxygen, not even at the 100 micron-level (Table 1).

Diakonis et al.[12] investigated experimentally the biomechanical effect of epi-off CXL at an hyperoxic environment using a continuous 30 mW/cm^2 irradiation and reported no significant effect. This data is in accordance to the here presented data, since the biomechanical testing was performed in 100 microns and deeper, for which our data shows no sufficient diffusion of oxygen for either atmospheric or hyperoxic conditions. In addition, the oxygen gas stream was directed onto the cornea without establishing a controlled enriched oxygen environment. Hill et al.[11] investigated the benefit of supplementary oxygen only for transepithelial CXL. A comparison is almost

impossible since the riboflavin imbibition solution had a 2.5-fold higher riboflavin concentration than for epi-off, leading to a totally different stromal riboflavin gradient and UV-absorption characteristics and, therefore, incomparable oxygen dynamics. In addition, it is well-known that the epithelium alone consumes nearly as much oxygen as stroma and endothelium together.[20, 21] Weng et. al[22] investigated the use of oxygen enrichment at 3mW/cm^2 with only insignificant superiority of the group with supplementary oxygen. This is in accordance to the here presented data, since at 3 mW/cm^2 a surplus of oxygen exists until a depth of 300 microns even under atmospheric oxygen conditions.

We found higher oxygen depletion rates with increasing irradiances and oxygen concentrations. This can somewhat be expected, since higher energy input causes higher consumption of available factors. In addition, the numerical model indicates that there is a strong depth-dependence of the oxygen depletion, following an exponential decay. This spatial decay agrees with the theoretical model proposed by Kling and Hafezi[23] to quantify the mechanical stiffening of CXL. However, to the best of our knowledge, no numerical model was calibrated on direct measurements of the oxygen concentration at different depths within the cornea during the CXL process. This information provides the basis for a precise quantification of the spatial distribution of oxygen depletion by CXL, which serves as a basis of models accounting for the photochemical kinetics of CXL.[11, 23]

One of the challenges of the numerical modelling concerns the boundary condition on the posterior surface of the cornea. Due to the ex-vivo nature of the experiments, the oxygen concentration in the aqueous humor is not regulated and is only affected by the environmental oxygen concentration (normoxic vs. hyperoxic). However, this diffusion process is also changing when the UV-irradiation depletes the corneal oxygen. The effect has been observed experimentally by direct measurement of the aqueous oxygen. To account for this effect, our model includes an additional boundary layer on the posterior side of the cornea to allow for the oxygen to diffuse from the aqueous humor. However, the oxygen diffusivity identified in this study is in the range of values previously published for human and rabbit corneas, which indicates that both the experimental measurements and numerical model produce pertinent data.[14, 24]

Another interesting issue arises from longer half-lives and lower depletion rates at a depth of 200 microns compared to 100 microns in a hyperoxic environment. Shorter times and higher depletion rates due to a higher UV-intensity[25] and more riboflavin[7] would be expected for 100 microns depth compared to 200 microns, similar to what we found in the atmospheric group. A suspected reason for these systematically longer half-lives and lower depletion rates at 100 microns may be the substantially higher oxygen diffusion from the surface into the anterior stroma under hyperoxic conditions.

From the point of view of clinical implication, our findings suggest that supplementary oxygen might increase the aerobic type II reaction enabling a potentially deeper and more effective CXL. This seems to be valid for all examined irradiances, however, as mentioned above, the optimum continuous UV-irradiance for epi-off CXL with supplementary oxygen must be assumed between 9 to 18mW/cm^2. For CXL at atmospheric oxygen conditions, the low oxygen availability can explain the better experimental outcome[8] for lower irradiances. On the other hand, similar clinical

results have been observed after CXL for irradiances[26] between 3 to 30 mW/cm^2. It is known, that all protocols achieve a CXL effect, although less and limited to the superficial corneal layers with higher irradiances. The only morphological difference, however, is the depth of the demarcation line.[27,28] It is remarkable, that lower irradiances produce deeper demarcations lines showing a good correlation with the oxygen kinetics found in this study. However, it is still unknown how much CXL is required to stabilize keratoconus.

Finally, the observation of lower oxygen availability at higher irradiances can explain the invalidity of Bunsen-Roscoe law for CXL. Shorter irradiations times with similar radiant exposures result in less time for oxygen re-diffusion and, therefore, lower total oxygen availability for CXL. However, experimental biomechanical measurements are required to proof this hypothesis.

A limitation of the study is the use of ex vivo porcine eyes which may not account for physiological consumption inside the cornea as well as for diffusion of oxygen from and into the aqueous humor. In addition, paired eyes would have allowed a better comparison between normoxic and hyperoxic environment, however due to logistical reasons at the abattoir this was not possible. Also, the sensitivity of the oxygen microsensor below 1% in corneal tissue limits conclusions for subtle differences for higher irradiances. The concern of potential oxygen leakage through the tunnel can be ruled out, since the re-diffusion time for the various depths differ substantially (Figure 4).

In conclusion, the here presented results provide new information on the oxygen kinetics during riboflavin-mediated CXL and can explain experimental and clinical findings on the effect of CXL with higher irradiances.

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Legends

Figure 1: Left: Photography with the inserted oxygen sensor inside the stroma at a depth of 200 micorns. Right: Schematic illustration of the channel creation. *D* represents the depth of the channel between 100 to 300 microns.

Figure 2: Data points af a measurement (green points) and the fit curve (red).

Figure 3a: Comparison of equilibrium oxygen concentration ± SD [%] at 100 microns depth. Blue bars represent measurements in a >95% oxygen environment, orange bars represent groups from an atmospheric environment.

Figure 3b: Comparison of equilibrium oxygen concentration ± SD [%] at 300 microns depth. Blue bars represent measurements in a >95% oxygen environment, orange bars represent groups from an atmospheric environment.

Figure 4a: Averaged oxygen concentrations during the UV-irradiation at different corneal depths (100, 200 and 300 µm, respectively) for the hyperoxic environment

Figure 4b: Averaged oxygen concentrations during the UV-irradiation at different corneal depths (100, 200 and 300 µm, respectively) for the normoxic environment.

Figure 5: The oxygen concentration calculated after identification of the parameters (solid lines) was compared to the experimental data (shaded area) for the different environments (hyperoxic and normoxic), CXL irradiances (3mW/cm^2 and 30mW/cm^2) and depths (100µm, 200µm, and 300µm). The shade area represents +/- one standard deviation around the average experimental curve.

Table 1: Representation of the equilibrium oxygen concentration in percent (upper number and half-lives of the oxygen decay in seconds (lower number) for the different depths and irradiations in hyperoxic and normoxic environment.

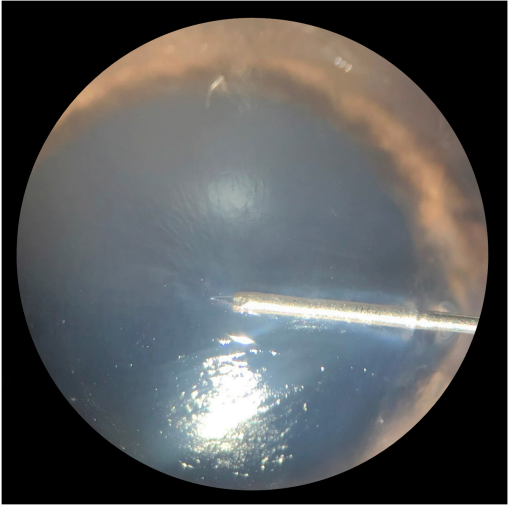
Table 2: Average absolute values of depletion rates and standard deviations in % oxygen/second for hyperoxic and normoxic environment in different irradiations and depths.

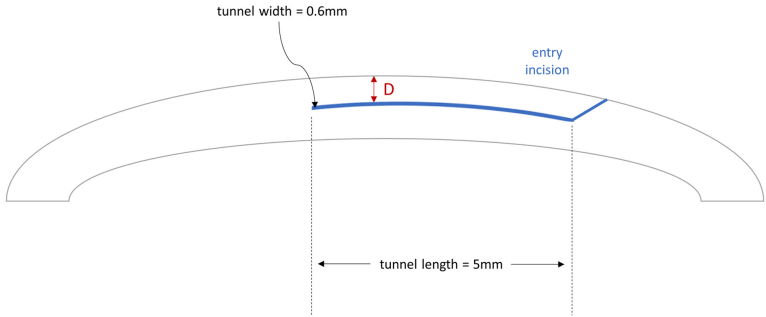
Table 1: Equilibrium oxygen concentration and decay half-lives

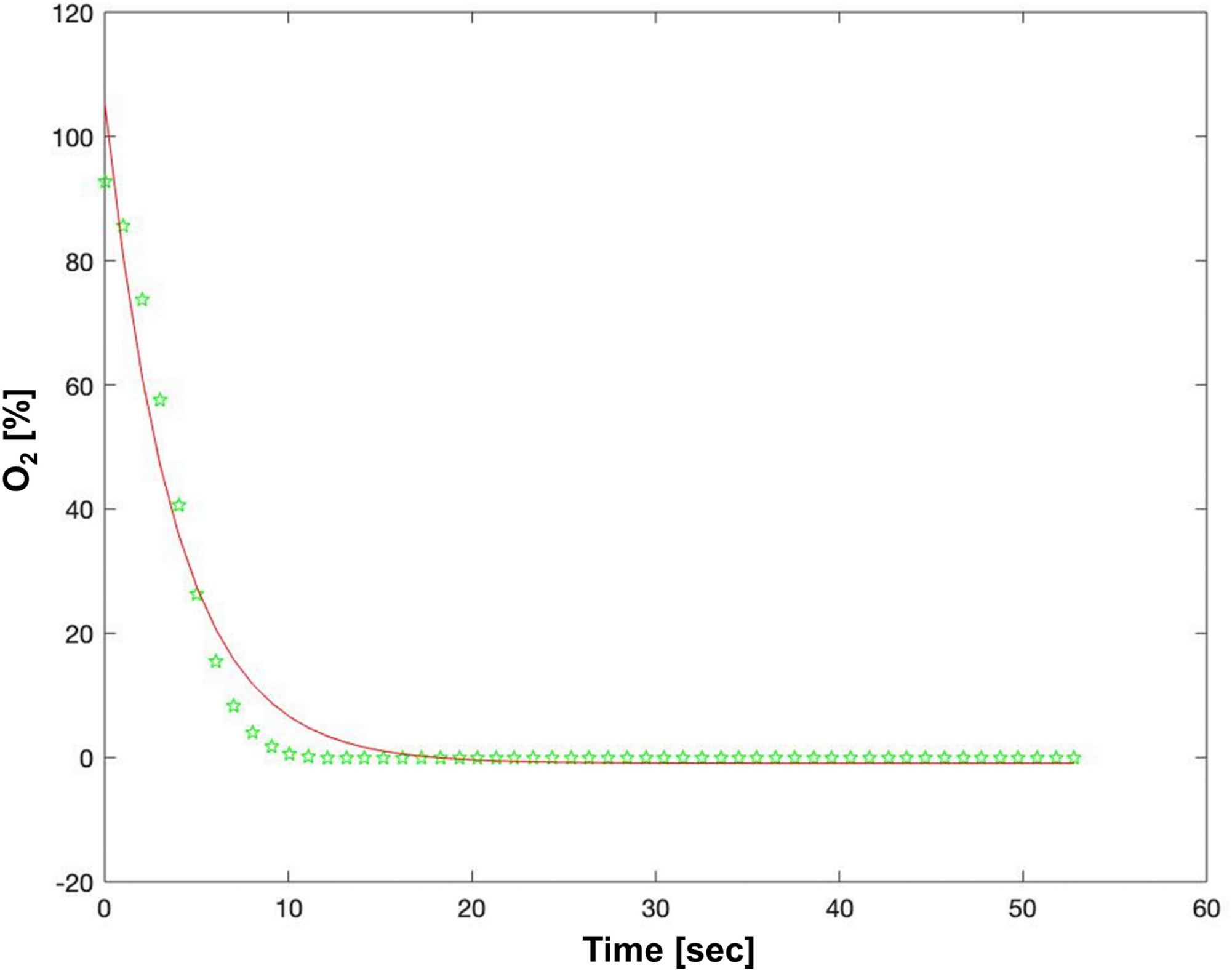
Hyperoxic	3mW/cm^2	9mW/cm^2	18mW/cm^2	30mW/cm^2
100 µm	50.2 ± 6.2 % 17.8 ± 6.7 sec	4.8 ± 3.1 % 8.9 ± 2.7 sec	< 1.0 % 3.2 ± 1.2 sec	< 1.0 % 1.8 ± 1.0 sec
200 µm	47.4 ± 6.6 % 15.3 ± 4.1 sec	2.6 ± 3.2 % 6.9 ± 1.5 sec	< 1.0 % 2.7 ± 0.6 sec	< 1.0 % 1.6 ± 0.5 sec
300 µm	39.7 ± 8.1 % 42.9 ± 27.6 sec	1.2 ± 1.8 % 15.4 ± 8.1 sec	< 1.0 % 6.1 ± 3.2 sec	< 1.0 % 3.9 ± 2.0 sec
Normoxic	**3mW/cm^2**	**9mW/cm^2**	**18mW/cm^2**	**30mW/cm^2**
100 µm	2.3 ± 2.5 % 4.8 ± 1.7 sec	1.0 ± 0.8 % 2.2 ± 0.9 sec	< 1.0 % 1.8 ± 0.8 sec	< 1.0 % 1.5 ± 0.8 sec
200 µm	1.1 ± 0.9 % 7.0 ± 4.0 sec	< 1.0 % 3.0 ± 0.6 sec	< 1.0 % 2.6 ± 0.5 sec	< 1.0 % 2.0 ± 0.6 sec
300 µm	< 1.0 % 9.2 ± 3.5 sec	< 1.0 % 3.9 ± 0.5 sec	< 1.0 % 3.1 ± 0.3 sec	< 1.0 % 2.9 ± 0.5 sec

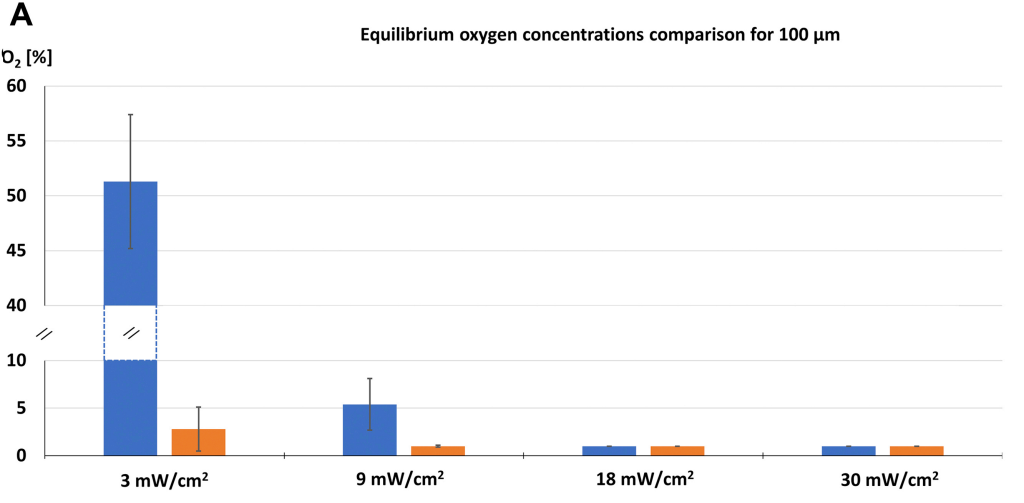
Table 2: Initial depletion rates

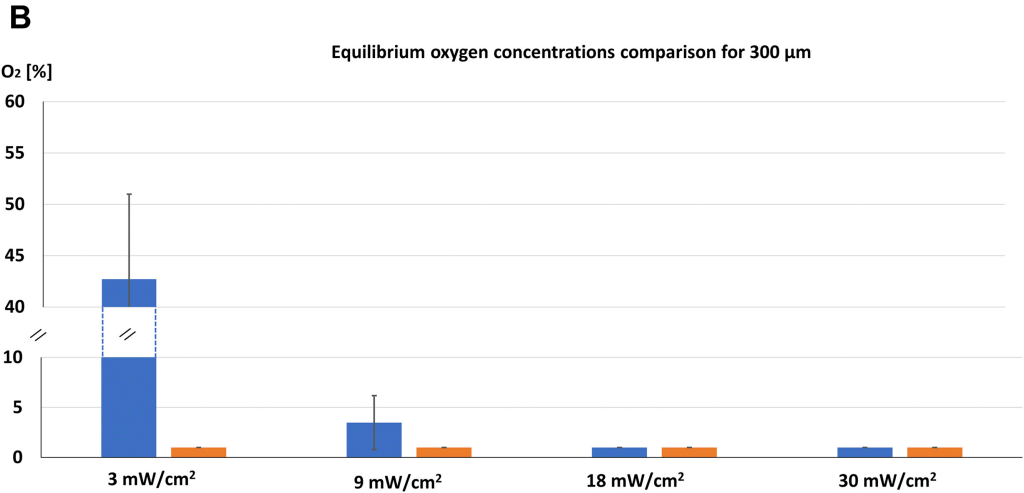
Hyperoxic	**3mW/cm^2**	**9mW/cm^2**	**18mW/cm^2**	**30mW/cm^2**
100 µm	1.9 ± 0.4 %O_2/sec	8.1 ± 1.6 %O_2/sec	27.7 ± 9.8 %O_2/sec	49.7 ± 15.0 %O_2/sec
200 µm	2.2 ± 0.8 %O_2/sec	11.1 ± 3.5 %O_2/sec	31.9 ± 9.3 %O_2/sec	57.3 ± 19.2 %O_2/sec
300 µm	0.9 ± 0.5 %O_2/sec	5.3 ± 2.8 %O_2/sec	14.5 ± 8.3%O_2/sec	21.7 ± 14.8 %O_2/sec
Normoxic	**3mW/cm^2**	**9mW/cm^2**	**18mW/cm^2**	**30mW/cm^2**
100 µm	2.6 ± 0.8 %O_2/sec	7.3 ± 2.4 %O_2/sec	10.2 ± 3.9 %O_2/sec	10.8 ± 12.2 %O_2/sec
200 µm	1.9 ± 1.0 %O_2/sec	4.4 ± 1.2 %O_2/sec	5.5 ± 1.4 %O_2/sec	6.7 ± 1.6 %O_2/sec
300 µm	1.1 ± 0.6 %O_2/sec	2.2 ± 0.45 %O_2/sec	2.5 ± 0.4 %O_2/sec	2.5 ± 0.2 %O_2/sec

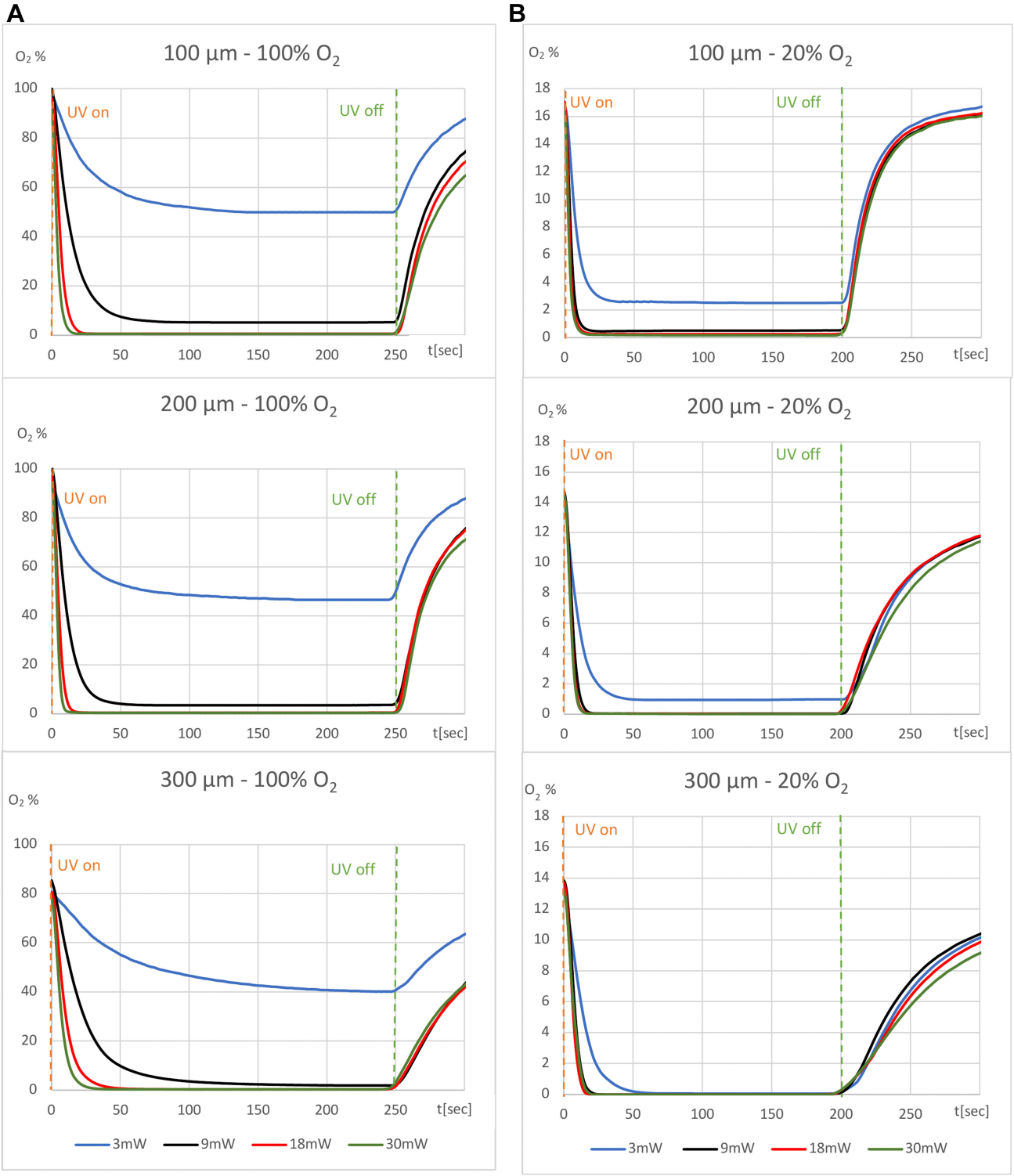



tunnel width = 0.6mm
entry
incision
D
tunnel length = 5mm


120
100
80
60
40
20
0
-20
O_2 [%]
0
10
20
30
40
50
60
Time [sec]


A
Equilibrium oxygen concentrations comparison for 100 µm
O2 [%]
60
55
50
45
40
10
5
0
3 mW/cm2
9 mW/cm2
18 mW/cm2
30 mW/cm2


B
Equilibrium oxygen concentrations comparison for 300 µm
O2 [%]
60
55
50
45
40
10
5
0
3 mW/cm2
9 mW/cm2
18 mW/cm2
30 mW/cm2


A
B
100 µm - 100% O2
100 µm - 20% O2
200 µm - 100% O2
200 µm - 20% O2
300 µm - 100% O2
300 µm - 20% O2
O2 %
UV on
UV off
t[sec]
3mW
9mW
18mW
30mW

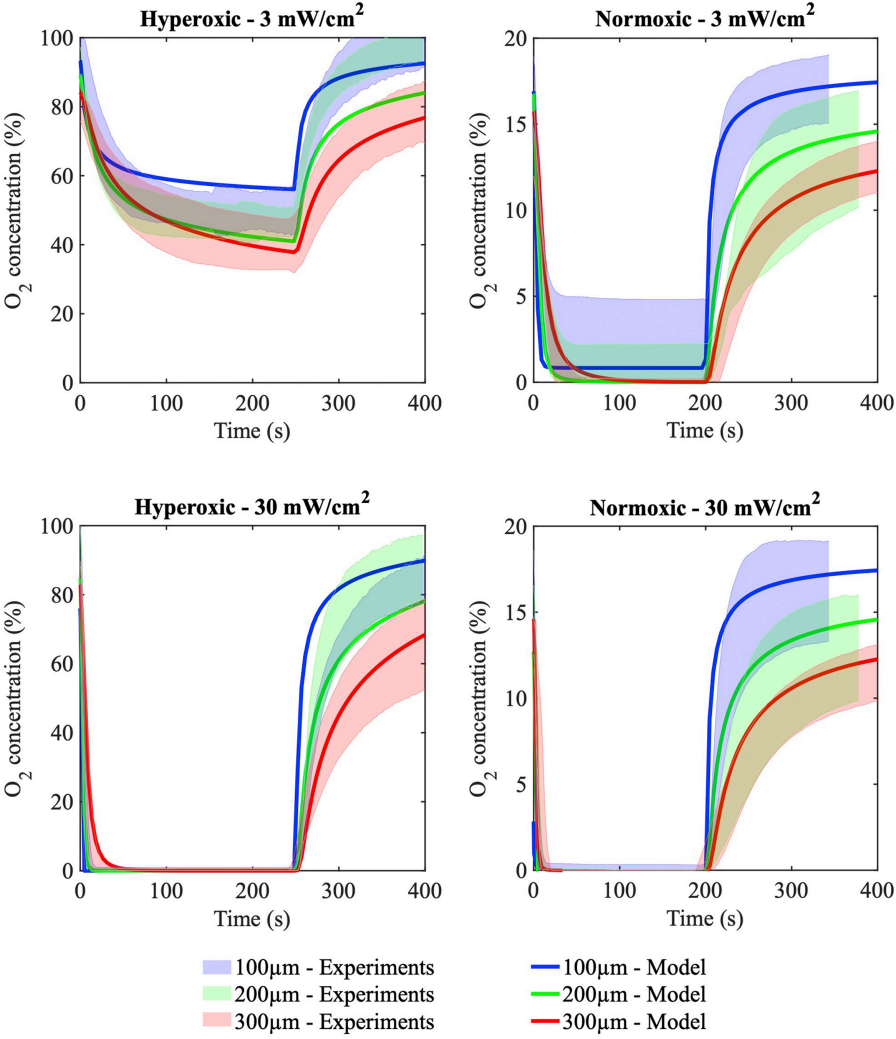

Hyperoxic - 3 mW/cm2
Normoxic - 3 mW/cm2
Hyperoxic - 30 mW/cm2
Normoxic - 30 mW/cm2
O2 concentration (%)
Time (s)
100µm - Experiments
200µm - Experiments
300µm - Experiments
100µm - Model
200µm - Model
300µm - Model

Table of contents

Oxygen appears to be the bottleneck of current CXL protocols, in particular when using higher irradiances than 3mw/cm^2. Supplementary oxygen can increase the stromal oxygen concentration and offers a pathway to potentially increase the efficacy of CXL.