Experimental and model analysis on the temperature dynamics during diode laser welding of the cornea

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Abstract. Corneal laser welding is a technique used clinically to induce the immediate sealing of corneal wounds. We present an experimental and model analysis of the temperature dynamics during diode laser-induced corneal welding, which is aimed at characterizing the mechanism of tissue fusion. *Ex vivo* tests were performed on porcine eyes in the typical irradiation conditions used for laser-induced suturing in cornea transplant. Three laser power densities (12.5 W/cm^2, 16.7 W/cm^2, 20.8 W/cm^2) were tested. The superficial temperature of the cornea was measured by means of an infrared thermocamera. Experimental data were compared with the results of a three-dimensional (3D) model of a laser-welding process in the cornea, solved by the use of the Finite Element Method (FEM). The model solution and experimental results showed good agreement. The model was thus used to estimate the temperature enhancement inside the corneal wound and to calculate the thermal damage inside the tissue. The results indicated a selective, spatially confined heating effect that occurred at operative temperatures (59 to 66 °C) close to intermediate denaturation points of the stromal collagen, before its complete disorganization. No significant heat damage to the region of the laser-treated wound was evidenced in the operative irradiation conditions of corneal welding.
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Keywords: Tissue welding; thermal model; cornea; diode laser; collagen denaturation.

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1 Introduction

Laser welding of the cornea is a technique that has been proposed since 1992[1] as a replacement for or as a supporting tool to conventional suturing procedure in ophthalmic surgery. It has been used experimentally in cataract surgery for the sealing of the corneal cut, and it was applied clinically for the first time in a transplant of the cornea in lieu of the continuous suture.[2] The laser procedure showed remarkable advantages compared to conventional suturing: it reduces postoperative inflammations and foreign body reactions; it provides an immediate sealing of the wound, thus preventing endophthalmitis; and it improves the subsequent healing process, as demonstrated in previous works on animal models.[3,4]

So far, various lasers have been proposed for corneal laser welding. Most of them had emission wavelengths in the near-infrared (e.g., 1.455-nm erbium fiber laser,[5] 1.9-μm diode laser[6,7]) and in the infrared spectral regions (10.6-μm CO_2 laser[8,9]) that are characterized by limited optical penetration depths in the corneal stroma, due to the high absorption of the water content of the tissue. Our approach is quite different, being based on the use of near-infrared diode laser radiation at 810 nm in association with the topical application of a solution of Indocyanine Green (ICG) to the corneal wound to be repaired. This dye is characterized by high optical absorption around 810 nm,[10] while the stroma is almost transparent at this wavelength. Photothermal activation of the stromal collagen is thus induced by laser radiation only in the presence of ICG, resulting in a selective welding effect that produces an immediate sealing of the wound edges and good mechanical strength.

The laser welding technique is based on an essentially photothermal process: local heating due to laser irradiation induces structural modifications and/or denaturation of the collagen fibers, followed by the formation of new bonds and interactions with adjacent proteins.[11] It is still not clear which mechanism leads to these new bonds and what kind of bonds are involved in this process, since both strongly depend on the

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type of tissue and on the temperature reached during the process. What is well known is that laser exposure time and temperature distribution inside tissue play primary roles in inducing collagen denaturation and a closure effect with good mechanical resistance and minimal side effects.[12] In order to promote a comprehensive analysis of the laser welding mechanism, it is thus very important to account for the temperature enhancement during laser treatment inside the tissue, by describing the spatial and temporal evolution of the associated thermal process.

In this paper, we present an experimental and model thermal analysis of laser welding of the cornea. We develop a mathematical model based on the bio-heat equation[13] which is solved by using the Finite Element Method (FEM). The predictive accuracy is verified by comparing the post-processing description of the temperature behavior at the air/cornea boundary surface with the results obtained from an *ex vivo* experimental study in which we set up infrared thermocamera measurements of the temperature rise on the external surface of a porcine cornea during a diode laser-welding procedure. After this verification, the model is used to describe the temperature rise inside the corneal wound and to estimate the thermal damage in the irradiated area and in adjacent tissues.

2 Materials and Methods

2.1 *Laser Welding Procedure*

Experimental and clinical experiences on diode laser welding of the cornea[3,14–16] have indicated that, after staining of the wound edges with a high-concentration solution of ICG in sterile water 12% w/w, effective tissue closure was achieved at very low laser power densities, typically around $16.7\ \mathrm{W/cm^2}$ in porcine cornea, emitted by an 810-nm AlGaAs diode laser. Overall laser irradiation time was found to be about 200 s for a 25-mm cut length (i.e., the typical perimeter of a transplanted corneal button). Laser light was delivered to the external perimeter of the cut by means of contiguous spots obtained by keeping the tip of a 300-μm-core fiber at a distance of about 1 mm from the surface of the cornea, with an exposure time of about 2 s for each spot. The technique that we used to deliver light energy was the so-called side irradiation, performed by keeping the fiber tip at a small angle with respect to the corneal surface. In doing so, the laser beam was able to penetrate deeply into the stroma (which is transparent to the diode laser wavelength), in a direction almost perpendicular to the wound walls, thus producing homogeneous irradiation and a welding of the cut edges.

2.2 *Optical Properties of ICG*

In order to study the temperature rise in the wound volume, the absorption coefficient of the pharmaceutical formulation of ICG (IC-GREEN, Akorn, Buffalo Grove, Illinois), which is used to enhance laser thermal effect, had to be measured. The ICG absorption curve depends greatly on concentration and on solvent[10]; thus, the absorption curve may change when the solution is absorbed by the stromal collagen. *Ex vivo* measurements were performed in order to estimate the ICG absorption coefficient value in corneal tissue at the diode laser wavelength (810 nm) and to characterize the spectral behavior of the solution. A full thickness cut 5 mm in length was produced by a precalibrated knife in freshly enucleated pig eyes. The cut was stained with the ICG solution, the preparation was left in place for 2 min, and the wound was then washed with abundant water, as is done typically during surgical procedures. The corneal portion around the cut was excised, positioned on a quartz sample holder, and studied with a spectrophotometer (Model V-560, Jasco Corporation, Tokyo, Japan). The optical absorption curve of the ICG-stained cornea showed a maximum at 799 ± 1 nm, which matched the diode laser wavelength quite well, as shown in Fig. 1. A reference spectra of the nonstained cornea was also acquired. Assuming that ICG penetrated in a thickness of about 100 μm inside the corneal collagen at the wound edges, we used this measured curve to estimate an ICG absorption coefficient of $150\ \mathrm{cm^{-1}}$ in the corneal cut. This value was used in our FEM model.

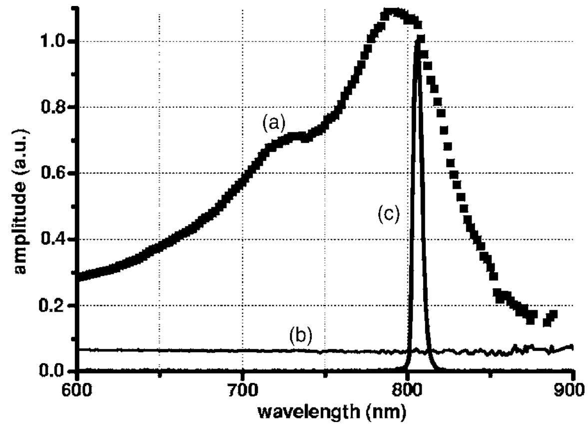

Fig. 1 Absorption curve of an ICG-stained porcine cornea (a) and of a native cornea (b). Diode laser peak wavelength is also shown (c).

2.3 *Infrared Camera Measurements*

Ex vivo measurements were performed on 15 porcine eyes obtained less then 6 h *post mortem* from a local slaughterhouse. Particular care was taken to enucleate the pig eyes before the usual washing procedure at 80 °C, as this could affect our measurements. A full thickness cut 2.75-mm in length was produced in each eye using a precalibrated knife in the periphery around the external perimeter of the corneal button. The cut was stained with the ICG solution and irradiated by laser light. The fiber tip (300 μm core diameter, with a numerical aperture of 0.24) was kept at a constant distance of 1 mm from the external surface of the cornea, as in typical surgical operations. A single spot was illuminated for 5 s, maintaining diode laser power at a constant value. Three different laser power emissions were tested: 60 mW, 80 mW, and 100 mW, corresponding to power densities of $12.5\ \mathrm{W/cm^2}$, $16.7\ \mathrm{W/cm^2}$, and $20.8\ \mathrm{W/cm^2}$ on the corneal surface, respectively. The intermediate value corresponded to the upper limit employed in surgical corneal laser welding. Two measurements were performed in each eye: (a) the three laser power densities were used to irradiate three different spots of the ICG-stained wound, and (b) the same irradiation conditions were used to illuminate a nonstained area, which was thus used as a control of the effect of the diode laser illumination on native stroma. During the measurements, the eyes were kept in a water bath at a constant temperature of 35 °C, and the posterior part of the eyeball was completely immersed in the water, while the cornea was kept in free contact with ambient air. This configuration simulated *in vivo* conditions and enabled direct measurement of surface temperature.

An infrared thermocamera (ThermoVision A20, FLIR Systems, Inc., Wilsonville, Oregon) was used to measure the temperature rise on the external surface of the cornea during the treatment. The camera was equipped with a 9-mm focal length germanium lens, which allowed a minimum working distance of 10 cm, resulting in a spatial resolution of 0.5 mm. The camera was controlled via computer, by the use of the ThermaCam Researcher Software. Once the object parameters were provided, such as the eye surface emissivity, direct measurement of the temperature was visualized and then stored for processing in the computer. The resulting thermal sensitivity of the system was 0.12 °C at 30 °C.

2.4 *Thermal Model*

The temperature rise inside the cornea during the laser welding treatment was evaluated by means of a model based on the solution of partial differential equations that describe heat transfer to the tissue and thermal propagation inside the cornea. The problem was solved with the FEM, by using commercial software (Comsol Multiphysics 3.2, Comsol AB, Sweden). The parameters and the coefficient values used in the model[17,18] are listed in Table 1.

A three-dimensional (3D) eye model was set up by assuming a spherical eye to be divided into three subdomains: cornea (7.0-mm inner radius and 7.7-mm outer radius), aqueous, and ICG-stained wound (positioned inside the cornea subdomain). Free convection at the interface with ambient air at room temperature was modeled. We supposed that the laser light propagated in a direction perpendicular to the corneal cut, as occurs in the "side irradiation" technique (see Fig. 2).

The bio-heat equation in space and time described the temperature profile in the eye:

$$\rho_n C_{pn} \frac{\partial T}{\partial t} - \nabla \cdot (k_n \nabla T) = Q, \tag{1}$$

where T is temperature (°C), t is time (s), ρ_n is density (kg/m^3), C_{pn} (J/kg°C) is heat capacity, and k_n (W/m°C) is thermal conductivity of the nth subdomain. Q(W/m^3) is the heat source term, i.e., Q is supposed to be null everywhere except in the ICG layer, being:

$$Q = \frac{\alpha P_0 \exp(-\alpha r)}{A_s}, \tag{2}$$

where α(m^{-1}) is the measured absorption coefficient of the ICG solution, P_0 (W) is the diode laser power output, A_s(m^2) is the spot area, and r (m) is the propagation length of the laser light. In Eq. (1), we omitted the heat source terms due to blood perfusion (the cornea is a nonvascularized tissue) and to metabolism (which is negligible, compared to laser heating).[17,19] The absorption coefficient was assumed to be constant during the laser treatment.

We considered that cornea, aqueous, and ICG-stained wound were at an initial temperature T_0=35 °C, while the air was at room temperature (20 °C). The optothermal parameters of cornea and aqueous were assumed to be the same. We modeled free convection at the cornea-air boundary (with a convection constant h_1=20 W/m^2°C) and at the cornea-aqueous interface (convection constant h_2=1000 W/m^2°C).[17,19] The other external boundaries were considered to be thermally insulated, while continuity was imposed between internal boundaries. Moreover, the following assumptions were adopted: thermal radiation emission at the tissue-air interface and reflection of laser light from the external surface of the cornea were disregarded; the thermo-optical parameters were considered to be constant during the process.

Calculations were also carried out to account for the heat damage occurring at the considered conditions of laser irradiation. By assuming that damage consists of the thermal denaturation of proteins, it is possible to estimate the damage function in terms of the Arrhenius integral[18,20]:

$$\Omega(\tau) = \ln\left[\frac{C(0)}{C(\tau)}\right] = \int_0^\tau A \exp\left[-\frac{E_a}{RT(t)}\right] dt, \tag{3}$$

where τ is the time duration of the heating treatment, $C(0)$ is the original concentration of undamaged tissue, $C(\tau)$ is the remaining concentration of undamaged tissue after time τ, R is the constant of gases, T is the temperature, E_a (J/mol) is an empirically determined activation energy barrier, and A is an

Table 1 List of model parameters and coefficients [Cornea parameters ρ, C_p, k, E_a, and ΔS are taken from the literature (Refs. 17 and 18)].

Parameter	Description	Numerical Value and Dimension
ρ	Cornea density	1062 kg/m^3
C_p	Cornea heat capacity	3830 J/kg °C
k	Cornea thermal conductivity	0.556 W/m °C
ρ_{air}	Air density (@ T=20 °C)	1.293 kg/m^3
$C_{p\ air}$	Air heat capacity (@ T=20 °C)	1005 J/kg °C
k_{air}	Air thermal conductivity (@ T=20 °C)	0.0257 W/m °C
α	ICG absorption coefficient (@ 810 nm)	150 cm^{-1}
P_0	Diode laser power output	60, 80, 100 mW
A_s	Spot laser	1.3 mm^2
E_a	Activation energy	106 kJ/mol
ΔS	Phase transition entropy	39 J/mol K
T_0	Cornea, ICG-stained wound, and aqueous temperature at t=0	35 °C
T_{air}	Air temperature at t=0	20 °C

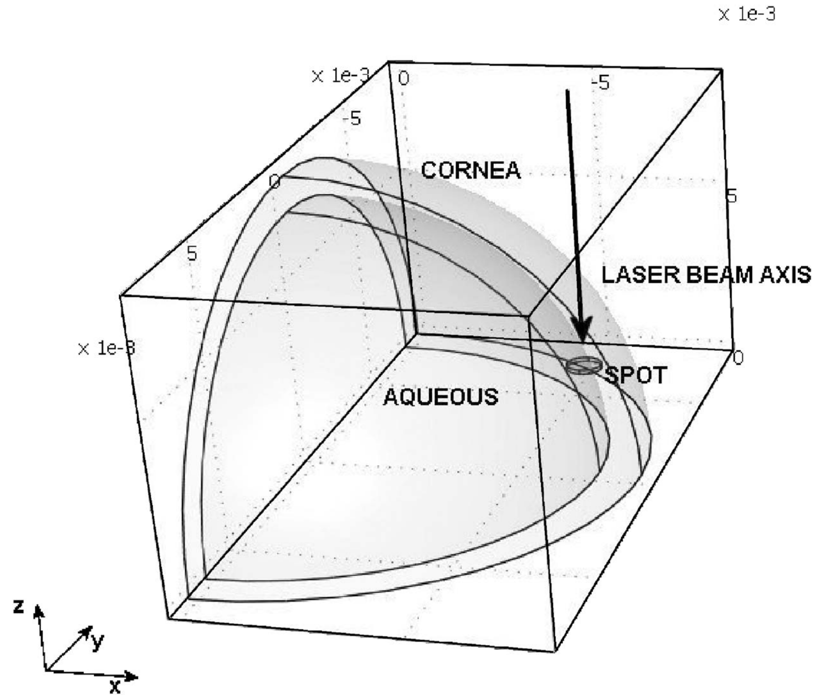

Fig. 2 Eye geometrical model during laser welding of a corneal cut.

empirically valued coefficient that is approximately:

$$A \cong \frac{k_B T}{h} \exp\left(\frac{\Delta S}{R}\right), \tag{4}$$

where k_B is the Boltzmann constant, h is the Planck constant, and ΔS (J/mol°C) is the entropy of activation. Values for E_a and ΔS used in the model are listed in Table 1.

An estimate of the Arrhenius integral thus gives an estimate of the damage induced in the tissue. Conventionally, $\Omega(\tau)=1$ indicates complete necrosis of the tissue and irreversible thermal damage.

3 Results

3.1 *Infrared Camera Measurements*

Measurements were first performed on nonstained porcine corneas: at a power density of $16.7\ \mathrm{W/cm^2}$ (the upper limit of clinical procedures) and after a 10-s exposure time, a temperature rise as low as $0.7\pm0.1\ ^\circ\mathrm{C}$ was recorded (averaged on 15 measurements) on the corneal surface. Slightly visible heat damage (evidenced by some tissue whitening) was found to occur when laser power greater than $41.6\ \mathrm{W/cm^2}$ was used and laser irradiation was maintained on the same area for longer than 10 s. The corresponding temperature enhancement was $\geqslant 30\ ^\circ\mathrm{C}$, as measured on the external surface.

In ICG-stained corneal wounds, all thermal images collected during laser irradiation showed heat confinement in close proximity to the irradiated area, as the heated zone radius was about twice the illumination spot, as measured at $16.7\ \mathrm{W/cm^2}$. For each laser power density (12.5, 16.7, and $20.8\ \mathrm{W/cm^2}$), 15 measurements of the temperature rise within the laser spot were detected. (Maximum values are reported in Table 2.) Averaged behaviors are illustrated in Fig. 3.

Table 2 Maximum temperature rise inside the ICG-stained cut and on the external surface, for different laser power densities and treatment times: calculated and measured data (mean value on fifteen measurements) and solutions of Arrhenius integral Ω (2 s) are reported.

	Calculated ΔT (°C) for 2 s Treatment Time		Ω (2 s)	Calculated ΔT (°C) for 5 s Treatment Time		Measured ΔT (°C) for 5 s Treatment Time	
	Inside the spot	On external surface	Inside the spot	Inside the spot	On external surface	Mean value on external surface	Standard deviation
$12.5\ \mathrm{W/cm^2}$	24.5	11.8	0.07	28.8	15.8	15.8	1.6
$16.7\ \mathrm{W/cm^2}$	31.2	14.2	0.19	38.6	18.9	18.4	2.5
$20.8\ \mathrm{W/cm^2}$	41.5	19.5	0.44	47.9	26.1	23.8	3.4

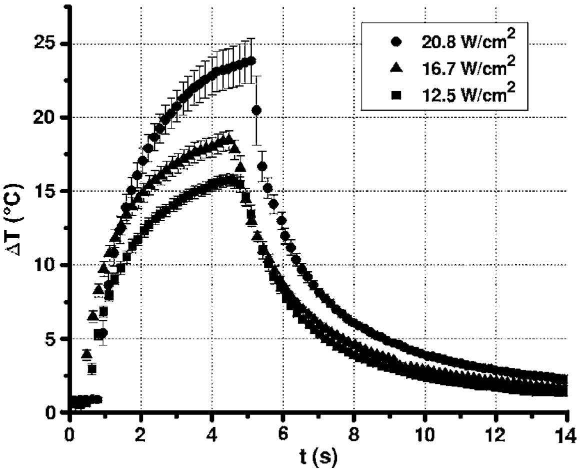

Fig. 3 Mean temperature rise measured on cornea external surface, for a 5-s single spot irradiation and three different laser power densities.

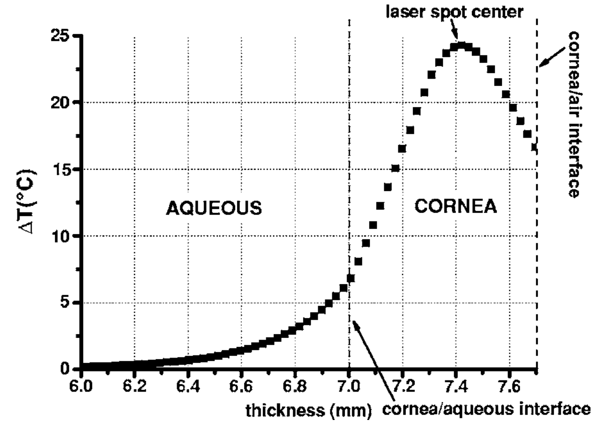

Fig. 5 Temperature rise distribution along radial distance from the center of the eye toward external ambient air. These calculated data refer to 12.5 W/cm^2 laser power density and 2-s treatment time. The ΔT maximum value is inside the cornea, in correspondence with the laser spot center.

3.2 *Thermal Model Results*

The bio-heat equation was solved by using the FEM. The results obtained for external corneal surface were compared with experimental data. There was excellent agreement between the experimental and calculated data (Fig. 4), confirming the assumptions made in the model. The model was thus used to study the temperature enhancement inside the wound, since it was impossible to perform direct measurement there. The analysis of the temperature distribution inside the eye indicated that the temperature radial distribution had a peak value located at about 250 μm from the corneal surface; it then dropped down toward the aqueous in about 1 mm (Fig. 5). Thermal damage due to laser irradiation was studied: the Arrhenius integral [eq. (3)] was calculated during laser treatment time for an exposure time $t=2$ s. The values (see Table 2) were found to be below the threshold of irreversible damage ($\Omega=1$) for clinically employed power density values.[20] The heat affected zone was well confined in the ICG-stained cut.

In order to find whether and when the heating process could reach saturation point during laser welding procedures, we applied the model to a longer exposure time of up to 50 s. In doing this, we assumed that ICG solution was stable under laser light. Calculated data showed that the induced temperature reached a plateau value after 10 s of irradiation (see Fig. 6).

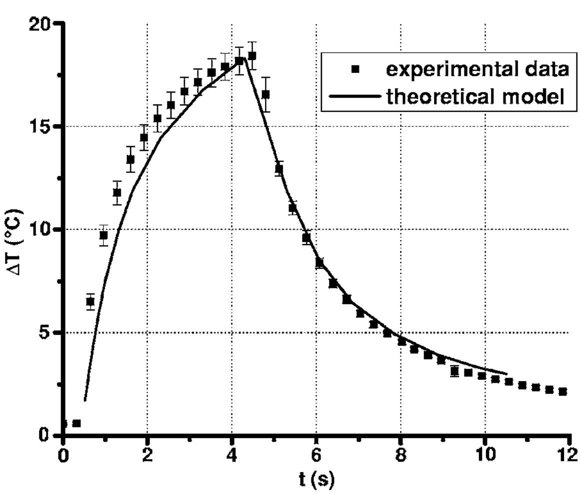

Fig. 4 Temperature rise of a laser-welded cornea external surface, calculated within the mathematical model (continuous line). Mean value experimental data are shown (symbols), as a reference. Calculations and data refer to a 5-s treatment time with 16.7 W/cm^2 laser power density.

4 Discussion

Experimental and simulation analysis of the temperature rise during laser welding of the cornea was carried out. Low power diode laser interaction with a porcine cornea was modeled, to reproduce the operative conditions of the laser-induced suturing of corneal wounds.[3] The present study enabled us to evaluate the temperature on the corneal surface in the region of the stained wound: we could compare the results

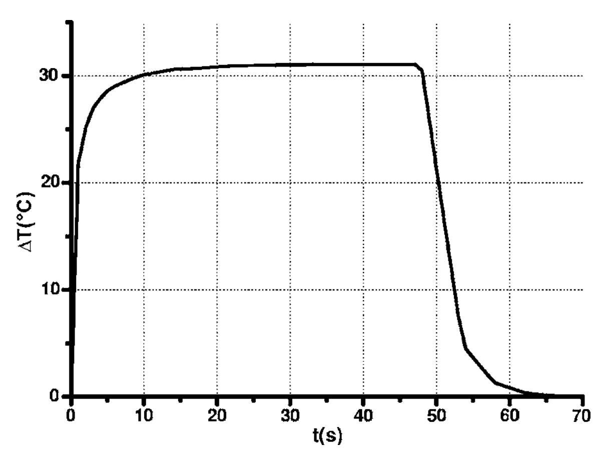

Fig. 6 Calculated temperature rise for a 50 s irradiation time of an ICG-stained cornea at a power density of 12.5 W/cm^2. Data refer to the maximum temperature inside the corneal tissue.

of experimental tests performed with an infrared (IR) camera, with simulations of the process, obtained by using the FEM. The results were found to be in excellent agreement, giving us the opportunity to characterize this particular thermal process on the whole and to study the effects of laser welding inside the tissue, where a direct measurement is unfeasible.

Both the thermocamera measurements and the predictive analysis showed a relatively modest temperature increase, which reached its maximum value inside the ICG-stained region. For a mean temperature of 35 °C in the living cornea, the maximum temperature peak due to photothermal interaction was in the 59 °C to 66 °C range, as calculated inside the stained wound for the operative irradiation parameters which, according to our experience, gave the best welding results in clinical procedures (i.e., laser power of 60 to 80 mW, corresponding to power density of 12.5 to 16.7 W/cm^2 in the irradiation spot, with 2 s of irradiation time on each laser spot). These temperature values were very close to the point at which early changes in the mechanical properties of the cornea can occur.[18,21] A consideration of the thermal denaturation processes of collagen thus has to be made. The main component of the corneal stroma is type I collagen, the temperature-dependent denaturation mechanism and melting temperature of which have been studied over the past few years. Experimental studies[18,21–24] have shown the presence of more than one denaturation point of type I collagen, which is associated with structural changes in the stromal fibers. In particular, differential scanning calorimetry (DSC) data[18] and second harmonic generation (SHG) imaging microscopy of porcine corneas,[21] supported by standard histological analysis, have pointed out that the collagen fibers of the corneal stroma undergo four distinct structural changes in the temperature range between 20 to 90 °C. The first change was found in the region between 30 °C and 45 °C and then at 53 °C, 65 °C, and around 76 °C. Researchers[21] suggested that, at these four denaturation points, a thermal disruption of stromal cross-links occurs, characterized by different order transitions, and that the reorganization of corneal collagen takes place in three distinct phases between 53 °C and 77 °C, resulting in a different strength of cross-links within corneal collagen. In this temperature range, collagen fibers are organized into bundles, which become denser as the temperature increases. The formation of these bundles, separated by cavities of a size that increases with the temperature, may be interpreted as being a consequence of the thermal disruption of cross-linking elements of the stroma. At temperatures higher than 80 to 90 °C, the collagen is completely denatured, and its structure is completely disorganized.

In view of this, our analysis indicated that an important factor for the success of laser welding procedures is the induction of structural modifications in native collagen fibers that correspond to low-temperature denaturation phases well below the limit of collagen disorganization. As was shown by our experimental observations and confirmed by the present photothermal model, temperatures near the second and third denaturation points are reached during typical surgery conditions (12.5 to 16.7 W/cm^2 laser power densities), and the objective result is an immediate sealing effect. When using higher laser power, higher temperatures are developed (e.g., about 77 °C with a 20.8 W/cm^2 power density); macroscopically, this corresponds to a poorer welding effect, with evident whitening and lack of transparency in the corneal stroma.

It should be noted that this behavior is a direct consequence of the association of ICG staining with 810-nm diode laser irradiation. The temperature dynamics would be completely different in the case of corneal welding induced by an infrared laser wavelength mainly absorbed by the water content of the stroma, without the application of any exogenous chromophore. It is clear that in such a case, the optical absorption would be localized in the proximity of the corneal surface, with a higher risk of superficial heat damage and lesser homogeneous temperature distribution in the corneal thickness. On the contrary, the application of ICG solution only in the cut edges resulted in a selective and localized welding effect, thus preventing temperature enhancement and, possibly, thermal damage in all those areas not stained with the ICG solution.

Direct thermocamera measurements on diode laser irradiation of unstained corneal tissue confirmed this figure. In the presence of ICG, our experimentally validated model indicated that the temperature rise was spatially well-confined in the ICG-stained wound and that local heat release was reliably controlled. As regards the latter, when solving the Arrhenius integral, we found that the fraction of damaged tissue was well below the threshold indicating irreversible thermal damage. This result confirmed previously reported studies on animal models:[3] microscopic observations and histological analyses never revealed the occurrence of thermal injuries in laser-welded rabbit corneas. A microscopy study on laser-welded porcine corneas is in progress: preliminary image analysis evidenced the absence of heat damage to the irradiated area.

When considering very long irradiation times (50 s) on the same spot, a constant temperature value was reached in about 10 s, indicating that ICG-mediated laser welding induced a heating process that leads to saturation. This dynamics of transient temperature is similar to the one studied in IR-laser irradiated collagen samples.[25,26] This behavior could be exploited in order to further increase the safety of the welding technique, by using even lower laser powers, but at the expense of longer application times. For example, a power density of 12.5 W/cm^2 could ensure that the temperature rise remains below 30 °C, but exposure times of 5 to 10 s per spot would be necessary in order to induce an effective corneal welding in each irradiated spot.

The cooling time history was also studied, showing that the cooling rate is relatively fast compared with the typical application times: in the case of retreatment, as sometimes occurs in operative conditions, the risk of heat accumulation is thus negligible.

5 Conclusions

This thermal analysis on the diode laser welding of porcine corneas has enabled us to quantify, in terms of space distribution and time evolution of the induced temperature rise, the encouraging phenomenological findings that we recorded in previous experimental and clinical studies regarding the effectiveness and safety of corneal laser welding procedures. In addition, the analysis could represent a starting point for forthcoming studies aimed at investigating, at a microscopic level, the mechanism of laser-induced welding of corneal tissue, in order to better characterize the structural modification that occurs near the second and third denaturation points of type I collagen (i.e., in the 59 to 66 °C range) under laser irradiation.

The practical consequence would be a more precise definition of the operative parameters, which could favor the diffusion of the clinical use of this procedure.

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